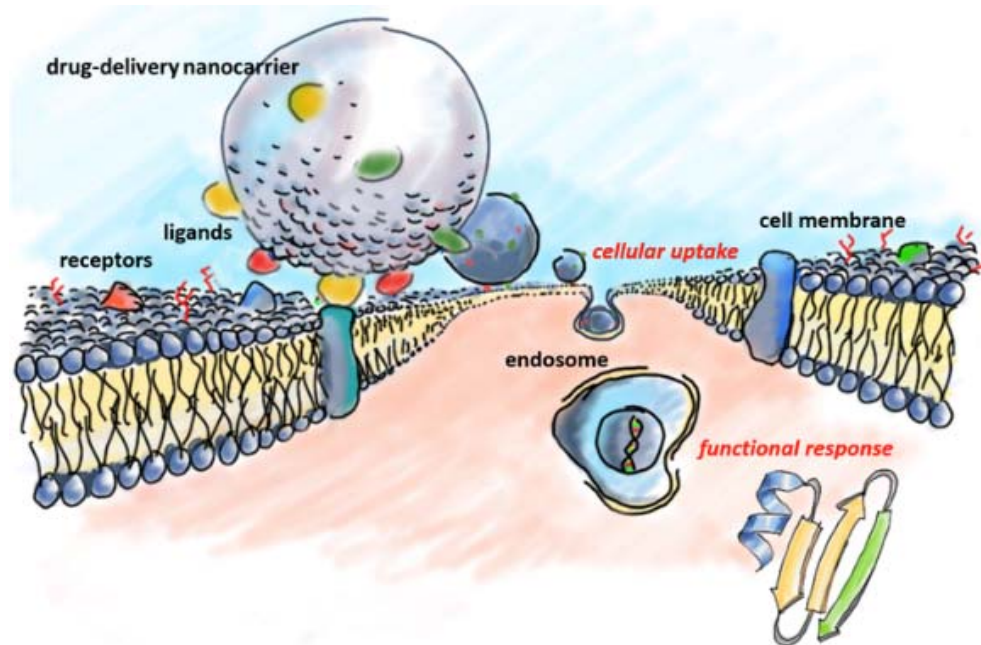


# ***Single nanoparticle analytics: from viruses via exosomes to drug delivery carriers***

---

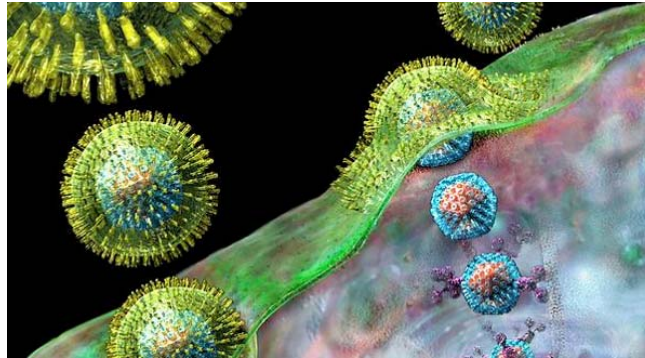
**Fredrik Höök**  
**Department of Applied Physics**  
**Biological Physics**  
**CHALMERS UNIVERSITY OF TECHNOLOGY**  
**Sweden**  
[fredrik.hook@chalmers.se](mailto:fredrik.hook@chalmers.se)



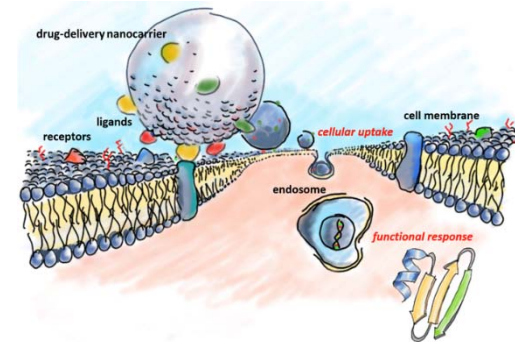
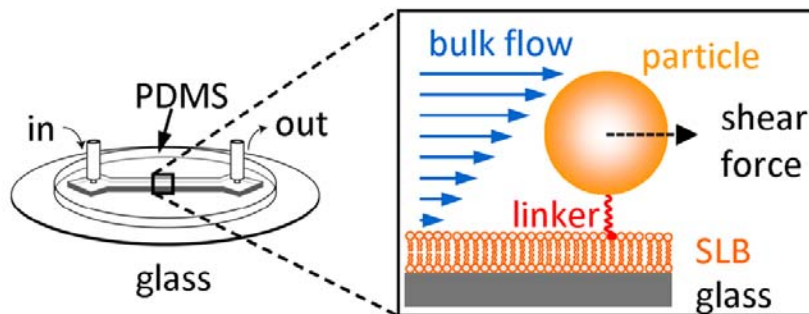
# SCIENTIFIC VISION

**Our research is focused on understanding the cell membrane:**

- How does the cell membrane control cellular function?
- How do viruses, exosomes and nanoparticles bind to and pass across the cell membrane?
- Can we use this understanding to combat infection and develop new drugs?

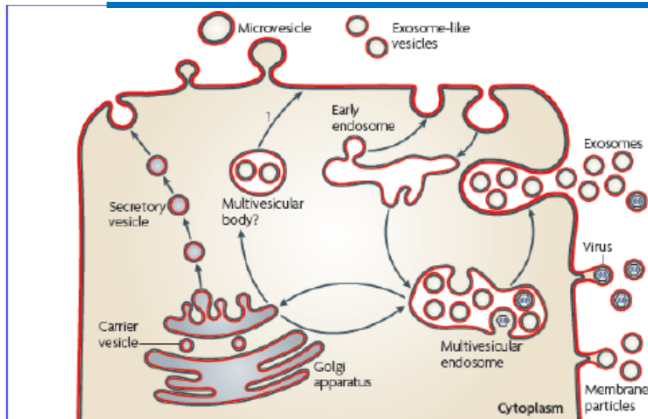


**For this purpose, we are actively developing new surface-based bioanalytical methods**



**We also actively seek new biologically, medically and pharmaceutically relevant questions to help steering our method development.**

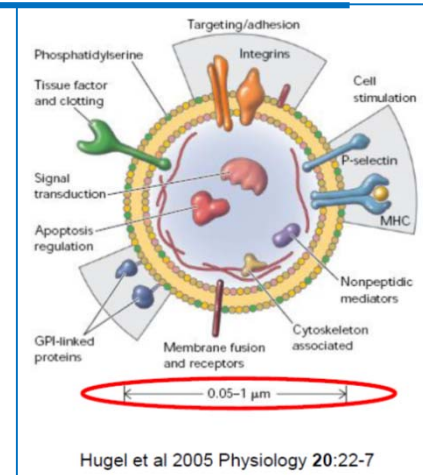
# Exosomes: a biological nanoparticle with high potential!?



Théry et al. *Nat. Rev. Immunology* 2009, 9:581-593

## Exosomes (50 - 100 nm)

- form in multivesicular bodies (MVBs),
- are secreted by cells after fusion of MVBs with the cell plasma membrane



Hugel et al 2005 *Physiology* 20:22-7

## Extracellular vesicles and exosomes play key roles in inter-cellular communication:

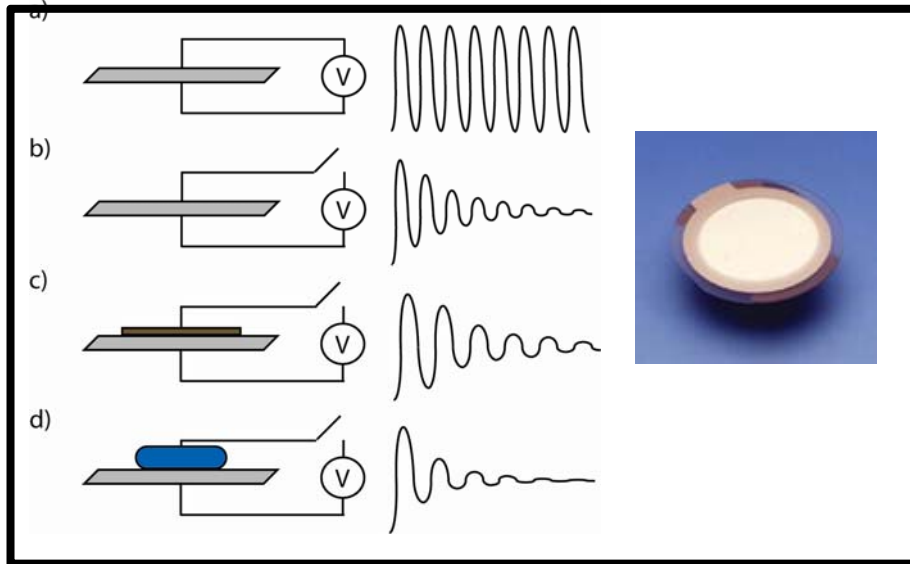
- Potential biomarker candidates in clinical diagnostics.
- Carriers in drug-delivery and gene-therapy applications.

## Due to their huge heterogeneity, their full exploitation depends critically on:

- New and complementary characterization methods that can help expanding the library of their distinct biologically relevant features.

# Label-free surface-analytical tools

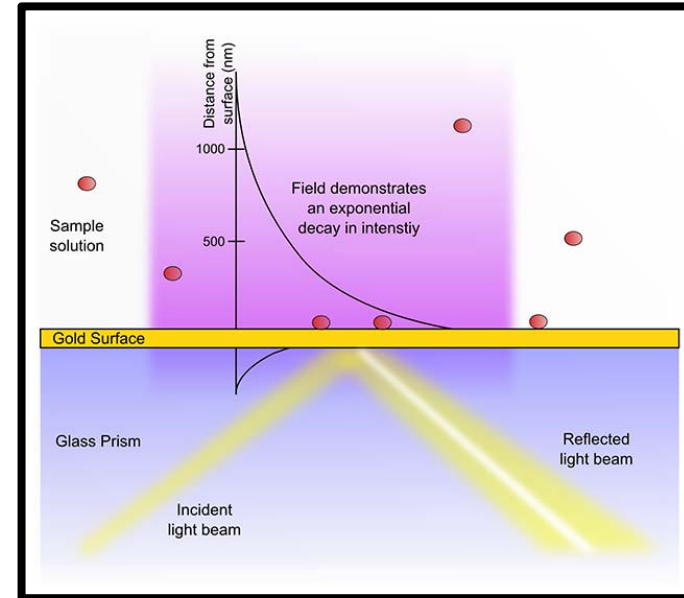
## Quartz Crystal Microbalance QCM-D™



$$\Delta m = g(\rho_{\text{film}}, d_{\text{film}}) \propto \Delta f$$

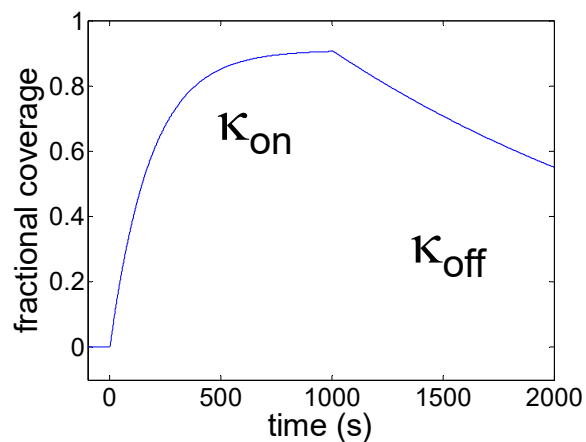
Dimension ~1 cm<sup>2</sup>

## Surface Plasmon Resonance: Biacore™

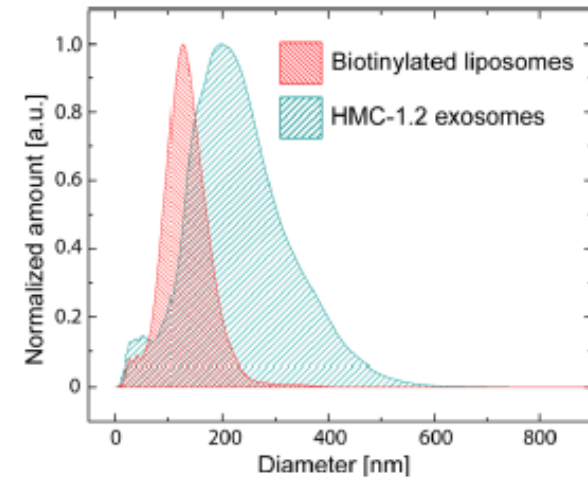
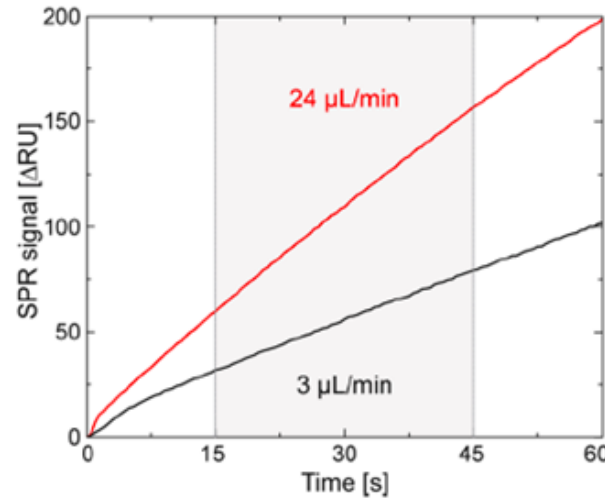
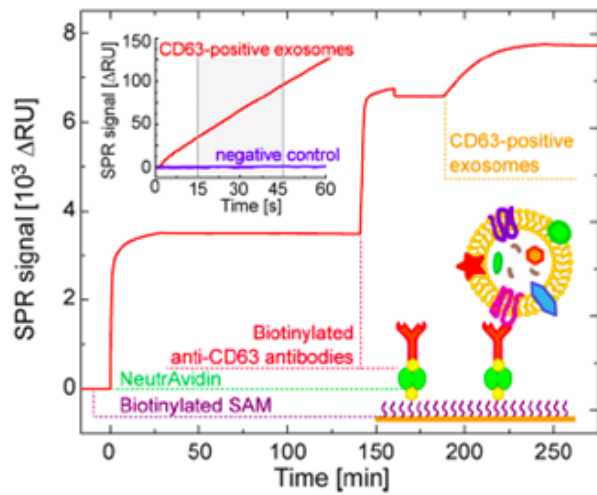


$$\Delta m = g(n_{\text{film}}, d_{\text{film}}) \propto \Delta \Theta$$

Dimension ~1 mm<sup>2</sup>



# Concentration determination of exosomes (in a biological fluid)



$$\Delta\Gamma(t) = \xi(D^2Q)^{1/3}C\Delta t$$

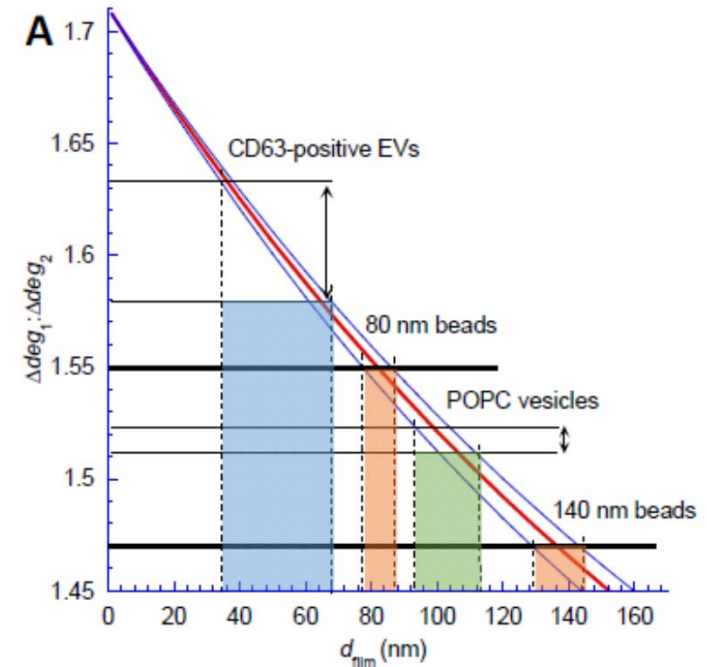
$$\Delta\Gamma = \frac{d}{S(dn/dC)[1 - \exp(-d/\delta)]} \Delta RU$$



$$C = \frac{\Delta RU}{\Delta t} \frac{d}{\xi(D^2Q)^{1/3} S(dn/dC)[1 - \exp(-d/\delta)]}$$

$$D = \frac{k_B T}{3\pi\eta d}$$

thickness determination ->  $d$  and  $D$

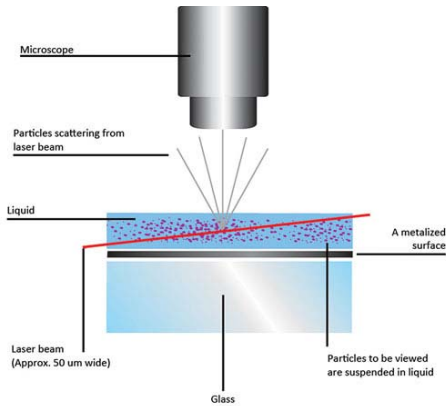


Rupert et al. **Anal. Chem.** 2014, 84, 6538  
 Rupert et al. **Anal. Chem.** 2016, 88, 9980



