Influence of a transmembrane domain on calcium-membrane interaction

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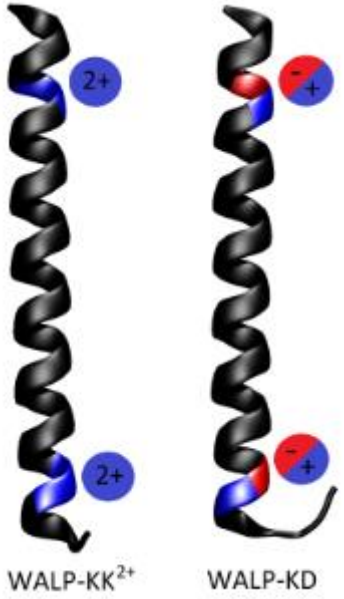
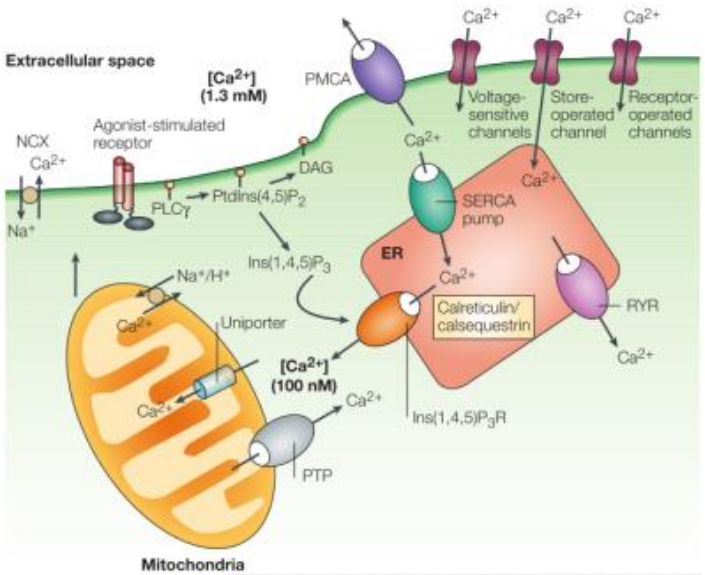
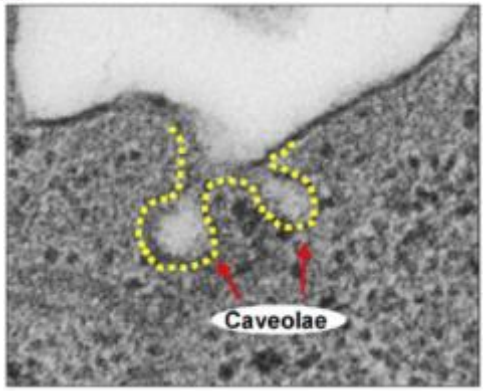
RBC Zreče, 20.5.2018



Ca^{2+} in cellular membranes

- Ca^{2+} in cell
  - ER ~mM
  - cytosol ~100 nM
  - Membranes ~?
- Ca^{2+} signaling
  - Ca^{2+} released near membranes
  - wide physiological concentration range
  - inner leaflet of plasma membrane
  - domains rich for cholesterol and sphongolipids
- Ca^{2+} binding influences the structure, function and association of membrane proteins





Orrenius, S. et al. *Nature reviews. Molecular cell biology*, 2003, **4**(7), 552–65.

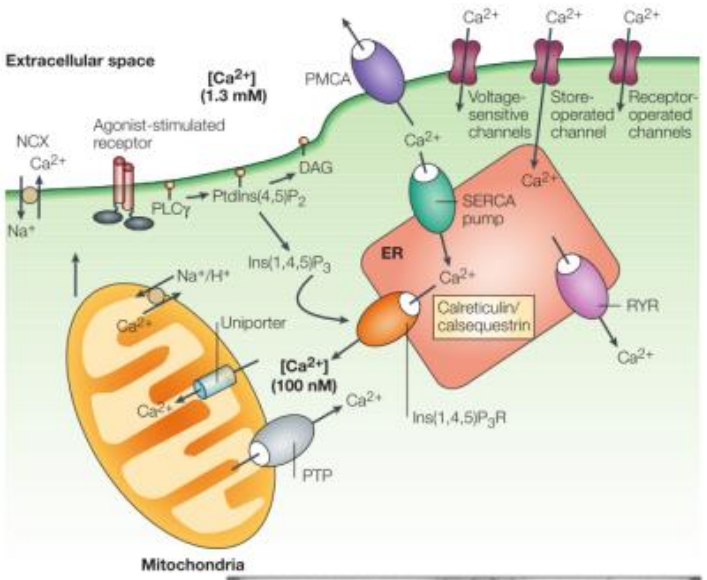
Pani, B., Singh, B. B. *Cell calcium*, 2009, **45**(6), 625-633

