March 22 Chalk Talk Press Release

Engineering the Alpha-Ketoglutarate Oxygenase FIH for Alternate Rebound Chemistry

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Alpha-ketoglutarate-dependent oxygenases functionalize complex biomolecules, including proteins. Their chemical reactivity parallels that of the P450 enzymes in many ways, suggesting that engineering the oxygenases could lead to novel chemistry. In the case of the enzyme factor inhibiting HIFα (FIH), engineering this enzyme to perform non-natural modifications would lead to bio-orthogonal labels for proteins. Herein we report the anion dependent substrate hydroxylation by FIH variants, in initial efforts to achieve non-natural peptide modification. Our results indicate that FIH can be engineered to bind exogenous anions and perform anion-selective hydroxylation chemistry, achieving the preliminary steps necessary for alternate rebound chemistry.
Hijacking His-tags to make functional multi-protein complexes

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Polyhistidine-tags have had a tremendous impact on biochemistry research by enabling simple and robust protein purification. This technology uses the high affinity of the histidine imidazole for divalent metals such as Ni(II) or Zn(II) chelated to beads to separate the His-tagged protein from other impurities. We have discovered that divalent metal binding to a His-tagged protein can drive assembly of functional chemoreceptor arrays. Native-like complexes of the His-tagged cytoplasmic fragment of the aspartate chemoreceptor (H$_6$CF) with its cognate kinase CheA and adapter protein CheW can be assembled in vitro, either by anchoring the chemoreceptor to Ni-NTA-functionalized vesicles or by including a molecular crowding agent, PEG8000. These complexes form extended hexagonal array structures, similar to the in vivo architecture. We have recently discovered that Ni(II), Zn(II), or Co(II) can mediate similar array formation in the absence of vesicles or molecular crowding agents. Moreover, deletion of the His tag prevents metal-mediated assembly: neither sedimentation nor kinase activity are observed. We hypothesize the metals bind to the His-tag, leading to receptor dimer formation and ultimately array assembly. Metal-mediated His-tag dimerization may prove a useful assembly method for other multi-protein or membrane protein complexes that require stabilization to mimic native architecture.
Solid-state NMR Detects Signaling-related Mobility Changes in Chemotaxis Receptors Assembled in Native-like Arrays

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Bacterial chemotaxis receptor serves as a tractable model for understanding the structural mechanism of transmembrane signaling. Chemoreceptor arrays allow bacteria to sense their environment and move toward attractants or move away from repellants. Binding of attractants to chemotaxis receptors inhibits kinase activity and stimulates methylation activity. The big question is how a signal travel 200Å to control the activity of the kinase bound at the receptor tip. Dynamic changes within the cytoplasmic domain of receptor have been proposed to play a role in signal transmission down to the membrane-distal tip. Our laboratory has used INEPT spectra in solid state NMR to specifically detect only the dynamic portions of the receptor cytoplasmic fragment (CF) in functional complexes with the kinase CheA and coupling protein CheW. The mobile regions are assigned, via biochemical and NMR strategies, to test proposals regarding changes in subdomain dynamics during signaling.