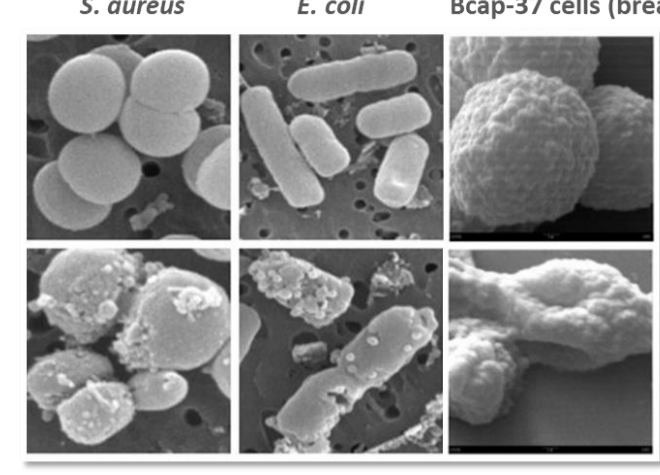


The "Sand in a gearbox" effect of antimicrobial peptides: beyond pore formation on model membranes and real bacteria cells

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Antimicrobial peptides: the « Sand in a gearbox » hypothesis



- Rapid and broad spectrum activity
- Selective toward bacterial membranes
- Kill pathogens by making their membrane permeable

~10 million bound peptides/cell

«Sand in a gearbox»

AMPs might perturb bilayer dynamics and interfere with cellular functions involving membrane proteins

