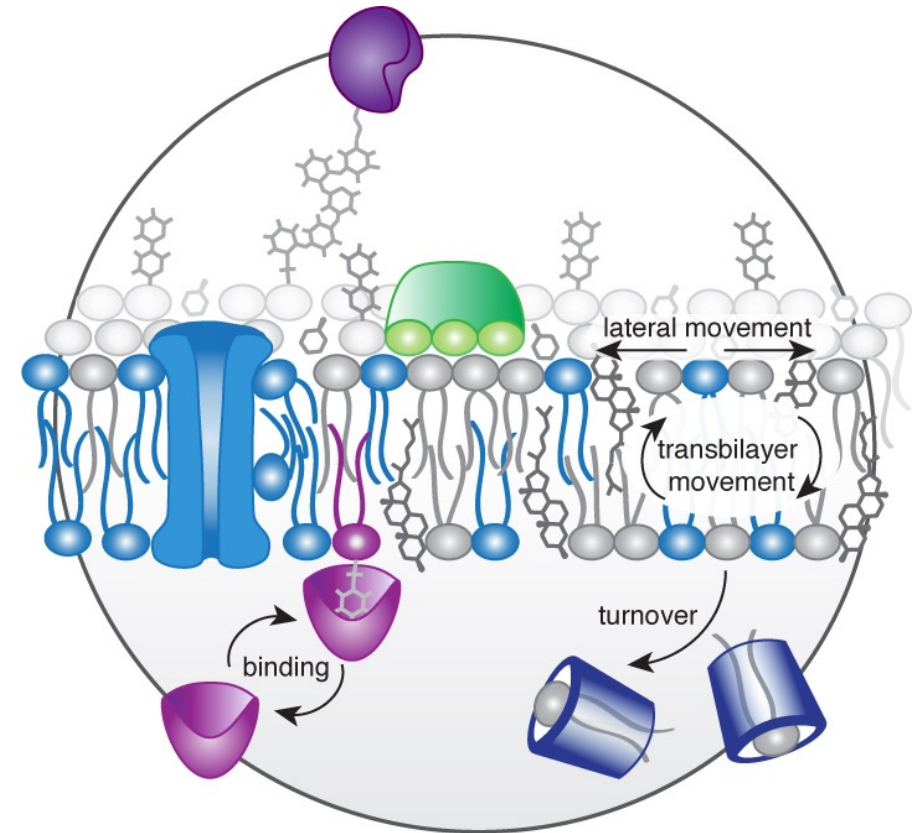


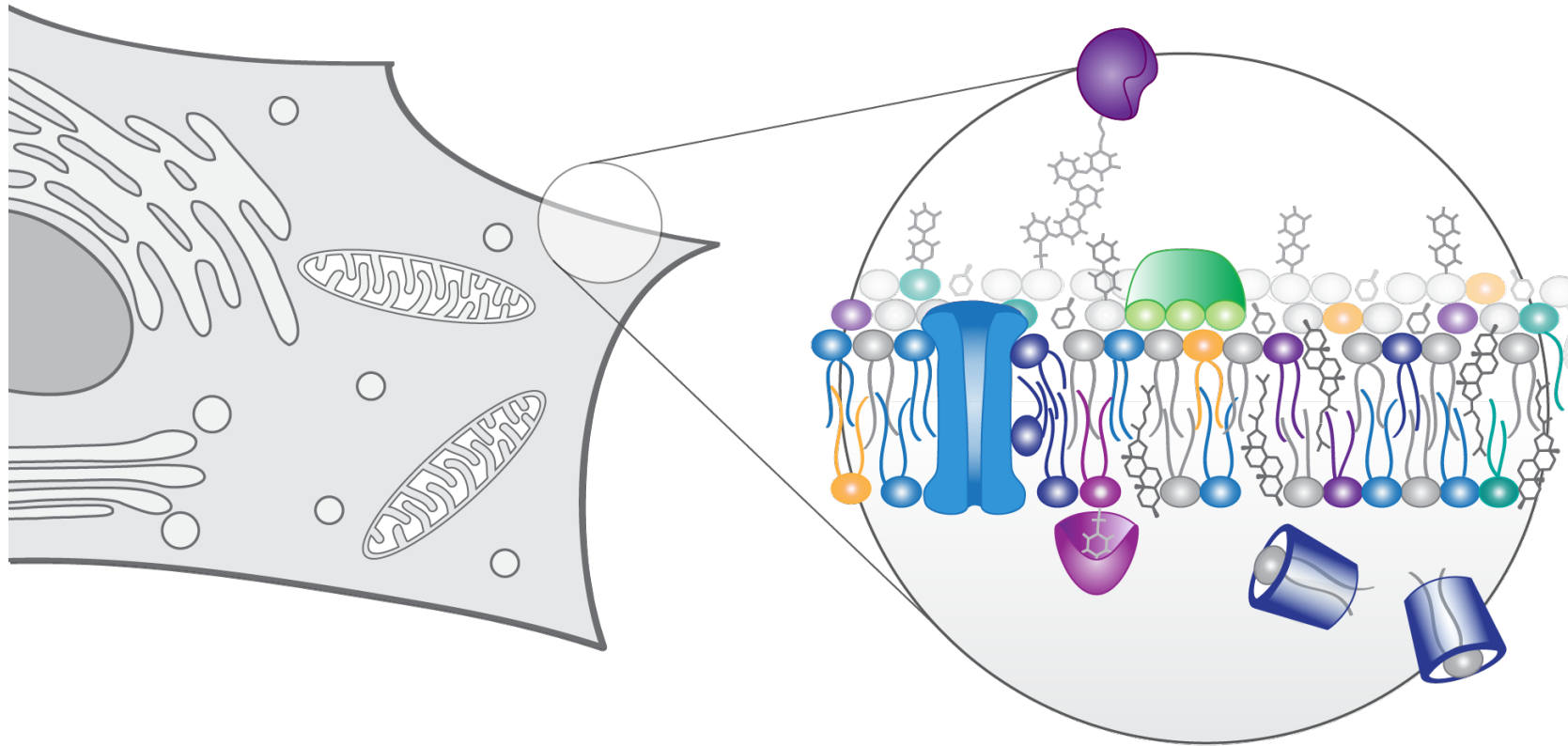
More than fat - Tracking the secret life of lipids

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07.10.2026



Biological membranes and lipid diversity



- semi-permeable barrier around cells
 - compartmentalization of biochemical reactions
 - essential for **structural integrity, signaling, and energy storage**
- **thousands of lipid species**

What is the biological function of lipid diversity?

Lipid dysregulation and disease

- High LDL cholesterol → plaque formation (atherosclerosis)
- Oxidized lipids trigger inflammation and vessel damage

Cardiovascular
disease

Cancer

- Rapidly growing cells reprogram lipid metabolism for membrane synthesis and signaling
- Certain lipids can promote tumor growth or metastasis

- Imbalanced lipid storage and signaling promote obesity and type 2 diabetes

Metabolic
disorders

Neurodege-
neration

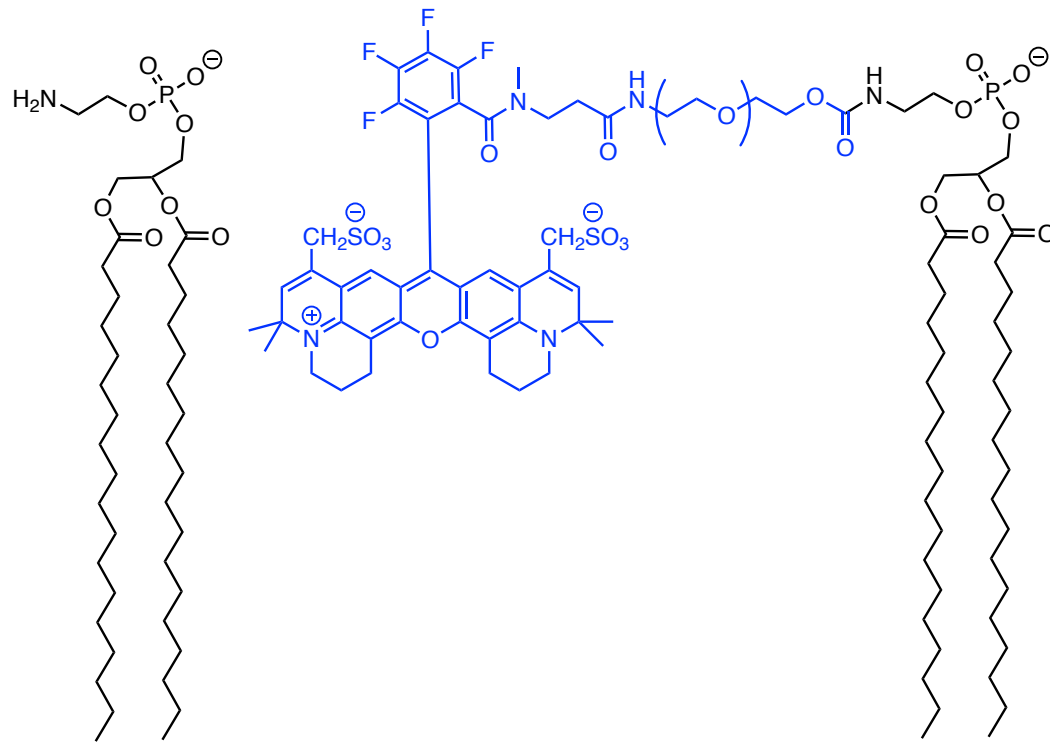
- Membranes become more rigid, oxidized, and less diverse
- Emerging role of altered lipid homeostasis in neurodegenerative disease progression

Lipid diversity and its impact on cellular processes

Observing lipids in cells is difficult...