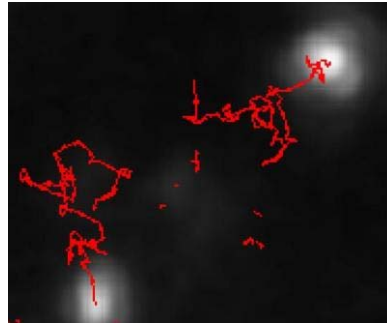
# 3D NTA

## Label-free Nanoparticle Tracking Analysis (NTA)



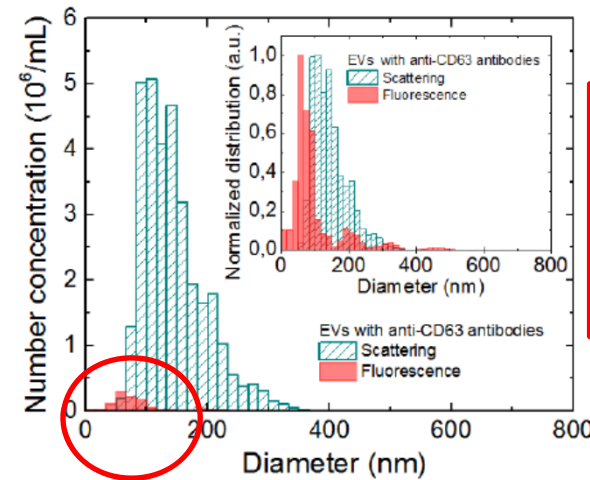
30 fps

10 μm

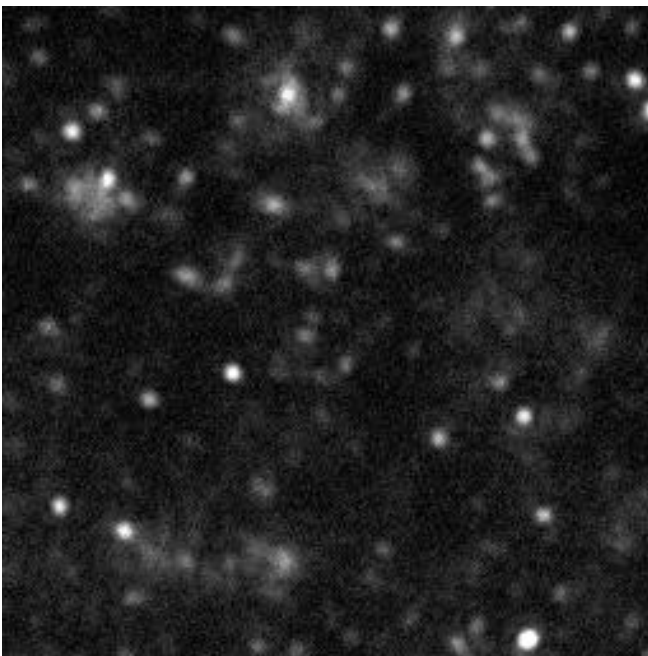


From Trajectories of D63 positive EVs  $\rightarrow D_{EV}$

$$D = \frac{k_B T}{3\pi\eta d}$$



CD63 positive:  
~5% of total #  
Mean size ~80 nm

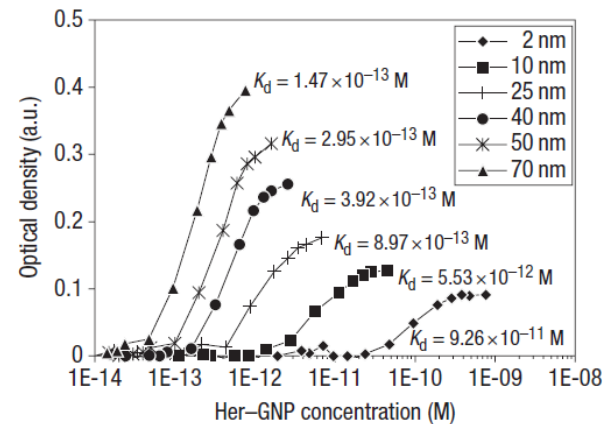
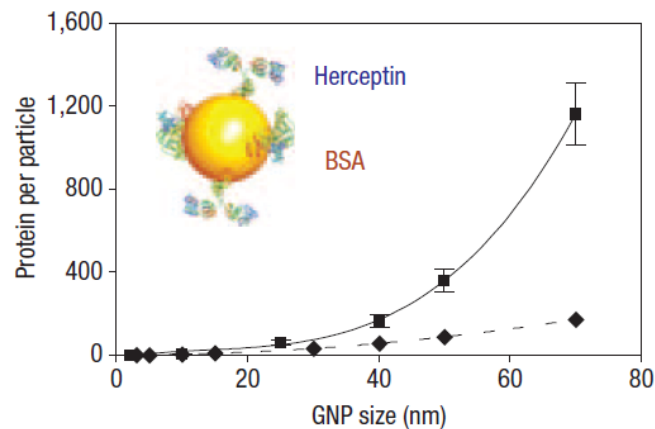
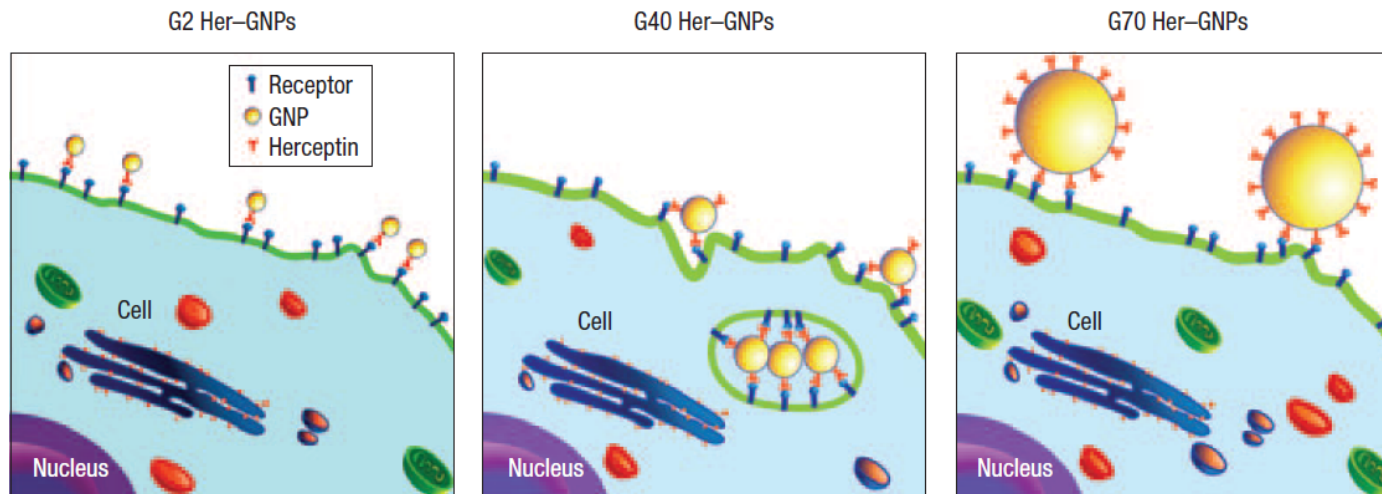


Simultaneous determination of both nanoparticle size and (bio)molecular content, e.g. ligand density, on the level of individual particles is crucial, but very complicated for these tiny and complex systems!

# Nanoparticle-mediated cellular response is size-dependent

WEN JIANG<sup>1,2</sup>, BETTY Y. S. KIM<sup>1,2,3</sup>, JAMES T. RUTKA<sup>3</sup> AND WARREN C. W. CHAN<sup>1,2\*</sup>

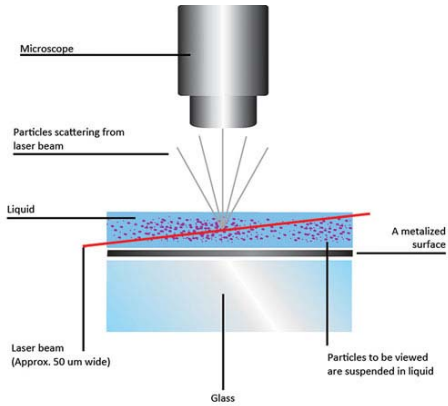
nature nanotechnology | VOL 3 | MARCH 2008 | [www.nature.com/naturenanotechnology](http://www.nature.com/naturenanotechnology)



$$K_d = k_{\text{off}} / k_{\text{on}}$$

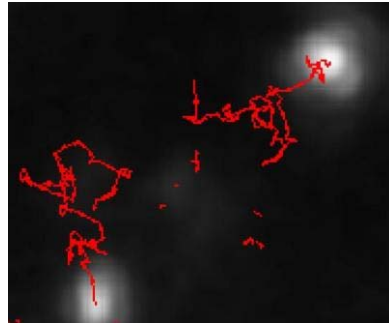
# 3D NTA

## Label-free Nanoparticle Tracking Analysis (NTA)



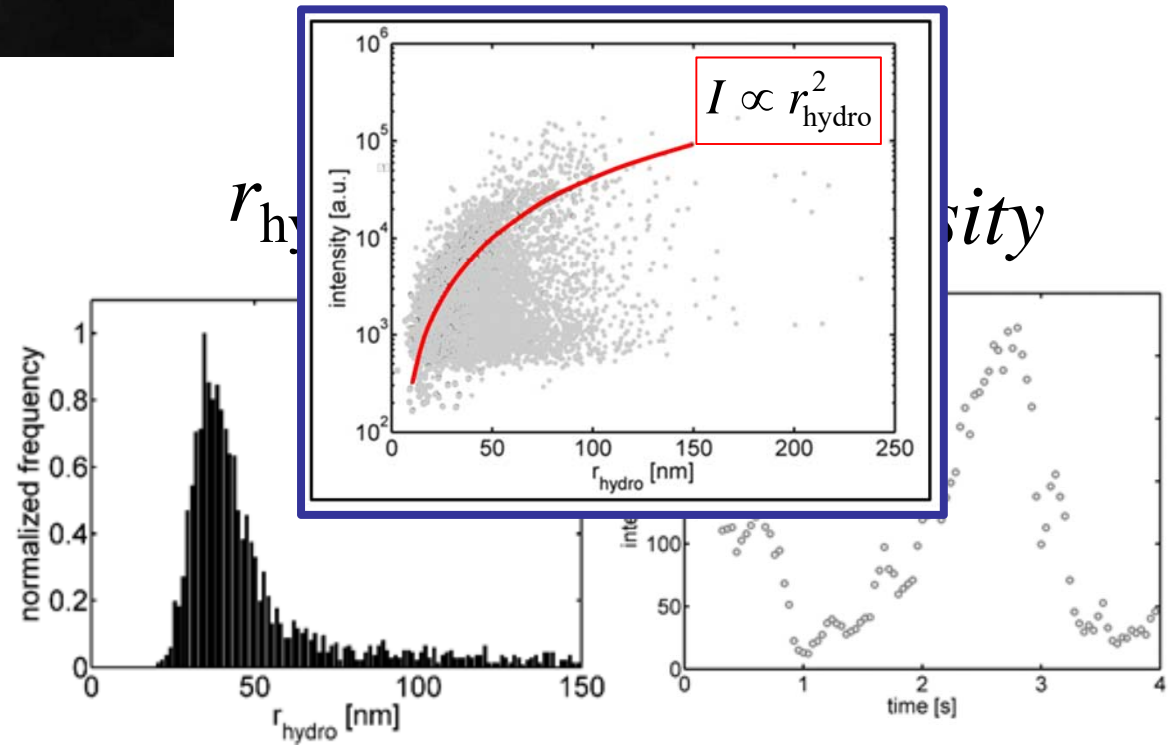
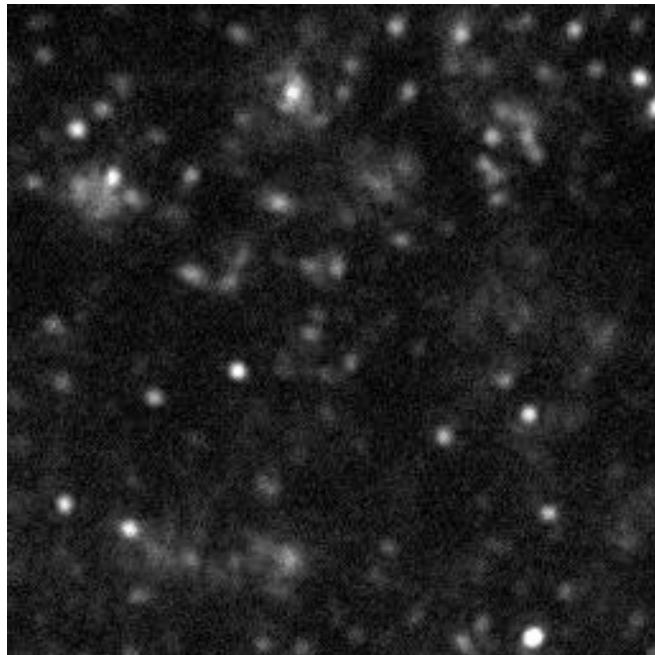
30 fps

