

The aim of this work is to investigate the character of the time-course of the blood plasma particles sizes and their concentrations in the samples during 30 hours after their preparation. For this purpose we use dynamic light scattering (DLS) that is a powerful technique for studying of the particle sizes in the solutions.

We study the fresh heparinized blood plasma samples obtained from the donors (n=10) of different ages. For all samples we obtain particle size distributions (PSDs) of the scattered light by DLS. Each PSD contains from three to five specific peaks (protein complexes). For each peak we observe the mean sizes changing during 30 hours after sample preparation. Then we calculate the volume concentration of particles of each sort using the Rayleigh-Gans-Debye approximation. The time-courses of plasma particles sizes and their concentrations represent the oscillations with decreasing amplitudes and non-regular periods. Such behavior of the system is typical for relaxation transition of the system to another state that is caused by the disbalance of the aggregation and degradation rates. The amplitude and period of these oscillations could be useful parameters for some disease diagnostics.

2314-Pos Board B6

Glass is a Viable Substrate for Atomic Force Microscopy of Membrane Proteins

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Since its invention in the mid-1980s, the atomic force microscope (AFM) has become an invaluable complementary tool for studying membrane proteins in near-native environments. Historically, mica is the most common substrate utilized for biological AFM. Glass being amorphous, transparent, and optically homogeneous has its own set of advantages over mica and has the potential to broaden the use of the AFM into fields that require high quality non-birefringent optical access. The use of silanized glass as AFM substrates has been reported as a means to fine tune surface chemistry. However, such coatings usually require hours of additional preparation time and can lead to increased surface roughness. In this work, we present a simple technique for preparing borosilicate glass as a substrate for two membrane systems: non-crystalline translocons (SecYEG) of the general secretory system from *E. coli*, and bacteriorhodopsin (BR) from *H. salinarum*. For both these membrane proteins, quantitative comparisons of the measured protein structures on glass versus mica substrates show agreement. An additional advantage of glass is that lipid coverage is rapid (< 10 minutes) and complete (occupying the entire surface). A goal is to study the bacterial export system using recently developed precision measurement techniques such as ultra-stable AFM.

2315-Pos Board B7

Detection of Protein Nanocrystals Based on the Reversibility of Crystallization

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A prerequisite for conventional X-ray protein structure analysis is the growth of crystals with a sufficient size in the range of several μm . This is a time consuming and not always successful process, challenging especially when working with membrane proteins. The recently developed technique of femtosecond X-ray crystallography enables structure analysis of crystals with a size in the nm range, thus the process of growing large single crystals can be avoided. Moreover femtosecond X-ray nanocrystallography is a potential method to overcome the radiation damage problem and to perform time-resolved structure analysis (1, 2). Nanocrystals are too small to be detected with an optical microscope, hence crystal growth cannot be monitored with common methods used in crystallography. A powerful technique to screen for nanocrystals is Second Order Nonlinear Imaging of Chiral Crystals (SONICC). This method is based on the principle of second harmonic generation and detects noncentrosymmetric ordered crystals (3). However, the instrumentation is not generally accessible yet. In addition, proteins ordered in higher symmetry crystal classes do not necessarily lead to a positive SONICC signal. In this work, a new method is developed to screen for nanocrystals based on the reversibility of crystallization. We show that dilution experiments performed with a crystallization robot and monitored by a crystallization imaging system enable the distinction between precipitation comprised of nanocrystals and precipitation caused by aggregated protein.

2316-Pos Board B8

Characterizing the Structure of Lipodisq for Membrane Protein Spectroscopic Studies

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Membrane protein spectroscopic studies are challenging due to the difficulty introduced in preparing homogenous and functional protein incorporated lipid system. Traditional membrane mimetics such as micelles or vesicles are proved to be powerful. Though, they all possess their own drawbacks. The lack of lipid bilayers of micelles could result in the increasing dynamics of membrane proteins and obtaining homogenous vesicles are not easy in the real applications. Recently, a nano-sized particle lipodisq was utilized to serve as a better membrane mimetic, it provides a lipid bilayer environment and homogenous samples. Furthermore, unlike nanodisc, lipodisq won't interfere the absorbance property of membrane proteins. Though lipodisq shows a high potential to become a good membrane mimetic to enhance the biophysical studies of membrane proteins, there is still lack of structural characterization of lipodisq in different lipid compositions that close to native lipid environment of membrane proteins. In this study, the formation of lipodisq nanoparticles using different weight ratio of 3:1 SMA polymer to POPC/POPG lipid was characterized using dynamic light scattering (DLS) and solid state nuclear magnetic resonance (SSNMR) spectroscopy. We achieved a physiologically relevant size (10nm) of lipodisq nanoparticles complex at weight ratio of 2.25:1 (3:1 SMA polymer : POPC/POPG lipid) and the transition phase from vesicle to lipodisq was characterized. These data were also compared with the corresponding data obtained for bicelles and micelles. This study will provide a proper path for the researcher working on membrane protein system to obtain pertinent structure and dynamic information on physiologically relevant membrane mimetic environment.

2317-Pos Board B9

Studying Distribution and Aggregation of TDP-43 in Mammalian Cells: A Comparison between Fluorescent Protein and Tetracycline Labelling Strategies

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Aggregation and deposition of TAR DNA-binding protein 43 (TDP-43) in motor neurons is a key pathological feature in nearly all amyotrophic lateral sclerosis (ALS) cases. However, little is known about the mechanism of TDP-43 aggregate formation, in particular how the protein structure changes over time and how this correlates with changes in cellular localisation. To date, a detailed analysis of the dynamic processes leading to TDP-43 aggregate deposits in live cells has not been reported. We have established neuronal cell lines that express the full length or C-terminal fragment (residue274-414) of TDP-43 tagged with a tetracycline (TC) motif that binds specifically to biarsenical dyes (FlAsH, ReAsH), and we are comparing this to a cell model expressing TDP-43 fused to green fluorescent protein (GFP). Unlike GFP which is 27 kDa in size and thus, much larger than the C-terminal fragment, the TC-tag and biarsenical dyes are relatively small (~1.1kDa); therefore, are less likely to interfere with the distribution and function of recombinant proteins. Using confocal microscopy and intermolecular FRET analysis, we demonstrate that biarsenical labelling technique enable the visualisation of TDP-43 localisation for the monomers and aggregate formed within live cells; in particular we can monitor the changes in distribution and aggregation as a function of time. Furthermore, using this approach we are able to compare, in a quantitative manner, the behaviour of ALS-associated variants of TDP-43 (A315T, M337V) that have been reported to possess enhanced aggregation as compared to the wild-type protein. Such models will prove useful towards investigating how changes in the structure of TDP-43 influences its localisation and aggregation under both physiological and cell stress conditions, and how these processes may relate to the pathogenicity and progression of ALS.

2318-Pos Board B10

Optimized Internalization of Fluorescently Labeled Biomolecules into Live Bacteria

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We have recently developed a method for delivering short DNA fragments and proteins labeled with bright and stable organic fluorophores into live *E. coli*. Cells are electroporated with a high-voltage pulse, recovered in a rich medium