Project number: **505868-1**

Project acronym: **NANOCUES**

Project full name: **Nanoscale surface cues to steer cellular biosystems**

Instrument type: **STREP**

Priority name: **NMP**

Final activity report

Period covered: from 2004-01-01 to 2006-12-31

Date of preparation: 2007-02-15

Start date of project: 2004-01-01 Duration: 3 years

Project coordinator name: Professor Bengt Kasemo
Project coordinator organisation name: Chalmers University of Technology, Gothenburg, Sweden

Revision: 0

Dissemination level: PU

The overall Science and Technology (S&T) objectives of the project (NANOCUES; Nanoscale surface cues to steer cellular biosystems) are to create a *knowledge base* and a *technical platform* that can be exploited in the design and development of ***nanobiotechnological processes and devices, tailored to steer/monitor cellular function.***

The project team consists of 7 leading groups in Europe, from six countries, representing the necessary interdisciplinary competence and the complementarity required for this purpose. The project includes all levels from materials design and preparation, *nanostructuring, chemical patterning, and biomolecular interactions up to the cellular level.* There are a number of obstacles which currently prevent a knowledge base and a technical platform from being realised. Some relate to the lack of suitable fabrication methods e.g. to produce reproducible nanopatterned non-fouling surfaces with functional arrays of macromolecules, some relate to a lack of suitable characterisation tools e.g. tools to characterize the interface between biomacromolecules and/or living cells and synthetic materials, others relate to a lack of understanding of molecular interactions at surfaces and of mechanisms and kinetics of cellular interactions at biointerfaces. The latter concerns both experimental and theoretical/mathematical modeling aspects.

The scientific and technical work plan addresses critical issues of the different types (fabrication, characterisation and understanding of molecular and cellular interactions. The main deliverables from this project will be

i) new fabrication tools for nano-scale patterned surfaces (topography and (bio)chemistry,
ii) new characterisation tools, and
iii) knowledge generated of the interaction of biological systems components with nano-scale patterns and structures.

This outcome will provide a platform for the design and implementation for directing cellular function via surface nano-scale molecular cues.

The contractors in the project are:
Chalmers University of Technology (Göteborg Sweden) (co-ordinating partner)
INano, Aarhus University (Aarhus Denmark)
University of Heidelberg (Heidelberg Germany)
University of Glasgow (Glasgow United Kingdom)
University of Bordeaux (Bordeaux France)
ETHZ (Zurich Switzerland)
Karolinska Institute (Stockholm Sweden)

Co-ordinator
Chalmers University of Technology
Professor Bengt Kasemo
Department of Applied Physics
Göteborg 41296
Sweden
kasemo@fy.chalmers.se
For more details go to the **Project Website www.nanocues.org.**

The goal of this project was to develop within a three year period significant understanding and technological advance as enabling technologies and knowledge base for the steering of cellular function via nanoscale surface cues. These significant breakthroughs in understanding and novel critical technologies addressed a number of current bottlenecks related to the design, fabrication and characterisation of functional surfaces. Together they provide a platform for the development of a range of cell-based devices.

