

8th Regional Biophysics Conference 2018 – Zreče, Slovenia

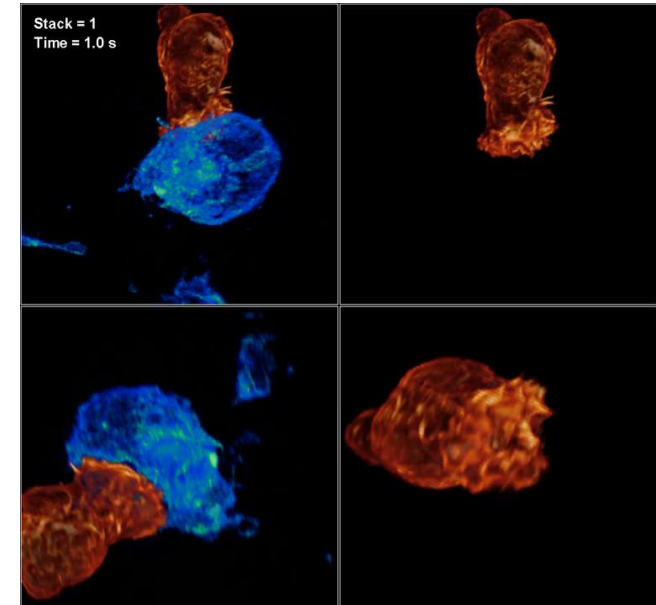
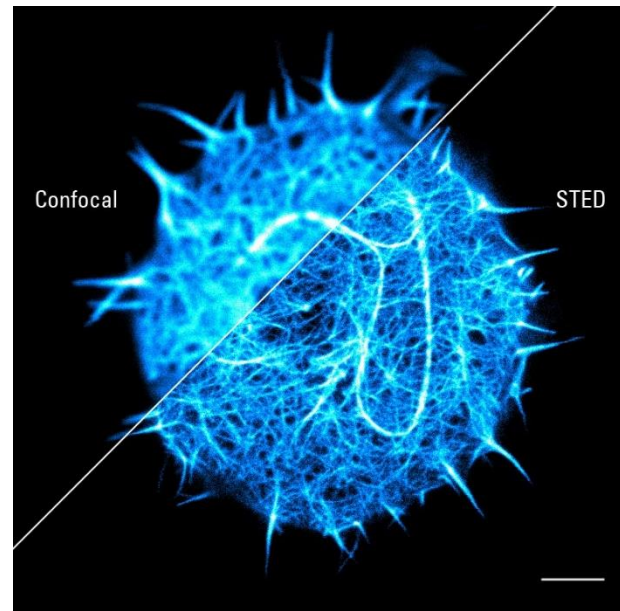
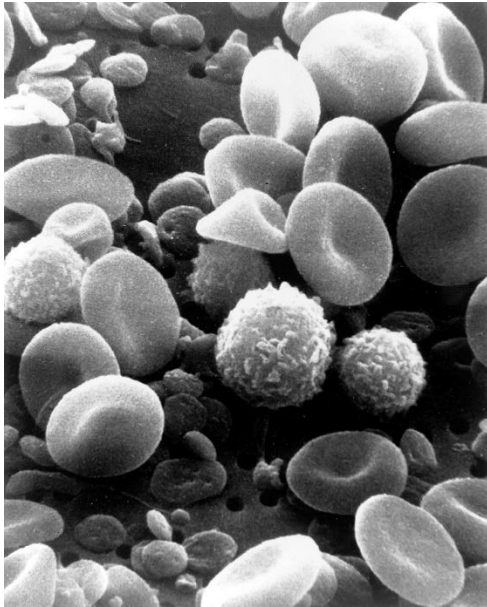
Advanced STED microscopy of the membrane organisation in activated T-cells

Iztok Urbančič

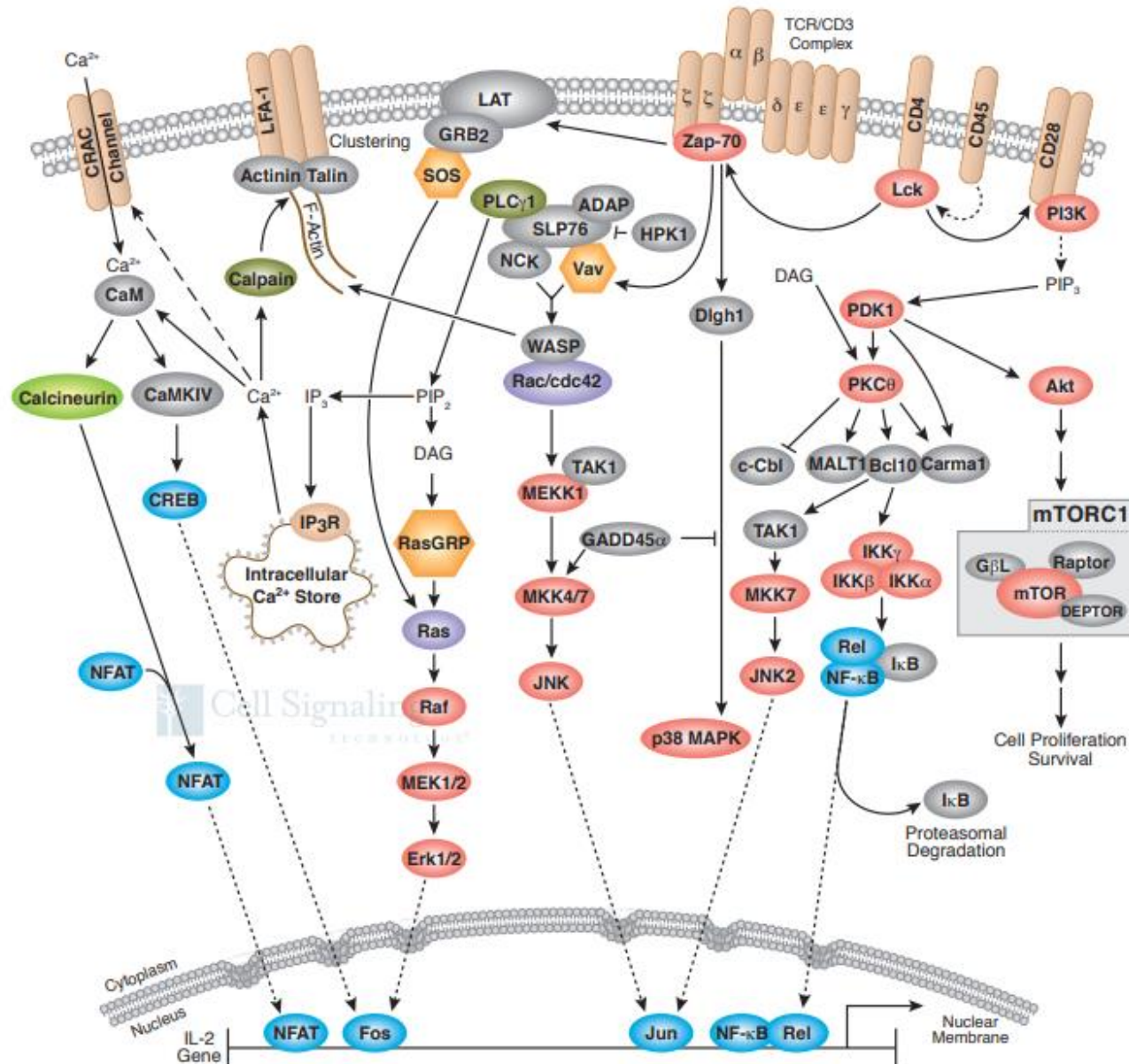
Nano-immunology - Eggeling Lab, MRC Human Immunology Unit,
Weatherhall Institute of Molecular Medicine, University of Oxford, UK

Laboratory of Biophysics, Condensed Matter Physics Department,
„Jožef Stefan“ Institute, Ljubljana, Slovenia

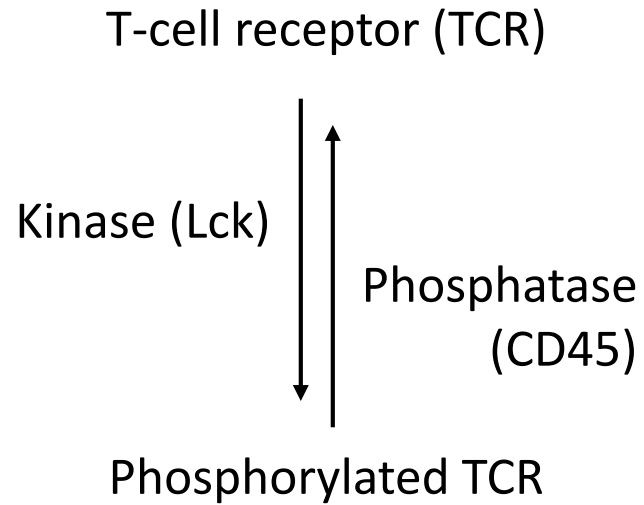
T-cells – key element of the immune system



