# MECHANISTIC INSIGHTS INTO CONTROLLED PEPTIDE-MEDIATED VESICLE FUSION

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**J.Heyrovský Institute of Physical Chemistry, Prague**

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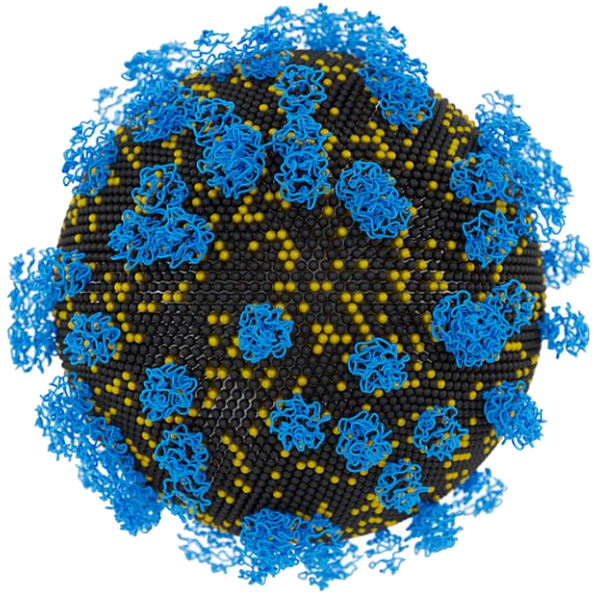
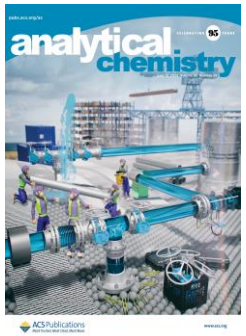
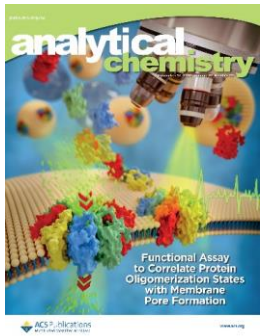


*Tomáš Belloň (IOCB Prague)*

# Focus of the team: areas of interest

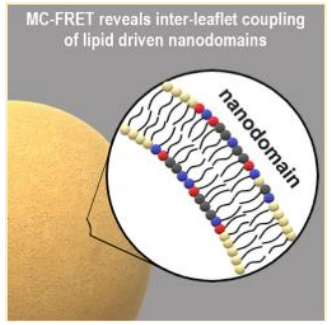
Development of new fluorescence methods and their applications in membrane biophysics



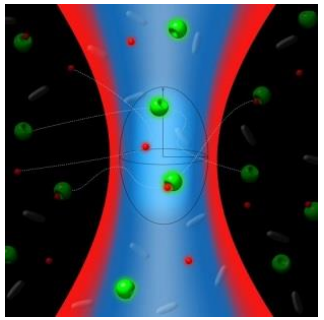

Šachl, R et al. *Anal. Chem.* **2020**, *92*, 14861–14866
Vandana Singh et al, Anal. Chem. **2023**

Tools to study membrane nanoscale organization and structure with single leaflet resolution



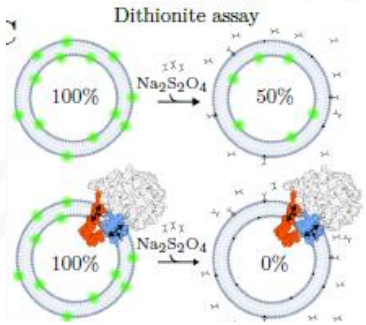

Vinklárek, IS et *J. Phys. Chem. Lett.*, 10, 2024–2030 (2019)

Tools to study membrane dynamics: Fluorescence (Cross) Correlation Spectroscopy (FC(C)S)



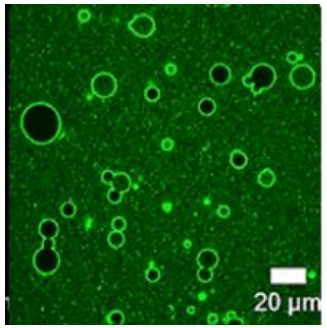
Amaro, M. et al. *Angew. Chemie 55*, 9411–9415 (2016)

Tools to study vesicle membrane fusion with single leaflet resolution

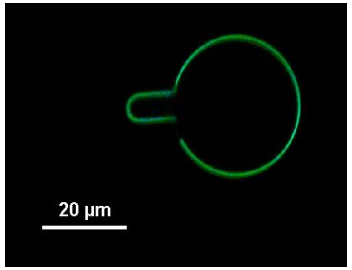



Koukalová, A et al *Nanoscale*, **4**, 19064–19073, (2018)
Mora, N. L. *Sci. Rep.* 2020, 10, 1–13

Our favorite systems: mimetics of cellular systems: SPBs, SUVs, LUVs, GUVs, GPMVs, aGUVs




Micromanipulation of GUVs: changing membrane tension and curvature




## Applying these ‘tools’ we study protein-membrane interactions:

1. **Programmed cell death** – from molecular level to human (the interaction of BCL-2 proteins with mitochondrial membranes.

   Mystek et al Biophys. J. in print., Lidman et al BBA 2016

2. **Protein translocation** across biological membranes

   Lolicato F. et al eLife 2024; Steringer et al eLife 2017

3. The mechanism **of membrane fusion** (RNA delivery into cells) and controlled transport of cell-penetrating peptides across asymmetric membranes – the mechanism of entrance

   Koukalová, A et al *Nanoscale*, **4**, 19064–19073, (2018); Mora, N. L. *Sci. Rep.* 2020, 10, 1–13

4. **The impact of membrane asymmetry** on the nanoscopic organization of lipid membranes - Nanoscale organization of (glyco)sphingolipids

   Sarmento, MJ et al, *Biophys. J.* 120, 24, 5530-5543 (2021)

5. **Inter-leaflet coupling** (transduction of signals across plasma membranes)

   Davidović, D *J. Phys. Chem. Lett.* **2023**, *14*, 5791–5797.

# Membrane fusion

**Alexander Kros**
**Leiden University**

**Petr Cígler**
**IOCB, Prague**

The mechanism of lipopeptide mediated **(SNARE derived)** membrane fusion

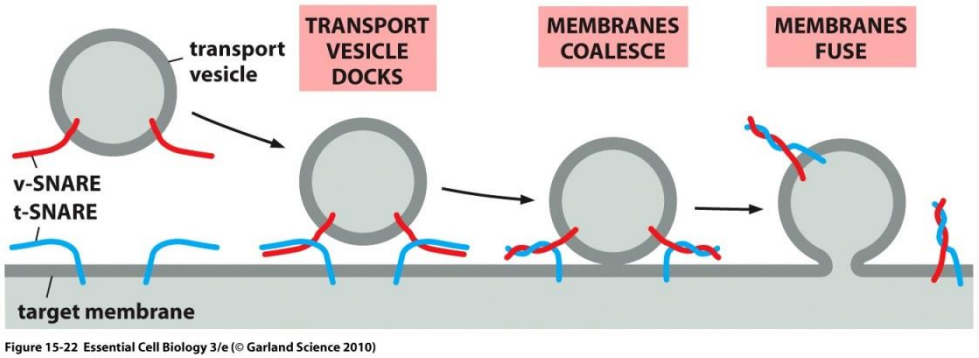
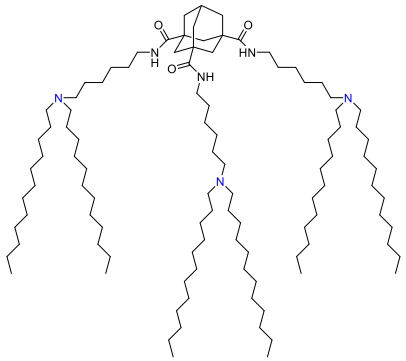



Figure 15-22 Essential Cell Biology 3/e (© Garland Science 2010)

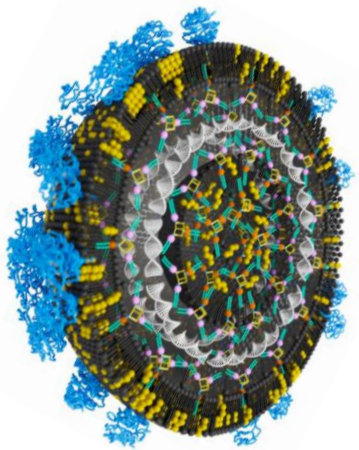
Van OS, WL; J. Control. Release 2024, 371 (May), 85–100.
Mora, N. L. *Sci. Rep.* 2020, 10, 1–13
Koukalová, A et al *Nanoscale*, **4**, 19064–19073, (2018);

