B Front page & List of Partcipants

Type of instrument **Integrated Project**

Proposal full title
High complexity peptide arrays: synthesis, read-out, management of data and applications

Proposal acronym **PeLaPri**

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Proposal summary page

Proposal full title
High complexity peptide arrays: synthesis, read-out, management of data and applications

Proposal acronym
PeLaPri

Research topic addressed
LSH-2002-1.1.1-1: Development of advanced array technologies

Proposal abstract
This project integrates interdisciplinary science from 7 countries (*among them 1 candidate state*) in order to achieve high complexity peptide arrays by new technologies developed for this purpose. The resulting system translates genomics data into physical products for scientific and industrial applications and will be commercialised by the SMEs involved. Combinatorial synthesis of peptide arrays is done with the help of high-resolution electric charge patterns and 20 different amino acid toners, i.e. solid particles that comprise the amino acids embedded into a "solid solvent". The charge patterns, which address these toner particles onto a solid support, will be generated by a 20-colour laser printer or a chip respectively. On the support, the amino acids are released simply by heating up the toner particles, which enables the amino acids to couple to the surface. Repeated coupling cycles result in the combinatorial synthesis of a high-complex peptide array, e.g. representing all proteins of a bacterial pathogen as overlapping peptides. In a model application these peptide arrays will be probed with sera from patients infected with pathogens in order to correlate the status of disease with peptide specific antibody staining. Read-out of binding events is done by labelling free detection based on a shift in the absorption spectra of colloidal nano gold particles upon reversible binding of ligands. Annotation and identification of specifically binding peptides is done with the help of the Swiss Prot database.

B.1 Scientific and technological objectives of the project and state of the art

Overview

	S&T objectives	State of the art
General	- physical products from genomics data - high complex peptide arrays - integration of labelling free detection - integration of data input and read-out - peptide/disease correlations	- genomics data - fragmented proteome data sets - oligonucleotide arrays - expensive peptide and protein arrays - low level of integration
Applications	- pathogene-peptidome arrays - Blood born viruses (hepatitis, HIV) - Respiratory viruses (influenza, RSV) - herpesviridae family (HSV1+2, EBV)	- hitherto inaccessible knowledge about viral peptides and antibody response correlated to patient's health status
Labelling free detection	- high degree of parallelisation - applicable to peptide arrays - very sensitive (low affinities)	- applicable to DNA arrays - not very sensitive - BiaCore: Low degree of parallelisation
Combinatorial synthesis of peptide arrays	- peptide array synthesis by means of dry biotoner particles: > 250 cm^{-2} - adapted Merrifield peptide synthesis	- peptide array synthesis by means of spotting technology: ~ 20 cm^{-2} - Merrifield peptide synthesis
Peptide laser printer	- 20 printing units - high precision printing by linear stages and optical alignment - automated washing unit	- laser printer technology established since 20 years - single pass technology and LED arrays (OKI)
Chip design	- array of pixel electrodes (~ 80 µm) - high voltage design (up to 90V) - integration of detection system	- conventional high voltage CMOS chip available - structures down to 10 µm possible
Data mining	- high quality proteome data sets - advanced, user-friendly analysis tools - dissemination by established and standardised public databases	- genomics data - proteome data sets available, but highly fragmented - data analysis not generally applicable - low level of standardisation

Table 1

S&T objectives, general:

Our objective is the development of a **platform that translates genomics data from databases into a physical product that allows for truly proteomics scale experiments in science and industry**. In order to achieve this, we want to develop a novel technology for the production of peptide arrays based on a new application of laser printing technique. A newly developed "peptide-laser-printer" or a chip respectively will be used to synthesise peptide arrays that assemble all proteins of a pathogen as overlapping peptides. In a model application these arrays will be stained by patient's antisera in order to find correlations of specifically recognised peptides to the status of disease. Read out of binding events is done preferentially by labelling free detection, especially in order to access low affinity binders. Proteome data sets and new data analysis tools will be provided by the Swiss-Prot group at the EBI. For data mining Swiss Prot database is employed. Applications of our "pathogen-peptidome-arrays" will address questions like:

- Which peptides corresponding to a pathogen's proteins are of diagnostic relevance?
- Is there a difference in pathogen-specific serum antibodies of patients with mild compared to severe symptoms, and if so which antibodies (which antigens) make the difference?
- Is there a difference in virus-specific serum antibodies of patients that cleared the virus compared to chronic infection, and if so which antibodies (which antigens) make the difference?
- Could we exploit this knowledge for fine tuning diagnosis?

S&T objectives, applications:

Dr. Shemer Avni (virologist, Ben Gurion University) will use her large collection of diagnosed sera in order to address these kinds of questions in viral models. We thereby expect hitherto inaccessible detailed knowledge about which viral peptides elicit an antibody response correlated to the patient's

health status and MHC polymorphism. Depending on the outcome, this data will help in fine diagnosis and prognosis of viral disease and / or hint to knowledge based design of viral therapy and vaccines. Viral diseases tackled this way will be the following:

- Blood born viruses: hepatitis B & C virus, and HIV.
- Respiratory viruses: RSV, adenovirus, parainfluenza 1, parainfluenza 2, parainfluenza 3, influenza A, influenza B, rhinovirus.
- Members of the herpesviridae family that cause persistence viral infections in humans: HSV1, HSV2, EBV and CMV.

Another scope of this study will be to apply the peptide array for screening of interactions between viral and host proteins. This information is very important for identifying host proteins targeted by viruses and understanding the pathogenesis of viral infections. In the future it will assist in the design of anti-viral therapy. Using the pathogen-peptidome array platform, the large collection of viral isolates from patients will be utilised as a source for structural and non-structural viral proteins to identify viral-host interactions. The various natural isolates from each virus type will enable us to gain information relevant to naturally occurring infections that is beyond small differences in subtypes of viruses. However, when the subtype of the virus is associated with the pathogenesis of the disease it will be included in the screening analysis. In addition, cloned viral protein will be employed (e.g. NS3, NS4, core and E2 of HCV) in order to compare to results obtained with peptide arrays.

S&T objectives, labelling free detection:

Antibodies and antigens are easily conjugated to colloidal nanoparticles of noble metals such as gold or silver through non-covalent ionic interactions. The changes in refractive index occurring at the surface of such probes upon biomolecular recognition of their binding partner, elicits a shift in the localised surface plasmon band in the absorption spectrum of the colloid, which can be exploited for the sensitive and direct read-out of the binding event (1). Moreover, the application of such principle allows for the real-time determination of the on- and off-rates of the interaction, i.e. allows a direct measurement of the affinity of the specific binding event (2). Label free detection with nanogold particles has been adapted to the DNA array format (3), but to date has never been applied to the read-out of protein or peptide arrays. Within this grant application, Dr. Englebienne (Free University of Brussels) will adapt this technology to the peptide array format. The ultimate goal of our research objective will be to gain the capacity to manipulate the nanocolloid conjugates characteristics so as to increase greatly their optical response to binding events. The final objective of the project is to design and build a small instrument within the forthcoming five years, in order to meet the demand of the market for user-friendly, labelling free, sensitive and reliable detection and analysis of binding events and affinities in peptide arrays. Emphasis will be placed in optimising the design of colloidal metal nanosensors for the transduction of biomolecular recognition into optical signals. To this aim, we plan first to study the relationship between both nanoparticle characteristics (i.e. size, shape, structure, type of metal, type of coating with peptides, number of conjugate layers), interparticle dynamic interactions (i.e. cluster size and structure, average density), and optical properties. In addition to the classical spectroscopic extinction (absorption) and surface plasmon resonance behaviour of the colloids, a new spectroscopic approach will be investigated, based on differential light-scattering (4).

Prof. Khlebtsov (physicist, Saratov State University), once again a known expert in the field, will contribute his expertise in optical detection of colloidal noble metal particles and bioconjugates needed for the signal detection in the array format (5, 6, 7). Besides his expertise in optics, Prof. Khlebtsov's group has also gained experience in the application of colloidal gold bioconjugates in solid-phased biomedical diagnostics, which complements the expertise of Dr Englebienne's team (8, 9).

State of the art, labelling free detection.

Labelling free detection using the surface plasmon resonance of colloidal gold is applicable to the measurement of a large range of affinities, including those of the hitherto hardly detectable low affinity binders (affinity < 10^{-6}M). Such high-throughput detection has been successfully applied in a clinical chemistry analyser (2). The technique has also been applied for the quantitative detection of a clinically relevant ligand in serum samples (10). However, such a system is only applicable to interactions occurring in solution, not at an array surface. As already mentioned above, noble nanoparticles have been successfully applied for biological detection (for recent review, see 11). Because such nanosensors have also been applied in solid-phased format for the detection of protein-protein (12) or DNA-DNA (13) interactions, it is reasonably expected that their application to peptide microarrays is an achievable goal. However, the development of current array applications didn't address in detail the optimisation of nanoparticle characteristics or optical detection. To date, there are no machines commercially available permitting the high-throughput determination of binding events by the colloidal gold labelling free detection on array surfaces. The BiaCore instrument, commercialised by BiaCore Inc. (formerly by Pharmacia) detects binding events by surface plasmon resonance on thin layer gold surfaces. However, the instrument accommodates for no more than 4 parallel determinations, which is a ridiculously low throughput when arrays are considered. Moreover, each experiment needs a careful optimisation of the reaction conditions because of the sometimes low accessibility of the ligand covalently bound to the sensor surface. Therefore, the detection system proposed in the present project offers a high level of inventiveness and fills a real gap in the currently available means for array detection and analysis.

S&T objectives, combinatorial synthesis of peptide arrays:

Combinatorial synthesis of peptide arrays is done by a modified colour laser printer or alternatively by a chip. The 20 different amino acids are embedded in a "solid solvent" (e.g. diphenyl formamide), thus producing 20 different toners. Electrostatic charge patterns, generated either by the laser printer or of the chip, address these toner particles (Fig. 1a) to a solid support. The amino acids are released simply by heating up the toner particles (Fig. 1b), which enables the amino acids to couple to the surface. Uncoupled material is washed away (Fig. 1c) with the terminal amino groups finally deprotected (Fig. 1d). Repeated coupling cycles according to the Merrifield synthesis result in the combinatorial synthesis of a peptide array. The novelty of this approach is due to the amino acid toner particles employed: heating up the toner particles changes the character of the solid toner matrix into a suitable solvent for a chemical reaction. A research report done by the European patent office, shows that this technology will be protected by a broad patent (patent applications DE19960346A1, EP1140977A2, US20020006672A1). Moreover a patent survey states the "freedom to operate", i.e. no other patent is expected to hamper commercialisation of this technology. This is of special

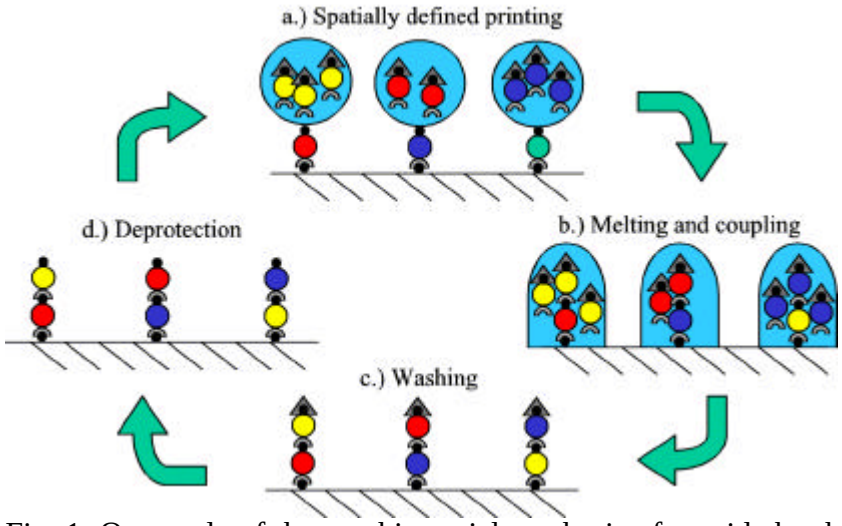

Fig. 1: One cycle of the combinatorial synthesis of peptide by the laser printer technology; repeating cycles lead to the complete array.

relevance for the commercialisation efforts to be headed by SMEs, given the fact that the promising field of array technology is mined by hundreds of patents.
The easiness and robustness of this method should allow for a significant improvement in the state of the art in this field. To date in the group of Dr. Frank Breitling (biochemist, DKFZ), 11 different amino acid toners were produced and successfully printed in high resolution by a normal laser printer. When heated for a few minutes, all of these printed toners lead to >100.000 spots in an area of 20 cm x 20 cm, with the mobilised amino acids coupled to the solid support in good yield and clearly separated from each other (Fig. 6). Since all the amino acids tried out so far could be incorporated into suitable toner particles, we expect to be able to synthesise >100.000 peptides in an area of 20 cm x 20 cm by the year 2004. With an ongoing improvement of toner particles done by experts in the groups of Prof. Knez (chemical engineer, University of Maribor) and Dr. Ose (electrical engineer, SME Tel-Tek), this complexity will be doubled every 18 months, with approx. 500.000 peptides synthesised in an area of 20 cm x 20 cm by the year 2007. Given the laser printer's unrivalled speed of addressing millions of pixels within seconds when compared to the spotting technology (see below), cost of array production should fall dramatically (>10x). This again should help in the commercialisation by SMEs.

State of the art, peptide arrays, protein arrays:
To date a maximum of 50.000 peptides can be synthesised in an area of 20 cm x 20 cm, based on the SPOT synthesis developed by Prof. Ronald Frank (14). These arrays are very expensive (approx. 6.500 € per 8.000 peptides, 15), mainly due to the slow spotting method employed. This contrasts with methods for the lithographic synthesis of nucleotide arrays, where much higher complexities are achievable (16). The complex chemistry involved hampered the initial efforts to adapt this concept to the synthesis of peptide arrays (17). Pellois et al. (18) solved this problem. However, the large number of coupling cycles needed in order to synthesise peptide arrays are still problematic (*each of the 20 amino acids has to be coupled individually to the solid support, i.e. 20 x 20 coupling cycles are needed for the synthesis of 20meric peptide arrays compared to only 4 x 20 coupling cycles for the synthesis of 20meric oligonucleotide arrays*). Another competing method is an ink-jet printer employed for the combinatorial synthesis of arrays. As to date, the ink-jet printer works fine for the synthesis of oligonucleotide arrays (19, 20). However, synthesis of high complexity peptide arrays with ink-jet printer has not been reported. This is probably due to the requirement for a relatively high viscosity solvent in peptide synthesis as well as basically the handling of liquids in the magnitude of few nano- or picolitres. In combination with the variability of 20 different amino acids this obviously clogs the piezo electric printing process employed in ink-jet printing. All the methods described are based on the 40-year-old Merrifield synthesis (21).
Protein arrays (22, 23, 24) are produced by spotting thousands of different recombinant proteins onto a solid support. Few of these very heterogeneous proteins retain their function during this process, moreover the amount of protein produced and spotted vary as well as the amount of contaminating material (*usually from E.coli*). These technical disadvantages contrast with advantages since larger protein fragments should retain more protein functions compared to peptides. Therefore we expect that both methods will complement one another in the future.

S&T objectives, peptide laser printer:
Dr. Güttler (physicist), Dipl. Ing. Henning (engineer), and Dipl. Inform. Hüttel (computer scientist) at the Fraunhofer Institute for Production and Automation (IPA) currently develop a peptide laser printer with 20 printing units, which will be able to print 20 different amino acid toners. The sample carrier is fixed to a high precision linear stage underneath the 20 different printing drums (see Fig. 2 and 8). The accuracy of moving the sample carrier relative to the printing drums and of synchronously controlling the LED units determines the positioning tolerance of the amino toner spots on the sample carrier. Since multiple layers have to be printed exactly on top of each other a small positioning tolerance (< 30µm) is crucial to synthesise the target number of peptides on the

carrier. The first instrument is currently under development and will be delivered by autumn 2003. A first proof of technical feasibility will then be possible. Objectives are to construct a second instrument having the following properties:

- **Increased printing accuracy**: While the first instrument is designed to print more than 100.000 peptides on a sample carrier of 20cm x 20cm, it is envisioned to increase the printing accuracy (positioning tolerance of the toner spots: < 20µm) in order to reach more than 500.000 peptides arranged in an array on the sample carrier by 2005.
- **Incorporation of a washing unit**: The chemical coupling and cleaning steps requires the development of the chemical reactor, with which the automated handling of the sample carrier and the efficient coupling of two process steps of essentially different speed (printing step: ~1-2min, washing step: ~ 40min) will be accomplished. The corresponding chemical reactor will be constructed by 2006.
- **Calibration**: While the first instrument is mainly intended to proof the technical feasibility and to experiment with, a basically easier handling is needed for the model application planned. Emphasis is placed on an easy calibration of the printer (necessary due to the high required accuracy) and a user-friendly control software, which both are planned by 2006.
- **Cooled nitrogen atmosphere**: Due to the sensitivity of the amino toner to oxygen, a cooled nitrogen atmosphere within the printer will significantly prolong the life of the expensive toner. This can be realised by 2005.

Together with the ongoing development of amino acid toners, this second instrument should be able to fulfil the milestones in the production of high complexity peptide arrays described above.

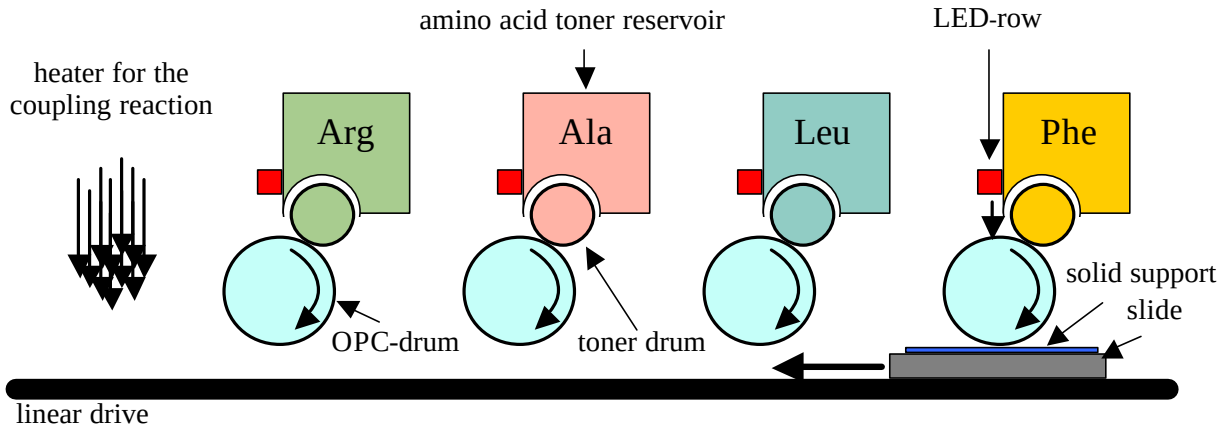

Fig. 2: Sketch of the peptide laser printer; only 4 of 20 printing units are shown

S&T objectives, chip design:

In addition to the laser printer described, a chip will be used in order to address the biotoner particles onto the chip's surface (Fig. 3). The use of the solid biotoner particles circumvents most problems that arise when dipping the chip into a solvent with the monomers dissolved within. With solid particles, there is neither flow of current in order to attract monomers to defined regions with its disastrous effects mediated by electrolysis, nor will the diffusion of monomers in liquids counteract the attraction to regions defined by the chip's polarisation. Furthermore, since the toner particles are directly deposited to the final location, there is no need for an alignment of the deposition with the existing peptide spots. Therefore, with this approach synthesis of peptide arrays with a spot diameter of approx. 80µm should be feasible by the year 2006. Initial experiments done in the groups of Dr. Breitling (DKFZ) and Prof. Lindenstruth (physics, University of Heidelberg) show that normal toner particles discriminate regions on an electrode's surface with only 30V applied as a difference in potential. Still that means that the chip has to be manufactured in a

commercial high-voltage CMOS process (up to 90V potential applicable), as it is used e.g. for automotive applications. Further advantages of the peptide chip approach are

- only very low quantities of chemicals are required
- high yield of peptide arrays due to the absence of mechanical alignment
- low risk of contamination due to the absence of mechanical interaction and the possibility of a self-contained implementation
- identification of array contents by chip ID or on-chip memory

Another objective will be a chip design that incorporates the read out of binding events by the year 2007. Underneath each electrode used in order to attract the solid amino acid particles, a photodiode will be incorporated. Thereby, light emitted by a labelled probe (chemoluminescence) will be easily attributed to a specific binding peptide. In addition our objective is the construction of a small instrument for the automatic synthesis of peptide arrays within the forthcoming five years, accommodating the chips and different toner reservoirs mentioned above.

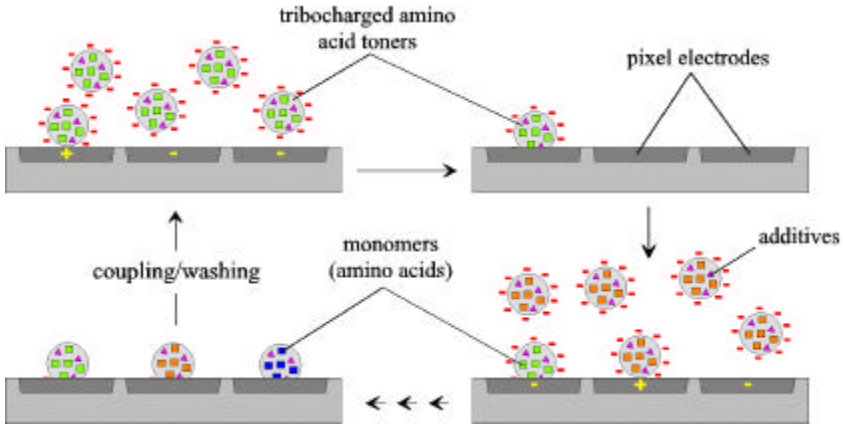

Fig. 3: Biotoner particles are addressed to the chip surface

State of the art, chip-design, laser printer:

To date, high voltage chip design (up to 90V applicable) enables electrode structures down to 10µm or less to be produced, well below the structures targeted within this grant proposal. The group of Prof. Lindenstruth (University of Heidelberg) has this expertise as well as for the design of chips with an extra array of photodiodes for in-silicon-read-out of binding events. The main tasks of this work will be to test and adapt consecutive chip designs for their selectivity in addressing biotoner particles and their ability to survive in the chemical surroundings needed for the process of combinatorial synthesis, binding of probe and read out.

The development of xerography (copiers) started in 1950. Laser printers are available now for about 20 years. Based on the recently developed single pass technology for colour laser printers (OKI C7000 series: The toner is directly applied to the paper, not to a subcarrier usually made from rubber), the Fraunhofer IPA currently develops a peptide printer suitable for the project as described. As conventional laser printers are not designed for printing several layers of toner particles one upon another with micrometer resolution, this is an essentially new application of this technique, requiring a construction of the printer different from commercial laser printers.

State of the art, combinatorial synthesis onto a chip's surface:

Southern (25) used an array of electrodes in order to remove tBoc protective groups at spatially defined acidified regions by means of electrolytic generated acids. Heller (26) used an array of electrodes on a chip surface in order to attract or repel monomers for combinatorial synthesis from solution (27). In both cases the liquid solvent used poses problems: The smaller the dimensions of individual electrodes, the more diffusion of monomers counteracts attractive or repellent forces. Moreover, acids and bases produced by electrolysis tend to destroy the material employed. Solid particles with the amino acids immobilised within circumvent these problems: Unless mobilised by heat diffusion of monomers does not occur and due to merely electrostatic interaction no current flow endangers the material employed.

S&T objectives, bioinformatics:
The bioinformatics packages in this proposal are focussed on three aspects:

- Experiment support by provision of high quality proteome data sets,
- Results analysis by construction of advanced analysis tools, and
- Dissemination by integration of results into established public databases.

The Sequence Database Group at the EBI already provides high-quality proteome data sets (28) for completely sequenced organisms. These sets include known alternatively spliced sequences and will be the basis for the definition of the peptide arrays for the organisms of interest. The normal Swiss-Prot curation activities will prioritise annotation of organisms of interest to the project. The result of the peptide array binding experiment will usually be protein sets, e.g. differentially binding proteins between two states of a system, e.g. patient versus control serum. The correct interpretation of these protein sets is very time-consuming, because the relevant knowledge is often distributed over many databases. The PREJUDICE system (Proteomic REsults JUdgement, Interpretation and Control Environment) will improve the efficiency of the analysis of results by providing optimised global and detailed views of the publicly available knowledge of individual proteins and protein sets. To facilitate the analysis of proteome sets in addition to individual proteins, we will create integrated views of these protein sets, emphasising shared properties, subclusters and potentially significant outliers. Finally, the integration of analysis results into the proteomics databases of the EBI Sequence Database Group will ensure a wide dissemination of these results and their availability to the scientific community beyond the duration of the project.

State of the art, proteomics databases, analysis, and dissemination:
Proteome Data sets: The Proteome Pages (28) and the accompanying proteome sets provide high quality, manually curated protein sequence data from Swiss-Prot, completed by automatically annotated protein sequences from TrEMBL and represent excellent reference sets for the definition of complete proteomes.
Proteomics data analysis: The advent of high-throughput technologies in proteomics, for example large-scale yeast-two-hybrid experiments, advanced methods for subcellular localisation determination, and quickly improving resolution and reliability of protein identifications through 2D gels, LC-MS, and protein arrays, provides a wealth of data of very different data types and data qualities. Data integration has become a key bioinformatics activity. In addition to the established manual, high quality curation of database entries, e.g. in Swiss-Prot (29), automated and partially curated approaches try to integrate data from different data sources, e.g. Genecards (30), the Proteome Pages (28), or RefSeq (31). These resources facilitate the retrieval of information on individual biological entities, but they do not support the analysis of protein sets according to user-defined criteria.
Many data mining and clustering tools are well-established, and often are available as web tools. Unfortunately, they are normally general tools, which require a lot of specific data preparation, e.g. the C4.5 algorithm (32), or are specialised towards a specific data type, e.g. microarray data (33). Currently, no publicly available tools allow the analysis of user-defined protein sets according to a broad variety of categories, from GO terms to promotor sites in the genomic upstream region, taking into account publicly available and user-supplied data.
Proteomics data dissemination: Due to the highly diverse nature of proteomics data and experimental technologies, the publicly available proteomics data, including protein and peptide binding data, is highly fragmented and hard to access. In addition to the curation efforts of established databases like Swiss-Prot, several efforts are currently being undertaken to define standards for the formatting and archiving of proteomics data, in particular the HUPO Proteomics Standards Initiative (34), PeDRO (35), and BioPAX (36). Building on the MGED (37) experience, the EBI is the driving force of the HUPO Proteomics Standards Initiative and is collaborating with both the PeDRO and Biopax initiatives. It is expected that proteomics data will become significantly more standardised during the runtime of this project.

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B.2 Relevance to the objectives of the LifeSciHealth Priority

Areas concerned

This grant proposal has its clear "centre of gravity" within the priority thematic area of research LSH-2002-1.1.1-1: Development of advanced array technologies. This is exactly what we plan to do, based on the novel idea to use solid particles that are simply melted after printing in order to activate amino acids hitherto immobilised within the particles. Combined with expertise in engineering, data mining and sophisticated labelling free detection methods, this should drastically improve the state of the art in the field, as shown by the achievable spot densities even of our first generation toners (Fig. 6). Thereby our focus is on the delivery of advanced array technologies for the analysis of large sets of proteins in the form of overlapping peptides with high precision and sensitivity, as requested by the call. Our project should help to fulfil the strategic objectives of the LifeSciHealth priority:

- It fosters the basic understanding of genomic information by translating genomics data into peptides by a novel tool, the laser printer,
- It develops the knowledge base by the use of these peptide arrays, e.g. for the exploration of the interaction of the human immune system with the proteins of pathogenic bacteria or viruses,
- It reduces the resources needed to decipher the function of genes and gene products relevant to human health and their interactions with each other by drastically reducing the cost for production of high complexity peptide arrays and
- It is intended to develop new and improved nanosised materials for application in novel diagnostics.

Through the model application planned (*deciphering the interaction of the human immune system with the proteins of pathogenic viruses by a high throughput proteomics technology*), our grant application also falls within the range of LSH-2002-1.1.1-2: Development and application of high throughput proteomics technologies for the generation of a large data set of protein-protein interactions. As requested by this call, our focus is to develop and apply high-throughput proteomics technologies (*arrays with >100.000 peptides*) for the identification of protein-protein interactions in complex biological samples by using patient's antibody sera.

Contribution to the scientific, technical, wider societal and policy objectives of the LifeSciHealth Priority

A huge set of genomics data is at hand, but to date no tools exist that would translate this data into an adequately large set of physically available proteins or peptides (see B1, state of the art). The integrated project applied for brings together a critical mass of resources and expertise (see below, B3) to reach the ambitious scientific and technical goal aimed at providing such a tool. Engineers, experts in bioinformatics, data mining and read out systems will add user friendly features that yield maximised information through integration when doing experiments in proteomic's scale (horizontal integration of a range of multidisciplinary activities). Within the project applied for we envision the construction of instruments doing automated synthesis of high complexity peptide arrays. The user will be able to define peptide sequences to be synthesised, e.g. fed in with the help of databases, thereby e.g. translating the whole genome of a pathogenic bacterium into its proteome corresponding to a set of overlapping peptides. Probing these "peptidome" arrays" with a patient's serum might hint to differential diagnosis and prognosis (*e.g. why do some patients clear the virus, others don't?).* We envision an automatic and sensitive read out within this instrument that preferentially relies on labelling free detection in order to find low affinity binders, compare affinities and reduce experimental variety through obviating of labelling. The very sensitive method chosen by us already works with DNA arrays. For subsequent data mining once again a relay to databases will be incorporated.

Besides its scientific impact, the technology employed is expected to be cheap, which should help in the commercialisation of the technology by the SMEs taking part in the consortium at a later stage. Therefore sectoral integration of actors from academia and SMEs and vertical integration of those involved in knowledge production through to technology development and transfer is envisioned within the project. This should lead to dissemination of this technology throughout Europe and might give industry a leading edge in "integrating post-genomic research into the more established biomedical and biotechnological approaches" (Commission's statement). Thereby the two main strategic objectives of the Commission are met:

- Strengthening the scientific and technological bases of industry and
- encourage its international competitiveness.

As a result of dissemination, we expect that our technology for translating genome data into practical applications might lead to an improvement of patient-oriented strategies (see B3, better diagnosis, cheaper diagnosis, novel therapeutics and antibiotics, research in the causes of disease).

B.3 Potential impact

Impact in reinforcing competitiveness

We plan to develop a novel tool that translates the huge but to date only virtual genomics data into a physical product: peptide arrays that represent e.g. all a pathogen's proteins. Only with tools like this post-genomic research can be integrated into the more established biomedical and bio-technological approaches by testing scientific hypothesis on a proteomics wide scale. As to date wider applications of proteomics technologies are not only hampered by complexity but also by the prohibiting costs, the tool mentioned should be easy to handle, including a low priced production. Combinatorial synthesis of high complexity peptide arrays could solve these problems. Based onto the routine Merrifield synthesis, the established laser printing technology, elaborated chip design methods and a sensitive method for labelling free detection, we think that our research will strengthen the competitiveness of the European economy in one of those areas "where the EU in the medium term intends to become the most competitive and dynamic, knowledge-based economy in the world capable of sustainable economic growth with more and better jobs and greater social cohesion" (Commission's statement). With the SMEs involved, a dissemination of this comparatively cheap technology to larger industry and to the scientific community is planned, with its preferential partners naturally in Europe. Therefore this technology might give "Research and Technological Development (RTD) in Europe as an essential element in the functioning of industrialised countries" (Commission's statement) a leading edge over its main competitors.

Impact on solving societal problems

We expect a whole range of future applications that will help in solving major societal questions, among them the battle against some diseases who's causes are still unknown. To date, nobody knows the cause of e.g. Parkinson's disease where dopaminergic neurons are specifically targeted and killed. One hypothesis would argue for an autoimmune reaction involved – which could be tested on a large scale with our high complex peptide arrays representing all human proteins. Compared to protein arrays, our peptide arrays should prove especially helpful in the analysis of T-cell reactions. If feasible, the readout of specific T-cell epitopes e.g. would have a huge impact on rational design of vaccines especially in the combat against malaria, tuberculosis and viral diseases. When testing a patient's immune reaction, we will pay special attention to childhood diseases, because here the clearest signals are expected (*less variety in the immune response*).

Other applications should lead to an improvement of patient-oriented strategies, e.g. when we learn about the difference in the immune response of patients that clear the virus compared to chronic disease. Also the outcome of bacterial and parasitic infections quite often is very different – our peptide arrays might read the difference in the immune response (antibodies and / or T-cells) in a truly large scale. Examples are infections by *Borrelia*, *Plasmodium*, *Mycobacter* and HCV where partial or complete immunity is known to occur. Thereby we might find hints for the design of better vaccines, for better diagnosis and for better prognosis with a potential impact on societal problems.

Yet another field where an impact on societal problems is expected will be the development of complex diagnostics. We envision an array assembling >100.000 peptides which initially were singled out due to their binding to one or a few from >1.000 patient's sera randomly chosen. Depending on the number of patient sera used for selection of peptides, within these >100.000 peptides most major (and some minor) diseases should be represented by peptides of diagnostic value. Physicians could probe "their" patient's sera with these arrays and learn to correlate diagnostic peptides with disease. Thereby many different diseases should be diagnosed by a simple and cheap array, which would be especially helpful when the underlying cause of the patient's illness is not clear. Once again maybe enigmatic diseases could be targeted by these arrays, provided these are the result of mixed causes. If one of these causes (*e.g. an otherwise harmless infection*) would be represented by the peptides of potential diagnostic value just mentioned, we should be able to find it.

Societal questions to be solved	How to be solved by the project
autoimmune reactions (e.g. involved in parkinson's disease?)	human peptidome arrays
rational design of vaccines (e.g. malaria, tuberculosis and viral diseases)	analysis of T-cell reactions, specific T-cell epitopes
improvement of patient-oriented strategies through differential diagnosis and prognosis (e.g. *Borrelia*, *Plasmodium*, *Mycobacter* and HCV)	pathogen peptidome arrays for antibody response
complex diagnostics	high complex arrays with selected peptides of diagnostic value
enigmatic diseases, mixed causes	complex diagnostics

Table 2

Overall innovation aspects

Besides or in combination with the translation of genomics data, the technology described could be used for the screening of kinase or protease substrates as well as for the screening of protein-protein and cell-peptide interactions. It is neither limited to L-peptide arrays nor to applications within the field of life sciences and health (Fig. 4). High complexity arrays of D-peptides could be used in a direct screen for leads, i.e. small molecules with potential therapeutic value (*e.g. D-peptides binding to HIV protease; compared to L-peptides D-peptides are less likely to be destroyed inside the body*) and for antibiotics. In the latter case, hydrophilic patches within a hydrophobic surrounding could accommodate small droplets of bacteria and patch-specific peptides, which might be released by light. If one patch-specific D-peptide mixture kills these bacteria, a potential antibiotic would have been identified. Even the screening for novel catalysts by incorporation of metal centres might benefit from peptide arrays. The resulting arrays of macromolecular bioinorganic assemblies have all the ingredients a potential catalyst should have: Metal ions, atoms or clusters eager to shovel electrons co-ordinated within many different protein-like structures. Even more distant applications are conceivable, with just a few to mention:

- Combinatorial printing of ceramic toners by a laser printer in order to screen for novel materials,
- Rapid prototyping of 3-dimensional structures in µm scale and
- Printing of organic chips onto polymers or paper.