Localisation

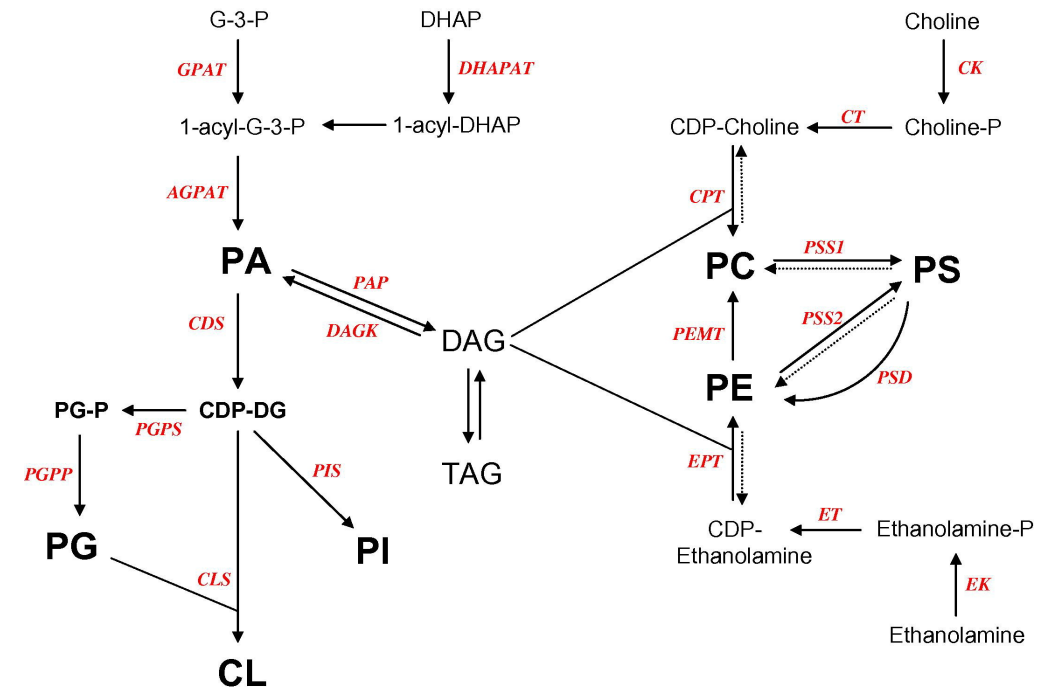
Lipids are small molecules



Structural changes affect physico-chemical properties and localisation

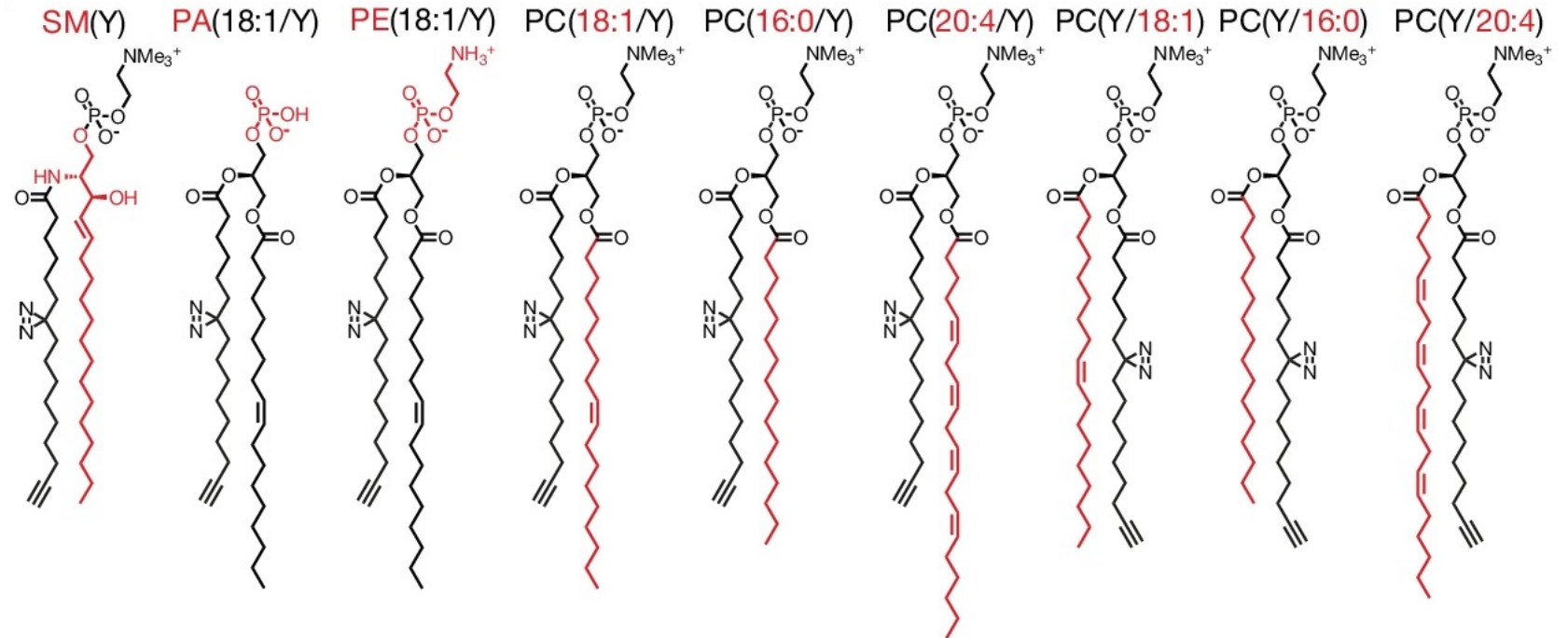
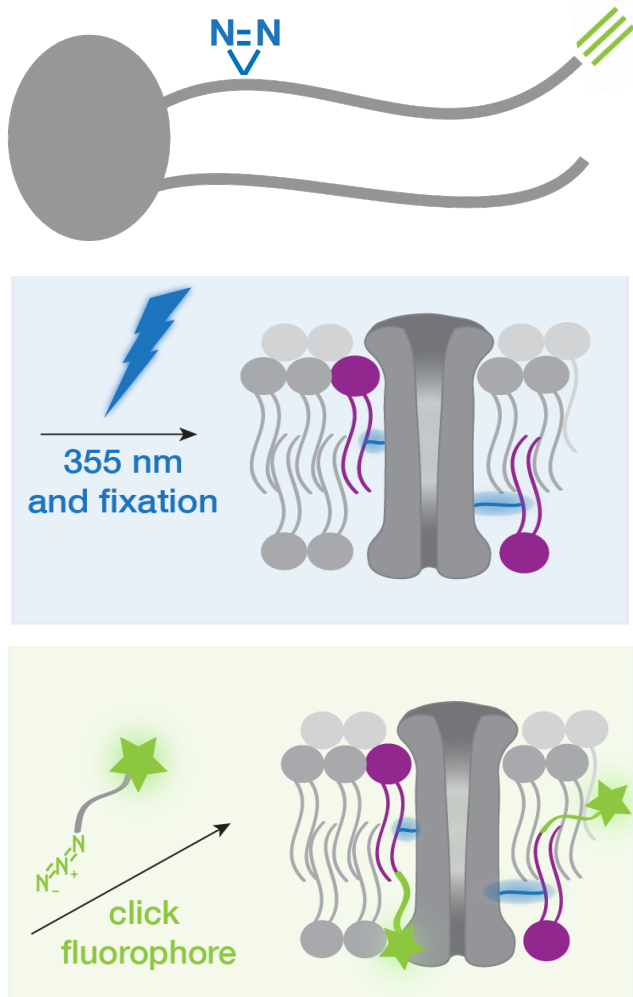
Perturbation

Lipids are not genetically encoded and rapidly turned over



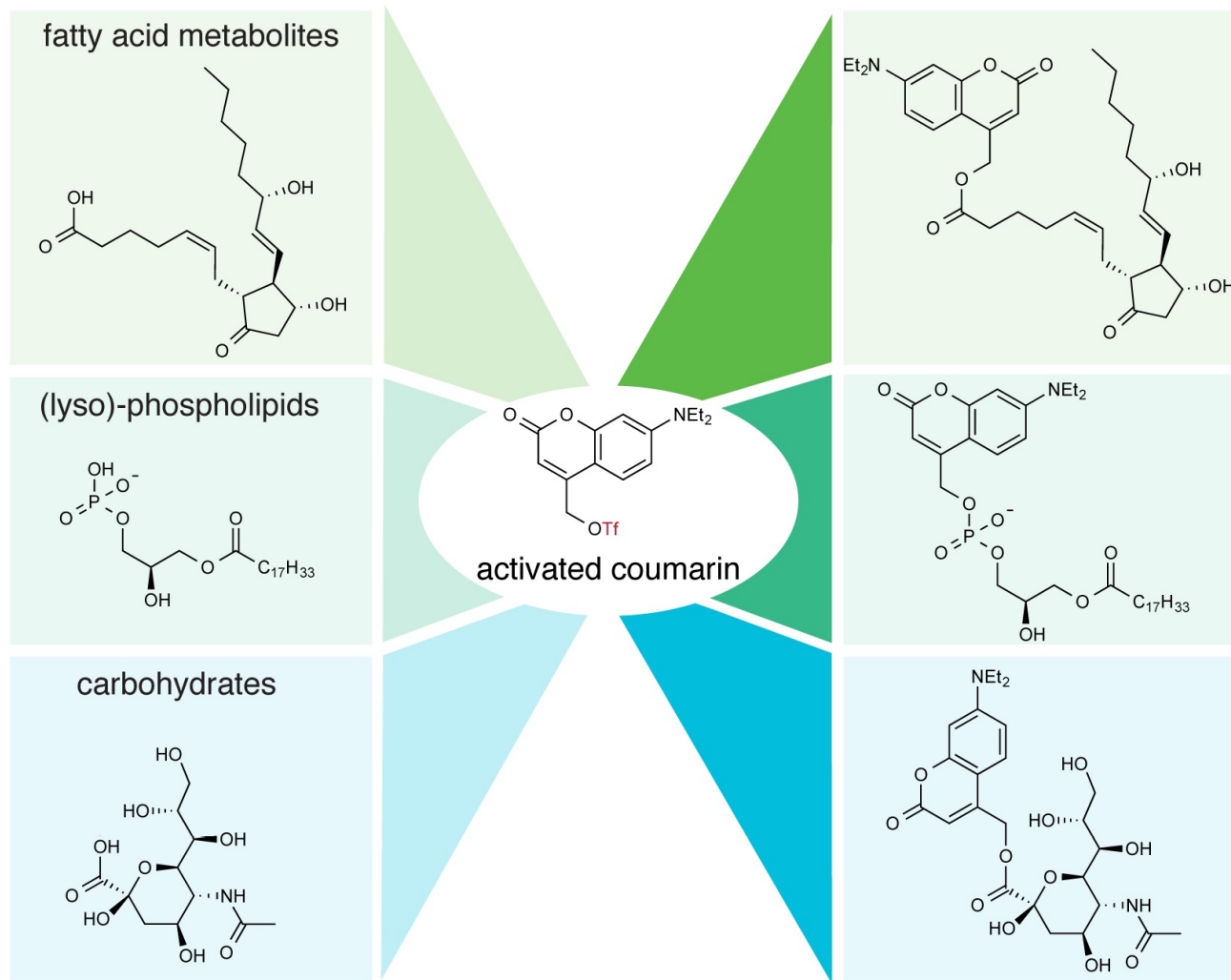
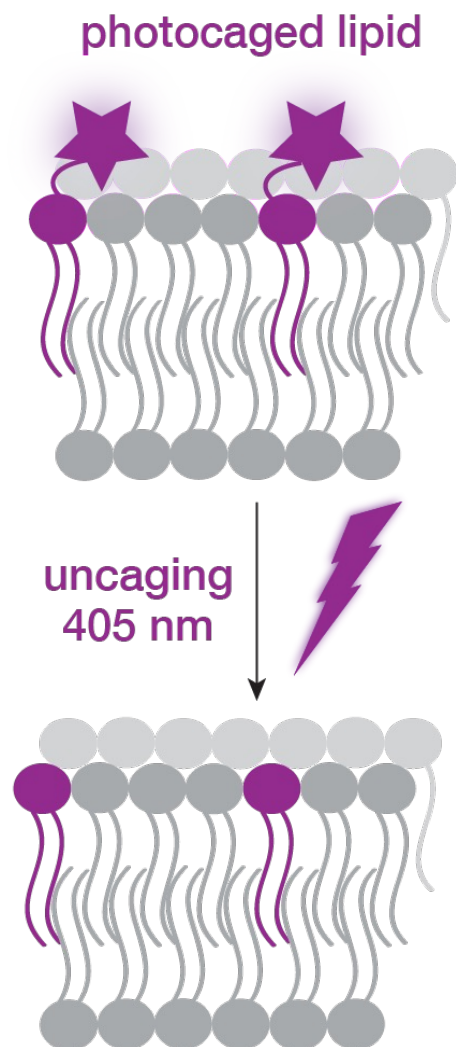
Interfering with lipid biosynthesis affects whole lipid classes/
alters the lipidome

