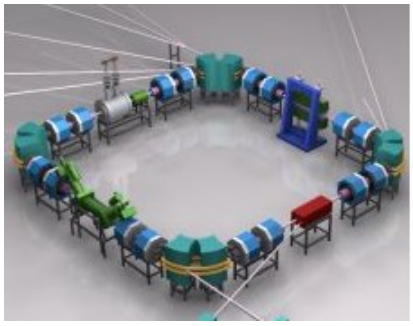
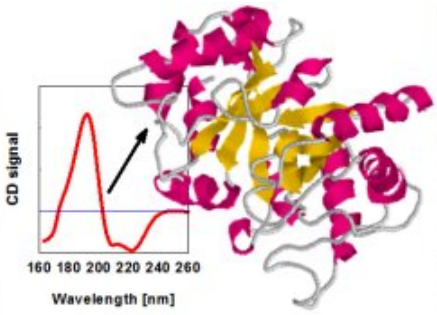
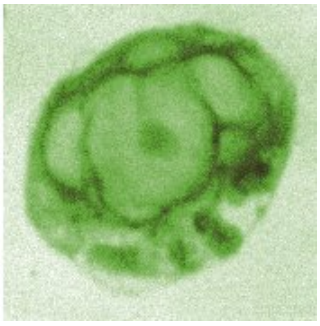
# Biology and Medicine with Low Energy Synchrotron Radiation
# &
# ISA Users meeting 2006

1st-3rd November 2006





University of Aarhus Conference Centre,
Building 1421 Nordre Ringgade, Aarhus, Denmark

This workshop is funded by

www.isa.au.dk/meetings/BioUsers06

# Table of Contents

# Programme

All talks will be held in room 246 on the 1st floor of the Conference Centre

| **Wednesday 1st November** | | |
|---|---|---|
| *09:00* | *Registration and Coffee* | |
| | *Session 1* | |
| 10:00-10:15 | User meeting starts<br>Introduction to ISA | Søren Pape Møller<br>*(University of Aarhus)* |
| 10:15-10:45 | Self limiting atomically controlled growth of ultra-thin films in Si | Per Morgen<br>*(University of Southern Denmark)* |
| 10:45-11:15 | A New Instrument for Studying Electron Molecule Interactions using a High Resolution Electron Transmission Technique | Richard Balog<br>*(University of Aarhus)* |
| 11:15-11:45 | Electron-phonon coupling on surfaces | Maria Jensen<br>*(University of Aarhus)* |
| 11:45-12:15 | Absolute photoionization cross sections of ions employing the merged beam technique at ASTRID | Caroline Banahan<br>*(Dublin City University)* |
| 12:15-12:45 | The route from molecules to nanostructures followed by SR photoemission | Bruno Domenichini<br>*(University of Bourgogne)* |
| *12:45* | *Lunch and Registration* | |
| | *Start of the "Biology and Medicine with Low Energy SR meeting and continuation of the Users meeting* | |
| | *Session 2* | |
| 14:00-14:15 | Welcome/Introduction | David Field<br>*(University of Aarhus)* |
| 14:15-15:00 | Synchrotron Radiation Circular Dichroism Spectroscopy: A New Tool for Structural and Functional Genomics | Bonnie Wallace<br>*(Birkbeck College, London)* |
| 15:00-15:45 | Probing radiation damage at the molecular level | Nigel Mason<br>*(The Open University)* |
| *15:45-16:15* | *Coffee Break* | |
| 16:15-17:00 | X-ray tomography of biological cells | Carolyn Larabell<br>*(Lawrence Berkeley National Laboratory)* |
| 17:00 | Poster Session<br>(ASTRID basement) | |
| 19:00 | Dinner<br>(7th Floor Physics Canteen) | |
| | User meeting ends. | |

## Thursday 2nd November

*Continuation of the "Biology and Medicine with Low Energy Synchrotron Radiation" meeting*

*Session 3*

| | | |
|---|---|---|
| 09:00-09:45 | Linear Dichroism | Alison Rodger *(University of Warwick)* |
| 09:45-10:15 | Glycoprotein-surfactant interactions. A calorimetric and SRCD study | Heidi Louise Bagger *(Novozymes/RUC)* |
| *10:15-10:45* | *Coffee* | |
| 10:45-11:15 | Nano-tomography and spectromicroscopy with the new BESSY X-ray microscope | Gerd Schneider *(Bessy, Berlin)* |
| 11:15-11:45 | Compact X-Ray Microscopy | Hans Hertz *(Royal Institute of Technology, Stockholm)* |
| 11:45-12:30 | Radiolysis of proteins in the solid state: what happens to the disulfide bonds? | Chantal Houee-Levin *(Orsay)* |
| *12:30-14:00* | *Lunch* | |

*Session 4*

| | | |
|---|---|---|
| 14:00-14:45 | Circular Dichroism study of polymorphic oligonucleotides | Jean-Claude Maurizot *(CNRS, Orléans)* |
| 14:45-15:15 | Atmospheric pressure photoionisation of biomolecules studied by mass spectrometry | Alexandre Giuliani *(Orsay)* |
| *15:15-15:45* | *Coffee* | |
| 15:45-16:15 | Protecting life from UV radiation: absorption spectra of known and potential UV screens | Andreja Zalar *(INRA, Versailles)* |
| 16:15-16:45 | On the influence of conformational locking of sugar moieties on the absorption and circular dichroism of nucleosides from synchrotron radiation experiments | Anne Sander Holm *(University of Aarhus)* |
| 16:45-17:30 | Biological Science is the art of the soluble - the role of X-ray sources in structure determination of biological specimen | Thomas Vorup-Jensen *(University of Aarhus)* |
| | Poster Session (ASTRID Basement) | |

## Friday 3rd November

*Practical details of techniques for CD and XM*

*Session 5*

| | | |
|---|---|---|
| 09:00-09:45 | Methods for SRCD data collections of Macromolecules | Frank Wien<br>*(Soleil Synchrotron)* |
| 09:45-10:30 | Sample preparation and cryo fixation in X-ray microscopy | Christian Knöchel<br>*(ISA, University of Aarhus)* |
| *10:30-11:00* | *Coffee* | |
| 11:00- | Demonstration of CD – bring your own sample | |

Meeting Ends

# Self limiting atomically controlled growth of ultrathin films on Si

Per Morgen

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The growth of films of only a few layers of material on Si surfaces has become the target for the development of industrial processes towards the next generations of Si based electronics. Obviously the processing and analysis of such systems is very demanding, and novel growth methods are looked for. The atomic layer deposition process (ALD) is an industrially viable process, and we have chosen to simulate it, by developing self limiting atomically controlled processes, working for insulator-, semiconductor-, and metal films on Si. All our work is done in an ultrahigh vacuum environment.

Access to synchrotron radiation facilities, using core-level and valence-band photoemission studies in-situ of the process steps, allow us to analyze details of the reaction dynamics and structural properties of the films during growth steps. Major issues are the film uniformity, composition and stability. Beyond this the electrical properties are important, such as the charge and defect densities, inside the film at the film-Si interface, and the band bending of Si. The purity and resistance of the film to further thermal device processing steps are similarly important.

Among the results obtained, we can list the growth of epitaxial metal systems showing quantum well behaviour, self limiting oxidation and nitridation processes of Nanometer and sub-Nanometer thick films, and sandwich structures of these. Examples of these processes and the resulting properties and characteristics will be discussed in the presentation.

Acknowledgements: This work has been performed in collaboration with many people, especially K. Pedersen, Ålborg, A. Bahari, Sari, Iran, and Z.S. Li, ISA, Århus. Support has been obtained from The Danish Research Councils, The Carlsberg Foundation, and from NanoS ApS.

# A New Instrument for Studying Electron Molecule Interactions using a High Resolution Electron Transmission Technique

**Richard Balog**, Peter Cicman, Nyk Jones, David Field

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A new experiment has been built to study the interactions of low energy electrons with molecules in the gas phase and condensed phase, with a special focus on collisions at very low electron energies (down to few meV). The electron source is a photoionisation source with a typical resolution of 1 meV in the incident beam, using SR from the ASTRID storage ring in Aarhus.

The experiment will be used to investigate chemical degradation and synthetic effects of electrons in condensed phase. Of particular interest are the radiation damage effects that cold electrons may induce in DNA and its constituent molecules. We will also study the effects of cold electrons on atmospheric pollutants such as freons. In further experiments we plan to irradiate pure ices and ice mixtures involving species such as $H_2O$, $NH_3$, methanol, $CO_2$, CO in order to discover what larger species, such as amino acids, may be created.

# Electron-phonon coupling on surfaces

**M. Fuglsang Jensen**[1], T. K. Kim[1], S. Bengió[1], Ph. Hofmann[1], Yu. Sklyadneva[2], A. Leonardo[2] and E.V. Chulkov[2]

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[2] Donostia International Physics Center (DIPC), 20018 San Sebastían / Donostia, Basque Country, Spain

The lifetime of excited electrons and holes has a pronounced effect on phenomena such as chemical reactivity and high temperature superconductivity. Recently, the reduction of the lifetime due to the electron-phonon coupling has attracted considerable attention.