10 μm 



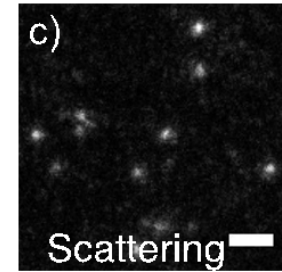
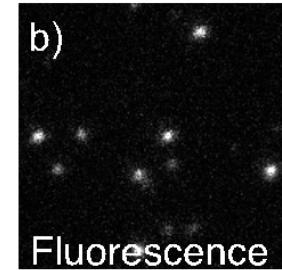
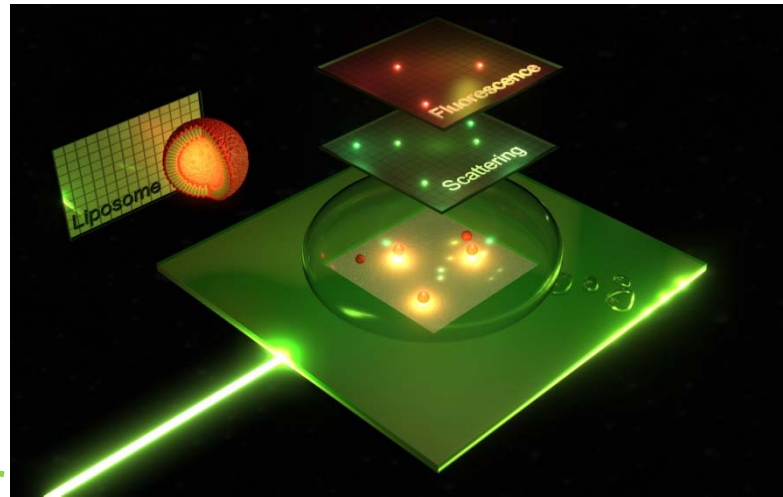
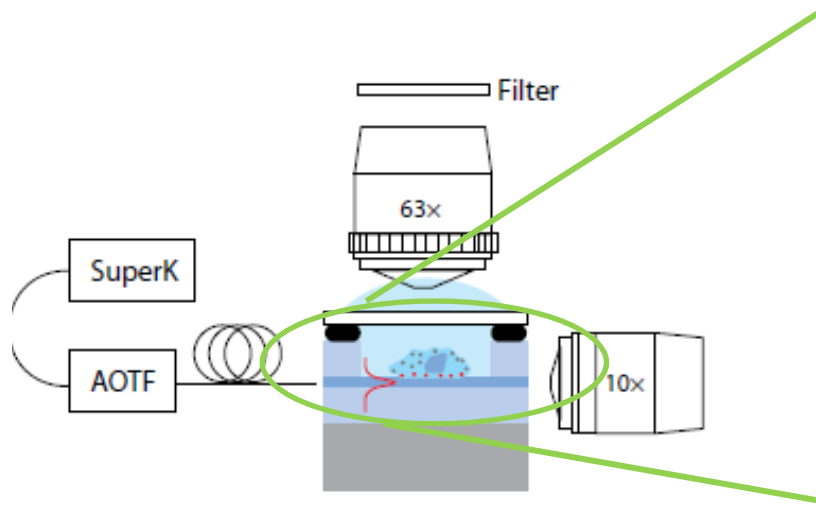
From Trajectories of D63 positive EVs  $\rightarrow D_{EV}$

$$D = \frac{k_B T}{3\pi\eta d}$$

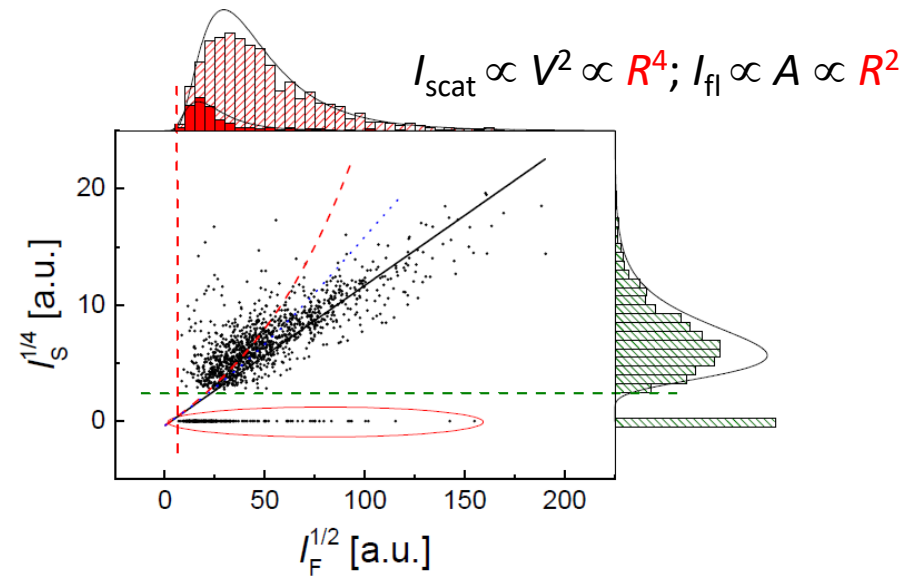




# Label-free imaging of lipid vesicles



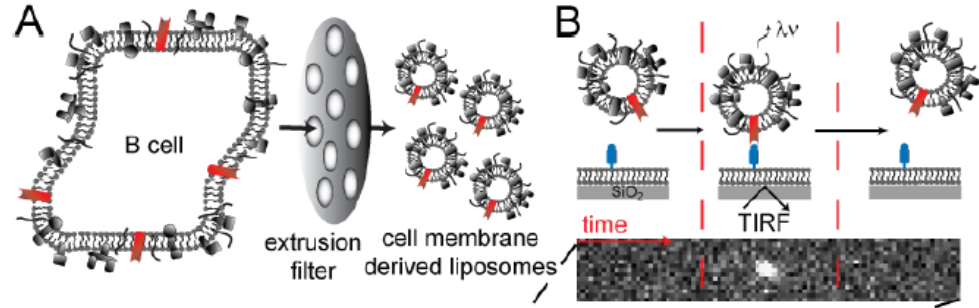
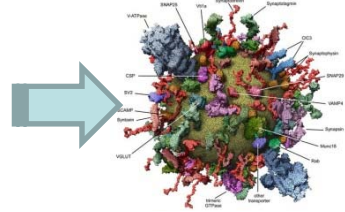
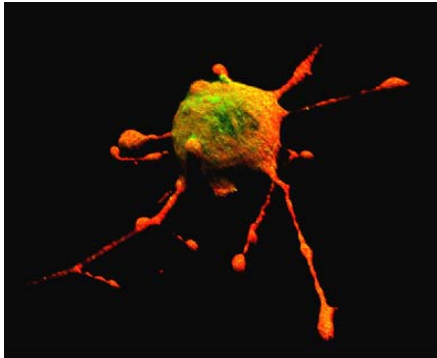
## Lipid-vesicle size determination (~100 nm)



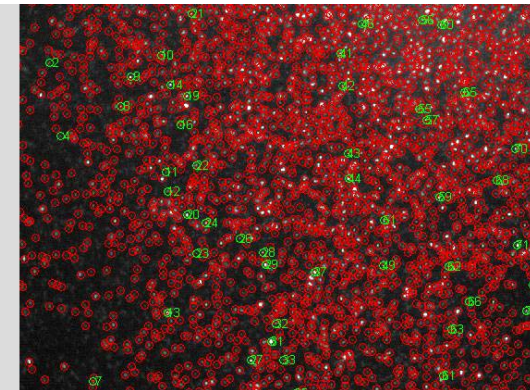
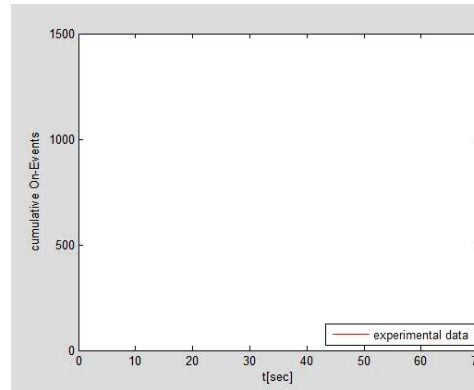
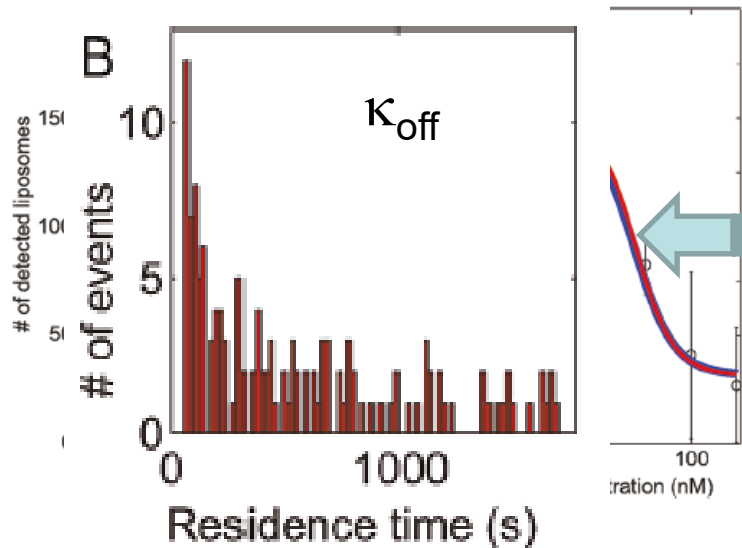
Björn Agnarsson et al. **ACS Nano**, 2015: 9: 11849.  
Patent application GSD / GU Holding / IKV

# Analysis based on single membrane proteins

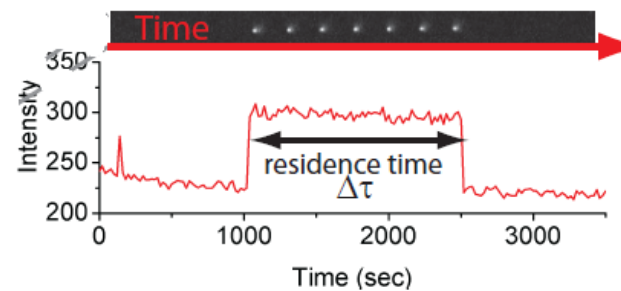
Cell-membrane derived liposomes (modified with a conventional membrane dye)



Histogram of residence times



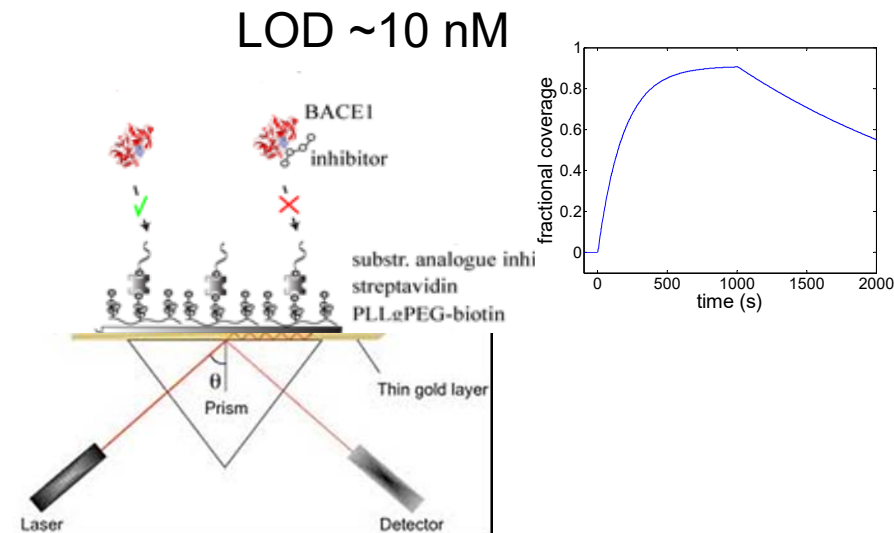
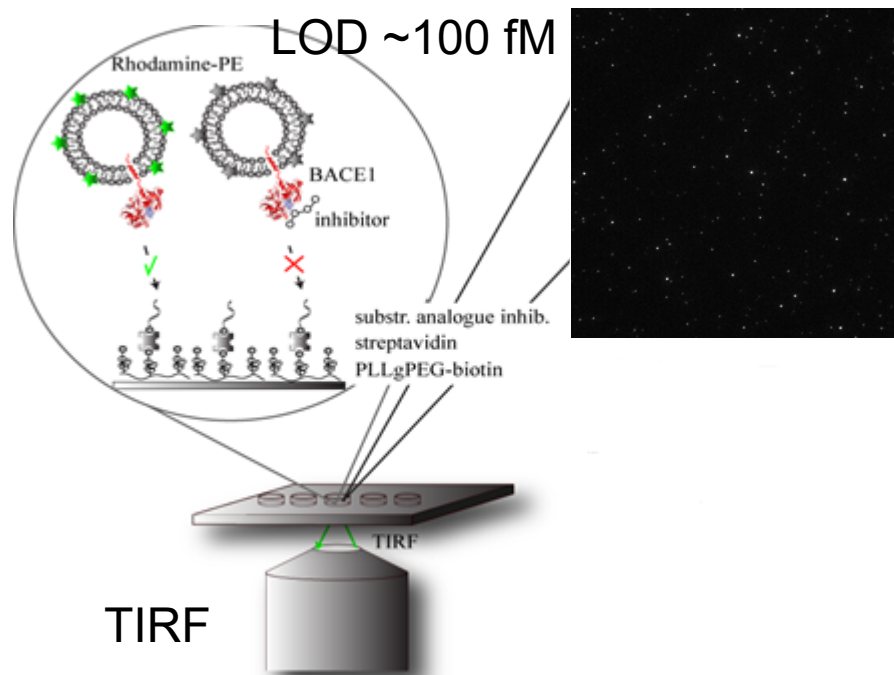
Residence time determination



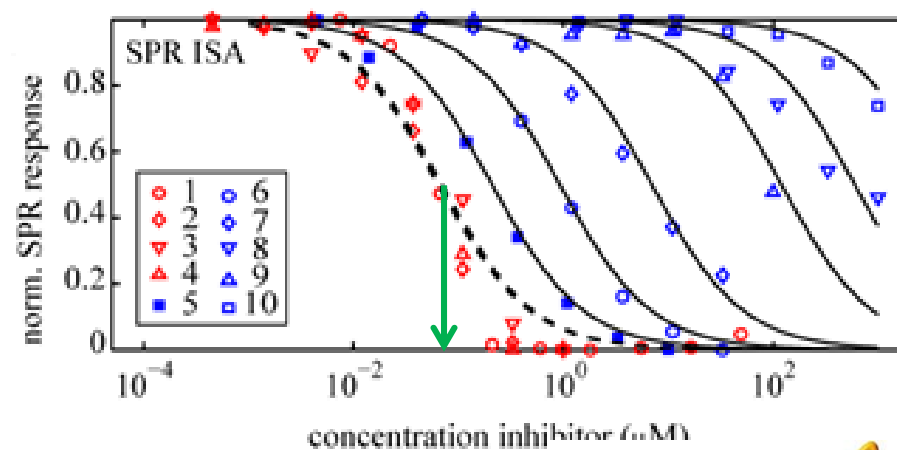
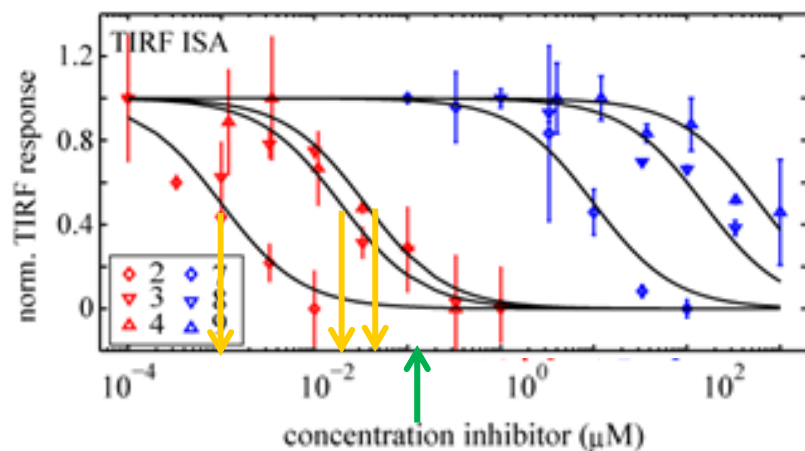
A. Gunnarsson  
Now @



