Herbert Wertheim College of Engineering
UNIVERSITY of FLORIDA

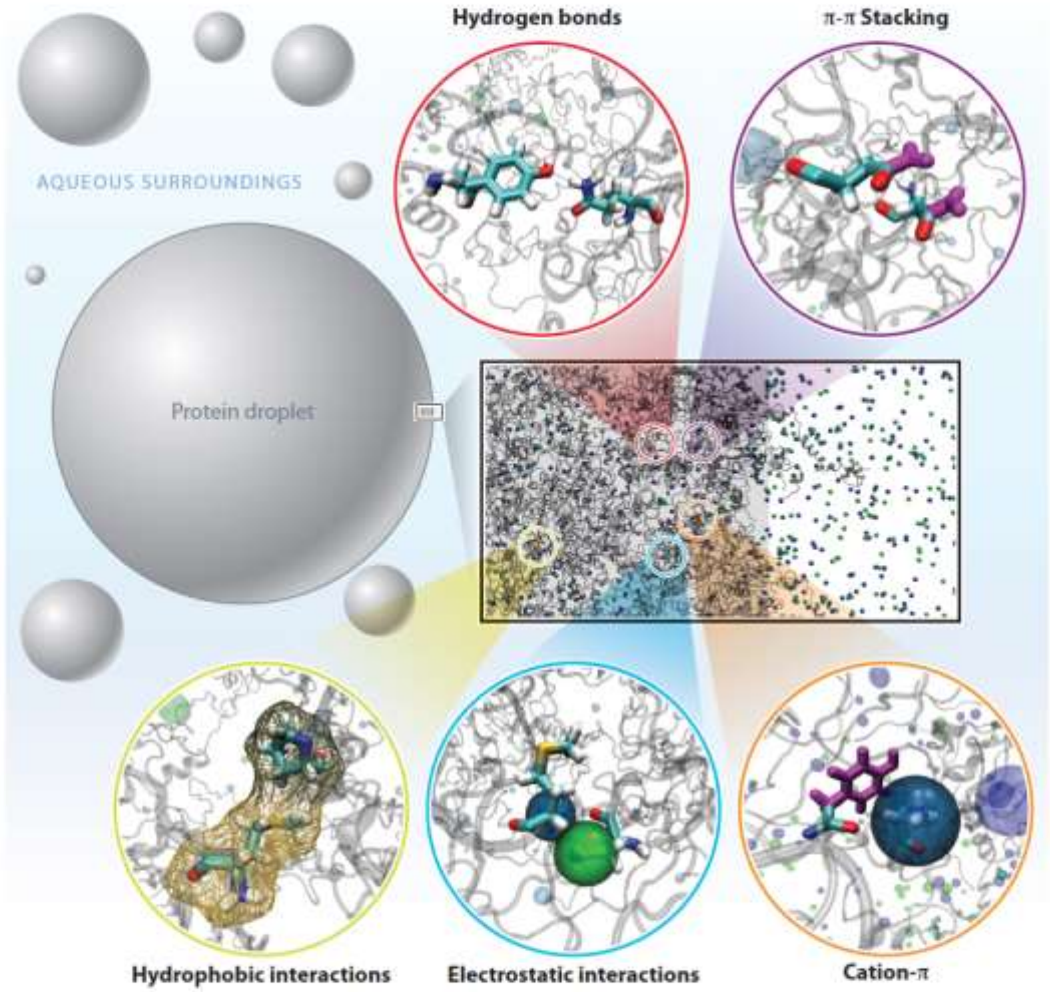
Department of Materials Science & Engineering

Charge density-driven demixing in multicomponent polyelectrolyte complex coacervates

Angelika Neitzel, Ph.D.
Rhines Rising Star Robert DeHoff Assistant Professor of Materials Science & Engineering
University of Florida

POWERING THE NEW ENGINEER TO TRANSFORM THE FUTURE

Coacervates form due to intermolecular interactions



Associative phase separation = coacervation

From Latin: *coacervāre* = to heap up, pile up

Cellular biomacromolecules (e.g., proteins, RNA) form *dense polymer droplets* = membraneless organelles (MOs)

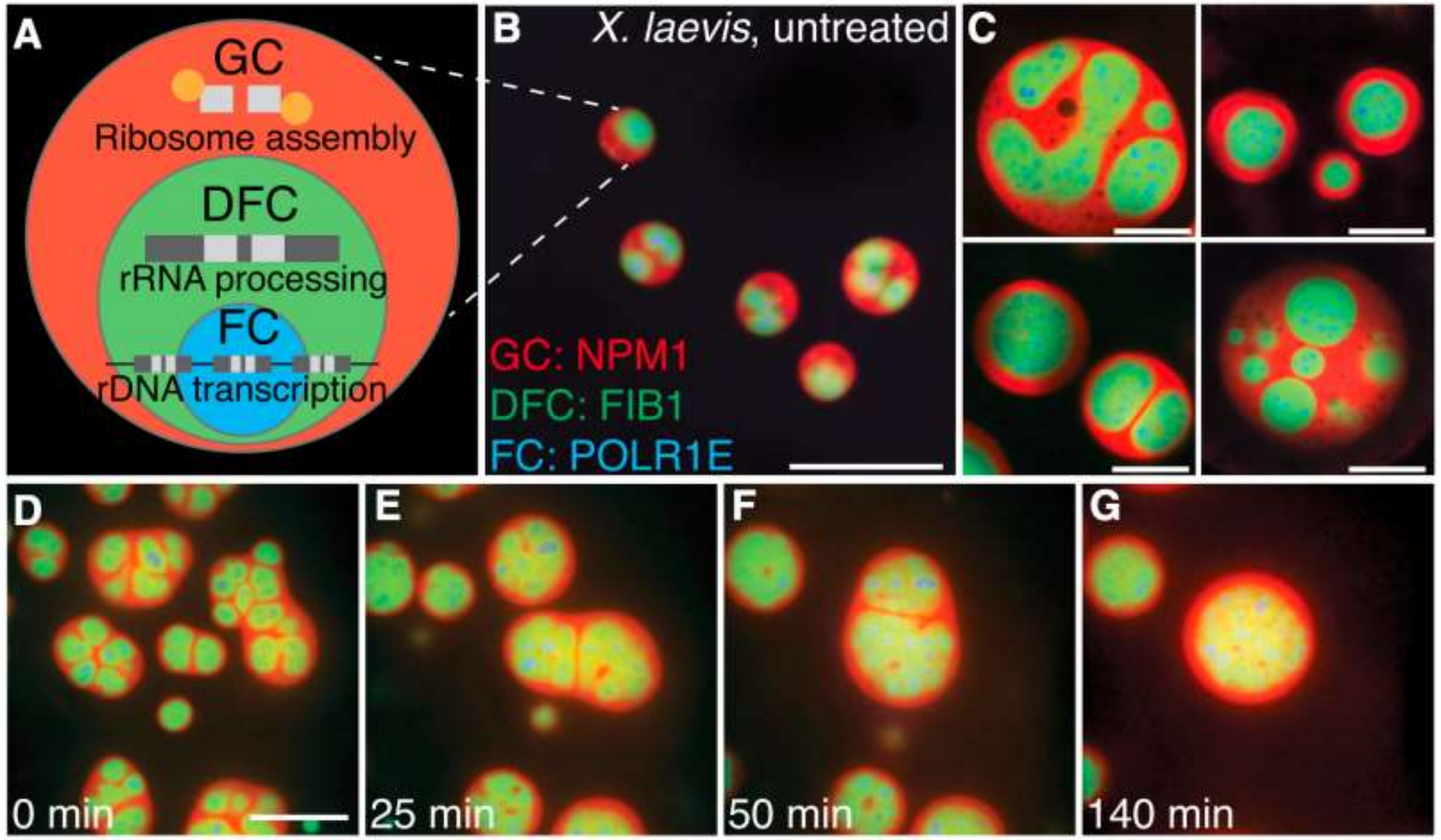
MOs compartmentalize and concentrate reagents and facilitate biochemical reactions within cells

No membrane → ability to rapidly assemble/disassemble in response to external stimuli

The internal structure of these *liquid-like* polymer phases continues to fascinate scientists

Dignon, D.L.; Best, R.B.; Mittal, J. *Annu. Rev. Phys. Chem.* **2020**, *71*, 53.

Not even 10 years ago it was shown that MOs are not homogeneous



MOs are composed of coexisting phases

Ribosome synthesis and assembly suggested to take place in nucleolar subcompartments

Compartmentalization of molecules and processes

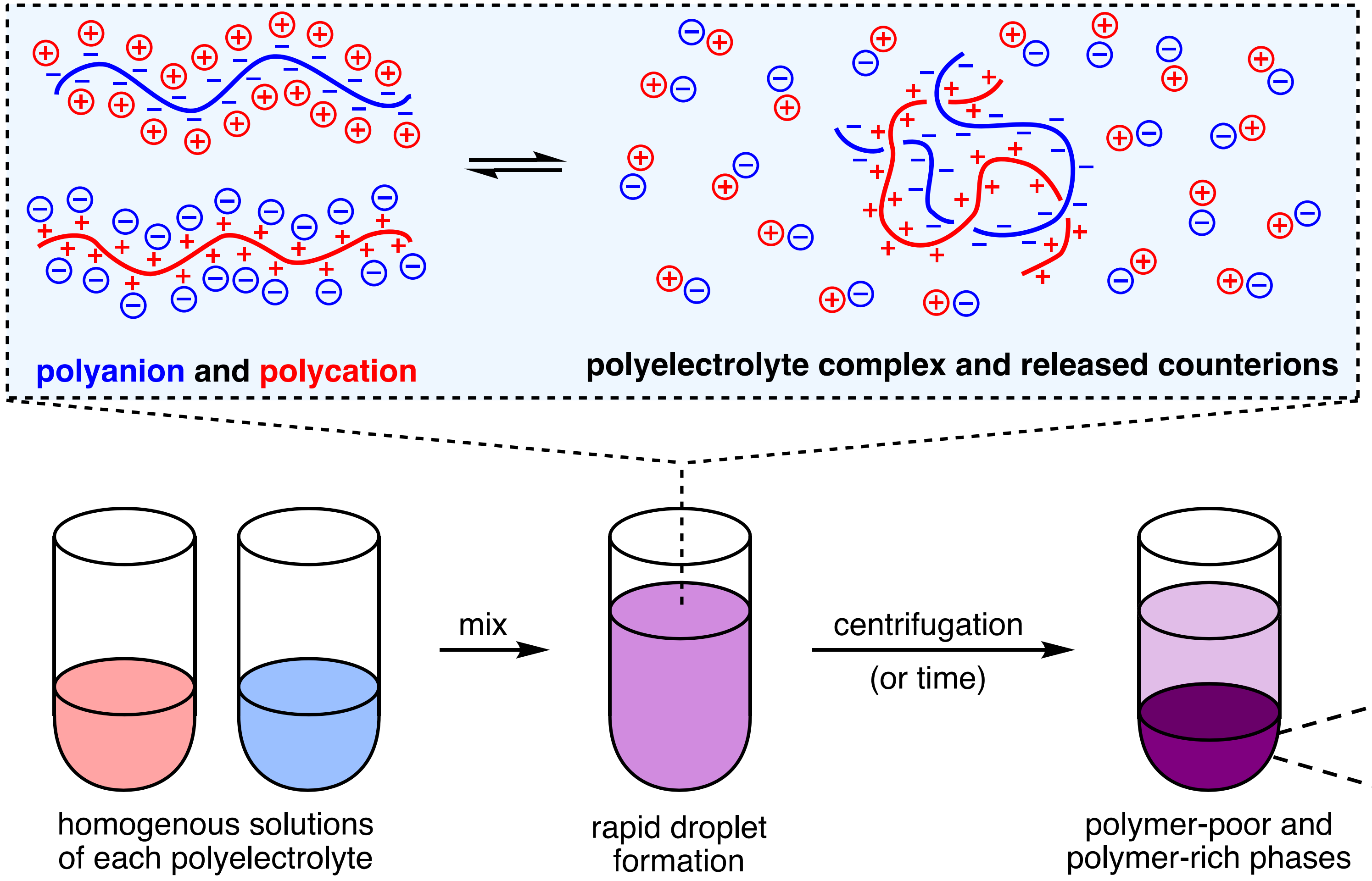
MOs can contain 100s of proteins and RNAs

Usually enriched in proteins with intrinsically disordered regions → ”basic polymer structure"

Simpler models for MOs can help us better understand these interesting structures

Feric et al. *Cell* **2016**, *165*, 1686.

Coacervation of oppositely charged polyelectrolytes serves as MO model



Association of polyelectrolytes (PEs) results in **liquid-liquid or solid-liquid phase separation** (**LLPS** and **SLPS**, respectively)

Polymer-rich phase = **polyelectrolyte complex (PEC)**
Polymer-poor phase = **supernatant**

Entropically-driven process predominantly due to the **restructuring of water molecules**

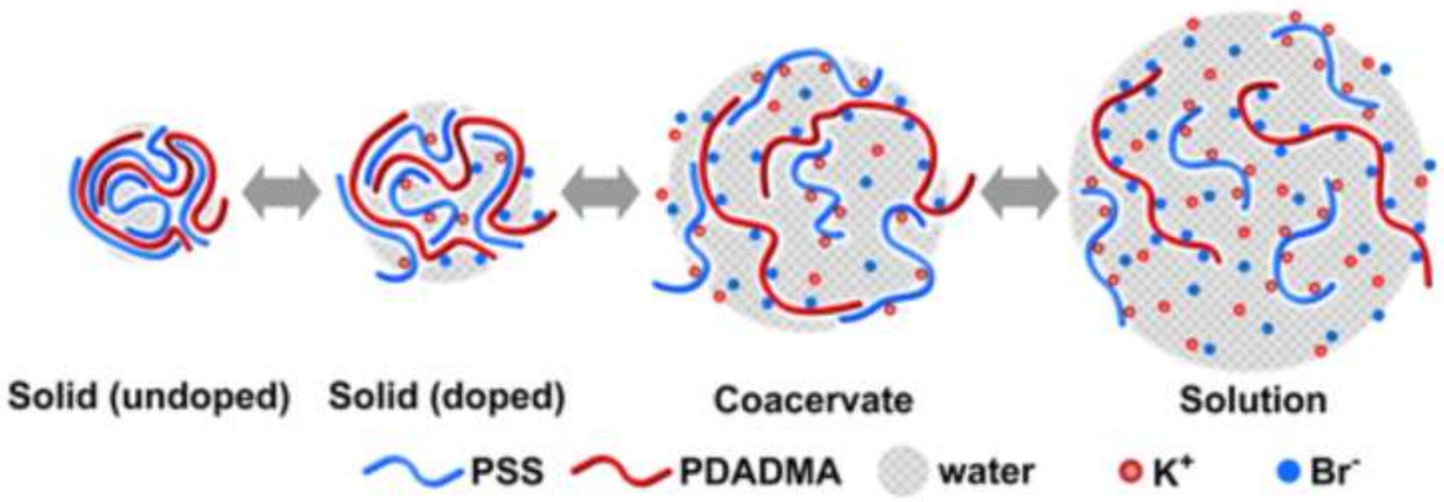
Properties of interest:
Density, interfacial tension, salt resistance

Electrostatic interactions are prevalent in biology—**other noncovalent interactions still matter**

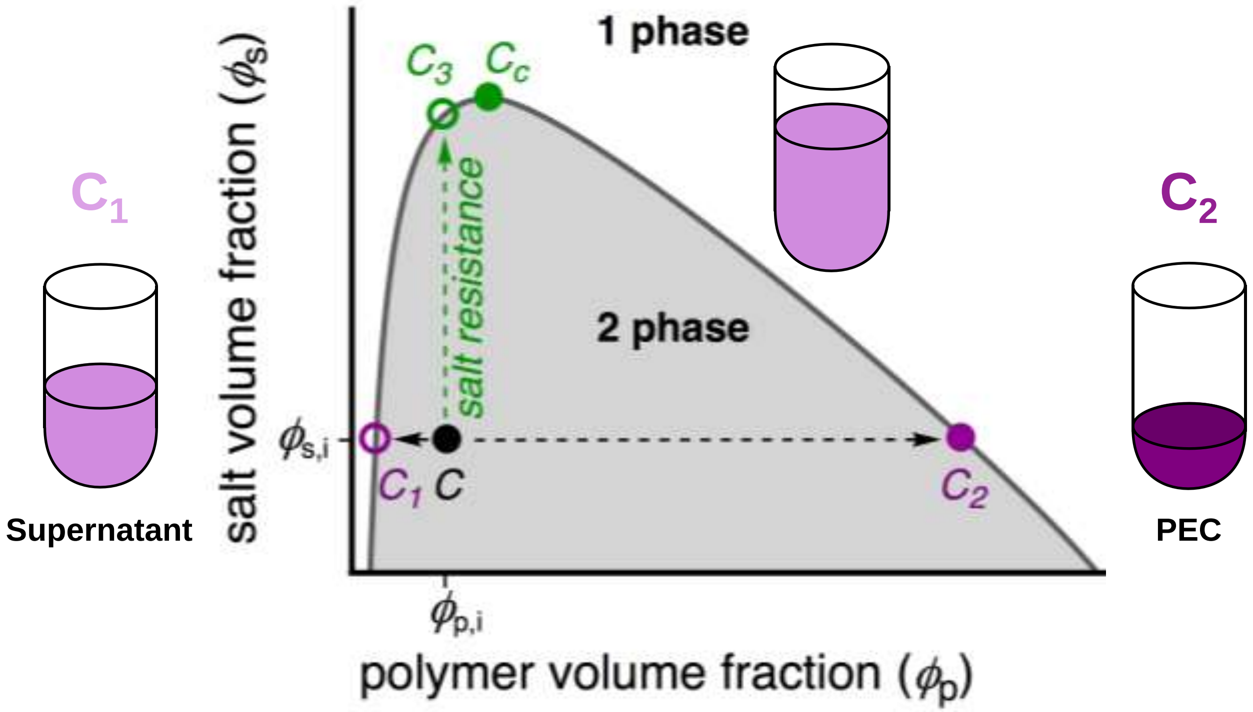
Neitzel, A.E.; De Hoe, G.X.; Tirrell, M. V. *Curr. Opin. Solid State Mater. Sci.* **2021**, *25*, 100897. Chen, S.; Wang, Z.-G. *Proc. Natl. Acad. Sci.* **2022**, *119*, e2209975119.

PECs are also referred to as “saloplastics”

Salt *screens* charge correlations → weakens attractions



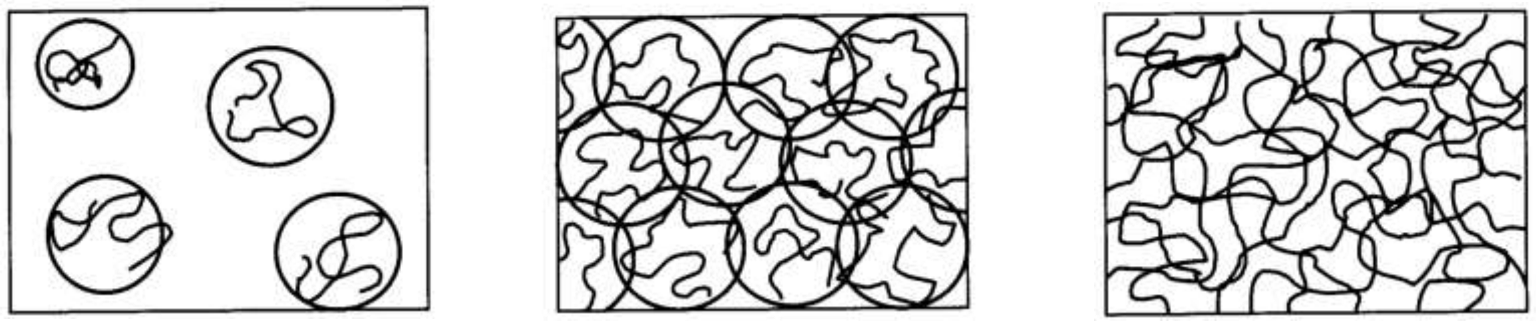
Generic phase diagram depicting coexisting phases



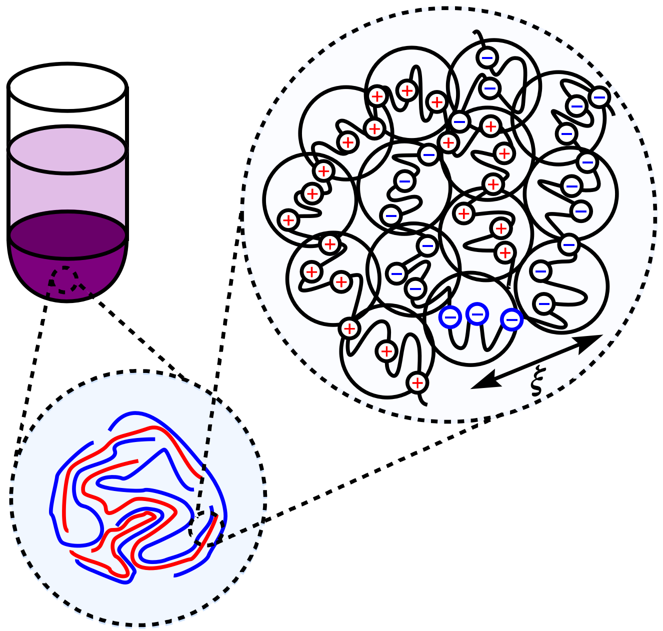
Thermoplastics are processed using temperature, saloplastics are processed using salt

Neitzel, A.E.; De Hoe, G.X.; Tirrell, M. V. *Curr. Opin. Solid State Mater. Sci.* **2021**, *25*, 100897. Wang, Q.; Schlenoff, J. B. *Macromolecules* **2014**, *47*, 3108–3116.

Theorists describe liquid PEC phase as melt of “electrostatic blobs”



Dilute $c < c^*$ | Overlap $c = c^*$ | Semidilute $c > c^*$



Semidilute solution = [polymer] above the overlap concentration c*

Overlapping blobs with correlation length ξ. Chain inside blob unaware of chains outside

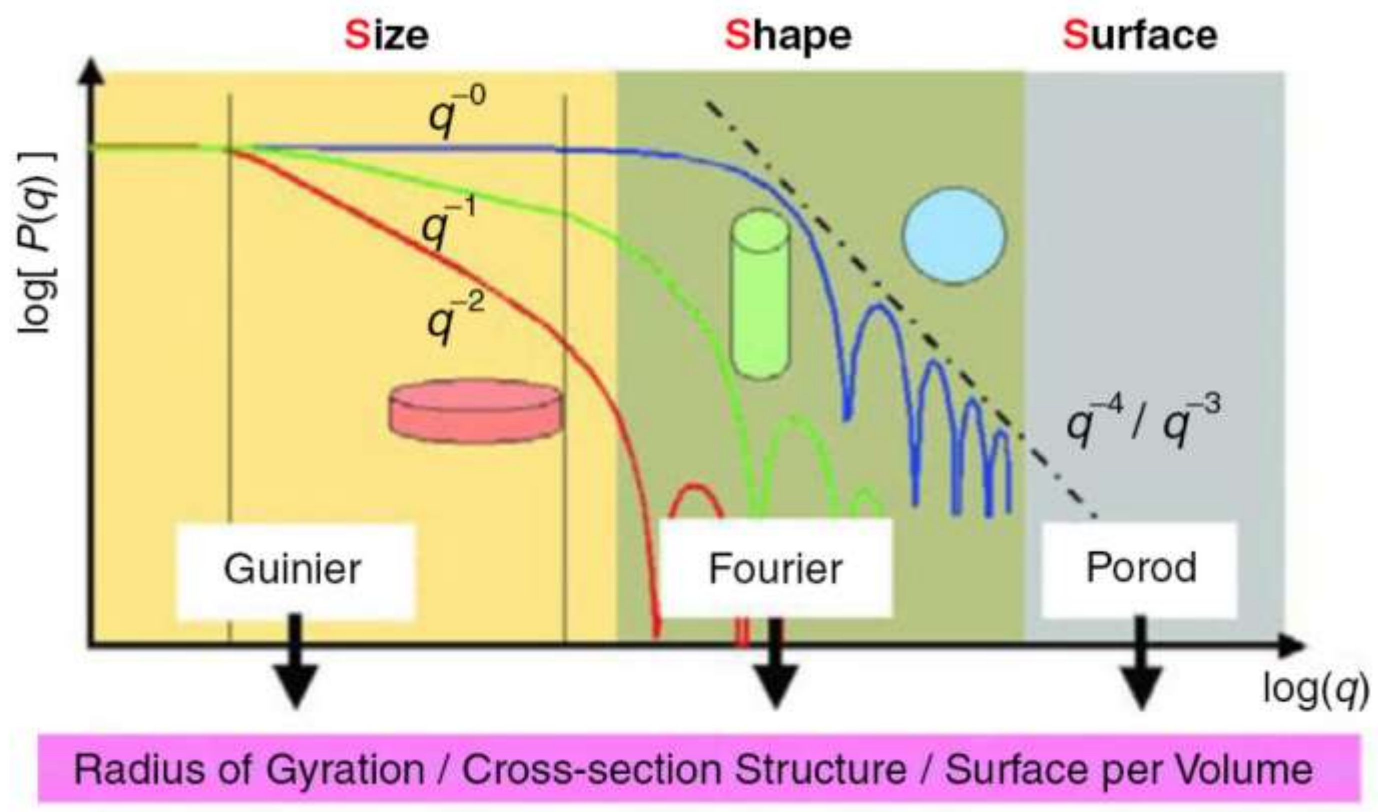
Chain conformation in blob scales as: $R_g \sim N^{\nu}$, where $\nu = \frac{1}{3}$ for bad, $\frac{1}{2}$ for θ, and $\frac{3}{5}$ for good solvent

Charge correlations between blobs is hypothesized to be the glue that holds PECs together

Rubinstein, M.; Colby, R. *Polymer Physics*; Oxford University Press: Oxford, 2003.

