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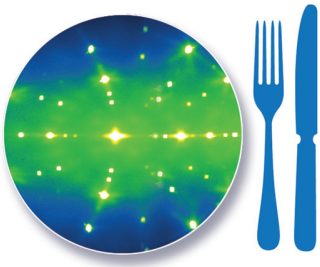

Neutrons and Food

Sydney, Australia
31 October – 3 November 2010

Sponsors

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Contents

www.nbi.ansto.gov.au/neutronsandfood

Overview

Scope

The application of neutron scattering to food-based systems is still in its infancy but has significant potential to understand the complex relationship between food structure, processing, rheology, nutrition, food quality and security.

The “Neutrons and Food” workshop seeks to identify the future scientific needs and opportunities in the application of neutron scattering to food science and to foster collaboration and networking between researchers.

The outcomes from this workshop will assist neutron scattering facilities to further adapt their infrastructure to the requirements of the wider scientific community and to enable potential users to develop collaborations with neutron scattering researchers.

Time	Sunday 31 October	Monday 1 September	Tuesday 2 September	Wednesday 3 September
8.00		Registration		
8.45				
9.00		Opening and welcome Introduction	Protein and complexes	Digestion and metabolic processes Drinks and beverages
10.30		Morning tea	Morning tea	Morning tea
11.00		Dairy	Protein and complexes	Where to from here?
12.30		Lunch	Lunch	Lunch
13.30		Lipids and fats	Glassy states	Visit ANSTO
15.00		Afternoon tea	Afternoon tea	
15.30		Networking session	Food packaging and food safety	
17.00	Arrival / registration	Poster session	Plant materials	
19.00	Welcome reception		Workshop dinner / harbour cruise	

Map


N
Amora Hotel Jamison
Bridge St
Lang St
Jamison St
Bond St
Margaret St
Curtin Pl
Hunter St
Wynyard Train Station
Western Distributor
Carrington St
Wynyard Ln
Shelley St
Erskine St
Lime St
Sussex St
Kent St
Clarence St
York St
George St
Pitt St
Barrack St
King St
Darling Harbour
Pier 26
Pitt St Mall
Pyrmont Bridge
Market St
100m

Programme

Day 1 - Monday, 1 November

9.00	Welcome by Adi Paterson, Chief Executive Officer, ANSTO Elliot Gilbert and Mike Davidson		

Introduction

Chaired by- **Mike Davidson**

9.15	Neutrons and food: what are the problems?	P. Lillford	University of York, United Kingdom
9.45	Neutron scattering – a natural tool for food science and technology research	E. Gilbert	ANSTO Bragg Institute, Australia
10.15	Discussion		
10.30	Morning tea sponsored by *Nestlé*		

Dairy session

Chaired by- **Hans Tromp and Rex Hjelm**

11.00	Quantitative models of casein micelle structure derived from SAXS and SANS	C. Holt	University of Glasgow, United Kingdom
11.30	Protein–lipid interactions at model membrane architectures	I. Koeper	Flinders University, Australia
11.50	Co–adsorption of β–casein and calcium phosphate nanoclusters (CPN) at hydrophilic and hydrophobic solid–solution interfaces studied by neutron reflectometry	T. Nylander	Lund University, Sweden
12.10	Discussion		
12.30	Lunch		

Lipids and fats session

Chaired by- **Tommy Nylander and Anna Sokolova**

13.30	Neutron scattering, hydrogenous materials and nutraceuticals	J. Katsaras	National Research Council, Canada
14.00	The effect of water on structuring of organic phases by mixtures of β–sitosterol and γ–oryzanol	W. Bouwman	Delft University of Technology, The Netherlands
14.20	Nanoaggregates of bile salt and cationic surfactant	J. Mata	ANSTO Bragg Institute, Australia
14.40	Discussion		
15.00	Afternoon tea		
15.30	Networking session		
17.00	Poster session		

Day 2 - Tuesday, 2 November

Protein and complexes session I

Chaired by- **Stephen Holt and Dominique Champion**

9.00	Neutron scattering studies of food structure: gelation, complexation and the effect of high pressure	R. H. Tromp	NIZO food Research, The Netherlands
9.30	The sweet taste of neutrons	S. Teixeira	Keele University, United Kingdom
9.50	Puroindoline binding to lipids and its relation to wheat endosperm structure	L. A. Clifton	ISIS Spallation Neutron Source, United Kingdom
10.10	Discussion		
10.30	Morning tea sponsored by *Nestlé*		

Protein and complexes session II

Chaired by- **Peter Lillford and John Katsaras**

11.00	Protein structure (SANS), water and protein dynamics (elastic and inelastic neutron scattering), and protein–lipids interface (neutron reflectivity)	C. Loupiac	Université de Bourgogne, France
11.30	Folding and dynamics of the digestive enzyme pepsin	D. Dee	University of Guelph, Canada
11.50	Antioxidant–protein interactions in phospholipid membranes	D. McGillivray	University of Auckland, New Zealand
12.10	Discussion		
12.30	Lunch		

Glassy states session

Chaired by- **Katy Wood and Susana Teixeira**

13.30	Protein structure and interactions in the solid state	S. Krueger	NIST, USA
14.00	Mobility in the vicinity of the glass transition: thermal and dynamical properties	D. Champion	Université de Bourgogne, France
14.20	The temperature and moisture dependence of protein dynamics in glycinin: a quasi–elastic neutron scattering study	A. Sokolova	ANSTO Bragg Institute, Australia
14.40	Discussion		
15.00	Afternoon tea		

Day 2 Programme continued next page →

Day 2 - Tuesday, 2 November continued

Food packaging and food safety session

Chaired by- **Susan Krueger and Jitendra Mata**

15.30	Need for neutron scattering techniques in packaging	R. Lee	PTIS, USA
16.00	Potential for neutron scattering in Food Safety	M. Davidson	University of Tennessee, Knoxville, USA
16.20	Discussion		

Plant materials session

Chaired by- **Herma Buttner and Ingrid Appelqvist**

16.40	Multi–scale structural characterisations of fatty acid multilayer tubes with temperature tunable diameter in bulk and at the air/water interface by coupling SANS and neutron reflectivity	A.L. Fameau	CEA/INRA, France
17.00	Discussion		
18.00	Depart from hotel to Pier 26, King St Wharf		
18.45	Embark for 19.00 departure		

Day 3 - Wednesday, 3 November

Digestion and metabolic processes session

Chaired by- **Duncan McGillivray and Al Paulson**

9.00	Bile physiology and physical chemistry in digestion: fundamental insights from small–angle neutron scattering	R. Hjelm	Los Alamos National Laboratory, USA
9.30	Starch granules under attack: multidisciplinary investigation of structural mechanisms governing starch digestion	J. Blazek	ANSTO Bragg Institute, Australia

Drinks and beverages session

Chaired by- **Wim Bouwman and Jaroslav Blazek**

9.50	Colloidal interactions involving condensed tannins in diluted systems: what problems can we solve through SANS?	A. Vernhet	Montpellier SupAgro, France
10.20	Discussion		

Where to from here?

Chaired by- **Rob McGreevy, Mike Davidson, Elliot Gilbert and Herma Buttner**

10.40	Open forum including morning tea
12.30	Lunch
13.30	Visit ANSTO

International Scientific Advisory Committee (ISAC)

Peter Belton
UEA, UK

Arjen Bot
Unilever, Netherlands

Jörg Fitter
Jülich, Germany

Rex Hjelm
LANSCE, USA

Peter Lillford
University of York, UK

Duncan McGillivray
University of Auckland, New Zealand

Phil Perkins
Bush Brothers, USA

V. Prakash
CFTRI, India

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Jitendra Mata
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Anna Sokolova
ANSTO

Rhiannon Still
AINSE

Cherylie Thorn
ANSTO

Michael Zettinig
ANSTO

Invited speakers

Elliot Gilbert
ANSTO,
Australia

Neutron scattering - a natural tool for food science and technology research

Elliot Gilbert is programme leader for Food Science at the Australian Nuclear Science and Technology Organisation (ANSTO) in Sydney, Australia and instrument responsible for the small-angle neutron scattering instrument, Quokka.

He was born in London, completed a first degree in Chemical Physics at Edinburgh University before moving to Australia to undertake a PhD in polymer physics. This is where he was first exposed to the idea of using neutrons to probe structure and dynamics in materials. After completing a postdoctoral fellowship with a major international company studying the formation of high-internal phase emulsions for explosive application, he subsequently became a fellow at Argonne National Laboratory in the United States where he investigated the structure of polymers in confined geometry.

He returned to Australia in late 2001 to lead the project for the design, construction and commissioning of the state-of-the-art small-angle neutron scattering instrument Quokka, at ANSTO.

Elliot's interests lie in soft condensed matter science and has investigated such diverse areas as phase separation in paraffins, the design of biocompatible ferrofluids for medical application and the nanostructure of composite materials for aerospace. He now has an increasing focus on naturally occurring materials.

Elliot devised, initiated and now leads a research group in the application of nuclear methods to investigate fundamental and industrial problems of national significance in food materials science. He recently published the first review of neutron scattering as applied to food-based systems in *Trends in Food Science and Technology* and is chair of the next international small-angle scattering conference in 2012.

Rex Hjelm
LANSCE,
United States of America

Bile physiology and physical chemistry in digestion: fundamental insights from small–angle neutron scattering

Rex Hjelm is a Senior Scientist at Los Alamos National Laboratory (LANL); in addition he holds positions at the Los Alamos Neutron Science Center, the Institute for Multiscale Materials Studies and is an Adjunct Professor of Chemical and Environmental Engineering, University of California, Riverside.

After his graduation from the University of California, Berkeley, and his Ph.D. in biology/biochemistry from the Johns Hopkins University, Rex was introduced to neutron scattering techniques as part of a Postdoctoral Fellowship at Portsmouth University (UK) and the Institut Laue Langevin (France) where he made one of the first determinations of the arrangement of proteins and nucleic acids in the basic chromosome unit, the nucleosome.

Later, Rex had a joint appointment between the University of Illinois and Argonne National Laboratory studying chromosome structure with electron microscopy and small-angle neutron scattering. It was during this period that Rex started to look at in-bile physiology and lipophilic molecular transport in-bile secretion and digestion, applying small-angle scattering methods.

Since joining Los Alamos National Laboratory, he has been responsible for the laboratory's Low-Q neutron scattering program and led various projects in soft matter. He now has135 published articles and 146 invited presentations in biophysics, physics, materials science, colloids and polymers, scattering instrumentation and methods and computer code development. In addition, Rex has edited major reports on neutron scattering facility instrumentation and instrument design and edited three volumes on composite polymer materials. Currently, he leads programs in viral structure, structural biology of molecular motors and the molecular response of polymers to stress using scattering and reflectometry techniques.

Carl Holt
University of Glasgow,
United Kingdom

Quantitative models of casein micelle structure derived from SAXS and SANS

Carl Holt received a broad education and research training in physical and biological sciences, which was reflected in his activities as a research scientist at the Hannah Research Institute and, latterly, at the University of Glasgow.

At an early stage in his career he developed the hairy casein micelle model to help explain aspects of casein micelle stability and structure and, with Lindsay Sawyer, went on to introduce the concept of caseins as rheomorphic proteins.

He played a leading part in the discovery and characterisation of thermodynamically stable calcium phosphate nanoclusters formed from amorphous calcium phosphate with one or more members of a group of secreted phosphoproteins or phosphopeptides, including caseins, osteopontin and fetuin sequences.

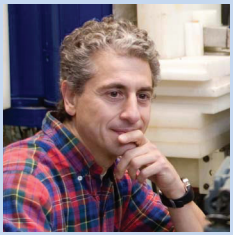
These discoveries have pointed the way to a new understanding of one of the structure-function relationships among unfolded proteins and have helped to uncover a novel mechanism in the control of biocalcification.