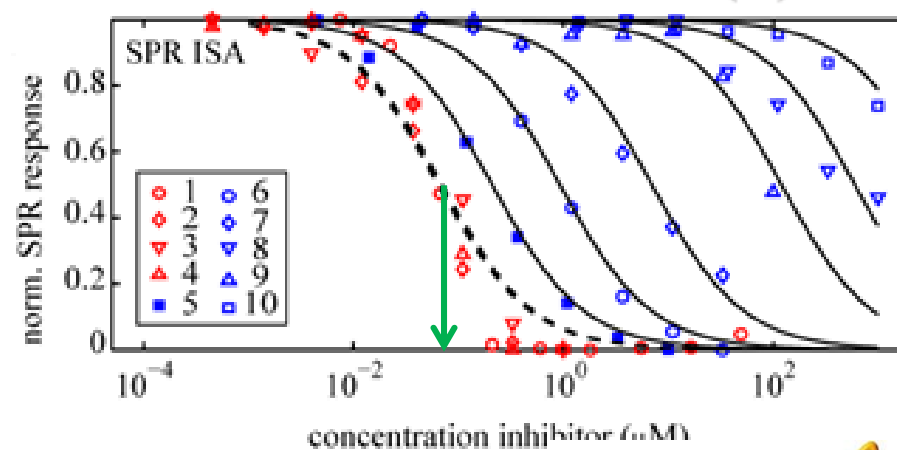
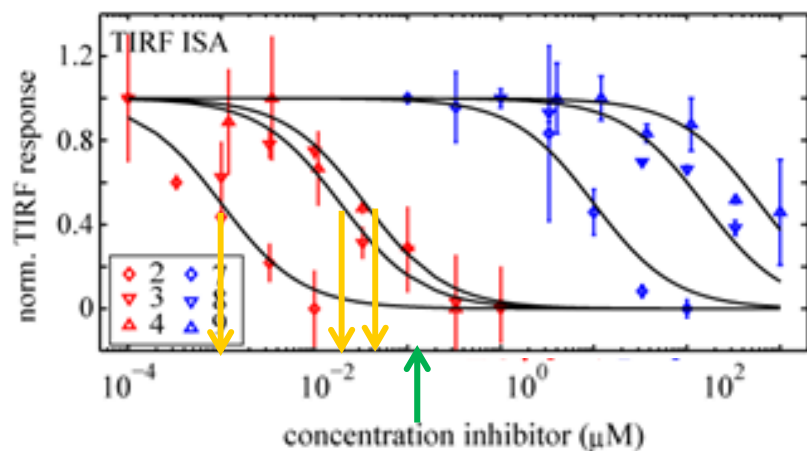
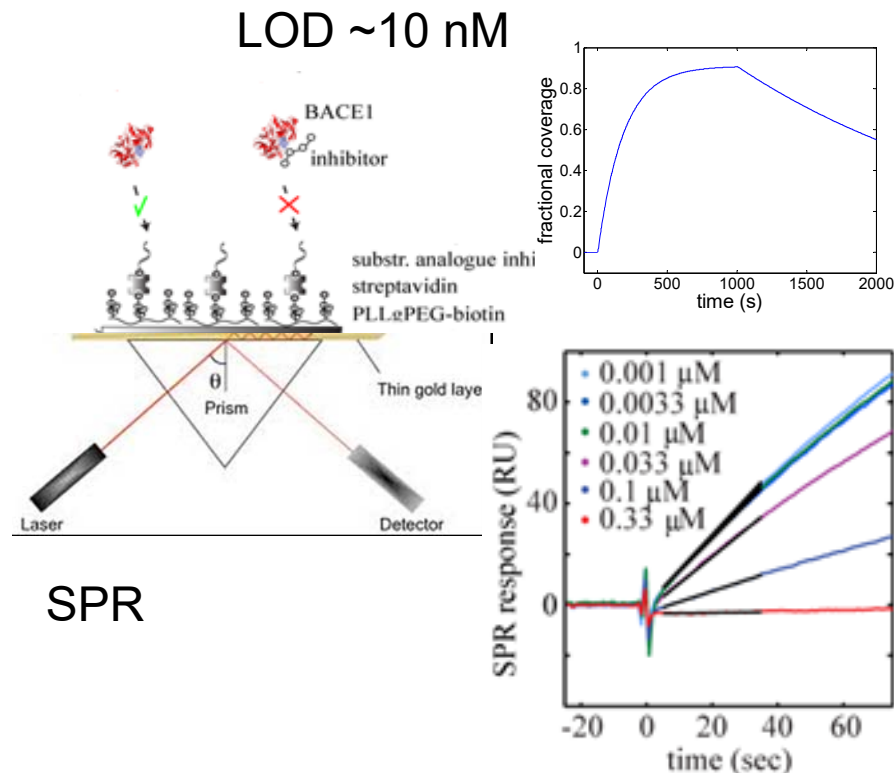
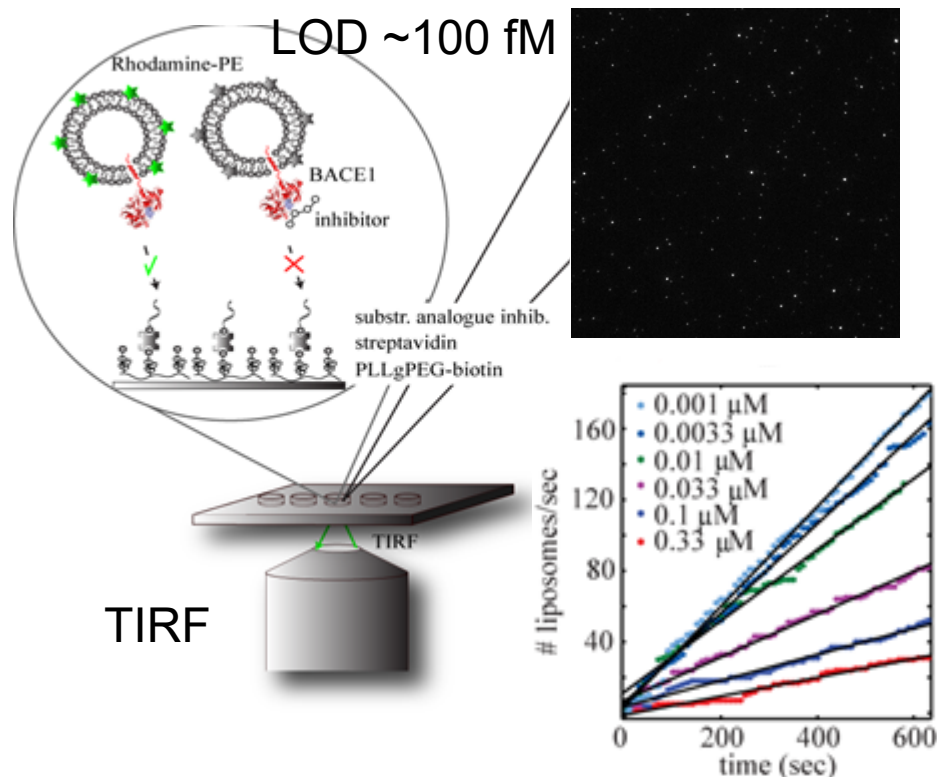
# Improving drug discovery by extending the dynamic range



SPR



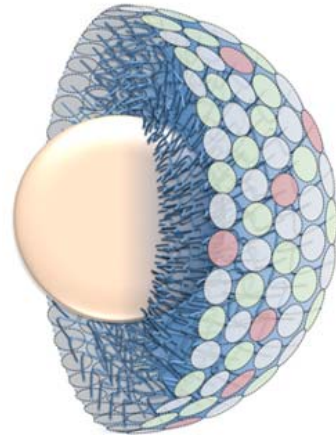
# Improving drug discovery by extending the dynamic range





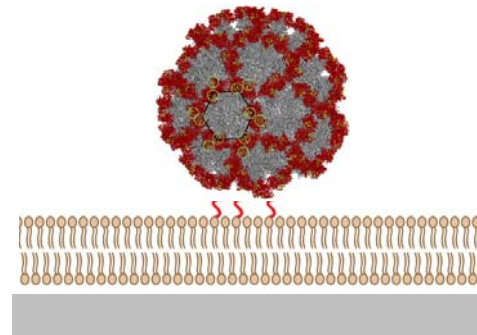
# Both nanoparticle size and specific multivalent binding must be considered

Non-specific DLVO-type Interactions need to be considered

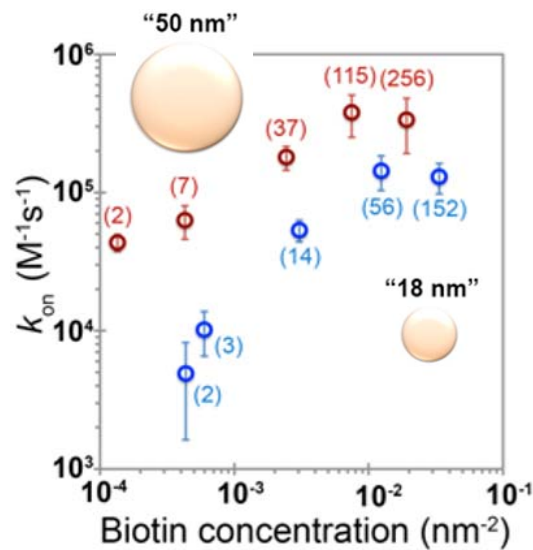


Core-shell nanoparticles

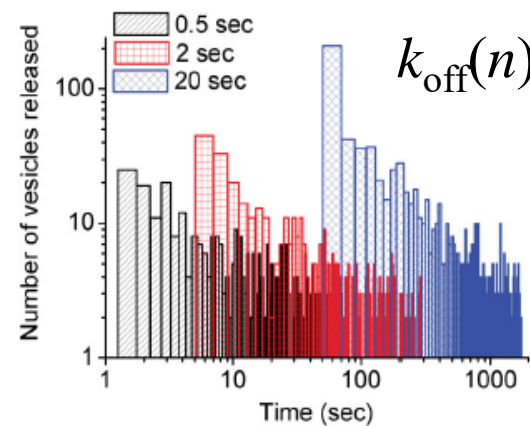
The residence time depends exponentially on the number of contact points



Anders Lundgren  
Now @  
Gothenburg Univ.



Lundgren, A. et al. **ACS Nano** 2016, 10, 9974.



$$k_{off}(n) \propto k_{off}(n=1)^n$$

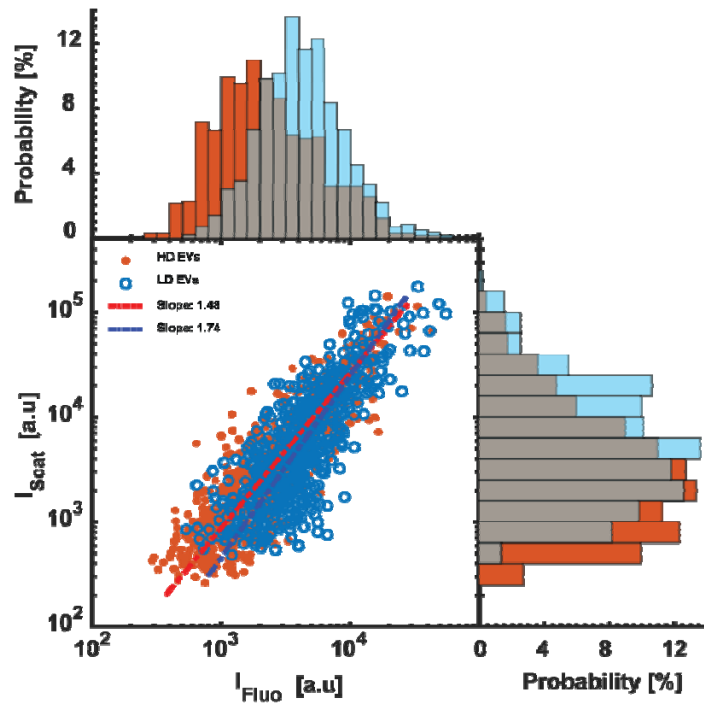
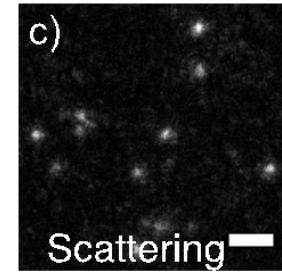
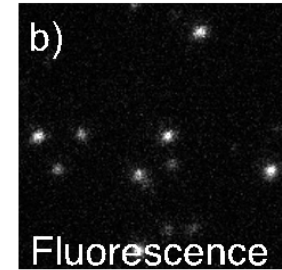
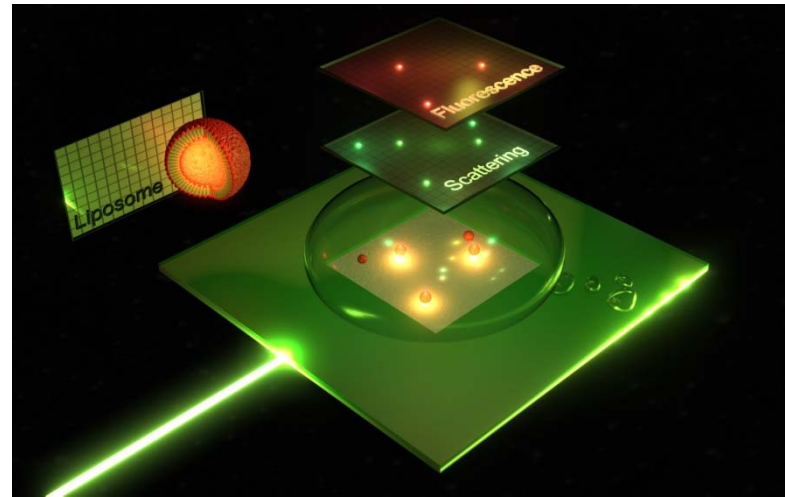
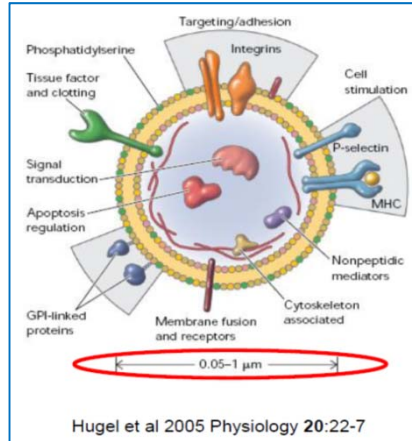
M. Bally et al **PRL** 2011, 107 (18)



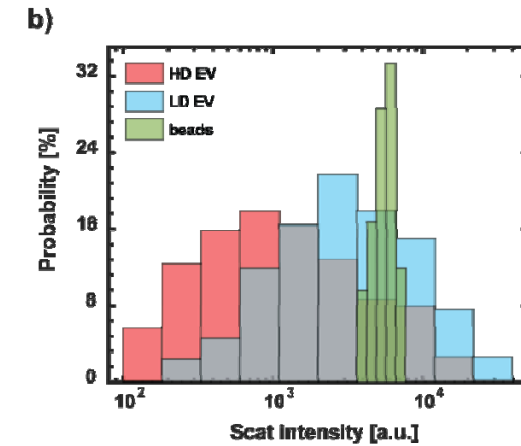
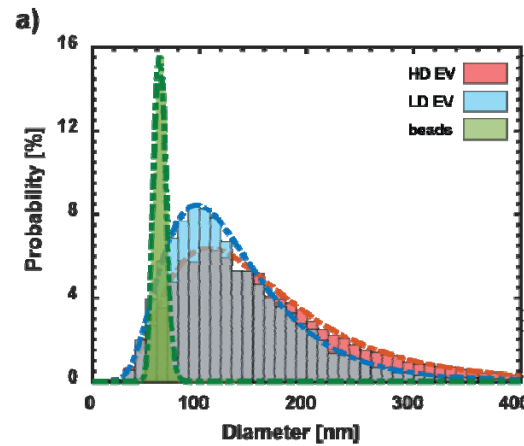
Marta Bally  
Now @  
Umeå Univ.

# Label-free imaging of lipid vesicles and exosomes

## Extracellular vesicles

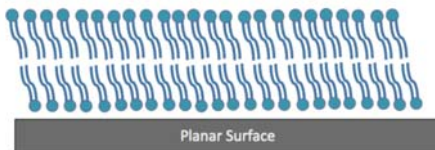
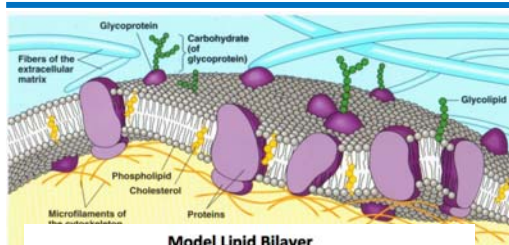


$$I_{scatter} \sim \Delta n^2 r^6 \rightarrow n_{exosome} \sim 1.38$$

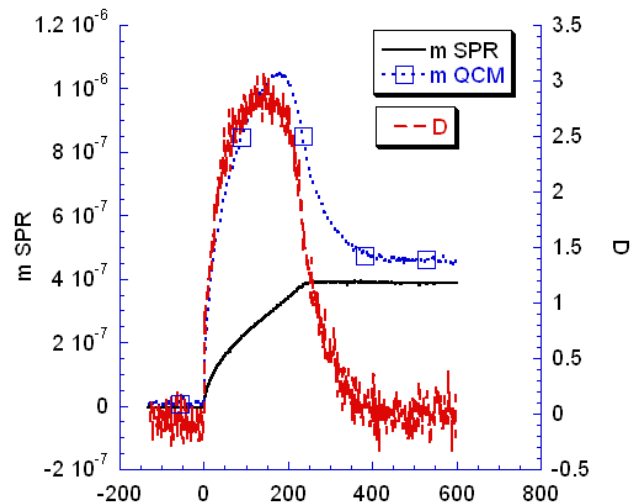
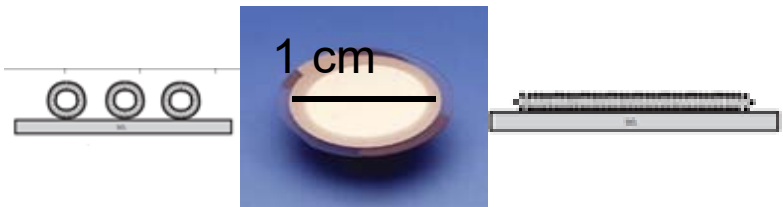


What is the nanoparticle size???

