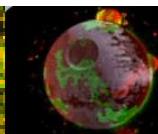
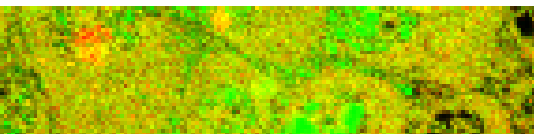
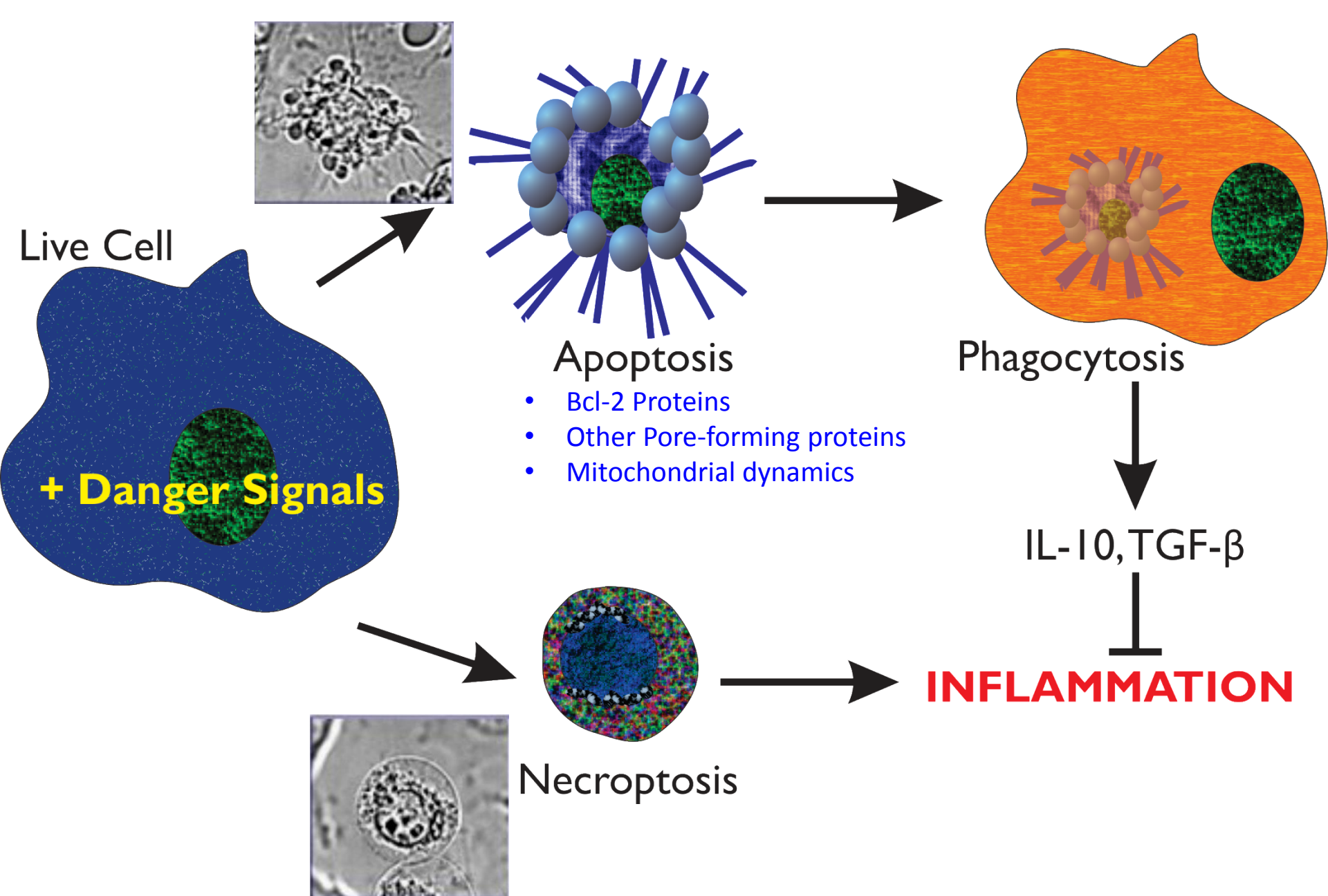


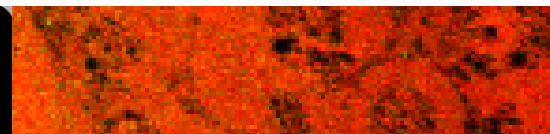
Single molecule methods to study cellular processes in inflammation

Prof. Dr. Ana J. García Sáez
Membrane Biophysics

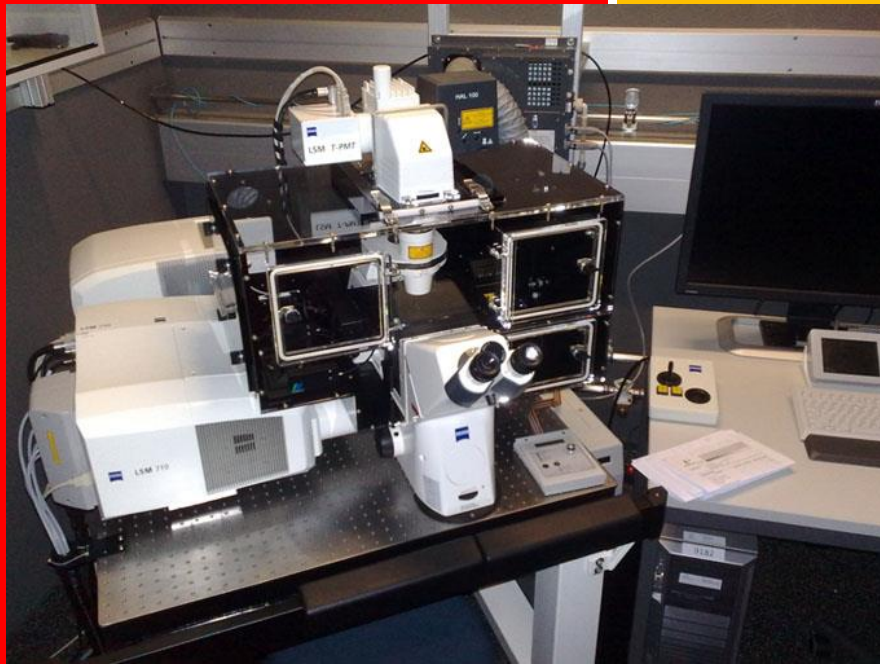




Membrane biophysics



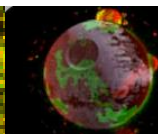
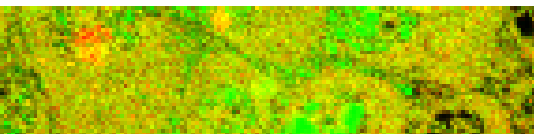
Confocal Microscopy