The mechanism of entry of lipid nanoparticles (loaded with RNA) with novel ionizable lipidoids into cells



**XMAN6**

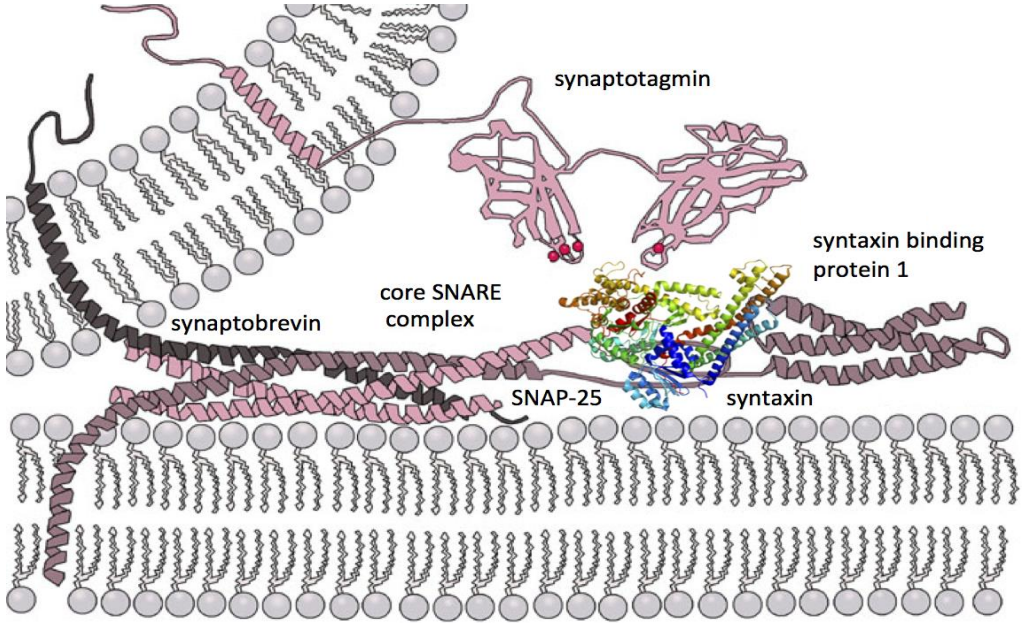
Lipidoid derived from adamantane molecule (low toxicity)



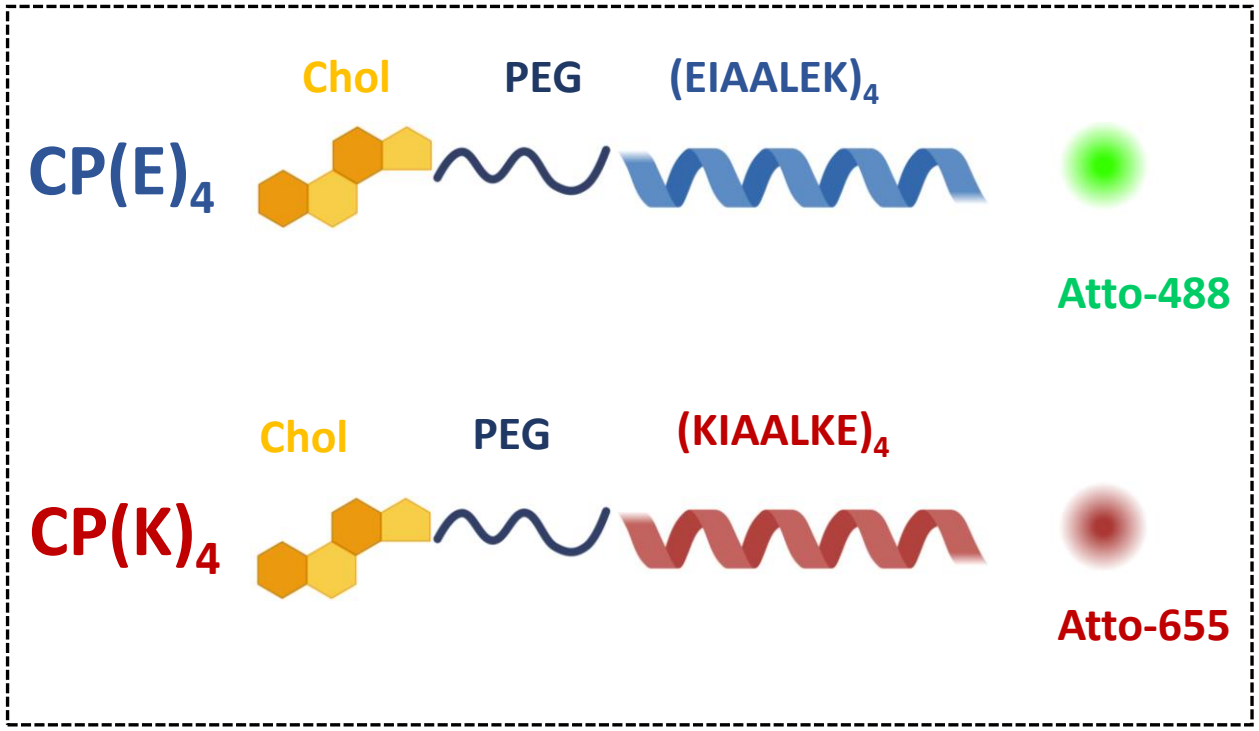
Hejdankova, Z. et al. Adv. Funct. Mater. 2021, 31 (47)