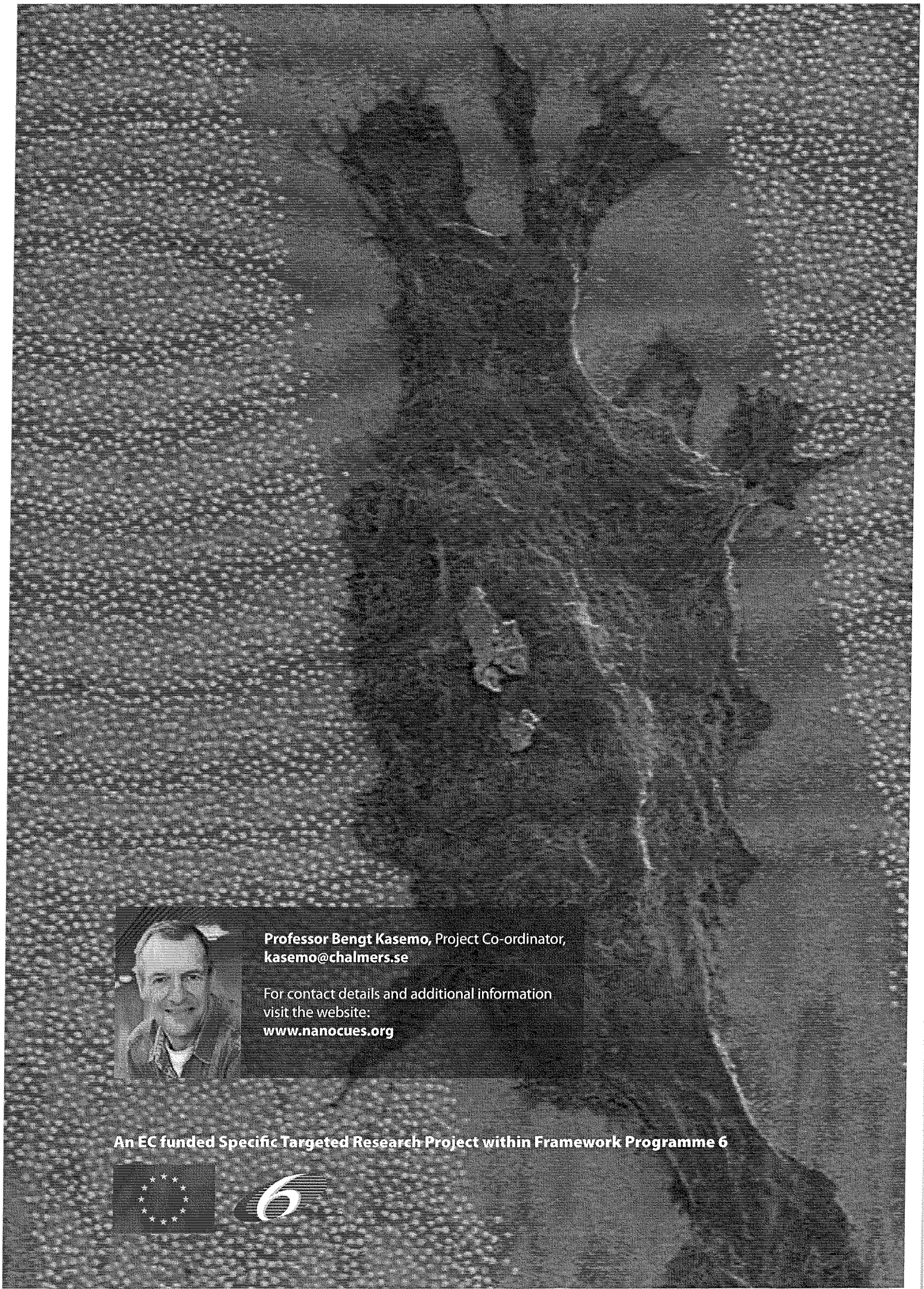
The project is divided into 6 workpackages, the first two were technology based and represent toolboxes of state-of-the-art and novel enabling technologies in nanoscale *fabrication* (WP1) and nanoscale *characterisation* of biointerfaces (WP2). The next two workpackages made use of the new enabling technologies to address critical issues of molecular/macromolecular surface interaction (WP3) and cellular interaction with surfaces (WP4). The fifth took care of management issues and the last, addressed issues of knowledge dissemination and exploitation. An important part of WP6 was be the identification and dialogue of key enterprises (of all sizes).

The methodology used in this project was to formulate specific questions (in WP3 and 4) and design appropriate model materials (WP1) with specific nanoscale architectures which addressed critical issues in the interaction of macrocomolecular and cellular biological systems at interfaces. Advanced nanobiotechnology was used (state of the art combined with new developments in key areas, WP1) to fabricate the substrates in sufficient quantities (several advanced nanofabrication methods are still limited in this respect) for simultaneous studies in a number of different biological systems. Additional developments in the area of biointerfacial characterisation (e.g., spectroscopy, imaging, kinetics) (WP2) provided new opportunities and techniques to go beyond the state of the art in molecular/macromolecular and cellular analysis. WP3 focussed on the interaction of molecular and macromolecular systems. Knowledge was developed that was useful both in interpreting cellular experiments in WP4 but also to develop the new fabrication tools in WP1. Example subprojects included the study of the influence of nanostructures on self-assembling systems and the study of protein crystallisation as a route to antibody arrays. WP4 was the main focus for the whole project. The goals were to study a number of different cellular functions (e.g. adhesion, communication and differentiation) and evaluate the potential to steer cellular behaviour via surface nanoscale molecular cues (in addition to the extracellular matrix (ECM) culture cues already present). These studies included "normal" cells and animal stem cells. Especially in the latter case a key question was if and how nanopatterned and functionalized surfaces could be used to influence or even steer stem cell differentiation and proliferation, an issue central both to therapeutic and diagnostic use of stem cells.

