Selective Transport Control in Biological and Biomimetic Nanopores

June 24 – 28, 2024, Monte Verità, Ascona, Switzerland

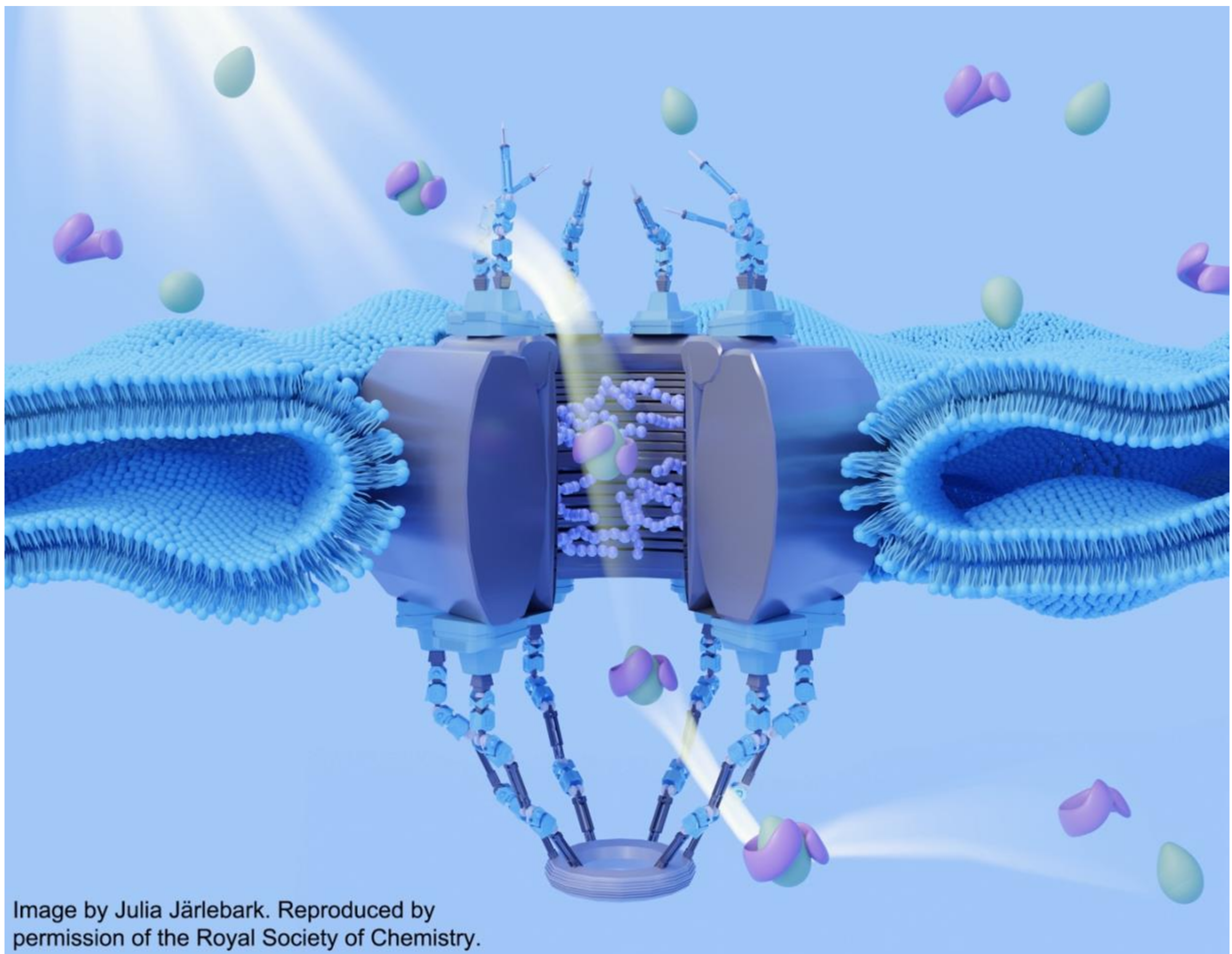


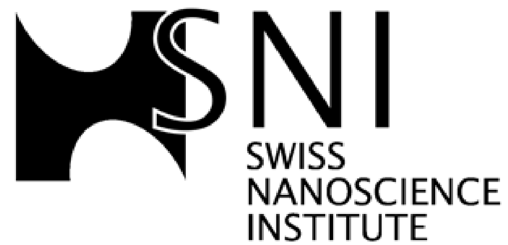
Image by Julia Järlebark. Reproduced by permission of the Royal Society of Chemistry.

Organizers: Roderick Lim (Biozentrum) and Karsten Weis (ETH Zürich)

This meeting is dedicated to the memory of Anton Zilman.

We thank our Sponsors:

Congressi
Stefano Franscini



EINE INITIATIVE DER UNIVERSITÄT BASEL
UND DES KANTONS AARGAU

NOVARTIS

BIOZENTRUM

University of Basel
The Center for
Molecular Life Sciences

Conference Program

Conference room: Hotel Monte Verità, room “Balint”, 1st floor.