# **SNARE** derived fusogenic lipopeptides with **minimalistic** structure

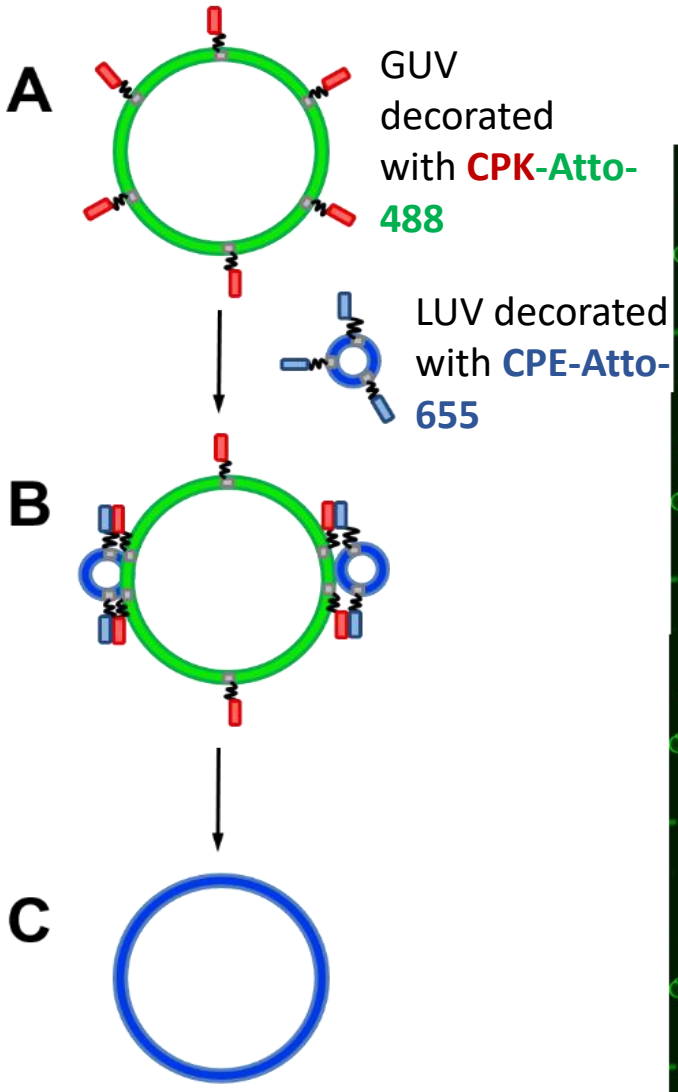
Fusogenic SNARE machinery




Complementary lipopeptides $CP(E)_4$ and $CP(K)_4$

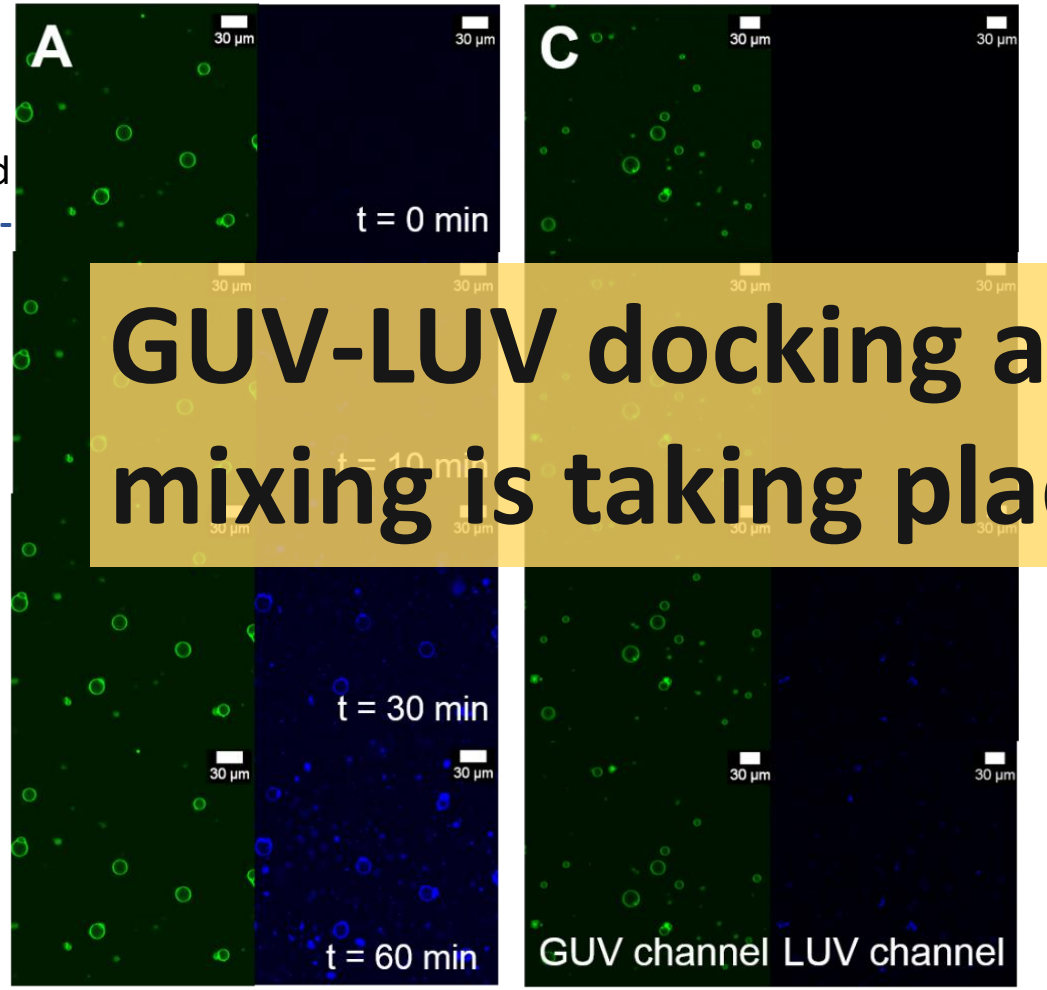


# Lipid docking and mixing assay between GUVs decorated with CPK-Atto-488 and LUVs decorated with CPE-Atto-655




**GUV-LUV docking**

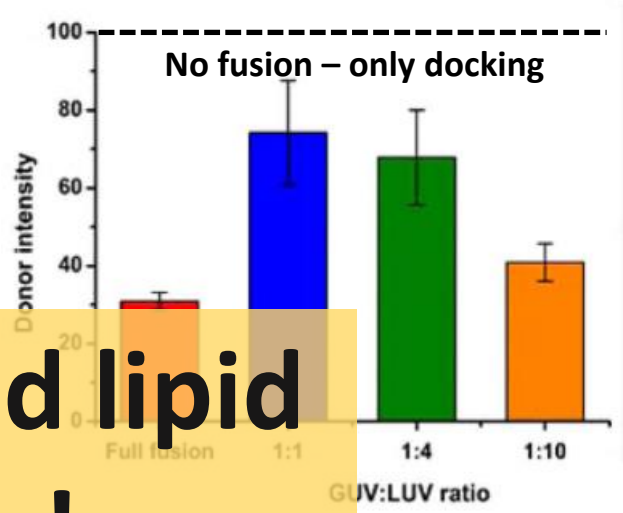
## Docking

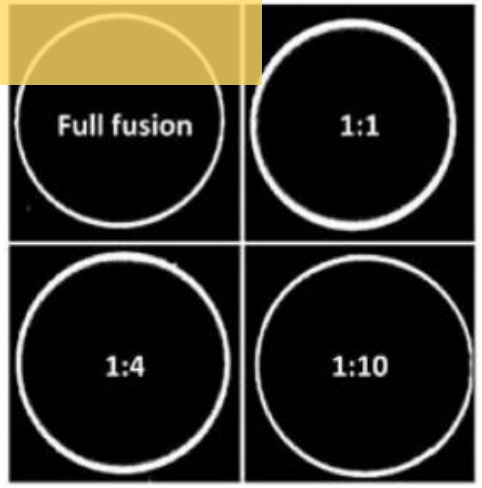



**GUV CP$_4$K$_4$ – LUV CP$_4$E$_4$**

**GUV – LUV**

## Lipid Mixing by FRET



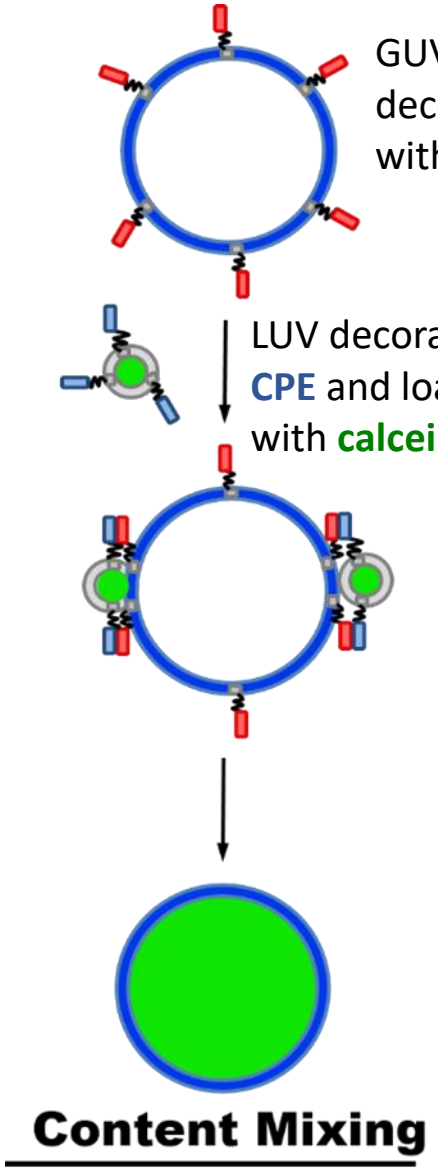



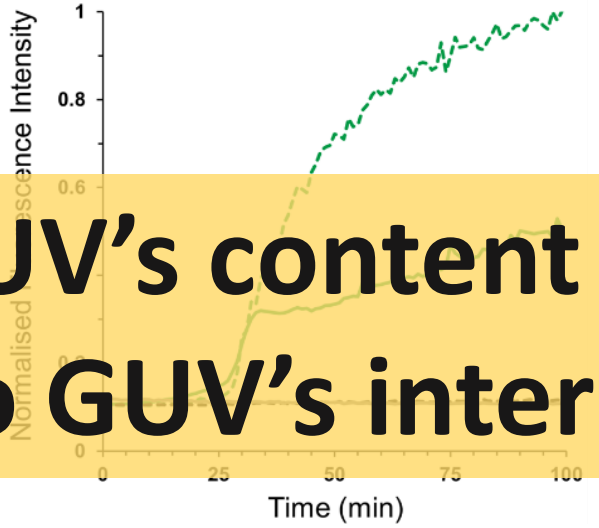

GUVs decorated with 0.5 mol% ATTO 488 DOPE (donor)
LUVs decorated with 0.5 mol% ATTO 633 DOPE (acceptor)

