Optical tweezers for *in vivo* DNA manipulation

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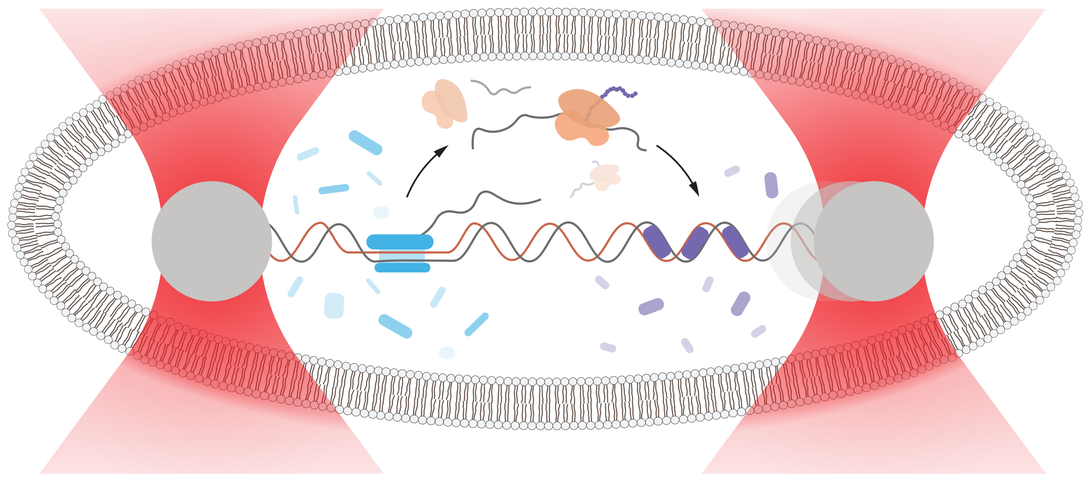
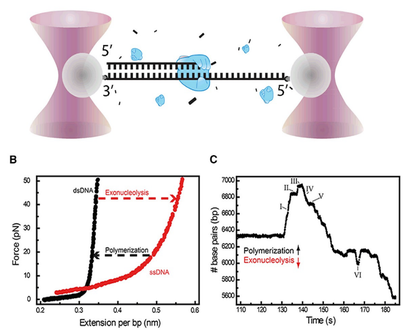
I. Vision

Creating an artificial cell, we strive to characterize and understand it as deeply as possible. Utilizing optical tweezers integrated with fluorescence spectroscopy and microfluidic techniques, I aim to build a system for in vivo manipulation of DNA. It will allow for a quantitative description of protein-DNA interaction inside the artificial cell during such fundamental processes of molecular biology as replication and transcription.

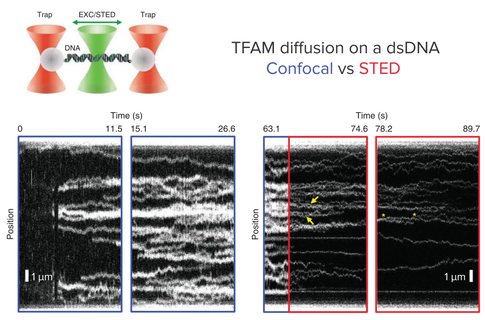


II. Optical traps in single-molecule DNA studies

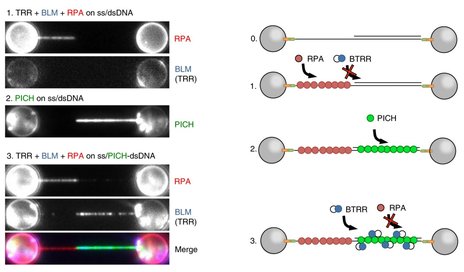
T7 DNA Polymerase switching between Exonucleolysis and Replication in force meaurements



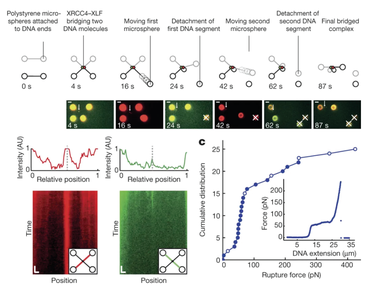
Protein dynamics on a densly covered DNA monitored using STED nanoscopy



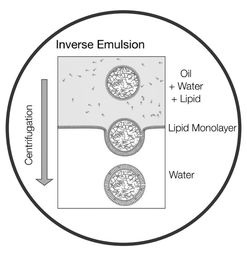
Studying DNA anaphase ultra-fine bridges by three-color imaging of associated protein factors