Day 0, Sunday 23rd of June

Afternoon from 15:00	Arrival & Check-in Hotel Monte Verità
18:00	Welcome Apéro with Pizza (Balcony, room Balint, 1st floor Hotel Monte Verità)

Day 1, Monday, 24th of June

09:10 – 09:30	Opening
09:30 – 10:15 CHAIR MORNING: Patrick Onck	**Michael Rout** *A tour of the pore: structural and functional mapping of the nuclear pore complex*
10:15 – 10:45	**Anders Barth** *Sub-10 nm tracking of nuclear transport receptors within isolated nuclear pore complexes*
10:45 – 11:15	COFFEE
11:15 – 12:00	**Aleksei Aksimentiev** *Toward all-atom simulations of the nuclear pore transport*
12.00 – 13:30	LUNCH
14:30 – 15:15 CHAIR AFTERNOON: Chenxiang Lin	**Andreas Dahlin** *Fully artificial nuclear pore complexes: Synthetic shuttle-cargo transport*
15:15 – 16:00	**Karsten Weis** *Nuclear pore assembly: how to punch a hole into the nuclear envelope*
16:00 – 16:30	COFFEE
16:30 – 17:00	**Christine Doucet** *Structure of assembling Nuclear Pore Complexes by correlative AFM-dSTORM*
19:15 – 20:45	DINNER

Day 2, Tuesday, 25th of June

09:30 – 10:15 CHAIR MORNING: David Cowburn	**Patrick Onck** *Residue-scale molecular dynamics of nuclear transport*
10:15 – 10:45	**Larisa Kapinos** *Kap-centric control of NPC selectivity and asymmetry in nucleocytoplasmic transport*
10:45 – 11:15	COFFEE
11:15 – 12:00	**Roderick Lim** *Dynamic organization of the nuclear pore complex permeability barrier*
12.00 – 13:30	LUNCH
14:30 – 15:15 CHAIR AFTERNOON: Siegfried Musser	**Cees Dekker** *Nuclear transport studies with a biomimetic approach*
15:15 – 16:00	**Pere Roca-Cusachs** *Transducing - and shielding - mechanical signals from integrins to the nucleus*
19:15 – 20:30	DINNER
20:30	Poster session AZ celebration

Day 3, Wednesday, 26th of June

09:30 – 10:15 CHAIR MORNING: Liesbeth Veenhoff	**Siegfried Musser** *Overlapping nuclear import and export paths unveiled by two-color MINFLUX*
10:15 – 10:45	**Maurice Dekker** *Nuclear transport at full amino-acid resolution*
10:45 – 11:15	COFFEE
11:15 – 12:00	**Chenxiang Lin** *Building nuclear pore mimics with DNA origami*
12.00 – 13:30	LUNCH
14:00	Bus departure EXCURSION
19:15 – 22:00	DINNER Ristorante Grotto Broggini
22:00	Bus departure (arrival Monte Verità 22:15)

Day 4, Thursday, 27th of June

09:30 – 10:15 CHAIR MORNING: Pere Roca-Cusachs	**Liesbeth Veenhoff** *The nuclear pore complex in ageing and disease – surveillance of FG-nucleoporins*
10:15 – 10:45	**Eva Bertosin** *Building the NPC from the bottom-up using DNA origami*
10:45 – 11:15	COFFEE
11:15 – 12:00	**David Cowburn** *New challenges in the nuclear pore complex*
12.00 – 13:30	LUNCH
14:30 – 15:15 CHAIR AFTERNOON: Aleksei Aksimentiev	**Fabien Montel** *Selective and directional transport through biomimetic nanopores*
15:15 – 16:00	**Barak Raveh** *Combining molecular and whole-cell models to reveal design features underlying nucleocytoplasmic transport*
16:00 – 16:30	COFFEE
16:30 – 17:15	**Yitzhak Rabin** *Aggregation of intrinsically disordered proteins: from nucleoporins to associative polymers*
17:15	Open discussion
19:15 – 20:30	DINNER

Day 5, Friday, 28th of June

09:30 – 10:15 CHAIR MORNING: Barak Raveh	**Rob Coalson** *Computational studies of cargo transport through the nuclear pore complex*
10:15 – 10:45	**Saskia Brugere** *Interplay between flux and selectivity within biomimetic nuclear pores*
10:45 – 11:15	COFFEE / Check-out
11:15 – 11:45	**Mareike Jordan** *LEM2-ESCRT filaments constitute a diffusion barrier during nuclear envelope reformation*
12.00	PACKED LUNCH
	Thank you for joining us and BUON VIAGGIO!