In addition to this fundamental research, he has worked as a consultant to the food and pharmaceutical industries and is a named inventor on two calcium phosphate nanocluster patents.

John Katsaras

NRC,
Canada

Neutron scattering, hydrogenous materials and nutraceuticals



John is a Principal Research Officer with the National Research Council (NRC) Canada and a Senior Scientist/ Distinguished R&D Staff with Oak Ridge National Laboratory (USA).

After undergraduate degrees (BA Psychology; BSc Biology) from Montreal's Concordia University, John Katsaras proceeded to the University of Guelph for graduate studies in Biophysics under the tutelage of the late R.H. Stinson.

After graduating in 1991, John accepted positions with R.M. Epand (McMaster University) as a Natural Sciences and Engineering Research Council of Canada postdoctoral fellow (McMaster University), and later with J. Dufourcq (CNRS, France) as a Poste Rouge Fellow.

In 1994 John returned to Canada to take up a position at AECL's Chalk River Laboratories. His arrival signified the beginning of the Canadian Neutron Beam Center's soft materials program, and the advent of an internationally recognised neutron based biomembranes initiative that has, in many ways, pioneered the application of thermal neutron scattering to lipid membrane biophysics.

Invited

Protein structure and interactions in the solid state

Susan Krueger
NIST,
United States of America

Susan obtained her PhD in physics from the University of Maryland in 1987 with a biophysics-related thesis, and subsequently became a research physicist at the National Institute of Standards and Technology (NIST) Center for Neutron Research (NCNR). During her work at the NCNR, her research interests have centred on structural studies of biological macromolecules in solution using both small-angle neutron scattering (SANS) and neutron reflectometry techniques. She developed a SANS data-acquisition software interface and took the lead in developing an improved neutron reflectometry technique for the study of single lipid bilayer systems supported on planar substrates. For this she received the Department of Commerce Silver Medal and the NIST Bronze Medal; and she was elected a Fellow of the American Physical Society.

Currently, she is involved in the development of a SANS data analysis and advanced modeling software suite of programmes, bridging the gap between computational chemistry methods and the calculation of a wide variety of biological scattering experiments covering both structure and dynamics applications. The biological systems of most interest to her studies include multi-component systems such as multi-subunit proteins, protein/lipid and protein/DNA complexes, as this allows for the strategic use of neutrons by performing contrast-variation experiments.

Susan is also interested in advancing the studies of single bilayer biomimetic membranes using neutron reflectometry, the ultimate goal being to study transmembrane proteins in suitable biomimetic environments. She also enjoys teaching the SANS technique, to new and potential users. Currently, Susan is Chair of the Neutron Scattering Science Advisory Committee, Oak Ridge, Tennessee.

Need for neutron scattering techniques in packaging

Ross Lee
PTIS™,
United States of America

Ross Lee is an experienced technology leader with over 36 years experience with the DuPont company (retired July 2009) spanning a wide variety of technology, product and new business developments including films, resins and innovative packaging systems. Many of these developments involved nanoscale coatings, particles and characterisations. In his most recent position, at DuPont, Ross was responsible for bringing new technology to packaging through open innovation and was instrumental in developing DuPont's alliances with Plantic, a provider of renewably sourced, thermoplastic starch for packaging and other applications, based in Victoria, Australia; and Scanbuy, a mobile enabled interactive technology provider. Ross was a recipient of DuPont's 2008 Sustainability Excellence award.

Ross is currently a Senior Business Associate with PTIS™ (Packaging & Technology Integrated Solutions, LLC) and an Adjunct Professor at Villanova University where he teaches green science, industrial chemistry and engineering entrepreneurship. He has a Ph.D. in Organic Chemistry from Michigan State University and a B.S. in Chemistry from the University of Rochester. Ross and his family reside in Chesapeake City, Maryland.

Neutrons and food: what are the problems?

Peter Lillford
University of York,
United Kingdom

Peter was trained as a chemist at King's College London, and after postdoctoral positions at Cornell and the San Francisco Medical Center, joined Unilever Research where he spent most of his career. He led Basic Research in Food Physical Chemistry and Materials Science and as Chief Scientist (Foods), was also responsible for research in Microbiology and Sensory Science.

He retired in 2001 and is currently a Visiting Professor in the Biology Dept. of the University of York., and the School of Engineering in Birmingham University. He is Chairman of Governors of the Institute of Food Research (UK), and is Chairman of the UK LINK Scheme in Advanced Food Manufacturing. He has held Fellowships with CSIRO (Australia), and consults for several multinational food companies.

He was Chairman of the UK Technology Foresight Programme for Food and Drinks and is also a former President of IFST. He was awarded the Senior Medal for Food Science by the Royal Society of Chemistry: and an Outstanding Achievement Award by the European Federation of Food Science and Technology; and is a Fellow of the International Academy of Food Science and Technology, The Royal Society of Chemistry and the Royal Society of Arts. He was made Commander of the British Empire (CBE) "for services to science and the food industry".

Camille Loupiac
Université de Bourgogne, France

Protein structure, water and protein dynamics, and protein-lipids interface. How neutron scattering experiments can target the behaviour of model food proteins?

Dr. Camille Loupiac obtained her PhD in Biophysical Chemistry in 1999 from the University Paris 11 (Orsay). During her PhD, she worked with Pr. Bernard Alpert and Dr. Serge Pin, in the “Laboratoire de Biologie Physicochimique” of the University Paris 7, on the structure-dynamics-function relationship of hemeproteins.

She received a strong background in the application and theory of a diverse number of biophysical techniques which include: NMR and Raman spectroscopies, X-ray and neutron scattering and laser flash photolysis. After her doctorate, she joined the CEA, and worked at the Laboratoire Léon Brillouin with Drs. Marie-Claire Bellissent-Funel and Patrick Calmettes on the development of neutron scattering (Small Angle Neutron Scattering and Inelastic Neutron Scattering) to study the structure and dynamics of protiens. In 2001 she obtained a post-doctoral position at the University of Illinois at Chicago in USA in Dr. Michael Caffrey’s group.

She learned more about the application of NMR spectroscopy to study protein structure and function, moreover on HIV membrane envelope proteins. There, Dr Loupiac also learned new techniques in molecular biology (segmental isotopic labeling, recombinant protein purification, mutagenesis assays). In 2003, she joined the ENSBANA at Dijon (now AgroSup Dijon), to become lecturer in food biochemistry. At the University of Burgundy, in EMMA team, she developed research on milk proteins functionalities, especially on globular proteins beta-lactoglobulin and caseins. Her main interest is to correlate proteins, structural and dynamics changes to their functional properties (gels, emulsions, powders, interfaces). Key parameters are environmental stresses (pH, salt, co-solvents, high pressure, heat, freezing). Favourite observation tools are spectroscopies (FTIR, fluorescence, NMR), DSC, light and neutron scattering.

Hans Tromp
NIZO,
The Netherlands

Neutron scattering study of food structure: gelation, coacervation and the effect of high pressure

Dr. R. Hans Tromp was born in Leiden, the Netherlands, in 1963. He graduated from the Department of Physical Chemistry of University of Leiden.

His thesis was an NMR study of counter ion dynamics in polyelectrolyte solutions. He had postdoctoral positions at the Physics Departments of the Universities of Bristol (Liquids Group of Prof. J. Enderby) and Cambridge, UK (Polymer & Colloids group of Prof. A. Donald).

He was a researcher at the Institute of Food Research in Norwich, UK, before becoming a senior scientist at NIZO food research, Ede, the Netherlands. His expertise is in phase separation, gelation, texture formation and stability of food biopolymer systems.

He is a part-time associate professor in the Physical and Colloid Chemistry Group at the University of Utrecht.

Aude Vernhet
INRA,
France

Colloidal interactions involving condensed tannins in diluted systems: what problems can we solve through SANS?

Aude was trained as a Food Scientist at the High National School of the Food Industries (ENSIA-Massy) and at the University of Technologies of Compiègne. After a phD in Enzymatic Processing, Bioconversion and Microbiology, of the University of Compiègne, she joined Montpellier SupAgro (an international centre for higher education in agriculture sciences) in 1993 as an assistant professor, in the Enology teaching team. She now supervises the Viticulture and Enology specialisation of the Engineer Diploma (Master's degree) of Montpellier SupAgro and of the "Vine and Wine" National Master.

Aude has developed her research activities since 1993 in the research joint unit Sciences for Enology (INRA/ Montpellier SuoAgro/University of Montpellier I). In that unit, she is responsible for the physico-chemical interactions axis. Her work is mainly directed towards the physico-chemical interactions involving the wine constituents and of their impact: (i) on the colloidal stability and quality of beverages; (ii) on the efficiency of the separation and stabilisation processes.

Talks

Neutrons and food: what are the problems?

Peter J. Lillford

University of York, UK.
Flagship Fellow CSIRO

Modern manufacturing practice and the requirements of consumers for novelty, means that we must understand more about how food components behave and what happens when they are assembled into the structures of finished foods. Without this, we can never design behaviour, but only rely on craft skills and the culinary arts.

How can Neutrons help?

By analogy with new knowledge obtained using industrial and synthetic materials, we can see the potential of the technique, but successful collaboration between physicists and food scientists depends on recognising the information that food science now needs, and the complexity of the raw materials it uses.

This introduction will examine some of the successes so far, involving small molecule solutes, biopolymers, colloidal systems and the ubiquitous solvent, water. Then we will examine what else might be done. The results from neutron scattering often require the development of alternative models before interpretation is possible.

It is obvious therefore, that the best food systems to be studied will be those to which other supporting measurement techniques can, or have been applied.

Some examples will be given.

Neutron scattering - a natural tool for food science and technology research

Elliot Paul Gilbert

ANSTO Bragg Institute, Locked Bag 2001 Kirrawee DC NSW 2232

The application of neutron scattering methods to understand the structure and dynamics in soft-condensed matter has a long history but until relatively recently, food-based systems were perceived to be too complex. However, the presence of higher flux facilities, with access to extended spatial and energy ranges, combined with greater computing power and enhanced modelling methods, have enabled food-based problems to be properly addressed.

Furthermore, the application of neutrons offers significant advantages over other avenues of characterisation, not only through contrast variation methods, which will be discussed, but also the highly penetrating nature of neutrons facilitates the ability for the radiation to be transmitted through complex sample environments.

The latter opens the opportunity to study industrially-relevant processes in real time. Understanding the structure and dynamics at the nano and atomic scale is essential to explain the macroscopic behaviour and functional properties in food. This talk will demonstrate how such methods can be employed with some recent examples [1].

References

[1] Neutron Scattering: A Natural Tool for Food Science and Technology Research, Amparo Lopez-Rubio and Elliot Paul Gilbert, *Trends in Food Science and Technology* 20 (2009) 576-586.

Quantitative models of casein micelle structure derived from SAXS and SANS

Carl Holt[1], **Kees de Kruif**[2], **Kazuhiko Kawasaki**[3]

[1] Department of Biochemistry and Cell Biology, FBLS, University of Glasgow, Glasgow, G12 8QQ.

[2] NIZO Food Research, P.O. Box 20, 6710 BA Ede, The Netherlands.

[3] Department of Anthropology, Pennsylvania State University, University Park, Pennsylvania 16802, USA.

Caseins are members of a paralogous group of secreted calcium-binding phosphoproteins (SCPPs) with a natively unfolded, or *rheomorphic*, conformation. SCPP genes are mostly found close together on the same chromosome with relatively well conserved synteny and exon structure. Nevertheless, orthologues tend to have divergent primary structures.