Lipid localisation using bifunctional fatty acids

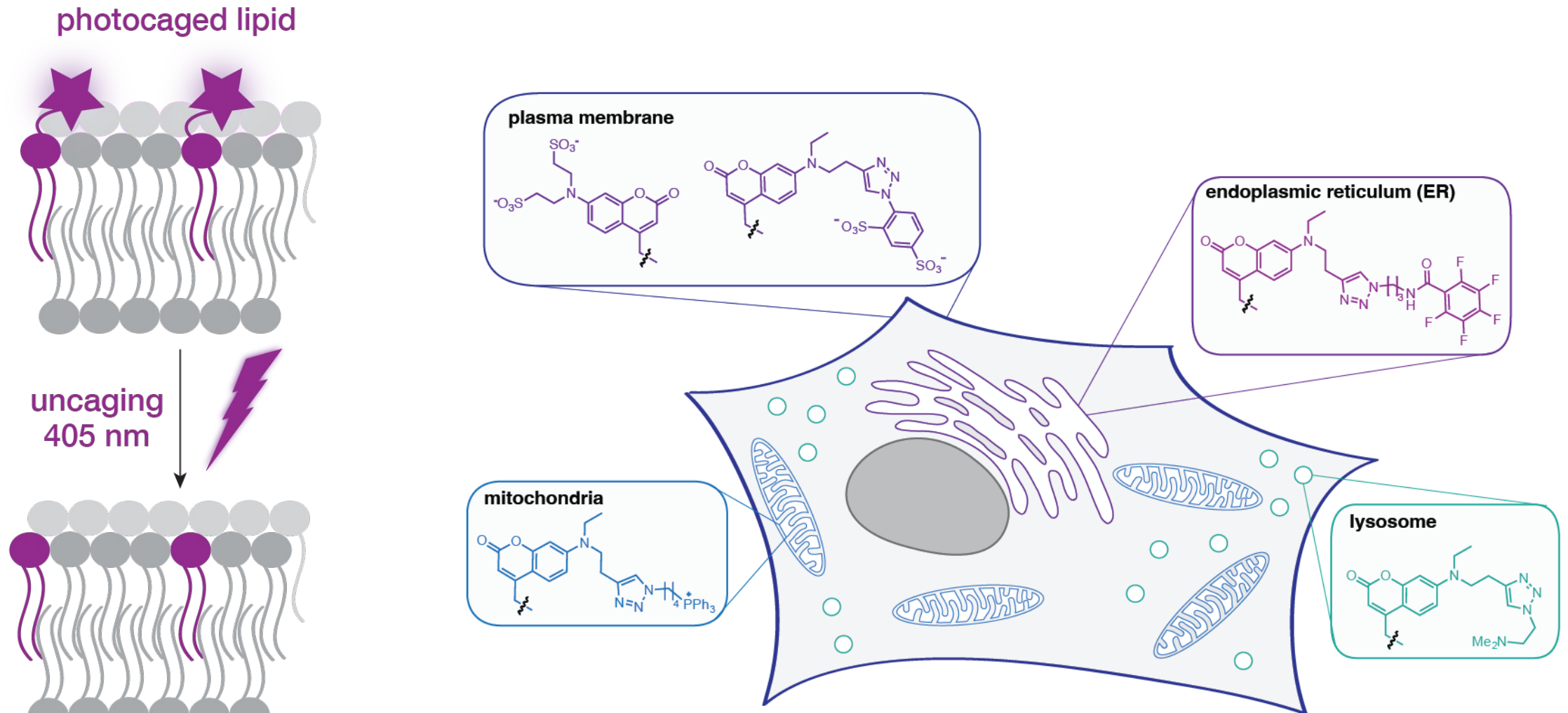


- Retrograde lipid transport occurs mainly through non-vesicular routes
- Flippases are involved in species-specific directional lipid transport
- Lipid metabolism is approximately one order of magnitude slower than lipid transport

Perturbations using photocaged lipids

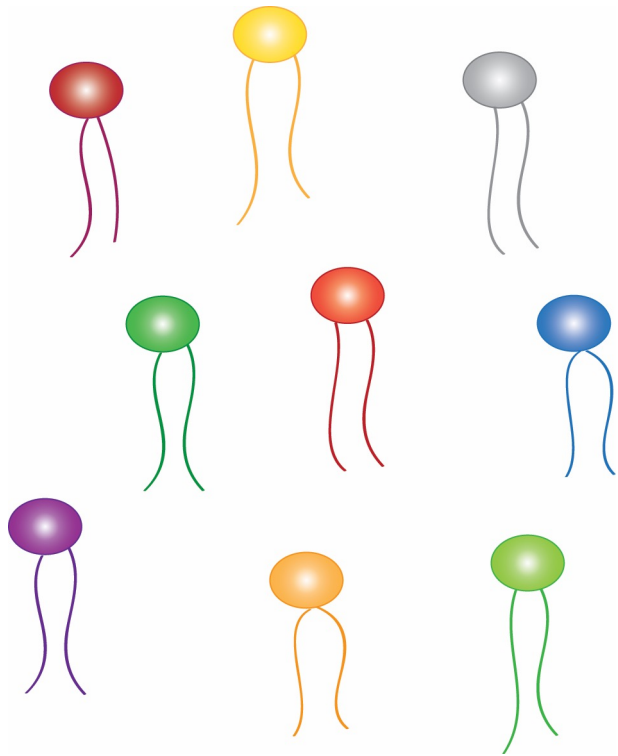


Perturbations using photocaged lipids



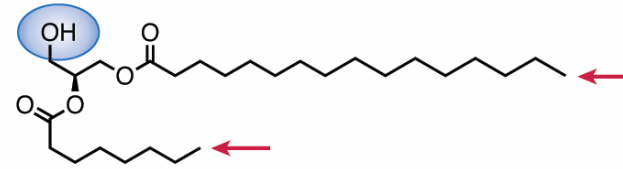
The chemical basis lipid diversity

Lipid classes

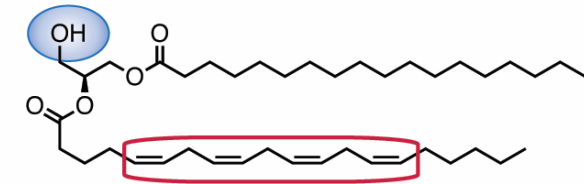


What are the chemical differences within a lipid class?

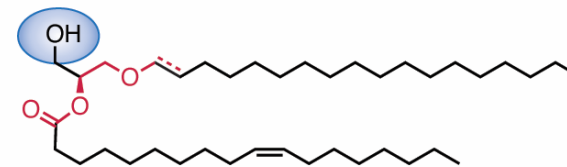
chain length



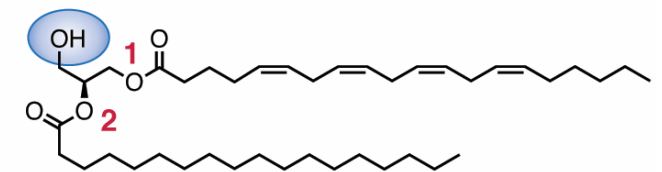
unsaturation degree



fatty acid linkage

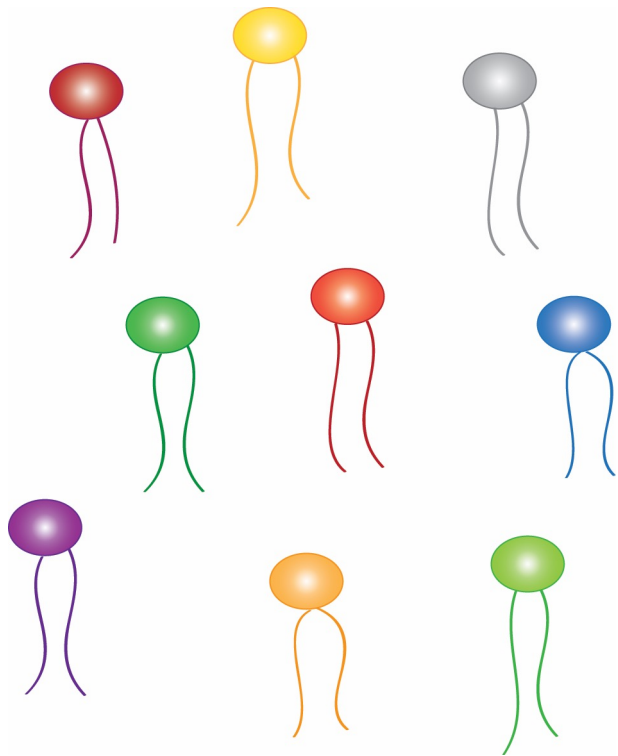


fatty acid position



Diacylglycerols as signaling lipids

Lipid classes



DAG effector proteins:

cPKCs ($\alpha, \beta I, \beta II, \gamma$)



nPKCs ($\delta, \epsilon, \eta, \theta$)



aPKCs (ζ, λ)



PKD



$\alpha 1/\beta 1$ - Chimaerin



$\alpha 2/\beta 2$ - Chimaerin



Ras GRP 1-4



DAG kinase α, β, γ



Munc13-1/ubMunc13-2



bMunc13-2/Munc13-3



● C1-domain (DAG binding)

