Abstracts

– Single molecule imaging and spectroscopy –

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INS spectroscopy study of Pt(II) and Pd(II) polyamine complexes displaying anticancer activity

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Pt-based antitumour drugs have been the target of intense research since Rosenberg's discovery of cisplatin (*cis*-$(NH_3)_2PtCl_2$). Their antitumour properties are based on selective interactions with DNA. However, a simple change in structure can drastically affect this cytotoxic activity. Pt(II) and Pd(II) complexes with two or three metal centres and aliphatic polyamines as bridging linkers, constitute a new class of third-generation drugs of great potential clinical importance.

This work reports an inelastic neutron scattering (INS) spectroscopy study of Pt(II) and Pd(II) chelates with biogenic amines. Good quality INS spectra were obtained from *ca.* 250 mg of compound, which is the smallest sample of a hydrogenous compound for which a successful INS interpretation has been reported. These INS spectra were completely assigned, in the light of DFT calculations and optical vibrational spectroscopy (Raman and FTIR) data. The cytotoxic properties of these complexes were previously evaluated [1-3], in view of gathering information on the *structure-activity relationships (SAR's)* ruling their biologial activity.

1. M.P.M. Marques *et al.*, *BBA (MCR)* **1589** (2002) 63.
2. L.J. Teixeira *et al.*, *J.Med.Chem.* **47** (2004) 2917.
3. S.M.Fiuza *et al., Letters in Drug Design and Development* **3** (2006) 149.

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Single fluorophore tracking of P-selectin-EGFP during exocytosis of Weibel-Palade bodies

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Weibel Palade bodies (WPB) are endothelial cell-specific secretory organelles that contain the leukocyte adhesion molecule P-selectin. WPB exocytosis delivers P-selectin to the cell surface in response to vasculature injury or inflammation. Little is known about the dynamics of P-selectin in the endothelial cell plasma membrane. The mobility of P-selectin-EGFP in the plasma membrane of human umbilical vein endothelial cells (HUVEC) under resting conditions and during delivery into the membrane following WPB exocytosis was studied. P-selectin-eGFP was transiently expressed in HUVEC by nucleofection and imaged using total internal reflection fluorescence microscopy. Using single fluorophore tracking we measured the lateral mobility of P-selectin, constitutively expressed at the plasma membrane under resting conditions ($0.075\pm0.09\mu m^2/s$, n=1537). Distribution of D_{lat} of the individual molecules showed two types of mobility: freely moving molecules and molecules with severely restricted mobility. 48hr post-nucleofection, P-selectin was found predominantly within WPB with few detectable single fluorophores on the plasma membrane. Following WPB exocytosis, evoked by ionomycin ($1\mu M$), the number of detectable single fluorophores on the membrane increased significantly. The majority of single fluorophores detected during WPB exocytosis were tracked in the first few seconds following exocytosis, within a $2\mu m$ radius of the point of fusion. The lateral mobility of secreted P-selectin at the site of WPB fusion was $\sim 0.12\mu m^2/s$ (n= 264 objects). Acutely secreted P-selectin, at the WPB release site, is more mobile than constitutively expressed P-selectin.

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Probing fast conformational dynamics of biomolecules by photoinduced electron transfer (PET)-FCS

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Detailed information about the dynamics of conformational fluctuations of proteins and nucleic acids in aqueous environment is imperative for a refined understanding of biomolecular recognition and folding pathways. We present data about structural changes of single biomolecules, i.e. oligonucleotides, and peptides determined under equilibrium conditions in solution occurring on time scales ranging from nanoseconds to milliseconds. The technique is based on quenching photoinduced electron transfer (PET) reactions between guanosine or tryptophan residues in proximity to selected fluorophores in combination with fast fluorescence correlation spectroscopy (PET-FCS). We demonstrate that PET-FCS can be used advantageously to study fast folding phenomena at the single-molecule level under thermodynamic equilibrium with nanosecond time resolution. Using PET-FCS we monitor folding transitions as well as conformational flexibility in the denatured state of a 20-residue protein (Trp-cage) and different mutants. Besides microsecond folding kinetics we reveal hierarchical folding of Trp-cage, hidden to previous experimental studies. Finally, we use the technique to study the influence of macromolecular crowding on polypeptide chain dynamics. Here our data suggest that within a cellular environment the early formation of structural elements in unfolded proteins can still proceed quite efficiently in spite of hindered diffusion caused by high macromolecular content.

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Purification of the retinal ABCA4 transporter for structural and functional studies

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ABCA4 is a member of the ATP binding cassette (ABC) superfamily of membrane transporters, localised to the disk membranes of photoreceptor cells. Mutations in the *ABCA4* gene are responsible for several autosomal recessive retinal degenerative diseases including Stargardt's disease. This suggests a role for ABCA4 in the visual cycle, most likely in the transport of a retinal derivative. Several substrates for ABCA4 have been proposed, including all-trans-retinal and the lipid conjugate, *N*-retinylidene-PE. The aims of our investigations are (i) characterise whether these compounds interact with ABCA4 and (ii) describe the structure of ABCA4 using electron microscopy (EM). The protein was engineered with a C-terminal His_{12} tag and expressed in insect cells using recombinant baculovirus. Unfortunately, non-ionic detergents could not extract more than about 5% of the ABCA4 protein. Only the detergents foscholine-14 and -16 were able to extract ABCA4 from insect cell membranes. The soluble protein was subjected to Ni-NTA chromatography and subsequent size exclusion chromatography, and a purity of more than 90% was achieved. The protein-detergent complex had an apparent molecular mass of 1075 kDa as compared to soluble molecular mass standards. The sample was detergent exchanged into dodecyl-β-maltoside to enable EM studies of the protein. Single particle analysis of the protein is now underway. Furthermore, the reconstitution of the purified protein into lipid vesicles is being undertaken using a detergent adsorption technique.