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Here, we describe discovery of two small molecules which specifically enhance catalytic efficiency of peptidase neurolysin. A computational approach was used to explore structure of neurolysin and identify a druggable surface pocket in its hinge region, followed by docking and ranking of 139,735 molecules from NCI DTP database. Top ranking compounds were subjected to pharmacological evaluation. Two structurally related compounds were identified which enhanced the rate of substrate hydrolysis by recombinant (human and rat) and mouse brain-purified neurolysins in a concentration-dependent manner. Neither the identified modulators nor dynorphin A(1-13), a competitive inhibitor, affected each other's affinity in modulating activity of neurolysin, suggesting that the modulators do not bind to the substrate binding site. Both modulators reduced K_m and increased V_{max} values for hydrolysis of the synthetic substrate by neurolysin in a concentration-dependent manner. The modulators had negligible effect on catalytic activity of thimet oligopeptidase, neprilysin, angiotensin converting enzyme (ACE) and ACE2, indicating that they are specific to neurolysin. Both modulators also enhanced hydrolysis of endogenous substrates, suggesting that their effect was not linked to the synthetic substrate. The identified molecules could be developed into research tools for evaluation of the (patho)physiological function of neurolysin. This study is one of few utilizing a structure-based approach for rational identification of enzyme activators, and by that it demonstrates applicability of this methodology for identification of allosteric modulators of other enzymes.

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Links of Conformational Sampling to Functional Plasticity and Clinical Phenotypes by Single Molecule Studies

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The dynamic exploration of conformational states, also termed conformational sampling, is known to govern all aspects of protein behavior from folding to function. The way however it encodes enzyme function - the capacity to accelerate the chemical step and plasticity of accepting structurally diverse substrates - are terra incognita for conventional bulk characterization but can be directly observed by single molecule studies(1-3).

To interrogate the functional and structural dynamics of individual enzymes we spatially confine them on arrays of native membrane like systems (liposomes or nanodiscs)(4). In addition to providing a native like environment - minimizing deleterious interactions with hard matter, these systems may act as 3D scaffold for the assembly of multiple regulatory bioelements. Our parallel single molecule readout allows us to directly observe, and quantify the extend of conformational sampling for multiple enzymes (P450 oxidoreductases and lipases) and consequently its dependence on regulatory inputs and mutations(5-7). Comparing the readouts on wild type enzymes with their pathogenic mutants with varying plasticity provides the intricate correlation between extends of conformational sampling and plasticity. Providing the first clues of conformational sampling acting as cue for functional and consequently clinical phenotype may pave the way for controlling plasticity and the de novo design of proteins with tailor made functionalities.

1. N. S. Hatzakis, *Biophys. Chem.* 186, 46 (2014).
2. T. Ha, *Nat Meth* 11, 1015 (2014).
3. M. F. Juetter et al., *Curr. Opin. Chem. Biol.* 20, 103 (Jun, 2014).
4. N. S. Hatzakis et al., *Nat. Chem. Biol.* 5, 835 (Nov, 2009).
5. N. S. Hatzakis et al., *J. Am. Chem. Soc.* 134, 9296 (Jun, 2012).
6. K. Bavishi, N. S. Hatzakis, *Molecules* 19, 19407 (Dec, 2014).
7. T. Laursen et al., *ACS Chem. Biol.* 9, 630 (Mar, 2014).

1962-Pos Board B106

Quantifying the Molecular Constraints Driving the Trimethoprim Resistance in *Escherichia Coli*

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Trimethoprim (TMP) is an antibiotic molecule that blocks nucleic acid synthesis by competitively binding to the dihydrofolate reductase (DHFR) enzyme [1]. We have previously identified DHFR mutations and the order they appear over the course of trimethoprim resistance evolution [2]. We have purified and characterized DHFR mutants that have up to five point mutations. For every mutant DHFR, we quantified trimethoprim affinity (K_i) and catalytic power (K_{cat}/K_m). We quantify epistatic interactions between resistance conferring mutations and the trade-offs between mutant proteins' catalytic powers and trimethoprim affinities [3]. Finally, we explain why some mutations appear earlier in the course of evolution.