The Ca-sensitive caseins comprise sequences encoded by 4 types of functional exons, namely signal peptide (SP), phosphate centre (PP) and Pro-, Gln-rich (PQ) peptides, usually separated by one or more flexible linker peptides (LPs). When classified in this way, all Ca-sensitive caseins contain1 SP, 0-3 PCs, a more variable number of LPs and 1 or 2 PQs. Casein peptides containing a PC but no PQ sequence, can sequester amorphous calcium phosphate to form a core-shell structure called the calcium phosphate nanocluster (CPN).
The CPNs are equilibrium particles of defined composition and size with a calcium phosphate core radius of about 2.4 nm.

On the other hand, PQ-containing SCPPs such as ODAM and amelogenin have a tendency to undergo self association driven by the formation of backbone H-bonded cross-β-sheets, resulting in amyloid fibres or plaque. In the caseins, these two characters are combined and controlled to produce colloidal, polydisperse, more-or-less spherical, particles called casein micelles. One other SCPP gene known to be involved in aspects of the control of mineralisation also has a PC exon and exons for PQ sequences suggesting that analogous colloidal structures formed from non-casein proteins might also exist.

It was once accepted that electron microscopy and SANS both showed that casein micelles were formed from protein subunits called submicelles with a diameter of about 18 nm. More recent electron microscopy and SAXS has shown only substructure of 4-6 nm size attributed to the micellar calcium phosphate.

The SANS feature at 0.35 nm^{-1} in D_2O-rich solvents was the principal support for the submicelle hypothesis but a simple calculation showed that it could alternatively arise from an interference effect from scattering between the nanoclusters, This suggestion will be developed to show how all the main features of SAXS and SANS curves and their variation with solvent and temperature can be fitted together in a coherent way by regarding the casein micelle as an assembly of hundreds of nanocluster-like structures.

The calcium phosphate nanocluster provides a link between the present function of caseins in milk and the antecedent function of certain related phosphopeptides able to sequester amorphous calcium phosphate in other biological fluids and tissues. The ancient function of a nanocluster-forming SCPP, dating back to the late Cambrian and marking a singular point in evolution, allowed soft and mineralised tissues to co-exist in the same organism with relative ease.

The function of the larger structure of the whole micelle is less clearly established but comparative studies with other SCPPs and their various roles in the control of calcification may help us to understand why casein micelles and not calcium phosphate nanoclusters are found in milk.

Protein-lipid interactions at model membrane architectures

Ann Falk[1], **Camille Loupiac**[2], **Ingo Köper**[1,3]

[1] Max Planck Institute for Polymer Research, Mainz, Germany

[2] Equipe EMMA, Université de Bourgogne, Dijon, France

[3] School of Chemical and Physical Sciences, Flinders University of South Australia, Adelaide, Australia

Protein-lipid interactions play an important role in a variety of fields, for example in pharmaceutical research, biosensing or in food science. However, the underlying fundamental processes that govern the interplay of lipids and proteins are often very complex and are therefore studied using model systems.
We have investigate the interactions between a model protein from milk, beta-lactoglubulin with model membrane architectures, both at the air-water interface as well as using solid supported lipid bilayer membranes.

The interfacial properties of the protein have been studied using a variety of surface analytical techniques, ranging from surface Plasmon spectroscopy to neutron reflectivity.

We could gain valuable information on different parameters, which influence protein-lipid interactions, namely the charge and the type of the lipids, the composition and structure of the membranes and the pH value of the surrounding media.

Co-adsorption of β-casein and calcium phosphate nanoclusters (CPN) at hydrophilic and hydrophobic solid-solution interfaces studied by neutron reflectometry

David Follows[1], **Carl Holt**[2], **Robert K. Thomas**[1], **Fredrik Tiberg**[3,4], **Tommy Nylander**[4]

[1] Physical and Theoretical Chemistry Laboratory, University of Oxford, Parks Road, Oxford OX1 3PJ, UK

[2] University of Glasgow, Department of Biochemistry and cell biology, Glasgow G12 8QQ, Scotland, UK

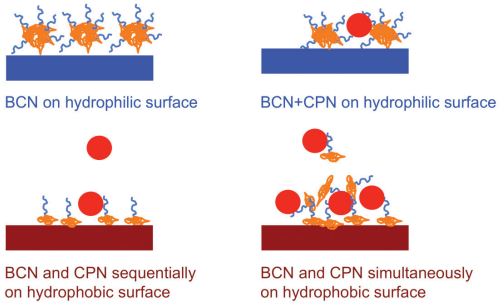
[3] Camurus AB, Ideon Science Park, Gamma Building, Sölvegatan 41, SE-223 70 Lund, Sweden

[4] Physical Chemistry, Department of Chemistry, Lund University, Box 124, S-221 00 Lund, Sweden

Neutron reflectometry was used to study the co-adsorption of calcium phosphate nanoclusters (CPN) and β-casein at hydrophobised and hydrophilic silica-water interfaces. The structural characteristics of the adsorbed layer were determined from neutron reflectivity curves to which multi-layer optical models was fitted.

The results showed that the calcium phosphate nanoclusters profoundly affected the rate of adsorption and structure of the interface compared to the adsorption of β-casein alone and for the hydrophobic interface the effects depended on the point at which the nanoclusters were added.

The main effect of the CPN is that the layer seems to be more compact, more irreversibly bound to the surface, and less susceptible to a selective proteolytic enzyme (endoproteinase Asp-N). It is proposed that the nanoclusters become surface active because whole β-casein molecules can replace one or more of the hydrophilic peptides in the shell of the nanoclusters.



Schematic illustration of the co-adsorption of BCN and CPN

Neutron scattering, hydrogenous materials and nutraceuticals

John Katsaras

National Research Council, Canadian Neutron Beam Center,
Chalk River Laboratories, Chalk River, Ontario K0J 1J0, Canada

Neutrons are neutral, non-destructive subatomic elementary particles found in all atomic nuclei, except hydrogen. Of particular interest, and unlike X-rays, is that neutrons interact strongly with nuclei, and the strength of their interactions varies dramatically and non-monotonically from element-to-element across the periodic table. This statement applies equally to isotopes of the same element (e.g. substitute ^{1}H for its isotope ^{2}H), and has been used to great advantage by biologists and polymer scientists who are typically studying materials inherently rich in hydrogen.

Over the past decade we have developed and characterised, using small-angle neutron scattering (SANS), the various morphologies assumed by the popular "bicelle" lipid mixtures as a function of lipid concentration and temperature. Of particular interest has been the spontaneous formation of unilamellar vesicles (ULVs). These self-assembled nanoscopic particles have attractive features which make them ideal for commercialisation as pharmaceuticals, contrast imaging agents and nutraceuticals.

These features are: (1) they are inexpensive, as they are made up exclusively of commonly available, low-cost phospholipids; (2) they can be functionalised (i.e. targeted); (3) they are highly stable providing long shelf-life and integrity when introduced into the body (i.e., extended circulation half-lives); and (4) they are easily adaptable to industrial scale production. The latter two features are distinct advantages over similar nanoparticles produced by traditional extrusion and sonication methods. An overview of neutron scattering in relation to hydrogenous nanoscopic particles of interest to food scientists/technologists will be the focus of the presentation.

The effect of water on structuring of organic phases by mixtures of β-sitosterol and γ-oryzanol

Wim G. Bouwman[1], **Vasyl M. Haramus**[2], **Arjen Bot**[3], **Ruud den Adel**[3], **Eckhard Flöter**[3], **Hassan Sawalha**[4], **Paul Venema**[4]

[1] Department Radiation, Radionuclides & Reactors, Faculty of Applied Sciences, Delft University of Technology, Mekelweg 15, NL-2629 JB Delft, The Netherlands

[2] GKSS Forschungszentrum Geesthacht GmbH, D-2054 Geesthacht, Germany

[3] Unilever Research & Development Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands

[4] Laboratory of Physics and Physical Chemistry of Foods, Department of Agrotechnology and Food Sciences, Wageningen University, Bomenweg 2, 6703 HD Wageningen, The Netherlands

The structure of oil-continuous products, such as margarine or butter, is based on a network of small crystallites of triglycerides (also known as triacylglycerols or TAGs). Surprisingly, few alternative structuring routes for food grade oils are known that are not based on the crystallisation behaviour of fatty acids [1]. One of the rare exceptions is the mixture of γ-oryzanol with β-sitosterol, which self-assembles in a helical ribbon, thus creating tubules with a diameter of ~7 nm and a wall thickness of ~1 nm. The tubules can aggregate to form transparent gels in triglyceride oils [2].

The mixture has also been used to form solid water-in-oil emulsions. The presence of water, however, modifies the assembly of the tubules in these systems. Small-angle X-ray scattering (SAXS) indicates that water interferes with the molecular ordering in these systems, leading to structures that give rise to 'normal' crystallographic reflections (as opposed to the scattering signal from the tubules). These different and bigger building blocks show complex phase behaviour as a function of temperature, and the processing conditions required to make a stable emulsion prove therefore rather critical.

Preliminary neutron scattering experiments at GKSS have been performed on emulsions containing sitosterol+oryzanol, water and decane to elucidate the structure of these tubules in organic phases further. Below is the SANS of the gel in deuterated decane.

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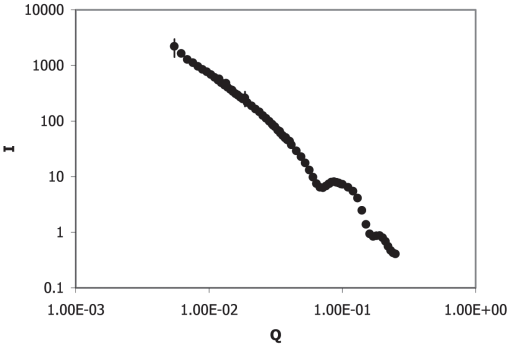

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Nanoaggregates of bile salt and cationic surfactant

Jitendra P. Mata[1], **P. Bahadur**[2] and **Elliot P. Gilbert**[1]

[1] ANSTO Bragg Institute, Locked Bag 2001 Kirrawee DC NSW 2232
[2] Department of Chemistry, V. N. S. G. University, Surat, Gujarat, 395007, India

The aim of this work is to study the interaction between a cationic surfactant and bile salt and so create the better understanding of the bile salt activity and its control in lipid digestion. Cationic surfactants are extensively used in many applications and formulations. Bile salts are negative charged, weak surface-active derivatives of cholesterol [1] and known for their role in lipid digestion [c.f.2]. The mixtures of bile salts with other amphiphiles are known to form mixed micelles [3] and liquid crystalline phases [4].

In our work the micellar characteristics of cetyltrimethylammonium bromides (CTAB) + sodium cholate (NaC) systems in the absence and presence of salts like NaBr, KBr and NaCl has been studied. Dynamic light scattering (DLS), small angle neutron scattering (SANS) along with viscosity has been use to understand the aggregation process. CTAB and NaC exhibit a strong interaction resulting in a mixed micellar system.
Our studies show the changes in the size of the mixed aggregates of the CTAB and NaC induced by the variation in temperature, salt counter ion, and salt concentration.

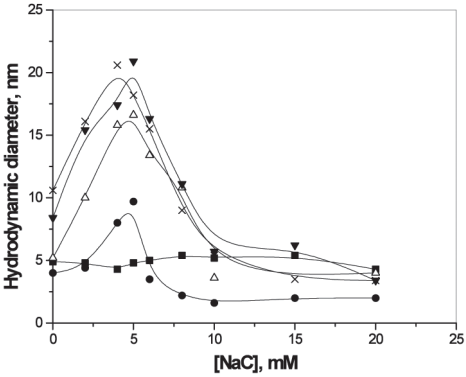

Figure 1
Hydrodynamic diameter of CTAB (25 mM) as a function of NaC concentrations in presence of NaBr at 25 ºC. [NaBr] (■) 0.0 M, (•) 0.05 M, (Δ) 0.10 M, (▲) 0.25 M, (X) 0.50 M

The addition of NaBr to this mixed micellar system results in the growth of extended aggregates. This growth occurs at lower NaC concentration (3 mM)

continued next page →

resulting in a peak in the aggregate size and in the viscosity of system. Further addition of NaC to the solution yields smaller micelles (Fig. 1). This behaviour is more predominant at low temperatures.