# Supported lipid bilayers



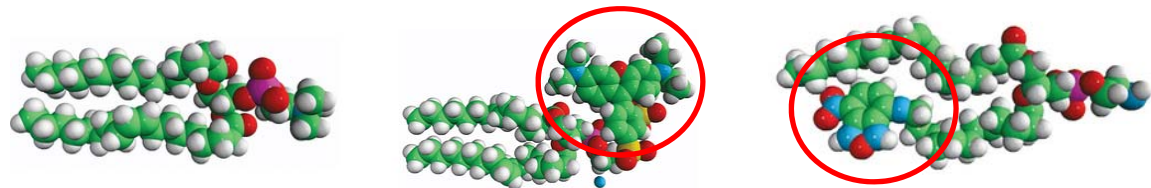
QCM-D™ : [www.q-sense.com](http://www.q-sense.com)



scattering microscopy



FRAP microscopy

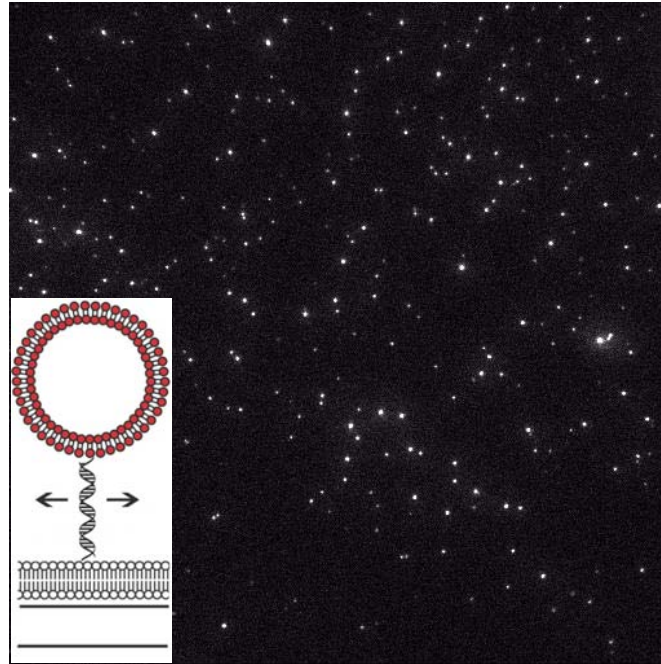




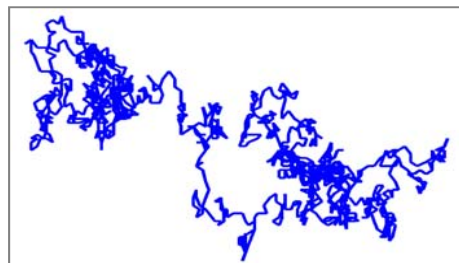
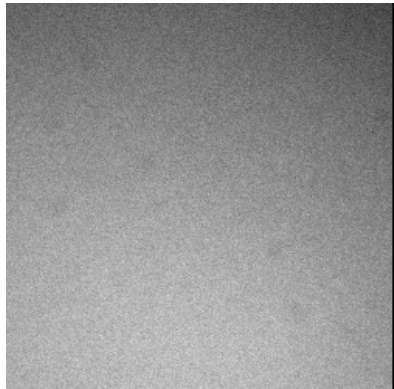
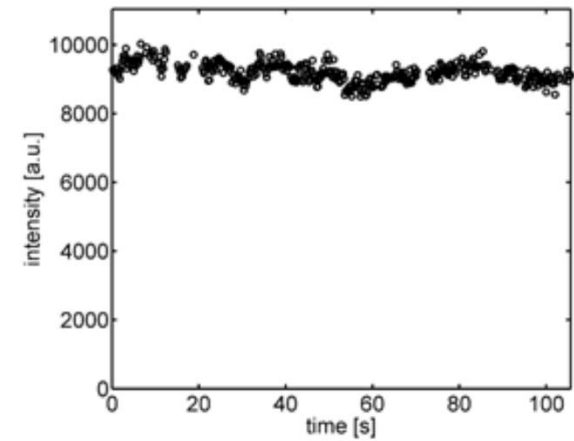
# Quantification of multivalent nanoparticle binding



Fluorescence microscope equipped with TIRF illumination

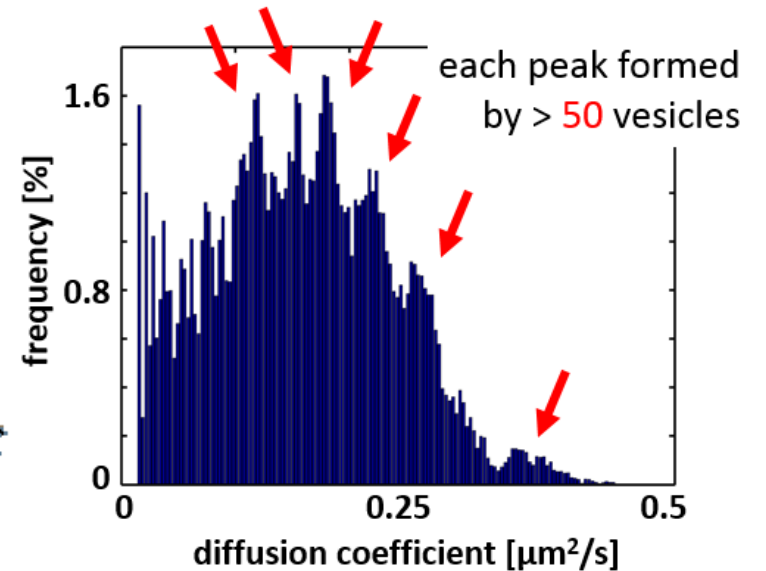


## Single vesicle intensity



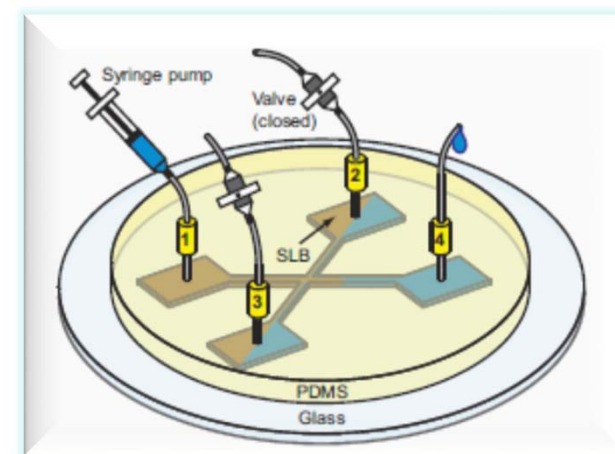
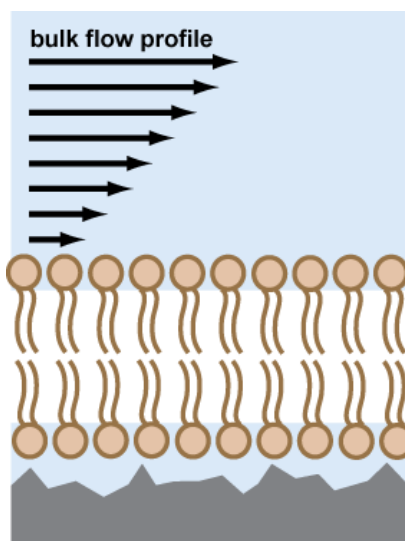
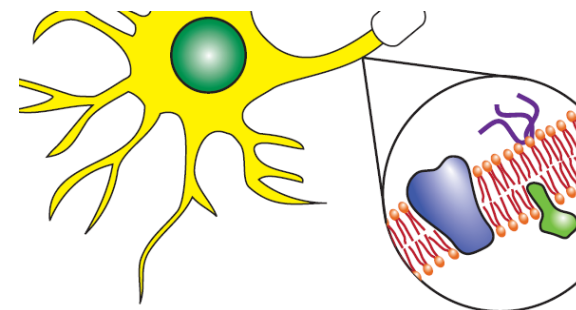
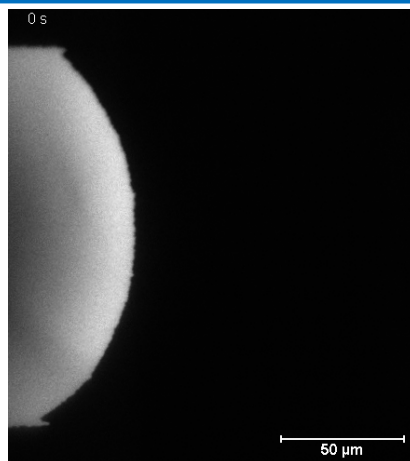
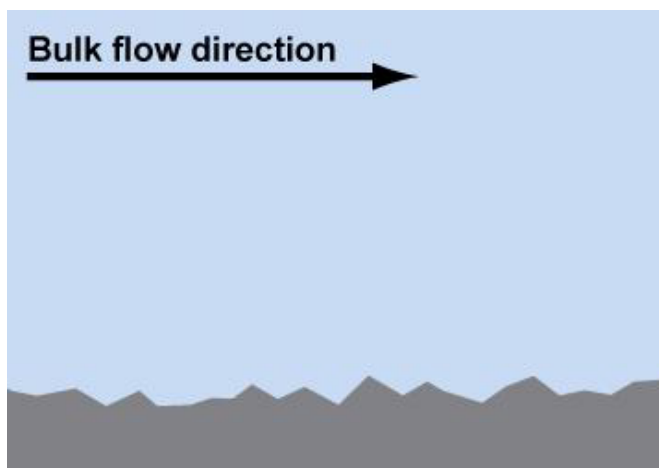
$$\langle MSD(\Delta t) \rangle = 4 \cdot D_{diff} \cdot \Delta t$$

## filtered





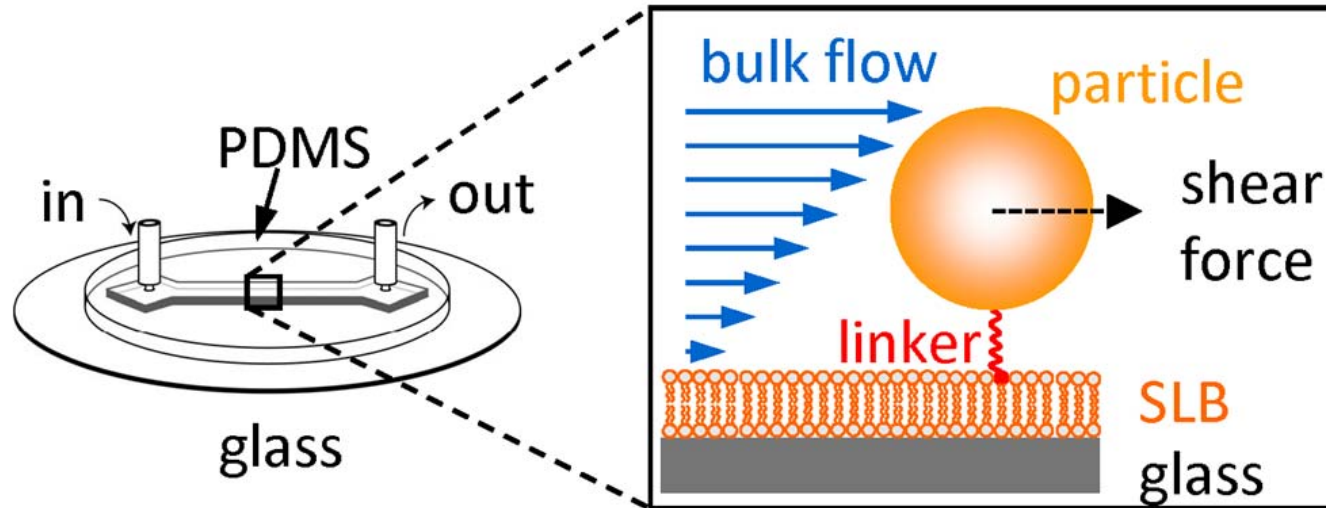
# Shear-driven supported lipid bilayers



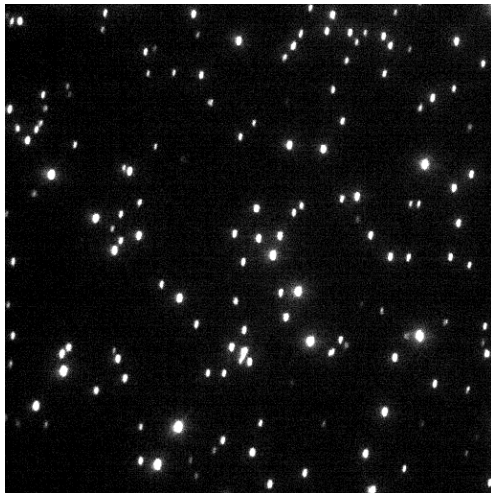
Peter Jönsson  
Now @ Lund Univ.

Jönsson, P. et al. *Biophys. J.* 2008; 95: 5334  
Jönsson, P. et al. *JACS* 2009; 131: 5294  
Jönsson, P. et al. *Langmuir* 2009; 25: 6279

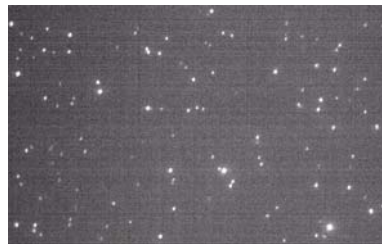
# "Two dimensional flow nanometry"



