Selective transport through biological and bio-mimetic nano-channels: mathematical modeling meets experiments

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1 Overview of the Field

Nuclear Pore Complexes (NPC's) are biological "nanomachines" that gate all traffic between the nucleus and the cytoplasm in eukaryotic cells. NPCs do not consume metabolic energy directly during the translocation process and seemingly do not undergo large scale molecular transitions from a 'closed' to an 'open' state. Nevertheless, they are directional and selective. A single NPC can selectively translocate thousands of molecules per second in both directions in the presence of vast amounts of background noise. Moreover, the NPC remains functional even when large portions of its components are deleted. Exquisite selectivity of the NPC, as well as the robust way in which it functions, provide inspiration for creation of cutting edge artificial nano-molecular sorting devices based on the same principles. Such artificial nano-devices can be used for single-mismatch DNA detection, protein sorting and concentration, and pathogen detection. First functional prototypes of such devices have been recently built. Thus, understanding the transport through the NPC is not only a fundamental biological question but also has far-reaching applications in nano-technology and nano-medicine. Understanding of the NPC also provides a 'stepping stone' for research on many other transporters, such as porins and secretion systems in bacteria. The mechanism of translocation through the NPC and, most importantly, its selectivity mechanism is still a subject of intense scientific debate. Experimental advances in single molecule microscopy, creation of in vitro bio-mimetic devices that mimic the NPC, and new structural data allow us now to tackle these questions in a quantitative manner. At the same time, recent advances in mathematical modeling of the transport through the NPC and related biological and artificial nano-channels have started to provide insights into the fundamental biophysical principles of NPC function. It has become clear that the key element in the selective transport through the NPC is the region of unstructured, natively unfolded proteins that line and partially occlude the NPC passageway and its nuclear and cytoplasmic orifices. While small molecules pass through the NPC unaided, larger molecules are selectively carried through by specialized transport factors that weakly and transiently bind these unstructured proteins. Moreover, artificial nano-channels functionalized with some of these unstructured proteins reproduce in vitro many important aspects of the selective in vivo transport through the NPC. A wealth of information has been gathered on 30 different proteins that constitute the NPC, including their in vitro behavior, assembly into a structural scaffold, and their in vitro and in vivo bulk and single protein transport rates.

2 Role of mathematical modeling

Mathematical modeling has proven to play an increasingly important role in the study of the transport through the NPC in particular, because many of the details of *in vivo* transport are in accessible to direct experimental measurements at this point. In the recent years, simplified models of diffusion through the NPC have provided important insights and the general framework for thinking about the transport through the NPC. The modeling of unfolded proteins is challenging and requires new conceptual mathematical tools. The mathematical modeling featured prominently in the workshop. Several presentations at the workshop discussed their efforts to capture the emergence of meso-scale order and function from underlying disorder. Importantly, the insights gained from these theoretical/computational studies on NPC can be applied to the design of bio-mimetic systems that can achieve highly regulated transport across biological or in vitro membranes

3 Presentation Highlights

Computational modeling of the FG nups and their assemblies

Most of the existing models of nucleocytoplasmic transport are phenomenological in nature. Thus detailed mechanistic model of transport are necessary for further progress.

Professor Yitzhak Rabin from Bar-Ilan University presented comprehensive study of the structure of yeast Nuclear Pore Complex (NPC) with a molecular theory that accounts for the geometry of the pore, the amino acid sequence and anchoring position of the unfolded domains of the FG-nucleoporins. The theory explicitly models the electrostatic, hydrophobic, steric, conformational and acid-base properties of the FG-Nups. The electrostatic potential within the pore, which arises from the specific charge distribution of the FG Nups, is predicted to be negative close to pore walls and positive along pore axis. The work shows that the positive electrostatic potential facilitates the translocation of negatively charged particles and the free energy barrier for translocation decreases with increasing particle hydrophobicity. The molecular theory also showed that the effects of electrostatic and hydrophobic interactions on the translocating potential are cooperative and non-equivalent due to the interaction-dependent reorganization of the FG-Nups in the presence of the translocating particle. The combination of electrostatic and hydrophobic interactions can give rise to complex translocation potentials displaying a combination of wells and barriers, in contrast to the simple barrier potential observed for a hydrophilic/neutral translocating particle.

