The objective of our research is to apply lessons learnt from biology in the manipulation of materials chemistry to laboratory studies aimed at generating oxides/metals with defined size and form. The focus of our work has been on peptide interactions with silica and zinc oxide. Detailed quantitative experimental studies together with molecular modeling studies have shown that G12 (GLHVMHKVAPPR) and GT16 (GLHVMHKVAPPRGGGC) control ZnO morphology by an adsorption mechanism that varies between crystal faces. In contrast, similar peptides, EM12 (EAHVMHKVAPRP) and EC12 (EAHVCHKVAPRP) were found to control ZnO formation via the retention of Zn(II) in solution. Differences between the behaviour of the two peptides arose from the availability of the sulphur atom for complex formation. A series of peptides identified by the phage display against specific sizes of amorphous silica particles showed relationships between fundamental properties of the peptides themselves (e.g. pI and hydrophobicity) and surface binding. The effect of single point mutations (Ala for His) in an individual silica binding peptide (KSLSRHDHHHH) were explored by experiment and modeling (MD and QM studies) and showed the importance of peptide flexibility for effective surface binding. Biochemical studies of a primitive
Award No: FA9550-06-1 0154

Title: “Studies of Peptide-Mineral Interactions and Biosilicification

Principal Investigator: Professor Carole C. Perry

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School of Science and Technology
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United Kingdom
Abstract
The objective of our research is to learn how biology is able to manipulate materials chemistry to produce oxides with defined chemistry and structure. The oxides chosen for study are amorphous silica whose numerous applications are dependent on physical form and structure, and zinc oxide, a wide band gap semiconductor. The project explored two distinct areas of biomimetic chemistry (1) peptide-mineral interactions, and (2) biosilification together with associated model studies. The ultimate objective of the first research topic is to produce a predictive model that explains why certain peptides, for example those generated by phage display methods, interact with and in many cases promote the formation of specific materials under mild reaction conditions. The ultimate objective of the second topic is to understand how biological organisms make silica and to use this knowledge to produce novel silica based materials in the laboratory using ‘simple’ but ‘functional’ molecules. The first topic has been studied for the duration of the project and the second topic for the first year of the funded period.

The report details our progress in both research topics and will include data acquired using a wide range of experimental techniques and our first computational studies performed in house as well as with our collaborators.

Highlights of the project include:

- The use of biopanning techniques at AFRL and latterly their development at NTU to identify 12-mer and 7-mer peptides that bind silica. The peptide sequences identified varied with silica particle size and preceeding thermal and chemical history.
- Detailed modelling of peptides associated with silica and identification of small peptide fragments that may be crucial for binding;
- Development of solid state peptide synthesis methods at NTU for the synthesis of the majority of peptides required for our synthetic and analytical studies. More than 30 different sequences from 3mers through to 17 mers have been successfully synthesised in the course of this project.
- Establishment of reliable, accurate and quantitative methods for the measurement of peptide concentrations in solution;
- Detailed experimental study of silica peptide interactions using a wide range of experimental techniques and using peptides identified from biopanning;
- Establishment of relationships between silica particle size and chemistry and molecular features of the peptides found to bind to the particles.
- Identification of ways in which silicic acid species can be stabilised in solution below pH 9 using specific molecular weight fractions of poly(1-vinylimidazole) and the effect of these polymers on silica formation;
- Establishing the range of shaped silicate materials that can be formed in the presence of azacyclics and the development of routes to monodisperse silica particles in the presence of these complexing/ charge balancing agents simply by changing the silica source;
- Identification of polysilicic acids that interact with molecules that have been found to promote silica formation.
- Development of experimental procedures for the generation of zinc oxide particles with single morphology for biopanning and subsequent studies of ZnO-peptide interactions;
- Detailed experimental and computational studies of ZnO-peptide binding (G series) that identified and quantified peptide adsorption on specific crystal faces as a route to control ZnO crystal morphology.
- Detailed experimental studies of peptide (E series) interactions with ZnO and its precursor LBZA identifying the importance of complexation in structure control.
- Establishment of reliable, accurate and quantitative methods for silicified plant cell wall analysis and their use in probing interactions between ‘Si’ in some form and proteins and carbohydrates in the cell wall of Equisetum arvense.
Status of Effort and Accomplishments:

Personnel Supported:

**PDRA personnel:**
(Dr S.V. Patwardhan, Dr H. Currie and Dr D. Belton) supported by the University from January 1st 2006 until 31st July 2006.

