

# Probing **lipid interactions** of plasma membrane proteins: a micropatterning approach



**Gergő Fülöp**

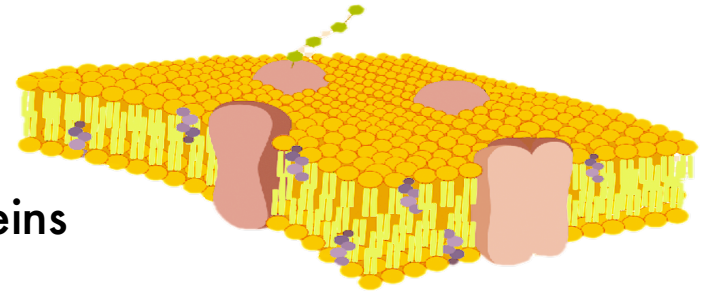
Applied Physics  
TU Wien

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?**

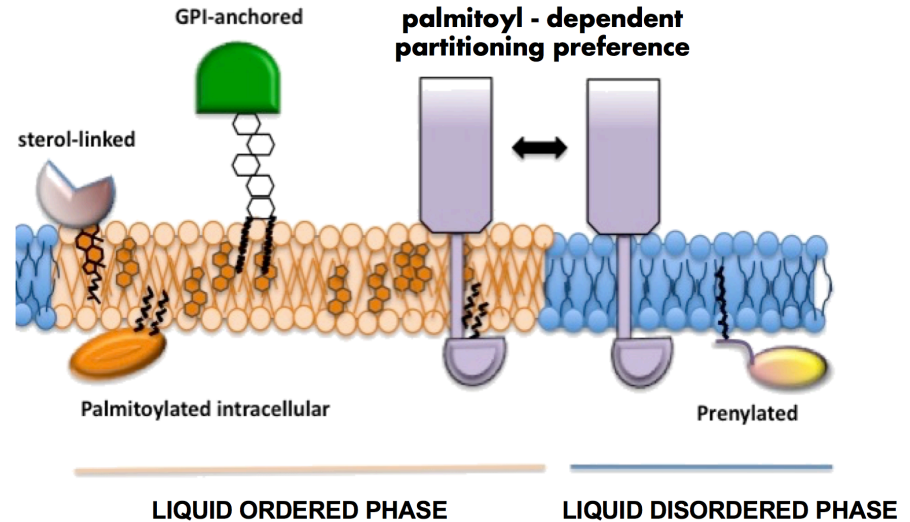
# Interplay of lipids and proteins in the plasma membrane

## Liquid ordered phase:

- sterol- and sphingolipid-enriched
- compartmentalize cellular processes

## Liquid disordered phase:

- rich in unsaturated fatty acids





# Interplay of lipids and proteins in the plasma membrane

## Liquid ordered phase:

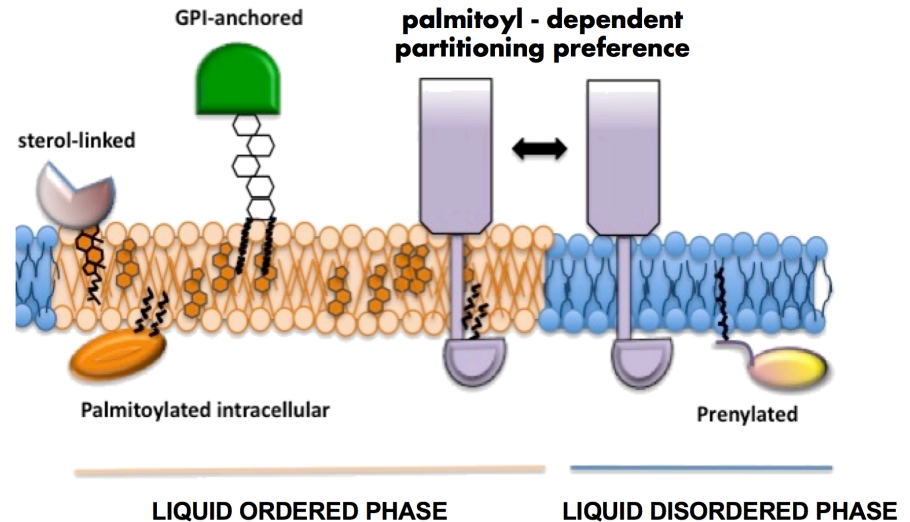
- sterol- and sphingolipid-enriched
- compartmentalize cellular processes

## Liquid disordered phase:

- rich in unsaturated fatty acids

## Protein palmitoylation:

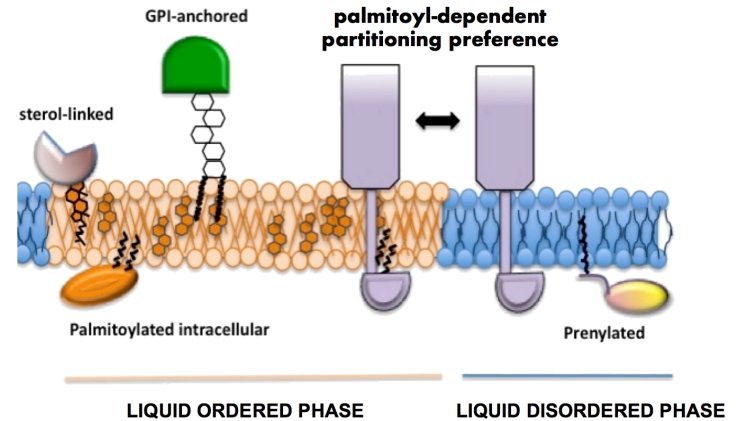
- posttranslational modification
- partitioning in the liquid ordered phase