The science and technology outcome from the project activity was of the form of a range of *methods* for nanofabrication, nanopatterning and nanocharacterisation and in the form of a *interpretative and predictive knowledge base* of interfacial interactions and mechanisms occurring at the (macro)molecular and cellular levels. The fabrication methodologies produced focussed on nanobiotechnology routes which combine self assembly and lithographic patterning to create large areas of nanopatterned surfaces combining inorganic and (macro)molecular components at the nanometre scale. New characterisation tool developed focussed on in situ techniques based on optical, scanning probe or acoustic read-out. Interfacial instrumentation combining existing techniques capable of measuring simultaneous on the same materials surface were produced. The knowledge base extends over a range of important research areas of relevance to the interaction/control of cells at interfaces. At the molecular level design parameters for molecular level artificial biointerfaces

e.g. based on biomolecule/lipid assembly were established. The focus at the cellular level was on stem cell interactions at molecularly patterned-surfaces in particular those relevant to neural and bone tissues. The research in the stem cell area included both experimental methods to make cell specific, "signalling" surfaces, and early attempts to develop mathematical modelling of stem cell evolution kinetics and patterns. Knowledge generated here will also be of direct relevance to the design of new and advanced biomaterial surfaces (e.g. bone anchored implants).

The knowledge and technical base generated is formed on a platform of smart biosurfaces and tissue culture systems for stem cell or any other type of cells. The stem cell technology is one of the single most exciting advances for the future treatment of a vast range of disorders and organ deficiencies, from musculoskeletal disorders to diabetes to genetic disorders. It also has promising potential for in vitro drug screening potentially reducing the need for animal experiments. The doctors of tomorrow may well be using stem cell based therapies to remove harmful future disorders from patients well before symptoms show. In reality however currently there is a desperate lack of knowledge over the mechanisms behind development (differentiation, proliferation, signalling patterns,..) in and between stem cells. There will follow an intense phase of study of stem cell systems leading on to new therapeutic and diagnostic approaches. Given the increasing importance of stem cell technologies as opposed to the traditional biomaterials and drug administration therapies, the technology and knowledge base generated within this project is optimised for cell culturing and tissue culturing of stem cells as well as for smart implant devices designed to direct stem cells to any given behaviour in vivo. It will be instrumental in studying and developing the stem cell platform for tissue engineering, biosensing (including environmental, health and safety testing) and diagnostic (e.g. pharmaceutical) markets. The long term innovation of the generic platform technology is related to the knowledge base and personnel 'know-how' that has been generated. That knowledge base helps overcome existing bottlenecks introducing radically new design potentials. The generic character of the developed methods and knowledge base gives them immediate potential for use in areas not specifically addressed in the project such as drug screening (lipid membrane platforms and stem cell therapies) and optimised substrates for biotechnology applications



Professor Bengt Kasemo, Project Co-ordinator,
kasemo@chalmers.se

For contact details and additional information
visit the website:
www.nanocues.org

An EC funded Specific Targeted Research Project within Framework Programme 6

Partners with special competence from several different areas are collaborating in this EC-funded project.

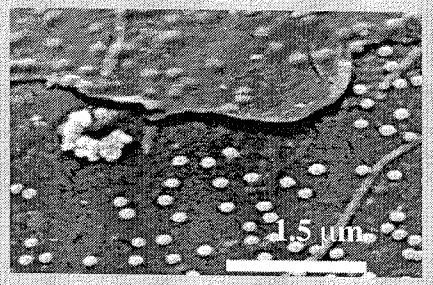
For further information contact any of the following:

Professor Bengt Kasemo Project Co-ordinator:
kasemo@chalmers.se
Dr. Staffan Sjödin Exploitation Manager:
vikan.ss@telia.com
Dr. Duncan Sutherland Scientific Manager:
duncan@inano.dk

For contact details and additional source of information visit the website: www.nanocues.org

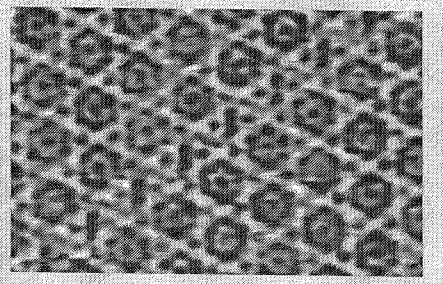
Chalmers (Gothenburg)