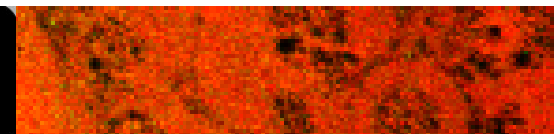
Single Molecule Microscopy

Atomic Force Microscopy

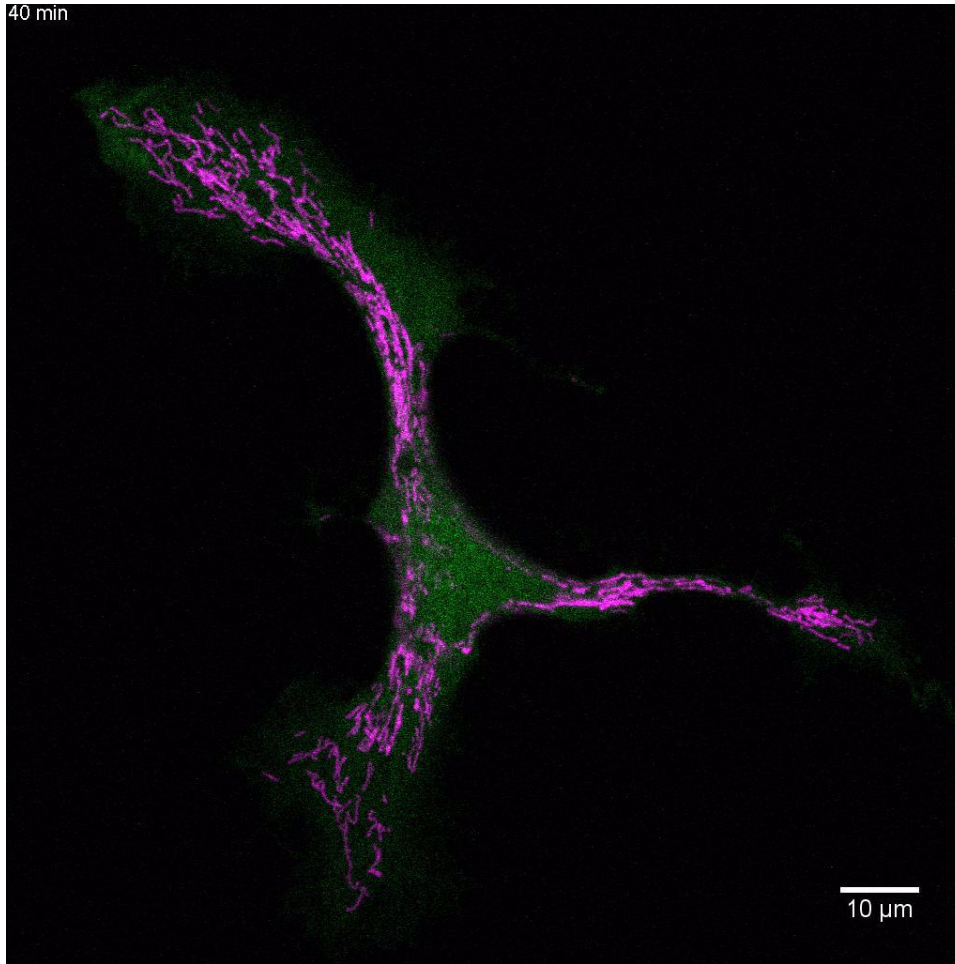
Functional
Microscopy



Membrane biophysics



Live Cell Imaging



Important results:

- **Localization and Morphological Effects At the Single Cell Level**
- **Kinetics of Slow Processes**

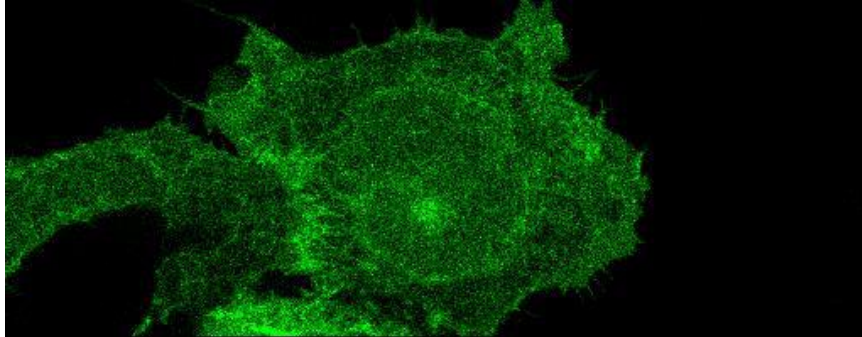
Staurosporine induced apoptosis of HeLa Cells overexpressing Bax-GFP (green) with stained mitochondria (magenta) .



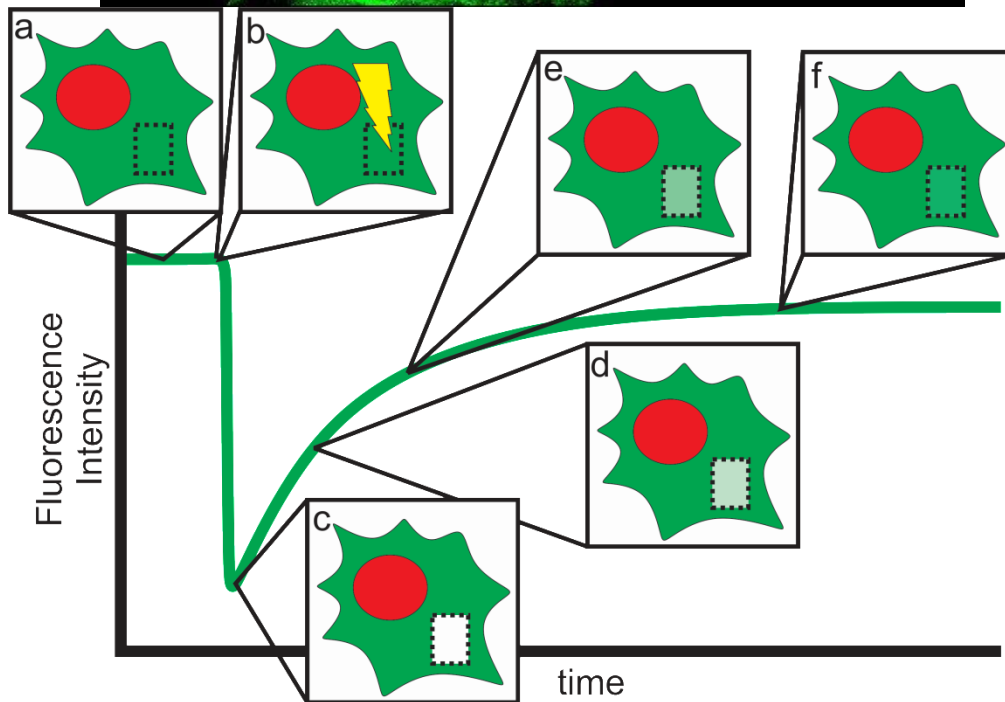
Aida Peña Blanco
Raquel Salvador Gallego
Begoña Ugarte Uribe



Fluorescence Recovery After Photobleaching (FRAP)



