Mapping the detailed composition (epitope exposure) of surface-adsorbed protein layers on biomaterials and nanoparticles – an alternative approach to biocompatibility and nanotoxicity.

Program Acronym: EpitopeMap

Steering Committee Acronym: EMRC
Additional Steering Committee Acronym: PESC

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Keywords: surface-adsorbed protein, adsorbed protein conformation, surface exposed epitopes, Ångström-level resolution, cell-surface interactions.

Abstract:
Interactions between cells and biomaterials determine the level of success of medical implants. A new paradigm for thinking about cell-biomaterial interactions is emerging, where it is the effect that the biomaterial has on the proteins that adsorb to the material upon contact with physiological solution that is important, rather than the actual nature of the surface itself. The important parameter is thus the conformation and structure of the adsorbed protein layer, and in particular, the very outer protein layer, as this is what the cells actually see. In this program we intend to bring together scientists working in the traditionally separate areas of biomaterials and nanoparticles, in order to develop and apply the most cutting-edge characterization techniques to understanding the nature of the surface-adsorbed protein layer on biomaterials and nanoparticles, and the effect of this on biocompatibility and nanoparticle toxicity. Envisaged highlights of the program include the exchange of ideas between the traditionally distinct research areas and the bringing together of a range of physical (characterisation and visualisation) techniques with biological and medical approaches to addressing the common goals, which will result in a great increase in the pace of understanding, a rational basis for risk assessment, and a reduction in the barriers to developing commercial applications of biomaterials and nanoparticles.

Previous and Current ESF applications:

- The principal applicants have also applied to organize an EMBO conference in 2007: Probing interactions between nanoparticles / biomaterials and biological systems – alternative approaches to bio-toxicity. Submitted October 2nd 2005.
- The contact person is a co-applicant on a SONS application: MOLFUN, Functional Single Polymer Molecules: Towards Molecule-Based Nanodevices, Submitted October 3rd 2005, Ref. No. 95.

Status of the relevant research:

The interaction of condensed materials with biological cells has long been a subject of scientific and practical interest. It has typically been assumed that the detailed chemical and physical properties of the surface determine the intra- and to some degree the inter-cellular processes via rather specific interactions. Such interactions, being a detailed property of a specific material and its surface preparation require rather thorough study in order to make a rational connection between the surface and the state (phenotype) of the cell. Given this picture, it is difficult to see how general paradigms, which are most often the remit of physical science, could emerge. However, this conception of surface-cell interactions is incorrect, and we believe that, besides leading to misinterpretations in various research programs, it has contributed to an unnecessary barrier between biological, biomedical and physical sciences.

It is now beginning to be understood that the nature of the biomaterial surface is not the important parameter in determining biocompatibility, but rather the nature of the outermost layer of the surface-adsorbed proteins, which is what the cells actually interact with. That is, the different organization of the proteins in the surface adsorbed layer may lead to a variety of peptide units (here termed “epitopes”) being expressed at the surface. In cases where the proteins have lost most of their tertiary structure, it is highly likely that peptide sequences (epitopes) that are not displayed at the surface by the native protein may in fact be presented at the surface by adsorbed proteins. Such surface expressions could contain novel epitopes that trigger various signalling pathways or even diseases. Thus, future approaches to understanding cell-biomaterial interactions should focus on characterizing the outer layer of the adsorbed proteins, or “epitope mapping”.

As nanoparticles become more and more prevalent, and applications of nanoparticles in medical devices and as drug delivery vehicles become commercial realities, human exposure to nanoparticle will increase drastically. Thus interest and concern regarding the safety of nanoparticles is an issue that is gaining attention, and for which very few answers exist at present. However, we note that the paradigm outlined above, where the role of the biomaterial surface may only be relevant in terms of the proteins it induces to adsorb, might have particular significance in understanding the interactions of nanoparticles with biological cells. Nanoparticles are simply biomaterials of smaller dimensions and higher curvature, thus the scientific issues are the same, so the EpitopeMap program will address both issues simultaneously.

The novelty of this program will be the bringing together of physical scientists (physicists, chemists etc.), with biologists and medical scientists, in order to address at a mechanistic and fundamental level how the nature of the adsorbed protein layer (on biomaterials and nanoparticles) controls the interaction of cells with the biomaterial or nanoparticle. A small workshop held in Dublin in August this year where these ideas were developed, was described by the Irish National Contact Point for Nanotechnology (Dr. Jenny Melia) as the best “Nano-workshop” she had attended all year, and she said that it was a major pity that the workshop was not open to the scientific community at large. Thus, the program proposed here would bring these ideas to a larger audience, which would promote a more rapid rate of understanding of the issues under investigation.

