Bilayer membrane solubilization by hydrophobic polyelectrolytes: a special case of ligand-mediated transitions

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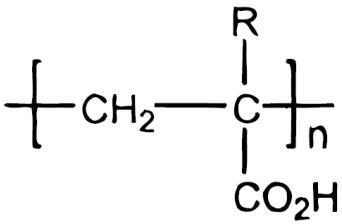
Hydrophobic polyelectrolytes

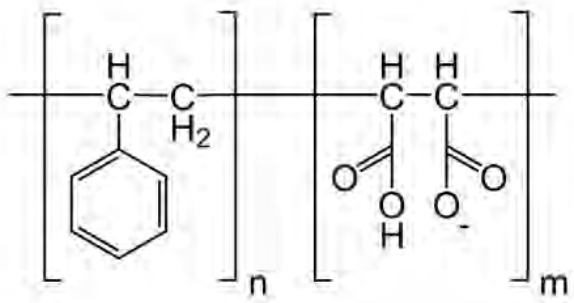
(with James Martín Robinson) - JL Martín Robinson & WKK, PNAS 120 (2023)

- Random/alternating arrangement of ionizable and hydrophobic repeating units.
- pH sensitivity.

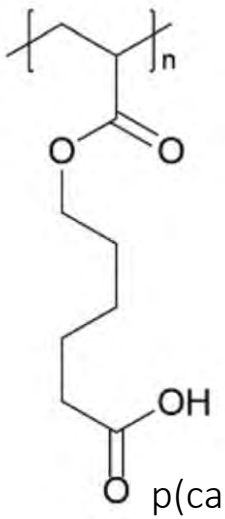


p(methyl acrylic acid) R=CH_3

p(ethyl acrylic acid) R=CH_2CH_3

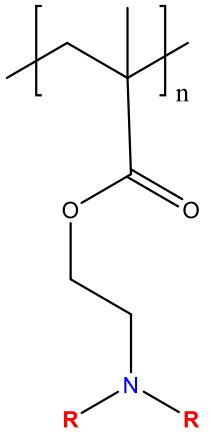


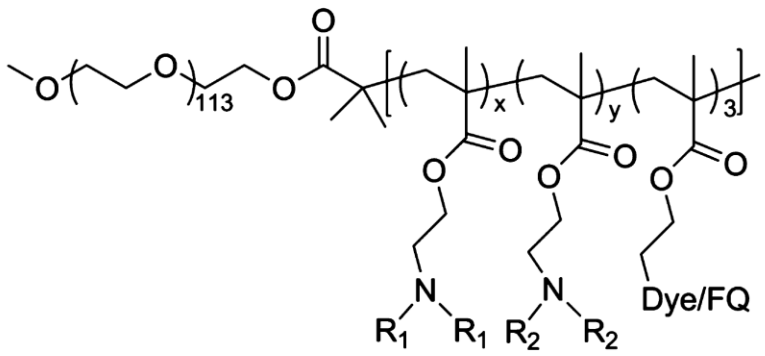
p(styrene maleic acid) SMA



PCPA

p(carboxypentyl acrylate)

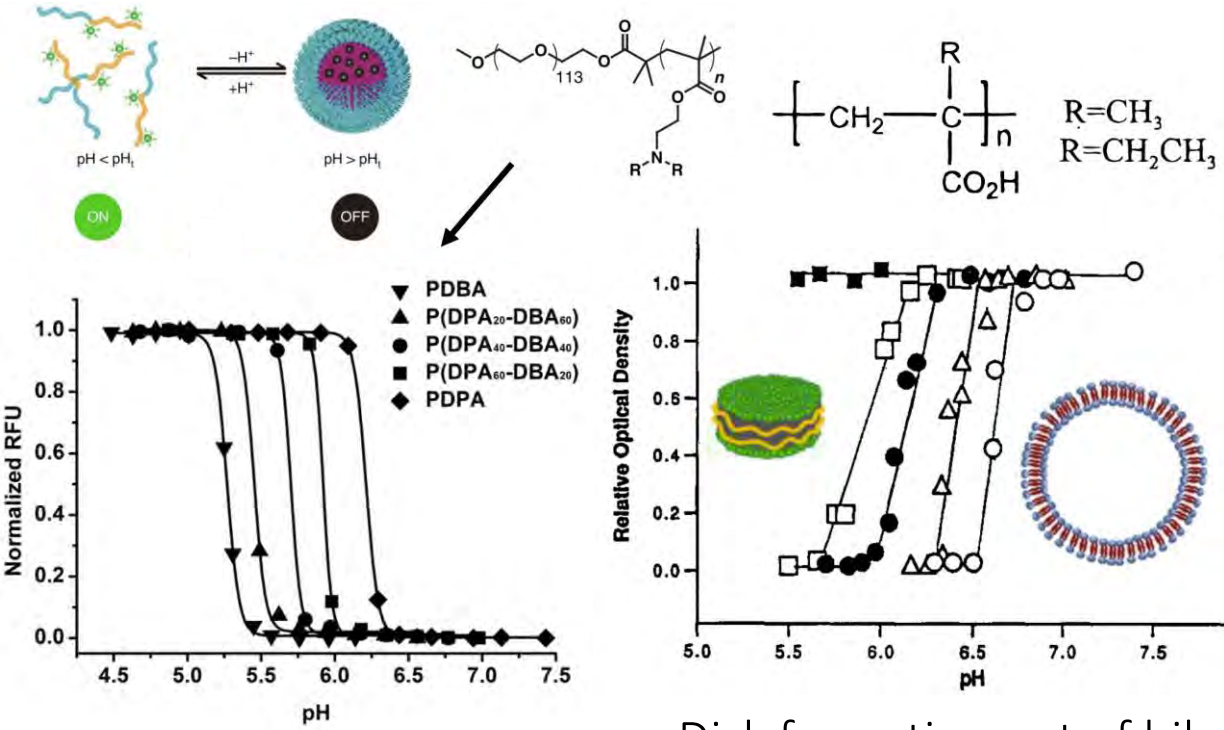




PEO-*b*-P(R_1-*r*-R_2-*r*-Dye/FQ)