- Dr Siddharth Patwardhan (in post until 11th January 2009) (working on studies of peptide mineral interactions)
- Dr Heather Currie (until December 31st 2006) (working on silicon involvement with carbohydrates within silicified plant cell walls).
- Dr David Belton (50% post funded by AFOSR for July-December 2006) working on model studies of silica formation in the presence of synthetic polyamine additives. Now a Medici Fellow looking to commercialise some of our silica results.
- Dr Valeria Puddu (May 2009-May 2010)
- Dr Laetitia Canabady-Rochelle (September-December 2009)
- Dr Olivier Deschaume (funded by EPSRC but involved in molecular modelling studies of silver dipeptide interactions and ZnO-peptide interactions)

**PhD students:**
- Graham Tilburey (24th September 2003- end November 2006 (working on model silica systems) thesis examined in 2007
- Mei Keat Liang (9th October 2006-8th October 2009) (working on peptide-mineral interactions) thesis examined 2010

**Project students:**
- Anubha Mithu, MSc Bioinformatics (24th September 2006- end November 2006) (Masters level project on ibuprofen encapsulation in silica nanoparticles and also performed a short project after her MSc finished on peptide modelling)
- Yuliya Nigmatullina (9th July 2006-8th October 2006, second year Cambridge University student) (working on solution based methods for quantification of silica-bound peptide).
- Deepti Lobi (July-August 2007, Year 12 Nottingham High School for Girls) (working on azamacroyclic materials)
- C. Barton (July-August 2008, Year 12 Nottingham High School for Girls) (oversight of all aspects of the group work)
- M. del mar Munoz (October 2008-February 2009, Erasmus exchange student from Tarragona University in Spain, peptide synthesis)
- M. Garton (October 2008-April 2009, final year Mchem project student, dipeptide/surface simulations)

Publications:

9. Annenkov, V. V.; Danilovtseva, E. N.; Likhoshway, Y. V.; Patwardhan, S. V. and Perry, C. C., Controlled stabilisation of silicic acid below pH 9 using poly(1-vinylimidazole). J. Mater. Chem., 18, 553-559

Papers in Press:


Papers in review:


Drafts of papers to be submitted:


PhD theses submitted to AFOSR:
1. G. Tilburey (2007) “Understanding molecular interactions in the precipitation and dissolution of silica and silicates under ambient conditions”

Interactions/Transitions:

Presentations by PI as invited speaker:

- March 2006, Edinburgh University, Chemistry
- June 2006, Advanced Functional Materials Workshop, Spain
- July 2006, Gordon Conference on Biominalisation, USA as discussion leader
- October 2006, Physical Chemistry, Oxford
- March 2007, Cambridge University, Chemistry
- May 2007, Irkutsk and Novosibirsk, Biology/Physics
- June 2007, ESF Workshop on Biominalization, Denmark
- August 2007, ACS, Boston
- October 2007, AO Foundation, Switzerland
- November 2007, Flowsyn Meeting (S.V. Patwardhan)
- April 2008, Physical Sciences, Keele University
- June 2008, Biominalization Workshop, Leeds (Geology) University
- July 2008, Ineos Healthcare, Warrington
- September 2008, Helsinki University, Finland
- October 2008, Limnological Institute, and Technical University, Irkutsk, Russia
- October 2008, National Nanotechnology laboratory, Lecce, Italy
- November 2008, Flowsyn Meeting, Cambridge
- January 2009, Biochemical Society Meeting, ‘Biotechnology II- from assembly to cell biology’, Cambridge, UK
- February 2009, Napier University, Bioscience
- April 2009, Simbioma, Mainz, Germany
- May 2009, Nottingham University, Mathematical Sciences
- January and October 2009, NTU (Chemistry, Bioscience)
- December 2009, MRS, Boston