Fas receptor-GFP overexpressed in plasma membrane of living cells

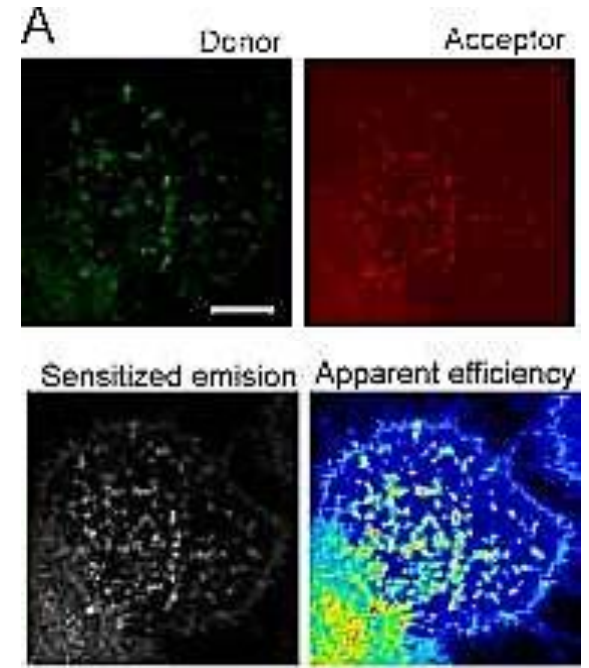
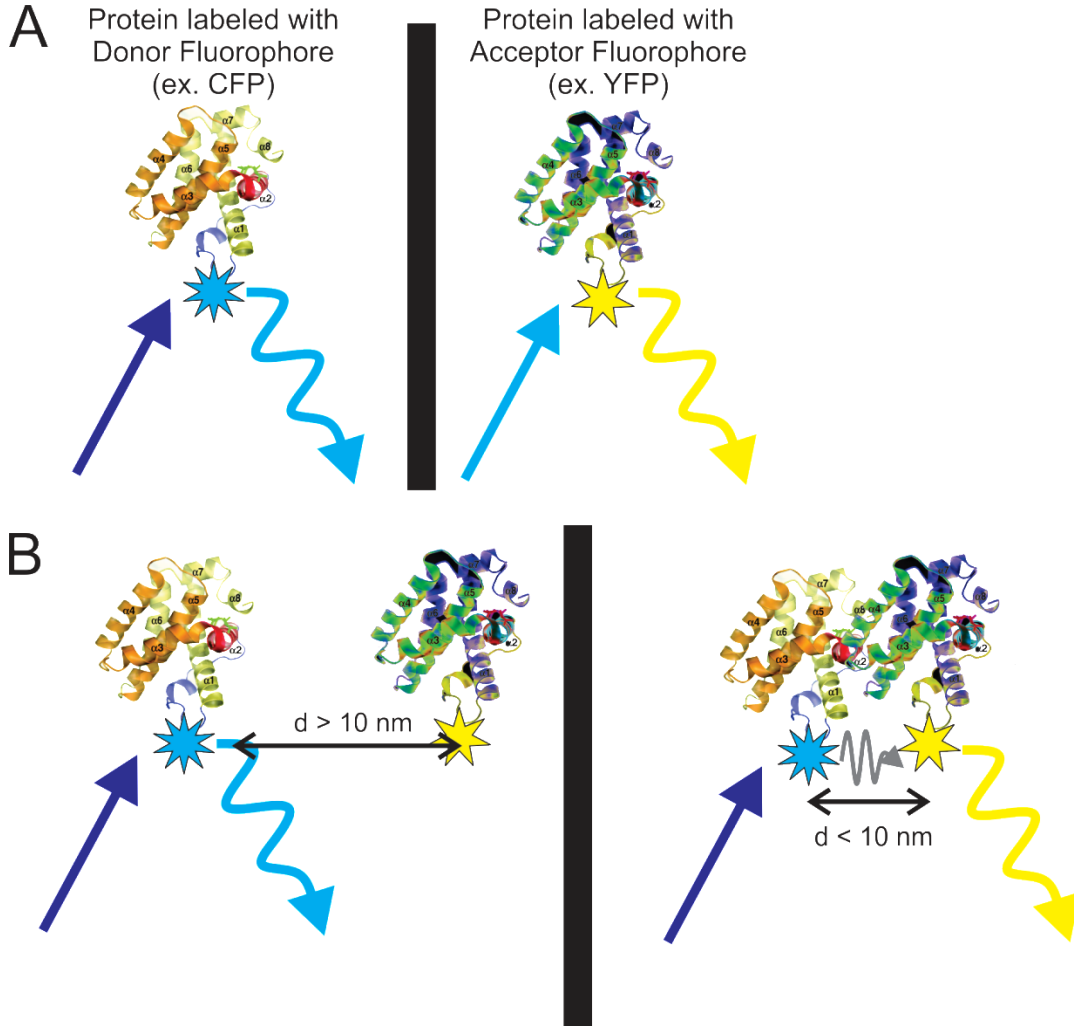


Important results:

- Diffusion
- Mobilities

Florencia Sanchez
Raquel Salvador Gallego

Förster Resonance Energy Transfer (FRET)



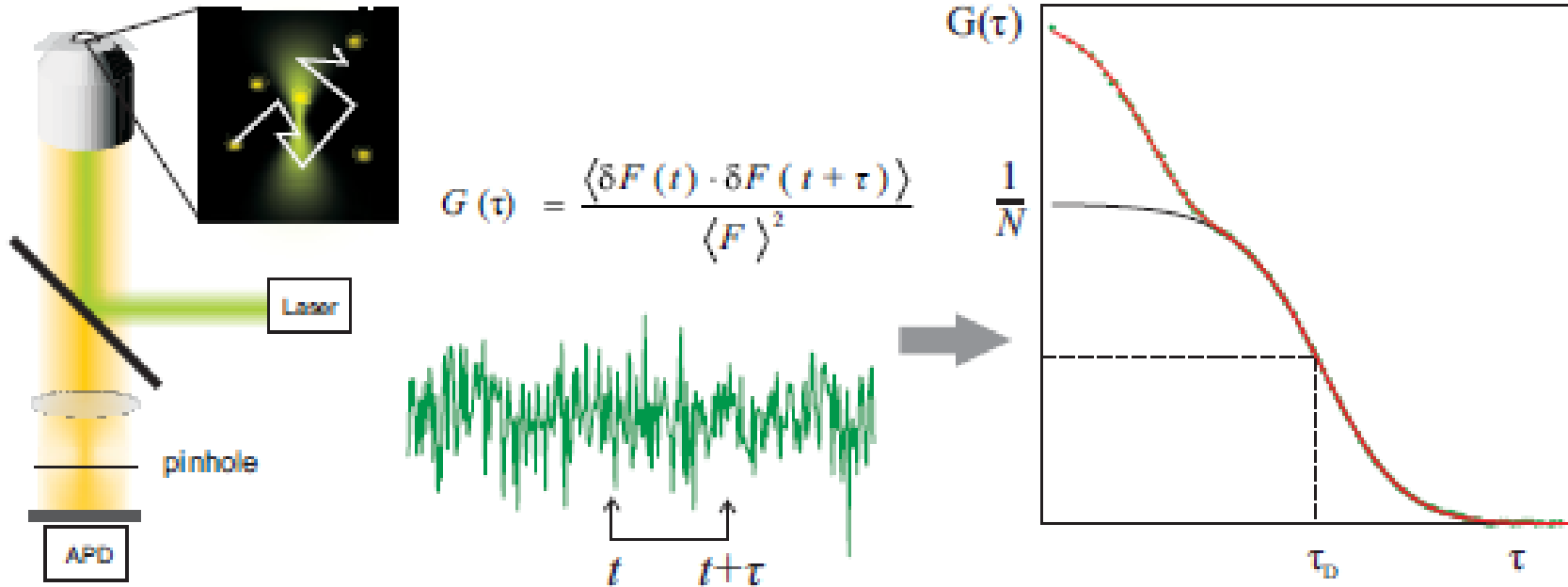
COS-7 cells incubated with Equinatoxin II-Alexa 488 and Equinatoxin II-Alexa 555.