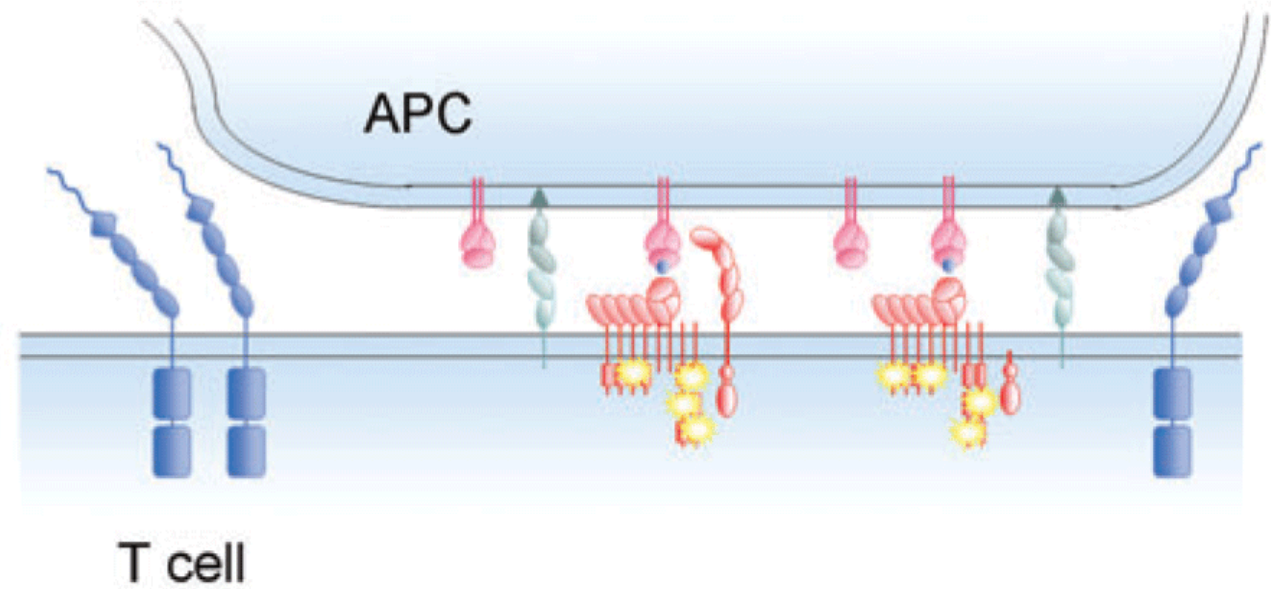
T-cell signalling



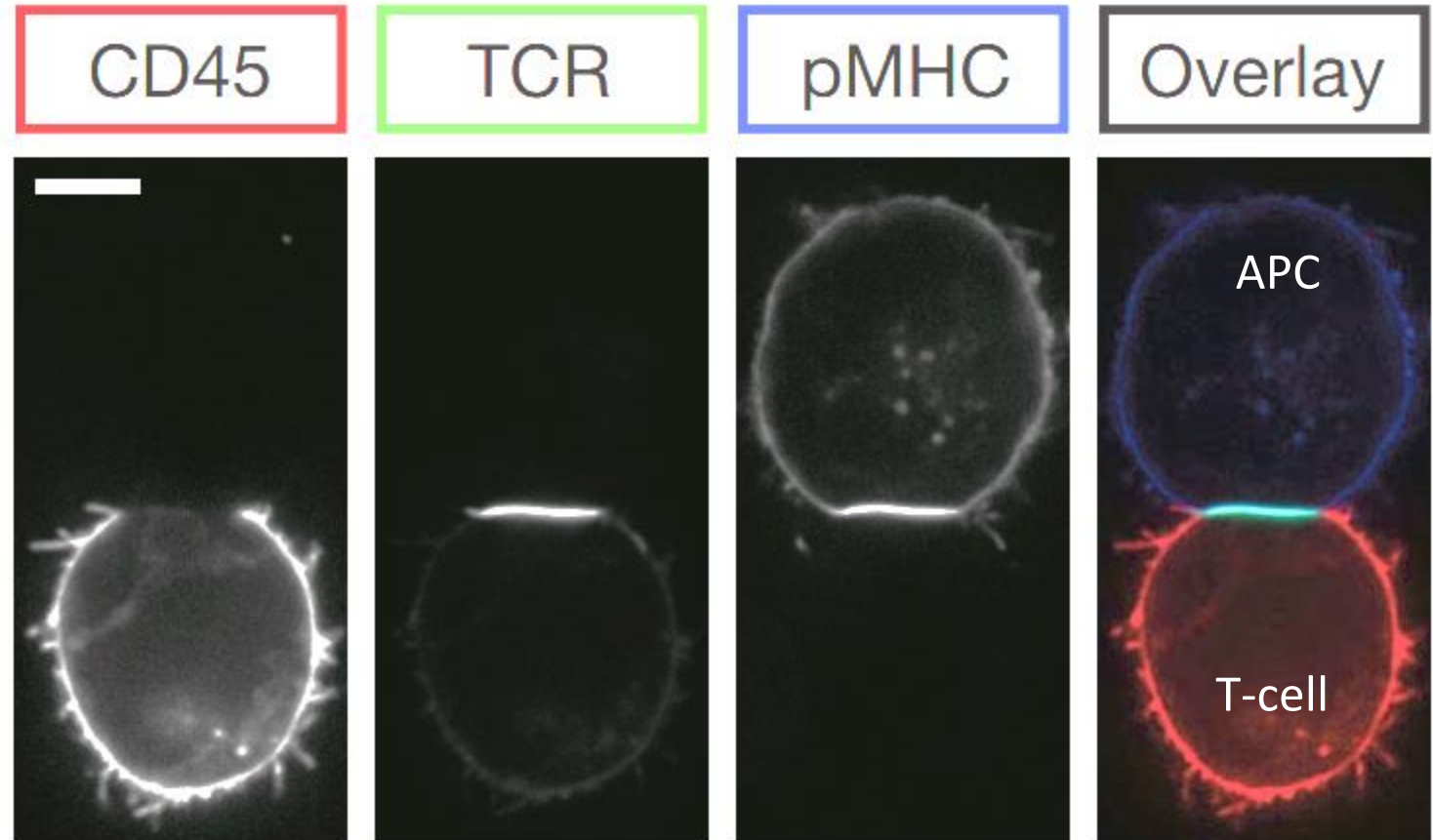
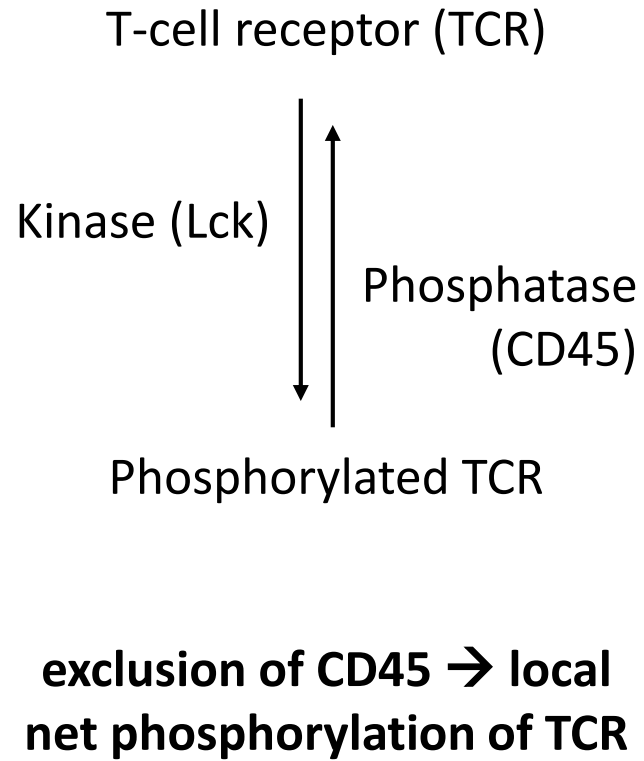
T-cell triggering – Kinetic-segregation model



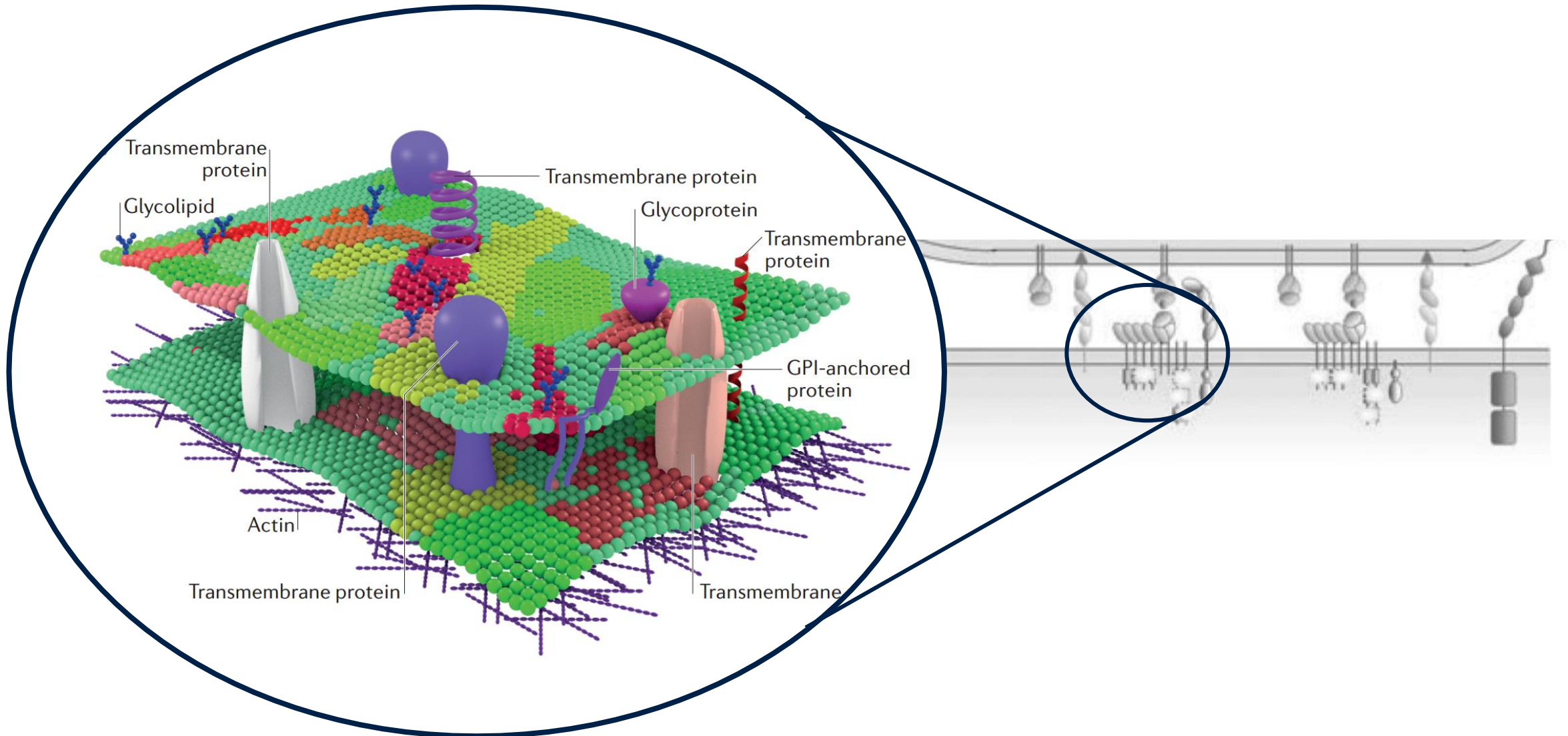
exclusion of CD45 → local net phosphorylation of TCR