# Problems and goals

## Problems:

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



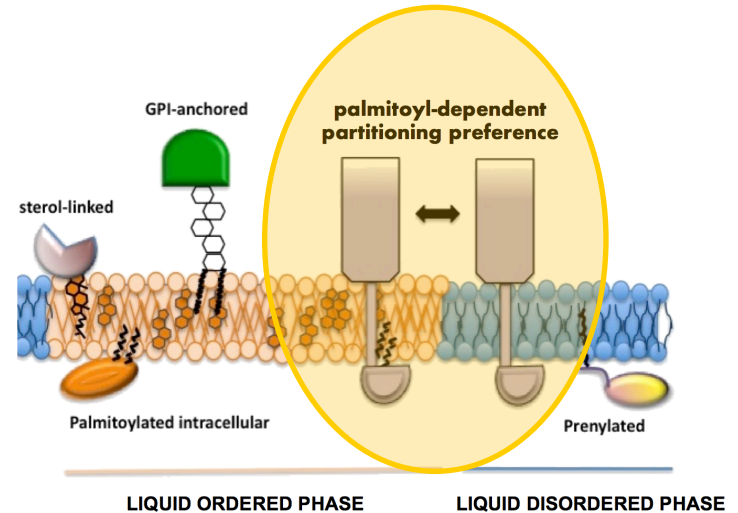
# Problems and goals

## Problems:

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

## Goals:

- recruit palmitoylated and depalmitoylated transmembrane proteins



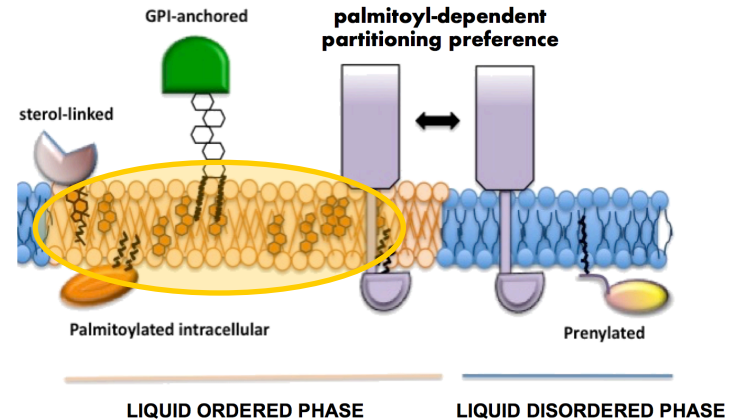
# Problems and goals

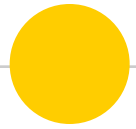
## Problems:

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

## Goals:

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



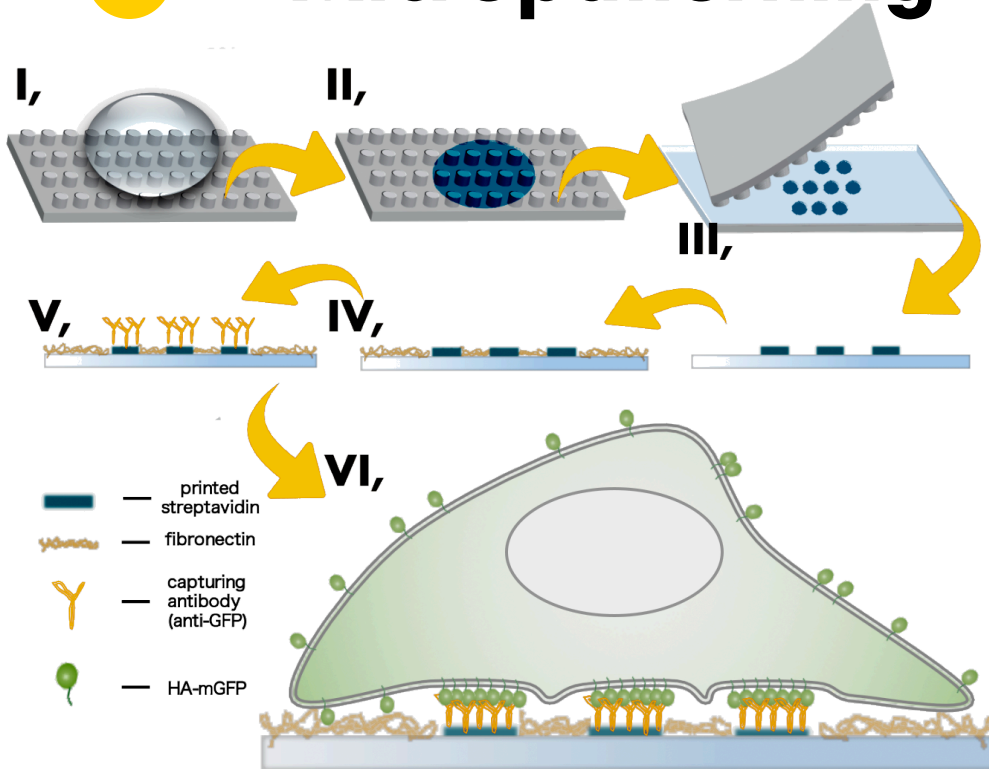


# **Micropatterning**



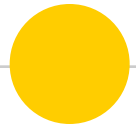


# Micropatterning

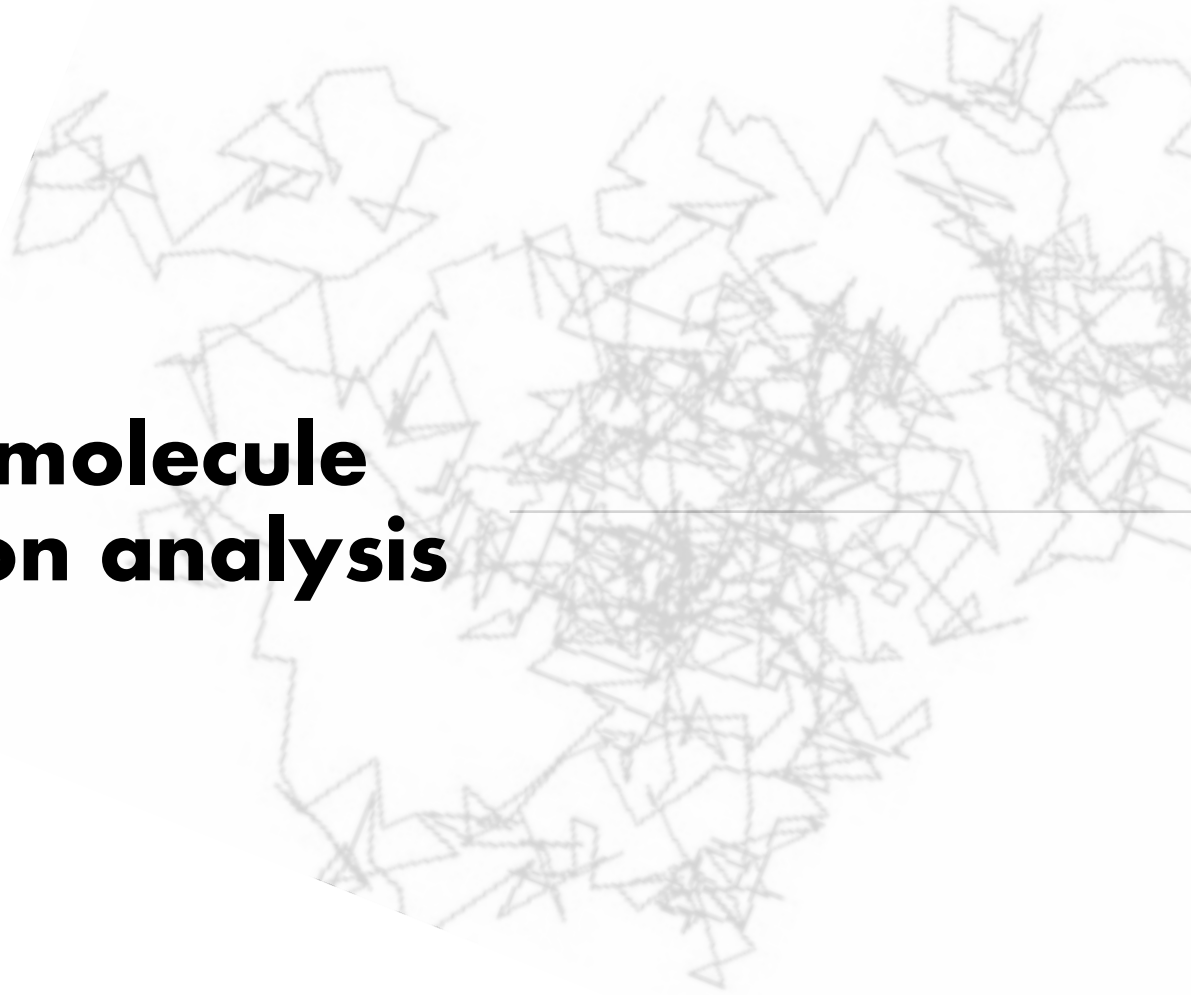


- I. incubation with streptavidin
- II. drying
- III. stamping/stamp removal
- IV. passivation with fibronectin
- V. biotinylated antibody attachment
- VI. cells are grown over the patterns

**Proteins in the plasma membrane can be immobilised by antibody patterns**

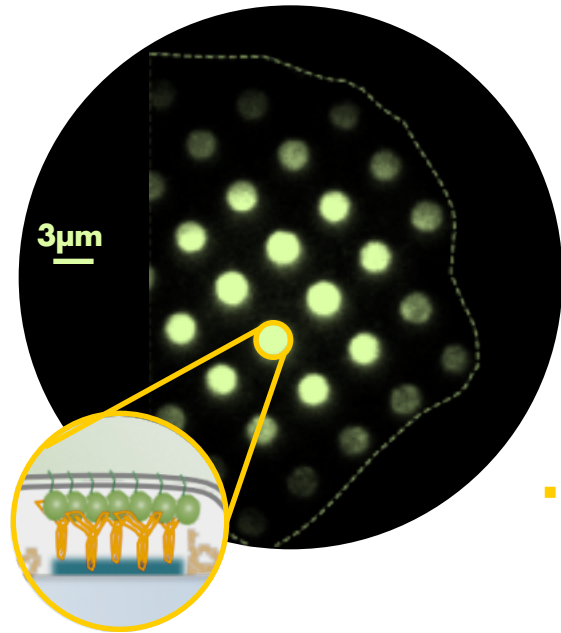
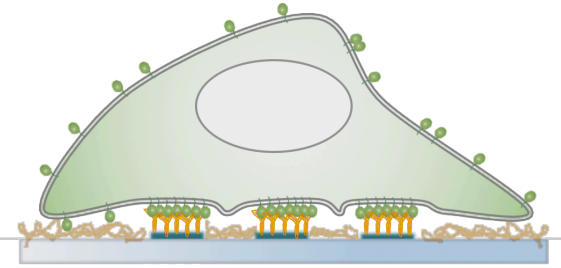