**GUV-LUV docking and lipid mixing is taking place!**

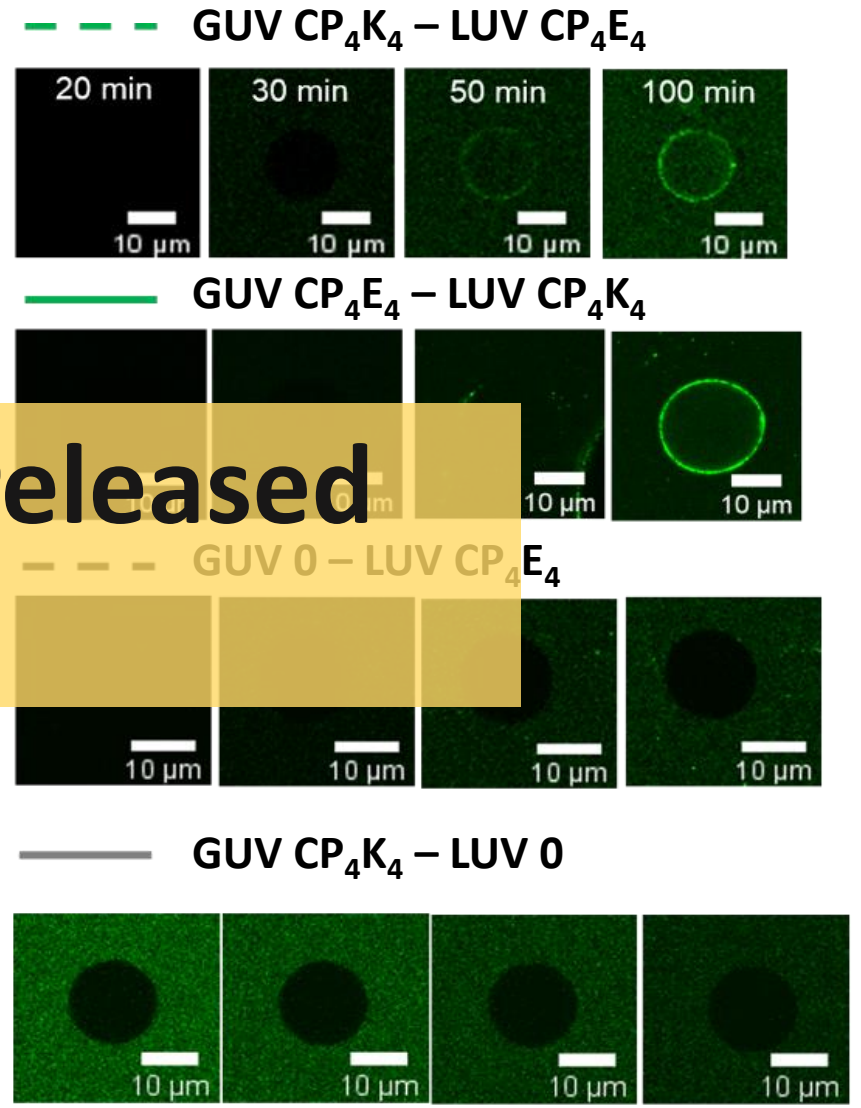
Mora, N. L. et al *Sci. Rep.* 2020, 10, 1–13

