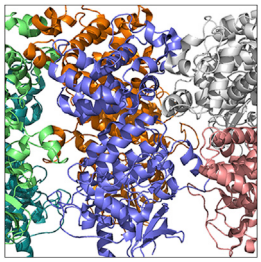
Cell

What's Your Favorite Crystal Structure?

Signaling Helix

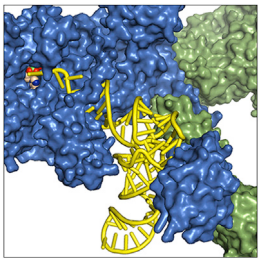


Hao Wu
Harvard Medical School

I stumbled into the crystal structure of Ire1 a few years ago when my lab had just solved the structures of several helical signaling oligomers formed by death domains. I was curious if others had observed similar structures in their systems and whether these higher-order oligomers might tell us something new about molecular mechanisms in signal transduction. Ire1 is a receptor critical for the unfolded protein response (UPR) in the endoplasmic reticulum (ER). It contains an ER luminal region that senses aberrantly folded proteins and a cytoplasmic region with a kinase domain and an RNase domain. Upon activation, the Ire1 RNase mediates nonconventional mRNA splicing to enable expression of transcriptional factors that activate the UPR.

The crystal structure of the Ire1 cytosolic domain reveals a striking oligomeric assembly, corroborating the observed activation of the Ire1 RNase in solution by oligomerization (Nature [2009]. *457*, 687–693). Symmetric back-to-back Ire1 dimers arrange into a filament through helical symmetry. Each kinase domain extends its activation loop to the next kinase domain, resembling a *trans*-autophosphorylation reaction. Remarkably, the RNase active site that is disordered in a dimer structure becomes ordered in the oligomer to create the mRNA-binding pocket. The oligomerization-promoted allosteric activation of the RNase made perfect sense to me, and an analogous mechanism may bring about kinase and caspase activation in the context of innate immune signaling. A picture is worth a thousand words.

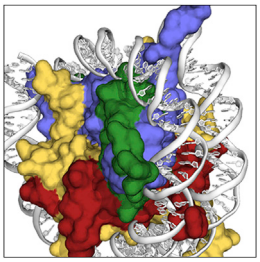
A Single Base Pair



Shigeyuki Yokoyama
RIKEN and The University of Tokyo

Reading the genetic code, which describes the rules relating amino acids to codons, relies on strict molecular recognition of both amino acids and tRNAs by aminoacyl-tRNA synthetases. Aminoacyl-tRNA synthetases must recognize key nucleotides far from the 3′ terminal adenosine that is ligated to the amino acid. The structures of alanyl-tRNA synthetase (AlaRS) in complex with $tRNA^{Ala}$ revealed how AlaRS selects $tRNA^{Ala}$ depending just on a single base pair G3•U70 (Nature [2014]. *510*, 507–511) and thereby unraveled what had been a mystery since 1988. A variant of $tRNA^{Ala}$ with A3•U70 can bind to AlaRS with nearly the same affinity, but aminoacylation is about hundred times slower than wild-type with G3•U70. The AlaRS•tRNA structure of the A3•U70 variant showed that the CCA sequence is bound in a site far removed from the catalytic site, which traps the 3′-adenosine. Moreover, G3•U70 is able to control the direction of the 3′-CCA. It is surprising that such high translation specificity is achieved by this unprecedented mechanism. The finding of this nonproductive binding mechanism may cause a paradigm shift in enzymology of not only aminoacyl-tRNA synthetases, but also other highly specific enzymes. Furthermore, rational engineering of aminoacyl-tRNA synthetases toward novel specificities for tRNAs may become possible in the field of synthetic biology, utilizing unnatural amino acids and base pairs in the central dogma.

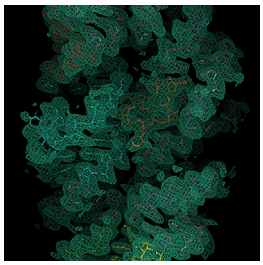
That Exact Moment



Karolin Luger
Colorado State University and HHMI

Everybody's all-time favorite structure has to be the DNA double helix because it exemplifies everything a high-impact structure should do: be aesthetically pleasing and explain complex biology. After this obvious choice, with full awareness of my bias, my favorite structure is the nucleosome. Obviously, the fact that I participated for 8 long years in the arduous process leading to a high-resolution model of the repeating unit of eukaryotic chromatin affects my choice. But on an objective scale, the nucleosome's beauty lies in its inherent 2-fold symmetry, its aesthetically pleasing proportions, and the way in which intricately intertwined histone helices generate a ramp that gently guides the DNA double helix into a superhelix. More importantly, the structure beautifully explains its function in organizing the entire eukaryotic genome: the histone octamer holds the DNA with just enough force to bend it around its perimeter yet allows its dissociation for key biological processes to take place. Its modular design lends itself to a dynamic response to regulate transcription, replication, and repair. Finally, the histone tails, the sites of numerous posttranslational modifications, are freely accessible for signaling and for promoting internucleosomal interactions (Nature [1997]. *389*, 251–260). The entire structure had to be manually fit into the electron density map, and so there was no single moment at which I first "saw" the nucleosome. But when I projected the structure for the first time at a Cold Spring Harbor meeting, it was greeted with a collective gasp. I remember that exact moment to this day.

