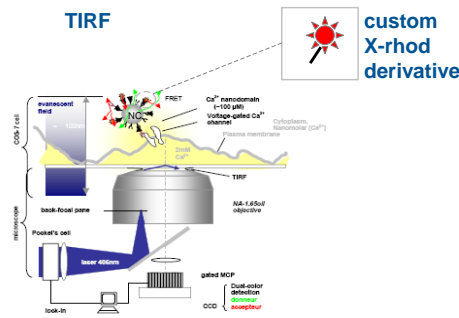


Abstract: NanoFRET sensors are based on the non-radiative energy transfer between a single functionalized semiconductor nanocrystal donor and a small number of organic acceptor fluorophores stably attached to the nanocrystal surface. In our project, we couple functionalized nanocrystals to a red-emitting BAPTA-based rhodamine-derived calcium (Ca²⁺)-sensitive probe. To visualize localized intracellular near-membrane Ca²⁺-concentration [Ca²⁺]_i domains nanoFRET sensors is addressed to the mouth of a Ca²⁺ channel, either by specific antibodies or by covalently binding a tag to the Ca²⁺ channel subunit that is recognized by a specifically modified rhodamine. We evaluated different techniques to load these sensors into the cytoplasm, including (i) osmotic shock of pinocytotic vesicles, (ii) bulk electroporation, (iii) transfection and (iv) single-cell electroporation. The [Ca²⁺]_i sensor is imaged by Total Internal Reflection Fluorescence (TIRF) microscopy that enables the selective excitation and background-free observation of a near-membrane optical section of less than 100 nm thickness. NanoFRET sensing combined to TIRF will enable the estimation of sub-resolution intracellular calcium concentration nanodomains *in vivo*.

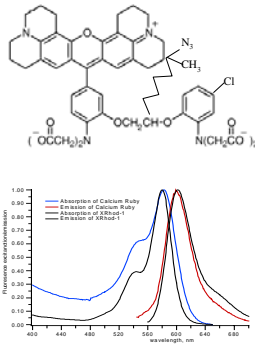
Aim of the project

- 1) NanoFRET sensor delivery inside cytoplasm by the least harmful technique.
- 2) Evaluation of their: intracellular targeting, biocompatibility and toxicity.

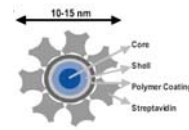
Methods



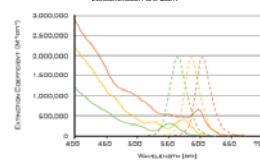
New Ca²⁺-sensitive dye



Qd structure and size:



Qd optical properties:

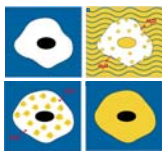


Advantages over conventional dyes:

- extremely photostable and high signal-to-noise ratio
- broad excitation and narrow emission spectra makes Qds ideal for multiplex applications and FRET

Strategies

•1 Osmotic lysis of pinosomes:



COS-7 or HEK-293 cells are placed in hypertonic loading reagent, along with the material to be transferred into the cells (yellow fluid, B), allowing the material to be carried into the cells via pinocytotic vesicles. In hypotonic medium, C) the pinocytotic vesicles burst, D) releasing their contents into the cytosol.

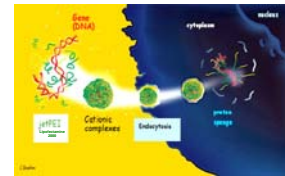
•2 Gene Pulser X-cell Electroporation System:



A high-intensity electric field transiently permeabilizes the membrane, enabling uptake of exogenous molecules from the surrounding medium.

After electroporation the cell membrane is expected to reseal in milliseconds to seconds.

•3 Transfection reagents: lipofectamine, nanofectine & jetpei

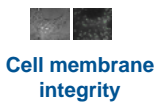


•4 Single cell electroporation

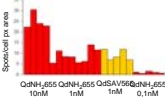


Results

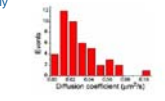
•1 QDSAV inside HEK cells



Qds per cell area

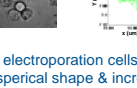
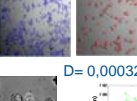


Diffusion coefficient distribution of QDSAV



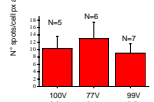
Courty et al. Nanoletters 6, 7 1492-5 2006

•2 QDCOOH inside COS-7 cells

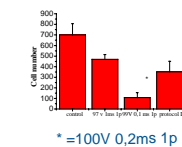


After electroporation cells showed a spherical shape & increased autofluorescence => cell death !!!

Qds per cell px area

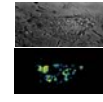


Cell loss after bulk electroporation

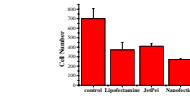


* =100V 0,2ms 1p

•3 QDSAV inside HEK cells

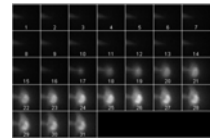


Cell loss after 'transfection'



Qds internalized by using nanofectine, lipofectamine & jetpei are aggregated, don't blink and are immobile

•4 Luciferase injection inside COS-7 cells



Conditions: 6-V 50x1ms stimulation 4ms delay. Whereas small organic dyes readily enter, Qdots are still resisting...

Conclusion

The osmotic lysis of pinosomes, compared to the other techniques we tested, is a successful tool to deliver single nanoparticles to the cytoplasm. In one step all cells are loaded under comparable conditions, the cell membrane is intact and nanoparticles are not aggregated and diffusible in the cytoplasm.

C. Luccardini¹, A. Yakovlev², S. Gaillard³, JM. Mallet³, A. Feltz² and M. Oheim¹

¹Neurophysiology & New microscopies laboratory, Inserm U603, 45 rue des Saints Pères, 75006 Paris, France

²Laboratoire de Neurobiologie ENS CNRS, UMR 8544, 46 rue d'Ulm, 75005 Paris, France

³Département de chimie, 24 Rue Lhomond, 75005 PARIS

Contact: camilla.luccardini@univ-paris5.fr