R_1	R_2
Et	Pe
Et	Pr
Pr	Bu
Bu	Pe

pH driven transitions involving HPE



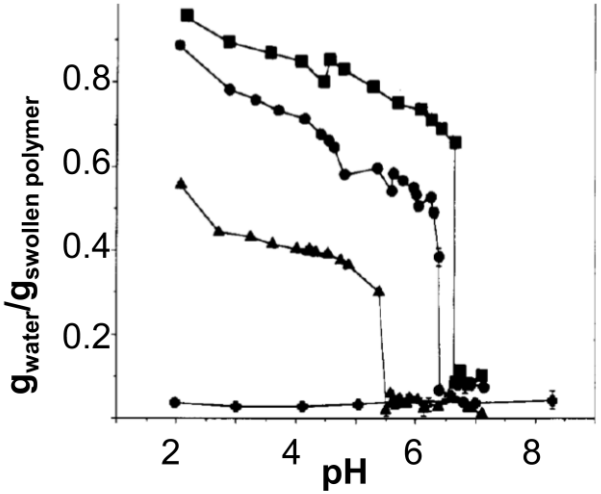
Micelle formation in diblock with 1 HPE block

X. Ma ea, JACS 2014.

Disk formation out of bilayer vesicles

Thomas, Tirrell ea Bioph. J. 1994; Acc. Chem. Res. 1992; JACS 1995

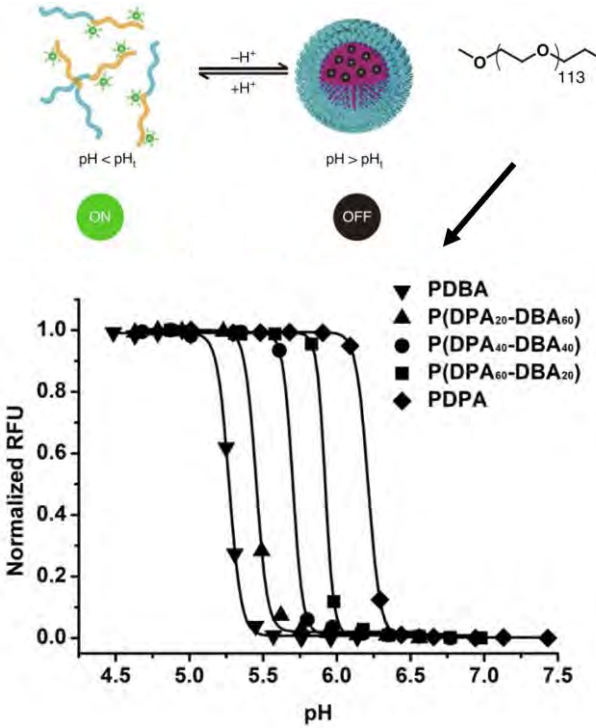
Similar behavior with SMA: Scheidelaar ea Bioph. J. 2016



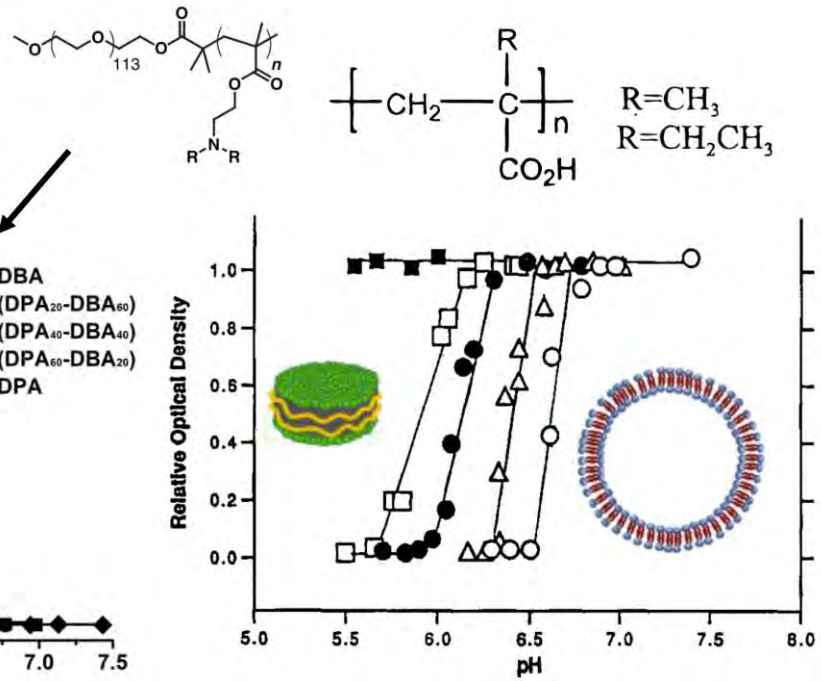
Swelling of weakly crosslinked HPE

Siegel ea, Macromolecules 1988; Adv Poly, Sci 1993

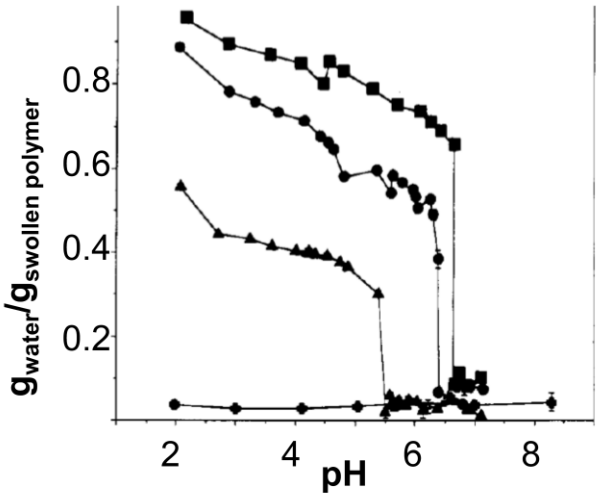
Transitions involving HPE are often SHARP / cooperative:



