Probing lipid interactions of plasma membrane proteins: a micropatterning approach

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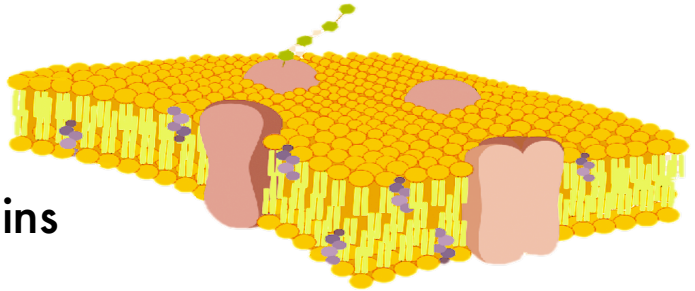
RBC 2018

Interplay of lipids and proteins in the plasma membrane



- Mammalian plasma membrane:
  - thousands of different lipids and proteins
  - local and temporal heterogeneity
  - lipids and proteins influence each other

Fundamental mechanisms
HOW? WHY? WHEN?

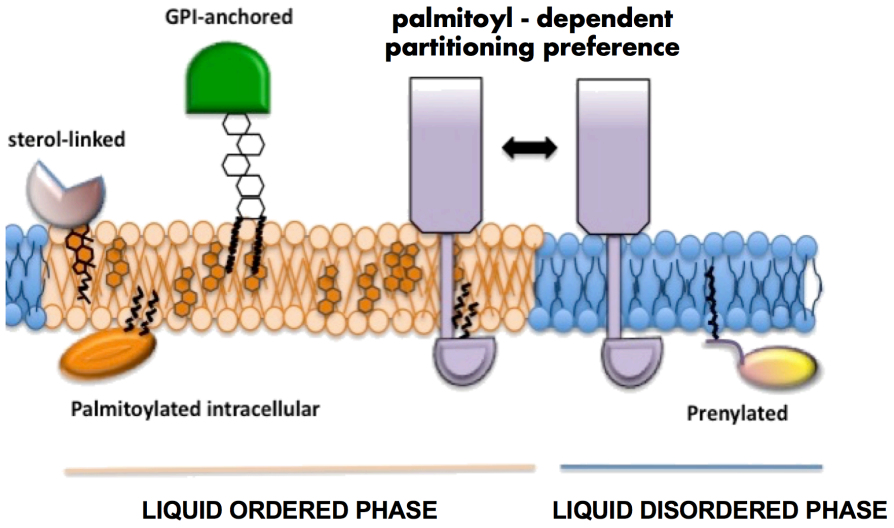
Interplay of lipids and proteins in the plasma membrane

Liquid ordered phase:

- sterol- and sphingolipid-enriched
- compartmentalize cellular processes

Liquid disordered phase:

- rich in unsaturated fatty acids



Levental et al. (2010), Biochemistry

Interplay of lipids and proteins in the plasma membrane

Liquid ordered phase:

- sterol- and sphingolipid-enriched
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Liquid disordered phase:

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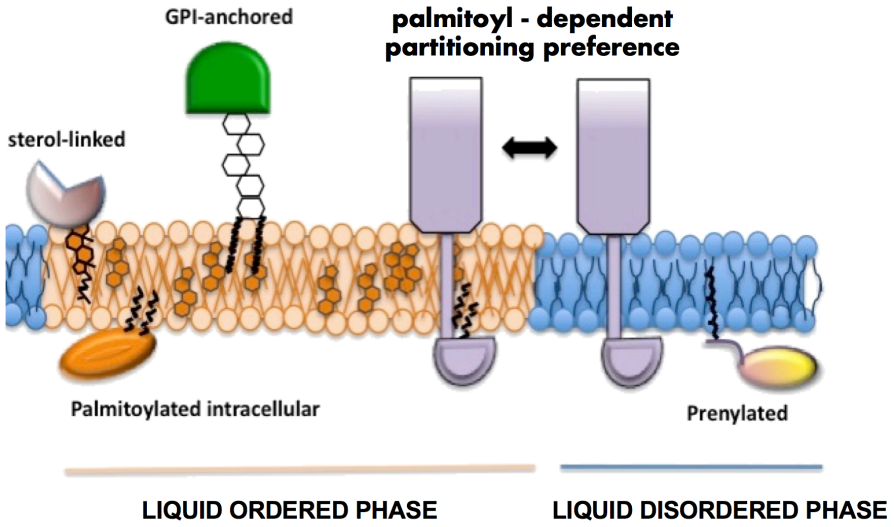
Protein palmitoylation:

- posttranslational modification
- partitioning in the liquid ordered phase

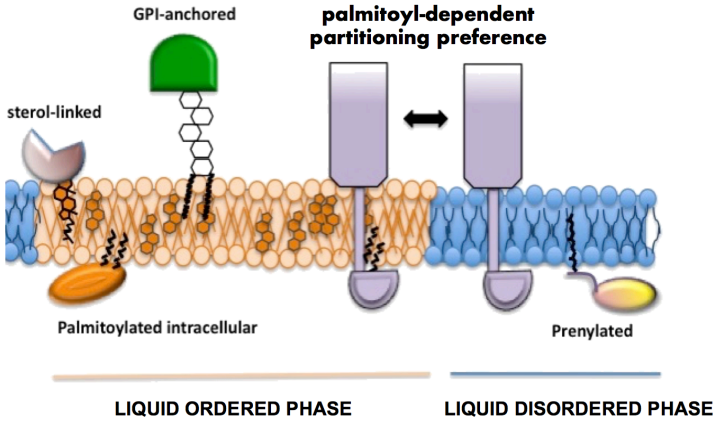


Levental et al. (2010), Biochemistry

Problems and goals

Problems:

