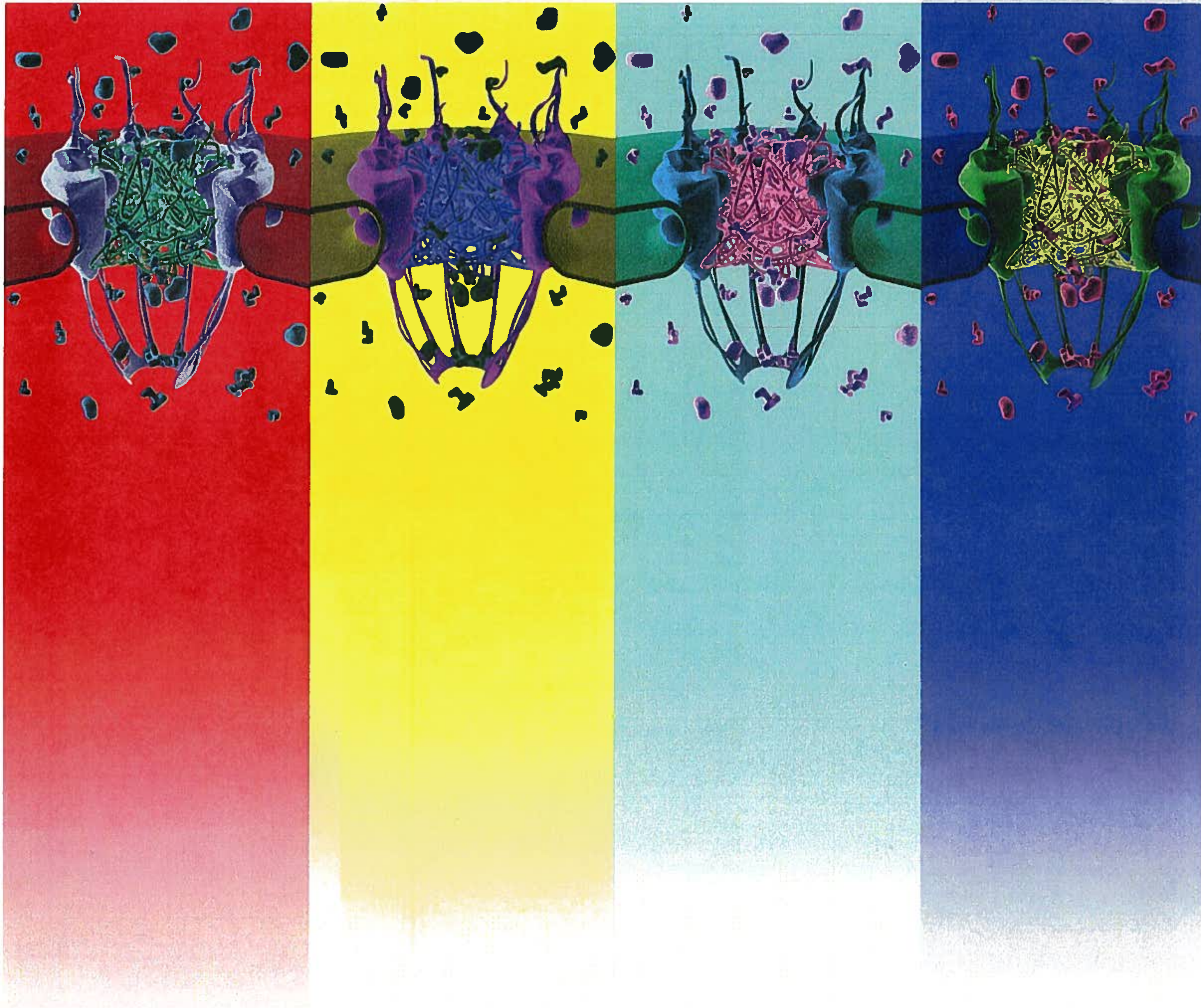
Workshop on

Nuclear Pore Complex

August 9-10, 2008 | Santa Fe, New Mexico, USA



Conference Proceedings

Workshop on Nuclear Pore Complex: Biology, Physics and Nanotechnology

Saturday, August 9, 2008

14:00-14:10
Workshop Opening

14:10-18:30
Afternoon Session

18:30-20:30
Banquet
Shared with the q-Bio conference

20:30
Posters and informal discussions

Sunday August 10, 2008

09:00-12:30
Morning Session

12:30-14:30
Lunch
Posters and informal discussions

14:30-18:00
Afternoon Session

18:15-19:15
Dinner

19:15-20:15
Open panel discussion and closing summary of the workshop

20:15
Posters and informal discussions

Conference Sponsored by the Center for Nonlinear Studies
Conference material created by: Adam Shipman, Ellie Vigil, Kelle Ramsey, and Charlotte Carter of the Center for Nonlinear Studies at Los Alamos National Laboratory.

* *Cover and website images provided by: Samir S. Patel.*

Saturday, August 9, 2008

14:00-14:10	**Workshop Opening** **ANTON ZILMAN, Conference Organizer** – *Welcome* **ADAM SHIPMAN, CNLS Conference Coordinator** – *Announcements*
14:10-15:00	**UELI AEBI (University of Basel)** *Nuclear Pore Complex, Structure, Conservation and Plasticity - An Update*
15:00-15:50	**SUSAN WENTE (Vanderbilt University)** *In Vivo Determinants of FG Nup Function*
15:50-16:40	**MURRAY STEWART (Cambridge University)** *Integrating mRNA Export*
16:40-16:50	**Break**
16:50-17:40	**MICHAEL REXACH (University of California at Santa Cruz)** *Distinct Structural Categories of Nucleoporin FG Domains*
17:40-18:30	**REINER PETERS (University if Münster)** *Reduction-of-dimensionality Model Revisited*
18:30-20:30	**Banquet** Shared with the q-Bio conference
20:30	**Posters and informal discussions**

In Vivo Determinants of FG Nup Function

Susan Wente

Department of Cell and Developmental Biology,
Vanderbilt University Medical Center

We have used budding yeast genetics to comprehensively define the requirements for FG Nups in nucleocytoplasmic transport. By in situ transport assay analysis of selected mutants, we have found that different transport receptors use distinct FG pathways. This suggests novel mechanisms for regulating transport at the level of the NPC.