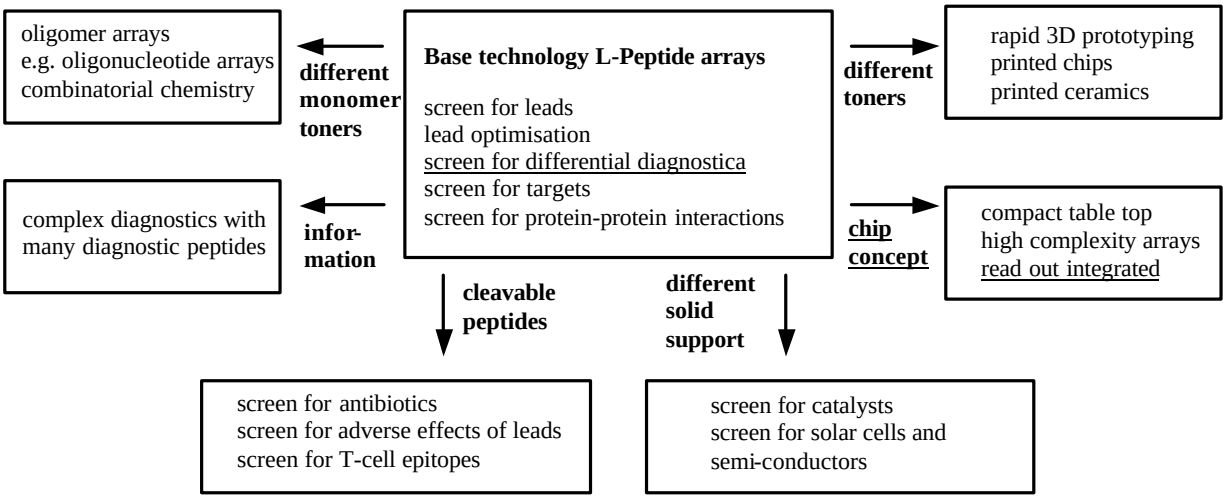


Fig. 4: General potential of the peptide array technology: Applications applied for in this grant are underlined

Exploitation and dissemination plans

The project planned builds upon segmented prior national activities (Table 1), that now are combined at the European level in order to add value. Each partner will be free to exploit previously existing knowledge and intellectual property on its own. However, there is a strong incentive of added value when combining successful activities into an integrated product. If possible, the partners involved will patent these values. Therefore, the consortium will agree and establish a transparent Intellectual Property (IP) policy that will promote careful consideration of the patenting and licensing of inventions or know-how before public disclosure (see B.4.1). However, as might be expected in such a competitive environment, outstanding 3rd party inventions are likely to occur. In order to accommodate for this, partners will be free to use their knowledge in combination with partners from 3rd parties, generally with the incentive of more value added (*e.g. use of different arrays / different methods for labelling free detection / different chips*). This last point is also very important in order to accommodate for conceivable difficulties arising in the realisation of one of the individual activities (*e.g. low sensitivity in labelling free detection*). After a three years period with the emphasis on science and development of technology we plan to integrate additional SME partners that will commercialise particle based array technology, the construction of machines as well as labelling free detection methods.

The SMEs will license previously existing intellectual property from the partners and intellectual property possibly originating from this integrated project. The SMEs mainly will concentrate on the construction of prototypes and on demonstration activities needed for successful commercialisation. Within this grant application, 11% of the funding allocated is foreseen for these SMEs, thereby bringing basic knowledge through to the application stage ("translational" approach), as repeatedly stated as the Commission's aims. Besides their financial involvement through demonstration activities, the SMEs chosen by Participants 1 to 9 to be added as well as Tel-Tek will drive the marketing and distribution of the technology and thereby ensure a mature perspective on the exploitation of the project results in form of new methods and materials. The benefits expectable were described in detail above, with the unique selling proposition given by the fact that to date no other technology rivals the one proposed here (see above B1, state of the art peptide arrays).

It is also essential that stakeholders and the diagnostics industry are fully informed of new intellectual property, its technological impact and how the new science can be brought to near-market. Therefore, we will set up a "User Industry Interface". This key node will interface with large industrial users and SMEs. It should be noted that at the companies level, there is a strong incentive to combine interdisciplinary expertise in order to benefit from a unique selling proposition mentioned above. However, since most single aspects of the technology described can be combined with aspects beyond this activity (*e.g. other arrays combined with labelling free detection*), all the actors will remain independent from each other.

This multinational collaborative will also serve as a platform for educational excellence, whereby the expertise of each participant will be disseminated throughout the co-operative. Obviously, this has many advantages. Programs that may be weaker in the field of e.g. combinatorial synthesis or engineering will be able to learn processes and have access to technologies. These same individuals can work to educate other groups who may not excel in labelling free detection or bioinformatics. This is the model used by many in business, where seminal ideas spring from a new found scientific knowledge and new insights into developmental processes, when each member of a team gain an understanding of many roles in the process. Therefore, we aim to arrange an at least annual meeting of the project's participants.

New scientific knowledge arising from the research will be disseminated in the primary literature and presented at national and international conferences. In addition to scientific publications, patent applications are aimed at as a matter of course. Especially for Activity 7 (Database), dissemination of knowledge is intrinsic because 3rd party users will access improved databases and analysis tools without any restrictions. As stated above, especially SMEs will exploit the results with a strong emphasis on the sale of arrays (*and machines yet to be developed*). Thereby customers (*scientists in*

industry and public science) will be free to use this technology in order to investigate genomics and proteomics data for their special purposes. Dissemination of technological knowledge with respect to commercialisation will also be ensued by means of workshops, that will be accomplished at national and international conferences, e.g. the annual meeting of HUPO or the "International Meeting on Proteome Analysis" in Munich.
As mentioned above (B3, Overall innovation aspects) more distant applications, e.g. of the 3D laser printer technology are conceivable, which are not covered by the patent application EP1140977A2. Therefore, an extra incentive for collaboration of partners is given within this project, because the partners closest in expertise surely will lead dissemination of applications beyond this grant application (*e.g. promotion of rapid prototyping of 3-dimensional structures in µm scale will be lead by Fraunhofer IPA*).

Added-value in carrying out the work at a European level
Our interdisciplinary approach is mirrored by the expertise of the participating scientists (virology, biochemistry, surface chemistry, combinatorial chemistry, physics, laser technology, optics, particle/powder technology, microelectronics, engineering, informatics, bioinformatics) from seven European countries (Germany, Norway, Slovenia, Belgium, Russia, UK, Israel). In order to harness the benefits from our novel array technology, these different expertises, originally funded and developed in different European countries, have to be integrated into one single project. Through the combination of high complexity peptide arrays, sophisticated read out technologies and bioinformatics tools, added value is achieved. None of these pieces alone would translate genomics data as efficiently as combined and thereby exploit the full potential of genome information with its applications to human health. Moreover, at national or regional level it would be difficult to find the whole range of expertise needed for the activity planned. Given the Commission's statement, that integrated multidisciplinary research, which enables a strong interaction between technology and biology, is vital in LifeSciHealth priority for translating genome data into practical applications, we consider our project as paradigmatic in respect thereof. Furthermore, we think that spreading the underlying concepts into the European research community will speed up applications of our technology not only in science, but also in industry and in a longer time scale in society (*e.g. through implementation of complex diagnostics*).

The account taken of other national or international research activities
The project proposed is based upon segmented prior national activities that now are combined at the European level in order to add value. The manpower within the subgroups directly involved and collaborations within the institutions involved contribute considerably to the research activity planned. According to a conservative esteem, the activity applied for will be embedded into a surrounding of >100 scientists within the subgroups directly involved and >200 scientists when considering the departments involved.

B.4 Outline implementation plan

The S&T objective of our approach is to integrate research and knowledge in different fields of expertise to reach the ambitious goal of a user-friendly novel tool that translates genomics data into high complexity peptide arrays with the help of databases, a tool that automatically reads out (*preferentially by labelling free detection*) and annotates specific binding events. The model application planned is the large scale deciphering of virus-specific immune reactions and the correlation of this data to the patient's immune status and prognosis. The different components needed for the implementation of this goal align themselves according to the expertise of the scientists involved. These components are grouped into larger entities (activities), which will function on their own in their majority, with the incentive of added value when combining the activities into a truly integrated project.

Activity 1 (Model applications, Ben Gurion University) comprises the different components needed for the model application planned. Instrumental in this activity is the large collection of carefully diagnosed sera Dr. Shemer Avni (Ben Gurion University) already has at her disposal. She will go on collecting these diagnosed sera and annotate whenever possible to each serum the patient's immune status and prognosis. In close coordination with Activity 7 (database), Dr. Shemer Avni will design the pathogen specific peptide arrays to be synthesised in Activity 6 (Peptide arrays). These arrays will be stained with the patient's sera in order to correlate staining patterns to immune status and prognosis (**Component 1-1, Pathogen peptidome array, Ben Gurion University**). Dependent on the output of peptide arrays achievable by the partners, we will identify dominant vaccinia epitopes within **Component 1-2 (Vaccinia epitopes)**. These epitopes will be identified with vaccinia peptidome arrays stained with the serum of immunised persons. Within **Component 1-3 (Pathogen- host interactions),** pathogen peptidome arrays are stained by labelled host proteins in order to identify viral peptides and thereby the corresponding proteins that interact with host proteins. Virus-targeted-host proteins can be further analysed by MALDI.

Activity 2 (Particle production) comprises different methods for manufacturing, analysis and testing of amino acid toners. In order to adapt the particles thus produced, iterative manufacturing and testing of particles within a commercially available laser printer (or with a chip) as well as conventional particle analysis is necessary. Formulation of amino acid toners and production of crude particles by mechanical milling is done within **Component 2-1 (Particle formulations, DKFZ)**. In accordance with the other components of Activity 2, particles are always tested within a laser printer (printing of particles, coupling of amino acids, achievable complexity). More sophisticated procedures for toner production are adopted in **Component 2-2 (Particles by RESS, DKFZ):** Fabrication of amino acid toner particles by RESS procedure (*rapid expansion of supercritical solutions*) and by air mill; **Component 2-3 (Particles by milling, Tel-Tek):** Fabrication of amino acid toner particles by milling and air classification. Estimation of charging by carriers or by direct charging and **Component 2-4 (Particles by PGSS University of Maribor):** Fabrication of amino acid toner particles by PGSS procedure (*particles from gas saturated solutions*).

Activity 3 (Peptide laser printer, Fraunhofer IPA) comprises the construction of a laser printer suitable for peptide synthesis, which is based on a crude first instrument developed before this grant application (*prospective delivery in summer 2003*). Within **Component 3-1 (Printing accuracy), Fraunhofer IPA** will improve the printing accuracy. A washing unit is incorporated into the instrument within **Component 3-2 (Washing unit, Fraunhofer IPA)**, while in **Component 3-3 (Calibration of the printer, Fraunhofer IPA)** the emphasis is on the calibration of the mechanical and electronic components of the printer to ensure a more precise positioning of the sample carrier relative to the printing drums (*needed for more accurate printing*). **Component 3-4 (Cooled**

nitrogen atmosphere, Fraunhofer IPA) finally comprises a cooled nitrogen atmosphere, which is needed due to the sensitivity of some toners to oxygen and water.

Activity 4 (Chip design, Kirchhoff Institute) comprises different chip designs. It further includes the mechanics and electronics required for test and application. It also includes a chip instrument device used for delivery of particles to the chip's surface. In **Component 4-1 (High voltage design, Kirchhoff Institute),** a high voltage design (up to 90V) and manufacturing of chips is envisioned in order to specifically address amino acid toners to the chip's surface. **Component 4-2 (Chip instrument, Kirchhoff Institute)** will be an electromechanical set-up accommodating the chips developed as Component 4-1 to demonstrate the ability to selectively and semi-automatically coat the chip electrodes with different toner sequences. A more sophisticated chip design is envisioned within **Component 4-3 (Nano gold chip design, Kirchhoff Institute)**, which will be combined with colloidal nano gold particles on the surface for label free detection (in cooperation with Activity 5). **Component 4-4 (Photodiode chip design, Kirchhoff Institute)** will include photodiodes arrayed underneath the defined surface regions. It will be implemented in deep sub-micron (0.35µm) technology, if the preceding feasibility studies turn out positive.

Activity 5 (Read out methods, University of Brussels & Saratov State University) comprises the development of methods and instruments for the detection of binding events. With the emphasis on labelling free detection, this is basically done by binding unlabelled ligands to colloidal nano gold particles, which leads to a very sensitive shift in the wavelength absorbed. Based on already existing expertise, within **Component 5-1 (Nano particles in solution, University of Brussels)** the development of colloidal nano gold or silver particles for labelling free detection in solution will be improved, thus serving as a reference for labelling free detection in the array format. In **Component 5-3 (Nano particles in array format, University of Brussels),** colloidal nano gold particles will be deposited onto a flat surface in order to accommodate our labelling free detection method to the array format. Therefore, particles of various sizes and shapes will be synthesised and evaluated for their optical properties in collaboration with Components 5-2 and 5-4. **Component 5-2 (Principles plasmon resonance, Saratov State University)** is aimed at the development of basic principles that determine parameters of plasmon resonant particles in the array format. Knowledge of these parameters is instrumental especially for a sensitive read out of binding events. In cooperation with Components 5-1 and 5-3, within **Component 5-4 (Nano particle labels, Saratov State University)** new technologies are developed that include the synthesis of nano particle labels with given properties and of markers with narrow size distribution and composite core/shell structures. An array reader, which is needed to detect the absorbance shift upon ligand binding, is developed in **Component 5-5 (Array reader, Saratov State University)**. This will be done in cooperation with Activities 1 and 6.

Activity 6 (Peptide arrays, DKFZ) integrates Activities 1, 2, 3, 4 & 7. Amino acid particles produced within Activity 2 are adapted to the laser printer (Activity 3) and to the chips designed within Activity 4 in order to synthesise high complex peptide arrays. Arrays will be designed in collaboration with Activity 7 and stained by sera collected within Activity 1. Within **Component 6-1 (Peptide arrays by a laser printer, DKFZ)** specific locations on a solid support will be addressed with a laser printer (Activity 3) and the particles produced in Activity 2. Within **Component 6-2 (Peptide arrays by a chip, DKFZ)** specific locations will be addressed with a chip (Activity 4) and the particles produced in Activity 2. In **Component 6-3 (Analysis of peptide arrays, DKFZ)** combinatorial synthesis of peptide arrays and analysis of coupling efficiency and yield will be done.

Activity 7 (Database, EBI) comprises the data management aspects of the project, from experiment support through result analysis to result dissemination. An existing service, the

Proteome Pages provided by the EBI Sequence Database Group, will be used to define well-annotated protein sets for the organisms of interest **(Component 7-1, Protein sets, EBI)**. The new PREJUDICE tool will support the set-oriented analysis of binding experiment results, significantly reducing this time-consuming task. The PREJUDICE development will start at the beginning of the project, so that a first tool will be available when the first experimental results are expected **(Component 7-2, PREJUDICE, EBI)**. Close collaboration with the EBI will ensure that all experimental results (Activity 1), after any intellectual property and publications have been assured, is made available to the scientific community through integration into existing and new EBI proteomics resources **(Component 7-3, Dissemination, EBI)**.

Activity 8 (Prototypes, SMEs) comprises demonstration activities, i.e. the construction of machines compatible with each other. Within **Component 8-1** (**Prototype Peptide laser printer, Fraunhofer IPA, SME),** a prototype machine capable of automatically synthesising peptide arrays will be constructed, with data fed in from databases. A second prototype machine will be constructed within **Component 8-2 (Prototype chip synthesis machine, KIP, Fraunhofer IPA; SME)**. This machine automatically will synthesise peptide arrays on a chip and read out binding events, preferably by labelling free detection. In order to accommodate the chip synthesis machine to labelling free detection in **Component 8-3 (Prototype Array reader, Saratov State University; SME),** a prototype array reader will be constructed for the sensitive detection of unlabelled ligand binding based on a shift in extinction.

Activity 9 (Administration, DKFZ) comprises the management of the integrated project. Financial management will be done by the DKFZ administration within **Component 9-1** (**Financial administration, DKFZ administration)**, while **Component 9-2** (**Scientific administration, DKFZ)** comprises the scientific management done within Dr. Breitling's group.

B.4.1 Research, technological development and innovation activities:
Integration of the project's activities

Fig. 5 shows the integration of different activities and components and their contribution to the project as a whole. Arrows symbolise the flow of information and knowledge generated by the partners that results into a novel technology for easy translation of genomics data into peptide array as tools for proteomics research. The project initially is dominated by research components (*production of particles; model applications; new method for labelling free detection; chip design*) and technological components (*construction of instruments and chips*). Indeed our interdisciplinary project spans a large spectrum from basic to applied research. In a second phase (years 4 & 5) the project's emphasis is shifted towards demonstration activities that should lead to commercialisation (**Activity 8, Prototypes**). Scientific and financial administration of the project is done within **Activity 9** (not shown).

A normal laser printer is used for iterative testing of the particles produced within **Activity 2 (Particles)**. Thereby printing, coupling of amino acids to the solid support and the spot resolution will be determined. Optimised particles are then used within the peptide laser printer constructed within **Activity 3 (Peptide laser printer)** in order to synthesise peptide arrays (**Activity 6, Peptide arrays**). Very similar particles from **Activity 2 (Particles)** are combined with different chip designs from **Activity 4 (Chip design),** once again with the goal of high resolution combinatorial peptide synthesis (Component 6-2).

The work done in **Activity 7 (Database)** will help in defining specific peptide arrays, e.g. an array representing all the pathogen's proteins as overlapping peptides. In a model application, these pathogenic *peptidome* arrays are stained with carefully diagnosed patient's sera from **Activity 1 (Model applications)**. Peptide specific signals are compared to protein specific signals in order to validate the data obtained (Component 1-3). Handling of the huge amount of data is done by read out systems, which are developed within **Activity 5 (Read out methods)**, preferably based on

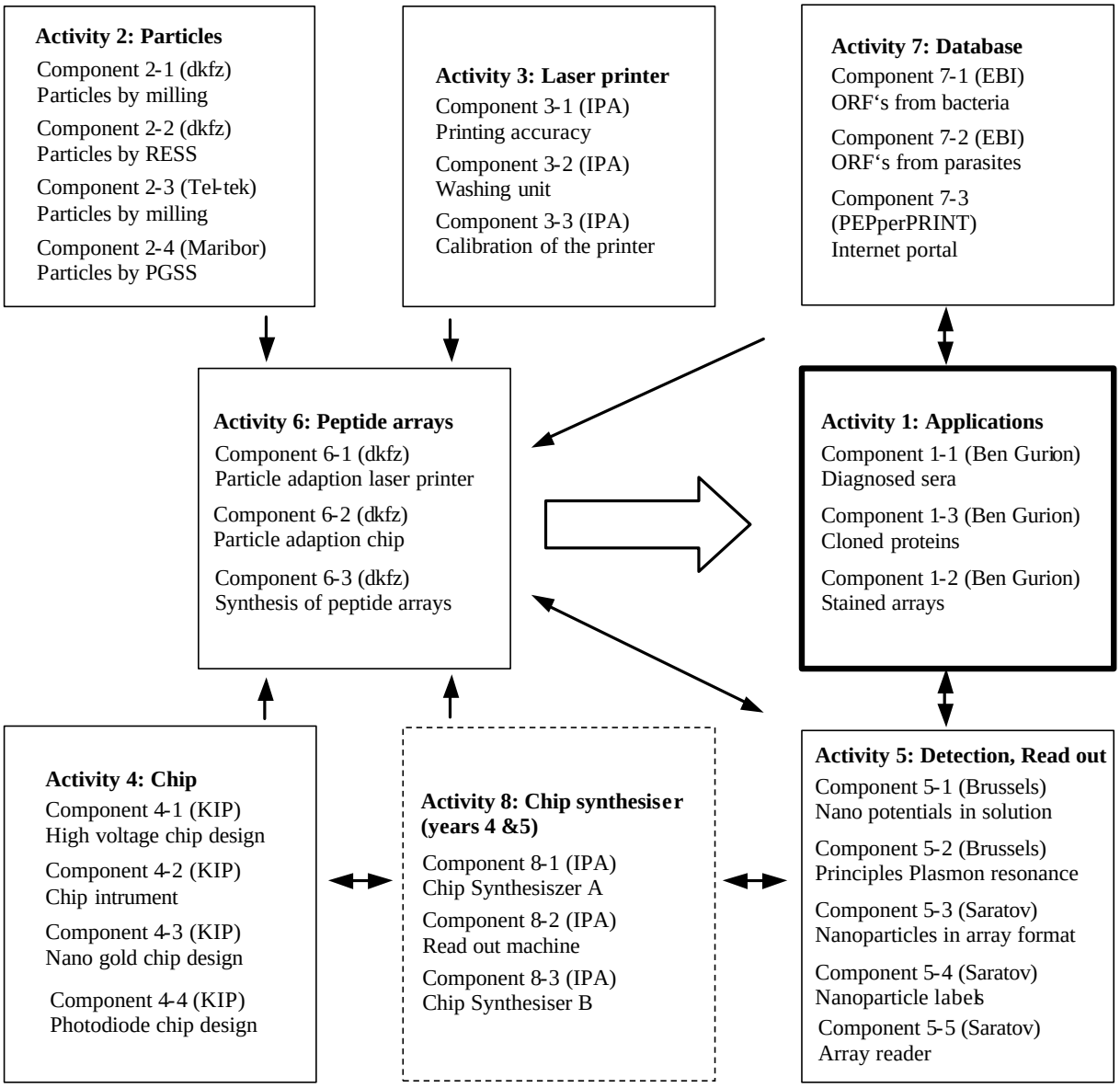


Fig. 5 Integration of the project's activities

labelling free detection (*especially for low affinity binders*). The prototype machines finally developed within **Activity 8 (Prototypes)** will lead to automation and commercialisation of our technology. They are instrumental in avoiding too much variety introduced by the experimenter and for handling the large set of data. For data mining, positive signals from applications are fed into databases (**Activity 7, Database**).

Management of knowledge and intellectual property

The knowledge emerging from this project including the previously existing knowledge needed for this integrated project will be made available to the partners involved in order to ensure integration of the work. The partners will share emerging intellectual property rights and declare their willingness to combine efforts for the exploitation of results and for the dissemination of knowledge. Within the contract to be signed, previously existing intellectual property rights, however, are excluded from commercial use. This is done in order to allow for combinations of technology not envisioned within this integrated project (*e.g. combining labelling free detection with other arrays*).

Exploitation of results

The project planned builds upon segmented prior national activities that now are combined at the European level in order to add value. Each partner will be free to exploit previously existing knowledge and intellectual property on its own. However, there is a strong incentive of added value when combining successful activities into an integrated product (*e.g. doing combinatorial synthesis of peptide arrays on a chip, with photodiodes arrayed underneath, combined with a read out and a link to databases*). If possible, the partners involved will patent these combinations of activities. However, as might be expected in such a competitive environment, outstanding 3rd party inventions are likely to occur. In order to accommodate for this, partners are free to use their knowledge in combination with partners from 3rd parties, generally with the incentive of more value added (*e.g. use of different arrays / different methods for labelling free detection / different chips*). This last point is also very important in order to accommodate for the (relative) failure of individual activities (*e.g. low sensitivity in labelling free detection*). After a three years period with the emphasis on science and development of technology, we plan to integrate additional SME partners that will commercialise

- Particle based array technology,
- Construction of machines and
- Labelling free detection methods.

These SMEs (*either one or more SMEs that line according to the sub projects*) will license previously existing intellectual property from the DKFZ and intellectual property possibly originating from this integrated project. The SMEs mainly will concentrate on the construction of prototypes and on demonstration activities needed for successful commercialisation (*prototypes chip synthesis machine, peptide laser printer, integrated read out machine etc*.). Besides their financial involvement through demonstration activities, the SMEs chosen by Participants 1 to 9 ideally should contribute know-how in marketing and worldwide distribution.

Disseminating of knowledge

- Mutual educational activities will be employed especially for dissemination of interdisciplinary knowledge within the groups involved. Therefore, at least annual meetings will be arranged.
- Especially for Activity 7 (Database) dissemination of knowledge is intrinsic because 3rd party users will access improved databases and analysis tools without any restrictions.
- In addition, scientific publications and patent applications are aimed at.
- As stated above, especially SMEs will exploit the results with a strong emphasis on the sale of arrays (*and machines yet to be developed*).
- Thereby customers (*scientists in industry and public science*) will be free to use this technology in order to investigate genomics and proteomics data for their special purposes. Workshops introducing the technology will be offered at national and international conferences in the corresponding scientific field.
- As mentioned above (B3, Overall innovation aspects), more distant applications are conceivable , which are not covered by the patent application EP1140977A2. Therefore an extra incentive for collaboration of partners is given within this project, because the partners closest in expertise surely will lead dissemination of applications beyond this grant application (*e.g. promotion of rapid prototyping of 3-dimensional structures in µm scale will be lead by Fraunhofer IPA*).

Detailed description of activities

Activity 1 (Model applications, Ben Gurion University)
Activity 1 comprises the different components needed for the model application envisioned. Our objective is to identify

- Pathogen-specific peptides that are relevant to viral interactions with the host,
- Peptides of diagnostic relevance that can be used for better diagnosis and prediction of disease progression of viral infections and
- Peptides and the respective antibodies of therapeutic relevance that might lead to better vaccination strategies against viruses.

Instrumental in this activity is the large collection of carefully diagnosed sera Dr. Shemer Avni (Ben Gurion University) already has at her disposal. She will go on collecting these diagnosed sera and annotate to each serum the patient's aetiopathology, if possible. In close coordination with Activity 7 (Database), Dr. Shemer Avni will decide on the pathogen specific peptide arrays to be designed. These arrays, synthesised within Activity 6 (Peptide arrays), will be stained with the patient's sera in order to correlate staining patterns with aetiopathology. Therefore we intend to:

- Design and apply the Pathogen-peptidome array to analyse humoral immune responses to viral infections,
- Identify the major neutralising peptide epitopes from vaccinia virus for synthetic vaccine design and optimisation and
- Analyse interactions of pathogen-peptides representing the virus' peptidome with various factors that affect pathogenesis.

Component 1-1 (Pathogen peptidome array, Ben Gurion University)
The objective of Component 1-1 is to use high complexity peptide arrays that represent all the pathogen's proteins as overlapping peptides (*pathogen peptidome array*) in order to analyse humoral immune responses to viral infections in unprecedented detail. The immune responses to viruses play an important role in clearing the virus and in the pathogenesis of viral infections. The sera at our disposal descend from patients that are infected with blood born viruses, with the emphasis on

- HCV (Hepatitis C virus),
- HBV (Hepatitis B virus) and
- HIV.

Infections with these viruses are wide spread and often result in chronic infections and the development of severe and lethal diseases, such as chronic active hepatitis, hepatocellular carcinoma (HBV and HCV) and AIDS (HIV). So far, no effective vaccine was developed for HIV or HCV. Our collection of sera includes a follow up (of more than 6 years) on many of the patients. Therefore we can compare

- The peptides reacting with sera from a single patient, in different stages of the diseases and
- The profile of antibodies (directed against viral epitomes) present in the sera of patients that managed to clear the virus versus antibodies profiles in the sera of patients that did not manage to clear the virus.

Since HCV and HIV go through multiple mutations during infection and during anti-viral therapy, this approach can be used to analyse those changes by reacting them with the peptides array. Furthermore, the detection of major viral peptide epitopes should assist in the design of vaccines and therapy against HCV, HIV and HBV, especially if peptide patterns correlating to better prognosis are identified.

Study design, patient population and clinical specimens:
Within Component 1-1 the HCV, HBV and HIV-proteomes will be screened with the sera described above. Peptide staining patterns will be correlated with

- the presence / clearance of the viruses,
- the detailed clinical diagnosis,
- MHC presentation,
- severity and
- outcome.

The findings expected from this data will enhance our understanding of important viral diseases of humans and contribute to the future development and assessment of new antiviral treatments and vaccines.

The first year of the study will be dedicated to the collection of additional sera and to the storage of a computerised data set, that will be used later in the analysis of the results from stained peptide arrays. The second and third year will be dedicated to the array-screen and analysis. The emphasis initially will be on HCV with HIV to follow. Depending on the capacity available and on the achievements within the consortium, we intend to apply the knowledge gained to the analysis of HBV, herpes viruses and the respiratory viruses, in that order.

Collection of samples; These studies will be carried out at the Laboratory for Clinical Virology, Soroka University Medial Center (Yonat Shemer-Avni) in collaboration with the Pediatric Infectious Disease Unit, Soroka University Medical Center and the Liver Clinic (David Greenberg; Ron Dagan will serve as a consultant in the study; the Pediatric ID unit and Emanuel Sikuler will serve as a consultant of the LC). We have already established ongoing collaborative studies of the epidemiology of viral respiratory tract infection and viral liver infections. The chronic blood born viral infections include sera collected from patients suffering from chronic infections of: HCV, HBV, and HIV with various clinical manifestations. Latent and chronic infections are the whole mark of human *herpesviridae*. On the basis of our frozen samples, we estimate that more than 400 patients can be enrolled in this part of the studies. Therefore, we shall employ RT-PCR and PCR (depending on the viral genome) for detection and monitoring of HCV, HIV, HBV and CMV (Cobas Amplicor, Roche diagnostics) in patient's sera and/or lymphocytes. The presence of HSV-1 & 2 and EBV, in clinical specimens, will be monitored by PCR using "real time PCR" (Roche). For HCV and HIV, phylogenetic analysis will be performed by direct sequencing of the PCR-products. The resulting sequences will be aligned, compared to the published sequences in the gene bank, and phylogenetic trees will be created. In addition, for part of the persons enrolled in the research, MHC polymorphism will be determined by PCR.

The pediatric study includes children (age up to 5 years), admitted to Soroka University Medical Centre with acute respiratory tract infections. These children undergo complete clinical and laboratory evaluation. The specimens are divided into aliquots upon receipt. Respiratory specimens obtained from children admitted for respiratory tract infections are studied directly and after culturing for the presence of RSV, influenza A and B, adenoviruses and parainfluenza 1,2,3 viruses by direct immunofluorescence assay, using commercial monoclonal antibodies. The study will expand over at least 3 successive years. It is expected that specimens obtained from approximately 2400 (800/year) children and 600 controls will be examined for the presence of respiratory viruses. In addition, sera are collected from the children. The respiratory viruses collected will be used at the second stage of the pathogen-proteomics studies. Part of these samples can be used to analyse respiratory infections in antibody-arrays, aiming at creating a chip that can diagnose all the common respiratory infections This can eventually be commercialised by a diagnostic company.

Component 1-2 (Vaccinia epitopes)

The objective of Component 1-2 is the identification of major neutralising peptide epitopes from vaccinia virus for synthetic vaccine design and optimisation. The attenuated vaccinia virus long-time used for immunisation against smallpox infection has severe manifestations in part of the population. Dr. Lobel established a research aiming at producing fully human neutralising monoclonal antibodies against vaccinia virus (by fusion of B-cells producing antibodies against vaccinia with transformed cell line) for immunotherapy and prophylaxis of smallpox. We intend to

use the vaccinia peptidome array to screen for peptides that are immunoreactive in both: Sera from people immunised against vaccinia and the monoclonal antibodies produced by Dr Lobel. The information gained will help to identify major neutralising epitopes to facilitate the design of synthetic vaccines. The advantage of this model is that the cultured human antibodies are cloned and the one identified as "strong-binders" by the peptidome array can be used directly *in-vitro* to test for viral neutralisation. The collection of sera and isolation of B-cells is already ongoing (these experimental protocols and the informed consent have passed the Helsinki committee at Ben Gurion University and assigned approval number 3339). We intend to start with this project in the fifth year.

Component 1-3 (Pathogen-host interactions)
The aim of Component 1-3 is to analyse pathogen- host interactions that affect pathogenesis. This work eventually might identify

- receptors for viral entry,
- DNA and RNA binding proteins and
- intra-cellular proteins that are targeted by viruses.

A specific pathogen array will be reacted with host-labelled-proteins and protein bound will be further analysed by mass-spectrometry. Furthermore, fluorescent RNA or DNA probes can be used to identify target peptides. For set up and fine tuning of this system, we intend to start with components known to bind to each other; e.g. the HCV-peptidome will be screened with the 5'NCR-RNA of HCV genome [that binds the helicase of HCV-NS3 (non-structural 3)], or NS3 protein tagged with GFP known to bind NS4. This study will start when the HCV-peptidome will be available (second to third year).

Activity 2 (Particle production, DKFZ, Tel-Tek, University of Maribor)
Manufacturing, analysis and (pre-)testing of amino acid toners are planned within Activity 2. The toners employed should fulfil several criteria:

- They must include (activated) amino acids to be coupled to the solid support,
- Amino acids must be embedded within a suitable "solid solvent" (*e.g. diphenyle formamide, diphenyl sulfoxide*),
- Only charged particles are transferred by a laser printer or addressed by a chip's surface (*need for charge control agents*),
- The particle's charge shouldn't decay (*need for charge stabilisers*),
- Particles of uniform size give better results (*transfer of particles depends on charge divided by mass, i.e. q/m*),
- Particle's ingredients should be stable (*e.g. non-activated amino acids activated on demand inside the particle's compartment*),
- Agglomeration of particles must be avoided by using anti-baking components (*e.g. different silicas or metal oxides*).
- The particles should resist mechanical stress inside the laser printer and
- None of the components mentioned should interfere with the coupling reaction.