Professor Ajay Gopinathan from UC Merced presented a ”bottom-up” approach to understanding the higher-order architecture formed by the FG nups using different methods - coarse-grained simulations and theoretical techniques used to describe polymer brushes in confined curved spaces. His results indicate that different regions or ”blocks” of an individual NPC protein can have distinctly different forms of disorder and properties and their bioinformatic analysis indicates that this appears to be a conserved feature across all of eukarya. Furthermore, this block structure at the individual protein level is critical to the formation of a unique higher-order polymer brush architecture. His results point to a novel form of protein architecture and gated transport in operation within the nuclear pore complex.

Dr. Barak Raveh from the University of California, San Francisco showed preliminary results from an integrative multiscale simulation of transport that dissected the spatiotemporal dynamics of transport through the NPC. Their approach allows to simultaneously consider the information from a multitude of experimental methods, which increases the fidelity of the resulting model by accounting for intrinsic experimental noise and inherent ambiguities in data interpretation. He discussed preliminary results for which he used coarse-grained Brownian Dynamics simulations implemented in their open source IMP package (salilab.org/imp) on a very large scale to explore the ”phase space” of transport models. Using hundreds of thousands of computational cores, available through the Google Exocycle project, they have mapped transport dynamics a function of a wide range of model parameters that describe the dynamic coupling between FG repeat domains, nuclear transport factors and cargo molecules during transport. Currently they are refining the model based on structural and dynamic data from in-cell NMR studies, knockout experiments and diffusion measurements, single molecule imaging and FRAP experiments, X-ray crystallography and high throughput in-vivo phenotypic assays.

Dr. Gnana Gnanakaran from the Los Alamos National Labs provided details of their study on intrinsically disordered proteins from NPC at atomistic resolution. He carried out atomic level molecular dynamics

simulations of single and multiple FG nups to unveil their dynamics and structures. As an important aspect of his studies of intrinsically disordered proteins (IDPs), his group made extensive comparisons between two popular water models, TIP3P and TIP4P-Ew, in combination with AMBER ff99sb force field to simulate different IDPs. They found that TIP4P-Ew combined with AMBER ff99sb force field gives better reproduction of experimental observations for FG nups. The reason is because of better solvation of the water model to the charged side chain of amino acid residues. However, he also identified a caveat of the current force field by studying the mutated form of FG nups. Also, the shape of IDPs is much closer to a rod-like shape regardless of whether they are extended or collapsed, which could be a signature of IDPs. Then, he explored the interaction of multiple FG nups to show that the arrangement of the FG nups can be different depending on the inter-chain distances. He identified the influence from the types of FG nups and inter-molecular spacing. FG nups showed a strong dependence of inter-molecular interaction strength on molecular spacing. However, inter-molecular interaction for one type is weaker compared to that of another type. While most of the FG nups of the two types are mainly disordered despite inter- and intra- molecular interactions, he observed noticeable beta-sheet formation at certain inter-molecular spacings, indicating a significant role between inter-molecular interaction and secondary structure formation

Prof. Anton Zilman from the University of Toronto presented a coarse grained model of the FG nups that treats the unfolded proteins and simple flexible polymers as a means to explain the observed contradictions in the in vitro behavior of the assemblies of the Fg nups and their interactions with the transport factors from which mechanisms of actual transport *in vivo* were being attempted to be inferred. This approximation was based on the in vitro experimental results showing that each individual elastic behavior FG nup is captured extremely well by the standard worm-like chain model. The transport factors were modeled as large particles that can interact attractively with the polymers and transiently bind to them. This simple coarse grained model was applied to the analysis of conformational changes induced by the transport factors in the in vitro layers of FG nup grafted to surfaces. It predicted three distinct regimes, governed by the interaction strength and the grafting density of the FG nups. At low grafting densities, the addition of the transport factors causes collapse, at intermediate densities - collapse and subsequent recovery, and at high concentration no collapse and limited penetration. All these regimes have been observed experimentally and the theory settles down the controversy and makes verifiable predictions regarding how to control the transitions from one behavior to another by parameter variation.

