2278-Pos Board B248

Reaction Mechanisms of Editing (Proofreading) by Leucyl-tRNA Synthetase Revealed by QM/MM Molecular Dynamics Simulations

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Aminoacyl-tRNA synthetases (aaRS's) play a critical role in decoding genetic information located on genome DNA sequence, through catalyzing attachment of their cognate amino acid to 3′-end of the specific tRNA. The fidelity of translation is assured by their strict discrimination of the cognate amino acids from non-cognate ones. However, in the case of valine, isoleucine, and leucine systems, it is difficult for the cognate enzymes to discriminate their specific amino acid; thus, the synthetases produce mis-aminoacylated tRNAs, such as Ile-tRNA^{Leu}. However, the mis-products are hydrolyzed through the ''editing'' reaction by the aaRS's.
In this study, we performed molecular docking simulations of the LeuRS•tRNA^{Leu} complex and an incognate amino acid (Val), using a novel algorithm referred to as the Fully Solvated Dynamical Docking (FSDD) scheme which is developed by our group (see the presentation by Hagiwara, Y., et al.). Thus, we have successfully identified ordered water molecules in its active site, and also, one of such waters has been found to be located at the appropriate position as the nucleophile.
Then, for the modeled structure of the LeuRS•Val-tRNA^{Leu} complex, we performed QM/MM calculations using our new interface program connecting QM (gamess) and MM (amber) engines, which has also been developed by us. Thereby, it has been found that LUMO is located on the reaction point on the substrate, suggesting that the water identified actually attacks the orbital as the nucleophile. In fact, as the distance between the substrate and the water (O atom) is decreased, the orbital energies of 2*p* electrons of the oxygen atom is elevated toward the energy level of HOMO. In the presentation, we will show recent results of our QM/MM-MD simulations performed to elucidate detailed reaction mechanisms of the editing.

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Redox activity and H_2 production upon glycerol fermentation in *Escherichia coli:* are hydrogenases reversible?

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The molecular hydrogen (H_2) can be produced through bacterial hydrogenases catalyzing the reaction of $2H^+ + 2e^- \rightarrow H_2$. Under fermentation of glucose *Escherichia coli* hydrogenases (Hyd) 3 and 4 are responsible for H_2 production depending on pH [1] and formate [2] and Hyd-1 and Hyd-2 operate as H_2 uptake hydrogenases. Hyd-3 was shown recently can work in reverse mode [3].
Redox activity and H_2 production are studied under glycerol fermentation by *E. coli* at acidic [4] and slightly alkaline pH. Redox potential was shown to decrease upon glycerol fermentation at pH 7.5. The H_2 production rate was ~5-fold less in *hybC* (Hyd-2) than that in *hyaB* (Hyd-1) mutant but it was lower than that in wild type strain or *hycE* (Hyd-3) and *hyfG* (Hyd-4) mutants. Interestingly, 0.5 mM *N,N′*-dicyclohexylcarbodiimide (DCCD) noticeably inhibited H_2 production in wild type and mutants used; at the same time DCCD inhibition of H_2 production rate was less and H_2 production was ~14 times higher in *hyaB* than in *hybC* mutant. The results point out that Hyd-2 plays a main role in H_2 production under glycerol fermentation; a participation of Hyd-1 is not ruled out although Hyd-3 and Hyd-4 don't operate under these conditions.
E. coli Hyd-2 might be concluded to work in reverse mode similar to Hyd-3 [3] but in different conditions.