We also studied the effect of other salts (KBr and NaCl) to investigate the counterion effect on the structural transition. Br^- ion shows strong growth compare to other counterions. A small shift in the peak position (3 mM to 5 mM) has been observed for NaCl. The structural transition of the mixed micelles is believed to depend on the localisation of the NaC molecule within the micelles along with screening effect of salt.

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Neutron scattering studies of food structure: gelation, complexation and the effect of high pressure

R. Hans Tromp

NIZO food research, Kernhemseweg 2, 6718 ZB Ede, The Netherlands

Neutron scattering, in particular at small scattering angles, is well suited for contributing to the understanding of food structure. The distances probed match the size of typical supramolecular structures, such as micelles and polymer complexes found in food. When H2O is replaced by D2O, neutrons have a penetration depth which exceeds that of light and X-rays, and offer therefore an advantage for the study of turbid food systems.

In this presentation an overview of small angle neutron studies on the structure of casein micelles and casein gels, both in the classical set up and using the spin echo (SESANS), will be given. In addition, investigations by neutron scattering of the structure of solutions of polysaccharides under high pressure, and complexed systems of polysaccharide and proteins (coacervates) will be discussed.

The sweet taste of neutrons

Susana Teixeira[1,2]

[1] EPSAM, Keele University, Keele, Staffordshire ST5 5BG, UK
[2] Institut Laue Langevin, 6 rue Jules Horowitz, 38042 Grenoble cedex 9, France

Humans have a sweet tooth. Our environment and habits changed dramatically from that of our primate ancestors, yet we are still hardwired to seek food with high energetic content. The exponential growth in the number of people suffering from obesity, diabetes, and a number of diseases related to the consumption of sugar is a major health concern. The current alternatives to sugar are predominately small synthetic sweeteners, which can have severe side effects.

Food biotechnologists now face a major challenge to engineer healthier, stable sweeteners that can be used in food, drinks and pharmaceutical applications. A number of taste receptors have been identified in the last decade, including the sweet taste receptor. When activated by binding to a sweet compound, receptors present in the taste bud cells trigger a signal that is ultimately interpreted as sweet taste, but the precise mechanism through which this happens for sweet proteins is unknown.

Extremely sweet proteins can be isolated from tropical fruit extracts: a number of them have been studied but few have reached the market. Expensive costs of production and instability at certain pH and temperature conditions have limited their industrialisation. Thaumatin, a sweet protein from a west African plant, has been approved for consumption in many countries. It is mostly used as a flavour modifier. The structural basis for its sweetness, and that of any other sweet proteins, is unknown.

Current evidence suggests that thaumatin triggers taste receptors through a large surface of interaction, where the electrical charge distribution is believed to be a major factor. Sweetness will therefore be strongly mediated by solvent and charge distribution in the protein structure.

These biophysical properties require accurate information on the structure down to the level of the positions of hydrogen atoms, the smallest found in biological samples. This is not easily done for biological molecules with most current techniques, either due to low sensitivity to hydrogen or difficulties found in sample

preparation. Recent developments in neutron sources and instrumentation, along with advances in preparation of isotope labeled samples, have brought biological samples into the realm of high resolution neutron studies.

Preliminary studies on the feasibility of using deuterium labeling and neutron crystallography at the Institut Laue Langevin to fully characterise protonation states, water molecule orientations and solvent accessibility of the sweet protein thaumatin have proven successful [1,2]. The results will be described and further studies will be discussed.

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Puroindoline binding to lipids and its relation to wheat endosperm structure

Luke Ashley Clifton[1], **Rebecca Green**[2] and **Richard Frazier**[3]

[1] ISIS Spallation Neutron Source, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0QX, UK

[2] Reading School of Pharmacy, University of Reading, PO Box 226, Whiteknights, Reading, RG6 6AP, UK

[3] Department of Food and Nutritional Sciences, University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP, UK

Wheat (*Triticum spp*) is the third largest produced cereal crop after Maize and rice. Puroindolines (Pins) are basic, amphiphillic and lipid binding proteins of ~13kDa, which posses unique Trp rich domains, which are fully conserved in Pin-a (WRWWKWWK) and partially in Pin-b (WPTKWWK). These seed defence proteins have been linked to the occurrence of soft texture in common wheat (*Triticum aestivum*) endosperm.

Endosperm texture (defined as soft or hard) is the key quality trait which defines the end use of wheat flour. We have examined the lipid binding behaviour of Pin-a, Pin-b+ and two Pin-b types from hard wheat varieties which contain single point mutations on the Trp rich domain of this protein, namely Pin-bH (Gly46 to Ser46 mutant) and Pin-bS (Trp44 to Arg44 mutant). This study aimed to examine the relationship between Pin-lipid binding and endosperm texture.

Neutron Reflectometry (NR) and Brewster Angle Microscopy (BAM) were used to characterise Pin-b adsorbed condense phase monolayers of anionic 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DPPG) [1]. NR profiles and BAM images of Pin-b+, Pin-bH and Pin-bS adsorbed monolayers showed that more Pin-b+ is able to penetrate the acyl chain region and disrupt the lipid domain structure of condense phase anionic films of DPPG than either Pin-bH or Pin-bS.

This data supported surface pressure and surface infrared measurements [2], suggesting that hard wheat mutations on the Trp loop of Pin-b change the interaction of this protein with the anionic lipid monolayers. Data also indicated that Pin-a is unable to penetrate the lipid acyl chain region of DPPG monolayers but shows high penetration of the lipid head group region. Interestingly, small

angle neutron scattering data showed that this protein spontaneously forms unique large prolate protein micelles in solution, suggesting surfactant like behaviour in solution.

These findings provide evidence towards the biochemical mechanism of wheat endosperm texturing suggesting a link between the composition of the Trp rich loop of Pin-b and Pin-lipid binding in determining this important wheat quality trait and also yields key insights into the relationship between protein composition, solution structuring and protein-lipid interactions.

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Protein structure (SANS), water and protein dynamics (elastic and inelastic neutron scattering), and protein-lipids interface (neutron reflectivity)

How neutron scattering experiments can target the behaviour of model food proteins?

Camille Loupiac

Equipe EMMA, Université de Bourgogne, Dijon, France

The food scientist is commonly confronted with the challenge of modifying the formulation of a food product. The objective may be to enhance the taste, texture or appearance of the food, to produce a product with a longer shelf-life or a healthier image, or to improve manufacturing efficiency by incorporating a cheaper ingredient or adopting a new processing technology. The speed with which these objectives can be accomplished depends on the level of fundamental understanding that exists on the key physico-chemical factors affecting products properties.

In the case of foods colloids, it is especially important to understand how the interfacial and aggregation behaviour of polymer constituents (polysaccharides, proteins, pectins etc) are affected by processing conditions (heat, drying, freezing, shear forces), or by molecular interactions with other constituents (fat, hydrocolloids, aroma, water etc). One of our goal is to improve insights into such factors by taking advantage of polymer science concepts and neutron scattering technique applications to such systems, to the systematic study of model food systems. Therefore we now have a strong collaboration between our research groups (ENSBANA, ILL, LLB, JCNS and Max Planck Institute) targeting by neutron scattering measurements, three different model systems:

1. **Interfacial behaviour** of pre-denatured whey proteins in solution: **neutron reflectivity**
   (P. Cayot, F. Cousin, I. Koeper)

2. Beta-lactoglobulin hydration: **thermodynamics** versus **dynamics**: differential scanning calorimetry and **incoherent neutron scattering measurements**
   (D. Champion, D. Russo, J-M. Zanotti)

3. Effects of **high pressure** on globular protein **structure** studied by **small angle neutron scattering**
   (M.S. Appavou, M. Bonetti, P. Calmettes)

Folding and dynamics of the digestive enzyme pepsin

Derek R. Dee[1], **Rickey Y. Yada**[1, 2]

[1] Biophysics Interdepartmental Group, and
[2] Department of Food Science, University of Guelph, Guelph, ON, Canada, N1G 2W1

Kinetically trapped and thermodynamically stabilised conformations of pepsin were characterised in order to relate stability with structure and dynamics. Pepsin initially forms as a zymogen containing an N-terminal, 44-residue prosegment (PS) domain, which is removed upon activation at acidic pH to yield native pepsin (Np). Using SANS and DSC it was observed that Np irreversibly unfolds at neutral pH and that a compactly folded denatured state (Rp) was more thermodynamically stable than Np [Dee, D. et al. (2006) *Biochemistry*, 45:13982]. It was found that the PS enhances the folding rate from Rp to Np by $\times 10^5$, stabilising the folding transition state by 14 kcal/mol. Upon catalyzing folding the PS is removed, the unfolding barrier increases and a kinetically trapped Np state remains suggesting a novel folding mechanism for pepsin [Dee, D.R., Yada, R.Y. (2010) *Biochemistry*, 49:365].

The internal dynamics of Np and Rp were studied using quasielastic neutron scattering (QENS), in order to compare the flexibility of these uniquely stabilised conformations. In the space-time window of the measurements (20 - 3 Å and 100 - 10 ps), both states displayed constrained diffusive motions of similar amplitude, although the motions in Rp occurred with less frequency, indicating that Rp was more rigid. Similarly, changes in Np dynamics when bound by pepstatin, an extremely tight binding inhibitor (K_i ~ 40 pM) were measured using QENS. It was found that the kinetically trapped Np is relatively flexible compared to the thermodynamically stable Rp, in contrast to results for another kinetically trapped enzyme, alpha-lytic protease [Jaswal, S.S., et al. (2002) *Nature*, 415:343], which was found to be highly rigid. Results of the above studies indicate that kinetic stabilisation is not correlated with global features, but instead may arise from local structural motifs. A mechanistic understanding of kinetic stability will be important for protein engineering.

Antioxidant-protein interactions in phospholipid membranes

Rachna Singh[1,2], **Laurence D. Melton**[1], **Jingli Zhang**[2], **David L. Worcester**[3], **Elliot P. Gilbert**[4], **Duncan J. McGillivray**[1,§]

[1] Department of Chemistry, The University of Auckland, Auckland, New Zealand
[2] Plant and Food Research Ltd., Auckland, New Zealand
[3] Division of Biological Sciences, University of Missouri-Columbia, Missouri, USA
[4] ANSTO Bragg Institute, Locked Bag 2001 Kirrawee DC NSW 2232
[§] Email: d.mcgillivray@auckland.ac.nz

Oxidative damage of cellular phospholipid membranes has been linked to a variety of disease pathologies, including cardiac disease, Alzheimer's and other ageing-related illnesses. The oxidation of unsaturated and polyunsaturated fatty acid chains found in membranes leads to alteration in their physical properties, which ultimately affect biological function and may lead to these diseases.

Polyphenols are naturally occurring phytochemicals present in a number of fruit and vegetables that are of interest for their anti-oxidative powers, for example in functional foods. These polyphenols inhibit lipid oxidation in cellular membrane surfaces, although the mechanism of this inhibition is not entirely clear. Moreover, the polyphenols have significant binding affinity for proteins, which can lead to the formation of soluble and insoluble protein-polyphenol complexes. Significantly, in the presence of casein proteins the oxidation inhibition the polyphenols in the membrane may be significantly enhanced. Thus the antioxidant pathway appears to involve these protein/polyphenol complexes, as well as direct antioxidant action by the polyphenol .