At the SGM3 beamline we use Angle Resolved Ultraviolet Photoemission Spectroscopy to study the electronic structure of solids. This technique is normally used for determining the band structure of a material, but for quasi two-dimensional electronic systems, such as surfaces, detailed information about the many body effects can be obtained.

The spectral linewidth of a surface state is inversely proportional to the lifetime of that state and it contains information about the many-body effects resulting in the decay of the photohole. The three interactions responsible for the decay are the electron-electron, the electron-defect and the electron-phonon interaction. However, the temperature dependence of the linewidth is dominated by the electron-phonon interaction. The linewidth is expected to depend linearly on the temperature with a slope mainly given by the electron-phonon mass enhancement parameter λ.

We have studied the strength of the electron-phonon interaction on Mg(0001) and on Al(001). Mg(0001) shows the expected behaviour and the resulting λ is in excellent agreement with the theoretical prediction. In the case of aluminium, however, the dependence at higher temperatures is not linear. In order to understand the whole temperature dependence, we have to take a fourth decay channel into account, which describes the scattering from thermally generated defects. This effect should generally be present. In fact, analysing recently published data from Au(111) along these lines permits us to reconcile the measured and predicted values for λ.

# Absolute photoionization cross sections of ions employing the merged beam technique at ASTRID

**Caroline Banahan**[1], Jean-Marc Bizeau[2], Finn Folkmann[3], John West[4].

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The past 20 years has seen a concerted effort both theoretically and experimentally to provide fundamental data on matter in its ionic form. Not only does this data test fundamental atomic theory but also, in particular, data on atomic ions is required for modelling interstellar and astrophysical plasmas and laboratory plasmas with their relevance to fusion reactors. However, providing absolute photoionization cross section measurements is a challenging task. This is due mainly to the difficulty in achieving a stable, dense beam of ions and the availability of intense light sources. Pioneering work carried out at the synchrotron radiation source (SRS) at Daresbury employing the merged beam technique [1,2] enabled the first photoionization cross section measurements of ions.

The undulator "Miyake" beamline at the 580 MeV storage ring ASTRID in the University of Aarhus provides two orders of magnitude greater intensity than the SRS, thus permitting cross section measurements of $10^{-19}$ $cm^{-2}$ or less to be recorded. This permits the study of ions of astrophysical importance e.g., C, N, O and Fe. Light from the undulator is incident on a Miyake monochromator and the output is merged over a distance of 50cm with a positive atomic ion beam. A magnetic deflector system located after the interaction region separates the parent and product ions into charge states for detection. A calibrated photodiode for the incident SR flux, together with a system of ion beam profile monitors allows measurement of absolute photoionization cross sections [3]. Ions are presently produced in a 10GHz all permanent magnet ECR source developed by CEA in Grenoble and used previously at the storage ring SuperACO [4].

The work carried out at ASTRID has provided absolute values of the cross section on several singly charged ions, see [3] for a review. Investigations along isoelectronic and isonuclear sequences [5,6] have also been performed and have provided a severe test for the many atomic codes used to predict ionic properties and many-body electron-electron correlations. Extension of the merged beam method to negative ion beams and molecular systems initiated, with the first measurement of the absolute cross section of the molecular ion $CO^+$ reported in [7], and negative ions Li- and Te- in [8,9] respectively.

[1] Lyon I C, Peart B, West J B and Dolder K 1986 *J. Phys. B: At. Mol. Phys.* **19** 4137

[2] Lyon I C, Peart B, Dolder K and West J B 1987 *J. Phys. B: At. Mol. Phys.* **20** 1471

[3] West J B, 2001 *J. Phys. B: At. Mol. Phys.* **34** R45

[4] J. M. Bizau, J.-M. Esteva, D. Cubaynes, F. J. Wuilleumier *et al* 2000 *Phys. Rev. Lett.* **84** 435

[5] Kjeldsen H *et al* 1999 *Phys. Rev. A* 62 020702(R)

[6] Andersen T, Kjeldsen H, Knudsen H and Folkmann F 2001 *J. Phys. B: At. Mol. Phys.* **34** L343

[7] Andersen P, Andersen T, Folkmann F, Ivanov V K, Kjeldsen H and West J B 2001 *J. Phys. B: At. Mol. Phys.* **34** 2009

[8] Kjeldsen H, Andersen P, Folkmann F, Kristensen B and Andersen T 2001 J. Phys.B 34, L353

[9] Kjeldsen H, Folkmann F, Jacobsen T S, and West J B 2004 Phys. Rev. A 69 050501(R)

# The route from molecules to nanostructures followed by SR photoemission

**Bruno Domenichini** and Sylvie Bourgeois

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Metallic nano particles are widely studied since several year for their outstanding reactivity which could find a lot of applications in several fields. However, it is very hard to define and control the risks related to the use of such materials. For instance, due to their large specific area, nano-particles should give rise to potential dust explosions if they are subjected to an ignition source. Besides, exposure to nano particles through air, water or soil is potentially highly dangerous for human beings and is almost impossible to avoid in any process involving these kinds of materials.

A way to both keep the remarkable properties of nano materials and limit the risks related to the use of such particles can be to use supported objects, which are processed on non-reactive substrates. However, although numerous synthesis methods are offered to perform supported nano structures on model surfaces, just a few of them allow the elaboration of small self-assembled structures on real surfaces such as metallic oxide ($TiO_2$, $Al_2O_3$, MgO, ...) particles.

An original way to obtain such supported nano objects containing metal atoms was recently developed in our lab. This innovative synthesis method can be defined as soft and controlled metallic organic chemical vapour deposition (MOCVD) growth. It is based on the decomposition of the phenomena that generally occur at the same time in such growths; Especially, in our method, the adsorption stage of the molecules which contain the atoms that we want to include in the final objects, the decomposition steps of such precursors as well as the final organization of the atoms as nano phases or nano objects occur during three different successive steps. This route allows us to obtain supported objects having extremely reduced sizes (containing some atoms only) and being thus very reactive. They can also be self-assembled on real surfaces, even on oxide powders.

The purpose of this talk will be to show how, using the tunable energy given by SX700 beamline, each elementary reaction occurring during synthesis can be separately studied. For instance, it will be shown that, through resonant phenomena, it is possible to exalt and thus detect signals which correspond to a very few amount of atoms and which should have been impossible to record without the enhancement due to resonance. Moreover, it will be shown that spectroscopic data can also allow a fast crystallographic characterisation of support surfaces. Besides, it will be pointed on the fact that the wide energy range offered by SX700 beamline makes this beamline highly effective for the characterisation of metal oxide solids.

# Synchrotron Radiation Circular Dichroism Spectroscopy: A New Tool for Structural and Functional Genomics

**B.A. Wallace**[1], A.J. Miles[1], J.G. Lees[1,2], L. Whitmore[1,2], F. Wien[1+], M. Radford[1], P.J. Evans[1], A. Abdul-Gader[1], T.Stone[1], S. Vrønning Hoffmann[3], R.W. Janes[2]

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Synchrotron radiation circular dichroism (SRCD) spectroscopy is an emerging technique for structural molecular biology [1-3]. Developments in instrumentation [4,5] and bioinformatics [6] mean that SRCD also has considerable potential for the future in Structural Genomics as a means of target selection [2,3], and for examination of a wide range of protein types, including glycoproteins [7] and membrane proteins [8],the latter being a category of proteins generally excluded from Structural Genomics programmes. SRCD also has potential applications in Functional Genomics as a high throughput, low sample-requiring screening method for defining function, as a means of identifying macromolecular interactions and ligand and drug complex formation [9], and for examining mutant proteins associated with disease [10,11]. These applications are all possible due to the additional information content in the low VUV wavelength data obtainable with intense synchrotron radiation light sources, compared with that present in spectra from conventional lab-based circular dichroism instruments [1,2] and the high signal-to-noise which enables the use of small amounts of protein samples [3]. In addition, the ability to monitor protein stability, conformational changes [12], and rapid protein folding in solution means that SRCD is capable of providing important structural information which is complementary to that obtainable by protein crystallography. These technical developments and advances in accuracy due to improvements in calibration methods [13,14] have been further enhanced by the availability of new computational tools to facilitate spectral processing, archiving and analysis (CDTOOL) [15], secondary structure analyses (DICHROWEB) [16], and the creation of a deposition data bank for CD spectra, the PCDDB [17,18], as well as a new SRCD reference data base covering fold and secondary structural space [19].