Component 2-1 (Particle formulations, DKFZ)
In order to produce particles according to the criteria just mentioned, iterative manufacturing and testing of particles within a commercially available laser printer or by addressing particles to a chip's surface is crucial. A fast pre-test will be done with the commercially available colour laser printer Oki C7200, whose components are the basis of the peptide laser printer constructed within Activity 3. Initially, the formulation of amino acid toners and production of particles planned within Component 2-1 is based upon pre-existing knowledge. Particles will be produced by mechanical milling and printed by the laser printer mentioned onto a suitable solid supports (*e.g. Whatman paper derivatised with amino groups*). Afterwards, the particles are melted for a few minutes with

the activated amino acids (*now mobilised within the former solid solvent*) coupling to the solid support. Residual free amino groups are blocked with acetic anhydride followed by deprotection of the non-permanent Fmoc-protection group, corresponding to the established Merrifield *synthesis*. As shown in Fig. 6, the thereby newly generated free amino groups are easily stained by bromophenole blue, giving a fast hint on the quality of the coupling reaction and on the achievable resolution of the particular formulation tested. Spot densities exceeding 700 spots/cm^2 are achievable even with the primitive 1st generation toner particles (*produced with a coffee mill*) and the relatively coarse Whatman paper employed. This compares favourably to the state of the art where a spotting machine employed to print 100 spots/cm^2 needs several days for the spotting process itself when a filter of 20cm x 20cm is to be completed. Within Component 2-1 (DKFZ), different toner formulations will be tested as shown in Fig. 6 and the knowledge gained will be shared with Tel-Tek and the University of Maribor, where more sophisticated methods for toner production will be employed. As described, the influence on coupling efficiency and spot resolution will be tested:

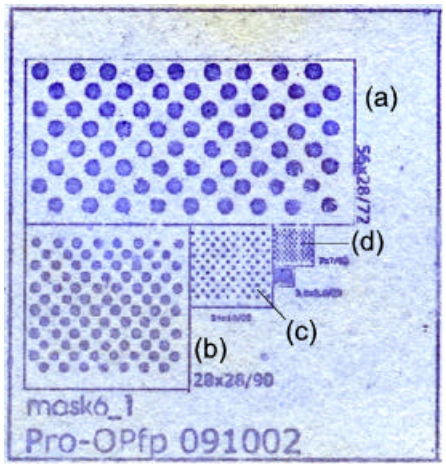


Fig. 6: Laser print of a Fmoc-proline pentafluorophenyl ester after coupling and staining with spot densities of: (a) 18 cm^{-2} (state of the art); (b) 56 cm^{-2}; (c) 196 cm^{-2}; (d) 784 cm^{-2}

- Of different solid solvents (*e.g. diphenyle formamide, diphenyl sulfoxide, 2-methoxy benzyl cyanide, formanilide*),
- Of different activated amino acids (*OPfp-ester, Anhydrides, Fluorides*),
- Of different activation methods within the particle compartment (*non-activated Fmoc-protected amino acids together with light-activatable photobases*),
- Of different negative charge control agents (e.g. sodium-bis-[2-(2-hydroxy-1-napthylazo) benzoic-acid]-aluminate(III) and Copy Charge NY VP 2351 from Clariant),
- Of different positive charge control agents (e.g. Copy Blue PR, Copy Charge PSY and Hostacopy Charge PX04 from Clariant)
- Of different charge stabilisers (e.g. Polymethylmethacrylate or Copy Level NCS from Clariant) and
- Of different particle's size and size distribution.

Component 2-2 (Particles by RESS, DKFZ)

More sophisticated procedures for toner production are adopted in Component 2-2 (DKFZ), where the fabrication of amino acid toner particles is done by RESS procedure (*rapid expansion of supercritical solutions* which is similar to PGSS, Fig. *7*) and by air milling.

Compared to the different milling methods, where usually sieves are employed to get evenly sized particles (*transfer of particles is governed by q/m, therefore a uniform size distribution of particles is instrumental*), RESS is a convenient and easy method that gives a good yield of particles with uniform size distribution. In addition, this method allows for the exclusion of H_2O and O_2 during the production of particles, impurities potentially disturbing the coupling reaction. However, probably not all the potential ingredients of amino acid particles specified within the description of Component 2-1 will dissolve in liquid CO_2. Therefore, the ingredients suitable for particle production by the RESS procedure will be identified within Component 2-2 and the particles thus produced will be tested as described for Component 2-1.

Component 2-3 (Particles by milling, Tel-Tek)

Component 2-3 (Tel-Tek) comprises manufacturing and analysis of toner particles by jet milling and air classification to generate narrow size distributions. One of the rationales behind this approach is the larger quantities yielded when compared to RESS and similar methods dependent on high pressure. More traditional methods of milling and/or air classification usually not only yield larger quantities, but they do so at a faster rate. This will help in fast iterative testing (described for Component 2-1) of different particle formulations in the commercially available colour laser printer Oki C7200, which is the basis of the peptide laser printer constructed in Activity 3. In addition to this, Tel-Tek will explore other methods for particle production with a potentially high yield. Examples include but are not limited to nano-crystallisation and emulsion-crystallisation.
In line with Tel-Tek's expertise, emphasis will be on electrostatic chargeability in different processes to be tested. Charge uniformity and strength is crucial for the accurate positioning of particles by addressing particles to oppositely charged regions spatially defined by a laser printer or a chip. Particles produced by Tel-Tek and the other partners will be tested by a range of different methods including charge deflection techniques, resistivity and discharge time. To start with, the emphasis will mainly be on charge deflection techniques.

Component 2-4 (Particles by PGSS, University of Maribor)
PGSS (*Particles from Gas Saturated Solutions*)-micronisation process [Weidner, E.; Knez, Z.; Novak, Z.: EU patent No. 0744992; US Patent No.6,056,791] is a novel method that uses supercritical fluids and high pressure to generate powder from various substances. The process is based upon the solubility of gases in liquids, which is usually quite high at elevated pressures. Large quantities of the dissolved gas (*between 5 and 50 % by wt*) change the properties of the liquid comprising the material destined to be micronised. Expansion of such a gas-saturated solution through a nozzle or other expansion device induces the compressed gas to evaporate and thereby rapid cooling of the solution. This leads to super saturation of dissolved solids with fine particles precipitated. By adjusting process parameters (*temperature, nozzle size and shape, CO_2 addition*), the particle size, particle size distribution, crystallinity and morphology can be adapted to specific requirements.
Figure 7 presents a schematic diagram of the process for production of particles from gas saturated solution. The equipment consists of a high-pressure part where the compressible medium/gas is dissolved in the substance to be micronised. The second part of the equipment is the expansion device, where the gas-saturated solution is rapidly depressurised through a nozzle. Finally, a spray tower is shown, where particles exceeding a diameter >10 µm are segregated. Smaller particles (1-10 µm) are collected at the bottom of the cyclone, while the finest particles (< 1 µm) are isolated from the gas stream with an electrofilter.

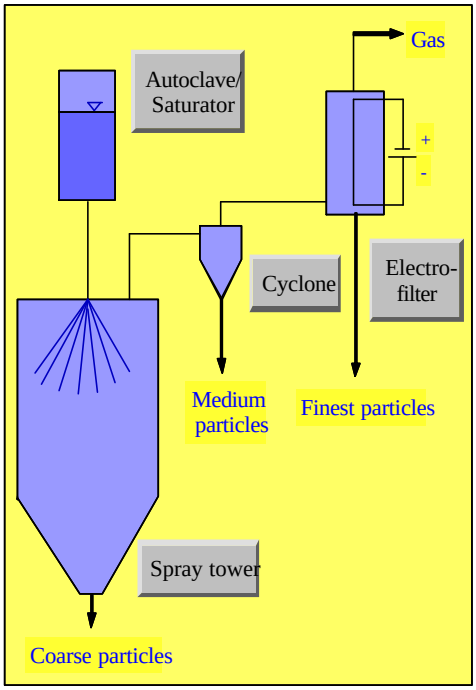


Fig. 7: Basic scheme of a PGSS process

Through the choice of the appropriate combination of solvent and operating conditions suitable for a particular compound, PGSS can eliminate some of the disadvantages of traditional methods of particle size redistribution in material processing. The advantages of PGSS over conventional methods for particle size reduction are numerous (*e.g. free of water and oxygen contamination, applicable for mixtures of substances, moderate pressures, low gas consumption, solvent-free powders, suitable for high viscous or sticky products, fine powders with narrow size distribution, different morphologies, easy scale-*

up, continuous operating mode that enables capacities of some hundred kg/h).

The characterisation of particles obtained will initially be done as described for Component 2-1 with the OKI C7000 series colour laser printer. Criteria are:

- The quantitative transfer of particles within the laser printer (*a large amount of particles printed*),
- The qualitative transfer of particles within the laser printer (*only defined regions covered by particles*) and
- The coupling reaction of amino acids to the solid support downstream to the printing process (described above in Fig. 6).

With smaller particles produced (*approx. 1µm*), however, chip electrodes produced within Activity 4 (Chip design) will be used as the testing device of choice instead of the laser printer. The rationale behind is the lower voltage applicable to chip electrodes (*9V, 19V, 90V for very smal1, small or larger chip structures respectively*) when compared to a laser printer *(>200V*). Due to this, average sized (*approx. 10µm*) commercially available toner particles give high background signals when addressed to defined areas by low voltage applied. Small particles, however, will be correctly addressed due to a much higher q/m ratio (*charging divided through mass*), which governs particle transfer. Also instrumental in particle characterisation will be Scanning Electron Microscopy (*determination of particle morphology and size*) and a particle size analyser (*size distribution*).

Activity 3 (Peptide laser printer, Fraunhofer IPA)

Activity 3 comprises the construction of a laser printer suitable for peptide synthesis, which is done at the Fraunhofer IPA. A first instrument currently under construction (*prospective delivery in summer 2003*) will serve as experimental platform. The developing units (Fig. 8) and the LED units are derived from a commercial laser printer (OKI C7000 series), which uses the single pass technology, i.e. the toner is directly applied to the sample carrier, not to a subcarrier usually made from rubber. To accommodate for the need of stable and partially automated peptide synthesis this first instrument will be developed further, thus designed to have a number of additional properties.

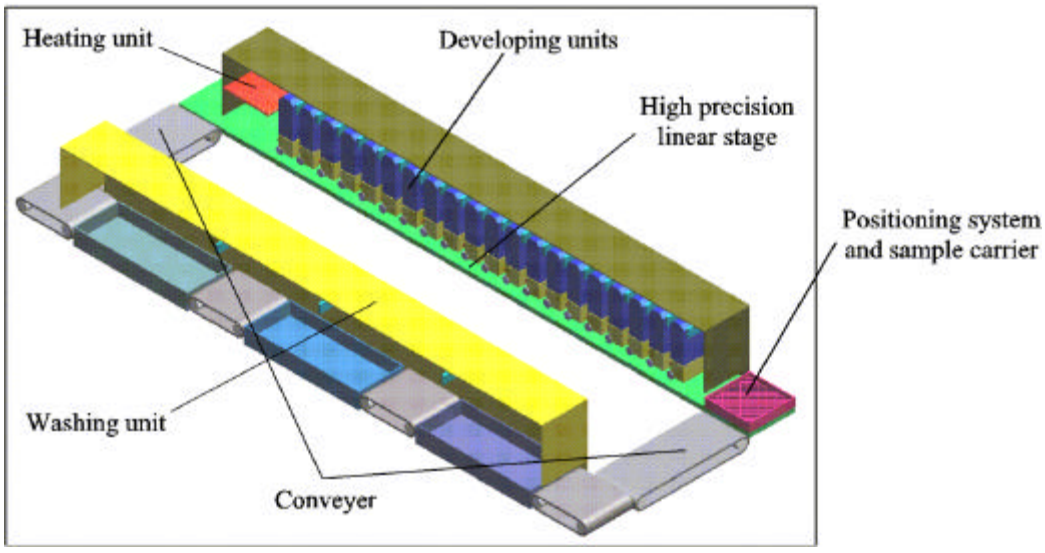


Fig. 8: Design drawing of the first instrument under construction with 20 developing units

Component 3-1 (Printing accuracy, Fraunhofer IPA)

The first instrument mentioned above is designed to allow for the combinatorial synthesis of more than 100.000 peptides onto a sample carrier made from glass (20cm x 20cm), which already excels the state of the art available. It is planned to increase the printing accuracy of this instrument in order to achieve more than 500.000 peptides by the year 2005 in an area of 20cm x 20cm. This

requires a positioning tolerance of the amino acid toner spots of approximately +/-20µm. Since printing with 20 different toners and therefore developing units is desired, the size of the machine exceeds 3m. Together with the required accuracy, this puts high demands on each part of the process chain approaching the limits of technical feasibility. Therefore, in order to increase the printing resolution, two directions of development are followed:

First the mechanical accuracy will be improved. This comprises the slide accuracy of the linear driving unit, the drive and the mounting of the printing drums and the positioning system of the sample carrier fixed onto the driving unit. An emphasis will lie on the positioning system which fulfils two tasks: Since after each printing step a chemical coupling and cleaning step is needed, the sample carrier has to be removed from the driving unit and precisely repositioned for the consecutive printing step with a tolerance of approximately +/-15µm. One possibility to achieve this is to implement an optical sensor system (camera) with an appropriate image processing; another is a tactile sensor system. Secondly, the mounting of the sample carrier has to ensure the contact of the stiff carrier (glass) with the printing drums. Even very small gaps of air will deter any toner transfer.

A second direction of development improves the hardware controller of the printer, which has to ensure the exact synchronisation (*tolerances of about 50 ms*) of the linear driving unit, the drive of the developing units and the LED units. Due to the required high accuracy, the mechanical and electronic components of the printer have to be calibrated. This has to be performed by adjusting the hardware controller. Due to the high frequency of data transmission (several MHz), a hardware solution is imperative. It is intended to use a Field Programmable Gate Array (FPGA) for this purpose, which will be programmed at the Fraunhofer IPA.

Component 3-2 (Washing unit, Fraunhofer IPA)

It is planned to integrate the chemical coupling and cleaning steps (*liquid chemistry according to Merrifield synthesis*) into the instrument described above by the year 2006. Therefore within Component 3-2, a chemical reactor appropriate for this purpose will be developed. The chemical coupling and cleaning of the peptide arrays comprises the subsequent application of six different chemicals with the arrays finally desiccated in order to prepare for the coupling of the next layer of amino acids. Therefore as sketched in Fig. 8, one straightforward approach employs six different trays that accommodate the different washing solutions needed. An alternative solution is based on a chemical reactor with chemicals loaded and removed successively. This concept already has been successfully realised at the Fraunhofer IPA in a number of electroplating facilities. It should be noted that the washing unit must accommodate two process steps of essential different speed. While the printing step takes about 1 - 2min, the chemical steps take about 40min. One way to increase the output of peptide arrays is to keep both processes continuously going on. This is done with 20-40 peptide arrays pooled for simultaneously washing. The coupling process immediately follows the printing process. Thereby the arrays are simply heated up for a few minutes leaving the arrays unsusceptible to mechanical handling. The complete process requires appropriate control software for the peptide printer as a matter of course.

Component 3-3 (Calibration of the printer, Fraunhofer IPA)

To achieve the required printing accuracy, the calibration of the mechanical and electronic components of the printer is very important, as mentioned above. To date, this has to be done by hand, i.e. a test printing is done, the relative distances of the toner spots on the sample carrier are measured with a microscope and the resulting corrections for each colour are entered into the control software. An easier and less laborious calibration procedure definitely is necessary. This task can be performed by an optical sensor system, which will then be used to position the sample carrier. Appropriate image processing software will be developed at the Fraunhofer IPA. An

alternative solution is to sufficiently increase the time intervals of recurrent calibration. This requires the improvement of the mechanical accuracy of the linear driving unit and the drive and mounting of the printing drums, as described in Component 3-1. Appropriate calibration software for any solution will be developed at the Fraunhofer IPA.

Component 3-4 (Cooled nitrogen atmosphere, Fraunhofer IPA)
Due to the relative sensitivity of the amino acid toner to heat, oxygen and water, decay of toner ingredients might require a frequent replacement of the development units with the expensive toner stored inside. A cooled nitrogen atmosphere within the printer should significantly prolong the lifetime of the expensive toner particles. This will be realised by a gas proof housing of the printer and a gas cooler, which allows the housing to be flooded with cooled nitrogen. A side effect of this air condition is the levelling of differences in temperature within the printer, which lowers thermal expansions and therefore improves mechanical accuracies. This will be realised by the year 2005.

Activity 4 (Chip design, Kirchhoff Institute)
Activity 4 comprises of different chip designs and suitability studies on different technologies for peptide array synthesis.

Component 4-1 (High voltage design, Kirchhoff Institute)
Within Component 4-1 a chip design is envisioned, that incorporates a few thousand electrodes on a die size of approximately 5mm x 5mm suitable to electrically attract amino acid toners to spatially defined regions on the chip's surface. The chip will feature a simple electrical interface in order to be controllable by a personal computer (see below, Component 4-2). In analogy to the laser printer, a charge pattern will be generated to address the toner particles by means of localised electrostatic interactions. Here, the charge pattern is produced by an array of individually triggered pixel electrodes arranged on the chip surface (Fig 9, a). Toner particles are charged by triboelectrification in a particle aerosol and hence adsorb specifically on uncharged pixel electrodes on the chip's surface (b), as particles and electrodes have the same charge. The first toner species is fixed by melting (d), and a second charge pattern is generated and brought into contact with a second species of toners (e, f). By 20x repeating this, the chip is completely covered by a first layer of all different amino acid toner particles (g). Spatially defined as the particles were glued to the chip's surface, the amino acids are released by melting and thereby couple to the chip's surface (h). At the end of a synthesis cycle, all residues are washed away, leaving the first layer of all different amino acids coupled at once (i). Iterative repetitions of this process eventually will result into a peptide array, e.g. 10x for an array of decameric peptides.

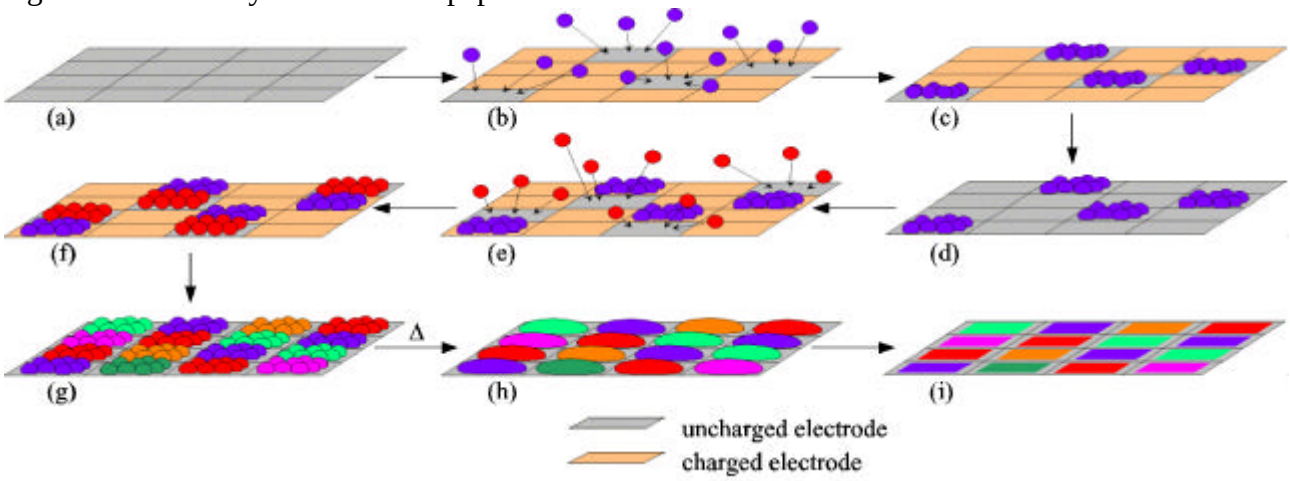


Fig. 9: Principle of the combinatorial peptide synthesis on a high voltage CMOS chip

Compared to the laser printer technology with working voltages between 200-1500V, a conventional chip design enables the application of voltages of only 5V and below. As we assume that the selectivity of the toner particle transition depends crucially on the interplay of electrostatic and kinetic energy, we aim to design a High-Voltage CMOS chip switchable on voltages of up to 90V. Commercial CMOS IC manufacturing technologies can be applied, since the electrodes comprise of "Pad"-structures. These "Pad"-structures are used to contact integrated circuits by wire bonding and are usually available as library elements supplied by chip manufacturers like Austria Microsystems. In general, chip electrode voltages exceeding 5V require special high-voltage chip designs, which are available in special HV-CMOS processes. These are especially used for automotive components, bearing the advantage of long term availabilitycompared to cutting edge computer technologies.

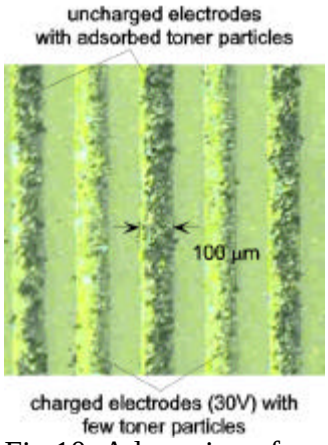


Fig 10: Adsorption of toner particles: Discrimination of charged and uncharged electrodes.

The chip design will be done in the Kirchhoff-Institute of Physics, followed by submission of the chip data to the chip manufacturer. In a first test run, only few chips will be manufactured to ensure their entire functionality as a matter of routine by means of electric and chemical tests at the KIP. Depending on the results gained from these tests, a second test run might appear to be essential to meet all prerequisites at this stage of the project. Finally, an engineering run with a large number of chips will be tackled. In parallel to the described electric tests, the chip designs will be examined with respect to the addressing of conventional toner particles. During the whole process, iterative modifications of the chip design are conceivable. Preliminary tests with conventional toner particles show that charged and uncharged electrode structures discriminate particles at voltages as low as 30V (Fig. 10), far below the 90V applicable in our high voltage design planned.

The use of solid particles circumvents most problems that arise when dipping the chip into a solvent with the monomers dissolved within. With solid particles, there is no flow of current (*e.g. in order to attract monomers from solutions to defined regions*) with its disastrous effects mediated by electrolysis, nor will the diffusion of monomers in liquids counteract the attraction of monomers to regions defined by the chip's polarisation. Therefore with this approach synthesis of peptide arrays with a spot diameter of approx. 80µm or less should be feasible by the year 2006.

Component 4-2 (Chip instrument, Kirchhoff Institute)

A small apparatus to accommodate the CMOS chips from Component 4-1 will be designed and constructed in the KIP (see sketch in Fig. 11). The apparatus will be employed in testing the selectivity of the test run chips and, if required, modified with respect to a subsequent continuous application with the engineering run chips. All different toner particles will be available in a corresponding particle reservoir (see amino acid acronym in Fig. 11), from which by demand triboelectric charging will occur. It is supposed that tribocharging will be obtained by means of an air jet within a Teflon-coated fluidised bed.

After tribocharging, the particles will be exposed to the chip's surface for addressing. For that purpose, the chip from Component 4-1 will be mounted to a carrier in chip-on-board (COB) technology. Another feature of the machine will be an automatic propulsion of the chip for transferring the chip from one reservoir to another. High precision alignment will be accomplished by means of linear stages and/or rotation stages. The apparatus is also envisioned to include a washing unit with reservoirs for solvents needed as well as for waste. Triggering of the apparatus will be managed by a laptop with the appropriate equipment (e.g. PCMCIA-DAQCards and LabView). A technical instructed student apprentice at the KIP will do the construction of this instrument.

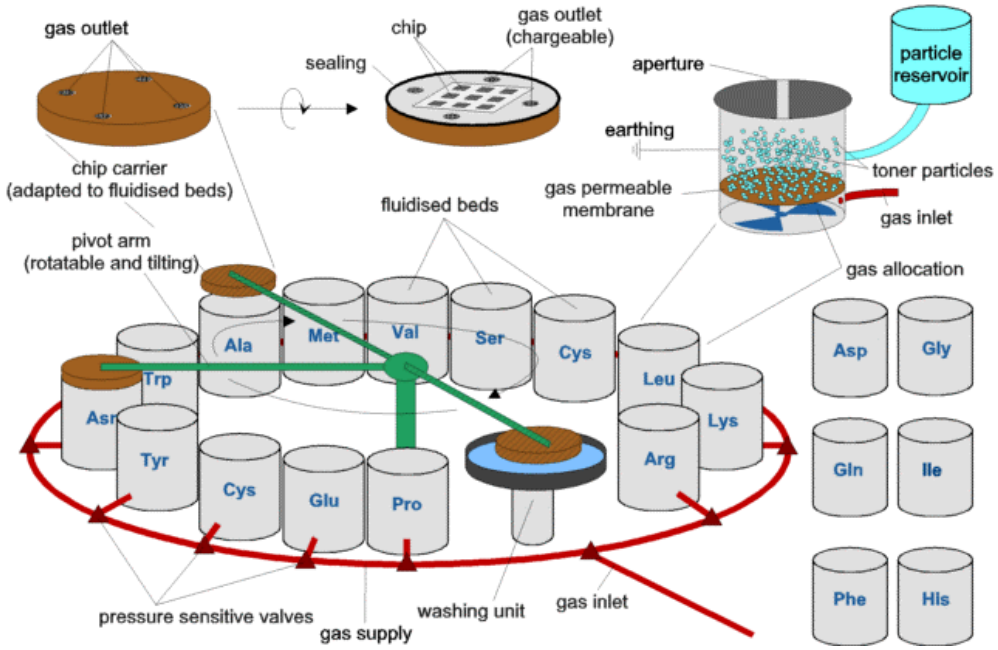


Fig. 11: Apparatus sketch for an automated chip synthesis of peptide arrays by the chip instrument

Component 4-3 (Nano gold chip, Kirchhoff Institute)
As mentioned in Component 5-3 (see below, Nano particles in array format), a method for the label free detection of binding events based on colloidal gold nano particles will be employed for the peptide arrays. We also aim to adapt this concept for the combinatorial peptide synthesis on the pixel electrodes of the chip surface. Therefore, we will integrate Component 5-3 into Component 4-1 by adsorption of the colloidal nano gold particles before synthesising the peptide arrays (Fig. 12).

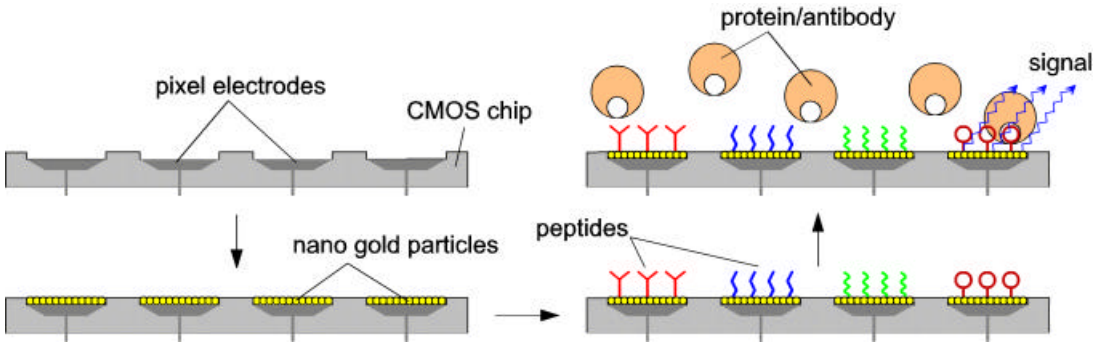


Fig. 12: Incorporation of Component 5-3 (colloidal nano gold particles) into Component 4-1 (synthesis of peptide arrays by means of a high-voltage CMOS chip)

As the nano particle layers are very thin and conducting, no significant changes in performance with respect to addressing the toner particles by means of electrostatic interactions is expected. However, slight variations of the surface chemistry will be envisioned. As the chip surface has to be terminated with amino groups for coupling of the amino acids, organic monolayers are designated for this purposes. On a conventional chip surface, the pixel electrodes are covered by a thin layer aluminium oxide, which enables the linking of organosilans followed by conventional methods of surface chemistry. By covering the electrodes with a thin layer of colloidal nano gold particles, linking has to result from adsorption of organic thiols, followed by the same conventional methods.

The merger of Component 5-3 (Nano particles in array format) and Component 4-1 (synthesis of peptide arrays by means of a high-voltage CMOS chip) will result in high complexity peptide arrays with ligand binding detectable by labelling free detection. Binding events induce a change in refractive index at the surface of such probes, which elicit a shift in the localised surface plasmon band in the absorption spectrum of the colloidal nano gold particles. Hence detection performance will strongly depend on the sophisticated coating of the particle's surface, which will be done in the University of Brussels (Component 5).

Component 4-4 (Chip with integrated photodiodes, Kirchhoff Institute)
Yet another chip design will be envisioned with respect to an integrated detection of binding events by means of conventional labelling of probes. Therefore, an array of photodiodes will be incorporated in the pixel electrodes of the CMOS chip design (Fig. 13), whereas the procedure will be the same as mentioned in Component 4-1.

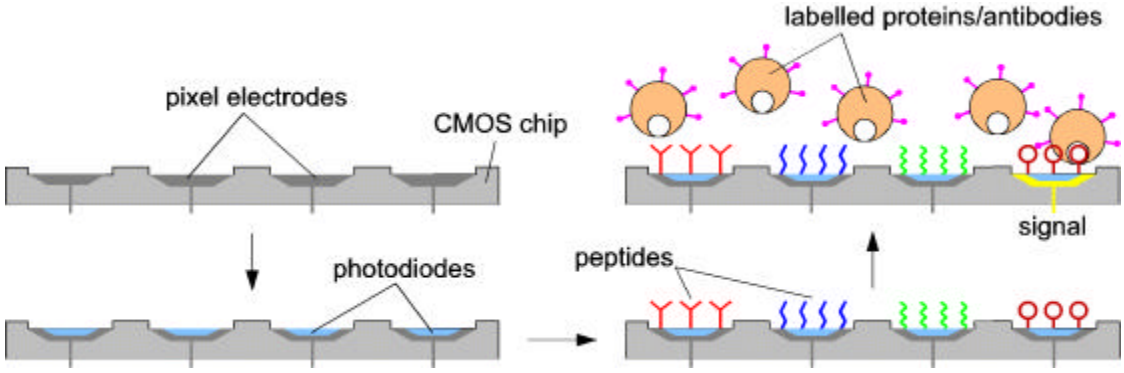


Fig. 13: Integration of photodiodes in the CMOS chip for detection of binding events of labelled probes

After exposure of e.g. peroxidase-labelled proteins or antibodies to the peptide array, the integrated photodiodes of this chip would be ideally suited for the spatially addressed detection of binding events by chemoluminescence. Compared to conventional detection systems, this concept physically links peptide sequences and binding events and thereby avoids time-consuming and circuitous image editing and recognition methods. Most beneficial, however, would be the massive amount of data reduction expected by these integrated read out circuits. The final revision of this chip will also be produced in an engineering run and will be thus available in larger quantities for other Activities.

Activity 5 (Read out methods, University of Brussels & Saratov State University)
Dr. Englebienne's laboratory is specialised in the application of colloidal metals in solution for the detection of molecular interactions in liquid format. The visible spectrum of such particles is characterised by a strong absorption band termed the localised surface plasmon resonance (LSPR), resulting from light absorption and scattering due to collective oscillations of conduction electrons induced by the incident electric field of light. Upon ligand binding the particle surface's refractive index changes, i.e. the visible spectrum shifts to the red and the absorbance decreases at the original LSPR peak, whilst the absorbance increases at longer wavelengths. This phenomenon is exemplified in Fig. 14, which shows difference spectra recorded with silver and gold colloidal particles coated with the same antibody, after interaction with increasing concentrations of the ligand. The high sensitivity shown in Fig. 14 exemplifies that nano colloidal gold particles are ideally suited for labelling free detection.

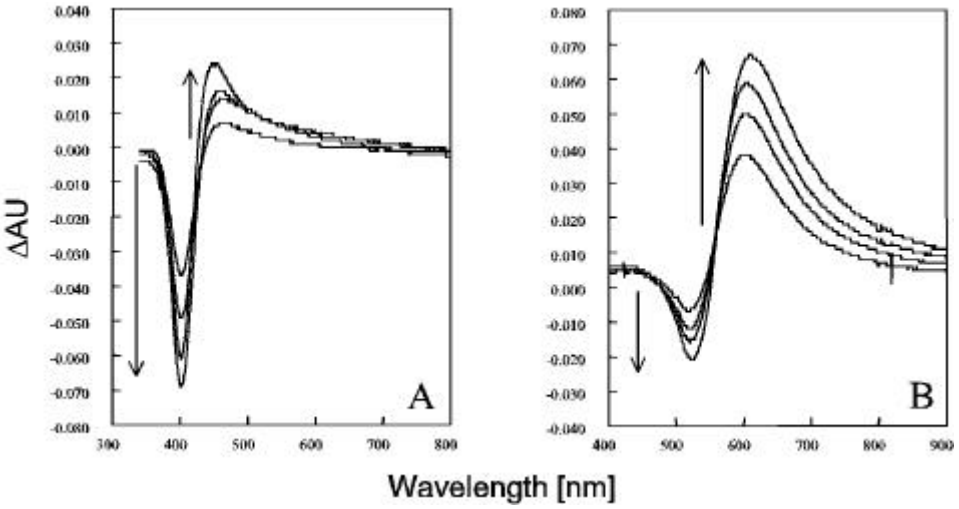


Fig. 14: Difference visible absorption spectra observed using silver (A) or gold (B) nanoparticles sensitised with the same antibody and respectively reacted for 5 min. with 1, 2, 5 and 10 nmol of ligand. The reference cell contains no ligand.

Component 5-1 (Nano particles in solution, University of Brussels)
As compared to the homodisperse spherical particles used to date in Dr. Englebienne's laboratory, recent reports in the literature indicate that other forms of colloids might increase the LSPR effect just described and thus the sensitivity of labelling free detection. We will therefore optimise synthetic processes for the production of colloidal gold and silver nanoparticles of various sizes and shapes. The synthesis of core-shell composites of gold-silver and silver-gold will also be considered. The particle shapes considered will include colloidal crystalline materials. The various materials synthesised will be characterised by UV-Vis spectroscopy and HPLC. They will be evaluated for their performance in surface plasmon resonance biomolecular recognition systems in liquid form used as models and available in our laboratory.

Component 5-2 (Principles plasmon resonance, Saratov State University)
The new particles resulting from Component 5-1 will require specific optical characterisation before a selection of the best performing material(s) producible. This Plasmon resonance characterisation of particles with various sizes and shapes is done within Component 5-2 by the Saratov laboratory, where a full evaluation of the optical properties (absorption and scattering) of the samples synthesised in Brussels will be performed in parallel. Evaluation of the sensitivity to changes in refractive index of the various samples in solution by experimental methods and confrontation to theoretical modelling will be assessed. The results obtained in this laboratory will be discussed regularly with Brussels (ULB) in order to allow fine-tuning of the synthetic procedures towards materials displaying the best optical properties.
The objective of components 5-1 and 5-2 is the optimisation of the synthesis of colloidal materials in solution displaying the best optical properties for surface plasmon resonance applications. The integration of both components is sketched in Fig. 15 below.