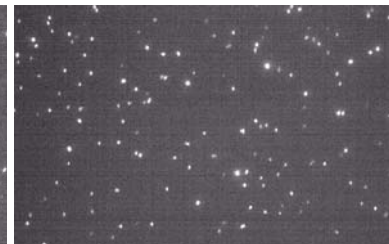
no flow



Applied shear force



$u=1 \mu\text{L}/\text{min}$

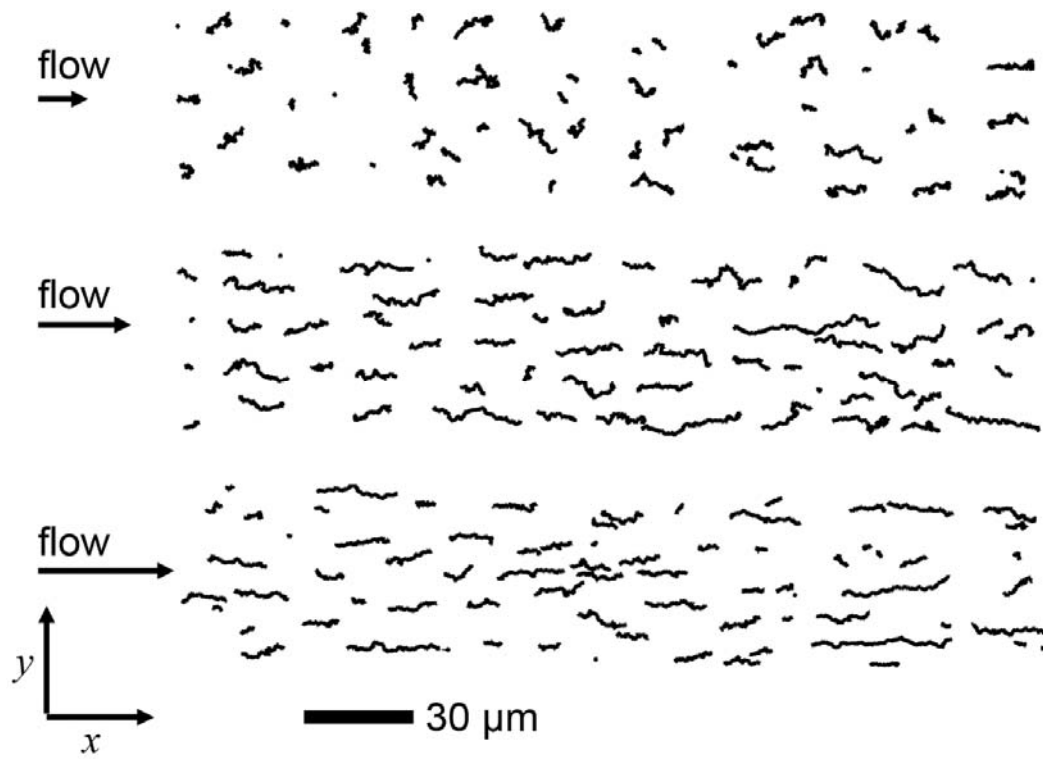


$u=2.5 \mu\text{L}/\text{min}$

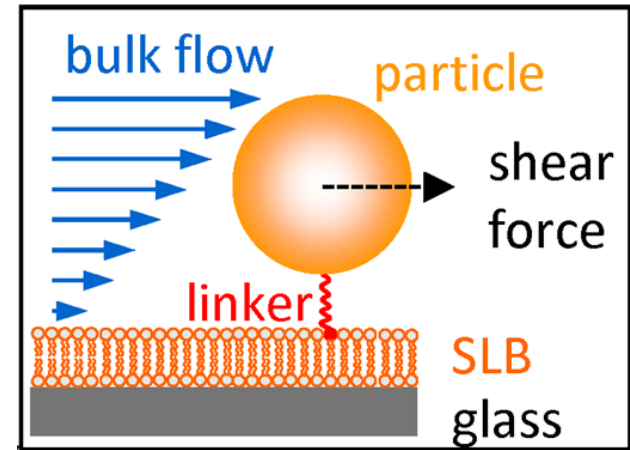
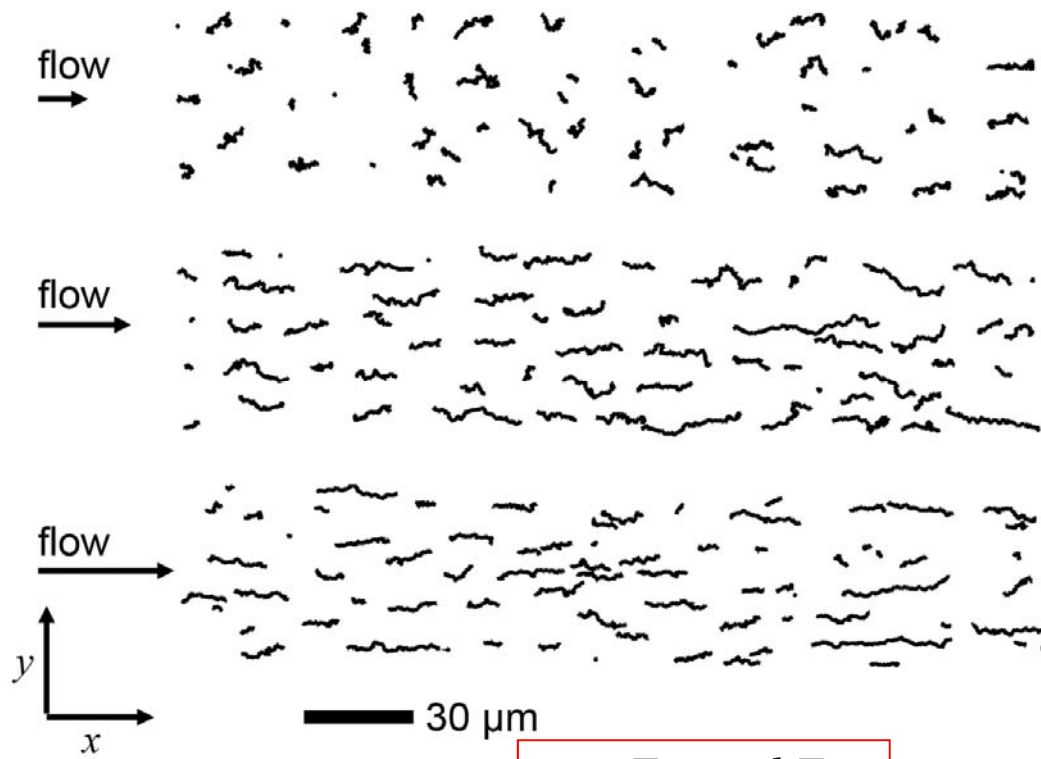


$u=5 \mu\text{L}/\text{min}$

# Data Analysis



# Data Analysis



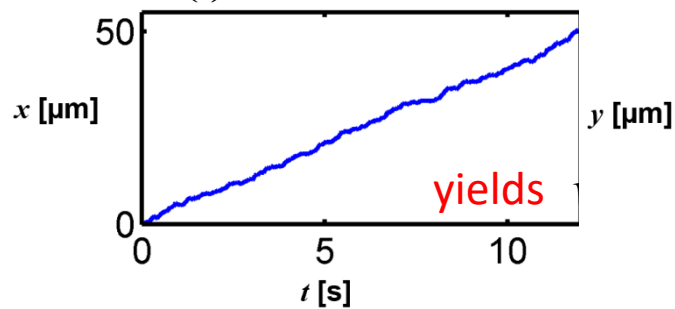
Einstein-Smoluchowski Eq.:

$$\frac{D}{k_B T} = \frac{v}{F_s(r_{\text{hydro}})}$$

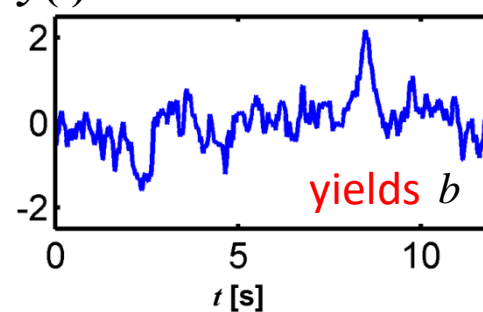
$$v_x \propto F_{\text{shear}} = b F_{\text{shear}}$$

$$b = D_{\text{link}} / k_B \cdot T$$

$x(t)$ : directed movement



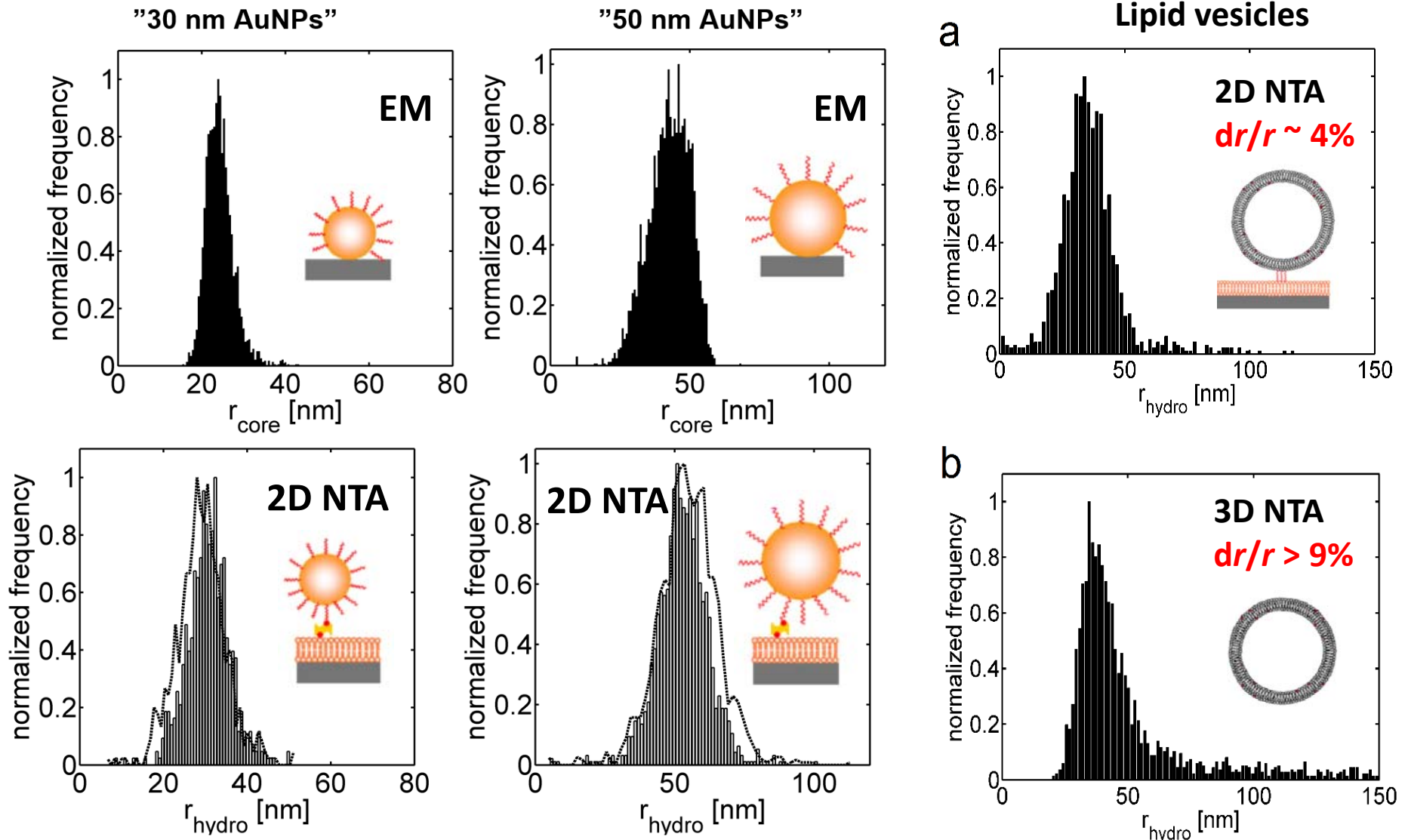
$y(t)$ : random movement



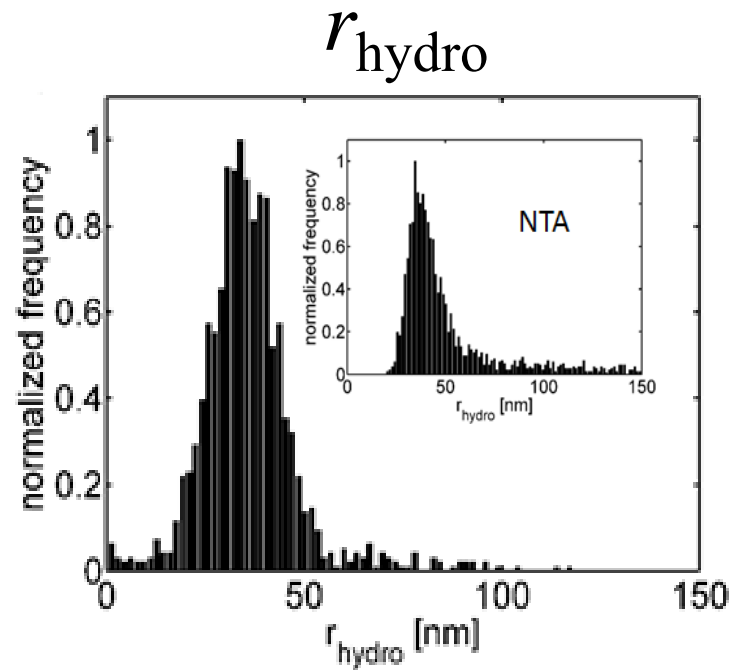


# Nanoparticle size determination

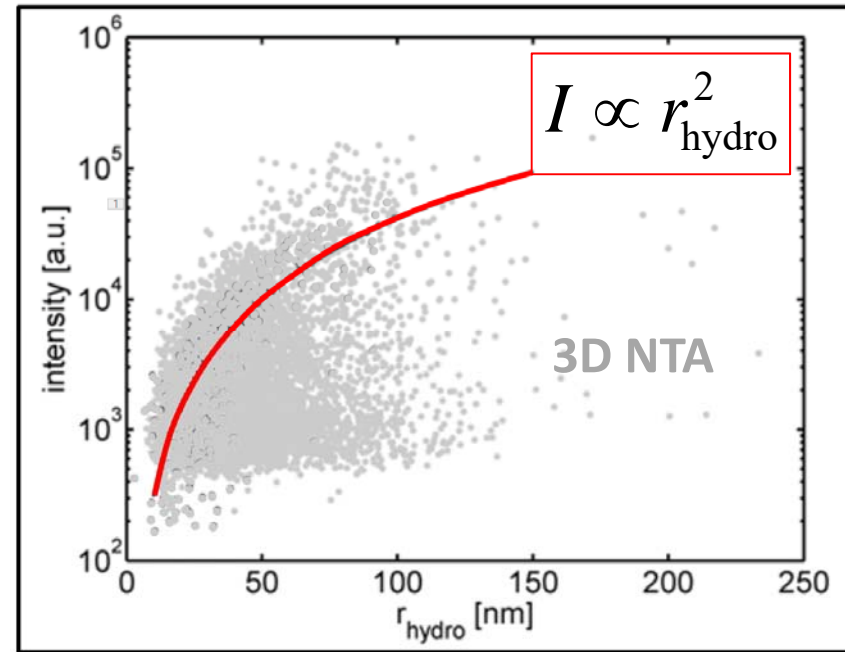
$$F_s(r_{\text{hydro}}) = A \cdot \eta \cdot v_0 \cdot r_{\text{hydro}} \cdot (r_{\text{hydro}} + \lambda)$$



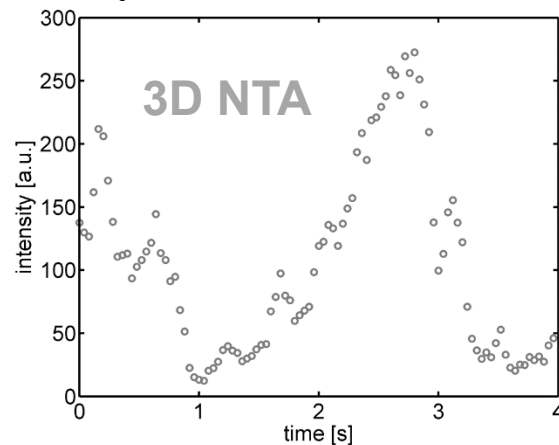
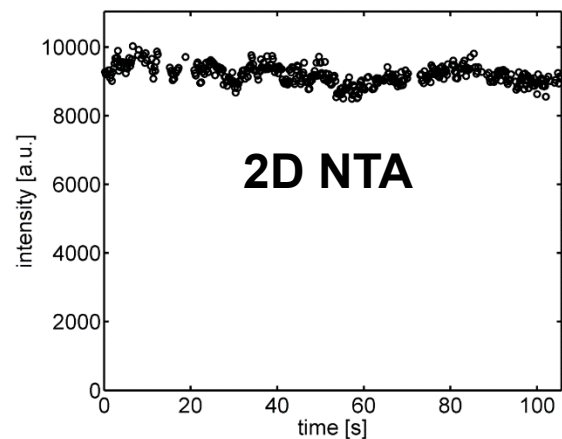
# Intensity vs. hydrodynamic radius



"74 nm lipid vesicle batch"