Integrating mRNA Export

Divyang Jani[1], Shiela Lutz [3], Neil Marshall[1], Christoph Brockmann[1], David Neuhaus[1], Milo Fasken[2], Anita H. Corbett[2], Ed Hurt[3] and Murray Stewart[1]

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The nuclear processing of mRNA and its subsequent nuclear export is mediated by an intricate series of protein:protein and protein:RNA interactions. Following transcription, any introns present must be removed and both the 5\' and 3\' ends processed before the material is ultimately exported through nuclear pores to the cytoplasm where translation takes place. MessengerRNA export is probably facilitated by a thermal ratchet mechanism, analogous to that seen in nuclear protein import, in which the assembly and disassembly of the mRNP export complex is critical. An extensive array of proteins participate in the various steps of the gene expression pathway, including the mRNA nuclear export factors Mex67 and Mtr2 together with a range of adapters and other proteins including nab2, Gfd1, Mlp1, Sus1, Sac3 and Cdc31. It is not currently known precisely manner how many of these components function in either assembling a competent export complex containing completely processed mRNA or in disassembling this complex in the cytoplasm. To address these problems, we are determining the structures of a range of complexes formed by these molecules during the course of the nuclear mRNA processing and export pathway and using these structures to engineer mutant proteins that can be used to help dissect the molecular details of this complex biological function. Nab2 is a multi-domain protein and its N-terminal domain interacts with both Mlp1 and Gfd1. The solution and crystal structures of the Nab2 N-terminal domain show it is constructed from a bundle of five helices and the Mlp1 and Gfd1 binding sites have been identified. Sac3 binds both Cdc31 and Sus1 in a short region called the CID domain. The structure of the Sac3:Cdc31:Sus1 complex shows the unusual way in which both Sus1 and Cdc31 envelop the long helical CID domain.

