Publishable Executive Summary

Background

The SANTS project, which stands for Synthesis and Application of Nanostructured Tethered Silicates, is designed to improve knowledge in nanoscale manufacturing and create new molecular materials for both biosensors and biocatalysed synthetic chemistry via the use of techniques inspired by natural biosilication processes. The project is based on the observation that diatoms, which are small unicellular algae, possess internal silica skeletons that are laid down due to the silica precipitating activity of specialised proteins called silaffins. These proteins have a highly repeating structure in which the repeated sequences are decorated with additional amine and phosphate groups. Whilst silaffins themselves are potent silica precipitants, synthetic peptides corresponding to these repeats will also act as silicating agents and will allow silica nanoparticles to be generated, provided phosphate is also supplied. Moreover, several polyamines, which can be viewed as peptide mimetics also act as silica precipitants. The biotechnological facility of these observations is that the biosilicates can entrap and immobilise a wide range of enzymes with dramatically higher efficiency than conventional immobilisation procedures, and that biosilica entrapment also serves to stabilise and protect the entrapped enzymes.

The primary objective of the NMP work programme was to promote real breakthroughs, based on scientific and technical excellence. Within the SANTS project two main areas were targeted:

- Biosensors - reagentless analytical devices employing intimately entrapped enzymes.
- Biocatalysis - functionalised supports with entrapped enzymes for green chemistry.

To fulfil these objectives a number of University partners have been chosen alongside two small to medium-sized enterprise (SME) companies. The University partners consist of the Universities of Leeds (coordinator), Alcalá, Warwick, Hull, Ghent, Crete and INSA (Toulouse). The two SME companies are C-Tech Innovation Ltd and Sarissa Biomedical Ltd. The project coordinator can be contacted using the following details:

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Final Project Overview:

Initially, a project logo was designed (shown below) and a website was constructed (www.sants-nanosilicates.com). The website has been used by all of the partners to share information and by the coordinators for posting new information and technical documents that can be accessed by all of the project partners. There is also an area of the website that can be accessed by the general public and any other outside entity which may be interested in the technologies that the SANTS project uses and has developed.

The project SANTS logo designed by Dr F. Neville, University of Leeds (Partner 1)

Production and characterisation of nanosilicate particles catalysed by R5, PEI and poly-L-lysine has successfully been achieved. As a result a number of common protocols were available to the project partners in the production of nanoparticles. The project progressed well from the generation of silica particles to the point where a number of enzymes (glucose oxidase, acetylcholinesterase, lipases, mannitol dehydrogenase and choline oxidase) could be entrapped or immobilised using silica matrices, or on electrode surfaces. Many potential sources of R5 peptide were scrutinised and a reliable supplier chosen so that high quality peptides could be supplied to all appropriate partners. R5 peptide with an N-terminal thiol was also sourced and supplied as well as R5 multimers and modified R5 peptides, bearing phosphate groups, extra basic group etc. Structure function studies were performed with the native and modified R5 peptides with respect to both nanoparticle formation and surface silication.

Attempts to ensure future use of R5 peptide would be much cheaper and more viable through the design of a recombinant R5 expression vector were partially successful. Maltose binding protein-R5 fusions precipitated silica but the isolated R5 multimers showed severe insolubility. Biosynthesis of R5 multimers and fusion proteins were achieved by Partner 2 and samples of these recombinant molecules were characterisation by Partner 1...

A microfluidic reactor for the production of R5 and PEI catalysed silica nanoparticles was developed that allowed the rapid production of nanoparticles that had been functionalised with enzyme. Silica monoliths were also been produced that contain enzyme functionalised silica nanoparticles. These monoliths were used to produce bi-enzyme functionalised microfluidic systems. R5, PEI and poly-L-lysine were investigated in the preparation of ultra-microbiosensors via the use of cross-linking agents. These devices were optimised and significant improvements made in their production consistency and stability. In particular Partner 2, 7 and 9 collaborated to understand the physicochemical basis of periodic structures on the ultra-microbiosensors, using FTIR and suppress these structures. R5 peptide was also used to successfully silicate the surface of gold electrodes where the R5 peptide was attached directly with the electrode via an N-terminal thiol group. Partner 1 showed that that this method could also be used to functionalise electrode surfaces with enzyme or to attach enzyme loaded thiol capped nanoparticles. Partner 1 also showed polymeric nanoparticles could be surface silicated to produce silica shell particles of narrowly defined sizes; enzymes could be entrapped within the silica shells and also nano-magnet cores (Ag coated Fe nanomagnets) to confer magnetic susceptibility.

