Abstract 1450

Tuning the Gas Binding Affinity of Cs H-NOX Using Tyrosine Analogs

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Caldanaerobacter subterraneus heme-nitric oxide and/or oxygen binding domain (Cs H-NOX) is a thermophilic heme protein that binds to small gas molecules including oxygen, nitric oxide, and carbon monoxide. Here, non-canonical amino acids (ncAAs) were utilized to either probe the local solvation environments in the protein or to modify the functionality of the protein. Specifically, the ncAA vibrational reporter 4-cyano-L-phenylalanine (pCNPhe) was site-specifically incorporated into the protein at a number sites using the Amber codon suppression methodology, including surface and buried sites in the protein. Temperature-dependent IR spectroscopy was then utilized to measure the temperature dependence of the nitrile symmetric stretching frequency of pCNPhe which was correlated to local solvation environment in conjunction with X-ray crystal structures of the protein constructs generated. Additionally, tyrosine analogs were individually incorporated at site 140 which is a tyrosine in the native protein structure. Y140 is involved in a hydrogen bonding interaction with oxygen bound to the heme iron. Thus modulation of the pKa of the phenolic hydrogen is predicted to impact the strength of this interaction and thus the affinity of the protein to oxygen binding. Results from probing local protein environments with ncAAs and modulating oxygen affinity will be presented.

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Abstract 1452

Modeling the binding of ω-conotoxin and other toxins to the N-type voltage-gated calcium channel

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Approximately 1.5 billion people in the world suffer from chronic pain, persistent pain that carries on for longer than 12 weeks despite medication or treatment. Management of chronic pain typically includes the use of non-steroidal anti-inflammatory drugs (NSAIDs) or prescription pain medications, including opioids. An alternative therapy derived from conotoxins, toxins released from marine predatory snails in the family Conidae, was approved for the treatment of severe chronic pain in 2004. This pharmaceutical has the trade name Prialt and is also known as ziconotide. Once ziconotide is in the human body, it acts as a channel blocker of the N-type voltage-gated calcium channels, also known as Cav2.2. The literature and related PDB files were manipulated using PyMol and Jmol to create a 3D-printed model to explain the molecular story behind how a particular conotoxin binds to a calcium-gated ion channel. Additionally, computer visualization tools were used to show how several related toxins from other organisms would be expected to dock to the calcium ion channel. The 3D-printed model highlights specific features that contribute to the ω-conotoxin (MVIIA) binding to the calcium channel alpha 1B subunit as described in the literature (PDB: 7MIX). These conotoxins have a very characteristic disulfide bond linkage pattern which plays a role in the correct folding of the peptide and stabilization of its structure. In MVIIA, the non-cysteine amino acids form unstructured loops affecting binding affinity and calcium channel-blocking activity. Of particular interest is the second loop located between Cys8 and Cys-15. It appears to be exceptionally important in directing selectivity toward N-type calcium channels and away from P/Q-type calcium channels. Ziconotide does not directly seal the entrance to the vestibule of the selectivity filter, but it blocks ion entrance by neutralizing the outer electronegativity and sterically hindering the ion access path to the entrance of the selectivity filter. Salt bridges are formed between Arg10 and Tyr13 on ziconotide and Asp664 of the channel. Four of the eight ziconotide-coordinating residues, Thr643, Asp1345, Lys1372, and Asp1629 in Cav2.2 are not conserved in other calcium channels which may explain the subtype specificity of pore blockage by ziconotide. The EEEE motif consisting of Glu314, Glu663, Glu1365, and Glu1655, determines the Ca2+ selectivity. Also included in the model are the receptor's alpha helices and bound calcium ion. The N terminus and C terminus of the receptor are labeled in blue and red respectively to orient the model. No crystal structures are available for ω-conotoxins bound to several other types of N-type calcium channels. To investigate the potential calcium channel blocking properties of conotoxins MVIIC,

GVIA, MoVIB (from the cone snail, Conus magus) and ω-agatoxin IVA (from the spider, Agelenopsis aperta), the computer-based tool ROSIE was used to simulate binding of these peptides to the Cav2.2 channel. As expected, the toxin shown in the crystal structure of 7MIX, bound best to the Cav2.2 channel. The toxins MVIIC, GVIA, and MoVIB bound with lower affinity. The agatoxin IVA did not have any relevant binding to this calcium channel. Overall, protein modeling allowed for a deeper understanding of how conotoxins bind to and block the calcium channel possibly leading to additional therapeutic approaches to pain relief.

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Abstract 1470
Probing the Pal-TolB Interaction in *E. coli*

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Peptidoglycan Associated Lipoprotein (Pal) is a protein found in the periplasmic domain of Gram-Negative bacteria, such as *Escherichia coli* (*E. coli*). In *E. coli*, Pal acts as a structural protein as it is tethered to the outer membrane (OM) through its lipid moiety and non-covalently binds to peptidoglycan (PG). Pal also associates with other OM and periplasmic proteins as part of the Pal-Tol complex, which is known to provide the bacterial cell with structural integrity and to play a crucial role in cell division by assisting in the constriction of the OM. For these reasons, we see Pal as an antibiotic target. To better understand the Pal-TolB interaction, which is known to be important for constriction, we created two site-directed mutants of Pal (K150E and G113P) with the goal of disrupting the Pal-TolB interaction. Recombinant wild-type and mutant proteins were expressed in *E. coli*. The effects of the mutations on the Pal-TolB interaction were determined using enzyme-linked immunosorbent assays (ELISA). Preliminary results of this study suggest that K150 and G113 dictate at least some of the Pal-TolB interaction. Future work will determine how mutations that decrease the Pal-TolB interaction affects cell division in *E. coli* cells.

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