- observations in **live cells** are controversial
- technical limitations
- lipid probes



Levental et al. (2010), Biochemistry

Problems and goals

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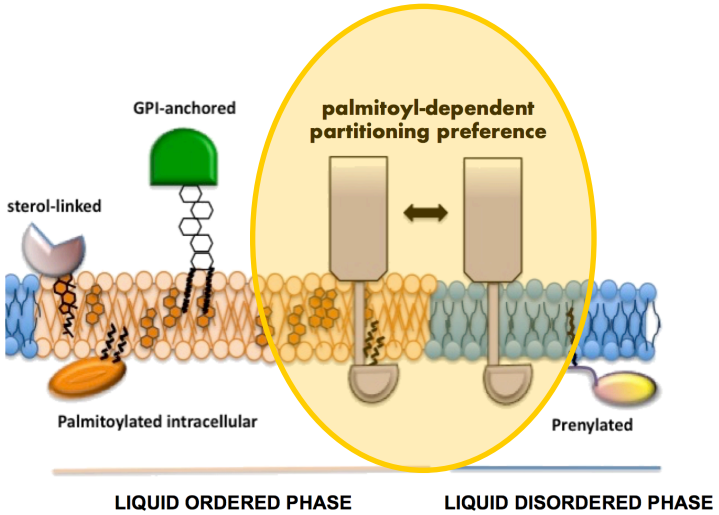
Goals:

- recruit palmitoylated and depalmitoylated transmembrane proteins



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Problems and goals

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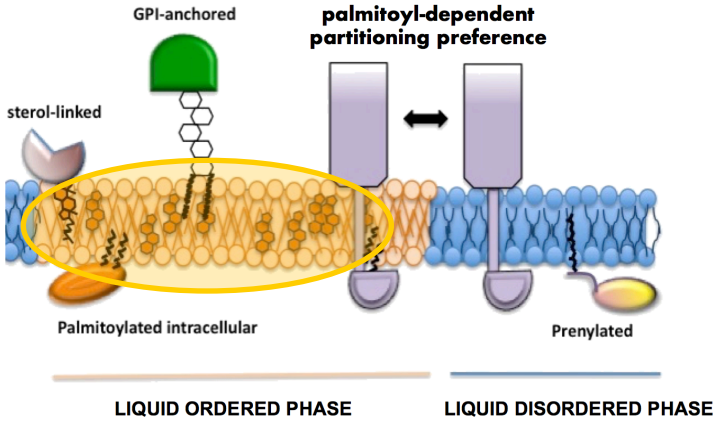
Goals:

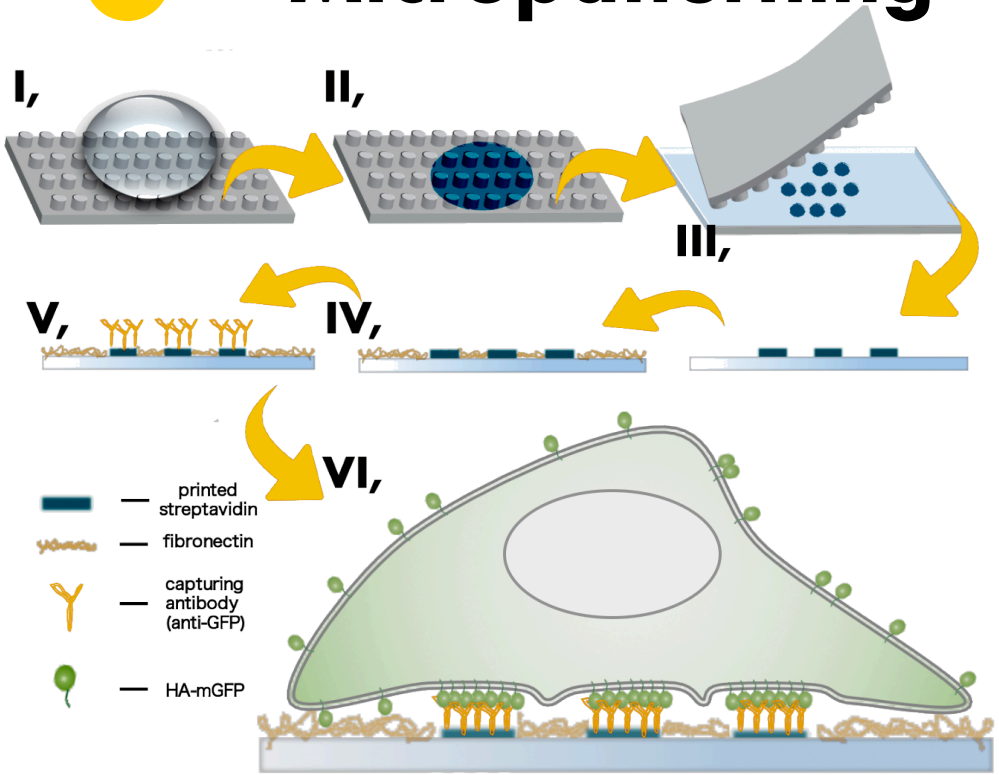
- recruit palmitoylated and depalmitoyltaed transmembrane proteins
- probe with lipids and proteins