Monday, 24th of June

A tour of the pore: structural and functional mapping of the nuclear pore complex

Michael Rout
The Laboratory of Cellular and Structural Biology, The Rockefeller University; USA

Nuclear Pore Complexes (NPCs) mediate the transport of RNAs and proteins between the cytoplasm and nucleoplasm. We have used an integrative approach to determine a structure for the entire 52 MDa yeast NPC, revealing the NPC's functional elements in unprecedented detail. The NPC is surprisingly modular, consisting of only 30 proteins of the nucleoporin family (Nups), which assemble into relatively rigid higher-order scaffold whose modules are held together by flexible connectors, imbuing the NPC with both strength and flexibility. The scaffold surrounds a central channel from which multiple intrinsically disordered Phe-Gly (FG) repeat motifs project which mediate selective nucleocytoplasmic transport through specific interactions with nuclear transport factors. I will describe how our latest findings allows us to rationalize key aspects of the architecture, evolutionary origins and transport mechanism of the NPC.

Sub-10 nm tracking of nuclear transport receptors within isolated nuclear pore complexes

Anders Barth[1], Javier Fernandez-Martinez[2,3], Michael P. Rout[4], Cees Dekker[1]
[1] Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, The Netherlands
[2] Ikerbasque, Basque Foundation for Science, Bilbao, Spain
[3] Instituto Biofisika (UPV/EHU, CSIC), University of the Basque Country, Leioa, Spain
[4] Laboratory of Cellular and Structural Biology, The Rockefeller University, New York, USA

While the structure of the scaffold ring of the nuclear pore complex (NPC) has been solved with near-atomistic resolution, a detailed understanding of the working principles of its central transporter is missing. A growing amount of evidence points towards the 'fuzziness' of the interaction between FG-Nups and NTRs as the key ingredient to establish an efficient, specific, and robust transport channel. However, these dynamics have remained difficult to visualize in vivo and in vitro.

Here, we study the interaction of the nuclear transport receptor Kap95 with isolated NPCs purified from yeast in a controlled in vitro environment using fluorescence microscopy. After immobilization of individual NPCs, we observe the binding of individual Kap95 molecules to the central transporter. Taking advantage of 3D-MINFLUX tracking microscopy, which offers unique isotropic spatial resolution of <10 nm and sub-millisecond time resolution, this assay allows us to follow the path of individual NTRs and resolve the heterogeneity of nucleocytoplasmic transport in unprecedented detail.

Rather than exploring the whole volume, we find that stably bound NTRs remain confined to sub-regions within the central transporter on the timescale of seconds with diffusion coefficients of ~30 μm2/s, reminiscent of the resident population of NTRs proposed by Kap-centric models.

Toward all-atom simulations of the nuclear pore transport

Aleksei Aksimentiev
University of Illinois at Urbana-Champaign, USA

The nuclear pore complex (NPC) serves as a gatekeeper, regulating the transport of biomolecules into and out of the nucleus. Determining the molecular mechanisms of nuclear transport regulation presents multiple challenges to both experimental and theoretical approaches. The recent explosion of structural and biophysical information about the NPC provides new opportunities for the computational study of nuclear pore transport. However, such studies remain very challenging. This talk will describe our lab's ongoing efforts to build a multi-resolution computational model of nuclear transport, with the ultimate goal of elucidating the molecular mechanism in all-atom detail.

Fully artificial nuclear pore complexes: Synthetic shuttle-cargo transport

Andreas Dahlin
Chemistry and Chemical Engineering, Chalmers University of Technology, Sweden

An established approach to learn more about native NPCs is to create artificial versions thereof, typically consisting of chemically modified solid state nanopores. These systems can reproduce the selective biomolecular transport remarkably well and provide new insights based on implementation of signal transducers, such as detection of single translocation events by chronoamperometry. Typically, purified native FG-Nups are grafted to nanopores tuned to be similar in size to the NPC scaffold.

Here we present a curiosity-driven project where we create “fully artificial” NPCs that contain synthetic polymers instead of FG-Nups. These synthetic polymers form a very strong barrier towards biomolecules in general, but certain other polymers can still bind to them by multivalent hydrogen bonds, which enables those “shuttle” polymers to translocate the pores. Furthermore, by conjugating “cargo” biomolecules to the shuttle polymers, a shuttle-cargo system can be created, i.e. the cargo only translocates when bound to a shuttle. The selectivity is remarkably high (no unspecific translocations detected), much more than in previous (very few) reports, and most likely even higher than native NPCs. We also show that shuttles can be prepared with clickable groups to selectively bind cargo. Furthermore, we show how the concept can be used to trap molecules in nanoscale cavities.