1. Wallace, B.A. (2000) Synchrotron radiation circular dichroism spectroscopy as a tool for investigating protein structures. J. Synch. Rad. 7:289-295.
2. Wallace, B.A. and Janes, R.W. (2001) Synchrotron radiation circular dichroism spectroscopy of proteins: secondary structure, fold recognition, and structural genomics. Curr. Opin. Chem. Biol. 5:567-571.
3. Miles, A.J., and Wallace, B.A. (2005) Synchrotron Radiation Circular Dichroism Spectroscopy of Proteins and Applications in Structural and Functional Genomics. Chem. Soc. Reviews 35:39-51.
4. Clarke, D.T., Bowler, M.A., Fell, B.D., Flaherty, J.V., Grant, A.F., Jones, G.R., Martin Fernandez, M.L., Shaw, D.A., Todd, B., Wallace, B.A., and Towns-Andrews, E. (2000) A high aperature beamline for vacuum ultraviolet circular dichroism on the SRS. Synch. Rad. News 13:21-27.
5. Wien, F., and Wallace, B.A. (2005) Calcium Fluoride Micro Cells for Synchrotron Radiation Circular Dichroism Spectroscopy. Appl. Spectr. 59:1109-1113.
6. Janes, R.W. (2005) Bioinformatics analyses of circular dichroism protein reference databases. Bioinformatics 21,4230-4238/
7. Cronin, N.B., O'Reilly, A., Duclohier, H., and Wallace, B.A. (2005) Effects of Deglycosylation of Sodium Channels on their Structure and Function. Biochemistry 44:441-449.
8. Wallace, B.A., Lees, J., Orry, A.J., Lobley, A. & Janes, R.W. (2003) Analyses of circular dichroism spectra of membrane proteins. Prot. Sci. 12:875-884.
9. Wallace, B.A. and Janes, R.W. (2003) Circular dichroism and synchrotron radiation circular dichroism spectroscopy: Tools for drug discovery. Biochem. Soc. Trans. 31:631-633.
10. Evans, P., Wyatt, K., Wistow, G.J., Bateman, O.A., Wallace, B.A., and Slingsby, C. (2004) The P23T Cataract Mutation Causes Loss of Solubility of Folded gD-Crystallin. J. Mol. Biology 343:435-444.
11. Wallace, B.A., Wien, F., Miles, A.J., Lees, J.G., Hoffman, S.V., Evans, P., Wistow, G.J., and Slingsby, C. (2004) Biomedical applications of synchrotron radiation circular dichroism spectroscopy: Identification of mutant proteins associated with disease and development of a reference database for fold motifs. Faraday Discussions 126:237-243.
12. Wallace, B.A. (2000) Conformational changes by synchrotron radiation circular dichroism spectroscopy. Nature Struct. Biol. 7:708-709.
13. Miles, A.J., Wien, F., Lees, J.G., Rodger, A., Janes, R.W., and Wallace, B.A. (2003) Calibration and standardisation of synchrotron radiation circular dichroism (SRCD) amplitudes and conventional circular dichroism (CD) spectrophotometers. Spectroscopy 17:653-661.
14. Miles, A.J., Wien, F., Lees, J.G., and Wallace, B.A. (2005) Calibration and Standardisation of Synchrotron Radiation and Conventional Circular Dichroism Spectrometers. Part 2: Factors Affecting Magnitude and Wavelength. Spectroscopy 19:43-51.
15. Lees, J.G., Smith, B.R., Wien, F., Miles, A.J., and Wallace, B.A.. (2004) CDtool – An Integrated Software Package for Circular Dichroism Spectroscopic Data Processing, Analysis and Archiving. Analytical Biochemistry 332:285-289.
16. Whitmore, L. and Wallace, B.A. (2004) DICHROWEB, An Online Server For Protein Secondary Structure Analyses from Circular Dichroism Spectroscopic Data. Nucleic Acids Research 32::W668-673.
17. Wallace, B.A., Whitmore, L., and Janes, R.W. (2006) The Protein Circular Dichroism Data Bank (PCDDB): A Bioinformatics and Spectroscopic Resource. Proteins: Structure, Function and Bioinformatics 62:1-3.
18. Whitmore, L., Janes, R.W.,& Wallace, B.A. (2006) Protein Circular Dichroism Data Bank (PCDDB): Data Bank and Website Design. Chirality 18:426-429.
19. Lees, J.G., Miles, A.J., Wien, F., and Wallace, B.A. (2006) A reference database for circular dichroism spectroscopy covering fold and secondary structure space. Bioinformatics, in press.

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# Probing radiation damage at the molecular level

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Ionizing radiation, derived from natural background radiation or arising from diagnostic and therapeutic techniques, (e.g. X-rays, radiotherapy, positron emission tomography) can produce a range of structural and chemical modifications of the DNA helix. Of these, double-strand breaks, where both strands of the helix are broken within a few base pairs, can lead to lasting damage via the production of chromosome aberrations, mutations and ultimately cell death. It is now known that the effectiveness of different ionizing radiations is critically dependent on the *patterns of ionizations* they produce on a nanometre scale, comparable with the diameter of the DNA helix. Theoretical track structure modelling is being used with increasing sophistication to simulate the distinctive patterns of ionizations produced by ionizing radiation. Such models reveal that much of the radiation damage is *site specific* with penetrating primary radiations (energetic photons or ions) producing nanometre sized clusters of ionisations at the end of the radiation track. Such patterning therefore suggests that initial radiation damage may be strongly influenced by the ionization patterns of specific molecules. Hence, in order to understand the mechanisms of radiation damage, it is essential to understand the interaction of different types of radiation with the constituent cellular molecules (DNA and its component nucleotides, nucleosides, phosphates, sugars and cellular water).

Radiation damage of DNA and other cellular components has traditionally been attributed to ionisation via direct impact of high-energy quanta or by complex radical chemistry (triggered by the primary ionizing radiation). However, recently Sanche and co-workers have challenged this explanation by suggesting that "*from a radiobiological perspective the abundant low-energy electrons and possibly their ionic and radical reaction products play a crucial role in the nascent stages of cellular DNA radiolysis*". They showed that strand breaks in DNA may be initiated by secondary electrons and is strongly dependent upon the target DNA base identity. As a consequence, they postulate that the localisation of low energy electrons on the nucleotide bases, through the formation of short lived negative ions, leads to molecular dissociation and then to single and double strand breaks in the DNA. Such research provides the fascinating perspective that it is possible that radiation damage may be described and *understood at an individual molecular level.* For example such a molecular picture may explain the well known correlation between electron attachment rates of many molecules and their carcinogenicity and may be used to suggest new compounds to be adopted in radiation therapy as treatment enhancing sensitizers. In this talk I will review the latest developments in this research and how it may lead to a new approach to the study of radiation damage.

# X-ray Tomography of Biological Cells

**Carolyn A. Larabell**[1,2], Mark A. Le Gros[2]

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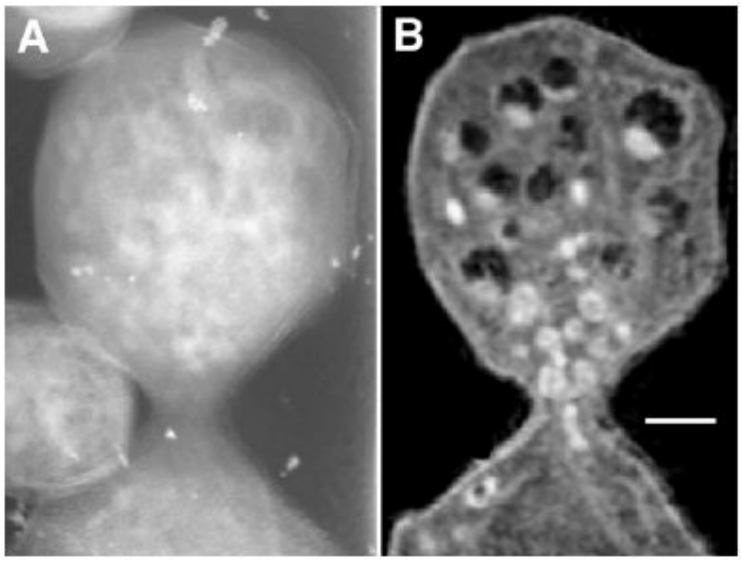
X-ray microscopy can image whole, hydrated, biological specimens up to 10 microns thick with a spatial resolution better than 50 nm. Soft X-ray microscopy uses photons with energies between the K shell absorption edges of carbon (284 eV, $\lambda$=4.4 nm) and oxygen (543 eV, $\lambda$ =2.3 nm). These photons readily penetrate the aqueous environment while encountering significant absorption from carbon- and nitrogen-containing organic material. In this energy range, referred to as the 'water window,' organic material absorbs approximately an order of magnitude more strongly than water, producing a quantifiable natural contrast and eliminating the need for contrast enhancement procedures to visualize cellular structures.