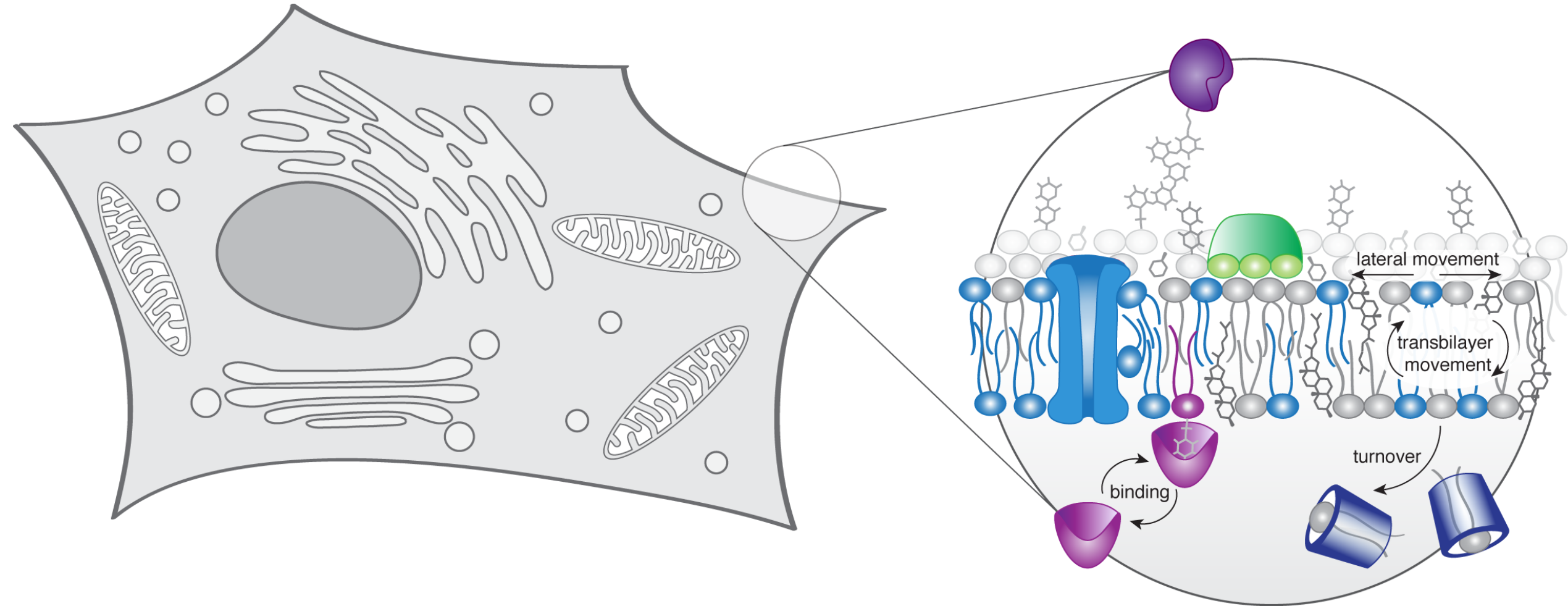
200 AA

Specificity needs to be encoded in the lipid signal

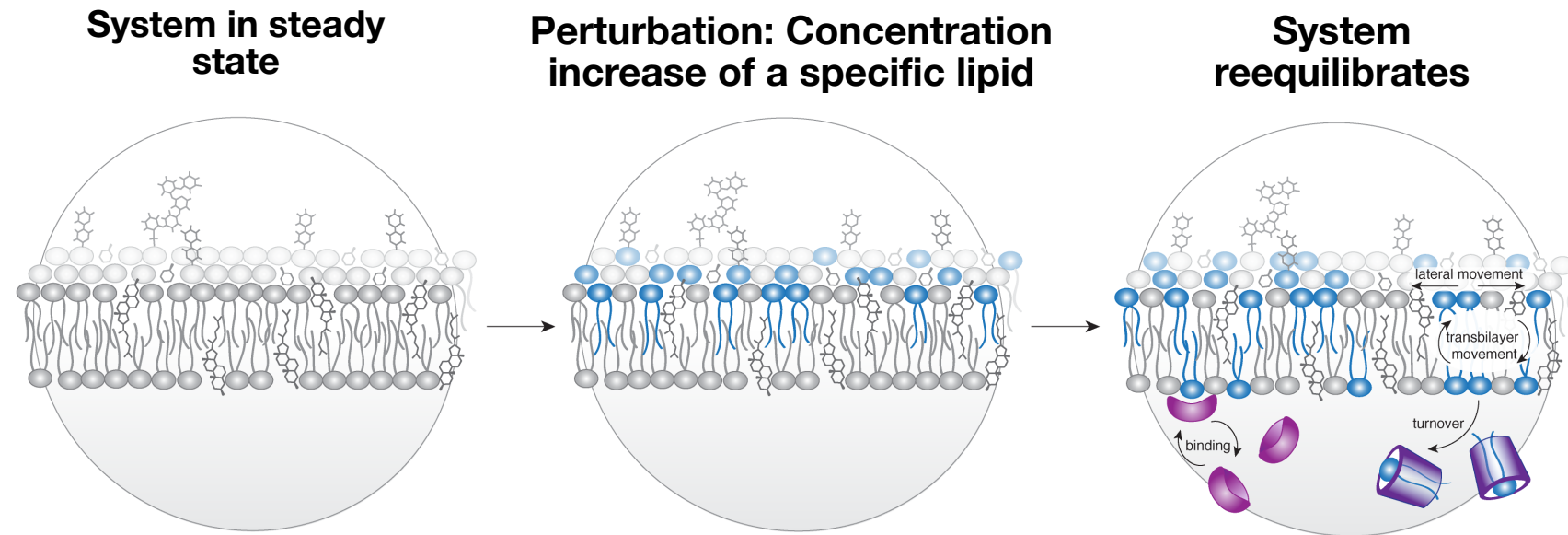
Hypothesis:

Chemical differences between individual DAG species affect cellular signaling.

How can lipids exert their function?

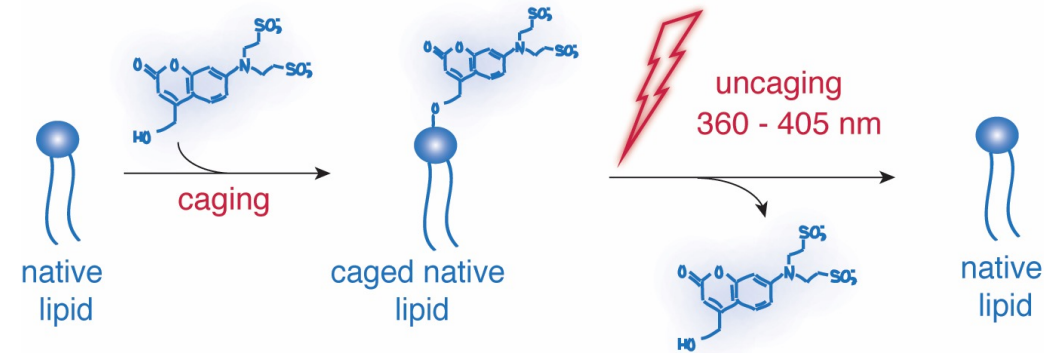


Experimental design: Live cell kinetic relaxation experiments



Quantitative lipid biochemistry in living cells

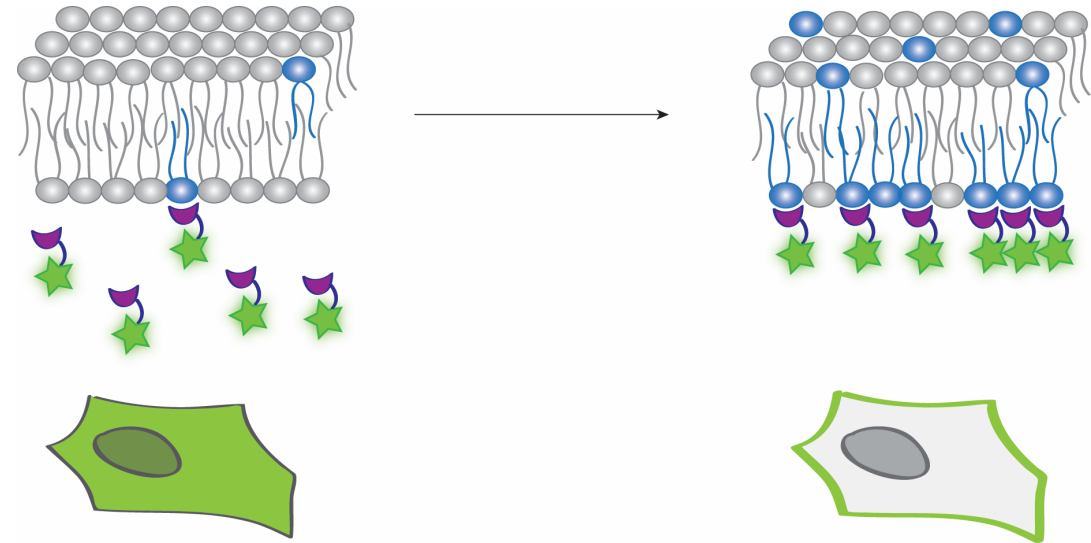
Native lipid probes:



- Photocage masks biological function
- Photocage prelocalizes probes
- No modification on the lipid after uncaging

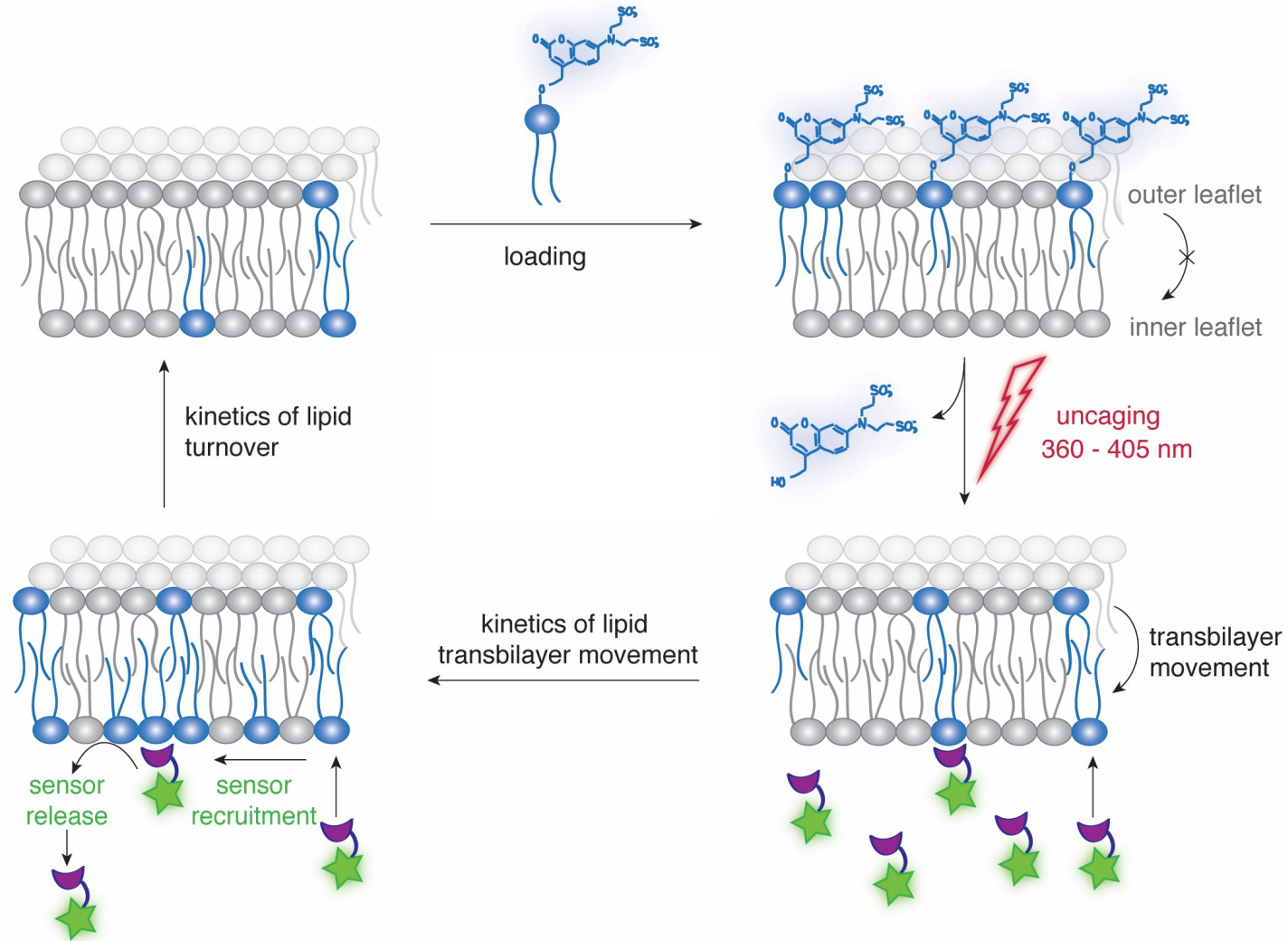
Translocation as a readout in the cell:

