1102-Pos Variolin Receptors for the Selective Binding of Anions

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Board B78

Assay studies previously reported in the literature have shown that variolins, meridianins, and meriolins inhibit protein kinases, including several which are responsible for the production of blood vessels in tumor tissues. These structurally similar classes of indole alkaloids also inhibit the growth of the P388 tumor cell line, and are active against herpes Simplex and the polio virus. Therefore, the properties of these biologically active compounds are an active area of investigation. We have investigated the absorbance and fluorescence properties of several variolin derivatives, and observed the selective binding of halide anions. According to the Job's plot, the binding stoichiometry is one to one. Electron-donating substituents such as the methoxy have shown a fluorescence enhancement, and quantum yields have been calculated to be much higher when the methoxy substitutent is attached. Fluorescence quenching occurs through a photoinduced electron transfer (PET) mechanism. The fluoride anion binds to the receptor via the N-H hydrogens, with the result being an increase of the PET. Proton NMR titration indicates the importance of these N-H bonds in the selective anion binding. Association constants obtained by using a Benesi-Hildebrand plot indicate that the fluoride anion has the greatest association constant, followed by Cl^- and Br^-, in accordance with the basicity of these anions. Consequently, enhanced selectivity for F^- occurs. Therefore, these variolin derivatives show the potential to be useful as fluorescent anion sensors.

1103-Pos Substrate Channeling in the Sulfate Activating Complex: Combined Continuum Modeling and Coarse-grained Brownian Dynamics Studies

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Board B79

This article describes modeling the whole channeling process via numerical solution of the time-dependent Smoluchowski diffusion equation, as well as Brownian dynamics. We have found that the synthesis of APS2- is not a diffusion-controlled process while the phosphorylation of APS2- is diffusion-controlled. Electrostatic plays the essential role in the APS2- diffusion. Furthermore, by combining with coarse-grained Brownian dynamics, substrate channeling process has been studied with reactions in multiple active sites. Our simulations provide a bridge for numerical modeling with Brownian dynamics to simulate the complicated reaction and diffusion biosystems and raise important questions relating with the electrostatically mediated substrate channeling in vitro and in vivo.

1104-Pos Biochemical and Biophysical Studies of the NS3-4A protease/helicase from Hepatitis C Virus

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Board B80

The NS3-4A protein is a bifunctional serine protease/helicase from the hepatitis C virus (HCV) that is responsible for several pro-translational processing of viral proteins. Therefore, NS3-4A has become a prime target for inhibitor design to combat the virus. In my poster, I will present current biochemical and biophysical data on my research pertaining to the progress towards gaining insight into how NS3-4A functions on its substrates.

Molecular Chaperones

1104.01-Pos Single Molecule Fluorescence Studies on the Hsp90

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Board B80.01

The molecular chaperone Heat-Shock-Protein 90 (Hsp90) is one of the most abundant Proteins in unstressed eukaryotic cells. It is involved in the folding and stabilization of several key regulatory Proteins as the tumor suppressor factor p53, stereoid hormone receptor and kinases. The ATPase-activity seems to be of crucial importance for its *in vivo* functions[1]. Recently it has become the target for a new type of anti-cancer drugs that specifically inhibit its ATPase-function[1,2]. The recently published crystal structures[3] of Hsp90 suggest, that the structural changes, which occur during the ATPase-cycle, are in the order of several nanometres and thus accessible with Fluorescence-Resonance-Energy-Transfer (FRET).

Conformational changes of the Hsp90 dimer were observed with single molecule FRET using total internal reflection microscopy. In combination with bulk FRET experiments we could show that - in contrast to previous assumptions - the dimer does only one ATP cycle before it dissociates. To be able to measure several complete ATPase-cycles we stuck the two Monomers together so that the dissociation constant is decreased significantly. The Mutant Hsp90 shows unchanged ATPase and Co-Chaperone-binding (Sba1) characteristics. The single molecule measurements with these modifications reveal further insights into the Hsp90 ATPase cycle.