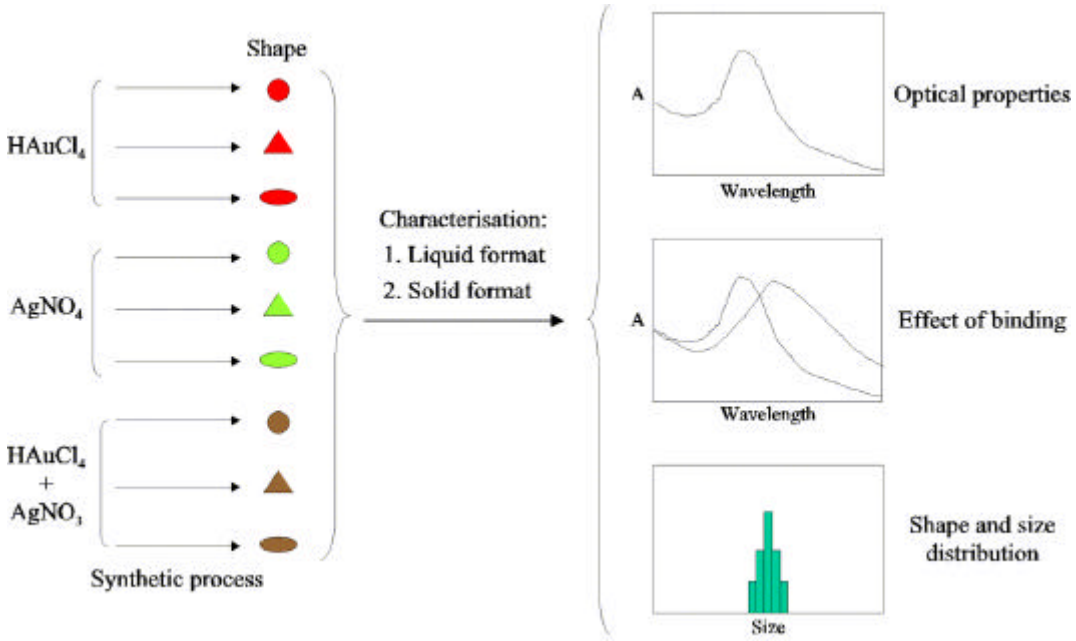


Fig. 15 Integration between components 5-1 and 5-2

Component 5-3 (Nano particles in array format, University of Brussels)

The conjugation of colloidal particles to proteins in solution is a straightforward process. Indeed, the particles remain negatively charged over a wide range of pH. Therefore, the selection of a suitable pH where the protein to be conjugated is surrounded by positive charges allows for a complete coverage of the particle surface by charge adsorption (Fig. 16). In order to do labelling free detection in the array format, however, the particles must be adsorbed to a surface first. Here, they serve as the solid support for the combinatorial synthesis of peptide arrays described in Activities 6-1 and 6-2. Thereby, discrete surface areas with nano particles displaying different peptides are generated.

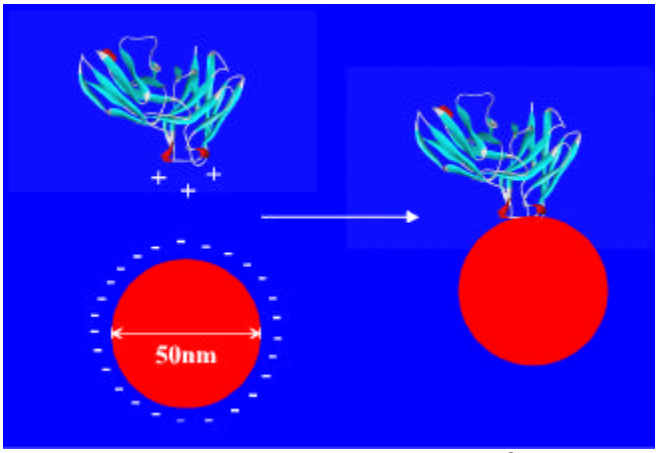


Fig. 16: Sketch showing the principle of charge adsorption for conjugating colloidal metal particles in solution

Within Component 5-3 we will adapt the synthetic procedures developed in Components 5-1 and 5-2 to the solid phase format. Various surfaces will be evaluated for their suitability (glasses and plastics displaying various refractive indexes). *In situ* synthetic methods such as lithography will be considered and compared with the film deposition of materials synthesised in solution. Yet another approach to be considered is schematically depicted in Fig. 17.

In a first step, the solid support is derivatised using ?-aminopropyl-triethoxysilane in order to introduce amino groups. The metal colloid is then adsorbed by charge on this layer, with the particle's surface deposition eventually helped by electrostatic charges switched on by the chip electrodes destined to comprise the array (Activity 4, Chip design). On the other side of the particles, further attachment of an anchor for peptide coupling occurs (mercaptopropionic acid is used in the example shown). Because the combinatorial synthesis of peptides (Activities 2 & 6) needs an amino group, the anchor is further derivatised by classical chemistry to yield the amino derivative. The different solid-phased materials thus produced will then be characterised by

spectroscopy and evaluated for their performance in biomolecular recognition models available in-house using surface plasmon resonance.

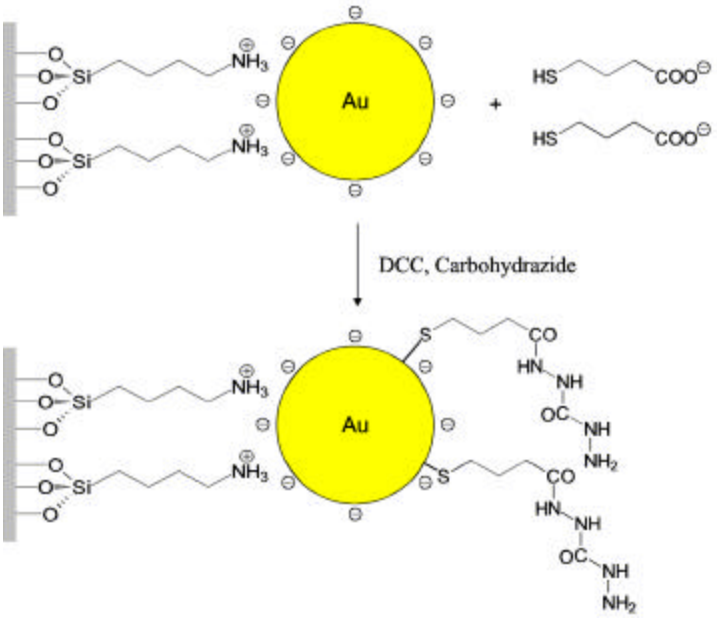


Fig. 17: Sequence of reactions for array construction with colloidal metal particles

Component 5-4 (Nano particle labels, Saratov State University)
Theoretical modelling of the optical behaviour of materials in solid phase and experimental evaluation of the samples provided by component 5-3 will be performed by the Saratov State University. Modelling of derivatisation effects on surface plamon properties of the colloids in liquid and solid phase and their influence on biomolecular recognition transduction will be done and verified in experimental models. A confrontation to the theoretical models developed will also be made in order to optimise the array performance in terms of evaluation parameters, size, shape and geometry. We expect some variation in the optical performances to occur as the result of the optimisation of synthetic procedures for alkyl derivatisation of the colloids and for adaptation to the peptide array system (covalent coupling of *in situ* synthesised peptides during array formation). Therefore, a full characterisation of the optical effects of the derivatisation procedures will be required. Integration of an instrument manufacture component will also be considered at this stage.
The objective of components 5-3 and 5-4 is to provide DKFZ and KIP with a surface plasmon resonance solid phase substrate susceptible to be adapted to the peptide array system. Furthermore, optical modelling and characterisation should allow the optimisation of the reader system development.

Component 5-5 (Array reader, Saratov State University)
This is done in collaboration with the DKFZ and KIP with the aim to integrate the sensor in the laser printer or chip array system. In particular, the effects of the amino acid toner particles used for peptide building and of coupling temperatures on the biochemical and optical performances of the colloidal substrate sensor will be evaluated. Necessary and iterative adaptations as a function of these various effects on the sensor performance will be considered. A model of array concept and performance is sketched in Fig. 18. It is envisioned to speed up the array reader by means of parallelisation, e.g. with the help of a matrix of photodiodes adjusted to the peptide arrays. The objective of this Component 5-5 is the complete development of an array sensor integrated into the peptide array generating components. At least, the partners should be able to use the sensing

instrument according to the model of biomolecular recognition considered and foresee its adaptation to other diagnostic models.

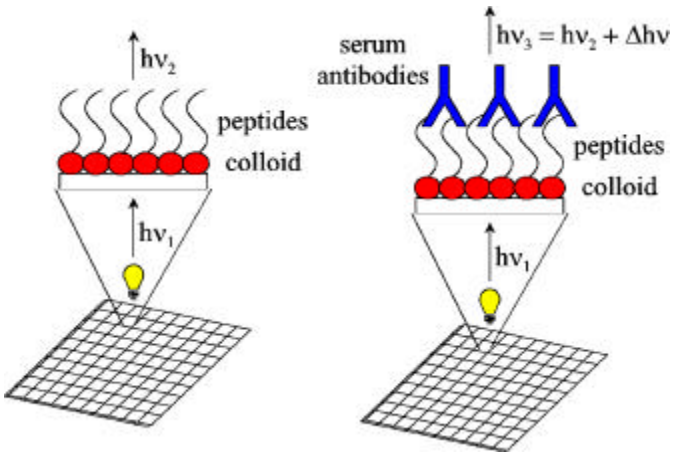


Fig. 18. Model of array concept and performance by surface plasmon resonance read-out.

Activity 6 (Peptide arrays, DKFZ)

Activity 6 integrates **Activity 1** (Model applications), **Activity 2** (Particle production), **Activity 3** (Peptide laser printer), **Activity 4** (Chip design) and **Activity 7** (Database) in order to synthesise peptide arrays and use them for the model applications planned.

Component 6-1 (Peptide arrays by a laser printer, DKFZ)

The laser printer constructed within Activity 3 and the particles produced in Activity 2 will be used for the combinatorial synthesis of high complexity peptide arrays. Initially the emphasis will be on proof of principle experiments, e.g. the chess board synthesis of two different peptides in increasing density that are stained by peptide-specific monoclonal antibodies. This kind of proof of principle experiments is an ongoing task done by DKFZ throughout the lifetime of the integrated project with the milestones and deliverables set in Part B7 and B8. Another objective within Component 6-1 is the synthesis of peptide arrays representing the proteomes of the viruses specified in Activity 1 in form of overlapping peptides. Data for the production of these arrays is derived from Swiss Prot database. Initially overlapping peptides shifted by 5 amino acids relative to each other will be represented on the array, with increasing time the shift narrowed to 2 and finally 1 amino acid. Simultaneously the peptide's length will be increased from 15 amino acids to 20 amino acids. These peptide arrays will be sent by mail to Dr. Shemer-Avni, who will test them with the patient's sera collected within Activity 1.

Component 6-2 (Peptide arrays by a chip, DKFZ)

Component 6-2 is similar to Component 6-1 just described with the difference of the using the chip (Activity 4) instead of a laser printer (Activity 3) in order to address amino acid particles (Activity 2) for combinatorial synthesis. As shown by the milestones and deliverables set in Part B7 and B8 we initially expect a significant slower progress compared to the laser printer. This is due to the comparatively low voltage applicable to chip structures making progress in this area much more dependent on progress in the sophisticated production of particles. Only small particles (approx. 1µm) are expected to have a q/m ratio high enough for reliably addressing particles to defined surface regions. Another reason for the initially slow progress is the relatively long time needed for chip design and testing. If achieved, however, this approach will lead to peptide arrays of extremely high density, because it exploits the huge preexisting knowledge in the miniaturisation of chip structures. As described for Component 6-1, the emphasis will be on proof of principle experiments, once again as an ongoing task done by DKFZ throughout the lifetime of the integrated project.

Component 6-3 (Analysis of peptide arrays, DKFZ)

In Component 6-3 (DKFZ), loading densities, coupling efficiency and overall yield with respect to the combinatorial synthesis of peptide arrays will be embraced. Instrumental in this is the DKFZ' expertise in surface modification and analysis. Emphasis will be on the deprotection of surface bound Fmoc-amino groups by piperidine followed by the routine photometric quantification of the dibenzofulven/piperidine adduct (λ = 301 nm). When coupling an Fmoc-amino acid to a surface or already synthesised peptides, this quantification enables us to continuously determine an overall coupling yield in a flow chamber equipped with an UV photometer. Staining of terminal amino groups with Bromophenol Blue will be employed for spatially defined determination of coupling yields by high-resolution image recognition. More sophisticated methods of surface analysis are available at the Institute of Applied Physical Chemistry in the University of Heidelberg: X-ray photoelectron spectroscopy (XPS) also allows overall quantification of coupling efficiencies and loading densities by determination of atomic compositions as well as the evaluation of film thicknesses. The latter is also enabled by ellipsometry in a static as well as dynamic mode. Infrared reflection absorption spectroscopy (IR-RAS) will be employed for qualitative and semi-quantitative surface analysis, which is particularly important with respect to the synthesis of amino-terminated organic layers on printer supports or the CMOS chips (Component 4). In summary, the enumerated methods will allow a more than sufficient characterisation and online analysis of the peptide arrays.

Activity 7 (Database, EBI)

Component 7-1 (Protein sets, EBI)

The EBI Proteome Pages (http://www.ebi.ac.uk/proteome) provide precomputed proteome analysis of completely sequenced organisms. As part of the description of each organism, a proteome set is provided which represents a high quality, non-redundant assembly of the proteome of the organism. The proteome sets are built on the manually annotated Swiss-Prot sequences and are complemented by automatically annotated sequences from TrEMBL or other, organism-specific databases, in particular Ensembl for eukaryotes. The proteome sets are based on Swiss-Prot format and contain detailed protein annotation where available. Alternatively spliced sequences are already contained in the proteome sets, and further sequence variations, e.g. conflicts between reports, or signal sequences, can be automatically extracted, thus providing a rich protein sequence set representing the currently known proteome of the organism. The curation efforts of the Swiss-Prot team at the EBI will prioritise curation according to the needs of the project.

Component 7-2 (PREJUDICE, EBI)

Results of the binding experiments performed with the constructed complex peptide arrays will usually be sets of proteins or peptides, e.g. differentially binding peptides between two states of a system, in particular patient versus control serum. The correct interpretation of these protein sets is very time-consuming, because the relevant knowledge is often distributed over many databases. The PREJUDICE system (Proteomic REsults JUdgement, Interpretation and Control Environment) will improve the efficiency of the analysis of results by providing optimised global and detailed views of the publicly available knowledge of individual proteins and protein sets. To facilitate the analysis of proteome sets in addition to individual proteins, we will create integrated views of these protein sets, emphasising shared properties, subclusters and potentially significant outliers. These views will be provided for multiple dimensions, for example functional category, subcellular localisation, InterPro domains as well as shared protein interaction partners, and will be highly configurable, with intelligent, data-dependent default settings. In addition, it will be possible to provide locally generated data and take this into account during the analysis. To provide optimised, data-dependent views of protein sets, we will apply data mining technologies, which have been successfully applied in the automatic annotation of protein sequences in the Sequence Database Group, for example the C4.5 algorithm. In the construction of the PREJUDICE tool, the EBI will bring to bear its long-standing experience in proteomics data integration and data mining.

Component 7-3 (Proteomics result disseminiation, EBI)
The Swiss-Prot database is currently a key resource for proteomics data. Its high level of manual curation, the extensive cross-referencing to other databases and the high level of standardisation makes it an integration nexus for publicly available data on proteins. However, not all kinds of data are suitable for integration into Swiss-Prot, either because of an excessive level of detail, or because the data structure is unsuitable for Swiss-Prot, in particular raw array data or protein interactions. The EBI is currently developing additional resources for proteomics data, in particular the IntAct protein interaction database. In the context of the Proteomics Standards Initiative of the Human Proteome Organisation, the EBI is actively participating in the development of international standards for Proteomics data. Through integration of the experimental results into public proteomics resources the EBI will increase the visibility of the project and ensure the availability of the experimental results during and beyond the project duration.

Activity 8 (for years 4 & 5, Prototypes, SMEs)
Activity 8 comprises the construction of prototype machines based on the know-how compiled by the Participants in the initial 3 years. Basically, these machines should be compatible with each other, e.g. in order to do labelling free detection with a peptide array synthesised on a chip's surface. Commercialisation of these prototypes will be done by SMEs to be added to the consortium, either by different SMEs that each develops one of the prototypes described below, or by one SME that commercialises the whole technology alone. The SMEs will finance their part of demonstration activities, with the construction work presumably done by Fraunhofer IPA by order of the SME Participant.

Component 8-1 (Prototype peptide laser printer, Fraunhofer IPA, SME)
Based on the first instrument available by year 3 that is described above (Activity 3, Peptide laser printer), a prototype peptide laser printer will be constructed. While the first instrument mentioned is mainly intended to prove technical feasibility and to experiment with, easier handling and a high degree of automation are crucial for the commercialisation intended. The machine will be designed to do synthesis of high complexity peptide arrays in a

- user friendly,
- error proof,
- high throughput and
- highly automated fashion.

Emphasis will be on an automated handling system (robots) combined with an optical or tactile sensor system to ensure the precise positioning of the sample carrier on the linear driving unit. A further important aspect is the user friendliness of the control software. As long as the peptide printer is regarded as an experimental instrument only specialists are supposed to operate the printer. For a prototype suitable for serial production a safe and easy handling is required. This is especially important for the simultaneous production of peptide arrays, i.e. for a production line. And last but not least, the software should intercept simple user errors or misentries (if possible) in order to avoid damage of the sample carrier or the machine.

Component 8-2 (Prototype chip synthesis machine, KIP, Fraunhofer IPA; SME)
Based on the instrument described in Component 4-2 (Chip instrument) for testing the CMOS chips, proving technical feasibility, and semi-automated array synthesis, it is envisioned that a SME will develop a corresponding prototype chip synthesiser for the fully automated synthesis of peptide arrays on the high-voltage CMOS chips within Component 8-2 for commercialisation. Compared to the peptide laser printer (Activity 3, Component 8-1), this apparatus is expected to be substantially smaller and hence easier to handle. While the laser printer will have to dispose 20 large toner cartridges, the chip synthesiser will employ 20 toner reservoirs for tribocharging in form of small coated tins as fluidised beds. Another feature is integrated into the CMOS chip itself, i.e. the

complete positioning necessary for the high-resolution peptide synthesis. This also will give the chip synthesiser a cutting edge compared to the complex and bulky assembly of linear stages as well as calibrating units in the laser printer. Summing up, the SME will design a fully automated prototype for the synthesis of peptide arrays on a chip, which will be safe, easy to handle (user friendly), error proof by means of robust and established structural elements, and particularly portable.

Besides the electronic controls done by a computer, the chip synthesiser for commercialisation will dispose a data read-out for binding events. Constituents will already be integrated into the CMOS chip itself (see Component 4-3 and 8-3, labelling free detection or Component 4-4, photodiodes), resulting in minor assimilation of the machine electronics needed. It is envisioned to incorporate the residual data read-out components in the mentioned computer for electronic controls.

Component 8-3 (Prototype Array reader, Saratov State University; SME)
In order to assimilate and standardise the labelling free detection system described in Component 4-3 and in Activity 5, a SME partner will design and engineer the prototype of an array reader for commercialisation. Depending of the insights in the fields of physics, mechanics, electronics, and image recognition we will gain in Activity 5, two standardised array readers for arrays by the peptide laser printer (Component 8-1) and arrays by the chip synthesiser (Component 8-2) will be envisioned. Therefore, the SME will enable the parallel detection of extinction shifts evoked by unlabelled binding events by means of arrays of photodiodes. The vast data stream expected will be processed by arithmetic preselection of apparent events, which will be caused by light scattering and/or background irradiation. Also, the SME will incorporate a wavelength filter in order to reduce the amount of "false" data.

Especially in the case of the chip synthesiser (Component 8-2), a close corporation of the SME(s) will be indispensable with respect to a fully integrated system as well as electronics and CMOS chip parameters. In case of the peptide laser printer, an autonomic system will be developed. Nevertheless, the SME will modulate the prototype array reader with respect to the printed array format as well as a periodical maximum spot density. This affords a flexible, broadly applicable system of one or more photodiode arrays grating an array surface. Data read out and output in both synthesis systems will be reversible, fully automated, comprehensible (e.g. in form of a graphical output), basically adaptable to other array formats, and therefore user friendly with respect to commercialisation.

Activity 9 (Administration, DKFZ)
Activity 9 comprises the management of the integrated project. Financial management will be done by the DKFZ administration within **Component 9-1** (**Financial administration, DKFZ administration)**, while **Component 9-2** (**Scientific administration, DKFZ)** comprises the scientific management done within Dr. Breitling's group.

B.4.2 Demonstration activities:
The project initially is dominated by research components (*production of particles; definition of ORFs; model applications; new method for labelling free detection; chip design*) and technological components (*e.g. proof of concept in chip design*). Indeed our interdisciplinary project spans a large spectrum from basic to applied research. Demonstration activities are only planned for a second phase, i.e. in years 4 & 5. At this time, technological and scientific progress is expected to have paved the way for stronger efforts in commercialisation that are envisioned within **Activity 8 (Prototypes)**. SME partners yet to be added to the consortium will lead and pay for demonstration efforts designed to prepare for commercialisation of peptide laser printer or chip technology. The demonstration activities planned are:

- **Component 8-1**: Construction of a **reliable prototype peptide laser printer** suitable for commercial synthesis of peptide arrays based on the preceding **Activity 3 (Peptide laser printer)**, which was done at Fraunhofer IPA,
- **Component 8-2**: Construction of a **reliable prototype** doing fully automated combinatorial synthesis of **peptide arrays on a chip's surface** based on the preceding **Activity 4 (Chip design)**, which was done at Kirchhoff Institute (KIP) and
- **Component 8-3**: Construction of a **reliable prototype** that integrates read out methods, preferably with labelling free read out of binding events based on the preceding **Activity 5 (Read out methods)**, which was done at the University of Brussels and the Saratov State University.

The SME partners yet to be added to the consortium will result from a EU wide search for SME partners of complementary competence (*prototyping, marketing, world wide distribution*). Another alternative are venture capital financed SMEs that hive off from the public institutes involved in the scientific work described.

Contingency planning for unexpected outcomes

For most of the subprojects described here, proof of principle experiments have been carried out successfully (*combinatorial synthesis of peptides by solid particles; addressing particles to chip's surface; labelling free detection by nano gold particles*). Still, exploring the undiscovered is always risky, i.e. unexpected outcomes are intrinsic in ambitious science. We accommodate for this in flexible parallel developments of technology that could substitute partial failure:

- Both the peptide laser printer and high voltage chip design can be used to address monomer toner particles for combinatorial peptide synthesis, yet each of these methods has its own merits (*e.g. free choice of solid support with the laser printer*);
- Different kinds of activated amino acids can be incorporated into toner particles, thereby reducing the risk e.g. of incompatibilities in solubility. Alternatively, inactivated and hence very stable amino acids will be activated inside the particle's compartment by light and a photobase;
- Different methods for production of toner particles are envisioned in line with the expertise of the scientists involved, once again with special merits for the different methods (*e.g. avoiding H_2O contamination with PGSS method*);
- The high risk project of labelling free detection by colloidal gold nano particles will be flanked by more conventional read out methods, e.g. an array of photodiodes either standing alone or integrated into a special chip design.

The flexible management of knowledge and intellectual property (see above) is also part of the contingency planning, especially in order to integrate outstanding 3rd party developments into our project. This point certainly also will influence the choice of the SME partner for demonstration activities. Due to the uncertainty in scientific and technological progress we plan a two-phase model for this grant application and we suggest an extra auditing (*done by EU referees*) preceding phase 2:

- Within years 1-3 emphasis will be on scientific research (phase 1), while
- Within years 4 & 5 in addition to scientific research additional SME partners will lead efforts for commercialisation (phase 2).

This two-phase model especially is chosen in order to accommodate for unforeseeable scientific and technological developments that will decide over economic success or failure (*e.g. rise of competing technologies; failure in milestone achievements*). Within 3 years it should be clear if the commercialisation planned has a sound basis or not.

B.4.3 Training activities:

The training activities specified below for the various partners within the project will have a large impact for the success of the project, especially since partners from very different disciplines are going to work closely together. It has been realised in previous meetings that it is indispensable to

get across the other's disciplines in order to find out basic things like e.g. a need for the chip's passivation against electrolyte solutions. Therefore, interdisciplinary meetings and workshops will be offered to inform the partners about basic aspects of their interdisciplinary work and progress of activities. These meetings are mostly in line with the Project Co-ordination Committee's meetings but also beyond the consortium in order to communicate

- Within consortium members and
- With outstanding 3rd party developments that might be comprised into the project.

The meetings are especially targeted to the large number of undergraduate and graduate students directly or indirectly involved in the Participant's groups.

Participant 1 (DKFZ)
The DKFZ will organise (mostly in line with the Project Co-ordination Committee's meetings) short (1 –2 days) interdisciplinary meetings in Heidelberg where mainly the project's Participants lecture basic principles underlying their work.

Participant 2 (Tel-Tek)
Tel-Tek performs a number of training activities in powder related subjects, and among other it arranges short courses in powder technology and related subjects. A number of students are every year doing final Master and Diplomas degrees and other projects, staying approximately 6 months. Students will be incorporated into the project, but will of course require special training, and given training in working in cross-discipline areas. Tel-Tek is also a Marie curie training site in powder technology, and trains both Norwegian and foreign PhD-students. Tel-Tek will organise workshops on its specialised fields to other participants.

Participant 3 (Fraunhofer IPA)
Within years 4 and 5 a prototype peptide laser printer based on the first instrument will be constructed by SMEs (Activity 8). Since the development of the printer until this stage is done at the Fraunhofer IPA a knowledge transfer to the involved SMEs is necessary. Therefore appropriate training activities will be organised at the Fraunhofer IPA which will accompany the development of the prototype within years 4 and 5. Parallel to this it is planed to organise workshops for potential users of the peptide laser printer at the DKFZ or the Fraunhofer IPA in order to support the commercialisation of this technique. The aims are to show potential users the benefits and to get them accustomed to this technique. Workshops about different subjects aiming especially at SMEs are frequently and successfully organised at the Fraunhofer IPA.

Participant 4 (KIP)
There are two areas of technology transfer, which require some staff training to be performed. On one hand the peptide chips need to be mounted and connected to the PCB using the low-cost-chip on-board technique. Appropriate protection has to be deployed (glob top), protecting the bond wires. During the years 4 and 5, when a large number of chips is being produced the appropriate knowledge transfer to the involved SMEs is required. On the other hand the peptide plotter device, built in this project during the first 18 months, is required to be operated by the staff of other partners, which are to be trained appropriately. Appropriate one or two day workshops are planned to be organised for this purpose.

Participant 6 (Univ. Brussels)
Training activities constitute an essential part of the success of our participation in the project. In order to fulfill its tasks, ULB will have to hire a full time post-doc collaborator from the start of the project which includes the optimization of synthetic procedures. The field of colloidal metals synthesis and use is new and very specific. Currently, no particular training is provided in this field in the curriculum of chemistry of biochemistry students. Consequently, appropriate training will be

made available by our laboratory to the new member. During the two last years of the project, the interactions with SMEs will also need further discussions, trainings and workshops, in order to brief their personnel to the technology developed during the first three years.

Participant 7 (Saratov)
Saratov scientific group has expertise in such an interdisciplinary field as Preparation, Optical Characterisation and Biomedical Application of Nanoparticle Markers. Our Institute (IBPPM RAS) in coordination with the Saratov State University would be able to host a specific short courses for graduate students and PhD's involved to the project.

Participant 8 (EBI)
The European Bioinformatics Institute (EBI) is performing a number of training activities, as high-level training to its staff, students and external guests is part of its mission: There are various conferences, seminars and workshops in the field of bioinformatics where members of the research community are informed about the projects and databases which are developed and maintained at the institute and teached how to use the tools and databases. (In 2002, for example, there has been the Proteomics Standards Initiative Meeting, an InterPro Workshop, a BioJava Boot Camp Workshop, a Human Genome Research Workshop about ethical, legal, and social implications, EU Advanced Practical Workshops and various seminars.)
Training of students is also part of the training activities of the EBI. There is an average number of about 10 undergraduate students per year who normally stay for a period of 6 months. Additionally, there is intensive PhD student training going on, coordinated by the EMBL PhD Programme, and the Marie Curie Training Sites Fellowship scheme in conjunction with the EBI offers short-term fellowships for PhD training in Bioinformatics awarded by the European Commission. The students are trained on project-related activities, so they are directly involved in the research projects.
Dr. Rolf Apweiler's Sequence Database Group at the EBI would be able to host a specific workshop for the PeptideLaserPrinter project in which participants could be trained in using the developed resources and tools, provided that there would be additional funding available (costs are about 9.000 € per 3-day workshop for 20 participants on site).

Participant 9 (BGU)
Since partners from very different disciplines are involved in the project, we intend to give an intense course in virology to the other participants. This training will take place either in the BGU, Beer Sheva or in any another place that will be chosen by the people involved. We intend to send our PhD students for training courses to EBI (Participant 8) and to the DKFZ (Participant 1). The training will help the bi-directional analysis and the management of data. This way the student will become familiar with processes done in EBI, and how to organize the data obtained from the pathogen-peptidome reaction with the sera, and the patients records.

Participant 10 (SME to be added to the consortium)
Participant 10 intends to commercialise the scientific achievements reached in years 1-3 of Participants 1-9. Therefore intensive and advanced training of technical personnel and researchers as well as industrial executives is needed in order to achieve these goals. Training must comprise most aspects of the technical, scientific and wider application oriented details of the technology developed.

B.4.4 Management activities:

The Integrated project will be managed by the different bodies explained in detail in section B.6. Decisions made by the Co-ordination Committee, the General Assembly of all Parties and the Project Co-ordinator respectively will be executed by the Project Office. The PeptideLaserPrinter project consists of different main tasks:

The scientific and technological co-ordination

Dr. Frank Breitling (Project Co-ordinator, DKFZ, Participant Nr. 1) will be responsible for:

- Co-ordination of scientific and technological developments in relation to the programme objectives and applications,
- Definition of project milestones: using the milestone reviews, the progress of the project can be critically reviewed and compared to the planning.
- Overall supervision and monitoring of the scientific programme on a workpackages level,
- Adjustment of the workplan and development of new work packages, deliverables and milestones for the next 18 months,
- Planning of future activities based on the input of the advisory council,
- Budget allocation,
- Launching of competitive call for proposals.

The administrative project management

One member of the project office referring to B6 will be located within the DKFZ administration (Heike Wester) and will be responsible for:

- Day-to-day project management (information and communication between all partners, facilitating the exchange of documents, optimisation of workflow, etc.)
- Helpline for partners' questions
- Supporting the co-ordinator as a mediator between all partners and the European Commission
- Organisation and moderation of partner meetings (kick-off, mid term, etc.)
- Communication with the Commission / project officer in charge
- Co-ordination of the project reporting

The financial and accounting management

One member of the project office will be located within the DKFZ administration (Financial Officer: Bettina Crispin, lawyer) and will be responsible for:

- bookkeeping
- allocation of money to Participants
- Budget distribution and controlling
- Preparation of cost statements
- Adjustment of the budget (breakdown of costs)
- Preparation of potential audits by the Commission

The management of gender issues

Dr. Barbera Bertram (women's representative at the DKFZ) will oversee the promotion of gender equality in the project.

The management of Innovation-related activities

Dr. Ruth Herzog, head of the department of Technology Transfer of the DKFZ, will be responsible for:

- Conception of a Technology Implementation Plan
- Dissemination of project results

- Organisation of workshops or conferences
- Support for Intellectual Property Protection with the help of lawyers (Dr. Breitling's long record in successful patent applications makes him the person of choice to co-ordinate intellectual property related issues in close contact with the DKFZ's department of Technology Transfer department, if approved by the General Assembly)
- Design and set up of webpages
- Publications
- Studies for the creation of spin offs
- Take-up measures
- Technology transfer possibilities

Further Tasks are:

- Project Controlling
- Management of Dissemination / Exploitation Activities
- Take up Measures
- Training activities

B.5 Description of the consortium

Description of the Participants and the main tasks attributed to them

In order to achieve the objectives planned within this integrated project Participants with complementary interdisciplinary expertise will combine efforts. Particle based combinatorial synthesis was invented in the German Cancer Research Centre (DKFZ), which is rated among the top 5 institutes world wide in the field of molecular biology. However, in order to implement such a technology beyond a first level of proof of principle, complementary expertise is needed in the fields of:

- particle technology,
- engineering,
- microelectronics,
- optics,
- read out methods and
- databases.

These scientific disciplines are certainly outside the DKFZ's core competence. Therefore other European institutes contribute these very diverse expertises and thereby add value. All of these institutes, as well as the individual scientists involved, rank high in their respective scientific fields, which should help in the achievement of the milestones planned. Thus a tool will be developed that translates the huge set of genome data into peptide arrays, which will enable scientists and industry to do experiments in a truly proteomics scale in their search for insight and practical applications. Therefore, in addition to the scientific impact, the technology to be developed is expected to have a large commercial impact, which in addition to SME Tel-Tek should arouse the interest of industrial partners to lead demonstration activities and finally commercialisation planned for years 4 & 5 within this project. Instrumental in this respect is the existence of intellectual property inside the DKFZ that spans the whole area of particle based combinatorial synthesis. Given the many patents mining the promising field of array technology, even more important is the freedom to operate expected to exist for our technology, which is due to the solid particle based novel approach.

Role, Contribution and Qualification of Participants

Participant 1 (DKFZ, German Cancer Research Centre, Germany)

DKFZ is among the world wide top 5 institutes in the field of molecular biology. The DKFZ department of Molecular Genome Analysis plays a leading role in genomics, i.e. in the deciphering of the human genome. Prof. Annemarie Poustka heads its > 70 scientists. Within this department **Dr. Frank Breitling** (biochemist) has a long record in successfull technology development. He co-invented the technology of recombinant antibodies, documented by a textbook and by the fact that most of his 12 patents to date resulting from his work are licensed to companies. One of them (US patent 5840479) should cover most antibody libraries used to date (*especially HuCal from Morphosys*). He also invented, proved and patented (EP1140977A2, US880688) the method for particle based combinatorial synthesis. To date 7 persons work on the development of peptide laser technology inside Dr. Breitling's group, with another 3 persons working with his main collaboration partner inside DKFZ, Dr. Ralf Bischoff, who is intimately involved in this and related projects (DKFZ department Molecular Biology of Mitosis). Given the cross-section know-how in genomics, proteomics and array related technologies existing in this department, Dr. Breitling is ideally suited in co-ordinating and integrating the project planned **Activity 6 (Peptide arrays)**. Furthermore Dr. Breitling's group meanwhile acquired a lot of expertise in the production of amino acid toner particles, an expertise needed for the accomplishment of **Activity 2 (Particle production)**. In addition to that DKFZ will do the financial and scientific administration of the project **Activity 9 (Administration)**.