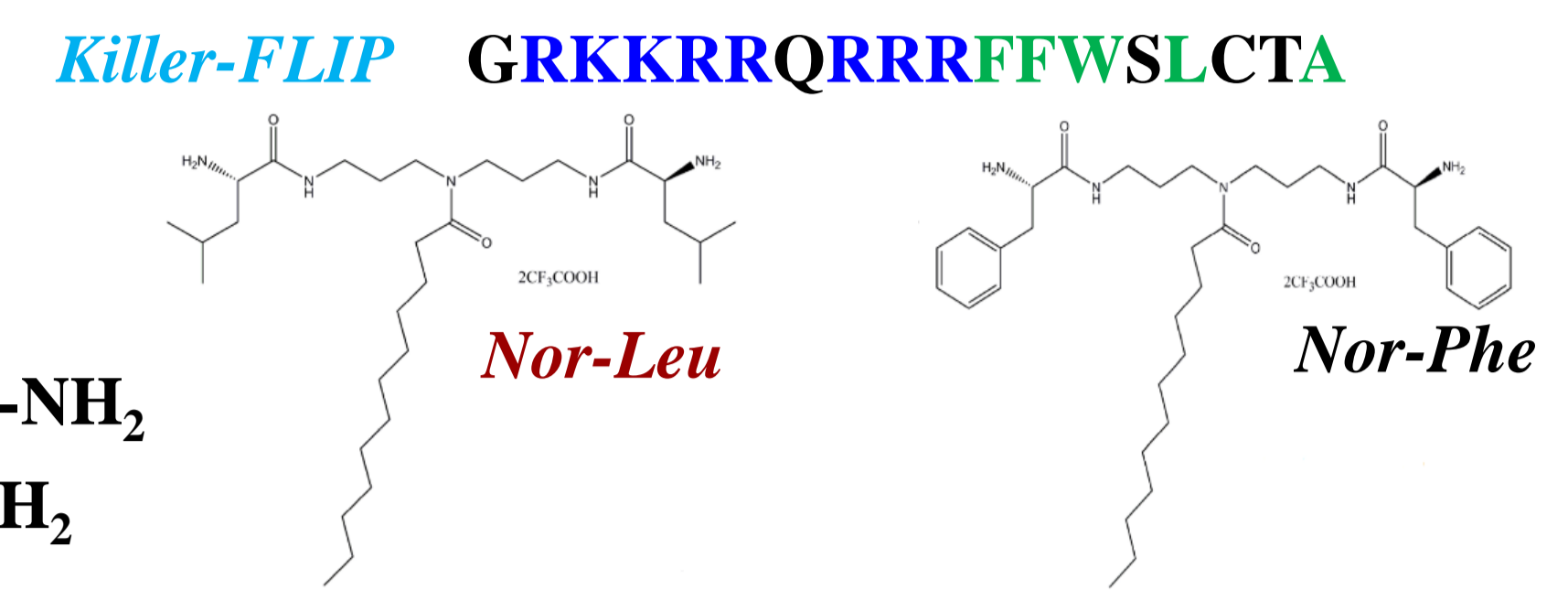


Systems investigated

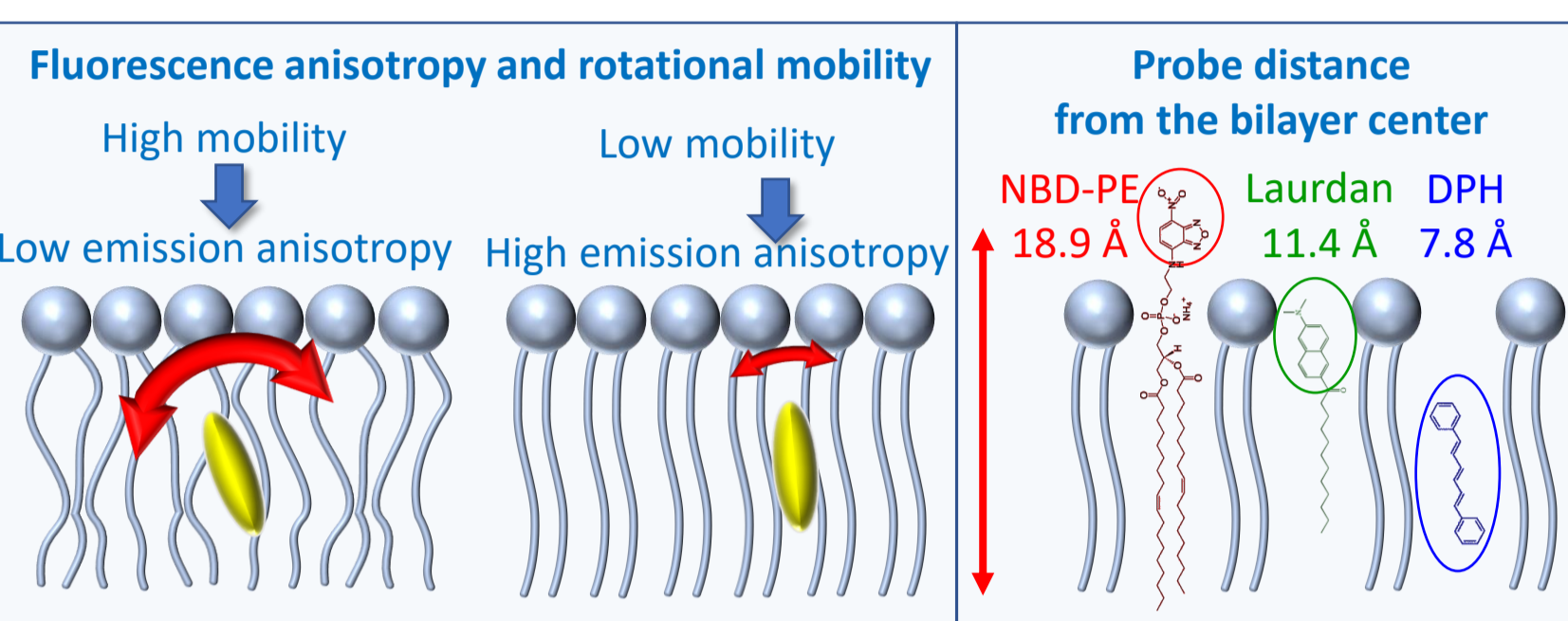
Antimicrobial peptides

PMAP-23 RIIDLLWRVRRPQKPKFVTWVWR
Alamethicin AcUPUAUAQUVUGLUPVUUQQFol
Magainin 2 GIGKFLHSAKKFGKAFVGEIMNS
LAH4 KKALLALALHHLAHLALHLAALKKA-NH₂
Melittin GIGAVLKVLTGLPALISWIKRKRQQ-NH₂