1. Schnell, J.R., H.J. Dyson, and P.E. Wright, *Structure, dynamics, and catalytic function of dihydrofolate reductase*. *Annu Rev Biophys Biomol Struct*, 2004. 33: p. 119-40.
2. Toprak, E., et al., *Evolutionary paths to antibiotic resistance under dynamically sustained drug selection*. *Nat Genet*, 2012. 44(1): p. 101-5.
3. Palmer, A.C., et al., *Delayed commitment to evolutionary fate in antibiotic resistance fitness landscapes*. *Nat Commun*, 2015. 6: p. 7385.

1963-Pos Board B107

The Catalytic Determinants of Streptococcal Pneumoniae IgA1 Protease are Formed by Multiple Domains

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Streptococcus pneumoniae (SPN) is a gram-positive bacterium which causes non-invasive infections, such as otitis media, as well as invasive diseases like pneumonia and meningitis. Despite the development of bacterial antibiotics and vaccines, exposure to SPN still leads to diseases in young, elderly, and immunocompromised individuals. Current vaccines have serious limitations due to the serotype variability and genomic plasticity of the bacterium and therefore, an ever-increasing frequency of multidrug-resistant strains have been reported. Immunoglobulin A1 (IgA1) represents 90% of the IgA within the human respiratory tract and multiple bacterial pathogens, including SPN, can produce an IgA1-specific protease (IgA1P) to inactivate this major component of mucosal immunity. IgA1P cleaves IgA1 at the hinge region and leads to removal of the Fc domain of IgA1, which is recognized by host clearance mechanisms. Because of the universal expression among different serotypes of SPN, IgA1P has been shown to be a potential target for new vaccine development. SPN IgA1P is unique with no sequence conservation to any other known protein, other than a conserved HEXxH Zn-binding motif found in many metalloproteases. We have discovered that the SPN IgA1P released from cells is active and our biochemical studies have revealed that the SPN IgA1P contains a well-folded C-terminal domain (CTD) that houses the third Zn coordinating residue, Glu1628. Furthermore, our data illustrates that the N-terminal domain (NTD) is primarily responsible for engaging IgA1 and the CTD does not have detectable affinity for IgA1 by itself. However, the CTD does play a role in facilitating the binding and is essential for the catalytic activity. Our discoveries shed light on the mechanistic details of this novel metalloprotease and potentially help in developing better vaccines.

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Pressure Modulation of the Enzymatic Activity of Phospholipase A2 - a Putative Membrane-Associated Pressure Sensor

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Phospholipases A2 (PLA2) catalyze the hydrolysis reaction of sn-2 fatty acids of membrane phospholipids and are involved in inflammatory responses to many neurodegenerative diseases through participation in receptor signaling and transcriptional pathways. We used pressure modulation of the PLA2 activity and of the membrane's physical-chemical properties to reveal new mechanistic information on the membrane association and subsequent enzymatic reaction (1). Although the effect of high hydrostatic pressure on soluble and integral membrane proteins has been investigated to some extent, its effect on enzymatic reactions operating at the water/lipid interface has not been explored, yet. This study focuses on the effect of pressure on the structure, membrane binding and activity of membrane-associated PLA2. To this end, high-pressure FTIR spectroscopy and high-pressure stopped-flow fluorescence spectroscopies were applied. The results show that PLA2 binding to model membranes is not significantly affected by pressure and occurs in at least two kinetically distinct steps. Followed by fast initial membrane association, structural reorganization of helical segments of PLA2 takes place at the lipid water interface. FRET-based activity measurements reveal that pressure has an inhibitory effect on the lipid hydrolysis rate. The reduced activity is due to a stronger compression of the enzyme-substrate complex in comparison to that of the transition state. Additionally, a decrease in membrane fluidity upon compression impedes conformational changes accompanying various reaction steps, thereby reducing the rate of the overall reaction. Our results provide novel information regarding molecular interactions in the course of PLA2 binding and lipid hydrolysis, and are important for understanding how such enzymes function under extreme environmental conditions, such as those in the deep sea where pressures up to the kbar-level are encountered.

- (1) Suladze S.; Winter R.; *J. Am. Chem. Soc.* 2015, in press.