Important results:

- **Interactions**

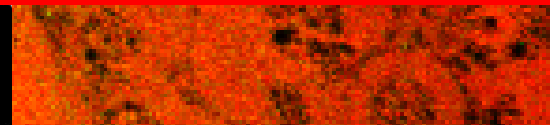
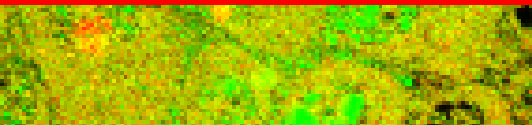
Kushal Das
Raquel Salvador Gallego

Fluorescence Correlation Spectroscopy

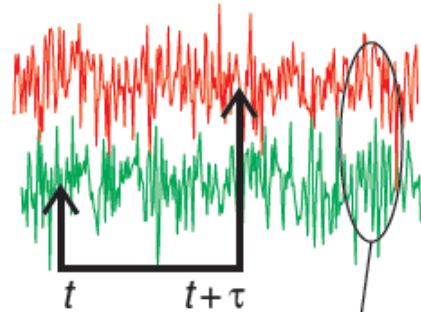
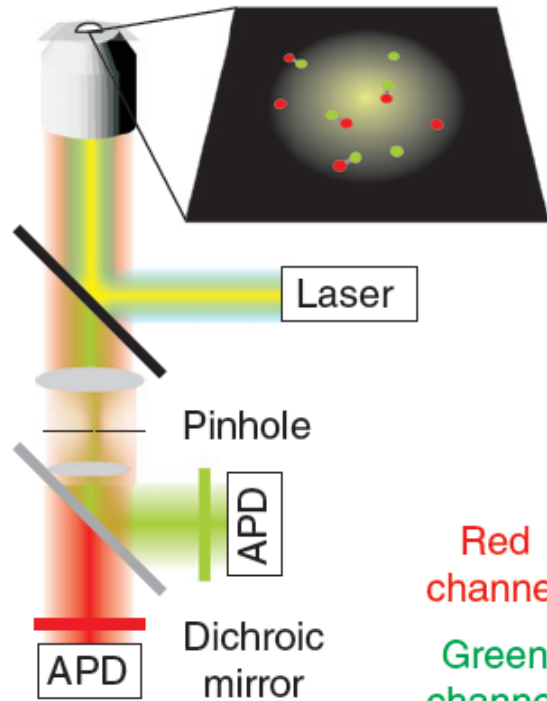


Important Results:

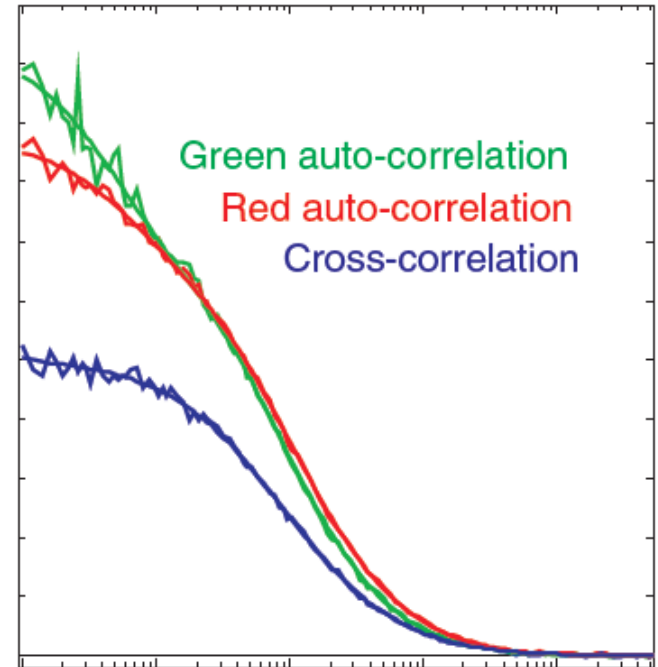
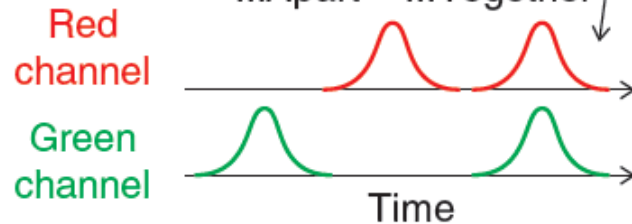
- Accurate concentration of particles
- Diffusion coefficient \rightarrow Size of a molecule



Two-color Fluorescence Cross Correlation Spectroscopy

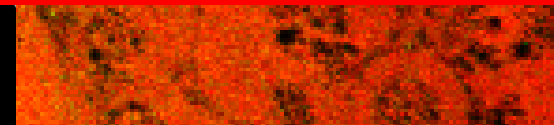
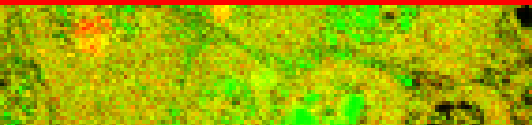


(b) ● ● ●
 ...Apart ...Together

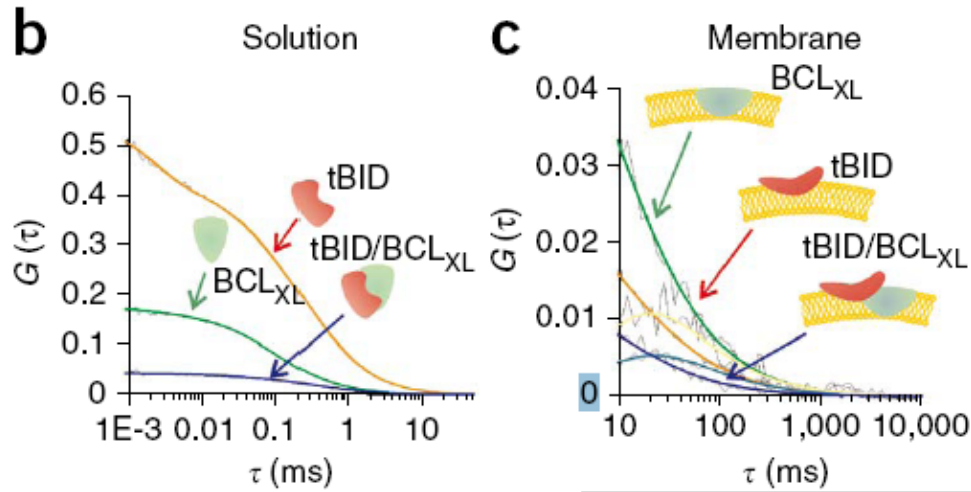


Important Results:

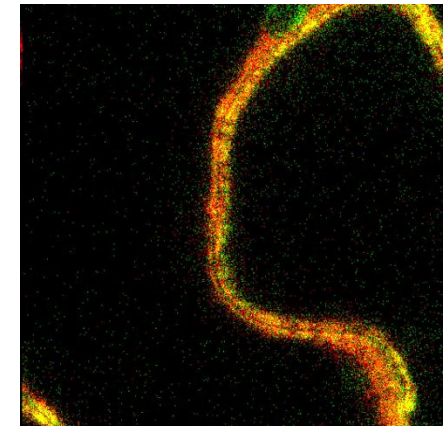
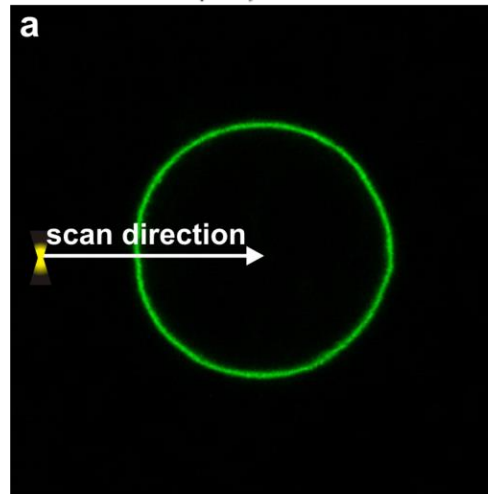
- Diffusion characteristics (D)
- Interaction (K_D)



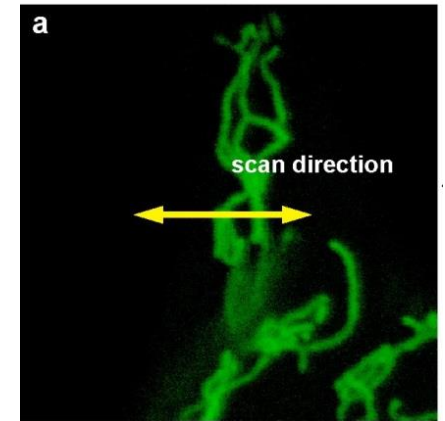
Fluorescence Correlation Spectroscopy



García-Sáez AJ, Ries J, Orzáez M, Pérez-Payà E, Schwille P (2009) *Nat Struct Mol Biol* 16:1178-1185.



