

structural and biophysical properties of HP1 *in vitro* as it interacts with differentially modified semi-synthetic chromatin. Nuclear magnetic resonance spectroscopy provides the selectivity to study HP1 in dense chromatin networks while resolving residue-specific biophysical changes. These results provide insight into the function of HP1 in near-physiological chromatin environments.

#### 2401-Pos

##### Dynamics Mediate Substrate Recognition and Remote Communication in a Peptide-Bond Forming NRPS Cyclization Domain

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<sup>1</sup>Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA, <sup>2</sup>Department of Computer Science, University of California Santa Cruz, Santa Cruz, CA, USA, <sup>3</sup>Dept Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA, USA, <sup>4</sup>Chemistry, University of Massachusetts Boston, Boston, MA, USA. Nonribosomal peptide synthetases (NRPSs) are large multidomain enzymatic systems that synthesize complex secondary metabolites from simple substrates like amino acids. The products often have invaluable pharmacological applications such as antibiotics and immunosuppressants. To assemble the products, condensation or cyclization (Cy) domains catalyze the formation of a peptide bond between two substrates each tethered to phosphopantetheinyl arms of an upstream donor or a downstream acceptor carrier protein (CP) domain. Cy domains also catalyze cyclodehydration of serine, threonine, or cysteine sidechains to produce heterocycles. For enzyme function, donor and acceptor CPs dock on opposite sides of a ~40 Å channel harboring the active site. The molecular determinants for substrate recognition, substrate introduction to the buried active site, and catalysis itself have proven difficult to determine. Using NMR relaxation dispersion, we demonstrate that the channel connecting the remote CP binding sites of the yersiniabactin synthetase cyclization domain Cy1 is subject to μs-ms dynamics, suggesting allosteric communication between the sites through a network of dynamic residues. By parsing transient distances provided by NOESY spectra, we determined a structural ensemble representing the conformational landscape of Cy1. We then demonstrate that the network of dynamic residues responds specifically to the presence of substrate when Cy1 is presented to its partner donor CP. We find that the response remodels the entire active site channel, thus confirming allostery. These studies throw light on how dynamics in Cy1 can simultaneously mediate substrate recognition and allosteric communication between partner CPs.

#### 2402-Pos

##### Molecular Responses of Mutagenesis in Nonribosomal Peptide Synthetase Cyclization Domains

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<sup>1</sup>Dept Biophys/Biophysical Chem, Johns Hopkins Sch Med, Baltimore, MD, USA, <sup>2</sup>Chemistry, University of Massachusetts Boston, Boston, MA, USA. Nonribosomal peptide synthetases (NRPSs) implement a modular architecture to assemble a series of substrates into secondary metabolites, often with medicinal properties. Engineering of hybrid NRPS systems has been an attractive approach in production of new pharmaceuticals due to their modular organization. Unfortunately, understanding the molecular mechanisms of NRPSs is challenging and artificial NRPSs are often beset by low product yields. Here, we focus on the cyclization domain from the yersiniabactin synthetase, which condenses and cyclizes salicylate and cysteine (each tethered to donor and acceptor carrier proteins), to form a precursor for the virulence factor yersiniabactin. Notably, enzymatic mechanisms inferred from mutagenesis interpreted through traditional biochemical and structural approaches are routinely revisited. Previous studies by Dowling, Schmeing, and coworkers have identified mutations in the active site of the cyclization domain leading to incorrect product formation and hence thought to contribute to catalysis through acid-base mechanisms and/or substrate positioning. However, our lab has shown that the native cyclization domain encompasses large-scale dynamics, departing from the accepted rigid model of this domain. Employing NMR, a technique sensitive to both structural and dynamic properties of a molecule, we revisited these mutations and observed a global molecular remodeling of the domain rather than expected local effects. In an effort to determine how these mutations impact domain communication, we probed the molecular response of one mutant to its substrate-loaded donor partner using *in situ* biochemistry with an NMR readout. Our results reveal that mutagenesis has global rather than local effects and emphasizes the

need for new approaches to interpret mechanisms of dynamic systems through mutagenesis.