Nuclear pore assembly: how to punch a hole into the nuclear envelope

Matthias Wojtynek[1], Jonas Fischer[1], Ashutosh Kumar[2], Katerina Radilova[1], Stefano Vanni[2] and Karsten Weis[1]

[1] Institute of Biochemistry, D-BIOL, ETH Zurich, Switzerland

[2] Department of Biology, University of Fribourg, Switzerland

The nuclear pore complex (NPC) is the central portal for macromolecular exchange between the nucleus and cytoplasm. In all eukaryotes, NPCs assemble into an intact nuclear envelope (NE) during interphase and more than 500 proteins have to come together to form a functional nuclear transport channel. The process of NPC biogenesis remains poorly characterized, and little is known about how NPC assembly leads to the fusion of the outer and inner NE. To date no factors have been identified that mediate this fusion event and only few nuclear pore assembly factors have been characterized. We will present our work on the transmembrane protein Brl1, an essential NPC assembly factor that is required for NE fusion in budding yeast. We will discuss how Brl1 is targeted to the NPC and present a model of how Brl1 together with its interaction partners triggers the fusion between the inner and outer NE.

Structure of assembling Nuclear Pore Complexes by correlative AFM-dSTORM

Anthony Vial, Emilie Costes, Luca Costa, Pierre-Emmanuel Milhiet, Christine Doucet
Centre de Biologie Structurale (CBS), Montpellier, France

Nuclear Pore Complexes (NPCs) are located at the fusion sites between the two concentric bilayers of the nuclear envelope (NE). The NPC core channel, which is highly symmetric across the NE, consists of three stacked rings that line the curved pore membrane. This structure is capped by two asymmetric domains: the nuclear basket and the cytoplasmic filaments.

In dividing cells, new NPCs assemble during interphase while the NE remains intact. This assembly process involves extensive membrane remodeling and the recruitment of hundreds of proteins. In humans, the assembly begins at the inner nuclear membrane, where a large protein structure forms. Subsequently, the two nuclear membranes bend and fuse, allowing the protein assembly to adopt its final conformation. The formation of such a symmetric complex in an asymmetric environment likely requires significant rearrangements of both the proteins and the nascent structure, although this process is not yet fully understood.

We employ super-resolution microscopy and Atomic Force Microscopy to elucidate the limiting steps and identify early intermediate stages of NPC assembly in NEs purified from human cultured cells. From these data, we propose a sequence of events, starting from the seeding of initial components to the sequential assembly of rings.

Tuesday, 25th of June

Residue-scale molecular dynamics of nuclear transport

Patrick Onck
Zernike Institute for Advanced Materials, University of Groningen, Groningen, Netherlands

The nuclear pore complex (NPC) facilitates selective trafficking of molecules between the nucleus and cytoplasm. The core of the pore, comprising disordered proteins rich in Phe-Gly repeats (‘FG-Nucleoporins’), forms a soft barrier against molecules larger than ~40 kDa, unless such molecules are bound to nuclear transport receptors (NTRs). Due to the large size and disordered nature of the NPC’s interior, the microscopic mechanisms responsible for selective transport have not been fully resolved yet.

To resolve the puzzling paradox of providing both transport and barrier functionality within a single protein complex, we developed a coarse-grained molecular dynamics framework at amino acid resolution. Recent results will be discussed of our quest to uncover the fundamental physical mechanisms that are responsible for selective transport. Both top-down and bottom-up approaches will be reviewed with special emphasis on essential, sequence-dependent molecular interactions.

Kap-centric control of NPC selectivity and asymmetry in nucleocytoplasmic transport

Larisa E. Kapinos, Joanna Kalita, Elena Kassianidou, Tiantian Zheng, Anton Zilman, Chantal Rencurel, and Roderick Y. H. Lim
Biozentrum, University of Basel, Switzerland

Nucleocytoplasmic transport (NCT) orchestrates the asymmetric partitioning of macromolecular cargoes within the nucleus and cytoplasm. This is governed by the nuclear pore complex (NPC), together with soluble importin and exportin receptors, collectively termed karyopherins (Kaps). Recent investigations have revealed a role of Kap enrichment at NPCs, particularly karyopherinβ1 (Kapβ1; importinβ1) and exportin 1 (XPO1; CRM1), in the re-enforcement of the NPC selective transport barrier, preventing nucleocytoplasmic leakage. Moreover, the containment of exportins within the nucleus is crucial for cellular homeostasis, with mislocalization leading to pathological conditions. Here, we have investigated the nuclear efflux of exportin 2 (XPO2; CAS) that delivers karyopherinα (Kapα; importinα), the cargo adaptor for Kapβ1, to the cytoplasm in a Ran guanosine triphosphate (RanGTP)-mediated manner. Our results show that the N-terminus of CAS attenuates the interaction of RanGTPase activating protein 1 (RanGAP1) with RanGTP to slow GTP hydrolysis, which suppresses CAS nuclear exit at NPCs. In sum, our work sheds light on how NCT asymmetry is established under a mechanism of Kap-centric control.

