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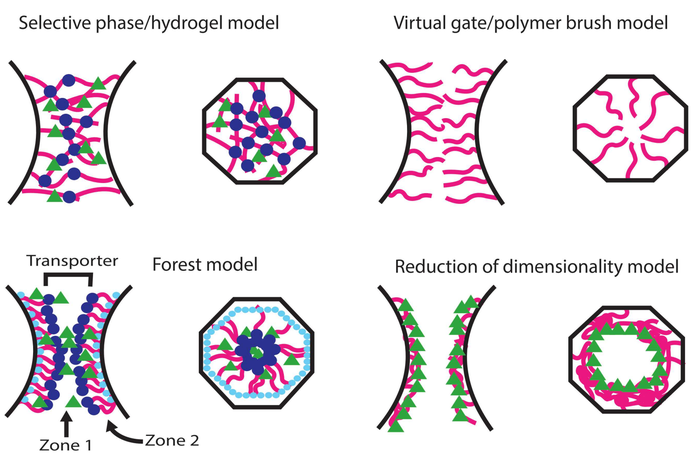
Microfludics to study rapid phase transition of disordered Nucleoporins

The nuclear pore complex (NPC) is a giant complex responsible for controlling the traffic of molecules between the nucleus and cytoplasm. The selective permeability barrier in the central channel of the NPC is filled with disordered proteins (IDPs) enriched in phenylalanine glycine (FG) residues, called nucleoporins (FG-Nups). Nuclear transport receptors (NTR) can interact with FG-Nups and mediate nucleocytoplasmic transport of cargo. *In vitro*, FG-Nups have been shown to phase separate into tough, gel-like states and into amyloid states with solid like properties, the role of which for *in situ* nuclear transport is widely discussed. However, the phase diagram of most proteins is complex and *in vitro* biochemical assays suffer from precise control over time and concentration. We developed a microfluidic device that enables us to trigger the formation of phase separated FG-Nups droplets with subsecond precision prior to optical interrogation. Additional laminar mixers are used to perfuse these droplets with cargo proteins and NTRs. We show that FG-Nups phase separate into liquid droplets that exhibit key features of the NPC permeability barrier: cargoes bearing a nuclear localization signal (NLS) bound to NTRs rapidly penetrate the droplets, while those without a NLS remain excluded. Thus, the phase-separated liquid FG-Nups droplets reconstitute the transport properties of the NPC.

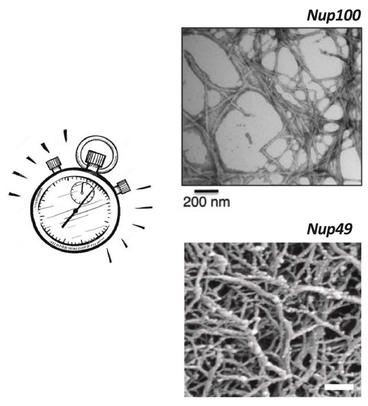
Introduction

The permeability barrier of the NPC

Several models of the nuclear transport mechanism have been proposed based on the macroscopic morphology and behavior of the FG-Nups [1].



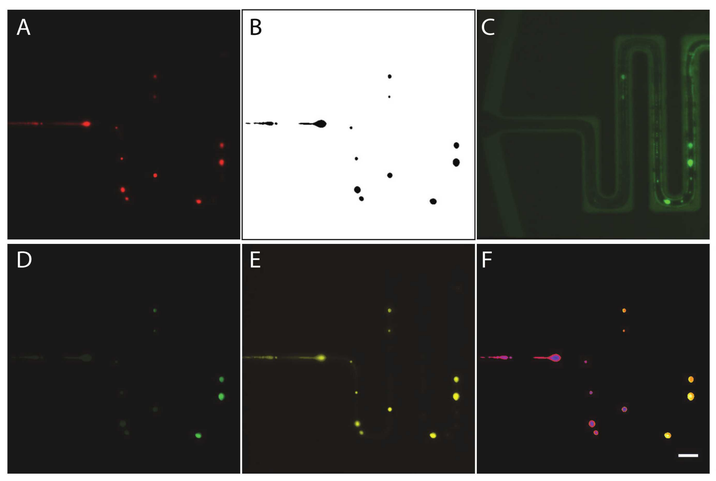
FG-Nups aggregate very quickly [2,3]



FG-Nups show different behaviors *in vitro* and *in vivo* under diverse conditions, which has provoked the generation of sundry models for NPC function.
***In vitro* (under biochemical conditions), FG-Nups can aaggregate quickly and exhibit fiber phenotypes.**

Analysis

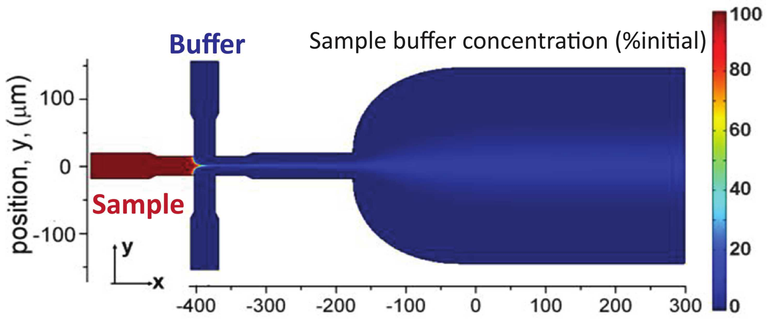
In order to investigate the interaction between the liquid droplets and a GFP cargo we designed a pipeline analysis to compute a ratiometric image.