A significant portion of work by Partner 2 centred upon the production and characterisation of expression vectors that will allow the preparation of Cal B and BCL lipases as well as mannitol dehydrogenase; this was successfully accomplished. These enzymes have significant industrial importance and the ability of the SANTS project partners to produce their own enzymes for entrapment studies will be greatly advantageous. Cal B was successfully produced in a yeast expression system and its activity was

compared to that of commercially available enzymes. Further work by Partner 2 involved the production of recombinant R5 and the production of expression vectors that successfully produced lipase-R5 fusion proteins which showed good activity and successfully catalysed biosilicate precipitation around the lipase-R5 fusion. The silica incorporated lipase-R5 was able to catalyse racemic resolution in a test esterification reaction with chiral substrates.

Finally, FTIR-Attenuated Total Reflectance and Raman spectroscopy were used to investigate the icclusion of poly-L-lysine and enzyme within silica nanoparticles and this was successfully shown. This work was also extended to include particles that were generated using R5 peptide and had enzymes entrapped. Carbon nanofibres (CNFs) in combination with poly(L-lysine) templated silica were also investigate for the design and development of highly stable electrochemical biosensor systems. Further work investigated the internal structure of silica nanoparticles using high resolution transmission electron microscopy, physical and chemical gas adsorption as well as mercury intrusion porosimetry. The latter porsimetry work was a result of a new collaboration between Partner 1 and researchers at BITS - Pilani-Goa (India) and is expected to lead to applications for EC funding to develop this area.

Work by Partner 3 led to the development of biosilica optical nano-sensors, predominantly using R5 mimics such as poly-L-lysine as silica precipitants. The most successful implementation of this approach was with O_2 sensing using entrapped $Ru(dpp)_3^+$ complex; these nanosensors could also be further doped with catalase to yield effective hydrogen peroxide sensors.

Within WP5 work was undertaken by Partner 4 to understand in detail the electrochemistry of redox enzymes entrapped in biosilica using advanced electrochemical kinetic approaches including rotating disk electrode measurements. Apparent Michaelis-Mentene kinetics parameters and true electron transfer rates form entrapped enzyme to electrode surface were described. Some of these efforts were a collaboration between Partner 4, Partner 3 and Partner 9 to examine in detail the ultra-microbiosensors that were fabricated.

Efforts were made to entrap MDH in biosilica to regenerate NAD(P)H; a substantial effort was made to successfully express MDH which included developing the expression vectors, optimising expression and purification procedures. As part of this endeavour, synthetic routes to high molecular weight NADH, via tagging with PEGs, were developed to allow its stable entrapment in biosilica. R5 biosilica was used to successfully entrap MDH and NADH-PEG although NADH regeneration with not shown.

Partner 6 developed a range of microfluidic reactors for both biocatalysic and biosensing applications; these contained a variety of biosilica immobilised enzymes including GOx, AChe plus ChO (as biosensor) and lipase. That last enzyme tested for 4 nitrophenyl butyrate hydrolysis since this class of enzymes (lipase) are extremely important for chiral biocatalytic syntheses needed for pharmaceuticals productions (see earlier). Flow and morphological parameters were optimised for these devices and both trapped silica nanoparticles and biosilica monoliths were produced; the latter were shown to be more stable and predictable. Along with Partner 6, Partner 8 assessed the efficacy of the microfluidic microreactors vs conventional sysntheses using lipase entrapped for biocatalysis. Finally, Partner 6 delivered a brief training course on microfluidic systems to the other partners.

In the final work package (WP8) Partner 7 worked extensively with almost all other partners to carry out a full analysis of the biomolecular interactions between the silica, precipitant (R5 and peptide mimics) and entrapped enzymes. Advanced biophysical techniques were employed including using FTIR and micro-Raman spectroscopy and electrochemical impedance spectroscopy (EIS). True molecular interactions were shown and characterised; the interaction between silica and biomolecules was clearly demonstrated to be specific and not just non-specific aggregation. Within this context, a stabilising effect on AChe and protection against protease activity were also demonstrated for biosilica entrapment. Silica/carbon-nanofibre /AChe nanocomposites were also produced and EIS measurements allowed calculation of enzyme rotational mobility to demonstrate that biosilica entrapped enzyme had the lowest

energy and hence greatest thermodynamic stability. Finally, FTIR measurements showed Si based oligonucleotide sensing and pave the way for specific DNA biosensors.

Adaptation of the natural silication process through the use of synthetic peptides and peptide mimics to catalyse nanosilica production has been fully explored by the Project SANTS partners. The materials produced have shown to be of use in the production of biocatalytic matrices, ultramicro-biosensors and nanoparticulate optical biosensors. Manufacturing protocols have been developed that allow the reproducible deposition of nanostructured surfaces for sensing and synthetic applications and also, the potential exists for the production of self-assembling and self-organising structures. This will lead to the development of a range of materials that will be of significant use in a range of industrially important areas.