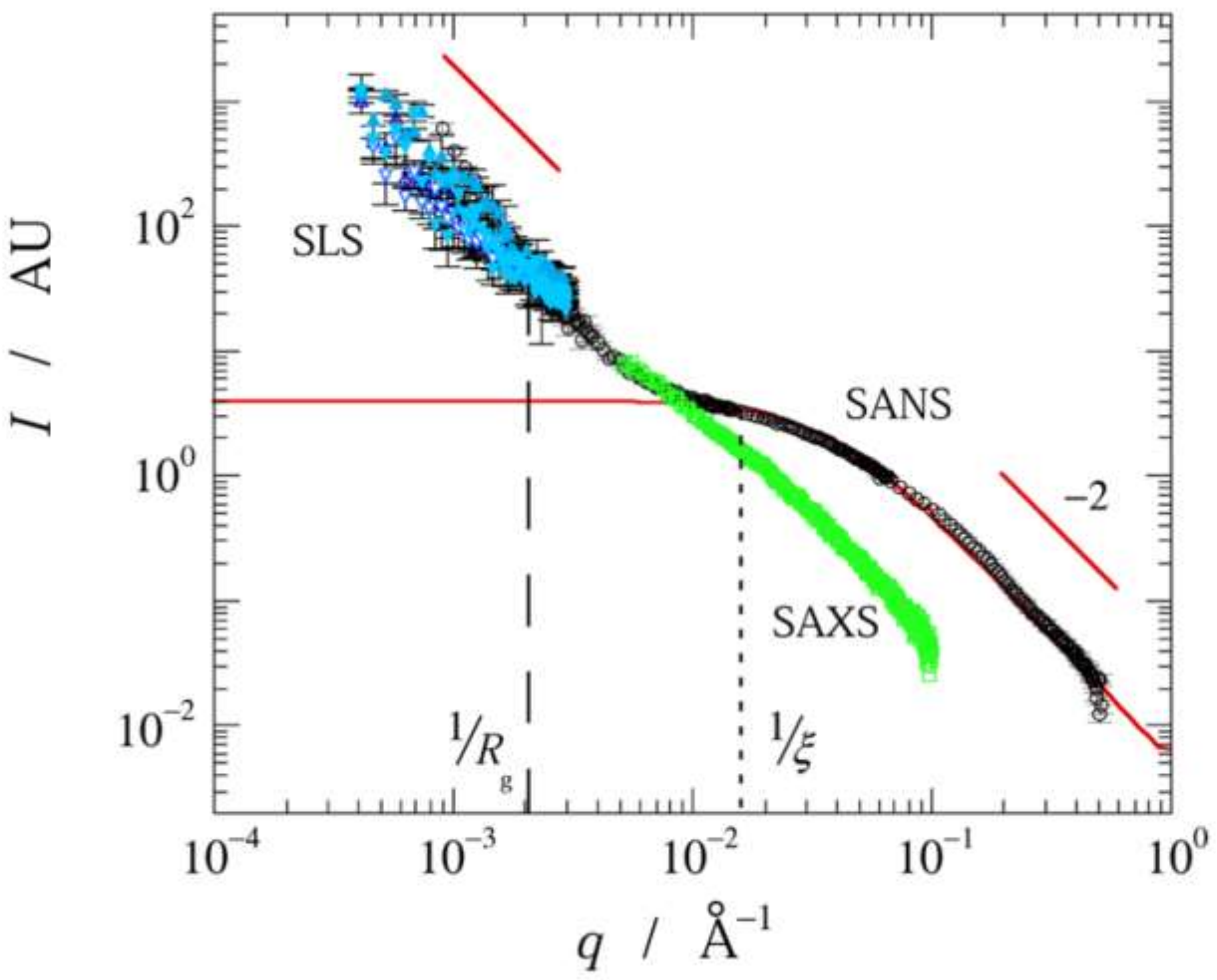
PECs structurally similar to neutral semidilute polymer solution

Small Angle Scattering (SAS) profiles for polymers



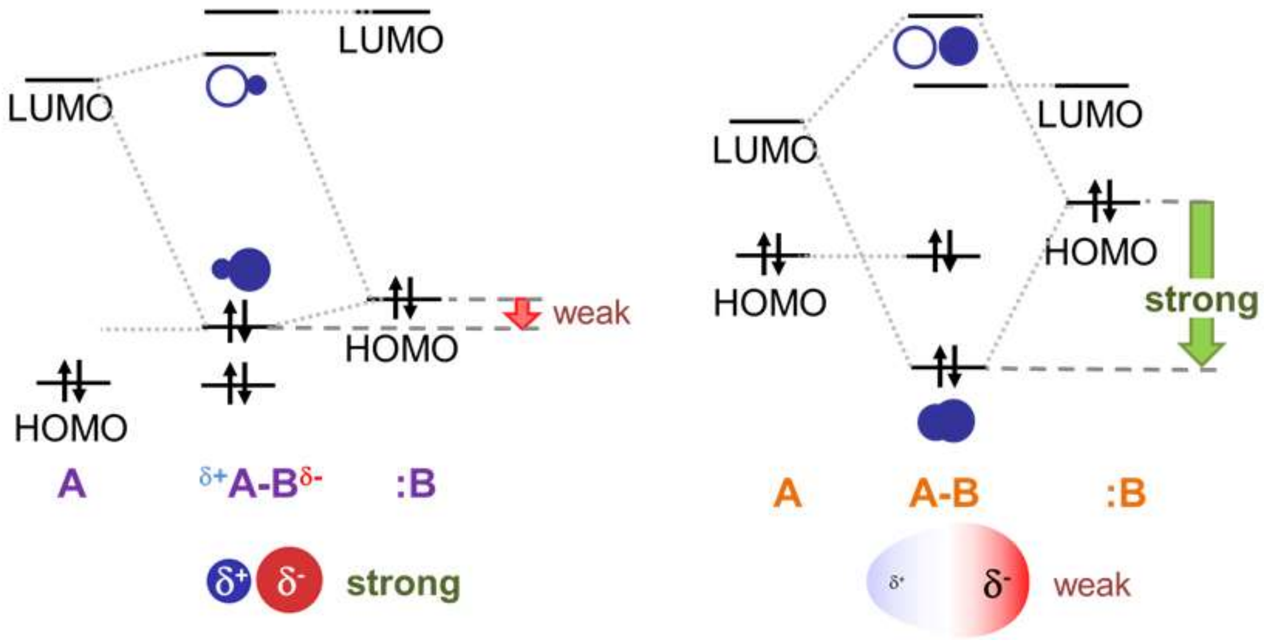
Ornstein-Zernike form: $G_{tot}(q) = \frac{G_{tot}(q=0)}{1+(q\xi_E)^{1/\nu}}$

PEC looks like blobs following random walk



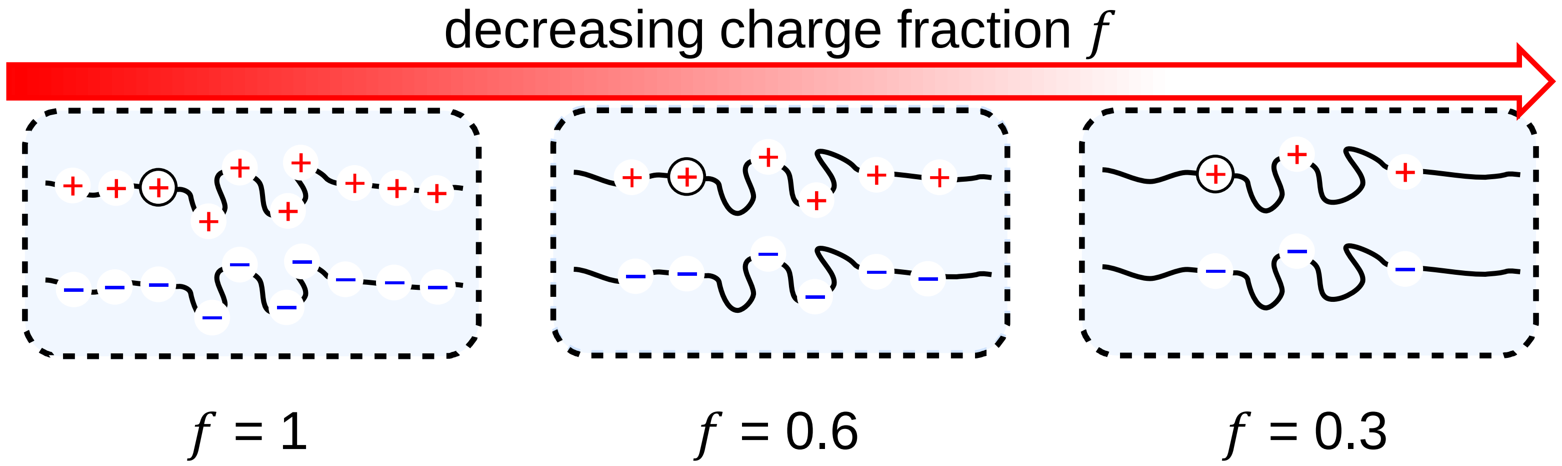
What is the relationship between PE charge fraction and PEC density?

There is a difference between pairs of non-polarizable and polarizable ion pairs



Cannot establish **universal** trends with salt!

Decrease number of charges per polymer chain instead to find universal relationship?

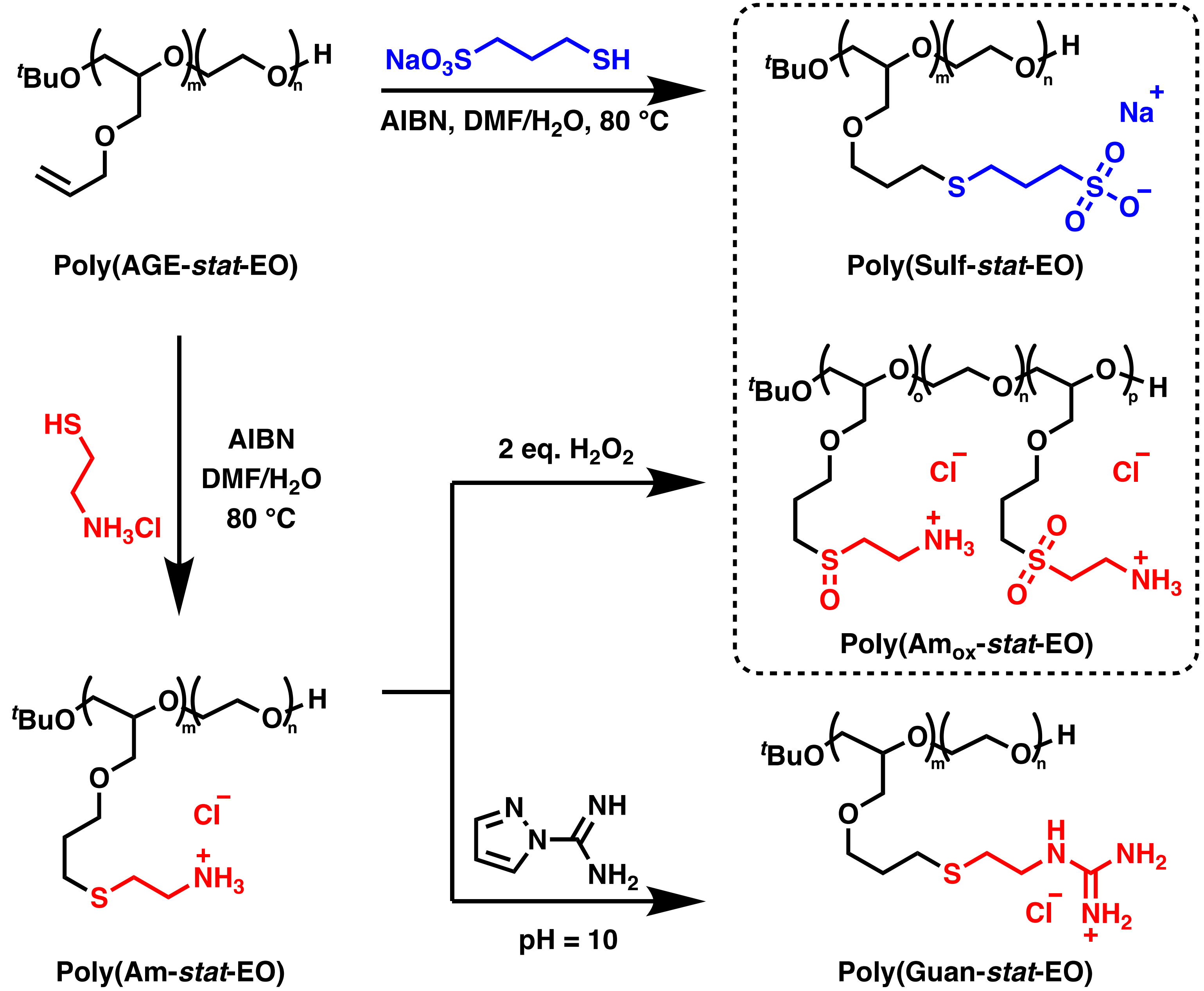


$$\phi \approx \frac{g}{\xi^3} \sim (uf^2)^{\frac{(3\nu-1)}{(2-\nu)}}$$

Does the density of the PEC phase (ϕ) change with charge fraction (f) as predicted by theory?

Rumyantsev, A. M.; Zhulina, E.B.; Borisov, O. V. *Macromolecules* **2018**, *51*, 3788.

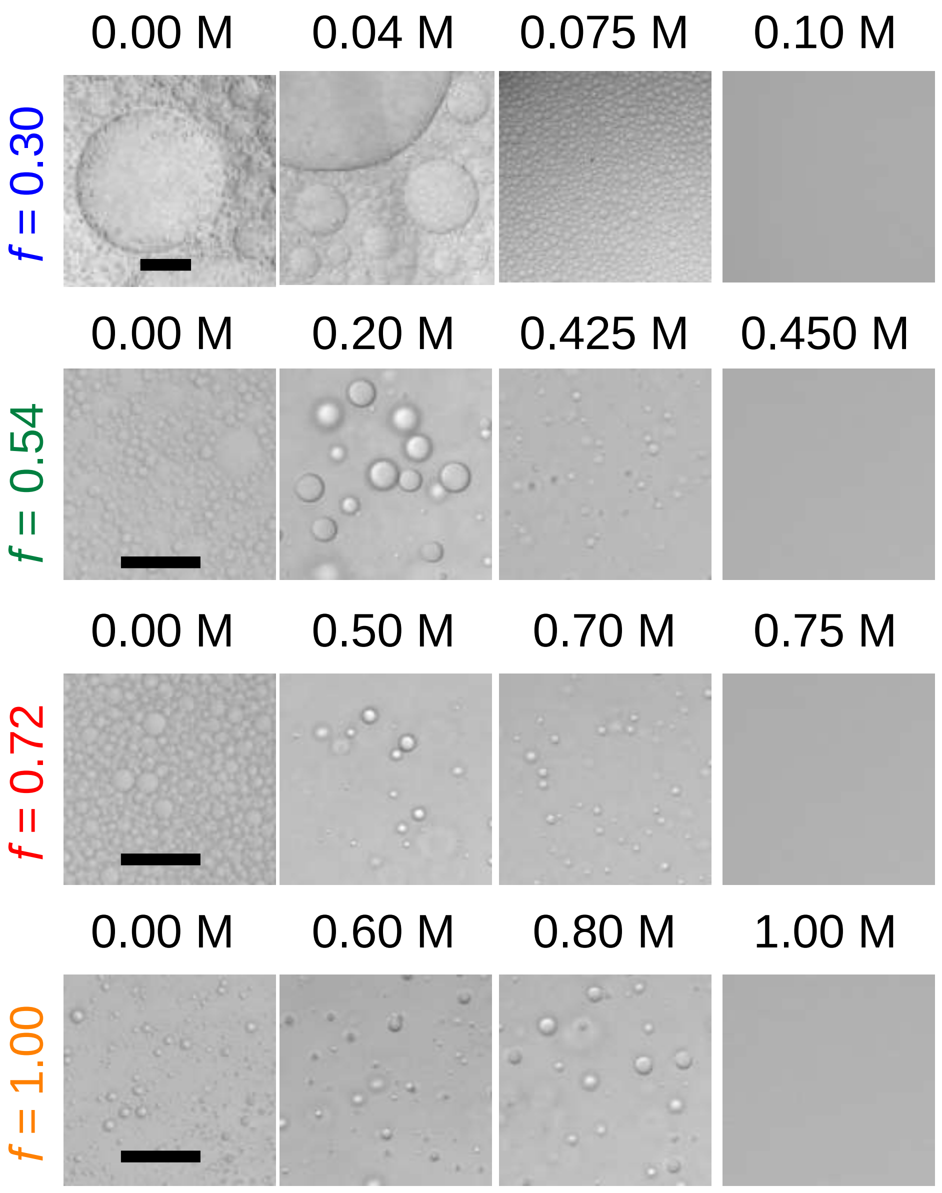
Modular polyether platform meets required design parameters

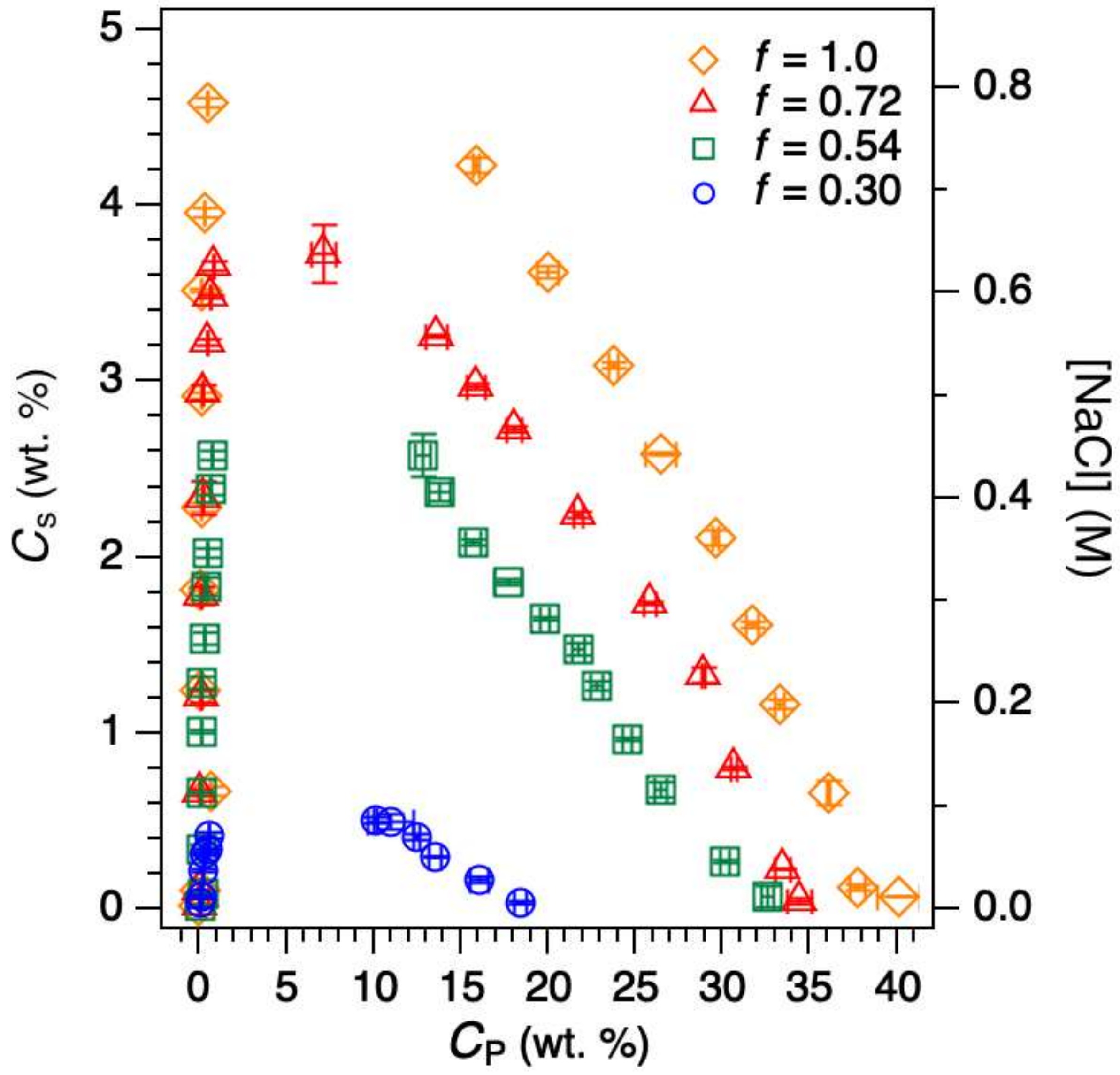


- ✓ Polyelectrolytes soluble in water and aqueous salt solutions at all f values
- ✓ Near ideally random microstructure
- ✓ Homologous polycations and polyanions (i.e., $f_+ = f_-$, identical N_n, $Đ$, and sequence)
- ✓ Other non-covalent interactions minimized (oxidation reduces hydrophobic interactions)

Probe phase behavior for f = 0.1–1.0

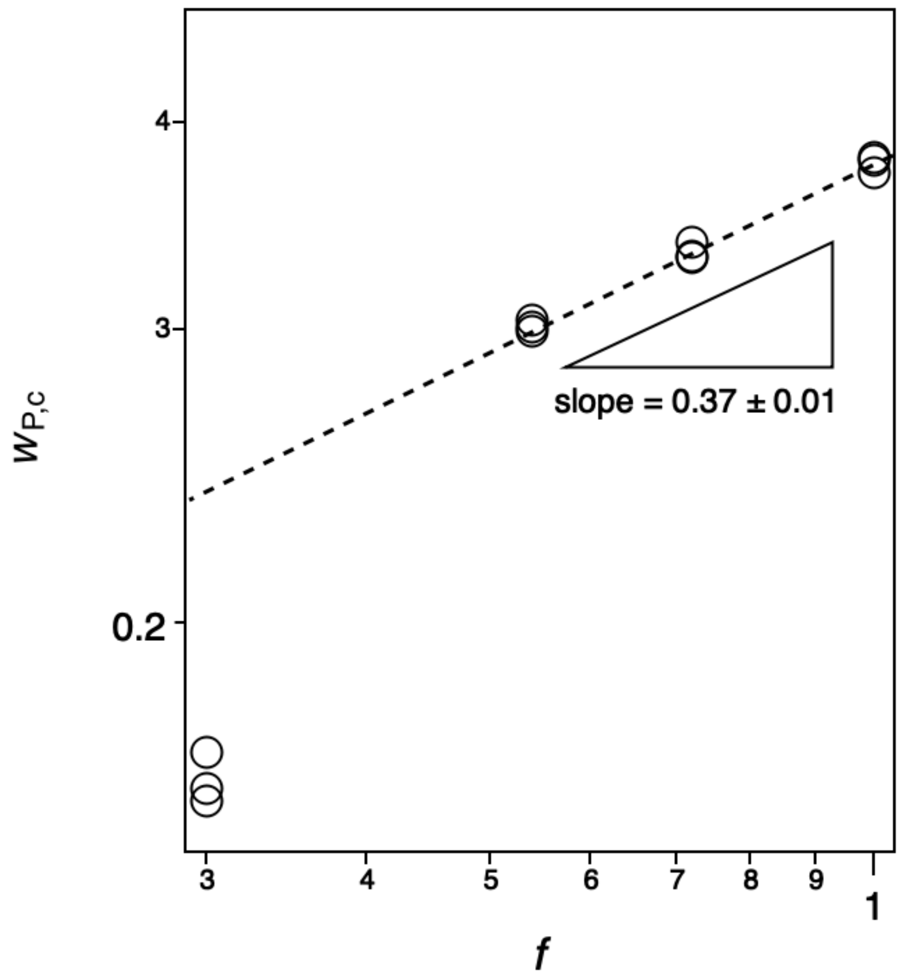
Single phase PECs binodal envelope shrinks with decreasing *f*



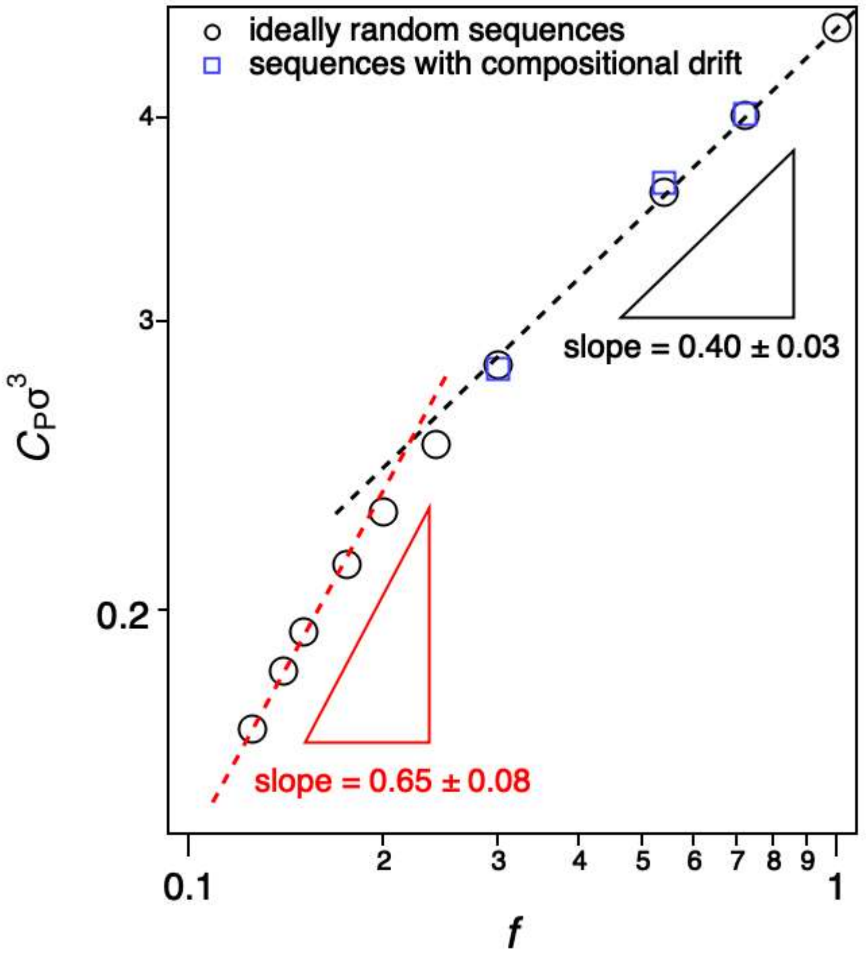


Agreement between experiment & simulations but…

Experiment



Theory & Simulations



From scaling analysis in the regime of $f \ll 1$:

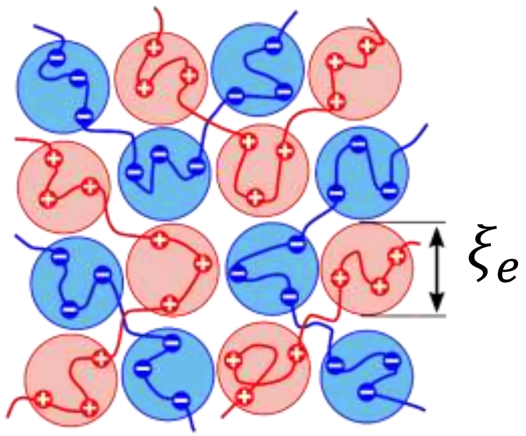
$$\phi \sim (f^2)^{\frac{(3\nu-1)}{(2-\nu)}}$$

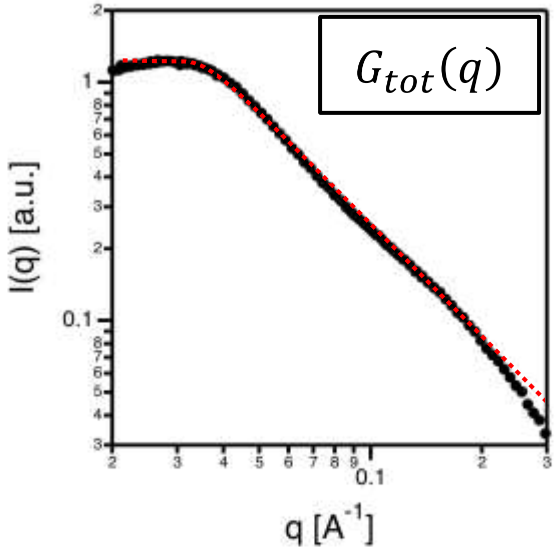
For theta solvent $\nu = \frac{1}{2}$, then

$$\phi \sim f^{2/3}$$

Theory is a more appropriate predictor at very low f → we need longer chains!