Dynamic organization of the nuclear pore complex permeability barrier

Roderick Lim
Biozentrum and Swiss Nanoscience Institute, University of Basel, Switzerland

Nuclear pore complexes (NPCs) mediate nucleocytoplasmic transport. However, the NPC permeability barrier remains unresolved at transport timescales. We show that this barrier is dominated by dynamic fluctuations of intrinsically disordered, phenylalanine-glycine nucleoporin domains (FG domains). These FG domain fluctuations radiate inwardly and associate with karyopherin receptors (Kaps) to form a central plug (CP). Systematic mutations in the NPC's FG domains that increase FG-domain entanglement or slow their collective motion, result in impaired nucleocytoplasmic transport *in vivo*. These *bona fide* NPC behaviors are not recapitulated by *in vitro* FG hydrogels, which show a large morphological heterogeneity at the nanoscale. Our data highlight the context-dependent properties of the NPC, where nanopore geometry, FG domain composition and arrangement, and CP-forming Kaps create a highly dynamic, pore-spanning permeability barrier that facilitates both selective transport and passive exclusion.

Nuclear transport studies with a biomimetic approach

Cees Dekker
Kavli Institute of Nanoscience, Delft University of Technology, The Netherlands

Nuclear Pore Complexes (NPCs) regulate all transport between the nucleus and the cytoplasm. Intrinsically disordered FG-Nups line the NPC lumen and form a selective barrier, where transport of most proteins is inhibited whereas specific transporter proteins can freely pass. The mechanism underlying this selective transport through the NPC is still incompletely understood.

We build bottom-up mimics of the NPC by attaching FG-Nups to nanopores in solid-state membranes and to DNA origami rings that can be inserted in lipid membranes. Subsequently, we study NPC structure and nuclear transport at the single-molecule level using ion current measurements, optical measurements using zero mode waveguides, phase condensates, and cryo-EM. These biomimetic NPCs are found to exhibit selective behavior that depends on the width of the pore. Using rationally designed artificial FG-Nups that mimic natural Nups, we show that simple design rules can recapitulate the selective import behavior of native FG-Nups.

Transducing - and shielding - mechanical signals from integrins to the nucleus.

Pere Roca-Cusachs
Institute for Bioengineering of Catalonia (IBEC), Universitat de Barcelona, Spain

Cell proliferation and differentiation, as well as key processes in development, tumorigenesis, and wound healing, are strongly determined by the properties of the extracellular matrix (ECM), including its mechanical rigidity and its composition. In this talk, I will discuss how mechanical force is transmitted from the ECM to the nucleus, and how this affects proteins in general, and transcription factors in particular, by controlling their shuttling between the cytoplasm and nucleus. Further, I will discuss how different matrix, integrin, and cytoskeletal proteins control whether the nucleus is exposed to, or shielded from, force transmission.

Wednesday, 26th of June

Overlapping nuclear import and export paths unveiled by two-color MINFLUX

Abhishek Sau[1], Sebastian Schnorrenberg[2], Ankith Sharma[1], Debolina Bandyopadhyay[1], Ziqiang Huang[2], Clara-Marie Gürth[3], Sandeep Dave[1], Siegfried M. Musser[1]*
[1]Department of Cell Biology and Genetics, Texas A&M University; College Station, TX, USA
[2]EMBL Imaging Centre, European Molecular Biology Laboratory; Heidelberg, Germany
[3]Abberior Instruments GMBH, Göttingen, Germany
*Presenting author

The nuclear pore complex (NPC) mediates nucleocytoplasmic exchange, catalyzing a massive flux of protein and nucleic acid material in both directions. A common assumption has been that distinct trafficking pathways for import and export would be an elegant solution to avoid unproductive collisions and opposing movements. Direct evidence of the three-dimensional (3D) nanoscale spatiotemporal dynamics of macromolecules within the NPC remains challenging to visualize on the timescale of millisecond-scale transport events. Here, we used 3D MINFLUX to identify the nuclear pore scaffold and then to simultaneously monitor both nuclear import and export, thereby establishing that both transport processes occur in overlapping regions of the central pore. Lengthy continuous tracks within the central pore region revealed a preference toward the pore periphery and circumferential movement was minimal indicating confinement within the permeability barrier. Diffusional movement within the pore was ~1000-fold slower than in solution, indicating a highly restricted environment, and was also further reduced during transport, consistent with either structural constraints or transient binding events. These results demonstrate that high spatiotemporal precision with reduced photobleaching is a major advantage of MINFLUX tracking, and that the NPC permeability barrier is divided into annular rings with distinct functional properties.