Curriculum Vitas Dr. rer. nat. Frank Breitling

Date of Birth: Jan 19th 1959 Place of Birth: Heidelberg, Germany Nationality: German

Education

1979-1985 Studies of Biology at the University of Heidelberg
1992 PhD, University of Heidelberg

Positions held

1992-1994 DFG-Fellowship, Dpt. Molecular Biol Mitosis, DKFZ
1995-1996 DKFZ-Fellowship, Dpt. Recombinant Antibodies, DKFZ
1997 Staff Scientist Institute of Mol. Genetics, University of Heidelberg
Since 1998 Staff Scientist, Dpt. Molecular Genome Analysis, DKFZ

Scientific and technical personnel involved

PD Dr. F. Ralf Bischoff (biophysics, protein chemistry) was right from the beginning involved in all the proof of principle experiments related to the peptide laser printer technology. Before that he discovered the proteins and protein functions of the main actors driving nuclear transport (e.g. Nature, 1991, 354:80-2), thereby opening a whole new field in molecular biology. Dr. Bischoff is an excellent expert in protein chemistry which makes him ideally suited to look at the peptide arrays from an applicator's view.

Dr. Volker Stadler (chemist, surface chemistry, nano technology) earned his degree awarded with a silk ribbon of excellence at the physical chemistry department of the University of Heidelberg. His expertise is instrumental in all the questions dealing with suitable solid supports, toner formulation, surface chemistry and activation of the coupling reaction on demand inside the particle's compartment.

Dr. Thomas Felgenhauer (chemist, surface chemistry) in the last year has specialised on the search for and testing of suitable charge control agents and charge stabiliser agents that are needed for the transfer of particles to the solid support.

Dr. Simon Fernandez (chemist, particle technology) has a long and successful record in the formulation of defined particles (*originally designed for pharmaceutical applications*). He is responsible for all the aspects dealing with the production of toner particles, e.g. by RESS method.

Dipl. Ing. Klaus Leibe (engineer) is responsible for all technical aspects of the work. He did the proof of principle experiments, which showed that a potential of 30V is sufficient to direct toner particles to a definable surface of an electrode (Fig. 10). This work will be expanded to the use of chips as solid support for peptide arrays synthesised by combinatorial synthesis.

Publications relevant to the project

- Breitling, F.; Poustka, A.; Groß, K.H.; Dübel, S. and Saffrich R. (2001) *Method and devices for applying substances to a support, especially monomers for the combionatorial synthesis of molecule libraries.* US patent application US880688.
- Breitling, F., Poustka, A., Groß, K.H. and Breitling, F. (2001) Verfahren und Vorrichtung zur Synthese, *in situ* Reinigung und Analyse von Träger gebundenen Oligomeren. German patent application DE10004659, international patent application WO2001056691
- Breitling, F. (2002) *Systems for the selection and identification of binders*. Habilitation, University of Heidelberg.

Participant 2 (SME Tel-Tek, Norway)

Tel-Tek is a SME research institute organised as a foundation mainly funded by the county of Telemark. The department of Powder Science and Technology (POSTEC) is headed by Prof Gisle Enstad. Tel-Tek has a close cooperation with Telemark University College, sharing at times personnel and equipment. When Norway in 2002 started its first centres of excellence in research, Tel-Tek was one of the final contestants, exactly in the area of powder technology. **Dr Sivert Ose**

(electrical engineer) has worked on electrostatic charging of particles both inside academia and for industry for more than 10 years. He is member of the EFCE WP on electrostatic charging in the process industries. He has also worked extensively on other characterisation methods, being Norwegian member of ISO/TC24/Sc4 (particle sizing) and in EFCE WP on characterisation of powders. Besides heading his group of 5 employees, he also has teaching responsibilities at Telemark University College. Tel-Tek's main expertise is the production of powders with an emphasis for this grant application on the electrostatic chargeability of particles, an expertise crucial for **Activity 2 (Particle production)**, especially when attempting to specifically address particles by low voltage to a chip's surface. Depending on the achievements within this integrated project the SME Tel-Tek will be involved in the commercialisation of the amino acid particle technology.

Curriculum Vitas Dr. Sivert Ose

Date of Birth: Apr 20th 1964 Place of Birth: Ørsta, Norway Nationality: Norwegian

Education

1989 Cand.Mag (equiv. to B.Sc.) University of Bergen, Norway (physics, electronics)

1992 Cand. Scient (equiv. to M.Sc.) University of Bergen, Norway (Physics, instrumentation)

2001 Ph.D., University of Southampton, UK (Elec. Eng, electrostatics)

Positions held

1991 Tel-Tek dept. POSTEC: research associate, scientist, senior scientist

Scientific and technical personnel involved

Ms. Anne Berdal has an M.Sc from Telemark University College in 1998. She has been working at Tel-Tek dept. POSTEC since as a research assistant/engineer. Main responsibilities has been laboratory work, data analysis etc.

Mr. Franz Otto von Hafenbrädl has an M.Sc from Telemark University College in 1994. He has been working at Tel-Tek dept. POSTEC since 1995 as engineer/research assistant. His main responsibilities have been particle production by air classification, laboratory work, data analysis etc.

Publications relevant to the project

- Sivert Ose, *Bipolar electrostatic charging of powders*, PhD-thesis, University of Southampton, 2001

Participant 3 (IPA, Fraunhofer Institute for Manufacturing Engineering and Automation, Germany)

The Fraunhofer Institute for Manufacturing Engineering and Automation (FhG-IPA) headed by Prof. Dr. Engelbert Westkämper and by Prof. Dieter Schraft is the second largest institute within the Fraunhofer Gesellschaft. To date IPA's 14 departments with its >250 employees has an annual turnover of >30m€. Fields of research are manufacturing engineering, automation technologies, and enterprise management. The research is mainly application orientated covering organisational and technological problems within all fields of production engineering for industrial enterprises. The institute's projects cover the development and application of methods, software, and devices as well as prototypes of machines and production facilities. Additionally, research is done on projects, which are sponsored by public research programs (the BMBF, the DFG and the European Union). The long-term aspect of public research is the industrial implementation from which enterprises especially SMEs are supposed to benefit. With its know-how and experience gained over many years and its technical equipment, the Fraunhofer IPA is able to deal with a great variety of manufacturing problems. The IPA's 5600sqm of laboratories include among others a:

- Laboratory for processing and disposal technology
- Laboratory for testing techniques and metrology

- Laboratory for microscopy, metallography
- Laboratory for process analytics
- Laboratory for sensor systems
- Laboratory for medical technology
- Laboratory for automation in biotechnology
- Laboratory for test and serial production for rapid-product-development
- Laboratory for coating technology

The departments "Technical information processing" and " Surface Engineering" are involved in Activity 3 (Peptide laser printer). The department "Technical information processing" is working in the fields of image processing, testing technique, metrology, rapid product development, and rapid prototyping since many years and has realised a large variety of projects for both private and public contractors. A new field within rapid prototyping are printing technologies including laser printing and wax jet printing. The focus of the laser printing technique lies on micro technology, i.e. on the production of high precision arrays and patterns with multiple layers. The device prototypes are developed and realised at the IPA.
The department of Surface Engineering is working in the field of industrial application of surface technologies (electroplating, plasma technologies). On the one hand coating processes are advanced and adapted to new materials, on the other hand customised chemical reactors and production facilities are developed and realised, for example mobile electroplating facilities for the printing industry. The department „Surface Engineering" has the expertise and experience to develop an automated chemical reactor for the Merrifield synthesis.

Curriculum Vitas Prof. Dr. Ing. Dr. h.c. Engelbert Westkämper
Date of Birth: Mar. 07. 1946 Place of Birth: Paderborn, Germany Nationality: German
Education
1967-1973 Studies of Engineering at the RWTH Aachen
1977 PhD, RWTH Aachen
Positions held
1977-1979 Staff member, MBB Inc., division of air crafts
1979-1983 Group leader, MBB Inc., headquarter
1983-1986 Head of the department "Manufacturing Engineering", MBB Inc., division of cargo planes
1987-1988 Head of the central division "Manufacturing Engineering", AEG, Inc.
1988-1995 Head of the Institute for Machine Tools and Production Engineering, TU Braunschweig
since 1995 Head of the Fraunhofer Institute for Manufacturing Engineering and Automation, Head of the Institute for Production Engineering and Factory Operation, TU Stuttgart

Scientific and technical personnel involved:
Dr. rer. nat. Stefan Güttler (scientific team leader, physicist) graduated in1996. He obtained his PhD 1999 about nonlinear time series analysis and signal processing at the Max-Planck-Institute for Physics of Complex Systems in Dresden. Since 2000 he is at the Fraunhofer IPA at the department "Technical Information Processing" where he works on process control, signal processing and the development and industrial application of physical technologies. He is responsible for the xerographic process (i.e. laser printing) to work with the required accuracy, i.e. to provide the concepts of the physical, mechanical, electrical and control requirements and to operate the peptide printer.
Bernd Biesinger:(mechanical engineer). He graduated in 1996 in mechanical engineering at the TU Stuttgart and is working in the department "Technical Information Processing" since then. He has

acquired a great experience in developing and realising customised machines. He will be engaged in the mechanical and electrical construction of the peptide laser printer.

Martin Gröning (electrical engineer) is working at the department since 2000. He is a hardware specialist and will develop the hardware controller of the peptide synthesis machine, i.e. programme the FPGAs for controlling the LED units and the driving units .

Axel Henning (mechanical engineer) graduated 1995 in Hannover in mechanical engineering and is working in the department since then. He is a specialist for developing mechanical solutions for customised machines and will be responsible for developing the high precision components of the machine.

Markus Hüttel (computer scientist)**:**is working at the department since 1995. He is a computer and software specialist and is responsible for the control architecture of the peptide printer, the development of the user software, and the interfaces to the hardware and the databases.

Peter Willems (technician) is working at the IPA for many years. He has a great experience in CAD construction, operating machine tools and assembling customised machines. He will work in the mechanical and electrical realisation of the peptide laser printer.

Nikolaus Zell (process engineer) is working in the department "Surface Engineering" since 1996. He is specialized in systems engineering and plant construction for customised surface technologies. He will be responsible for developing the washing unit.

Publications relevant to the project:

- S. Güttler , A. Henning und M. Hüttel *"Konstruktion und Realisierung einer Synthesemaschine für Peptidarrays"*, Jahresbericht 2002, Fraunhofer IPA, Januar 2003.

Participant 4 (KIP, University of Heidelberg, Kirchhoff Institute for Physics, Germany)

The subproject will be lead jointly by Prof. Dr. Lindenstruth and Dr. U. Trunk, the head of the interdisciplinary ASIC Laboratory in house. The main focus of the institute in this project is the development of the Peptide Chips, their readout and integration. The chair of computer science concentrates on the development of readout and control systems for scientific applications, ranging from microelectronic development to large scale high performance computing systems and leading to the delivery of the complete working system. Together with the ASIC Laboratory under the lead of Dr. Ulrich Trunk a variety of chips were produced, including mixed signal analogue/digital designs with high performance readout and processing systems. The latest chip in preparation includes more than 5 million transistors in the deep submicron UMC 0,18µm process. It combines 21 10-Bit, 10 MHz ADCs with four 120 MHz processors and several 2.5 GBit readout links. Working prototypes exist.

Curriculum Vitas Prof. Dr. Volker Lindenstruth

Date of Birth: October 8th , 1962 Place of Birth: Frankfurt/Main Nationality : German, US Green Card

Education

1983-1989	Studies of Physics at the Technical University Darmstadt.
1989-1993	Graduate student at Gesellschaft für Schwerionenforschung in Darmstadt focusing on the development of a multi processor readout system for heavy ion physics experiments.
1993	Graduate at the University Frankfurt
1993-1995	Post Doc, Lawrence Berkeley National Laboratory, award a two year Humboldt fellowship in computer science.

Positions held

1995-1998 Permanent scientific staff at the UC Space Sciences Laboratory. In summer 1998 Prof. Lindenstruth founded iCore technologies, a US corporation focusing on the development of high-speed computer interconnects.

1998 Chair of Computer Science (Ordinarius), University Heidelberg and Director of Kirchhoff Institute.

Curriculum Vitas Dr. Ulrich Trunk

Date of Birth: February 20th , 1967 Place of Birth: Heidelberg Nationality : German

Education

1989 Vordiplom (equiv. to B.Sc.) in Physics, University of Heidelberg, Germany

1995 Diplomphysiker (equiv. to M.Sc. in physics) University of Heidelberg, Germany

2000 Dr. rer. nat. (Ph.D. in physics) at University of Heidelberg, Germany

Positions held

1996-1997 DFG scholarship for experimental particle physics and instrumentation. Development of the HELIX128 readout chip used in the HERA-*B*, ZEUS and HERMES experiments at DESY.

2001 Post-Doc at MPI for nuclear Physics, Heidelberg, Germany. Responsibe for the development of the Beeetle readout chip for the LHC*b* experiment at CERN, Geneva, Switzerland.

2001- Staff member of Heidelberg University and head of the ASIC laboratory. Responsible for the Beetle and OTIS readout chips for the LHC*b* experiment at CERN and several smaller projects.

Scientific and technical personnel involved

Dr. Venelin Angelov, Microelectronics, FPGA Design
Dr. Heinz Tilsner, Microelectronics, FPGA Design
Marcus Dorn, Engineer, chip design software, tooling, submission support
Ralph Achenbach, Engineer, ASIC test laboratory, test infrastructure, (automatic) bonding, packaging
Werner Lamade, Engineer, Mechanic development, CNC

Publications relevant to the project

- A MIMD-based Multi Threaded Processor. By F. Lesser, J. de Cuveland, V. Lindenstruth, C. Reichling, R. Schneider, M.W. Schulz Presented at HotChips 2001, Stanford, August 9-13
- Characterisation of a Radiation Hard Front-End Chip for the Vertex Detector of the LHC*b* Experiment at CERN. By N. van Bakel, M. van Beuzekom, H.J. Bulten, E. Jans, T. Ketel, S. Klous, H. Snoek, H. Verkooijen (NIKHEF, Amsterdam), D. Baumeister, S. Lochner, E. Sexauer (Heidelberg, Max Planck Inst.),
- M. Feuerstack-Raible, U. Trunk (Heidelberg U.), N. Smale (Oxford U.). To be published in Nucl.Instrum.Meth.A
- An ASIC for Hartmann-Shack Wavefront Detection. By D. Droste et al.. Published in IEEE Journal of Solid State Circuits. Vol. 37. 2002.
- A Self-Calibrating Single-Chip CMOS Camera with Logarithmic Response. By M. Loose et al.. Published in IEEE Journal of Solid-State Circuits, Vol. 36, 2001

Participant 5 (University of Maribor, Slovenia)

One of the fields defined by the University of Maribor as its key areas is represented by **Prof. Zeljko Knez** (chemical engineer) who not only serves as the university's vice chancellor, initiated technology transfer of the University of Maribor, but also heads the Laboratory for Separation Processes with its 12 employees. One of the major research fields of the Laboratory is high pressure technology, where research is performed on following fields:

- mass transfer and phase equilibrium studies,
- extractions with dense gases,
- supercritical fluid chromatography,
- enzymatic reactions in dense gases
- material processing with supercritical fluids like PGSS micronisation and
- aerogel synthesis.

Crucial for **Activity 2 (Particle production)** is the know how and expensive equipment in the production of particles by a newly developed process PGSS (particles from gas saturated solutions). Prof. Knez' expertise is documented by 54 original scientific articles and 33 patents and patent applications. The advantages of PGSS over conventional methods for particle size reduction makes this expertise a corner stone in the achievement of the milestones planned.

Curriculum Vitas Prof. Dr. Zeljko Knez

Date of Birth: Aug 26th 1954 Place of Birth: Maribor (Slovenia) Nationality: Slovene

Education

1977	B. Sc., University of Maribor, Slovenia
1979	M. Sc., University of Ljubljana, Slovenia
1984	Ph.D., University of Maribor, Slovenia

Positions held

1977-1981	Pinus - Chemical Works, Maribor, Slovenia
1981-1983	Assistant lecturer in Separation Processes, Department of Chemical Engineering, Faculty of Technical Sciences Maribor,
1985 -	Establishment of the Laboratory for Separation Processes at University of Maribor
since 1989	Professor for separation processes and mass transfer
1999 -	Vice Rector for research, University of Maribor
July 2002 -	Rector at the University of Maribor

Scientific and technical personnel involved

Dr. Mojca Skerget (chemical engineer; Scientific team leader) has scientific expertise in the design of high-pressure processes like PGSS, phase equilibria and transport properties.

Dr. Zoran Novak (chemical engineer) has broad experience in material processing with supercritical fluids like PGSS.

A **PhD student** works on a related project.

Publications relevant to the project

- Weidner, Eckhard, Knez, Zeljko, Novak, Zoran. Verfahren zur Herstellung von Partikeln bzw. Pulvern = Process for preparing particles or powders, Europäisches Patent Nr. EP 0 744 922 B1, 15.10.1997.
- Weidner, Eckhard, Knez, Zeljko, Novak, Zoran. Process for the production of particles or powders : United States Patent US006056791A, Patent Number 6, 056,791, Date of patent May 2, 2000.
- Kerz, Janez, Srziz, Stanko, Knez, Zeljko, Senzar-Boziz, Petra. Micronization of drugs using supercritical carbon dioxide. *Int. j. pharm.*, 1999, vol. 182, no. 1, 33-39.
- Weidner, Eckhard, Knez, Zeljko. Herstellung von pulverförmigen Feststoffen : Enge Partikelgrössenverteilung durch überkritische Fluide. *Chem.-Anl. + Verfahr*., 1996, 4, 108-111.
- Funke-Kokot, Klementina, König, Axel, Knez, Zeljko, Skerget, Mojca. Comparison of different methods for determination of the S-L-G equilibrium curve of a solid component in the presence of a compressed gas. *Fluid phase equilib*., 2000, vol. 173, 297-310.

Participant 6 (Free University of Brussels, Belgium)

Dr. Patrick Englebienne's group inside the department of Nuclear Medicine (headed by Prof. Michel Verhas) has extensive expertise and experience in the synthesis of "intelligent materials"

and their application in clinical diagnostics and high-throughput techniques for biomolecular recognition. The intelligent materials synthesized and applied by this laboratory to biochemical techniques comprise conducting polymers (water-soluble and colloidal) and colloidal noble metals. Besides small laboratory equipment (automatic pipettes, vortex mixers, magnetic stirrers etc.), the laboratory is fully equipped for chemical syntheses and purification of colloidal metals and their derivatives (water purification, hoods, oven, specific glassware, drying equipment, evaporation, concentration and extraction, centrifugation, HPLC). The laboratory has also direct access to the optical instrumentation required by the project (UV-visible spectroscopy) and automated biomolecular interaction analysis in liquid format (Cobas-mira plus instrument with computer control). Therefore together with Prof. Khlebtsov's complementary expertise (see below, Participant 7), Dr. Englebienne and his group are ideally suited for the planned **Activity 5 (Detection of binding events)**.

Curriculum Vitas Dr. Patrick Englebienne

Date of Birth: Apr 3rd 1949 Place of Birth: Charleroi (Belgium) Nationality: Belgian Citizen

Education

1969	Pharmaceutical Technologist degree, Paul Pastur University, Charleroi
1983	B. S. Chemistry, Pacific Western University, CA
1985	Ph. D. Biochemistry, Pacific Western University, CA

Positions held

1969 - 1971	Lecturer of Sciences, Mureke College (Rep. Of Burundi)
1971 - 1976	Head of Laboratory, Nuclear Medicine Dept., Free University of Brussels, Brugmann Hospital
1976 - 1986	Scientific Director, Center for Research and Diagnostic in Endocrinology, Kain, Belgium
1986 - 1990	Scientific Director, Sopar-Biochem, S.A., Brussels
since 1988-	Senior Research Associate, Dept. of Nuclear Medicine, Free University of Brussels
1990 - 1998	Founder and Managing Director, Analytical and Pharmaceutical Expertise (APE) Associates, S.A., Ghislenghien, Belgium
2003	Head and founder of the Biocybernetics Unit, Laboratory of Experimental Medicine, Free University of Brussels

Scientific and technical personnel involved

Dr. Pierre Bergmann has an outstanding experience in labelling free detection methods.

Publications relevant to the project

- Englebienne, P., Van Hoonacker, A. and Verhas, M. Surface plasmon resonance: methods and biomedical applications. *Spectroscopy*, in press.
- Englebienne, P., Van Hoonacker, A. and Verhas, M. High throughput screening using the surface plasmon resonance effect of colloidal gold, *Analyst*, 126 (2001) 1645-1651.
- Englebienne, P., Van Hoonacker, A. & Valsamis, J. Rapid homogeneous immunoassay for human ferritin in the Cobas-Mira using colloidal gold as the reporter reagent. *Clinical Chemistry* 46 (2000) 2000-2003.
- Englebienne, P. *Immune and Receptor-Assays in Theory and Practice*. Boca Raton:CRC Press, 1999, 392pp.
- Englebienne, P.: Synthetic materials capable of reporting biomolecular events by chromic transition. (Feature Article) *J. Materials Chemistry*. 9 (1999) 1043-1054.

Participant 7 (Saratov Universtity, Russia)

Prof. Nikolai Grigorevich Khlebtsov has published 120 scientific works, including 61 peer-reviewer papers in Russian and international scientific Journals. His expertise (optical biosensors based on nanosized structures) and his group's equipment (UV-VIS spectroscopy, Static and Dynamic Laser Light Scattering, Light and Electron Microscopy, ELISA Lab Equipment) makes

him ideally suited for the optics part of **Activity 5 (Detection of binding events)**, which he will collaborate together with Dr. Patrick Englebienne. Prof. Khlebtsov's detailed expertise is in:

- light scattering by small particles,
- optics of nanosensors,
- optics of disperse systems,
- electrooptic effects in suspensions and high polymer solutions,
- dynamic light scattering,
- fractal clusters,
- biophysics of microbial populations,
- thermodynamics of high polymer solutions,
- Fortran programming and
- calculation of light scattering,
- absorption by using various approximate and state-of-art methods and
- Experimental research using static and dynamic light scattering, UV-Vis spectrosopy, electrooptical, biophysical and electron microscopy methods.

Curriculum Vitas **Prof. Dr. Nikolai Grigor'evich Khlebtsov**

Date of Birth: Dec 5th 1949 Place of Birth: Astrakhan Region (Russia) Nationality: Russian

Education

1972 B. S., Radiophysics and Electronics, Saratov State University, Russia
1980 Ph. D., Physical and Mathematical Sciences, Saratov State University, Russia
1996 Doctor of Physical and Mathematical Sciences, Saratov State University, Russia

Positions held

1973-1978 Associate Professor at the Chair of Physics, Saratov Medical Institute, Russia
1978-1982 Senior Researcher, Department of Chemistry, Saratov State University, Russia
1982 – to date Head of Biophysics Group at the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS),
1996 – to date Professor of Optics Chair, Physical Department, Saratov State University, Russia

Scientific and technical personnel involved

Ms. Irina S. Zaitseva, Junior Staff Scientist at the Laboratory of Physical Chemistry of Cellular Structure, IBPPM RAS. Graduated from the Tumen State University in 1987. 1999-2001 – Post-graduate student of Institute of Biochemistry and Physiology of Plants and Microorganisms, RAS. Field of research: study of the efficiency of production of antibodies against haptens *in vivo* by the use of antigens conjugated with colloidal gold and *in vitro* by selection from synthetic antibody bacteriophage display libraries.

Dr. Vladimir A. Bogatyrev, Senior researcher of Biophysics Group at the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS). Graduated from the Saratov State University in 1980, PhD (candidate of biological sciences, 1995). Field of research: Electro-optical effects in microbial suspensions, synthesis and application of colloidal-gold biomarkers. Has about 90 scientific publications.

Dr. Lev A. Dykman, Head of Immunotechnology Group at the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia. Graduated from the Saratov Medical Institute in 1985. PhD of Biology (1996). Field of research: immunology; immunochemistry; microbiology; colloidal chemistry and optics of gold sols. Has about 70 scientific publications.

List of selected publications of IBPPM RAS Group

- Khlebtsov N.G., Bogatyrev V.A., Dykman L.A., Melnikov A.G. Spectral extinction of colloidal gold and its biospecific conjugates. *J. Colloid Interface Sci*. 1996, **180**: 436-445.

- Dykman L. A., Bogatyrev V. A. Colloidal gold in solid-phase analysis. A review. *Biochemistry* (Moscow) 1997, **62**: 350-356.
- Dykman L.A., Bogatyrev V.A. Use of the dot-immunogold assay for the rapid diagnosis of acute enteric infections. *FEMS Immunol. and Med. Microbiol*. 2000, **27**: 135-137.
- Dykman L. A., Krasnov Ya. M., Bogatyrev V.A., Khlebtsov N. G. Quantitative immunoassay method based on extinction spectra of colloidal gold bioconjugates. *Saratov Fall Meeting 2000: Optical Technologies in Biophysics and Medicine II*, *Proc. SPIE*, 2001, **4241**: 37-41.
- Bogatyrev V. A., Dykman L. A., Krasnov Y. M., Plotnykov V. K., Khlebtsov N. G Differential light scattering spectroscopy for studying biospecific assembling of gold nanoparticles with proteins or oligonucleotide probes. *Colloid J.* 2002; **64**: 671-680.
- Khlebtsov N.G., Maksimova I. L., Tuchin V. V., Wang L. Introduction to light scattering by biological objects. In: *Handbook of Optical Biomedical Diagnostics* [V.V. Tuchun, ed.], Bellingham, Washington DC, 2002, pp. 31-167.

Participant 8 (EBI Hinxton, Swiss Prot database, UK, EU institution)

The Sequence Database Group of **Dr. Apweiler** is part of the European Bioinformatics Institute (EBI), Cambridge, UK, a non-profit academic organisation that forms part of the European Molecular Biology Laboratory (EMBL). The EBI is a centre for research and services in bioinformatics. The institute manages databases of biological data including nucleic acid, protein sequences and macromolecular structures. The mission of the EBI is to ensure that the growing body of information from molecular biology and genome research is placed in the public domain and is accessible freely to all facets of the scientific community in ways that promote scientific progress. (See http://www.ebi.ac.uk/). Dr. Rolf Apweiler has been working on the Swiss-Prot database since 1987, and in 1994 he became leader of the Swiss-Prot group at EBI, UK. He started the TrEMBL, InterPro, GOA, Proteome analysis and CluSTr projects at the EBI. Since 2001 he is also in charge of the EMBL nucleotide sequence database. He became, together with Peter Stoehr, in 2001 the leader of the newly formed Sequence Database Group at EBI, which has been expanding to well over 90 biologists and programmers.

Dr. Apweiler has published over 70 papers and is a frequent invited speaker for lectures and tutorials at universities, companies, and conferences. He is responsible for the coordination of all activities of his group, which includes overall system design for the databases, budgetary management, supervision of group members, and overseeing all projects to ensure their progress and scientific quality.

Curriculum Vitas **Dr. rer. nat. Rolf Apweiler**

Date of Birth: Sep 8th 1963 Place of Birth: Geilenkirchen Nationality: German

Education

1984 - 1990	Studies of Biology at the University of Heidelberg
1994	PhD, University of Heidelberg

Positions held

1987 - 1991	Swiss-PROT Database Curator, European Molecular Biology Laboratory (EMBL), Heidelberg
1991 - 1994	Research Scientist, Diabetes Division, Department of Medical Research, Boehringer Mannheim
since 1994	Group Leader, European Bioinformatics Institute (EBI), Cambridge, UK

Other experience and professional memberships

1997-present	Associate Member, Nomenclature Committee of IUBMB (The "Enzyme Commission")
1997-present	Member, HUGO (Human Genome Organisation)
1998-present	Member, ISCB (International Society for Computational Biology)
2001	Member, NIH BISTI pre-NPEBC review panel

2001 Member, Germany Ministry of Research Bioinformatics review panel
2001–present Council Member, HUPO (Human Proteome Organisation)
2001-present Member, FlyBase advisory board
2001–present Member, Expert committee on "Bibliometric Mapping of Excellence in the Area of Life Sciences" of the European Commission

Scientific and technical personnel involved

Dipl.-Ing. Henning Hermjakob, Senior Computer Scientist and Group Coordinator (bioinformatician), is after working on tool development and integration of external data for Swiss-Prot and TrEMBL now managing the IntAct protein interaction database project and has started up the HUPO Proteomics Standards Initiative.

Dr. Manuela Pruess, Annotation Coordinator (biologist), is in the Sequence Database Group at EBI responsible for ensuring the quality and quantity of the data content of several databases and for monitoring project progress in the group and general project management.

Publications relevant to the project

- Boeckmann B, Bairoch A, Apweiler R, Blatter M, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M. The Swiss-Prot protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Research 2003;31:365-370.
- Mulder NJ, Apweiler R, Attwood TK, Bairoch A, Barrell D, Bateman A, Binns D, Biswas M, Bradley P, Bork P, Bucher P, Copley RR, Courcelle E, Das U Durbin R, Falquet L, Fleischmann W, Griffiths-Jones S, Haft D, Harte N, Hulo N, Kahn D, Kanapin A, Krestyaninova M, Lopez R, Letunic I, Lonsdale D, Silventoinen V, Orchard SE, Pagni M, Peyruc D, Ponting CP, Selengut JD, Servant F, Sigrist CJA, Vaughan R, Zdobnov EM. The InterPro Database, 2003 brings increased coverage and new features. Nucleic Acids Research 2003;31:315-318.
- Pruess M, Fleischmann W, Kanapin A, Karavidopoulou Y, Kersey P, Kriventseva E, Mittard V, Mulder N, Phan I, Servant F, Apweiler R. The Proteome Analysis database: a tool for the in silico analysis of whole proteomes. Nucleic Acids Research 2003;31:414-417.
- Hermjakob H, Apweiler R. TEMBLOR - Perspectives of EBI database services. A presentation for the ESF workshop Data integration in functional genomics and proteomics. Comp Funct Genom 2002;3:47-50.
- Orchard S., Kersey P., Hermjakob H., Apweiler R. The HUPO Proteomics Standards Initiative meeting: towards common standards for exchanging proteomics data. Comparative and Functional Genomics2003;4:16-19

Participant 9 (Ben Gurion University, Israel)

Ben-Gurion University of the Negev was established 30 years ago to spearhead development of Israel's southern region. The University actively promotes hi-tech industry, agriculture, health services and education in the region. BGU is home to more than 15,000 students and it is the bridge between academia and industry by promoting academic research with commercial applications as the key to economic development in the Negev. **Dr. Yonat Shemer Avni**, the head of the laboratory for Clinical Virology of Soroka Medical Center and a member of the Dept. of Virology at the BGU, is an expert in human viral diagnosis. She did research and clinical work on many pathogens (*chlamydiae*, herpes viruses, blood born viruses, etc.) and the immune responses toward them. The molecular research in the laboratory is focused on protein-protein interactions of viruses (e.g. HCV, measles virus). Her laboratory for viral diagnosis serves the entire southern region of Israel. Seven trained laboratory technicians are in charge of the detection of viruse infections by various methods: culturing, detection of specific antibodies in sera and boody fluids, PCR and genotyping of viruses by sequencing. She therefore is ideally suited for the work planned within **Activity 1 (Model applications)**. All diagnosed sera and viral isolates are routinly kept frozen. As part of the Mediacl Academy Center the laboratory is operating in close collaboration with the medical stuff of the hospital. In addition, Dr Shemer is an adviser in "Rosetta Genomics" genomic-data-analysis company.

Curriculum Vitas

Date of Birth: May12th 1952 Place of Birth: Israel Nationality: Israeli

Education

1972 - 1975 B.Sc. Faculty of Natural Sciences, Ben-Gurion University Of the Negev, Beer Sheva. *Thesis Title*: " Isolation and Polypeptides Characterization of Varicella-Zoster Virus

1978 - 1981 M.Sc Faculty of Natural Sciences, Ben-Gurion University Of the Negev, Beer Sheva

1983 - 1984 Ph.D. Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva

1986 - 1989 *Thesis Title:* "The effect of interferon-gamma and TNF on *C. trachomatis* development in Hep-2 cells

1990 - 1992 Post Doctorat at the Weizmann Institute, Rehovot

Positions held

1988 - 1993 Research associate, subjects: Cytokines-Chlamydial interaction

1989 - 1990 Co-Investigator in research on *Chlamydia pneumoniae*. Rapid diagnosis of chlamydia

1990 - 1991 Investigator, Dept. of Virology, Faculty for Health Sciences, BGU, Project *C.pneumoniae*

1987 - 1995 Instructor. Ben-Gurion University, Medical School, Beer-Sheva

1992 - 1996 Co-investigator in M.E.R.I.D. project "Natural history of chronic viral hepatitis

Since 1995 Lecturer, BGU, Beer-Sheva, Head of Molecular Diagnostic and Research of Viral Hepatitis

1995 - 2001 Head of the Laboratory for Molecular Diagnostic and Research of Viral Hepatitis

Since 2001 Soroka University Medical Centre and BGU, Head of the laboratory for Clinical Virology.

Scientific and technical personnel involved

Department of Virology and Microbiology & Immunology:

M.D. PhD., Leslie Lobel, scientist, will be in charge of Component 1-2, together with Dr Shemer. He has already established a research to isolate and produce fully human neutralizing (patent No.62259) monoclonal antibodies against vaccinia virus for immunotherapy and prophylaxis of smallpox. In concert with this project he will use these human monoclonal antibodies to identify major neutralizing epitopes to facilitate the design of synthetic vaccines;

PhD, Angel Progador, scientist involve in Component 1-3. He is a leading scientist from the Department of immunology with strong background in peptide activation of T-cells and antigen presenting cells; he will be involved in the research of cellular immune responses

Physicians at BGU and Soroka University Medical Centre:

Head of the Paediatric Infectious Disease Unit, **Ron Dagan, M.D.** medical consultant for Component 1-1

Head of the Liver Disease Unit, and vice of the Dean, **Emanuel Sikuler, M.D**. medical consultant for Components 1-1, 1-2 and 1-3.

Publications relevant to the project

- Shemer Y, Leventon-Kriss S, Sarov I. Isolation and polypeptide characterization of Varicella-zoster virus. *Virology*; **106**:133-140, 1980.
- Shemer-Avni Y, Lieberman D. *Chlamydia pneumoniae*-induced ciliostasis in ciliated bronchial epithelial cells. *J Infect Dis* May; **171**(5):1274-8, 1995.
- Elsana S, Shemer-Avni Y, Sikuler E, Sarov B, Naggan L, Bar-Shany S, Gilon E, Yaari A, Orgel M, Frazer GM, Margalith M. Prevalence of specific IgA and IgM anti-HBc antibodies compared with HBV DNA in the sera of HBsAg chronic carriers. *J Hum Virol* Nov-Dec; **1**(1):52-7, 1997
- Shemer-Avni Y, el Astal Z, Kemper O, el Najjar KJ, Yaari A, Hanuka N, Margalith M, Sikuler E. Hepatitis C virus infection and genotypes in Southern Israel and the Gaza Strip. *J Med Virol* Nov; **56**(3):230-3, 1999
- Benharroch D, Shemer-Avni Y, Levy A, Myint YY, Ariad S, Rager B, Sacks M, Gopas J. New candidate virus in association with Hodgkin's disease. *Leukemia and Lymphoma* **44**:605-610, 2003

Additional as-yet-unidentified Participants

Participant 10+ (Small and medium enterprise SME, Europe)

The prospective additional SME Participant 10+ will be included into the consortium only for years 4 and 5 according to the results of an EU wide competitive call (compare to B7, budget). This SME will concentrate on the construction of prototypes, i.e. on demonstration activities needed for successful commercialisation. Within this grant application

- 5% of the funding is allocated for SME Participant 10+ yet to be added,
- plus another 5% for SME Participant 2 (Tel-Tek).