SAS with neutrons reveals the length scale of Coulomb repulsions

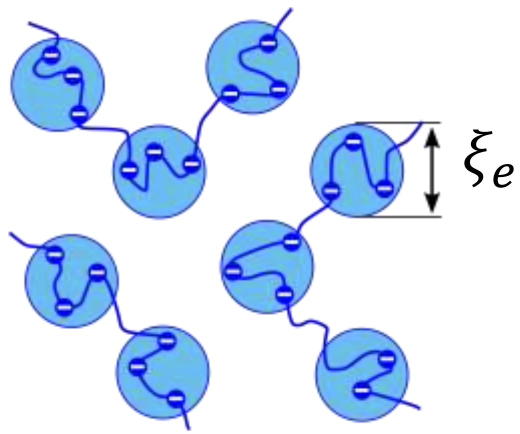


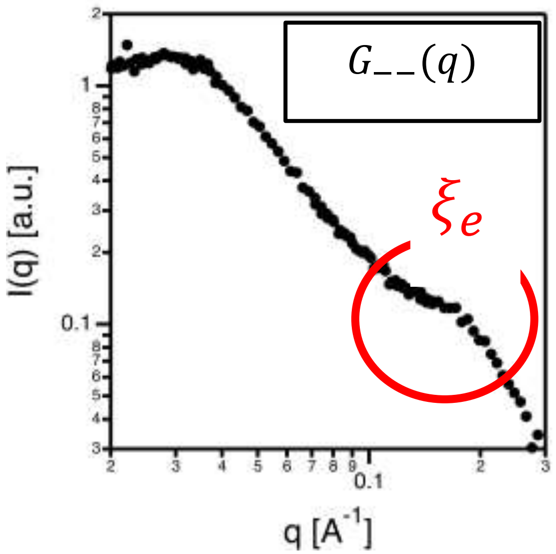


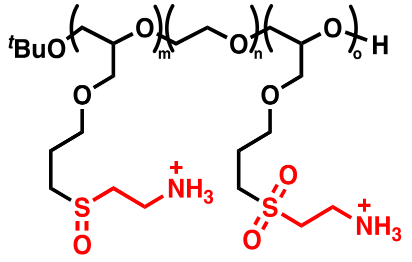
Deuterate polycations

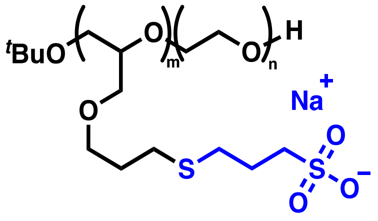
Contrast-match to solvent

Scatter from polyanions

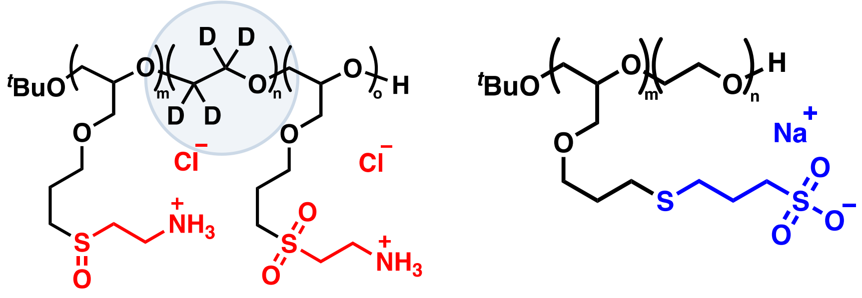








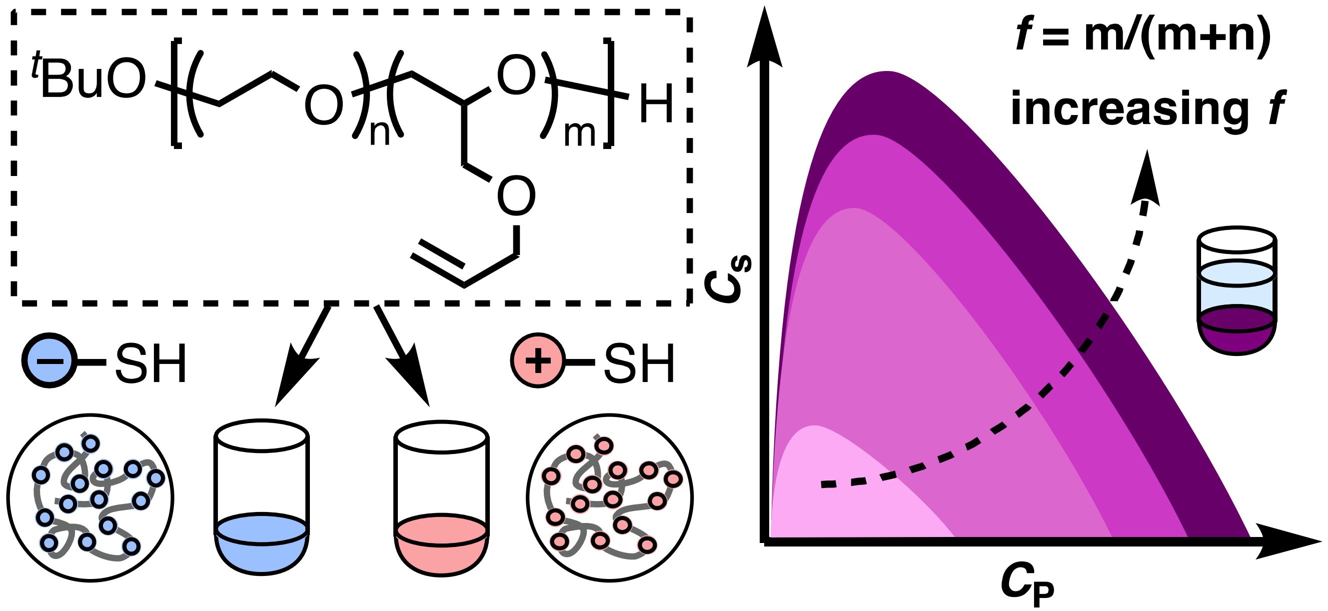
$N_n = 186; f = 0.30, Đ = 1.15$



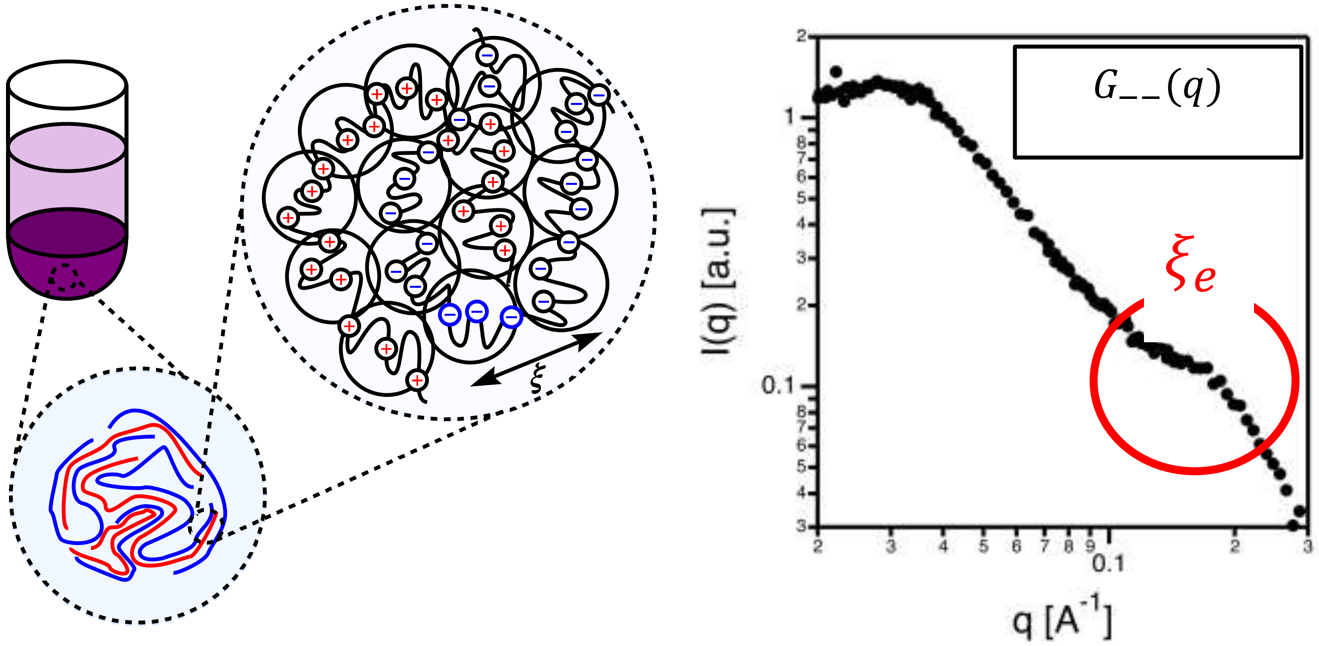
$N_n = 206;\ f = 0.31;\ Đ = 1.07$

Fang, Y. N.; Rumyantsev, A. M.; Neitzel, A. E.; Liang, H.; Heller, W. T.; Nealey, P. F.; Tirrell, M. V.; de Pablo, J. J. *Proc. Natl. Acad. Sci.* **2023**, *120*, e2302151120.

Phase behavior and structure of single phase PECs resolved



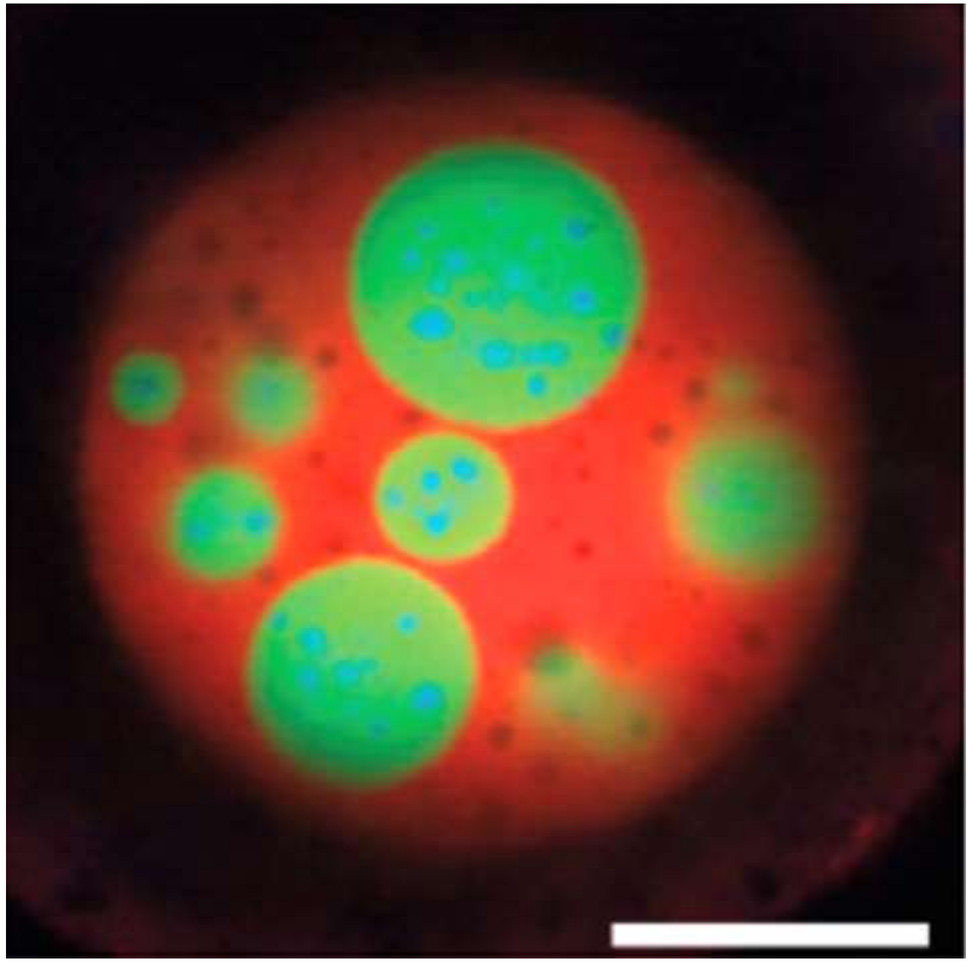
Neitzel, A. E.; Fang, Y. N.; Yu, B.; Rumyantsev, A. R.; de Pablo, J. J.; Tirrell, M. V. *Macromolecules* **2021**, *54*, 6878−6890.

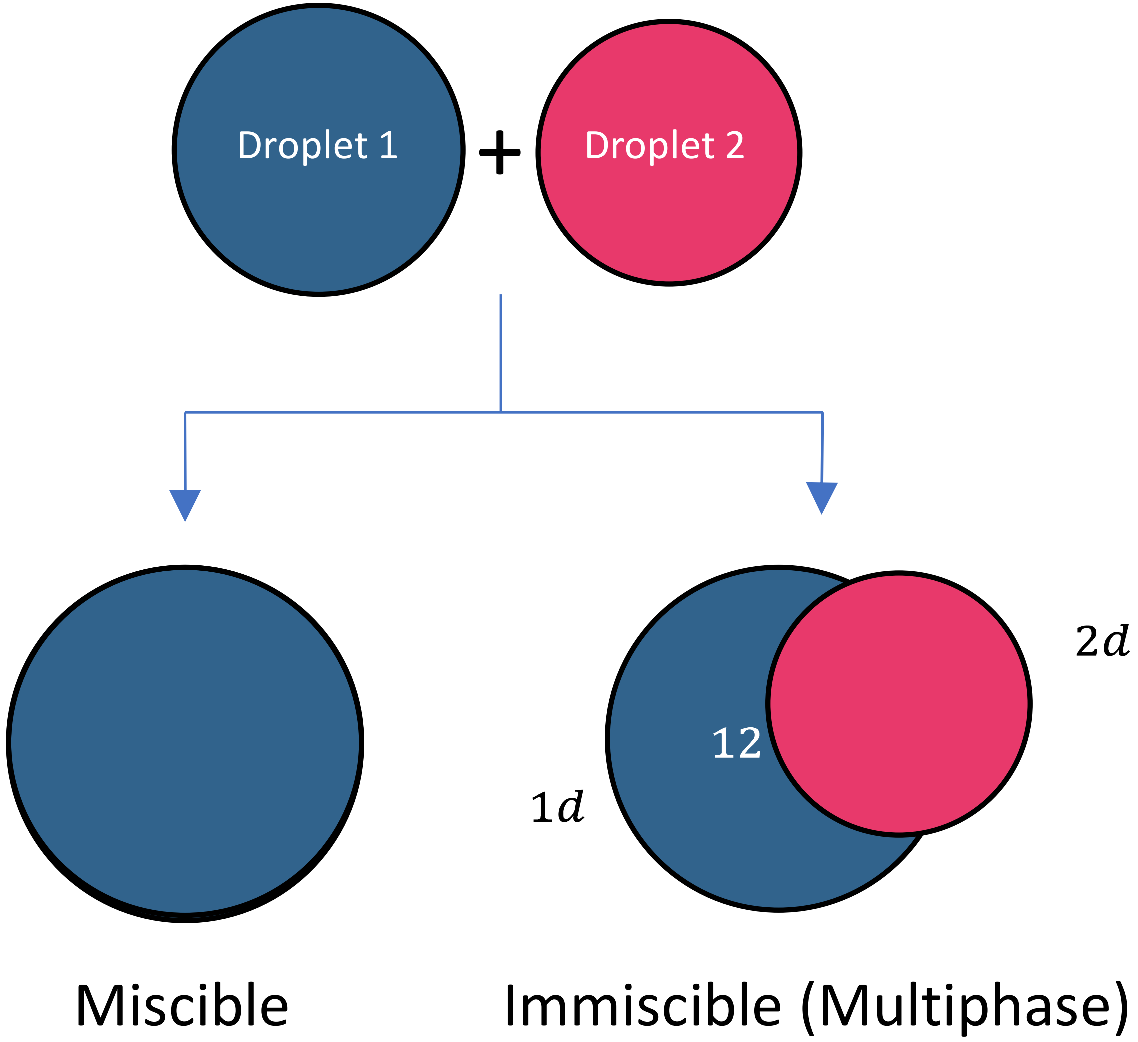


Fang, Y. N.; Rumyantsev, A. R.; **Neitzel, A. E.**; Liang, H.; Heller, W. T.; Tirrell, M. V.; de Pablo, J. J.; *Proc. Natl. Acad. Sci.* **2023**, *120*, e2302151120.

With these data in hand, we can tackle more complicated multicomponent PEC systems

Nested PEC structures arise due to immiscibility of two or more PECs

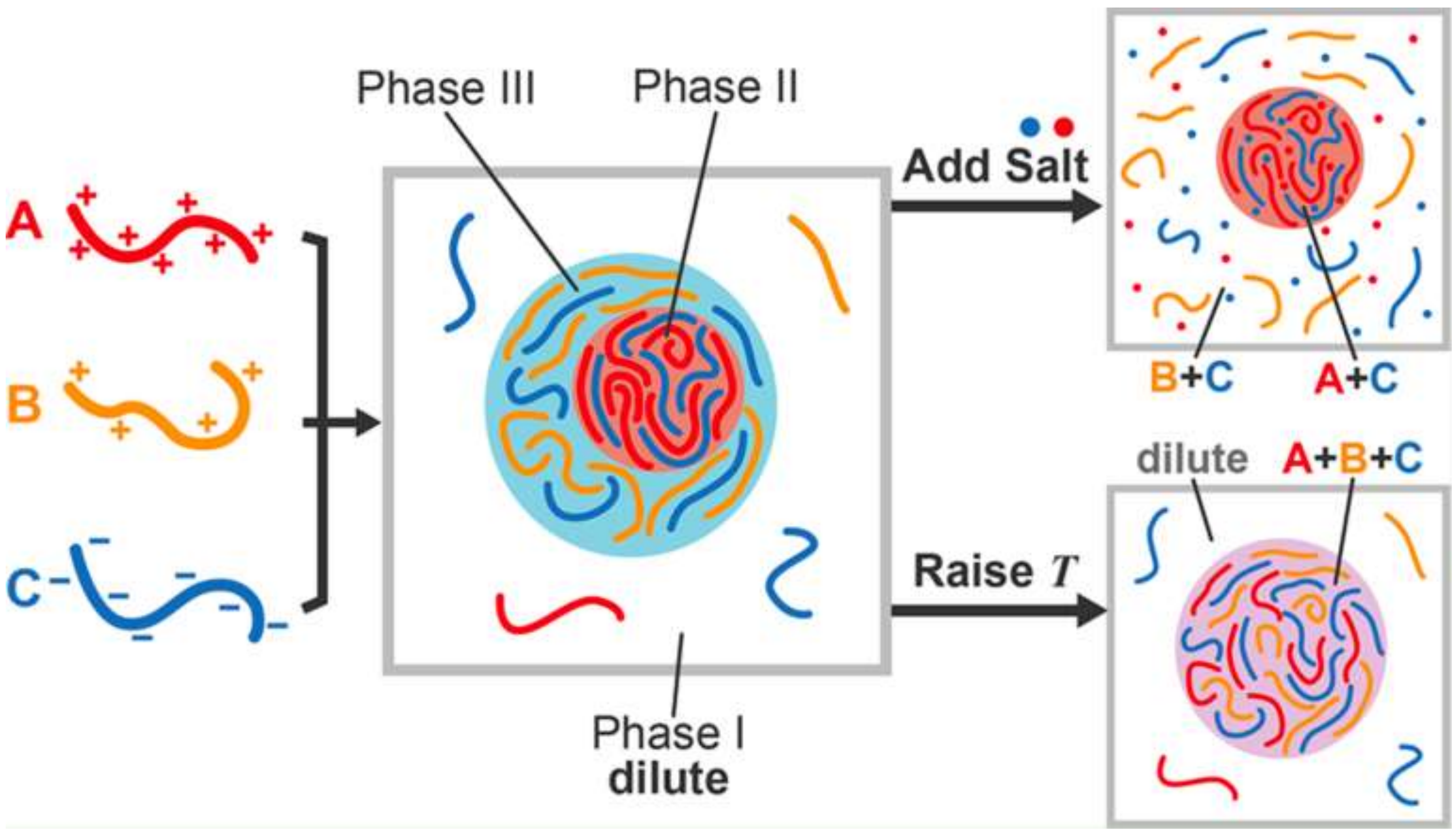




Generally, mixing ≥ 2 dissimilar polymers results in immiscibility; **is this surprising/interesting?**

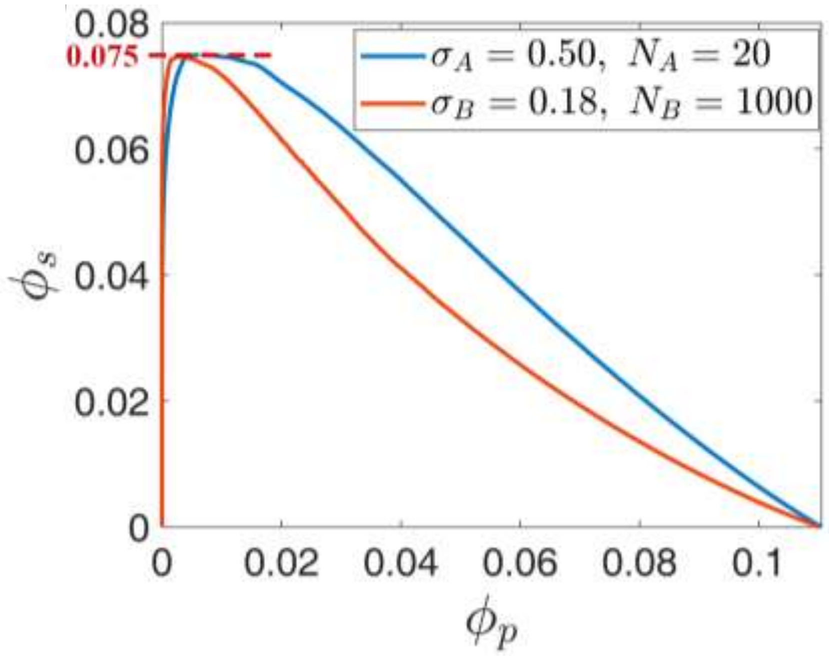
Chen et al. *ACS Macro Lett.* **2021**, *10*, 1041.

Theory predicts that charge density differences *alone* can drive demixing



$$\chi'_{\mathrm{eff},\,ij} = \boxed{\chi_{ij}} + \frac{1}{2}\left(\sigma_i^2 - \sigma_j^2\right)^2 \int \mathrm{d}\mathbf{q}\, a(\mathbf{q})\, g^2(\mathbf{q})$$

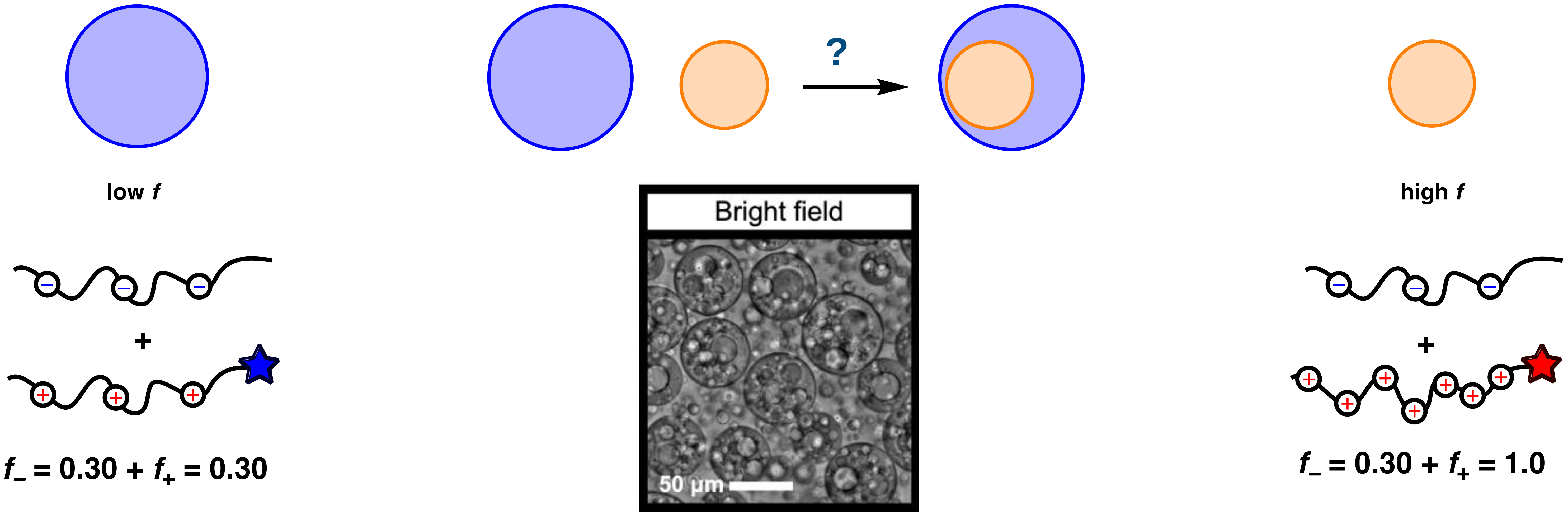
Flory-Huggins — electrostatic contribution



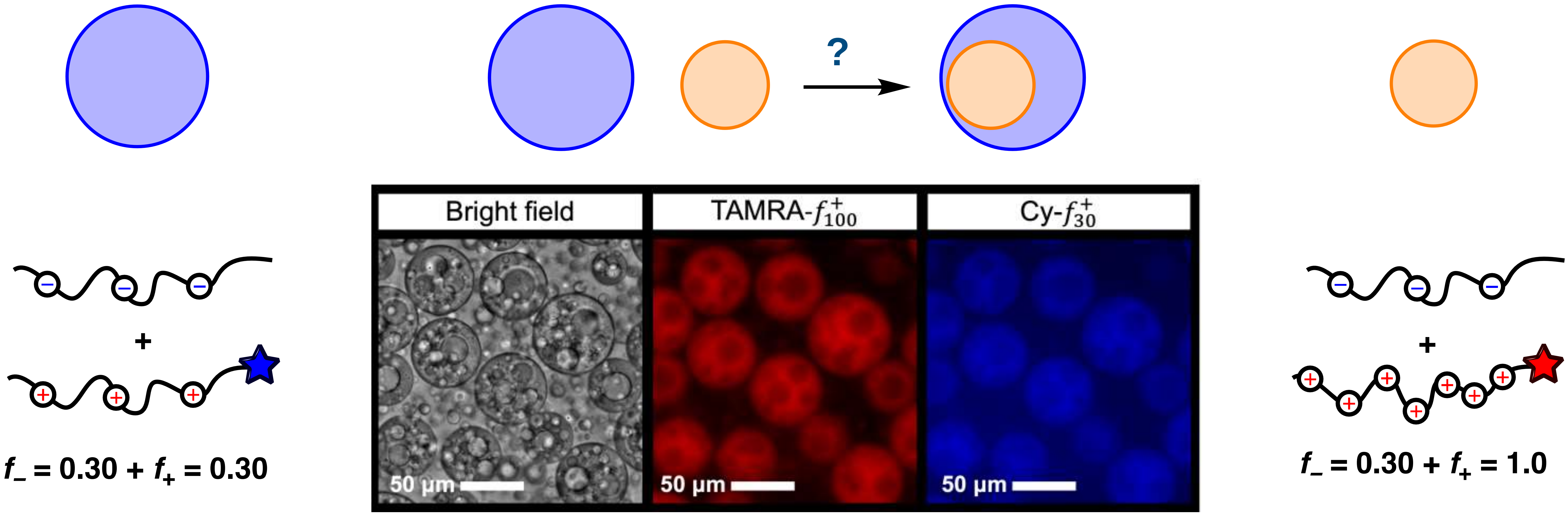
Interesting AND platform of variable f polyelectrolytes is ideally suited to corroborate this!

Chen et al. *ACS Macro Lett.* **2021**, *10*, 1041.