Levental et al. (2010), Biochemistry

Micropatterning

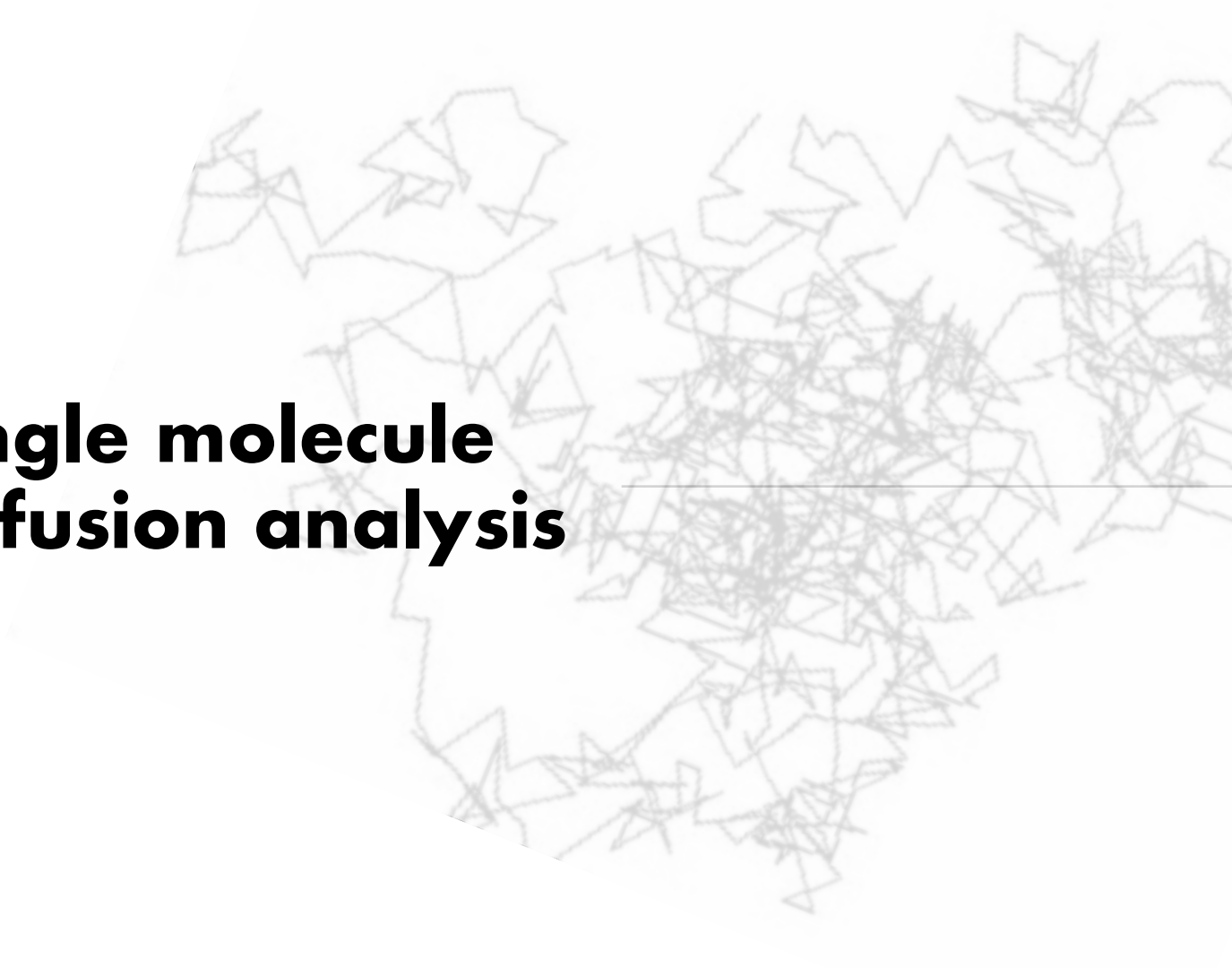
Micropatterning



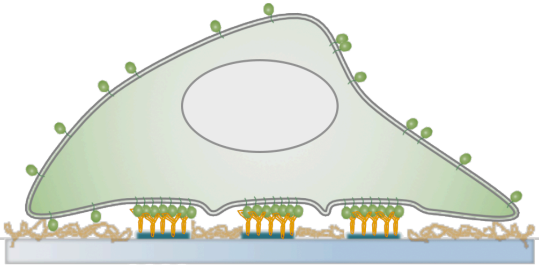
I. incubation with streptavidin
II. drying
III. stamping/stamp removal
IV. passivation with fibronectin
V. biotinylated antibody attachment
VI. cells are grown over the patterns