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Protein-Ligand Interactions I

2280-Pos Board B250

Localizing Fatty Acid Binding Sites on Human Serum Albumin by 2D-NMR

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The most abundant protein in plasma, human serum albumin (HSA), is the principal carrier for endogenous lipophilic compounds and lipophilic drugs. Non-esterified long chain fatty acids (FA) are its primary physiological ligand, with multiple binding sites of varying affinities. Detailed structural understandings of HSA-ligand interactions are vital during the development of drugs. HSA ligand interactions have been studied by various biophysical methods: x-ray crystallography, fluorescence spectroscopy, and nuclear magnetic resonance (NMR). Numerous crystal structures of HSA, with and without drugs and/or FAs, have been published. Compared to crystallography, our solution state NMR studies are focused on the more physiologically relevant state. Previously, we identified three high affinity and several low affinity FA binding sites on HSA by 1D-NMR at low resolution. Now, we are correlating crystallographic data with high-resolution 2D-NMR spectra of 13C-methyl-labeled oleic acid (OA). Our 2D-HSQC spectra show the 13C-methyl group of OA bound to HSA. The unique microenvironments that each methyl group experiences in the binding pockets gives rise to different NMR signals of varying intensities. These correspond to the different affinities that HSA has for OA, and our 2D-NMR spectra clearly differentiate the three highest affinity-binding sites from six lower affinity-binding sites. We have identified Sudlow's Drug Sites by utilizing the known FA competitors and Sudlow's site binders: warfarin and diazepam. By expanding this drug competition strategy with ibuprofen and diflunisal, we have identified additional FA binding sites. The highest affinity FA binding sites do not have known competitors and these sites are identified with the use of site-directed mutagenesis of HSA. In summary, we are reporting an approach for studying the interactions of FAs with HSA and FA-competitive drugs in a site-specific manner through the use of state-of-the-art 2D-NMR techniques.

2281-Pos Board B251

Calcium-Dependent Interactions of Calmodulin with Calcineurin: Evidence for Domain-Reversal in Target Recogition

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Cardiac hypertrophy results from stress on cardiomyocytes, which contributes to heart failure. Constitutive activation of calcineurin (CaN), a dimeric Ser/Thr phosphatase, induces hypertrophic responses. Ca^{2+}-CaM is essential for CaN activation; however, the mechanism of activation is not fully understood. Recent reports have raised new questions regarding the stoichiometry of the CaM:CaNp complex, *in vitro* and *in vivo*. A recent structure showed CaM forms a novel 2:2 complex with the CaM-binding domain of CaN (CaNp, 2R28.pdb).
Hydrodynamic methods were used to determine the stoichiometry of the complex formed between Ca^{2+}-saturated *Paramecium* CaM_{1-148} (PCaM) and CaNp. Stokes radius (R_s) and sedimentation coefficient (s) values were consistent with a 1:1 complex. At higher protein concentration, ^{15}N T_2 relaxation rates of $(Ca^{2+})_4$-^{15}N-$PCaM_{1-148}$ in the presence of CaNp or melittin were compared. Results support a model in which CaM associates with CaN with a 1:1 stoichiometry. Under the same solution conditions, $(Ca^{2+})_4$-$PCaM_{1-148}$ has a very high affinity for CaNp (estimated K_d of 1 pM).
The response of individual PCaM residues to CaNp binding was studied. ^{15}N-HSQC spectra of ^{15}N-$(Ca^{2+})_4$-$PCaM_{1-148}$ showed that the addition of CaNp caused dramatic peak shifts. Backbone assignments for ^{13}C,^{15}N-$(Ca^{2+})_4$-$PCaM_{1-148}$ in the presence of CaNp were determined with standard 3D NMR experiments. This showed that residues from *both* domains of CaM experience conformational changes when bound to CaNp; however, the N-domain was perturbed to a greater extent. This is unusual when compared to all 17 known compact CaM-target structures, where the C-domain of CaM preferentially serves as a target-docking site. Support by AHA & UI CBB Predoctoral Fellowships to SEO, NIH R01 GM57001 to MAS.