# Content mixing assay between GUVs and LUVs decorated with CPE / CPK and loaded with calcein



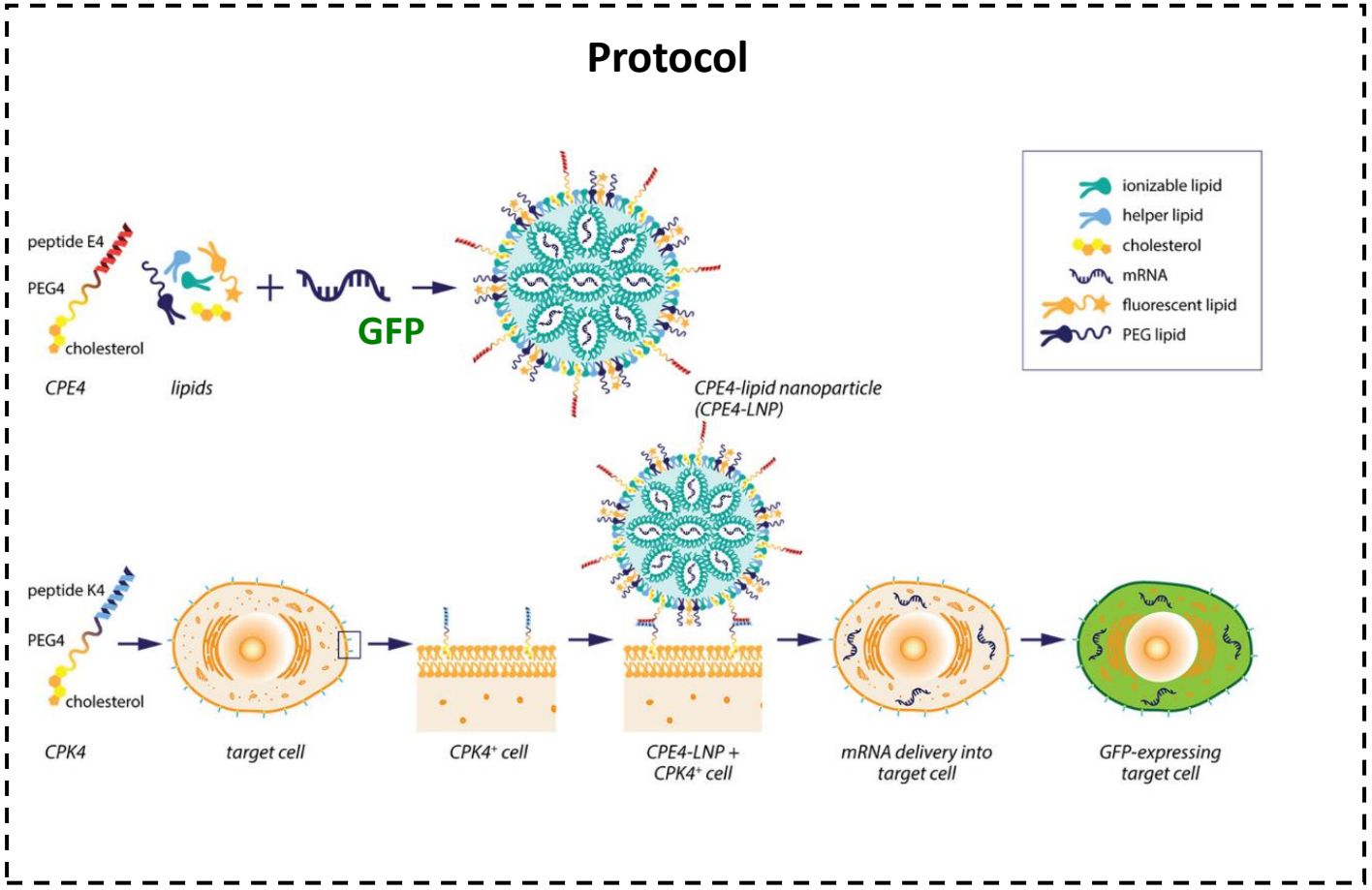



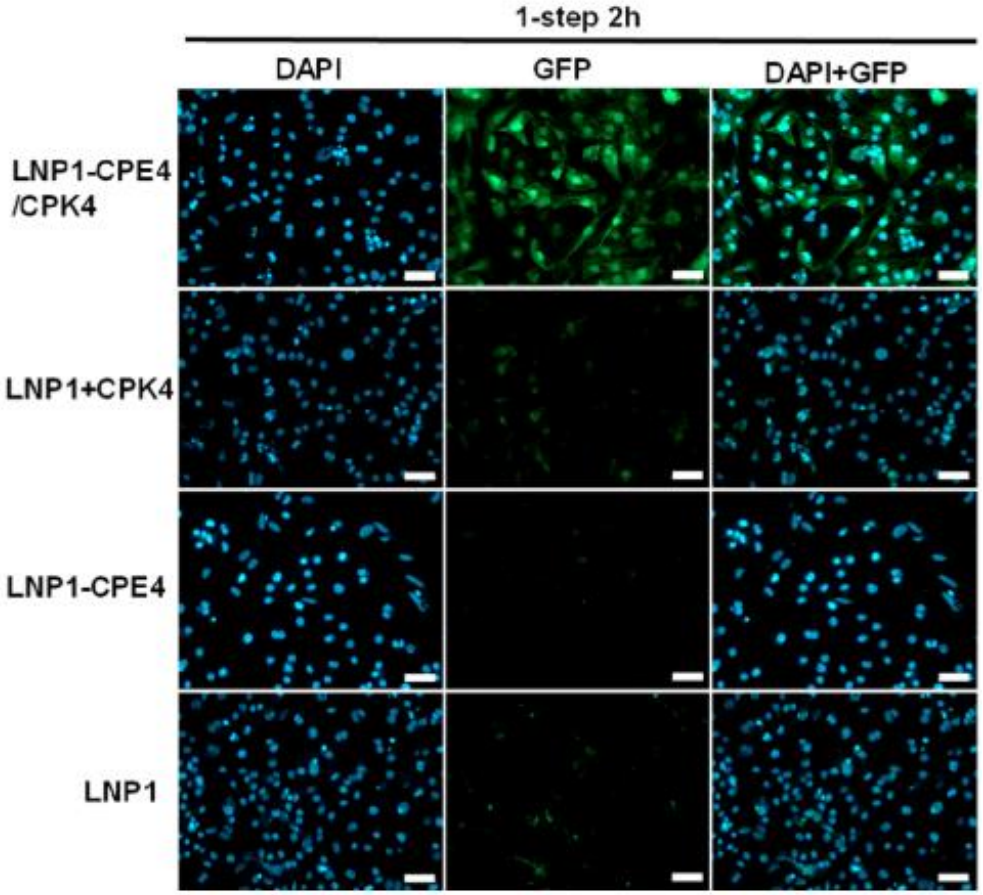

**LUV's content is released to GUV's interior.**




Mora, N. L. et al *Sci. Rep.* 2020, 10, 1–13

# Enhancement of mRNA transfection in cells by ionizable liponanoparticles (LNPs) decorated with CPE / CPK



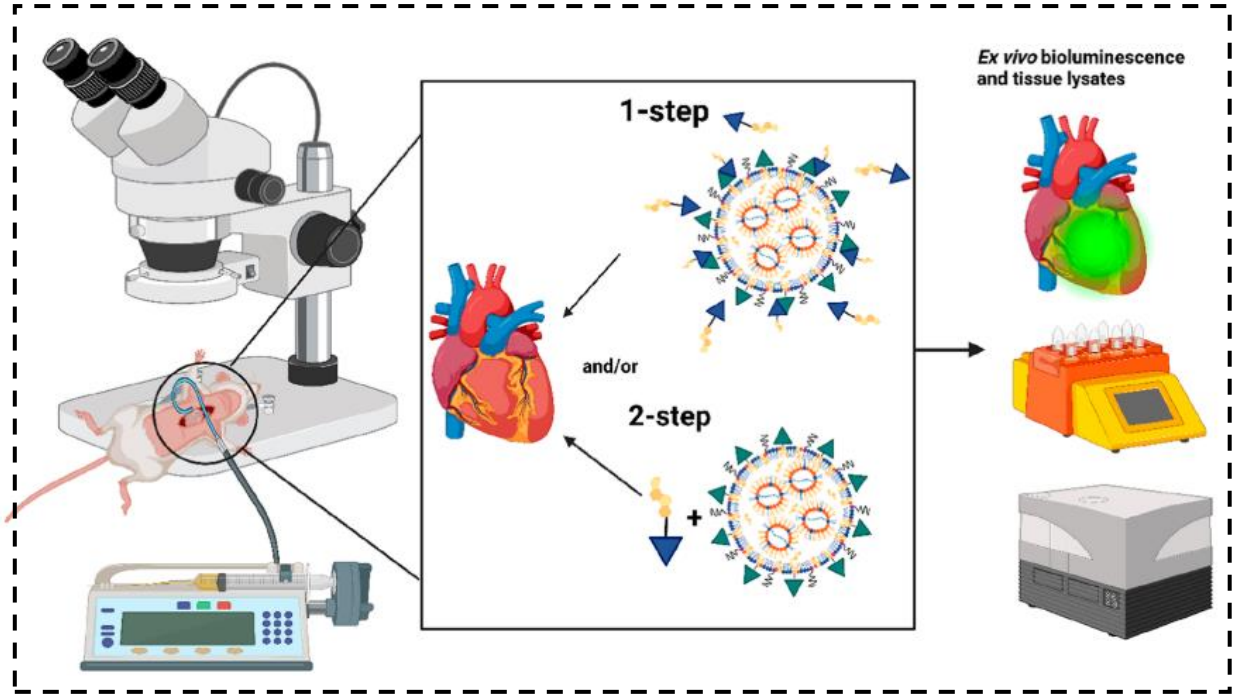




Enhanced mRNA-GFP transfection efficiency in cell-derived cardiomyocytes

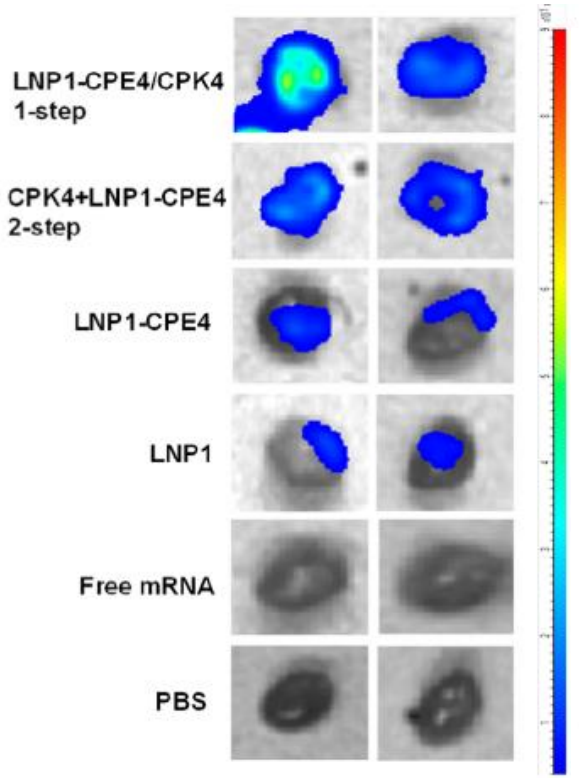
Zeng, Y t al, Nanoscale 2023, 15 (37), 15206–15218; Zeng, Y. ACS Nano 2023, 17 (23), 23466–23477.

# Enhancement of LNP-mediated mRNA delivery upon intramyocardial injection by CPE / CPK lipopeptides

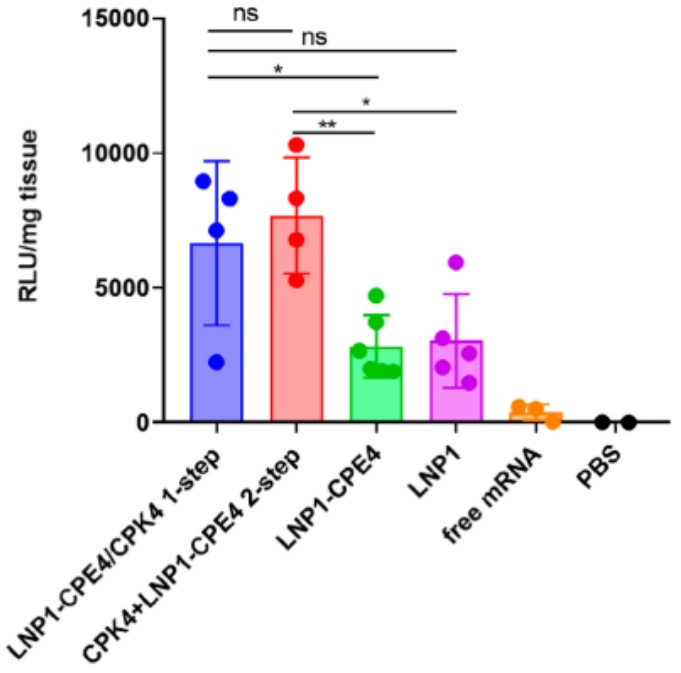
Delivery of mRNA-luciferase (fluorescent) into the heart muscle




