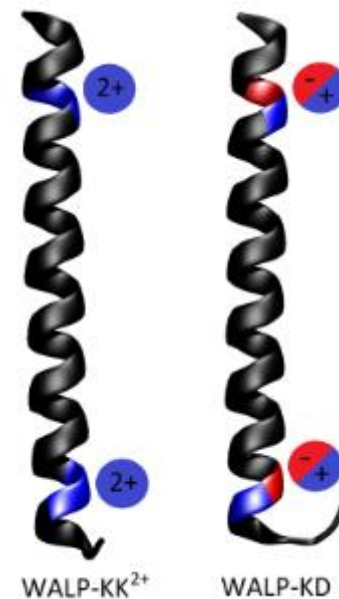


Influence of a transmembrane domain on calcium-membrane interaction

Adéla Melcrová

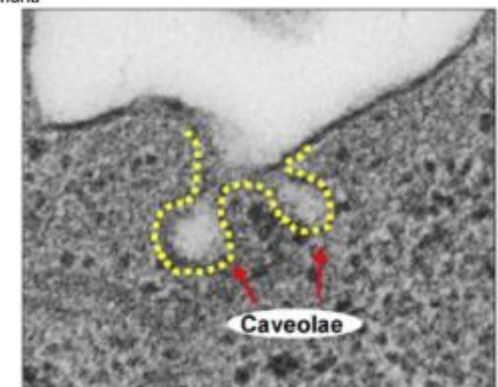
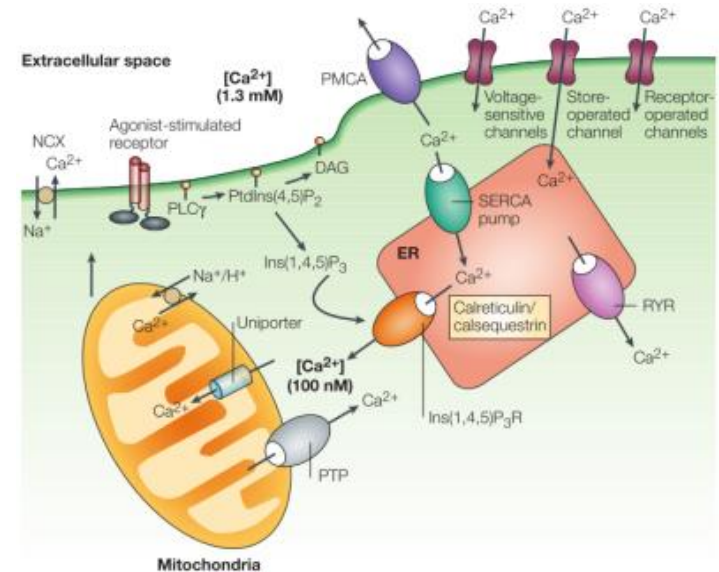
J. Heyrovský Institute of Physical Chemistry, Prague

RBC Zreče, 20.5.2018



Ca²⁺ in cellular membranes

- Ca²⁺ in cell
 - ER ~mM
 - cytosol ~100 nM
 - Membranes ~?
- Ca²⁺ signaling
 - Ca²⁺ released near membranes
 - wide physiological concentration range
 - inner leaflet of plasma membrane
 - domains rich for cholesterol and sphingolipids
- Ca²⁺ binding influences the structure, function and association of membrane proteins