Thereby these SMEs will bring basic knowledge through to the application stage with

- SME Participant 10+ concentrating on prototyping and
- SME Tel-Tek concentrating on particle technology.

Successful commercialisation of a novel technology depends on:

- The benefits a scientist, collaborator or customer expects (*there should be a market for it*),
- Its price (*it shouldn't cost a fortune*),
- The easiness of handling (*it should be customer-friendly*),
- Strong intellectual property rights (*nobody else should be able to market the technology*),
- The availability, the freedom to operate (*no intellectual property rights standing against it*),
- Marketing (*potential users should know about it; they should know it works*),
- Industry, especially SMEs, that promotes the technology (*people that market the*
- *technology*) and
- A unique selling proposition (*the product should compare very favourable to competitor's products*),

Therefore besides expertise in **prototyping** the SME Participant 10+ planned to be added to the consortium ideally should have complementary expertise in

- **marketing** and
- **worldwide distribution** networks

in order to ensure use of the project results. With the prerequisite of a functioning peptide array technology yet to be developed by the other Participant's "scientific" expertise within years 1-3 of this grant applied for, potentially all the criteria for commercialisation specified above are met with the candidate SME Participant 10+ just described.

A company like Grohmann engineering (Germany) would be an ideal candidate SME Participant 10+ comprising all the "commercial" expertise specified above, i.e.

- broad know how and full infrastructure for prototyping,
- a world wide distribution and service network and
- (to a lesser extent) expertise in marketing.

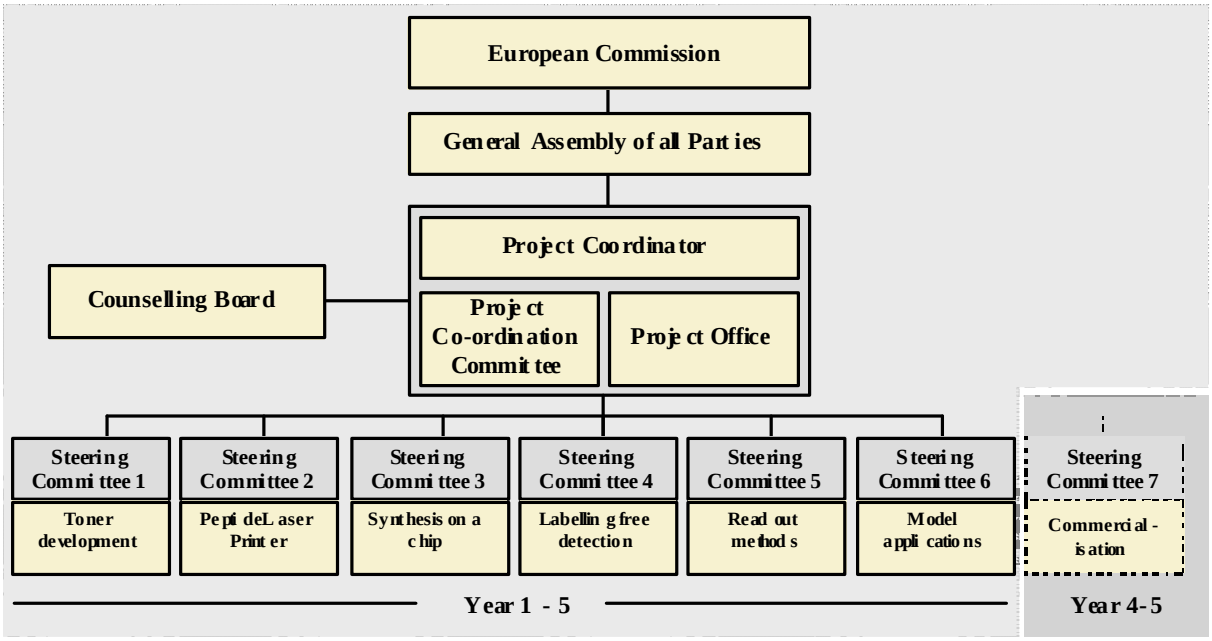
However, this is no absolute requirement. Instead two different SME Participants 10+ could combine their commercial expertise in order to commercialise our array technology. Thereby one SME Participant could be selected to have a strong position in marketing and worldwide distribution focused on proteomics and biomedical research tools (e.g. Clontech). The other SME partner then could be selected solely due to their "prototyping expertise". This model would allow for the inclusion of a venture capital financed SME offspring, which would descend from the public science institutes involved. Indeed Dr. Frank Breitling and his partners already won a 20.000€ awarded 2nd place for their commercialisation concept within the business plan competition Genius Biotech Award organised by the federal state of Baden-Württemberg, while Dr. Patrick Englebienne is the co-founder of a small company commercialising colloidal nano particles for labelling free detection.

B.6 Description of project mamagement

Organisation

The management of this project will be kept simple and straightforward also taking into account the specific requirements of an Integrated Project.

Organisational Structure



Steering Committee		1	2	3	4	5	6	7
1	University Maribor	X						
2	Tel-Tek	X						X
3	Frauenhofer IPA							X
4	Universität Heidelberg			X		X		
5	DKFZ (Deutsches Krebsforschungszentrum)	X	X	X	X		X	X
6	University Brussels				X	X		X
7	University Saratov				X	X		X
8	EBI						X	X
9	University Ben Gurion						X	
10+	SMEs							X

Fig. 19: Organisational structure

The involved organisations are working together in seven different subprojects and will be represented through corresponding Steering Committees. Members of the different Steering Committees are shown in the table.

Organisational Elements

The organisational structure for the project management basicly consists of six elements. These different parts will be specified below:

Organisational unit: General Assembly

Tasks: Is the highest decision-making body in the consortium and comprises all project partners, who are also eligible to vote on propositions presented to the General Assembly. The decisions of the general Assembly are legally binding to all Parties in the Project-related matters. The General Assembly will only decide questions of fundamental importance, i.e.: Budget, Premature project ending, Exclusion and acceptance of new partners, Structure and restructuring of the Sub-projects, Alteration of the Consortium Agreement, Annual implementation plan and Liability related issues

Members: All Parties shall be entitled to send one voting representative to the General Assembly.

Organisational unit: Project Co-ordinator (Dr. Frank Breitling)

Tasks: Heads both, the Project Co-ordination Committee and the Project Office. The Project Co-ordinator will check scientific content and legal implications of the reports and audits creating the subproject teams. If the project partners fail to provide necessary information or provide information or documents that are in any way incomplete or incorrect in form or content, the Project Co-ordinator can, with the approval of the Project Co-ordination Committee, refuse to authorise payment of the sub project's project share.

Organisational Unit:Project-Coordination Committee

Tasks: Is the central body and responsible for the day to day operational management of the project and decides all the matters necessary for the scientific and administrative management of the project as well as for the management of innovative related activities. The Project Co-ordination Committee oversees the different subprojects. In close cooperation with the Project Co-ordinator, the Project Co-ordination Committee is responsible for the overall supervision of the scientific content and budget as well as the coordination of all activities carried out by the project partners and the various sub projects. The Project Co-ordination Committee shall have the right to set up Panels to advise and support it in the proper management and co-ordination of the Project. These Panels have an advisory role only.

A comprehensive annual implementation plan for the entire project is formulated by the Project Co-ordination Committee to be submitted to the General Assembly for approval.

Members: The Participants will delegate five members into the Project Co-ordination Committee, whereby some Participants jointly delegate one member when appropriate (related work packages and expertise).

Organisational Unit: Project Office

Tasks: The Project Office will carry out the following tasks:

Co-ordinating the formulation of the annual implementation plans (organizing meetings, reviews, audits, reporting, public relations…), guaranteeing adequate administrative project controlling (consortium agreements, legal issues, internal calls, changes in consortium..) and taking care of financial and budgetary matters (financing, controlling, cost statements..).

Members: One member each of the project management team will be responsible for the management of finances and administration. Another member will have a scientific background in order to do scientific-technical project controlling (18,5 working hours per week) and will be located within the Co-ordinator's group.

Organisational Unit: Steering Committees

Tasks: The Steering Committees integrate different activities into subprojects and co-ordinate the activities of the project partners in the sub projects to ensure effective controlling of assigned scientific tasks and budget matters. Each Steering Committee sets out the contribution of its respective sub project to the overall project in an annual implementation plan and presents this proposed plan to the Project Coordination Committee. As figured above (Organisational structure) the Number of Steering Committees is corresponding to the number of subprojects: Six (in the first three years) and seven (in year four and five). The **Subproject** level is based on scientific tasks or areas of specialization and ensures efficient management of the project and ensures efficient management of the project. The project will consist of 7 sub projects, with several tasks each.

Members: It consists of a representative of each project partner (two or three members) with one vote each. Decisions in the Steering Committee depend on a simple majority (in cases of a stalemate the Project Co-ordination Committee will decide). The Steering Committee elects a Chairperson from among its members who heads the sub project and acts as its administrative coordinator, who is responsible for reporting to the Project Co-ordination Committee.

Organisational Unit: Counselling Board

Tasks: In order to allow for an outside view of problems and chances foreseeable a Counselling Board is planned that advises the Project Co-ordination Committee on questions like: Shift of emphasis in scientific and technological developments, Choice of SMEs and Involvement of 3rd party developments.

Members: 2 to 5 external scientific experts, to be suggested by the Steering Commitees.

Project management

The processes are based on a central unit, which is responsible for installing and maintaining the agreed standards of project management. This central unit consisting of

- the project coordinator,
- the project office and
- the coordination office

It is not possible to lead a large-scale project under scientific objective for the purpose of a fine control centrally operationally since the necessary information only incompletely or lately would happen and associated communication would paralyse the project. Installing and maintaining the standards means therefore, that the day-to-day controlling and operative steering of the progress will be done decentralized within the steering committees or subproject level, respectively. The relevant information are transmitted at regular intervals to the central project unit, where they are processed and forwarded according to the communication and decision making agreements. The processes and structure of the project management is basically drafted in Fig. 20.

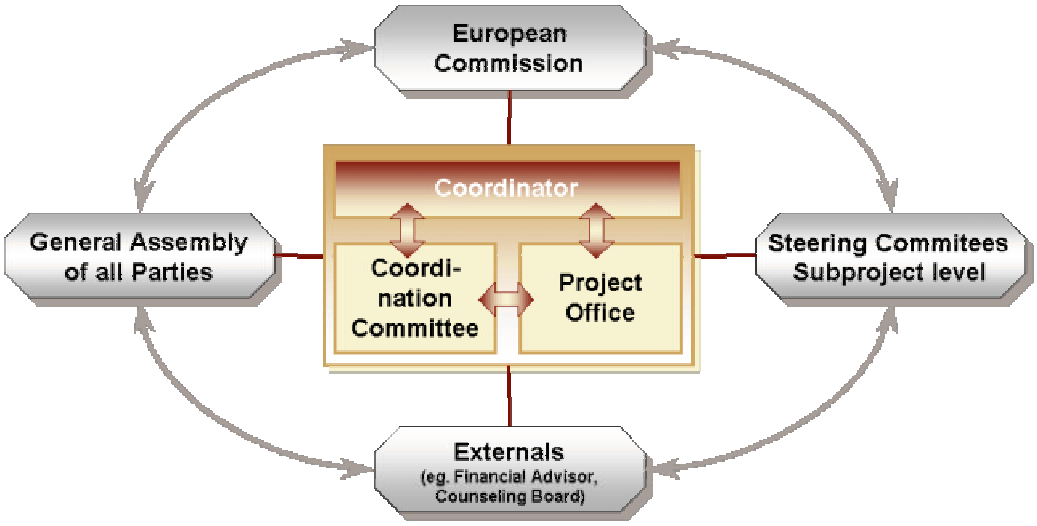


Figure 20: Project management structure

- Task 1: Communication management

  Substantial success factors for large-scale projects are the management of project communication and the knowledge. A goal is to create by purposeful project communication an orientation for all project-taken part and by the availability of knowledge to avoid double work as well as the project progress to accelerate. Most important task is in this range thus the dressing and distribution of subproject-spreading knowledge. Based on a uniform technical communication platform the information and knowledge in regulated processes and suitable form are receiver-oriented made available and archived.

- Task 2: project controlling and escalation management

  This field covers mainly the planning and monitoring of the overall project. The realisation of the project aims within time, budget and quality shall be guaranteed.

  The achievement of milestones and project deliverables, consortium agreements, such as ratios for gender equality and ethnical equalization as well as budget and resources consumption has to be continuously balanced between plan and realization, adapted according to new requirements and changes of relevant project conditions and prepared in form of standardized project reports.

  Furthermore this task involves the analysis and dressing of the relevant project information as a support for the respective decision-making bodies. On the one hand the firmly scheduled meeting of the decision-making bodies are informatively prepared. Beyond that it applies in the case of defined deviations between project planning and project implementing, the change of basic conditions and objectives or on request of individual members of the project organization to call the responsible project committees and to collect and prepare all decision relevant information.

- Additional Task 3: formal project organisation and bookkeeping

  This field covers the formal preparation and support of the project committees. In addition belong the supply of the organizational basic conditions for project meetings, dispatching of invitations, the logging of substantial meeting results, the supply and monitoring of the technical mechanisms and communication instruments as well as the formal finance management.

The agreed methodical standards and planned instruments within the major tasks are described in the following passages.

Project communication

How the knowledge emerging from this project including the previously existing knowledge needed for this integrated project will be made available to the partners involved, including 3rd party members is described within chapter B4.1.

The results of meetings as well as relevant information out of the daily work will be made available to the whole consortium via a databank within a web-based project management tool. All decisions made above sub-project level will be published automatically in this databank by the project office or the respective steering committee. All organisational and budgetary project data as well as the standardised controlling reports are published there, too. The publishing of specific information gathered on sub-project level is facultative, but it is planned to offer a web-based discussion platform (Fig. 21) so that all information are stored centrally and accessible for the project memb xer SME's.

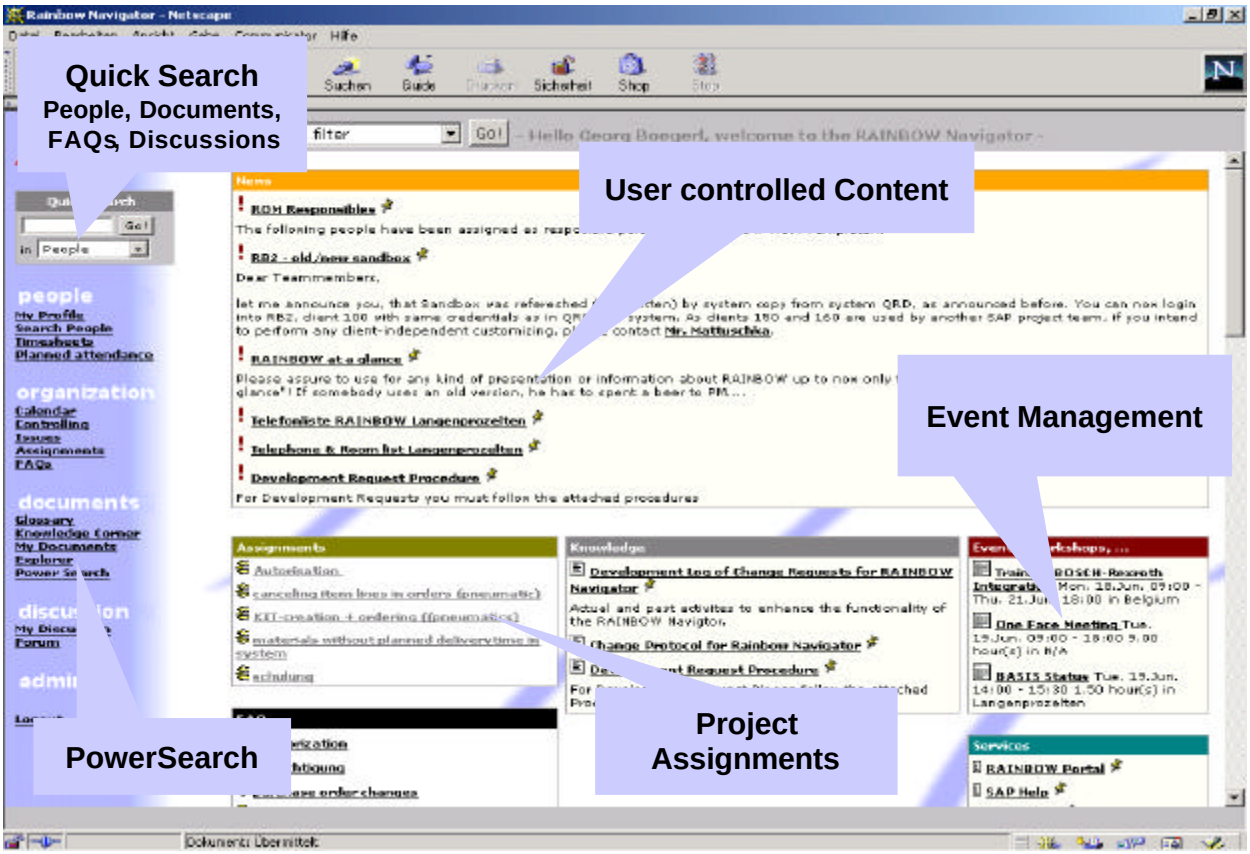


Fig. 21: Web-based discussion platform

Project controlling and escalation management

- Planning s and to external parties like involved

  Each Steering Committee sets out the contribution of its respective sub project to the overall project in an annual implementation plan and presents this proposed plan to the Project Coordination Committee. A comprehensive implementation plan for the entire project is then formulated by the Project Co-ordination Committee to be submitted to the General Assembly for approval. If decisions of fundamental importance, like described in chapter 1.1 force a modification of the annual planning, the general assembly can dispose a new planning process or an actualisation of the existing plan within the actual year. In every other case, the annual plan is binding for all project members.

- Supervision and Reporting

  In close cooperation with the Project Co-ordinator, the Project Co-ordination Committee is responsible for the overall supervision of the scientific content and budget as well as the coordination of all activities carried out by the project partners and the various sub projects. The Project Co-ordination Committee shall have the right to set up Panels to advise and support it in the proper management and co-ordination of the Project. These Panels have an advisory role only.

  The Chairperson of the sub project collects all audits, reports and other information in the sub project and ensures that the sub project partners fulfil their legal and contractual obligations towards the Commission and the other project partners.

  Every 6 months, status review meetings are carried out at the sub project level within the individual Steering Committees during which overall progress with respect to the implementation plan and the use of resources are reviewed. The Chairperson of each Steering Committee forwards a standardized status report to the Project Co-ordination Committee within 14 days after the status review meeting. If within 3 weeks after receipt the Project Co-ordination Committee does not raise objections, the minutes are considered approved.

  If agreed tolerances between planed and realized project progress or budget are equalled an extraordinary report including suggestions for closing the gabs and a detailed forecast has to be submitted to the Project Co-ordinator within 3 weeks.

  After the Co-ordinator and, where necessary, the Project Co-ordination Committee have checked their scientific content and legal implications, all these materials and documents are forwarded to the Commission and especially in the latter phases of the project to external parties like the SME's as well.

  Further participant's responsibilities for flow of information and reporting (*e.g. confidentiality, Co-ordinator's reporting to EU*) will be regulated by the Consortium Agreement to be signed (see B.4.4, responsibilities).

  The Project Co-ordination Committee can refuse to accept documents and receipts if they do not meet the legal requirements of the Commission or correspond to decisions taken previously by the General Assembly. In addition, the Project Co-ordination Committee can request the Steering Committee to provide a report within 21 days on the current status of the sub project or to make available additional information to supplement a status report. If the project partners fail to provide necessary information or provide information or documents that are in any way incomplete or incorrect in form or content, the Project Co-ordinator can, with the approval of the Project Co-ordination Committee, refuse to authorise payment of the sub project's project share.

- Escalation

  The Project Co-ordination Committee will decide only in cases of disagreement inside the Steering Committee and in cases where the milestones and deliverables set in B7 and B8 are significantly missed according to agreed tolerances (eg. Time gab more than2 month, budget consumption more than 20 % over plan, quality target missed). In the latter case the Project Co-ordination Committee can ask for a General Assembly where a decision on budget reduction and reallocation to the other partners and / or withdrawal of partners is made.

All described controlling information like the project plan, regular controlling reports, audit reviews, escalation results, etc will be stored and published in the above described project tool.

The specific instruments for the controlling are therefore focused on establishing a reporting and planning standard as well defining specific values of pan deviations which automatically engage controlling or escalation processes. These instruments will be developed on basis on the following reporting structure which is agreed upon by the consortium members.

The report parts should normally not exceed the length of two pages, to ensure that essential information will be displayed clearly and concisely. For every part of the reporting responsibilities within the sub level or steering committee will be defined. The central consolidation and aggregation of the plans will be done or supervised by the project office and the project coordinator, respectively.

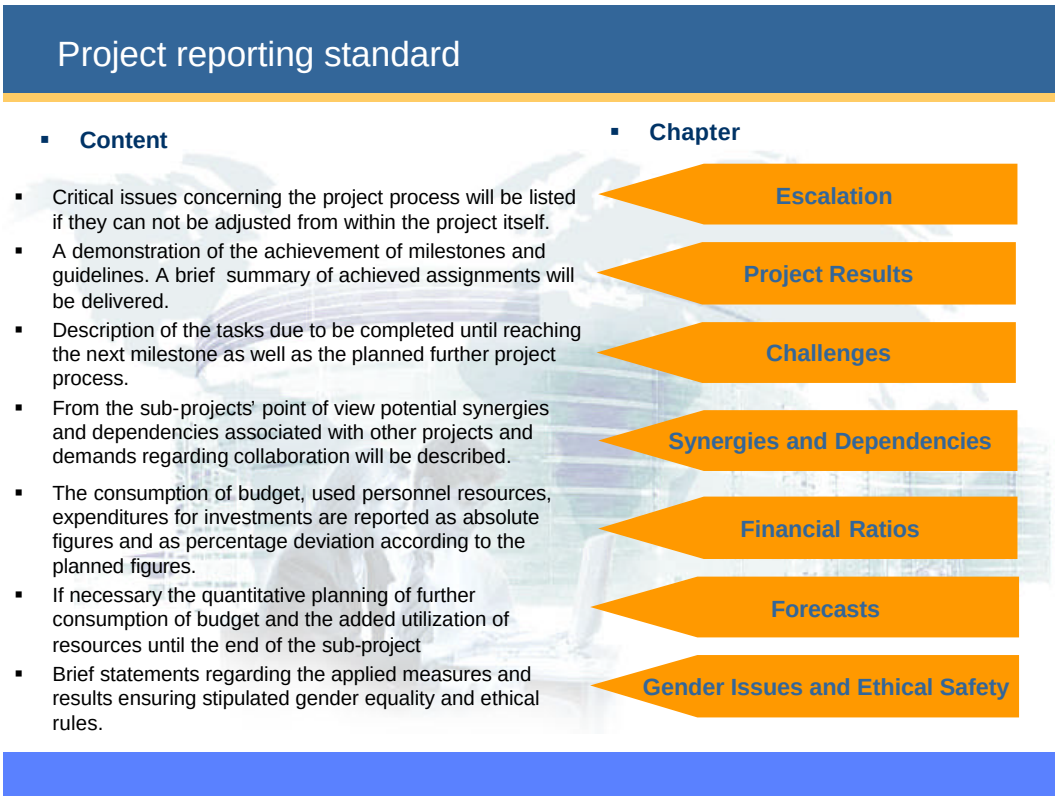


Fig. 22: Project reporting standard

Financial auditing, management of finances

Local experts approved by the Commission will do financial auditing of the partners involved. The Co-ordinator will allocate 100% of the money needed for that from the budget for management costs (7% of the budget). However, in order to avoid exaggerating costs for financial auditing, a financial cap will be agreed upon by all partners within the Consortium Agreement (*2.500 € per year and participant for financial auditing*).

Quality Management

In relation to each of the activities the planned project comprises a series of individual research and technological activities where clear milestones for the measurement of progress are feasible.

- The work progress in the development of the peptide laser printer (Activity 3, IPA) will be demonstrated by its accuracy in repeatedly addressing defined regions onto a solid support (spot resolution; repeated addressing of the same spots). Initially normal commercially available colour toner particles will be used, with the distance of different toner deposits easily measurable.
- The same kind of testing will be applied with the different chip designs (Activity 4, KIP). The resolution in addressing toner particles onto the chip's surface will be measured.
- Amino acid toner particles will be tested as shown above, where once again the resolution of spots, this time with amino acids coupled to the solid support is the most important test for scientific progress (Activity 2, DKFZ, University of Maribor, Tel-Tek). In addition the average size of particles (and even size distribution) as well as yield are important parameters for the measurement of scientific progress in Activity 2.
- For research in labelling free detection (Activity 5, Universities of Brussels and Saratov) the most important parameters are sensitivity and the transformation of this method into array format (once again the resolution of spots is the most important parameter).
- Within Activity 1 (Ben Gurion University) the number of diagnosed sera collected as well as the number of virus proteins cloned and expressed are important milestones.

Knowledge Management/Management of Intellectual Property

DKFZ currently presumes that the technology named "peptide laser printer" has a broad patent coverage. The following patent applications have already been validated: DE19960346A1, EP1140977A2, US20020006672A1.

In order to examine the related intellectual property rights, the DKFZ's Technology Transfer department recently ordered an appropriate experts opinion (focusing on high complexity peptide arrays) in respect of the necessary "freedom to operate".

In the forthcoming years DKFZ will widen the focus on additional fields of technologies ermerging from the "peptide laser printer". It is planned to conduct this research and development in collaboration with SMEs anticipated to join the consortium for years 4 & 5. As to being an exclusive user of the pre-existing and future intellectual property rights the SME's will sense a strong incentive to commercialise DKFZs technology.

Except for the intellectual property right mentioned above, the partners involved will be the applicants of emerging intellectual property rights. Futher more, they declare their willingness to take on any efforts for the exploitation of results and for the dissemination of knowledge. In respect to the fact that commercialisation of the technology is very much intended, SMEs fit well into the consortium.

Dr. Breitling shows an outstanding track record as a successful inventor. A lot of patents are based on his research and development at DKFZ. These contributions show that he should be the person of choice - if approved by the General Assembly - to co-ordinate intellectual property related issues in close contact with the DKFZ's Technology Transfer department.

The Consortium Agreement to be signed must state that previously existing intellectual property rights, however will be excluded from commercial use. This ensures the possibility of combining technologies not envisioned within the scope of this integrated project (e.g. combining labeling free detection with other arrays).

Any secret knowledge that will result from the collaboration including the previously existing knowledge will have to be shared by partners involved mainly inside the Steering Committees. This is crucial to ensure a proper work flow among the partners.

It should be noted that the peptide laser printer as well as chip technologies or labelling free detection related issues might show additional commercial applications in the future (e.g. 3D rapid prototyping; screen for ceramics based catalysts). Whenever it is financially reasonable the consortium will file patent applications to protect any ideas of the participants of the collaboration. The participants closest in expertise will always be assigned a leading role in this process.

Exploitation and Dissemination

Efficient communicational means will ensure the effective exploitation of the outcomes of the research project.
The identification of pilot users/end users of project results will be vital to the successful search for SME partners. Within the German Cancer Research Centre a large number of the former has already expressed interest in *high complexity peptide arrays*. The peptide laser printer core technology is currently being developed within this institution. Depending on milestone achievements these scientists will be included in order to disseminate the technology beyond the consortium. The same process will be led by the remaining participants throughout their institutions/surroundings.

The large scientific interest in the described technology (see: B.3) that has already been expressed by potential partners/customers leads to the assumption that the consortium's capacity for take up measures will be saturated by this search itself. Should this assumption prove wrong for any reason, a search for partners throughout the European Union (including NAS) will be conducted. Existing networks will be utilized by participants' departments (e.g. Prof. Annemarie Poustka, DKFZ, is involved with several EU consortia as well as the German Genome Research Network, NGFN).

An additional competitive search throughout the European Union (including the NAS) will be conducted in order to add further SME Participants to the consortium. This search will be based on technology profiles/technology offers jointly written by the consortium's Participants, e.g. through the Network of Innovation Relay Centres. Furthermore a number of companies with potential interest in the described technology has already been identified (e.g. Qiagen, Jerini Biotools, Oxford Gene Technology) and partly been contacted (e.g. Roche Diagnostics, Eppendorff).

Yet another alternative involves venture capital financed Start Up companies closely linked to the scientific centres. This opportunity certainly strongly depends on the situation of the financial markets (which at present tends to show great weakness and unpredictable developments). It will be mainly distribution partners (e.g. Roche Diagnostics) who will account for technology marketing in this case. A business plan has already been written to further commercialization of the peptide laser printer core technology. This plan won the 20.000€ awarded 2nd place in the highly competitive business plan competition "Genius Biotech Award" (awarded by the German state of Baden-Württemberg). Generally speaking it is expected that within the first years of this integrated project especially the European added value (advanced products through integrated complementary expertise) will generate an extra incentive for SMEs in the commercialization of the described technology.

Integration of participants

The character of the integrated project planned lends itself especially to a modular organisation where on a time scale sub-modules are integrated to increasingly larger modules with finally SMEs commercialising the technology or at least parts of it (*depending on the achievements of deliverables and the number of SMEs involved*). Within the first years the emphasis is on frequent meetings in Steering Committees that assemble the partners working on related sub-modules. In this phase for the majority of sub modules DKFZ is involved except for read out related modules, where Universities of Brussels and Saratov play a leading role. Regular meetings of the Project Co-ordination Committee will spread the information to the other Participants.

With time these sub-modules grow to larger entities, which is reflected by the number of Participants in the meetings, especially in years 4 & 5 when with commercialisation driven by SMEs the partner's expertise is combined (Steering Committee 7). Integration of SME Participants during the lifetime of the project is done mainly via Steering Committee 7. The prospective additional SME Participants will be included into the consortium only for years 4 and 5 and will focus mainly on demonstration activities, i.e. the construction of prototypes. In order to anticipate these activities financial resources will be reserved in the budget. The selection of specific SMEs to be added to the project will be agreed upon in a General Assembly. To date we propose a financial involvement of the SMEs for years 4 & 5 in demonstration activities leading to prototype machines in order to speed up commercialisation of the technology. The emphasis (*which machines – e.g. emphasis on laser printer or chip synthesis machine*), however, should mainly depend on the milestone achievements in year 3.

B.7 Project resources

B.7.1 IP Project Effort Form.

Figure 23 shows the global project planning, integrating the different subprojects. The plan will be detailed within the annual project planning by specific GANTT-Charts.

Workplan	activity (component)	budget in 1.000€	re-source	done by	year 1	year 2	year 3	year 4	year 5
number of diagnosed sera collected	1 (1-1)	647	B.Gur. Univ.	B.Gur. Univ.	HCV: 50 HBV: 50	HCV: 100 HBV: 100	HCV: 150 HBV: 150	HCV: 200 HBV: 200	HCV: 350 HBV: 350
model applications virus peptide arrays	1; 6; 7 (1-1; 6-3; 7-2; 7-3)		EU & BGU	B.Gur. Univ.		1st	2nd	3rd	4th
vaccinia epitopes	1 (1-2)		EU & BGU	B.Gur. Univ.		1st	2nd	3rd	4th
pathogen / host interactions	1 (1-3)		EU & BGU	B.Gur. Univ.			1st	2nd	3rd
resolution of spots with amino acids coupled to support	2 (2-1)	460	EU & DKFZ	DKFZ	200 spots/cm², 15 amino acids	300 spots/cm², 20 amino acids	350 spots/cm², 20 amino acids	400 spots/cm², 20 amino acids	500 spots/cm², 20 amino acids
particles by RESS	2 (2-2)		EU & DKFZ	DKFZ		yes	particle diameter of 20µm	particle diameter of 10µm	particle diameter of 8µm
particles by milling & classification	2 (2-3)	250	EU & Tel-Tek	Tel-Tek		yes	particle diameter of 20µm	particle diameter of 15µm	particle diameter of 10µm
particles by PGSS	2 (2-4)	466	EU & Maribor	Maribor	design of PGSS process	optimisation of PGSS process	scale up, particle diameter of 20µm	economic evaluation, diameter of 10µm	particle diameter of 8µm
conceptual design and construction of a basic peptide laser printer	3 (3-1)	583	EU & IPA	IPA	design	construction	improvement	improvement	
improvement of the printing accuracy	3 (3-1; 3-3)		EU & IPA	IPA	experiments, existing instrument	experiments 100 000 dots	experiments 200 000 dots	experiments 500 000 dots	easier handling
laser printer, automatic washing	3 (3-2)		EU & IPA	IPA		design	design, construction	experiments / demonstration	easier handling
calibration of the printer	3 (3-3)		EU & IPA	IPA		experiments	design, construction	easier handling / demonstration	easier handling
cooled nitrogen atmosphere	3 (3-4)		EU & IPA	IPA			design, construction	experiments / demonstration	
chip addressing toner particles	4; 6 (4-1; 6-2)	634	EU & KIP	KIP, DKFZ		area of 80x80µm	area of 80x80µm	area of 40x40µm	area of 20x20µm
chip instrument	4 (4-2)		EU & KIP	KIP		yes	improved	semi automatic	
nano gold chip design	4; 5 (4-3; 5-2)		EU & KIP	KIP, Bruss.			yes	improved sensitivity	
chip with integrated photodiodes	4 (4-4)		EU & KIP	KIP			yes	area of 40x40µm	area of 20x20µm
Synthesis of particles and labelling free detection in solution	5 (5-1)	642	EU & Bruss.	Bruss.	Synthesis	Synthesis and optimal selection			
Characterisation and SPR modelling	5 (5-2)		EU & Bruss.	Bruss.	Theoretical Modeling	Experimental characterisation	Integration		
Nanoparticles in solid-phase format	5 (5-3)	438	EU & Saratov	Saratov		Transfer from liquid to solid	Demonstration	Prototype in plate reader	Integration with printer
nanoparticle label optical characterisation in solid-phase	5 (5-4)		EU & Saratov	Saratov	Theoretical modeling	Experimental characterisation	Characterisation demonstrated	Integration with plate reader	
array reader	5 (5-5)		EU & Saratov	Saratov			Design	Prototype	Integration with SMEs
resolution of spots printed with laser printer	6 (6-1)	549	EU & DKFZ	DKFZ	200 spots/cm², 15 amino acids	300 spots/cm², 20 amino acids	350 spots/cm², 20 amino acids	400 spots/cm², 20 amino acids	500 spots/cm², 20 amino acids
resolution of spots addressed by chip	6 (6-2)		EU & DKFZ	DKFZ			200 spots/cm², 15 amino acids	300 spots/cm², 20 amino acids	400 spots/cm², 20 amino acids
peptide array, resolution of spots	6 (6-3)		EU & DKFZ	DKFZ		200 spots/cm², 15 amino acids	300 spots/cm², 20 amino acids	350 spots/cm², 20 amino acids	400 spots/cm², 20 amino acids
definition of proteome sets	7 (7-1)	636	EU & EBI	EBI	Tool available	3 sets defined	6 sets defined	12 sets defined	20 sets defined
PREJUDICE protein set analysis tool	7 (7-2)		EU & EBI	EBI	Tool specification	Prototype	Production tool	Optimisation & maintenance	Maintenance
proteomics results dissemination	7 (7-3)		EU & EBI	EBI			Interaction data integration into IntAct	Result data into Swiss Prot	Result data into Swiss Prot
peptide laser printer	8 (8-1)	350	EU & SME	SME				1 array overnight	Prototype
chip synthesis machine	8 (8-2)		EU & SME	SME				1 chip array overnight	Prototype
prototype array reader	8 (8-3)		EU & SME	SME				read out for 100 000 pixels	Prototype

Fig. 20: Global project planning

IP Project Effort Form
Full duration of project (Total workforce*)

Project acronym - PeLaPri

	Partner 1 DKFZ Germany	Partner 2 Tel-tek (Norway)	Partner 3 IPA Germany	Partner 4 KIP Germany	Partner 5 Univ. Maribor (Slovenia)	Partner 6 Univ. Brussels (Belgium)	Partner 7 Univ. Saratov (Russia)	Partner 8 EBI (United Kingdom)	Partner 9 Univ. Ben Gurion (Israel)	Partner 10 SME Prototype	Total Partners
RTD/Innov. activities											
Activity 1									360		360
Activity 2	60	78			120						258
Activity 3			74								74
Activity 4				86							86
Activity 5						144	300				444
Activity 6	60										60
Activity 7								72			72
Total research	120	78	74	86	120	144	300	72	360		1.354
Demonstration activities											
Activity 8										120	120
Total demonstration										120	120
Training activities											
Activity 1	4										4
Activity 2		4									4
Activity 3			2								2
Activity 4				4							4
Activity 5						10					10
Activity 6									12		12
Activity 7									12		12
Total training	4	4	2	4		10			24		48
Management activities											
Activity 9	48										48
Total management	48										48
TOTAL ACTIVITIES	172	82	76	90	120	154	300	72	384	120	1.570

* EU funded plus non-EU funded

B.7.2 IP management level justification of resources and budget.