The experiments presented here were performed at the Advanced Light Source using the full field transmission X-ray microscope, XM-1. This microscope employs a bend magnet X-ray source and zone plate condenser and objective lenses. The condenser zone plate acts as a monochromator and the X-ray images are recorded directly on a cooled, back-thinned 1024x1024 pixel CCD camera. The sample holder was a rotationally symmetric glass tube; the region containing the sample was 10 microns in diameter with a wall thickness of 200 nm. Live cells were loaded into the tube, rapidly frozen by a blast of liquid nitrogen-cooled helium gas, and maintained at –140 degrees C by a steady flow of cold helium gas. The image sequence spanned 180 degrees and consisted of either 45 images spaced by 4 degrees or 90 images spaced by 2 degrees. The images were aligned to a common axis and computed tomographic reconstruction was used to obtain the 3-D X-ray linear absorption coefficient. Volume rendering and animation of reconstructed data was performed using the 3-D program, Amira. The tomographic reconstructions generate 3-D images at approximately 50 nm isotropic resolution and reveal high fidelity views of the internal architecture of cells.

The high penetrating power, coupled with a near absence of reflection at the interface of dissimilar materials, makes X-rays an ideal probe for studying cellular morphology and examining the location of labeled proteins in single cells. We have used this imaging approach to reveal remarkable details of the nuclear and cytoplasmic architecture of fully hydrated whole cells. We have also localized molecules in the nucleus and cytoplasm of whole, hydrated cells using immunogold labeling protocols. We are developing additional labels uniquely suited to x-ray imaging to enable simultaneous localization of multiple proteins. Using the x-ray linear absorption coefficient, quantitative information about cellular structures and molecular distributions can be obtained from the reconstructed data.




**X-ray tomography of whole yeast cells.** (A) Single projection image of a rapidly frozen budding yeast. (B) Computer-generated section through a tomographic reconstruction of the raw data shown in (A). Bar = 1 um.

# Linear Dichroism

**Alison Rodger**,[1] Cedric Dicko[2]

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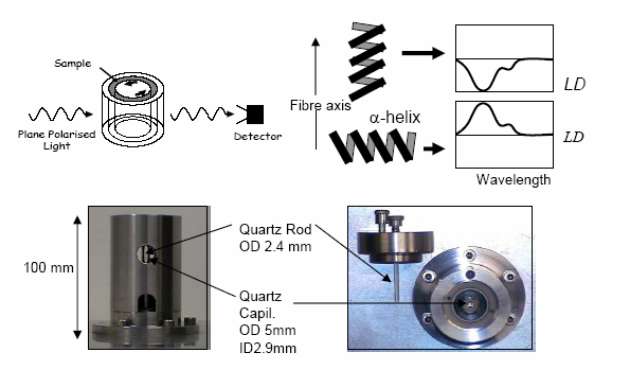
Understanding the organisation of molecules in naturally occurring ordered arrays (*e.g.* membranes, protein fibres and DNA strands) is of great importance in understanding their biological function. Unfortunately few biophysical techniques provide detailed structural information in these non-crystalline systems. Ultra-violet, visible and infrared linear dichroism (LD) has the ability to provide such information and may hold the key to understanding molecular mechanisms of such fundamental biological processes as amyloid fibre formation, and membrane protein folding. This complements the secondary structural information provided for such molecules by circular dichroism (CD) spectroscopy. The lecture and associated poster will show how LD and CD data can be used to understand more about the formation and function of fibrous proteins with particular application to tubulin, FtsZ (a bacterial analogue of tubulin), actin and tubulin. This work has become possible following recent work at Warwick on instrumentation for LD spectroscopy. The key factor has been the design of new capillary Couette cells (room temperature and thermostatted) which reduce sample requirement from ~2000 μL of 100 μM of monomers, *e.g.* amino acids or nucleic acid bases (say ~70 μg), to only ~20 μL (~700 ng) per experiment.

Recent work on spider silk fibres undertaken at both Warwick and ISA show the potential for using LD to study fascinating but otherwise intractable systems. A brief description of how a synchrotron LD system was established will be given and data will be presented to show when it is better to use a bench top and when a synchrotron set up.




## References:

Rodger, A.; Marrington, R.; Geeves, M.A.; Hicks, M.; de Alwis, L.; Halsall, D.J.; Dafforn, T.R. "Looking at long molecules in solution: what happens when they are subjected to Couette flow?" *Physical Chemistry Chemical Physics*, **2006**, *8*, 3161–3171

Dafforn, T.R. and Rodger, A "Unravelling the configuration of protein fibres and membrane proteins" *Current Opinion in Structural Biology*, **2004**, *14*, 541–546

Marrington, R.; Small, E.; Rodger, A.; Dafforn, T.R.; Addinall, S.; "FtsZ fibre bundling is triggered by a calcium-induced conformational change in bound GTP" *Journal of Biological Chemistry* **2004**, *47*, 48821–48829

Marrington, R.; Dafforn, T. R.; Halsall, D.J.; Rodger, A. "Micro volume couette flow sample orientation for absorbance and fluorescence linear dichroism" *Biophysical Journal*, **2004**, *87*, 2002–2012

# Glycoprotein–Surfactant Interactions. A Calorimetric and SRCD Study

**Heidi Louise Bagger**[1,3], Søren Vrønning Hoffmann[2], Claus Crone Fuglsang[3] and Peter Westh[1]

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One challenging mission in biotechnology is to maintain biological active proteins and enzymes in the presence of surfactants. Owing to their amphiphilic nature, surfactants tend to adsorb at interfaces to minimize the contact of their hydrophobic part with water. This tendency also leads to unspecific binding of surfactants to the interface of proteins in solution. Surfactant addition therefore makes protein-solvent contacts thermodynamically more favorable which explains their solubilizing properties (prevention against surface adsorption, aggregation and precipitation of proteins). The disadvantage of surfactant binding is however that non-compact protein structures become favored with subsequent destabilization of the native (biological active) state.

The combination of calorimetric and spectroscopic techniques has proven to be a very strong tool to elucidate the relation between protein-surfactant interactions and consequential structural transitions. In our studies we use Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy to obtain information of the structural changes occurring in the heavily glycosylated enzyme Peniophora lycii Phytase (Phy) when it is exposed to varying concentrations of sodium dodecylsulfate (SDS). To investigate the effects of protein glycosylation on the surfactant induced decrease in protein stability we made comparative investigations of the enzymatically deglycosylated variant of this enzyme (dgPhy). It turned out that the glycan mantle does not hold a strong potential to protect the peptide structure from denaturation. On the basis of Isothermal Titration Calorimetry (ITC) data the limited effect of protein glycosylation is explained by very weak glycan-SDS interactions. Analysis of the binding characters (binding strength, -enthalpy and -numbers) of Phy and dgPhy reveals that the relative affinity of the glycan and peptide moieties are rather different - carbohydrates bind much less surfactant.

# Nano-tomography and spectromicroscopy with the new BESSY X-ray microscope

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X-ray microscopy is a powerful imaging technique with many applications in materials, environmental and life sciences. Among many scientific questions in life sciences, the cell nucleus which is a vital and complex organelle is still a mystery. How the DNA it contains and its associated proteins are arranged and packaged to fit within this ~10 µm diameter organelle is unknown. The normal cellular contingent of DNA is ~105 fold longer than the nuclear diameter. Some DNA compaction (~6 fold) can be accounted for by its wrapping around nucleosomes. The nucleosomal fiber is itself probably folded to yield a thicker fiber of ~30 nm thickness, providing ~7 fold more compaction. How this "30 nm fiber" is folded to achieve the further necessary compaction is unknown. Other questions of packaging concern how much "free" space for diffusion is available in the nucleus.

The interaction of x-rays is element specific, therefore, x-ray nano-tomography can be used to quantify the packing density of organic material. However, different proteins or molecular structures cannot be distinguished directly in x-ray microscope images. This problem is solved by the availability of specific fluorescent probes detectable by fluorescence microscopy. Thus the two imaging modalities are complementary. Since fluorescence and x-ray microscopy permit analysis of whole cells, it is possible to investigate the same cell in both microscopes. These correlative studies are ideally suited to x-ray microscopy because of its ability to image cells in 3D. We expect to develop a widely applicable technique that, as applied to nuclear structure, will yield significant new insights.

3D x-ray microscopy - pioneered at BESSY – has found numerous applications worldwide. To further improve 3D x-ray imaging towards sub-10 nm spatial resolution and to increase the usable photon energy range by phase contrast methods, progress has to be made in x-ray optics, instrumentation and theory. In the talk, the current status and future aspects of x-ray microscopy at 3rd generation electron storage rings and the upcoming Free Electron Lasers with their fs-pulses will be discussed.

# Compact X-Ray Microscopy

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X-ray microscopy in the water-window region ($\lambda$ = 2.3-4.4 nm) is an attractive technique for high-resolution imaging. In this wavelength region state-of-the-art optics has demonstrated sub-20 nm resolution and the sample preparation techniques are maturing. However, present operational x-ray microscopes are based on synchrotron radiation sources, which limit their accessibility. Many biological investigators would benefit from having the x-ray microscope as a tool among other tools in their own laboratory. For this purpose we demonstrated the first compact x-ray microscope with sub-visible resolution.[1] The microscope operates at $\lambda$=3.37 nm (methanol-liquid-jet laser plasma source) with zone plate optics and a W/B4 multilayer condenser.