Single molecule imaging techniques

Three presenters used novel single molecule microscopy methods to investigate nucleocytoplasmic transport. Prof. Siegfried Musser from Texas AM Health science Center, College of Medicine presented work developed in his lab with Li-Chun Tu and Dr. Guo Fu and in collaboration with Prof. Anton Zilman from University of Toronto on efficient nuclear transport of large cargos. Using single molecule fluorescence microscopy, Prof. Musser's team showed that a large cargo with four M9 signal sequences requires multiple transportin molecules for efficient transport, while a single NTR promotes binding to the NPC but not transport. Surprisingly, the interaction time depended only weakly on the number of bound NTRs, indicating that the additional interactions provided by multiple NTRs are brief. Particle tracking, avidity calculations and theoretical modeling based on diffusion in a potential well suggested that the FG-network on the cytoplasmic side of the pore is characterized by a low effective concentration of free FG-repeats and a millimolar FG-transportin affinity, and served as the initial docking site for NTR-cargo complexes. Prof Musser's team postulated that multiple NTRs allow a large cargo to migrate through a centrally located permeability barrier by providing the binding energy necessary to permeate the barrier, yet the energetic cost of displacing the barrier prevents a strong overall interaction, which leads to rapid escape. They conclude that FG-NTR affinities vary significantly in different environments due to modulation of bare binding energies by the entropic cost of displacing FG-polypeptides and, possibly, by an enthalpic cost of breaking FG-FG interactions to accommodate a cargo complex within the FG-network.

Prof. Weidong Yang form department of Biology at Temple University presented the study of nucleocytoplasmic transport by SPEED microscopy. Prof. Yang's lab employed single-point edge-excitation subdiffraction (SPEED) microscopy to address several important questions: a) what is the nuclear export kinetics, three-dimensional (3D) pathway, and selectivity step of individual messenger RNA:protein complexes (mRNPs) transiting NPC b) wether mRNPs occupy distinct or overlapping physical regions of the NPC.

Yang and co-workers employed single-molecule fluorescence microscopy with an unprecedented spatiotemporal super-accuracy of 8 nm and 2 ms combined with Monte-Carlo simulations to unveil these mechanistic fundamentals of nuclear mRNP export in live human cells. The team found that mRNPs exiting the nucleus are decelerated and selected at the center of the NPC, and adopt a fast-slow-fast diffusion pattern as they translocate through the NPC. Approximately 34% of all mRNPs successfully transited during their brief, 12-ms interaction with the NPC. This fraction is very close to the 50% export efficiency of their export receptor Tap-p15. A 3D mapping of export routes for individual mRNPs indicated that mRNPs primarily interact with the periphery on the nucleoplasmic side and in the center of the NPC, without entering the central axial conduit utilized for passive diffusion of small molecules, and eventually dissociate on the cytoplasmic side.

Prof. Enrico Gratton, from University of California, Irvine presented his work in collaboration with Dr. Luca Lanzano (UCI) and Dr. Francesco Cardarelli (Italian Institute of Technology in Pisa) on Super-resolution by feedback imaging. Prof. Gratton discussed current models proposed for the opening and closing of the pore. He presented recent experimental evidence from his lab which pose strong constrains about possible mechanisms of translocation. Prof. Gratton also described a nanoimaging technique that has a very high time and spatial resolution. This technique is based on the observation of single molecules using feedback imaging. Using this approach Gratton's team observed the translocation of particles in great detail that reveal possible mechanisms of action of the nuclear pore complex.

In vitro systems

Several presenters used advanced biophysics and surface science approaches to study the components of the NPC transport mechanism *in vitro*.