# **Single molecule diffusion analysis**





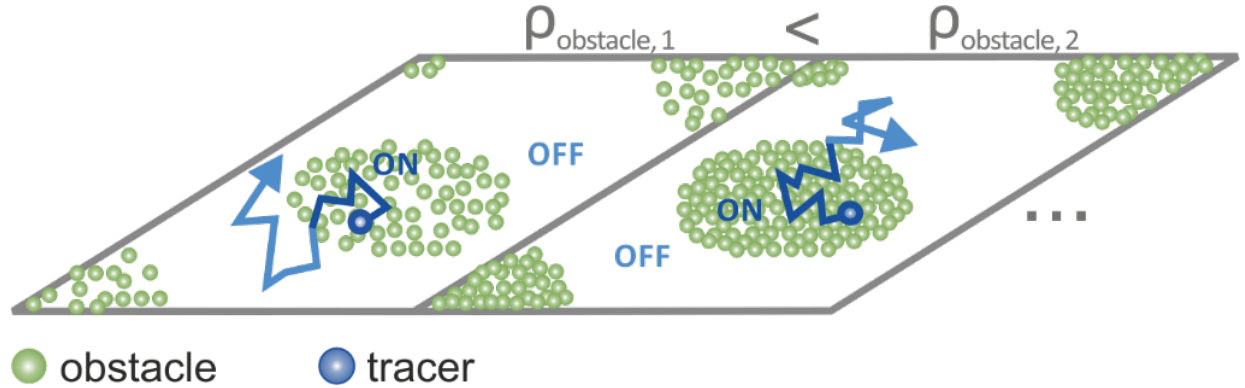
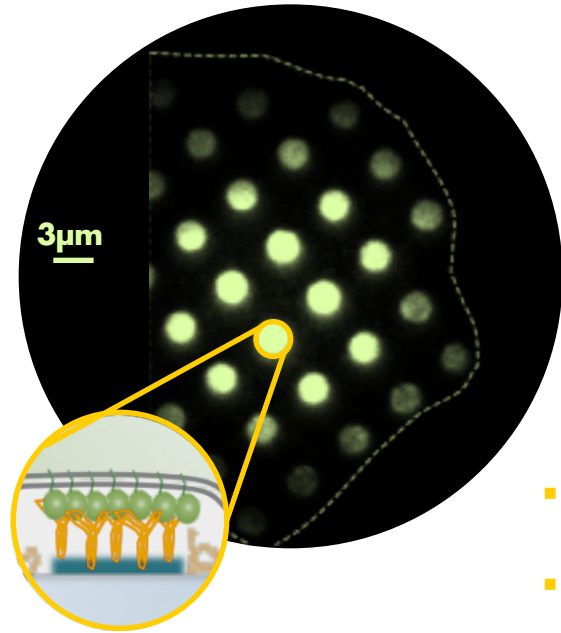
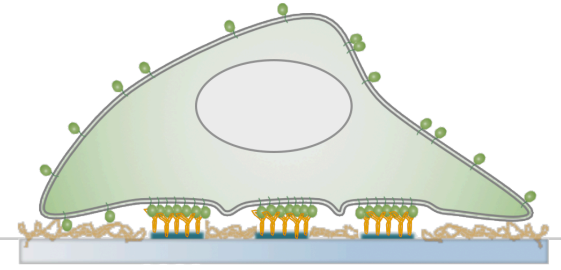
# Single molecule **diffusion** analysis



- the image of immobilised protein pattern is captured



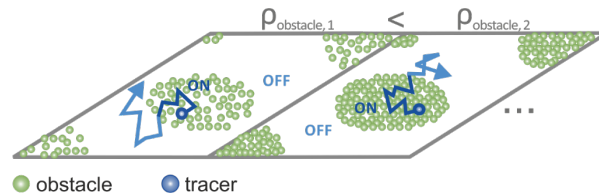
# Single molecule **diffusion** analysis



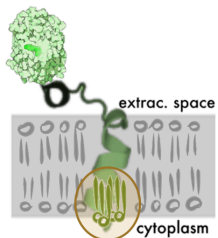
- the image of immobilised protein pattern is captured
- the trajectory of diffusing lipids are recorded
- mobility of lipids at ON and OFF area serves useful information