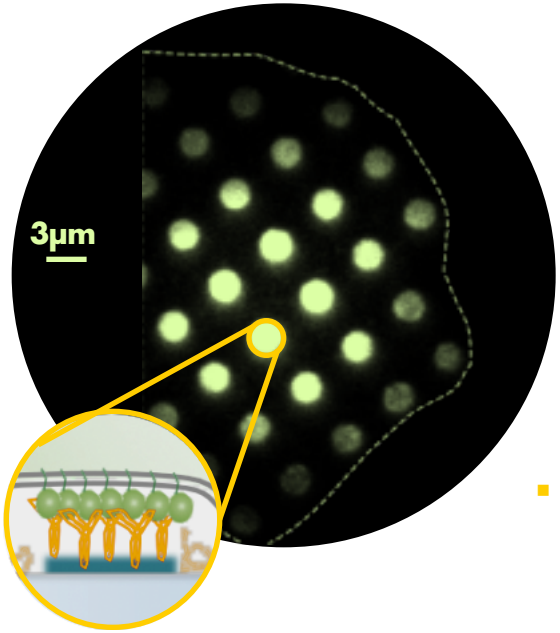
Proteins in the plasma membrane can be immobilised by antibody patterns

Single molecule diffusion analysis



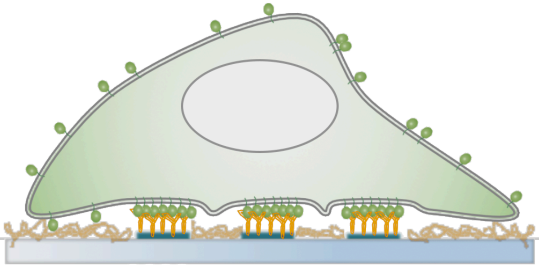
Single molecule diffusion analysis

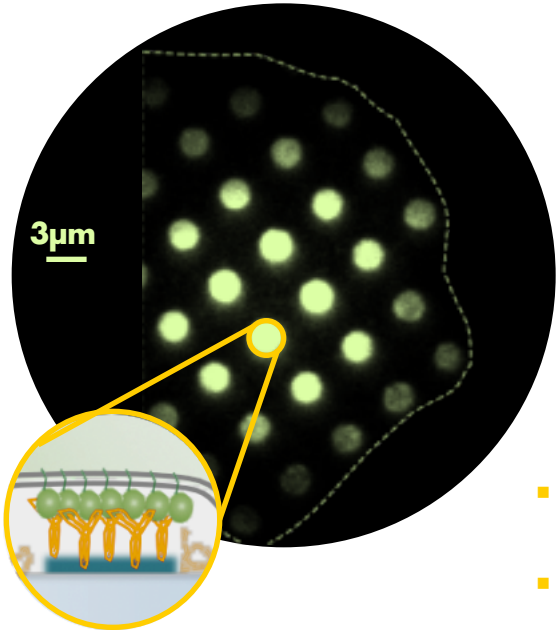


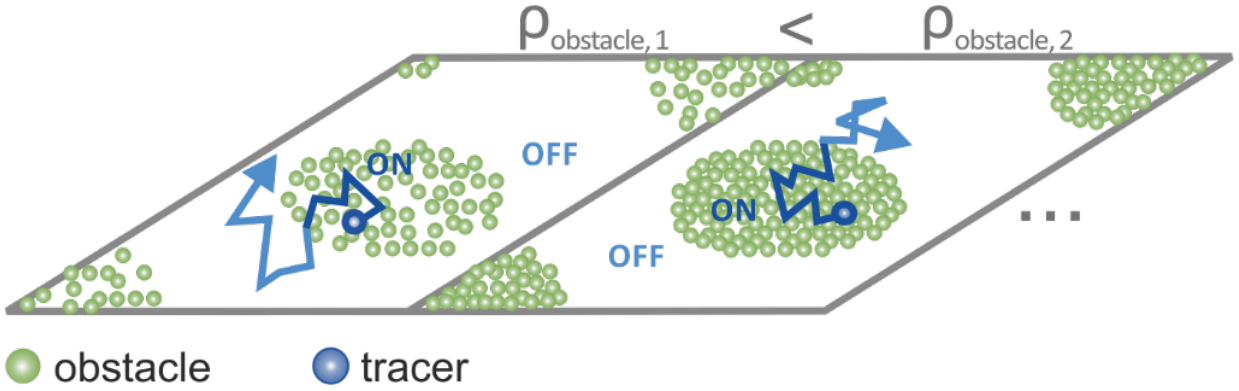


- the image of immobilised protein pattern is captured

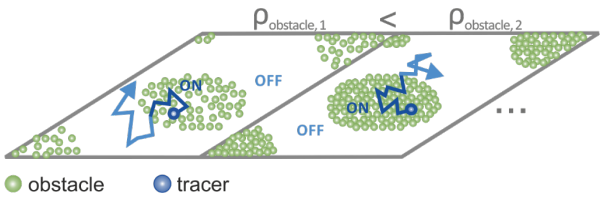
Single molecule diffusion analysis





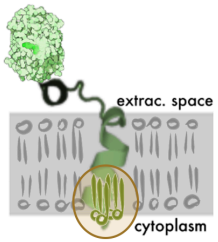


- the image of immobilised protein pattern is captured
- the trajectory of diffusing lipids are recorded
- mobility of lipids at ON and OFF area serves useful information