Micelle formation in diblock with 1 HPE block



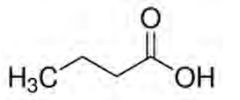
Disk formation out of bilayer vesicles

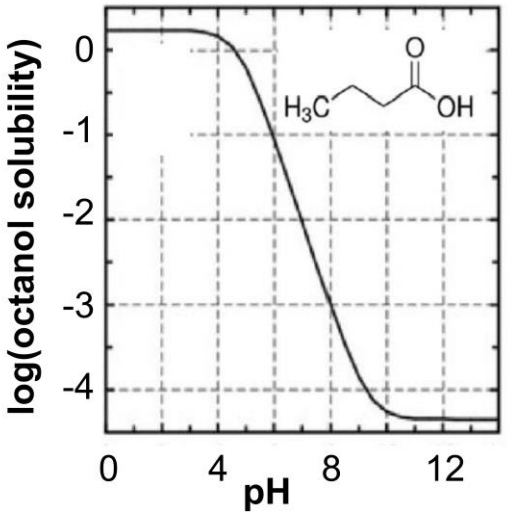


Swelling of weakly crosslinked HPE

Transitions occur within ~0.2 pH units.

Compare partitioning of butyric acid between water & octanol





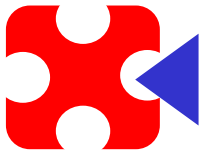
[S. Rayne ea, Flavour and Fragrance J. 2015]

What causes cooperativity in transitions involving HPE?

Hypothesis: coupling between HPE conformations and ionization - similar to oxygen binding by hemoglobin (?)

MWC theory: J. Mol. Biol. **12,** 88, (1965)

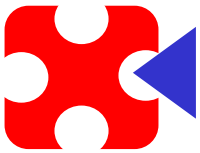
Hemoglobin



Ground state –
weak ligand affinity

oxygen

Excited state
strong ligand affinity
conformation penalty



Ground state –
weak ligand affinity

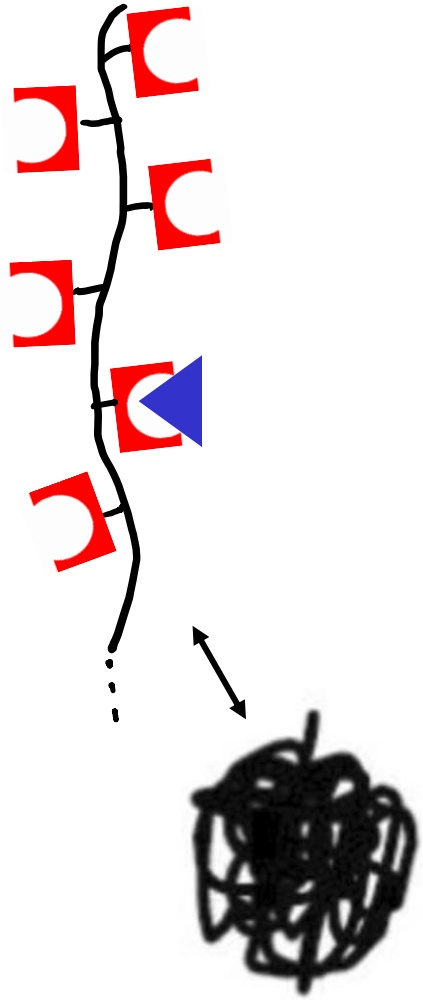
Hemoglobin

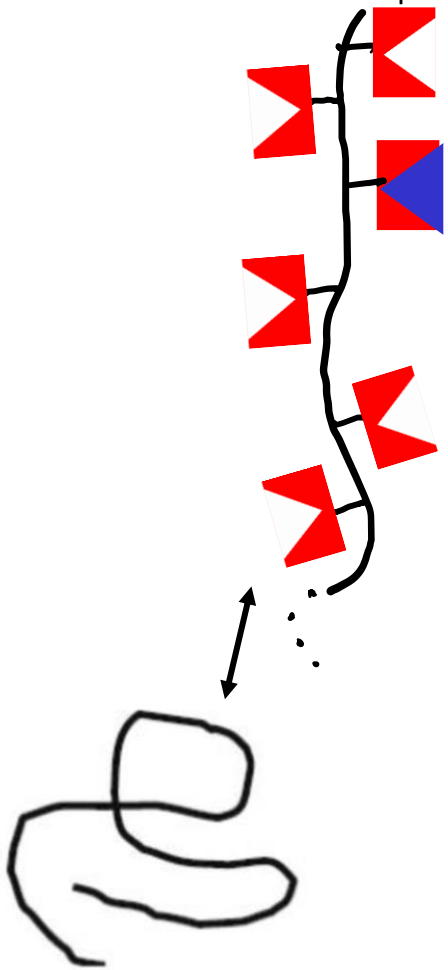
oxygen

Excited state
strong ligand affinity
conformation penalty

HPE

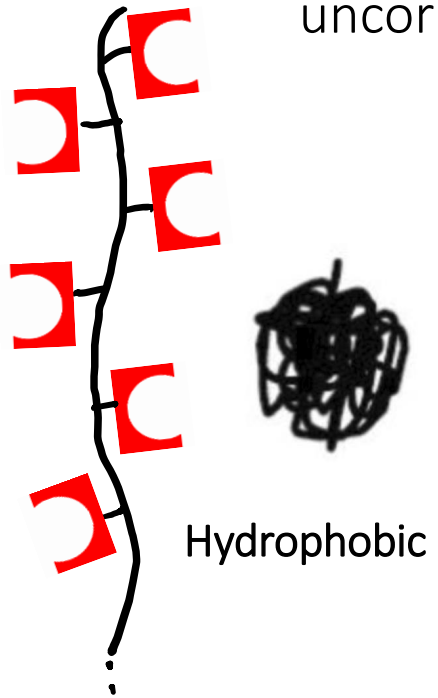
Proton or
Hydroxyl ion





(Grand canonical) statistical weights of aqueous and hydrophobic conformations:

