Films made of intrinsically disordered phenylalanine-glycine repeat domains as nanoscopic model systems of the permeability barrier of nuclear pore complexes.

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Macromolecular exchange between cytosol and nucleosol is performed across the permeability barrier of nuclear pore complexes (NPCs). The permeability barrier arises from a meshwork-like assembly of natively unfolded domains (FG repeat domains) [1]. These domains are grafted at high density to NPC walls [2] and are rich in phenylalanine–glycine (FG) repeats. Transport across the permeability barrier is selective: molecules bigger than 5 nm in diameter [3] need to be bound to a nuclear transport receptor (NTR) for efficient transport [4]. The mechanism behind transport selectivity, however, is currently poorly understood and a matter of intense debate.
We have developed ultrathin FG repeat domain films as a label free model system of the 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 (30-40 nm) [5]. By using a toolbox of biophysical characterization techniques (quartz crystal microbalance with dissipation monitoring, ellipsometry, and atomic force microscopy), we observed that NTRs can efficiently enter, permeate and leave FG repeat films. Moreover, we could quantify the binding of NTRs to FG repeat domains and the impact of cargo on binding. NTR binding to the film changes neither the mechanical properties nor the morphology of the FG repeat domain assembly. These results extend our understanding of the interaction of FG domain assemblies with NTRs and contribute important information to refine the model of transport across the permeability barrier.

[1] Denning, D., Patel, S., Uversky, V., Fink, A., and Rexach, M. *Proc Natl Acad Sci USA* **100**(5), 2450–2455 (2003).
[2] Strawn, L., Shen, T., Shulga, N., Goldfarb, D., and Wente, S. *Nat Cell Biol* **6**(3), 197–206 (2004).
[3] Mohr, D., Frey, S., Fischer, T., Güttler, T., and Görlich, D. *EMBO J* **28**(17), 2541–2553 (2009).
[4] Görlich, D. and Kutay, U. *Annu Rev Cell Dev Biol* **15**, 607–660 (1999).
[5] Eisele, N., Frey, S., Piehler, J., Görlich, D., and Richter, R. *EMBO Rep* **11**(5), 366–372 (2010).