Friday, March 15, Session III

L9

PROTEIN CHARACTERISATION USING DLS – MEASUREMENT PRINCIPLE AND TECHNOLOGY TYPICAL APPLICATIONS, LIMITATIONS AND TROUBLESHOOTING, THE NEW ZETASIZER NANO

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Malvern Instruments offers a wide-array of exciting instrumentation for the biopharmaceutical/biotechnology and academic industries. This presentation gives a scientific overview of the new Malvern platforms and what they deliver to the life-science researcher. The topics covered are: The New Zetasizer ZSP, the Archimedes and the Viscosizer. The Viscosizer and Archimedes are two new systems in the Malvern Instruments portfolio, and offer an orthogonal approach to protein analysis. The Viscosizer offers low-volume sizing of small molecules >200 Da in complex, aqueous and non-aqueous, solvents and measures viscosity accurately at the water viscosity threshold. The Archimedes generates accurate size/mass distributions and particle counting data, for molecules >50nm with clear distinction between monomer and dimer, allowing the scientist to screen for biotherapeutic product contaminants/aggregates quantitatively. The new addition to the Zetasizer range, the ZSP, offers further advances in technology for the accurate sizing and zeta potential measurements of proteins. This offers the researcher more protein characterisation information, resulting in increased confidence when selecting protein candidates for large-scale expression, crystallisation or interaction studies. Altogether, these technologies complement our existing market-leading products and ensure that Malvern Instruments continues to offer leading edge protein analysis instrumentation.

L10

ANTIBACTERIAL PEPTIDES INTERACTING WITH MODEL MEMBRANES. SPECTROSCOPIC STUDY

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Naturally occurring antimicrobial peptides (AMPs) represent one successful form of chemical defense of eukaryotic cells against bacteria, protozoa, fungi, and viruses [1]. Many of them have been already isolated, thousands of their synthetic analogs were synthesized and a broad spectrum of their antimicrobial, anticancer and antiviral activities was proven [2,3]. Despite large number of known AMPs and their therapeutic potency, exact mechanism of action remains a matter of controversy. There is a consensus that these peptides selectively disrupt cell membranes and it is believed that their amphiphatic structure plays an important role in this process.

Novel antibacterial peptide halictine 1 (Hal-1) from the venom of the eusocial bee *Halictus sexcinctus* was recently described and some of its analogs was synthetized [4]. For our purpose we selected some of analogs with already known biological activities to study influence of specific modifications of amino acid sequences on their behavior in the presence of membrane mimicking environment. Thus, we studied the influence of increasing amphipathicity, Arg/Lys exchange or of restricting the ability to form -helical structure. Using various spectroscopic techniques including circular dichroism (CD), infrared(IR) and fluorescence spectroscopy we studied structural changes of chosen peptides induced by 2,2,2-trifluoroethanol (TFE) as a helix promoter, sodium dodecyl sulphate (SDS) as a very simple membrane model as well as interaction with more appropriate models represented by phospholipid bilayers mimicking mammalian and bacterial membranes in order to clarify differences in their biological activities and to suggest the mechanism of the action.

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L11

CHARGE TRANSPORT THROUGH DNA/DNA DUPLEXES AND DNA/RNA HYBRIDS: COMPLEX MECHANISM STUDY

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The position of oligonucleotides among biological and man-made polymers is unique due to its ability to preserve, transfer, and transmit information. Charge transfer through oligonucleotides may serve as an indicator of changes in many physical and chemical properties or to repair some of their lesions. Oligonucleotides conduct electric charge via various mechanisms and their characterization and understanding is very important and complicated task. In this work, experimental and theoretical approaches (time-resolved and steady state fluorescence spectroscopy, melting point measurements plus Density Functional Theory) were combined to study charge transport processes in short DNA/DNA and RNA/DNA duplexes with virtually equivalent sequences. The optical spectroscopy measurements revealed higher values of charge transport through RNA/DNA hybrids. The experimental results were consistent with the theoretical model - the delocalized nature of HOMO orbitals and polarons, base stacking, electronic coupling and conformational flexibility form the conditions for short distance charge transport processes in RNA/DNA hybrids via a coherent charge transport mechanism. In contrast, more localized polarons and molecular orbitals without significant electronic overlap between adjacent base pairs in DNA/DNA duplexes create less effective conditions for charge transport that can occur via incoherent multistep random transport mechanism.

Friday, March 15, Session IV

L12

PLANT NUCLEASE TBN1 INVOLVED IN APOPTOTIC PROCESSES BLOCKS ITS ACTIVE SITE BY A SURFACE LOOP – SIGN OF REGULATORY FUNCTION?

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Tomato multifunctional nuclease (TBN1; UniProt accession no. Q0KFV0), which belongs to the nuclease type I family, plays an important role in specific apoptotic functions, vascular system development, stress response, and tissue differentiation in plants [1]. Furthermore, TBN1 exhibits anticancerogenic properties [2]. The enzyme posseses endonuclease and exonuclease-like activity on ds and ss RNA and DNA and on structured RNA, with production of 5'-mono- and oligonucleotides [3]. TBN1 consists of 277 aminoacids with a molecular mass of 31.6 kDa (about 37 kDa when glycosylated).

Structures of wild type TBN1 and mutant N211D were solved by our group by the means of X-ray crystallography [4]. Molecules of TBN1 form super-helices generated by crystal symmetry, where contacts are provided by the active site of one molecule and a surface loop of a neighboring molecule. This motif is conserved in all the known crystal structures of the enzyme but the rest of crystal packing differs across different crystallization conditions. Formation of intermolecular contacts in crystals suggests the way of assembly of molecules into oligomers in solution. The interaction of the active site and the surface loop is best