Prof. Ralf Richter from CIC biomaGune, Spain, presented work developed in his lab with Nico Eisele and in collaboration with Prof. Dirk Gorlich, Dr. Steffen Frey and Dr. Aksana Labokha from Max Planck Institute for Biophysical Chemistry, Germany on the assembly kinetics and morphology of FG nucleoporin domain meshworks and the implications for the functionality of the nuclear pore permeability barrier. Team of Prof. Richter recently created ultrathin films - end-grafted monolayers - of nucleoporin FG repeat domains as nanoscale model systems of the nuclear pore permeability barrier. The films reproduce the mode of attachment and the density of FG repeats in NPCs, and exhibit a thickness that corresponds to the nanoscopic dimensions of the native permeability barrier. They tailor-made these films, and characterized them with a combination of biophysical surface-sensitive techniques, to understand how nucleoporin FG repeat domains self-organize in confined spaces (such as the interior of the nuclear pore complex) and how they interact with nuclear transport receptors. To understand the functional roles of cohesiveness, Richter and co-workers have assessed how the formation kinetics, morphology, dynamics, and mechanical properties of FG repeat domain monolayers are affected by a varying degree of FG domain cohesiveness. Based on the analysis of their data in terms of classical polymer theory concepts, they propose the formation of a compact FG domain assembly that fills the entire pore - a compacted entangled and/or crosslinked meshwork - as a key design principle for a functional permeability barrier. Tuning of inter-chain interactions emerges as a robust and effective tool to optimize functionality, and should be useful as a design rule for the engineering of man-made species-selective filtering devices.

Dr. Steffen Frey from Max Planck Institute for Biophysical Chemistry discussed the previously proposed "selective phase model" in which the FG repeats interact with one another to form a sieve-like barrier that can be locally disrupted by the binding of nuclear transport receptors (NTRs), but not by inert macromolecules, allowing selective passage of NTRs and associated cargo. He presented direct evidence for this model in a physiological context. By using NPCs reconstituted from Xenopus laevis egg extracts, they showed that Nup98 is essential for maintaining the permeability barrier. Specifically, the multivalent cohesion between FG repeats is required, including cohesive FG repeats close to the anchorage point to the NPC scaffold. The data seem to exclude alternative models that are based solely on an interaction between the FG repeats and NTRs and indicate that the barrier is formed by a sieve-like FG hydrogel.

Professor Lim from the Biozentrum at the Basel University presented a study of the role multivalent interactions important in the targeting of the cargo-carrying karyopherin (Kap) receptors to the intrinsically disordered FG- nucleoporins of the nuclear pore complex (NPC). He noted that the fact that multivalent Kap-FG interactions facilitate cargo translocation is paradoxically at odds with the de facto notion that binding avidity hinders mobility. He demonstrated and discussed how the balance is struck between Kap selectivity

and mobility such that transport speed, efficiency and bi-directionality are optimally maintained in the NPC. He showed the results on brushes with kaps with collapse and recover and the diffusion coefficients and dwelling times of NTRs with different number of binding sites on surfaces. If the density of the binding sites is too low - they do not move and fall off quickly, if it is too high they are stuck and cannot move.

Prof. Fabien Montel from University of Paris, Diderot and CNRS presented the work he did with his colleagues from Complex Matter and Systems Laboratory Prof. Jean-Marc Di Meglio and Prof. Loc Auvray on DNA transport in functionnalized nanopores. They studied the mechanism of export through real and biomimetic NPCs by using electrical, optical and mechanical single molecule techniques associated with super-resolution optical microscopy. Using an adaptation of zero mode wave guide field localization to probe nanopore translocation (developed in close collaboration with Virgile Viasnoff, MBI Singapore) and super-resolution algorithm (FIONA), Montel and co-workers could detect with one nm accuracy the exit of the nanopore. Preliminary results show that it is possible to achieve live imaging of the complete translocation with a time resolution of 5 ms (compatible with indirect observations of transport in the NPC) for nucleic acid translocation through functionalized nanopores. They have also successfully tested a setup to control the transport by applying osmotic and hydrostatic pressure.

Other nanochannels

Professor Ulrich Keyser from Cambridge University presented a clever way to test the theories of selective transport through nanochannels using optical traps colocalized with a nanopore, which allows him to manipulate the potential.Living organisms crucially depend on the transport of molecules across their membranes. Evolutionary pressures have led to the development of highly specialized protein channels with complex potential landscapes for optimized transport. It is known that the so-called channel-facilitated diffusion relies on specific binding sites within the channel. However, the underlying physical mechanisms are still under intense investigation. Direct experimental tests on membrane proteins are extremely challenging due to the fast dynamics at the nano scale. To address these issues they introduced a novel, synthetic model system at the microscale to mimic channel-facilitated transport. Keyser's team showed that particle flux is increased beyond the limit of free diffusion by introducing an attractive and tunable binding potential created by holographic optical tweezers. They found that an optimal potential depth enhances the diffusive current by a factor of three. This novel experimental approach opens the way for a general understanding and optimization of passive transport processes through nanochannels.