Distinct Structural Categories of Nucleoporin FG Domains

Justin Yamada[1], Vladimir Uversky[2], V.V. Krishnan[3], Edmond Y. Lau[4], Joshua Phillips[5], Michael E. Colvin[5] and Michael F. Rexach[1]

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[2]Center for Computational Biology and Bioinformatics, Indiana University School of Medicine,
[3]Department of Applied Science, UC Davis, California
[4]Chemistry, Materials, Earth and Life Sciences Directorate, Lawrence Livermore National Laboratory, California
[5]Center for Computational Biology, School of Natural Sciences, UC Merced, California

The segregation of nuclear and cytoplasmic components defines eukaryotic cells. In their nuclear envelope, the nuclear pore complex acts as a gated molecular filter that regulates the exchange of macromolecules between the cytoplasm and the nucleoplasm. Acting as the gatekeepers, the FG nucleoporins deploy highly flexible and structurally disordered domains to sort which macromolecules may pass back and forth. Although a structural analysis of these domains is needed to understand their function, progress has been limited because classic structural biology methods such as X-ray crystallography provide little information on these structurally dynamic proteins. We are using computational modeling and biophysical measurements, first to characterize the dimensions and shape adopted by these disordered domains, and second to address how their FG motifs and/or the regions between motifs influence their ensemble of structures. We find that most FG domains in yeast nucleoporins adopt loosely knit globular configurations on average, rather than highly extended configurations. The FG motifs appear to function as intra-molecular cohesion elements that compact the FG domains. The results suggest that part of the molecular filter at the nuclear pore complex center is formed by a tightly stacked array of cohesive FG domains in loosely knit globular configurations.

Reduction-of-dimensionality Model Revisited

Reiner Peters

Institute for Medical Physics and Biophysics & Center of Nanotechnology, University of Münster, Germany

The nuclear pore complex (NPC), a keystone of the eukaryotic building plan, surprises us with an apparent paradox: High-speed translocation through tight binding. We have previously suggested [7] that the nuclear transport paradox is based on reduction of dimensionality (ROD) because molecular sliding reconciles binding (in one direction) with mobility (in orthogonaldirections). Here, we critically test the ROD model against own data obtained by single-molecule studies [2;5;8] 4Pi microscopy (1;3), and artificial nanopores with NPC-like transport specificity [4] as well as against pertinent literature data [6;10]. The thus specified ROD model [9] has the following features: 1) At physiological (micromolar) concentrations of nuclear transport receptors (NTR) the NPC binds large numbers of NTRs and NTR-cargo complexes (100-200/NPC) in dynamic equilibrium. 2) NPC-bound NTRs collapse the FG domains of the NPC to yield one coherent, thin (~10-20 nm height), quasi-fluid FG/NTR bilayer coating the walls of NPC transport channel and filaments. 3) The FG/NTR bilayer consists of a flexible and dynamic outer layer of collapsed FG domains and an inner monomolecular of NTRs and NTR-cargo complexes. 4) NTRs, although attached to the FG layer by multiple hydrophobic interactions, are mobile in the bilayer plane. 5) Empty NTRs pick up cognate cargos at one entrance of the transport channel and deliver them at the other. 6) NTR-cargo complexes preassembled in cytosol or karyosol replace empty NTRs on exposed parts of the FG/NTR bilayer because of a higher affinity for FG motifs. 7) The release of NTR-cargo complexes from the FG/NTR bilayer is triggered by interaction with RanGTP (import) or hydrolysis of Ran-bound GTP (export). 8) Translocation through the NPC of molecules which do not bind to NTRs is restricted because the FG/NTR bilayer reduces the free cross-section of the NPC transport channel to ~10nm. The profound implications of the ROD model for nucleocytoplasmic transport and the design of nanopore devices [4] will be discussed.