Luminiscence images of mice hearts



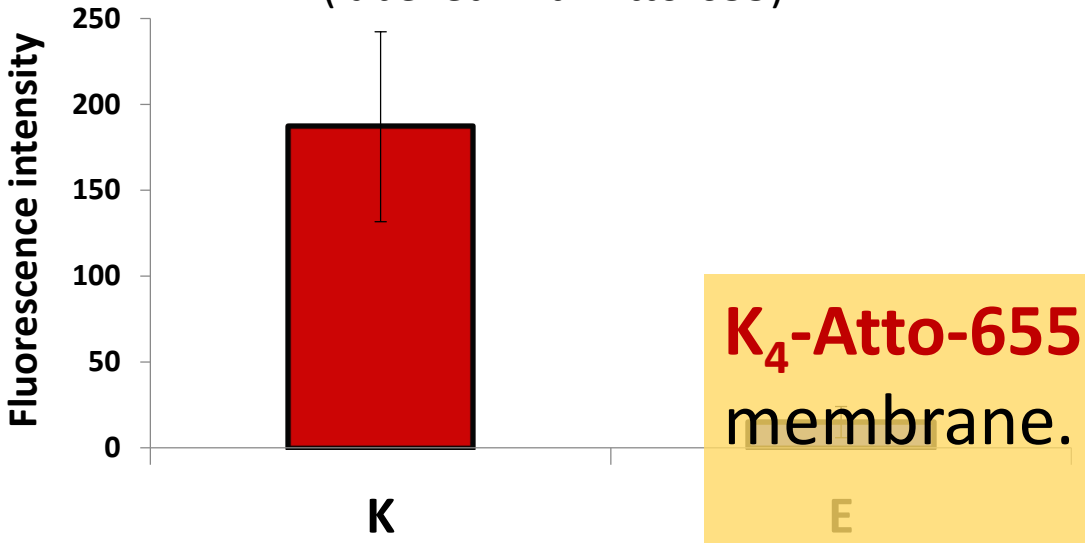

Luciferase activity in heart lysates




Zeng, Y t al, Nanoscale 2023, 15 (37), 15206–15218; Zeng, Y. ACS Nano 2023, 17 (23), 23466–23477.

# Interaction of $E_4$ and $K_4$ with DOPC/DOPE/Chol (50/25/25) membranes

Fluorescence intensity of membrane bound peptides
(labelled with Atto-655)




***D* = 9.18 ± 0.93 µm²/s**

***D* = 130 ± 9.4 µm²/s**

Peptide diffusion coefficient at the membrane

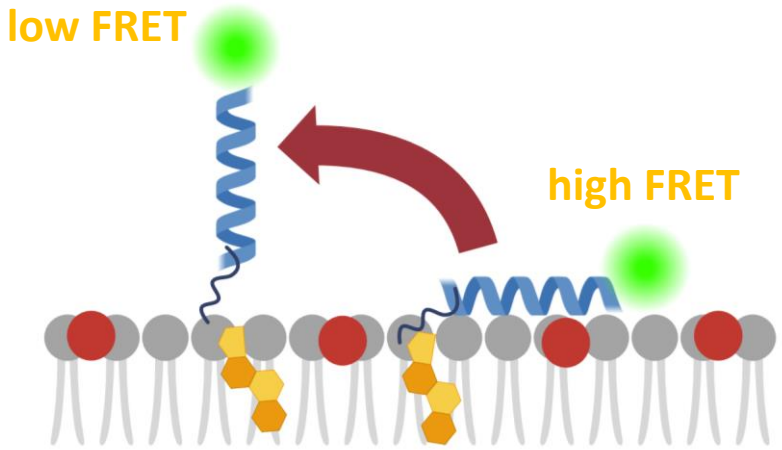
- GUVs incubated with **$K_4$-Atto-655** exhibit **6.5 times higher intensity** than those incubated with the same concentration of $E_4$-Atto-655!

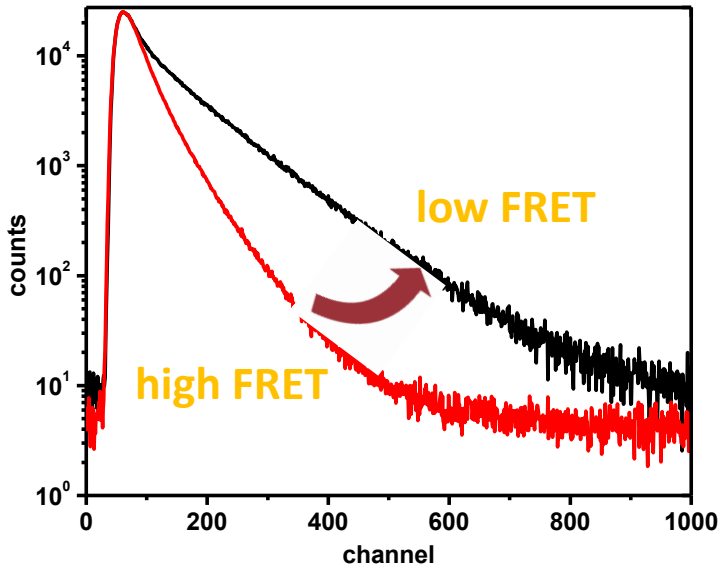
**$K_4$-Atto-655** strongly interacts with the membrane.

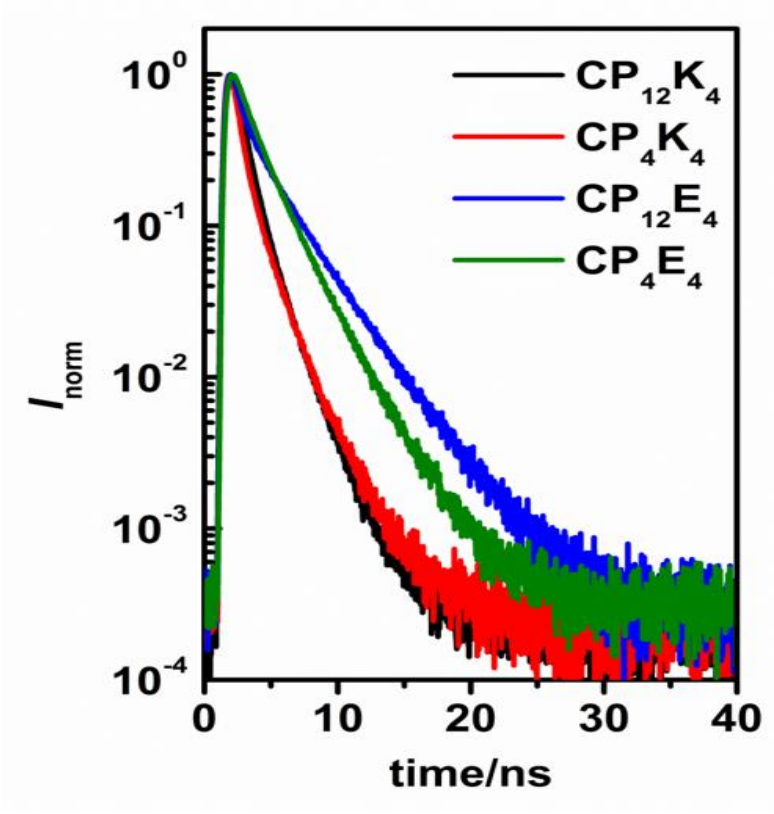
**$E_4$-Atto-655** exhibits much less interaction.

- The diffusion of $K_4$-Atto-655 is slow and the diffusion coefficnet value is typical for a membrane associated molecule.
- **The diffusion of $E_4$-Atto-655** is too fast for a membrane associated molecule.

# Distance of $E_4$ / $K_4$ in $CPE_4$ / $CPK_4$ from the lipid-water interface

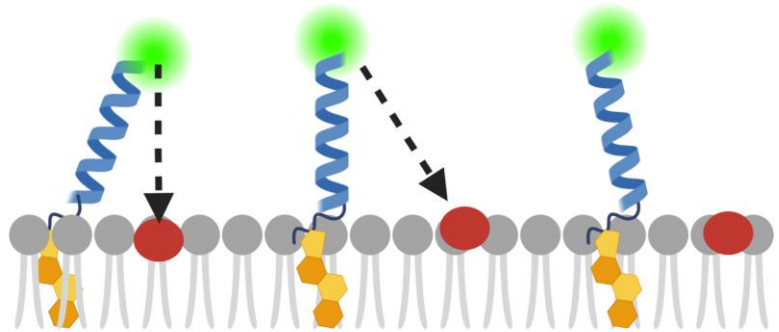


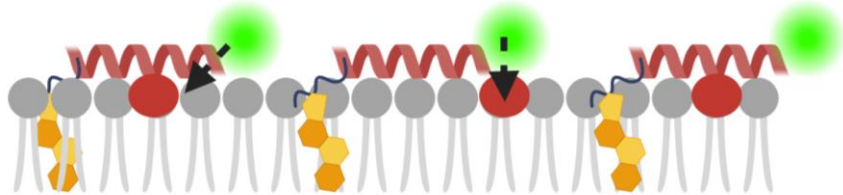




| Lipopeptide | Distance (nm) |
|---|---|
| $CP_{12}K_4$ | 2,2 ± 0,2 |
| $CP_4K_4$ | 2,3 ± 0,2 |
| $CP_{12}E_4$ | 6,7 ± 0,6 |
| $CP_4E_4$ | 6,0 ± 0,5 |