Nuclear transport at full amino-acid resolution

Maurice Dekker, Henry de Vries, Koen Wortelboer, Harm Jan Beekhuis, Maarten van Oosterhout, Erik van der Giessen, Patrick Onck
Zernike Institute for Advanced Materials, University of Groningen, The Netherlands

The phase state of the selective permeability barrier of the nuclear pore complex (NPC), comprising multiple intrinsically disordered phenylalanine–glycine (FG) repeat proteins, is not known and under intense debate. We have developed a coarse-grained molecular dynamics model of the yeast NPC, consisting of the yeast scaffold with all 10 different FG-Nups tethered at their experimentally-resolved anchor positions. We combined this NPC model with our amino-acid-scale representation of Kap95, which enabled us, for the first time, to study nuclear transport at full amino-acid resolution. We showed that the organization of the FG-Nups in the permeability barrier contains a large variation in protein density, with the cohesive GLFG-Nups forming a ring-like assembly at the center of the NPC that is sustained by highly dynamic FG–FG cross-links. Nsp1 is an essential component of the selective permeability barrier, by forming a low-density percolated meshwork that spans the central channel. Our simulations demonstrate that NPCs are able to accommodate large amounts of NTRs (> 100) without significantly altering the FG-Nup protein distribution while still maintaining a high degree of dynamics. By tracking individual Kaps through the NPC, we found that the Kap transport trajectory is mediated by a highly-dynamic interplay between FG-repeat binding and steric hindrance featuring Kap–FG interaction life times on the order of nanoseconds.

Building nuclear pore mimics with DNA origami

Chenxiang Lin
Department of Cell Biology, Nanobiology Institute, Yale University, USA

In eukaryotic cells, myriad molecular machineries control the formation of membrane-bound compartments and the molecular transport amongst them. However, evolution hands us these beautiful end products without a user manual, making these sophisticated systems difficult to dissect or re-engineer. Our research seeks to unlock mechanistic details of cellular organization and dynamics at the single-molecule level by establishing cell-free platforms that robustly recapitulate the structure and dynamics of membranous compartments and protein complexes. Specifically, we use DNA nanotechnology, an emerging technique that programs supramolecular assembly in three dimensions, to build various biomimetic constructs with precisely controlled geometry, motion, and molecular placement. In this talk, I will share our progress on building an adaptable framework for building nuclear pore mimics. We show that the DNA-origami-based reductionist NPC mimics, termed NuPODs (nucleoporins organized on DNA), reproduce the basic selective barrier function and nucleoporin dynamics of the immensely complicated NPC. By tuning the nucleoporin composition, density, and spatial arrangement as well as the nanopore size and dynamics, we aim to unlock structure-function relationship of the NPC that is difficult to study using conventional methods. Our ongoing effort to build NuPODs with ever-increasing complexity has led to better understandings of the nuclear import mechanism of HIV-1 capsids as well as the width-dependent selectivity of NPC central channel.

The nuclear pore complex in ageing and disease – surveillance of FG-nucleoporins

Liesbeth Veenhoff
European Research Institute for the Biology of Ageing, University Medical Centre Groningen, The Netherlands

The function of the Nuclear Pore Complexes (NPCs) is intimately connected to the hallmarks of ageing and defects in NPCs are linked to diverse ageing-related human diseases. In my talk, I will discuss our work on the surveillance of the intrinsically disordered FG-nucleoporins or FG-nups. Away from the NPC, FG-Nups readily form condensates and aggregates, and it is not well understood how this behavior is surveilled in cells. Such surveillance is important in the timeframe between the synthesis of FG-Nups and their assembly into mature NPCs, and under conditions of stress such as in ageing and in several aggregating pathologies. I will present data to show how the yeast FG-Nup Nsp1 and a human molecular chaperone from the DNAJ family alter the properties of FG-Nup condensates *in vitro* and in cells. Emerging from a genome-wide analysis of intrinsically disordered proteins in yeast and from the analysis of specific mutants, we are learning how the primary amino acid sequence relates to these surveillance functions. Lastly, I will discuss how this data contributes to a larger emerging theme of a tightly regulated interplay between FG-Nups and other native and disease-related IDPs, nuclear transport receptors and the classical protein quality control system.

Building the NPC from the bottom-up using DNA origami

Eva Bertosin, Anders Barth, Eli O. van der Sluis, and Cees Dekker
BN Department, TU Delft, The Netherlands

The nuclear pore complex (NPC) is a massive protein system constituting a permeable barrier on the nuclear envelope. It consists of an octagonal ring-like scaffold filled with intrinsically disordered proteins rich in FG repeats (FG-Nups). This mesh is crossed by hundreds of cargo-bound nuclear transport receptors (NTRs) translocating between the nucleus and the cytoplasm in a milliseconds time scale.

Due to the high number, intrinsic flexibility, and fast motions of the NPC components, it is challenging to resolve the physical mechanism underlying the transport through the barrier. To overcome this issue, we developed biomimetic bottom-up approaches to reconstitute the NPC selectivity.

To this end, we use the technique of DNA origami to build structures with similar shapes and sizes as the NPC scaffold. The DNA origami lumen can be functionalized with FG-Nups, allowing control over the position, number, and types of Nups involved in the system. We can integrate the DNA origami pores into lipid bilayers to study NTR transport through membranes. Moreover, we developed DNA origami nanopores that can expand and contract upon the addition of an external trigger. This will allow us to understand how NTR transport is affected by changes in the diameter of our biomimetic NPCs.