Membrane-active compounds

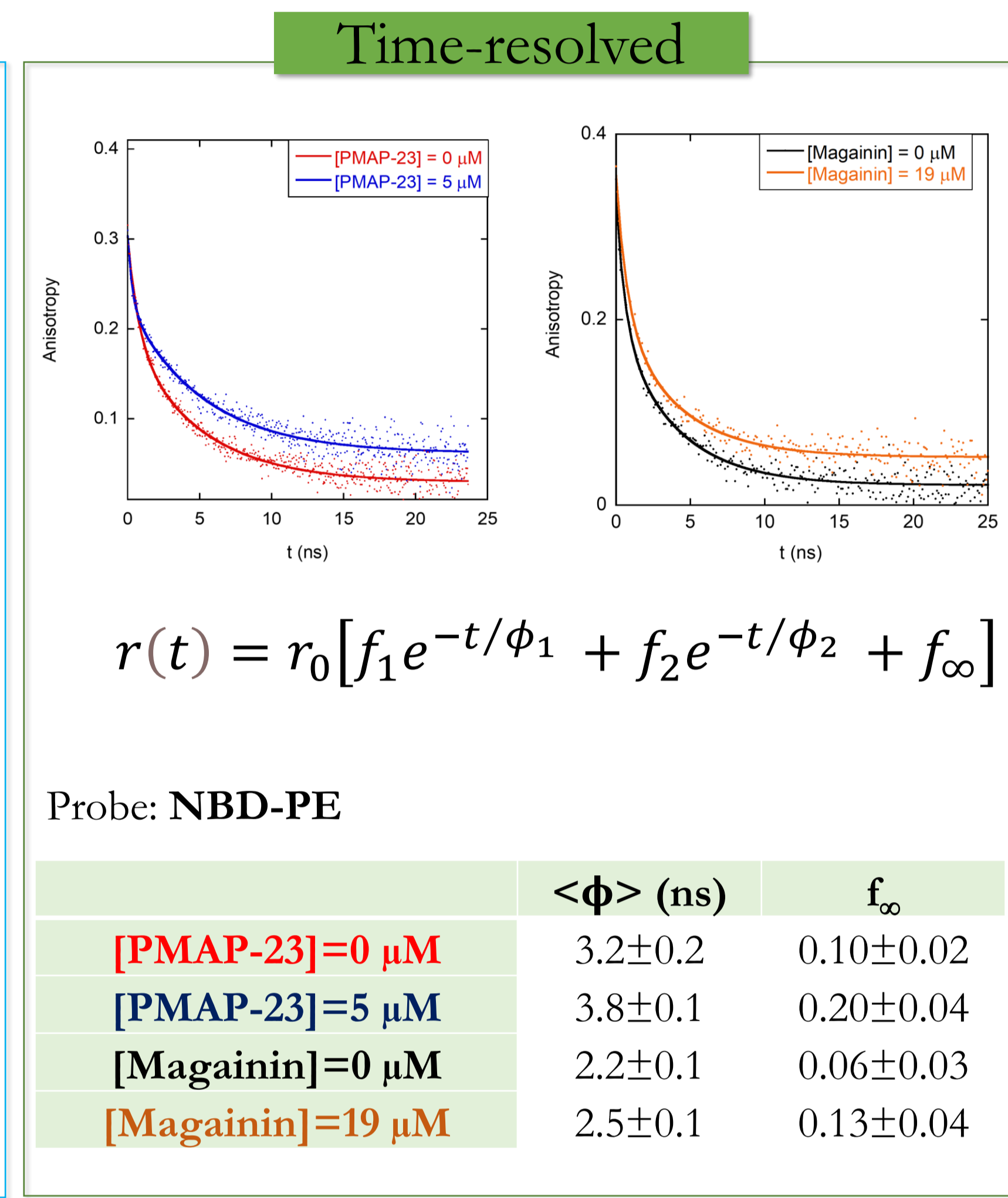
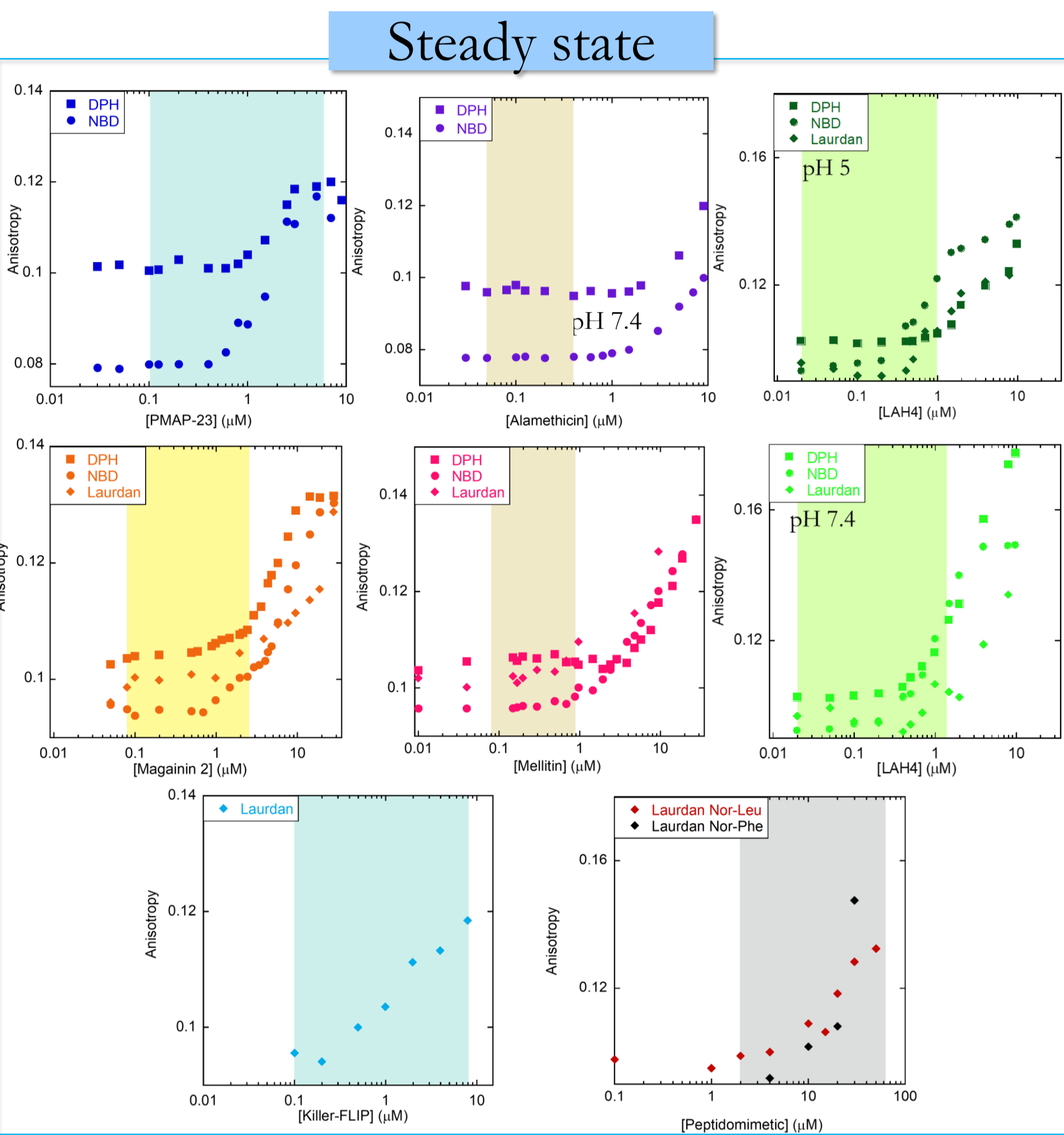