Other presentations given by group members:


Organisation of International Meetings

- MRS Easter 2007 “The nature of design-utilizing Biology’s portfolio”, with Dr R. Ulijn, Manchester University, Dr R. Naik (AFRL) and Professor K. Shiba (Tokyo)

New funding on research complimentary to that funded by AFOSR:

- Royal Society Joint Project Grant with Dr Annenkov, Limnological Institute, Irkutsk (2006-2008)
- INTAS/EU project with collaborators in Irkutsk, Novosibirsk and Helsinki (2006-2008)
- EPSRC funding for Symposium T at the Easter 2007 MRS meeting with Dr Ulijn (Manchester)
- NIH with David Kaplan, Tufts University and Bruce Rutherford, Washington State University; 2007-2012
- EPSRC; collaborating with David Kaplan, Tufts University and Rajesh Naik, AFRL; 2007-2010
- EPSRC with Rein Ulijn, Manchester University for MRS Spring Meeting, 2007.
- EU Biomintec (2008-2012) with many partners in Europe

Consultative and advisory functions

- Steering Committee for the HEFCE funded proposal “Chemistry for our Future”, an initial £3.6m pilot study as part of a projected £28m spend. (2006)
- Panel member of DFG Review Session for “Centres of Excellence/ Graduate Schools” (2006)
- Member of RSC Benchmarking Group for Chemistry (2006)
- Elected trustee and Council member of the Royal Society of Chemistry (2007-2011)
- Member of RSC audit committee (2007-2011)
- Ineos Healthcare (July 2008-July 2009)
- Chair of RSC Audit Committee (2009-2011)
- Elected chair elect of the United Kingdom’s Heads of Chemistry (2008)
- Chair, UK Head’s of Chemistry (2009-2011)
- Member of EPSRC College 2003-, renewed by invitation in 2010)
- Grant proposal and fellowship reviewing for a range of government funded bodies and charities both within the UK and around the world.

Transitions/Collaborations in place with other scientists funded in this programme are as follows:

- **Professor David Kaplan**: several papers on joint studies arising from our AFOSR funded projects have been published with more in the pipeline. One of the PDRAs initially funded on this grant went to join the Kaplan group. One of the PhD students funded by this grant went to the Kaplan group as a PDRA. We have gained joint funding from NIH and funding from the UK engineering and Physical Sciences Research Council where some of the project work is performed in the Kaplan labs in Boston with members of my team spending periods of time in his laboratory to learn about molecular biology and generate silk based chimeric proteins.

- **Dr Rajesh Naik**: We have collaborated on the biopanning studies against silicas with defined size and surface characteristics produced from Airforce and other (Smith and Nephew) funded work and other materials throughout the project. Dr Naik and I were joint organisers of Symposium T at the Easter 2007 MRS meeting with, Dr Uljijn (Manchester) and Professor Shiba (Tokyo). We have gained funding from the UK engineering and Physical Sciences Research Council with Dr Naik as a named collaborator working on the development of novel routes to chimeric proteins and their composites with nanoparticles. We have enjoyed access to unpublished data from the Naik group and some of the scientists at AFRL and have hosted a visit of Dr Rajiv Berry to the laboratory in 2010.

