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Molecular machines: Structure, dynamics and therapeutics

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Our work concerns the structure and function of two large biomolecular machines, the nuclear pore complex and the photosynthetic apparatus, as well as the nature and magnitude of the forces involved in molecular recognition. Other efforts are placed on the fabrication of single-molecule motion detectors based on capacitance modulation and the development of ligand-recognizable DNA vectors with enhanced nuclear import capability for human gene therapy.

Nucleocytoplasmic transport

Nuclear pore complexes (NPCs) are large and highly elaborate structures that allow the passive diffusion of small molecules and the directed, receptor-mediated transport of macromolecules across the nuclear envelope. To obtain structural information on the pores, we use the scanning force microscope (SFM), which works by scanning a sharp tip over a sample to sense its surface topography at nanometer resolution. In addition, the SFM can be used as a force spectrometer to exert and measure forces on a picoNewton scale. We utilise the latter mode to measure and characterise interaction forces between components of the NPC-associated soluble transport machinery at the single-molecule level. The information obtained is used to understand the different steps of transport and, more generally, to get detailed insight into the energy landscape that controls molecular interactions under applied force. Transport kinetics is studied on intact nuclei by time-resolved fluorescence imaging and fluorescence recovery after photobleaching, and the results are analysed in terms of different transport models. Other studies concern the interdependence of directed transport and passive diffusion through the pores.

DNA vectors with enhanced nuclear import capability for human gene transfer

The ability to efficiently import exogenous DNA molecules into the nucleus of eukaryotic cells is critical to the success of gene delivery methods in clinical applications. Most DNA plasmids, however, are too large to diffuse through the NPC channels and, since they lack import recognition signals, they cannot be transported actively. To overcome this limitation, we are modifying the DNA by including repetitive, high-affinity binding sites for transcription factors, which can cross through the

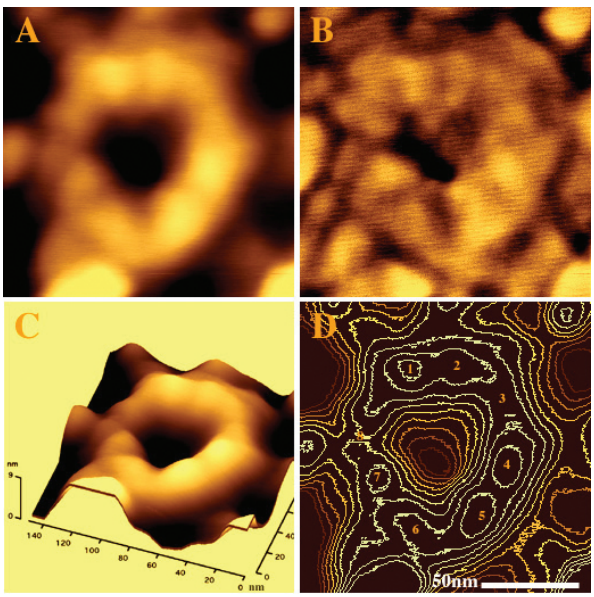

Fig. 1 *The cytoplasmic face of the NPC as imaged by high-resolution tapping mode SFM. (A) Height image (B) Phase image (C) Three-dimensional rendering (D) Contour map.*

NPC via several dedicated receptor-mediated import pathways. Adding binding sites for the inducible transcription factor NFkB, increased nuclear import efficacy of DNA plasmids by 12-15-fold and transgene gene expression levels by up to ~250-fold. Both nuclear targeting of the DNA and transgene transcription could be regulated by exogenous stimulators that modulate the intracellular distribution of NFkB. This system is now being investigated for its ability to preferentially transfect cancerous cells in which the NFkB proteins are constitutively active. In parallel, we are searching for other transcription factors whose expression is limited to certain tissues or organs to endow the system with a higher degree of in vivo specificity.