Novel elements and specific objectives of the EpitopeMap Program:

The central idea of the project is that nanoparticles (or indeed also macroscopic surfaces) rarely exist in their bare form in physiological conditions. It is certainly almost unknown for a flat surface to be free of protein in plasma, unless exceptional efforts (such as grafting specific polymers to it) are taken. It is well known that a rather substantial layer of highly disorganized protein is adsorbed to hydrophobic surfaces. Hydrophilic particle surfaces also adsorb proteins but this tends to lead to less disruption of the protein structure. Similar remarks apply also to metals and metal oxide surfaces. There is already

extensive experience in the medical device industry of the different biological responses to implants with these types of surface. Thus, we postulate that the potential “toxicity” of nanoparticles is related to the proteins that they surface-adsorb, and the effect on the conformation of these proteins. Thus it is necessary to really understand nanoparticles in a physiological environment (plasma), in terms of the identity and detailed nature of the protein adsorbed to the nanoparticle and the nature of the (coated) nanoparticle dispersion (usually aggregated in some form) that is presented to the cells. It is these complex fluid properties that determine the biological response, and to monitor these *in situ* requires some of the best that biocolloidal, biophysical and instrumental scientists can offer.

In order to probe the composition and structure of the outermost protein layer, a range of complementary techniques will be used, some of which have not been previously applied to the study of adsorbed protein layers. Adsorbed proteins will be identified using modern proteomics methods with high-resolution mass-spectroscopy as the key technology. Key physical characterization techniques to be used are neutron and x-ray reflectometry, surface force balance measurements, surface plasmon measurements, and ellipsometry, supported by more routine surface characterization. The basic procedure in structural measurements will be adsorption of a protein layer (singly or from ‘model’ and real serum). While the highest structural resolution can be obtained by using a flat surface, the surface plasmon technique, small angle scattering techniques and classical protein techniques will be used for direct exploration of adsorption on nanoparticles. Solid-state NMR will be applied to these systems for the first time. An important feature of the surface plasmon experiments will be the development of methods coupling plasmon excitations to near field microscopy. The nature of the adsorbed protein will also be addressed using classical techniques such as limited proteolysis and backbone amide protein exchange in combination with mass spectrometry and NMR spectroscopy, studies of stability towards denaturation, as well as mapping of exposed epitopes using monoclonal antibodies.

In addition, modelling and theory will be required to translate and interpret experimental data in terms of epitope maps expressed by the adsorbed protein. Computational studies will use for example, the Connolly molecular surface to model nanoparticle surfaces. Taking into account multiple protein layers, polymer melt dynamics and other relevant factors will enable modelling and correlation of the experimental findings.

Specific objectives of the EpitopeMap program include:

- To understand how relevant (serum) proteins associate with and subsequently organize on biomaterial and nanoparticle surfaces;
- To express, in simplified form relevant to biology, the oligopeptides exposed on the protein layer (those parts furthest away from the biomaterial or nanoparticle surface, but closest to the cell-surface to which the particle is attached). Currently we see the surface as a collection of surface-expressed epitopes (oligopeptidic fragments that are biologically active) that can be represented as an ‘epitope map’;
- To develop a range of labelled (fluorescent labelling, isotope labelling, radiolabelling etc.) proteins, as well as a range of bioconjugated biomaterials and nanoparticles containing specific peptide sequences, such as the RGD sequence known to be involved in cellular adhesion processes;
- To promote the development of a precise experimental link between the different presentations of proteins that have been adsorbed onto biomaterials or nanoparticles and cell signalling pathways and other intra and inter-cellular processes;
- To develop new methods and protocols for studying protein-biomaterial and protein-nanoparticle interactions, such as solid state NMR, neutron reflectivity and surface plasmon spectroscopy:
- To model the likely epitope exposure based on unfolding of proteins at surfaces, using for example Connelly surfaces;

- To develop a correlation between the display of key ‘epitopes’ on the outer surface of the protein layers and key disease-related pathways such as apoptosis;
- Development of well-characterized and (sufficiently) stable dispersions of nanoparticles in appropriate protein mixtures (e.g. Serum and ‘model’ serum) as a fundamental basis of subsequent work with cell lines;
- Reproducible full characterisation of the composition of the adsorbed protein layer on the nanoparticles upon exposure to appropriate (serum, model serum and cellular extracts) protein mixtures;
- Preparation of a series of deuterium labelled samples, e.g. albumin, fibronectin, S100G using recombinant methods, for determination of their conformation upon adsorption to the various types of nanoparticles and surfaces. Preparation of ^{15}N-labelled samples for domain swapping studies;
- Characterization (to the limits of modern surface science) of the precise organization (density, orientation, correlations) of amino-acid units at the surface of the adsorbed protein layer using partially deuterated proteins and surface reflectometry, and comparison to simpler methods such as ellipsometry;
- Characterization of the structural organization of adsorbed protein using limited proteolysis and site-specific measurements of amide proton exchange rates;
- Study the structural stability of the adsorbed protein by calorimetry and spectroscopy. In a partially unfolded protein, a number of epitopes may be accessible to cells;
- Simplification and quantification of the description of the outer surface of the adsorbed protein layer; description of the outer presentation of the protein layer in terms of the biologically effective units (‘epitopes’), and a novel means to assess the composition via ‘epitope maps’;
- Determination of whether proteins adsorbed to nanoparticles are 3D domain swapped and whether the presence of nanoparticles of different types promotes other canonical kinds of misfolding events;
- Development of simplified methods to make surface epitope maps using antibody and other binding assays, and calibration of these against the state of the art scattering and reflectometry methods;
- Protein adsorption studies using tube flow, serum replacement (equivalent to CSTR), and packed column experiments in conjunction with radiolabelled proteins. Determination of the kinetics, equilibria and reversibility of adsorption and changes in the structure and biologic function of adsorbed proteins;
- Direct examination of the forces acting between nanoparticles (coated or uncoated with protein) and surfaces coated with adsorbed proteins using the surface force balance.
- Understanding of how optical excitation (as a function of wavelength appropriate for practical applications, e.g. titanium dioxide valence-conduction band excitations) of nanoparticles affects the nature of the adsorbed protein layer;
- Quantitative exploration of the adsorption of proteins on tuneable nanostructures using surface plasmon related techniques coupled to near field microscopy;
- Formalization of the relationship between nanoparticles with adsorbed protein layers to protein layers on flat surfaces made from identical materials, and audit of the conclusions being drawn from nanoparticle studies alone by use of large radius of curvature particles.