Trace dye-labeled polycations introduced to probe demixing with 3 PEs

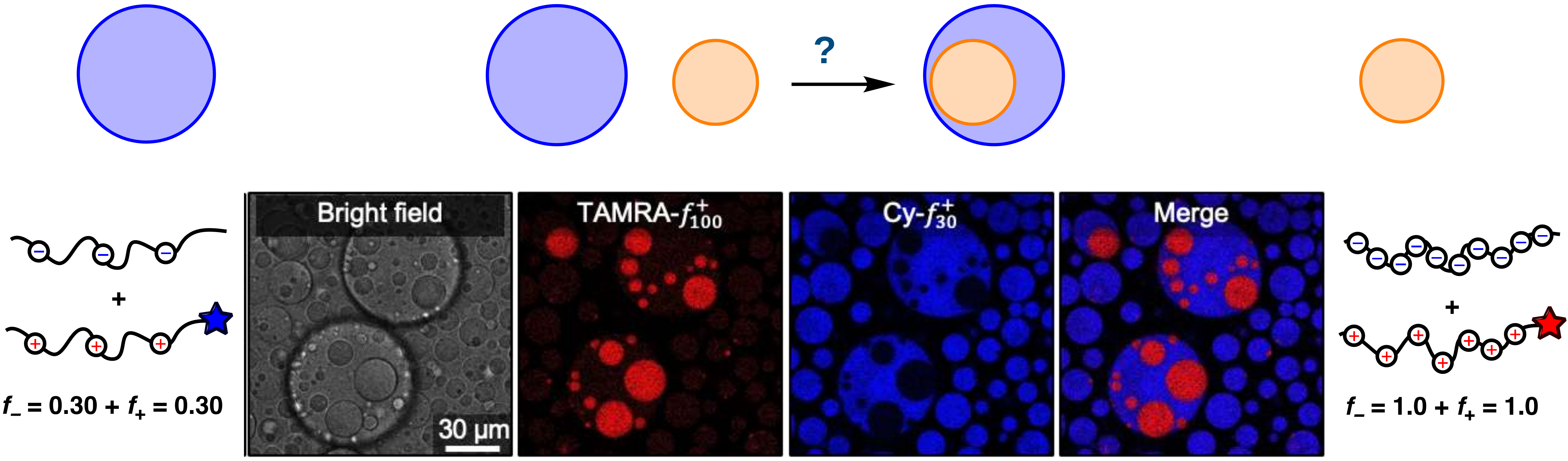


Trace dye-labeled polycations introduced to probe demixing with 3 PEs



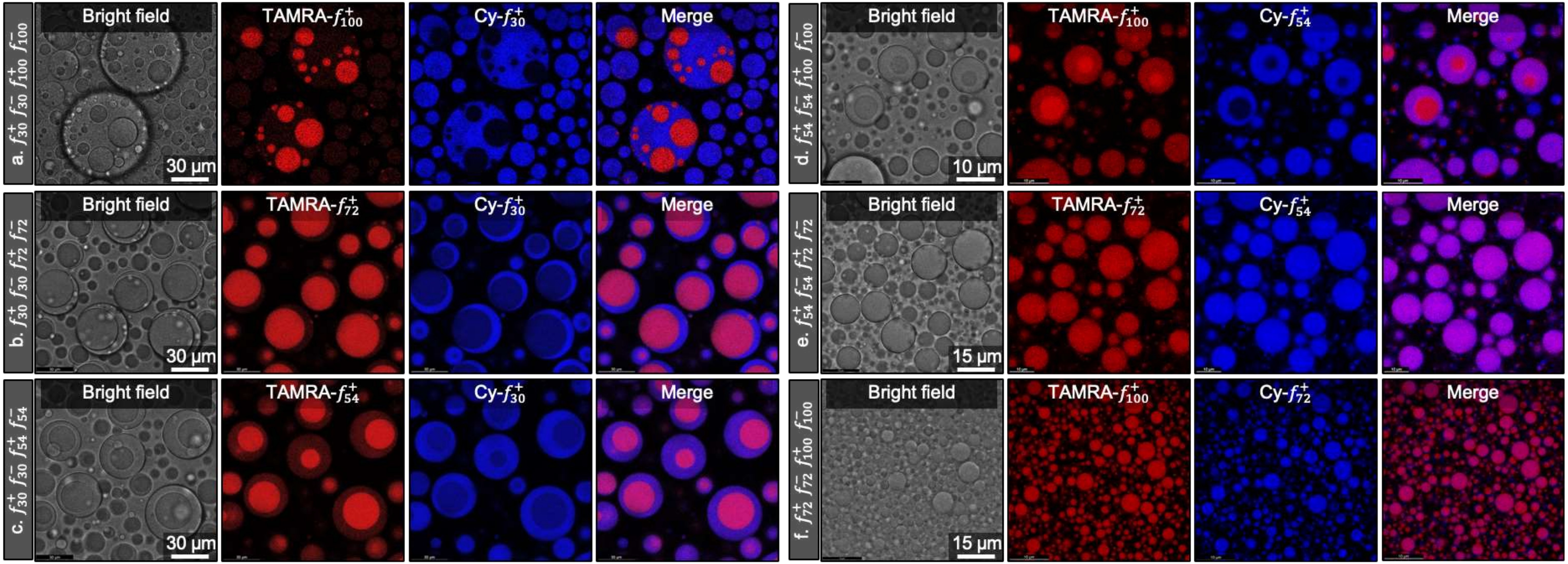
Vesicles rather than multiphase polyelectrolyte complex coacervates observed

Mixture of PE pairs mismatched in charge density form nested structures

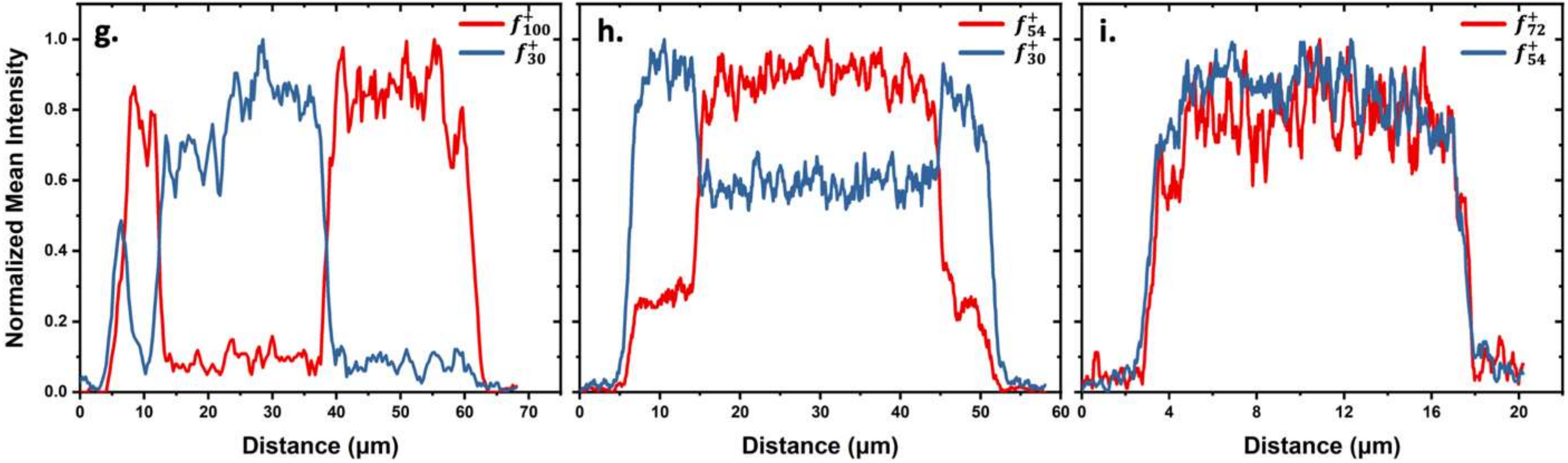


At the highest achievable charge density difference with our system little intermixing observed

Four of the six symmetric PEC pairs exhibit demixing

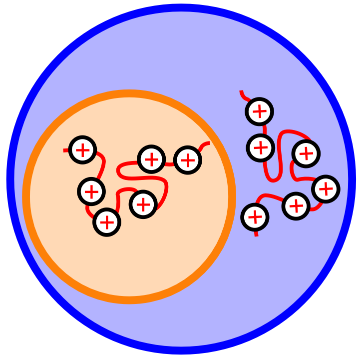


Line intensity profiles quantify intermixing of polycations in droplets



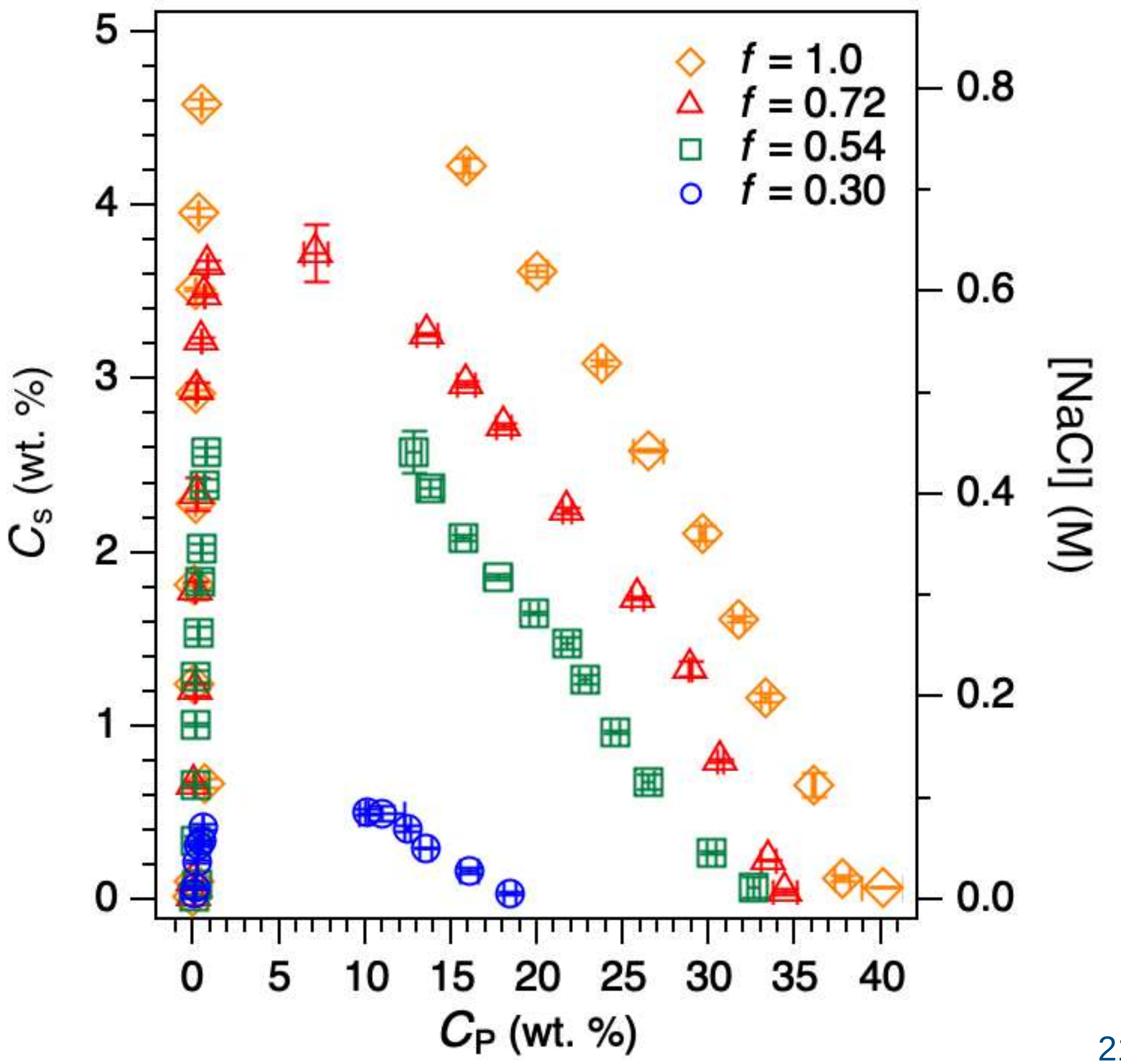
Higher Δf results in little intermixing of polycation between inner and outer droplets

Polycation partition coefficients track with PEC density differences

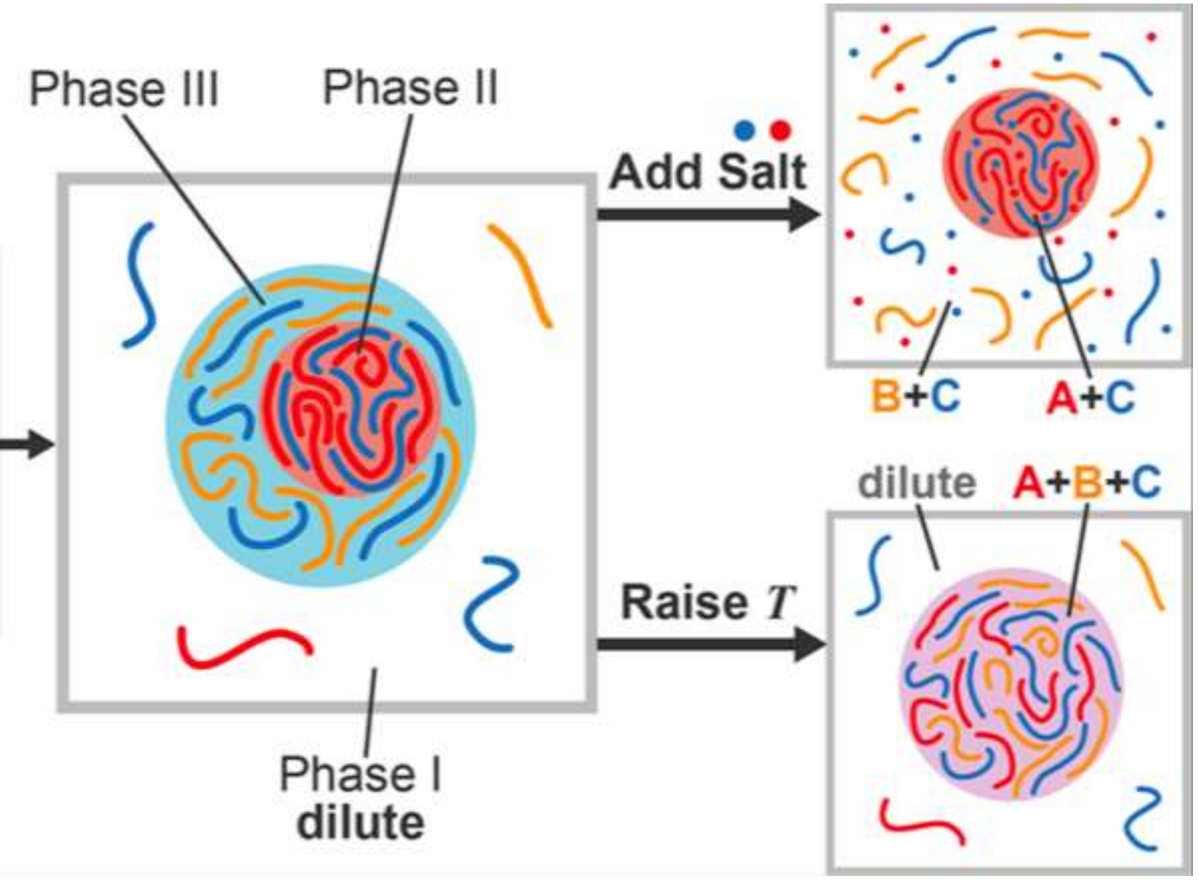


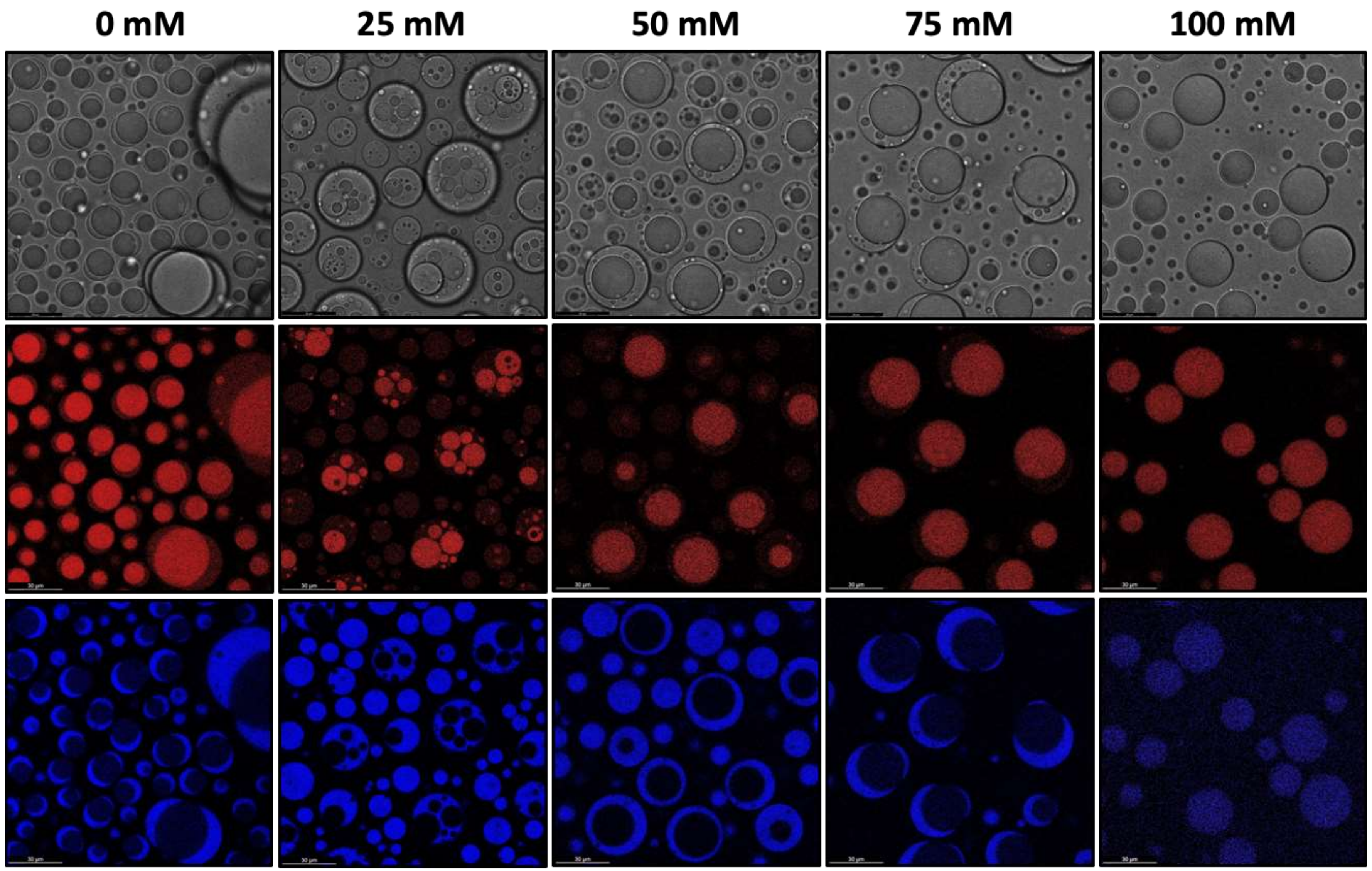
$$K_{P,b^+} = \frac{[f_b{}^+]_{\text{inner}}}{[f_b{}^+]_{\text{outer}}}$$

$f_a^+ \, f_a^-$ (a)	$c_{P,a}$ (wt%)	$f_a \, f_b$ (a/b)	Δf $(f_b - f_a)$	$\Delta c_{P,a/b}$ (wt%)	$K_{P,b}$
30	16.1	30/54	24	14.1	3
		30/72	42	17.3	5
54	30.2	54/72	18	3.2	—
		54/100	46	7.4	2
72	33.4	72/100	28	4.4	—
100	37.8	30/100	70	21.7	9.5

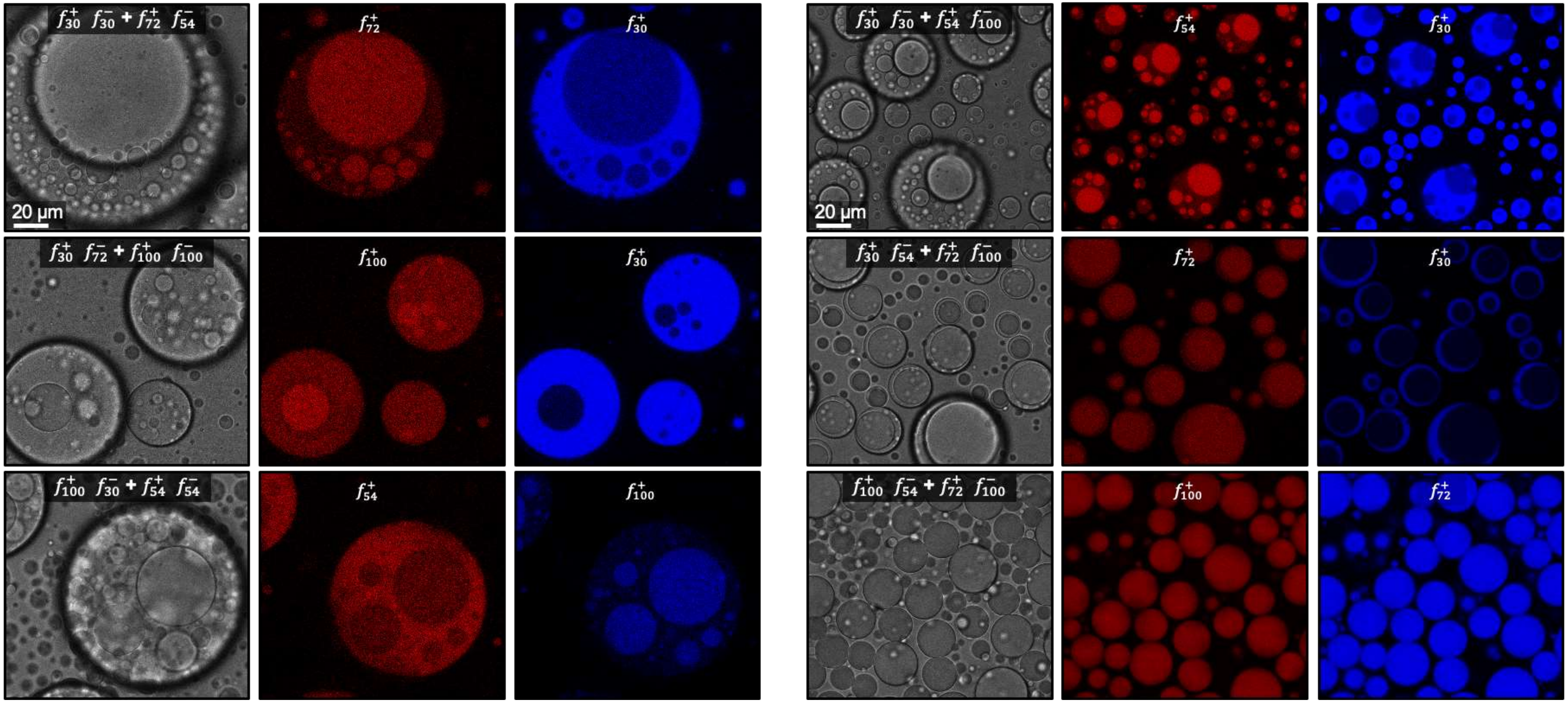


Addition of salt leads to dissolution and uptake of outer droplet



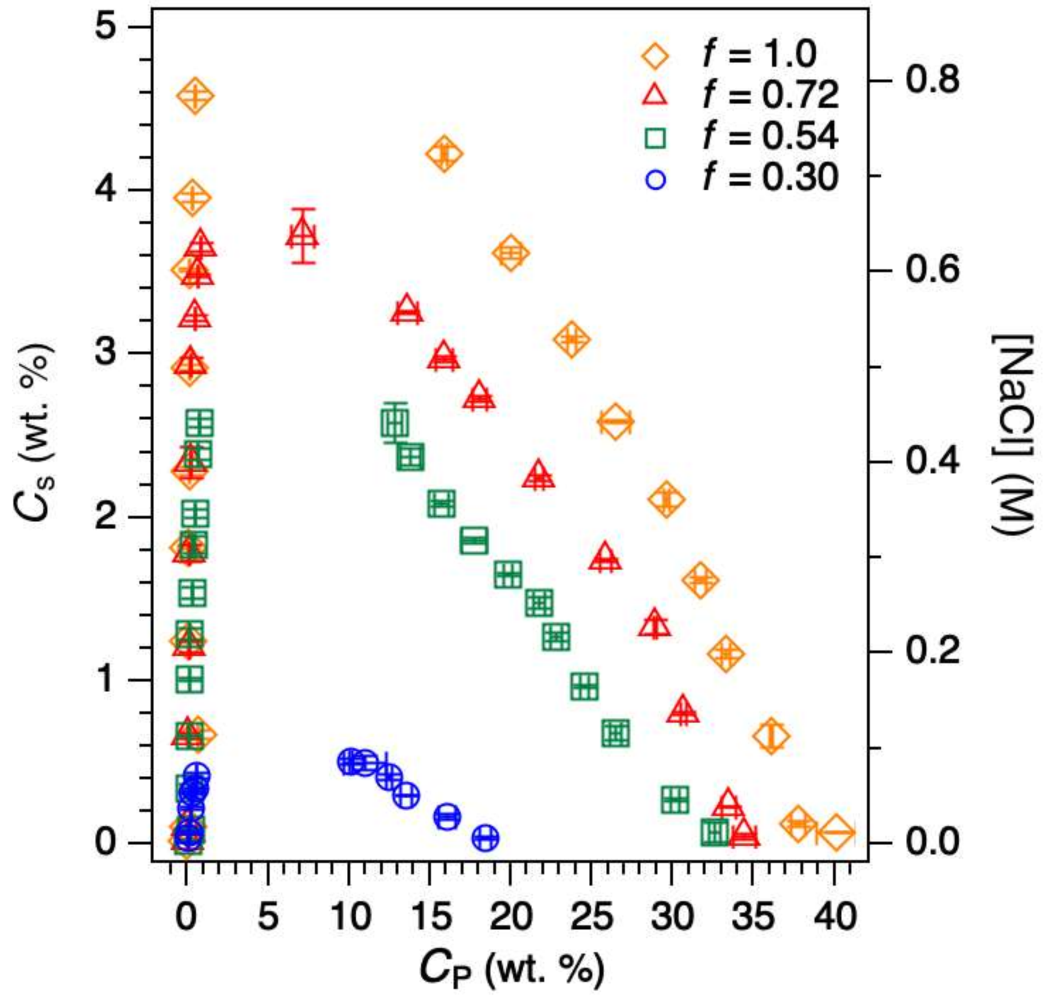


Asymmetric combinations also produce multiphase PECs



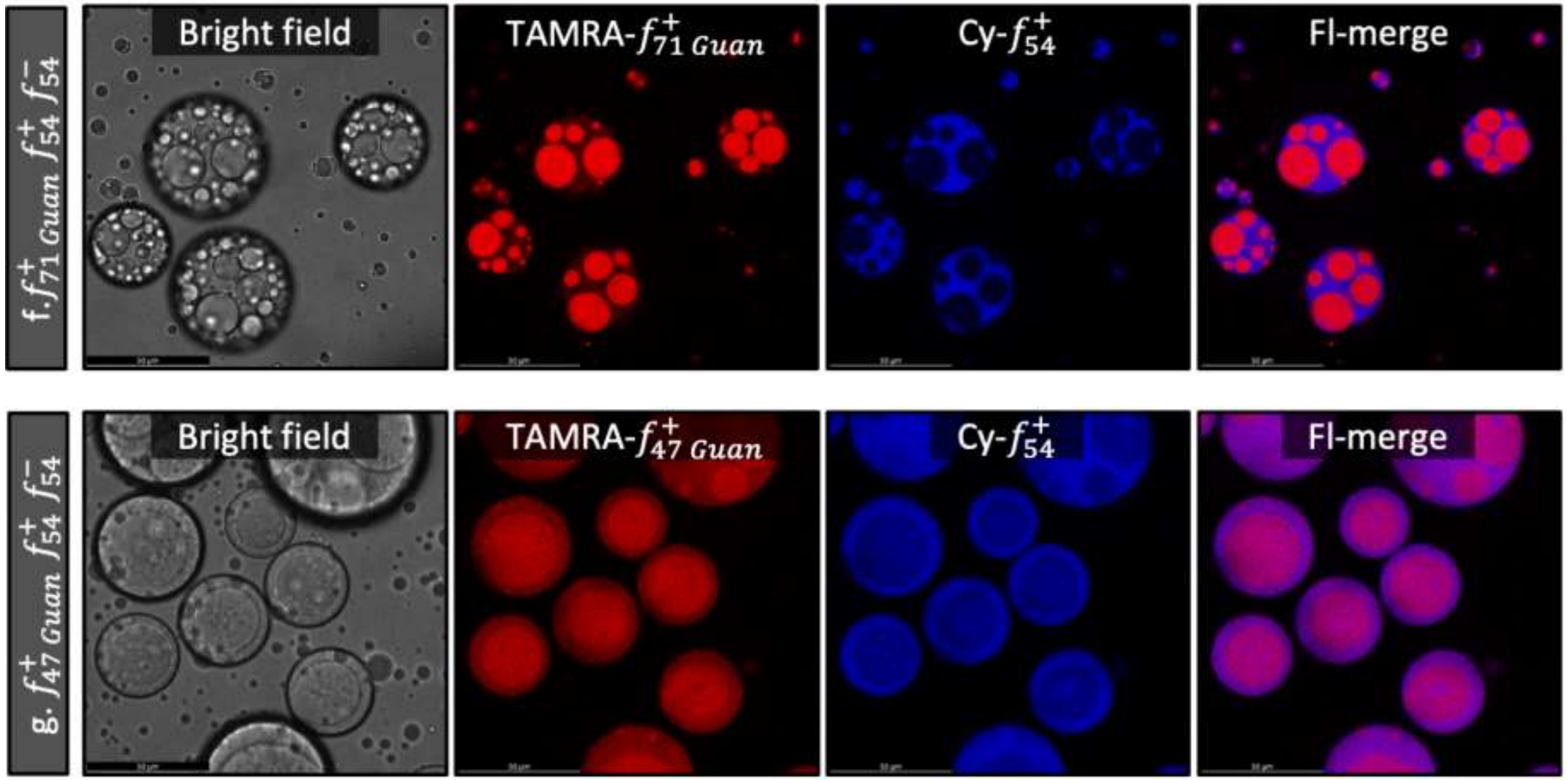
Of 36 possible combinations 32 produce multiphase PECs

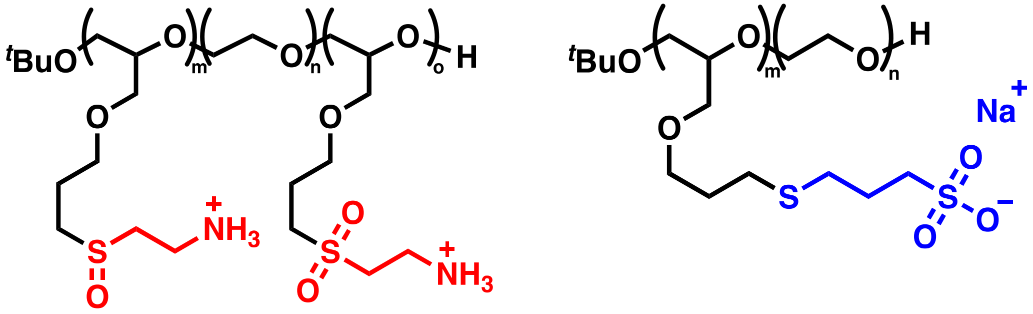
$f_a f_b$	Δf	Multiphase	Miscible
$f_{30}\,f_{100}$	70	18	1
$f_{30}\,f_{72}$	42	17	2
$f_{30}\,f_{54}$	24	17	2
$f_{54}\,f_{100}$	46	16	3
$f_{72}\,f_{100}$	28	15	4
$f_{54}\,f_{72}$	18	14	5