Professor Ayce Asatekin from Tufts University used experimental insights to design artificial nanoporous materials for molecular separations. Her talk focused on the fabrication of polymeric nanopores inspired by the structure of biological pores, with nm-scale diameters and hydrophobically functionalized pore walls. To manufacture these high-aspect ratio hydrophobic, cylindrical nanopores having diameters as low as 5 nm, they used conformal vapor deposition of fluorinated polymeric layers into porous track-etched polycarbonate membranes. The resultant selectivity of these membranes for pairs of small molecules of similar size, but of different hydrophobicity, arises from solute-pore wall interactions emphasized by confinement. Increasing selectivity was observed as pore diameter decreased and as the surface of the pore became more hydrophobic. Cylindrical pores provided higher selectivity than bottleneck-shaped pores having the same minimum diameter. A maximum selectivity of 234 was achieved between mesitylene and phloroglucinol by the best performing membrane. Membranes with small fluorinated pores exhibited an effective cutoff based on the polar surface area of the molecules, with limited correlation with solute size. This technology could lead to a new generation of membrane separations based on specific interactions. Finally, she discussed discuss some future plans for manufacturing polymeric systems that simulate nanopores by polymer self-assembly.

Professor Tim Albrecht from the University College, London discussed the ion transport through artificial nano-channels. Current rectification is sometimes taken as evidence for ion-selective transport through nanochannels and usually rationalized based on a combination of electrostatic and geometric effects. In the presence of more than two current sources in the system, for example in a three-electrode device or when one supposedly insulated surface is in fact not, apparent rectification effects can also occur. However, these are clearly not rooted in selective ion transport. Rather, they are the result of alterations in the current distribution in the sensor cell, for example due to changes in the charge transfer properties of the electrode surfaces involved. They have studied these effects both theoretically and experimentally, and found significant effects on key parameters of sensor operation, such as the pore current and the local electric field, and the current

noise.

Biological functions of the NPC

Professor Murray Stewart from Medical Research Council, UK provided biological introduction on several aspects of the function of the Nuclear Pore Complex. The principal function of the nuclear envelope is to separate transcription from translation. This enables transcripts to be processed in the nucleus before being translated in the cytoplasm. It is therefore critical that ribosomes are excluded from the nucleus and that transcripts are retained until fully processed, after which they can be exported to the cytoplasm through nuclear pores. The pores themselves must prevent ribosomes from entering the nucleus while enabling transcripts to be exported. The barrier function necessary to exclude ribosomes is generated by FG nucleoporins and probably also retards the import and export of smaller molecules such as proteins and tRNAs. Consequently transport factors are necessary to accelerate the movement of these molecules between the nuclear and cytoplasmic compartments. It is important to distinguish between kinetic processes and sorting processes. All nuclear transport pathways are based on three basic steps: (i) formation of transport-competent cargo-carrier complexes in the donor compartment; (ii) equilibration of the cargo-carrier complexes between the compartments through nuclear pores; and (iii) disassembly of the cargo-carrier complex in the acceptor compartment, thereby preventing the return of the cargo. Overall directionality is therefore provided by the assembly and disassembly of the cargo-carrier complex and not by passage through the pore itself. Consequently nuclear transport can be considered as an example of a thermal ratchet or rectified Brownian movement. Both the assembly and disassembly of the cargo-carrier complexes are often catalyzed by peripheral pore components and, in the case of transcripts, by several pore-associated complexes, such as the TREX-2 complex. The TREX-2 complex and Nab2 function to generate export-competent mRNA particles and so link nuclear export to preceding steps in the gene expression pathway. The structure of these proteins and complexes has enabled mutants to be generated to define how each functions.