Envisaged highlights of the program include the exchange of ideas between the traditionally distinct research areas of biomaterials and nanoparticles, as well as the bringing together of a range of physical (characterization and visualization) techniques with biological and medical approaches to addressing the common goals, which will result in an exponential increase in the pace of understanding, and a reduction in the barriers to developing commercial applications of biomaterials and nanoparticles.

Facilities and expertise which would be available to the program:

Program Research Groups, Personnel, and key skills

Ireland	*University College Dublin:* Prof. Kenneth Dawson Dr. Iseult Lynch Dr. Dominic Zerulla Prof. Stephen Pennington Prof. Dolores Cahill	Synthesis, characterization, surface modification of range of surfaces /nanoparticles, Physical characterization in dilute and biophysical conditions, Biocolloidal stability, Novel characterization techniques, Interaction of surfaces and nanoparticles with cells – phenotype and genotype response, cytoskeletal structure, State-of-the art proteomics/genomics facilities, Identification of proteins involved in cellular processes, Largest characterized antibody bank in Europe.
Sweden	*Lund University:* Prof. Sara Linse Dr. Tord Berggård *Linköping University:* Prof. Uno Carlsson Prof. Bengt-Harold Jonsson	Expertise in biophysical chemistry of proteins, protein structure, folding, misfolding, ligand binding, protein chemistry, protein-protein interactions, role of non covalent interactions in proteins, Expertise on methods and physical basis of protein interactions and large scale protein isotope labelling. Expertise in protein biochemistry, adsorption and conformation. Protein adsorption and orientation in the light of fluorescent probes: mapping of the interaction between site-directly labelled proteins and silica nanoparticles.
UK	*Oxford University:* Prof. Jacob Klein Dr. Robert Thomas *Nottingham Trent University:* Prof. Carole Perry	Developed the Neutron specular reflection technique to a level where it is now one of the most effective techniques for probing structure and composition at wet interfaces, Molecular Force Probe expertise in micro/nanoparticle-surface measurements, Neutron reflectivity. Biomaterials chemistry with emphasis on understanding the relationships between surface chemistry and topography on wetting and protein adsorption.
Germany	*Ludwig-Maximilians Universität:* Prof. Joachim Rädler Dr. Wolfgang Parak	Liposome nanoparticle transfection expertise, Novel nanoparticle tracking techniques (e.g. FCS), Physico-chemical characteriation cell-nanoparticle interaction, Visualization techniques - confocal, fluorescence.
France	*Université Paris-Sud:* Prof. Dominique Langevin *University Paris 11, CNRS:* Prof. Patrick Couvreur	Soft nanoparticles, nanoparticle characterization, Biocolloid stability, Surface adsorption kinetics. Synthesis & conception of new colloidal carriers, cell culture, synthesis and characterization of nanospheres, labelling of oligonucleotides.

Switzerland	*Swiss Federal Institute of Technology:* Prof. Beat Mayer Prof. Viola Vogel	Development of new methods of solid-state NMR, Application of solid-state NMR to materials and biological systems, Biologically oriented materials, Bionanotechnology - engineering principles of biological nanosystems for the development of new technologies.
Italy	*Universiti degli Studi di Siena:* Dr. Annalisa Santucci *University Eastern Piedmont:* Prof. Mario Cannas	Expertise in proteomic techniques, 2D gel- electrophoresis, and biological systems, Two-step elution of human serum proteins from different glass-modified bioactive surfaces. Expertise in the molecular interactions between host tissues and biomaterials: protein adsorption, Hemocompatibility, inflammatory response to implants.
Bulgaria	*Institute of Biophysics*: Prof. George Altankov	Cell-biomaterials interaction in response to the materials surface properties - characterisation of tissue compatibility of materials, Organization of provisional extracellular matrix by living cells on biomaterials interface.
Netherlands	*University of Leiden:* Prof. J. Fraaije	Theoretical investigation of the thermodynamics of ion binding in solution, protein adsorption and ion co-adsorption. The emphasis is on charge regulation effects.