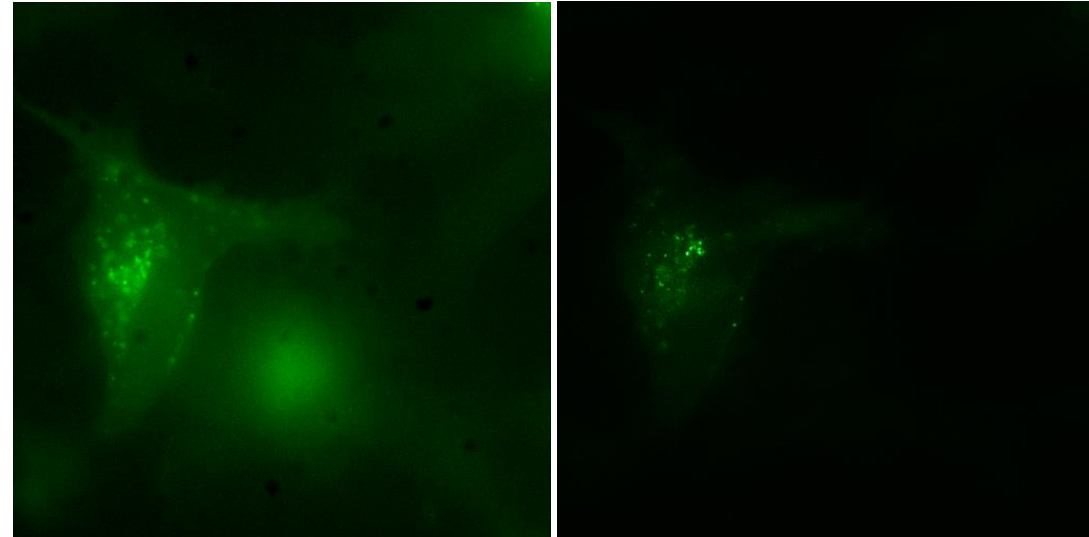
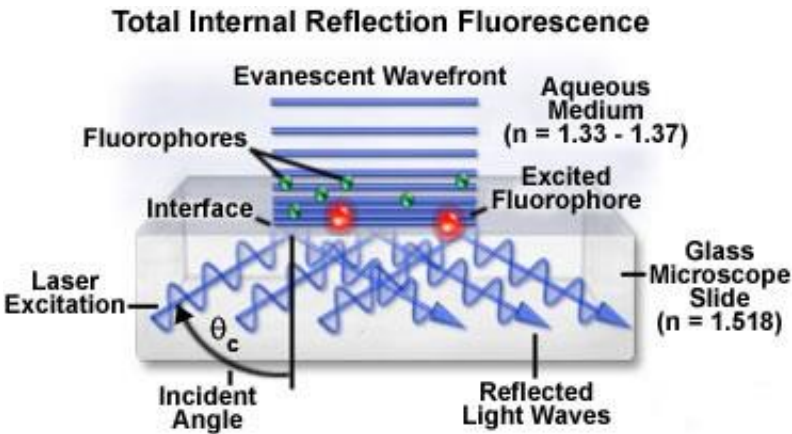
Plasma Membrane



Mitochondria

Stephanie Bleicken
Joseph Unsay

Total Internal Reflection Fluorescence (TIRF) Microscopy



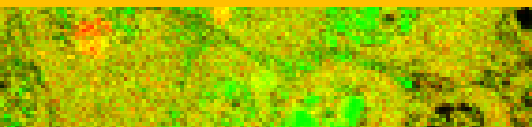
Epifluorescence

TIRF

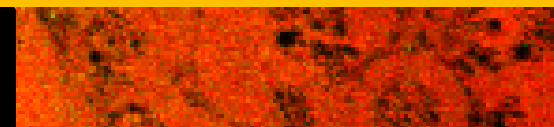
VEGF receptor 2-GFP
overexpressed in HEK cells

Important Results:

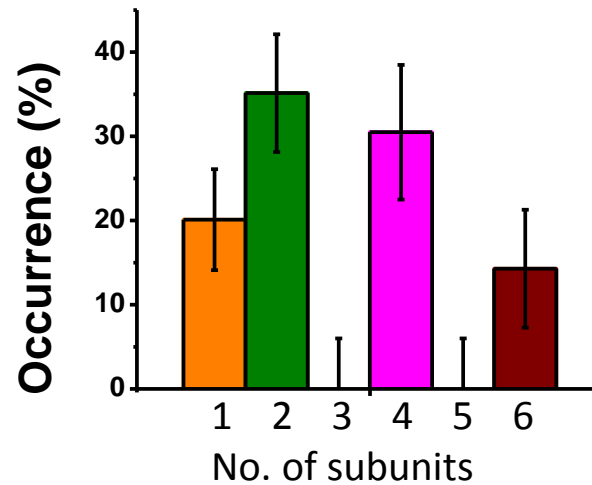
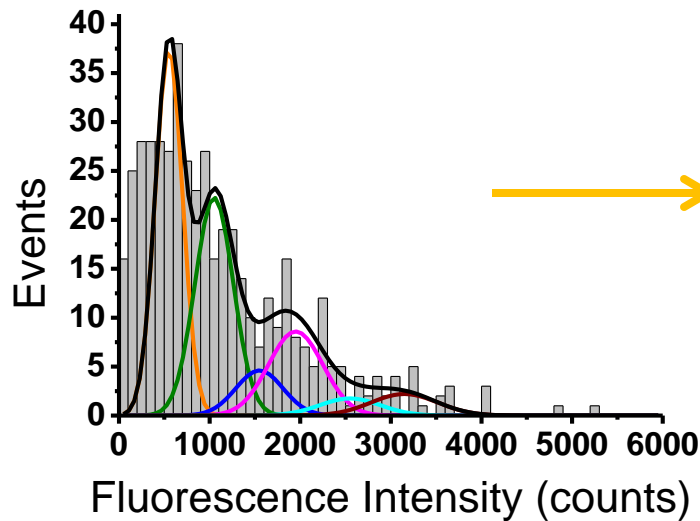
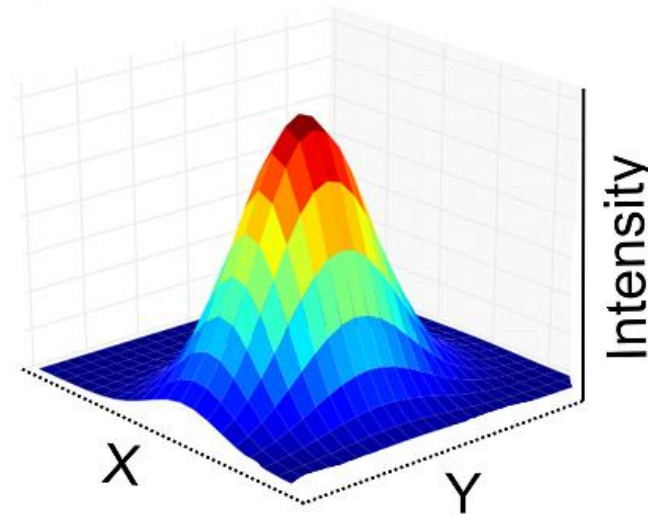
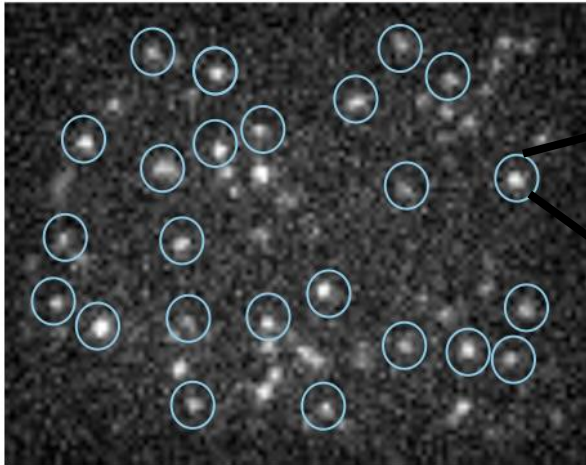
Interactions and Dynamics ONE MOLECULE/PARTICLE AT A TIME