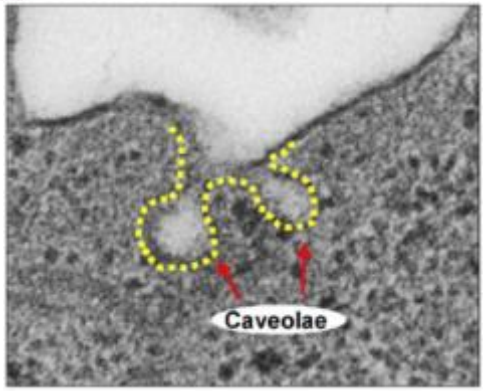
Ca^{2+} in cellular membranes

Already known:

- Ca^{2+} binding to phospholipid membranes
- Changes in membrane biophysics upon Ca^{2+} binding

Ca^{2+} binding and membrane proteins??





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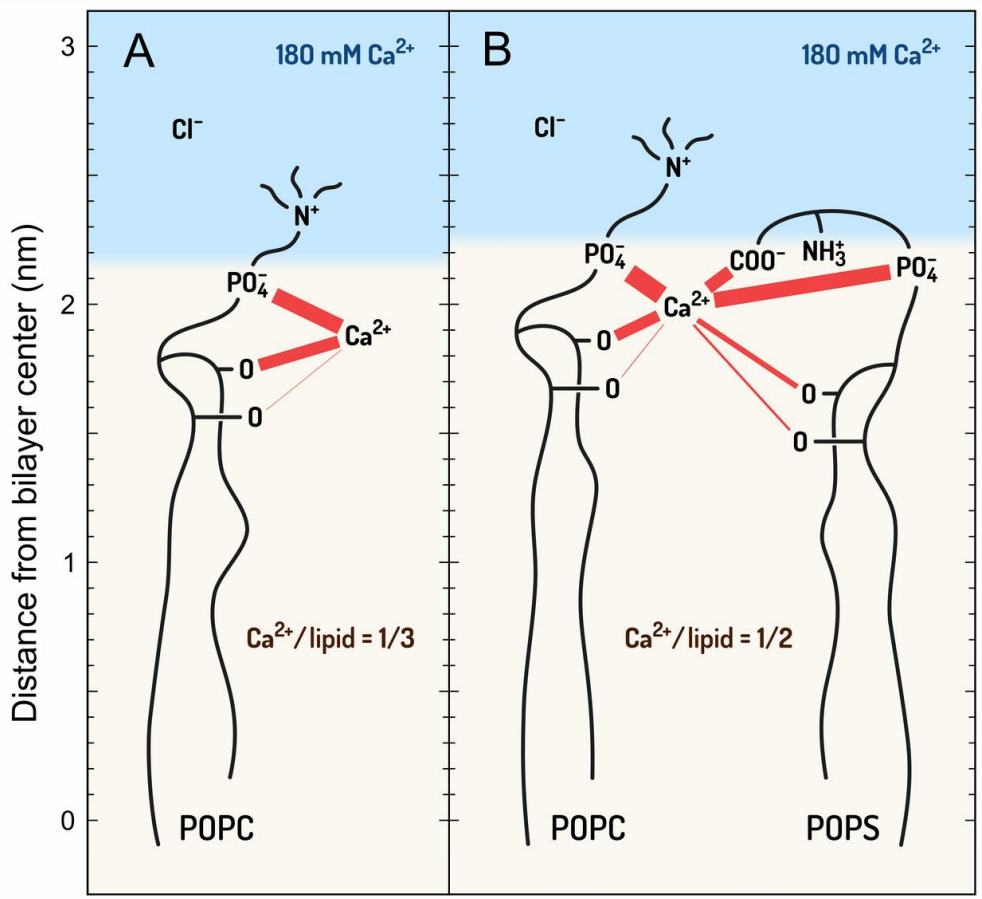
Binding of Ca^{2+} into phospholipid membranes