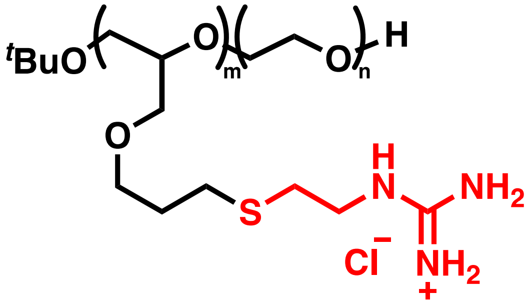


Miscibility strongly correlated with similar PEC density = similar water content

Hydration differences b/w PECs are more powerful than Δf alone



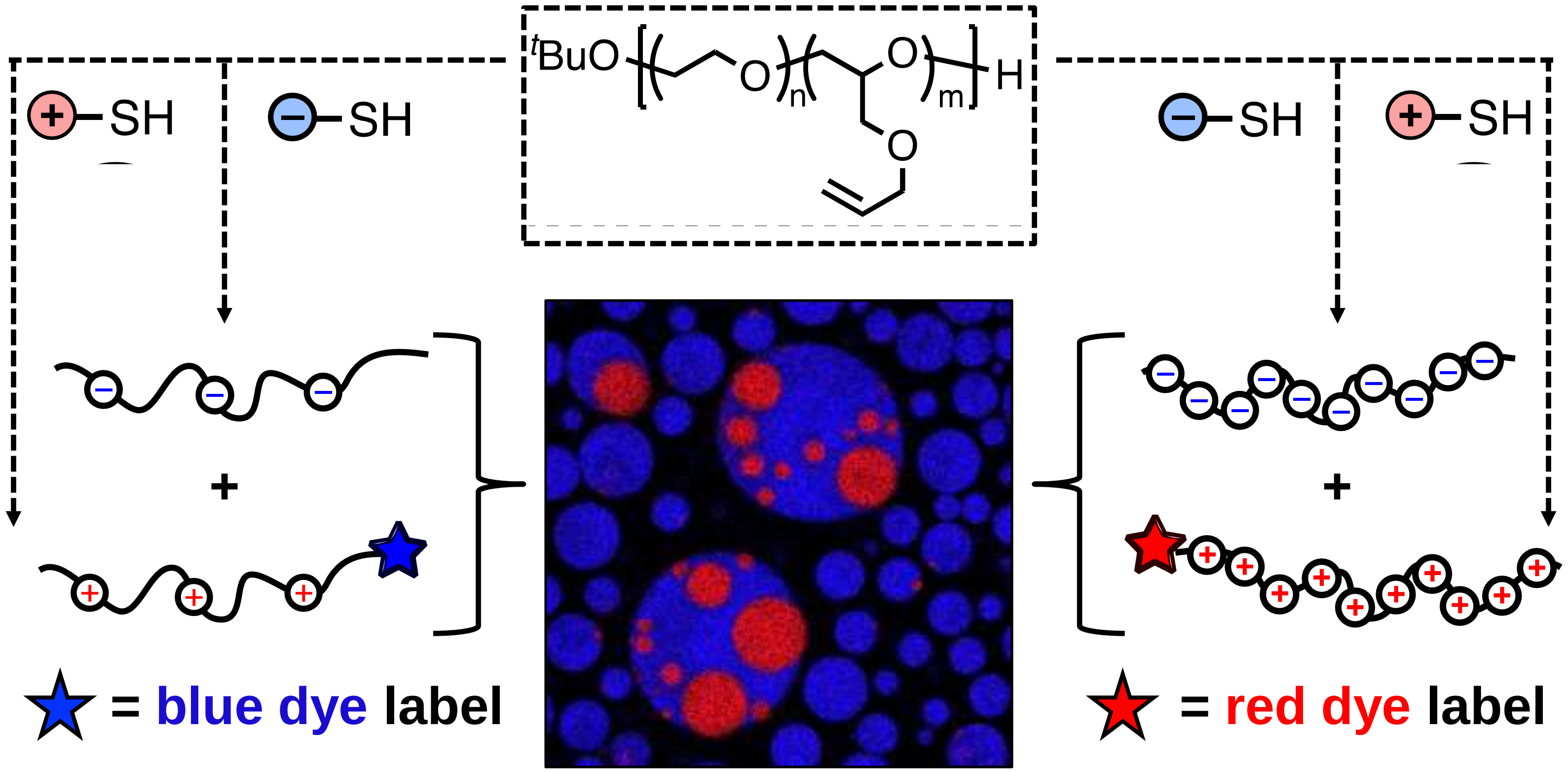




Poly(Guan-*stat*-EO)

3-component PE mixtures of similar f-values will demix due to hydration differences

Summary and acknowledgements



Prof. Artem Rumyantsev (now NCSU)

Yan Fang (U Chicago)

Dr. Aman Agrawal (U Houston)

Dr. Heyi Liang (U Chicago)

Dr. Boyuan Yu (U Chicago)

Prof. Alamgir Karim (U Houston)

Prof. Juan de Pablo (U Chicago)

Prof. Matthew Tirrell (U Chicago)

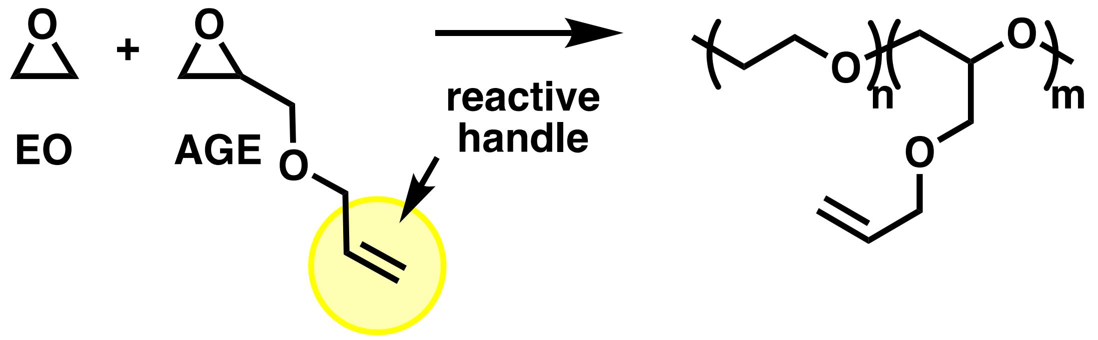






CHIMaD

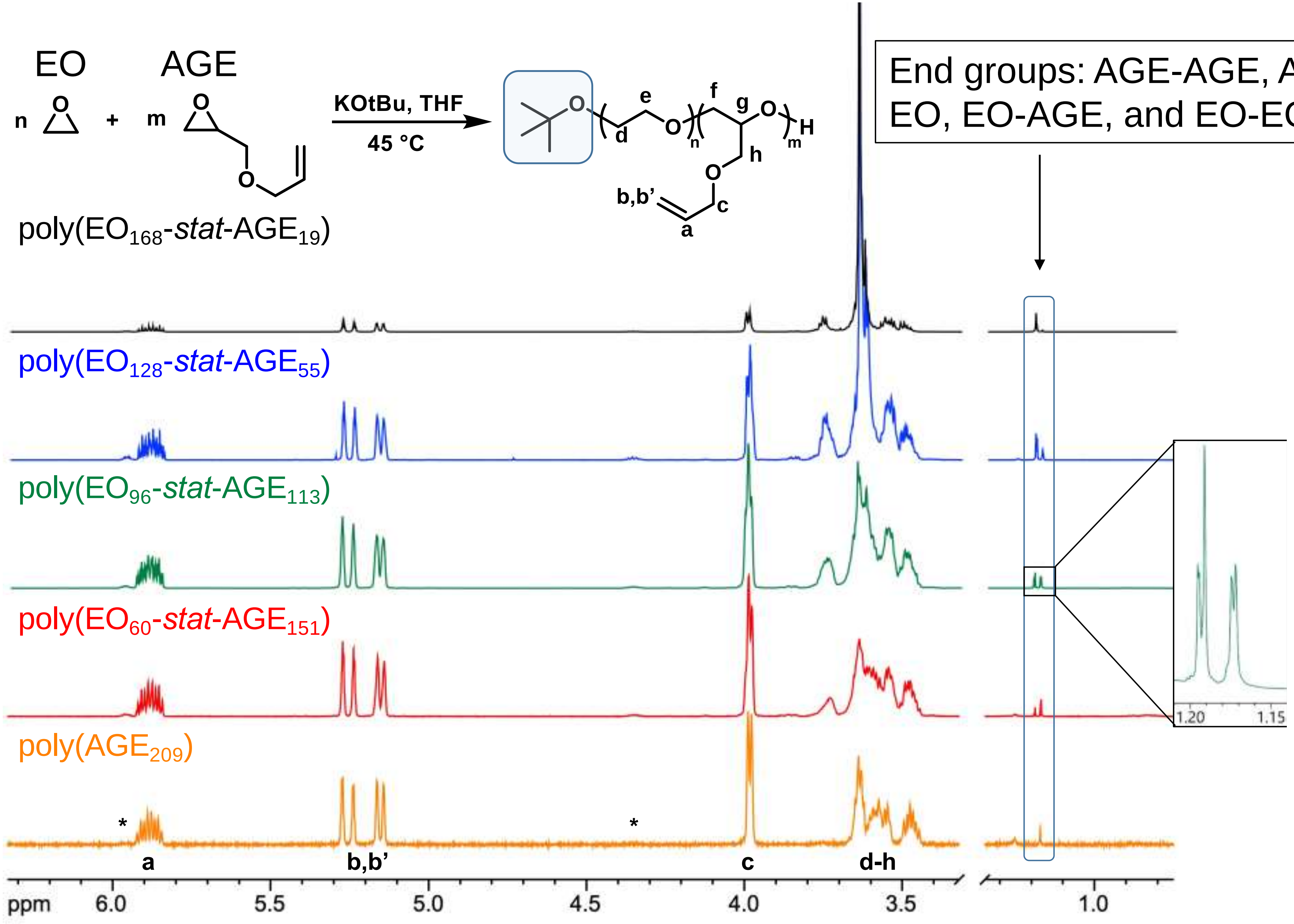
Air- and moisture-free synthetic methods are a valuable skill



1. Polyethylene oxide (PEO) → water-soluble
2. EO/AGE copolymerize near ideally randomly
3. Postpolymerization modification of AGE → **homologous** polyanions and polycations
4. Anionic polymerization**:** synthesis of long and well-defined polymer chains
5. EO comonomer: minimal chemical complexity introduced

Anionic polymerization setup at Argonne National Laboratory

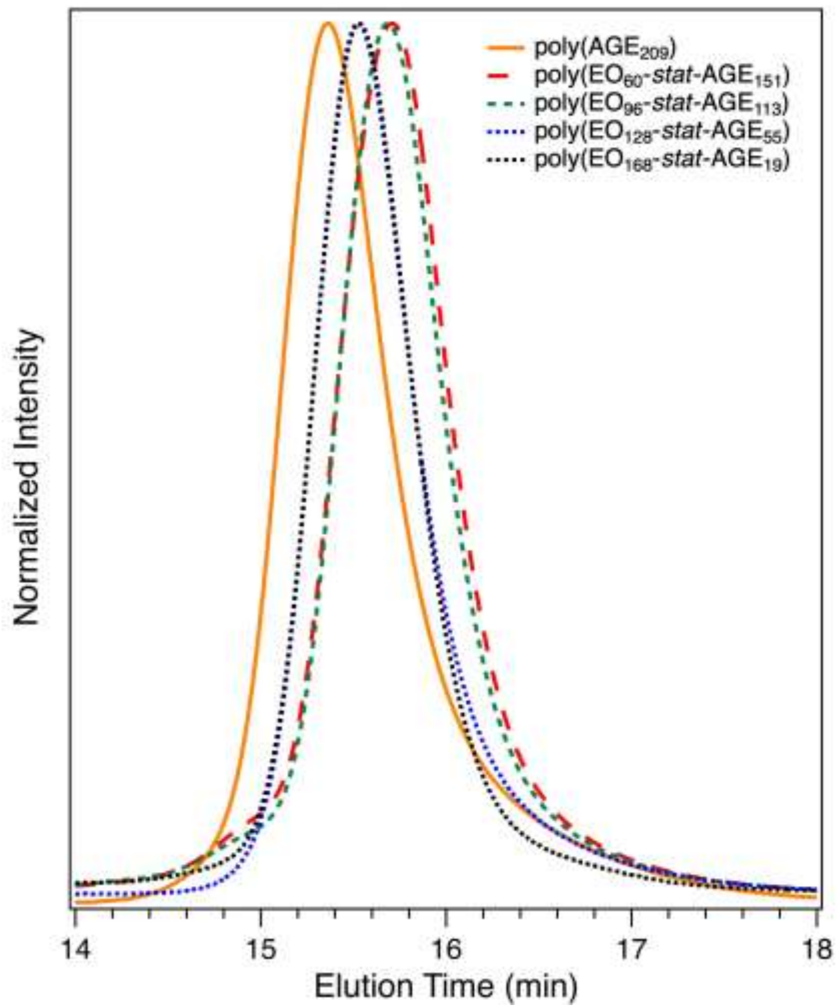
Molecular characterization shows well-defined polymers of variable *f*



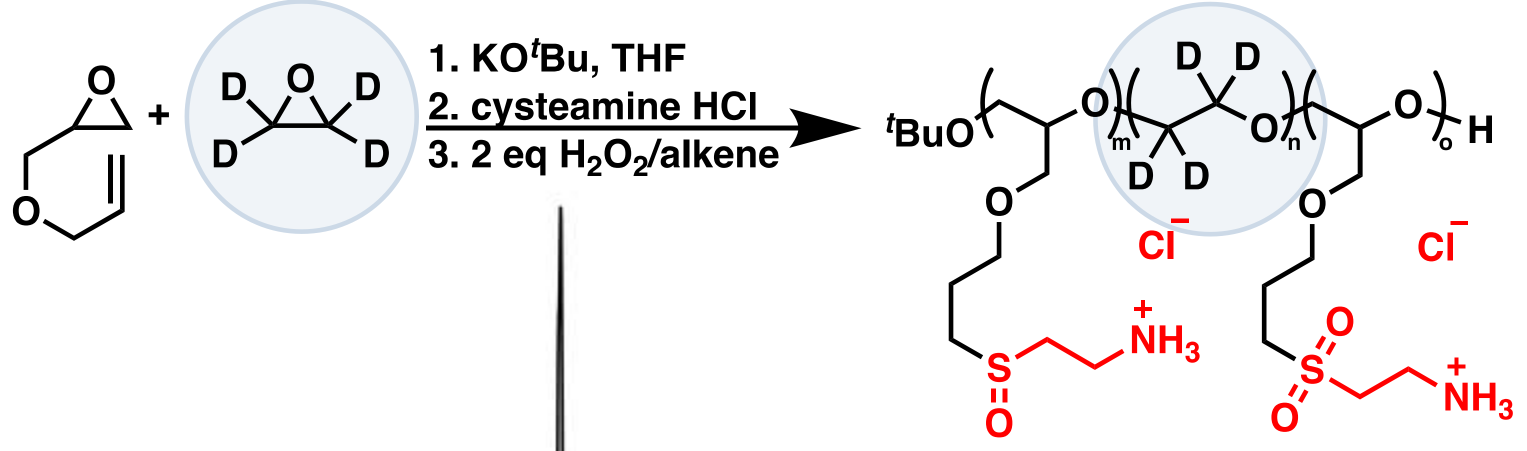
End groups: AGE-AGE, AGE-EO, EO-AGE, and EO-EO

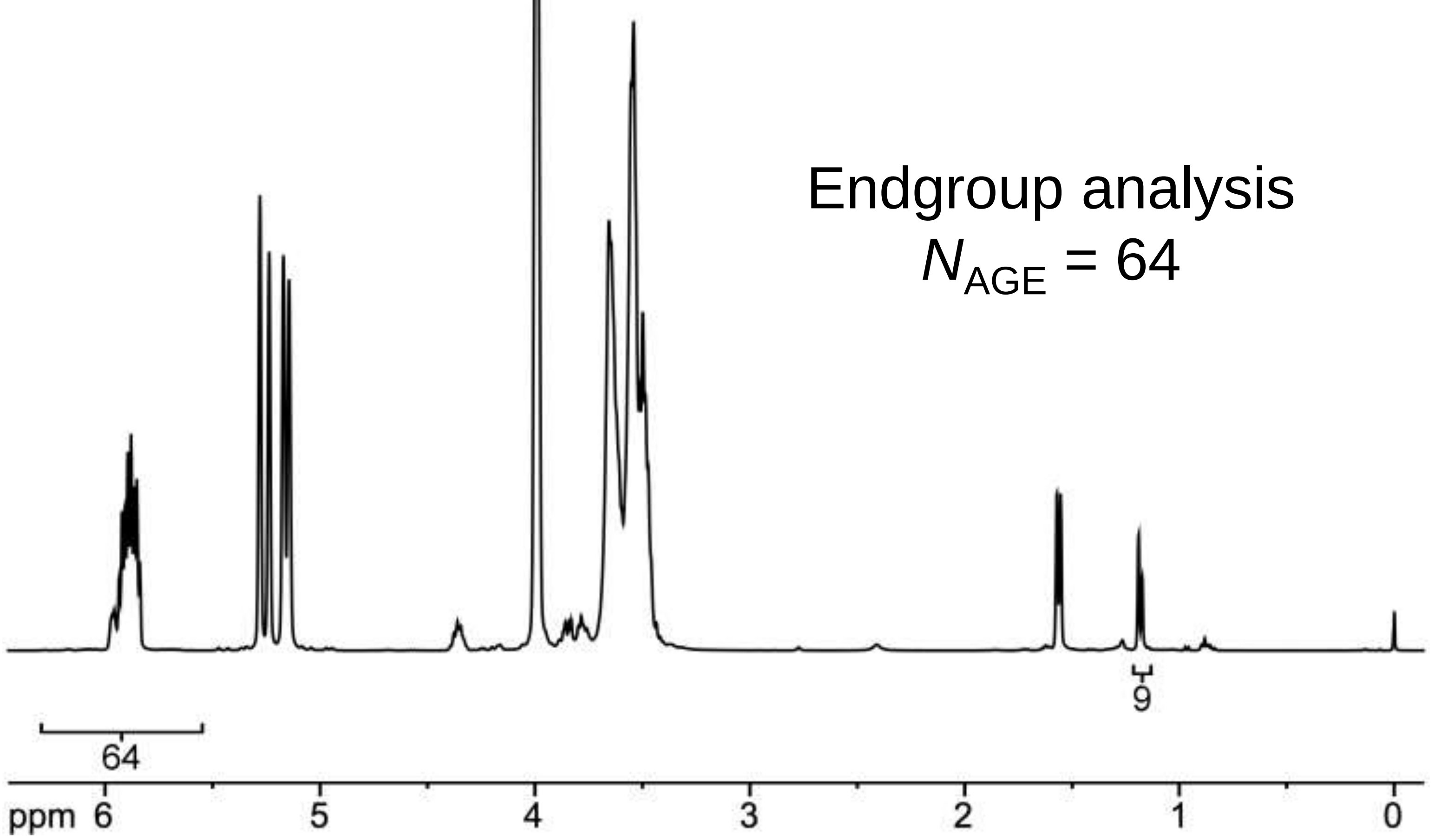
M_n calculated from [1]H NMR spectral end group analysis

Đ calculated from DMF SEC



d_4-Ethylene oxide provides partially deuterated polyelectrolyte

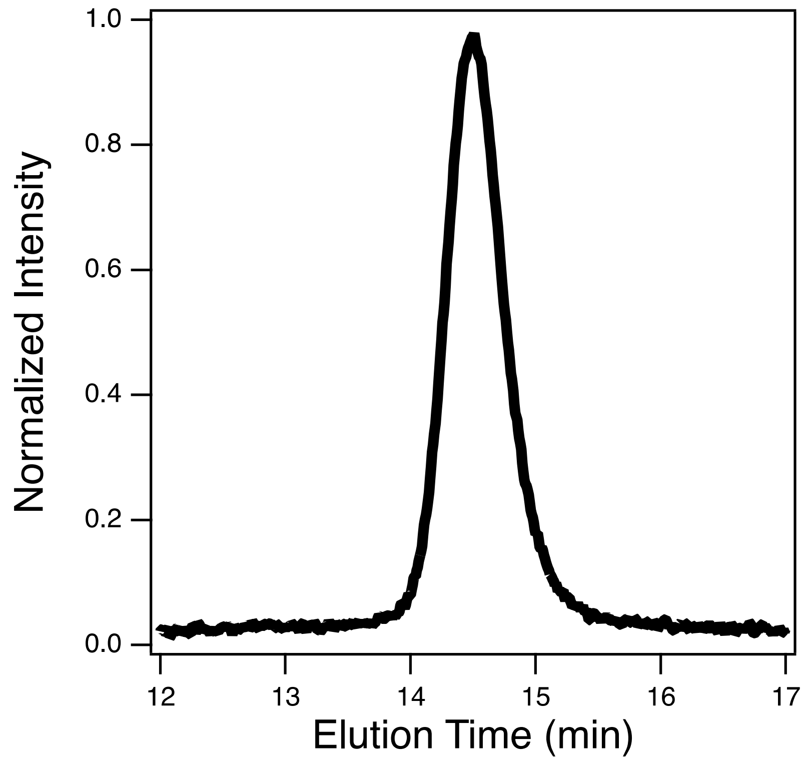




Endgroup analysis
$N_{AGE} = 64$

^{1}H NMR ($CDCl_3$) spectrum of *neutral* poly(AGE-*stat*-d_4-EO)

THF SEC-MALLS

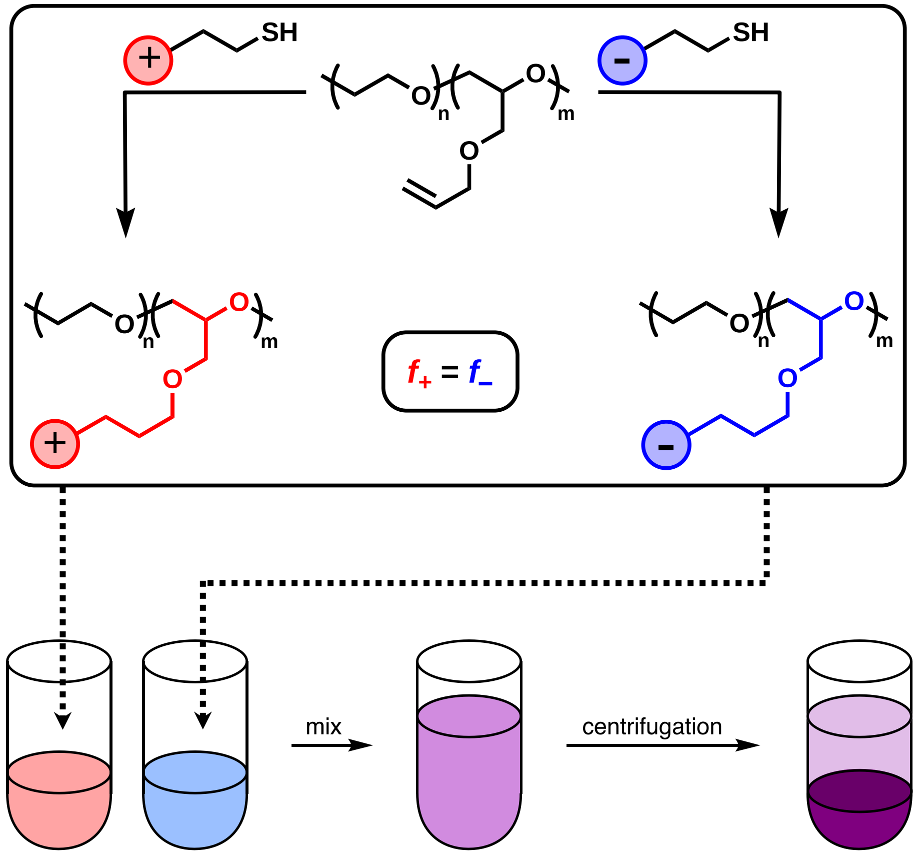


$M_{w,MALLS}$ = 14 kg/mol
$Đ$ = 1.05

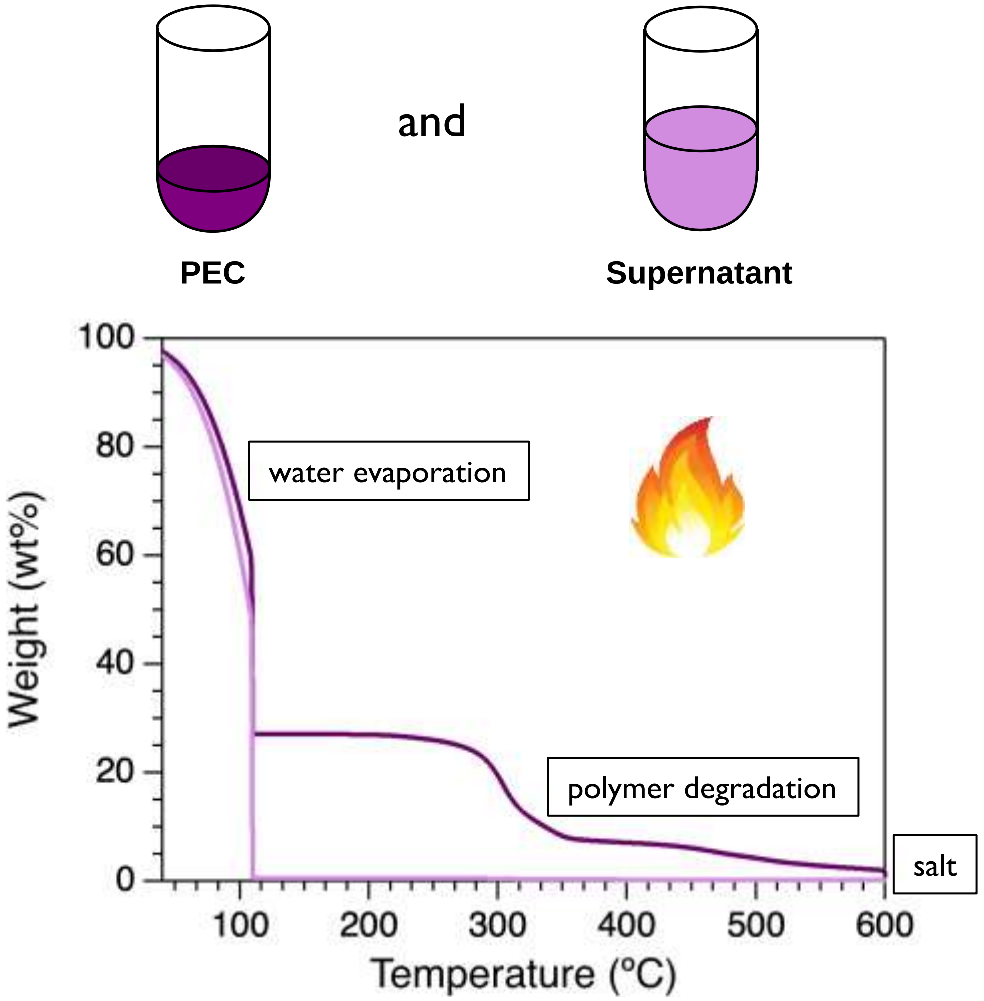
$N_{n,total} = 206$, $f_{AGE} = 0.31$
poly(AGE_{64}-*stat*-d_4EO_{142})

Click chemistry provides homologous polyelectrolytes for study

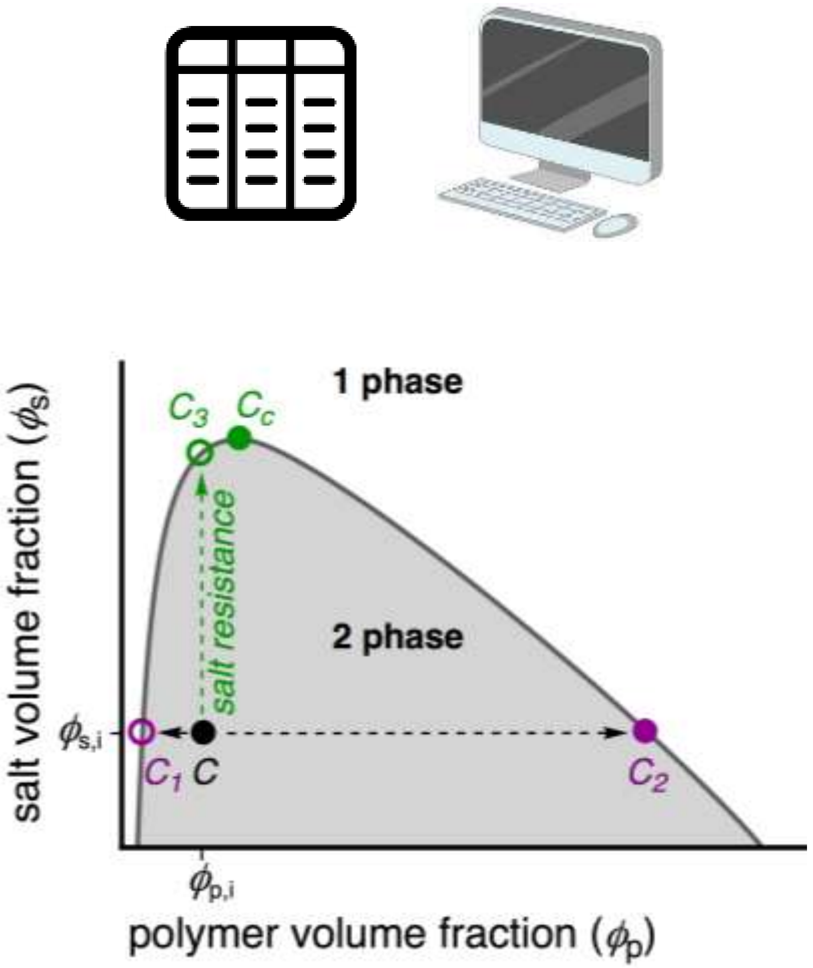
1: Functionalize neutral copolymers and prepare polyelectrolyte complexes