Here we discuss neutron and X-ray scattering results from phospholipid membranes, looking at the positioning of two examples of polyphenolic antioxidants in phospholipid membranes, quercetin and phloretin, the antioxidants' impact on the membrane organisation, and the interaction between antioxidant and extra-membranous protein. This information sheds light on the mechanism of antioxidant protection in these systems, which may be used to understand biological responses to oxidative stress.

Protein structure and interactions in the solid state

Joseph Curtis[1], **Hirsh Nanda**[1] and **Susan Krueger**[1]

[1] NIST Center for Neutron Research, NIST, 100 Bureau Drive, Stop 6102, Gaithersburg, MD 20899-6102

The role of freezing in food preservation is to prevent the growth of microorganisms and to slow chemical reactions to preserve the quality, nutrient content, texture, flavour and colour of foods. The food industry must change continuously in response to consumer demands for a greater variety of high quality, convenient products at economic prices. As such, traditional methods of food preservation such as freezing need continual improvements to keep abreast of advances in technology. Thus, the freezing process and its affects on water, proteins, lipids, carbohydrates, vitamins and minerals in food must be well understood. For instance, it has been inferred in some cases that proteins frozen in ice are adequately stable, correctly folded, and not affected by the freezing or thawing process. However, experiments to probe the structure of proteins in water ice are limited. While many proteins maintain their secondary structure in ice, it is thought that cold-denaturation of proteins upon freezing occurs for many systems, thus leading to a reduction in viable protein or a coincident increase of protein aggregates.

SANS is uniquely qualified to study the structure of proteins in the liquid and solid phases that are biotechnologically relevant for proteins. We have studied a model protein, lysozyme, in both the liquid and water ice phases to determine its gross-structure, interparticle interactions and other properties. These properties have been studied under a variety of solution conditions before, during, and after freezing. We have also performed contrast variation experiments to determine which features of the scattering are from the protein and which are from the ice structure. Results for lysozyme at concentrations ranging from 1 mg/mL to 100 mg/mL in solution and water ice with NaCl concentrations ranging from 0M to 0.4M will be presented and implications for food science and engineering will be discussed.

Mobility in the vicinity of the glass transition: thermal and dynamical properties

D. Champion[1a], **C. Loupiac**[1], **M. Gumiero**[1], **D. Russo**[2], **D. Simatos**[1], **J.M. Zanotti**[3]

[1] Equipe EMMA, Université de Bourgogne, AgroSup Dijon - Dijon, France
[a] Correspondance: d.champion@agrosupdijon.fr
[2] CNR, INFM & CRS/soft, c-o Institut Laue Langevin, Grenoble, France
[3] Laboratoire Léon Brillouin, CEA-CNRS, Saclay, France

Knowledge of dynamical processes occurring in low hydrated biological materials is important to predict the preservation of their function or so-called “shelf life” of Glassy materials are particularly sensitive because of the disorder at a molecular level induced by immobilisation of the liquid structure. The dynamics which are still possible in the glass, such as mobility of short range order or residual water mobility play a crucial role, and may induce structural evolution during storage.

The aim of the present contribution is to explore the molecular mobility in the vicinity of the glass transition, by using several complementary techniques in order to explore different time scales; and to discuss the relative mobility between water and solutes. Measurements of thermal (differential calorimetry) and dynamic (neutron scattering) properties were compared for frozen sucrose solutions where most of the water is in ice form, and for beta-lactoglobulin mixtures at different water contents. The exchange of water for deuterium allows the distinction between the dynamic of solute and water. Our findings also showed the difference of protein stability as a function of co-solute (sucrose or sodium chloride) used before dehydration.

The temperature and moisture dependence of protein dynamics in glycinin: A quasi-elastic neutron scattering study

Anna V. Sokolova[§], **Gordon J. Kearley**, **Elliot P. Gilbert**

ANSTO Bragg Institute, Locked Bag 2001 Kirrawee DC NSW 2232
[§]E-mail: asl@ansto.gov.au

The soy protein, glycinin, has been studied as a function of moisture content due to its importance to the food industry and its range of application as an ingredient. Several techniques (SAXS, FTIR, differential scanning calorimetry (DSC) and ^{1}H and ^{13}C NMR) have been applied to investigate the structure, dynamics and moisture-dependence of the thermal transitions [1].

Further, additional detail has been gained by correlating the macroscopic transitions with the changes in dynamics on the nanoscale by the use of Quasi Elastic Neutron Scattering (QENS). This technique probes the details of the low-energy diffusive relaxation processes of the various hydrogen-bearing structural units of the protein on the ps to ns timescales. Previous experiments as a function of humidity have revealed that at room temperature the water and the protein appear to be almost immobile in the low-moisture (less 25%) phase and mobile in the high-moisture phase, but with no evidence of free water for moisture contain less then 35% within the accessible time domain.

In the present study we determine more accurately the moisture-level at which the change in the dynamics occurs and correlate this with the macroscopic transition, which provides additional information on the dynamics close to the transition. Also we not only determine activation energies for the various processes, but we find how these depend on moisture content. The latter is of central importance because the QENS data reveal that there is no separate relaxation of the chemically-distinct parts of the protein/water system, but a sub-diffusion of the system as a whole on this timescale, which seems to be characteristic of entanglement. It is difficult to understand the details of this relaxation for such a complex system, but it is clear that moisture plays a crucial role. We have described the complex non-linear dependence of relaxation processes on temperature.

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Need for neutron scattering techniques in packaging

Ross A. Lee

[1] PTIS™ Packaging and Integrated Solutions, LLC
[2] Chesapeake City, Maryland

Today's leading edge technology in packaging continues to demand the ability to reproducibly characterise nanocoatings, nanoreinforced materials and nanostructures. Applications include improved barrier for food quality over the life of a product, improved mechanical and thermal properties at the lightest possible weight, and the ability to sense and communicate with the consumer via sensors and printable electronics (batteries included). A brief overview of these areas will be presented emphasising the need for better ways to characterise and "see" this game-changing "nanoscape" that will be required to enable the advances expected in the future.

Utilisation of neutron scattering in microbiological food safety and quality

P. Michael Davidson and **Federico Harte**

Department of Food Science and Technology, 2605 River Drive, University of Tennessee, Knoxville, TN 37996-4591

Major goals driving current research, development and innovation in food microbiology include the rapid detection and control pathogenic microorganisms in foods, the identification and control microorganisms causing food spoilage and reduced shelf-life of products, and the development of probiotic cultures to improve health. Neutron scattering has potential to assist in understanding of the interaction of microorganisms with foods in several of these areas.

For example, in the area of food safety and spoilage, deuterium labeling could help in elucidating intracellular mechanisms and targets of action of intervention technologies at the sub-second and nano scales. Additionally, neutron scattering could assist in developing effective encapsulation procedures for antimicrobials and determining the effect of intervention procedures on cell membrane structure and morphology.

A new thrust in food microbiology is the study of biofilms nano-structure properties and their relationship to population dynamics on processing equipment and more complex living systems. Certain beneficial microorganisms have been extensively studied as probiotics. These microorganisms are often encapsulated for delivery. These techniques could assist in developing methods to enhance viability of probiotics during the encapsulation and trigger precise delivery processes and targets. Finally, neutron scattering has potential to answer basic questions about novel processing technologies such as high pressure by assisting in determining mechanism of inactivation and determining the influence of processes on quality or integrity of products in real time.

This presentation will focus on an overview of microbiological food safety and some specific examples of how neutron scattering might be used to advance investigations in selected areas of microbiological food safety. A discussion on determination of how best to utilise neutron scattering to answer questions in food microbiology safety and quality will follow.

Multi-scale structural characterisations of fatty acid multilayer tubes with temperature tunable diameter in bulk and at the air/water interface by coupling SANS and neutron reflectivity

A.L.Fameau[1,2],*, **F. Cousin**[2], **F. Boué**[2], **B. Novales**[1] and **J.P. Douliez**[1]

[1] Biopolymères Interactions Assemblages, INRA, rue de la Géraudière, 44316 Nantes, France.

[2] Laboratoire Léon Brillouin, CEA Saclay, 91191 Gif sur Yvette Cedex, France.

In the current context development of green chemistry for industrial applications, we have recently shown how to use specific counter-ions to solubilise in aqueous media long chains fatty acids and their hydroxylated derivatives from plants, which make them good candidates to form new classes of surface active agents. Here we report on the formation of multilayer tubular structures made of 12-hydroxy stearic acid (Figure 1) [1]. The ethanolamine salt of that fatty acid exhibits two interesting but unexpected properties: (i) the outer diameter of tubes Dtube is temperature-dependent and varies from 600nm to 5µm; (ii) foams made with these tubes are very stable.

To understand the temperature dependence of Dtube, we studied the effect of the nature of the counter-ion. We used various hydroxyalkylamines and obtained in all cases tubes with a temperature tuneable diameter, but the dependence is different for each counter-ion. We systematically measured the structural parameters (the bilayer thickness, the interlayer repeat distance) at different scales and thermodynamical parameters by coupling phase contrast microscopy, DSC and SANS as function of the temperature. The temperature influences the elastic fluctuations in the lamellar stack, which tunes Dtube at the micron scale. However, surprisingly, the structural evolution does not follow the thermodynamical phase transitions.

In order to understand why such systems yield extremely stable foams, we studied the behaviour of the multilayer tubes at the air/water interface by neutron reflectivity that provides the bilayer thickness and interlayer spacing. Remarkably, our results show that the multilayer tubes are adsorbed at the interface and the same temperature dependence of the Dtube as in bulk is observed.

In summary, although the mechanisms responsible for the increase of the tubes diameter remain yet to be understood, our exhaustive study allows us to obtain multilayer tubes with desired sizes, at a given temperature, by a simple change of the nature of the counter-ion. This can be done either in bulk or at an interface.

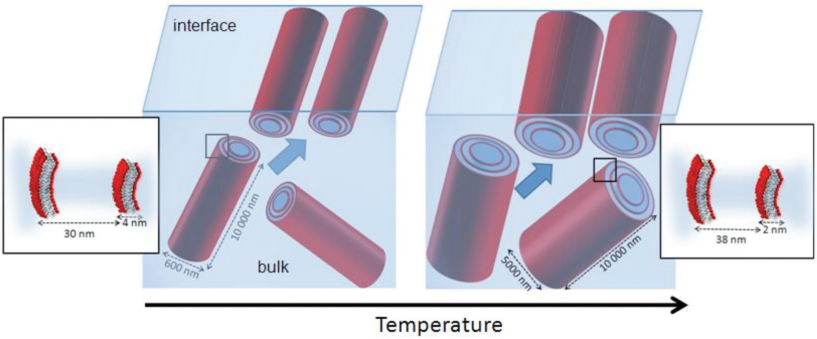

Figure 1:

Structure of the tubes in bulk and at the air/water interface as a function of the temperature.

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Bile physiology and physical chemistry in digestion: fundamental insights from small-angle neutron scattering

Rex P. Hjelm

Los Alamos Neutron Science Center, Los Alamos National Laboratory, Los Alamos, New Mexico, 87545, USA

Bile transports lipophilic materials, such as cholesterol, from the liver to the intestine. Once in the intestine, bile emulsifies dietary fats and aids in the action of lipases in hydrolysing dietary triglycerides and facilitates the adsorption of insoluble digestion products into the intestinal lumen.