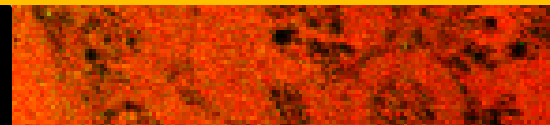
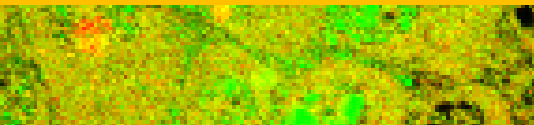
Membrane biophysics



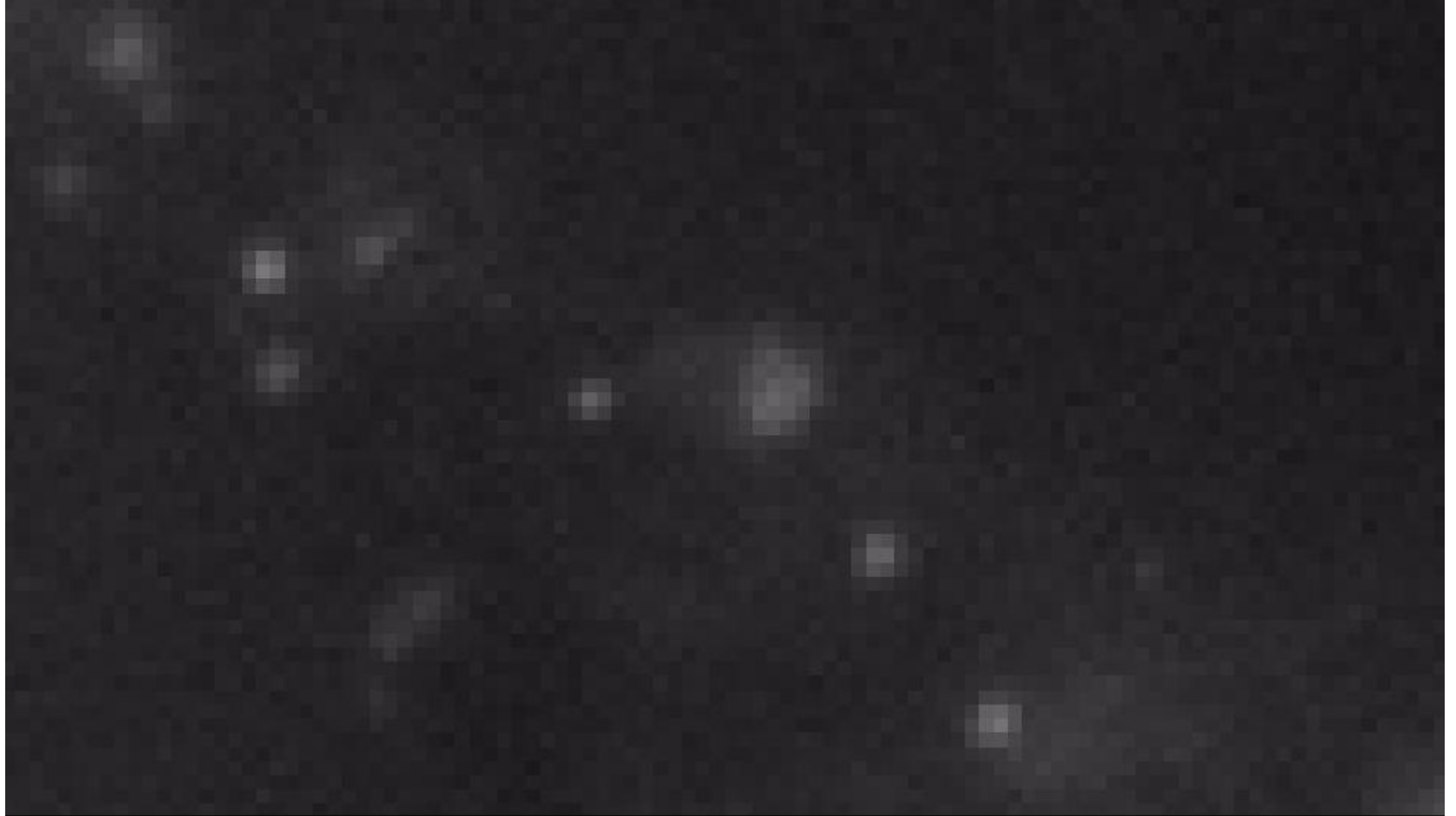
Stoichiometry of Particles using TIRF



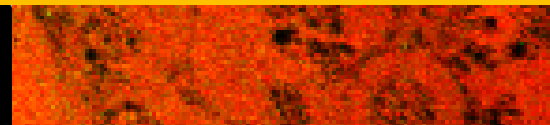
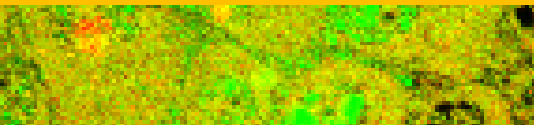
Katia Cosentino
Eduard Hermann
Yamunadevi Subburaj



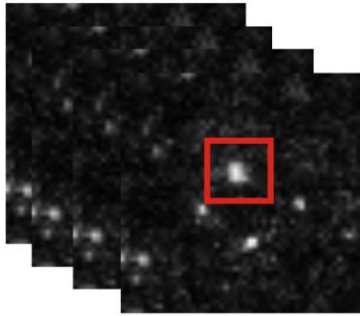
Particle Tracking in TIRF



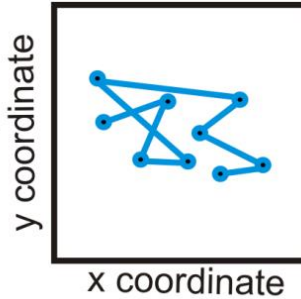
COS-7 cells incubated with Equinatoxin II-Alexa 488