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1104.02-Pos Single Molecule Imaging Of Chaperonin Functions Using Zero-mode Waveguides

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Board B80.02

Chaperonin GroEL, which is composed of two rings, mediates protein folding while co-chaperonin GroES binds to each ring of GroEL. It has been unclear more than a decade whether two GroES-molecules bind to both rings of GroEL simultaneously during the chaperonin reaction cycle. A single molecule fluorescence (SMF) imaging technique is expected to be able to resolve this controversy. While GroES is expressed at the concentration of 5uM in the living-cell, conventional SMF imaging by total internal reflection fluorescence microscopy (TIRFM) is not available due to high background fluorescence intensity of 5 uM fluorescent dye-labeled GroES. On the other hand, zero-mode waveguides (ZMW), which is nano-holes arrays lined up on a metal-clad quartz glass, can reduce the background intensity by three orders of magnitude than that of TIRFM. We therefore fabricated ZMW and confirmed it was suitable for SMF imaging of association and dissociation of Cy3-labeled GroES to GroEL immobilized on the ZMW. The histogram of durations from binding to release showed that GroES released from GroEL after two sequential steps, which agreed with previous studies. Next, the solution containing 500 nM Cy3-GroES and 500 nM Cy5-GroES, 2 mM ATP, denatured protein as substrate was introduced. On the position of the Alexa488-GroEL preassigned, Cy3-GroES and Cy5-GroES appeared and disappeared repeatedly. It will be discussed how GroES binds to double rings of GroEL in the meeting.

1104.03-Pos ADP Regulates Interaction Between *Escherichia Coli* Chaperonin GroEL And Its Cofactor GroES

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Board B80.03

GroEL is an *Escherichia coli* chaperonin which is composed of two heptameric rings stacked back to back. GroEL interacts with its cofactor GroES and assists protein folding in an ATP dependent manner. Because of negative cooperativity between two rings in GroEL, it is widely believed that GroES interacts with the rings of GroEL alternatively; *i.e.* an asymmetrical 1:1 complex (termed bullet-shaped complex) is considered to be the only form of GroEL-GroES complex during the reaction cycle. However, a symmetrical 1:2 GroEL-GroES complex (termed football-shaped complex) has been observed by electron microscopy and chemical cross-linking. Therefore, it is still controversial whether GroES bounds both rings of GroEL simultaneously in the physiological condition. In this study, we monitored GroEL-GroES interaction in solution using fluorescence resonance energy transfer (FRET). We found that nearly half of GroEL-GroES complexes formed football-shaped ones during the reaction cycle. Similar result was obtained using fluorescence correlation spectroscopy (FCS). Next, we found that presence of ADP with one-fifth of concentration of ATP halves the amount of football-shaped complexes. To clarify how ADP inhibits the formation of football-shaped complex, we carried out FRET assay using inorganic phosphate analog (beryllium floride, BeF_x) and ATP hydrolysis defective GroEL mutant (D398A) in the presence or absence of ADP. These results showed that ADP prevents the association of ATP to the *trans*-ring of GroEL and, as a consequence, the second GroES cannot bind to GroEL in the presence of ADP.

1104.04-Pos Structural Characterization of a Peptide Bound to GroEL

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Board B80.04

The E.coli molecular chaperone GroEL and its co-chaperone GroES function together as essential cellular machinery which assists protein folding in an ATP-dependent manner. The double-ring GroEL tetradecamer encapsulates non-native substrate proteins into the central cavity. It is intriguing that about 30% of *E.coli* proteins are misfolded in a GroEL-deficient cell and these affected proteins have diverse structures and functions. Extensive structural and biochemical studies have been directed to unravel the principles of the GroEL-substrate interaction and the mechanism by which GroEL assists protein folding. One of the approaches to address the substrate promiscuity is to characterize multiple GroEL-substrate complexes.

We have used various NMR techniques to study the binding of a peptide to the GroEL substrate binding domain, apical domain (a.a. 191–345). To map the peptide binding site, we studied HSQC (heteronuclear single quantum correlation) spectra of the GroEL apical domain in the absence and presence of the peptide. Residues with altered chemical shifts were identified, and a peptide binding site was proposed to be located between helix H and I. To study the conformation of the GroEL-bound peptide, we used transferred Nuclear Overhauser Effects, and found that the peptide adopts a helical structure. We also use fluorescence technique to locate the peptide's interacting surface to GroEL.