We have recently developed a flexible, compact x-ray microscope operating at $\lambda$ = 2.48 nm. This wavelength should provide improved imaging of thicker structures compared to the $\lambda$ =3.37 nm microscope in Ref. 1. The microscope is based on a 100 Hz liquid-nitrogen-jet-target laser-plasma x-ray source[2], in-house fabricated diffractive condenser optics[3], in-house fabricated 25 nm Ni zone plates[4], and CCD detection. The sample holder is positioned in a helium atmosphere with silicon nitride membranes separating it from the vacuum in the condenser and imaging module. Initial images of test objects show structures down to 30 nm lines and spaces.

This presentation will discuss sources, the diffractive optics, multilayer condensers, imaging properties, systems' issues, and initial imaging with the compact microscopes.

**References**

1. M. Berglund et. al., J. Microsc. **197**, 268 (2000).
2. P. A. C. Jansson et. al., Rev. Sci. Instrum. **76**, 043503 (2005).
3. S. Rehbein et. al., J. Vac. Sci. Technol. B **22**, 1118 (2004).
4. A. Holmberg et. al., XRM 2005, Himeji (2005).

# Radiolysis of proteins in the solid state: what happens to the disulfide bonds?

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The radiosensitivity of sulfur functions in proteins is well known. When proteins are in solution, the disulfide bonds are reduced by hydrated electrons whereas the thiol functions are oxidized by OH radicals. The thioethers are also sensitive. They can be either desulfurated or oxidized to their sulfoxide form.

When proteins are irradiated in the solid state, results are much less clear. As for disulfide bonds experiments disagree. Observation using synchrotron radiations indicate that the disulfide bonds trap electrons giving disulfide anions. EPR results show the formation of RSS• perthiyl radicals. Calculations by quantum chemistry methods are in agreement with elongation of the SS bond and confirm that disulfide anions are more stable than perthiyl radicals. Analysis of final products shows the reduction of the disulfide to thiol functions.

Thus it seems that the fundamental processes of radiation chemistry of the sulfur functions are not understood.

# Circular Dichroism study of polymorphic oligonucleotides

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Circular Dichroism is a particularly valuable technique for study of nucleic acids because they are polymorphic, that is, they can assume a variety of secondary structures. To illustrate this point we present a study on the polymorphism of oligonucleotides which can adopt several types of conformation. The oligonucleotides were made of three blocks of 12 nucleotides joined by two sequences of four T. The four T can form hairpin allowing the blocks of 12 residues to form base pairs, base triplets, and in some conditions base quadruplets. With this system four types of structure were observed:

- Single stranded unorganised conformation.

- Double stranded conformation with a dangling single stranded arm.

- Intramolecular triplex with non isomorphic stretches of G*G:C and T*A:T triplets. The oligonucleotides were designed to allow the third strand to be either parallel or antiparallel to the GT strand. Difference were observed in the behaviour of the oligonucleotides depending on the orientation of the third strand (parallel or antiparallel)

- Quadruplex structure with two dangling duplex arms. The quadruplex part is due to the dimerisation and folding back on itself of the $G_4T_4G_4$ part of two oligonucleotides.

Each structure exhibits a characteristic circular dichroism spectrum.

Circular dichroism is used to follow transitions between these various conformations. The transitions between the forms and the stabilities of the different structures depend on the temperature, the nature of the salt (for example $Mg^{++}$ or $K^{+}$), the orientation of the third strand, and in some cases of the oligonucleotide concentration.

These oligonucleotides are very attractive models for further studies on the comparative binding of small molecules on various conformations of nucleic acids. This binding can also be studied using induced circular dichroism signal.

# Atmospheric pressure photoionisation of biomolecules studied by mass spectrometry

**Alexandre Giuliani** [1,2], Aïcha Bagag [2], Olivier Laprévote [2]

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Recently developed, atmospheric pressure photoionisation (APPI) has rapidly attracted interest from the analytical chemistry community. Indeed, the technique has proven successful coupling of mass spectrometry with separation techniques such as liquid chromatography. It is based on the formation of a heated pneumatic spray, which is subsequently irradiated by VUV photons. Ions are generated either after light absorption or by chemical ionisation. The technique allows all kind of solvents to be used, even the most non-polar organic solvent and it is insensitive to salts.

These features position APPI as a credible alternative to electrospray for the analysis of non-polar compounds. Moreover, due to the particular ionisation mechanisms, the method possesses interesting capabilities for structural analysis.

Since photoionisation occurs at atmospheric pressure and in the presence of solvent, the technique allows probing the role of the medium upon photoionisation. From a fundamental point of view, atmospheric pressure photoionisation may help understanding basic mechanisms that occur during and immediately after the ionisation process.

An overview of the applications of atmospheric pressure at fixed wavelength to the analysis of biomolecules will be given, going from the fundamental ionisation mechanisms to the applications. The advantages expected from the use of synchrotron radiation are then discussed.

# Protecting life from UV radiation: absorption spectra of known and potential UV screens

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Ultraviolet (UV) radiation is deleterious for life. The spectrum of solar UV emission is generally divided into four regions: the UV-A (315-400 nm), the UV-B (280-315 nm), the UV-C (200-280 nm) and the VUV (<200 nm). Neither VUV nor UV-C reaches the surface of the present-day Earth, thanks to filtration by the stratospheric ozone layer, which accumulated from oxygen produced by photosynthesis. The UV absorption spectrum of DNA shows peaks at 260 nm and 190 nm with continuously increasing absorption throughout the VUV. DNA does not absorb at wavelengths greater than 300 nm, but other cellular compounds do, sometimes leading to free radical formation and thus to indirect degradation of DNA and other cellular components. Before the accumulation of atmospheric oxygen and ozone, organisms were obliged to evolve mechanisms for resisting short wavelength UV radiation. One of these was probably through the synthesis of UV screens.

Using synchrotron radiation, we determined the UV absorption spectra of known and potential UV-screens, isolated from phylogenetically distinct groups of organisms. These spectra, which included the VUV, the UVC, the UVB, and part of the UVA regions, allowed us to compare the potential of these natural substances to protect DNA. Of particular interest are flavonoids, which are phenolic compounds from plants, having absorption spectra that closely resemble that of DNA. We show, using mutants in *Arabidopsis*, that plant seeds are uniquely equipped to withstand UV radiation, thanks to flavonoids that are deposited in the seed coat.

# On the influence of conformational locking of sugar moieties on the absorption and circular dichroism of nucleosides from synchrotron radiation experiments

**Anne I. S. Holm**[1], Esben S. Worm[1], Tapas Ckakraborty[2], B. Ravindra Baby[3], Jesper Wengel[3], Søren Vrønning Hoffmann[4], Steen Brøndsted Nielsen[1]

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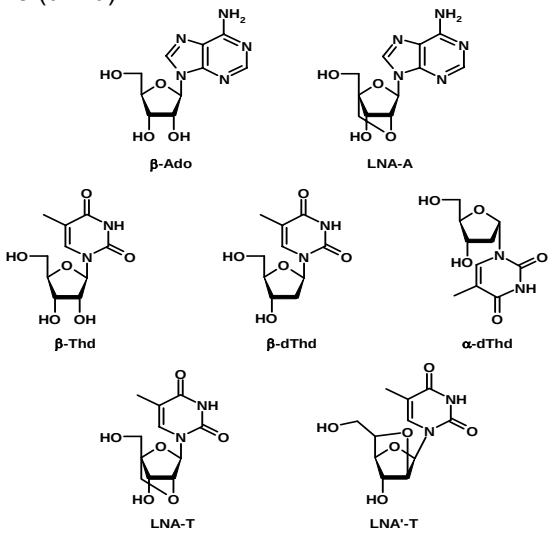
Circular dichroism (CD) spectroscopy is extensively used in conformational analysis of optically active biological molecules, such as proteins, peptides and nucleic acids in the solution phase. Nucleic acid duplexes of A and B type structures exhibit characteristic positive and negative peaks at specific wavelengths in the ultraviolet region of their CD spectra. A change in relative intensity of these peaks due to chemical modification at any nucleotide site is qualitatively related to changes in A-B type structural pattern. In general, the overall structure of the nucleic acid duplexes is determined by conformations of the flexible ribose/deoxyribose sugar rings. To understand the origin of the CD spectral changes due to chemical modifications at specific sites of nucleic acid duplexes, it is essential to investigate the spectra of modified nucleotides.

Nucleic acids in which one or more of the nucleotide sites are altered incorporating methylene bridges between O2' and C4' atoms of their ribose sugars, popularly known as locked nucleic acids (LNAs), have attracted a lot of attention in recent years. Oligonucleotides containing such modified sugar moieties exhibit remarkable helical thermal stability when hybridized to complementary DNA or RNA without detectable reduction in sequence selectivity. Furthermore, the melting temperature of a modified duplex is enhanced by about 4–9 $^{o}$C for incorporation of each modified nucleotide compared to unmodified duplexes.