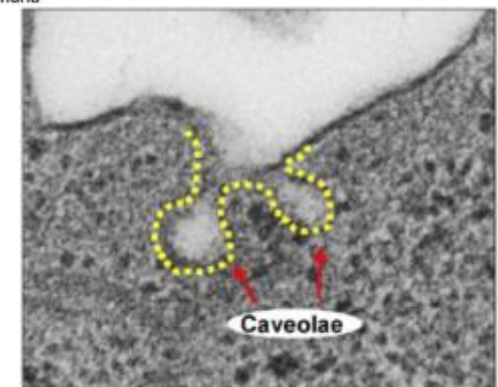
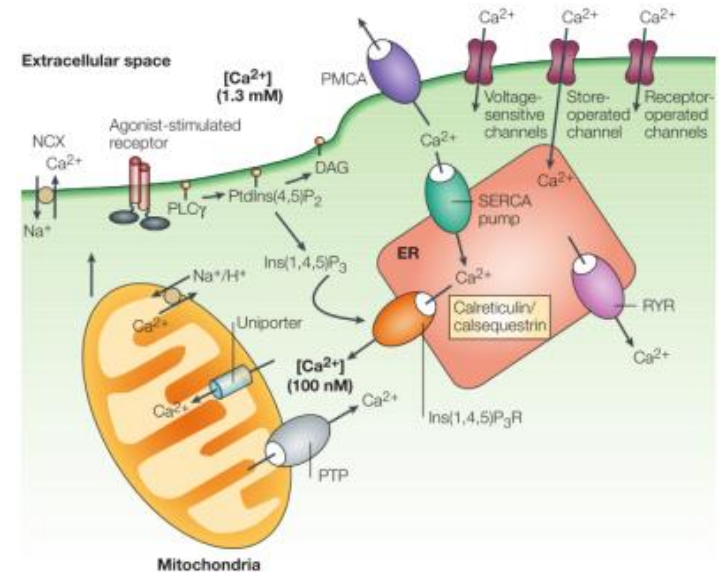


Ca²⁺ in cellular membranes

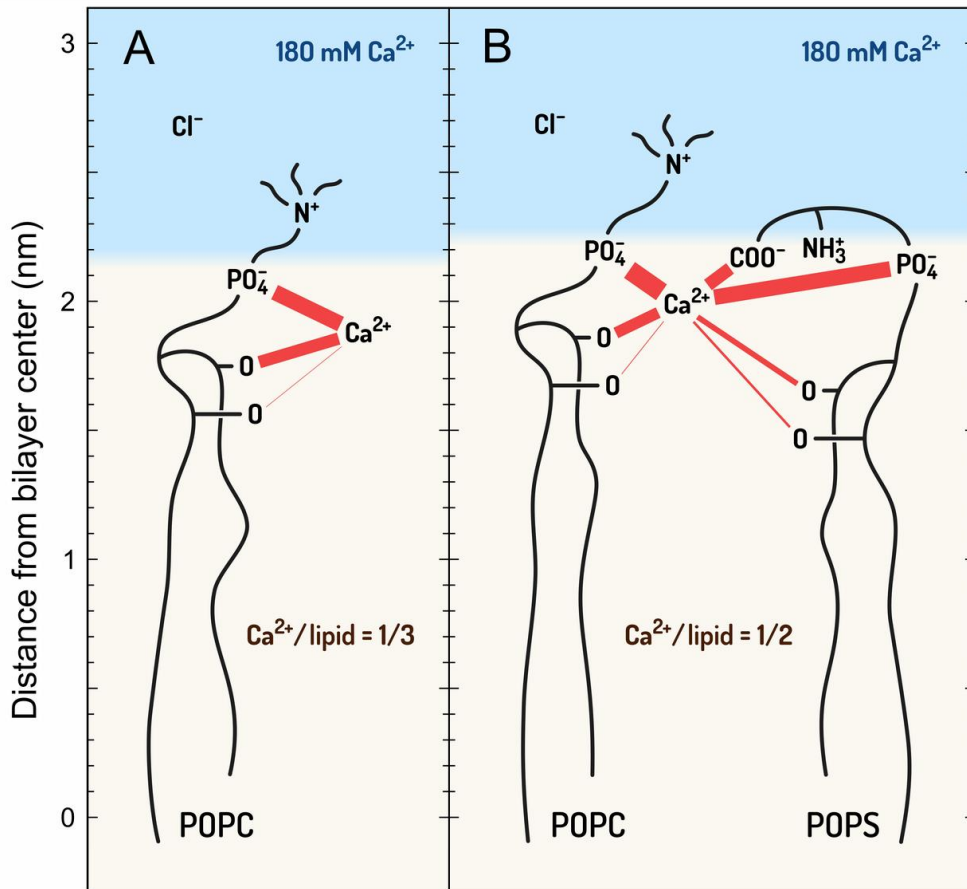
Already known:

- Ca²⁺ binding to phospholipid membranes
- Changes in membrane biophysics upon Ca²⁺ binding

Ca²⁺ binding and membrane proteins??