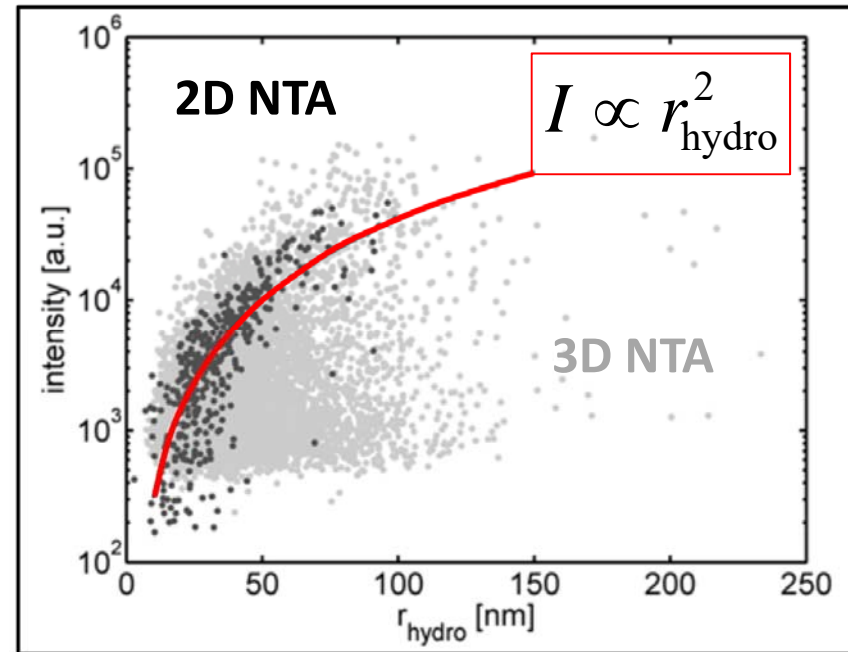
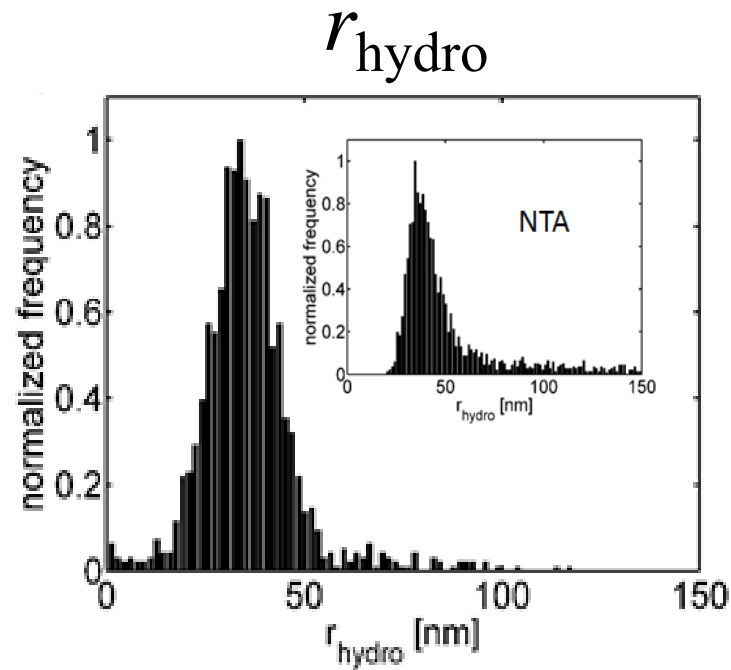


Intensity

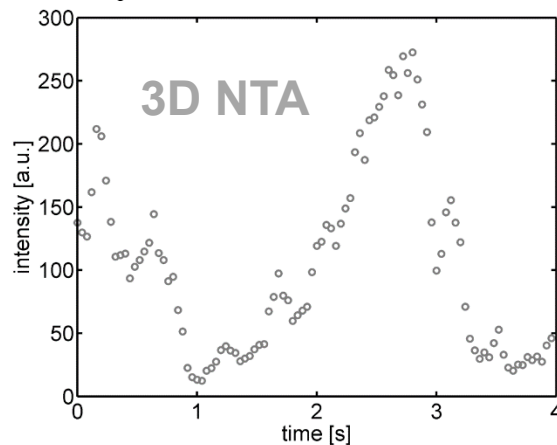
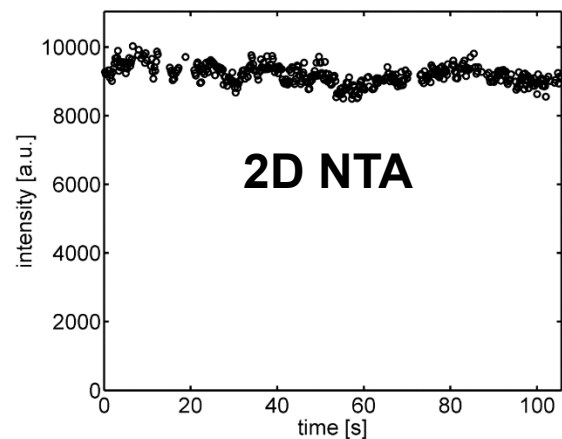


# Intensity vs. hydrodynamic radius

"74 nm lipid vesicle batch"



## Intensity

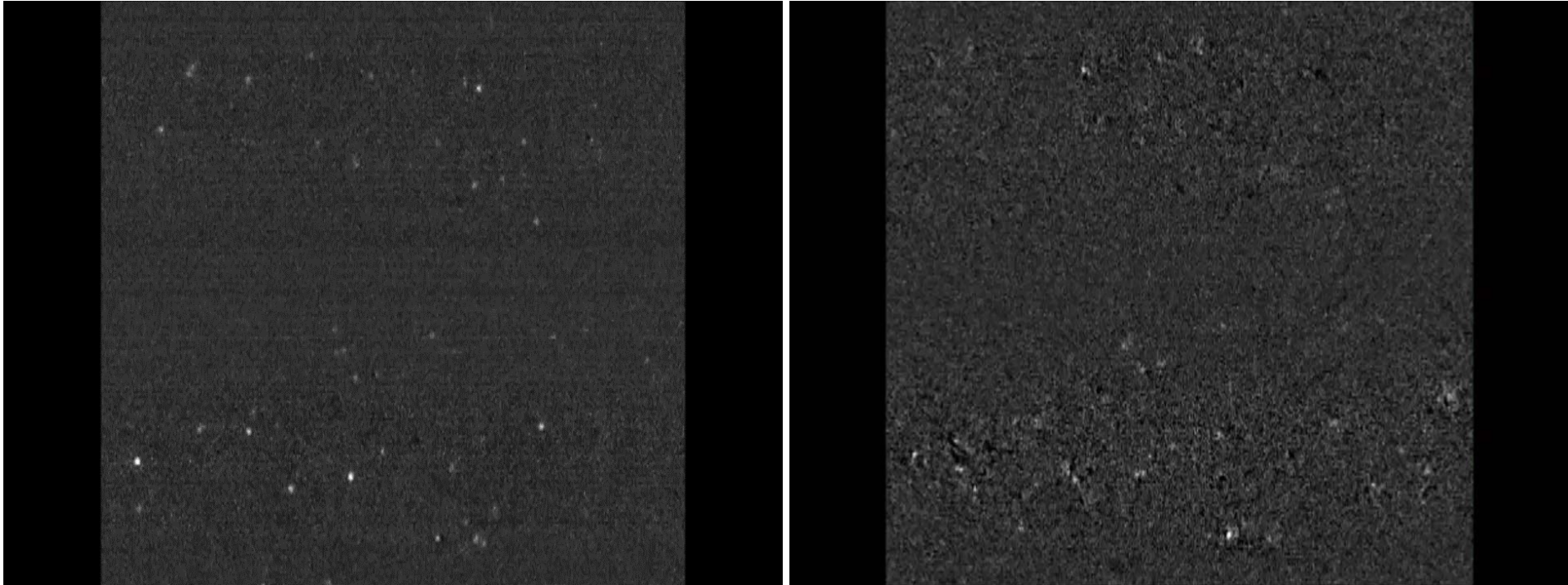


# Outlook: Correlating light scattering to size

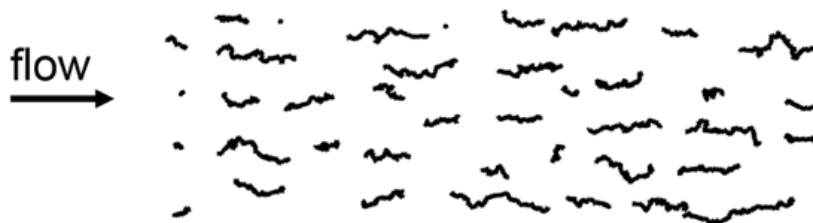
Fluorescence

$$I_{\text{scatter}} \sim \Delta n^2 r^6$$

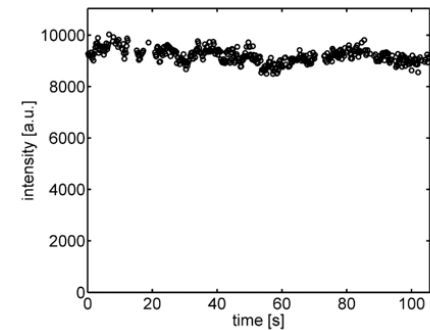
Scattering



Size of each nanoparticle



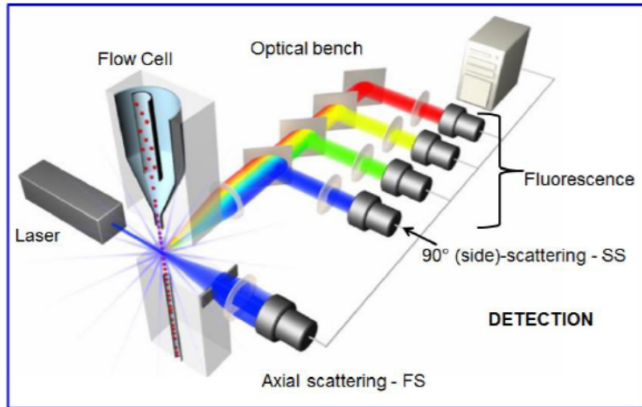
Intensity (fluorescence & scattering)



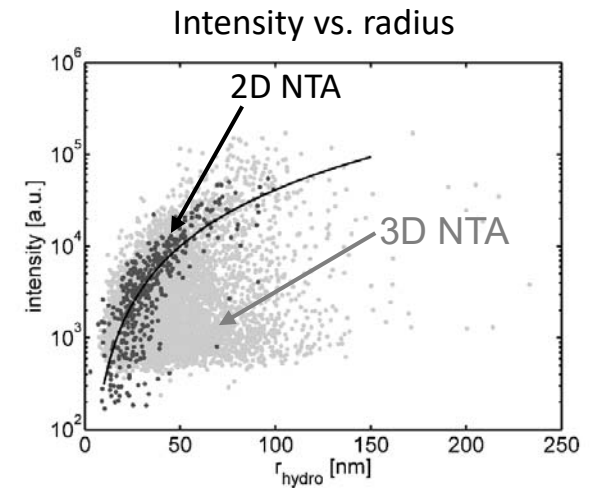
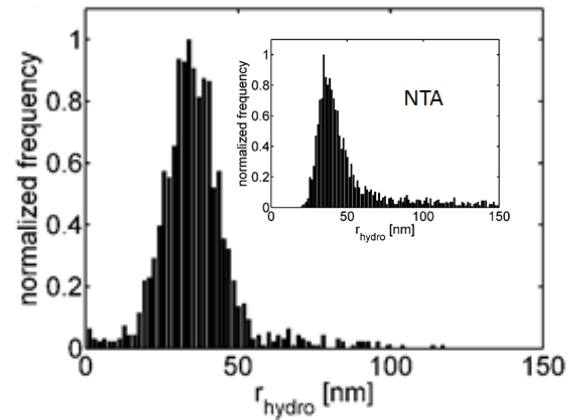


# Outlook: Nanoparticle Flow Nanometry

## Flow cytometry

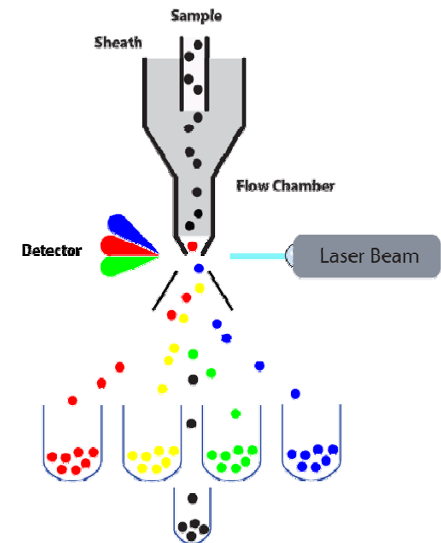
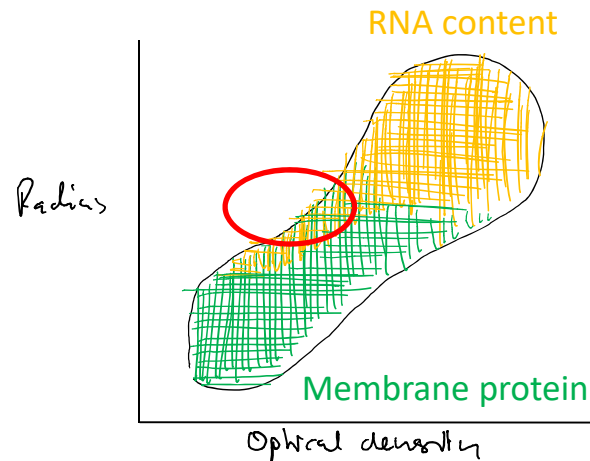
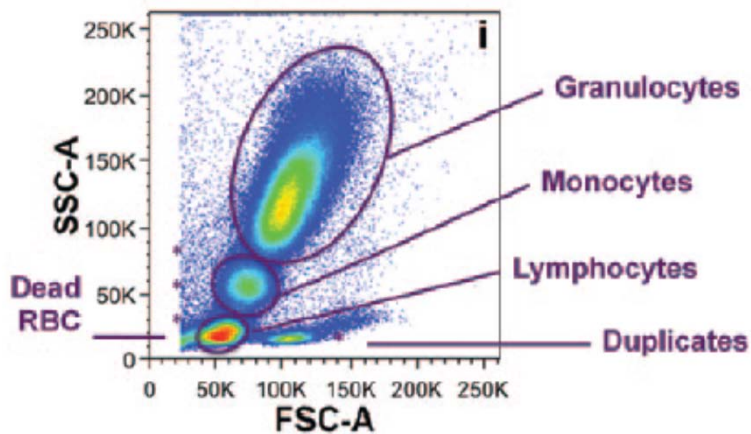


$$F_{\text{shear}}(R) = A \cdot \eta \cdot R \cdot v_{\text{fluid}}(R) \Rightarrow R$$



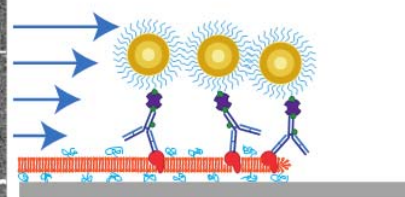
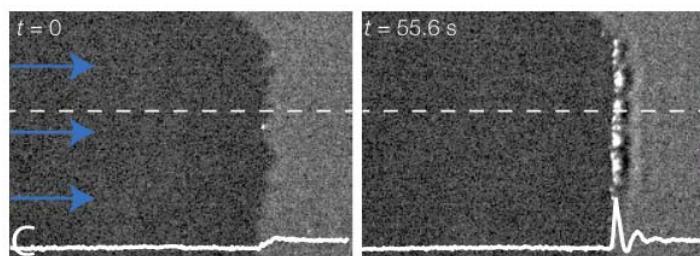
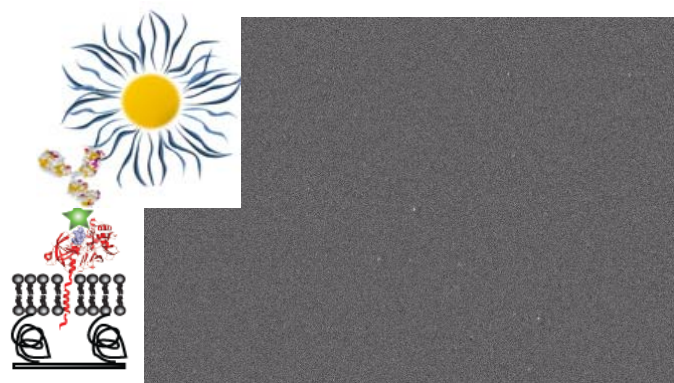
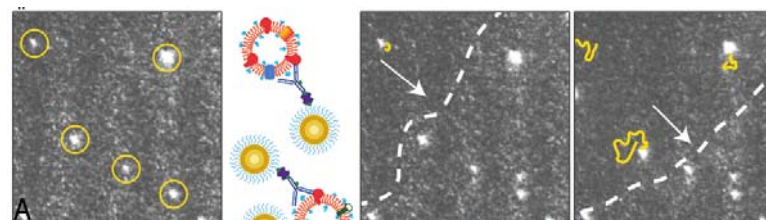
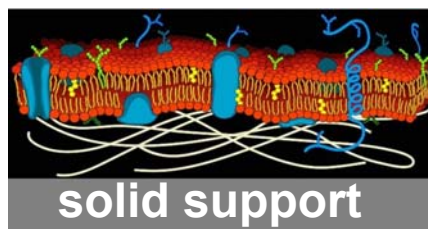
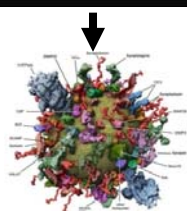
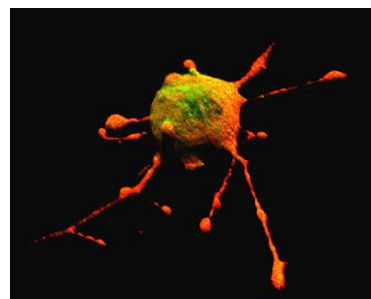
“Flow-Cytometry like” analysis of single nanoparticles