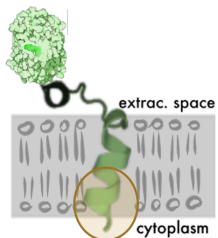
# Selection of materials



## Obstacle molecules



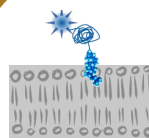
Hemagglutinin  
(HA)-mGFP



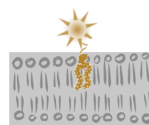
$\Delta$ palm-HA-mGFP

**Micropatterning**

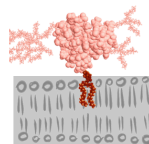
## Tracer molecules



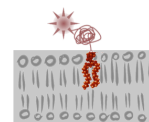
Cholesterol-  
PEG-KK114



Sphingomyelin-  
ATTO594



CD59



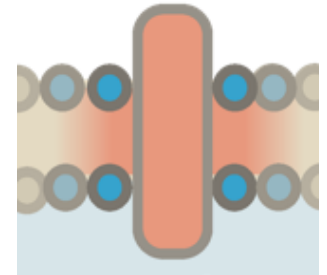
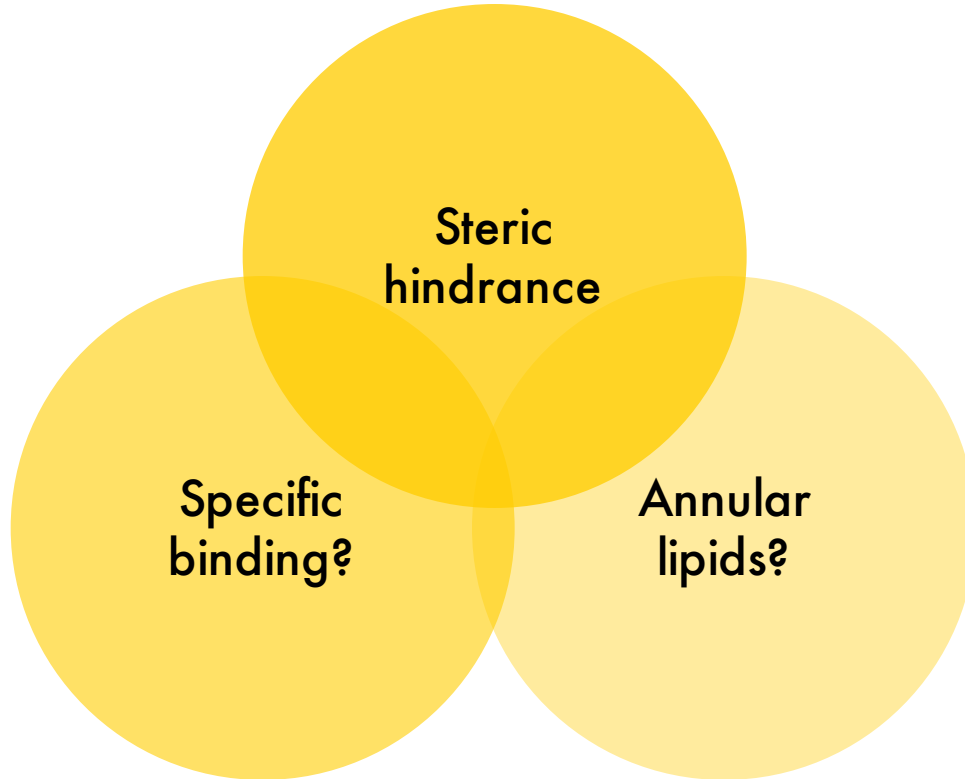
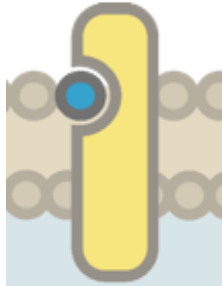
DOPE-PEG-  
KK114

liquid ordered phase  
probes

liquid disordered  
phase probe

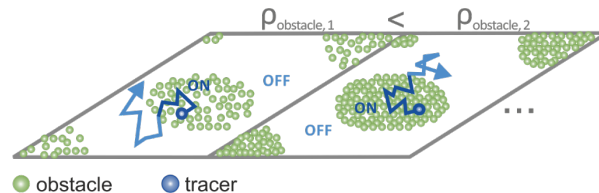
**Single molecule diffusion analysis**

# Possible scenarios

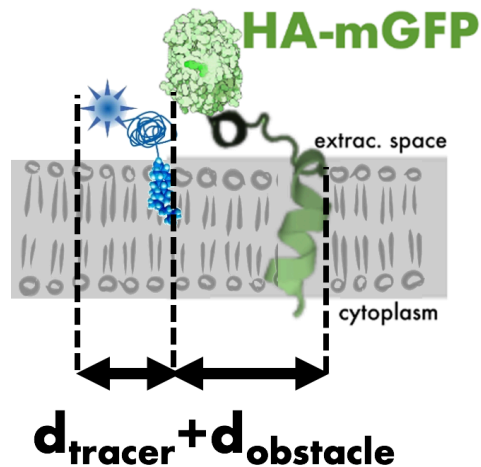
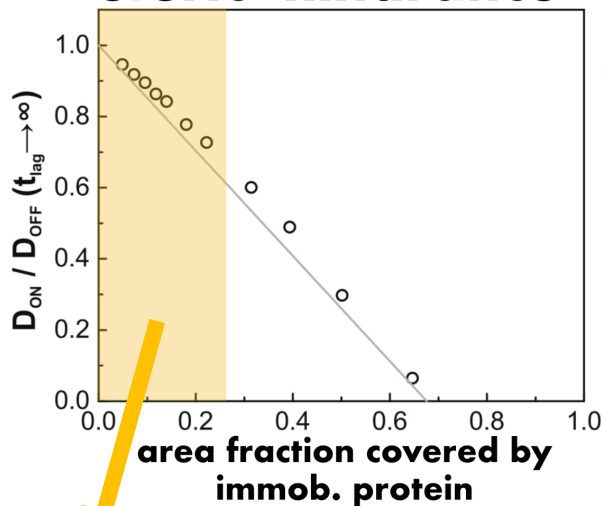