Program Resources and Key Equipment

Ireland	University College Dublin	Synthesis facilities, mass spectroscopy, NMR, UV and fluorescence spectrometers, rheometers, Surface plasmon Raman Spectroscopy, dynamic light scattering, 3-D DLS, State of the Art Proteomics Centre: MALDI-Tof / Tof mass spectrometer with autoloader for high throughput MALDI-MS/MS and off-line liquid chromatography (LC) MS/MS workflows. Linear ion trap electrospray mass sprectrometer and a linear ion trap electrospray mass spectrometer coupled to a Fourier Transform Ion Cyclotron Resonance detector, ProteinChip Facility, Microarray & Robotics, Nucleic Acid Analysis, Confocal Microscopy and Digital Imaging.
Sweden	Lund University	CD spectrometer, UV/VIS spectrophotometer; Fluorescence spectrometer, stopped-flow kinetics fluorescence instrument, surface plasmon resonance instrument, isothermal titration calorimeter, differential scanning calorimeter, 600, 500 and 360 MHz NMR spectrometers. Higher-field NMR spectrometers (800 and 900 MHz) available at the Swedish NMR Center at Göteborg University. Equipment for molecular biology, protein expression and purification and limited proteolysis: sterile bench, PCR, autoclave, incubators, centrifuge, sonication, manual &

		automated gel electrophoresis, chromatography.
	Linköping University	High-resolution 2D 1H-15N NMR Fluorescence Spectroscopy
UK	Oxford University	State-of-the art Surface-force balances (SFB) and associated laboratories, AFM, Dedicated Molecular Force Probe, Surface-force balance (SFB), Unlimited access to in-house Surface Analysis Facility including X-ray reflectometry system, spectroscopic ellipsometry, BET surface adsorption system, dedicated scanning probe microscopies, drop shape and contact angle goniometers, Neutron reflectivity, Neutron and X-ray scattering, Unlimited access to Neutron reflection facilities at the spallation source at ISIS (outside Oxford), the high flux beam reactor at Grenoble, and neutron sources in U.S.
	Nottingham Trent University	Synthesis of SAMs (self-assembled monolayers), Quartz crystal microbalance, Grazing angle Infrared Spectroscopy, Contact angle measurements.
Germany	Ludwig-Maximilians Universität	SAXS (Small Angle X-ray Scattering), 4-Circle X-ray Diffractometer, X-ray Reflectometer, Confocal Fluorescence Microscope, Fluorescence Correlation Spectroscopy, Fluorescence microscope with sensitive CCD camera, Bio-chemical lab und cell culture lab; Mobile UHV-chamber for *in-situ* growth studies using X-ray diffraction.
France	University Paris-Sud	Contact angle, static and dynamic (adsorption kinetics), Ellipsometry, X-ray reflectivity (thickness of surface layer), Brewster angle microscopy (surface imaging), Light (elastic and inelastic) & small angle X-ray scattering.
	University Paris 11	Visualisation techniques for study of *in-vitro* protein-rejecting properties of nanoparticles, Fluorescence and confocal microscopies.
Switzerland	Swiss Federal Institute of Technology	600 MHz solid-state NMR Bruker spectrometer, 500 MHz solid-state NMR Varian Infinity+ spectrometer, 400 MHz solid-state NMR Bruker spectrometer, 300 MHz solid-state NMR Varian Infinity+ spectrometer, 300 MHz solid-state NMR Chemagnetics Infinity NMR spectrometer, 220 MHz solid-state NMR Varian Infinity+ spectrometer, Epifluorescence Microscope, Fluorescence resonance energy transfer, Protein fluorescent-labelling.
Italy	Universiti degli Studi di Siena	Secondary Ion Mass Spectrometry with Time of Flight Analyzer (ToF-SIMS), Proteomics, 1D, 2D gel electrophoresis, Optical Microscopy, Gas Chromatography with Flame Ionization Detector

	University Eastern Piedmont	(GC-FID). Electron Microscopy, Slide preparation techniques, Cell culture facilities, Hemocompatibility, histology, immunohistology.
Bulgaria	Institute of Biophysics	Basic cell biology techniques, methods for studying cell-biomaterials interaction *in vitro*, processing with blood, histological and immunofluorescence techniques, cell culture, studies on surface distribution of integrin receptors, experience in receptor-mediated endocytosis. Purification of cell attachment proteins like fibronectin, vitronectin and collagens, affinity chromatography and other chromatographic techniques, ELISA, RIA and related bioassays, gel electrophoresis, cell adhesion and aggregation, experience with human fibroblasts, lymphocytes and different cell lines, synthesis of gelatin microspheres by emulsion-polymerization technique, microcarrier cell cultures.
Netherlands	University of Leiden	Theory and simulation techniques, Biopolymer Physics.

