each other when compared to the intrinsic propensities of a mostly unperturbed arginine in the tripeptide GRG. A conformational analysis based on experimentally determined J-coupling constants from heteronuclear NMR spectroscopy and amide I' band profiles from polarized Raman spectroscopy reveals that nearest neighbor interactions stabilize extended β-strand conformations at the expense of polyproline II and turn conformations. The results from MD simulations with an CHARMM36m force field and TIP3P water reproduce our results only to a limited extent. The use of the Ramachandran distribution of the central residue of GRRRG in a calculation of end-to-end distances of polyarginines of different length yielded the expected power law behavior. The scaling coefficient of 0.66 suggests that such peptides would be more extended than predicted by a self-avoiding random walk.

## Platform: Protein Dynamics and Allostery I

**480-Plat**

**Emerging Experimental Probes for the Spatial and Temporal Resolution of Protein Dynamics in Enzyme Catalysis**

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Macromolecular dynamics is an integral part of our understanding of protein function. This talk will focus on temperature dependent hydrogen deuterium exchange by mass spectrometry (TDHDX-MS) as a tool to uncover spatially resolved thermal networks that connect protein/solvent interfaces to reaction centers. When TDHDX-MS is combined with time and temperature dependent Stokes shifts measurements, experimental evidence emerges for site-specific protein quakes as the source of the thermal activation of active site chemistry. A background of equilibrium sampling among a wide range of protein substates acts in concert with protein quakes, to enable precise positioning of active site components with regard to their anisotropic thermal conduits. This behavior provides a unifying model for both enzymatic catalytic rate enhancement and allosteric regulation. Supported by NIGMS.

**481-Plat**

**Hdxms Reveals the Role of Dynamics on the Serine Protease Activity of the Urokinase-Type Plasminogen Activator (UPA)**

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The urokinase-type plasminogen activator (uPA) is a key regulator of cellular processes such wound healing, angiogenesis and anticoagulation, among others. This protein is a serine protease synthesized as inactive single-chain zymogen that becomes active after a very specific cleavage between K158 and I159. The uPA catalyzes the rate limiting step of anticoagulation: the activation of peri-cellular plasminogen into the active serine protease plasmin. Even though the chemistry behind this reaction is well understood with a Ser/Asp/His catalytic triad, the dynamics of the protein and how dynamics control the activity remain unknown. The uPA is formed by an EGF domain, a Kringle domain, a 27-residue linker region and a serine protease domain. The structure of Full-length uPA has not been solved, however, there are structures available for the EGF and Kringle domains (the Amino terminal fragment, or ATF), and the serine protease domain separately. Whether the ATF interacts through the linker with the protease domain remains unknown, but the full-length protein is 6-fold more active (kcat) towards plasminogen when the ATF is present. We hypothesized that there is through-linker dynamic allostery within uPA. To test our hypothesis, we evaluated the changes in dynamics of uPA by Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS). We compared the protease domain alone to full-length uPA, the single chain to the two chain, and receptor (uPAR)-bound to free. The HDX-MS results help to resolve which regions of the protease domain are allosterically regulated.

**482-Plat**

**Essential Dynamics that Drive Sars-Cov-2 Spike Conformational Changes**

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The ongoing COVID-19 pandemic caused by the SARS-CoV-2 virus has prompted the need for rapid development of effective vaccines to attenuate this global emergency. The Spike glycoprotein present on the surface of this virus is known to elicit immune response and has been a prime candidate for vaccine design. This trimeric protein plays a critical role in infection where its Receptor Binding Domain (RBD) moves upwards from the rest of protein making it free to bind with the host ACE2 receptor. This is followed by S1-S2 subdomain disengagement and cell entry. Structural understanding of such inter-domain motions is important for obtaining an effective molecular handle over this protein, and in turn, exploiting it towards improved immunogen development. We performed large-scale molecular dynamics simulations of the soluble form of the Spike in both 'down' and 'up' conformations of the RBD, and employed Principal Component Analysis (PCA) of the inter-residue correlated fluctuations to extract major collective modes that dominate the functional dynamics. We observe that there is significant influence of coupled dynamics connecting the RBD, the N-terminal Domain (NTD) and the S2 region that can drive RBD transitions. Moreover, both RBD and NTD sample disparate rotational space between the Up- and Down-RBD conformations, that can affect antibody interactions. Since the Spike is densely glycosylated, simulations were also performed for the dominant Spike glycoform to elucidate the effect of glycans on these essential dynamics. Our results further provide a mechanistic rationale for the allosteric modulations that distinguish the predominant D614G viral Spike form which has been shown to have enhanced infectivity and RBD-up probability.

**483-Plat**

**Direct Observation of the Catalytically Relevant Fully Folded-Locally Unfolded Equilibrium in Peroxiredoxin Q of Xanthamonas Campestris**

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Peroxiredoxins (Prxs) are ubiquitious peroxidases found in all living organisms. All peroxiredoxins share a conserved catalytic site containing a 'peroxidatic' cysteine which reduces peroxide to water while being converted to a Cys-sulfenate. A subset of Prxs called 2-Cys Prxs then either locally unfold and form an internal disulfide between the peroxidatic and a second 'resolving' cysteine, or become hyperoxidized, forming a Cys-sulfinate which inactivates the protein. The balance between resolution and hyperoxidation is thought to be key to the hypothesized role of some 2-Cys Prxs as a 'floodgate' regulating peroxide concentration. A significant body of work has been devoted to studying hyperoxidation as well as the resolution rate for a variety of 2-cys peroxiredoxins. Despite this, the dynamics of the local unfolding process and how it is tuned for function remains an open question. Here, we have used a combination of nuclear magnetic resonance (NMR)-based methods to characterize the solution dynamics of peroxiredoxin Q from *Xanthamonas campestris*. A 17 kDa monomer, *Xc*PrxQ is an ideal candidate for study by NMR, a technique largely unsuitable for typical peroxiredoxins, which form large oligomers. In addition to clear evidence of intermediate exchange obtained by a model-free analysis of $^{15}$N spin-relaxation rates and $^{1}$H-$^{15}$N Het-NOE values, we have directly measured through chemical exchange saturation transfer (CEST) experiments that catalytically active, dithiol *Xc*PrxQ, exchanges with a locally-unfolded state at a rate of 72 $s^{-1}$. We have additionally characterized the hydrogen exchange behavior of *Xc*PrxQ, which reveals a tightly folded core that persists through local unfolding. To our knowledge, this study is the first evidence of a 2-cys Prx exchanging with an unfolded state while in its dithiol form, and the first measurement of the fully folded-locally unfolded equilibrium.

**484-Plat**

**Combining Single-Molecule Fluorescence and MD-Simulations to Delineate the Kinetics and Regulation of Proteins**

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Protein conformational kinetics and its regulation occur on many time- and length-scales. While for example post-translational modifications act very locally, specific protein-protein interaction typically affect larger domains, and global changes (like crowding) affect the whole protein (complex) non-specifically.

In a first part we use a combination of single-molecule FRET, FRET-FCS, ns-FCS and MD simulations to delineate the timescales for information transfer from the hydrolysis event in the nucleotide binding site of the heat shock protein 90 (Hsp90) to large conformational changes in the Hsp90 dimer[1]. This