Binding of Ca^{2+} into phospholipid membranes



- Studied compositions:
 - 100% POPC
 - POPC/POPS (80:20 mol:mol)
- $\text{PO}_4 > \text{COO}^- > \text{C=O}_{\text{sn-2}}$
- reduction of membrane hydration, lipid mobility, and lateral inter-lipid distance

TM peptide and Ca²⁺ binding

- **Artificial TM peptides:**

- **WALP-KK2+**

GTSTSKKWW(LA)₈LWWKKFSTS

- Biologically relevant (positive-inside rule)
- Might repel Ca²⁺ ions from membranes

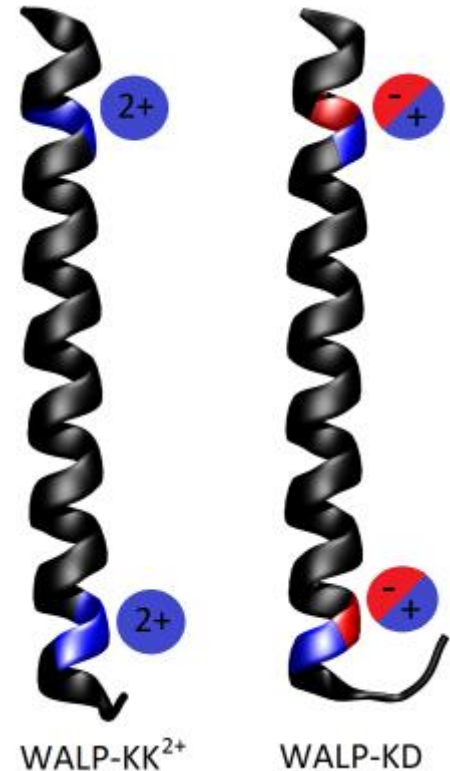
- **WALP-KD**

GTSTSKDWW(LA)₈LWWKDFSTS

- Zwitterionic
- Should not repel nor attract Ca²⁺ ions

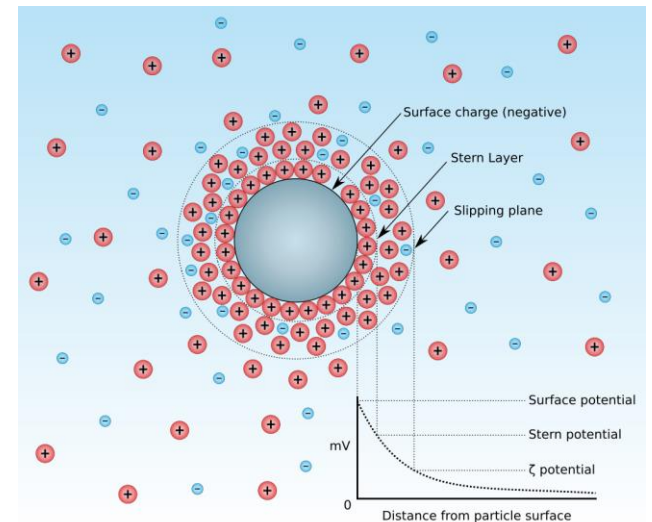
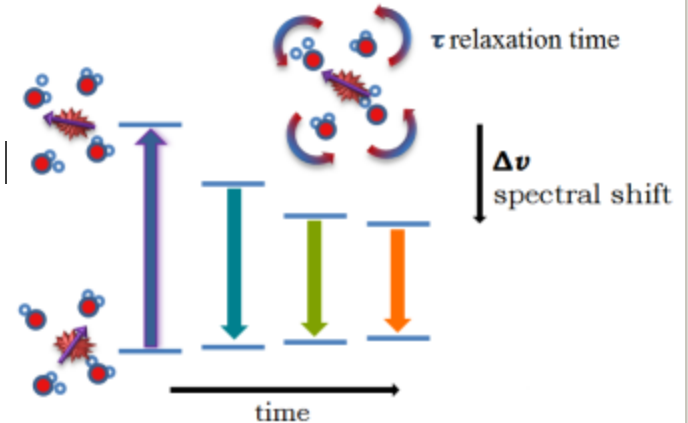
- **Questions:**

- Influence of TM α -helix on the Ca²⁺ binding to the membranes??
- Impact of positive charge of the peptide on Ca²⁺ binding??