In this talk I will show absorption and SRCD spectra of nucleosides having purine (adenine) and pyrimidine (thymine) bases, and we analyze the spectral effect for incorporation of methylene internal locking in the sugar moiety between O2' and C4' and between O2' and C5'. The compounds chosen for study are shown below. The importance of the nucleobase being in its $\alpha$ or $\beta$ position (nucleobase down or up relative to the C5' atom of the sugar ring orientation was also investigated for thymidine (dThd).



# Biological Science is the art of the soluble - the role of X-ray sources in structure determination of biological specimen

Thomas Vorup-Jensen

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The structure of biological materials is an integral part of supporting their function. However, high-resolution imaging of biological specimen such as cells and molecules is often dependent on experimental conditions that, at least in part, affect the structure of specimen under investigation. In this seminar I will present results from studies with synchrotron radiation and other sources of X-ray radiation that allow for the determination of the structure of molecules and cells under conditions close to physiological.

# Methods for SRCD data collections of Macromolecules

Frank Wien

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# Sample preparation and cryo fixation in X-ray microscopy

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Biological specimens in an aqueous environment up to a thickness of 10 microns can be investigated in soft X-ray microscopy with a resolution below 30 nm. This method utilises the natural contrast between carbon rich organic material and water to generate a visible contrast within the images. Some care has to be taken to prevent the image quality being deteriorated by sample movement and radiation damage. While these effects are not critical for some specimens, special fixation methods have shown to be necessary in other samples. Besides well known procedures including the treatment of the sample with glutaraldehyde or formaldehyde, cryogenic fixation methods seems to be a worthwhile alternative.

Recently a sample stage for the investigation of specimens at cryogenic temperature has been established at the Aarhus X-ray microscope. In this setup material prepared on sample holders are plunge frozen in liquid ethane. After their transfer into the microscope these ice embedded samples are imaged under vacuum. Alternatively samples can still be investigated under room temperature and atmospheric pressure in a thin water layer.

On the basis of some first experiments the sample preparation procedures for both chemical and cryogenic fixation at the Aarhus microscope will be described in more detail. Advantages and disadvantages of the methods will be addressed. Furthermore an overview of gold labelling techniques for X-ray microscopy will be given.

# List of Posters

**P1** **Synchrotron Radiation Circular Dichroism (SRCD) Spectroscopy of Membrane Proteins**
Ali Abdul-Gader

**P2** **Mass spectrometry for structural analysis of biomolecules**
Aïcha Bagag, Alexandre Giuliani and Olivier Laprévote

**P3** **Low Energy Electron Transmission through Acetic Acid and Fluorobenzene**
Peter Cicman, Richard Balog, Nyk Jones, David Field

**P4** **Testing the structure-process-properties in spider silks**
Cedric Dicko

**P5** **Reactive Interactions of Singly and Doubly Charged Molecular Ions with Surfaces**
L.Feketeová, T.Tepnual, F.Zappa, V.Grill, P.Scheier, J.Žabka, J.Roithová, A.Pysanenko, J.Jašík, I.Ipolyi, Z.Herman and T.D.Märk

**P6** **Biomolecular membrane mimics VUV spectroscopy by high resolution synchrotron radiation**
P J Gomes, P A Ribeiro, M Raposo, N J Mason, S V Hoffmann, P Limão-Vieira

**P7** **SRCD study of conformation of small peptides and their metal complexes**
Béla Gyurcsik, Attila Jancsó, Tamás Gajda, Jiří Šebek, Jaroslav Šebestík and Petr Bouř

**P8** **Synchrotron Radiation Circular Dichroism spectroscopy of the Late Embryogenesis Abundant Protein LjARG2**
Sven Haaning

**P9** **ASTRID 2**
Niels Hertel, Søren Pape Møller, Jørgen Nielsen, Nyk Jones

**P10** **SRCD studies of biomolecules using UV1**
Anne Sander Holm

**P11** **Synchrotron Radiation Circular Dichroism spectroscopy reveals a new structural transition in the muscle protein tropomyosin**
Robert Janes, Robin Maytum

**P12** **Radiobiology with Synchroton Radiation at NSRL**
Shiping Jiang

**P13** **Synchrotron radiation CD spectroscopy at BESSY: Latest developments**
Jan Lengefeld, Peter Baumgärtel, Avinash Kane, David Hertzog, Armin Hoffmann, Gerd Reichardt, David A. Horsley, Robert Seckler, Olgica Bakajin and Benjamin Schuler

**P14** **Circular dichroism spectra of proteins**
Andrew Miles

**P15** **Tortuosity Flow Parameter in Scaffolds from Synchrotron Tomography**
Jens Vinge Nygaard, Luigi Bregnant, Tina Mygind, Cody Bünger, Flemming Besenbacher.

**P16** **Voltage gated sodium channels**
Matthew Radford

**P17** **The Analysis and Interpretation of Synchrotron Radiation Circular Dichroism Data**
Timothy Stone

**P18** **The Protein Circular Dichroism Data Bank (PCDDB): A Bioinformatics & Spectroscopic Resource**
Lee Whitmore

# Atmospheric pressure photoionization - mass spectrometry for structural analysis of oligonucleotides

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The potential of atmospheric pressure photoionization was investigated for the structural analysis of dinucleotides. These compounds are the smallest subunits of nucleic acids that bear sequence information ; their characterization by mass spectrometry is potentially useful in structural studies of nucleic acids.

Almost all the ionization techniques were employed for the analysis of nucleobases, nucleosides nucleotides and oligonucleotides in mass spectrometry, except for the atmospheric pressure photoionisation (APPI). APPI is a new ionization method that has proven successful coupling with mass spectrometry. It has attracted these last years a growing interest for the analysis of biological molecules. Hitherto, APPI was applied to the analysis of various compounds such as lipids[1], pharmaceutical drugs[2], peptides[3] or PAH.[4] In this work, we report a complete study related to the mechanisms of ionization in APPI of the oligonucleotides, which are relatively polar compounds. In source fragmentations were first studied with the UV lamp switched off, that is to say under thermospray conditions. It is shown that, in this mode of operation, fragmentations are minor. Then, the fragmentations patterns of these biomolecules have been monitored in dopant-assisted APPI for different dopants at various amounts. In dopant-assisted APPI, protonated and deprotonated molecular ions dissociate to yield to several characteristic products. The fragmentation pathways are directed by the site of protonation leading to elimination of protonated base, generally the 3'-terminus base. Major yields of sequence ions are observed with the quasi-molecular ions. Reaction mechanisms accounting for the observed products are proposed.

Atmospheric pressure photoionization mass spectrometry proved to be an efficient method for the analysis of oligonucleotides. This technique provides very informative mass spectra and these results confirm that the ionization mechanism in APPI is quite complex and may involve reactive species such as electrons or radicals. Consequently, APPI allows for the effect of the solvent on the photoionisation mechanism to be studied.

[1] S.S Cai and J.A Syage. "Comparison of Atmospheric Pressure Photoionization, Atmospheric Pressure Chemical Ionization, and electrospray Ionization Mass Spectrometry for analysis of lipids", *Anal. Chem.*, **78**, 1191-1199 (2006)
[2] Y.Hsieh and G wang, "Integration of atmospheric pressure photoionization. Interface to HPLC-MS/MS for pharmaceuticals analysis", *Am. Pharmaceut. Rev.*, **7**, 88-93 (2004)
[3] A.Delobel, F.H Halgand, B. Laffranchise-Gosse, H snijders and O. Laprévote, "Structural characterization of Phosphatidylcholines by atmospheric pressure photoionization mass spectrometry", *Anal. Chem.*, **75**, 5961-5968 (2003)
[4] T.J Kauppila, T. Kuuranne, E.C Meurer, M.N Eberlin, T. Kotiaho and R. Kostiainen, "Atmospheric Pressure Photoionization Mass Spectrometry. Ionization mechanism and the effect of solvent on the ionization of naphtalenes", *Anal. Chem*., **74**, 5470-5479 (2002)

# Low Energy Electron Transmission through Acetic Acid and Fluorobenzene

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A new experiment has been built to study the interactions of low energy electrons with solids. The electron source is the photoionisation source used in numerous gas phase experiments in earlier work [1,2], with a typical resolution of 1 meV in the incident beam, using SR from the ASTRID storage ring in Aarhus. Electrons are formed into a beam and focused by a 4-element electrostatic zoom onto the substrate. The sample to be studied is laid down on tantalum substrate, presently cooled by liquid nitrogen. Electron currents at the substrate at very low energy are of the order of hundreds of femtoamps. The energy of the electron beam can varied from a few meV to 10eV. The entire system may be immersed in an axial magnetic field of strength ~$2x10^{-3}$ T. The experiment also includes a custom built trochoidal electron monochromator, which enables the use of much higher electrons current (nA). Using a mass spectrometer it is possible to study electron induced chemistry in the sample.