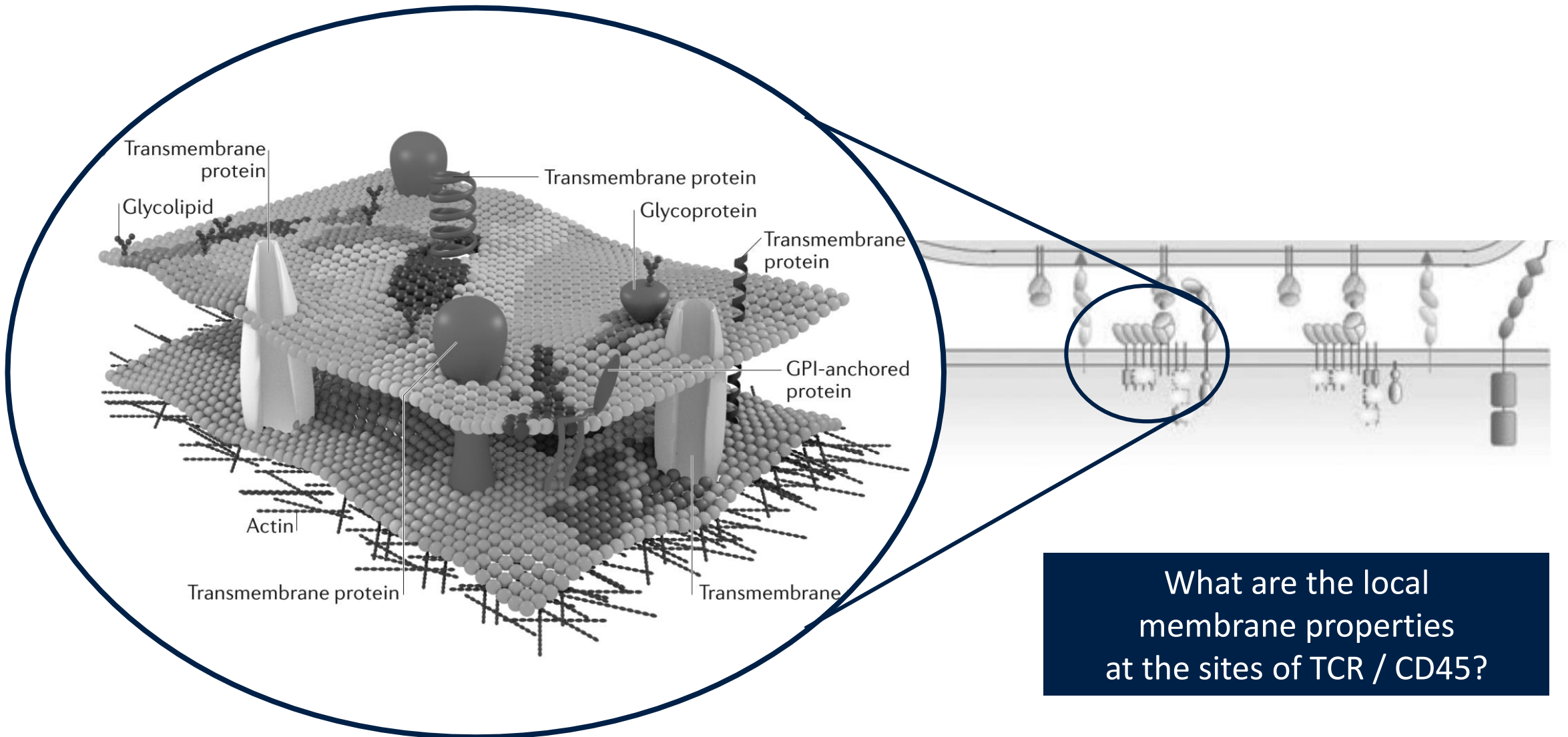
T-cell triggering – Kinetic-segregation model



Roles of the lipid environment in T-cell signaling?

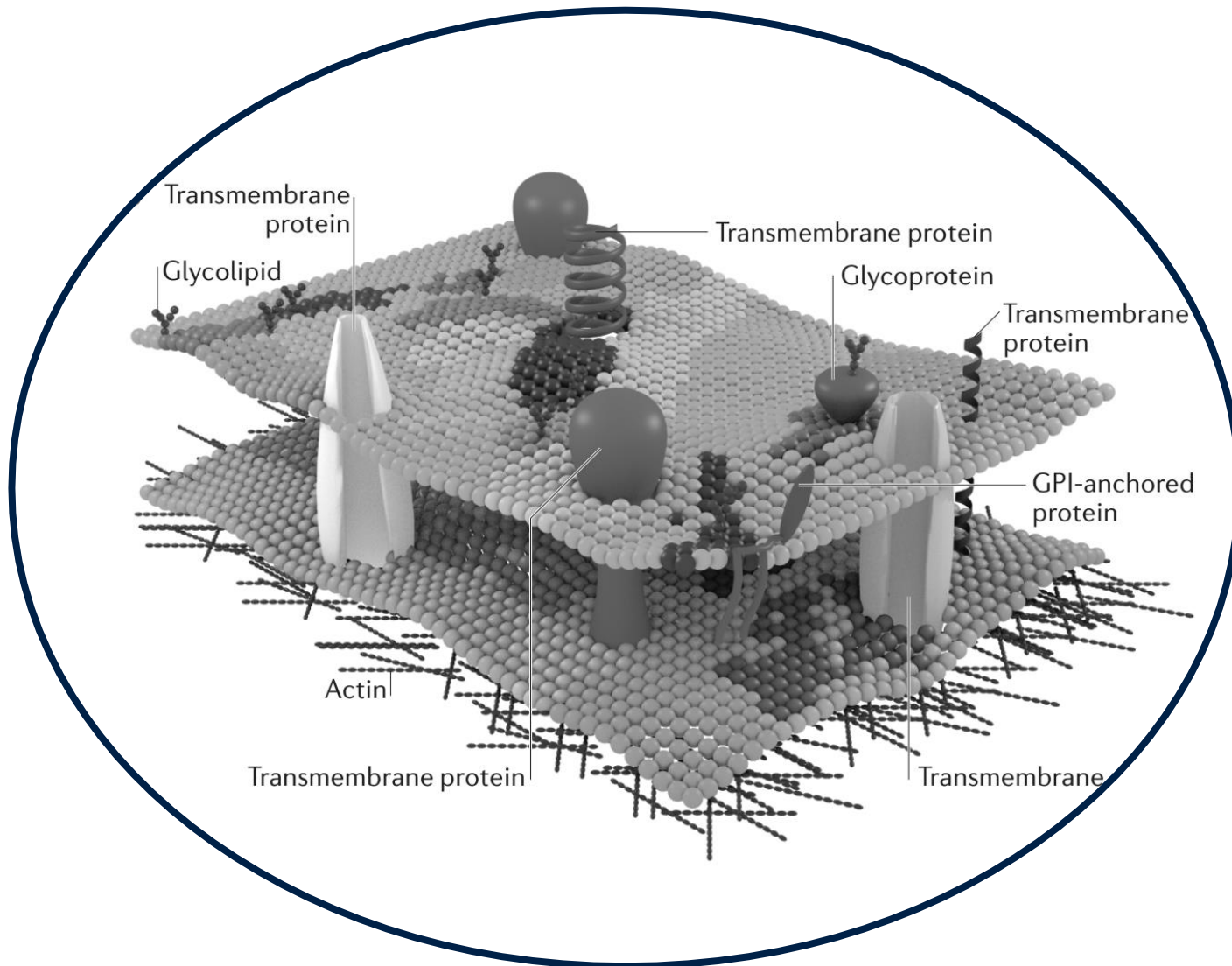


Membrane organisation in T-cells?



What are the local membrane properties at the sites of TCR / CD45?

Local membrane properties to investigate in T-cells



Lipid order

Fluorescence microspectroscopy (spectral imaging) with polarity-sensitive probes

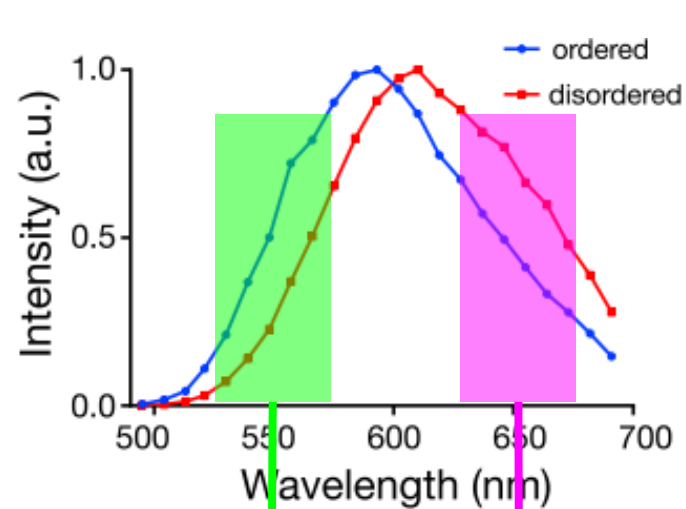
Diffusion rate

Fluorescence correlation spectroscopy (FCS)