$CPE_4$



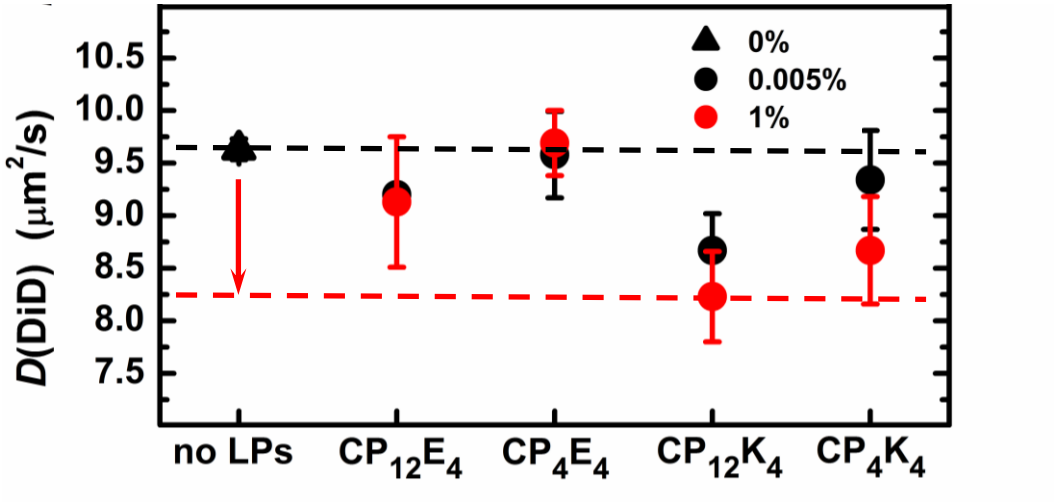
$CPK_4$



- $E_4$ is largely exposed to the bulk
- $K_4$ is found at the lipid-water interface

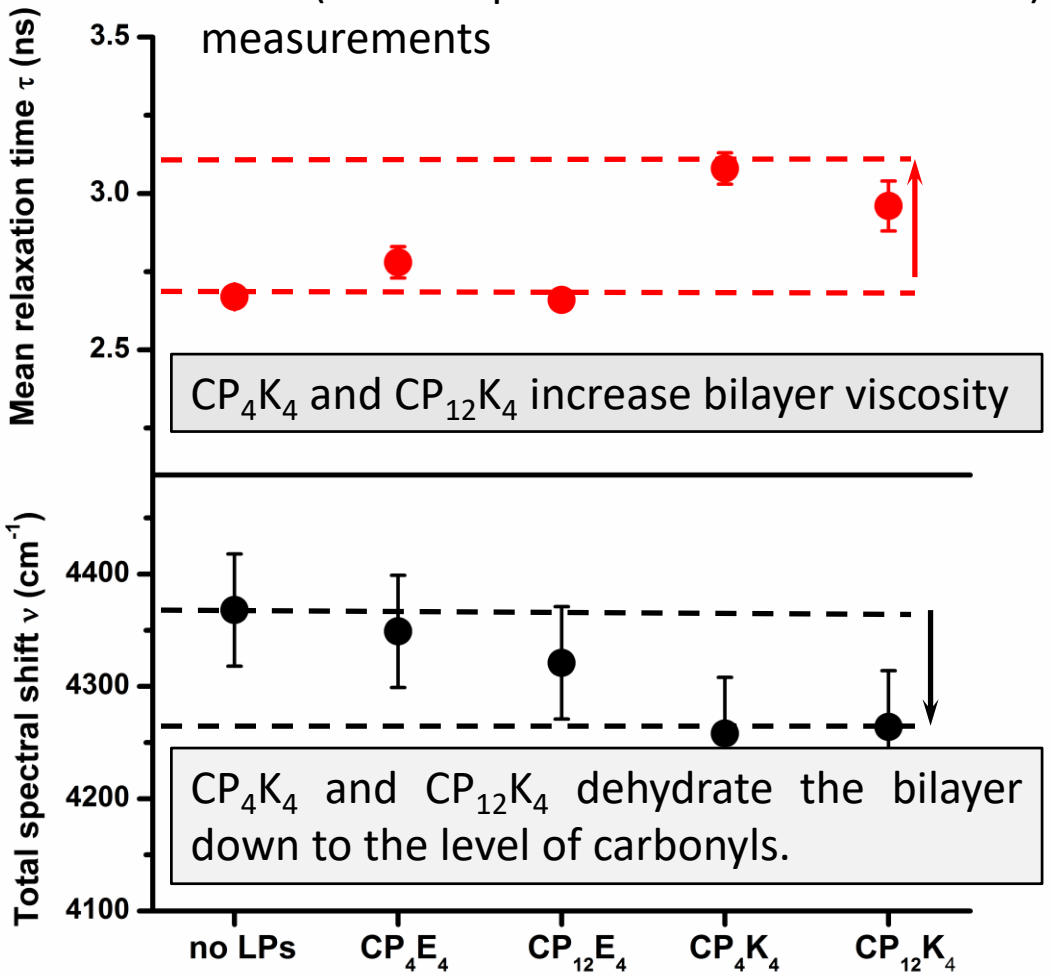
# Implication I: $CPK_4$ but not $CPE_4$ modulates bilayer properties

Membrane diffusion measurements by **FCS**

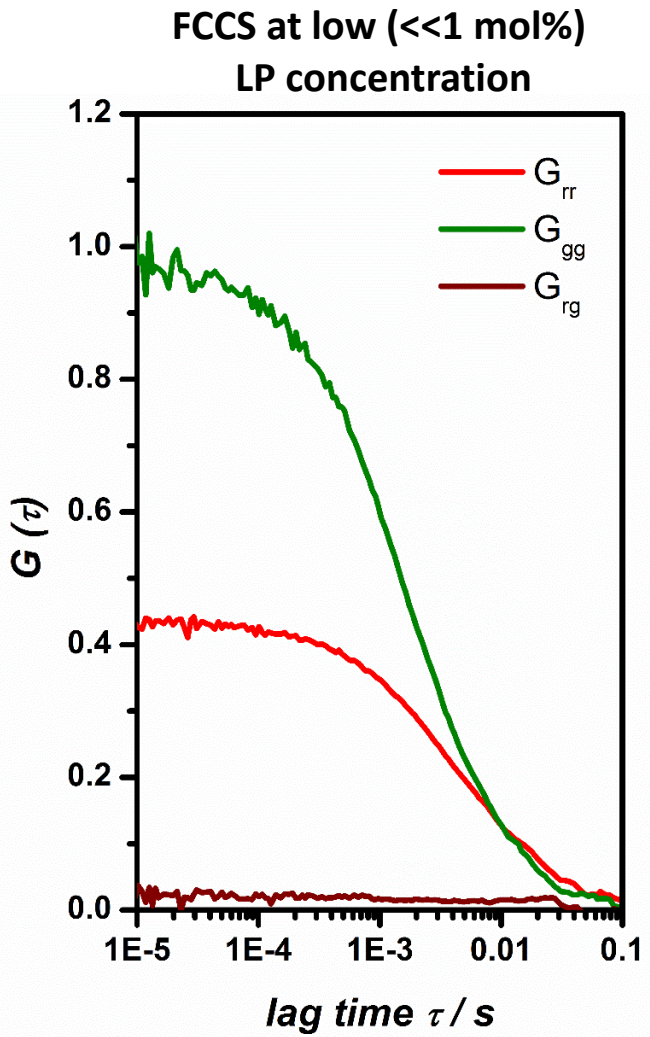



- **Diffusion** of the lipid tracer DiD becomes impeded after addition of $CP_4K_4$ and $CP_{12}K_4$ but not $CP_4E_4$ nor $CP_{12}E_4$.
- $CP_4K_4$ and $CP_{12}K_4$ increase bilayer viscosity.