Membrane viscosity: fluorescence anisotropy



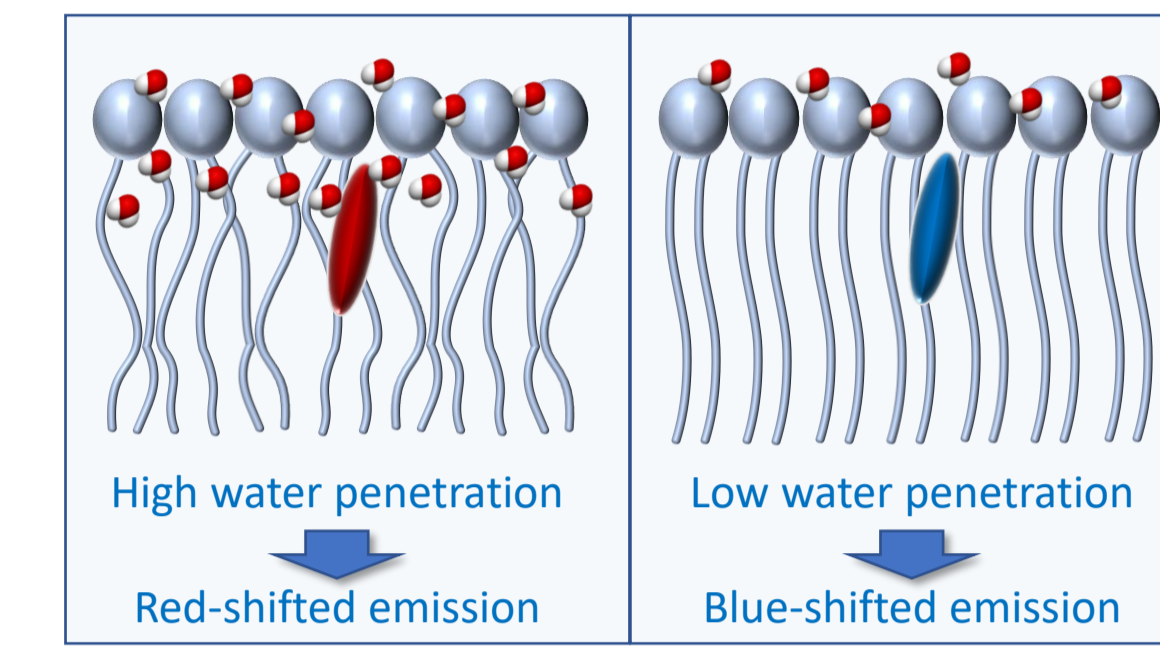
▪ **Steady state**
 Increase in anisotropy: decreased mobility of the probes

▪ **Time-resolved**
 Increase in both the average rotational correlation time and in the fraction of residual anisotropy.