Fabrication/preparation: Electron-Beam Lithography, Photo Lithography, Colloidal Lithography, Thin Film Deposition, nano-technology laboratories, coating/plasma/etching techniques, bio-functional supported biomembranes, adsorbed or tethered vesicles with incorporated membrane proteins, 2D cell/tissue scaffolds
Analytical Tools: AFM, ToF-SIMS, XPS, FTIR, fluorescence micro-scopy, QCM-D, SPR, HRTEM, SEM mathematical modelling, stem cell cultures, bio-arrays
Principal Investigator: Bengt Kasemo



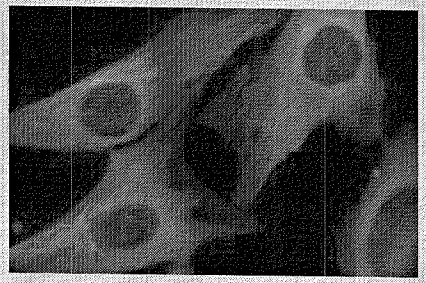
Centre of Cell Engineering (Glasgow)

Fabrication/preparation: Nano- and microlithography, Electron-beam lithography, Nanoimprint Lithography, hot embossing, phase demixing nanostructuring, contactprinting 3D-nano-imprinting, 3D-nano-micro patterned templates for tissue engineering
Analytical Tools: SPM, RAS for surface adsorption, interferometric methods, video time lapse microscopy, microinjection tracktion force microscopies, ultrasensitive polarisation microscopy, fluor-escence immunocytochemistry, array work to detect gene expression, motion analysis of cells, biocompatibility testing
Principal Investigator: Adam Curtis



Institut Européen de Chimie et Biologie (Bordeaux)

Fabrication/preparation: Supported lipid bilayers (SLB), protein-lipid assemblies, protein 2D crystals, protein production, fusion proteins, protein chemistry, nano-bio-technological lab
Analytical Tools: TEM, cryo-SEM, electron tomography, AFM, fluorescence microscopy, Brewster angle microscopy, NMR, X-ray crystallography, QCM-D, SPR, SPR Imaging
Principal Investigator: Alain Brisson

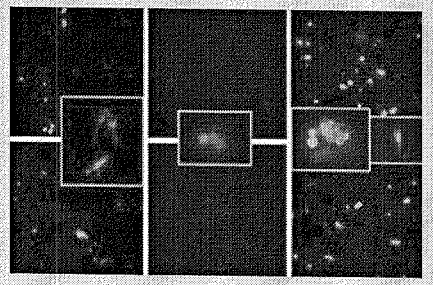


iNANO (Aarhus)

Fabrication/preparation: Nanotechnology laboratories, biotech-nology laboratories, class I and class II cell culture facilities, Thin Film Deposition, access to photolithography and Electron-Beam Lithography, hot embossing, classified animal facility
Analytical Tools: SPM (AFM/STM), SPR, fluorescence microscopy, QCM-D, Cryo-SEM/SEM (X-Ray), Small-Angle X-ray Scattering (SAXS), class II FACS/flow facility, in vitro monitoring of cell function, gene transfer technology, in vitro mineralisation assays, ectopic bone formation assay, reflection contrast microscopy, Q-PCR, pre-osteo-blastic and mesenchymal stem cells of human and nonhuman origin
Principal Investigator: Flemming Besenbacher

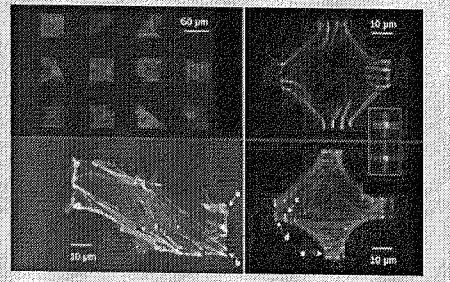
University of Heidelberg

Fabrication/preparation: Sub-micron photolithography, Electron-Beam Lithography, chemical nanolithography, sputter deposition facilities, polymer coating, state-of-the-art synthetic chemistry laboratory, clean room facilities, cell culture lab
Analytical Tools: XPS, electron projection microscopy, SEM, access to soft X-ray beam line (BESSY II), X-ray microscopy, Sum Frequency Generation, in vitro monitoring of cell spreads, gene transfer technology, in vitro mineralisation assays, ectopic bone formation assay, reflection contrast microscopy, Q-PCR, Pre-osteoblastic and mesenchymal stem cells of human and nonhuman origin
Principal Investigator: Michael Grunze