1104.05-Pos Trigger Factor Assisted Protein Folding

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Board B80.05

Trigger Factor (TF) is an important catalyst of nascent peptide folding and possesses both peptidyl-prolyl *cis-trans* isomerase (PPIase) and chaperone activities. TF has a modular structure, containing three domains with distinct structural and functional properties. The N-terminal domain of TF is important for ribosome binding and the M domain carries the PPIase activity. However, the function of the C-terminal domain remains unclear and the residues or regions directly involved in substrate binding have not yet been identified. In our previous studies we have obtained experimental evidence that TF is effective as a molecular chaperone and its chaperone activity is distinct from its PPIase activity both *in vitro* and *in vivo*. Here, guanidine induced equilibrium and kinetic folding of a variant of green fluorescent protein (F99S/M153T/V163A, GFPuv) was studied. Using manual mixing and stopped-flow techniques, we combined different probes, including tryptophan fluorescence, chromophore fluorescence and reactivity with DTNB, to trace the spontaneous and TF-assisted folding of guanidine denatured GFPuv. We found that both unfolding and refolding of GFPuv occurred in a stepwise manner and a stable intermediate was populated under equilibrium conditions. The thermodynamic parameters obtained show that the intermediate state of GFPuv is quite compact compared to the denatured state and most of the green fluorescence is retained in this state. By studying GFPuv folding assisted by TF and a number of TF mutants, we found that wild-type TF catalyzes proline isomerization and accelerates the folding rate at low TF concentrations, but retards GFPuv folding and decelerates the folding rate at high TF concentrations. This reflects the two activities of TF, as an enzyme and as a chaperone. A general mechanism of TF assisted protein folding is discussed..

1104.06-Pos Copolymer Surfactant-Assisted Disaggregation of Denatured Proteins depends on Surfactant

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Board B80.06

Intracellular accumulation of misfolded or unfolded proteins is a well established as a part of the pathogenesis of certain human diseases, particularly thermal injury and neurodegenerative conditions. Effective therapies to refold or eliminate these proteins are needed. Certain biocompatible multiblock copolymer surfactants are capable of disaggregating and refolding denatured proteins. This research project was designed to investigate, using Rayleigh light scattering methods, whether disaggregation and refolding of heat denatured proteins was dependent on the structure of the surfactant, protein or both. We examined the refolding of two thermally denatured proteins: lysozyme and bovine serum albumin (BSA) in response to three different surfactants: Poloxamer 108 (P108), Poloxamer 188 (P188), and Tetronic 1107 (T1107). We observed that these three surfactants are capable of reducing the size of denatured protein aggregates and T1107 was more effective than the smaller triblock surfactants. By comparison, we observed that equimolar 10 kDa polyethylene glycol (PEG), a monoblock hydrophilic polymer, was incapable of disaggregating these proteins. Finally, we observed that surfactant disaggregation of chemically denatured Carboxypeptidase A restores its catalytic activity. These findings indicate that amphiphilic surfactant copolymers are efficient in disaggregating proteins and are more effective than hydrophilic polymers.

RNA Folding

1105-Pos Nucleotide Analog Interference Mapping of Ligand Mediated Conformational Changes in the SAM Riboswitch

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Board B81

Riboswitches are RNA genetic regulatory elements. Upon binding a small molecules, such as a metabolite, a conformational change occurs that either promotes or precludes continued transcription or translation. Here we investigate the mechanism of structural stabilization by S-adenosylmethionine in the SAM I riboswitch using NAIM(Nucleotide Analog Interference Mapping). The results with A and G analogs suggest the conformational change to the SAM bound form is related to pseudoknot formation.

1106-Pos Two-dimensional Infrared Spectroscopy Of RNA Folding

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Board B82

The phenomenon of RNA folding has been studied with a variety of methods, including molecular dynamics simulations, temperature jump kinetics, and NMR spectroscopy. Many questions remain unanswered, including how base pairing and base stacking interactions influence folding and what mechanism the folding follows. These interactions have characteristic vibrational signatures that can be used to provide detailed structural information about RNA strands during folding events. In this study, we simulate the 2DIR signal of several RNA motifs along their folding trajectories. Novel pulse configurations are used to improve the resolution of cross-peaks. By following the evolution of the spectra as the RNA unfolds, we are able to see how the coupling interactions change. The different model strands have distinct spectra, making 2DIR a powerful tool for more detailed structural analysis of RNA folding. We use our results to propose possible folding pathways and discuss the use of more detailed models to describe the coupling interactions in the future.