DNA bridging by XRCC4 and XLF investigated on a quadro-optical trap setup

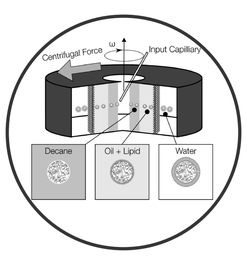


III. Production of the GUVs



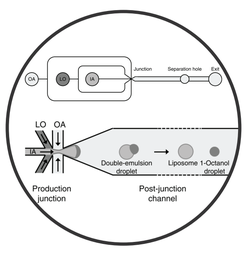
Inverse emulsion

Water-in-oil emulsion droplets are pushed through the lipid monolayer formed on the interface of lipid-in-oil and aqueous solutions to produce liposomes.



cDICE

An improved version of the inverse emulsion. Here, the water-in-oil droplets are produced with a capillary. The encapsulation efficiency and size monodispersity are higher.



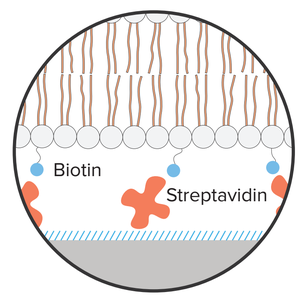
OLA

A microfluidics-based approach, where the double emulsion droplets of equally small size are flow focused. The dewetting process leads to the separation of the oil droplet from the liposome.

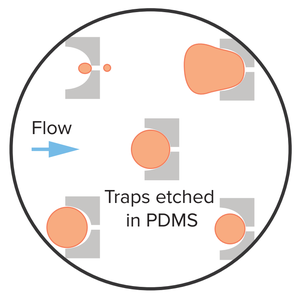
IV. Confinement of the liposomes

If the liposomes are freely floating in the solution during the experiment, they will get trapped. This would obstruct successful bead manipulation. Possible solutions to this problem are:

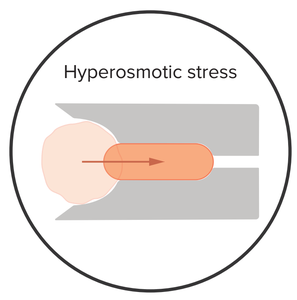
Immobilization



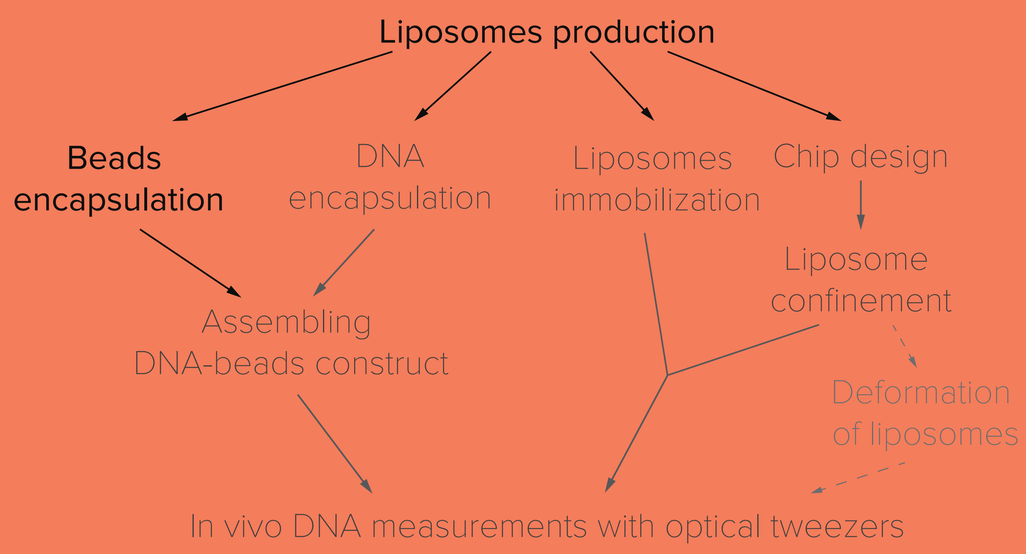
Trapping



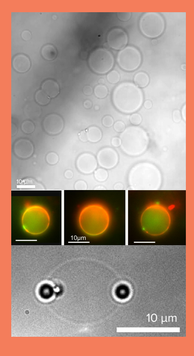
Deformation



V. Research roadmap



VI. Results and outlook



The inverse emulsion has a high yield and was already used for beads encapsulation (bottom figure). Nevertheless, **cDICE** is the most prospective candidate for GUV production due to its fast automated liposome production rates, high encapsulation efficiency and a lower degree of shear stress exerted on the inner solution. The latter is particularly important to prevent DNA damage.

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