Selective Transport of Functionalized Nanocarriers into Biomimetic and Natural Nuclear Pore Complexes

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1. Current State of Research in the Field: Nuclear pore complexes (NPCs) form the sole passageways between the nucleus and cytoplasm in eukaryotic cells (1). Each ~50 nm-diameter NPC ensures that only specific proteins such as transcription factors gain access to the nucleus (2) while promoting the export of staggeringly large messenger ribonucleoproteins (mRNPs; ~100 MDa complex of proteins and mRNA) out of the nucleus for subsequent protein translation in the cytoplasm (3). In general, this process is known as nucleocytoplasmic transport.

The key components of the NPC machinery comprise of a group of nuclear pore proteins (i.e., nucleoporins or Nups) that contain large intrinsically disordered domains that are rich in phenylalanine-glycine (FG)-repeat motifs (i.e., FG domains) (4). A priori, the FG domains pose a physical barrier to the passage of macromolecules (>40kDa). Exclusive access is given to soluble transport receptors (known as karyopherins or Kaps, importins or exportins) that ferry specific cargoes through the NPC by exerting biochemical binding interactions with the FG domains (5).

Towards this end, the Lim Lab has contributed extensively to understanding how Kap-FG domain binding imparts selective transport control, which include:

- *(i) resolving the nanomechanical response of the FG domains to Kap-binding in vitro(6-8);*
- *(ii) constructing a biomimetic NPC that reconstitutes NPC speed and transport selectivity (9);*
- *(iii) reconciling Kap-FG domain binding kinetics with FG Nup conformational changes (10, 11);*
- *(iv) exploiting multivalent Kap-FG domain interactions for controlling selective diffusional search processes in two-dimensions (12).*

However, it is unknown how very large cargoes such as mRNPs are exported through the tight confines of the NPC channel. Several outstanding questions remain, including: Does the NPC deform structurally in a diaphragm-like manner to accommodate large objects (1)? Do large mRNPs deform and translocate as a "string of beads" (3)? How does multivalent Kap-FG domain binding influence this process (10)? Other pathological correlations include: (i) large viruses that "hijack" Kaps to infiltrate NPCs and enter into the nucleus (13); and (ii) the aberrant transcription of target genes leading to mRNP upregulation and increased nuclear crowding in cancer (14). Not surprisingly, it remains formidable to study such processes *in vivo*. This owes largely to the complex interplay of cargo vs. NPC nanomechanics, biochemical interaction kinetics, and NPC structure.

To gain insight into this process, we propose a step-wise, bottom-up strategy that uses artificial "cargoes" as a means to study how large, deformable objects enter into the NPC via interactions with the FG domains. Specifically, we will exploit nanometer-sized 3D supramolecular hybrid polymer vesicles that allow for the insertion, encapsulation, and attachment of active compounds and biomolecules (15,16). Functionalizing these so-called "nanocarriers" (17) with Kaps will promote their molecular recognition by the NPC as authentic cargoes. Such fundamental insight may lead to applications that use nanocarriers for delivering therapeutic agents into the nucleus e.g., gene delivery.

2. Research Proposal Our project requires a step-wise characterization of the various physicochemical attributes of the vesicles, how they interact with the FG domains, and visualizing their translocation

through biomimetic NPCs and finally in NPCs under physiological conditions. An interesting aspect lies with constructing vesicles of various materials (polymers, lipids), sizes and deformability (i.e., elasticity) to explore the most effective properties that lead to optimal NPC penetration. Conversely, this will reveal insight as to the intrinsic structural-nanomechanical properties of the NPC itself. **Importantly, this study takes advantage of established expertise and experimental capabilities in both collaborating labs:**

Lim Lab: atomic force microscopy (ARTIDIS-AFM), high speed-AFM (HS-AFM), surface plasmon resonance (SPR), optical tweezers (OT), fluorescence microscopy (FM), fluorescence recovery after photo-bleaching (FRAP), single particle tracking (SPT), biomimetic nanopores (BN), cell biology and nuclear transport assays

Palivan Lab: combination of proteins, enzymes, and mimics with supramolecular polymer assemblies, design of active surfaces, nanoreactors and artificial organelles (18), light scattering (LS), spectroscopy (FTIR, fluorescence), fluorescence correlation spectroscopy (FCS), and fluorescence cross correlation (FCCS), electron paramagnetic resonance (EPR), Brewster angle microscopy, ellipsometry.

Our project is anticipated to proceed in the following stages:

Vesicle construction, fluorescence and Kap-functionalization:

- protein expression and purification (Lim)
- vesicle construction using various polymers (Palivan)
- vesicle Kap-functionalization (Palivan)

Vesicle characterization:

- size distribution to be determined by LS, TEM and AFM (Lim and Palivan)
- vesicles stability and functionalisation efficiency by LS, LSM and FCS (Palivan)
- vesicle stiffness to be measured by ARTIDIS-AFM (Lim)
- Kap-FG domain binding affinity and kinetics measured by SPR and FCS, FCCS (Lim and Palivan)

Vesicle biomimetic transport and tracking:

- Vesicles up-take in cell lines by LSM, TEM (Palivan)
- on FG domain surfaces by OT, FM and SPT (Lim)
- through FG domain-functionalized BN (Lim)

Vesicle NPC transport:

- bulk transport into isolated nuclei measured by FRAP (Lim)
- single vesicle transport at the single NPC-level by HS-AFM (Lim)
- NPC structural determination by TEM (ZMB; CINA; or other collaborators)

3. Necessity of interdisciplinary collaboration: This challenging project requires the collaboration and combined expertise in nanoscience, soft matter physics, biochemistry, and physical chemistry. RL is an expert in the area of the NPC, nanoscience methodology and biological soft matter. CGP is involved in the design and characterization of new nanoscale hybrid systems by combining self-assembling copolymers with biological molecules (proteins, enzymes, mimics).

4. Impact Distilled into its most basic form, our study will reveal how the physico(bio)chemical properties of vesicles dictate their transport in confined spaces e.g., nanopores. This has undoubtedly very interesting nanoscale physics and is important in terms of biological NPC function. With the knowledge gained, our work can, for instance lead to applications where vesicles may be used to deliver therapeutic agents (e.g., drugs that intervene with pathological activity) via the NPCs into cell nuclei (19).

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