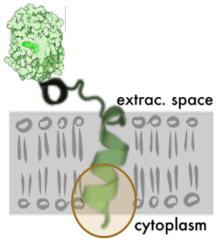


Selection of materials

Obstacle molecules



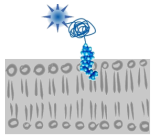
Hemagglutinin (HA)-mGFP



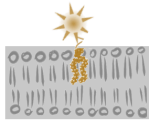
Δpalm-HA-mGFP

Micropatterning

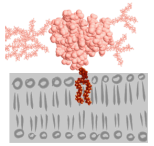
Tracer molecules



Cholesterol-PEG-KK114

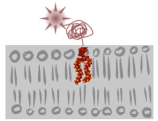


Sphingomyelin-ATTO594



CD59

liquid ordered phase probes

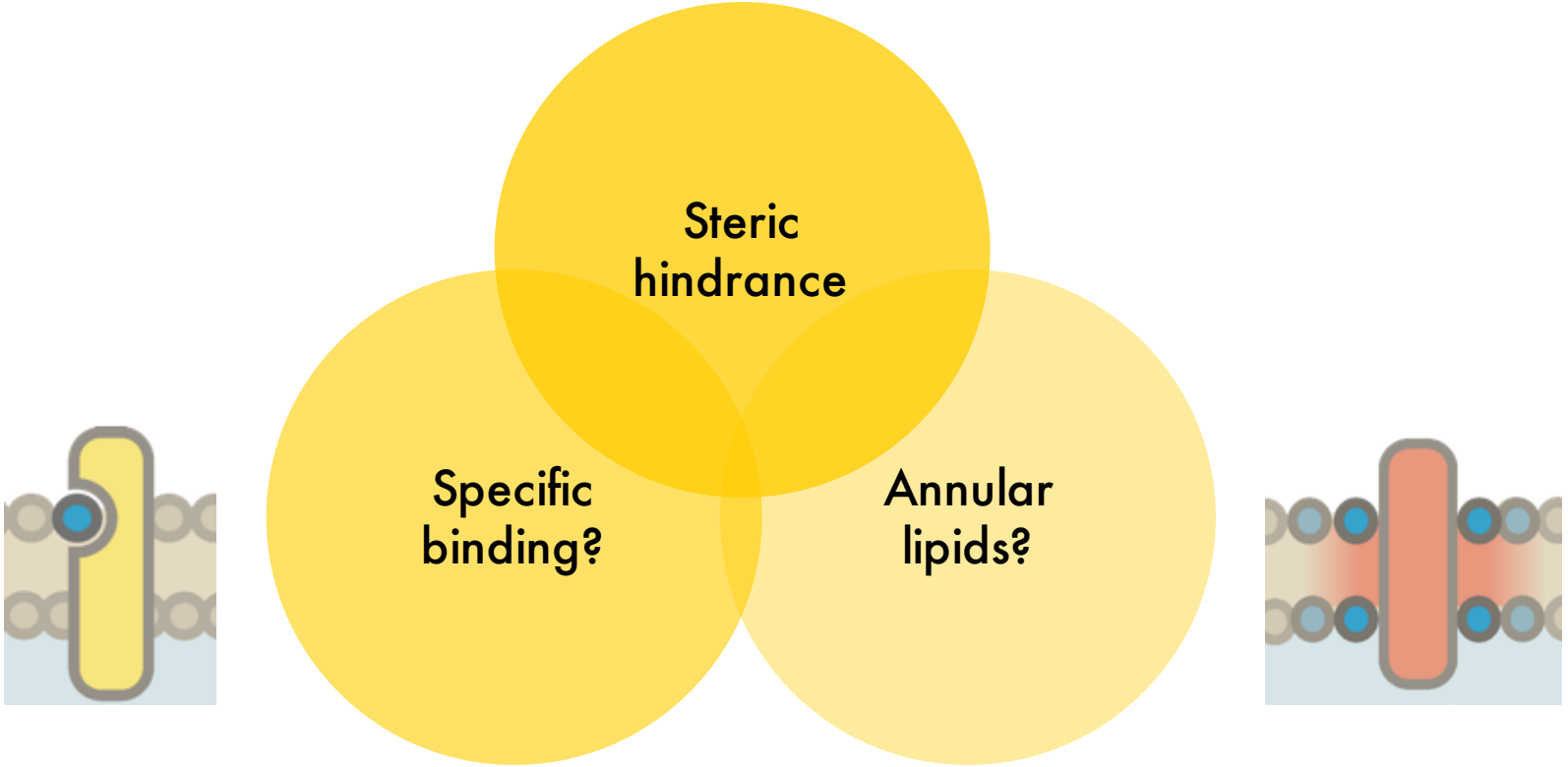