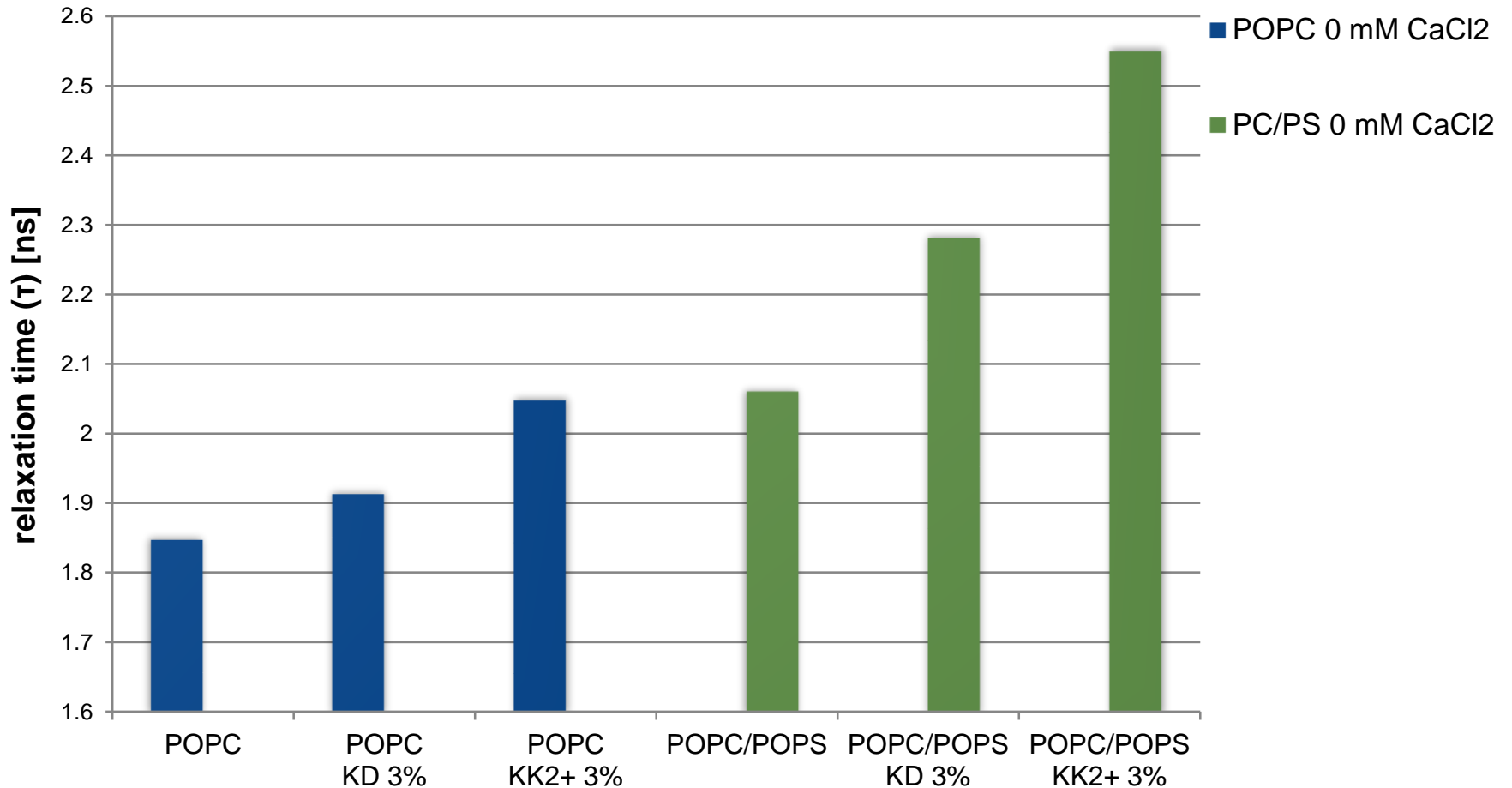


Methods

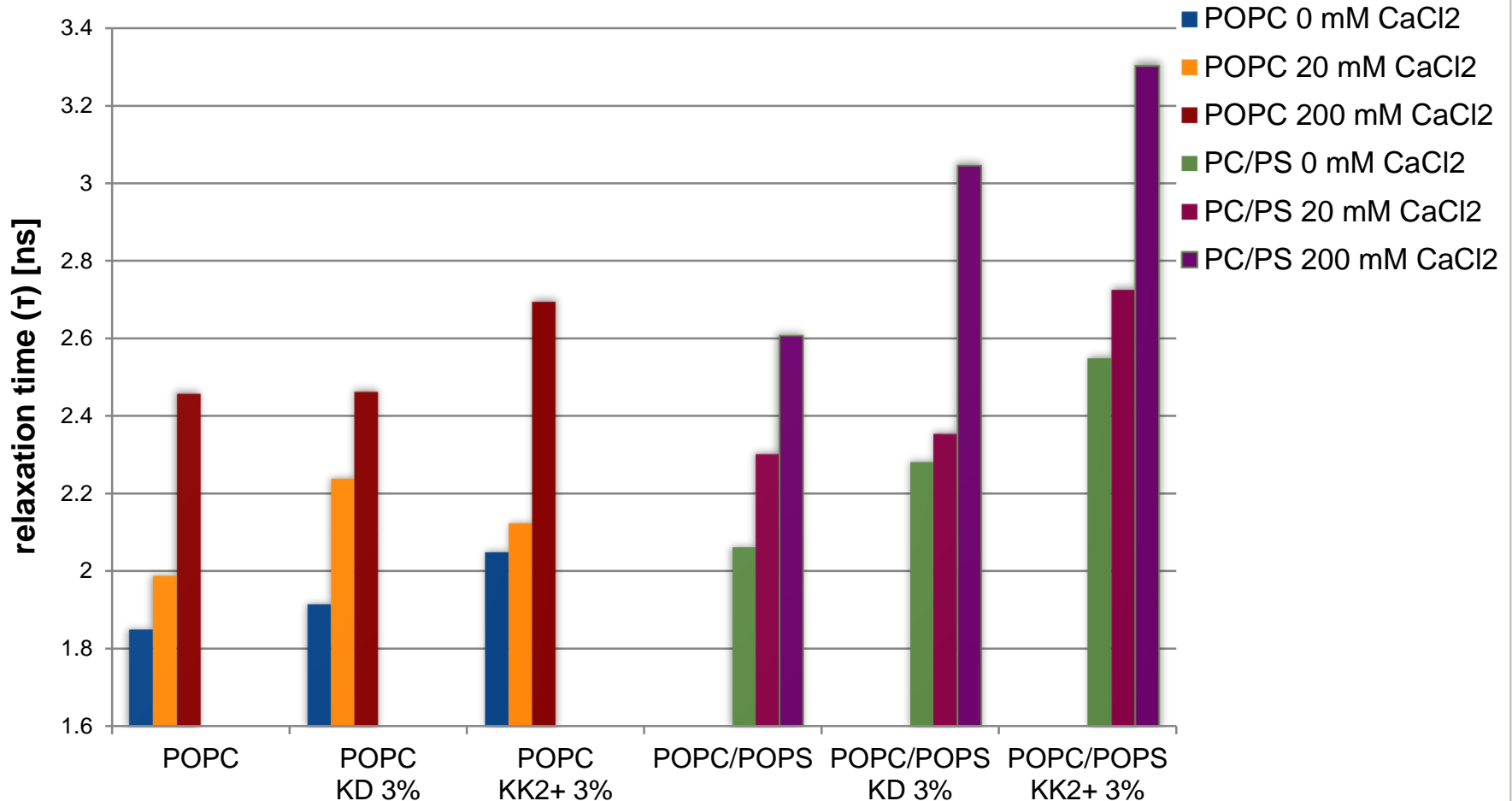
- Time dependent fluorescence shift
 - Laurdan fluorophore at carbonyl level
 - Local hydration and mobility of lipids
- Zeta potential
 - Charge near the membrane surface



