SIXTH FRAMEWORK PROGRAMME

Project Number.	**013523 (NMP4-CT-2004-013523)**
Project Acronym.	**NAS-SAP**
Project Title.	**Nano-Arrayed Systems Based on Self-Assembling**
Proteins	
Instrument.	**STREP**
Thematic Priority.	**Priority 3 'NMP'**

Executive Summary

Period Covered:	**from month 13 to month 24**
Date of Preparation:	**April 1st 2007**
Start date of project:	**April 1st 2005**
Duration:	**36 months**
Project Coordinator:	**Professor Simon M. Cutting**
Proj. coord. Organisation:	**Royal Holloway and Bedford New College**

Publishable Executive Summary

Nanobiotechnology is an important and exciting discipline falling under the new science of nanotechnology. This field emerges from physics-based nanotechnology and harnesses all that we currently know from biology and particularly the molecular biosciences. At the forefront of these technologies are the study of molecular motors, protective coatings and novel delivery vehicles. In each case what we have learnt from nature can be applied to industrial use, whether this be electronics, medicine or the pharmaceutical industry.

Microroganisms have been studied extensively and offer a number of attributes that are of interest to the nanoscientist, these include the bacterial flagellum, the eukaryotic cytoskeleton, molecular glues or adhesions to name a few examples. The NAS-SAP consortium is focused on the surface structures of bacteria, specifically, the so-called, S-layers of bacteria and the spore coats of endospores.

S-layers are normally homogenous layers of self-assembled protomers. These self-assembled molecules form a single layer covering too many bacteria. Although the precise function of these surface (S) layers is not fully understand we do know they are important for protection of the microbe from environmental insult as well as adhesion. In most cases the protein components are known and vary in size from about 60-100 kDa. High-resolution imaging methods have been used to demonstrate the crystalline arrangement of S-layer nanoarrays. From an application point of view individual S-layer proteins can be made to self-assemble alone or in a variety of solid substrates as well as on phospholipids vesicles. Clearly, this is importance for the deliberate and precise arrangement of molecules tethered to S-layer proteins, as well as the packaging of pharmaceuticals and vaccines.

Spores are dormant life forms that are metabolically inactive bioentities yet able to resist, in a desiccated state extreme environmental conditions particularly temperature extremes reaching as high as 95^{o}C. The genetics of bacterial spore formation are well understood but far less about the spore structures, especially the spore coat. The coat forms the egg-shell covering and is comprised almost exclusively of protein subunits. In some spore-forming species as few as 4-6 different subunits form the spore coat while in others as many as 35. In any event at least some of the spore coat subunits are known to self-assemble. This self-assembly process is of course important and the scientist can ask how this is similar to S-layers, are the basic processes the same. What is the order of self-assembly and can more than two subunits self-assemble. Further, do these structures offer any protective value and might this be utilised in nanobiotechnology. Bacterial spores are being used for delivery of antigens and in some cases as vaccine vehicles. Here their intrinisic heat-stability and robustness make them attractive as vaccines for developing countries.

The NAS-SAP consortium is comprised of 9 European partners including 2 SMEs. Broadly speaking the consortium comprises 3 groups, an S-layer group, a spore group, and an applications group consisting of 2 SMEs. In another context the consortium contains bacterial geneticists, molecular biologists, protein biochemists and bioimaging specialists.

The long term goals of NAS-SAP are to understand the functional properties of S-layers and identify unique and novel applications for S-layer nanoarrays and S-layers assembled onto liposomes or related platform substrates. Similarly, yet more ambitious, with bacterial spores we plan to identify all the protein constituents of the spore coat of one representative, *B. subtilis*, and then determine how to exploit surface coat proteins for antigen display. In addition, using the unique imaging resources and biophysical expertise now present in the NAS-SAP consortium we will determine how the surface of bacterial spores appears topographically. Applications of this type of topographical analysis might be in fingerprinting spores that have been used in acts of bioterrorism.

Year 2 has provided a number of project achievements. The self assembly of S-layer proteins has been studied in depth including the fusion of S-layers to a major birch pollen antigen. This is a prototype for a potential novel adjuvant therapy to Type 1 allergy. The results of this work have been published in a high impact factor medical journal. For the first time, the S-layer of an important human pathogen, *Clostridium difficile* has been studied. This bacterium is the cause of *Clostridium difficle*-associated diarrhea (CDAD) and is a major cause of hospital illnesses as well as fatalities.

The S-layer proteins have been characterised and shown to reassemble. The lattice arrays are now being studied.

The spore coat proteins have also been studied in depth and a set of insertional mutants created in a large number of spore coat genes. In addition, an intensive screen of the interactions between spore coat proteins has been made with evidence of a number of previously unknown protein-protein interactions. Together with imaging analysis this has enabled the first steps towards understanding the topography of the spore coat. In addition, we now have tentative evidence of spore coat interactions of the CotA polypeptide suggesting that it can self-assemble. This work will continue.

The final year will focus mostly on applications and functionalisation studies of the S-layers and spore coats. Substantial progress has already been made with this on the construction of spore coat and S-layer proteins to novel immunogens. These will be tested for their capacity as vaccines as well as other applications such as diagnostic kits etc.