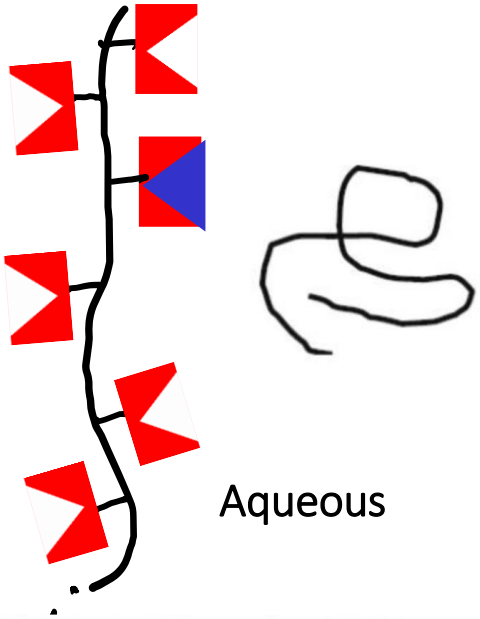
uncorrelated ionization of max M groups



Hydrophobic

$\Xi_H \approx 1$

Proton or
Hydroxyl ion



Aqueous

$\Xi = \Xi_{aq} + \Xi_H$

$\Xi_{aq} = \exp(-\beta G_H)\ (1 + 10^X)^M$

Hydrophobic penalty

$X = pH - pK_a$ acidic groups

$X = pK'_a - pH$ basic groups

$G_H = M\, g_H(x)$ x ≡ composition

Fraction in hydrophobic state

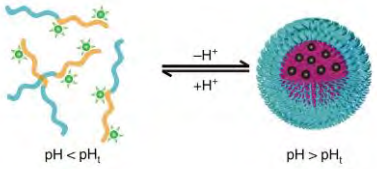
$$f_H = \Xi_H/\Xi = \left(1 + \exp(-\beta G_H)(1 + 10^X)^M\right)^{-1} \text{ and } f_{aq} = 1 - f_H.$$

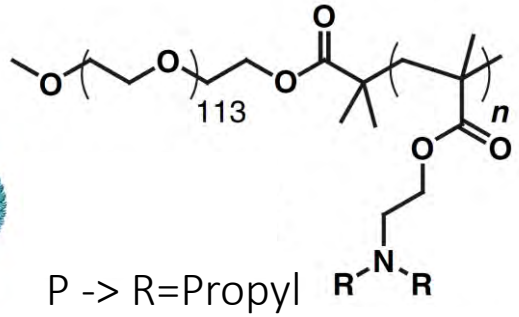
Ionized fraction $\theta = \dfrac{10^X}{1 + 10^X} f_{aq}$

pH driven micellization:

Take micelle as hydrophobic conformation.