## nano-FACS

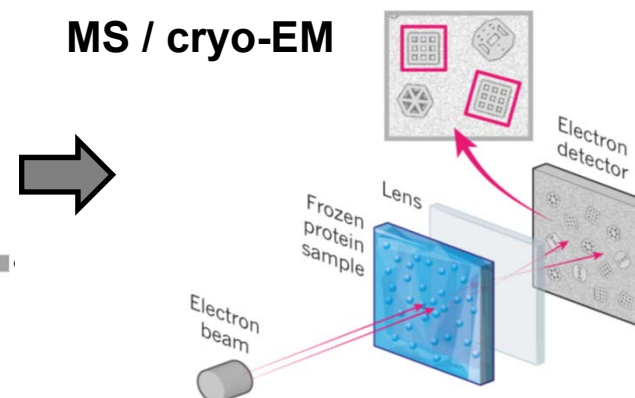


# Outlook: Local enrichment of membrane proteins

Increased local concentration for ligand binding studies and/or membrane-protein crystallization

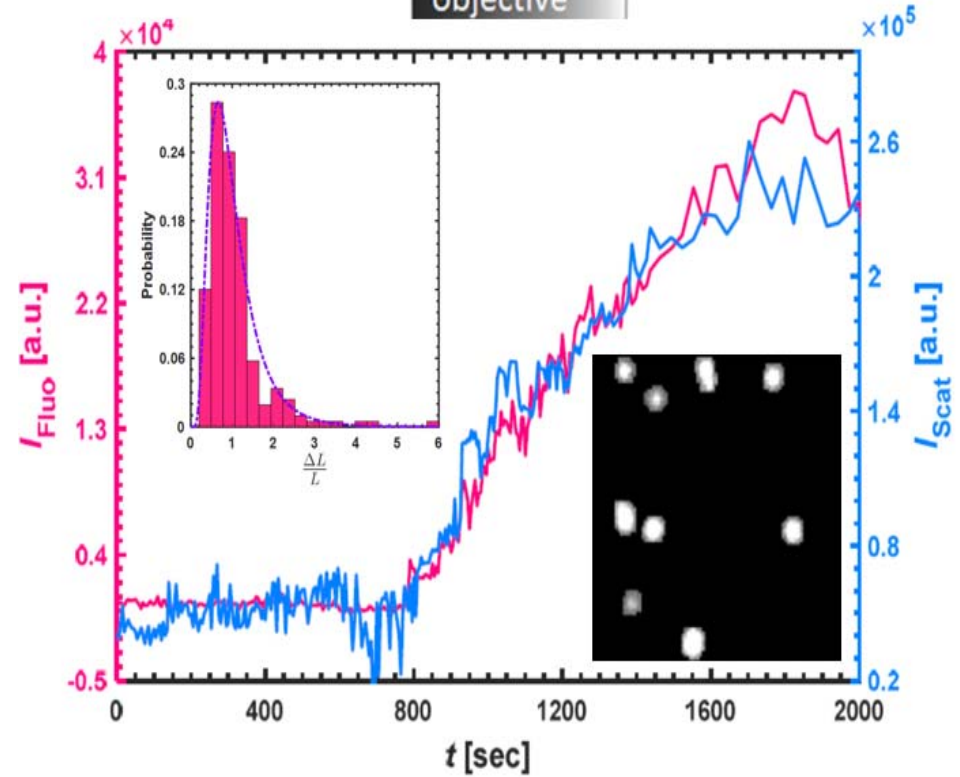
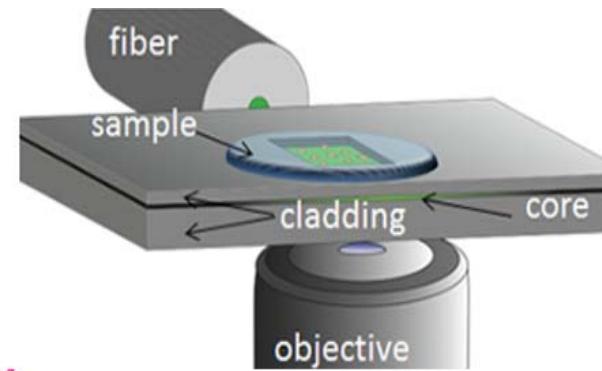
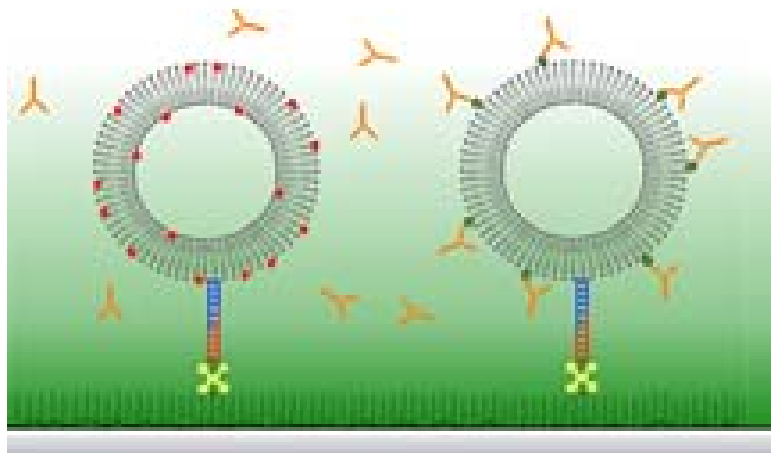


MS / cryo-EM

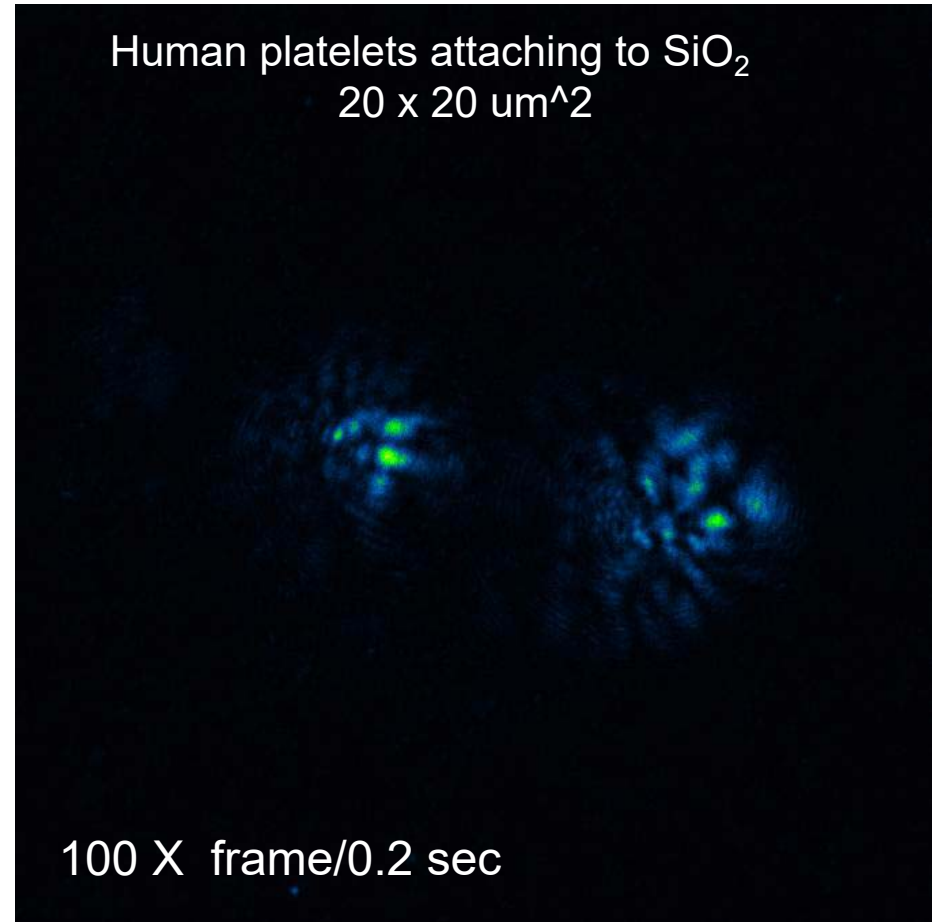
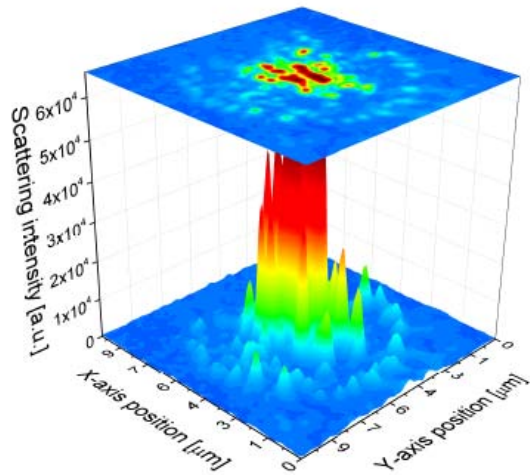
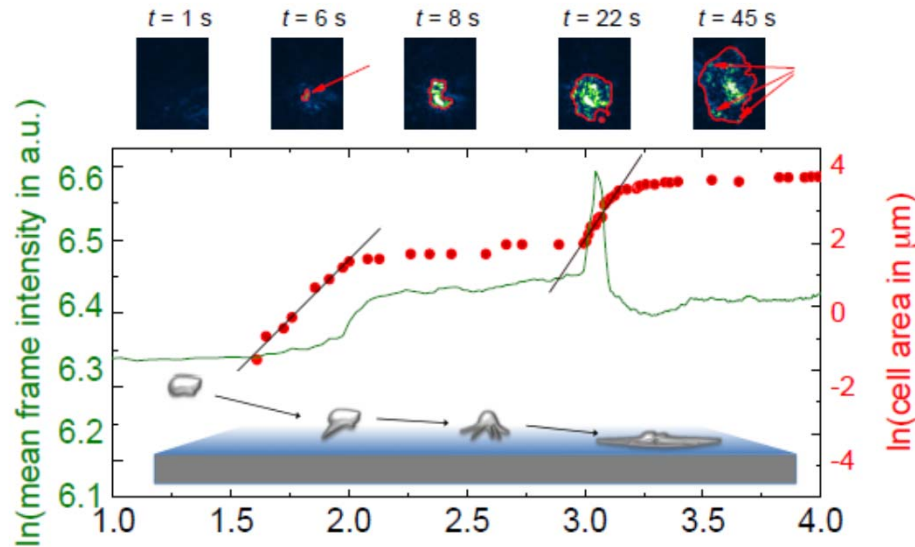
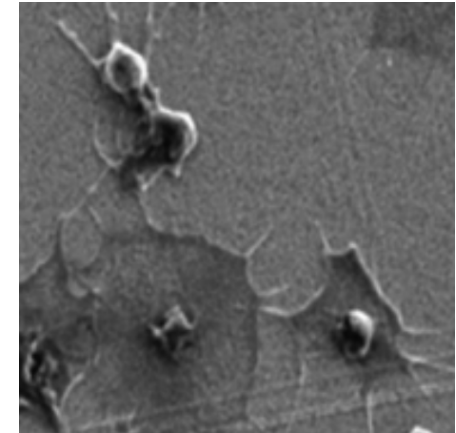


Pace, H et al., *Anal Chem*, 2015, 87:9194  
Lundgren, A. et al. *Nano Letters* 2018, 18: 381

# Outlook: Label-free monitoring of protein-binding to individual nanoparticles

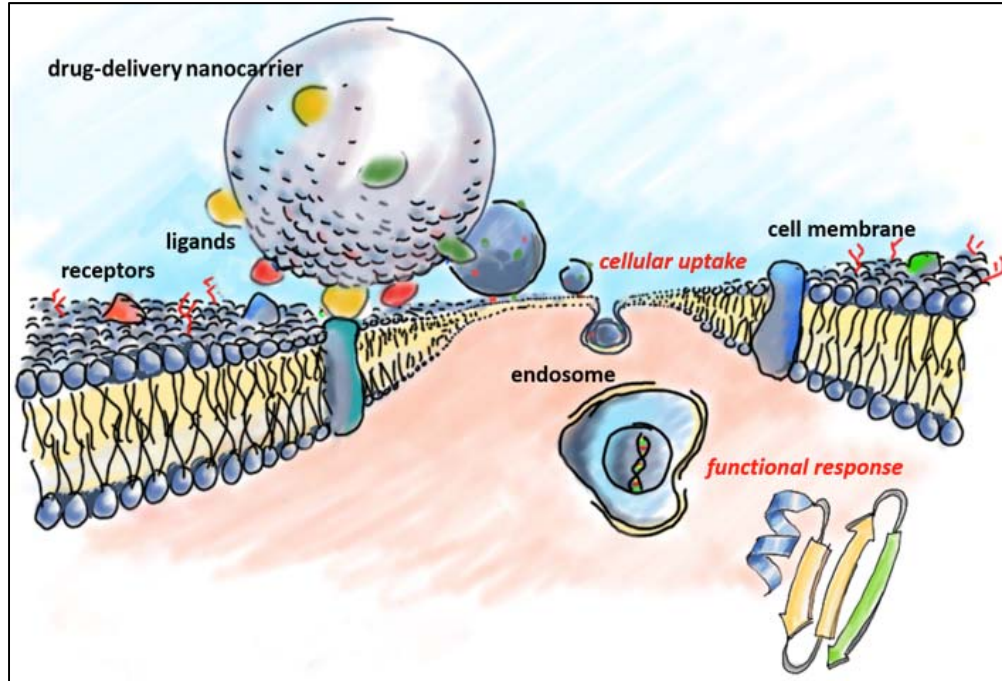


# Outlook: cell-surface interactions





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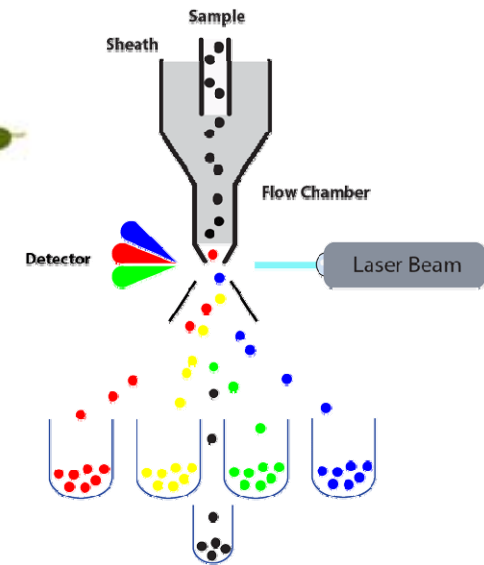
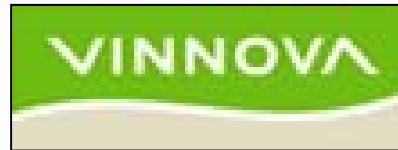


Marcus  
Wilhelmsson  
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