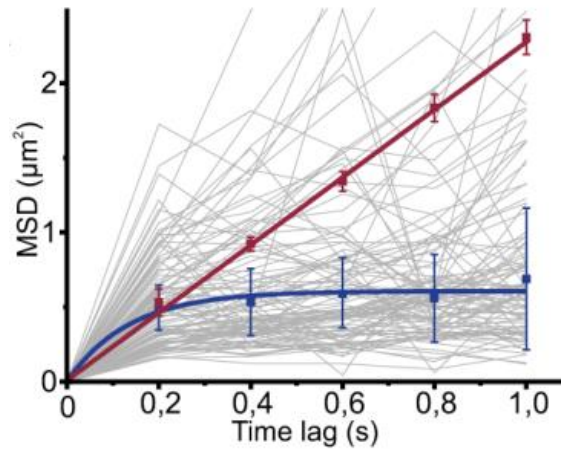
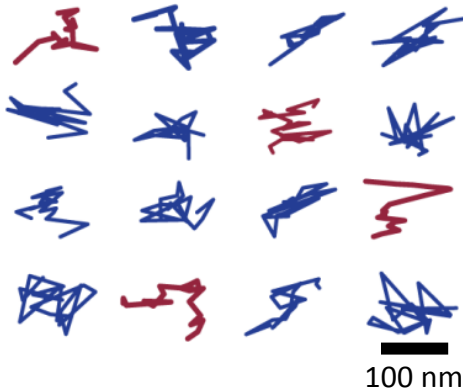
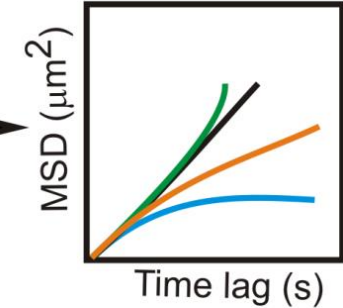
Detection
over time



Trajectory
determination



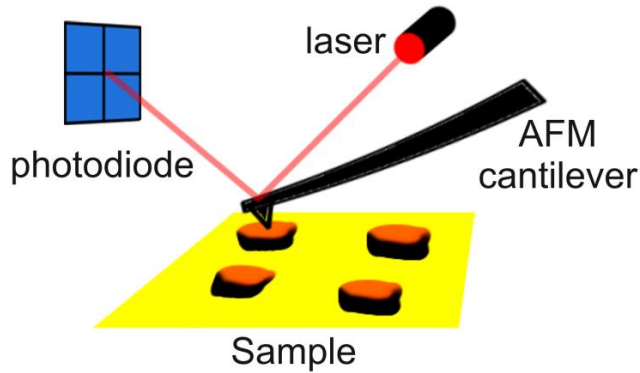
Trajectory
analysis



Brownian motion

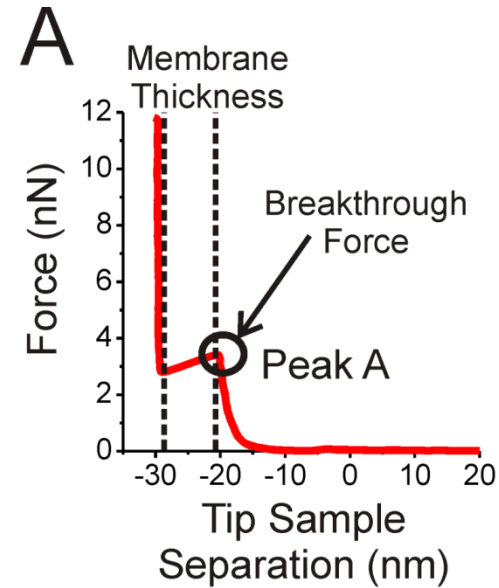
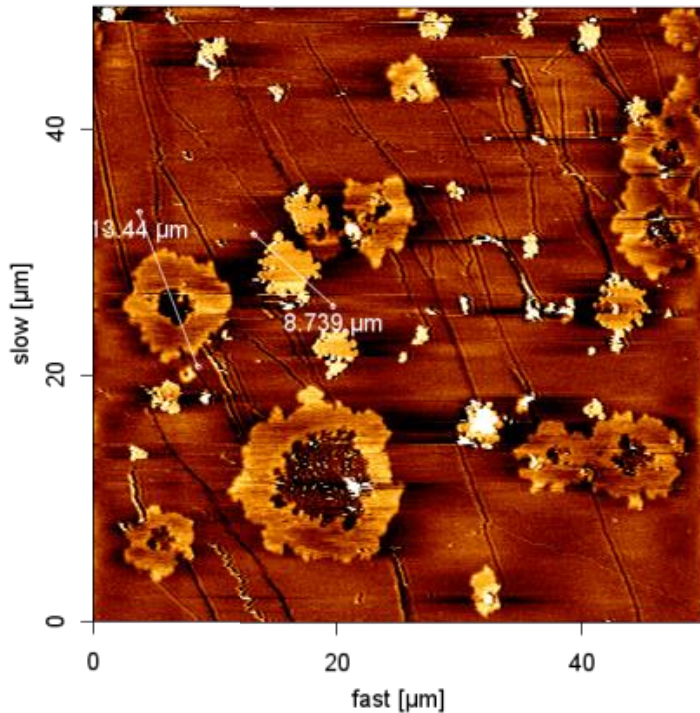
Confined diffusion

Katia Cosentino
Yamunadevi Subburaj

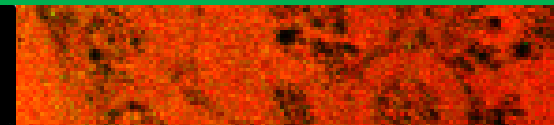
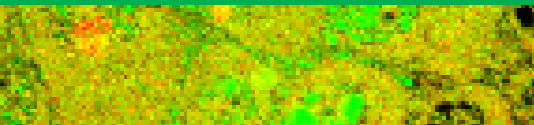


Important Results:

- High-resolution imaging (nm to sub- μm in xy, and nm in z)
- Force measurements



Katia Cosentino
Joseph Unsay



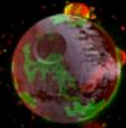
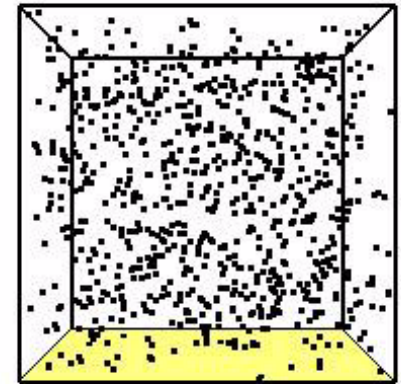
Other Capabilities

- **Biochemistry**

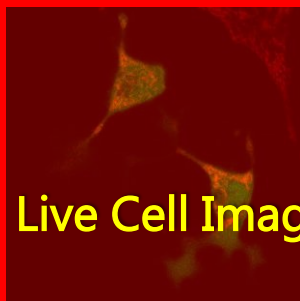
- Full range of biochemistry techniques for cloning, and protein purification
- Expertise in cell culture

- **Computational Approaches**

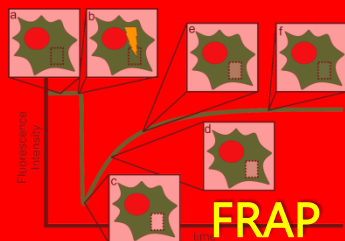
- Software development for data analysis
- Particle-based simulations