# Simulations

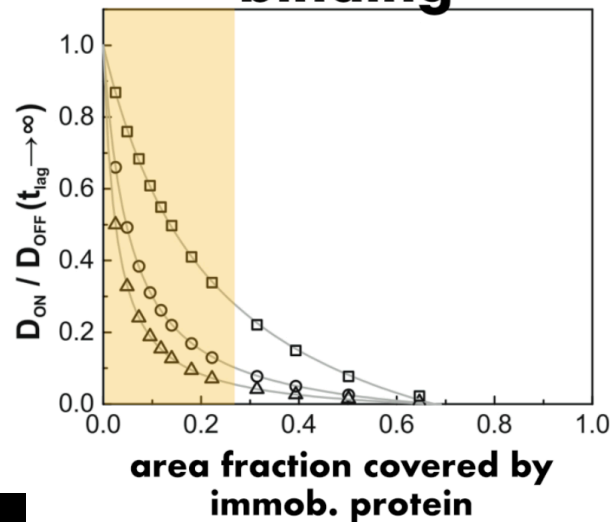


## steric hindrance

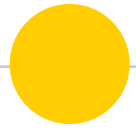


**tracer plus obstacle size  
can be calculated**

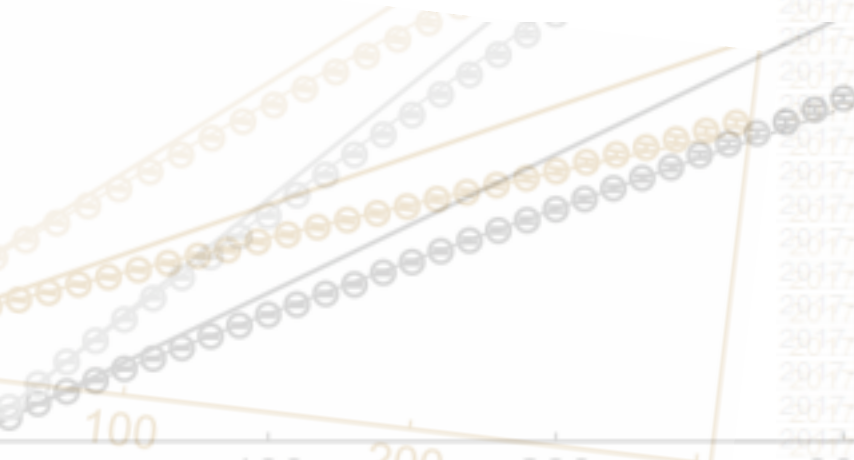
## binding



SM1_c0_OFF_high_Dthresh_0.trc	1729.39771	1.03833
SM1_c6_ON_high_Dthresh_0.trc	1467.98917	1.02715
SM1_c7_ON_high_Dthresh_0.trc	1148.5819	1.02382
SM1_c8_ON_high_Dthresh_0.trc	2436.56788	0.98534
SM1_c9_ON_high_Dthresh_0.trc	3651.43403	0.91924
SM1_c10_ON_high_Dthresh_0.trc	1508.38113	0.96107
SM1_c11_ON_high_Dthresh_0.trc	2268.37157	0.9849
SM1_c12_ON_high_Dthresh_0.trc	1874.18738	1.00512
SM1_c13_ON_high_Dthresh_0.trc	2688.65519	0.95765
SM1_c14_ON_high_Dthresh_0.trc	2129.17463	1.01309
SM1_c15_ON_high_Dthresh_0.trc	1320.92097	0.97354
SM2_c1_ON_high_Dthresh_0.trc	2638.13522	0.96319
SM2_c2_ON_high_Dthresh_0.trc	2216.66303	0.91945
SM2_c3_ON_high_Dthresh_0.trc	2439.09487	0.90671