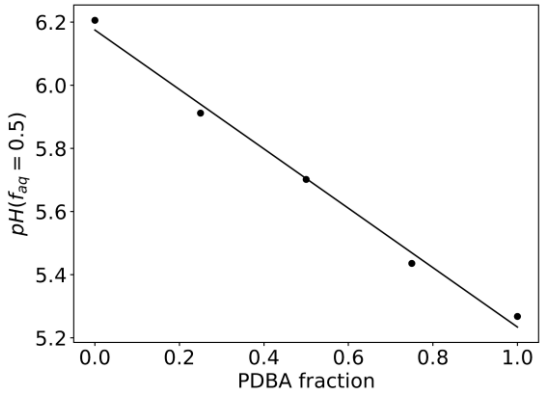
Soluble state ‘aqueous’ conformation

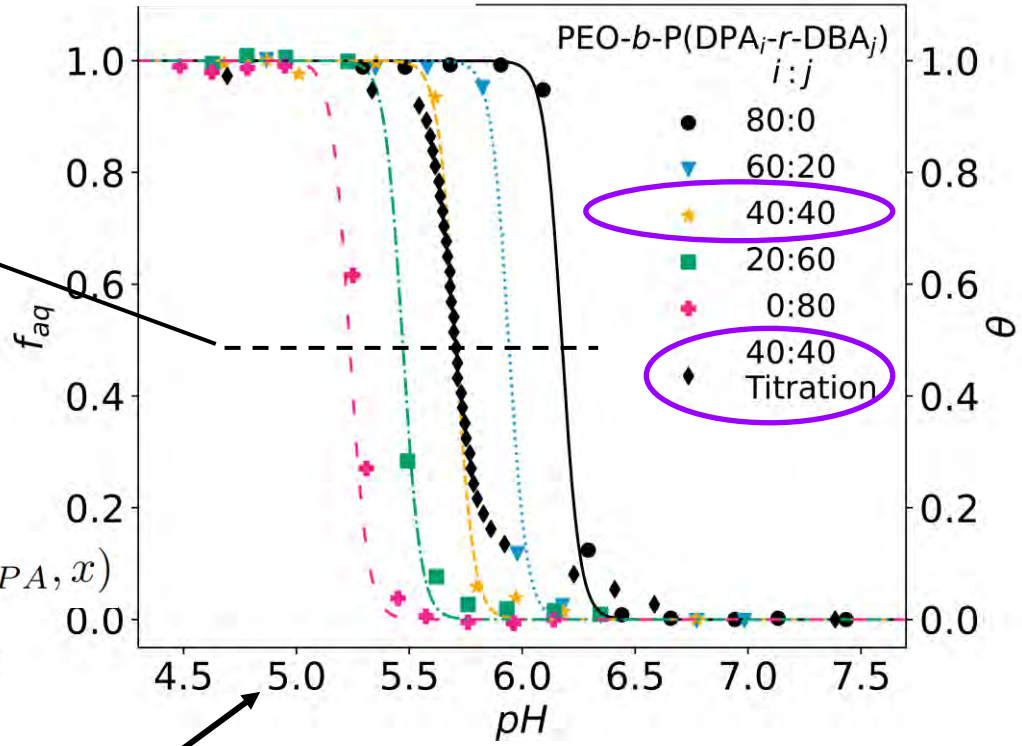




P -> R=Propyl

B -> R=Butyl





$$pH_{\text{micellization}} = pK'_a - 0.4343\beta f(g_{DBA}, g_{DPA}, x)$$

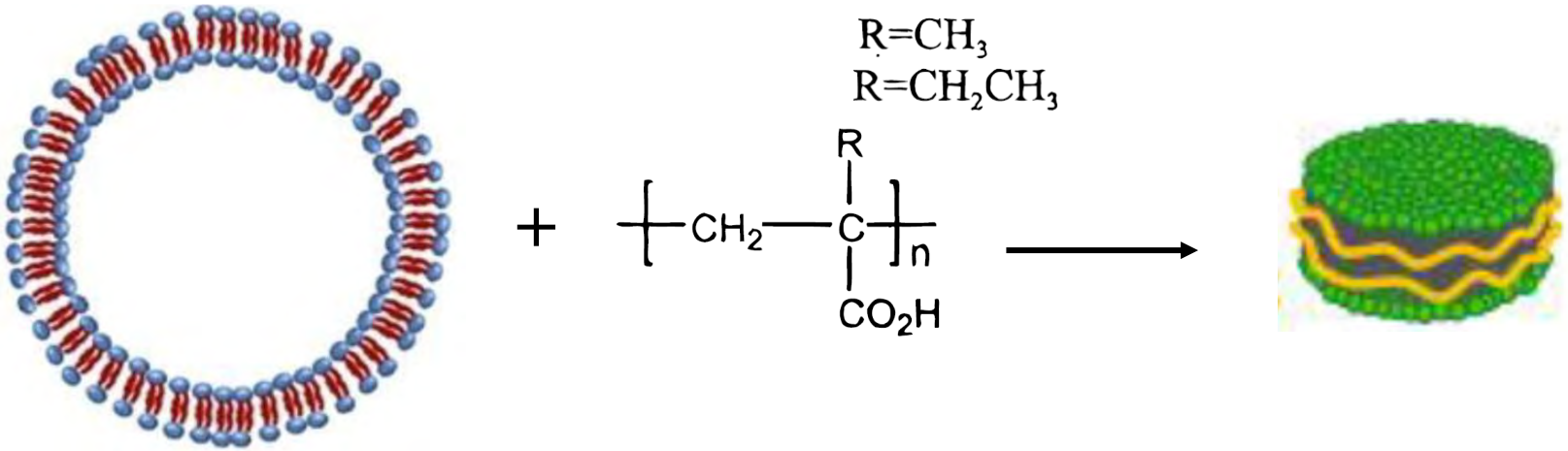
$$f(g_{DBA}, g_{DPA}, x) = x g_{DBA} + (1 - x) g_{DPA}$$

Calculate fractions, ionization state

Good agreement with M ≈ 11 (# ionic groups per chain ≈ 80)

Data: X. Ma ea, JACS 2014.

HPE – induced membrane solubilization by disk formation



Define third state for HPE: 'Disk' conformation where hydrophobic groups stick into rim of the disk and ionic groups (mainly) orient towards aqueous side

$$\Xi_D = \exp(-\beta G_{HD})\ (1 + 10^{pH-pK_a})^{M_D}$$

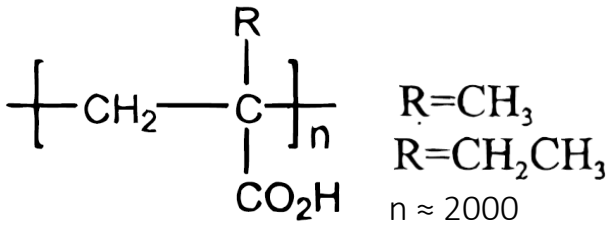
work to create interface
disc rim – hydrophobic groups

Max # ionized groups near rim
$M_D \leq M$

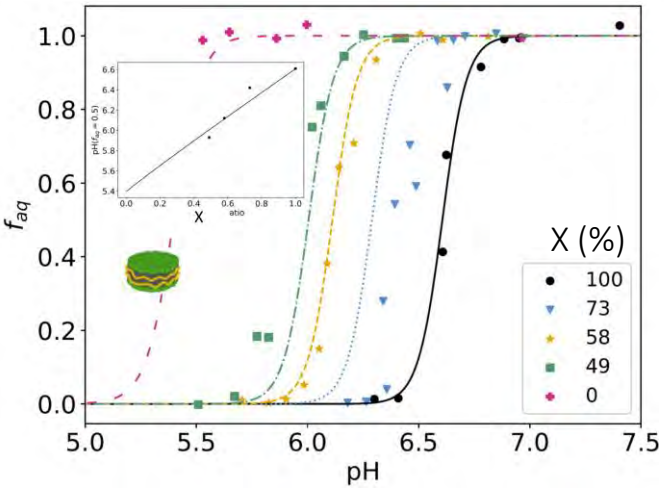
Aqueous-disk transition in pMAA - r - pEAA with variable EAA fraction X