Expected benefit from European Collaboration in this area:

Nano-toxciology is an arena that is not yet established as a mature discipline, with its own paradigms, methodologies, protocols, and instruments. It is of such importance and complexity that it will have to be built rapidly using all of the experience and resources of contiguous disciplines. In the EpitopeMap proposal, we build on the knowledge gained from extensive studies of biomaterials interactions with living material, and extend this to the case of nanoparticles. We believe that the risk of nanoparticles in contact with living tissue is the same as that inherent in the contact of biomaterials in general with living tissue – namely the effect of the materials on the proteins that adsorb onto the surfaces. The rapid development of these areas will represent a strong competitive advantage for Europe, especially in terms of understanding and controlling the risks associated with nanoparticles, and the development of a European Research Platform on Nanoparticle-Cell Interactions.

EpitopeMap comprises a cross-disciplinary alliance of leading research groups from Europe (and North America via the programs' International dimension) working on an ambitious long-term program that aims to develop a detailed understanding of the nature of the exposed surface of the adsorbed protein layers on biomaterials and nanoparticles, i.e. the surfaces that cells actually come into contact with. The network encompasses competences, skills and knowledge from physical and biophysical chemistry, through sophisticated experimental techniques, to biological function, drawing expertise from both the biomaterials and nanoparticles communities. The cross-disciplinary nature of the network is central for the programs' success, and offers excellent opportunities for training of young scientists in conducting research at the interface between the physical sciences and the life sciences.

Due to the fact that the area of nanoparticle-cell interactions is a newly emerging discipline, the focus of the EpitopeMap program on researcher mobility and training via the longer term research visits (up to 6 months) will result in a concerted effort to train PhD students and post-docs in a cross-disciplinary manner by exposing them to areas not directly related to their PhD field (i.e sending physical chemistry students to biology labs and vice versa, and having biomaterials people work with nanoparticles and vice versa), thus widening their knowledge-base and providing access to new ideas, methodologies and applications. EpitopeMap, with its fundamental and challenging approach is expected

to attract top students in Europe, who will work in an environment that supports experimentation and stimulates the generation of new ideas. The availability of staff trained in these areas will represent a key asset for Europe in terms of competitiveness, the development of a knowledge-based economy, and in developing a dynamic attractive European Research Platform to help keep European researchers in Europe.

Responsible development of nanotechnology, and public dialogue
The recent communication “Towards a European strategy for nanotechnology” highlighted the possibility that without a serious communication effort, nanotechnology innovations could face an unjust negative public reception. The public trust and acceptance of nanotechnology will be crucial for its long-term development and to allow the EU and its’ citizens to profit from the potential benefits, and this trust can be fostered by scientists. We believe that a Program such as EpitopeMap, which focuses on directing excellence in science and technical execution to the question of nanoparticle biocompatibility and safety, will be perceived as a strong and responsible commitment to the public need (trust). A large emphasis will be placed on the communication of the results from this program, both in top-quality scientific journals, but also in a publicly accessible way, through attendance at science-week events and public outreach programs. All students and postdocs will be encouraged to present their work to non-scientific audiences as well as scientific ones.

Development of standards
The EpitopeMap program intends to generate a large body of high quality scientific information, subject to the strictest protocols, on the fundamental mechanisms of interaction between proteins and biomaterials / nanoparticles. Both the data (and the protocols involved in its collection) will contribute to the development of standards that can be used by regulators. Besides this the EpitopeMap program offers the possibility to evolve design criteria leading to safe engineered nanoparticles and biomaterials. We believe that the development of standards at every level of the program will be a key priority.

European context:

While the fields of Biomaterials and Nanoparticles are both dynamic fields, there are no concerted pan-European efforts either in COST or in the ESF Programs or Themes, working in the areas of biocompatibility, nanotoxicity or to determine the role of the adsorbed protein conformation in these processes. The applicants currently have an application in for the final NMP call of FP6, called NanoInteract, which intends to develop discipline-independent “platforms” (composed from the state-of-the-art methodologies in different fields, physics, chemistry, biophysics and others) to characterize the state and location of nanoparticles at every stage of their interaction with cells. Thereby we will understand how nanoparticles interact with, enter, and are subsequently transported around cells. We will determine the effects of nanoparticles’ final location on the most complete range of cellular processes to be studied in this context, and analyse them for potential mechanisms of disease, both known and new.

One extremely important aspect of the NanoInteract proposal is the detailed characterization of the state of nanoparticles *in situ* (including in the physiological environment). This is important because we know that particles in physiological environments (plasma) are coated with proteins, and may also associate and aggregate in various manners, not previously controlled or characterized. This aspect will also be emphasized in the EpitopeMap Program, thus bringing the importance of standards and protocols to the wider European Community, and ensuring that research efforts in this area are **reproducible and meaningful**. Additionally, this program will build on the knowledge

of the more established Biomaterials field, and use this knowledge to accelerate the development of the European Platform on Nanoparticle Interactions with Living Tissue.