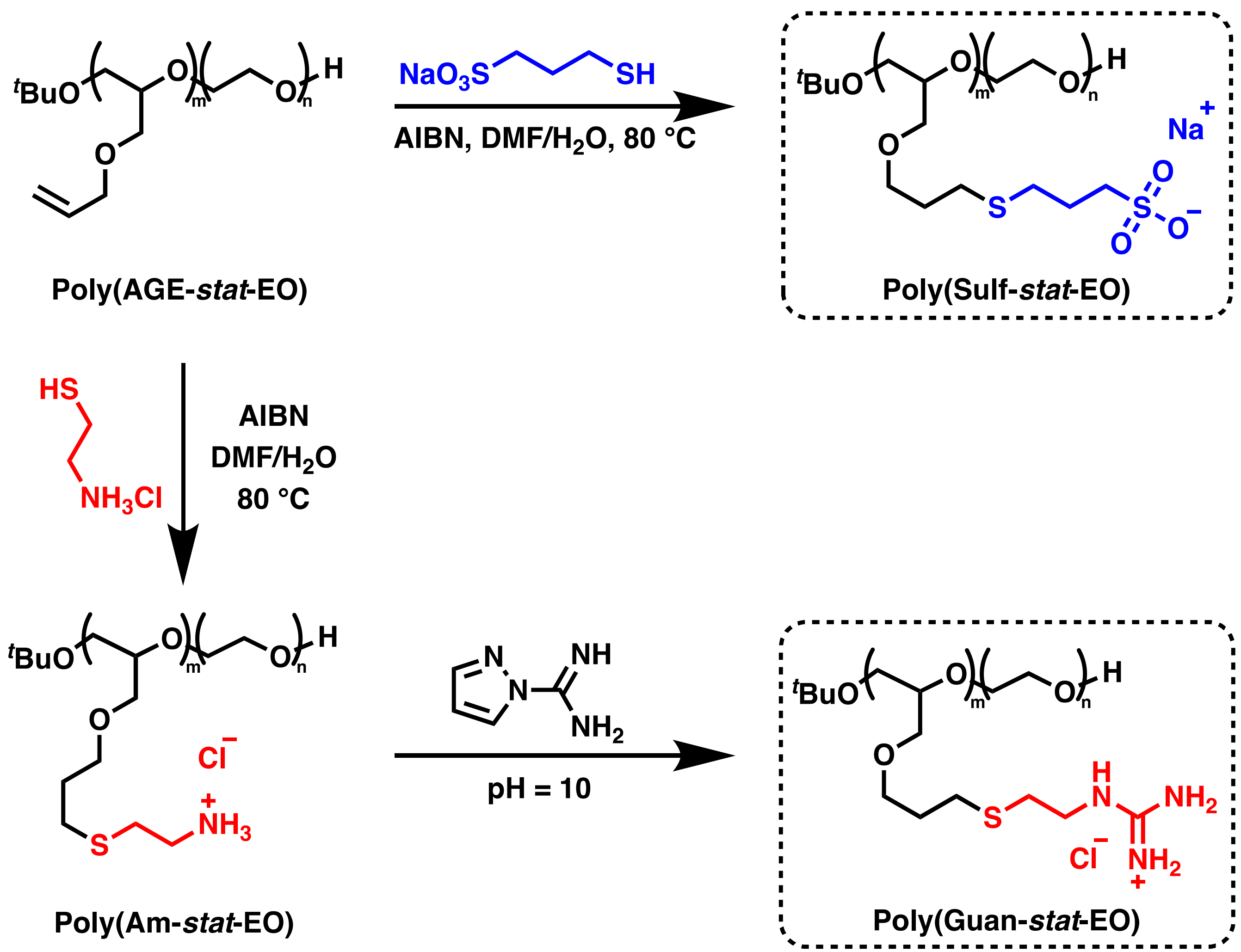
2: Separate the polyelectrolyte complex from the supernatant phase and burn

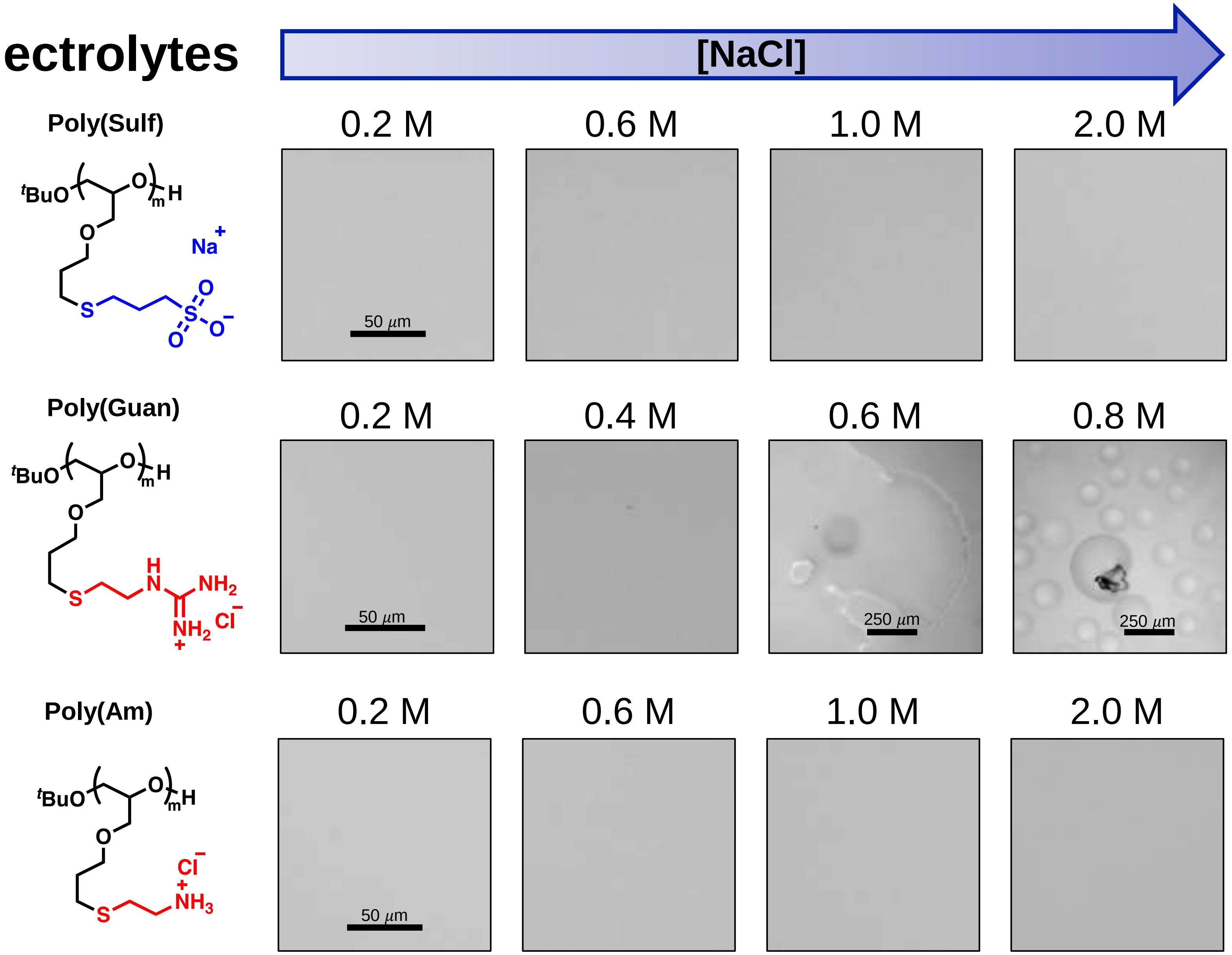


3: Quantify wt % polymer and salt in both phases at different [NaCl] and plot binodals



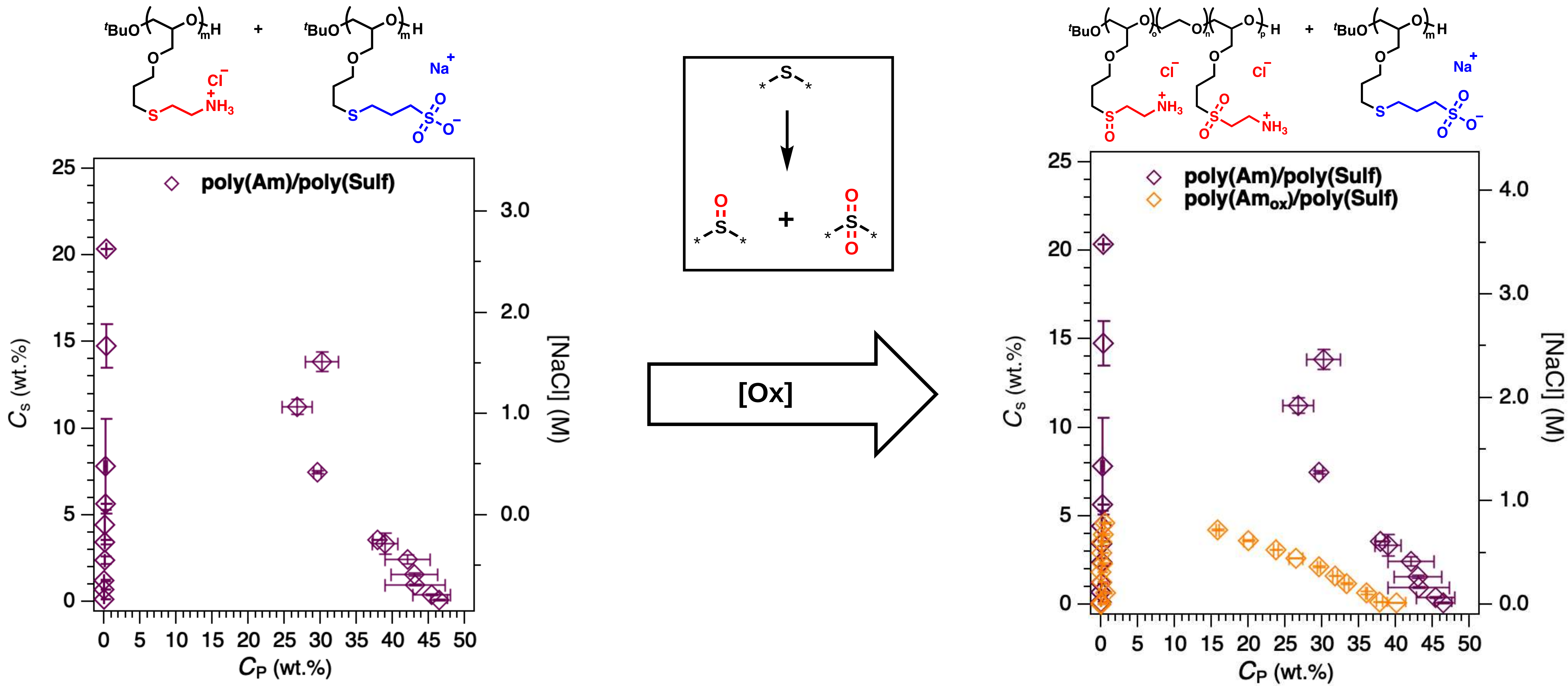
Gen 1: Synthesis of pH-independent (co)polyelectrolytes





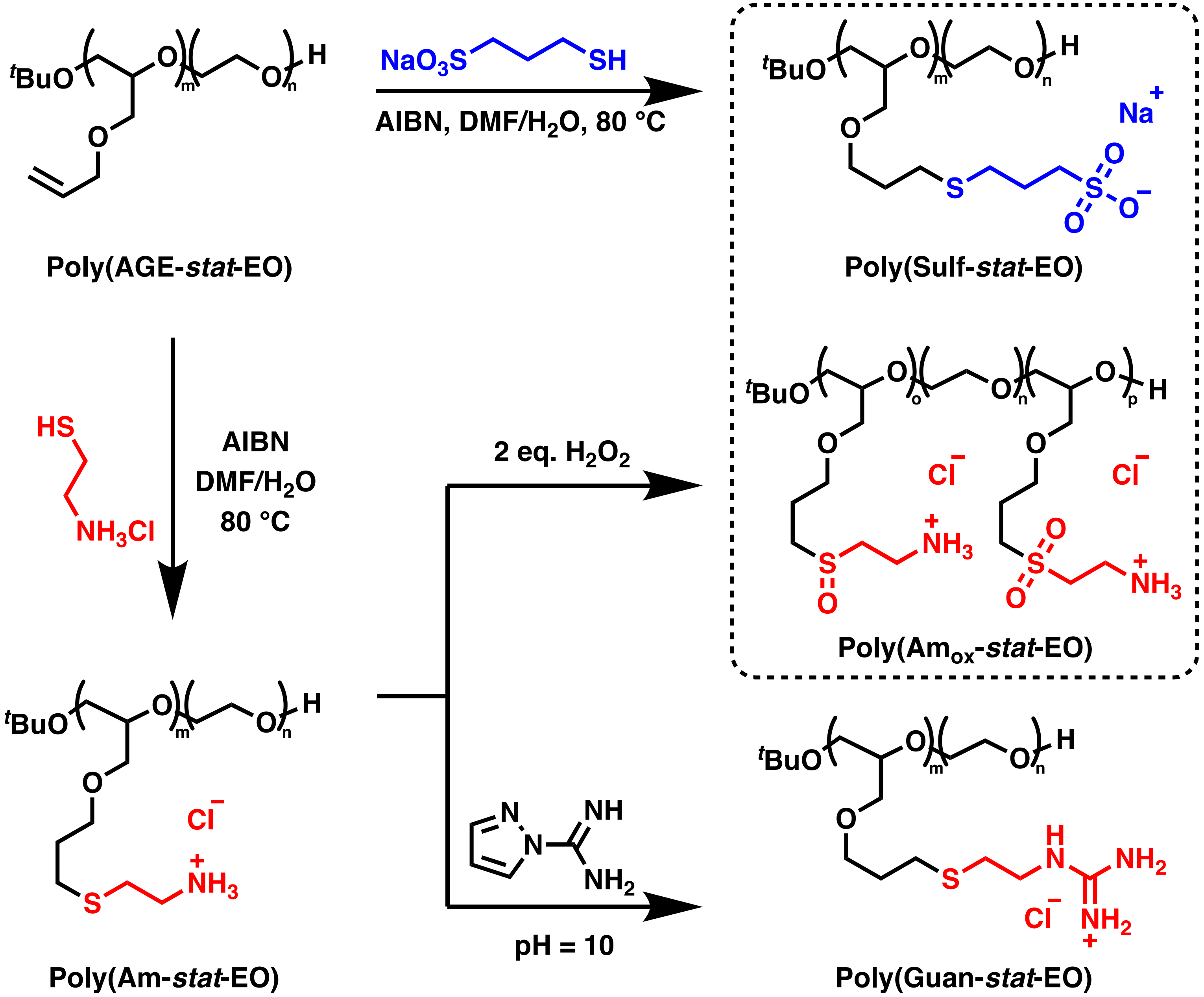
Want phase separation due to **complexation** of oppositely charged polymers → can't use Guan

Other intermolecular interactions are coming into play



Lou et al. *ACS Cent. Sci.* **2019**, *5*, 549.

Finally, we arrive at a system that meets all requirements!

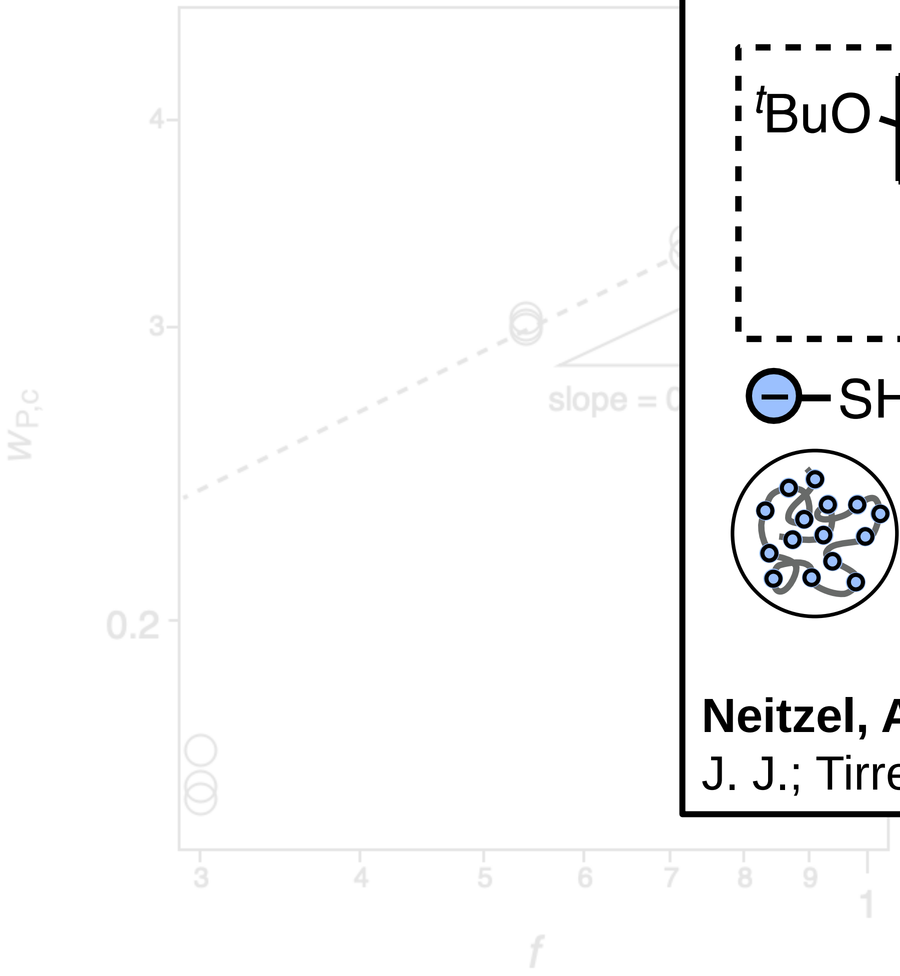


- ✓ Polyelectrolytes soluble in water and aqueous salt solutions at all f values
- ✓ Near ideally random microstructure
- ✓ Homologous polycations and polyanions (i.e., $f_+ = f_-$, identical N_n, $Đ$, and sequence)
- ✓ Other non-covalent interactions minimized (oxidation reduces hydrophobic interactions)

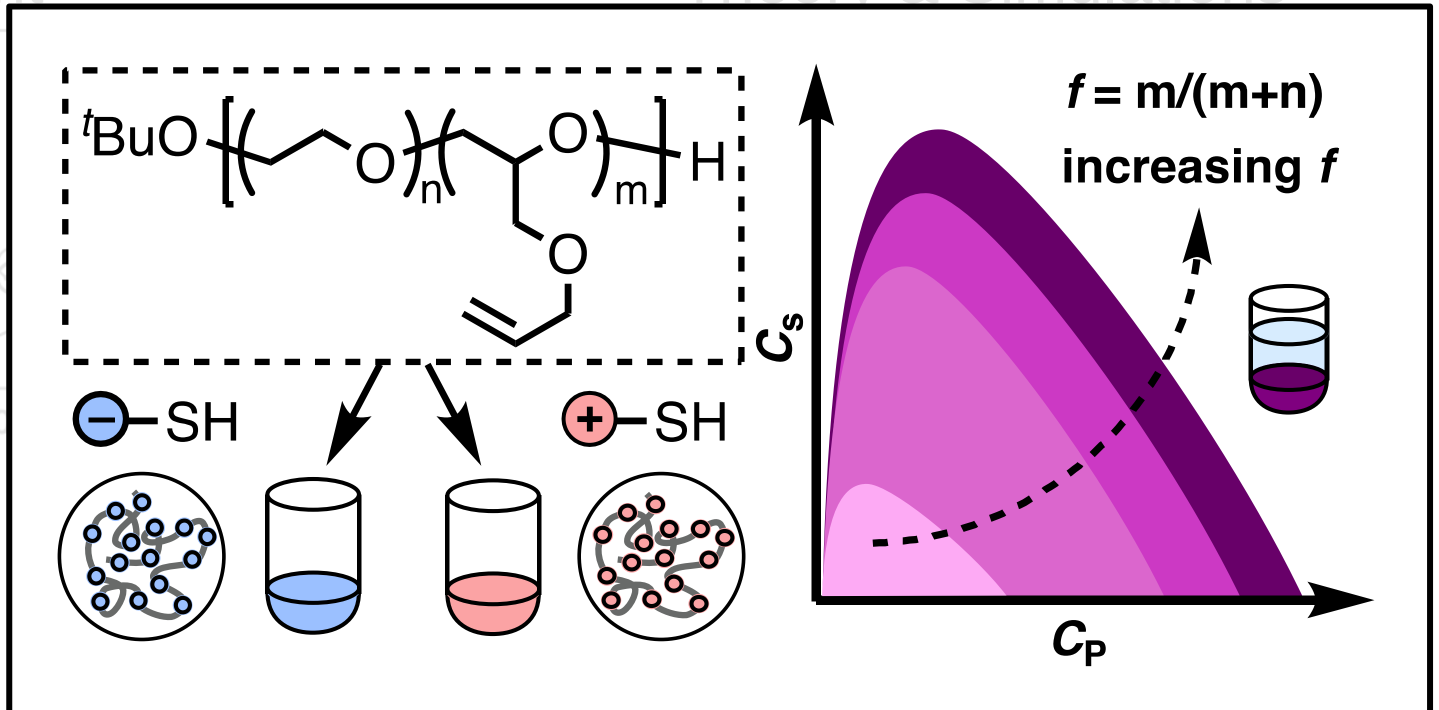
Probe phase behavior for f = 0.1–1.0

Agreement between experiment & simulations but…

Experiment



Theory & Simulations



Neitzel, A. E.; Fang, Y. N.; Yu, B.; Rumyantsev, A. R.; de Pablo, J. J.; Tirrell, M. V. *Macromolecules* **2021**, *54*, 6878−6890.

From scaling analysis in the regime of $f \ll 1$:

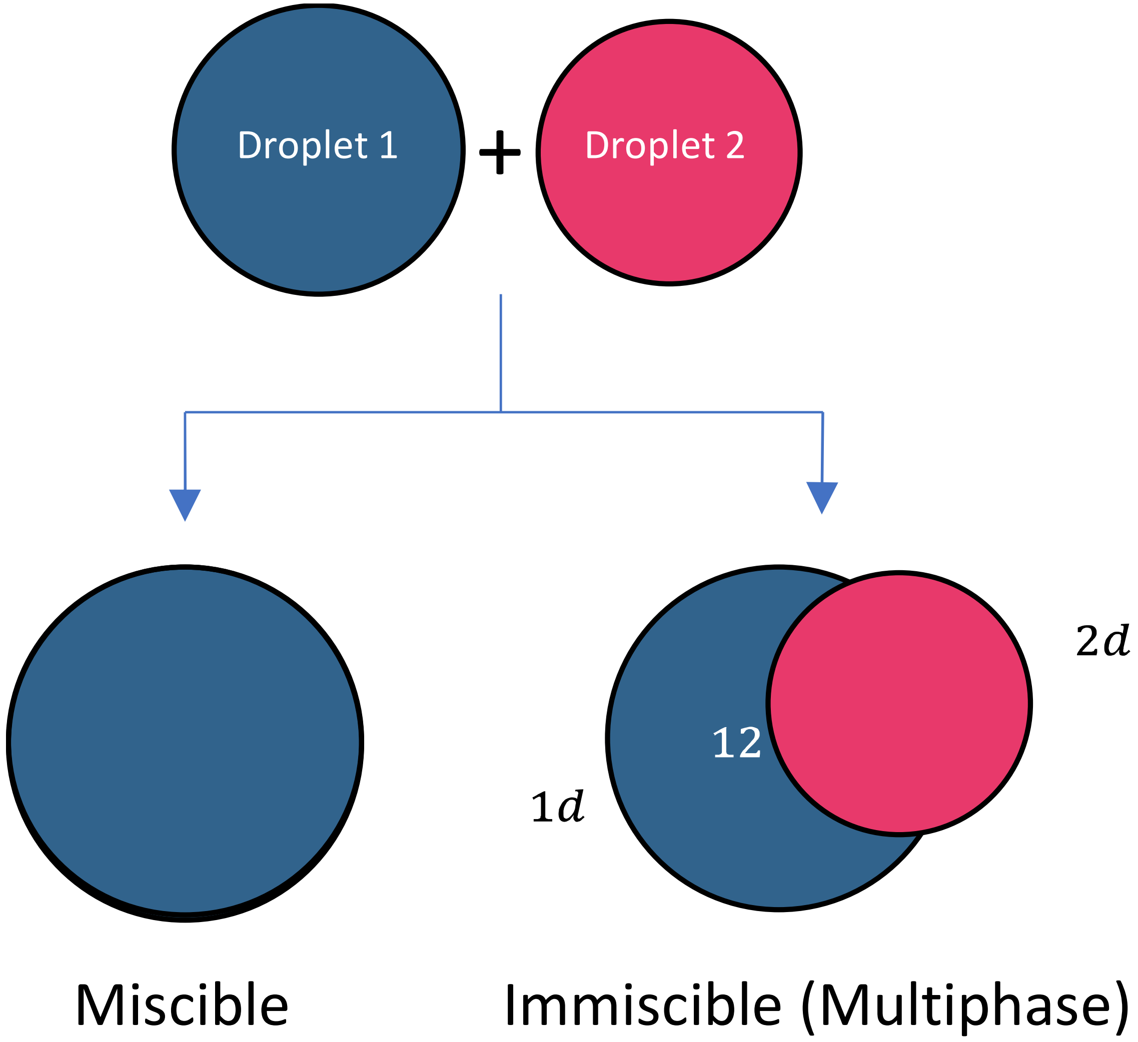
$$\phi \sim (f^2)^{\frac{(3\nu-1)}{(2-\nu)}}$$

For theta solvent $\nu = \frac{1}{2}$, then

$$\phi \sim f^{2/3}$$

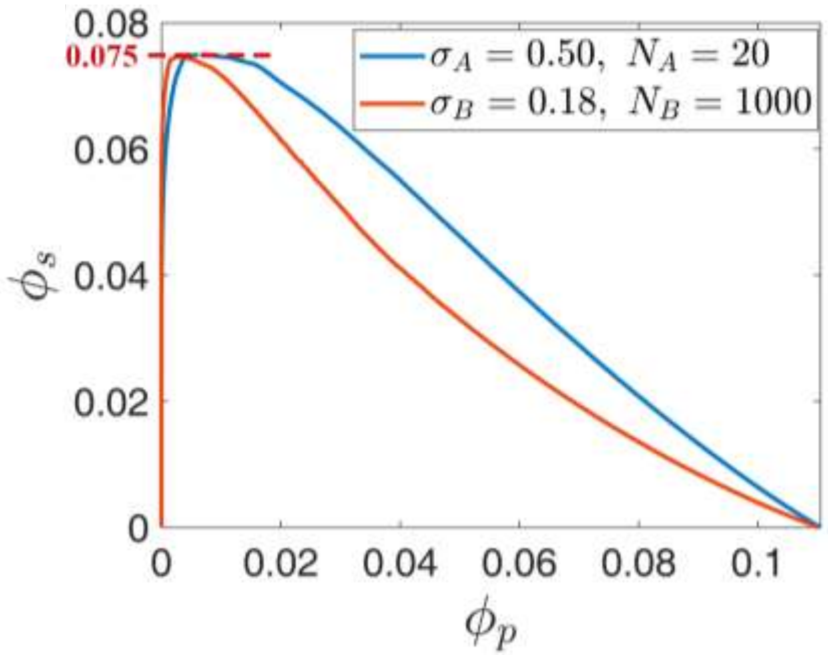
Theory is a more appropriate predictor at very low f → we need longer chains!