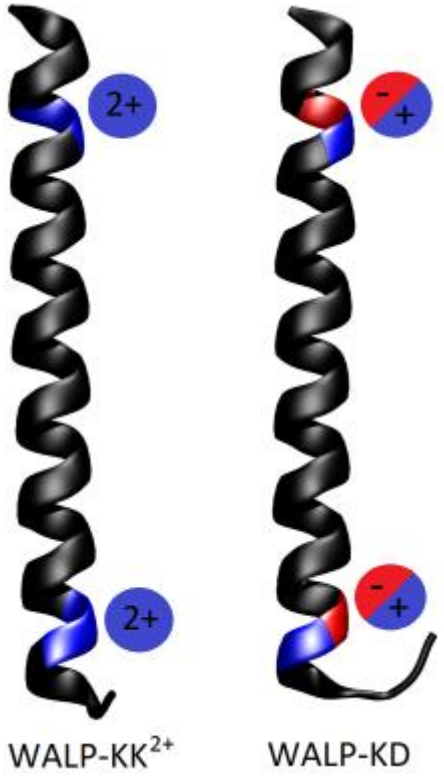


- Studied compositions:
  - 100% POPC
  - POPC/POPS (80:20 mol:mol)
- $PO_4 > COO^- > C{=}O_{sn\text{-}2}$
- reduction of membrane hydration, lipid mobility, and lateral inter-lipid distance

Melcrova et al. *Scientific Reports*, 2016, **6**, 38035

TM peptide and Ca^{2+} binding

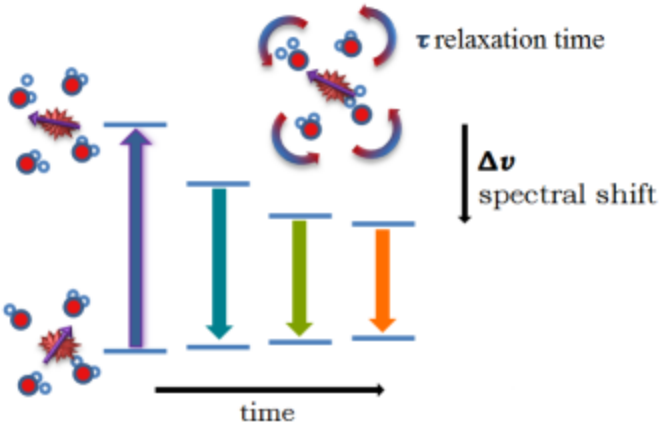
- **Artificial TM peptides:**
  - **WALP-KK2+**
    GTSTS**KK**WW$(LA)_8$LWW**KK**FSTS
    - Biologically relevant (positive-inside rule)
    - Might repell Ca^{2+} ions from membranes
  - **WALP-KD**
    GTSTS**KD**WW$(LA)_8$LWW**KD**FSTS
    - Zwitterionic
    - Should not repel nor attract Ca2+ ions

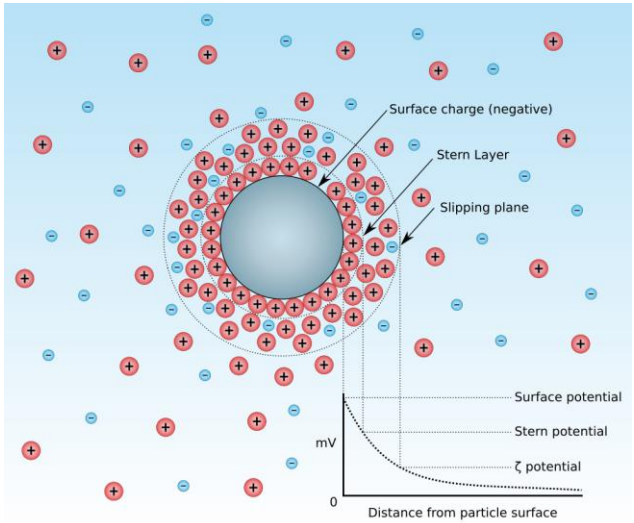


- **Questions:**
  - Influence of TM α-helix on the Ca^{2+} binding to the membranes??
  - Impact of positive charge of the peptide on Ca^{2+} binding??