1. Arkhipov, A., J. Hueve, M. Kahms, R. Peters, and K. Schulten. 2007. Biophys. J. 93:4006-4017.
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3. Hueve, J., R. Wesselmann, M. Kahms, and R. Peters. 2008. Biophys. J. 95:877-885.
4. Jovanovic-Talisman, T., J. Tetenbaum-Novatt, A. S. McKenny, A. Zilman, R. Peters, M. P. Rout, and B. T. Chait. 2008. This meeting.
5. Kubitscheck, U., D. Grunwald, A. Hoekstra, D. Rohleder, T. Kues, J. P. Siebrasse, and R. Peters. 2005. J. Cell Biol. 168:233-243.
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7. Peters, R. 2005. Traffic 6:421-427.
8. Peters, R. 2007. Annu. Rev. Biophys. Biomol. Struct. 36:371-394.
9. Peters, R. 2008. Submitted.
10. Tokunaga, M., N. Imamoto, and K. Sakata-Sogawa. 2008. Nat. Methods 5:159-161.

Sunday August 10, 2008

09:00-09:50 **SIGFRIED MUSSER (Texas A & M University)**
What Can Single-molecule Studies Tell Us About Nuclear Transport?

09:50-10:40 **MICHAEL ELBAUM (Weizmann Institute of Science)**
Surprising Simplicity in the Thermodynamic System for Active Transport

10:40-10:50 **Break**

10:50-11:40 **RODERICK LIM (University of Basel)**
On the Generality of Selective Gating Beyond the Nuclear Pore Complex

11:40-12:30 **MICHAEL BRENNER (Harvard University)**
A Potential Electrostatic Contribution to the Nuclear Pore Complex Selectivity

12:30-14:30 **Lunch**
Posters and informal discussions

14:30-15:20 **DIRK GÖRLICH (Max Planck Institute for Biophysical Chemistry)**
Reconstitution of the Permeability Barrier of Nuclear Pores

15:20-16:10 **KLAUS SCHULTEN (University of Illinois at Urbana-Champaign)**
Gating Mechanisms of the Nuclear Pore Complex Studied Through Molecular Dynamics

16:10-16:20 **Break**

16:20-17:10 **TIJANA JOVANOVIC-TALISMAN (Rockefeller University)**
Artificial Nanopores That Mimic the Selectivity of the Nuclear Pore Complex

17:10-18:00 **ANTON ZILMAN (Los Alamos National Laboratory)**
Selective Transport Through Narrow Channels - Nuclear Pore Complex and Beyond

18:15-19:15 **Dinner**

19:15-20:15 **Open panel discussion and closing summary of the workshop**

20:15 **Posters and informal discussions**

Surprising Simplicity in the Thermodynamic System for Active Transport

Ronen Benjamine Kopito and Michael Elbaum

Department of Materials and Interfaces,
Weizmann Institute of Science

The nuclear pore plays a central role in cellular regulation in all eukaryotes, and the general principles of operation appear to be similar in yeast, plants, and animals. At the same time, it elicits a physical interest for its capacity to separate the macromolecular content of the cell by a mechanism of chemically selective transport. Kinetics of nucleocytoplasmic transport have been studied in a number of configurations and using various model systems. We have used cell-free nuclei reconstituted in *Xenopus* egg extract. In this system the cytosolic volume is essentially infinite in comparison with the nuclear volume. The cytosol thus behaves as a thermodynamic reservoir for all transport factors, in the sense that they are not depleted by nuclear accumulation. This permits an analogy to classic enzymology in interpreting transport measurements, and we find a surprisingly simple behavior[1]. Accumulation kinetics of a model fluorescent cargo protein are first-order, characterized by an initial rate and saturating value. Moreover, the saturating nuclear concentration depends on the cytoplasmic concentration according to a curve that describes equilibrium receptor-ligand binding. The same nuclear to cytoplasmic concentration ratio is achieved whether by accumulation, i.e. import, or by dilution, in which case the transport cargo exits the nucleus. This demonstrates an important thermodynamic stability. At saturation there is a balanced bidirectional flux of the cargo, dependent on its interaction with the "import" receptor. A phenomenological model explains these results on the basis of equilibration of the receptor–cargo complex between the cytosol and nucleus, plus a competitive reaction with RanGTP in the nucleus only. We now build a first-principles transport model that predicts these and other observations. We suggest that the import–export "delivery" paradigm of nuclear transport should be replaced by one of accumulation–depletion. Furthermore, the capacities of the nuclear transport system for selectivity and accumulation are largely separable. This has important implications for synthetic mimics of the nuclear pore[2].

