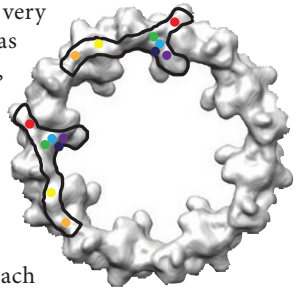
NPCs, super-resolved



Determination of the structure of very large protein assemblies, such as the nuclear pore complex (NPC), remains quite challenging because current methods used to determine their structures *in situ* lack the resolution needed for detailed molecular assignment. To address this, Ellenberg and colleagues have developed a methodological approach that combines super-resolution fluorescence microscopy with single-particle averaging. They used their method to examine the organization of the NPC scaffold primarily composed of multiple copies of the Nup107–160 subcomplex. By combining thousands of super-resolution images of single nuclear pores to generate an average image, it was possible to map the average position of a given fluorescent label in the NPC with a precision of 0.1 nm and an accuracy of 0.3 nm. The Nup107–160 subcomplex is believed to be structurally homologous to that of the yeast Nup84 subcomplex, which crystallographic, electron microscopic and biochemical work have revealed to be Y-shaped. The authors first attempted to identify members of the complex by using fluorescent-labeled antibodies. The data suggested that the stalk (Nup133 and Nup107) and the Nup160 arm are located at the periphery of the nuclear pore, with the second, Nup85–Seh1 arm reaching into the center. Further examination using Nup-GFP fusions and dye-coupled anti-GFP nanobodies indicated that the long stalk of the Y was oriented perpendicularly to the direction of transport through the NPC. When examined in conjunction with the cryo-EM structure of the human NPC, the data support a head-to-tail arrangement of the Nup107–160 complex in the cytoplasmic and nucleoplasmic rings of the pore, with 8 or 16 copies of the complex in each ring, and do not support previously proposed fence-like or lattice models. This methodology could bridge the gap between atomic resolution techniques and label-free *in situ* methods. (*Science* http://dx.doi.org/10.1126/science.1240672, published online 11 July 2013) *MM*

A funnel-like viroporin

The integral membrane protein p7 from hepatitis C virus (HCV) can self-assemble into a large cation channel, or viroporin, which is required for viral replication and is a potential anti-HCV drug target. Chou and colleagues have now determined the NMR structure of the large hexameric p7 channel reconstituted in micelles. Each monomer consists of three helices (H1–H3), and the monomers intertwine to form a tightly packed channel, with H1 and H2 forming the channel interior. Each monomer interacts not only with its direct neighboring monomers but also with the +2 and +3 monomers, thereby forming an unusual funnel-like structure. To define the elements involved in cation conductance and gating, the authors identified highly conserved polar residues in the channel interior. Asn9 (His9 in some strains) has affinity for monovalent and divalent cations, and forms a ring that was proposed to serve as a broad selectivity filter at the narrow end of the funnel. Replacement of His9 with alanine caused a large reduction in channel conductance. The Ile6 ring at the funnel's narrowest point forms a hydrophobic gate that is thought to prevent water passage. Arg35 forms a positively charged ring at the wider, C-terminal end of the channel and was proposed to bind and obstruct anions at the pore entrance while allowing unidirectional cation diffusion. As expected, mutation of Arg35 to a negatively charged residue hindered the diffusion of cations into the pore and reduced conductance. The available NMR data suggest that adamantine-derived drugs bind six equivalent hydrophobic pockets between the pore-forming and peripheral helices. As channel activation may involve structural rearrangements, the binding of adamantine derivatives might allosterically inhibit cation conduction by restricting movements of the three helical segments, thereby preventing the channel from opening. More rigorous testing will be required to validate this attractive model. (*Nature* **498**, 521–525, 2013) *AH*

DNA pol in action

Error-free DNA replication, and thus genomic stability, critically depends on the ability of DNA polymerases to discriminate correct from incorrect dNTPs during base-by-base extension of the complementary strand. This dNTP-substrate discrimination, known as enzyme fidelity, has been extensively studied with the model eukaryotic DNA polymerase pol β. Functional assays combined with crystal structures of ternary complexes of pol β with DNA and a dNTP positioned opposite the template base suggest that a complementary incoming dNTP promotes conformational changes of active site residues that enhance nucleotide incorporation. This 'induced fit' mechanism is supported by kinetic analyses but is not well characterized structurally because substrate analogs that prevent catalysis are traditionally used to capture reaction intermediates for crystallography. Now, Wilson and colleagues provide a rare glimpse of active complexes containing natural substrates. By soaking ternary-complex crystals in $MgCl_2$ to initiate nucleotide insertion and freezing them at various time points during catalysis, they obtain snapshots of the active site that reveal differential effects of correct or incorrect dNTP incorporation. Among their most striking findings are that pol β maintains its 'closed conformation' after correct but not incorrect dNTP insertion and that pyrophosphate (PPi), a product of the nucleotidyl transfer reaction, and its associated Mg^{2+} remain bound to the active site after phosphodiester-bond formation. In contrast, misincorporation promotes an 'open' conformation and PPi release. Moreover, a transient third metal-binding site that is observed after correct dNTP incorporation is absent from mismatched ternary complexes, though its function is presently unclear. The finding that pol β ternary complexes show substrate-dependent conformational differences both during and after catalysis suggests that active site configuration can be altered by dNTP misincorporation to restrain subsequent chain elongation, thereby enhancing enzyme fidelity. (*Cell* **154**, 157–168, 2013) *BM*

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