Methods

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

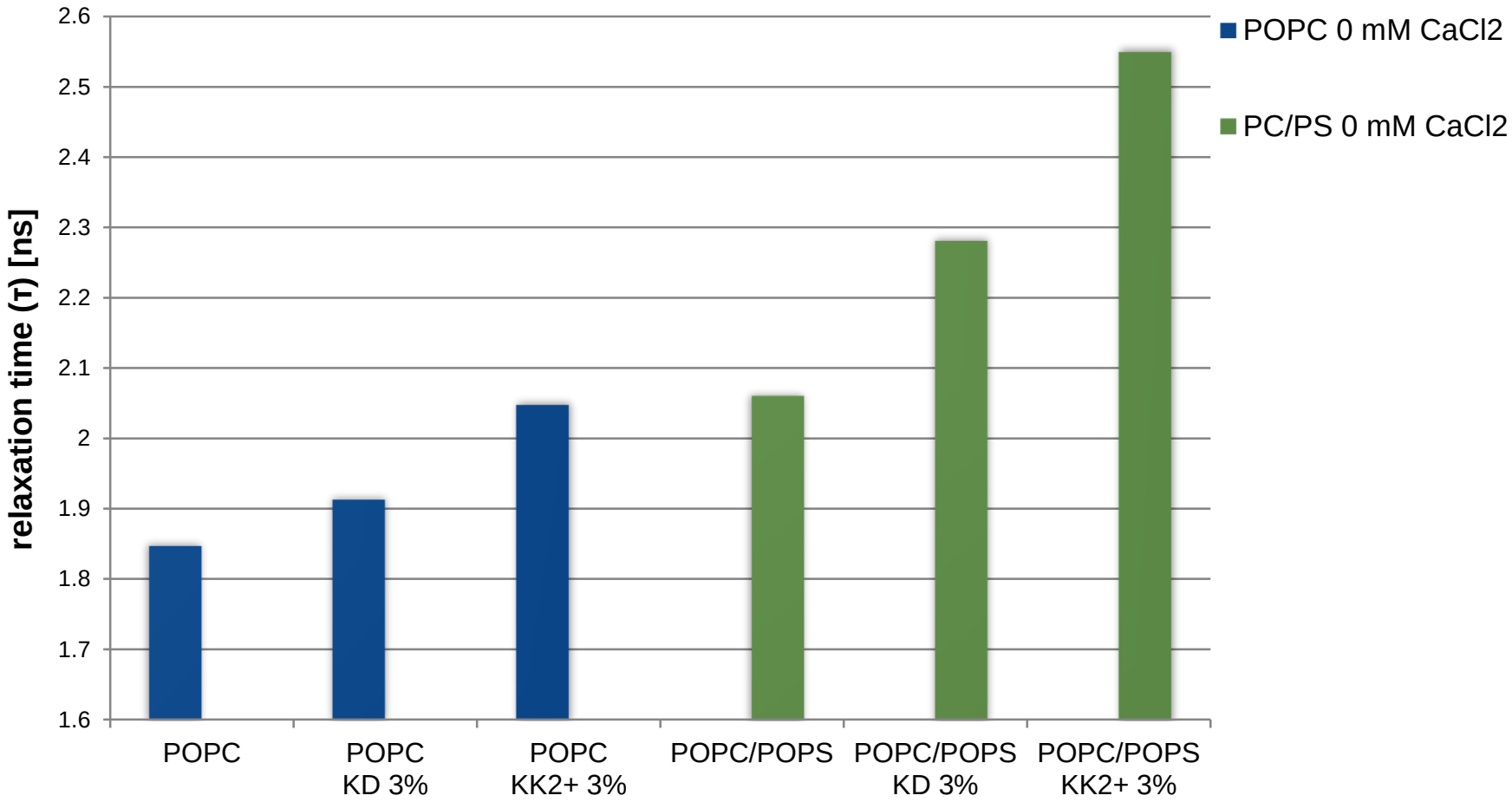