Low lipid concentration

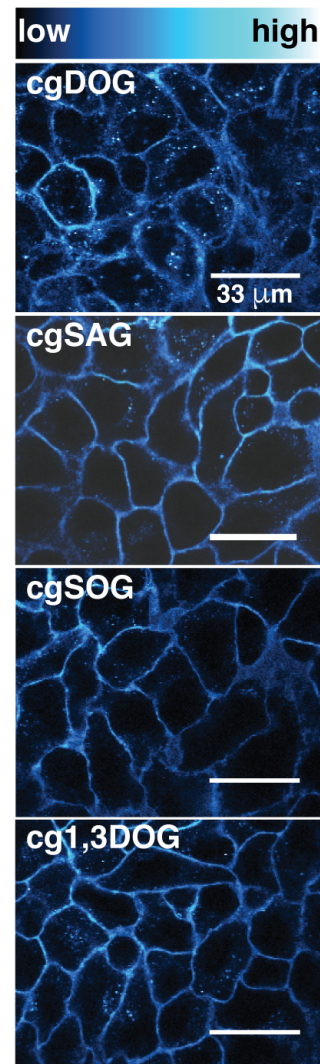
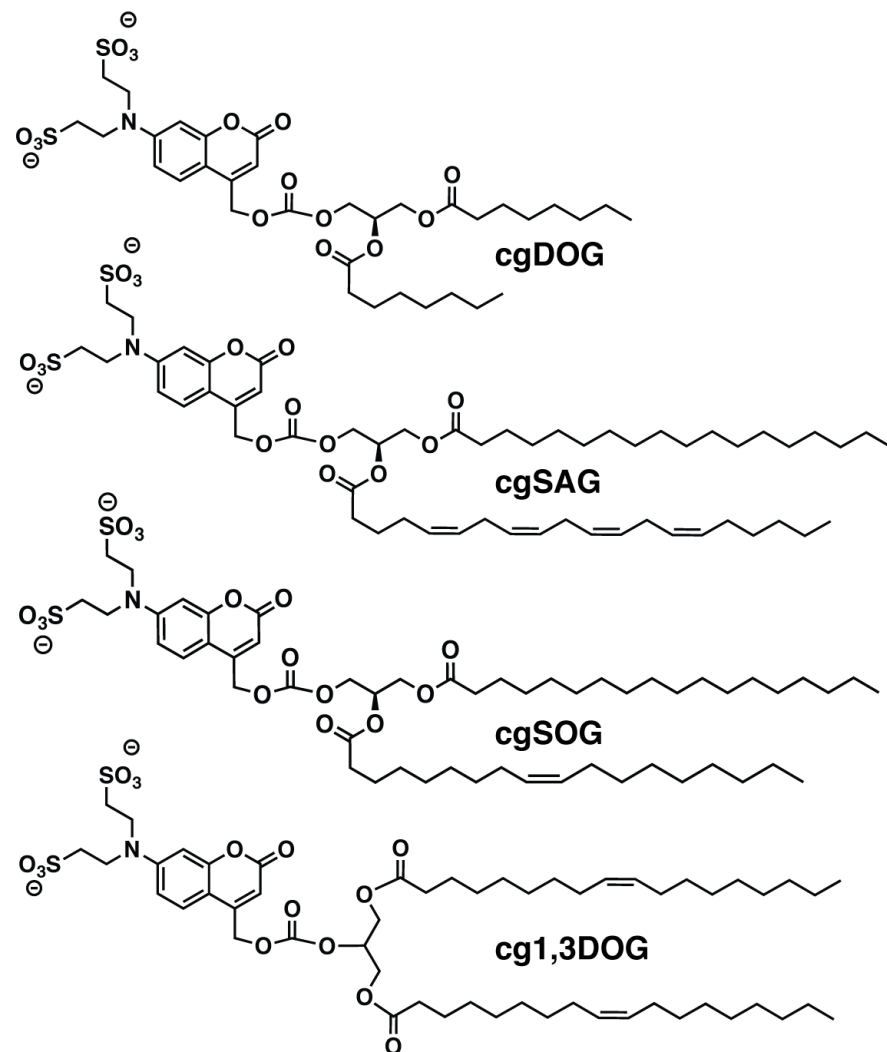


- FP-tagged lipid binding protein expressed in the cell
- Recruitment to the PM after uncaging

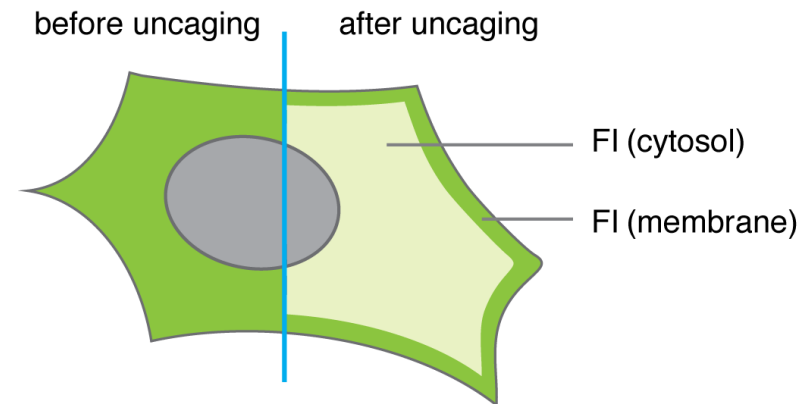
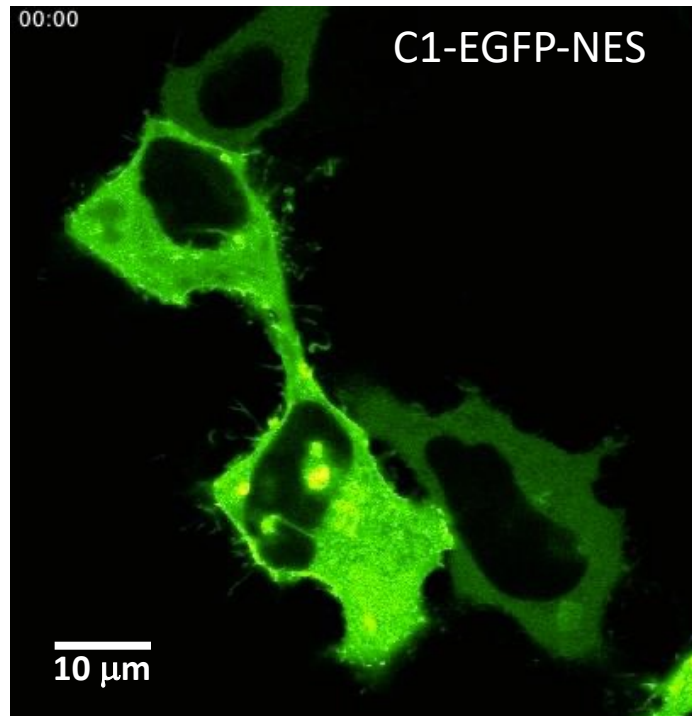
An assay for lipid dynamics and turnover



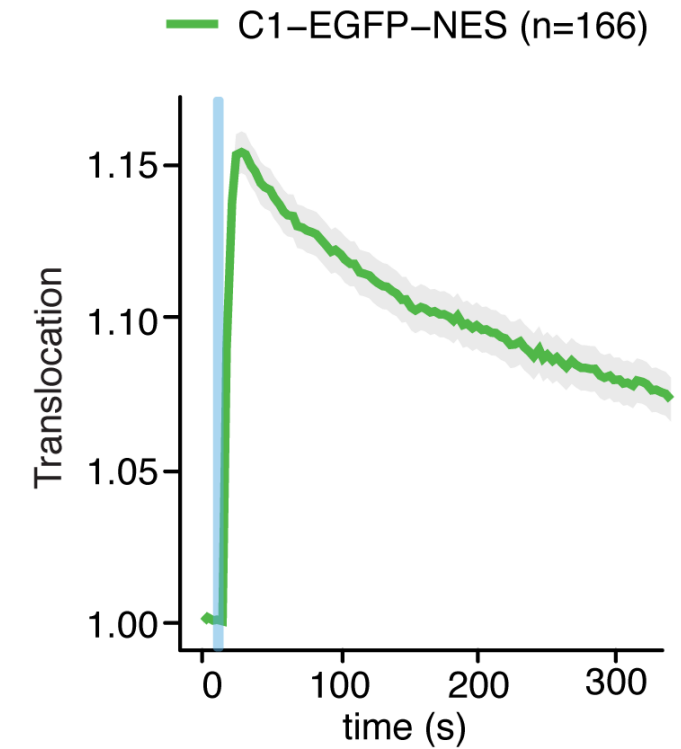
Caged DAGs localize to the outer PM leaflet



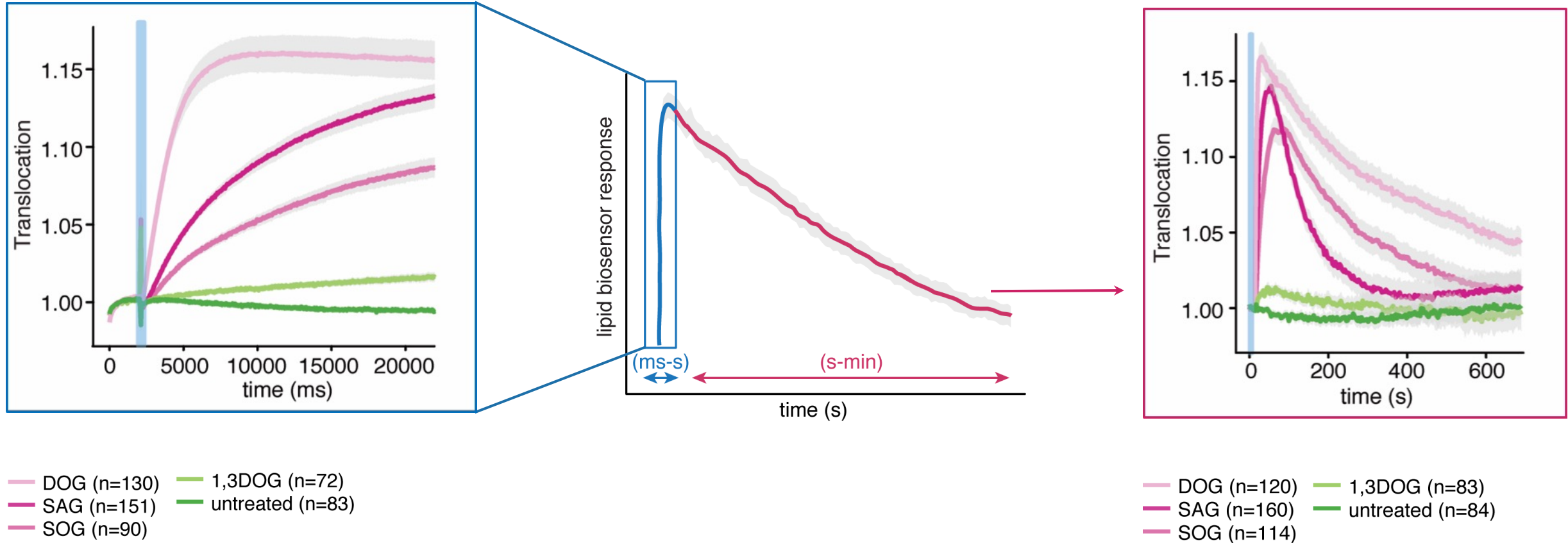
A novel translocation probe for DAG sensing



$$\text{Translocation efficiency} = \frac{FI/FI_0 \text{ (membrane)}}{FI/FI_0 \text{ (cytosol)}}$$