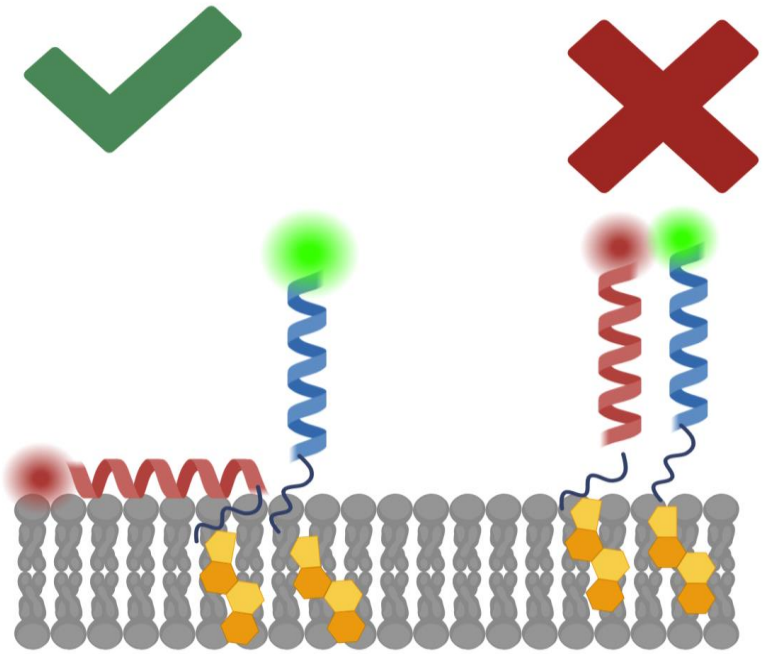
**TDFS** (Time-Dependent Fluorescence Shift) measurements



# Implication II: $CPK_4$ and $CPE_4$ do not form heterocoils within the same membrane

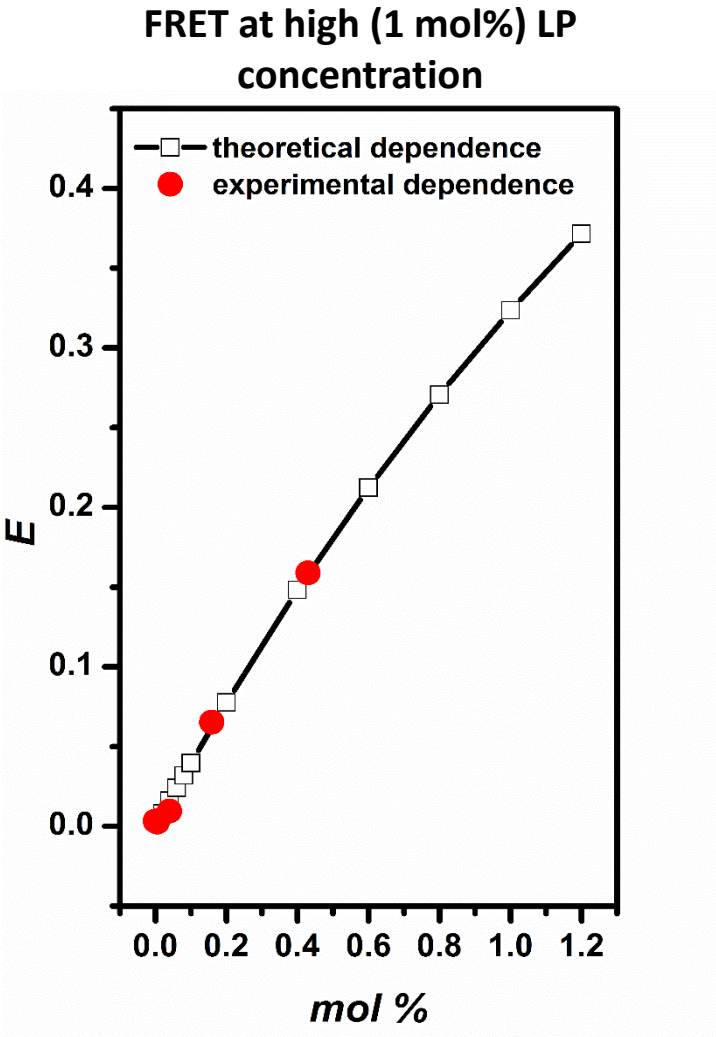



No detectable lipopeptide hetero-coiling at low concentrations by **FCCS**

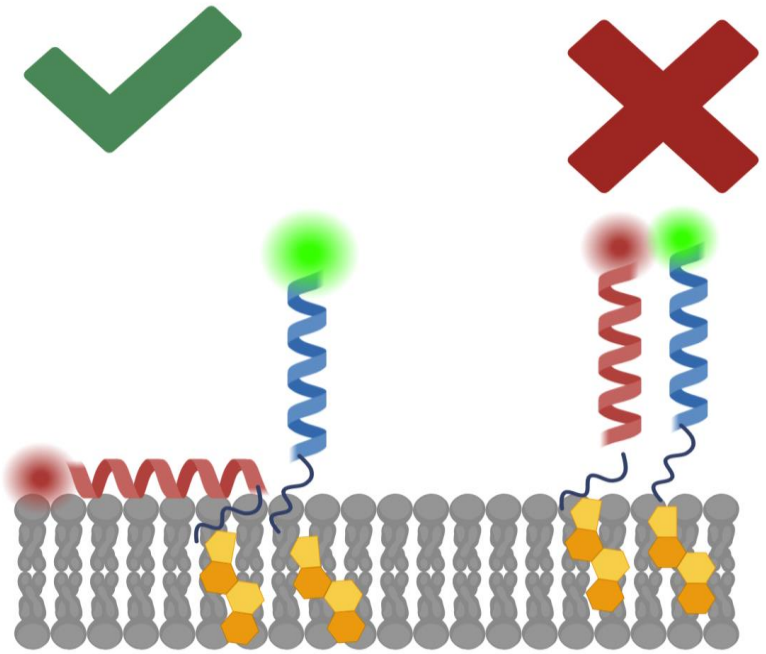


**This different orientation of $CPE_4$ and $CPK_4$ inhibits the interaction** within the same bilayer. It maintains the number of free $CPE_4$ and $CPK_4$ monomers in the bilayer **at a high level**, thereby **facilitating** the fusion.

# Implication II: $CPK_4$ and $CPE_4$ do not form heterocoils within the same membrane




No detectable lipopeptide hetero-coiling at high concentrations by **FRET**

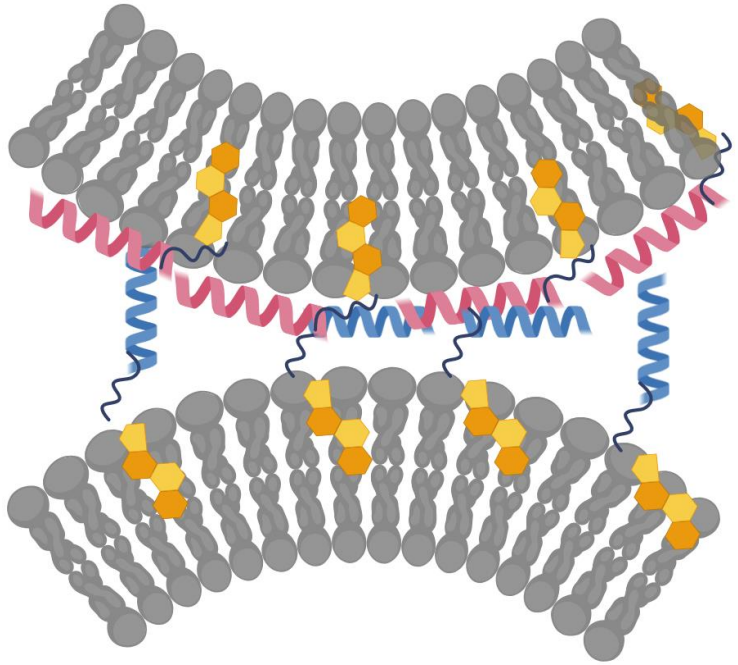


**This different orientation of $CPE_4$ and $CPK_4$ inhibits the interaction** within the same bilayer. It maintains the number of free $CPE_4$ and $CPK_4$ monomers in the bilayer **at a high level**, thereby **facilitating** the fusion.

# Working model for the lipopeptide-mediated vesicle fusion

- **The main roles of $CP_nK_4$** are 1) to **disrupt the bilayer** and **stimulate it for undergoing fusion** and 2) to **interact** with **$CP_nE_4$**.

- **The main roles of $CPE_4$** are to work as **lipid anchors**. The peptide moieties are exposed to the bulk and ready to interact with **$CP_nK_4$.**



# Acknowledgements

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GAČR
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DFG

AMULET

J. Heyrovský Institute
of Physical Chemistry

# Thank you for your attention

Department of Biophysical Chemistry