We present experimental data using the photoionization source for electrons transmission through solid samples of three molecules: acetic acid, fluorobenzene, and water. In the case of water, the sample appears transparent to the incoming electrons, and the transmitted current is nearly the same as that in the absence of the sample. For the other two molecules, the electron transmission is quite different. In fact, for comparable sample thickness (e.g. about 90 monolayers) the transmitted current through water is about 40 times higher than that for acetic acid and about 6 times higher than for fluorobenzene up to 300 meV. The transmission curves also show different structures for the different molecules. These structures evolve with increasing amount of sample irradiation. Although the number of electrons hitting the sample during the scans is estimated to be typically more than five orders of magnitude smaller than the number of molecules deposited and interrogated on the substrate, the changes in the structures in the transmitted current for low energies (up to 300 meV) are very clear and show sharp energy dependence.

After irradiation, the samples were heated and mass spectra recorded. These mass spectra showed no evidence of any chemical change in the sample after irradiation. However, it is clear that even a very small number of electrons with low electron energy is able to induce changes in the sample, as detected in the transmission experiment. This remarkable qualitative feature requires explanation, not yet forthcoming.

**References**

[1] S. V. Hoffmann, S. L. Lunt, N. C. Jones, D. Field, J.-P. Ziesel, Rev. Sci. Instrum. **73** (2002), 4157-4163
[2] D. Field, S. L. Lunt, J.-P. Ziesel, Acc. Chem. Res. **34** (2001), 291-298

# Reactive Interactions of Singly and Doubly Charged Molecular Ions with Surfaces

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Interest in studying collisions of molecular ions with surfaces has been growing rapidly in the past twenty years. Some of the recent interest has been directed to the study of processes stimulated by the impact of slow ions of energy up to 100 eV. In this energy regime the relative collision energy and the energy transferred is within an order of magnitude of energies of chemical bonds. Thus, slow ion-surface interaction studies can provide useful information regarding the nature of both the projectile and the surface, as well as the characteristics of ion-surface interactions. In our work, reactive collisions of slow ions have been studied in an effort to investigate the ion-surface interaction processes involving reflection, surface-induced dissociation (SID), charge exchange reactions (CER) and surface-induced reactions (SIR) and the concomitant energy transfer in ion-surface collisions.

Besides being of fundamental importance, ion-surface reactions are also relevant for a number of technological applications including such different fields as secondary ion mass spectrometry, reactions of ions with aerosols in the Earth's atmosphere and in the interstellar medium all the way to plasma processing of advanced materials and plasma-wall interactions in electrical discharges and fusion plasmas. Here the detailed and quantitative knowledge of the elementary processes in the gas phase and of the interaction of gas phase particles with the plasma walls are necessary as an input for edge plasma modelling and for various diagnostic techniques.

This work presents experimental data on several studies of singly and doubly charged ions ($SF_4^{2+/+}$, $C_7H_8^{2+/+}$, $C_7H_7^{2+/+}$, $C_7H_6^{2+}$ and $C_2X_n^+$, where X=H, D and n=2-5) which interact with a number of targets including stainless steel, carbon tile and diamond surfaces in an energy regime below about 100 eV. These data resulted from a joint study between two laboratories: the laboratory of the Institut für Ionenphysik und Angewandte Physik in Innsbruck and the Čermák Laboratory in Prague. Employing a tandem mass spectrometer (BESTOF) constructed in Innsbruck, the mass spectra of the product ions were measured at different collision energies of the projectile ions. The relative abundance of the product ions as a function of the incident projectile ion energy (so called CERMS curve) was determined. An extension to this was performed in scattering experiments using a beam scattering machine (EVA II) constructed in Prague. The mass spectra, translational energy distributions, and angular distributions of the product ions were measured. The survival probabilities for a range of incident energies and angles were estimated.

Detailed conclusions with respect to SID and other processes and how these differ for singly and doubly charged ions as well as the dominant channels for interactions will be described in the poster. Many results are already published or are about to be published [1-5].

[1] J. Jašík, J. Žabka, L. Feketeová, I. Ipolyi, T. D. Märk and Z. Herman, J. Phys. Chem. A 109 (2005) 10208.
[2] J. Jašík, J. Roithová, J. Žabka, A. Pysanenko, L. Feketeová, I. Ipolyi, T. D. Märk and Z. Herman, Int. J. Mass Spectrom., Chava Lifshitz Memorial Issue 249-250 (2006) 162.
[3] L. Feketeová, T. Tepnual, V. Grill, P. Scheier, J. Roithová, Z. Herman and T. D. Märk, J. Phys. Chem. (*to appear*).
[4] L. Feketeová, F. Zappa, T. Tepnual, V. Grill, P. Scheier and T. D. Märk, Int. J. Mass. Spectrom. (*submitted 2006*).
[5] L. Feketeová, F. Zappa, V. Grill, P. Scheier and T. D. Märk, J. Chem. Phys. (*submitted 2006*).

# Biomolecular membrane mimics VUV spectroscopy by high resolution synchrotron radiation

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In order to quantify the risk of radiation damage, several models have been developed to study the effect of radiation on cellular material. Such models require a detailed understanding of the underlying interactions between the primary radiation and the cellular environment. Our understanding of radiation damage within cells, and thence mutagenesis, therefore depends upon our detailed knowledge of the spectroscopy and dissociation dynamics of the biomolecular constituents. There is up to now little or no information on how the electronic states and thence fragmentation pathways of several biological relevant molecules are influenced by their local environment, e.g. by different phases (solid, liquid or gas). Therefore, cross-sectional results for these interactions are highly relevant to the use of radiation in medicine.

The inclusion of the layer-by-layer (LbL) technique has recently been developed as a promising method for the fabrication of functional molecular heterostructures [1, 2]. Since the interactions occurring in biological systems are essentially ionic interactions and the hydrogen bonding patterns are the same as those observed in LbL films of polyanilines [3 - 6], such films are also potentially good mimics of biological membranes. As part of a large project to study the spectroscopy and the effects of radiation in biomolecular structures, we have already investigated several molecular targets in both gas and more recently in the condensed phases [7 - 9]. Some of these molecules include relevant initiation reaction of the lipidic peroxidation that takes place in the double layer of the cellular membrane and parts of DNA adenine substitution molecules.

[1] G Decher, *Science*, **277**, (1997) 1232.
[2] O N Oliveira Jr, M Raposo, A Dhanabalan, *Handbook of Surfaces and Interfaces of Materials* (New York: Nalwa, H.S., Ed.; Academic Press), Vol.4 (2001).
[3] M Raposo, R S Pontes, L H C Mattoso, O N Oliveira Jr, *Macromolecules*, **30**, (1997) 6095.
[4] R S Pontes, M Raposo, C S Camilo, A Dhanabalan, O N Oliveira Jr., *Phys. Status Solidi A*, **173** (1999) 41.
[5] M Raposo, O N Oliveira Jr, *Langmuir*, **16**, (2000) 2839.
[6] M Raposo, O N Oliveira Jr, *Langmuir*, **18**, (2002) 6866.
[7] R Mota, R Parafita, M J P Maneira, N J Mason, G Garcia, P A Ribeiro, M Raposo and P Limão-Vieira, *Radiat. Prot. Dosim.*, (2006) in press.
[8] R Mota, R Parafita, A Giuliani, M-J Hubin-Franskin, J M C Lourenço, G Garcia, S V Hoffmann, N J Mason, P A Ribeiro, M Raposo, P Limão-Vieira, *Chem. Phys. Lett.*, **416** (2005) 152.
[9] P Limão-Vieira, A Giuliani, M-J Hubin-Franskin, J Delwiche, R Parafita, R Mota, D Duflot, J-P Flament, E Drage, P Cahillane, N J Mason, S V Hoffmann**,** *Chem. Phys.*, **324** (2006) 339.

# SRCD study of conformation of small peptides and their metal complexes

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Electronic absorption and synchrotron radiation circular dichroism (SR CD) spectra of the protonated, basic and zwitterionic forms of L-alanyl-L-alanine (AA) were measured in aqueous solutions and interpreted with the aid of time-dependent density-functional (TD DFT) computations. The role of the conformation, molecular charge and interaction with the polar environment in formation of the spectral profiles is discussed.