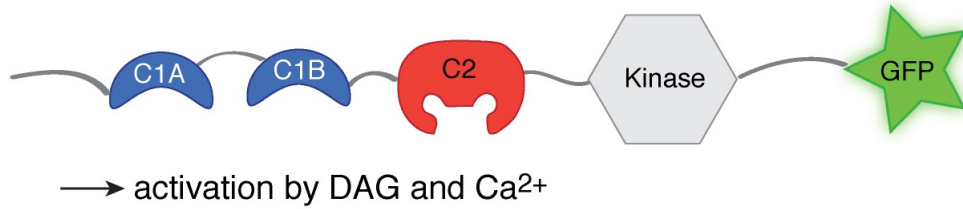
Lipid dynamics vary for different DAG species



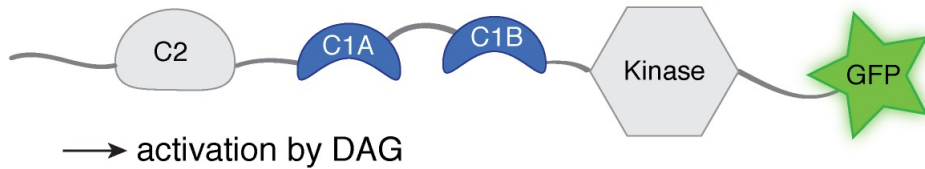
The finding implies that the function of signaling lipids can not be understood on the level of lipid classes

PKCs as DAG effector proteins

classical PKCs (α, β, γ)

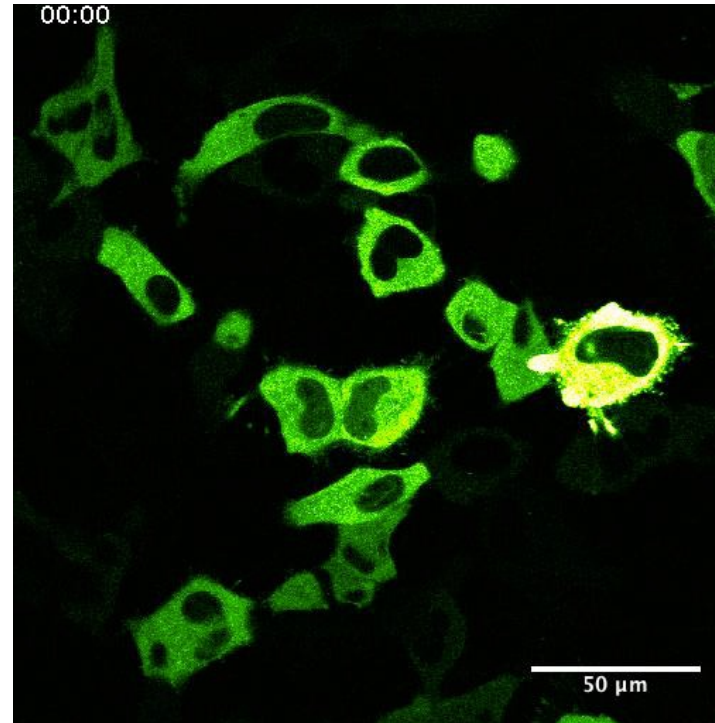


novel PKCs (δ, ϵ)

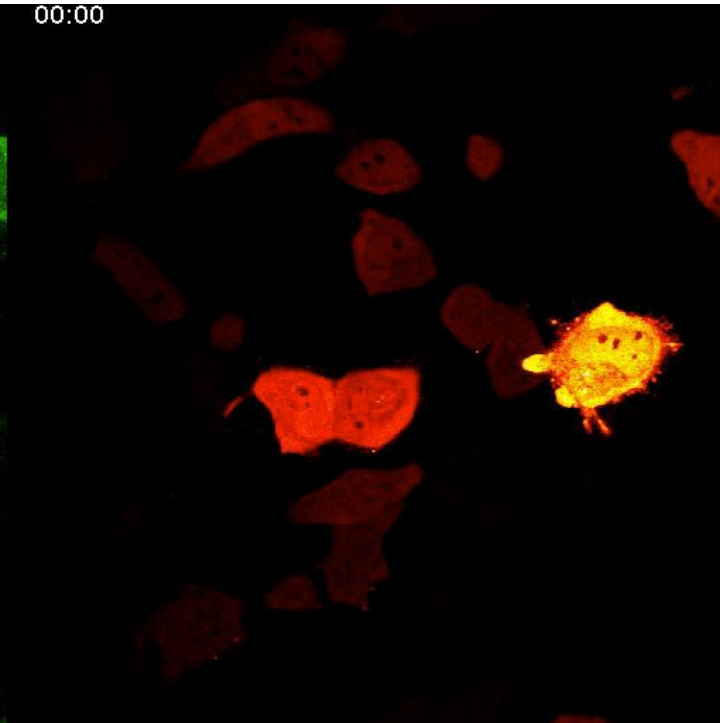


SAG uncaging in cells expressing:

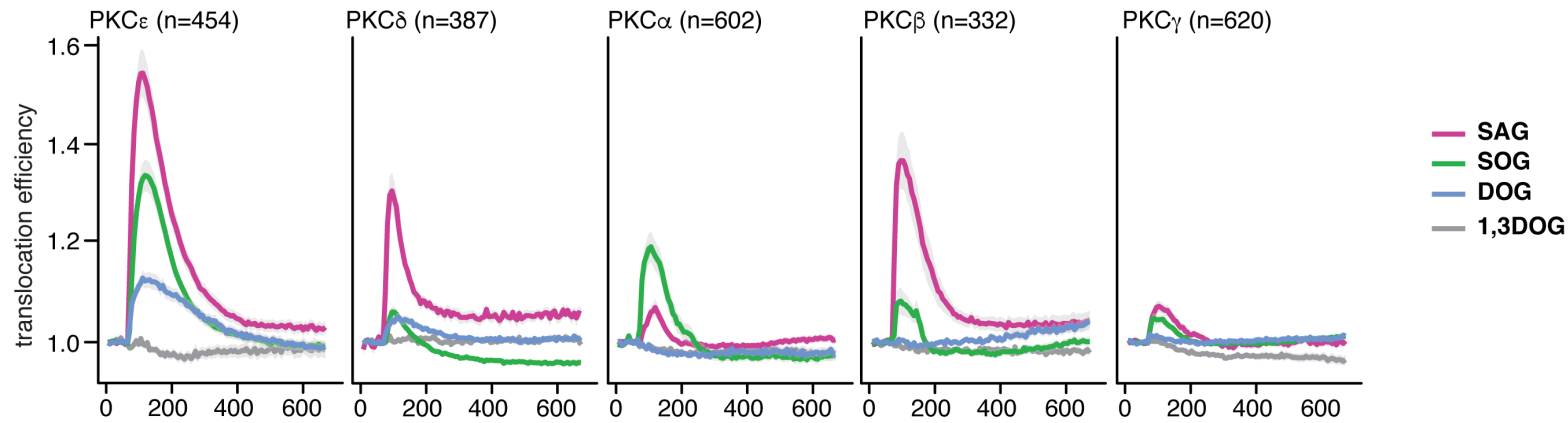
PKC α -EGFP



RGECO (intensiometric Ca^{2+} sensor)

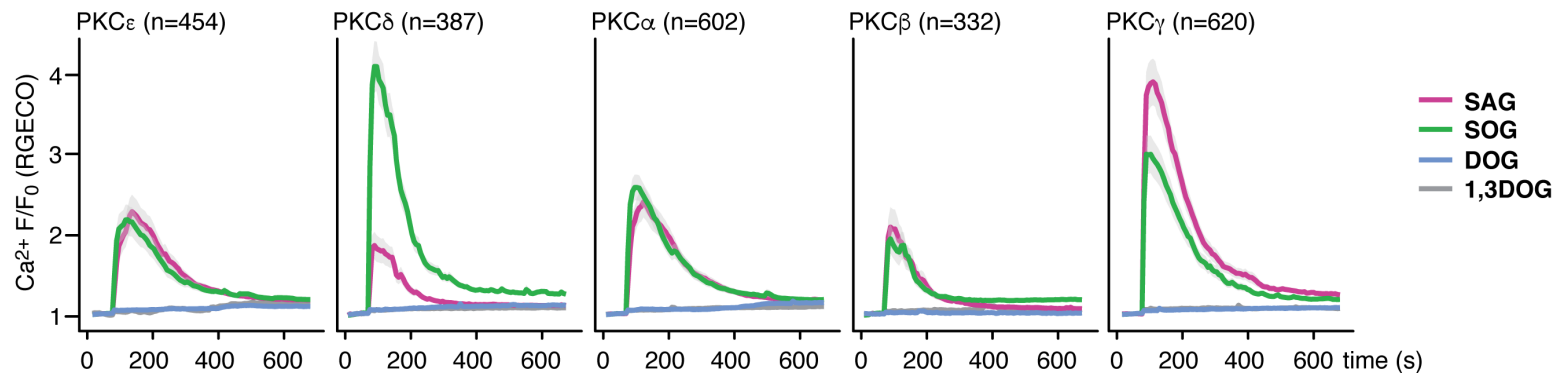
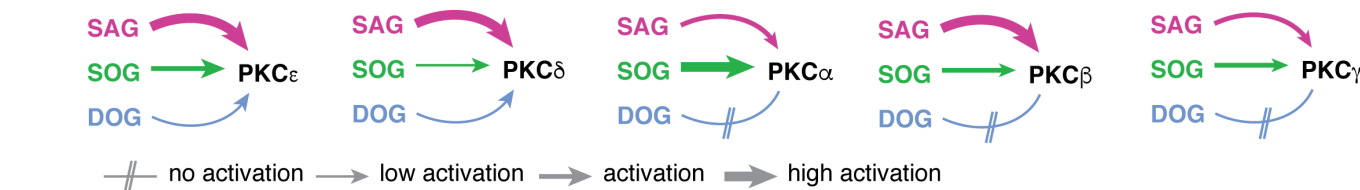


PKC responses depend on DAG species

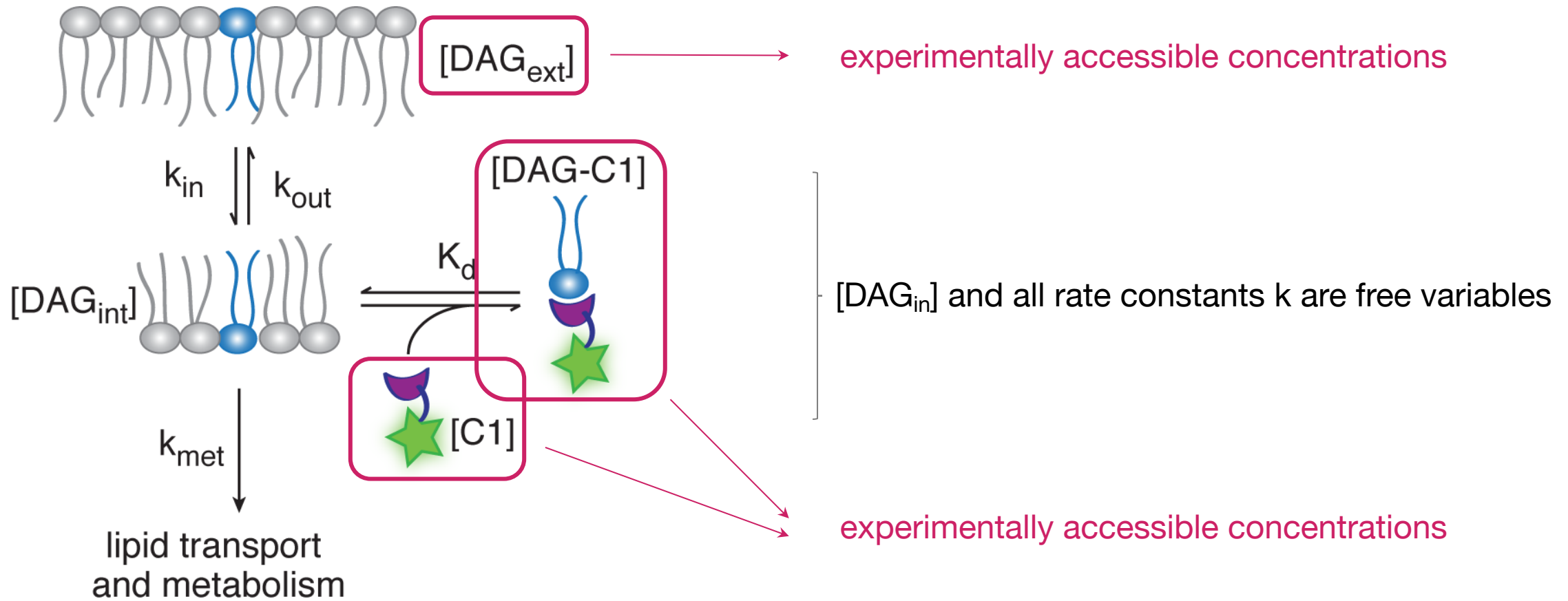


- different recruitment patterns for all DAGs
- short chain DAG does not recruit classical PKCs