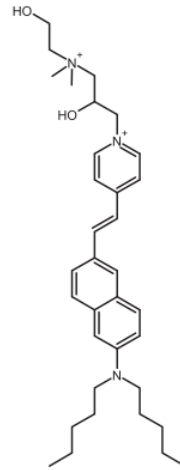
Diffusion modes

Spot-variation (super-resolution)
STED-FCS

Lipid order? Polarity-sensitive probes



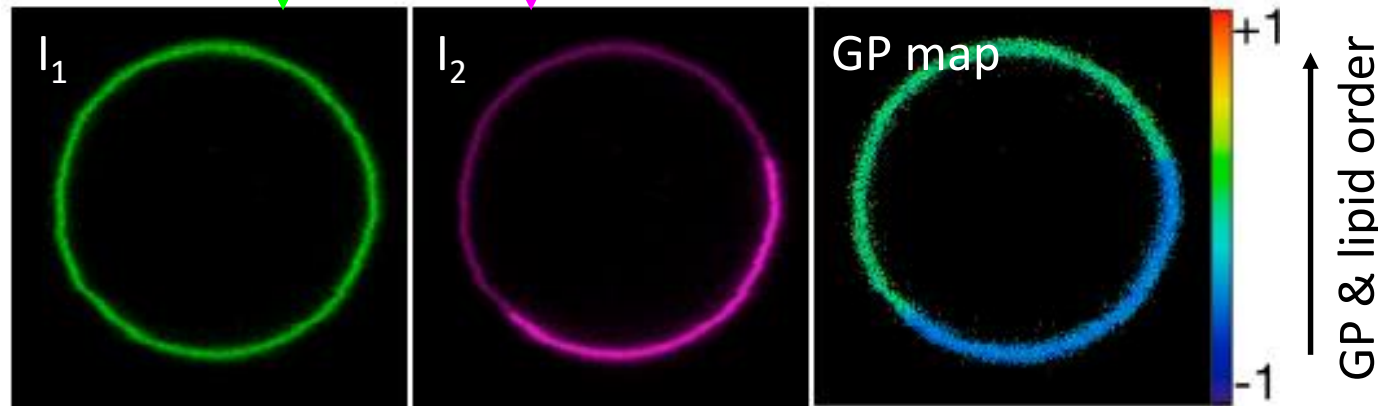
Di-4-ANEPPDHQ



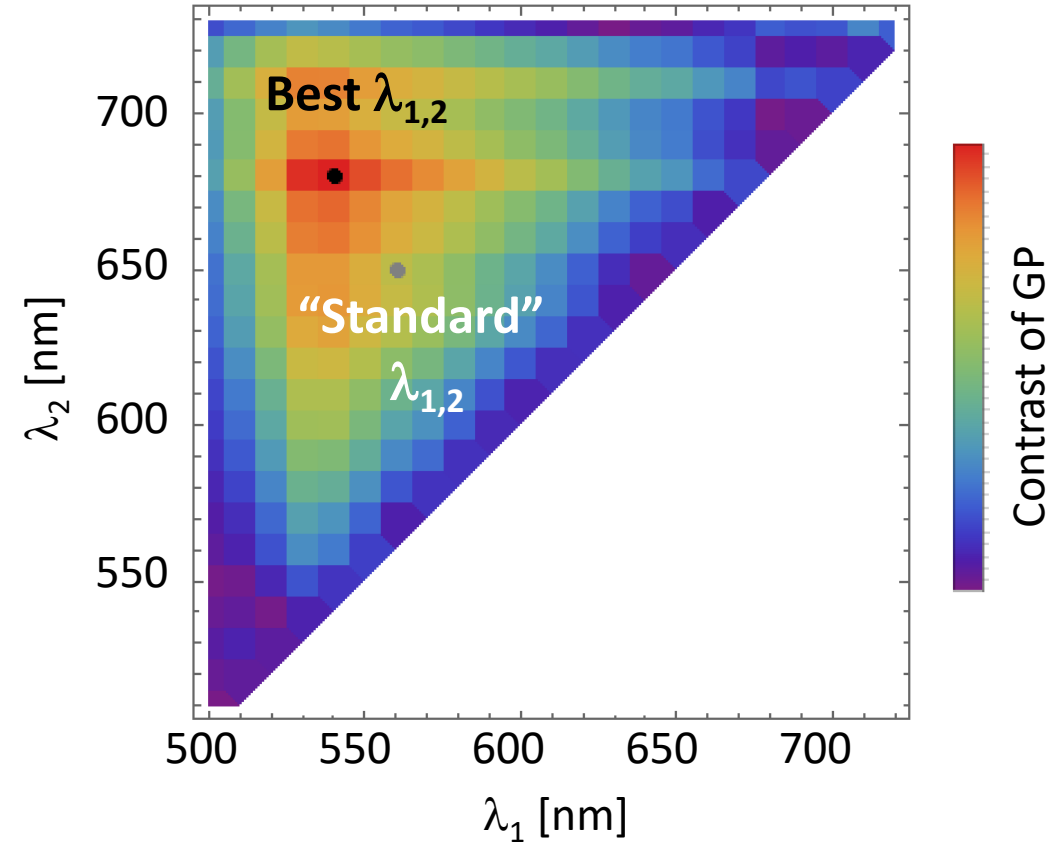
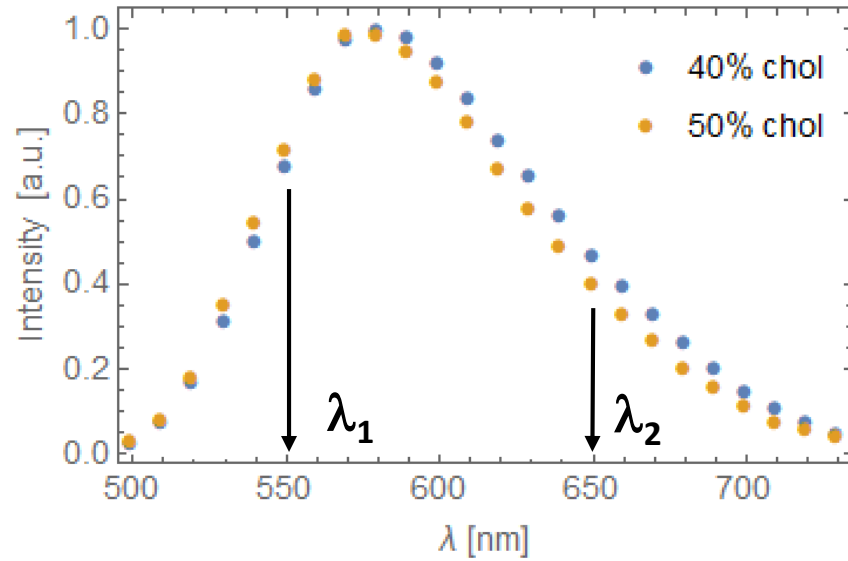
Such probes **red-shift** their emission spectrum in more polar environment, e.g. **disordered membranes**

Empirical index of lipid order:
Generalised Polarisation (GP)

$$GP = \frac{I_1 - I_2}{I_1 + I_2}$$

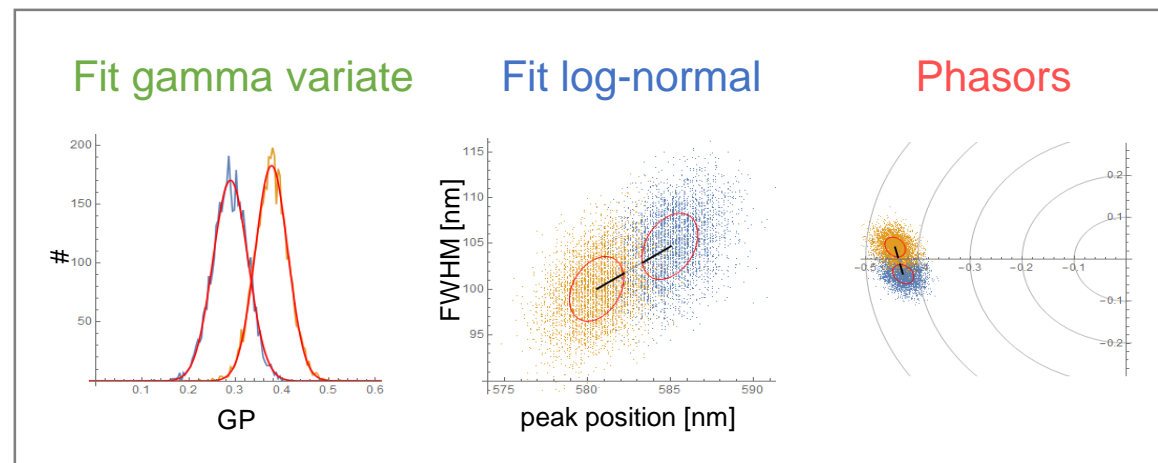
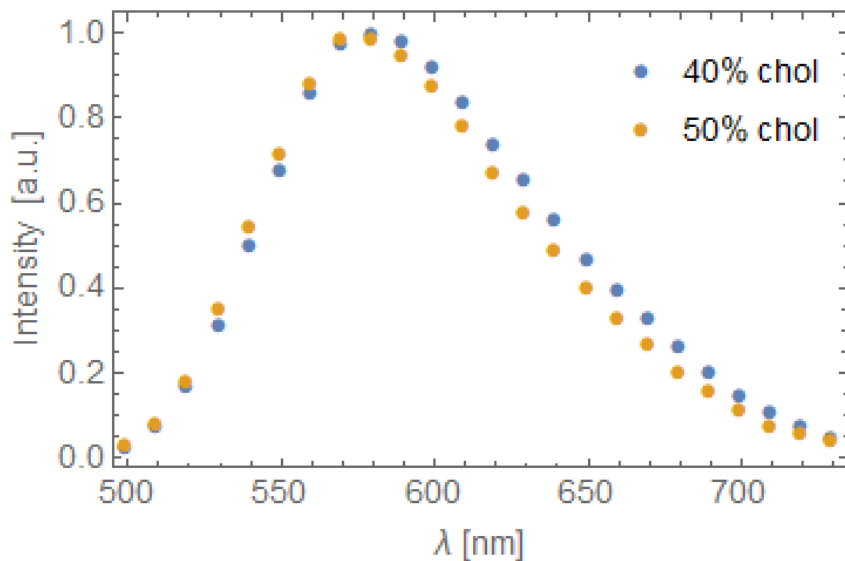