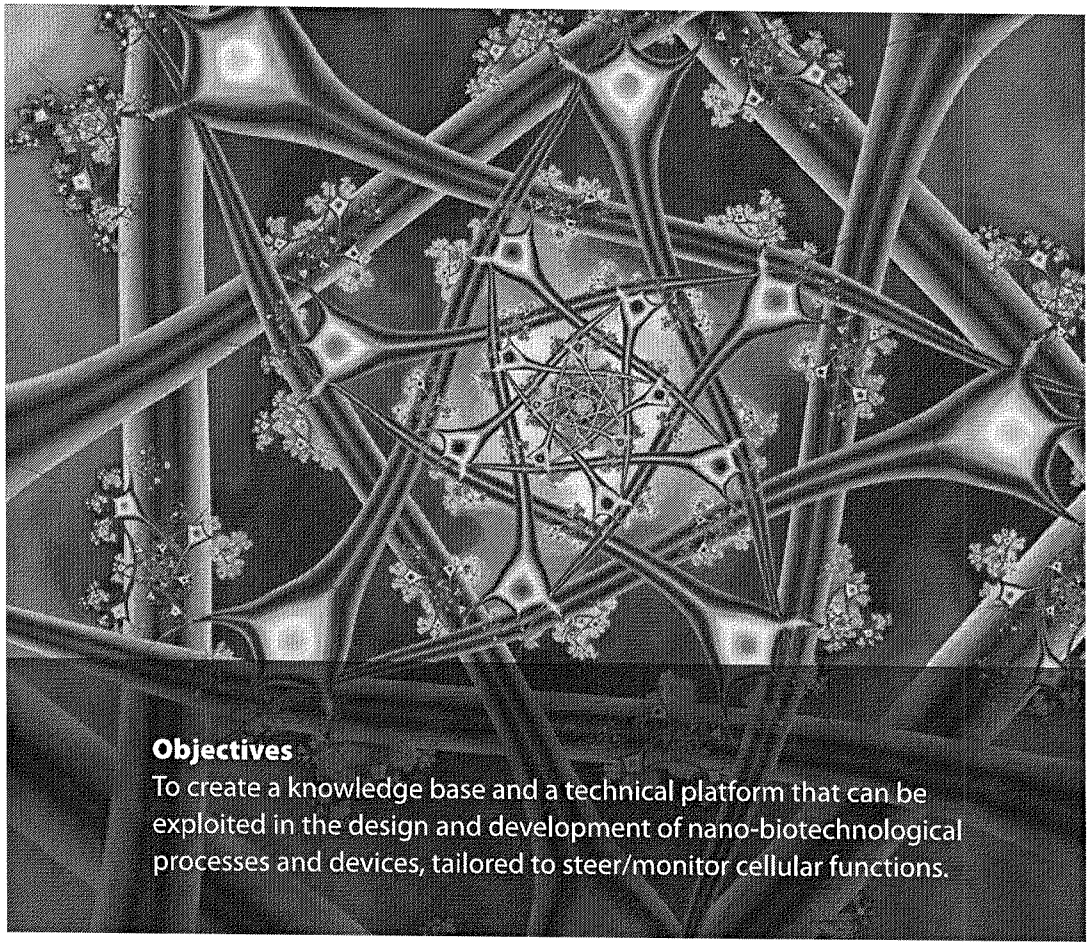
Karolinska Institute (Stockholm)

Fabrication/preparation: Biotechnology laboratories, cell culture labs
Analytical Tools: Protein chemistry techniques, molecular biology techniques, cellular biology techniques, transgenic animal models, cell transplantation animal models of Parkinson's disease, neuro-chemistry techniques, histology techniques, behavioural analysis
Principal Investigator: Ernest Arenas



ETH Zürich

Fabrication/preparation: Micro- and nanofabrication lithography, electrochemistry, molecular self-assembly, chemical patterning
Analytical Tools: XPS, ToF-SIMS, AFM, FTIR, Optical waveguide techniques, SPR, QCM-D, fluorescence microscopy
Principal Investigator: Marcus Textor



Objectives

To create a knowledge base and a technical platform that can be exploited in the design and development of nano-biotechnological processes and devices, tailored to steer/monitor cellular functions.

