Single molecule methods to study cellular processes in inflammation

Prof. Dr. Ana J. García Sáez
Membrane Biophysics
• Bcl-2 Proteins
• Other Pore-forming proteins
• Mitochondrial dynamics

+ Danger Signals

Live Cell

Apoptosis

Phagocytosis

IL-10, TGF-β

INFLAMMATION

Necroptosis

Membrane biophysics
Confocal Microscopy
Single Molecule Microscopy
Atomic Force Microscopy

Functional Microscopy

Membrane biophysics
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).
Fluorescence Recovery After Photobleaching (FRAP)

Fas receptor-GFP overexpressed in plasma membrane of living cells

Important results:
- Diffusion
- Mobilities

Florence Sanchez
Raquel Salvador Gallego
**Förster Resonance Energy Transfer (FRET)**

**A**
- Protein labeled with Donor Fluorophore (ex. CFP)
- Protein labeled with Acceptor Fluorophore (ex. YFP)

**B**
- d > 10 nm

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

Important Results:
- Diffusion characteristics (D)
- Interaction ($K_D$)
Fluorescence Correlation Spectroscopy

Total Internal Reflectance Fluorescence (TIRF) Microscopy

VEGF receptor 2-GFP overexpressed in HEK cells

Important Results:
Interactions and Dynamics ONE MOLECULE/PARTICLE AT A TIME
Stoichiometry of Particles using TIRF

- Confocal Microscopy
- Single Molecule Microscopy
- Atomic Force Microscopy

Katia Cosentino
Eduard Hermann
Yamunadevi Subburaj

Membrane biophysics
Confocal Microscopy

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Particle Tracking in TIRF

COS-7 cells incubated with Equinatoxin II-Alexa 488
Important Results:

- High-resolution imaging (nm to sub-\(\mu\)m in xy, and nm in z)
- Force measurements
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
Atomic Force Microscopy

Live Cell Imaging

Confocal Microscopy

Single Molecule Microscopy

Stoichiometry

Tracking

FRET

FCS

FCCS

Hi-Res Imaging

Force Spectroscopy

FRA P

Biochemistry + Computational Capabilities

Membrane biophysics
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