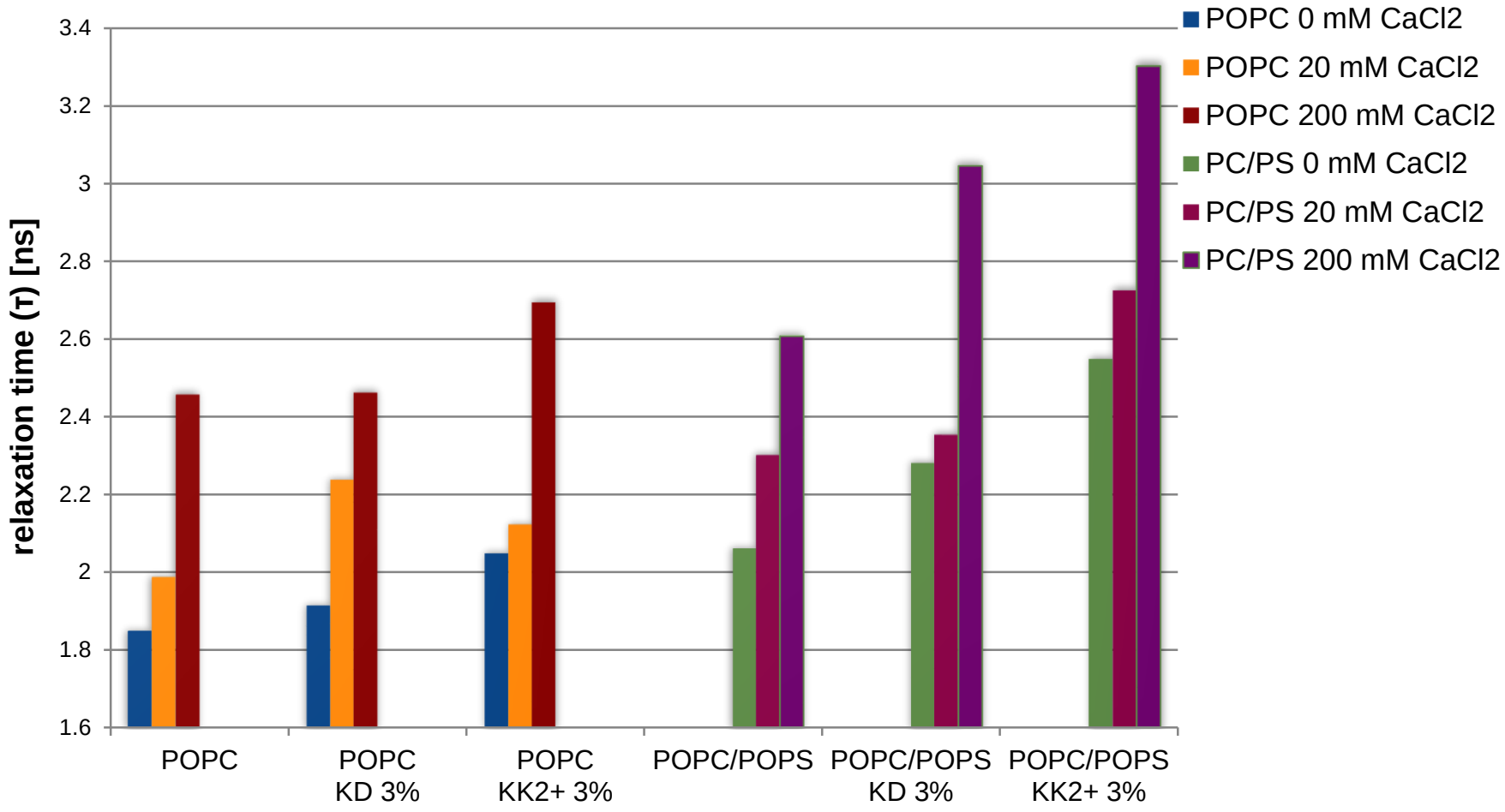
Jurkiewicz, P. et al. *Journal of fluorescence*, 2005, **15**(6)

