Mimicking the Nuclear Pore Complex with Solid-State Nanopores

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We use solid-state nanopores to mimic the nuclear pore complex (NPC), an essential protein complex that act as gatekeeper for molecular transport between the nucleus and the cytoplasm in eukaryotic cells. The crucial element responsible for the selective function of the NPC, is it's central channel filled with a class of intrinsically disordered proteins called nucleoporins (FG-Nups). The intriguiging mechanism of selective FG-Nup-mediated transport is however still unclear.

In our work, we fabricate and characterize biomimetic NPCs by covalently attaching Nsp1, a model FG-Nup from Saccharomyces *cerevisiae* (yeast), to the surface of a solid-state nanopore and probe the transport of ions and yeast transport receptor (Kap95). We observe a clear difference between the diameter-dependent conductivity of bare pores, pores with Nsp1, and pores coated with a Nsp1-S mutant where hydrophobic amino acid residues (F, I, L, V) were replaced with the hydrophilic Serine (S). The transport receptor Kap95 is found to translocate across the Nsp1 biomimetic nanopores, whereas transport of inert proteins such as tCherry was severely hindered. Nsp1-S mutant pores however lose the selectivity and allow translocation of both Kap95 and tCherry.

Our coarse-grained molecular dynamics simulations show that Nsp1 forms a highly dense central plug, in contrast to the uniform and significantly less-dense protein distribution of Nsp1-S. Our results identify a strongly sequence-dependent spatial structure of the disordered FG-Nups that affects the conductance and emphasis the key role of hydrophobic interactions in establishing the NPC's selective permeability [1].

Finally, to understand the effect of FG Nups density and spatial arrangement, we assembled the Nsp1 and Nsp1-S within a scaffold DNA origami ring. These rings were docked onto the solid-state nanopore to probe the conductance blockade and the clear difference in conductance was observed for varying protein densities [2].

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