Bile is a complex, mixed colloid of lipid carriers, the major component of which is phosphotidylcholine, and detergents, the bile salts. The bile salts consist of a cholesteric core, containing two or three hydroxy groups, and a hydrophilic, carboxylate head group, which in humans is commonly conjugated with either glycine or taurine. Physical chemical studies of the lipids-bile salts phase map using small-angle neutron scattering show that the particle morphology is extremely sensitive to its lipid and bile salt content. Thus, significant changes in the particle size and shape occur as the bile is formed in the bile duct, concentrated in the gall bladder and subsequently diluted again in the small intestine. At the highest concentrations the particles are globular mixed micelles with an overall size of 50 Å.

These elongate as the concentration is reduced, becoming rod-like with diameter about 50 Å with a radial core-shell structure with the core lipid fatty tails arranged radially and the head groups forming the shell. The bile salts are at the interface between the shell and core with the hydrophilic parts facing outward. At sufficiently low concentrations, as in the intestine, the mixed micelles transformed into single bilayer vesicles.

The introduction of the lipid hydrolysis product, monoolein, does not change this picture and points to a role of the bile salts in preserving the functional shapes. These results give insight on the physiological function of bile and on the rules governing the self-assembly of bile particles in the hepatic duct and the small intestine.

Starch granules under attack: Multidisciplinary investigation of structural mechanisms governing starch digestion

Jaroslav Blazek[1,2] and **Elliot Paul Gilbert**[1]

[1] ANSTO Bragg Institute, Locked Bag 2001 Kirrawee DC NSW 2232
[2] Food Futures Flagship, North Ryde Riverside Corporate Park, 5 Julius Avenue, North Ryde, NSW 2113, Australia

Starch is not only the most important carbohydrate in the human diet but is also a key ingredient in animal feed and has many industrial applications outside the food industry such as in paper manufacture, packaging and the production of biofuels. Therefore, understanding the factors that control the kinetics and extent of enzymic digestion of native starches is essential to ensure fitness-for-purpose for such a diverse range of end uses.

Most *in-vitro* studies of granular starch digestion have been limited to samples for which aliquots have been removed from the reaction mixture at various time intervals and freeze-dried to be subsequently characterised using a range of techniques. In this study, we report the first neutron scattering study of enzymic digestion of granular starch in which data have been collected in real-time and in-situ. We have utilised a range of complementary powerful analysis techniques such as X-ray diffraction, small-angle X-ray scattering, differential scanning calorimetry and microscopy to determine the structural changes of six commercial starches of varying crystal structure and amylose content over six orders of magnitude in spatial resolution.

We find that in the course of digestion, the lamellar peak intensity gradually decreased and low-q scattering increased and these trends were more substantial for A-type than for B-type starches. These observations may be explained by preferential digestion of amorphous growth rings.

Consideration of the changes in the molecular densities of the three granular regions in the course of digestion, alongside a range of other characteristics among starches from different botanical origin indicates that the enzymic susceptibility is not determined by the lamellar nanostructure of the semi-crystalline growth ring but, to a great extent, by the opportunity of access through granular pores and channels.

Colloidal interactions involving condensed tannins in diluted systems: what problems can we solve through SANS?

Aude Vernhet[1], **Céline Poncet-Legrand**[1] and **Bernard Cabane**[2]

[1] UMR 1083 Sciences for Enology, INRA - Montpellier SupAgro- UM1, 2, place Pierre Viala, F 34060 Montpellier, France.
[2] PMMH, UMR 7636 CNRS-ESPCI-P6-P7, 10 rue Vauquelin, 75231 Paris cedex 05, France.

Physico-chemical interactions, occurring in solutions or at interfaces, take a primary part in wine stability, clarification and taste. They can lead to the formation of stable/metastable assemblies or induce hazes and precipitates, detrimental to wine quality. They also influence the efficiency/selectivity of treatments applied to ensure wine clarity and stability.

The part played by such interactions in the overall organoleptic perception of wines is still unclear, but it is usually admitted that colloidal equilibria are decisive. An improved understanding of these interactions is thus a key point to: (i) elucidate the relationships between wine composition, organoleptic properties and colloidal stability; (ii) develop new processes and design low fouling-materials for wine elaboration and stabilisation. The wine constituents primarily involved in colloidal phenomena are polyphenols, polysaccharides and proteins.

This presentation will focus on interactions involving polyphenols in solutions, and more especially flavan-3-ol polymers (condensed tannins) that constitute with anthocyanins the major polyphenols in red wines. To study flavan-3-ol interactions it was first essential to determine their conformations and their colloidal behaviour in aqueous solutions. Using Dynamic light scattering (DLS) experiments coupled with separative techniques we measured the incidence of flavan-3-ol structure and solvent conditions on their solubility and colloidal stability, and we determined the phase diagrams for flavan-3ol solutions in water-ethanol solutions.

We present structural information (conformations in solution, overall dimensions, shapes) for condensed tannins and aggregates, obtained through small angle X-ray and neutron scattering (SAXS and SANS). Information given by these studies will be discussed first. We also report the use of DLS and SAXS to investigate (i) the respective ability of wine polysaccharides to prevent flavan-3-ol aggregation; (ii) interactions between flavan-3-ols and proline-rich proteins. Finally we discuss how the use of SANS with the contrast variation method could help us in identifying complex or aggregate structures.

Posters

Neutrons and X-rays reveal the cause of a rotting issue

S.Ali[1,2], **S.Teixeira**[1,3], **R.W. Pickersgill**[2]

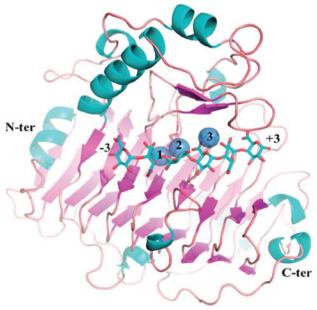
[1] Deuteration Laboratory, Institut Laue-Langevin, 6 Rue Jules Horowitz, BP 156, F-38042, Grenoble Cedex 9, France

[2] School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, UK, E1 4NS

[3] EPSAM, School of Physical and Geographical Sciences, Keele University, Keele, Staffordshire, ST5 5BG

Bacillus subtilis Pectate lyase (BsPel) is a secreted microbial enzyme that plays a pivotal role in 'plant pathogenesis. It is a potent virulence factor of pathogenic bacteria causing soft-rot disease and affecting a wide range of crops. Soft rot disease is one of the most severe post-harvest diseases of potatoes worldwide. BsPel acts by breaking down the very stable pectin network, a major component of the plant cell wall, via a β-elimination reaction. A proposed catalytic mechanism by Pickersgill et al. (Seyedarabi et al., 2010) features a conserved arginine acting as a base at pH 8 and three calcium ion binding sites.

At present the major mechanistic question is the protonation state of this active site arginine, which at physiological pH (7.0) is expected to be protonated. For it to abstract a proton to initiate the reaction is therefore quite unusual and likely to result from a local shift of pKa that has yet to be proven. A number of approaches have been used to study the structure of BsPel and the active site residues in particular. We have produced perdeuterated BsPel, crystallised it, and collected both Neutron and X-ray data on the same crystal sample. The results of a joint X-ray/neutron crystal structure refinement will be presented and the conclusions will be discussed.



Above: Cartoon representation of the parallel β-helix architecture of BsPel with β-strands and α-helices represented as magenta arrows and cyan helices, respectively. The cyan/red liquorice bonds represent bound hexasaccharide (pectin I). The oligosaccharide binds to the surface of the β-sheet known as PB1. The reducing end of the hexasaccharide binds toward the C-terminal end of the parallel β-helix. The three bound calcium ions are shown as blue spheres.

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A neutron scattering study of acid-induced casein micelle aggregation

S. Botegård[1,2,§], **T. Nylander**[2], **M. Paulsson**[2], **K. Schillen**[2], **W. Bouwman**[3], **J. Plomp**[3]

[1] European Spallation Source, Sweden;
[2] Lund University, Sweden;
[3] Delft University of Technology, The Netherlands
[§] Email: sofie.botegard@esss.se

Neutron scattering is an ideal tool to use when studying complex dispersed systems such as milk. This is due to neutrons ability to penetrate solutions that are not transparent for light and the fact that hydrogen scatters neutrons, and that the scattering is different from that of deuterium. This makes neutron scattering a particularly important tool within life science.

This work aims to relate the nano- and microscopic structures formed in milk based products to macroscopic effects such as gel strength and syneresis. For this purpose we used SESANS (Spin-Echo-Small-Angle-Neutron-Scattering), which allows following the formation of structure at a wide length scales from 10nm-20mm. The objective of the current study is to reveal the aggregation process of casein micelles caused by a decrease in pH. A convenient way to achieve this decrease in pH is to add GDL (glucono-δ-lactone). This compound discomposes with time, which causes a decrease in pH and the kinetics of this process is depending on the concentration of GDL.

A better understanding of the gel network formed during acidification is important for a range of food products, like yoghurt and cheese. To improve the quality of the casein gel network, whey protein can be added and in this study we therefore investigated the effect of whey protein on the gel network structure. To capture the process in the native casein system and thereby avoid the effect of heat treatment, raw milk was used rather than milk reconstituted from milk powder.

At milk pH the SESANS result was interpreted as a stabilised casein micelle size of 0.3mm in diameter, and as the pH decreased (due to the added GDL) casein micelle aggregates are formed and their size steadily increased up to 15mm. The results also showed that whey protein strongly promote gel formation and stability of the formed aggregates.

Spin-echo small-angle neutron scattering for the study of food systems

Wim G. Bouwman[1], **Jeroen Plomp**[1], **Chris P. Duif**[1], **Franck P. Duval**[2] and **Arjen Bot**[2]

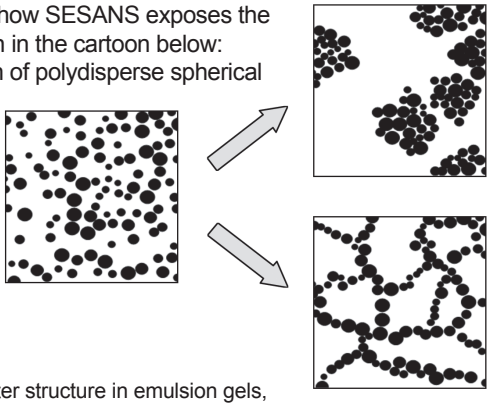
[1] Department Radiation, Radionuclides & Reactors, Faculty of Applied Sciences, Delft University of Technology, Mekelweg 15, NL-2629 JB Delft, The Netherlands

[2] Unilever Research & Development Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands

Systems of practical relevance to the food industry are often hard to investigate non-invasively. This is caused by the fact that most food emulsions are opaque and soft materials. The relevant length scales are often micrometers. Spin-echo small-angle scattering (SESANS) operates at these length scales and benefits from the high penetrating power of neutrons [1,2]. SESANS yields directly the scattering length density correlation function, which facilitates visual data-analysis [3].

With SESANS we investigated the fat droplet structure of different emulsion gels after storage at fixed temperature or after temperature cycling. Upon temperature-cycling, it is found that the fat droplet clusters increase in size, next to the droplets themselves getting larger as well.

We present a basic model to show how SESANS exposes the processes occurring in the emulsion in the cartoon below: The emulsion is initially a dispersion of polydisperse spherical fat droplets. After cycling they can aggregate into larger droplets as indicated in the higher cartoon, or into network, as indicated in the lower cartoon. The difference in structure comes out clearly in the density correlation function (as measured by SESANS).



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A soluble nanoscale self-assembled complex from starch, protein and lipid for healthy nutrient delivery

D. Bhopatkar, **A. Shah**, **G. Zhang**, **O.H Campanella** and **B.R. Hamaker**

Interaction among food ingredients significantly affects nutritional and sensory quality of food products. Evidence of interaction between starch, protein and fatty acid and their ability to produce a self assembled nano-scale complex was first reported by our laboratory. The complex produced from interaction of normal corn starch, whey protein and different fatty acids was identified by an unusual increase in the cooling peak viscosity determined by rapid visco-analyzer (RVA). Viscosity and viscoelastic properties of the complex were also determined in a pasting cell attached to a rheometer.