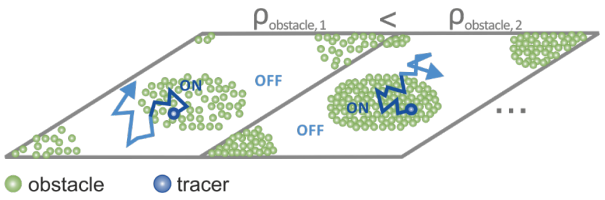
DOPE-PEG-KK114

liquid disordered phase probe

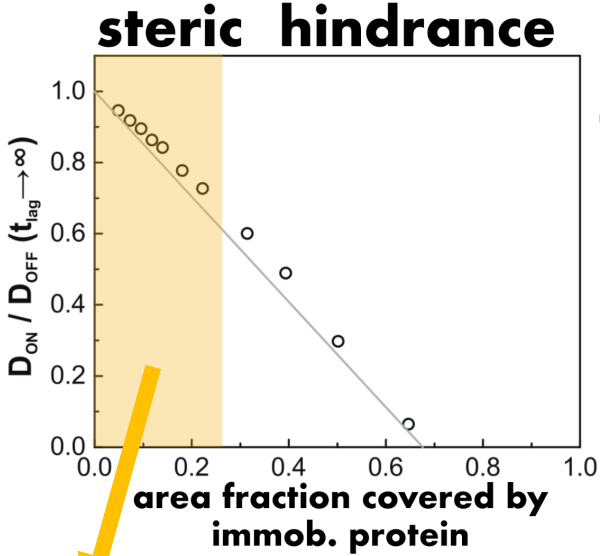
Single molecule diffusion analysis

Possible scenarios

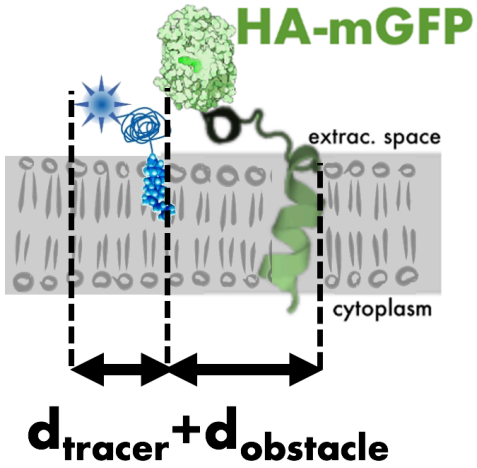




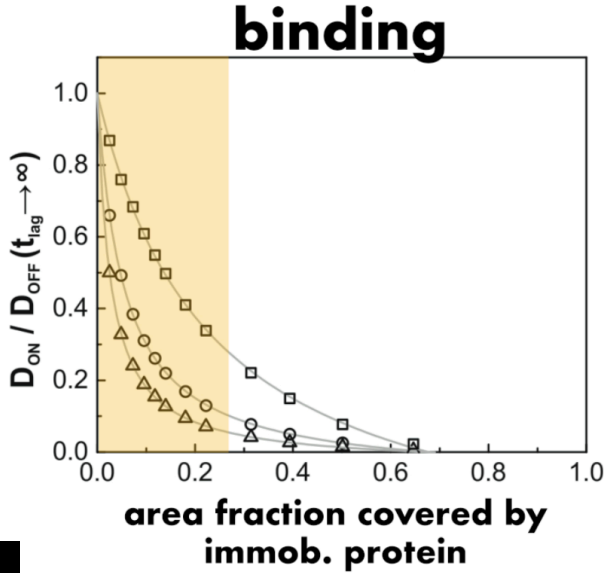
Simulations



regime experimentally achievable

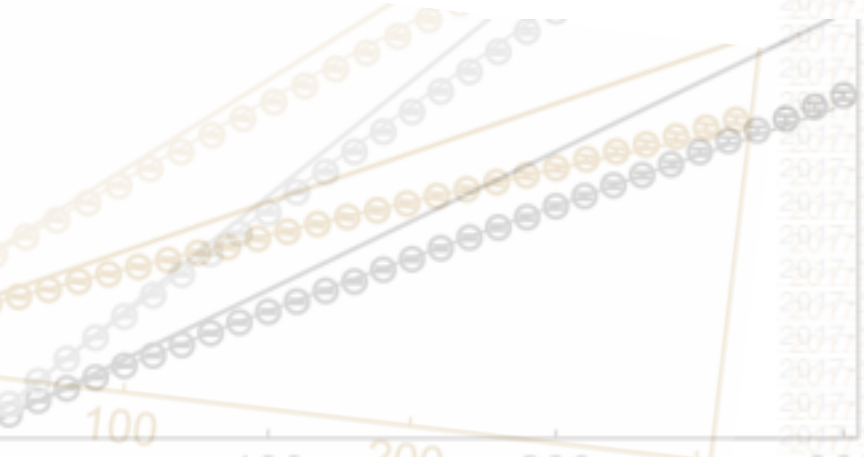


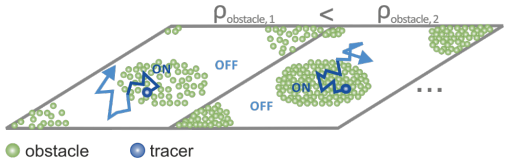
tracer plus obstacle size can be calculated