Transition pH

$$pH_{DA} = pK_a + \frac{0.4343\beta f(\Delta g_{EAA}, \Delta g_{MAA}, x)}{1 - \frac{M_D}{M}}.$$



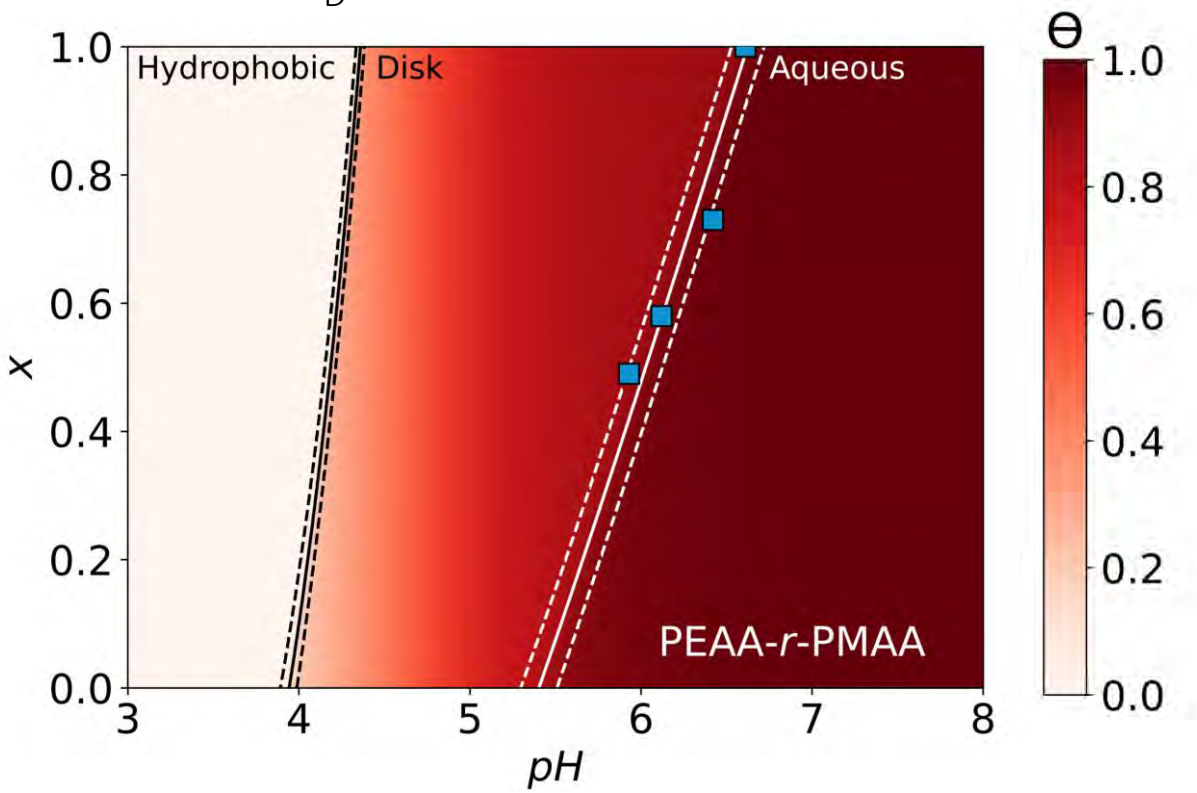
n ≈ 2000



Data: Tirrell ea Bioph J 1994, JACS 1995

Lines = theory with M-M_D ≈ 6

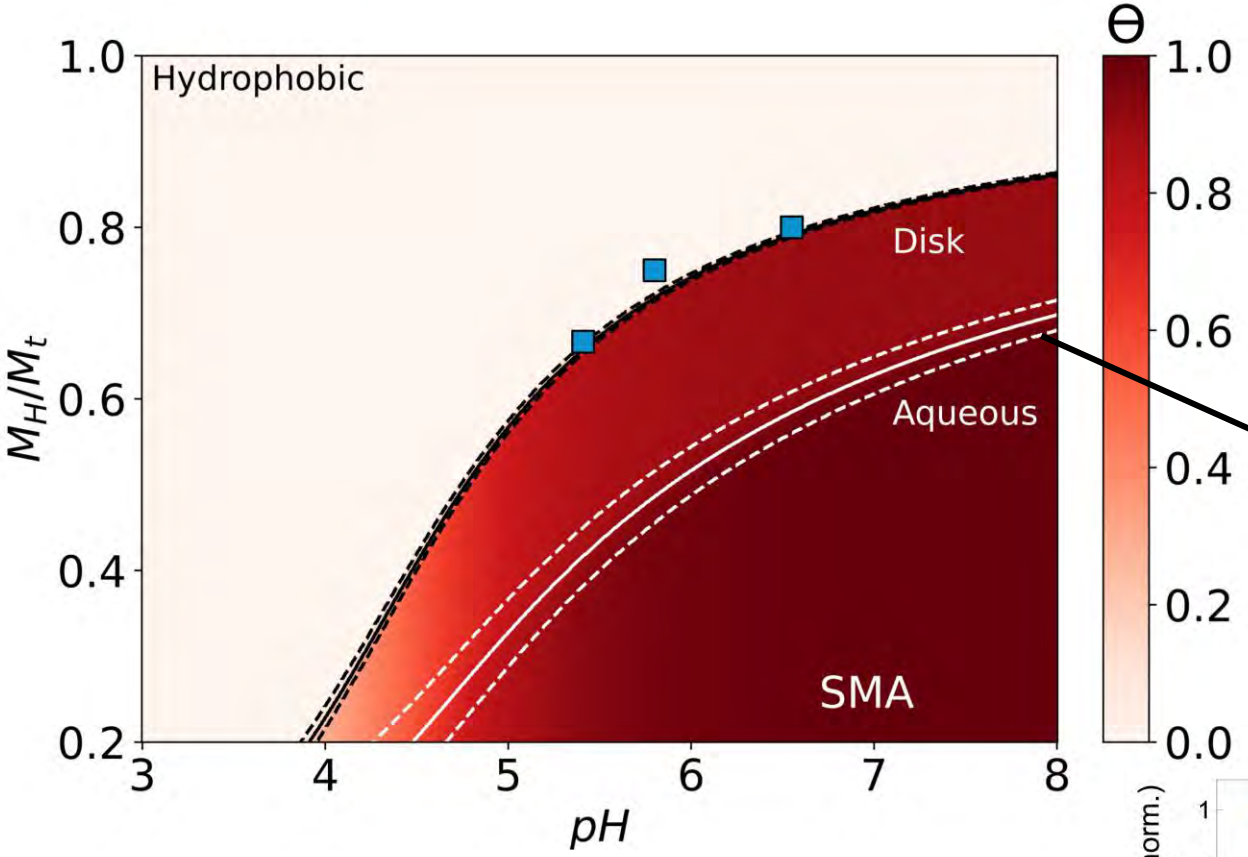
Assume M_D=0.9M

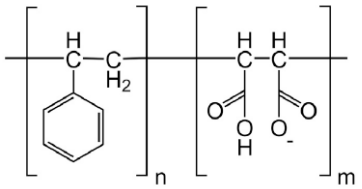


Assume $G_H = 2G_{HD}$