2282-Pos Board B252

Structural Basis for Phosphate Stabilization of the Uniquely Coordinated 2Fe-2S Cluster of the Outer Mitochondrial Membrane Protein MitoNEET.*

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MitoNEET is the first outer mitochondrial membrane protein shown to contain a pH labile redox active 2Fe-2S cluster (1). Additionally, we found that phosphate at physiological concentrations (10 mM) stabilized the cluster from release by ~10-fold over a broad pH range from $5.0 \leq pH \leq 7.5$. The structure

obtained using phosphate buffer (pH 7.0, 1.75Å diffraction, R_{factor}=18%) showed the presence of a bound phosphate anion interacting with His87, a key residue in cluster release (1). The phosphate also interacts with the N-terminus of a symmetry-related dimer (Figure). None of these interactions were previously reported (2-4). We attribute the increased stability to the novel interactions, suggesting that the rate of cluster release could be modulated by interaction with a phosphate or phosphate moiety, such as a phosphorylated protein or peptide.

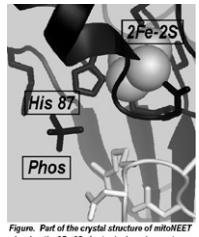

Figure. Part of the crystal structure of mitoNEET showing the 2Fe-2S cluster (spheres), a cartoon representation of the backbone (black is one dimer and white a symmetry related dimer) and specific amino acids (as labeled) that interact with the bound phosphate anion (black tetrahedron).

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2283-Pos Board B253
Potent Inhibitors Of c-Myc-Max Dimerization Through Multivalent Binding To The Intrinsically Disordered c-Myc Monomer
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We have shown that selective inhibitors of dimer formation between the oncogenic basic-helix-loop-helix-leucine zipper (bHLHZip) trancription factor c Myc and its bHLHZip partner protein Max act by binding to the intrinsically disordered (ID) c Myc monomer. Multiple, independent sites for inhibitor binding were found along c-Myc bHLHZip. We exploited the multiplicity of these sites to generate novel compounds capable of multivalent binding to c-Myc. Despite their disordered protein target, these molecules bind purified c-Myc with low nano-molar affinity, which is orders of magnitude tighter than that of c-Myc's obligate heterodimerization partner Max. The inhibitors effectively disrupt c-Myc-Max dimerization and specific DNA binding; they also inhibit growth of c-Myc overexpressing cancer cell lines in vitro.

2284-Pos Board B254
Dynamic Analysis of Beta-lactamase Ligand Recognition
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A serious public health threat today is the emergence of pathogens that are resistant to commonly used antibiotics. One of the mechanisms of acquired drug resistance is the bacterial production of beta-lactamases, which break down these antibiotics. Currently used beta-lactamase inhibitors are not effective at targeting the 700 types and new mutants of beta-lactamases. Beta lactamase is therefore an important drug target in combating antibiotic resistance. Beta lactamase inhibitory protein (BLIP) is an effective inhibitor of TEM-1 and SHV-1, but binds and inhibits the two variants with different affinities. We hypothesize that elucidating the mechanism whereby the differential binding results will guide the design of new peptide inhibitors based on the BLIP structure. Molecular dynamics simulations are performed to examine the binding properties of BLIP and BLIP based peptides to TEM-1 and SHV-1 beta lactamase. These simulations on the complex will guide the design of new peptides.

2285-Pos Board B255
The Role Of Dynamics On Binding Specificity And Promiscuity
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Protein - protein interactions regulate and control a wide range of physiological processes. Protein interaction networks abound with proteins that have only a few connections while a small number of proteins, which are called hub proteins, are highly connected. Recent reports suggest that hub proteins are structurally and dynamically different from non-hub proteins. Hub proteins tend to be more disordered than other proteins. Further, binding partners of hub proteins are also observed to have higher levels of disorder. Here we perform a detailed examination of hub and non-hub proteins using the Gaussian Network Model (GNM) to elucidate the role of intrinsic dynamics for binding diversity and promiscuity.