New challenges in the nuclear pore complex

David Cowburn
Albert Einstein College of Medicine, Bronx, NY, USA

The Nuclear Pore Complex (NPC)'s static ring structure is well understood, but its central transporter (CT), presents a paradigm challenge to modern structural biology. How does a totally disordered spaghetti of protein chains provide selective transport to a set of nuclear transport proteins (NTRs)? We have answered this is part as a constrained concentrate of the nucleoporin disordered chains providing a physical condensate with a high concentration of NTRs, with weak interactions and fast diffusion based on specific measurements of affinity and disorder (Hayama, J Biol Chem 282 4555 '18; Cowburn, Biochem Soc Tran 51 871 '23) in vitro, and this is expanded by spatiotemporal mapping (Raveh et al. this conference) providing a mechanism for selective vs passive transport. What can in vitro biophysical experiments (NMR, ITC, SAS etc) further characterize these interactions and help resolve controversies about the internal motions within the CT, and the organizationof the specific components within the tube, and their interactions with NTRs/cargoes.

Selective and directional transport through biomimetic nanopores

Fabien Montel

Laboratoire de Physique ENS de Lyon (CNRS, UMR 5672), Lyon, France

Biological nanopores are uncanny molecular machines that perform a large variety of cellular function ranging from sorting of biomolecules, to building of the cellular osmotic pressure and folding of newly synthetized proteins. Their performance measured through their energetic efficiency, directionality or selectivity have no equivalent among artificial systems. During the past years we have focused on one of this nanopore called the nuclear pore complex whose consume chemical energy in order to drive the transport of proteins, DNA and RNA inside or outside of the nucleus. In this talk I will present the results of our mimetic approach based on single molecule transport experiments in nanofluidic systems to understand its selectivity and directionality.

References:

1] Thermally switchable nanogate based on polymer phase transition.
Kolbeck PJ, et al. NanoLetters 2023, 23, 11, 4862–4869

2] Experimental study of a nanoscale translocation ratchet.
Molcrette B, et al. PNAS 2022, 119 (30) e2202527119

Combining molecular and whole-cell models to reveal design features underlying nucleocytoplasmic transport

Barak Raveh
Faculty of Computer Science and Engineering, The Hebrew University of Jerusalem, Israel

The Nuclear Pore Complex (NPC) facilitates rapid and selective nucleocytoplasmic transport of molecules as large as ribosomal subunits and viral capsids. It is not clear how key properties of this transport arise from the system components and their interactions. To address this question, we relied on our recent Bayesian metamodeling approach (Raveh et al., PNAS 2021) to couple an integrative coarse-grained Brownian dynamics model of transport through a single NPC and a kinetic model of Ran-dependent transport in an entire cell (Raveh et al. bioRxiv 2024). The microscopic modelparameters were fitted to reflect experimental data and theoretical information regarding the transport, without making any assumptions about its emergent properties. The resulting integrative map of nucleocytoplasmic transport is validated by reproducing several features of transport not used for its construction, such as the morphology of the central transporter, rates of passive and facilitated diffusion as a function of size and valency, *in situ* radial distributions of pre-ribosomal subunits, and active transport rates for viral capsids. The map suggests that the NPC functions essentially as a virtual gate whose flexible phenylalanine-glycine (FG) repeat proteins raise an entropy barrier to diffusion through the pore. Importantly, this core functionality is greatly enhanced by several key design features, including 'fuzzy' and transient interactions, multivalency, redundancy in the copy number of FG nucleoporins, exponential coupling of transport kinetics and thermodynamics in accordance with the transition state theory, and coupling to the energy-reliant RanGTP concentration gradient. These design features result in the robust and resilient rate and selectivity of transport for a wide array of cargo ranging from a few kilodaltons to megadaltons in size. By dissecting these features, our integrative map provides a quantitative starting point for rationally modulating the transport system and its artificial mimics.

Aggregation of intrinsically disordered proteins: from nucleoporins to associative polymers

Yitzhak Rabin

Department of Physics, Bar-Ilan University, Tel Aviv, Israel

We use computer simulation to study aggregation of intrinsically disordered proteins modeled as inhomogeneous polymers of made of non-sticker and sticker beads. We discuss the kinetics of network and droplet formation in such systems and explore the difference between aggregation of grafted and free associative polymers.

Computational studies of cargo transport through the nuclear pore complex

Rob D. Coalson
Department of Chemistry, University of Pittsburgh, USA

Coarse-grained Molecular Dynamics simulations are performed on a model of the Nuclear Pore Complex (NPC) interior that is resolved at the amino acid level. The main goal of these calculations is to assess the validity of the Kap-centric Model of transport through the NPC. In particular, the role of karyopherin receptors (kaps) in directing the transport of Nuclear Transport Factor 2 (NTF2) receptors through the pore is studied. Both quasi-equilibrium properties (e.g., steady state distributions of nucleoporin monomers and both Kap and NTF2 receptors) and nonequilibrium fluxes of receptor molecules transporting through the pore are computed. As regards transport properties, emphasis is placed on the manner in which receptor flow is distributed across several lanes that differ in their radial distance from the pore center.