Predict ionization state

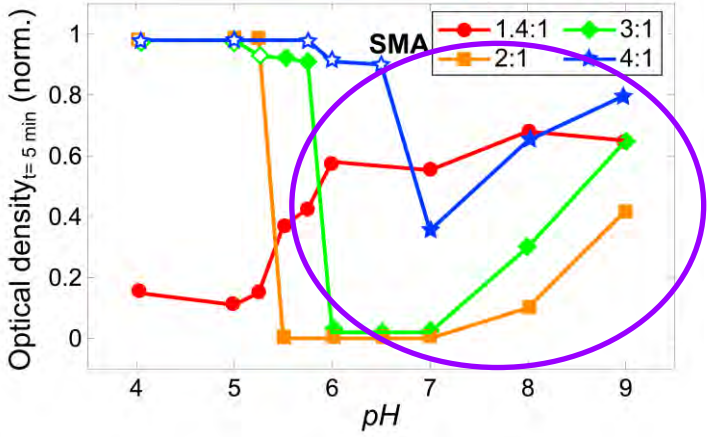
Hydrophobic-disk transition in SMA (~50 monomers)





n+m ≈ 50

Experimental (likely) Aq – disk transition:



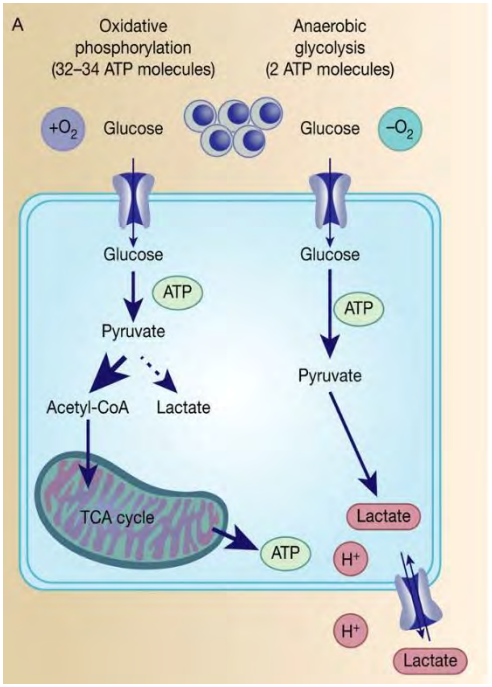
Aqueous-disk transition predicted cooperativity =(M – M_D)

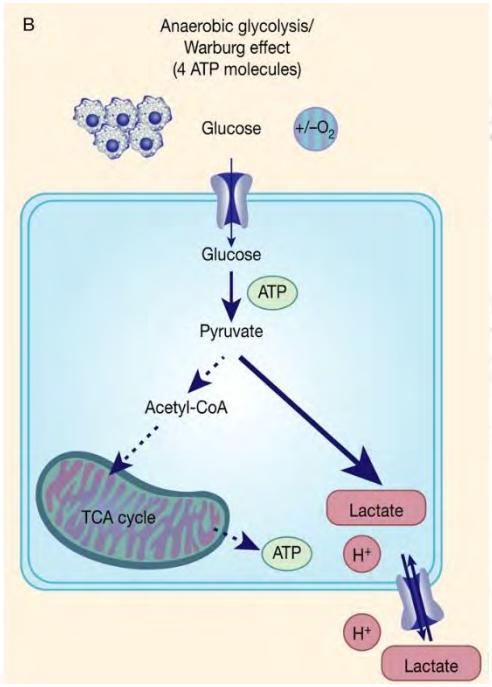
Hydrophobic – disk has cooperativity M_D