- **Dr. Hendrik Heinz (Akron)**: Following extended discussions at the MRS meeting in San Francisco (2007) Dr Patwardhan spent two weeks at Dr Heinz’s laboratory learning about the modelling of materials surfaces. A visit of Dr Heinz to the laboratory in 2008 has enabled us share our understanding of silica chemistry so that he, in conjunction with scientists from AFRL can develop a much better model of the silica surface (this has been ongoing through telephone conferencing) and joint publications are being prepared.
Externally Funded Visitors to the Laboratory:

- Dr Kate McGrath (Royal Society Easterfield lecture tour and summer visit 2006)
- Dr Ken-Ichi Sano (funded by Japanese Government, September-October 2006)
- Dr V.A. Annenkov, Dr. E.N. Danilovtseva and Dr S.V. Zelinskiy, 2006-2008 (Royal Society and INTAS funded projects, several visits).
- Dr A. Prabune, NCL Pune (6 months funded by Indian Government); February-August 2008

New Discoveries etc.

Honors/ Awards

CCP elected as Fellow of the Royal Society of Chemistry
CCP elected as Trustee and Council Member of the Royal Society of Chemistry
SVP travel grant for ACS meeting/ visit to Akron
CCP elected as next 'Chair of Heads of Chemistry' UK (2009-2011)
SVP appointed to Lectureship in Chemistry at NTU (2009-)
SVP associate editor of the new journal ‘Silicon’, CCP as advisory editorial board member
MKL joint first prize winner at the Royal Society of Chemistry Industry Study Tour, July 2008
SVP appointed to Lectureship in Chemistry at Strathclyde University (2010-)
Appendix 1: Published papers and posters
Appendix II: PhD thesis abstracts

G. E. Tilburey “Understanding Molecular Interactions in the precipitation and Dissolution of Silica and Silicates under Ambient Conditions”

Abstract: The bio-geo-chemical silicon cycle provided the inspiration for investigations to be carried out into the formation and dissolution of silica. This thesis is concerned with understanding the molecular interactions occurring between silicon species and small organic molecules with emphasis on the dissolution and formation of silica in vitro. Three model systems have been employed to investigate molecular interactions occurring during silica formation; two supersaturated orthosilicic acid containing solutions generated from dipotassium tris(1,2-benzenediolato-0,0)silicate (KSiCat), an unbuffered tetramethoxysilane (TMOS) system and globally undersaturated silicon systems. The later two were developed in this thesis. These model systems enabled use to investigate the molecular interactions between bioinspired additives and different silicon species. Bioinspired polyelectrolytes and small organic molecules were investigated and shown to interact in a completely differently manner with similar silicon species.

The role of hydroxyl groups in silica formation was investigated using alkanediols. It has been hypothesised in the literature that Si-O-C bonds may template silica formation in vivo. Investigation into the interactions between hydroxyl functionalised molecules and supersaturated solutions of orthosilicic acid. No evidence was found for the formation of Si-O-C bonds and it was found that hydrogen bonding does not have a significant effect on the formation of silica.

Finally, an investigation was carried out into the effect of azamacrocyclic molecules in silica formation. Two supersaturated silica forming systems which utilised; KSiCat and TMOS were used. Inorganic-organic hybrid needle-like tetragonal prisms were formed when KSiCat was used which was shown to be a displacement reaction of the potassium ions by cyclam to form a 1:1 cyclam:SiCat layer like structure. In the unbuffered TMOS system azamacrocyclic molecules with ≥14 atoms were found to have a significant effect on the kinetics of silica formation. Through the control of pH, mono-dispersed solutions of silica spheres of different sizes could be stabilised through a charge neutralisation mechanism.

M.K. Liang “Studies of Peptide-Mineral interactions”

Abstract: The studies of peptide-mineral interactions presented within this thesis aimed to identify and understand the effect(s) induced by peptides/selected motifs on amorphous silica and crystalline zinc oxide (ZnO) formation. The effect of imidazole functionality on silica formation was studied using polyvinylimidazole (PVI) and polyhistidine (P-His). The effect of zinc oxide-binding peptides (ZnO-BPs) on the morphology and formation of ZnO were studied using G-12 (GLHVMHKVAPPR) and EM-12 (EAHVCHKVAPRP), and their derivatives, GT-16 (GLHVMHKVAPPRGGGC) and EC-12 (EAHVCHKVAPRP) respectively. The influence of these additives on reaction kinetics, their effect on the precipitates, and their level of incorporation into the precipitates were investigated. This series of studies revealed three common characteristics of peptide-mineral (ZnO-BPs-ZnO and imidazole-silica) interactions.