Spectral sensitivity – pitfalls of GP

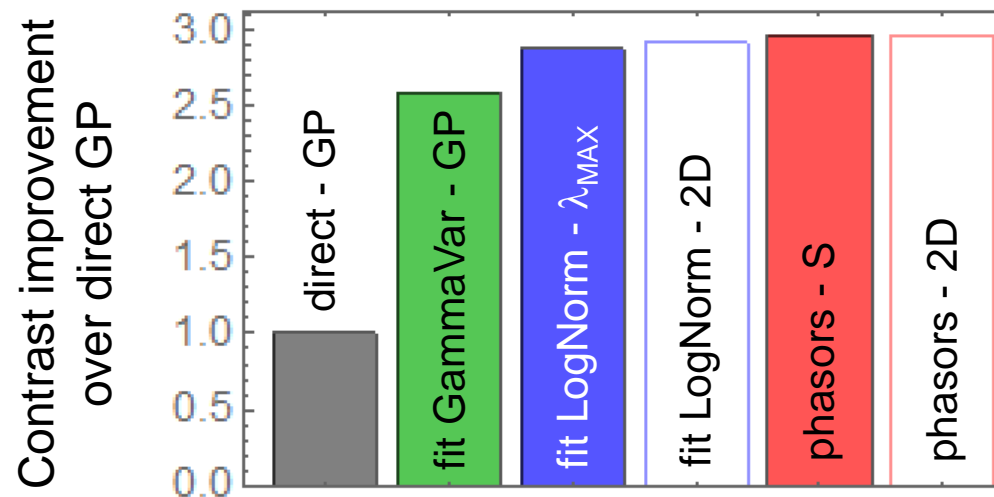


GP contrast depends on the λ -pair

Maximising spectral sensitivity

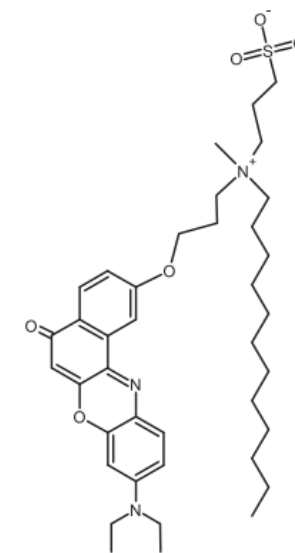


Using all spectral datapoints helps



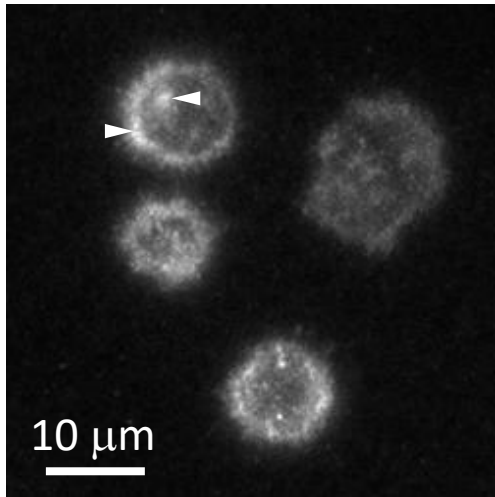
Lipid order in activated T-cells

Spectral imaging
with polarity-sensitive membrane probe NR12S

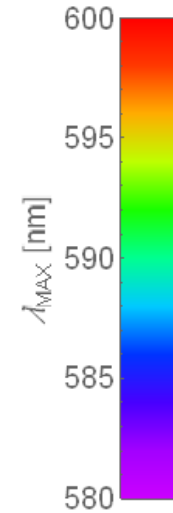
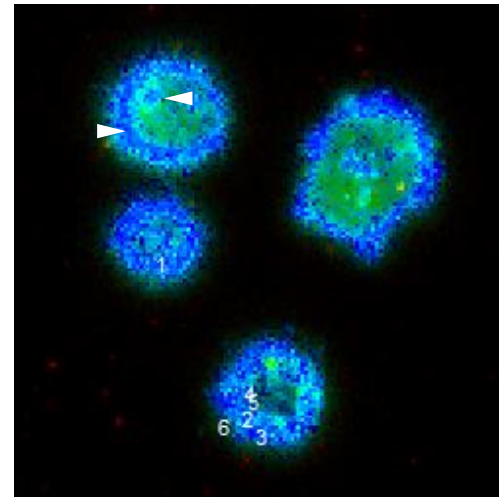


Lipid order in activated T-cells

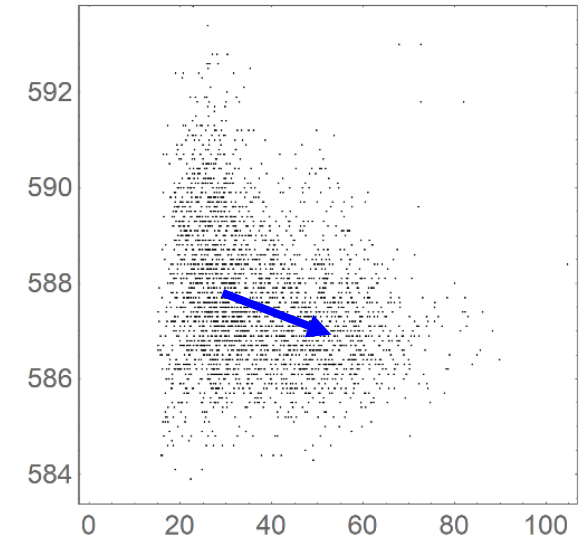
α TCR-633 intensity



NR12S peak position

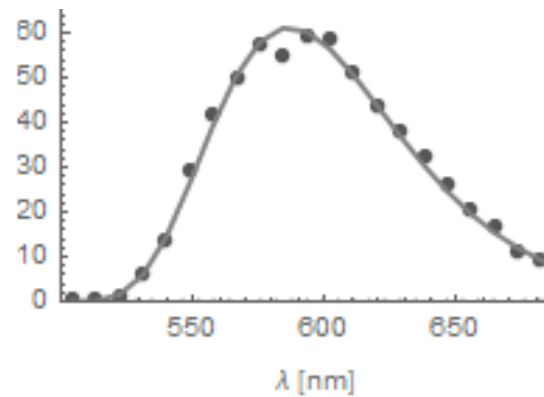


NR12S λ_{MAX} [nm]