Courtesy of: Kris Northern.

Examples of future applications

- *new smart implant materials for improved biocompatibility*
- *tissue engineered therapeutics*
- *cell-based biosensing*

Critical issues

- *materials design and preparation*
- *nanostructuring*
- *chemical patterning*
- *characterisation*
- *understanding of biomolecular interactions*

All these issues are of crucial importance in the design and fabrication of surfaces intended to achieve a specific function in a biological environment.

Transfer of knowledge

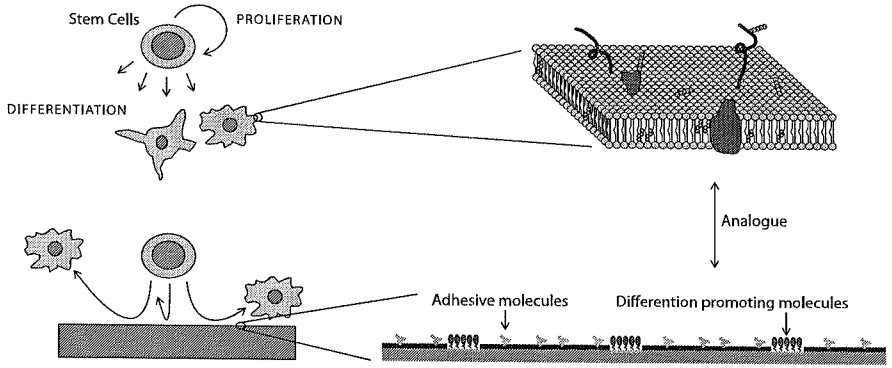
The time span from basic research, as represented by this project, to clinical therapies and commercial products is very long. Therefore, the strategy has been to actively expose the results of the research to industries, in order to expedite the process of knowledge transfer from academic research to economic growth and welfare. The ambition is that within a few years the findings in NANOCUES will form the basis for more large scale and focused projects, with a clear emphasis on concrete applications in different areas.

Project budget

The total project budget is approximately 2.4 Million €. Significant contributions have been added to this budget from the participating groups.

Time schedule

The formal time schedule for the project is three years (2004–2006).



Schematic representation of a simple nanostructured artificial biointerface (courtesy of Duncan Sutherland).

Science and technology

The overall Science and Technology objectives of this project have been to create a knowledge base and a technical platform that can be exploited in the design and development of biotechnological processes and devices, tailored to steer/monitor cellular functions. The project includes all levels from materials design and preparation, nanostructuring, chemical patterning, characterisation and biomolecular interactions up to the cellular level. There are a number of obstacles that currently prevent a knowledge base and a technical platform from being realised. Some relate to a lack of suitable fabrication methods, e.g. to produce nanopatterned non-fouling surfaces with functional arrays of macromolecules, some relate to a lack of suitable characterisation tools, e.g. tools to characterize the interface between living cells and synthetic materials. Others relate to a lack of understanding of molecular interactions at surfaces and of mechanisms of cellular interactions at biointerfaces as well as lack of mathematical models. The scientific and technical work in the project addresses critical issues of different types (fabrication, characterisation and understanding of molecular and cellular interactions). The main deliverables from this project are

i) new fabrication tools for nano patterned surfaces,
ii) new characterisation tools, and
iii) increased knowledge about the interaction of biological systems components with nanoscale patterns and structures.

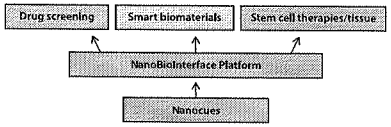
This outcome will provide a platform for the design and implementation of surfaces for directing cellular function via nano-scale molecular cues.