Firstly, a specific functionality of the biomolecule was responsible for the effect induced while a supporting functionality enhanced the effect. The imidazole group of PVI and P-His catalysed the condensation of monosilicic acid but the peptide backbone and more flexible imidazole enhanced the catalytic capability of P-His with respect to PVI having a similar concentration of imidazole groups. The presence of G-12 and GT-16 reduced the aspect ratio of ZnO crystals formed via an adsorption-growth inhibition mechanism. However the addition of a GGGC-tag on GT-16 weakens the adsorption of GT-16 on the (10-10) face of ZnO crystals. This gave rise to selective adsorption of GT-16 on the (0002) face, with a greater reduction of the crystal aspect ratio. For the EM/EC-12 peptides, metal ion complexation that leads to a delay/suppression of ZnO formation was higher for EC-12 compared to EM-12 and was caused by the more efficient complexation of Zn²⁺ with the peptide containing cysteine.

Secondly, additives can interact with different species in the reaction. Imidazole interacts with neutral monosilicic acid via hydrogen bonds but protonated species of imidazole interact with anionic polysilicic acid via electrostatic interactions. Although EM-12 only interacts with Zn²⁺ in solution, EC-12 was able to interact reversibly with the solid phases formed in the course of reaction.

Thirdly the type of interaction and interacting species determine the level of additive incorporation and its effect on the concentration of reactants remaining in solution when equilibrium is reached. Peptide-surface interactions generally
result in incorporation of the peptide into the solid phase (G-12, GT-16, and EC-12) as opposed to the situation where complexation of reactant species in solution shown by EM-12 did not result in peptide incorporation.

The use of peptides/motifs (biomolecules) isolated from combinatorial libraries for silica and ZnO synthesis has been shown to be a promising approach for morphology and reaction control. It is particularly exciting when their use can be extended to the generation of ZnO which has a non-biological origin. By understanding the phenomenal complexity of the behaviour of biomolecules in (bio)mineralising systems, the ground rules in their interactions (with species in (bio)mineralising systems) can be generated and exploited for the synthesis of novel nanomaterials with properties tailored for specific applications.
Appendix III: unpublished papers
Appendix IV: Example abstracts of project student reports

The encapsulation of sodium ibuprofen in silica particles
K. Anubha Mithu, MSc project (summer 2007)

Abstract: The synthesis of Sodium-Ibuprofen / silica has been achieved by using sodium-metasilicate precursor, non-steroidal anti-inflammatory drug sodium-Ibuprofen and phosphate buffer (500 mM) in the presence of pentaethylenehexamine. Furthermore, in a separate set of experiment silica samples were prepared using sodium meta-silicate precursor in presence of pentaethylenehexamine and phosphate buffer (500 mM) to observe the effect of buffer on the morphology of silica nanoparticles. Both drug loaded and drug free silica nanoparticles were characterized by FTIR spectroscopy (Fourier transform infrared spectroscopy) and SEM (scanning electron microscope). The experimental results show that the loading of sodium-Ibuprofen into silica decreases with the increase in the pH and the highest loading achieved is 17%. In addition, scanning electron microscope images of silica prepared in presence of different volume of phosphate buffer (500 mM) showed an increasingly granular structure with increase in the volume of phosphate buffer. In order to observe the efficiency of this drug carrier/vehicle, release experiments must be performed in presence of simulated gastric and proximal intestinal fluid. This method of encapsulation of Sodium-Ibuprofen can also be used to encapsulate macromolecules, vitamins, nanoparticles, pesticides etc.