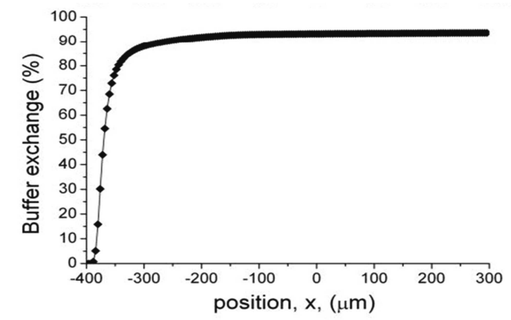


- Red signal from FG-Nups droplets
- Mask obtained from the red channel (FG-Nups)
- Green signal from the cargo complex
- The green signal is confined only in the region of interest.
- Sum of red signal from FG-Nups droplets and thresholded green channel
- Colorimetric image of the ratiometric analysis

Microfluidics

Our goal is to trigger and control phase separation of IDPs as fast as possible, so that even early phase separation events can be studied reliable. Laminar flow mixers implemented on a microfluidic device can be used for rapid buffer exchange coupled with immediate optical interrogation to visualize over time potential phase separation processes on a microscope [4].





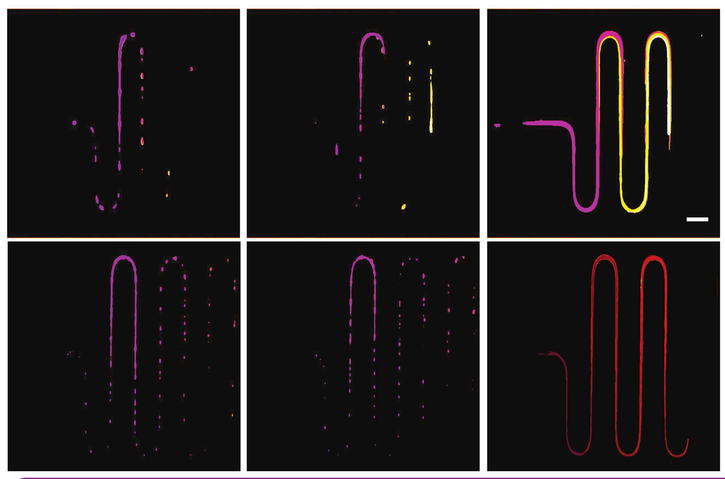
Rapid buffer exhange

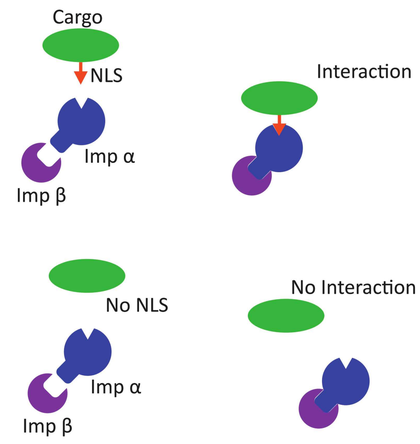
$$\tau_{mix} = \left(\frac{w_f^2}{\pi^2 D}\right)$$

D: diffusion coefficent of the sampple buffer
Wf: width of the sample stream solution

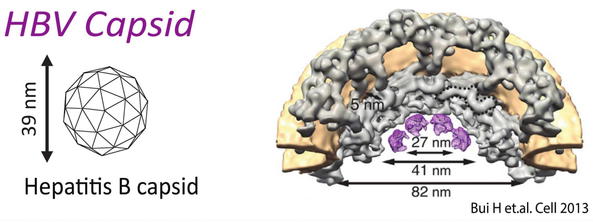
The liquid state of the FG-Nups shows NPC-like permeability barrier properties

To study FG-Nups liquid droplets functionality, they were perfused with cargo-GFP/ Importin α/Importin β.