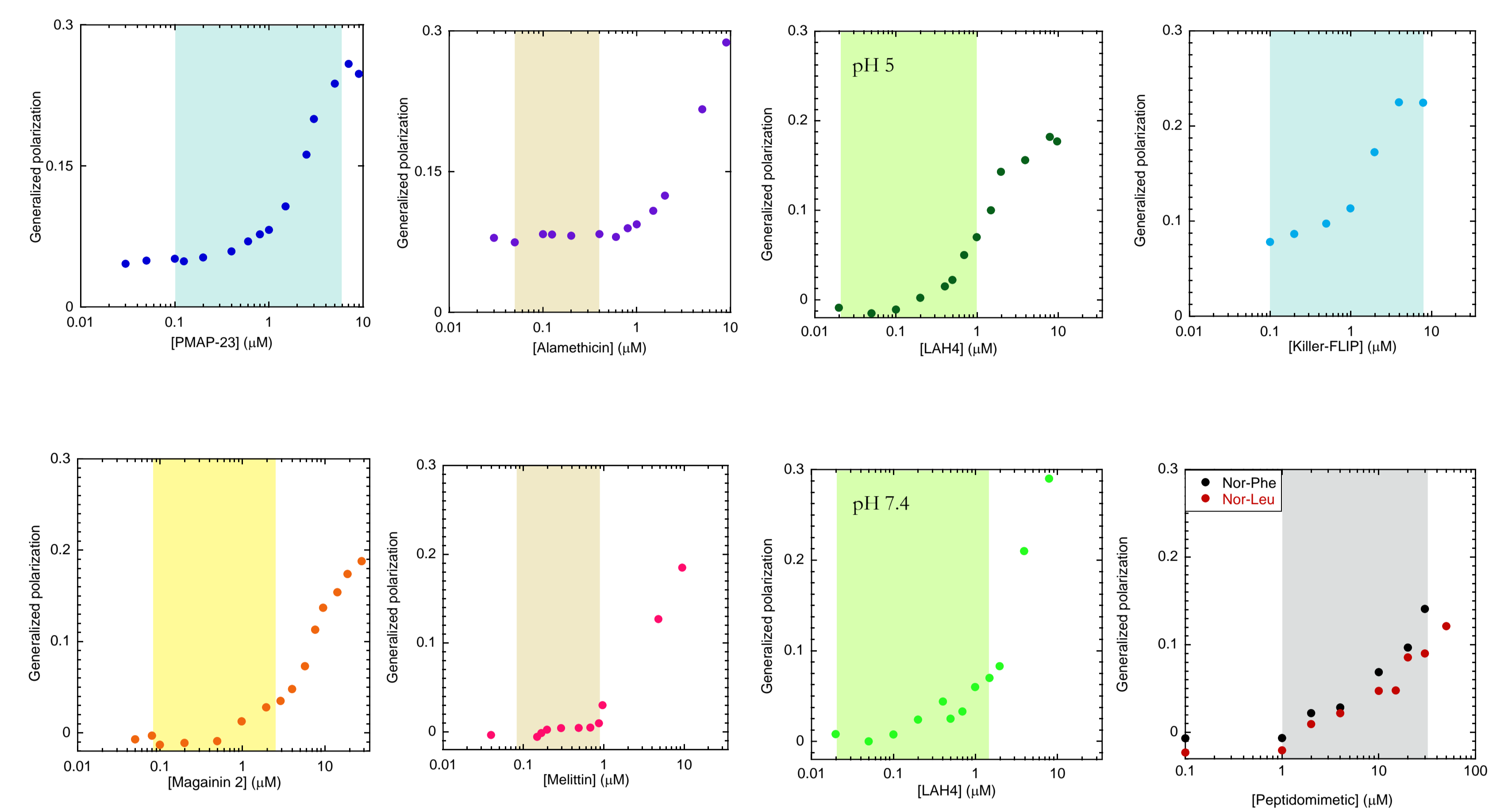
Probes motions are slower and more hindered: increase of membrane viscosity

Membrane hydration: generalized polarization

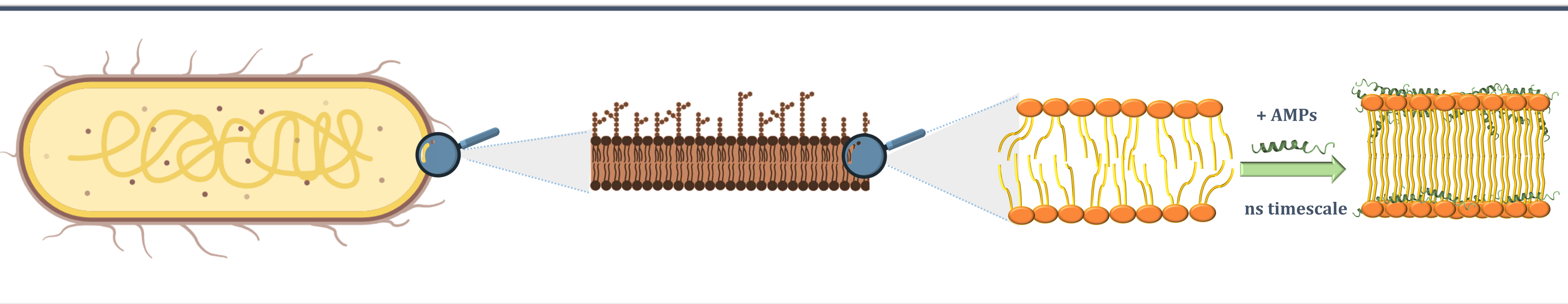


Red shift of Laurdan emission spectrum of 50 nm when water molecules surround the naphthalene moiety.