Proposed activities, key targets and milestones of the EpitopeMap Program:

- Each year a number of both short visits (up to 15 days) and exchange (research) visits (up to 6 months) will be funded to promote the research activities of the program and to advance the exchange of knowledge, and access to different techniques, between the collaborating groups.
- Workshops for program collaborators on specific aspects of the program will be held in years 1 and 4. Possible topics for discussion at workshops include “What can biomaterials research teach nanoscientists regarding biocompatibility?”, “State-of-the-art techniques and their application to determination of the conformational state of surface-adsorbed proteins” or “Development of novel approaches to understanding the conformation of surface-adsorbed proteins”.
- An International School will be organized in Year 3, where the topic will be “Proteins as the drivers of biocompatibility - methods for determining the explicit nature of the adsorbed protein layer”. Key scientists from each of the collaborating groups will give lectures on their area of expertise, with particular emphasis on the novel techniques used to determine protein conformation upon adsorption to materials, including instrumental techniques such as neutron reflectivity, solid state NMR and plasmon spectroscopy, and biological techniques such as limited proteolysis, proteomics, mass spectroscopy, and radiolabelling. This school will be the first in the nanotechnology area on the topic of interfacing physical chemistry approaches with classical protein science and newer emerging technologies such as proteomics. The course will provide the necessary physical chemistry background for biologists, and the necessary protein and basic biology background for physical chemists, resulting in a truly inter-disciplinary school that will prepare young scientists for the challenges of research at the interface between chemistry, physics, biology and technology which is becoming increasing necessary in today’s research environment.
- A Program Conference will be organized in Year 5, which will bring together all of the participants, as well as other International researchers to whom the topic is of interest. The proceedings of the workshop will be published as a book on this topic, as to date there are no books dealing with this topic. The principal applicant has contacts in Springer Publishers, where he produced a CD-ROM based teaching tool called “The Dynamic Cell”.

Program Duration: 60 months

Budget estimate, by type of activities and per year of the program:

Budget Estimate (all in €)

	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Steering Committee meetings	6,000	6,500	7,000	7,500	8,000
Grants for short visits	8,875	8,875	3,550	7,100	5,325
Grants for research visits	60,600	80,800	60,600	60,600	60,600
School or workshop	25,000	-	45,000	30,000	-
Conference	-	-	-	-	45,000
Publicity & websites	4,000	3,000	3,000	3,000	3,000
Data / Report Preparation	4,000	3,000	2,000	1,000	1,000
Coordinator expenses	1,000	1,000	1,000	1,000	1,000
Administration costs	1,000	1,000	2,000	1,000	2,000
ESF Administration costs (7.5%)	8,286	7,813	8,554	8,340	9,445
Total	118,761	111,988	122,604	119,540	135,370

Total 5 years: □ 608,238

Justification of the Budget:

Grants for short visits and research visits: In each case we have assigned a large amount of the budget to these visits as they constitute an important aspect of the program's focus – the short term visits to promote exchange of ideas between the senior scientists, and the research visits to promote cross-disciplinary education and training of students and postdocs, and to enable sharing of facilities and techniques between the program collaborators. In each case the maximum costs per visit have been used as an estimate (€ 1775 for short visits, and €10,100 for the research visits). However, it is clear that the actual number of visits financed per year depends on the lengths of the visits.

School or workshop: Workshops will be held in years 1 and 3, and will be held in conjunction with Steering Committee meetings or relevant European Meeting/Conference. A school will be held in Year 3, offering a unique interdisciplinary training opportunity for the Program Collaborator students and others in this new and developing research area.

Data / Report Preparation / Administration costs: Instead of hiring a program manager, it has been decided to use as much as possible of the Program money for research and training purposes, and to set aside a small amount to cover the costs involved in preparing the mid-term and final reports, as well as costs involved in preparing materials for publicity and publication, and organization of the school, workshops and conference.

Coordinator expenses: These are kept to a minimum, and are intended to cover travel related to the program undertaken by the co-ordinator.

CURRICULUM VITAE

Kenneth A. Dawson

Resume

Name: Dawson, Kenneth A.
Grade: Professor and Chair of Physical Chemistry
Year of appointment at University College Dublin: 1992

Academic degrees (Institution and year of completion): BSc, (QUB) (1980); MSc Mathematics (QUB) (1981); DPhil (University of Oxford) (1984)

Positions held prior to University College Dublin: Teaching Assistant, Mathematics, Queen's University Belfast 1980-1981 Tutor in Mathematical Methods, University College Oxford, 1983-1985 Junior Dean, University College Oxford, 1982-1985 Weir Junior Research Fellow , University College Oxford, 1983-1984 Research Visitor, Institute Haute Etudes Scientific, Paris, 1983 Visiting Lecturer, Theoretical Chemistry, University of Ulm, West Germany 1984 Lindemann Fellow, 1986-1987 Associate Fellow in Atomic & Solid State Physics, 1986-1987 Materials Science Postdoctoral Fellow Cornell University, 1985-1988 Strategic User at the Cornell National Supercomputer Centre, 1987-1990 Assistant Professor of Chemistry, University of California, Berkeley 1989-1992 Adjunct Professor of Biophysics, University of California, Berkeley, 1989-present Chair of Physical Chemistry, University College Dublin 1992-present Weir Fellow (Oxford).

Research Interests:

- Fundamental (theoretical, simulation and experimental) principles of Soft Matter and Colloidal Science, particularly in relation to dynamically arrested systems.
- Novel theoretical and simulation methods of statistical mechanics, and relations to complexity 'Complexity'.
- Applications of soft matter and dense colloidal system to biology, and biomaterials.