Resources needed

The following tables give a list of resources needed to carry out the project. Extra consumables, equipment and travel expenses are specified according to Participants and Activities. In addition to B.7.1 IP Project Effort Form (person-months per partner) the cost of personnel resources are specified.

Consumables

Consumables for the execution of the Activities 1 to 8 with the different partners cover: chemicals, solvents, reagents, kits, dispensaries, laboratory stuff.

For Partners 1, 2, 3, 4, 5, 6, 7, 8, 9: **20.000€ per year.**
For Partner 4: **10.000€ per year.**

Extra consumables are specified for the following partners:

Participant 1:	Fmoc-amino acids, activated Fmoc Amino acids, solid solvents like diphenyl sulfoxide for each year (2.000 € + 4.000 € + 4.000 € = 10.000 €)
Participant 2:	no extra consumables
Participant 3:	Additional toner cartridges and chemical detergents (5.000 € per year = 25.000 €)
Participant 4:	3x (MPW chip mfg. 5x5 mm^2 Europractice AMS CXZ (50V) = 15kEUR) 45kEUR + 1x (Engineering Run AMS CXO/ CXL CMOS HV 90V 2M,2P,HR 60kEUR) + 1x (MPW chip mfg. 10 x 10 mm^2 Europractice AMS C35 (5VIO) 60kEUR) + 1x (Engineering Run AMS C35 (5VIO) 85kEUR) (15kEUR + 30kEUR** + 60kEUR + 60kEUR + 85kEUR) **Funded by DKFZ
Participant 5:	Special chemicals (1.200€ per year), PGSS apparatus spare parts (year 2, 40.000€)
Participant 6:	none
Participant 7:	Specific chemical reagents for gold and silver particle labelling in year 1-5 (1.500 € x 3 = 7.500€), laboratory glass, office and computer supplies, software licenses, computer and miscellaneous disposable supplies, for each year (1.100€ x 5 = 5.500 €)
Participant 8:	Office supplies, software licenses, computer supplies/misc. disposables, miscellaneous software for each year (3.000 € + 2.100 € + 2.205 € + 2.315 € + 2.430 € = 12.050 €)
Participant 9:	For each year: molecular diagnosis materials (17.000€), HLA typing (16.000€).
Participant 10:	f*or peptide laser printer:* Fmoc-amino acids, activated Fmoc Amino acids, solid solvents like diphenyl sulfoxide for each year (2.000 € + 4.000 € + 4.000 € = 10.000 €) *for Chip synthesis machine:* Fmoc-amino acids, activated Fmoc Amino acids, solid solvents like diphenyl sulfoxide for each year (1.000 € + 2.000 € + 2.000 € = 5.000 €)

Equipment

Equipment is specified for the following partners:

Participant 1:	for production of toner particles (*Activity 2; RESS procedure*): **Sitek machine** (66.000 €), **high-pressure HPLC pump** (30.000€) for testing of toner particles in the laser printer (*Activity 2; particles*): **OkiC7200 colour laser printer** & special designed cartridges (5.000 €), for production of peptide arrays (*Activity 6; synthesis of peptide arrays*): LapTop or Macintosh **Computer** controlling 3D printing patterns (3.800 €), **Spectrometer** (5.000€) for training activities: **Projector** (3.500 €),

Participant 2:	Modifications to existing equipment (running in other atmospheres (N2), changes to electrostatic characterisation equipment and others (10.000€) for testing of toner particles in the laser printer (*Activity 2; particles*): **OkiC7200 colour laser printer** & special designed cartridges (5.000 €),
Participant 3:	Mechanical and electronic components and materials for the peptide laser printer and the automatic washing machine (linear and resolving motors, power electronics, controller electronics, mechanics)(80.000 €)
Participant 4:	**none**, all required equipment already present, such as Computer infrastructure, complete full custom, semi custom, SOC, EDA software (Cadence, Mentor etc.) Chip handling equipment, 2 bonders (Delvotec), Wafer prober (Suess), T&M equipment pool with more than 20 units (chip tester, high speed oscilloscopes, Logic analysers, Waveform Generators)
Participant 5:	for analysing and producing particles (*Activity 2; particles*): **High pressure autoclave** (approx. 200ml, 1.000 bar) and **Spray tower**(50.000€) **High pressure pumps** (21.000€) **Particle size analyser** (100.000€) for testing of toner particles in the laser printer (*Activity 2; particles*): **OkiC7200 colour laser printer** & special designed cartridges (5.000 €),
Participant 6:	characterization of the particles requires specific equipments, namely a **nephelometer** (scattering characteristics, 50,000€), and a **plate reader** (evaluation of the optical response of the colloids on solid phase 34,000€)
Participant 7:	**A workstation for theoretical modelling** in year 1 (6000€), **UV-VIS Diode Array spectrophotometer and Lap top computer controlling spectrophotometer** in year 1 **(**15000€+3000€=18.000€), **Dynamic Light Scattering Particle Sizer (PD2000) and controlling computer Pentium IV (2-4 GHz),** (alternatively: Cultronics N4+, Malvern, or PhotoCorr FC) in year 1 (40.000 €), **On-Desk Transmission Electron Microscope** in year 2 (Philips, resolution 0.1 nm, 100.000€)
Participant 8:	Two **workstations** in year 1 and one in year 4 (6.000 € + 3.470 € = 9.470 €)
Participant 9:	**Real-Time-PCR machine** (year 2, 60.000€), computers (year 1; 2000€).
Participant 10:	**Lap top computer** for Chip synthesis machine (4.000€) **Lap top computer** for read out machine (4.000€) machine parts for peptide laser printer (150.000€) machine parts for Chip synthesis machine (50.000€) machine parts for array reader (50.000€)

Innovation related activities

Participant 1:	Cost for patent applications of patent families EP1140977A2, US20020006672A1 and DE10004659 per year (26.000€) Cost for patent auditing by a patent attorney (once 8.000€) Feasibility studies for the creation of spin-offs (once 5.000€) Assessment of take-up activities (once 5.000€)

Travel and subsistence

Participant 1:	Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)
Participant 2:	Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)
Participant 3:	Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)
Participant 4:	Travel and subsistence for 2 persons for 5 years (5 x 3.000 € = 15.000 €)
Participant 5:	Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)
Participant 6:	Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)

Participant 7: Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)
Participant 8: Travel and subsistence for 1-2 persons for 5 years (4.500 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 18.070 €)
Participant 9: Travel and subsistence for 1-2 persons for 5 years (3.000 € + 3.150 € + 3.305 € + 3.470 € + 3.645 € = 16.570 €)
Participant 10: Travel and subsistence for 2 persons for 2 years (8.000 €)

Personnel resources

Participant 1: one engineer over 5 years (= 301.500€)
one technical assistant over 2 years (85.600€)
(+ 0,25 administrator over 4-5 years (see management activities))
(+ 1 scientific administrator over 4-5 years (see management activities))
Participant 2: one engineer over 4 years (220.000€) & one scientist over 2,5 years (150.000€)
Participant 3: Six scientists (one physicist, two mechanical engineers (different fields of specialisation), one electrical engineer, one computer scientist, one process engineer)
Participant 4: 1,5 FTE (one PostDoc plus one PhD student over 5 years (65.000€ per 1,0 FTE person and year)
Participant 5: 1 engineer over 5 years (=351.000€)
1 technical assistant over 5 years (=243.000€)
Participant 6: one post-doc over 5 years (=280,000€)
4 x 1 FTE guest scientists from Saratov each over 6 months (= 112.000 €)
Participant 7: two technical assistants over 5 years (salaries of 3000€ per year = 30.000 €), two technicians over 5 years (salary of 3.600 € per year = 36.000€), one programmer over 5 years (salary 3.600€ per year = 18.000€).
Participant 8: one programmer over 5 years, an additional half programmer position for the first year, 0.1 position for systems support over 5 years (120.000 € + 86.625 € + 90.955 € + 95.505 € + 100.280 € = 493.365 €)
Participant 9: one technician over 5 years (120.000), 2 PhD students (90.000€).
Participant 10: four engineers over 2 years (= 540.000€)
one technician over 2 years (= 97.000€)

Management activities

Participant 1: 0,25 personnel for 4 years for administrative management (= 45.000 €)
1 personnel for 4 years for scientific management (= 232.000€)
2.500€ per year for financial auditing (=12.500€ in five years)
10.000€ in year 3 for the implementation of competitive calls by the consortium to find new SME-participants (EU wide search)
Participant 2: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 3: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 4: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 5: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 6: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 7: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 8: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 9: 2.500€ per year for financial auditing (=12.500€ in five years)
Participant 10: 2.500€ per year for financial auditing (=5.000€ in years 4 & 5)

Overheads

Participant 1: 20% (flat rate for indirect costs)
Participant 2: 20% (flat rate for indirect costs)
Participant 3: full cost model (FC) (real indirect costs)
Participant 4: 20% (flat rate for indirect costs)
Participant 5: full cost model (FC) (real indirect costs)
Participant 6: 20% (flat rate for indirect costs)
Participant 7: 20% (flat rate for indirect costs)

Participant 8: 20% (flat rate for indirect costs)
Participant 9: 20% (flat rate for indirect costs)
Participant 10: full cost model (FC) (real indirect costs)
Travel and subsistence
Travel and subsistance : calculated based upon a standard trip within the European Union at a unit price of around 1666 Euro per trip and per person

EU grant driven mobilisation of the critical mass of resources and integration into a coherent project

Within the Participant's groups additional resources are mobilised that exceed the resources applied for in this application. However, at least as important for the achievement of critical mass is the Participant's complementary interdisciplinary expertise, which is combined to add value. This will be done through this integrated project whereby resources funded by the EU enable scientists to combine and adapt their different expertise in order to implement the Activities planned (see above, Fig. 5). Detailed description:

Additional resources and integration, Participants 1, 2 and 5:

Within the German Cancer Research Centre (DKFZ) an additional 6 scientists, 1 technician and 3 PhD students have worked and work on the development of peptide laser printer core technology and on related issues. Within the groups of Participants 2 (Tel-tek) and 5 (University of Maribor) an additional 5 scientists have specialised in different methods for the production and characterisation of small particles and their behaviour (*e.g. powdered dyes attracted to electrostatic metal surfaces*), with expensive machines needed for that already existing. Thereby these groups together achieve a critical mass of equipment, workforce, pre-existing expertise and know-how in order to implement Activity 2 (*toner production*) & the particle part of Activity 6 (*combinatorial synthesis of peptide arrays*).

Additional resources and integration, Participants 1, 3 and 4:

An engineer from Fraunhofer IPA has already constructed a first modified laser printer that could be employed for the combinatorial synthesis of arrays. The Kirchhoff Institute (KIP) 5 operates an ASIC division with integrated laboratory design centres. At any time point a team of more than 20 scientists working on the various >5 chip projects pursues the projects. Common meetings and workshops ensure the proper integration and collaboration amongst those projects, e.g. designed for use in particle accelerators. The institute's permanent staff maintains the laboratory and design software infrastructure. In addition both institutes have professional mechanical engineering equipment at their disposal together with highly skilled workforce (*e.g. different turning lathes, chip-bonding and testing machines*). Thereby a critical mass (*know-how, equipment, workforce*) is generated to implement Activity 3 (*peptide laser printer*) and Activity 4 (*high voltage chip and instrument for delivery of particles*). Combined with the particle-related Activities (see above), these Activities are integrated within the DKFZ to implement Activity 6 (*combinatorial synthesis of peptide arrays*).

Additional resources and integration, Participants 6 and 7:

At the Universities of Brussels and Saratov complementary know-how (*production of nano colloidal gold particles and ccd camera or similar read out methods respectively*) for labelling free detection based on nano colloidal gold particles has been elaborated that is instrumental for the implementation of Activity 5 (*detection of binding events, labelling free detection*). Both institutes have a successful record of collaboration proven by shared publications. The surrounding of these institutes (*3 scientists, equipment for synthesis, characterization of colloids (spectroscopy, HPLC) and application to diagnostics (automated clinical analyzer)*) back the integrated project planned by

additional resources and know-how relevant to the project. In Dr. Englebienne's and Prof. Khlebtsov's groups one and 4 additional post-doc scientist works on related subjects respectively.

Additional resources and integration, Participants 1, 8 and 9:

The Soroka Medical Center serves the entire southern region of Israel in human viral diagnosis. Additional resources, equipment and workforce is used to diagnose, collect, test and store a large reservoir of diagnosed patient's sera that is at our disposal in order to implement Activity 1 (*model applications*). At the Ben Gurion University and the DKFZ many more scientists with a biological or medical background are employed that potentially should be interested in our technology beyond the model applications planned.

More than 50 additional employees take care of the Swiss-Prot database, the largest provider of protein sequence data, a free resource indispensable not only for our model applications planned, but also for molecular biology as a whole, the InterPro database of protein families and domains, and ten more ambitious database projects. The integration of these group's equipment, additional workforce, expertise and know-how will help to design and produce "peptidome arrays" representing the entire genome of a pathogen that are stained by patient's sera. Moreover sequences derived from specifically stained peptides will be fed into the Swiss-Prot database thereby helping in data mining.

Additional resources and integration, Participants 10+:

In a second phase (years 4 & 5) the project's emphasis will shift from research to commercialisation efforts headed by SMEs to be added to the consortium before the year 4 (*demonstration activities, prototypes, Activity 8*). Participants 1 to 9 would contribute and integrate their intellectual property and know-how in order to produce prototypes of commercial value (*e.g. a small machine for combinatorial synthesis of peptide arrays that houses >20 toner reservoirs and a programmable chip with photo diodes arrayed underneath the surface for read out of binding events*). In order to add value the SMEs complementary expertise and know-how mainly should reside in prototyping, complementary products, marketing and worldwide distribution networks. The SMEs additional resources would be their 65% shares in demonstration activities. Candidate SME participants are Qiagen, Eppendorff, Grohmann Engineering or OGT. Eventually in collaboration with some of those SMEs just mentioned, venture capital financed SMEs could concentrate on prototyping.

Conclusion

As detailed above the overall financial plan for the project will mobilise additional resources and will combine a very large spectrum of complementary interdisciplinary expertise and know-how in order to obtain the critical mass of resources needed to develop and commercialise an integrated tool for doing experiments on a proteomics scale. With the huge scientific and economic interest in such a low priced, versatile, reliable, easy, robust and user-friendly tool we anticipate successful commercialisation efforts in the second phase of the integrated project planned.

B.8 Detailed implementation plan – first 18 months

a) Introduction, 18-month implementation plan

Objectives, general

The objective of our approach is to integrate research and knowledge in different fields of expertise in order to finally develop a user-friendly novel tool that

- ⇨ translates genomics data into **high complexity peptide arrays** with the help of **databases**,
- ⇨ thereby enables **experiments in a truly proteomics scale**,
- ⇨ automatically **reads out binding events** preferentially by **labelling free detection** and
- ⇨ annotates specific binding events once again with the help of **databases**.

The **model application** planned is

- ⇨ the **large scale deciphering of virus-specific immune reactions** and
- ⇨ the **correlation of this data to the patient's immune status** and prognosis.

Central achievement within the first 18 months

Within the first 18 months we plan to reach the ambitious **goal of producing a first high complexity peptide array**, which is only possible due to the fact that a first peptide laser printer will be available late in summer 2003, i.e. before the start of the project applied for.

Activity 2, particles

Central in the achievement just described will be the production of **solid amino acid toner particles** that are delivered to their destiny

- ⇨ by means of a **laser printer** or
- ⇨ simply addressed to a **chip's** surface by electrostatic forces.

However, in order to have these particles addressed by a chip's surface or printed by a laser printer they must fulfil several criteria, which are tackled in different workpackages:

- ⇨ they should be **evenly charged** (WP2.3),
- ⇨ they should have a **narrow size distribution** (WP2.2, WP2.4),
- ⇨ **particles should be small** (WP2.2 - WP2.4) and
- ⇨ all the **particle's ingredients** needed for that **shouldn't hamper the consecutive chemical reactions** (WP2.1).

Other criteria for particle production are

- ⇨ **yield** (WP2.2 – WP2.4) and
- ⇨ to exclude O_2 and H_2O in order to **avoid decay of activated amino acids** and thereby preserve the toner (WP2.2, WP2.4).

The last points are especially important for the model applications planned (WP1.1):

- ⇨ the more peptide arrays in good quality are achievable (and affordable),
- ⇨ the more patient's sera can be correlated e.g. to the patient's immune status and
- ⇨ the more viral diseases can be tackled.

Activity 3, 4 & 6, delivery systems and combinatorial

Within the first 18 months the project's **emphasis** in terms of achieving a high complexity peptide array certainly is **on the peptide laser printer** (WP3.1 – WP3.3), which is mainly due to the long time needed for design, submission and delivery of a **chip suitable for addressing solid particles** (WP4.1). The

peptide synthesis

development of the peptide laser printer will focus

- ⇨ on an **instrument that is accurate** (WP3.1, WP3.3) and
- ⇨ has a **washing unit** integrated (WP3.2)

in order to allow for the synthesis of a sufficient number of high complexity peptidome arrays needed for the model application planned (WP1.1).

The **chip approach** will have to tackle the inherent difficulty of the comparatively low voltage that is applicable to the tiny chip structures. We therefore start with comparatively "big" **chip structures** (80ym x 80µm) due to the advantage of these "coarse structures" to **accommodate up to 90V** (WP4.1). In this range the particles produced in WP2.1 – WP2.4 should be addressable to spatially defined chip structures. This will be done with the help of a **chip instrument** (WP4.2) **that houses the chip** from WP4.1 **and the amino acid particles** produced in WP2.1 – WP2.4.
With the chip (WP4.1) and chip instrument (WP4.2) available we will try to address especially small particles (WP2.2 & WP2.4) with a lower voltage applied. Depending on the particle's quality we thereby might be able to define conditions where **chips with smaller structures** and lower voltage applied could be used.

Activity 6, combinatorial peptide synthesis

For **combinatorial synthesis of high complexity peptide arrays** (WP6.3) the particles mentioned above (WP2.1 – WP2.4) are employed in a chip instrument (WP6.2) or peptide laser printer (WP6.1). Particles are

- ⇨ **printed to a solid support**,
- ⇨ **heated in order to mobilise the activated amino acids** comprised in the particles and
- ⇨ the resultant **coupling reaction to the solid support** analysed

in terms of

- ⇨ **spot-density**,
- ⇨ **selectivity** and
- ⇨ **coupling yield**.

By taking into account the pre existing expertise we should be able to **synthesise roughly 100.000 peptides per 20cm x 20cm by M12**, thereby supplying Participant 9 with a first test set of pathogen peptidome arrays needed for the model application planned (WP1.1).

Activity 4 & 5, read out systems

Read out systems and especially **labelling free detection** (WP5.1 – WP5.7) can only be integrated with high complexity peptide arrays at a later stage, because a number of principle questions dealing with

- ⇨ sensitivity of the **labelling free detection method** employed (WP5.1 – WP5.4),
- ⇨ suitable **forms and materials of the nano colloidal particles** used (WP5.1, WP5.2, WP5.7) and
- ⇨ **basic optical principles** (WP5.5, WP5.6)

have to be tackled first.
Therefore in the first 18 months the emphasis in this field of research will be on the **transfer of the labelling free detection method to the array format** (WP5.3). The integration of an array of photodiodes into the chip will be tackled at a later stage.

Activity 1 & 7, model applications and bioinformatics

As repeatedly stated above the high complexity peptide arrays synthesized within WP6.3 will be used to screen for

- ⇨ the **large scale deciphering of virus-specific immune reactions** and
- ⇨ the **correlation of this data to the patient's immune status** and prognosis (WP1.1).

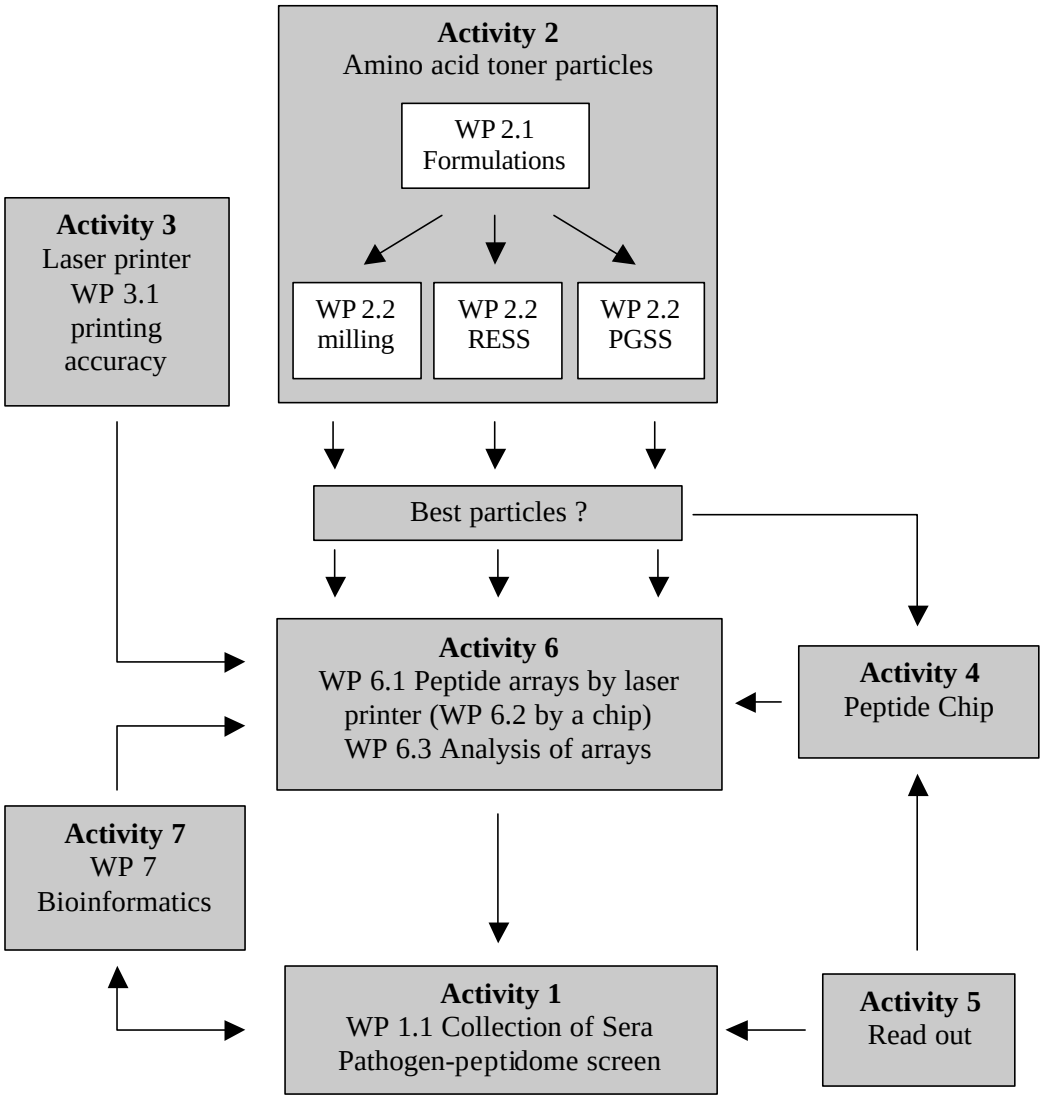
The peptide sequences of these virus-specific peptidome arrays will be fed into the laser printer by a kind of three-dimensional printing process with consecutive layers of amino acid toners printed and coupled to the solid support. The **sequence information** for this will be derived from **Swiss-Prot** with the help of **tools and databases developed** (WP7).

b) Timing of the different workpackages

Work-package No[I]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
WP1.1: Pathogen-peptidome screen												D1.1[II]						D1.2 D1.3
WP2.1: Particle formulations			D2.1.1															
WP2.2: Particles by RESS						D2.2.1												
WP2.3: Particles by milling													D2.3.1					D2.3.2
WP2.4: Particles by PGSS						D2.4.1												D2.4.2 D2.4.3
WP3.1:Printing accuracy																		D3.1
WP3.2:Washing unit																		D3.2
WP3.3:Calibration of the printer																		D3.3
WP4.1: Peptide chip								D4.1.1[III]										D4.1.2
WP4.2: Chip instrument												D4.2.1						D4.2.2
WP5.1:Nanoparticles in solution				D5.1.1		D5.1.2												
WP5.2:Principles plasmon resonance										D5.2.1								
WP5.3:Nanoparticles in array form																		D5.3.1
WP5.4: Spectral properties of markers												D5.4.1						
WP5.5: Solution of the optimisation problem												D5.5.1						
WP5.6: Multiparticle scattering problem																		D5.6.1
WP5.7: Gold nanoparticles and -rods																		D5.7.1
WP6.1:Peptide arrays by a laser printer												D6.1.1[IV]						
WP6.2:Peptide arrays by a chip																		D6.2.1
WP6.3:Analysis of peptide arrays																		D6.3.1
WP7: Bioinformatics			D7.1			D7.2						D7.3						D7.4 D7.5
WP9: Administration	D9.3	D9.1				D9.2												

[I] **Workpackage number: WP 1 - WP n**
[II] **Data concerning the persons involved available without personal details**
[III] **Submission of first prototype peptide chip**
[IV] **Previously exisiting laser printer**

c) Graphical presentation of the components with their interdependencies



Significant risks

The technology described in this grant application comprises 7 different ambitious Activities. Therefore, partial failure of one or more developments cannot be excluded leading to a project fraught with relative risks.

Activity 2, particles

Generation of the amino acid toner particles might result in

- ⇨ **particle sizes** or
- ⇨ **size distributions**

insufficient for selective and efficient addressing to the laser printer support or to the chip's surface.

- ⇨ **Agglomeration** and
- ⇨ **uneven triboelectric charging**

could hamper the use of amino acid particles.

It is also conceivable, that one of the

- ⇨ **additives** necessary for accurately defined charging (charge stabilisers, charge control agents) **cannot be embedded** in the particle's matrix by the methods applied.

Amino acid derivatives might be

- ⇨ **instable** or
- ⇨ **decay** inside the particle matrix.

The **efficiency of the coupling reaction** might be

- ⇨ **inadequate** or
- ⇨ **unpredictable**.

Activity 3, 4 & 6, delivery systems and combinatorial peptide synthesis

Difficulties in the production of amino acid particles as described above might result in problems with the

- ⇨ particle's **spatially defined delivery** to the supports

by means of

- ⇨ the **peptide laser printer** or
- ⇨ the high voltage **CMOS chip**.

Agglomeration, uneven charging, deficient size as well as size distribution might lead to a

- ⇨ slight and **unselective toner transfer**,
- ⇨ which **hampers the array complexity** aimed at.

In addition to that, the working voltages of the CMOS chip (up to 90V) might be

- ⇨ **insufficient** to the obligatory high **transfer selectivity**.

Altogether these risks would decrease the **achievable complexity** of peptide arrays.

Activity 6, combinatorial peptide synthesis

The peptide synthesis adapted from the well established Merrifield solid-phase synthesis will be accomplished by means of solid toner particles. Amino acid monomers are release d from the melted particles at elevated temperatures (70-80°C) and thereby react with surface-bound amino groups. It is possible

- ⇨ that the **sophisticated methods of peptide chemistry will conflict with these conditions** and
- ⇨ hence result in **degradation of chemicals** and
- ⇨ of **coupling yields**.

These detrimental effects might also originate from the additives mentioned above, if they **compete the coupling reaction**.

Activity 4 & 5, read out systems

As yet, the label free detection based on colloidal gold nanoparticles has not been applied to peptide arrays, therefore unforeseen conflicts and risks are conceivable.

- ⇨ **Unspecific protein adsorption** as well as
- ⇨ **deficient optical properties**

might lead to a

- ⇨ **low detection sensitivity** or
- ⇨ a **small signal-to-noise ratio** respectively.

Therefore, a relative failure of the labelling free detection of binding events on the peptide arrays might happen.

Activity 1 & 7, model applications and bioinformatics

The model applications planned within Activity 1 might be inadequate for the results envisioned. The specific viral infections targeted might induce

- ⇨ **irrelevant humoral immune responses** or
- ⇨ **large background noise**.

In the field of bioinformatics, we will have to struggle with

- ⇨ **misentries** and blanks in a vast amount of data.

However, a further significant risk seems not to burden this part of the project, since proteomics and genomics data for comparable low complexity viral genomes should be available.

Contingency planning for unexpected outcomes

For most of the subprojects described here, proof of principle experiments have been carried out successfully. Still unexpected outcomes are intrinsic in ambitious science. We accommodate for this in flexible parallel developments of technology that could substitute partial failure as specified below:

Activity 2, particles

Proof of principle experiments have been done successfully for the production of amino acid particles. Still we develop several interchangeable methods for particle production:

- ⇨ WP2.1 & 2.3, **Milling procedures**,
- ⇨ WP2.2, **RESS procedure** and
- ⇨ WP2.4, **PGSS procedure**.

In addition special emphasis is on the particle's chargeability (WP2.3).

Activity 3, 4 & 6, delivery systems and combinatorial peptide synthesis

Two interchangeable systems for delivery of amino acid toner particles to the solid support are being developed:

- ⇨ WP3.1 – WP3.3, **peptide laser printer** and
- ⇨ WP4.1, a **chip** (and WP3.2, a **chip instrument**)

For both systems proof of principle experiments have been done successfully. As for the peptide laser printer a first instrument will be available in summer 2003.

Activity 4 & 5, read out systems

The most promising read out system certainly would employ labelling free detection, because thereby in principle not only low affinity binders but also absolute values for affinities could be determined. However, to date all of these systems suffer from low sensitivity or delicate handling. We therefore not only comprise in our project

- ⇨ WP5.1 – WP5.7, Component 4-3: methodology for **labelling free detection**,

but also more conventional methods for read out relying on labelling,

- ⇨ either employing an **array reader** (Component 5-5),
- ⇨ or **integrating read out** of chemoluminescence **within the chip design** (Component 4-4).

Activity 1 & 7, model applications and bioinformatics

In our model application planned we want to correlate:

- ⇨ the patient's antibody response towards virus-specific peptides
- ⇨ with the patient's immune status among other things.

It is known from the literature:

- ⇨ that antibodies are important in blocking viral entry into cells and
- ⇨ that different individuals cope differently with viral infections.

