MS14.02.03 AUTOMATED SCATTERING CURVE MODELLING OF PLASMA MULTIDOMAIN PROTEINS. S. J. Perkins, Dept. Biochemistry and Molecular Biology, Royal Free Hospital Sch. Med., Rowland Hill St., London NW3 2PF, UK

Solution scattering analyses of multidomain proteins have been much enhanced by a recently-developed method of constrained scattering curve fits. The domains are represented by known atomic coordinates from homologous proteins. Scattering curves are calculated using a previously calibrated procedure. This process can be automated in order to generate a range of sterically allowed structures which are then evaluated systematically. Error limits on the ensuing models can be determined. Three types of applications have been developed to date for single proteins, glycosylated proteins and protein-protein complexes: (1) Bovine immunoglobulin G was analysed in terms of two Fab subunits and one Fc subunit to show that an extended planar structure gave good fits. The six-domain structure of the Fc subunit of human immunoglobulin E was studied to show that only bent non-linear Fc structures satisfied the experimental scattering curves. (2) The heavily glycosylated sevendomain cell surface protein, carcinoembryonic antigen (>50% carbohydrate) was represented by an adaptation of the crystal structure of the two-domain protein CD2. This yielded an extended model that satisfied the scattering data. (3) Homodimers and homotrimers of the bacterial cytosol amide sensor protein AmiC, which is structurally related to two-domain monomeric periplasmic binding proteins, were modelled to show that compact associations of the monomers accounted for their scattering curves. The heterodimeric complex of tissue factor and factor VIIa, which are two- and four-domain plasma proteins implicated in blood coagulation, were modelled to result in a compact complex. While unique structures cannot be determined by scattering analyses, application of the constraints inherent in the use of known crystal structures for scattering curve fits usually permitted a limited family of multidomain structures to be determined in each case. In combination with other information in relation to known ligand binding sites, these studies can provide useful biological insights on the steric accessibility or exposure of domains in multidomain proteins and their complexes.

MS14.02.04 EYE LENS TRANSPARENCY OR COLD CATARACT : A QUESTION OF PROTEIN INTERACTION POTENTIALS. Annette Tardieu[1], Marc Malfois[1], Françoise Bonneté[1] & Luc Belloni[2]. [1]Laboratoire de Minéralogie Cristallographie, Tour 16, Case 115, 4 Place Jussieu, F-75252 Paris Cedex 05, France; [2]CEA-DRECAM, Service de Chimie Moléculaire, Centre d'Etudes de Saclay, 91191 Gif-sur-Yvette Cedex, France

Interaction potentials between the eye lens proteins, the crystallins, account for eye lens optical properties. Alpha-crystallin repulsive interactions control lens transparency whereas gamma-crystallin attractive interactions are responsible for a fluid-fluid phase separation with decreasing temperature, known as cold cataract.

Small angle X-ray scattering was used to construct crystallin phase diagrams. Experimental structure factors were measured in a variety of physico-chemical conditions : pH, ionic strengh and temperature. Theoretical structure factors, calculated using the numerical methods developped in liquid state and colloïd physics, allowed us to determine the best fit parameters of the attractive and repulsive potentials. Relationships with macromolecular properties could be inferred. The protein charge generates a coulombic repulsive potential that is a function of pH and Debye length. The attractive Van der Waals forces are sufficient to account for the gamma-crystallin phase separation. Entropic effects, however, contribute to the attractive potentials at high ionic strengh.

MS14.02.05 NEW DIRECTIONS IN MUSCLE DIFFRACTION USING INSERTION DEVICE BEAMLINES AT SYNCHROTRON SOURCES. Tom Irving, BioCAT, Illinois Institute of Technology

The modern concept of the mechanism of muscle contraction is based on structural, mechanical and biochemical studies, all indicating that relative sliding between the two filament types in the sarcomere depends on cyclic ATPase-coupled crossbridge interactions between myosin heads on the thick filaments and actin in the thin filaments. Fiber diffraction has historically and continues to play a key role studiying this system because of its ability to study muscle fibers under hydrated, physiological conditions, in fact even in the living state. Furthermore, it has the ability (as yet only partially realised) to detect global changes in sarcomere structure at the physiologically relevent millisecond and sub-millisecond time scale. Small angle X-ray diffraction of muscle make unusally high demands on X-ray sources and optics. Over 20 year ago Ken Holmes and Gerd Rosenbaum performed the first muscle experiments using a storage ring as a source of synchrtron radiation motivating the development of the first synchrotron X-ray diffraction facility. Since then, static and limited time-resolved experiments using synchrotron sources have told us much of what we know about muscle contraction. The future promise of time resolved X-ray diffraction studies of muscle can be realized in studies that produce full two-dimensional diffraction data at high time resolution during rapid mechanical transients from cellular level preparations. The major impediment to reaching this goal has been lack of flux. I will present a personal overview of experiments that have been made possible by high intenisty insertion device beamlines at CHESS, Daresbury, and an innovative beamline on the KEK Main ring in Tsukuba and where I think the field will be going with the advent of undulator based beamlines at ESRF, Spring-8 and the Advanced Photon Source.

MS14.02.06 MICROPHASE SEPARATION IN BULK AND SURFACE-ADSORBED BINARY ALKANE SYSTEMS. E.P. Gilbert, P.A. Reynolds and J.W. White, Research School of Chemistry The Australian National University Canberra. ACT 0200. AUSTRALIA.

Certain binary mixtures of n-alkanes, when quenched from the melt to room temperature, undergo spontaneous demixing from the solid solution to form a lamellar microphase[1]. In this 'bulk' system, demixing is essentially longitudinal, the molecules slipping along their long axes to separate into lamellar components which neutron scattering can easily detect when isotopic substitution of one of the components is employed[2].

STM studies have shown that when these mixed hydrocarbons are spread onto a clean graphite surface from a non-polar solvent, only the longer chain is imaged. This preference indicates a strong demixing enthalpy in the two-dimensional layer.

We have investigated microphase formation in binary $C_n:C_{36}$ systems (20<n<34) not only in the bulk but also in which the two-component protonated/deuterated melt is quenched inside the gallery structures present in exfoliated graphite. In the latter system, it is possible for phase separation not only to occur in the longitudinal direction, as in the 'bulk', but also in a transverse direction due to the graphite surface forces.

We report here how the microphase structure and its rate of formation are determined by the presence of the graphite substrate, alkane composition, isotopic labelling, quench temperature and chain-length mismatch[3].

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