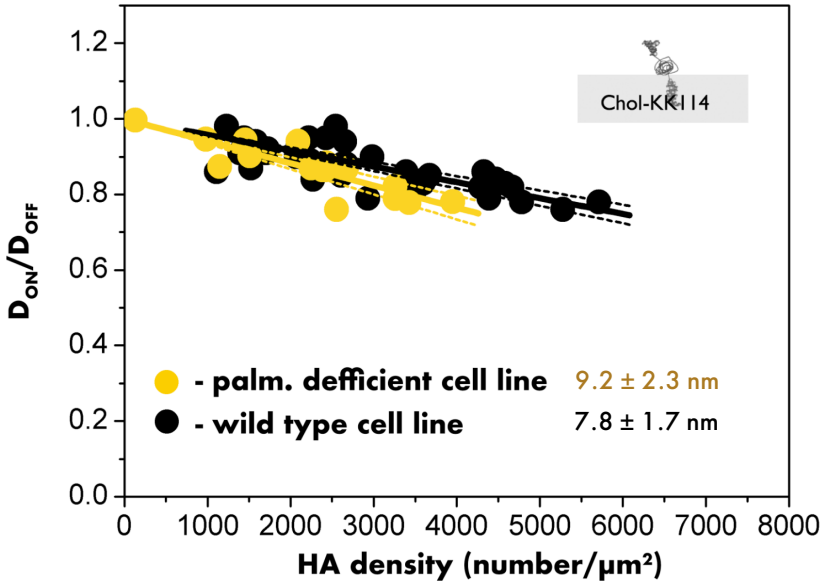
Arnold et al.