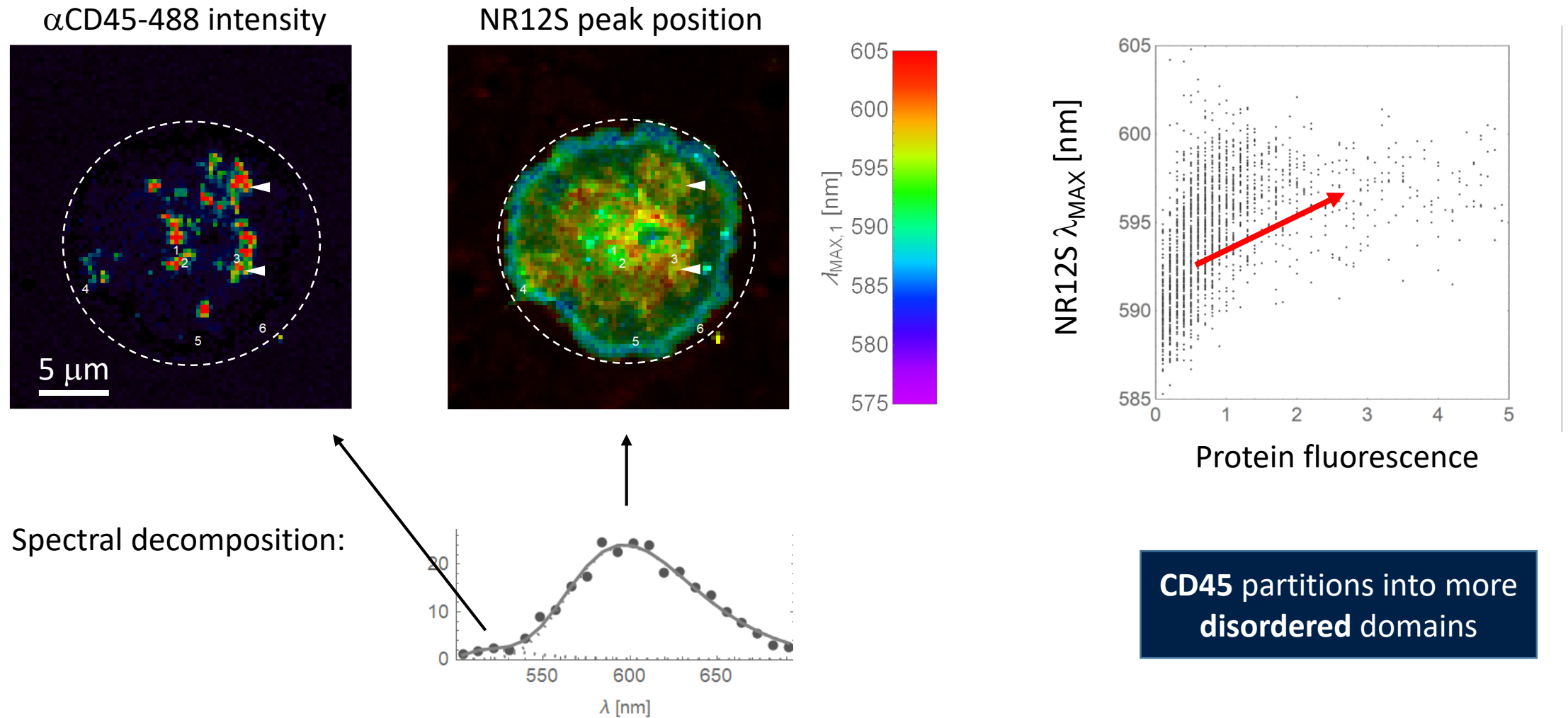


Protein fluorescence

TCR concentrates in more ordered domains



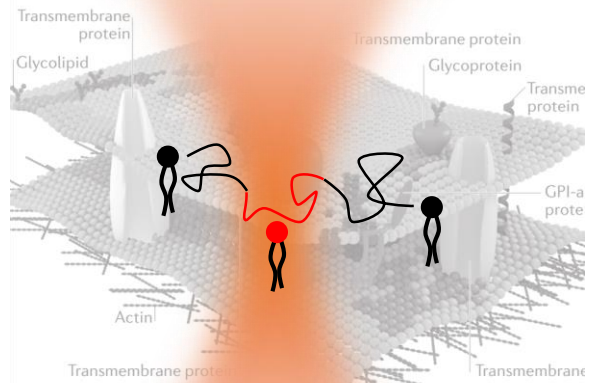
Lipid order in activated T-cells



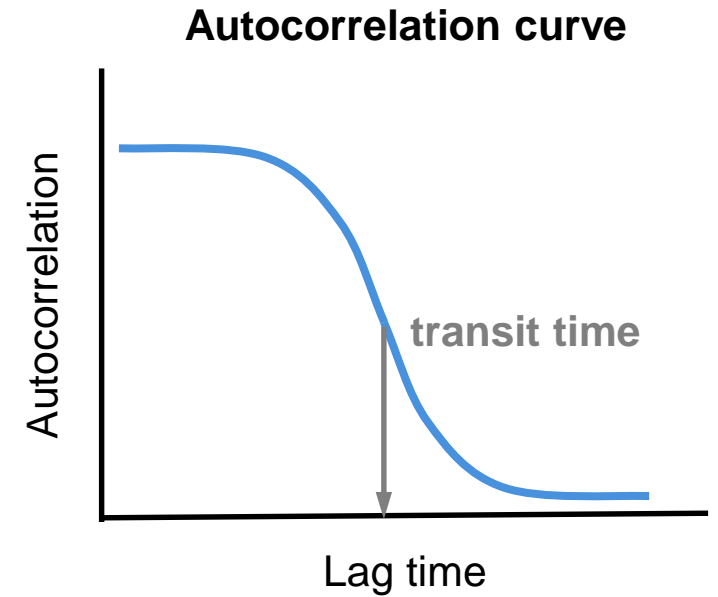
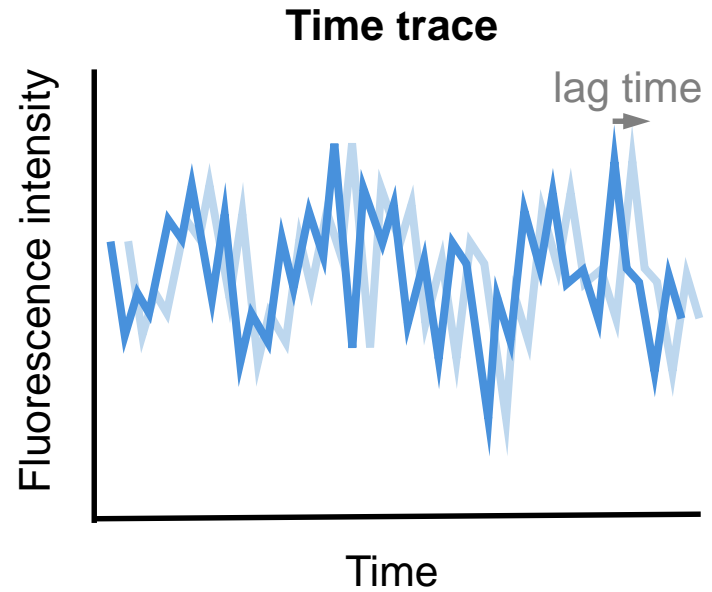
Does the membrane order correlate with the lipid diffusion rates?

Fluorescence correlation spectroscopy (FCS)
with Atto647N-DPPE

Fluorescence Correlation Spectroscopy (FCS)

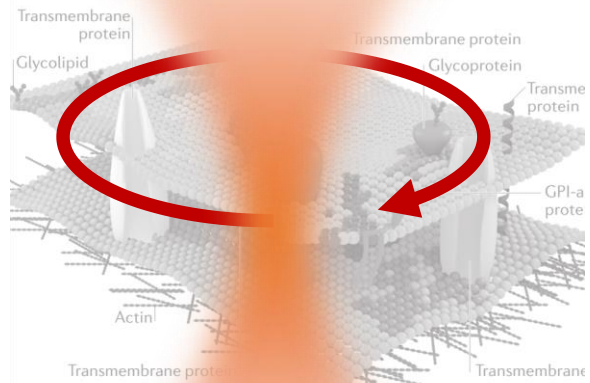


Excitation beam



Diffusion coefficient (D)

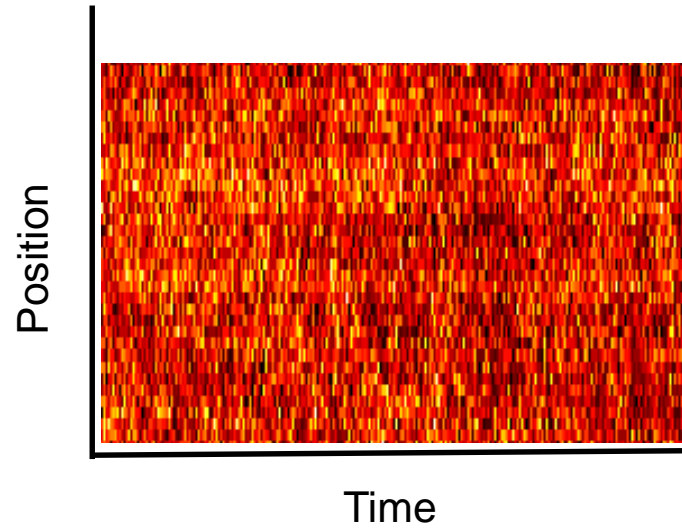
Scanning FCS



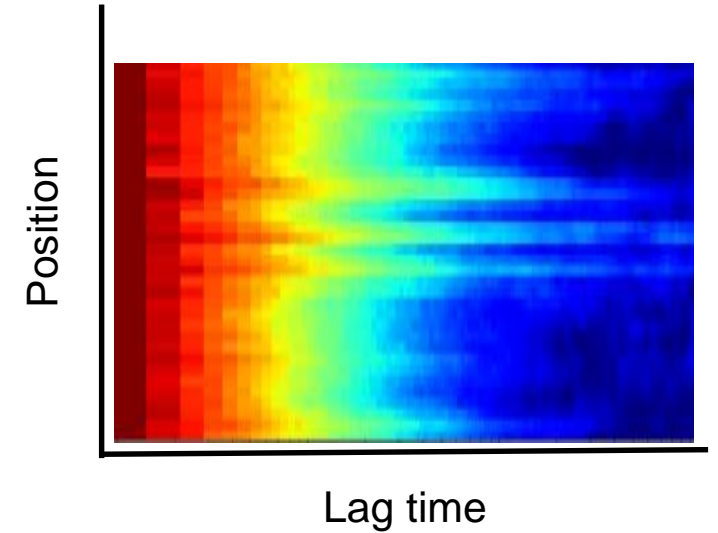
Excitation beam



Time traces

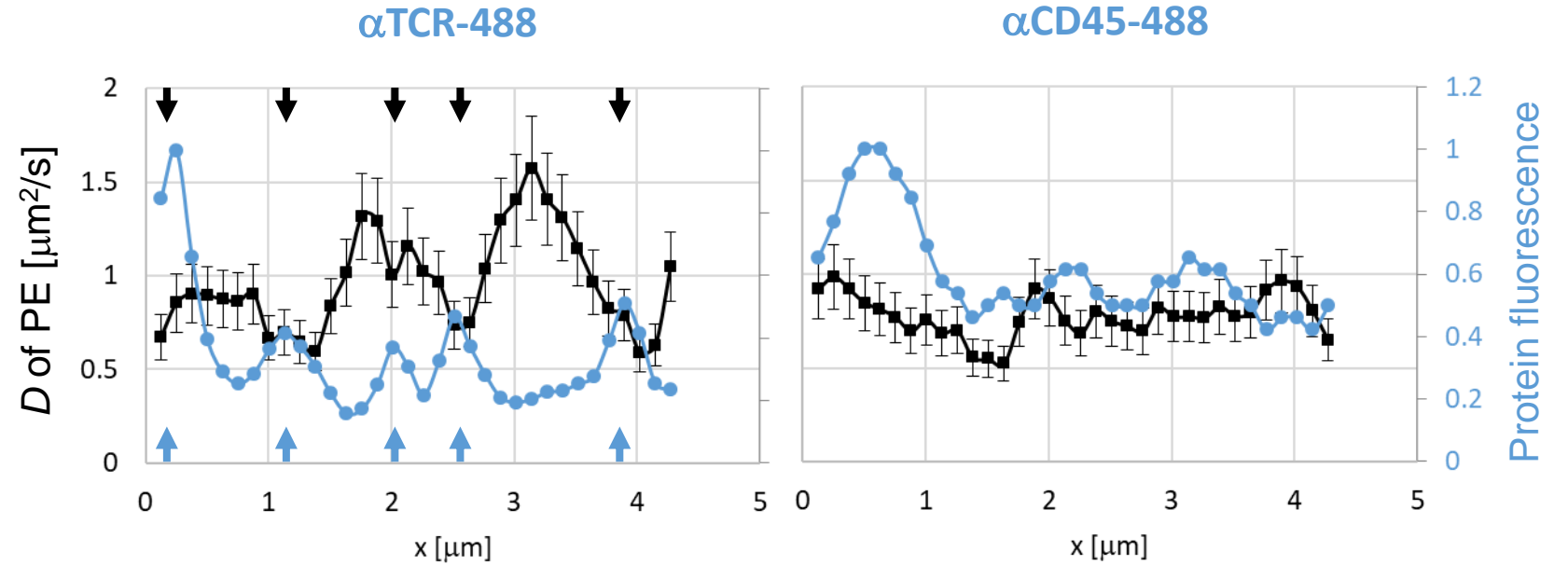
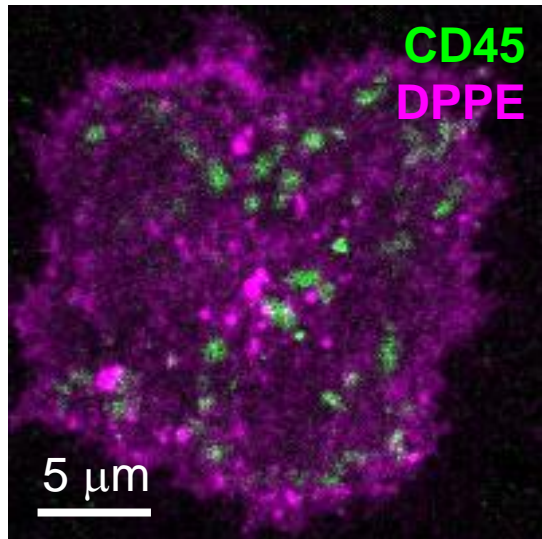


