NATURE-INSPIRED NEXT GENERATION NANOSORTERS FOR PROTEIN PURIFICATION

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Creating a new class of synthetic membranes with the high selectivity of biological membranes while maintaining large permeation fluxes is the holy grail of membrane science and technology. In Nature, cells have evolved many separators (machines) to select, concentrate and purify water, ions and proteins. In particular, the Nuclear Pore Complex (NPC) is a macromolecular complex that efficiently fractionates proteins between the cell nucleus and cytoplasm in all eukaryotic cells[1]. Its architecture is well understood and described in the literature,[2-5] yet the molecular transport mechanism remains unclear. Transport across the NPC is fast, energy-dependent (to give directionality) and often receptor-mediated. While small molecules pass through the NPCs unchallenged, large macromolecules (>40 kDa) are excluded unless assisted by transport factors collectively termed Karyopherins (Kaps). The translocation of proteins/RNAs occurs through the specific affinity and binding between Kaps and particular nuclear pore complex proteins (nucleoporins) called FG-Nups, which share a degenerate multiple-repeated "Phe-Gly" motif. Because FG-Nups are the major component of the selective gating mechanism, we first investigated the nanomechanical properties of cysteine-modified Nsp1 using the volume force mapping technique of atomic force microscopy (AFM). From single molecule AFM on a sparse Nsp1 surface, we estimated structural parameter as persistence length and contour length. In an attempt to better understand the transport and the selective process under crowding conditions, we then used quartz crystal microbalance with dissipation (QCM-D). Nsp1 and truncated variations of it were immobilized on QCM-D sensors. The binding and unbinding of Kap95, other binding proteins, as well as control proteins, was studied in order to investigate specificity and effect of competitive binding. Finally, we coupled Nsp1 to maleimide functionalized PS-b-PEO membranes and characterized them through X-ray photoelectron spectroscopy. Inspired by Nature, we aim to gain sufficient understanding of the molecular scale engineering principles behind nuclear transport to allow us to design the next generation of synthetic selective nanosorters capable of purifying any protein that we desire.

References

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