$$GP = \frac{I_{435nm} - I_{500nm}}{I_{435nm} + I_{500nm}}$$



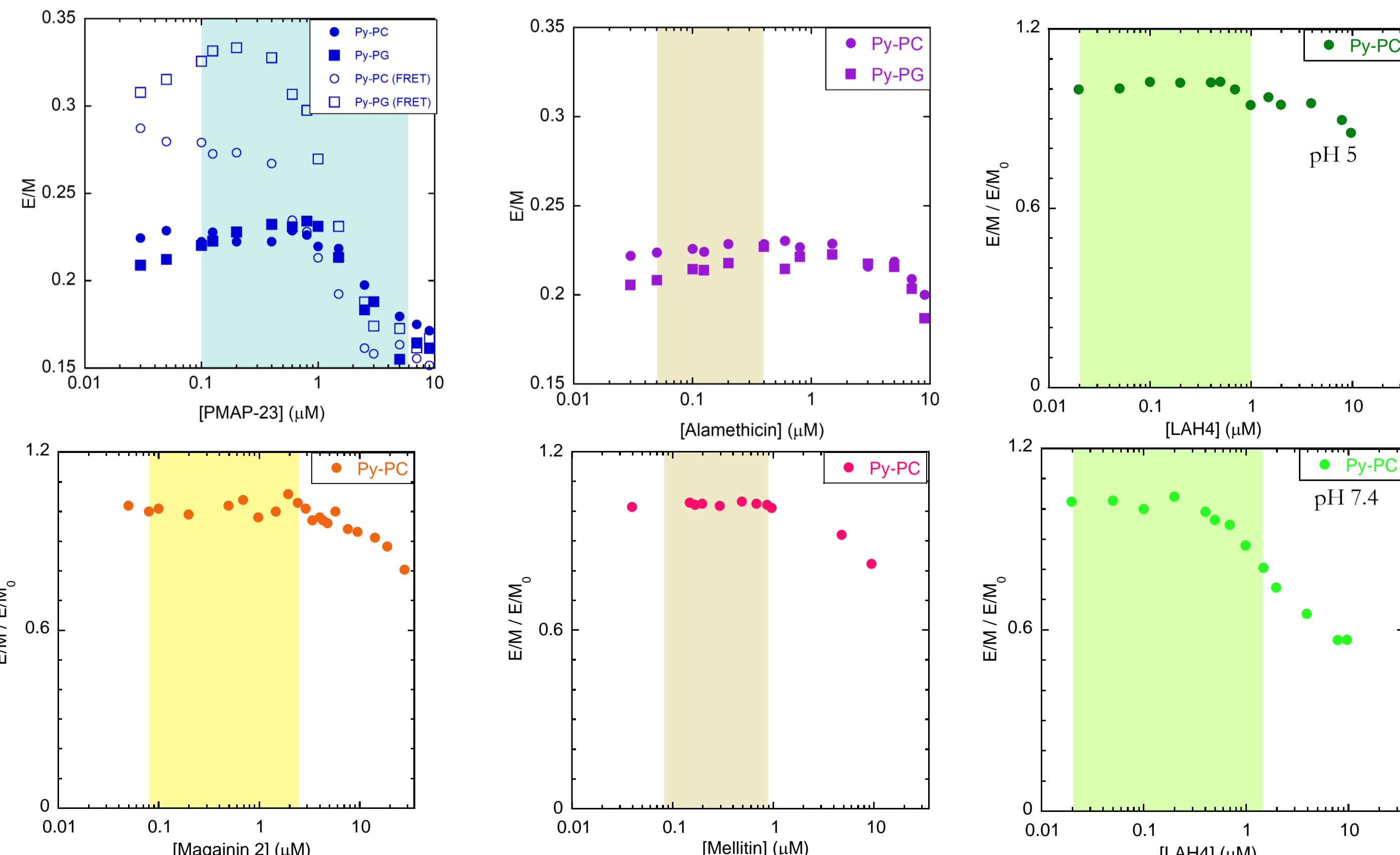
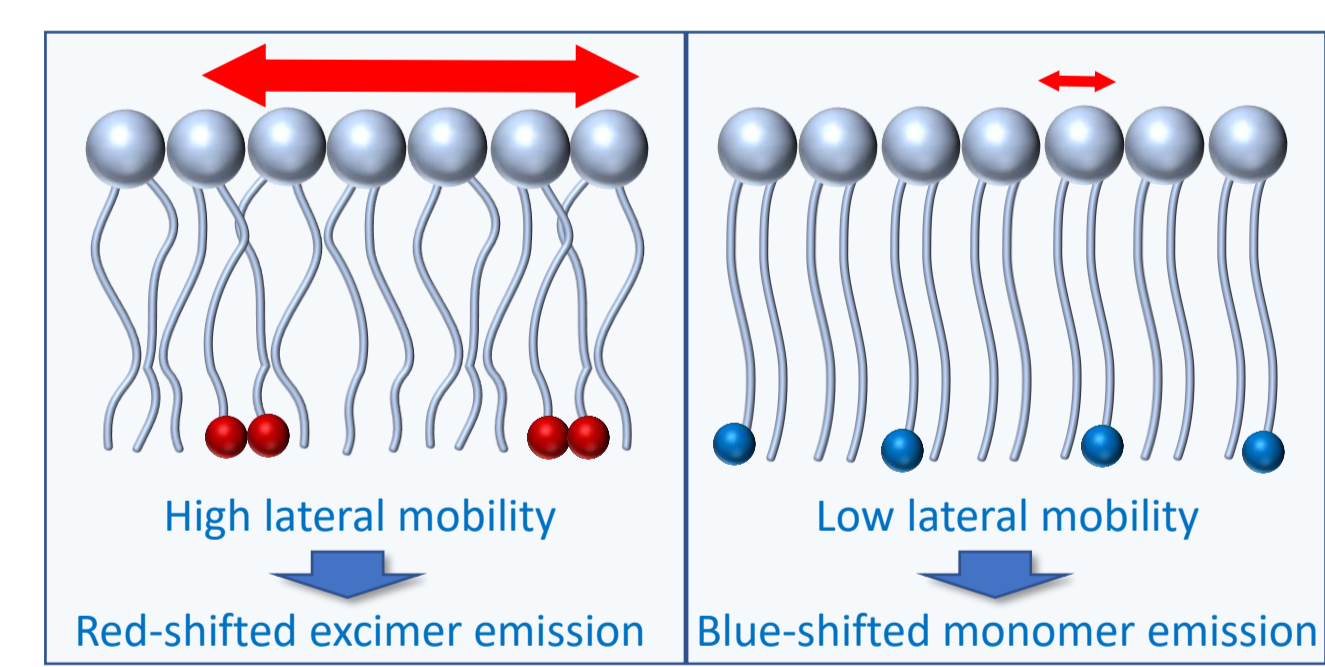
Reduction of the degree of water penetration inside the bilayer for all the compounds