Characterisation of the complex was performed by size exclusion chromatography. SEC-MALS data indicate that the complex has a molecular weight in the range of 6-8 million Da with a radius of gyration 20 to 70 nm. The fact that it is a water soluble complex makes it a valuable vehicle for nutraceutical delivery. However, various aspects of this nano-scale complex are still unresolved and need further investigation. Some of them are, possibility of improving complexation for its industrial application, effect of salt on the complexation, driving forces involved in formation and stabilisation of three component complex, and potential for carrying a fourth valuable hydrophobic constituent.

In this work we investigated properties of the nanocomplex during its formation in a rheometer. Formation of the nanocomplex was performed several fatty acids (oleic, palmitic, linoleic and conjugated linoleic acids). The effect of protein concentration, shear rate and temperatures were evaluated.

The effect of salt and ionic strength on the complexation was also evaluated for its potential application in real food systems. The three component complex was prepared in a diluted system by mixing dialyzed supernatant from high amylose corn starch (50% amylose), dialyzed b-lactoglobulin and different fatty acids in a 20:2:1 weight ratio. Solutions of 3 different kosmotropic anions (SO_4^{2-}, Cl^-,CH_3COO^-) and a chaotropic anion (SCN^-), ranging from 0.2mM to 1mM, were added into the mixture during complex formation. Structural properties of the complex in presence of various anions were elucidated using size exclusion chromatography and multi angle laser light scattering attached to the SEC system. Rheology was used to further investigate the properties of the ternary complexes prepared with the different salts.

Results indicated that high amylose corn starch provides a viable alternative to achieve higher complexation, thus enhancing the feasibility of industrial application. Absence of ions does not hinder the formation of the three component complex. Increase in concentration of the kosmotropic anions resulted in increased molar mass, hydrodynamic radius and compact structure of the ternary complex. However, increased concentration of chaotropic anions resulted in a complex with loose structure. We hypothesise that increase concentration of kosmotropic salt promotes increased involvement of individual components in the complex resulted in a more compact structure. The ability to alter the structural property of the complex provides an opportunity to manipulate the carrying capacity of the ternary nano-complex and enhance its functionality.

A micelle forming protein; Puroindoline A, which shows adsorption behaviour in the presence of phospholipid membranes

M Sanders[1,2], **Luke Clifton**[3], **Richard Frazier**[2] and **Rebecca Green**[1]

[1] Reading School of Pharmacy, University of Reading, PO Box 226, Whiteknights, Reading, UK, RG6 6AP.

[2] Department of Food and Nutritional Sciences, University of Reading, PO Box 226, Whiteknights, Reading , UK, RG6 6AP

[3] ISIS Spallation Neutron Source, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK, OX11 OQX

Integral membrane proteins have a tendency to aggregate in solution, which unlike the ordered hydrogen bond molecular arrangements found in crystals is at least partly driven by electrostatic interactions in exposed hydrophobic regions. Surfactants show similar behaviour in solution however these small amphiphillic molecules form uniform and well-ordered structures that are known as micelles. Several small proteins and peptides that are membrane or membrane associated have amphiphillic charge distribution that is similar to the charge distribution found in lipids and surfactants, however they do not regularly show the spontaneous formation of a micellular structure. Here we describe the presence of a protein micelle for the lipid binding protein Puroindoline A that also disassembles and inserts in the presence of a charged lipid model membrane.

Using a wide range of analytical techniques we have shown that Puroindoline A spontaneously forms uniform prolate micelles in solution; which then disassembles when exposed to non-ionic surfactants and anionic phospholipids. Using Small Angle Neutron Scattering (SANS) and Dynamic Light Scattering (DLS), we have characterised the low-resolution structure of the aggregate, as well as the disruption of the micellular structure by the non-ionic surfactant dodecyl-b-maltoside.

Previous studies using surface pressure measurements have described Pin A adsorption at the air/liquid interface however using neutron reflection we have shown that Pin A not only adsorbs to the surface but also inserts into a floating lipid monolayer. Furthermore using Attenuated Total Internal Reflection Infrared Spectroscopy (ATIR-FTIR) in conjunction with a planar bilayer model we have shown that Puroindoline A also affects the order of the phospholipid in a bilayer structure.

Attraction versus repulsion: from proteins to model colloids - and back?

Kitty van Gruijthuijsen, **Peter Schurtenberger**, **Anna Stradner**

Adolphe Merkle Institute, University of Fribourg, CH-1723 Marly, Switzerland

A way to control food's complexity is to break it down into its smallest constituents, to understand the behaviour of the individual components, and then start mixing those again. Globular proteins – often referred to as 'biocolloids' in the colloid community – represent an important class of food ingredients. There are several examples of proteins that show typical colloidal features like simple hard spheres behaviour, or the appearance of a temperature dependent short-range attraction. In addition they can carry pH-dependent residual charges, which result in repulsive interactions whose range can be tuned by the ionic strength.

Depending on the exact nature of the resulting interaction potential protein suspensions exhibit a rich variety of different phases and states. We can thus observe fluid-fluid phase separation and gelation [1], stable cluster phases [2] or equilibrium gels [3] to name only a few. To study the influence of the individual contributions to the interaction potential on the structural and dynamic properties of the proteins in various phases, we use colloidal model systems with tuneable interaction potentials. We now work on an aqueous synthetic colloid/polymer-mixture, where we can vary the range and depth of the depletion-induced attraction and of the electrostatic repulsion. The colloids are an order of magnitude larger than typical proteins. This enables us to study their bulk structure with scattering techniques such as small-angle neutron scattering and diffusing wave spectroscopy.

Moreover, although at the resolution limit of microscopy, it enhances the information obtained from real space measurements. We validate the model system in the presence of only attractions or repulsions. We then gradually increase the range of the repulsion. We describe the phase behaviour at the transition from a shorter- to a longer-ranged repulsion compared the range of the attraction.

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Diffusing-wave spectroscopy investigation of sheared acid-milk gels

Y. Hemar[1], **A. Raudsepp**[2] and **K. W. Fenkel**[2]

[1] Chemistry Department, the University of Auckland, New Zealand
[2] Previously at School of Chemical and Physical Sciences, Victoria University of Wellington, New Zealand

Diffusing-wave spectroscopy (DWS) is a dynamic light scattering based technique, which exploits the multiple scattering of the scattered light. The technique is ideal for the study of the dynamics of turbid media such as milk. DWS measurements are fast (typically in the order of seconds) and the technique is non-invasive and non-intrusive. This makes DWS a suitable method to investigate turbid food materials when subjected to external stressors such as heat [1] or shear flow [2].

In this paper, DWS is used to study the mechanical behaviour of acid-milk gels as they undergo shear flow [3]. The measurements are performed using DWS in both the backscattering and the transmission configurations, and the acid-milk gels are sheared in a Couette or a cone-plate geometries attached to a conventional stress-controlled rheometer. At low shear rates, DWS measurements showed a high shear-band near the surface of the moving cone of the cone-and-plate geometry. At intermediate shear rates, a plug-flow with shear-band at the stationary plate of the con-and-plate geometry is observed. At very high shear rates the flow becomes homogeneous. These findings were confirmed by Nuclear Magnetic Resonance measurements on the same samples sheared in a Couette geometry. A mechanistic explanation based on the effect of shear on the microstructure of acid-milk gels is offered.

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Observing the thermal behavior of the self-assembling gels based on the molecular interaction at different temperatures

Kuo-Chih Shih, **Hsi-Mei Lai**

Department of Agricultural Chemistry, National Taiwan University

Polypseudorotaxane (PPR) is composed via the host-guest interaction that several beta-cyclodextrins (β-CDs) could thread onto one polymer chain. When Pluronic®, a copolymer of polyethylene glycol (PEG) and polypropylene glycol (PPG), is mixed with β-CD, the selective formation of PPR between β-CD and the PPG segments results in different assembled structures depending on the β-CD and Pluronic® concentrations.

At sufficient concentration, the PPR segments could act as cross-linking points and lead to gelation. PPR gels feature *in situ* gelling, time-dependent shear thinning and thermoreversible sol-gel transition, thus are potential to be used as injectable hydrogels for drug delivery or as carriers for bioactive compounds in food system. Also, our previous studies shows that the smaller the crystallites are, the higher gel strength and thixotropy of the PPR gels can be obtained.

However, the effects of molecular interactions on the thermal behaviour of PPR gels are not clear so far. Here, we suppose that the shape of PPR is worm-like and its length is proportional to the degree of complexation. The temperature corresponding to the maximum degree of complexation (T_{max}), dependent on the solvents and Pluronic® composition of PPR, is proportionally related to the sol-gel transition temperature (T_{tr}) of its PPR gel. Lower T_{max} occurs when the solvent contains citric acid, which inhibits the hydrogen bonding between β-CDs and PPR, or when the Pluronic® is with lower PG/EG ratio, which reduces the complexation stability.

However, β-CD based PPRs easily form large aggregates even at low concentrations and a relatively dilute solution of PPR is required in case that the PPRs self-assemble with each other. Therefore, SANS is superior to SAXS considering that higher contrast between the solute and solvent could be obtained. Therefore, we propose to use SANS to verify our hypothesis on how changes in the degree of PPR complexation dominate the sol-gel transition of PPR gels during heating.

Exploration of complex structures

Mihály Makai

KFKI Atomic Energy Research Institute
Email: makai@aeki.kfki.hu

The Budapest Neutron Research Center (BNC) operates a research reactor of 10 MW power. There are 13 experimental sites around the reactor and neutrons are utilised to explore micro structure of liquids, solutions, as well as solid matter. Technical details are available at the web site www.BNC.hu.

One of the research areas is the investigation of the structure of new chemical compounds. A recent endeavor of chemists is to make fullerene derivatives suitable for forming determined spatial structures such as polymeric stars with fullerene cores. The spatial structure of such materials is explored at BNC by means of small angle neutron scattering (SANS).

Another structure of interest is that of micelles. The aim of the measurements is to explore the thermal stability of the micellar structure, and to obtain solubility characteristics of the nano-perticles.

The stability of fullerenes in aqueous dispersion without addition of any stabilisers is also a hot research topic. We know very little of the molecular forces stabilising the fullerene. SANS is appropriate for studying that interaction.

Lipid vesicles in dilute dispersion of model lipid POPC have been studied by small-angle neutron scattering. The dispersions were prepared by extrusion through filters of different pore sizes. The experimental data were treated by a newly developed model, which allowed us to determine the proportions of different kinds of vesicles, unilamellar and multilamellar, of up to four bilayers, and determine their structural and hydration parameters.

For the first time, the structural change of the hydration parameters in function of the vesicle radii has been characterised for unilamellar, and for multilamellar vesicles as well.

In the molecular interactions, the hydrogen bound plays a central role. It is well known that by altering the hydrogen-deuterium ratio fundamental biological processes can be influenced. The isotope effect is a handy tool in the study of the various hydrogen bounds, especially in solved systems.

Another efficient tool of studying the molecular interaction is to explore binary and ternary solutions. Altering the rate of a component, the solved material may take various different forms and those forms are studied, among others, by neutron scattering.

In photosynthesis, light energy is harvested by antenna complexes, which ‘funnel’ the dilute photon flux for photochemistry. The excitation energy is transferred from the light harvesting complexes (LHCs) towards the photochemical reaction centres for energy conversion. In green plants, this complex process occurs in a multilamellar membrane system. The natural system is constituted by essentially identical thylakoid membranes, which - in the lateral direction - contain a large number of photosynthetic complexes and redox components.