Present Research Oriented International Activity:

- President of the European Colloid and Interface Society
- Co-ordinator DASM Marie Curie Research Training Network
- Partner CIPSNAC Marie Curie Research Network;
- Co-ordinator of EU proposal NanoInteract, submitted to FP6 September 2005.
- Executive Board of Centre of Excellence in La Sapienza, Complex Matter
- Editorial Board, Current Opinion in Colloid and Interface Science and Physica

RESEARCH OUTPUTS: 215 PAPERS. EXAMPLES INCLUDE:

- Allen, LT, Fox EJ, Blute I, Kelly ZD, Rochev Y, Keenan AK, Dawson KA, Gallagher WM, Interactions of soft condensed materials with living cells: phenotype/transcriptome correlations for the hydrophobic effect, *Proc Natl Acad Sci U.S.A*., 2003, 100, 6331-6336
- Dawson, K. A., The glass paradigm for colloidal glasses, gels and other arrested states driven by attractive interactions, *Curr. Opinion Colloid Interface Sci.*, 2002, 7, 218
- Lynch, I., Blute, I.A., Zhmud, B., MacArtain, P., Allen, L.T., Tosetto, M., Byrne, H.J., Farrell, G.F., Gallagher, W.M., Dawson, K.A. Correlation of the adhesive properties of cells to N-Isopropylacrylamide / N-tert-Butylacrylamide Copolymer Surfaces with changes in the surface structure using Contact Angle Measurements, Molecular Simulations and Raman Spectroscopy. *Chem. Mater*. 2005, 17, 3889-3998.
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- Allen, L. T., Tosetto, M., Miller, I., O'Connor, D., Penney, S.C., Lynch, I., Keenan, A. K., Pennington, S.R., Dawson, K.A., Gallagher, W.M. Surface induced changes in protein adsorption and implications for cell-surface response. Submitted to *Biomaterials* 2005.

CURRICULUM VITAE
Sara Linse.

Resume
Name: Linse, Sara
Born: April 30, 1962.
Grade: Professor and Vice-Chair of Biophysical Chemistry, Lund University, Sweden.

Sara Linse is a member of the Royal Swedish Academy of Sciences, and of the international committee for calcium-binding proteins in health and disease. She is the winner of the Arrhenius Medal of the Swedish Chemical Society, the Hugo Theorell Award by Swedish Biophysics Society, and is distinguished as an Excellent Researcher of the Swedish Research Council. She studies protein structure and folding, misfolding, ligand binding, protein chemistry, protein-protein interactions, the role of non-covalent interactions in proteins. She has developed experimental methods and software that are used world-wide. She is considered a world-leading expert on protein-calcium interaction and co-operativity by scientists in the field. Among her achievements are 1. The unravelling of the molecular details of HAMLET – a protein-fatty acid complex that selectively kills tumor cells. This complex was first isolated from human milk and the molecular foundation was proven using conversion experiments with pure components (oleic acid and milk-derived or recombinant a-lactalbumin). 2. Development of a valuable method for determining the domain organisation of proteins and its use in the discovery of the hexa EF-hand domain. 3. The discovery of redox regulation of an anti-apoptotic neuronal protein. 4. The discovery of a link between the calcium and phosphoinositide signalling. 5. The first discovery of a calcium-binding site in an EGF-like module. This was discovered for the anticoagulant protein C and has been followed by a large number of examples by her own and other laboratories. 6. The notion of the link between the phenomena of protein reconstitution and 3D domain swapping. This was based on the finding of a domain swapped EF-hand protein that could also be reconstituted from exactly the same units as were trading places in the swapping event. Inspection of other systems revealed that this is a common theme. 7. The discovery that protein surface charges are important for binding of ionic ligands even if not directly involved in ligand coordination at specific binding sites. This is due to the long-ranged nature of electrostatic interactions that may be utilized by the proteins for efficient channelling of ions to their binding sites. The result was unexpected by the research community and in contrast to the commonly accepted picture at the time that only directly coordinating groups were of importance for metal-ion binding to proteins. 8. The discovery of novel aspects of electrostatic interactions in proteins such as saturation effects and a charge regulation mechanism. 9. The unravelling of the calcium-binding modes of calmodulin. This had been debated for decades when she showed very clearly that each of the two globular domains bind calcium with positive cooperativity within the domain but independently of the other domain. 10. The first high-resolution structure of a hexa EF-hand protein. Prof Linse has also been actively involved in teaching. She has lectured quantum chemistry, reaction kinetics, optical and NMR spectroscopy, and biophysical chemistry. She has organised and developed courses at undergraduate and graduate level.