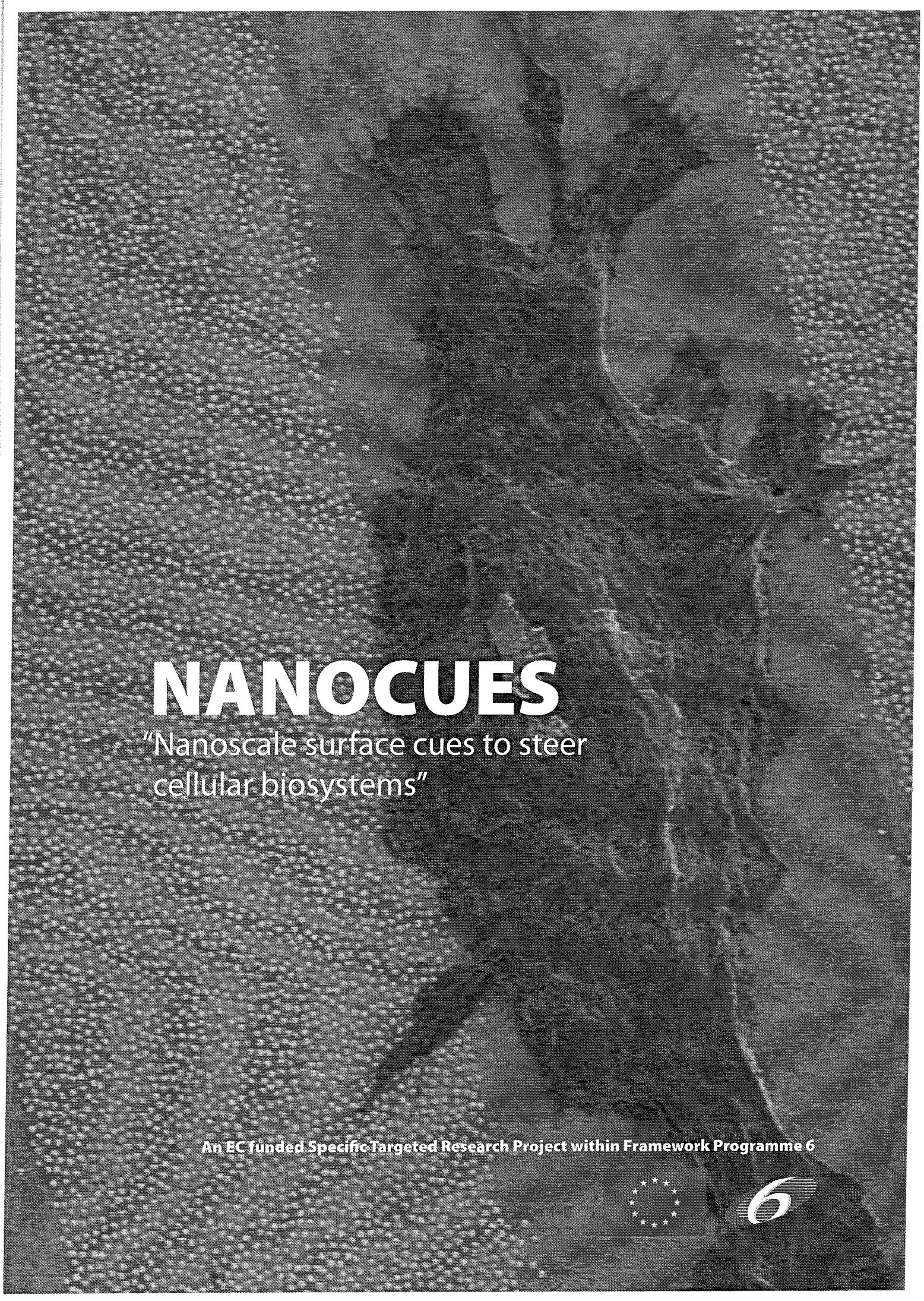
Project format

The project is a Specific Targeted Research Project (STREP) within the NMP theme of the European Commission's 6th Framework Programme (FP6). Such projects are relatively focused and are aimed to address scientific or technical topics that are of generic interest for enhancing the competitiveness of European industry.

Commercialization of results

The NANOCUES project has developed a platform of technology and knowledge that can be used in the design and implementation of artificial biocompatible surfaces. The focus is on controlling cellular systems with nanostructured interfaces based on specific interactions and molecular recognition. In particular the first demonstrator projects will be in the area of stem cell technology. The consortium aims to form the core of more comprehensive projects in the FP7, and the European Technology Platform on NanoMedicine, each addressing a particular market area. In order to ensure that an appropriate IPR (intellectual property rights) strategy is adopted for each particular market the consortium will invite one or more industrial partners to join the project team (one per potential market area) both to direct demonstrator style research projects towards industrially relevant areas, but also to include the industrial partners early in the IPR strategy formulation.





NANOCUES

"Nanoscale surface cues to steer cellular biosystems"

An EC funded Specific Targeted Research Project within Framework Programme 6