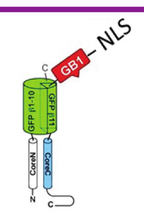


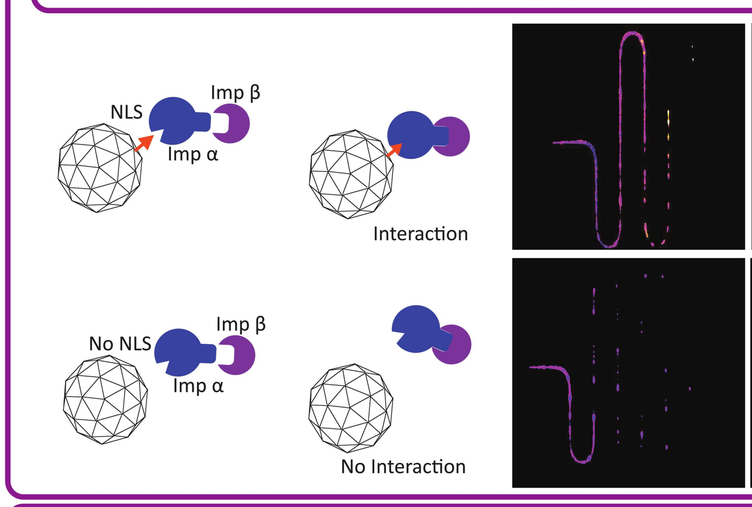


HBV Capsid



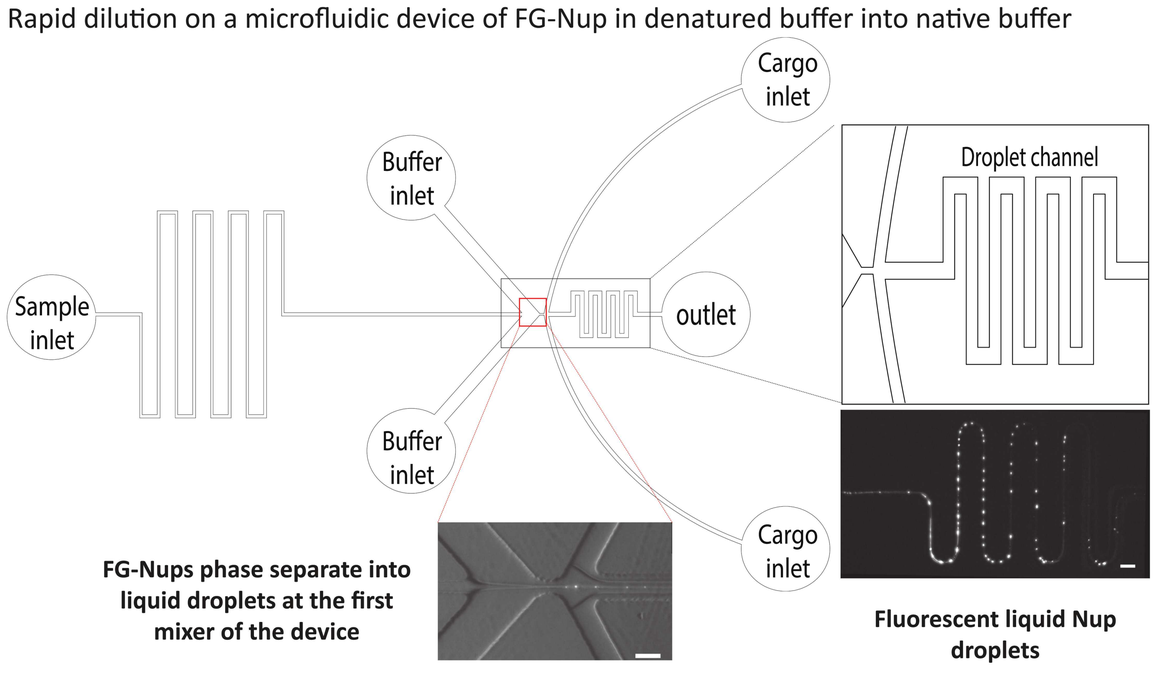
We engineered a HBV core protein with an exposed NLS able to bind with NTR





Microfluidics to cach the early stage of FG-Nups

Rapid dilution on a microfluidic device of FG-Nup in denatured buffer into native buffer

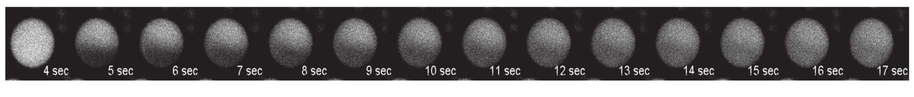


FG-Nups phase separate into liquid droplets at the first mixer of the device

Fluorescent liquid Nup droplets

Coalescence of two FG-Nups droplets

FRAP



Nup droplets are dynamic

Summary and outlook

- *Microfluidic device that provides rapid phase transition coupled with subsequent perfusion and direct optical interrogation, prior to the rapid formation (further maturation) of solid states (gels/amyloids etc.)*
- *Liquid FG-Nup droplets show NPC like permeability properties*
- *Permeability barrier properties shown across 1 order of magnitude cargo sizes*
- *Penetration of large viral mimics appears to be very fast*

References

1. Wälde, Sarah, and Ralph H. Kehlenbach. "The Part and the Whole: functions of nucleoporins in nucleocytoplasmic transport." Trends in cell biology 20.8 (2010).
2. Halfmann, Randal, et al. "Prion formation by a yeast GLFG nucleoporin." Prion 6.4 (2012).
3. Milles, Sigrid, et al. "Facilitated aggregation of FG nucleoporins under molecular crowding conditions." EMBO reports 14.2 (2013).
4. Lemke, Edward A., et al. "Microfluidic device for single-molecule experiments with enhanced photostability." Journal of the American Chemical Society 131.38 (2009).
5. Bui, Khanh Huy, et al. "Integrated structural analysis of the human nuclear pore complex scaffold." Cell 155.6 (2013).

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