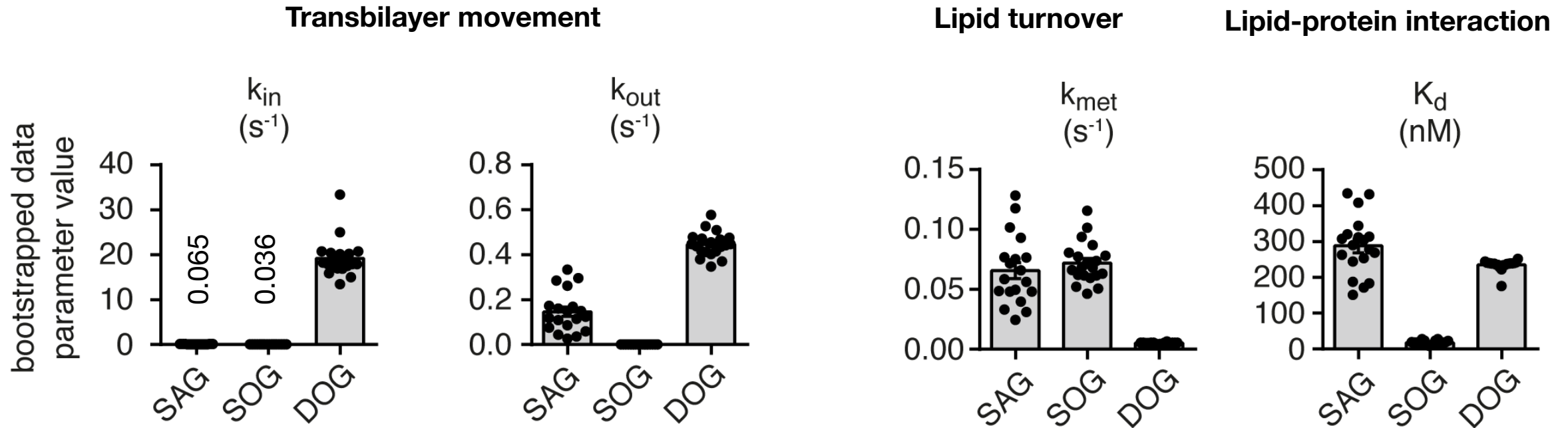
→ Lipid diversity provides a basis for modulation of signaling events



A quantitative model for DAG signaling

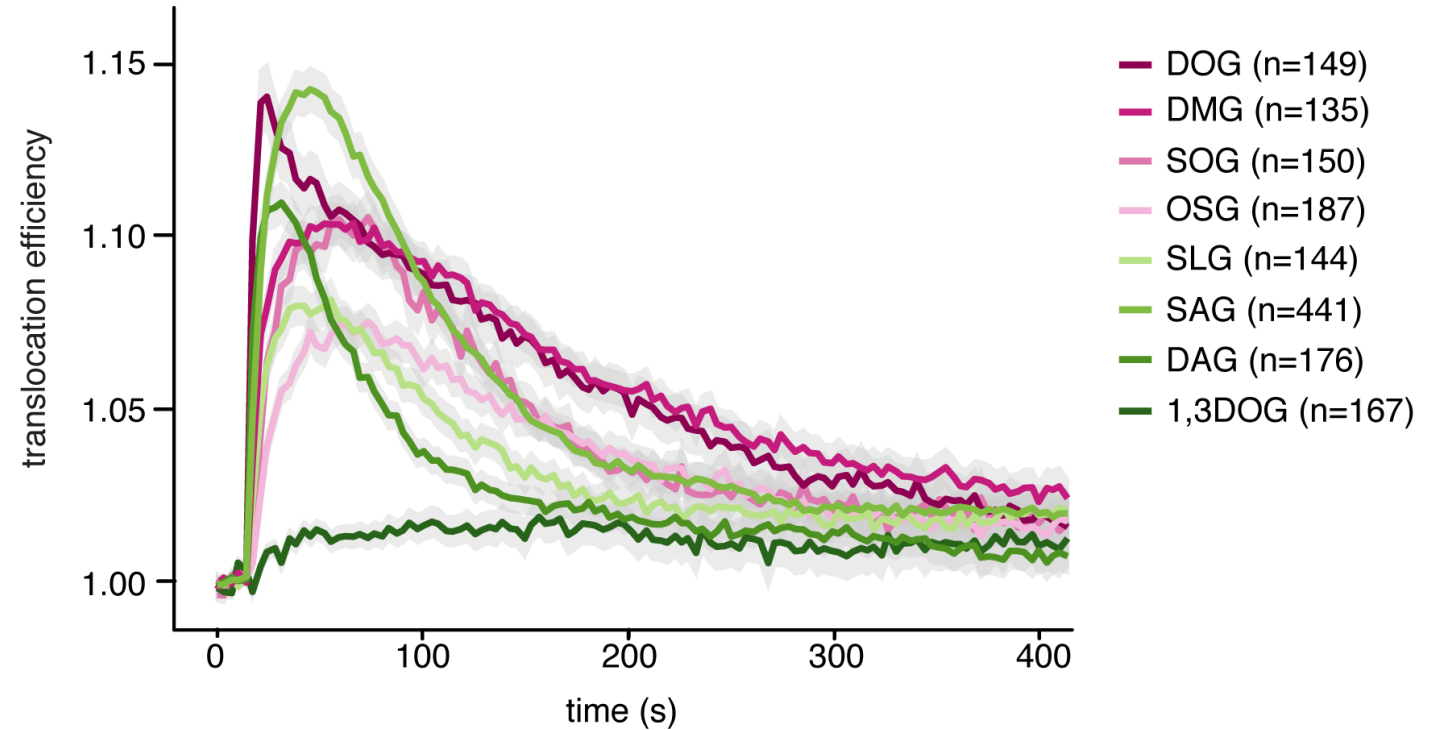
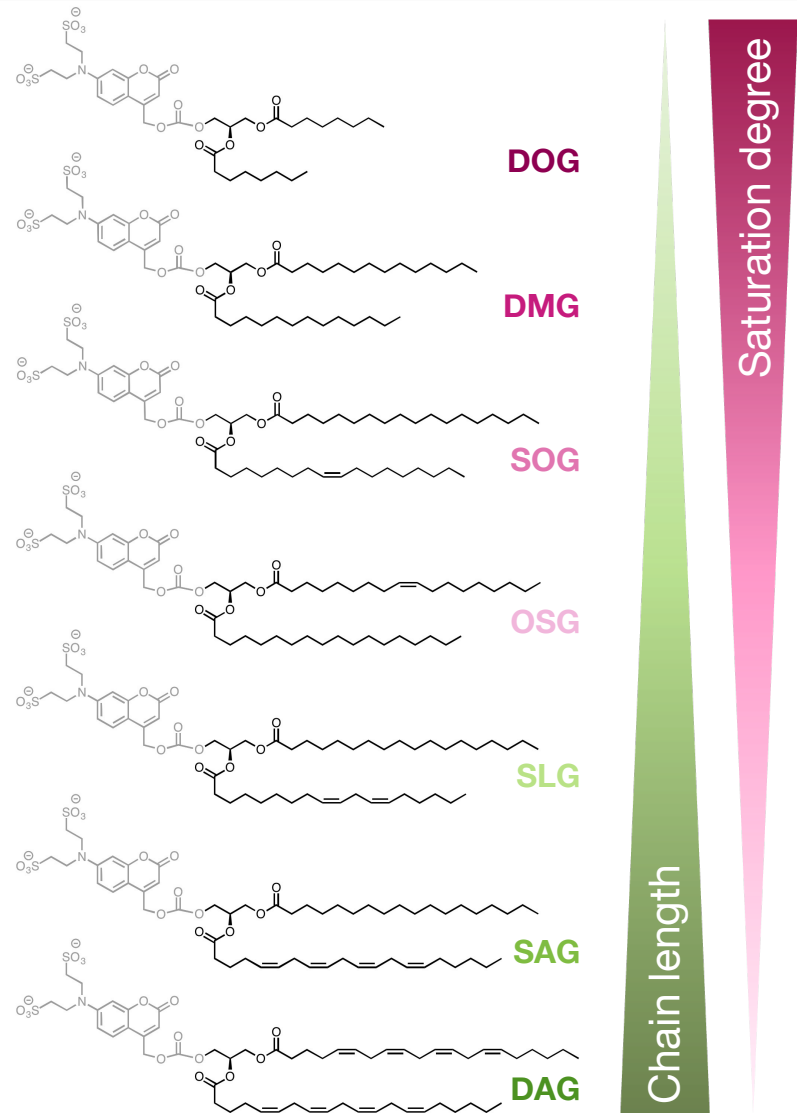


Quantification of kinetic rate constants and lipid-protein interaction



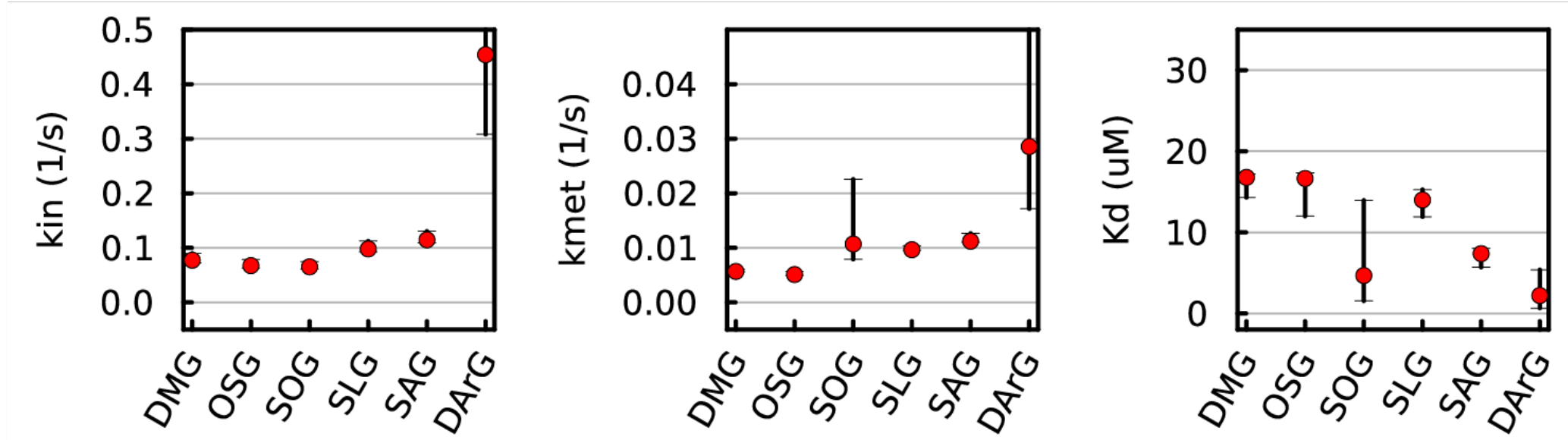
- DOG has a significantly higher k_{in}
- SAG is the only lipid with a meaningful k_{out}
- K_d varies by orders of magnitude