Autocorrelation carpet



Spatial variability of D

Diffusion rates of PE-lipids in activated T-cells

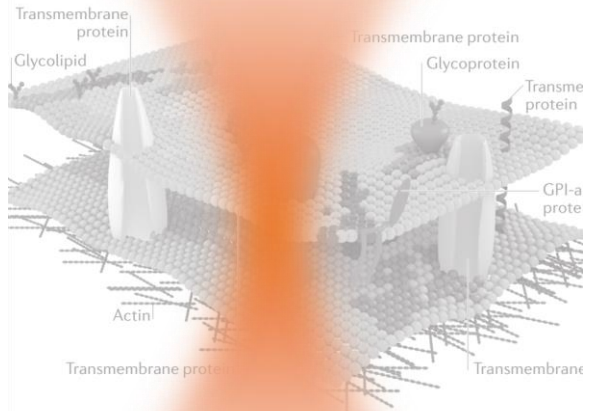


Slowed-down lipid diffusion at
TCR

Do lipids diffuse freely at the sites of TCR / CD45?

Spot-variation super-resolution STED-FCS
with Atto647N-sphingomyelin

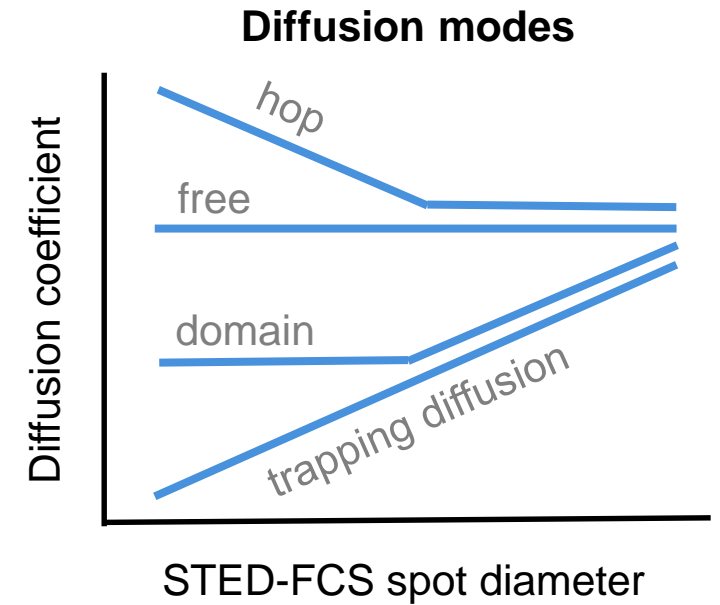
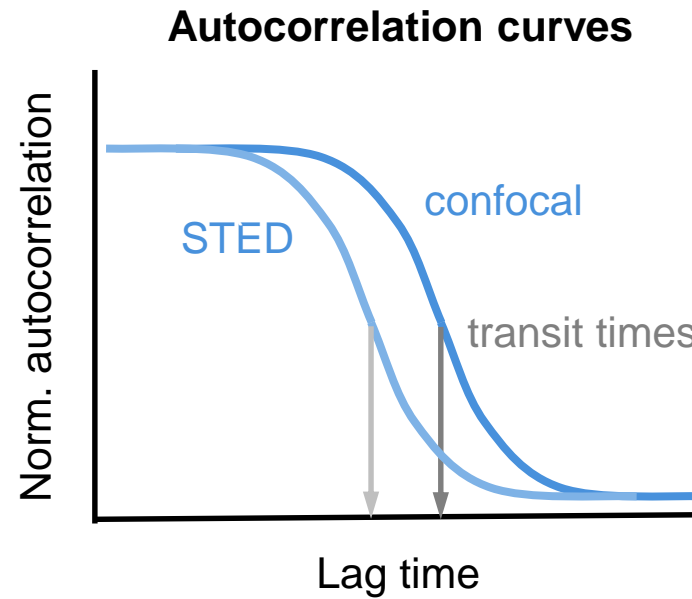
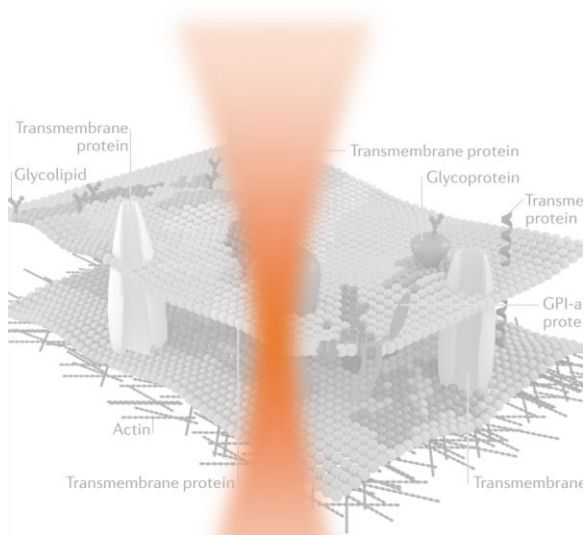
Lipid diffusion modes? Spot-variation STED-FCS



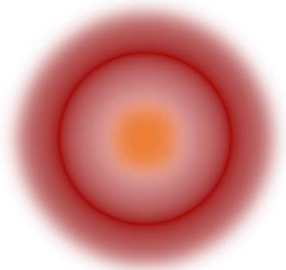
Excitation beam



Lipid diffusion modes? Spot-variation STED-FCS

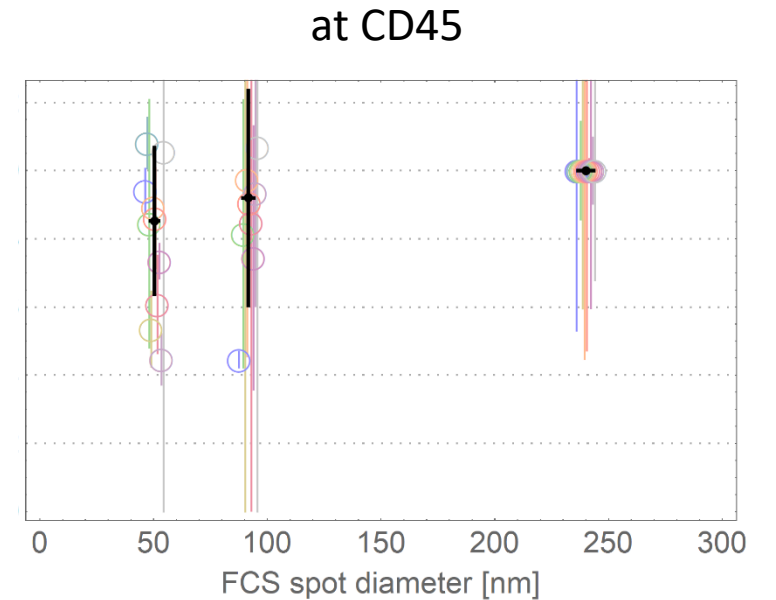
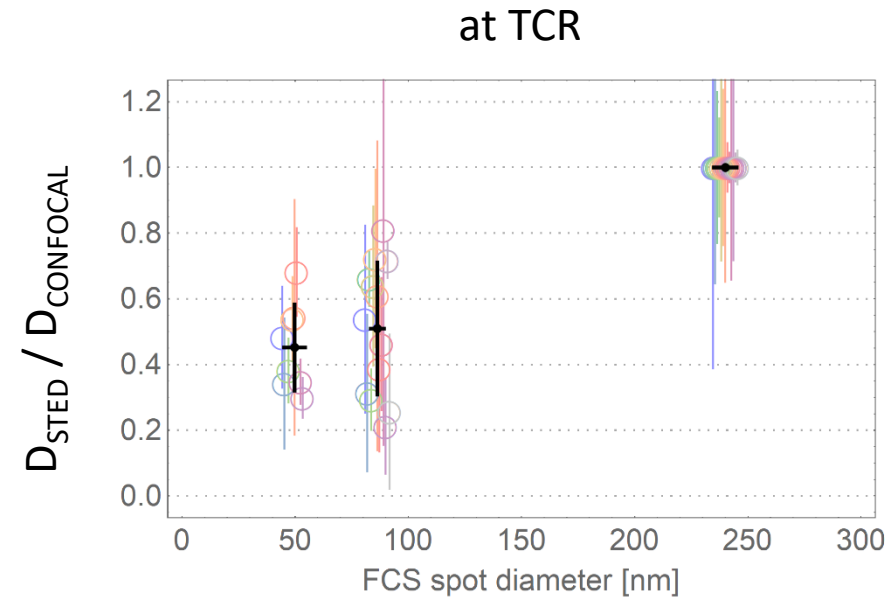
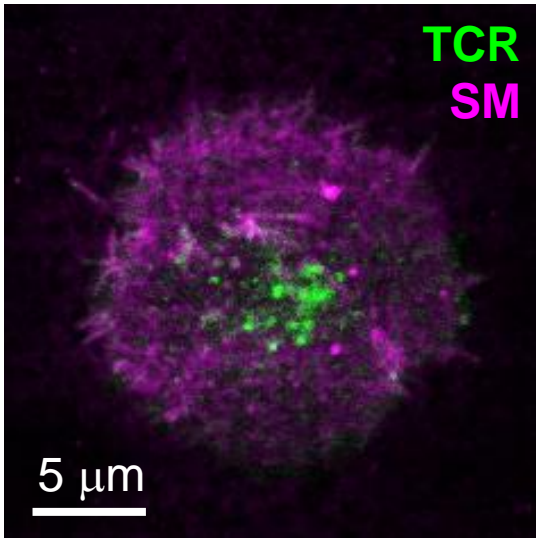