RESEARCH OUTPUTS: 93 PAPERS. EXAMPLES INCLUDE:
- Conversion of alpha-lactalbumin to a protein inducing apoptosis. M. Svensson, A.K. Mossberg, A. Håkansson, S. Linse. & C. Svanborg. *Proc. Natl. Acad. Sci. USA 97*, 4221-4226 (2000).
- Calbindin D28k exhibits properties characteristic of a Ca^{2+} sensor. T. Berggård, S. Miron, P. Önnerfjord, E. Thulin, K.S. Åkerfeldt, J.J. Enghild, M. Akke & S Linse. *J Biol Chem. 277*: 16662-16672 (2002).

• An extended hydrophobic core induces EF-hand swapping. M. Håkansson, J. Fast, A. Svensson & S. Linse. *Protein Science 10*, 927-933 (2001).
• Structural basis for the negative allostery between calcium and magnesium binding to calbindin D_{9k}. M. Andersson, A. Malmendahl, S. Linse, I. Ivarsson, S. Forsén & L. A. Svensson. *Protein Science 6,* 1139-1147 (1997).
• The Role of Protein Surface Charges in Ion Binding. S. Linse, C. Johansson, P. Brodin, T. Grundström, E. Thulin and S. Forsén. *Nature 335*, 651-652 (1988).

Envisaged Steering Committee members:

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France	Prof. Dominique Langevin, Laboratiore de Physiques des Fluides, Université Paris Sud
Germany	Prof. Joachim Rädler, Center for Nanoscience, Ludwig Maximillians Universität, Munich
Italy	Dr. Annalisa Santucci Dipt. Biologia Molecolare, Universiti degli Studi di Siena
Ireland	Prof. Kenneth Dawson (Chair of the Steering Committee) School of Chemistry and Chemical Biology, University College Dublin
Netherlands	Prof. J. Fraaije Faculty of Mathematics and Natural Sciences, University of Leiden
Sweden	Prof. Sara Linse Department of Biophysical Chemistry, Lund University
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UK	Prof. Robert Thomas, Prof. Jacob Klein Theoretical and Physical Chemistry, Oxford University Prof. Carole Perry, Interdisciplinary Biomedical Research Centre, Nottingham Trent University,

International Dimension:

USA	Dr. David A Schultz Department of Physics, University of California at San Diego, La Jolla, CA Prof. Robert Latour, Clemson University, Rhodes Engineering Research Center, South Carolina Prof. Jannette Carey Department of Chemistry Princeton University, New Jersey

The International Dimension of this program is described in detail below. Each of the international collaborators represents a key area in this project, and while no existing funding arrangements exist, contacts between the groups exist (or are in the process of being established) in order to complement the ESF program. Over the next few months concerted efforts will be made to apply for joint US-EU funding to enable exchange of researchers between these groups and to consolidate the collaborations.

Plasmon resonance: A large part of the work of EpitopMap will be to use plasmon resonance to investigate the conformation of surface-adsorbed proteins, as well as to investigate how optical excitation of nanoparticles affects the nature of the adsorbed protein layer. **Dr. David Schultz** has expertise in the production and use of plasmon resonant particles (PRPs), and introduced colloidal silver PRPs as optical reporters in typical biological assays. PRPs are ultrabright, nanosized optical scatterers, which scatter light elastically and can be prepared with a scattering peak at any color in the visible spectrum. PRPs are readily observed individually with a microscope configured for dark-field microscopy, with white-light illumination of typical power. PRPs can be surface coated with standard ligands, as target-specific labels in an *in situ* hybridization and an immunocytology assay, and can replace or complement established labels, such as those based on radioactivity, fluorescence, chemiluminescence, or enzymaticycolorimetric

detection that are used routinely in biochemistry, cell biology, and medical diagnostic applications.

Molecular Modeling of Protein-Surface Interactions: Molecular modeling provides an extremely valuable tool to investigate these interactions. While there exist very few groups in Europe actively working on simulation and modelling of protein-surface interactions, this is an active field in the US. The addition of a strong modelling group to the EpitopeMap program will offer a unique training opportunity for the European students. **Prof. Robert Latour** uses both quantum mechanical and molecular mechanics/dynamics based modeling approaches to investigate how molecular structure and molecular functionality influence protein-surface binding behavior. Results from these studies provide guidance for the design of biomimetic structures to control and manipulate biological response.

Limited Proteolysis: An important aspect of understanding the structure of surface-adsorbed proteins will be the use of limited proteolysis, where the surface-exposed epitopes (peptide sequences) will be cleaved for identification, and subsequent re-construction of models of surface-adsorbed protein structure. **Prof. Janette Carey** is considered to be a world-expert in the field of proteolysis, and is using this to understand protein structural hierarchies, stabilities, and folding. She has used the technique to determine the domain organisation and functional units in a number of proteins including the DNA-binding Arg and Trp repressors. She demonstrated that proteolytic fragments of TrpR too small to exhibit stable secondary or tertiary structures in isolation could acquire such structures upon reconstitution with each other. The reassembly reaction regenerated a native-like structure in an obligately ordered series of steps which she speculated might reflect the order of steps in the folding pathway of intact TrpR, a hypothesis that is still under experimental evaluation. She will be visiting Prof. Sara Linse (the co-applicant) in Lund from Nov 2006 for one year, as the Tage Erlander Guest Professor, sponsored by the Swedish Research Council.