Lipid lateral mobility: excimer formation

- Excited-state dimer formation is diffusion-limited:
 Information on the lateral diffusion of pyrene-labelled lipids

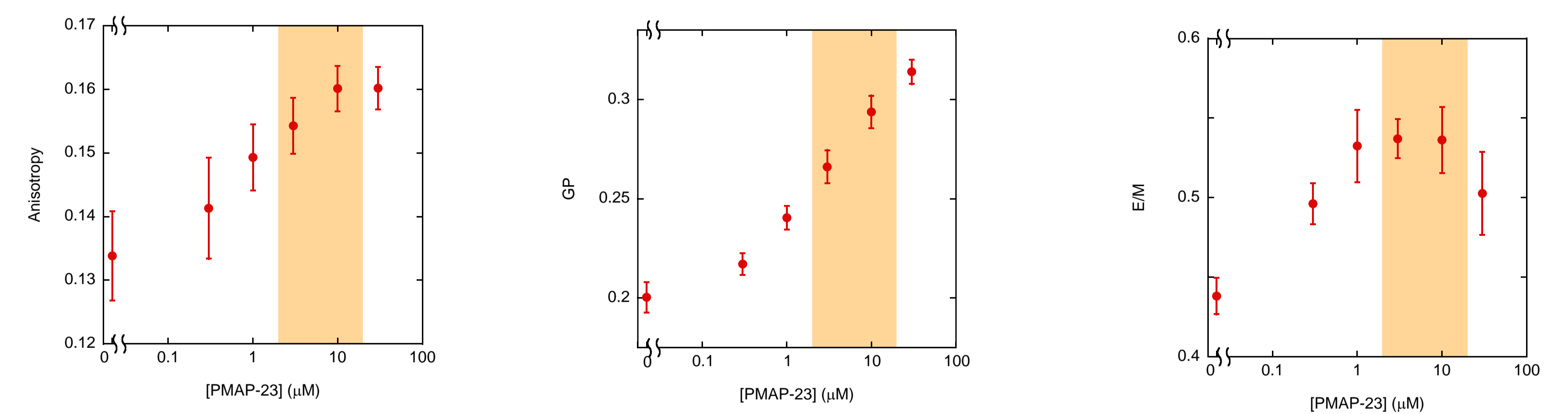
$$\frac{\text{Excimer } (\lambda_{em} = 475nm)}{\text{Monomer } (\lambda_{em} = 397nm)}$$