Interplay between flux and selectivity within biomimetic nuclear pores

Saskia Brugère[1], Philippe Guégan[2], Camille Raillon[3], Sébastien Mailfert[4], Didier Marguet[4], Fabien Montel[1]

[1]Laboratoire de Physique (UMR 5672 CNRS-ENS de Lyon), France

[2]Institut Parisien de Chimie Moléculaire (UMR 8232 CNRS - Sorbonne Université), France

[3]Laboratoire Physiologie Cellulaire & Végétale (CEA Grenoble), France

[4]Centre d'Immunologie de Marseille Luminy (Inserm U631 - CNRS UMR6102), France

The Nuclear Pore Complex (NPC) controls the exchanges between the cell nucleus and the cytoplasm. Its remarkable selectivity is due to the presence of a network of intrinsically unstructured proteins (FG-nups) inside its central channel. While thousands of molecules evolve in the cytoplasm, solely few proteins with specific features can cross the NPC. Recent theoretical studies predict a non-trivial relation between the affinity for the pore and the flux of molecule. In cellulo approaches are limited to tackle these subjects because of the complexity of the natural system. Our project aims to study these phenomena thanks to a biomimetic system. To this end, we use synthetic nanoporous membranes grafted with hydrophobic tunable polymers mimicking the FG-nups networks. Flux of molecules are determined by an optical detection which allows us to measure the effective diffusion constant of proteins through the pores. Affinities of the different proteins for grafted and ungrafted surfaces are determined by Biolayer Interferometry. We show that selectivity depends mainly on the affinity of the proteins for the pore surface and not on the size of the molecules. We develop a simple model to describe our results and highlight the facilitated diffusion observed when proteins interact with the nanopores.

LEM2-ESCRT filaments constitute a diffusion barrier during nuclear envelope reformation

Mareike Jordan
Max Planck Institute of Molecular Biology and Genetics, Dresden, Germany

At the end of mitosis, the nuclear envelope (NE) reassembles from growing sheets of double membrane that eventually result in catenoid-shaped holes, constituting an energetic minimum that prevents spontaneous fusion. Since these holes even form around spindle microtubules that are still connected to chromatin, the nucleus is already sealed for diffusion at this stage. In 2020, we demonstrated that the NE receptor LEM2 and the filament-building ESCRT proteins mediate NE reformation at these sites of closure. Using cryo-electron tomography, I could now generate a structure of these assemblies that gives evidence of how the NE holes are sealed: The transmembrane protein LEM2 is placed with a tight repeat along filaments forming flexible ring-shapes with a diameter of about 120 nm. Thus, LEM2's intrinsically disordered domain can form a dense meshwork within the hole, likely preventing proteins from diffusing through the NE. This architecture strongly resembles the FG-repeats of nuclear pores, which form a permeability barrier with their same phase separating properties. My structure represents a short snapshot of a structurally diverse and highly dynamic process; further fusion of these holes requires downstream remodeling, likely performed by recruiting other co-polymerizing ESCRT proteins.

Poster Session, Tuesday, 25th of June 2024

Poster 1	**Aina Albajar Sigalés,** Cellular and molecular mechanobiology, Institute for Bioengineering of Catalonia (IBEC), Spain	The mechanical regulation of nucleocytoplasmic transport: a molecular approach
Poster 2	**Kevin Baumann / Lukas Beckert,** Biozentrum, University of Basel, Switzerland	Reconstitution of ultrawide DNA origami nanochannels in droplet interface bilayers
Poster 3	**Yuliya Kuchkovska,** Biozentrum, University of Basel, Switzerland	Mechanical force impacts the asymmetry of nucleocytoplasmic transport
Poster 4	**Ahmed Hassan Mahmoud,** Biozentrum, University of Basel, Switzerland	The nuclear import receptor importin-β (Kapβ1): a novel regulator of actin cytoskeleton dynamics
Poster 5	**Jesper Medin,** Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Göteborg, Sweden	Using Polymer Brushes as a Selective Diffusion Barrier for Cargo Transport
Poster 6	**Marc Molina Jordán,** Cellular and Molecular Mechanobiology, Institute for Bioengineering of Catalonia (IBEC), Spain	Study of the role of substrate stiffness and force transmission to the nucleus in nucleocytoplasmic transport and nuclear pore conformation

Poster 7	**Shuo Wang,** Faculty of Applied Sciences/Department of Bionanoscience, Delft University of Technology, The Netherlands	Droplet interface bilayer assay as a new platform to visualize transport through mimics of nuclear pore complexes and peroxisome pores
Poster 8	**Mareike Jordan**, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany	LEM2-ESCRT filaments constitute a diffusion barrier during nuclear envelope reformation