1. R.B. Kopito & M. Elbaum (2007) Reversibility in nucleocytoplasmic transport. Proc Natl Acad Sci USA 104:12743-8.
2. see accompanying poster of Y. Caspi.

On the Generality of Selective Gating Beyond the Nuclear Pore Complex

Roderick Y.H. Lim

M.E. Müller Institute for Structural Biology, Biozentrum, and
The Swiss Nanoscience Institute, University of Basel, Switzerland

Biological machines are touted to offer novel technological strategies provided that their mechanisms can be understood and replicated outside the cell. This serves as an impetus in our lab to resolve the *modus operandi* of the nuclear pore complex (NPC) and how it regulates macromolecular traffic between the nucleus and the cytoplasm. As a physical pore ~ 50 nm in diameter, the biological marvel of the NPC lies in its ability to restrict or promote cargo translocation via biochemical selectivity and not size exclusion *per se*. Moreover, because the NPC does not seem to clog *in vivo*, this suggests that it exerts a repulsive force field (i.e. barrier) around its near-field to effectively repel non-specific macromolecules - in spite of the molecular complexity of the cellular environment.

Considering that nuclear transport occurs at molecular length scales, our work involves integrating the key constituents of the NPC gating mechanism (i.e. the natively unfolded phenylalanine-glycine (FG)-rich domains) to nanostructures, in order to closely mimic the geometry and contextual details of the NPC (e.g. the FG-domains are surface tethered on one end and not free floating in solution). Importantly, this affords us the ability to carefully scrutinize any nanoscopic effects that may be relevant to the NPC e.g. the polymer brush-like behavior of Nup153. By combining such a multidisciplinary approach with *in situ* NPC studies, we have correlated the nanomechanical responses of the FG domains to the biochemical interactions that govern nuclear transport in order to obtain insight as to how the NPC gating mechanism is regulated. Based on this understanding, I will further postulate and discuss the possibility of how a more general mechanism of selective gating may be replicated using synthetic polymers and non-karyopherin based "transport receptors" in the design of "smart" nanopores and membranes

Gating Mechanisms of the Nuclear Pore Complex Studied Through Molecular Dynamics

Klaus Schulten, Lingling Miao, Tim Isgro

Theoretical and Computational Biophysics Group, University of Illinois, Urbana-Champaign

Nuclear pore complexes (NPCs) are selectively gated pathways between nucleoplasm and cytoplasm. While small molecules can diffuse freely through NPC, large molecules (> 40 kD) can pass only when bound to transport receptors. The NPC central channel is filled with disordered proteins, rich in phenylalanine-glycine (FG) repeats, referred to as FG-nups. FG-nups control the selective transport through NPCs, the underlying mechanism being still unknown. To elucidate this mechanism, we have modeled FG-nups tethered to a plane surface. Coarse-grained molecular dynamics simulations show that arrays of FG-nups at a density found in the NPC form random brush-like structures of multi-protein bundles. Many of the FG-repeats are found on the surface of the bundles, offering a favorable environment for transport receptors that exhibit FG-repeat binding spots also on their surface. Brush-height, Phe-Phe pair distribution, Phe-Phe pair dissociation, and the effect of Phe->Ser mutation are monitored in detail.

Artificial Nanopores that Mimic the Transport Selectivity of the Nuclear Pore Complex

Tijana Jovanovic-Talisman[1], Jaclyn Tetenbaum-Novatt[2], Anna Sophia McKenney[2], Anton Zilman[3], Reiner Peters[4], Michael P. Rout[2], Brian T. Chait[1]