However, we don't know *a priori* which viral infections will give clear correlations. Therefore we plan to analyse several viral pathogens, with the data for the combinatorial synthesis delivered by Swiss-Prot. The low complexity viral genomes should allow for the production of a sufficient number of peptide arrays for initial experiments even without automatic production procedures at hand.

d) Detailed work description broken down into workpackages

Workpackage list (18 month plan)

Work-package No	Workpackage title	Lead participant No	Person-months	Start month	End month	Deliv-erable No
WP1.1	Pathogen-peptidome screen	9, BGU	16	M0	M18	D1.1 D1.2
WP2.1	Particle formulations	1, DKFZ	10	M0	M18	D2.1.1
WP2.2	Particles by RESS	1, DKFZ	8	M0	M18	D2.2.1
WP2.3	Particles by milling	2, Tel-Tek	24	M0	M18	D2.3.1 D2.3.2
WP2.4	Particles by PGSS	5, Maribor	24	M0	M18	D2.4.1- D2.4.3
WP3.1	Printing accuracy	3, IPA	28	M0	M18	D3.1.1
WP3.2	Washing unit	3, IPA	6	M12	M18	D3.2.1
WP3.3	Calibration of the printer	3, IPA	3	M15	M18	D3.3.1
WP4.1	Peptide chip	4, KIP	19	M0	M18	D4.1.1 D4.1.2
WP4.2	Chip instrument	4, KIP	8	M3	M18	D4.2.1 D4.2.2
WP5.1	Nano particles in solution	6, ULB	12	M0	M6	D5.1 D5.2
WP5.2	Principles plasmon resonance	6, ULB + Saratov	8	M7	M10	D5.2.1
WP5.3	Nano particles in array format	6, ULB	16	M11	M18	D5.3.1
WP5.4	spectral properties of markers	6, Saratov	15	M0	M18	D5.4.1
WP5.5	Solution of the optimisation problem	6, Saratov	20	M4	M18	D5.5.1
WP5.6	multiparticle scattering problem	6, Saratov	15	M8	M18	D5.6.1
WP5.7	gold nanoparticles and nanorods	6, Saratov	40	M6	M18	D5.7.1
WP6.1	Peptide arrays by a laser printer	1, DKFZ	6	M8	M18	D6.1.1
WP6.2	Peptide arrays by a chip	1, DKFZ	2	M16	M18	D6.2.1
WP6.3	Analysis of peptide arrays	1, DKFZ	2	M12	M18	D6.3.1
WP7	Bioinformatics	8, EBI	22	M0	M18	D7.1- D7.5
WP9	Administration	1, DKFZ	18	M0	M18	D9.1.1- D9.3.1
	TOTAL		322			

Deliverables list (18 month plan)

Deliverable No	Deliverable title	Delivery date	Nature	Dissemination level
D1.1	Collection of sera from HCV, HBV, HIV chronic patients	M12	R	PP
D1.2	Identify virological / prognostic markers of sera/patients	M18	R	RE
D1.3	Results from pathogen-proteome array screen	M18	R	PP
D2.1.1	Component mixtures identified for the achievement of D6.1	M3	D	PP
D2.2.1	RESS procedure for particle production established	M6	D	PP
D2.3.1	Production of 20 µm particles with narrow size range	M12	P	PP
D2.3.2	First adaption of existing technology to measure charge classification	M18	D	PP
D2.4.1	Phase equilibrium data for single components comprised in amino acid toner particles and CO_2	M6	R	PP
D2.4.2	Optimal operating parameters for PGSS micronisation process using component mixtures	M18	R	PP
D2.4.3	Influence of operating parameters on properties of produced particles	M18	R	PP
D3.1	An operative peptide laser printer having all basic properties for experiments and advancements	M18	R, P	PU
D3.2	Concept and CAD design of the washing unit	M18	R, O	RE
D3.3	Report about calibration strategies for the printer	M18	R	RE
D4.1.1	Submission of first prototype peptide chip	M8	R	PP
D4.1.2	Final report of qualification of peptide chip prototype	M18	O	PP
D4.2.1	Preliminary chip instrument for toner deposition	M12	D	PU
D4.2.2	Final report and demonstration materials of Peptide Plotter	M18	O	PU
D5.1.1	Synthesis-Preliminary report	M4	R	PP
D5.1.2	Synthetic materials in solution	M6	D	RE
D5.2.1	Characterisation and performance of materials in solution	M10	D	RE
D5.3.1	Solid-phasing and derivatisation – Preliminary data	M18	R	PP
D5.4.1	Spectral properties of markers	M12	R	PU
D5.5.1	Calculated “optimisation” curves	M12	R	PU
D5.6.1	FORTRAN codes for multiparticle scattering	M18	R	PP
D5.7.1	Colloidal gold conjugate preparations	M18	O	PP
D6.1.1	20 different amino acid toners printed	M18	D	PP
D6.2.1	Combinatorial synthesis of peptide arrays	M18	D	PP
D6.3.1	Addressing particles to a chip’s surface	M18	D	PP

D7.1	Specification for proteome sets and PREJUDICE tool	M 3	R	PP
D7.2	Proteome sets for target organisms	M 6	O	PP
D7.3	PREJUDICE prototype	M 12	D	PU
D7.4	PREJUDICE production version	M 18	O	PU
D7.5	Specification of proteomics data submission formats	M 18	R	PP
D9.1	Communication links between Participants	M2	O	PP
D9.2	Internet platform for Participants	M6	P	PP
D9.3	Participant’s data at financial administration	M1	O	CO

Description of Activity 1 (18 month plan, Participant 9, Ben Gurion University)

Workpackage number	**1**	**Start date or starting event:**		**M1**			
Participant id	**9**						
Person-months per participant	**54**						

Objectives are to identify pathogen's peptides that are relevant to viral interactions with host. This contributes to

- ⇨ the knowledge on viral infections,
- ⇨ improved diagnosis,
- ⇨ prediction of disease progression and
- ⇨ vaccination against viruses.

In accordance we intend to:

1.1. Apply the pathogen-peptidome array to analyse humoral immune responses to viral infections

1.2. Identify the major neutralising peptide epitopes from vaccinia virus for synthetic vaccine design and optimisation.

1.3. Analyse peptidome-wide different interactions of pathogen-peptides with various factors that affects pathogenesis.

Description of work

Sera from patients with various infectious diseases will be collected along with detailed data about the person and the pathogenesis of the disease. The sera collected for each viral infection will be reacted with the corresponding peptidome-array. We expect that insertion of non-relevant peptides to each peptidome array as a negative control will help to tune the system and the experiments done. The screen results will be analysed together with participant 8 and 1, for fine-tuning of the experiments.

Deliverables

D1.1: The data concerning the persons involved in the research will be made available in a report without any personal details.

D1.2 & D1.3: The screen results and the correlation to the patient's status will be published in reports and finally in scientific publications.

Milestones and expected result

Depending on the results obtained from the initial peptidome screen, decisions concerning:

1) fine-tuning of the peptidome array and
2) conditions for screening, will be made.

The first HCV-peptidome array will be used as a case study and then the rest of the peptidome-arrays will follow. We expect:

- ⇨ To publish the results.
- ⇨ To contribute to the design of vaccines for HIV, HCV, small pox, and later to other viruses.
- ⇨ To gain knowledge that will help to design drugs against these viruses.
- ⇨ Be able to collect data that will enhance the design of pathogen-peptodome chip for diagnosis.

Description of Activity 2 (18 month plan, Participants 1, 2 & 5, DKFZ, Tel-Tek & Maribor)

Workpackage number	**2.1 & 2.2**	**Start date or starting event:**		**M1**			
Participant id	**1**						
Person-months per participant	**18**						

Objectives

- ⇨ Identification of component mixtures suitable for amino acid toners
- ⇨ Production of amino acid toner particles by RESS procedure

Description of work

The toner particles needed for this work need to fulfil several criteria:

- ⇨ they must be chargeable in order to be printed by a laser printer or be addressed by a chip,
- ⇨ they must keep their charge upon storage,
- ⇨ the charge control agents and the charge stabilisers needed for that must not disturb the consecutive chemical reactions,
- ⇨ they should comprise still active or activateable amino acids,
- ⇨ the solid solvent should be suitable for the consecutive chemical reactions.

The influence of the components to be tested will simply be analysed by successive mixing of components, melting, freezing and mechanical milling. The resulting particles will be tested

- ⇨ with the OkiC7000 printer and
- ⇨ a consecutive coupling reaction of amino acids to the solid support.

RESS procedure adds yet another requirement onto the components employed for particle production:

- ⇨ The components must dissolve in CO_2 under high pressure to yield a supercritical solution.

RESS procedure, as well as PGSS procedure (WP2.4), however, are especially suited for the production of homogenous and very small particles with a very favourable size distribution.

Deliverables

D2.1.1: Month 6: Suitable component mixtures identified for the achievement of D6.1
D2.2.1: Month 3: RESS procedure for particle production established

Milestones and expected result

The identification of suitable components for the production of amino acid particles paves the way for their use in the peptide laser printer (Activity 3) or in the chip instrument (Activity 4). Depending on the component's suitability for RESS or PGSS procedure, emphasis in particle production will be shifted from milling procedures.

Workpackage number	**2.3**	**Start date or starting event:**		**M1**			
Participant id	**2**						
Person-months per participant	**22**						

Objectives
Manufacture 20µm particles for use in peptide laser printer or chip instrument

Description of work
In the first 18 months, the focus will be on producing toner particles by air classification and air milling. The objective is to produce some larger amounts of toner particles of narrow size distributions that can be employed as toners in the laser printer (Activity 3) or the chip instrument (Activity 4). The objective is to produce narrow sized particles of nominally 20 µm in 2004 that can be used for combinatorial peptide synthesis, and below 10 µm in 2005. The main problem expected is the production of very narrow size classes.
More important, we will investigate the chargeability of the particles, using different charge stabilisers and charging methods. The ultimate test system for these amino acid toners will be the OkiC7200 laser printer with the standard set by commercially available colour toners. For this technique it is crucial to that the charge-to-mass-ratio (q/m) is more or less equal for all particles. Experience and experimental results show that this can be difficult to achieve. Often particles can have both positive and negative charge, which is detrimental to the selectivity and efficiency. The particles will be tested for charge uniformity by using a charge deflection apparatus, together with a laser-doppler meter. This will give information about the size dependent charging. In addition, particles of different charges can be collected for analysis in order to investigate and correct differences.

Deliverables
D2.3.1: 200g of 20 µm toner particles (20 different amino acids) for use in the peptide laser printer or chip instrument (Activities 3 & 4)
D2.3.2: Adaptation of our existing deflection measurement apparatus for the testing described. First results on chargeability of the toner particles

Milestones and expected result
- 18 months: Production of 20 µm particles
- 18 months: Determination of the practical limitations to the technique in terms of yield
- 18 months: Determination of the practical limitations to the technique in terms of narrowness of size distribution
- 18 months: The technique should be established and the first results available.

This milestone would enable the particle part for combinatorial peptide synthesis. However, 20µm particles probably would only work with the peptide laser printer (Activity 3) and with the 90V chip design (Activity 4).

Workpackage number	**2.4**	**Start date or starting event:**		**M1**			
Participant id	**5**						
Person-months per participant	**24**						

Objectives

Optimise high-pressure PGSS micronisation process for the mixture of components comprised in amino acid toner particles

Description of work

- ⇨ Solid-liquid phase transition of each of the components that are comprised within amino acid toner particles will be determined with a modified capillary method or using a high-pressure view cell. In this work dense gas (CO_2) under high pressure is used to dissolve solid components resulting in a *supercritical* solution.
- ⇨ Furthermore, preliminary PGSS micronsiation experiments of component mixtures will be performed, where optimal operating parameters will be determined. The influence of operating parameters on the morphology and size distribution of obtained particles will be determined. Particles obtained will be analysed by microscopy, size analyser, by their ability to develop (i.e. being printed) within colour laser printer Oki C7200 and by their ability to perform successive coupling reactions for combinatorial peptide synthesis.

Deliverables

Report on:
D2.4.1: Phase equilibrium data for single components comprised in amino acid toner particles and CO2; the Pressure-Temperature (PT) trace of three phase Solid-Liquid-Gas (SLG) line
D2.4.2: Specification of optimal operating parameters for PGSS micronisation process when using component mixtures
D2.4.3: Influence of operating parameters on properties of produced particles

Milestones and expected result

Characterisation of particles produced and determination if the particles fulfil the criteria. If they do this milestone would enable the particle part for combinatorial peptide synthesis. Depending on the particle's size and q/m (see WP 2-3) these particles could be used in combination with a chip (Activity 4; WP4-1 and 4-2):

- ⇨ that attracts particles with 90V (coarse chip structures, approx. 80µm x 80yµm),
- ⇨ that attracts particles with 19V (smaller chip structures, approx. <20µm x 20yµm), or
- ⇨ that attracts particles with 5V (very small chip structures, approx. <5µm x 5yµm).

Thereby this milestone influences decisions on chip design (Activity 4).

Description of Activity 3 (18 month plan, Participant 3, IPA)

Workpackage number	**3**	**Start date or starting event:**		**M1**			
Participant id	**3**						
Person-months per participant	**37**						

Objectives

1. Conceptual design and construction of a peptide laser printer
2. Conceptual design of the washing unit
3. Decision about the calibration procedure for the printer

Description of work

Workpackage 3.1:

In the first 18 months a new peptide laser printer based on the first instrument will be designed and constructed. Compared to the first instrument it has the following additional properties:

1. Improvement of the mechanical accuracy to provide the prerequisites for a higher printing accuracy. This comprises the improvement of the slide accuracy of the linear driving unit, the drive and the mounting of the printing drums.
2. Improvement of the hardware controller to provide the prerequisites for a higher printing accuracy and to integrate the calibration procedure.

This work requires the conceptual design, the CAD design and complete construction of the laser printer including the development or advancement of the hard- and software. As result an operative peptide laser printer having all basic properties will be available. Using this instrument first experiments to improve the printing accuracy will be made.

Workpackage 3.2:

In the first 18 month the conceptual design of the washing unit will be made. First the details of the chemical process (Merrifield synthesis) will be specified together with Participant 1 (DKFZ). Based on this knowledge a strategy for the automation of the chemical process will be worked out. This includes the efficient coupling of the printing step (~1 min) and the chemical process step (~40min). Based on this work a CAD design of the washing unit will be made. This work will be done parallel to the construction of the printer.

Workpackage 3.3:

Using the instrument described in workpackage 3.1 first experiments concerning the calibration procedure of the printer will be made within the first 18 month. In detail: the accuracy obtained by the calibration procedure of the peptide laser printer and the time intervals at which recurrent calibrations are necessary will be studied in experiments. Based on these results strategies for a calibration procedure meeting the requirements of accuracy and easy handling are worked out.

Deliverables

3.1.1: An operative peptide laser printer having all basic properties for experiments and advancements.
3.2.1: Concept and CAD design of the washing unit
3.3.1: Report about calibration strategies for the printer

Milestones and expected result

- 18 months: An operative peptide laser printer having all basic properties

Together with the amino acid particles from Activity 2 this milestone allows for the production of high complexity peptide arrays

- 18 months: Concept and CAD design of the washing unit

Together with the amino acid particles from Activity 2 this milestone allows for the production of a significant number of high complexity peptide arrays needed for extending Activity 1 to other viral genomes / testing more antibody sera

- 18 months: Decision about the calibration strategy for the printer.

Together with the amino acid particles from Activity 2 this milestone allows for the production of peptide arrays of higher complexity needed for extending Activity 1 to other viral genomes / testing more antibody sera

Description of Activity 4 (18 month plan, Participant 4, KIP)

Workpackage number	4.1	Start date or starting event:		M1			
Participant id	4						
Person-months per participant	19						

Objectives

1. Development of a microchip for the synthesis of peptide arrays on the chip surface
2. Manufacturing of the microchip
3. Test and qualification of the first microchip

Description of work

Based on the existing on-going work in the area of peptide chips, the detailed requirements for the first microchip will be evaluated and set during the first 3 months of the project, together with first implementation studies. In particular high-voltage designs, the implementation of the electrode control on the chip as well as the exact electrode layout for the composition of the peptide array will be studied. The technology envisioned is the AMS 0,8µm 90V CMOS process.

During the remaining time until the submission of the chip in Month 8, the full custom chip layout will be implemented together with the necessary infrastructure for controlling the electrodes on the chip. An appropriate simulation infrastructure and libraries will be generated for the further use throughout the project. In parallel, an appropriate electrical test infrastructure for the chip will be prepared.

About 20 chips are expected back from the first multi-project wafer run. They will be tested electrically and chemically by using the test infrastructure and then employed for toner deposition, using the chip instrument (WP 4.2).

After completion of the testing and toner deposition, appropriate specification and design documentation is produced for further use throughout the project.

Deliverables

1. D4.1.1, Month 8: Submission of first prototype peptide chip
2. D4.1.2, Month 18: Final report of qualification of peptide chip prototype

Milestones and expected result

No Milestones within first 18 months, expected results are the qualification of the first peptide chip manufactured and tested.

Workpackage number	**4.2**	**Start date or starting event:**		**M1**			
Participant id	**4**						
Person-months per participant	**8**						

Objectives

1. Development of mechanical infrastructure for handling of peptide chips and toner deposition
2. Computer assisted control of infrastructure and micro chips

Description of work

The peptide microchips developed in WP 4.1 will be received bare die as required for the later toner deposition. However appropriate infrastructure, such as low-cost chip-on-board bonding and control of the chips is required as well as their mechanical handling. The appropriate mechanical infrastructure, PCBs for Chip-on-board bonding, including deposition of glob top, will be developed in this work package as well as a simple mechanical apparatus for the semi-automatic exposition to different toner particles.
In order to prepare for automatic control first appropriate computer interfaces are also developed in this task.

Deliverables

D4.2.1: Month 12: Preliminary chip instrument for toner deposition
D4.2.2: Month 18: Final report and demonstration materials of the chip instrument

Milestones and expected result

Results expected from this WP are the mechanical infrastructure for handling the microchips developed in WP 4.1. This infrastructure will be the baseline for the first peptide array synthesis on top of the peptide chips. The experience gained therein will be used for the final design of the peptide plotter during the latter part of the project.

Description of Activity 5 (18 month plan, Participants 6 & 7, Univ. of Brussels & Saratov)

Workpackage number	**5.1**	**Start date or starting event:**		**M1**			
Participant id	**6**						
Person-months per participant	**12**						

Objectives

- ⇨ Optimise synthetic processes for the production of colloidal silver and gold particles of various sizes and shapes.
- ⇨ Optimise synthetic processes for the production of composite particles made of silver and gold

Description of work

Different reduction processes will be examined in standard mixtures made of either gold or silver salts, or both respectively, and the nucleation mechanisms evaluated. The sizes of the particles obtained will be assessed by spectroscopic measurements and microscopy. The reduction conditions will then be adapted in order to generate particles of various sizes and shapes. Ratios of silver to gold salts will also be varied in order to generate particles containing different ratios of the composites.
Other processes such as light maturation will be used to change the form of the particles from spheroids to crystalline materials.

Deliverables

D5.1.1: Preliminary report on synthetic developments
D5.1.2: Final report and demonstration materials

Milestones and expected result

One meeting with Saratov in order to evaluate progress in terms of optical properties.
Depending on the (continuous) characterisation of the materials synthesised, guidelines will be drawn for further development of nanoparticles.
Results expected from this WP are nano-sized particulate materials of various sizes, shapes and compositions suited for sensitive labelling free detection of binding events.

Workpackage number	**5.2**	**Start date or starting event:**			**M7**		
Participant id	**6**						
Person-months per participant	**8**						

Objectives
Full characterization of particulate materials developed in WP1

Description of work
The materials generated in WP1 will be fully characterised in terms of shape, size, composition and physical properties in close collaboration with Saratov. The materials deemed to provide the best optical characteristics will be applied in model systems for surface plasmon resonance biomolecular recognition in solution. The model systems are antibody-ligand and binding protein-ligand interactions. The binding proteins and antibodies will be coupled to the particles, and the interactions will be followed up by the high-throughput screening system we have adapted to our clinical chemistry analyser.

Deliverables
D5.2.1: Demonstration materials and report.

Milestones and expected result
Expected results from this WP are the application of optimised materials to surface plasmon resonance biomolecular recognition in solution.
Comparative evaluation of theoretical and experimental optical performances with actual results in applications constitute regular milestones allowing to adapt progressively the optimisation process.

Workpackage number	**5.3**	**Start date or starting event:**		**M11**			
Participant id	**6**						
Person-months per participant	**16**						

Objectives

- ⇨ Selection of array substrates for particle deposition.
- ⇨ Adaptation of synthetic procedures to solid phase.
- ⇨ Derivatisation of particles for further linkage to peptides.

Description of work

Selection of a suitable array substrate for particle deposition (glass, plastics) and comparative evaluation of the solid-phase performances by both spectroscopic and surface plasmon resonance biomolecular recognition model systems in a plate reader. Comparative evaluation of different particle deposition methods on substrates: simple adsorption, lithography, adsorption on derivatised substrate. Further derivatisation of solid-phased particles for adaptation to the peptide array format.

Deliverables

This WP will extend over the 18 months period and a preliminary report will be delivered at M18 (D5.3.1).

Milestones and expected result

The comparative evaluation of the particles solid-phased by different methods and the availability of reaction sites on their surface for further derivatisation will indicate the orientations to be followed in the sensor design.

A preliminary sensor array surface is expected, suitable for further optimisation.

Workpackage number	**5.4**	**Start date or starting event:**		**M1**			
Participant id	**7**						
Person-months per participant	**15**						

Objectives

- ⇨ Development and detailed study of two-layered model of biomarkers based on silver or gold nanoparticles.
- ⇨ Development and detailed study of multi-layered model of biomarkers

Description of work

The optical properties of nanoparticle biomarkers will be studied in terms of a two-layered spherical model with a gold (silver) core and a homogeneous polymer shell. One has to calculate the absorption and differential light scattering spectra for complete set of model parameters: The particle size, the polymer shell thickness and its refractive index. As a two layered model is an idealisation, a multilayered non-homogeneous model will be developed in application to colloidal gold or silver biomarkers.

Deliverables

D5.4.1: spectral properties of markers (and demonstration materials)

Milestones and expected result

One meeting with Brussels in order to evaluate progress in terms of optical properties of the marker.
An atlas of absorption and light scattering spectra will be prepared and used as guideline for optimisation of marker's properties.
Results expected from this WP are Fortran codes and illustrative spectra within 300-1000 nm.

Workpackage number	**5.5**	**Start date or starting event:**		**M4**			
Participant id	**7**						
Person-months per participant	**20**						

Objectives

- ⇨ Solution of the optimisation problem (optical response as a function of the particle size) for two-layered model (adsorption of polymer onto bare particle)
- ⇨ Solution of the optimisation problem for multilayered model (adsorption of polymer onto conjugated particle possessing a primary adsorbed polymer layer)

Description of work

This WP consists of two parts. The first part is the solution of the optimisation problem. In other words, we would like to answer the following question: What particle size results in a maximal differential optical signals related to biospecific adsorption of target molecules on conjugate surface.
The second part of this WP deals with solution of multiparticle scattering problem for plane arrays. The main objective is to elucidate interrelation between interparticle spacing and arrangement on the one hand and the optical responses on the other.

Deliverables

D5.5.1: Calculated “optimisation” curves, which illustrate the dependence of changes in absorption and scattering as functions of the particle size of bare gold and silver particles.

Milestones and expected result

Expert visit to ULB. Expected results from this WP are the theoretical recommendations for optimal choose of nanoparticle size.

Workpackage number	**5.6**	**Start date or starting event:**		**M8**			
Participant id	**7**						
Person-months per participant	**15**						

Objectives

- ⇨ Development of FORTRAN codes for multiparticle scattering problem solution.
- ⇨ Modification of public-domain codes for the case of interacting conjugates (noble metal core and a polymer shell).
- ⇨ Illustrative calculations of spectra for linear chains and plane arrays.

Description of work

Development of two sets of codes. The first type of codes solve the fixed-orientation problem (i.e. when the incident light and a particle array are fixed in a laboratory coordinate system. The second code packages will be applicable to clusters with random orientations

Deliverables

D5.6.1: This WP will extend over the 10 months period and a preliminary report will be delivered at M18.

Milestones and expected result

Two packages of tested codes will be prepared for use by ULB group.

Workpackage number	**5.7**	**Start date or starting event:**		**M6**			
Participant id	**14**						
Person-months per participant	**40**						

Objectives

- ⇨ Synthesis and characterisation (UV-VIS spectroscopy, Dynamic Light Scattering and Transmission Electron Microscopy) of gold nanoparticles in the range 5-90 nm
- ⇨ Verification of optical models for colloidal gold and silver bioconjugates
- ⇨ Synthesis of gold nanorods with different aspect ratio (diameter/length)
- ⇨ Experimental study of size, shape, and optical properties of gold nanorods
- ⇨ Development of theoretical models and comparison of experimental and calculated spectra

Description of work

This work comprises of: (1) synthesis of nanoparticle conjugates and (2) their characterisation by 3 optical methods (absorption and static light scattering spectroscopy, DLS and TEM). Our primary goal is to develop a simple experimental technique for the quantitative evaluation of thickness of polymer shells adsorbed. Using this estimations, we could calculate the extinction and scattering spectra and compare them to the experimental measurements. This comparison should result in a clear understanding of the main factors determining optical properties of nanoparticle labels.

In addition to ball-shaped particles, synthesis of nanorods will be developed. Nanorods with different aspect ratio will be prepared and characterised using TEM, UV-VIS spectroscopy, and DLS. We are going to study the dependence of experimental spectra on the nanorod size, aspect ratio, and polymer additives to colloid solution. To simulate adsorption and scattering spectra, we shall use the T-matrix code along with a simplest dipolar model.

Deliverables

D5.7.1: Colloidal gold conjugate preparations

Milestones and expected result

The results expected are:

- ⇨ Description of methods for controlled preparation of nanorods
- ⇨ Experimental data on spectral properties of labels based on nanorods conjugates with biopolymers.
- ⇨ Conclusion on possibilities and limitations of simple optical models for simulation and prediction of label properties.

Description of Activity 6 (18 month plan, Participant 1, DKFZ)

Workpackage number	**6**	**Start date or starting event:**		**M8**			
Participant id	**1**						
Person-months per participant	**10**						

Objectives

- ⇨ Getting amino acid toners printed with a peptide laser printer
- ⇨ Combinatorial synthesis of peptide arrays
- ⇨ Getting amino acid toners addressed to a chip's surface

Description of work

Toner particles produced within Activity 2 must be delivered to their defined location on the solid support's surface. We plan to do this either with a peptide laser printer (Activity 3), or by specifically addressing solid particles with a chip (Activity 4). Due to the long time needed for chip design and due to the lower voltage applicable to the chip's structures, we expect that the emphasis of Activity 6 within the first 18 months will be on the laser printer's side. The corner stone experiment within this time will be the combinatorial synthesis of two different chess-board-patterned peptides stained with a monoclonal antibody.

Deliverables

D6.1: Month 18: 20 different amino acid toners printed in a resolution of 200 spots per cm^2
D6.2: Month 18: Combinatorial synthesis of peptide arrays with the resolution of 150 peptide spots per cm^2
D6.3: Month 18: Addressing normal toner particles to a chip's surface, preliminary experiments with amino acid toner

Milestones and expected result

With all 20 different amino acid toners printable by a laser printer combinatorial peptide synthesis can be done (together with instruments from Activities 3 & 4). This is the prerequisite for model applications planned within Activity 1. Depending on the timing of the milestone described here, Activity 1 will be shifted towards staining peptidome arrays by patient's sera.

Description of Activity 7 (18 month plan, Participant 8, EBI)

Workpackage number	**7**	**Start date or starting event:**		**M1**			
Participant id	**8**						
Person-months per participant	**72**						

Objectives

- ⇨ Providing comprehensive proteomics data management
- ⇨ Ensuring optimum coverage of the target proteome by the peptide arrays through high quality proteomics datasets
- ⇨ Efficient analysis of binding experiment results through advanced analysis tools
- ⇨ Effective, long-term dissemination of proteomics results.

Description of work

In the early phase of the project we will adapt the existing Proteome Sets to the requirements of the project, e.g. by replacing the currently provided native sequences with the cleaved versions, and by focussing curation on the target organisms.

In the startup phase of the project, the main component of this work package, the PREJUDICE proteomics analysis tool, will be specified in cooperation with the project partners charged with performing the binding experiments to optimally need the analysts' requirements. A specification of the prejudice system will be written, with a focus on the data exchange format for input data and analysis results, and the user interface. The specification will be provided according to established industry standards, e.g. XML for data exchange formats, and UML diagrams and textual use cases for the user interface definitions.

The prototype will be available when the result data sets become available. The prototype PREJUDICE server will allow to upload protein sets of interest, including additional, user-defined properties. The members of the protein set will be visually represented and highlighted if they posess a currently selected property, e.g. a Swiss-Prot keyword, GO term, or user-defined property. Multiple protein sets can be displayed in parallel to allow the comparison of sets. The analysis can be performed according to Swiss-Prot keywords, GO terms, or subcellular location. For each of these dimensions, additional data can be provided by the user. Analysis results can be provided for download, e.g. via XML. The PREJUDICE system will be set up to provide data confidentiality through industry standard technology, e.g. Secure Socket Layer (SSL). The prototype will be demonstrated to project partners, and feedback will be documented.

To achieve the PREJUDICE production version, additional data types will be added to the system. These will include InterPro domains, chromosomal location, and transcription factors extracted from Ensembl. Depending on data availability in public databases, we will include additional data types, e.g. identification of the proteins on the same 2D gel, as provided by Swiss-2D-PAGE.

In cooperation with international partners inside and outside this project, the standardisation of proteomics data representation will be pursued, and appropriate repositories will be created. Efficient tools will be developed to support the peptide array data submission and integration of results into proteomics resources.

Deliverables

D7.1: Month3: Specification for proteome sets and PREJUDICE tool
D7.2: Month 6: Proteome sets for target organisms
D7.3: Month 12: PREJUDICE prototype
D7.4: Month 18: PREJUDICE production version
D7.5: Month 18: Specification of proteomics data submission formats

Milestones and expected result

- ⇨ Workshop to define requirements for proteome sets and PREJUDICE analysis tool.
- ⇨ Workshop to define proteomics data formats and submission procedures

Description of Activity 8 (18 month plan, Participant 10+, SME)

This Activity only starts with year 4.

Description of Activity 9 (18 month plan, Participant 1, DKFZ)

Workpackage number	**9**	**Start date or starting event:**		**M1**			
Participant id	**1**						
Person-months per participant	**19**						

Objectives

- ⇨ Scientific administration management of information flow,
- ⇨ Administrative and financial management.

Description of work

Establishing of scientific and administrative communication links between Participants

Deliverables

D9.1: Month 2: Establishing of communication links between Participants
D9.2: Month 6: Establishing of an internet platform for Participants
D9.3: Month 1: Linking Participant's data to financial administration

Milestones and expected result

Kick-off meeting of Participants, 1st meeting of General Assembly

B.9 Ethical, safety and other EC-policy related issues

B.9.1 Ethical aspects

Our proposed research does not involve any of the items written under section B.
This work will be carried out in full accord with all national and European Union requirements. Experimental protocols involving human material (blood sera) are solely done by Participant 9 (Ben Gurion University) and will be conducted only after the research plan has passed the Helsinki committee at the Soroka Academic Medical Centre, BGU and each person enrolled in the research signed before head an informed consent. Nevertheless, special care will be taken to conduct the major research when ever possible, using sera that were taken for necessary medical reasons.
All the other work does not need authorisation of any national or European Union body with respect to its ethical implications. Other than the general responsibility for ensuring that data in the public domain (Activity 7, Database) are accurate and inoffensive, and that data are correctly attributed to their authors and that we have proper permission to use data from third parties, there are no particular ethical issues that need to be addressed.

a) Specify if your project involves:

Does your proposed research involve:	**YES**	**NO**
• Human beings	YES	
Persons not able to give consent		NO
Children	YES	
Adult healthy volunteers	YES	
• Human biological samples	YES	
Human embryonic stem cells in culture		NO
Human foetal tissue/human foetuses		NO
• Personal data or genetic information	YES	
• Animals (any species)		NO
Transgenic animals		NO
Non- human primates Dogs, pigs, cats,		NO
• Release into the environment of genetically modified micro-organisms or plants		NO

b) Confirm that the proposed research does not involve:

Our research activity:

- does not aim at human cloning for reproductive purposes,
- does not intend to modify the genetic heritage of human beings, which could make such changes heritable[1],
- does not intend to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer, and
- does not involve the use of human embryos or embryonic stem cells.

[1] Research relating to cancer treatment of the gonads can be financed.

B.10 Gender issues

B.10.1 Participation of women and gender action plan

Answer to the following questions:

- Are there women directly involved:
  - in the scientific management of the project? YES
  - in the scientific partnership as scientific team leader in the project? YES
- % of women scientists involved in the project[1]:
  - Early researchers (less than 4 years after graduate)? 30%
  - Experienced researchers (minimum 4 years after graduate or having a PhD)? 20%

Women's participation in research will be encouraged both as scientists/technologists and within the evaluation, consultation and implementation processes,
Incorporation of women in research will have an input on the research done and on the collaborative efforts of the 9 groups. Most of our projects are done in fields that are considered usually as "man's job", in Faculties where traditionally most of the students are men. Therefore, we intend to take special care to incorporate women in our projects. Assertion will be strengthened by the following decisions:

- Overall, as many women as possible will be recruited.
- Women with appropriate qualifications will get an advantage.

A special effort will be made in order to meet women's need:

- No consideration will be taken as to the family status of the candidates, on the contrary, women with children will be encourage to participate, and PhD female-student mothers to children will be given addition grant to meet their special needs.
- We commit ourselves to make sure, -even when in the different Institutes it is not the norm, that women will be encouraged to work within the frame of a "flexible hours" and when ever required and possible, work from home.

B.10.2 Gender aspects in research.

Answer to the following questions:

• Does the project involve human subjects?	YES	
• Does the project use human cells / tissues / other specimens?	YES	
• If human subjects are not involved or human materials not used, does the research involve animal subjects or animal tissues / cells / other specimens *(as models of human biology/physiology)* in such a way that it is expected that may have implications for humans?		NO
• Does the project use collection of data related to human subjects, human materials, animal subjects or animal materials	YES	

Are gender/sex differences with respect to the research documented in the literature?		NO

Our research is aimed at exploring the humoral immune responses to various human pathogens. Although no information is available with respect to gender differences in response to infections by the pathogens HCV, HBV and Herpes infections, we intend to consider this issue, especially considering the immune status of pregnant women (there are some hints on an influence of pregnancy when analysing other infectious agents, in particular malaria). Therefore the results obtained from the pathogen-proteome array screen will not only be analysed by criteria like

- severity of infection,

[1] Definitions according to the FP6 mobility & Marie Curie activities.

- viral load,
- host and viral genotype,

but will include

- gender and especially
- pregnancy as well.

Trained statistical analysts will be integrated in the research in order to test the contribution of each of the above mentioned criteria. The gender-contribution to the humoral immune responses, observed for each pathogen, will be examined. The proposed research comprises a very powerful screening methodology. Therefore, we expect to be able to contribute to the community the knowledge if differences by gender in the humoral immune responses toward a specific pathogen should exist. Even subtle differences between man, pregnant female and non-pregnant in immune responses can be evaluated. Consequently, new knowledge that will point to either possibility is expected.

EUROPEAN COMMISSION
Directorate-General RTD

Priority Life sciences, genomics and biotechnology for health

Please write in the box the name, full postal address and fax number to which this acknowledgement of receipt should be sent ⇨	Dr. Frank Breitling Deutsches Krebsforschungszentrum (DKFZ) Im Neuenheimer Feld 280 69120 Heidelberg FAX +49 6221 423460 e-mail: f.breitling@dkfz.de Tel: +49 6221 424744
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The LifeSciHealth Priority is pleased to acknowledge receipt of your proposal:

To be completed by Proposal Coordinator
Proposal title: High complexity peptide arrays: synthesis, read-out, management of data and applications. Proposal acronym: PeLaPri

To be completed by Commission
Date of reception Time of reception Proposal number

You are kindly requested to quote this proposal number and acronym in all future correspondence relating to this proposal. Please ensure that all your partners are also made aware of this proposal number.

Your proposal has not yet been checked for eligibility. If eligible, your proposal will be evaluated and it is expected that the final result of the evaluation will be communicated to you within three months of the deadline for submission of proposals.

On behalf of the Commission we thank you for your proposal and your interest in the LifeSciHealth Priority.