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 causes by the disbalance of the aggregation and degradation rates. The amplitude and period of these oscillations could be useful parameters for some decease 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 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 secretary 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 Tetracysteine 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 tetracysteine (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

and thoroughly washed to remove any non-internalized fluorescence. Internalized molecules can be tracked at the single-molecule level, and both single-cell and single-molecule FRET can be measured.
We have explored the effect of electroporation voltage on the internalization efficiency of short DNA fragments and proteins, and have found a linear relationship. Hence, an appropriate voltage can be selected depending on the application of interest, such as whether single-molecule or ensemble measurements are desired. In addition, we have optimized the buffer and salt conditions for electroporation, in terms of maximizing internalization efficiency whilst preserving protein integrity.
Various conditions have been tested for cell washing, including the use of salt and detergent in the washing buffers. The medium used for cell recovery after electroporation has also been noted to affect the efficiency of cell washing. A significant improvement in the removal of non-internalized fluorescence has been achieved by cell filtration. In the case of proteins prone to aggregation, cell filtration has also been found to remove any high-molecular weight species from the cell suspension.
Finally, we have found free dye to be internalized at much higher efficiency than the labeled biomolecules, and hence it is important to remove any contaminating free dye from the samples used for electroporation. We have optimized the methodology for determining and minimizing the amount of free dye in our samples, and have obtained samples that show less than 1% free dye contamination, which is at the level of background autofluorescence.

2319-Pos Board B11
Photo-Activated Crosslinking Mass Spectrometry for Studying Biomolecular Interactions
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The structural and mechanistic analysis of biomolecular interaction is important for understanding the molecular basis of a wide range of biological phenomenon. Relatively weak interactions between functional molecules may play crucial roles in regulating highly networked and dynamically controlled biological systems. This type of interaction, however, is more challenging to study because of its transient nature. As a general solution to the problem, we propose the use of chemical crosslinking in combination with mass spectrometry and apply it to protein-protein interactions. Ultraviolet-activatable crosslinker molecules that are incorporated to the protein of interest can be utilized to capture transient interactions under a physiological condition by forming covalent bonds between interacting molecules in close proximity via carbene chemistry. The crosslinked sample is then enzymatically digested and analyzed by liquid chromatography-mass spectrometry. High mass resolution analysis ensures identification of crosslinked peptide species and enables spatial mapping onto available structural models obtained from crystallography or NMR spectroscopy. The proposed methodology is demonstrated with a model system of cytochrome c and its oxidase, where we find multiple binding modes and explore their possible role in controlling enzymatic activity.

2320-Pos Board B12
Protein Resilience and Fluorescent Protein Resistance to Photobleaching
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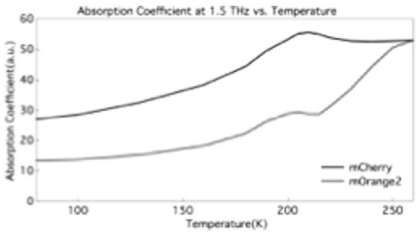
Fluorescent proteins (FPs) are ubiquitous in biophysics. To simultaneously tag many different biomolecules and allow excitation at lower wavelengths, FP's have been developed with excitation and fluorescence at wavelengths $>$ 550 nm, however these red fluorescent proteins are more susceptible to photobleaching [1].



A possible reason for this is a decrease in the structural stability of the beta barrel, but X-ray B-factor measurements do not indicate this stability change. Zaccai introduced a measure of protein stability called resiliency [2], derived from the temperature dependent atomic mean squared displacement measured by neutron scattering. These facility-based measurements require ~100 mg of protein, so are not conducive to systematic testing of changes in resiliency with mutation. However table top THz optical absorption measurements have shown the same temperature dependence as [3]. Using this method we find the resiliency of mCherry is 2.5 times higher than mOrange2 consistent with mCherry's higher stability and longer irreversible photobleaching time constant (3.46 s) compared to mOrange (0.26 s).
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2321-Pos Board B13
Microsecond Conformational Dynamics of Cytochrome C Revealed by Two-Dimentional Fluorescence Lifetime Correlation Spectroscopy
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Characterization of the folding process is a long-standing central issue in protein science. Single-molecule spectroscopy, especially that in combination with fluorescence resonance energy transfer (FRET), has been utilized as a powerful tool to explore the conformational heterogeneity of proteins and its transition dynamics on the sub-millisecond to second timescales. However, observation of the dynamics on the microsecond timescale is still challenging. Elucidation of protein dynamics in the microsecond region is very crucial to understand elementary processes of not only folding but also various biological functions of proteins. We recently developed a new single-molecule technique to quantitatively examine the microsecond dynamics of biomolecules based on fluorescence lifetime correlation analysis. This method, two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS) [1,2], was applied to the spontaneous conformational transition of cytochrome c (cyt c) in this study. One fluorophore, Alexa546, was covalently attached as a FRET donor to the single free cysteine residue of cyt c located in the C-terminal region. The temporal change in the donor fluorescence lifetime due to FRET between the donor and heme was then analyzed to evaluate the conformational transition dynamics of cyt c. We show that 2D FLCS reveals diverse conformers of cyt c and provides unambiguous information about their microsecond transition dynamics. This work demonstrates the high capability of 2D FLCS to elucidate the complex conformational transition dynamics of proteins.
References
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2322-Pos Board B14
Measuring Protein Structural Heterogeneity with Two-Dimensional Infrared Spectroscopy
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Protein structure and heterogeneity is particularly difficult to measure due to lack of experimental techniques that combine structural sensitivity and sub-microsecond time resolution. Two dimensional spectroscopy is a new optical technique that measures protein structure and dynamics with ultrafast time resolution. The delocalized backbone C=O (Amide-I) vibrations reflect the global secondary structure of the protein. A 13C=18O isotope label on a residue red-shifts its frequency by ~60 cm-1, isolating the site from the main amide band. The label provides a unique spectroscopic handle on the structure (distances), heterogeneity, and hydrogen-bonding environment (solvent exposure) of the labeled residues, and the ultrafast time resolution is able to distinguish between different fast-exchanging conformational states.
We apply this new method to NTL9, a 39-residue α/β mini protein, by isotope labeling five different sites, including a dual-label across a type-I beta-turn. The structural interpretation is enabled by spectral simulations based on a recent Markov state model (MSM) built from millisecond-long molecular dynamics trajectories. Structures are assigned by matching the measured frequencies and lineshapes to simulated spectra for each Markov state. The excellent qualitative agreement between theory and experiment provides a solid set of structural constraints. We find a number of sub-states with different configurations, particularly in flexible regions of the protein, such as the type-I beta turn. Specifically, we find a significant population of bulged turn configurations. The results show that residues in the first and last turns of the helix exhibit multiple hydrogen-bonding environments reflecting the greater solvent-exposure within these regions of the backbone. Finally, the lineshapes serve to characterize the flexibility and stability of the backbone at the different sites. We find that β-strands remain relatively rigid whereas the turn and helix regions show increased flexibility, qualitatively matching b-factors extracted from crystallography.

2323-Pos Board B15
Conformational Equilibrium between the Sub States of the Acidic Denatured State of ACBP Determined by NMR Chemical Shifts and Metadynamics
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NMR Chemical shifts have recently gain renovated attention as a probe for both the structure and the dynamics of proteins. The structure and the dynamics of