2286-Pos Board B256
Phospho(enol)pyruvate Binding to Thermus thermophilus Phosphofructokinase
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ATP-dependent type 1 prokaryotic phosphofructokinase (PFK) catalyzes the conversion of fructose-6-phosphate (F6P) and MgATP to fructose-1,6-bisphosphate and MgADP. This enzyme is allosterically inhibited by phosphoenolpyruvate (PEP) and exists as a homotetramer with the four active sites formed along one dimer-dimer interface and four allosteric sites formed along the other. The overall structures of PFK's from E. coli, Bacillus stearothermophilus, and Lactobacillus delbruekii are very similar, and the sequences have high percent identity and similarity with Thermus thermophilus PFK. Thermus thermophilus PFK (TtPFK) comes from a gram negative bacterium that is an extreme thermophile. One of the interesting features of TtPFK is an unusually tight PEP binding (Kd=1.3 ± 0.02mM at 250C pH 8) compared to PFK's from E. coli (300 ± 10mM), Bacillus stearothermophilus (60 ± 4mM), and Lactobacillus delbruekii (24 ± 2mM). Since no structural data are available for TtPFK to date, a structure of a highly homologous PFK from a moderate thermophile Bacillus stearothermophilus was used to predict the residues important for PEP binding. From the sequence alignment and the PEP-bound D12A BsPFK structure, three non-conserved residues were identified in the allosteric binding site that may be responsible for tight binding: R55, N59, and S215. To quantify the contribution of these residues to PEP binding, single and double mutations were made to the corresponding residues in BsPFK, EcPFK, and LbPFK. Kinetic data showed that these mutations weakened the binding of PEP to different extents. In case of N59D/S215H, the PEP binding affinity was 10-fold lower N59D showed a 100-fold decrease in PEP binding affinity and R55G showed a 700-fold decrease in PEP binding. Furthermore, these mutants showed a slight increase in coupling, which means that although the binding of PEP becomes weaker, its ability to inhibit was somewhat improved. Supported by NIH grant GM33261 and Welch Foundation grant A1543.

2287-Pos Board B257
Distinguishing Interactions Responsible for Phospho(enol)pyruvate Binding from Interactions that Communicate Allosteric Inhibition in E. coli Phosphofructokinase
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The notion that substrate affinity is modified via interaction of effector ligands is a fundamental characteristic of allostery. In order to further understand the complexities of this phenomenon, this study investigates the role that functional groups in phospho-enol-pyruvate PEP play in ligand binding and allosteric propagation. Thermodynamic linkage analysis enables the nature and magnitude of allostery to be determined, allowing allosteric action to be distinguished from allosteric ligand binding affinity. By using this type of analysis to compare the inhibition caused by PEP to the inhibition imposed by PEP analogs upon binding to E. coli PFK (EcPFK), the importance of specific functional groups of PEP to ligand binding and/or allostery can be ascertained. EcPFK displays a much weaker binding affinity for the PEP analog phosphoglycolate (PG) compared to PEP. However, PG is still able to inhibit EcPFK to an extent comparable to that of PEP. This finding suggests that the methylene group that is absent in PG plays a larger role in ligand binding than in allosteric propagation. The chemical structure of phosphonoacetic acid (PA) is very similar to that of PG except for the deletion of the oxygen that bridges the phosphate group to the rest of the molecule. When the inhibition of EcPFK by PA is examined, a binding affinity comparable to PG is observed but a substantial loss in allostery is also observed. These data suggest that the bridging oxygen in PEP contributes substantially to the propagation of allosteric signal. Funding is provided by NIH grant GM33261 and Welch Foundation grant A1543.

2288-Pos Board B258
Weak Binding of Phospho(enol)pyruvate to Phosphofructokinase from Lactobacillus delbrueckii
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Most prokaryotic phosphofructokinases (PFKs), like those from Bacillus stearothermolphilus (BsPFK) and E.coli (EcPFK), exhibit both tight binding affinity for the allosteric inhibitor phosphoenolpyruvate (PEP) and strong inhibition. PFK from Lactobacillus delbrueckii subspecies bulgaricus (LbPFK), which is similar in structure and sequence to BsPFK and EcPFK, exhibits weak PEP binding, with the strength of the inhibition being indeterminate. D12, found along the active site interface, is conserved in over 150 prokaryotic PFKs,