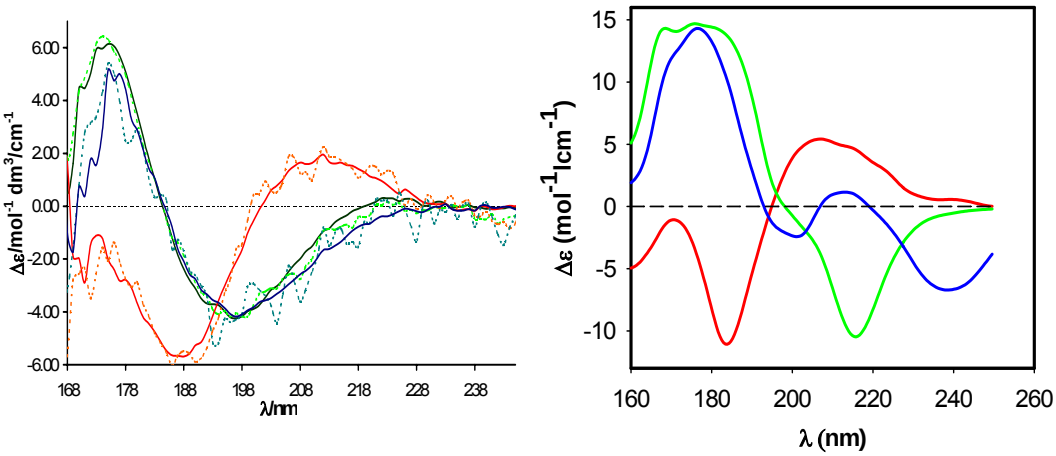


*Figure 1. The measured and the calculated SRCD spectra of the different forms of the Ala-Ala dipeptide. The pH of the measured solutions are: red - 1.43, green - 6.17, blue - 12.1. These pH values correspond to different protonation states of the Ala-Ala dipeptide, which has two pKa values around 3.64 and 8.25. (ref.: P. Reddy, K. Nightingale, Indian J. Chem. 39A, 1157 (2000).*

Addtionally, Zinc(II) and copper(II) complexes of the peptide Ac-His-Lys-His-Lys-His-$NH_2$ (Ac-HKHKH-$NH_2$) mimicking the minimum consensus sequence of the extramembranal metal binding site ( $(HX)_n$, n = 3-6) of eucariotic zinc transporters have been studied by combined potentiometric and spectroscopic (UV-VIS, CD, NMR and EPR) investigations.

The CD spectra in the UV region reflect the conformational changes of the peptide backbone. The pH-dependence of the observed CD intensity at 217 nm has been correlated with the species distribution in the copper(II)-pentapeptide system. The complexes $Cu_2H_{-2}L$, $Cu_2H_{-3}L$ and $Cu_2H_{-4}L$ have identical CD spectra in the UV-region, supporting that they differ only in the protonation state of the farther ε-amino groups of lysines, not influencing the backbone conformation. The increase of the CD intensity parallel with the formation of $Cu_2H_{-5}L$ and $Cu_2H_{-6}L$ indicates notable conformational change of the peptide backbone, which may result in a longer copper-copper distance, and thus decreased antiferromagnetic interaction between the metal ions. This can explain why the dimeric species formed at physiological pH are EPR silent, while those formed at high pH exhibit EPR spectra.

# Synchrotron Radiation Circular Dichroism spectroscopy reveals a new structural transition in the muscle protein tropomyosin

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Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy was used to examine the unfolding of the well-characterised α-helical coiled-coil muscle protein tropomyosin (Tm). The higher photon flux available from synchrotron radiation sources enabled circular dichroism studies to be undertaken into the vacuum ultra-violet (VUV) wavelength region, inaccessible to conventional equipment. The data were collected on the UV1 beamline at ISA, Aarhus, Denmark over the wavelength range 168 to 270 nm. Analysis using singular value decomposition reveals a previously uncharacterised transition centred on a positive ellipticity peak at around 190 nm, prominent in data in the VUV wavelength range 168 to 200 nm. This transition does not follow the same thermal profile as the classic negative 208 and 222 nm peaks, characteristic of α-helical structure, which are seen using conventional CD instruments. This CD spectral region has been linked to super-secondary/tertiary structure elements of protein topology. Significantly for α-skeletal tropomyosin this new transition provides evidence of a previously unresolved structural change that takes place in the physiological temperature range (30 to 37 oC). This is of potential biological significance as recent proposals have suggested tropomyosin flexibility and hence structure is fundamental to its function in muscle regulation.

# Synchrotron radiation CD spectroscopy at BESSY: Latest developments

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The experimental station for circular dichroism (CD) spectroscopy at BESSY has a modular setup, which allows both determination of CD signal, birefringence and insertion of a microfluidic rapid mixing device for continuous flow kinetic experiments. At present two beam lines at BESSY can operate in the spectral range needed for CD on biomolecules as proteins and polysaccharides. Therefore, the station is moveable and can be installed either at the beam line of Dipole 12.1 with a 3 m NIM (normal incident monochromator) or at the Undulator U125/2 with a 10 m NIM.

When measuring CD spectra with short pathlengths and moderate protein concentrations the CD signal can be comparatively small. In such a case baseline artefacts that arise from imperfections in the optics must be considered. To reduce these baseline artefacts we extended the CD experimental station at BESSY to the method of Dual Polarisation Modulation.

We have used kinetic SRCD experiments to probe the collapsed unfolded state of the small cold shock protein Csp*Tm* under near-native conditions. This regime is physiologically most relevant, but difficult to access experimentally, because the equilibrium signal in ensemble experiments is dominated by folded molecules. The collapsed unfolded molecules were transiently populated with a microfluidic rapid mixing device. CD kinetics were measured by scanning the synchrotron radiation beam along an observation channel. Different positions in the channel thus correspond to different times after mixing, with a dead time of 0.4 ms. From our results a significant beta-structure content of the collapsed unfolded state can be deduced

# Tortuosity Flow Parameter in Scaffolds from Synchrotron Tomography

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Open, porous foams made of biodegradable polymers are used as templates for the reconstruction of bone in tissue engineering. Bone is developed by seeding stem cells into porous foams and cultivate the cells prior to implantation of the whole construct. When these cells are grown in culture, a good distribution of cells, oxygen, and nutrients throughout the scaffold is achieved by a dynamic fluid flow through the microstructure. The result of bone forming stem cells cultivated at dynamic conditions for 21 days in a 3D scaffold is shown in figure 1. It is the layout of the random microstructure that determines the local environment to each growing cell. Several structural parameters, such as porosity, tortuosity, surface area, and pore diameter determine the fluid flow conditions and must be characterised in order to predict the cell growth conditions. These parameters are also closely related to the permeability of the foam.

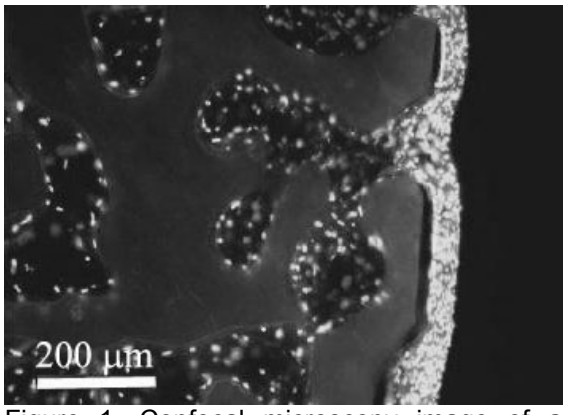


Figure 1. Confocal microscopy image of a PLGA scaffold cultured with human stem cells (small bright dots).

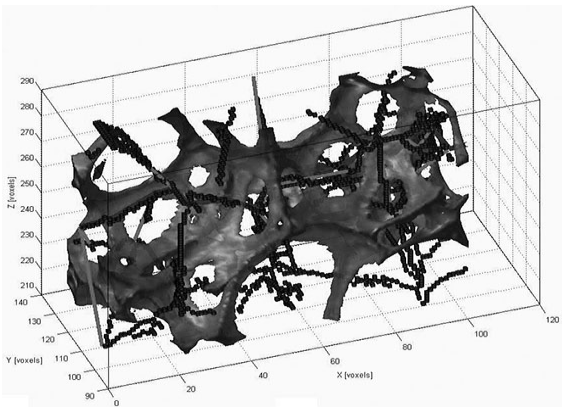


Figure 2. Flow paths with minimum flow resistance reconstructed from a skeletonization of the inverse microstructure.

This study considers the tortuosity parameter. Sheidegger (1963) defines tortuosity as the ratio of the real length that the fluid travels inside a medium to the thickness of the medium. In this work it is suggested that the tortuosity can be estimated from pure geometrical considerations. The travel path of the fluid is approximated by the skeletonisation of the gasseous phase of the foam described by a graph,as shown in figure 2.

Figure 3 shows the results from a 1,5x1,5x1,5 mm polyurethane foam. The tortuosity distribution is calculated in 3 perpendicular planes. The measured value on a macroscopic sample is 1,65.

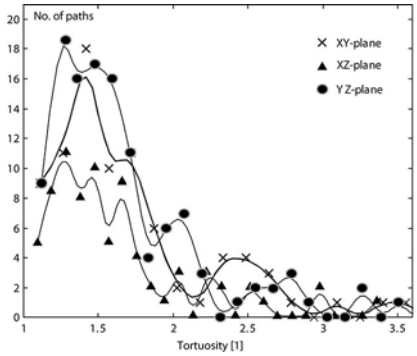


Figure 3. Calculated tortuosity distribution.

Sheidegger, A.E., The Physics of Flow through Porous Media, 3rd ed., Univ. Toronto Press, Toronto (1963).

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