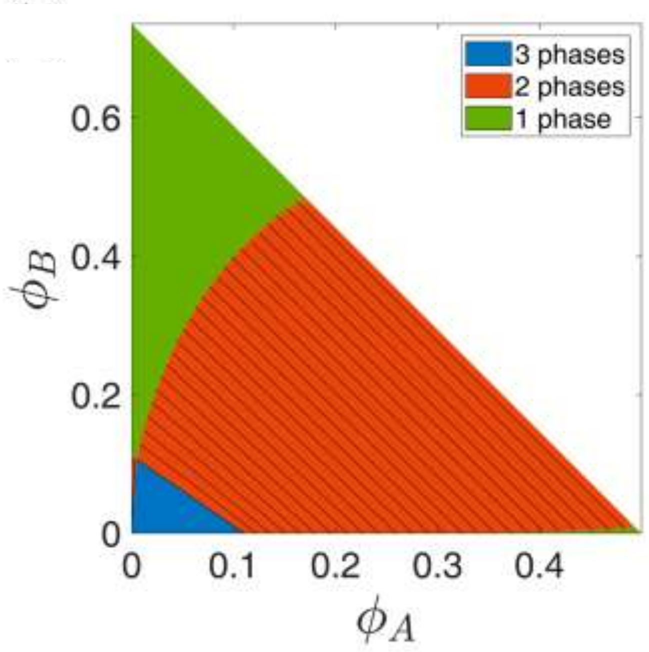
Gibbs free energy of mixing has ΔH term dependent on electrostatics



$$\chi'_{\mathrm{eff},\, ij} = \boxed{\chi_{ij}} + \frac{1}{2}\left(\sigma_i^2 - \sigma_j^2\right)^2 \int \mathrm{d}\mathbf{q}\, a(\mathbf{q})\, g^2(\mathbf{q})$$

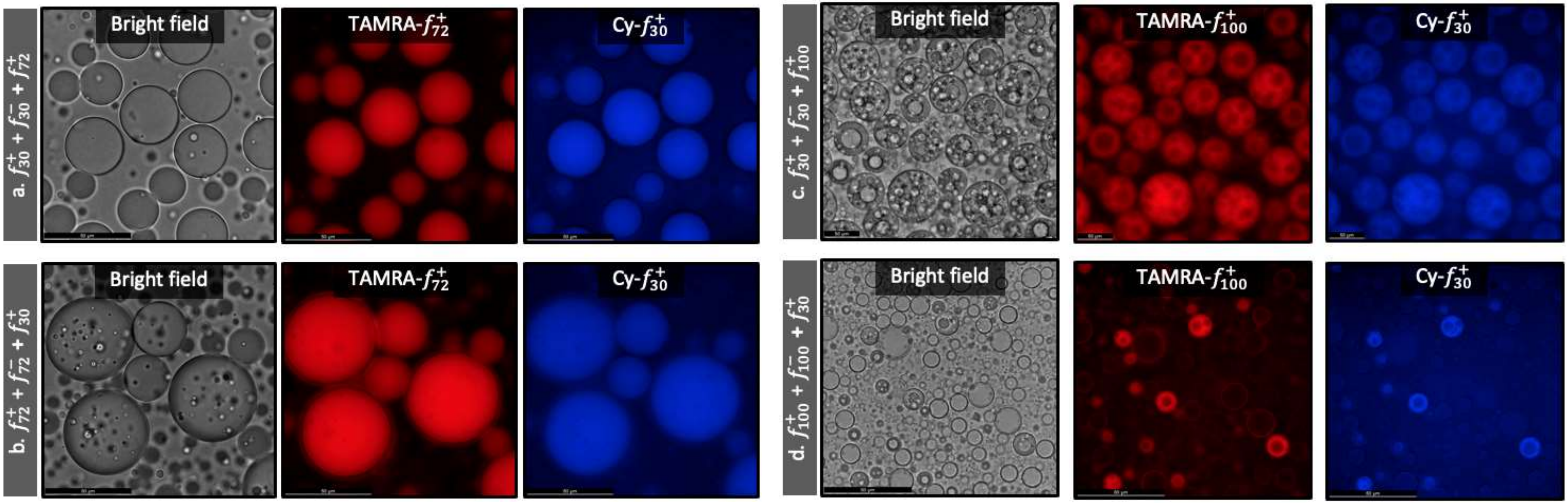
Flory-Huggins — electrostatic contribution





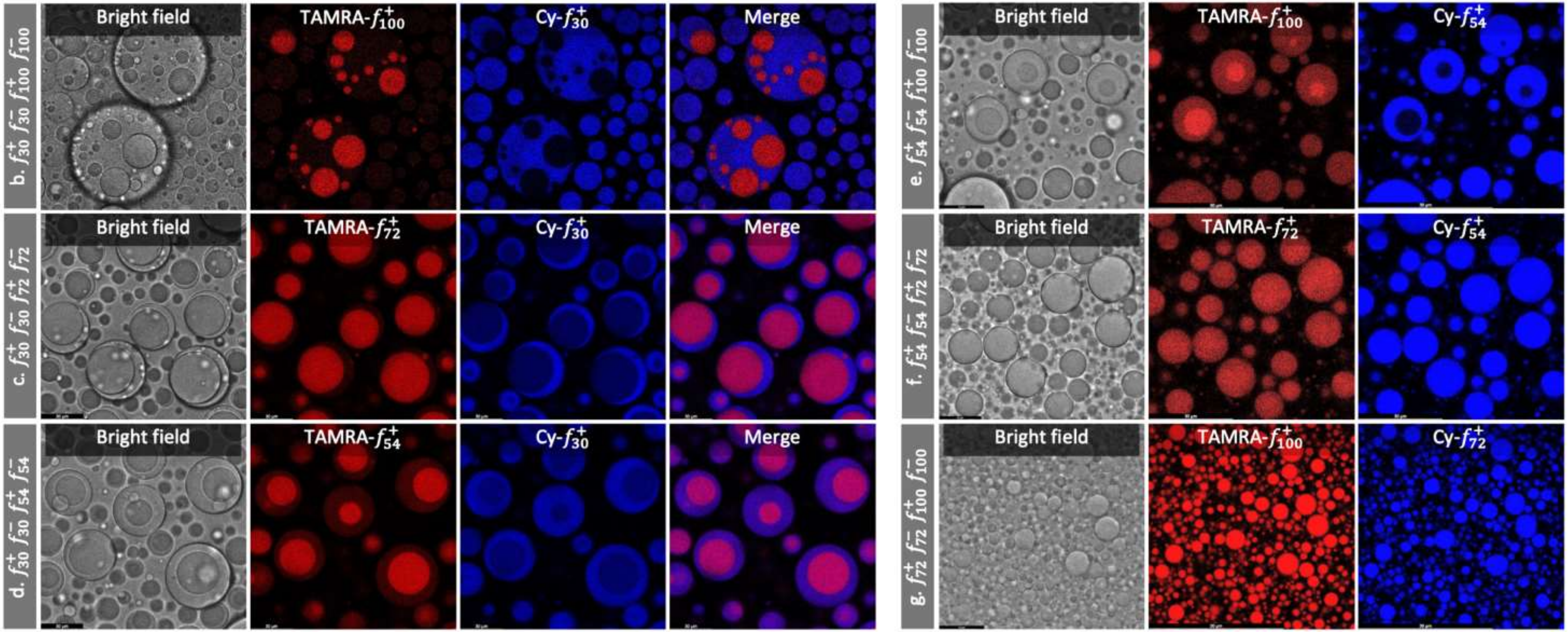
Are charge density differences in a homologous system enough to drive demixing?

Combination of three PEs produces promising result until…

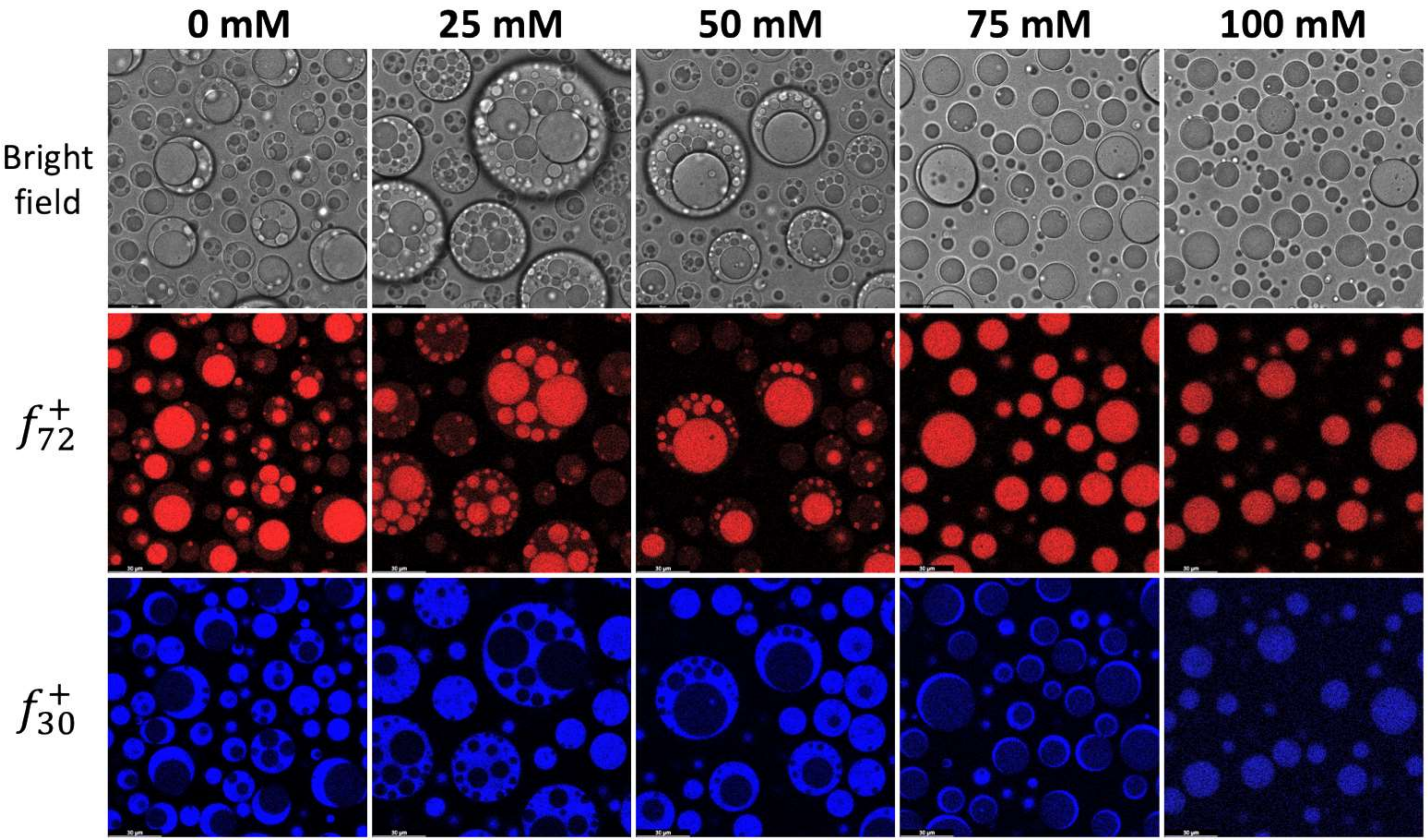


No coexisting polyelectrolyte complex phases, instead vesicles with water in the core

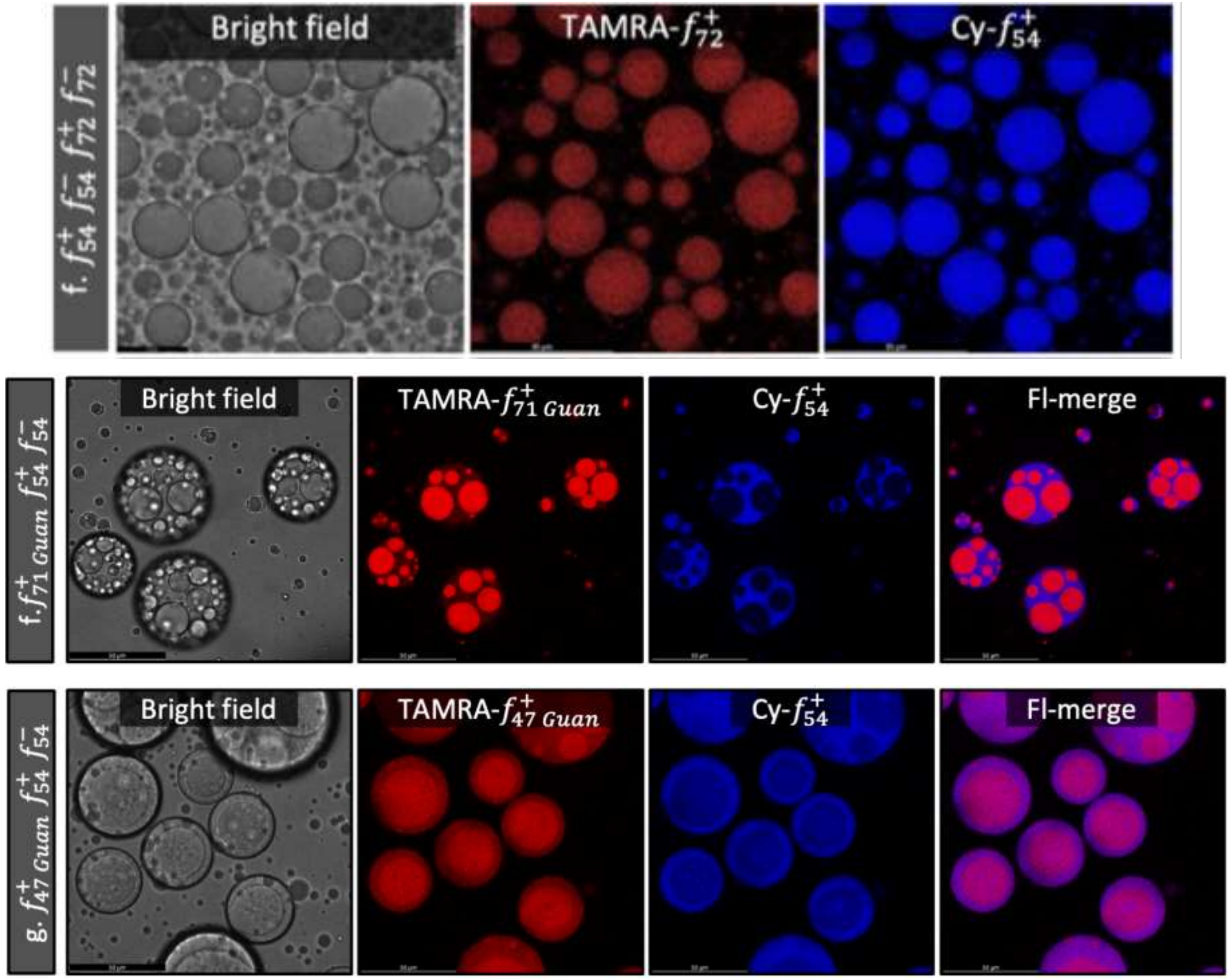
Well…if you can't do it with 3, try it with 4!

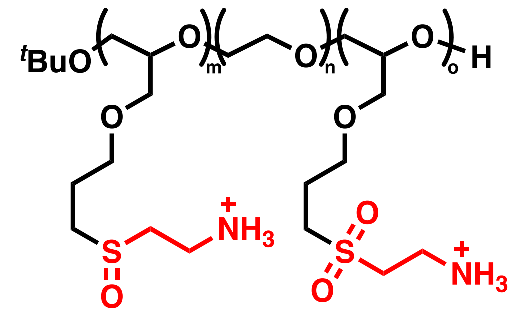


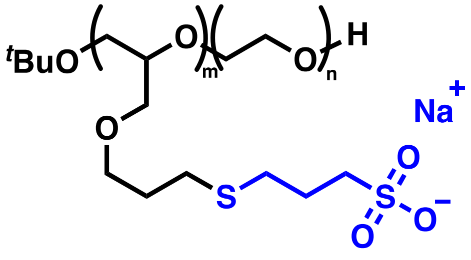
Salt can be used to selectively dissolve the outer droplet

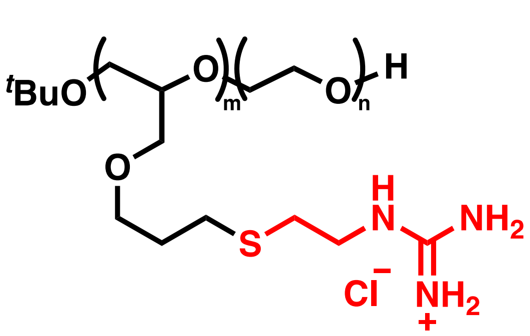


Specific ion effects are powerful due to differential hydration

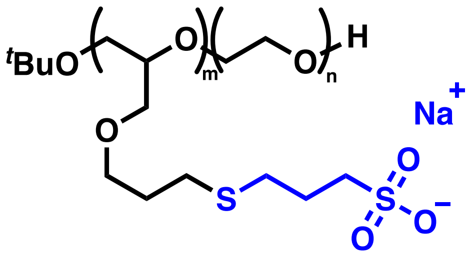




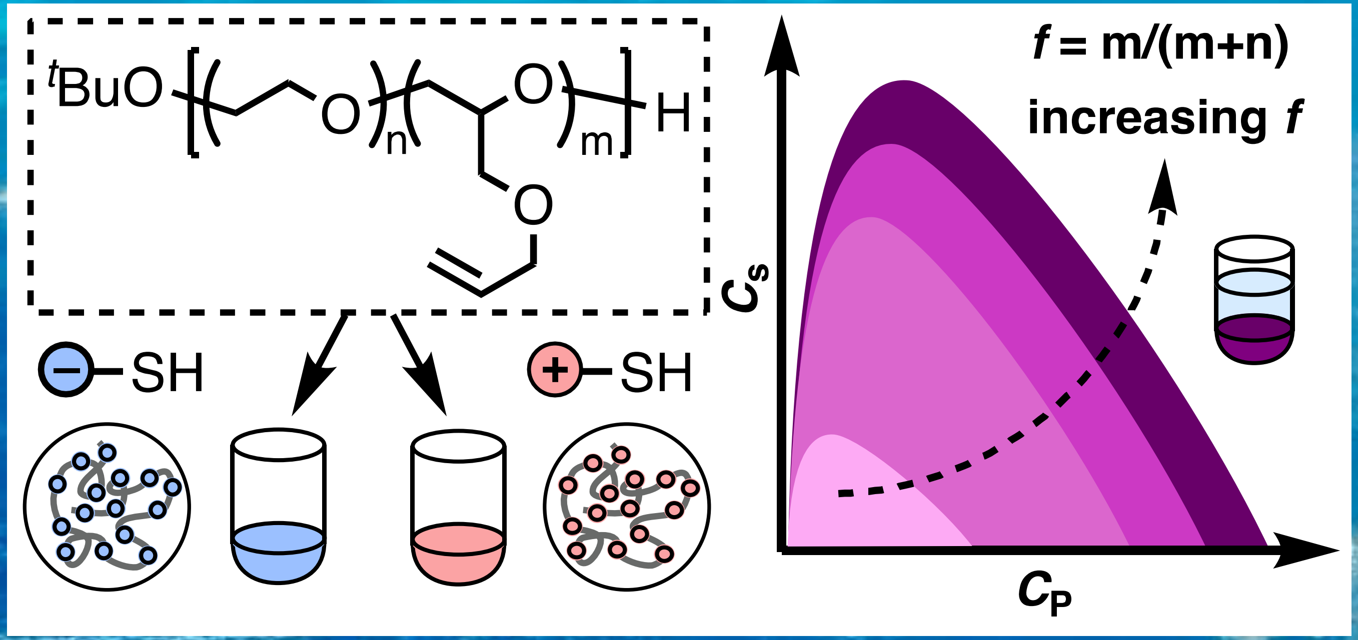




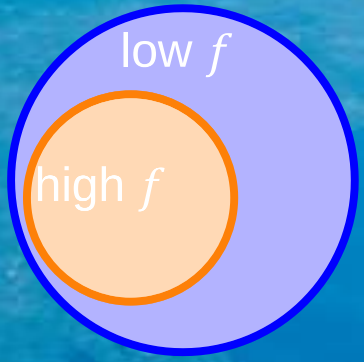
Poly(Guan-*stat*-EO)

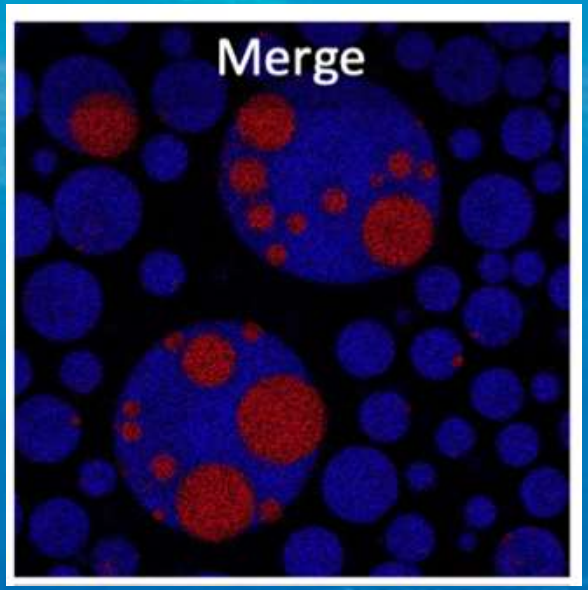


Solvation of ammonium-sulfonate greater than solvation of guanidinium-sulfonate



Neitzel, A. E.; Fang, Y. N.; Yu, B.; Rumyantsev, A. R.; de Pablo, J. J.; Tirrell, M. V. *Macromolecules* **2021**, *54*, 6878−6890.





Agrawal, Karim, Tirrell, and Neitzel. In preparation for publication





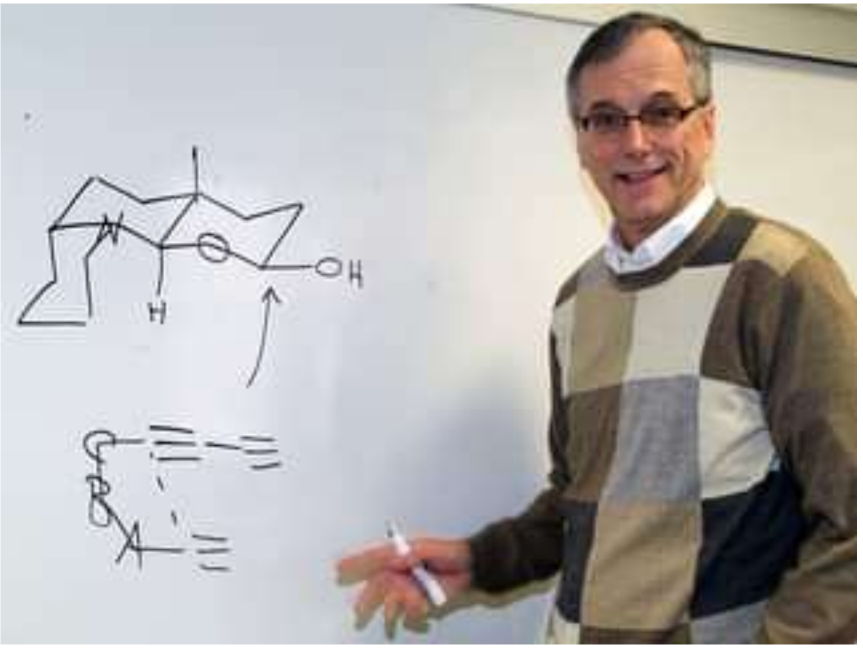


CHiMaD

Bachelor

Master PhD

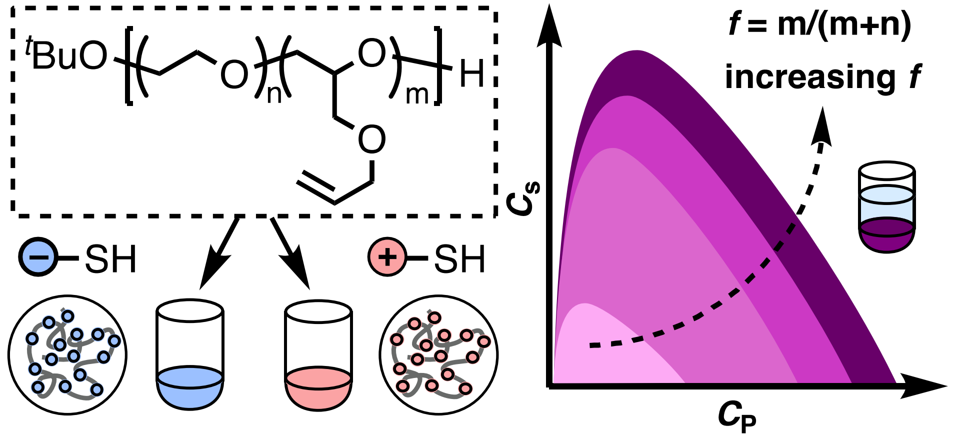
Postdoc PI Emeritus Prof



Thank you for your time and attention

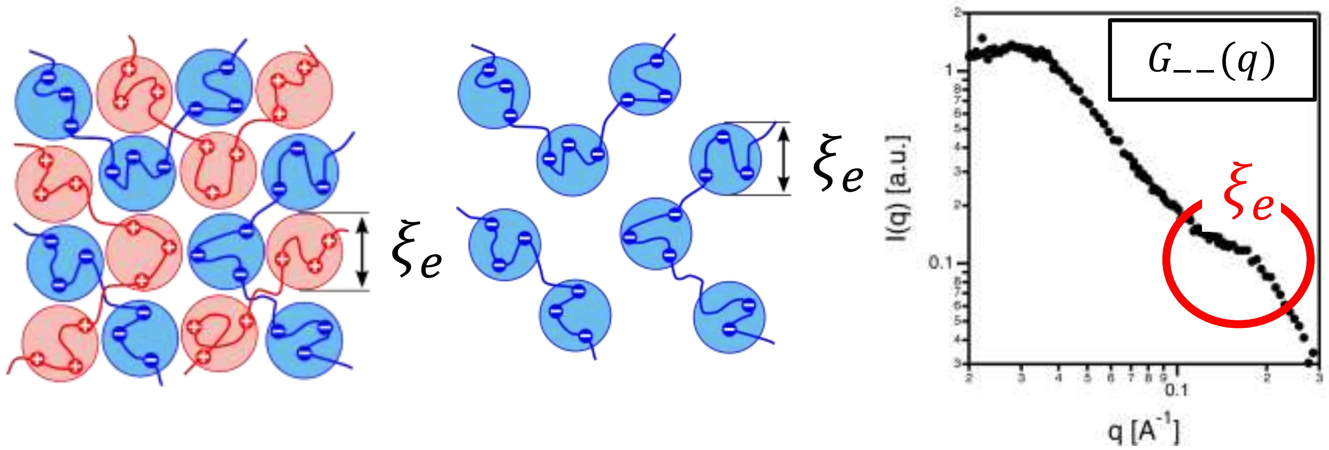
Skillful synthesis enables systematic studies in polymer physics

PEC phase behavior across broad range of charge densities



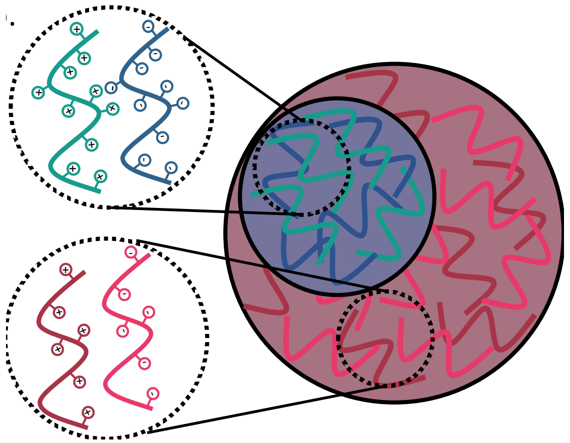
Neitzel, A. E.; Fang, Y. N.; Yu, B.; Rumyantsev, A. R.; de Pablo, J. J.; Tirrell, M. V.* *Macromolecules* **2021**, *54*, 6878.