Data from Scheidelaar ea Bioph. J. 2016

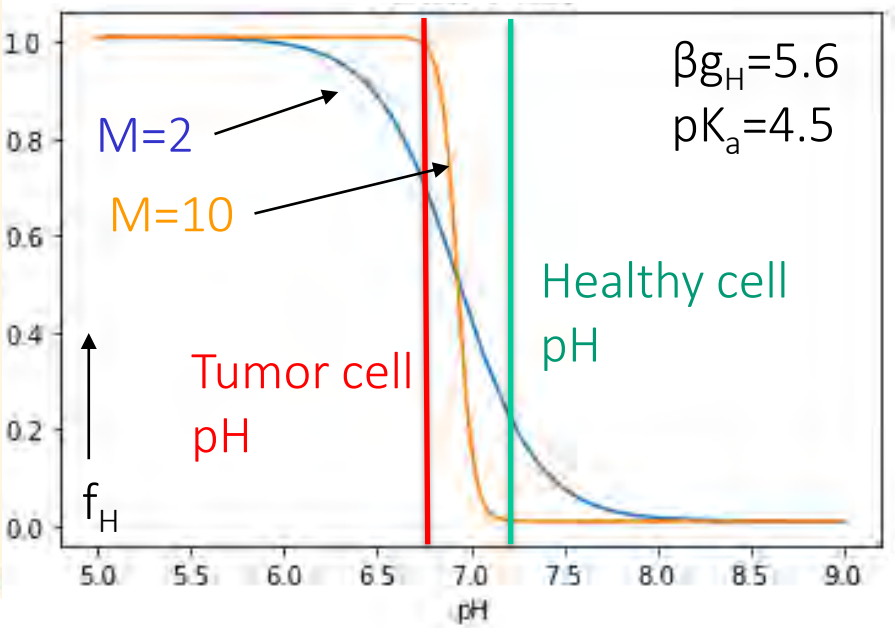
HPE as anti-tumor agents

Warburg effect: tumor cells often are in fermentation-like metabolic mode and have ~0.5 units lower extracellular pH than healthy cells





Membrane solubilization / permeation



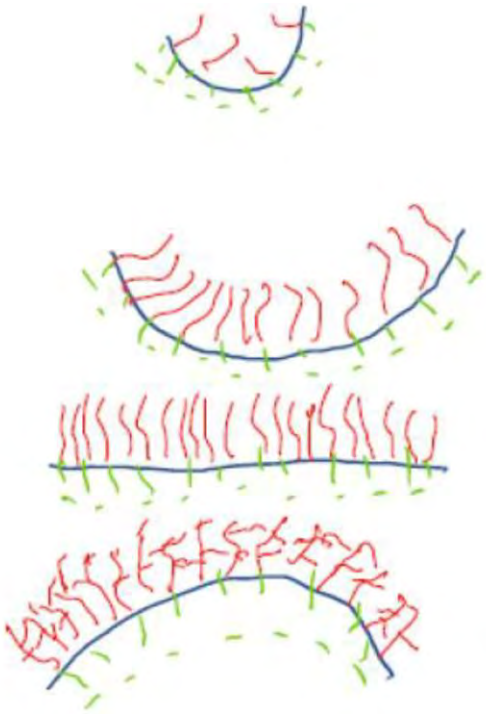
Healthy: pH ≈ 7.2 – 7.4

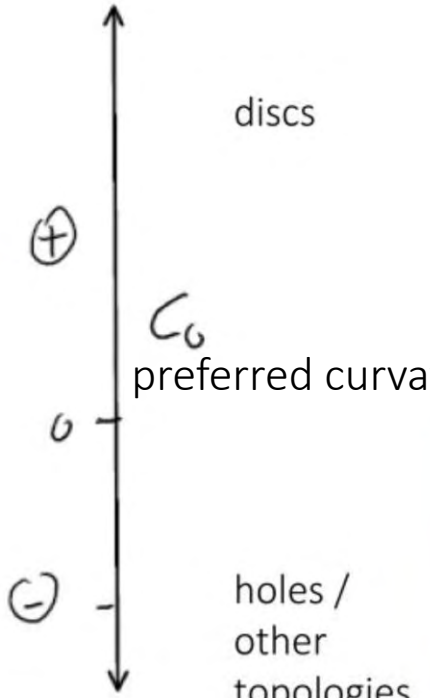
Tumor: pH ≈ 6.5 – 6.9

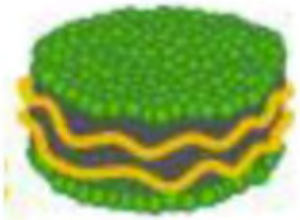
It should in principle be feasible to tune transition pH + cooperativity so that only tumor cells are affected by **disk formation or permeation**.

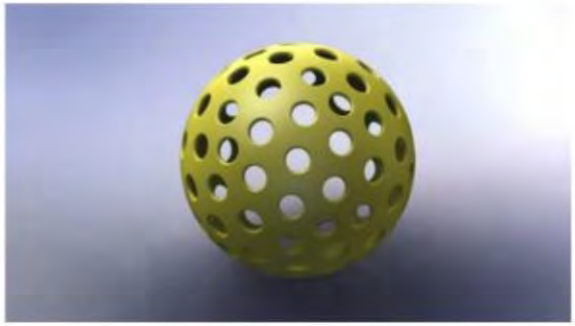
-> that would not require complex chemical targeting strategies!

Outlook:
‘Topology of membrane destruction’ & HPE architecture









Take-home

- Cooperative transitions in ('simple') HPE driven by pH consistent with MWC model for allosteric interactions.
  For details see JL Martín Robinson & WKK, PNAS 120 (2023)

- Requirement: small number of conformational states / reservoirs

- Solubilization of bilayer membranes can occur from a hydrophobic state of HPE as well as from an aqueous state.

- -> switchable materials by small ligand concentration variations

- -> potential for shattering tumor cells by HPE

THANK YOU

and:

Bas van Ravensteijn, Kanvaly Lacina, Dominique Thies, Neshat Moslehi, Wessel Custers, James Martín Robinson, Isabelle Meijer,
Mies van Steenbergen, Jan Groenewold, MBB group, Fri brainstorm group, FCC.