Development of Solution Based Methods for Quantification of Silica Bound Peptides.
Yuliya Nigmatullina (summer 2006)

Abstract: A range of UV absorption, colorimetric and fluorometric methods were investigated for their suitability for the detection of a range of small peptides (12mers) bound to silica surfaces. Of the three methods tested in depth, UV absorption, CBQCA and fluorescamine, the method using fluorescamine was the safest to use, fluorescence development was the quickest and molecules containing low numbers of primary amines (such as peptides) could be quantified. The ideal experimental conditions for the detection of peptides such as YITPYAHRLGGN, include a ca. 15:1 molar ratio of fluorescamine to peptide, incubation for 1 hour with the mineral sample, centrifugation at 13000 rpm for 10 minutes followed by reading the fluorescence with a 360nm excitation filter and a 465nm emission filter. It was found that of the many peptides explored, each had a slightly different complexation behaviour with the fluorometric agent making it essential for all future studies to calibrate for the specific peptides being investigated.

Molecular modelling and MD simulations of inorganic material binding peptides.
K. Anubha Mithu (3 month project after completion of MSc studies, autumn 2007)

Abstract: Molecular modelling, molecular dynamics simulations and ClustalW analysis of a range of 12-mer peptides identified by the Naik group at AFRL as binding to silica (many different forms and preprocessing conditions), titania and an iron-platinum alloy were performed. Commercial Sybyl software was used to produce energy minimised structures and the acid/base properties and spatial functionality of the peptides explored. For the silica binders, two classes of peptides could be identified that either contained basic amino acids including histidine, arginine and/or lysine or the peptides contained large numbers of hydrophobic amino acids or polar amino acids. A common feature observed for all peptide material systems studied was that the functionality tended to be only on one side of the backbone of a particular peptide.

Studies of Silver-dipeptide interactions using MD and MC simulations.
Michael J. Garton, (final year MChem Project 2008-2009)

Abstract: Classical molecular dynamics (MD) and Metropolis Monte Carlo (MC) simulations were performed to investigate the adsorption orientation, mode and affinity of a number of dipeptides with respect to the (111), (110) and (100) surfaces of silver. The atomistic description of the system, which for MD included explicit solvent, was based on a customised version of the pcf forcefield, the SPC water model and parameters available from literature and prior work. MC calculations in vacuum to obtain optimal adsorption orientations proved fruitless without a solvent, whilst resulting adsorption energies were used to shortlist dipeptides for MD. Following 2 ns MD simulations, peptide chemical group–surface distances, surface–water and peptide–water radial distribution functions, and interaction energies for all system components were analysed. Surface adsorbed water plays a critical role in the peptide adsorption mechanism as the hydrophilic chemical groups have an affinity for the three solvation layers shown. Introduction of hydrophobic sidechains and their position changed affinity for the (111) surface compared with a strongly interacting glycyl glycine and this was verified experimentally. Preferential interaction
with (110) surface by glycyl alanine was observed in simulation but not confirmed by experiment. Dipeptides interacted with the surfaces primarily through the carboxyl oxygens or backbone groups.

Microwave assisted synthesis and characterisation of mineral-binding peptides
Maria del mar Munoz Serrano- (foreign exchange student, final year project, September 2008-January 2009)

Abstract: Solid state peptide synthesis aided by microwave heating was used to synthesise a range of peptides previously identified by Phage display as binding to a range of metals and oxides. The synthesis of 16 peptides by Fmoc chemistry was attempted and the products characterised by reverse phase HPLC and NMR spectroscopy. 75% of the synthesised peptides were produced at purity levels higher than 80% and required no further clean up before use. Of these, with 50% were synthesised at purities higher than 90%. An initial study of silver precipitation in the presence of one of the peptides showed that differently shaped crystals could be obtained using the various peptides.