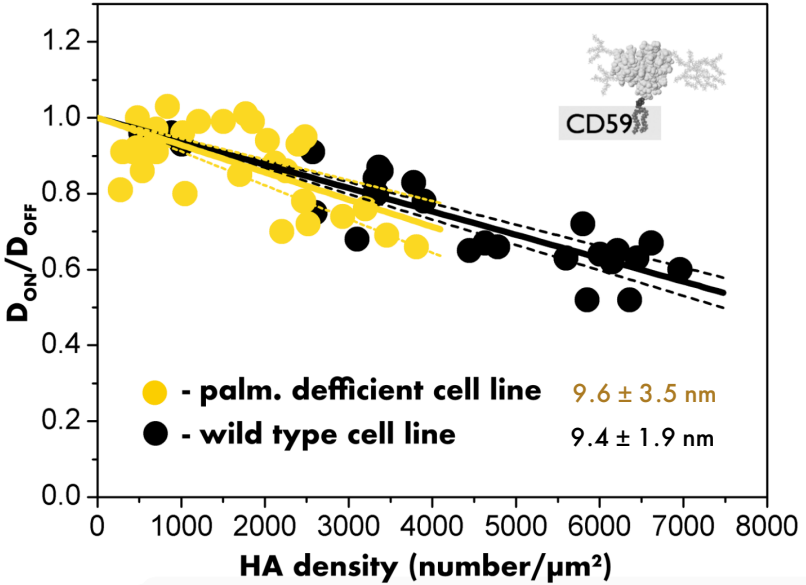
Results



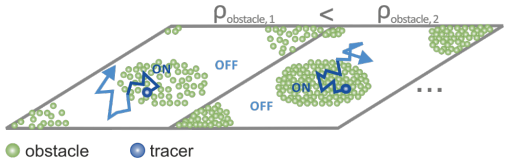


Rel. diffusion of Chol-KK114 and CD59

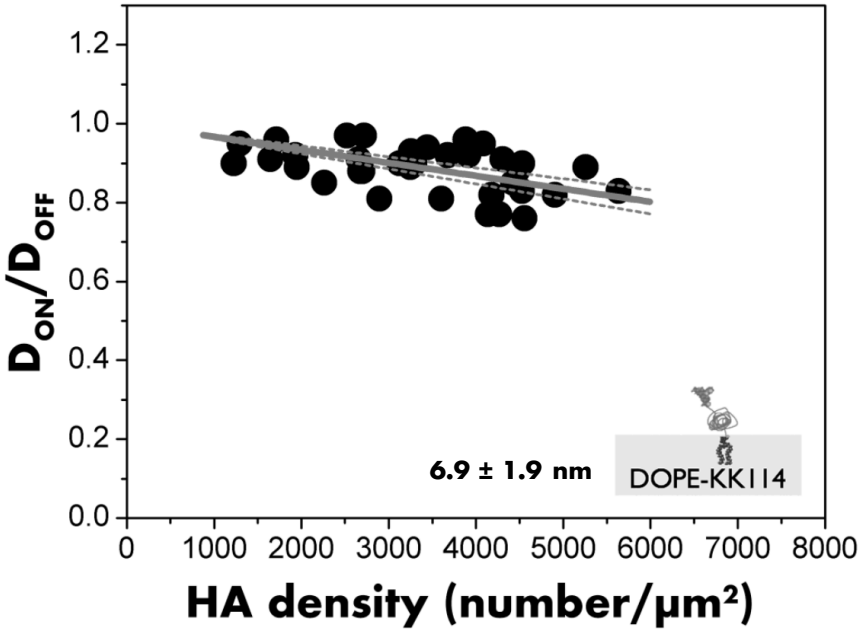




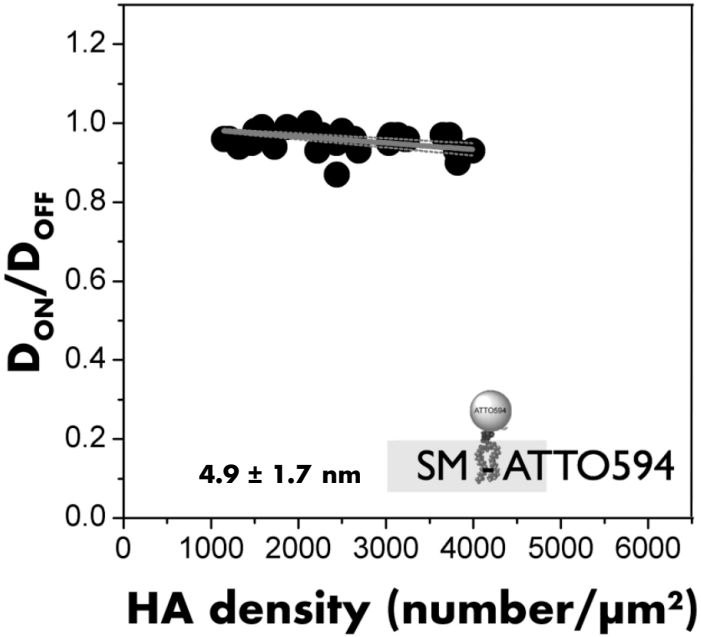
- Palmitoylated and wild type cells show the same behaviour



Rel. diffusion of DOPE-KK114 and SM-ATTO594

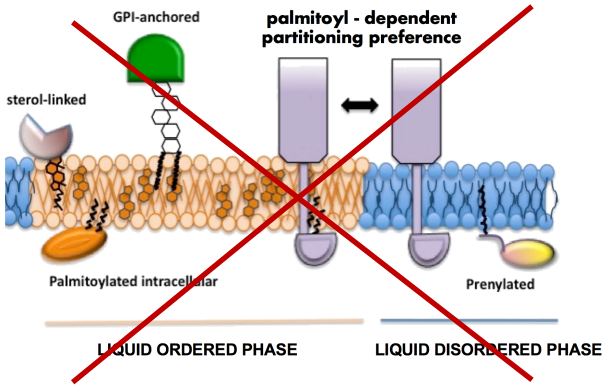


DOPE-KK114 shows similar behaviour like Chol-KK114



SM-ATTO594 shows minor decrease of rel. diffusion

Apparent size calculated from diffusion data



Immob. protein	Diffusing tracer	$D_{ob}+D_{tr}$	Probable cause
HA-mGFP	CD59	9.4 ± 1.9 nm	steric hindrance (ectodomain)
	Chol-KK114	7.8 ± 1.7 nm	steric hindrance (ectodomain)
	DOPE-KK114	6.9 ± 1.9 nm	steric hindrance (ectodomain)
	SM-ATTO594	4.9 ± 1.7 nm	?
HAΔpal-mGPF	CD59	9.6 ± 3.5 nm	steric hindrance (ectodomain)
	Chol-KK114	9.2 ± 2.3 nm	steric hindrance (ectodomain)

Conclusion & Acknowledgements

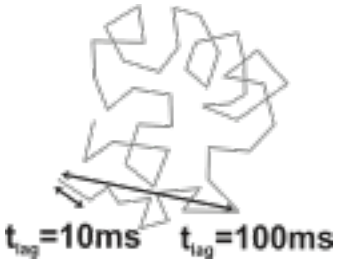
- Palmitoylation of HA-mGFP did not have a significant effect on cholesterol or CD59 diffusion
- Cholesterol and DOPE shows similar diffusion behavior, albeit they have different membrane partitioning preference
- ATTO594 labeled lipids are good tracer candidates, further experiments are needed (DOPC-ATTO594, DSPC-ATTO594)

Thanks!

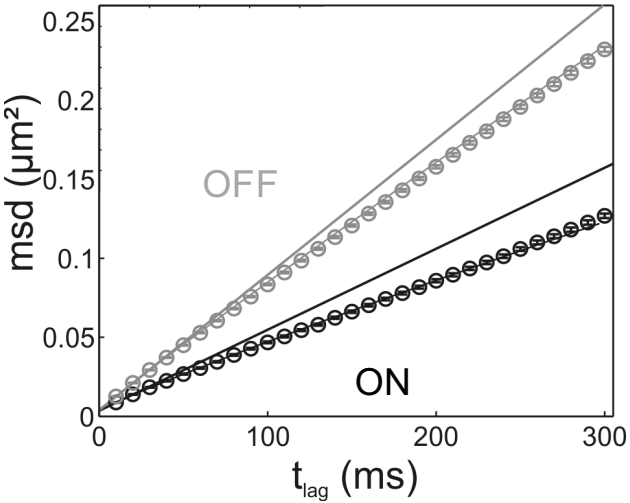
Any questions?

Special thanks to E. Sevcsik, M. Brameshuber, A. Arnold, G. Schütz, A. Honigmann and N. Matsumori.

Single molecule diffusion analysis



$$msd = 4Dt_{lag} + 4\sigma^2$$



- the pattern of immobilised proteins is recorded
- the trajectory of diffusing lipids are recorded
- diffusion constants of lipids at ON and OFF area are yielded
- overlaying the two source of data can result in useful information