Ms. Rebecca Adams from Prof. Susan Wente's lab at the Vanderbilt University discussed effects of the genetic deletions of different FG nucleoporins on the transport through the NPC. Using Saccharomyces cerevisiae, they have determined that deletion of the FG domains of nuclear pore proteins located exclusively on the cytoplasmic face of the nuclear pore complex results in an mRNA export defect. Genetic experiments have suggested that the mRNA export defect is due to defective mRNP remodeling, and our current hypothesis is that cytoplasmic FG nucleoporins function as a scaffold to position the exporting mRNP in close proximity to mRNP remodeling factors.

Professor Rick Wozniak from the University of Alberta spoke about the role of the Nuclear Pore Complexes in sculpting the chromatin landscape and gene silencing.

4 Outcomes, Impact and Scientific Progress Made

While several models have been proposed, we still do not understand how fast and selective transport through the NPC can be mechanistically tied to the conformation and the dynamics of the unstructured proteins with the NPC channel. This question is not only of fundamental biological importance, but it also has direct practical relevance for realizations of artificial man-made bio-mimetic devices. As often in a developing interdisciplinary field, large part of the challenge is to ask the right questions and the workshop was designed in a maximally interactive fashion in order to help developing such questions.

Although many workshops explore applications of physical principles in biology, the uniqueness of the current workshop lies in that it successfully brought together a very diverse group of researchers that focused on well defined set of systems and questions one hand, while keeping an eye on general organizing principles arising from the study of these specific systems. On the experimental side the participants included cell biologists, biochemists, biophysicists and nanoscientists. On the theoretical/computational side - physicists and, computational biologists with expertise in molecular dynamics simulations, statistical physics, and mathematical modeling of transport. The organizing committee included a theoretical physicist (Zilman), an experimental biophysicist (Elbaum), a computational biologist (Gnanakaran), and an experimental biophysical chemist (Jovanovic-Talisman). The participants also came from considerable geographical diversity, with 11 from USA/Canada, 8 from Europe/Middle East. About 15% of the participants (including postdocs and

students) and organizers (Jovanovic-Talisman) were women. About 40% were either junior researchers or untenured young faculty.

The multidisciplinary program of the workshop started to be realized at the previous, highly successful, workshop held in Albuquerque, New Mexico in July 2010 (http://cnls.lanl.gov/npc2), which almost exclusively focused on the transport through the NPC. Partial support by ICAM has been an important factor in the workshop success and the current workshop became possible as a consequence of important achievements of the previous one. Despite some overlap in terms of covered topics and participants, the current workshop deepened and broadened the discussion of existing questions, expanded the subject matter, and most importantly introduced completely new topics and concepts with the emphasis on systematic integration of mathematical and physical models with experimental results for the NPC and bio-mimetic channels. One major successful outcome of the workshop is the development of the direct working interaction between theorists and experimentalists, and establishment of new collaborations, especially between young investigators.

Several important scientific advances were made at the workshop:

1.Computational and theoretical modeling of the local nano-scale organization of the confined flexible filaments in the NPC and artificial channels. Several talks presented computational models of the FG nup organization on multiple scales - from the atomistic models all the way up to high level theoretical models based on polymer physics, providing important novel insights into what factors govern NPC transport efficiency and selectivity. Integrating of these multiple scale models and into a coherent theory, and validation and testing by comparison with the detailed experiments, will be the challenge for the next few years.

2.Connection of the novel high precision single molecule measurements and other optical techniques to mathematical models. One key experimental technique that will provide data that can be interfaced with the models is the single molecule fluorescence techniques. The presented talks included new and enhanced resolution methods that allow us to dissect the correlated motions of the FG nups and transport factors as well as detailed interpretation of the dynamic data with quantitative theoretical models. Such combination has started to provide insights about the spatial organization of the FG nups and their involvement in the transport that is not available by any other method.

3. Emerging principles of design of artificial nanochannels for nanotechnology. Another significant portion of the workshop were reports of artificial nanochannels designed for technological goals such as high precision molecular separations and testing the theoretical models. Such systems provided inspiring and refreshing look at the transport through the NPC.

To summarize, the workshop was characterized by exceptionally high level of scientific interactions and discussions. During the presentations and the discussions several important issues have been clarified and new outstanding questions for the field have been formulated. The committee has been formed to prepare the next meeting for the emerging field, represented by the participants, to be held in Switzerland in 2015.