Confocal Microscopy



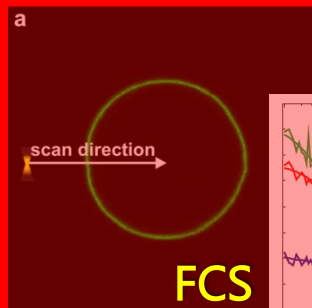
Live Cell Imaging



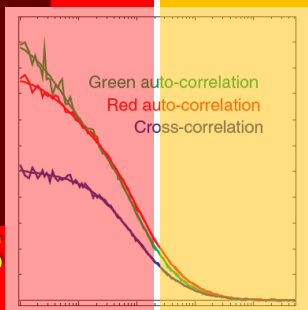
FRAP



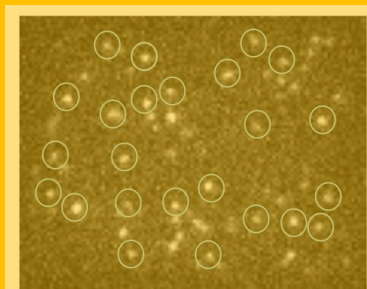
FRET



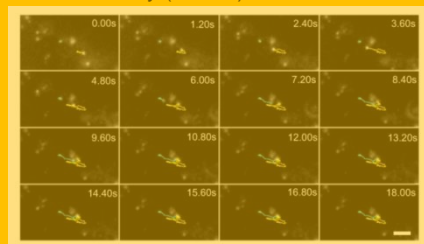
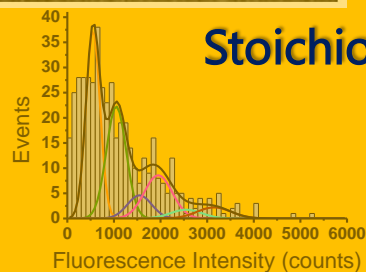
FCS
FCCS



Single Molecule Microscopy

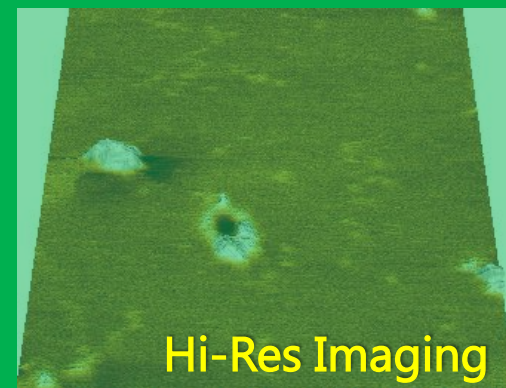


Stoichiometry

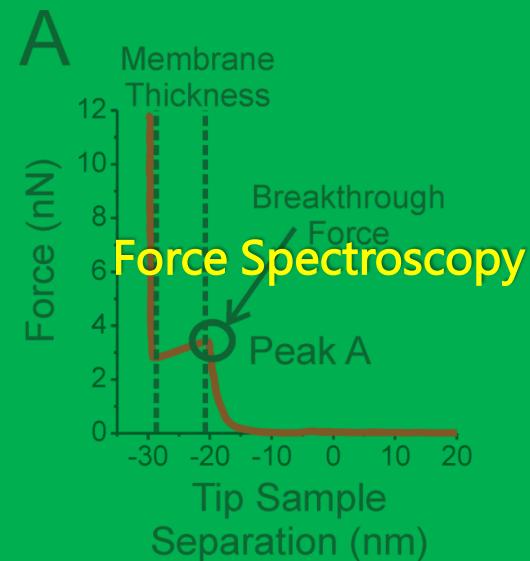


Tracking

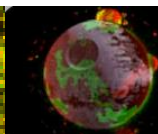
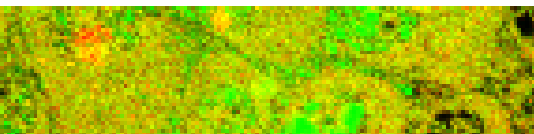
Atomic Force Microscopy



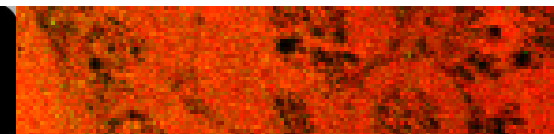
Hi-Res Imaging

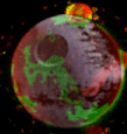


Biochemistry +
Computational
Capabilities



Membrane biophysics





Membrane biophysics



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ana.garcia@uni-tuebingen.de

Group website: <http://www.ifib.uni-tuebingen.de/research/garcia-saez.html>

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Joseph Unsay



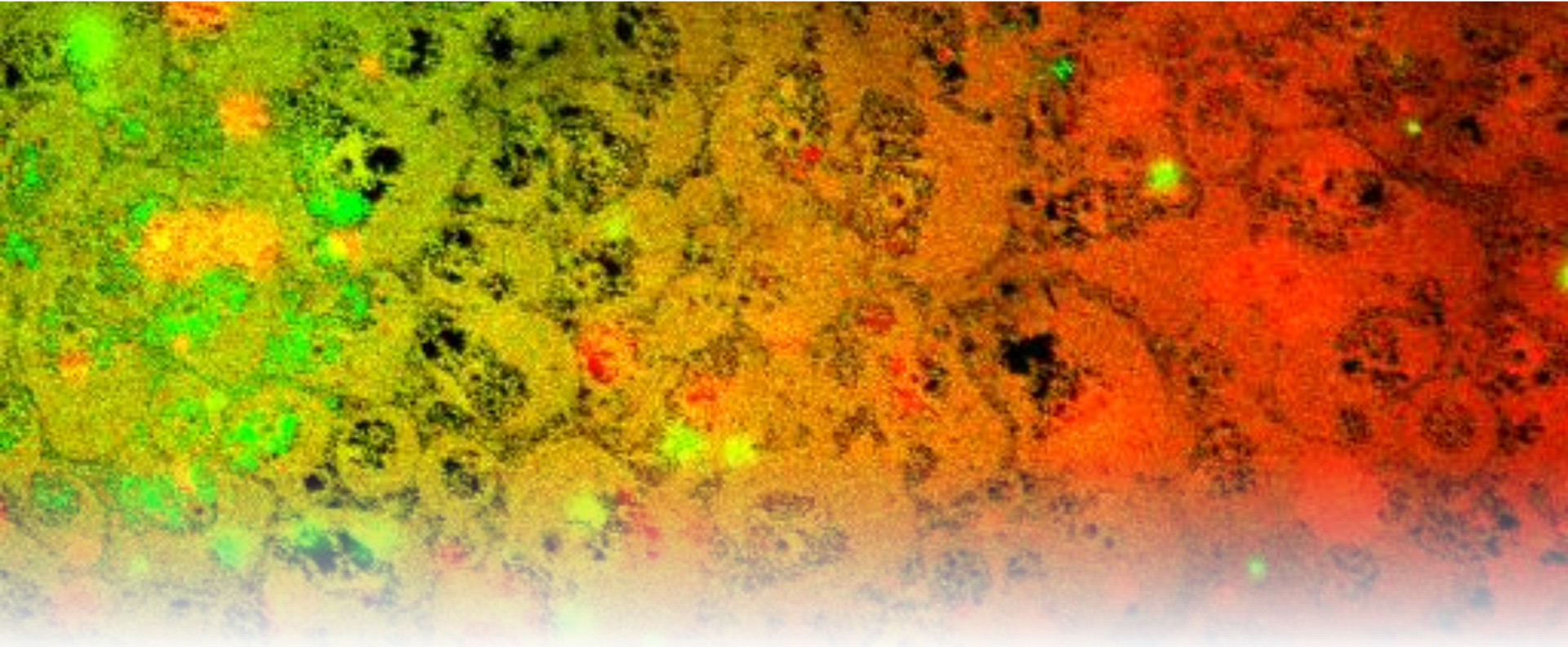
MAX-PLANCK-GESELLSCHAFT

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UNIVERSITÄT
TÜBINGEN





Membrane biophysics



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