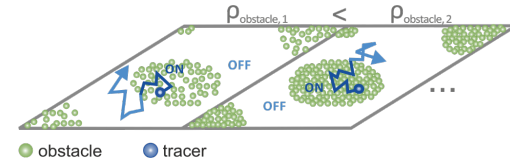


# Results

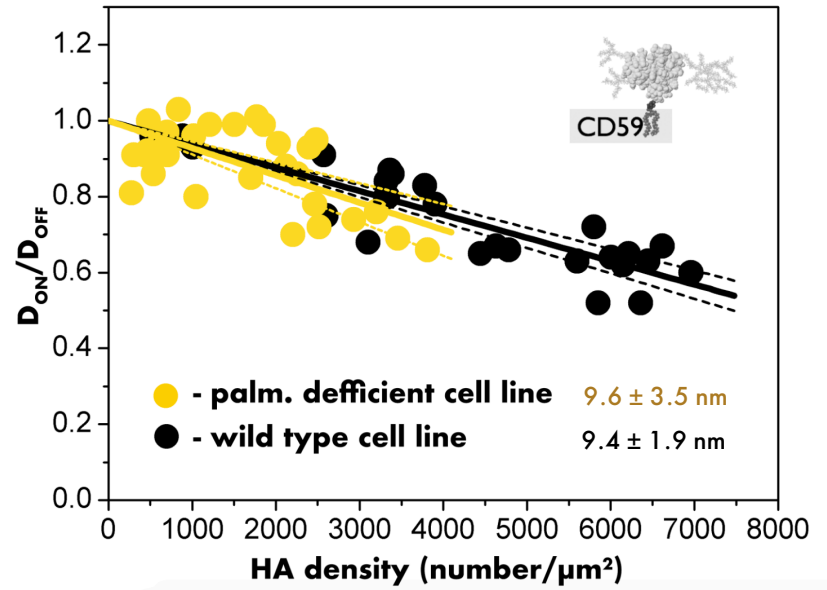
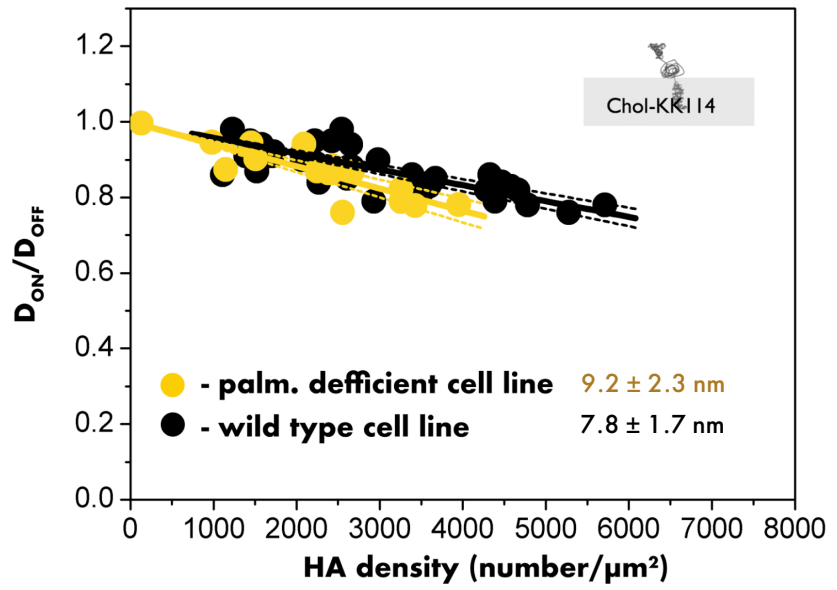


2017-11-16_SM2_c5_ON_high_Dthresh_0.trc	3140.54961	0.96483
2017-11-16_SM2_c6_ON_high_Dthresh_0.trc	3732.98833	0.88395
2017-11-16_SM2_c7_ON_high_Dthresh_0.trc	2969.00686	1.00664
2017-11-16_SM2_c8_ON_high_Dthresh_0.trc	2489.01745	0.87934
2017-11-16_SM2_c9_ON_high_Dthresh_0.trc	2379.75293	0.87255
2017-11-16_SM2_c10_ON_high_Dthresh_0.trc	4125.16899	0.95132
2017-11-16_SM2_c11_ON_high_Dthresh_0.trc	2989.16279	0.96191
2017-11-16_SM2_c12_ON_high_Dthresh_0.trc	3796.04865	0.95883
2017-11-16_SM2_c13_ON_high_Dthresh_0.trc	3061.85398	0.99222
2017-11-16_SM2_c14_ON_high_Dthresh_0.trc	3221.55508	0.94877
2017-11-16_SM2_c15_ON_high_Dthresh_0.trc	3377.02403	0.96165
2017-11-16_SM2_c16_ON_high_Dthresh_0.trc	3419.16277	1.08125
2017-11-16_SM2_c17_ON_high_Dthresh_0.trc	3242.01282	0.98811
2017-11-16_SM2_c18_ON_high_Dthresh_0.trc	4055.48894	0.99483





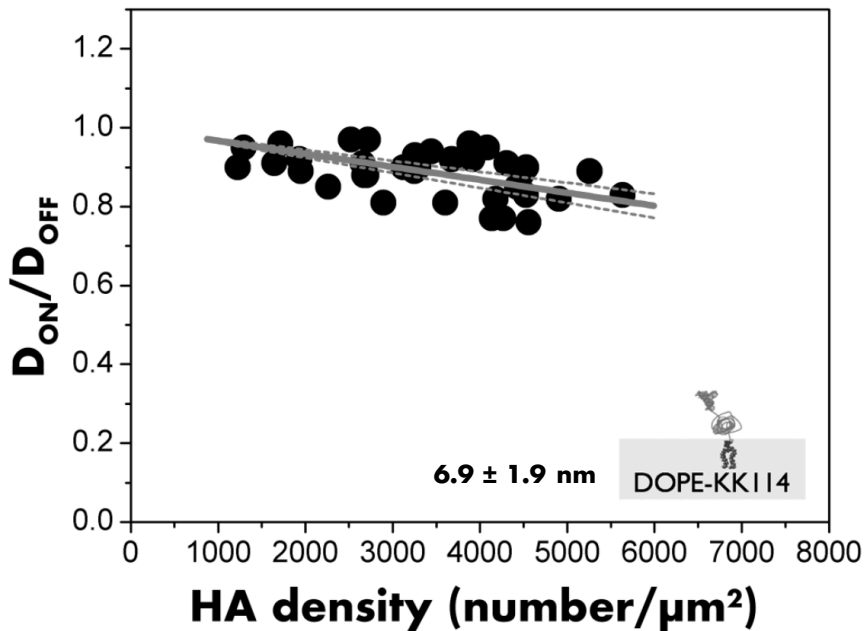
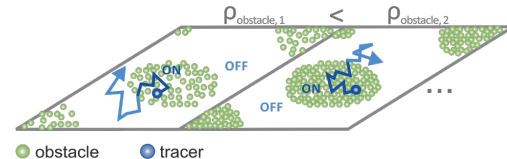
# Rel. diffusion of Chol-KK114 and CD59