en.wikipedia.org/wiki/Zeta_potential

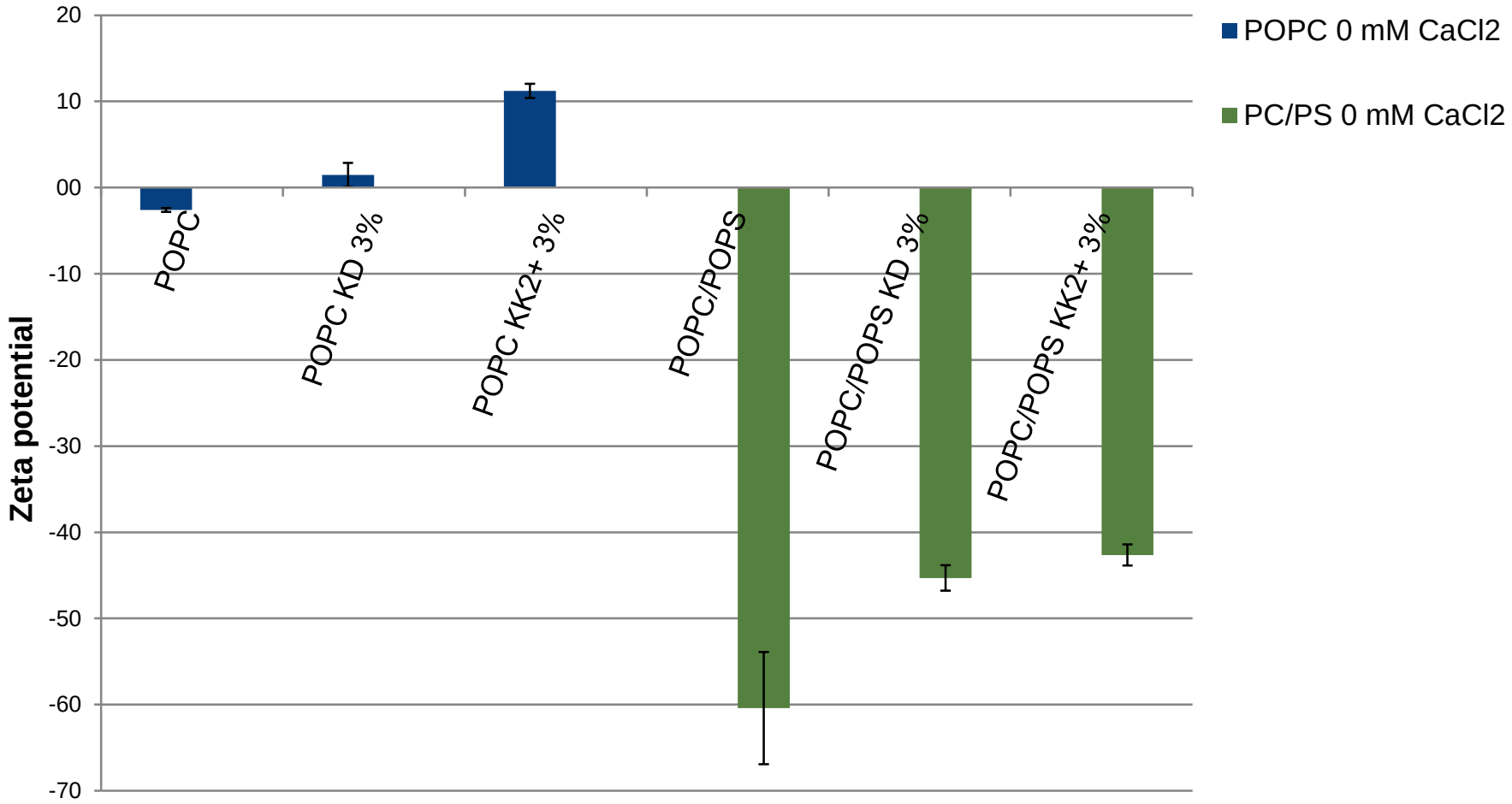
Impact on membrane rigidity – peptide



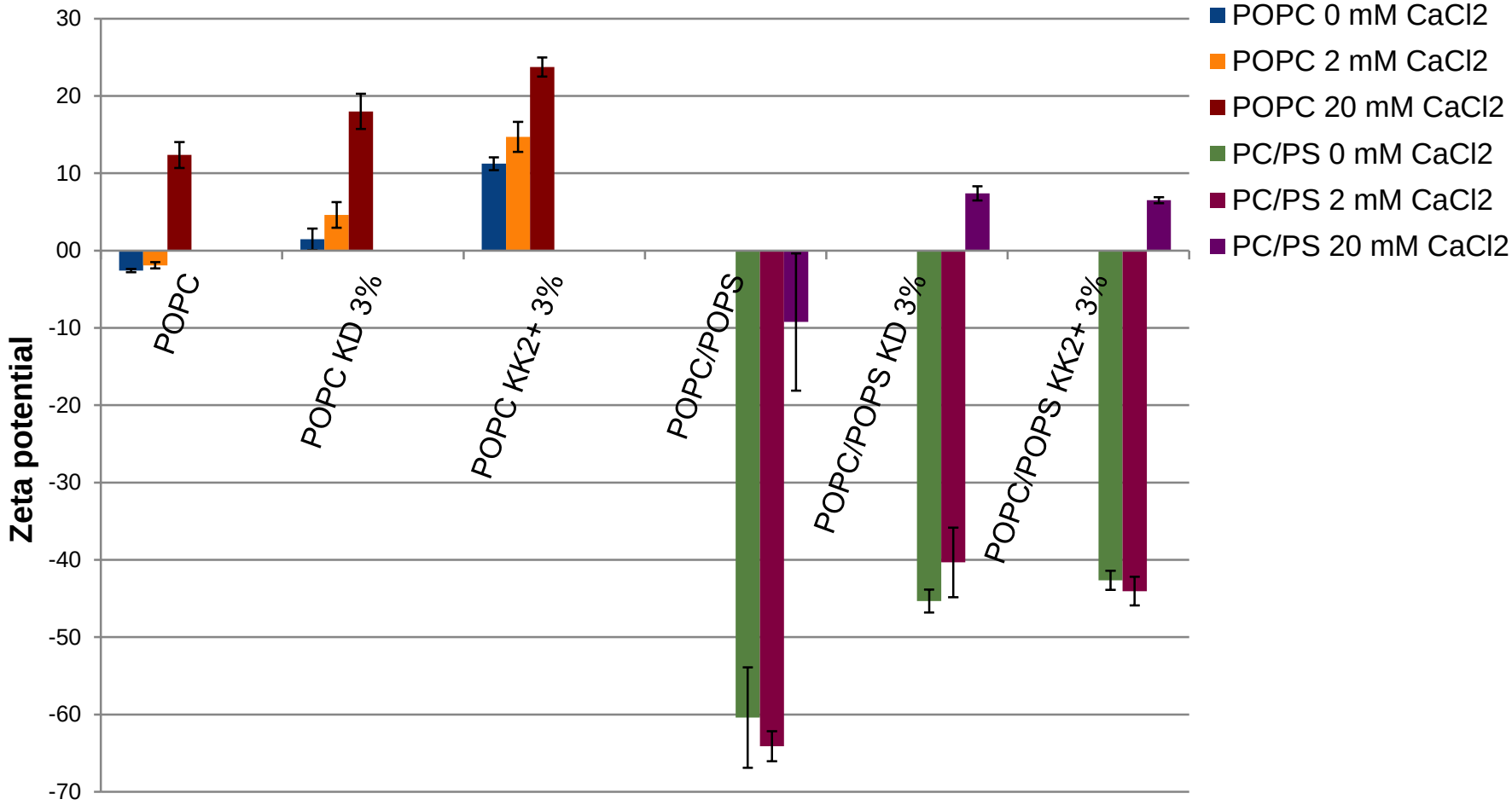
Impact on membrane rigidity – Ca^{2+}



Charge on LUVs – peptide



Charge on LUVs – Ca^{2+}



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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  - Daycare for children

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