Reduction of the lipid lateral diffusion observed with all the compounds analyzed

Membrane dynamics in live bacterial cells

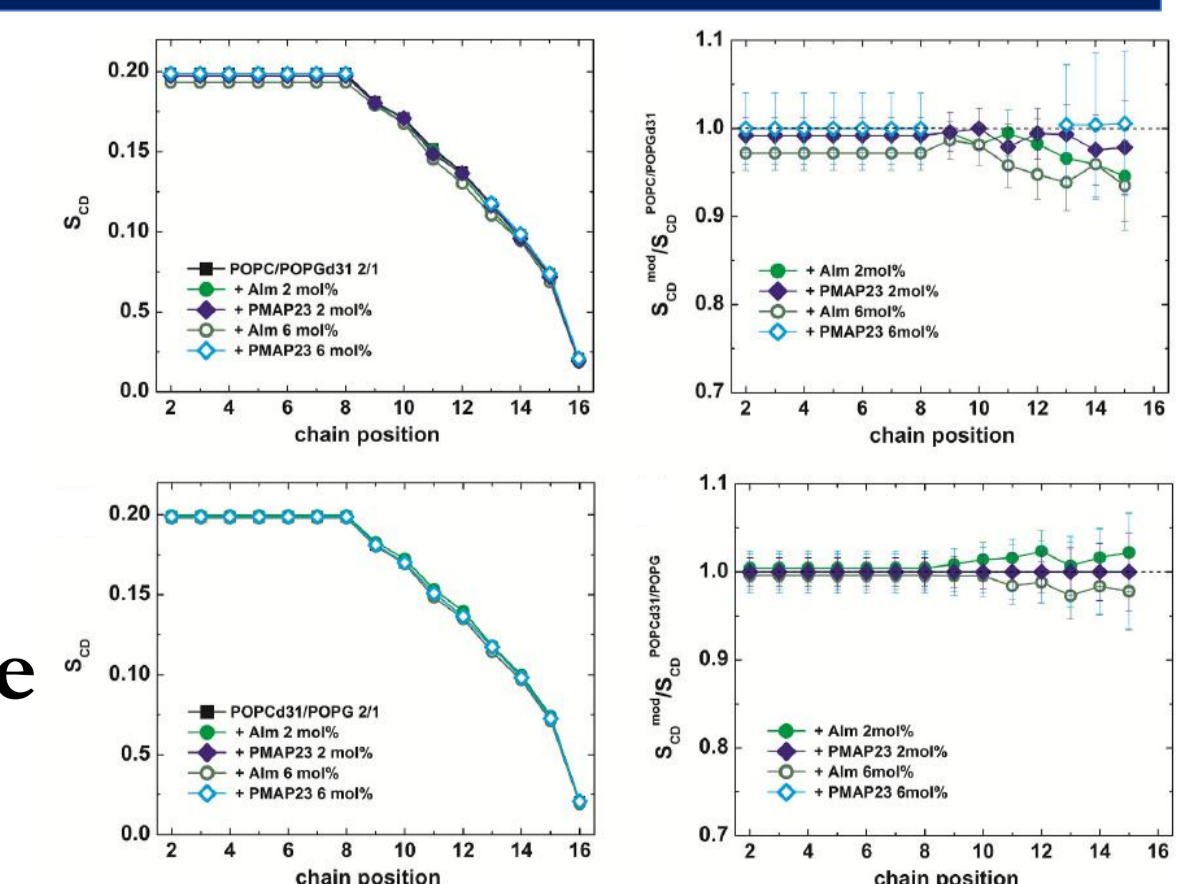
- PMAP effects were measured on the dynamics of membranes of live *E. coli* cells



Increase in DPH anisotropy and laurdan GP, and an initial rise, followed by a decrease in pyrene excimer to monomer intensity ratio.

Lipid order parameters: ²H ssNMR spectroscopy

- ²H ssNMR spectroscopy studies to measure peptide effects on lipid order parameter of POPC/POPG 2/1 liposomes.



No significant peptide effects were observed on the order parameter of the zwitterionic or anionic lipids.

All the compounds induce an increase of membrane viscosity, a reduction of water penetration and of lipid lateral mobility. The overall effect is a membrane stiffening of the vesicle.

PMAP-23 affects membrane dynamics also in real, live cells