Capacitance-based single-molecule motion detector

We are developing a novel electrostatic device to follow the motion of single particles at nanometer resolution and

millisecond time scales. The device is based on the change in capacitance, ΔC, induced by a conducting particle moving between the plates of a miniaturised capacitor. The distance between the plates is designed to vary in one direction such that ΔC becomes a function of the particle's position and a record of its 1D motion can be obtained. The feasibility of the device has been confirmed by both numerical simulations and experiments made on a scaled-up prototype. Two configurations are being fabricated. The first has a planar geometry and is based on silicon micromachining technology. The second relies on capillary structures and is made by laser-pulling a square quartz micropipette to a sub-micron diameter and evaporating a metal layer on two opposite faces to define the capacitor plates. Applications include measurements of diffusional and directed macromolecular motions, determination of dielectric constants, molecular sorting, and nanopumping.

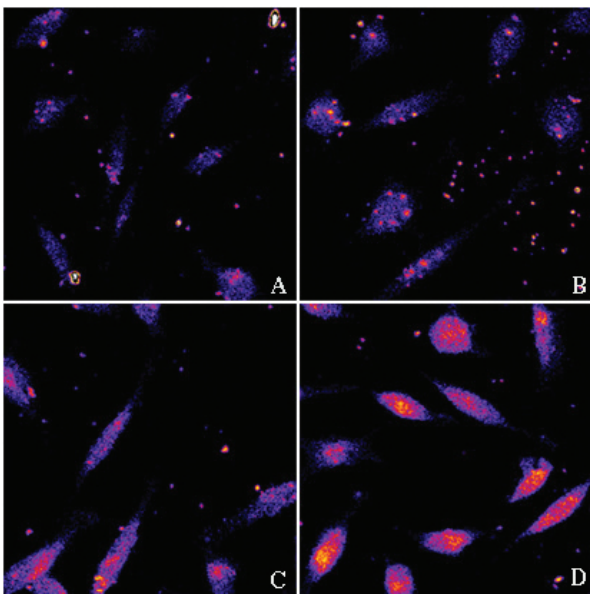

Fig. 2 *Nuclear import of control and modified pGL3 plasmids as monitored by confocal fluorescence microscopy. HeLa cells were incubated with rhodamine-labelled pGL3 (A, B) or kB-pGL3 (C, D), in the absence (A, C) or presence (B, D) of TNF-α.*

Structure and dynamics of photosynthetic membranes

In higher plants, the major photosynthetic complexes (PS I, PS II, LHC II, cytochrome b_6/f and the C_0/C_1 ATP synthase) are segregated into distinct morphological and functional domains within the thylakoid membranes. This segregation is critical for efficient utilisation of light energy by the photosynthetic apparatus and its protection against photoinhibition. Our research aims at the understanding the effects of light and other parameters on this segregation, in situ. For these studies, we use de-enveloped chloroplasts, which have intact thylakoids and are photochemically active. SFM is used to image the thylakoids at nanometer lateral resolution, which enables to resolve individual protein complexes within the membranes. Surface rearrangements and complex redistribution induced by different effectors can then be followed and structural changes can be correlated to photosynthetic activity by time-resolved measurements of chlorophyll fluorescence. Future plans include in situ measurements of electrostrictive volume changes in PS I and PS II reaction centres by microsecond force-sensing microscopy and measurements of light-induced LHC II migration by fluorescence energy transfer.

Selected Publications

Nevo, R., Markeiwicz, P., Kapon, R. Elbaum, E. and Reich, Z. (2000) High-resolution imaging of amphibian NPCs by AC scanning force microscopy. Single Mol. 1, 109-114.

Mesika, A., Gregoreva, I., Zohar, M. and Reich, Z. (2001) A regulated, NFkB-Assisted import of plasmid DNA into mammalian cell nuclei. Mol. Therapy 3, 653-657.

Kapon, R., Nevo, R., Shahar, D. and Reich Z. (2001) From single complexes to single molecules using tapping mode scanning force microscopy and capacitance-based motion detection. Single Mol. 2, 85-89.

Reich, Z., Kapon R., Pilpel, Y., Nevo, R., Zmora, S. and Scolnik, Y. (2001) Scanning force microscopy in the applied biological sciences. Biotechnol. Adv. 19, 451-485.

Zohar, M., Mesika, A. and Reich, Z. Analysis of genetic control elements in eukaryotes: transcriptional activity or nuclear hitchhiking? (2001) BioEssays (in press).

Kaftan, D., Brumfeld, V., Nevo, R., Scherz, A. and Reich, Z. Thylakoid structure and unstacking studied in situ by scanning force microscopy. Submitted to EMBO J.

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