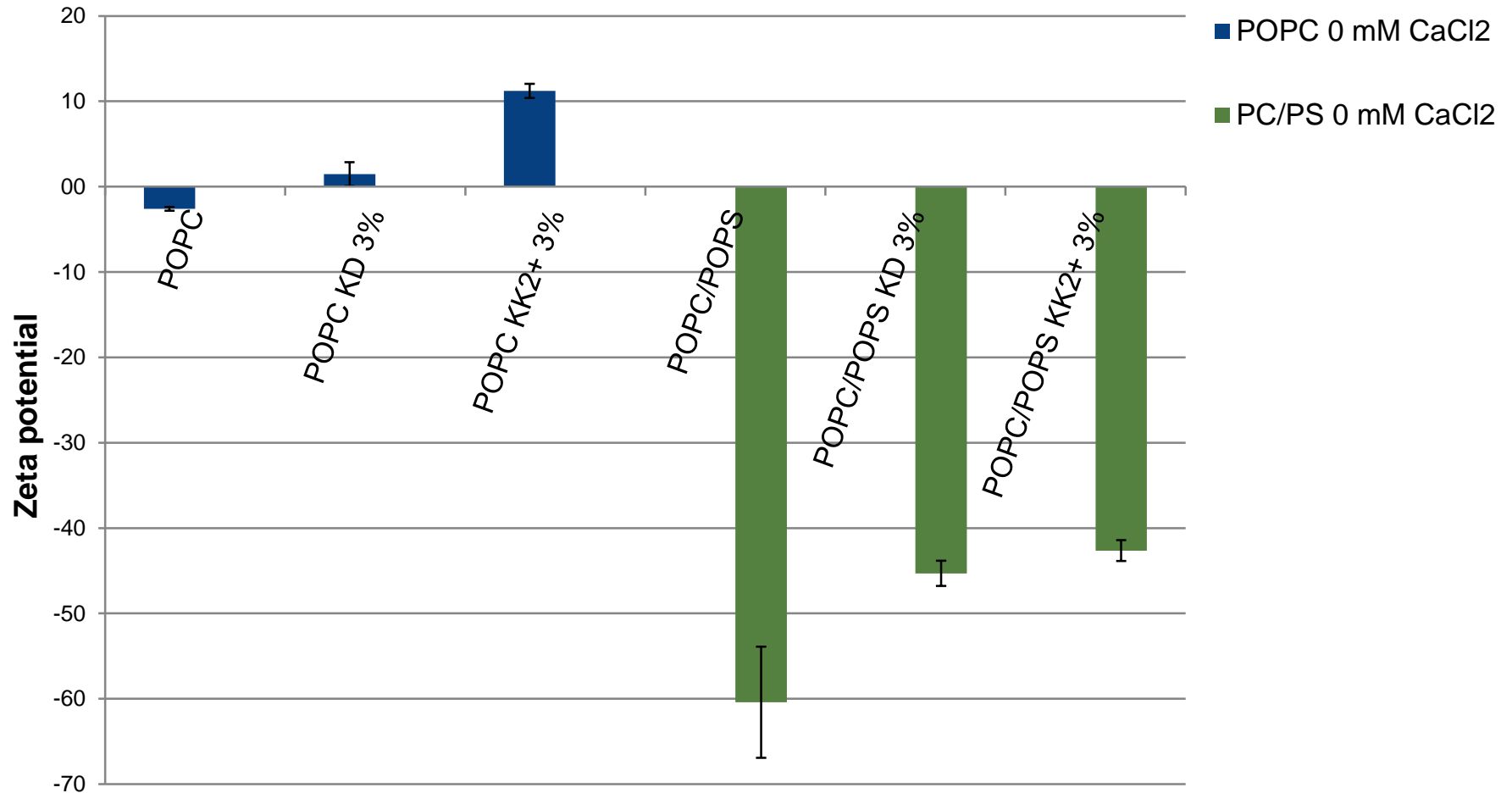
Impact on membrane rigidity – peptide



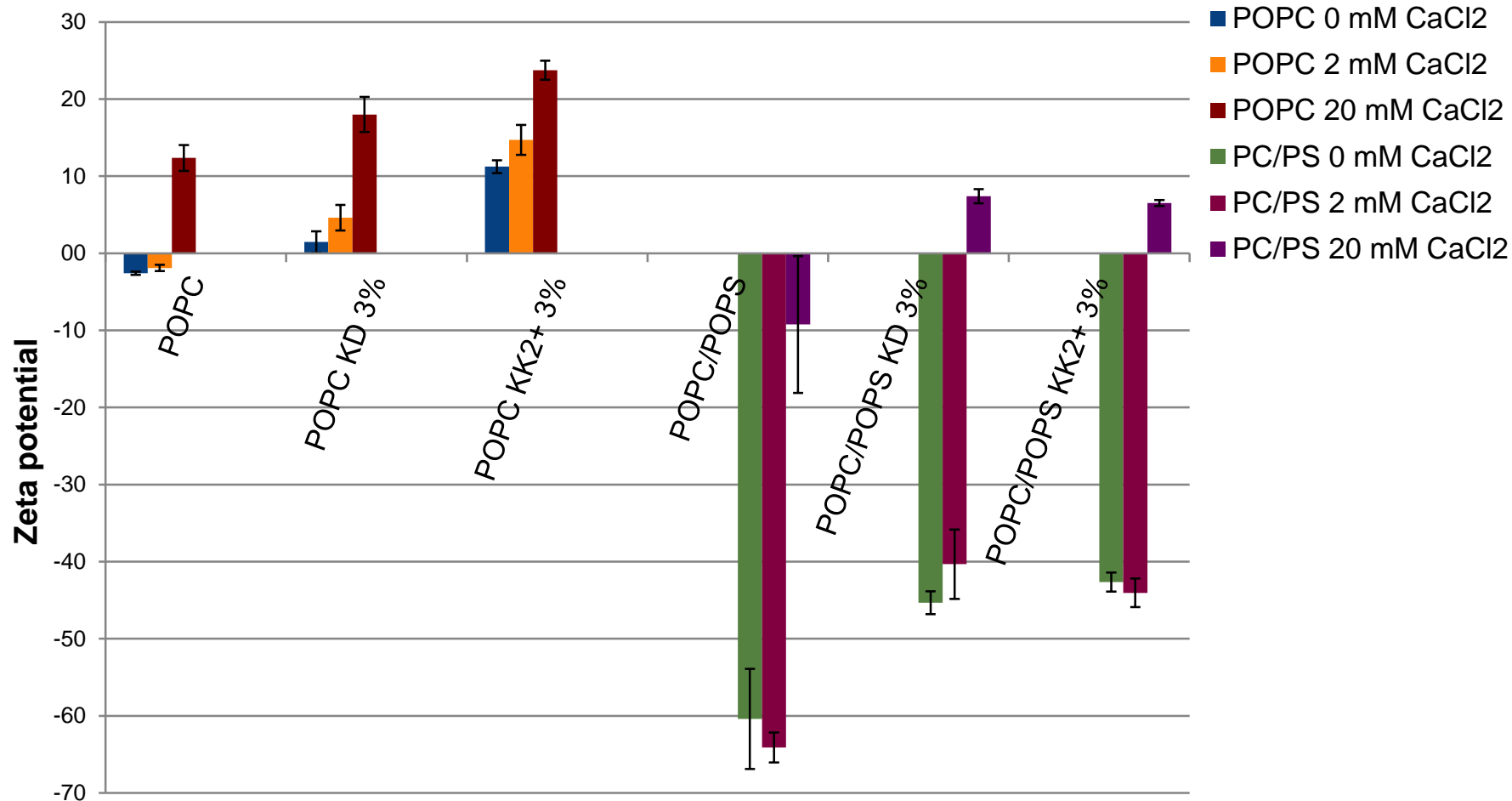
Impact on membrane rigidity – Ca²⁺



Charge on LUVs – peptide



Charge on LUVs – Ca²⁺



The impact of TM helix on membrane and its Ca^{2+} affinity

- Rigidifying effect of peptides
 - WALP-KK2+ > WALP-KD
 - Bigger effect on POPC/POPS
- Charge near the membrane surface
 - POPC, POPC/POPG – only WALP-KK2+ increases zeta pot.
 - POPC/POPS – also WALP-KD increases zeta pot.
 - POPS headgroup reorientation
- Membrane affinity for Ca^{2+} ions is not affected by the peptides presence
- No suppressing impact of positive charge of WALP-KK2+ on Ca^{2+} binding

Hof Fluorescence group

JH Institute of Physical Chemistry, Prague



- Piotr Jurkiewicz
- Martin Hof

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CHARLES
UNIVERSITY
IN PRAGUE



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Martiny Roeselové

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- Charles University in Prague, SVV project
- Martina Roeselova Memorial Fellowship
 - Daycare for children

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