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Nuclear pore complexes (NPCs) act as effective and robust gateways to the nucleus, allowing the passage of only selected macromolecules across the nuclear envelope. Despite their large size (~50 MDa) and elaborate structure, NPCs are comprised of a core scaffold that defines a ~30 nm diameter passageway between the nucleus and cytoplasm and anchors proteins termed FG nups, whose natively disordered domains line the passageway. The FG nups are packed densely enough to form an effective barrier to the diffusion of most macromolecules. However, cargo-carrying transport factors overcome this barrier by transient binding to the FG nups. To test whether no more than a passageway and a lining of FG nups are sufficient for selective transport, we designed a functionalized membrane that incorporates just these two elements. We demonstrate that this membrane functions as a nanoselective filter, efficiently passing transport factors and transport factor-cargo complexes that specifically bind FG nups, whilst significantly inhibiting the passage of proteins that do not bind. Important determinants of efficient selectivity include passageway diameter, binding strength to FG nups and competition between binding and non-binding macromolecules. We show that this artificial system recapitulates most key features of NPC-mediated trafficking.

Selective Transport Through Narrow Channels: NPC and Beyond

Anton Zilman

Theoretical Biology and Biophysics Group and Center for Nonlinear Studies, Los Alamos National Laboratory, New Mexico, USA

Proper functioning of living cells requires continuous selective transport of molecular signals between cell nucleus and the cytoplasm. This transport is carried by Nuclear Pore Complexes (NPC), which perforate the nuclear envelope. NPC is an efficient transport machine which is able to selectively transport only certain molecular species while effectively filtering others, even very similar ones. Moreover, NPC can selectively transport its corresponding cargoes in the presence of vast amounts of non-specific competition. It functions without direct input of metabolic energy and without large scale transitions from an 'open' to a 'closed' state during the transport event. Recently, artificial molecular sorting nano-devices have been built that mimic the structure and function of the NPC, which hold a great potential for applications in nano-technology and nano-medicine.

Mechanisms of selectivity and efficiency of transport through the NPC and related artificial devices are still unknown. I present a theoretical model to explain the mechanisms of selectivity of transport through the NPC and artificial nano-channels, which contains only two essential ingredients: - i) transient trapping of the cargoes inside the channel (e.g. due to transient binding to moieties inside the channel) ii) competition between the transported molecules for the limited space inside the channel. The theory provides the mechanism for selectivity based on the differences in the times of transport through the channel between different molecules. The theory also explains how the specific molecules are able to efficiently filter out the non-specific competitors – and proposes a mechanism for sharp molecular discrimination. The theoretical predictions account for previous experiments on transport through the Nuclear Pore Complex and artificial nano-channels and have been experimentally verified in ongoing experiments.

POSTER

Yaron Caspi

Department of Materials and interfaces,
Weizmann Institute of Science

In an attempt to understand the physical mechanism of transport selectivity through the central channel of the Nuclear Pore Complex, we have constructed a chemical mimic of the facilitated diffusion process. The chemical mimic is based on the hydrogen bonding polymer pNIPAM (polyIsopropylacrylamide). PNIPAM serves both as a carrier molecule, which can take an ssDNA piggyback through the channel, and as a thin coat on the channel walls, which creates the required surface interactions. When the surface interactions were strong enough, an increased diffusion rate through the coated channels of the pNIPAM-ssDNA complex over the ssDNA alone was observed.

POSTER

Geza Ambrus-Aikelin

The Scripps Research Institute

Nucleocytoplasmic transport of macromolecules is a fundamental process of eukaryotes. Translocation of most proteins and many RNAs between the nucleus and cytoplasm is carried out by shuttling receptors of the importin-beta family. The identification of leptomycin B, a small molecule that inhibits nuclear export by binding to the importin-beta homologue CRM1 enabled researchers to decouple nuclear export from import and to study cellular events that were either impossible or impractical earlier. Analogously, a molecular probe that inhibits nuclear import would also be a valuable tool for the study of nucleocytoplasmic transport across the nuclear pore

In an effort to identify a small molecule inhibitor of nuclear import we have set up a permeabilized cell nuclear transport assay using the nuclear localization signal of the SV40 T antigen as cargo. We have screened approximately 29,000 peptidomimetic compounds in the form of compound mixtures and identified two compounds that inhibited nuclear import levels significantly. These two compounds are alpha-helix mimetics that do not inhibit transport in mediated nuclear import. Binding studies are under way to determine their molecular targets and their apparent affinities to them.

Workshop on Nuclear Pore Complex — Participant List

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