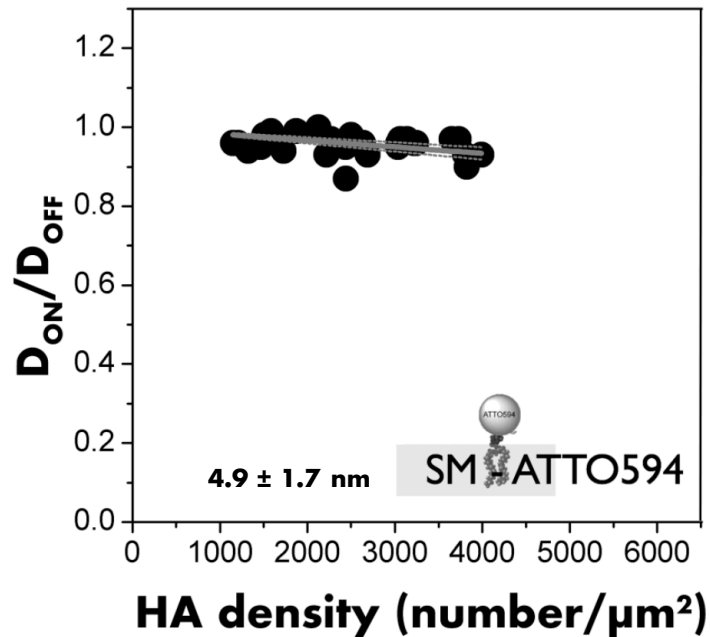
- Palmitoylated and wild type cells show the same behaviour



# Rel. diffusion of DOPE-KK114 and SM-ATTO594



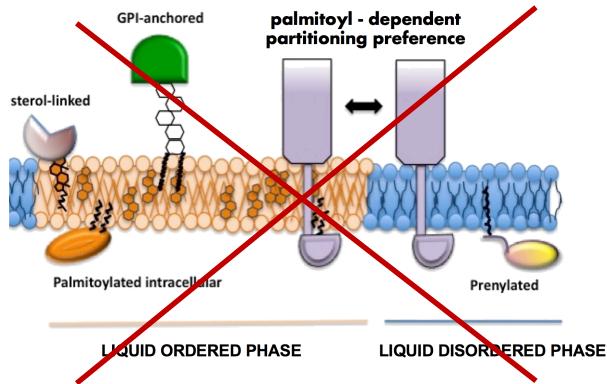
DOPE-KK114 shows similar behaviour like Chol-KK114



SM-ATTO594 shows minor decrease of rel. diffusion



# Apparent size calculated from diffusion data



Immob. protein	Diffusing tracer	$D_{ob}+D_{tr}$	Probable cause
<b>HA-mGFP</b>	CD59	$9.4 \pm 1.9$ nm	steric hindrance (ectodomain)
	Chol-KK114	$7.8 \pm 1.7$ nm	steric hindrance (ectodomain)
	DOPE-KK114	$6.9 \pm 1.9$ nm	steric hindrance (ectodomain)
	SM-ATTO594	$4.9 \pm 1.7$ nm	?
<b>HA<math>\Delta</math>pal-mGFP</b>	CD59	$9.6 \pm 3.5$ nm	steric hindrance (ectodomain)
	Chol-KK114	$9.2 \pm 2.3$ 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?**

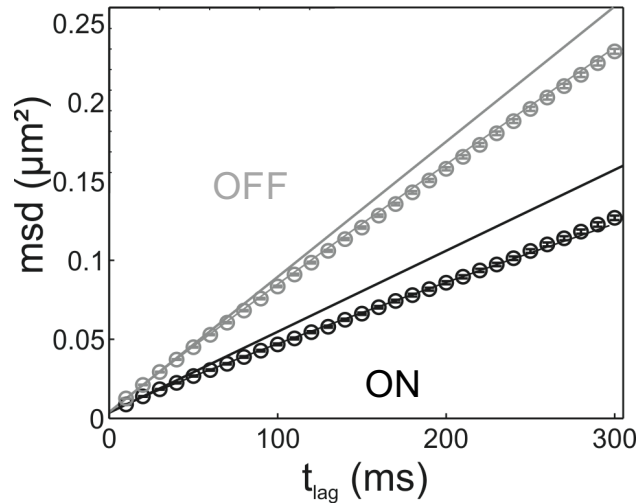
Special thanks to E. Sevcsik, M. Brameshuber, A. Arnold, G. Schütz, A. Honigmann and N. Matsumori.



# Single molecule **diffusion** analysis



$$\text{msd} = 4D t_{\text{lag}} + 4\sigma^2$$



- 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