Effective excitation beam



Depletion beam

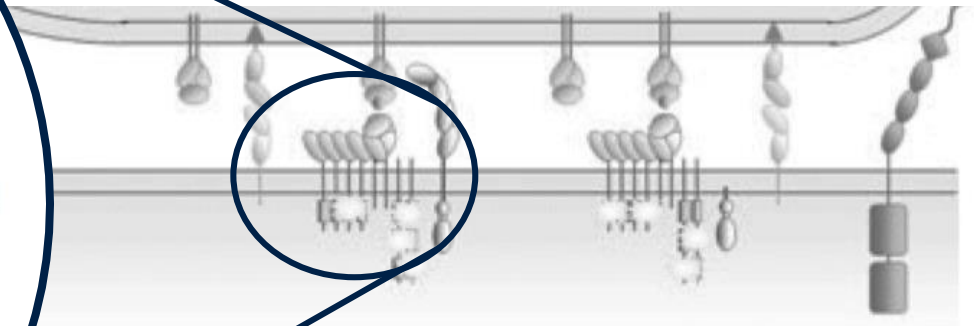
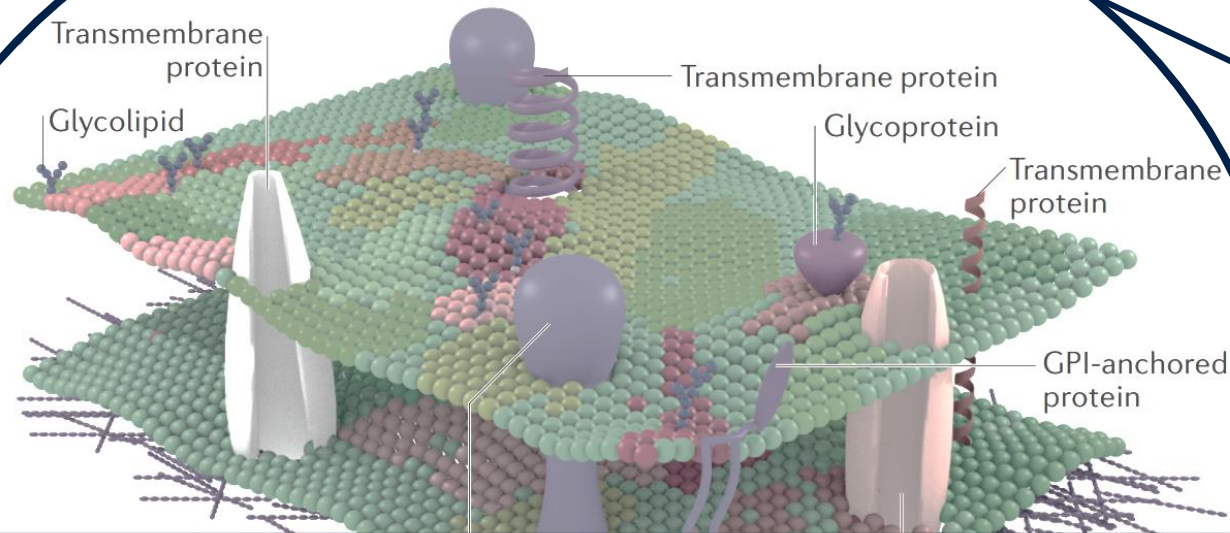
Diffusion modes of sphingomyelin (SM) in activated T-cells



Trapping diffusion at TCR

Free diffusion at CD45

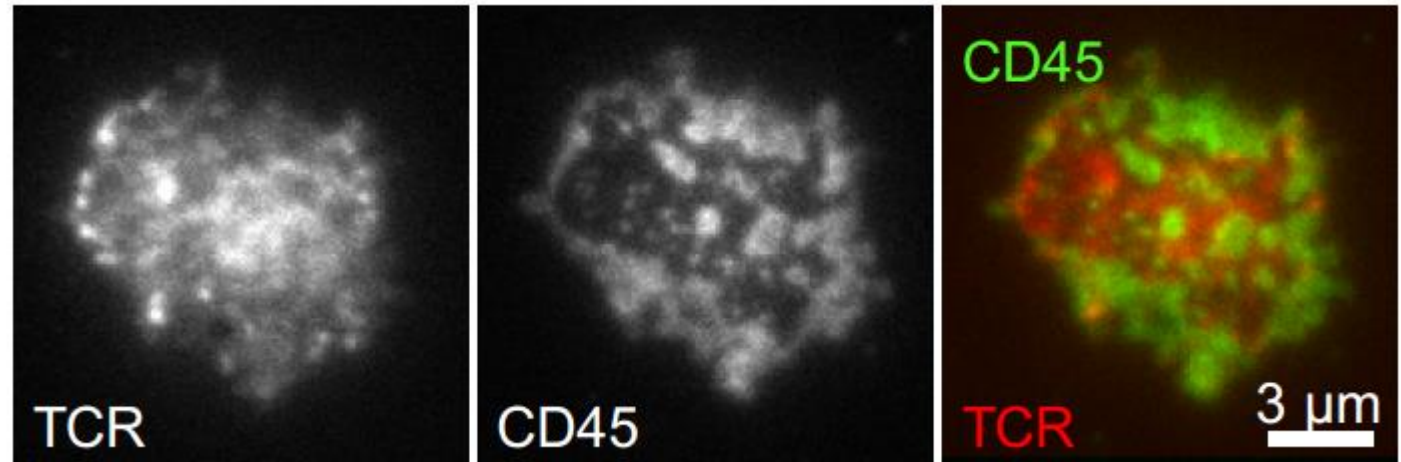
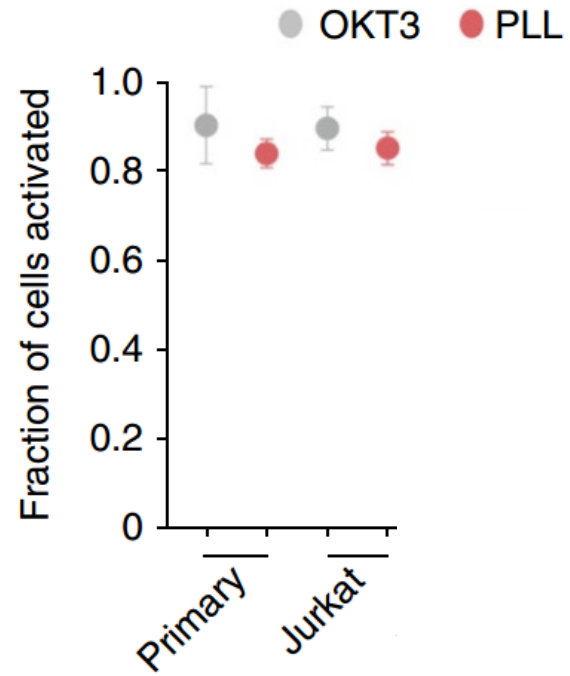
Membrane properties of activated T-cells



Membrane property	at TCR	at CD45
Lipid order	ordered	disordered
PE diffusion rate	slowed down	-
SM diffusion mode	trapping	free

Does lipid phase separation drive, assist, or follow the segregation of proteins?

Triggering of T-cells on poly-L-lysine (PLL)



Many thanks to ...

Eggeling & Fritzsche Labs

Christian Eggeling

Erdinc Sezgin

Falk Schneider

Francesco Reina

Silvia Galiani

Dilip Shrestha

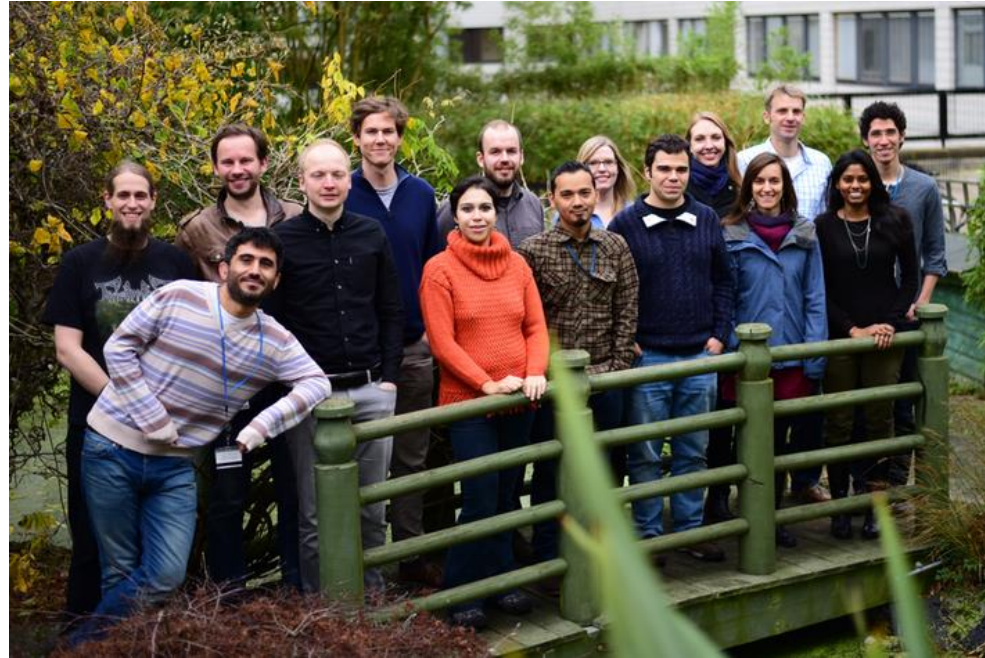
Huw Colin-York

Katharina Reglinski

Liliana Barbieri

Marco Fritzsche

Tess Stanly



Davis Lab

Simon Davis

Ana-Mafalda Santos

Edward Jenkins

Wolfson Imaging Centre

Christoffer Lagerholm

Dominic Waithe

Pablo Hernandez-Varas



MARIE CURIE ACTIONS



European
Commission

Horizon 2020
European Union funding
for Research & Innovation