Structure of casein micelles under high-pressure

Andrew J. Jackson[1,2] and **Duncan J. McGillivray**[3,§]

[1] NIST Center for Neutron Research, 100 Bureau Drive, Gaithersburg, MD 20899, USA
[2] Department of Chemical Engineering, University of Delaware, Newark, DE 19716, USA
[3] Department of Chemistry, The University of Auckland, Auckland 1142, New Zealand
[§] Email: d.mcgillivray@auckland.ac.nz

The casein micelle is a complex consisting of low-structure casein proteins (α-, β- and κ-caseins), existing in a loose and highly hydrated framework which stabilises nanocrystals of insoluble colloidal calcium phosphate (CCP) in solution, and are a major protein constituent of milk. The detailed structure of the ~300 nm and roughly spherical micelles, including the existence of any sub-structure, and the disposition of the CCP within the micelle, are questions that have been incompletely addressed by numerous groups, particularly using small angle neutron and X-ray scattering.

Of further recent interest has been understanding of how the casein micelle structure responds to high pressures, and the reversibility of these changes. When subjected to high pressures it is well reported, primarily through light scattering measurements, that the casein micelle structure breaks down (with consequent reduction in the solution turbidity). Knowledge of how the micelle breaks down under applied may give more information on the internal organisation of the micelle, while there is significant interest the role of the CCP in stabilising the protein structure.

We report on detailed structural work performed using (ultra-) small angle neutron scattering ((U)SANS) in multiple isotopic contrasts, which has allowed us insight into the casein micelle structure over three orders of magnitude in length scales. The casein micelles were then subjected to up to 350 MPa of applied hydrostatic pressure while being continually measured in situ, allowing us to gain the first detailed structural information on the nature of the casein micelle break-down, and the extent of reversibility of the observed changes. This information gives insight into the as-yet unresolved issue of whether the casein micelle is made up of smaller “sub-micelle” fragments and provides a basis from which to further investigate the effects of environmental conditions (for example, pH and temperature) on the protein complex’s structural stability.

Effect of recovery method and co-drying with genstein on the characteristics of reconstituted antioxidative milk peptides

Supanida Pattorn[1,2], **Rickey Y. Yada**[3], **Parichat Hongprabhas**[1]

[1] Department of Food Science and Technology, Kasetsart University, Bangkok, Thailand
[2] Department of Food Science and Technology,Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathumthani, Thailand
[3] Department of Food Science, University of Guelph, Ontario, Canada

Milk proteins have been recognised as dietary ingredients providing a rich source of antioxidative peptides. However, protein/peptide aggregation is a common phenomenon encountered during manufacturing process, transportation and storage (Zhou et al., 2008).

The aggregation can result in dramatic changes not only in food functionalities but also antioxidant capacity. This study investigated the effect of three factors that induced milk peptide aggregation. Those factors are peptide recovery method (EtOH precipitation or isoelectric precipitation), pH of peptide solution prior to freeze-drying and soy isoflavone genistein during drying on the physicochemical properties and antioxidant capacity of reconstituted tryptic hydrolysed milk peptide. Hydrolysis of Ca lactate milk protein by trypsin increased antioxidant capacity of the hydrolysates determined by TEAC assays from 0.05 to 0.72 mmol TE/mg protein.

The recovery methods by isoelectric precipitation to fractionate hydrophobic milk peptides from milk protein hydrolysate resulted in higher yield (42% of initial protein) of milk peptides with higher antioxidant capacity in both $ORAC_{FL}$ and TEAC values (1.24 and 1.15 mmol TE/mg protein, respectively) than did EtOH precipitation ($p<0.05$). The peptides from EtOH precipitation were mainly hydrophilic.

Moreover, freeze-drying and the presence of genistein during the preparation did not affect antioxidant capacity of reconstituted isoelectric precipitated peptides. On the other hand, freeze-drying and pH of peptide solution prior to freezing in lactose solution also affected aggregation of EtOH precipitated peptide and their antioxidant capacity, especially under acidic pH of 2.0. The aggregation of peptides caused a decrease in free SH group contents, resulting in a decrease in antioxidant capacities.

continued next page →

Nonetheless, EtOH precipitated peptide and genistein could have synergistic effects on antioxidant capacities. This study suggested that the aggregation of peptides during recovery and drying did not reduce antioxidant capacities of hydrophobic peptides from isoelectric precipitation but reduce antioxidant capacities of hydrophilic peptides from EtOH precipitation.

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The technique of ultra-small-angle neutron scattering at OPAL and its application in food science

Christine Rehm

ANSTO Bragg Institute, Locked Bag 2001 Kirrawee DC NSW 2232

Ultra-Small-Angle Neutron Scattering (USANS) allows determination of microstructures of complex systems covering length scales in the range of 100 nm to 10 μm. For instance, complex fluids, containing structures and complexes in nanometer and much larger length scales, have widely varying physical properties and are extensively used in food (e.g. ice cream, mayonnaise, milk), cosmetic/personal care, pharmaceuticals and drug delivery. In these length-scales, which are inaccessible to standard pinhole-SANS measurements, lie some of the organisational features that dictate the bulk rheological and stability properties of solutions. Therefore, a USANS technique (in parallel with isotopic substitution) enables the detailed study of such systems and their internal organisation.

Extending the experimentally measurable length scales currently accessible at the ANSTO OPAL reactor through the already existing SANS instrument, Quokka, through a new USANS instrument, Kookaburra, will allow the characterisation of microstructure over 4 orders of magnitude in size (1 nm to 10 μm). In this contribution we will discuss technical parameters of the Kookaburra USANS instrument as well as applications of USANS in food science.

Effect of modified starches from non-conventional sources in the preparation of custard dessert

Amruta Bawane and **D.C.Saxena**[§]

Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology Longowal-148106 (Punjab) India
[§] Email: dcsaxena@yahoo.com

Objective:

To modify the starches from different non conventional sources viz. sweet potato, buckwheat, water chestnut by dual modification, their characterisation and their effect on properties of custard prepared from them thereof.

Methodology:

Starches from different non conventional sources viz. sweet potato, buckwheat and water chestnut were isolated and their physicochemical, pasting and rheological characterisation was done. Effect of dual modification (Hydroxypropylation cross linking) of all starches from different sources was carried out. Further, custard was prepared from native as well as modified starches. The effect of different starches on quality parameters of custard (hardness, consistency, viscosity, mouthfeel and other sensory evaluation) were analyzed.

Results and conclusion:

The physicochemical and rheological properties of starches varied significantly upon modification. Dual modified starches formed clear gels except buckwheat starch. After hydroxypropylation crosslinking the peak viscosity increased from 5871-6288 RVU in case of sweet potato starch, whereas contrarily, it decreased in case of water chestnut and buckwheat starch. The final viscosity too exhibited the same trend. In case of sweet potato starch, it increased from 2697- 3033 RVU whereas it was decreased in case of buckwheat and water chestnut.

The custard prepared from native as well as modified starches, when qualitatively evaluated, showed that the custard prepared from dual modified starches were more viscous as compared to those prepared from their native counterparts. The viscosity of the custards prepared from modified starches increased from 1180 (in native) to 3510 cP. to was found to be in the range of - of the custard prepared from hydroxypropylated sweet potato starch was observed to be higher viz. 3510 cp. Back extrusion test performed with the help of Texture analyzer revealed that modified starches resulted in hard textured custards which would help in using lesser amount of starch for custard preparation.

Contacts

Benu Adhikari
University of Ballarat
b.adhikari@ballarat.edu.au

Sandra Ainsworth
Dairy Innovation Australia
sainsworth@dairyinnovation.com.au

Salyha Ali
ILL
ali@ill.fr

Ingrid Appelqvist
CSIRO
ingrid.appelqvist@csiro.au

Joseph Bevitt
ANSTO
joseph.bevitt@ansto.gov.au

Jaroslav Blazek
ANSTO
jaroslav.blazek@ansto.gov.au

Sofie Botegård
European Spallation Source, ESS, AB
sofie.botegard@esss.se

Wim Bouwman
Delft University of Technology
w.g.bouwman@tudelft.nl

Herma Buttner
ANSTO
herma.buttner@ansto.gov.au

Osvaldo Campanella
Purdue University
campa@purdue.edu

Dominique Champion
Université de Bougogne
d.champion@agrosupdijon.fr

Jayani Chandrapala
University of Melbourne
jayanic@unimelb.edu.au

Cissie Chen
The University of Auckland
cissie_sharp@hotmail.com

Tochukwu Collins Chimara
Lagos State University
chitochukwu@hotmail.com

Luke Clifton
STFC
Luke.Clifton@STFC.ac.uk

Mike Davidson
University of Tennessee
pmdavidson@utk.edu

Kees De Kruif
NIZO food research
dekruif@nizo.nl

Derek Dee
University of Guelph
ddee@uoguelph.ca

Anne-Laure Fameau
CEA/INRA
anne-laure.fameau@nantes.inra.fr

Mike Gidley
University of Queensland
m.gidley@uq.edu.au

Elliot Gilbert
ANSTO
elliot.gilbert@ansto.gov.au

Federico Harte
University of Tennessee
fede@utk.edu

Yacine Hemar
University of Auckland
y.hemar@auckland.ac.nz

Rex Hjelm
Los Alamos National Laboratory
hjelm@lanl.gov

Carl Holt
University of Glasgow
cholt002@udcf.gla.ac.uk

Stephen Holt
ANSTO
stephen.holt@ansto.gov.au

John Katsaras
Oak Ridge National Laboratory
John.Katsaras@nrc.gc.ca

Ingo Koeper
Flinders University
ingo.koeper@flinders.edu.au

Susan Krueger
NIST
susan.krueger@nist.gov

Hsi-Mei Lai
National Taiwan University
hmlai@ntu.edu.tw

Ross Lee
PTIS
ross.lee.usa@gmail.com

Peter Lillford
University of York
PL8@york.ac.uk

Camille Loupiac
Agrosup Dijon,
Universite de Bourgogne
c.loupiac@agrosupdijon.fr

Mike Makai
KFKI AEKI
makai@aeki.kfki.hu

Jitendra Mata
ANSTO
jitendra.mata@ansto.gov.au

Duncan McGillivray
University of Auckland
d.mcgillivray@auckland.ac.nz

Robert McGreevy
STFC
robert.mcgreevy@stfc.ac.uk

Tommy Nylander
Lund University
Tommy.Nylander@fkem1.lu.se

Lydia Ong
University of Melbourne
lon@unimelb.edu.au

Supanida Pattorn
Kasetsart University
daow@hotmail.com

Allan Paulson
Advanced Foods and
Materials Network
allan.paulson@dal.ca

Siew Young Quek
The University of Auckland
sy.quek@auckland.ac.nz

Christine Rehm
ANSTO
christine.rehm@ansto.gov.au

Dharnesh Chandra Saxena
Sant Longowal Institute of
Engineering & Technology
dcsaxena@yahoo.com

Anna Sokolova
ANSTO
anna.sokolova@ansto.gov.au

Susana Teixeira
Keele University and I.L.L.
s.c.m.teixeira@natsci.keele.ac.uk

Cherylie Thorn
ANSTO
cherylie.thorn@ansto.gov.au

Ray Trejo
University of Tennessee
rtrejo1@utk.edu

Hans Tromp
NIZO food research
Hans.Tromp@nizo.nl

Kitty van Gruijthuijsen
University of Fribourg
kitty.vangruijthuijsen@unifr.ch

Aude Vernhet
Montpellier SupAgro
vernhet@supagro.inra.fr

Katy Wood
ANSTO
kathleen.wood@ansto.gov.au

acts

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Notes

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Australian Nuclear Science and Technology Organisation
New Illawarra Road, Lucas Heights NSW 2234
www.ansto.gov.au