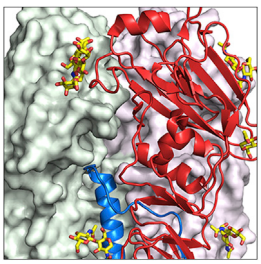
The 3D Beauty of RNA



Jennifer Doudna
UC Berkeley and HHMI

As a graduate student in the late 1980s, with a backdrop of Madonna tunes and post-punk fashion, I was fascinated by the hypothesis that RNA played a starring role during early evolution. Perhaps this agile molecule had both stored genetic information *and* catalyzed the chemical reactions that ensured its replication, enabling evolution to get going. Thinking that the three-dimensional structure of RNA was key to understanding the function and possible origins of ribozymes, I worked with then-student Jamie Cate to determine the crystal structure of the P4-P6 domain of the *Tetrahymena* self-splicing ribozyme (Science [1996]. *273*, 1678–1685). I'll never forget my sense of wonder as the first electron density maps revealed the beauty of the RNA structure: its coiled helices were dramatically bent to form a distinctive swan's-neck shape, with magnesium ions nestled in the center to hold together phosphate backbones that would otherwise spring apart due to electrostatic repulsion. At 2.8 Å resolution, the 160 nucleotide P4-P6 structure was brimming with exciting details. Recurrent motifs, including the tetraloop-receptor interaction, the A-A platform, A-minor contacts, and ribose zippers, were subsequently observed in complex RNAs ranging from the ribosome to riboswitches, setting the stage for many functional studies and mechanistic insights. But for me, the first electrifying moments of seeing the P4-P6 RNA structure emerge from initially noisy electron density remains a standout in my career.

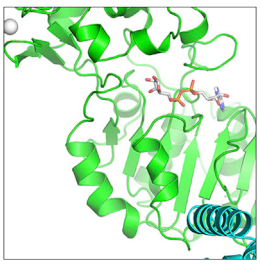
Flu Escapes Immunity



Ian Wilson
The Scripps Research Institute

The crystal structure of influenza virus hemagglutinin (HA) in 1981 revolutionized our thinking on how animal viruses bind and enter host cells and how they evade our immune system (Nature [1981]. *289*, 366–373 and 374–378). At the time, the trimeric HA glycoprotein from the 1968 Hong Kong pandemic virus was considered a very large protein (200 kDa) for structural studies. Given its composition of 25% glycosylation by weight, novel methods were required to solve its structure, using only one heavy atom derivative with 3-fold symmetry averaging and solvent flattening (>80% solvent). Its now classic fold was composed of a jelly roll globular head for receptor binding and a largely helical stem housing the fusion machinery. Mapping of natural mutations from 1968 to 1977 on the HA structure uncovered four major antigenic sites, elucidating how the virus can escape immune recognition and why we need a seasonal vaccine. It was a stunning moment as the structure came to life and explained a huge amount of influenza virology and biology. The recent discovery of broadly neutralizing antibodies to flu has now uncovered key sites of vulnerability on the HA of this virus and has given hope for structure-based design of a universal vaccine. Remarkably, 33 years after the first structure of HA, fascinating insights on this viral glycoprotein are still being revealed.

Visualize Longevity Modulator



Rui-Ming Xu
Chinese Academy of Sciences

The discovery in 2000 that Sir2 and related proteins (sirtuins) are NAD-dependent histone/protein deacetylases was a landmark achievement following more than a decade's effort of genetic and biochemical characterization of yeast Sir2 function in transcriptional silencing. Sirtuins are found in all three kingdoms, but their sequences give no hints of their 3D structures and biochemical mechanisms. The first structures of Sirtuins, an archaeal protein in complex with NAD and an apo form of human SIRT2, revealed a prominent NAD-binding Rossmann-fold domain (Cell [2001]. *105*, 269–279). NAD was bound in an inverted orientation compared to the canonical binding mode. This, together with the presence of a zinc-binding domain, immediately pointed toward the locations of the acetyllysine-binding channel and the active sites for deacetylation and nicotinamide cleavage. Now the catalytic mechanisms have been worked in considerable detail with the help of many structural studies. In addition, the mechanisms regulating sirtuin activities have begun to emerge, with the determination of the Sir4-bound structure of Sir2, which appears to lock the regulatory and catalytic domains of Sir2 in a defined configuration to allow productive deacetylation reactions. Mammalian SIRT1 shares a similar domain structure with Sir2, and SIRT1 has been implicated in many physiological and pathological processes, including aging, cancer, and cardiovascular and metabolic diseases. There has been keen interest in developing small-molecule modulators for SIRT1, an area where X-ray crystallography can make further contributions.