#### 2403-Pos

##### An Allosteric Signaling Governs the CRISPR-Cas9 Function

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<sup>1</sup>Department of Bioengineering, Univ Calif Riverside, Riverside, CA, USA, <sup>2</sup>Dept Pharmacology, Univ Calif San Diego, San Diego, CA, USA, <sup>3</sup>ENS LYON, Lyon, France, <sup>4</sup>Dept Chemistry, Yale Univ, New Haven, CT, USA, <sup>5</sup>Chemistry & Biochemistry, Univ Calif San Diego, La Jolla, CA, USA. CRISPR-Cas9 is a bacterial adaptive immune system that recently emerged as the centerpiece of a transformative genome editing technology. This system is based on the single Cas9 protein, which can be programmed with single guide RNAs to site-specifically target and cleave any desired DNA sequence bearing a short recognition region, known as protospacer adjacent motif. Here, all-atom molecular dynamics simulations reveal the important role of allostery in enabling regulation and function in CRISPR-Cas9. We disclose an allosteric mechanism in which the DNA recognition activates the catalytic function at distal sites of the CRISPR-Cas9 complex. In this mechanism, the binding of a short recognition sequence acts as an “allosteric effector”, establishing a tight communication network (and increased correlated motions) between the catalytic domains. Analysing the communication pathways within the system allowed the identification of residues responsible for the information relay. Mutating two of these residues (K775A and R905A) was shown to decrease unselective cleavages of partially complementary DNAs, opening new avenues for modulating the activity of CRISPR-Cas9 systems. Overall, these findings contribute in clarifying the mechanistic function of this unique genome-editing tool, while also offering key insights for future efforts aimed at improving its selectivity.

#### 2404-Pos

##### Positive and Negative Substrate Interference Supported by Coinciding Enzyme Residues

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Chemistry Department, Umea University, Umeå, Sweden. Enzymes bridge the time-scale gap between chemistry and biology and are as such essential for cellular viability. A fundamental property of enzymatic function inside the complex and crowded cellular milieu is recognition of correct over in-correct substrates. This is particularly challenging when chemically related substrates such as ATP and GTP should be discriminated. Specificity can, in principle, be accomplished with positive interference with the correct substrate and negative interference with in-correct substrates. Here we present the atomic mechanism for ATP versus GTP selectivity of the small enzyme Adenylate kinase. The finding was enabled through a combination of protein NMR spectroscopy, x-ray crystallography and synthetic organic chemistry. We discovered that while ATP activates the enzyme by triggering a massive conformational change, the enzyme is arrested in an inactive conformation in its complex with GTP. Unexpectedly, we found that a set of residues that are vital for ATP dependent activation also are responsible for stabilizing the inactive GTP-complex. Hence, this set of residues has evolved to accomplish positive interference with ATP and at the same time negative interference with GTP. From a structure-function analysis of a key hydrogen bond formed between ATP and the enzyme, we discovered that this single hydrogen bond is essential for substrate selectivity. This particular aspect of ATP recognition is conserved throughout the entire family of eukaryotic protein kinases thus highlighting its importance.

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#### 2405-Pos

##### The Placement of Vibrational Probe Labeled Substrates to the Phosphopantetheine Arm of the E.Coli Acyl Carrier Protein for Site Specific Vibrational Spectroscopy

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<sup>1</sup>Chemistry, Haverford Coll, Haverford, PA, USA, <sup>2</sup>Haverford Coll, Haverford, PA, USA, <sup>3</sup>Dept Biology, Haverford Coll, Haverford, PA, USA. The study of acyl carrier proteins (ACP) can provide unique insights into bacterial bio-synthetic pathways due to their integral role of ferrying intermediate substrates to desired enzymes' active sites. Understanding bacterial biosynthesis can broaden our abilities to synthesize unnatural bioproducts using the cell's machinery. e.Coli's fatty acid ACP is a model for understand the broad