Links of conformational sampling to functional plasticity and clinical phenotypes by single molecule studies

Moses, Matias Emil; Thodberg, Sara; Bavishi, Krutika; Eiersholt, Stine; Li, Darui; Stamou, Dimitrios; Møller, Birger Lindberg; Laursen, Tomas; Hatzakis, Nikos

Published in:
Biophysical Society. Annual Meeting. Abstracts

DOI:
10.1016/j.bpj.2015.11.2144

Publication date:
2016

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
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 neurolysin in a concentration-dependent manner. Neither the identified modulators nor dynorphin A1-13, 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 Km and increased Vmax 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, neuropilin, 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.

1961-Pos Board B105
Links of Conformational Sampling to Functional Plasticity and Clinical Phenotypes by Single Molecule Studies
Mattias E. Moses1, Sara Thodberg2, Krutika Bavishi2, Stine Eiersholt3, Dana Li4, Dimitrios Stamou4, Birger L. Møller4, Tomas Laursen4, Niels Jørg S. Hatziakis5
1Chemistry, Nanoscience Center University of Copenhagen, Copenhagen, Denmark, 2Plant and Environmental sciences, Plant Biochemistry, Copenhagen, Denmark.

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. 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 extent 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.


1962-Pos Board B106
Quantifying the Molecular Constraints Driving the Trimerophim Residues in Escherichia coli
Yusuf T. Tamer

Molecular Biophysics, UT Southwestern, Dallas, TX, USA.

Trimerophim (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 trimerophim resistance evolution [2]. We have purified and characterized DHFR mutants that have up to five point mutations. For every mutant DHFR, we quantified trimerophim affinity ($K_d$) and catalytic power ($K_m/V_{max}$). We quantify epistatic interactions between resistance conferring mutations and the effects between mutant proteins' catalytic powers and trimerophim affinities [3]. Finally, we explain why some mutations appear earlier in the course of evolution.


1963-Pos Board B107
The Catalytic Determinants of Streptococcal Pneumoniae IgA1 Protease are Formed by Multiple Domains
Ying-Chih Chi1, Agnieszka A. Kendrick1, Jeremy Rahkola1, Edward N. Janoff2, Elan Z. Eisenmesser3, 4Biochemistry and Molecular Genetics, University of Colorado Denver Anschutz medical campus, Aurora, CO, USA, 2Division of Infectious Diseases, University of Colorado Denver Anschutz medical campus, Aurora, CO, USA.

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 (IgAIP) to inactivate this major component of mucosal immunity. IgAIP cleaves IgA1 at the hinge region and has a broad spectrum effect against removal of the Fe domain of IgA1, which is recognized by host clearance mechanisms. Because of the universal expression among different serotypes of SPN, IgAIP has been shown to be a potential target for new vaccine development. SPN IgAIP 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 IgAIP released from cells is active and our biochemical studies have revealed that the SPN IgAIP 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.