A structure-reactivity study on DAG-C1 interactions



Both saturation degree and chain length influence DAG kinetics in biological membranes

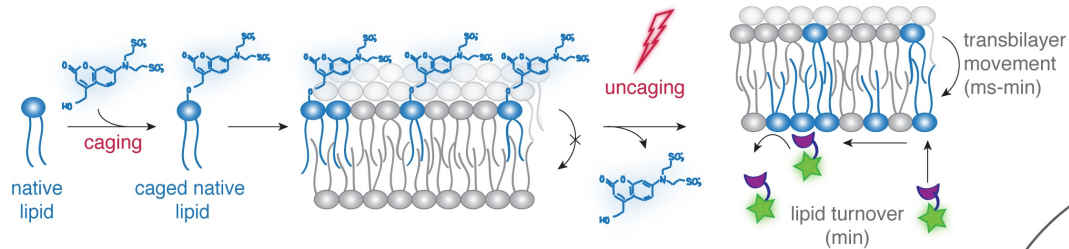
Quantifying single cell diacylglycerol signaling kinetics



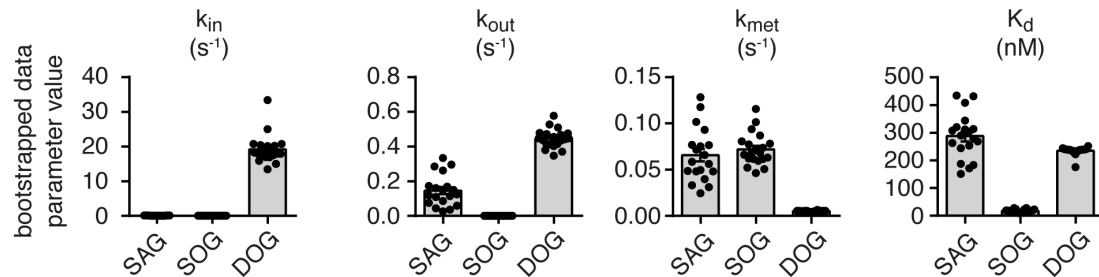
- Most parameters are identifiable, except for k_{in} for DArG
- There is a trend of higher k_{in} rates with increasing acyl chain unsaturation
- The inferred K_d values indicate that C1 effector protein affinities to DAGs are species specific

Summary

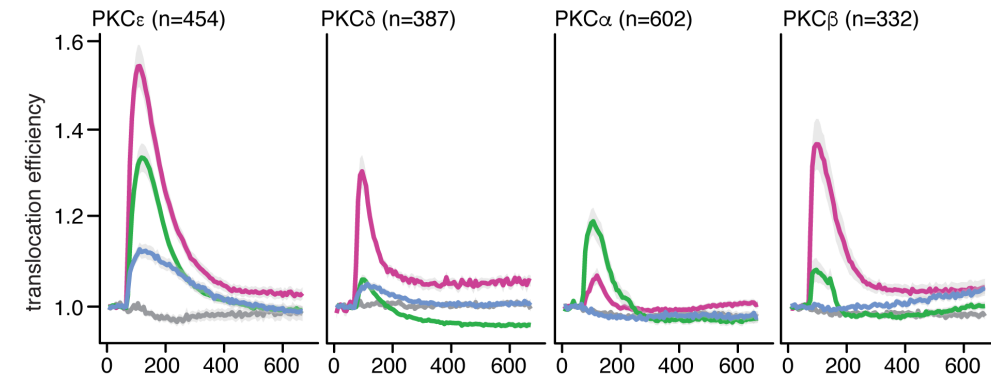
New tools to study native lipids in living cells



Mathematical modelling allows determination of rate and binding constants



Differential recruitment of effector proteins

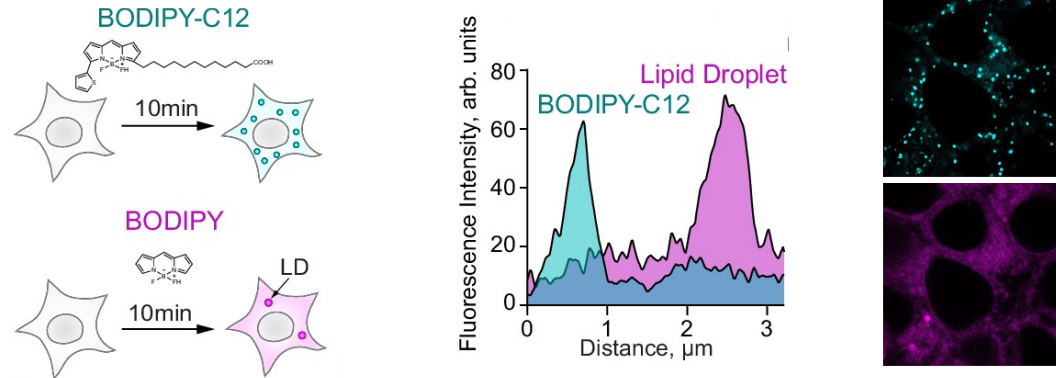


- Subtle chemical differences change rate and binding constants by orders of magnitude
- These differences are sufficient to explain differential recruitment of diacylglycerol binding proteins

→ **Lipid diversity provides a molecular basis for specification of signaling events**

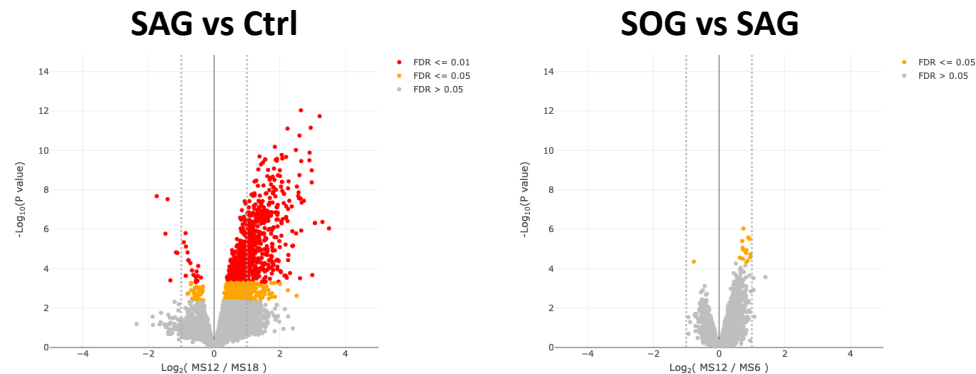
Current Research in the Schuhmacher Lab at EPFL

Lipid-based probes for peroxisomes

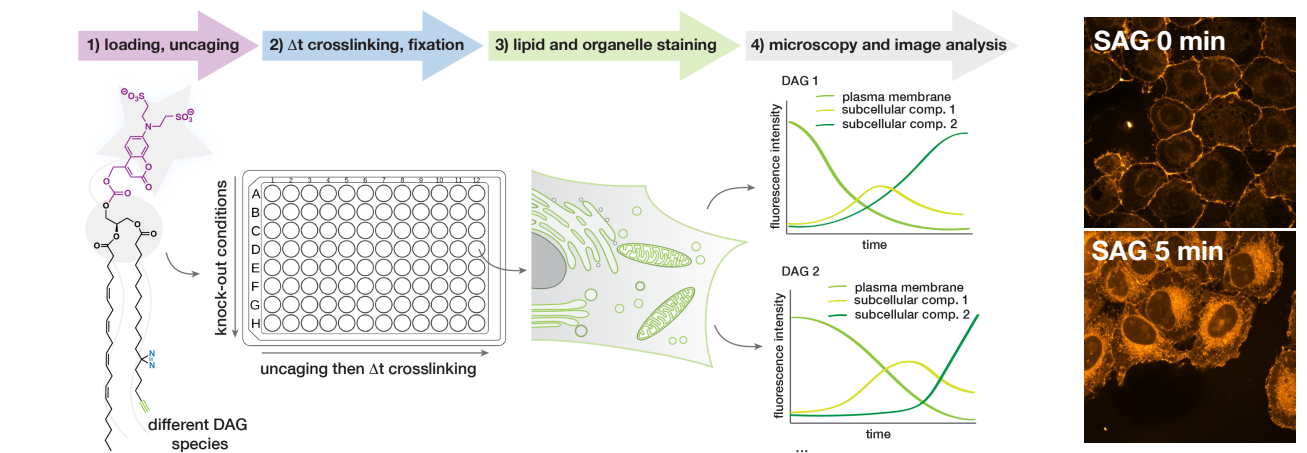


Korotkova, D., ... **Schuhmacher, M.** & Amen, T. (2024). *Nature Communications* 15(1), 4314
 Howman C., ..., **Schuhmacher M.**, Zalckvar E., Kaganovich D., Amen T. (2025). *JoVE*, (226)
 Long, A. J., Candeias, D., Coveña, N. F., Reymond, L., **Schuhmacher, M.**, ... & Amen, T. (2026). *Bio-protocol*, 16(5)

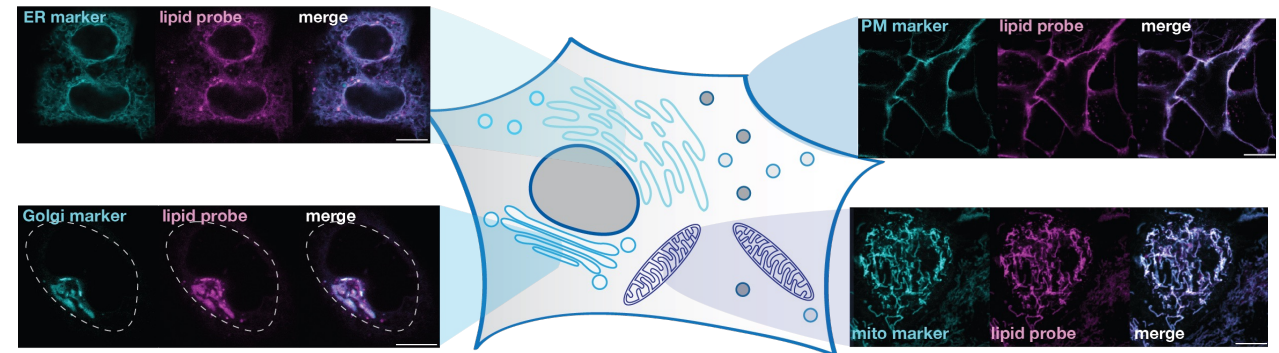
Analysis of species-specific lipid signaling



Tracking lipid movement and metabolism



Leaflet-specific modulation of lipid levels





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