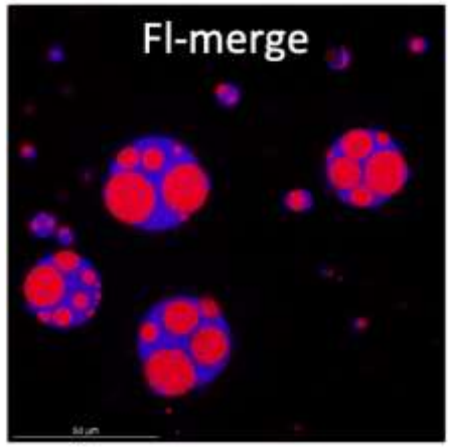
Scattering Evidence of Charge Correlations in Polyelectrolyte Complexes



Fang, Y. N.; Rumyantsev, A. R.; Neitzel, A. E.; Liang, H.; Tirrell, M. V.;* de Pablo, J. J.* **Under Review** at *Proc. Nat. Ac. Sci.*

Charge density-based pairing drives PEC multiphase formation



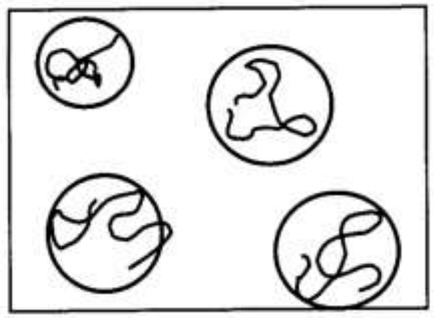


Agrawal, A.; Neitzel, A. E.;* … ; Karim, A.*; Tirrell, M. V.* **In preparation.**

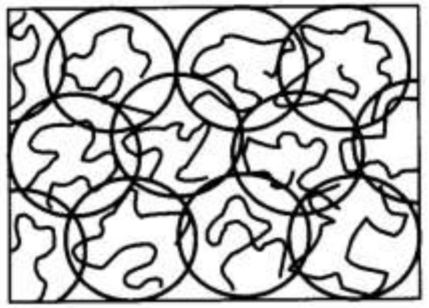
Our group will use synthesis as a tool to explore how morphology of charge-complexed polymers can be controlled over several length scales of structure and drive at new solid-state structures

Structure of polymer solution ⟷ Polymer solution properties

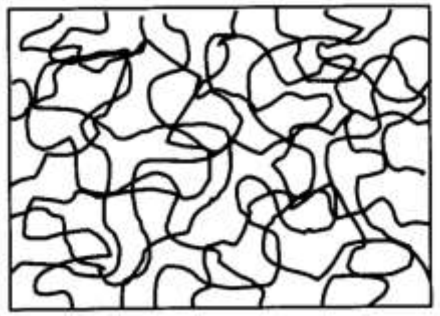
Scaling relationships: qualitative understanding of universal features of polymer solutions and melts



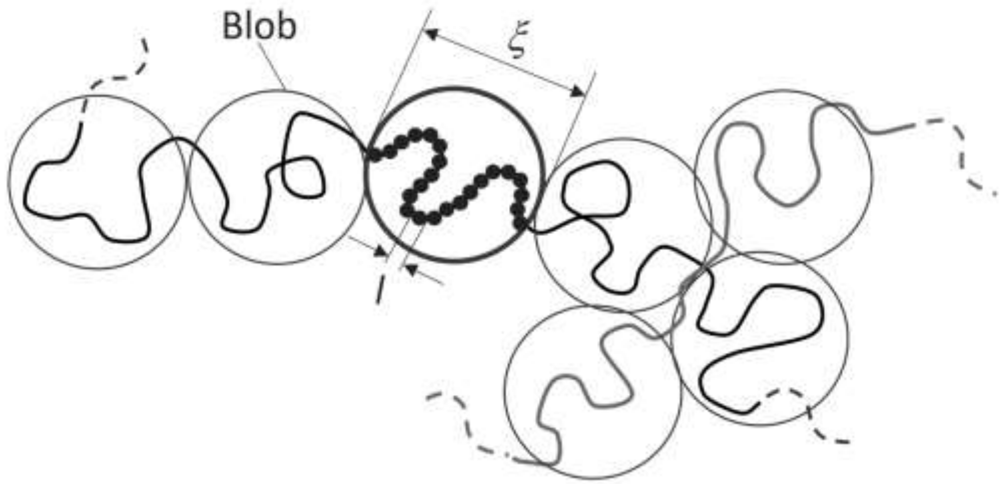
Dilute
$c < c^*$



Overlap
$c = c^*$



Semidilute
$c > c^*$



PECs are semidilute solutions, polymer concentration is above the overlap concentration c*

Pictured as overlapping blobs with correlation length ξ. Chain inside blob unaware of chains outside

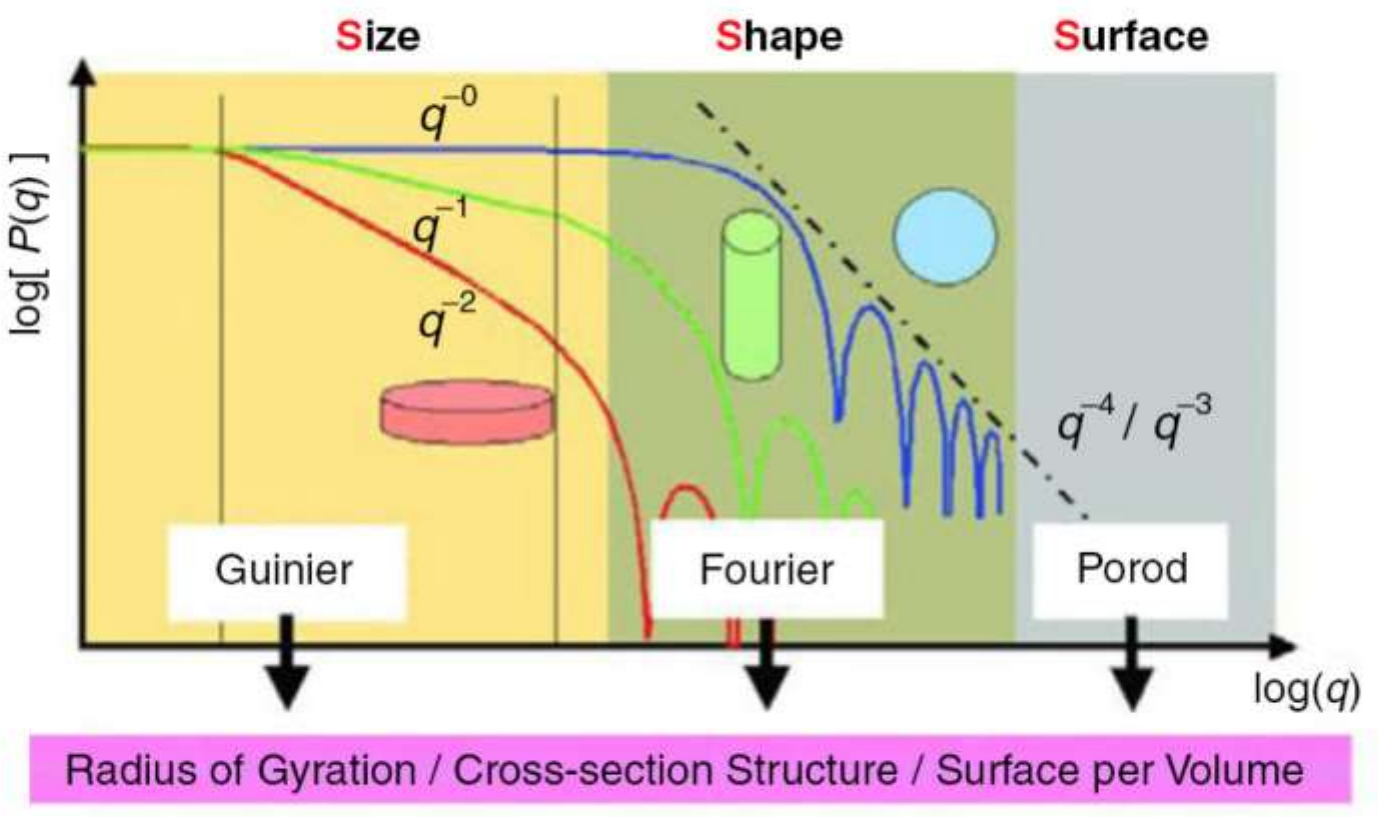
Chain conformation scales with solvent quality: $R_g \sim N^{\nu}$, where $\nu = \frac{1}{3}$ for bad, $\frac{1}{2}$ for θ, and $\frac{3}{5}$ for good solvent

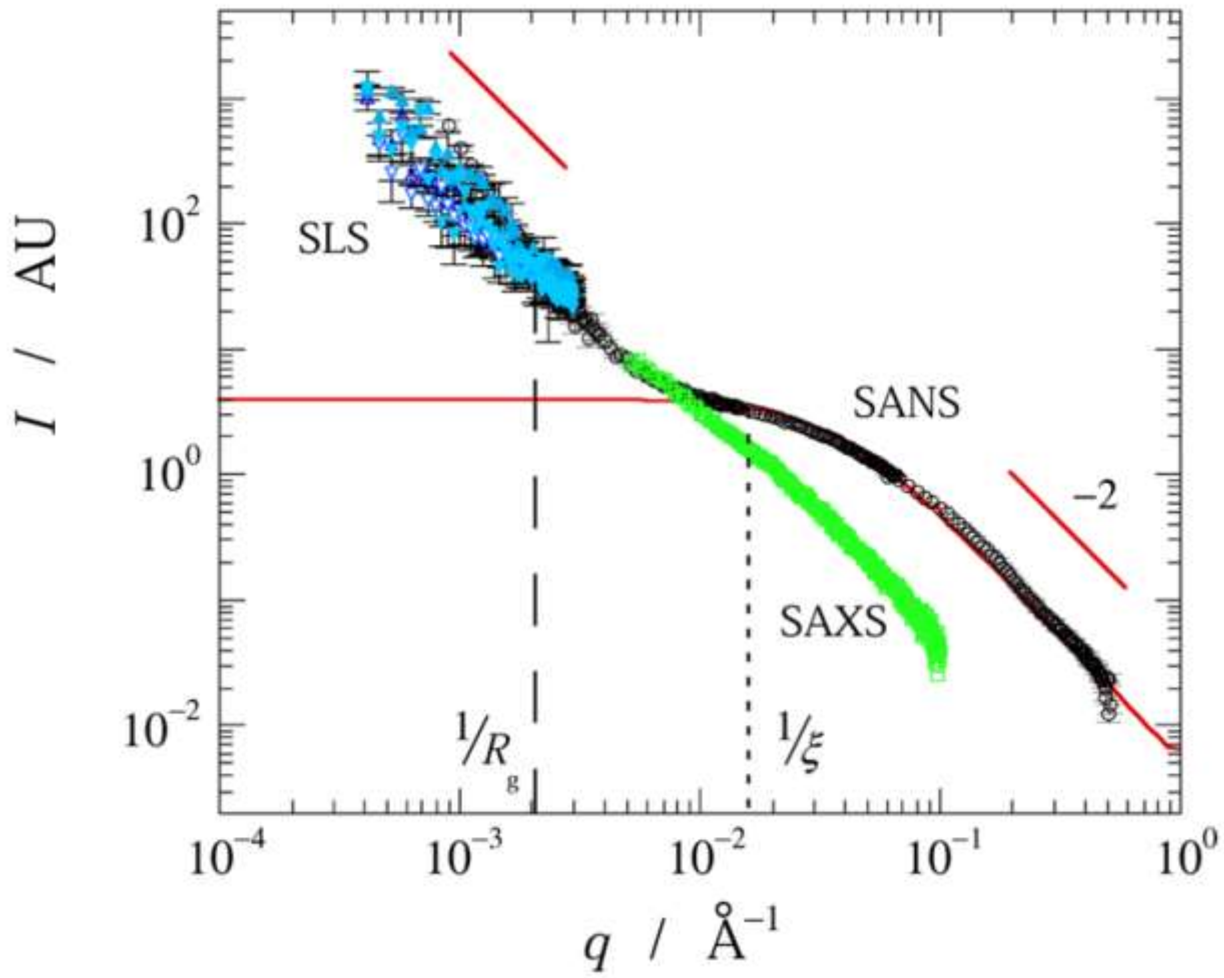
Each macromolecule is a chain of blobs following random walk statistics in 3D (“drunken sailor”)

Rubinstein, M.; Colby, R. *Polymer Physics*; Oxford University Press: Oxford, 2003.
https://polymerdatabase.com/polymer%20physics/BlobModel.html

Structure of polymer solutions is studied using scattering

Small Angle Scattering (SAS) from neutral, semidilute polymer solutions using X-rays or neutrons





Ornstein-Zernike form: $G_{tot}(q) = \frac{G_{tot}(q=0)}{1+(q\xi_E)^{1/\nu}}$

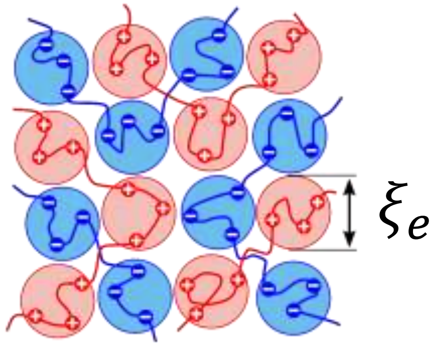
For $q = 1/\xi_E$: $G_{tot}(q) = \frac{G_{tot}(q=0)}{2}$

PECs are structurally similar to semidilute solutions of neutral polymers

Boldon, L.; Laliberte, F.; Liu, L. *Nano Reviews* **2015**, *6*, 25661. Spruijt, Leermakers,…Cohen Stuart; *Macromolecules* **2013.**

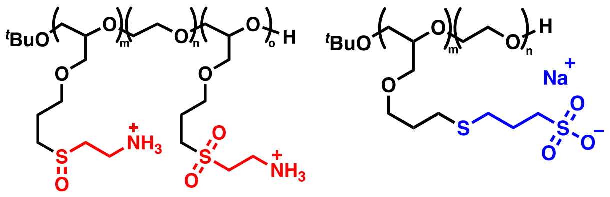
Important length scale in PECs: electrostatic blob size

Positional correlations in the spatial arrangement of *electrostatic* blobs: negative blobs are preferentially surrounded by positive blobs and vice versa

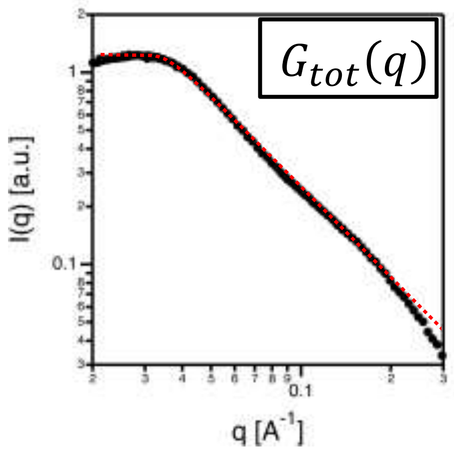


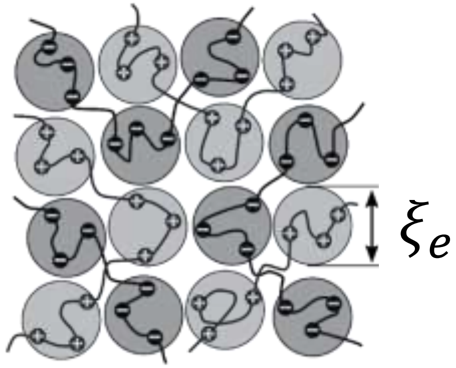
Experimental evidence for charge correlations in PECs still outstanding

SANS from anionic **and** cationic blobs



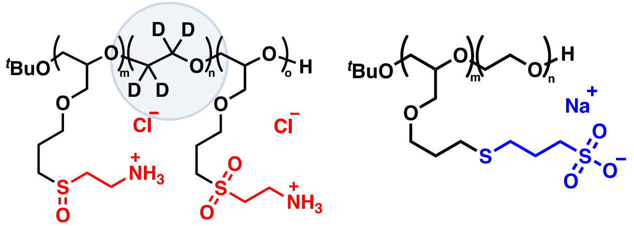
$N_n = 186$
$f = 0.30$



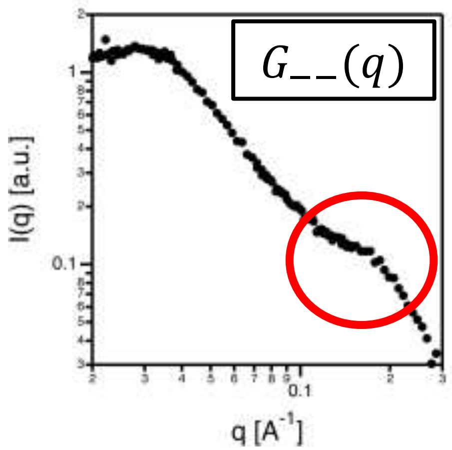


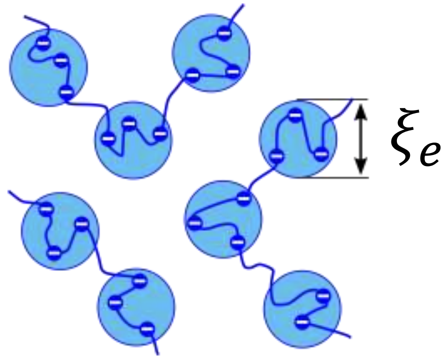
Ornstein-Zernike scattering profile

SANS from anionic blobs **only**



$N_n = 206$
$f = 0.31$





Shoulder due to charge correlations

First experimental evidence of positional charge correlations in polyelectrolyte complex coacervates

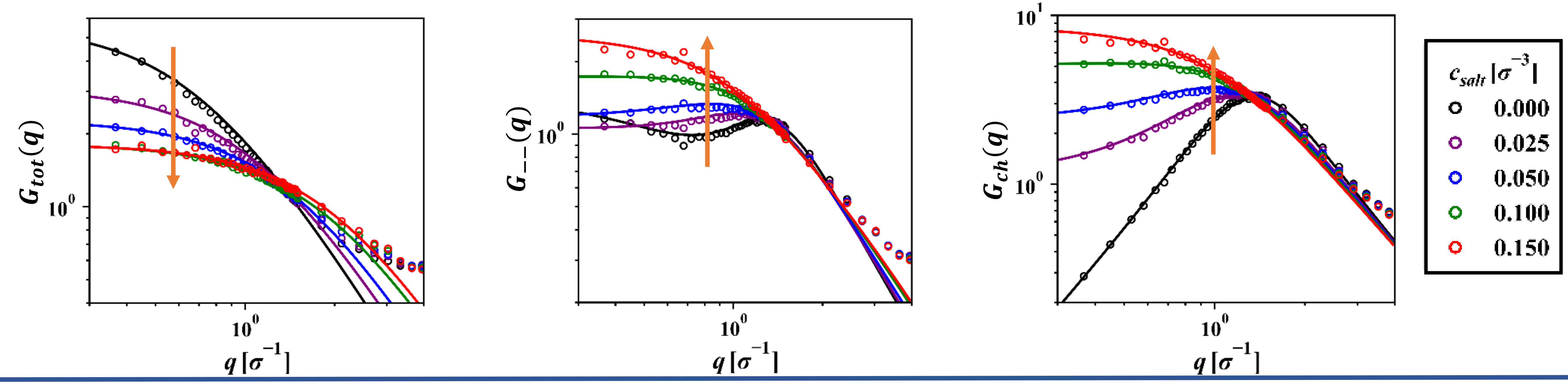
Big deal for theorists!

Borue and Erukhimovich. *Macromolecules* **1988**, *21*, 3240

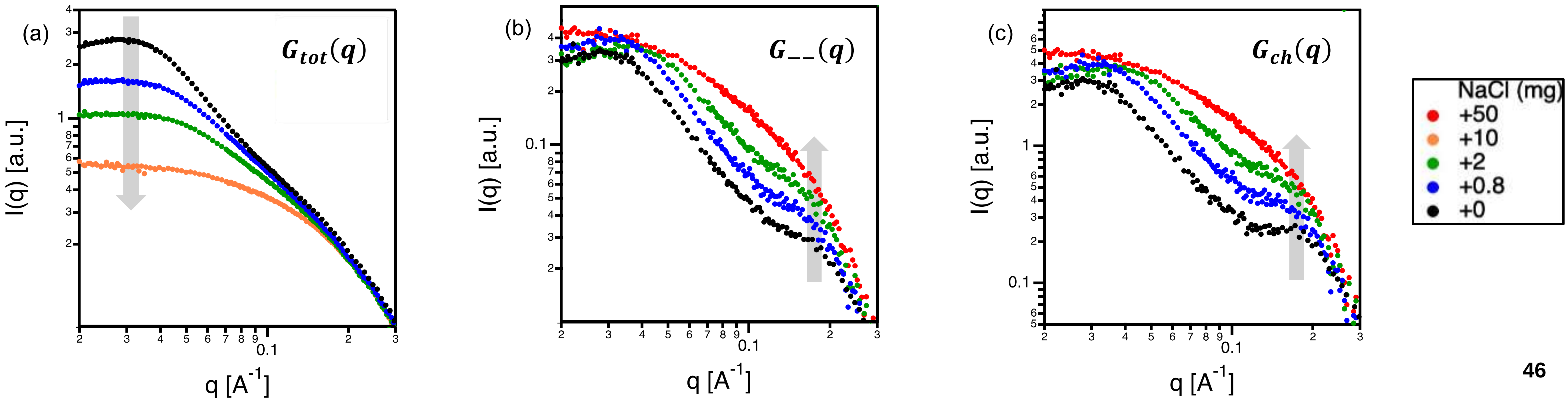
Theory, simulations, and experiment agree

Theory & Simulations

Scattering from All Polymers | Scattering from Polyanions | "Charge Contrast" Scattering

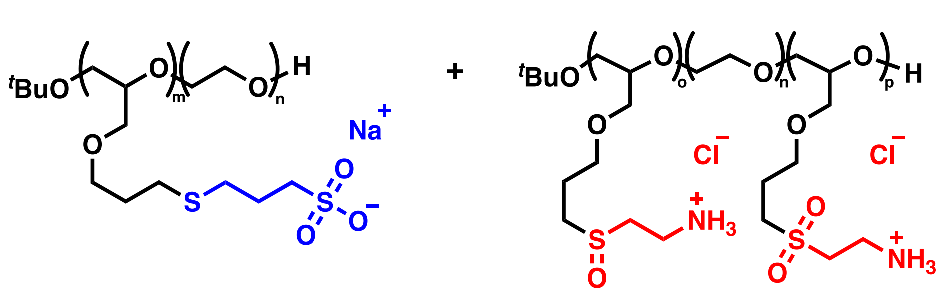


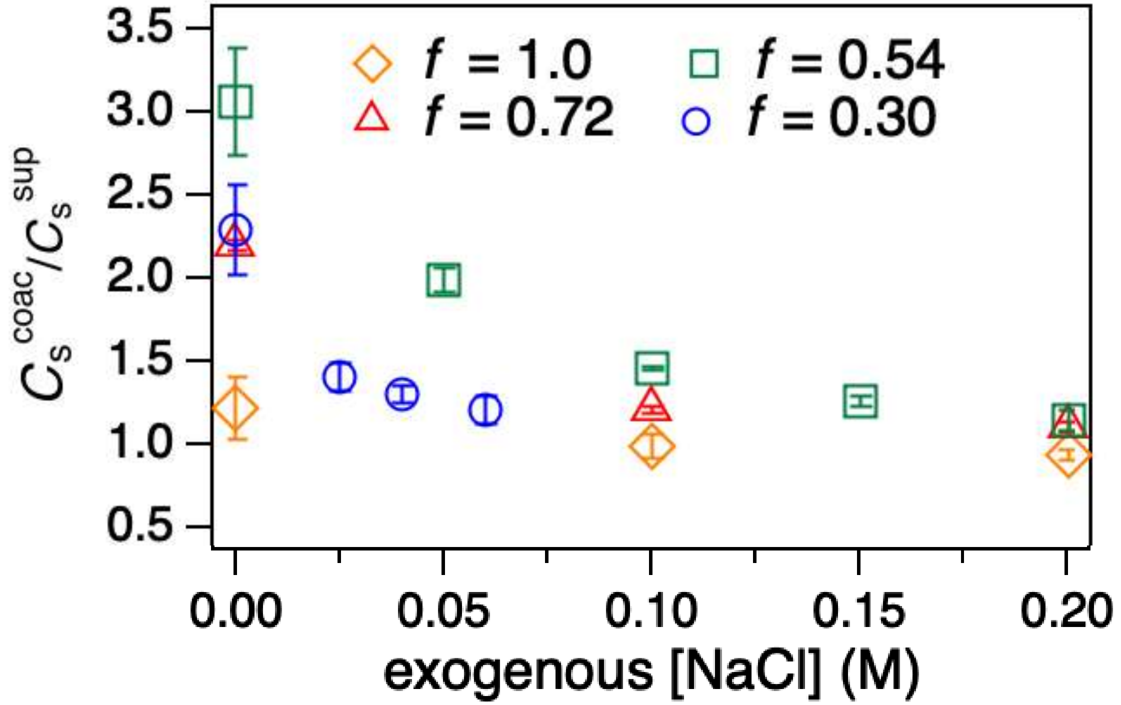
Experiment



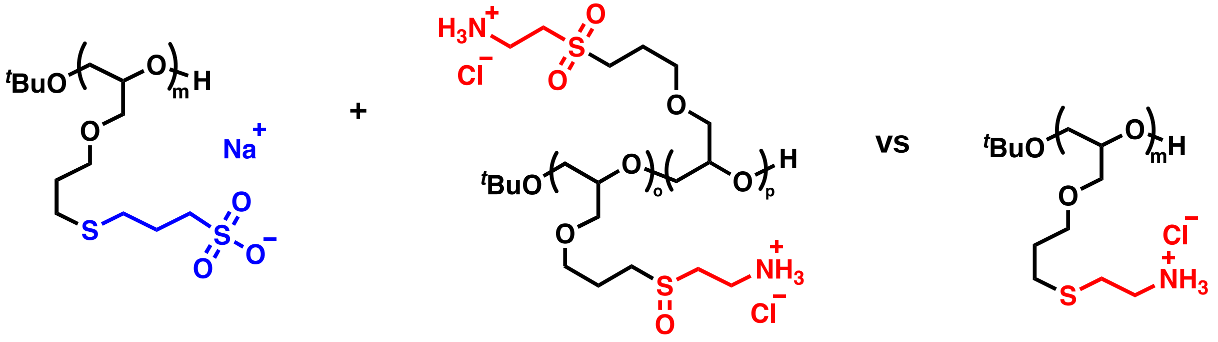
Salt partitioning impacted by f and polymer chemistry

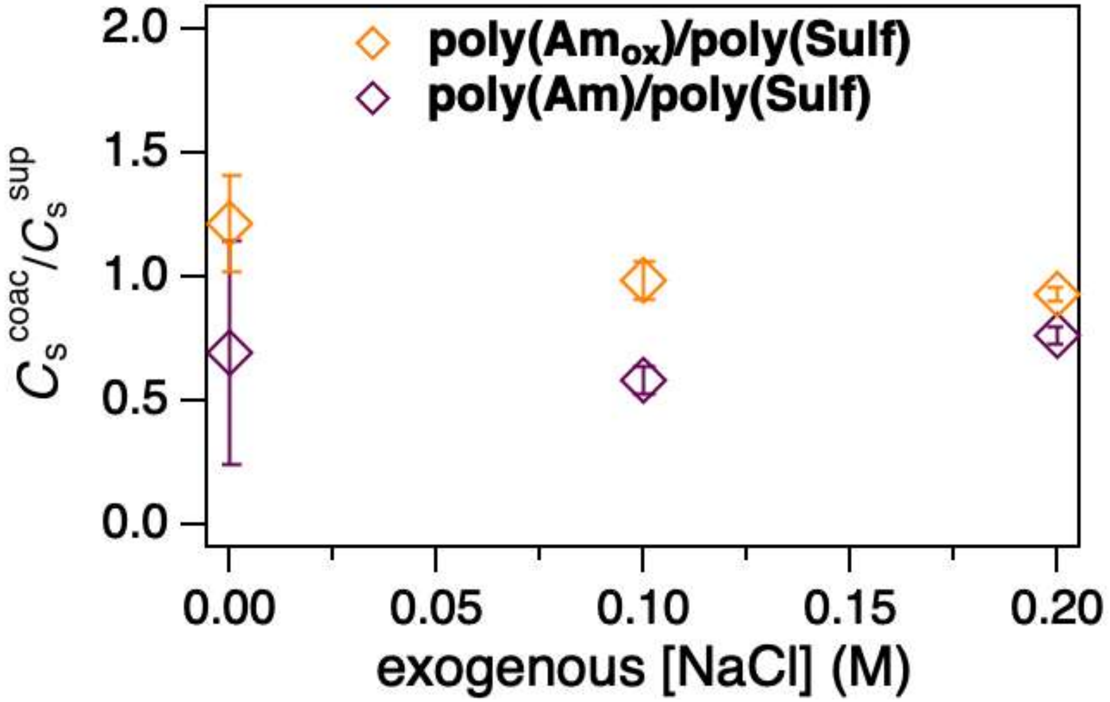
Changing charge density





Changing polycation hydrophobicity



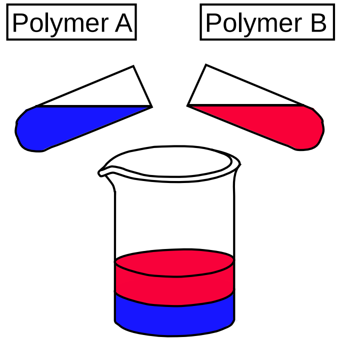


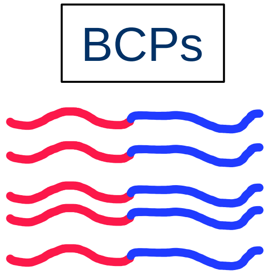
Charge density, polarity, and solvation ability impact salt partitioning

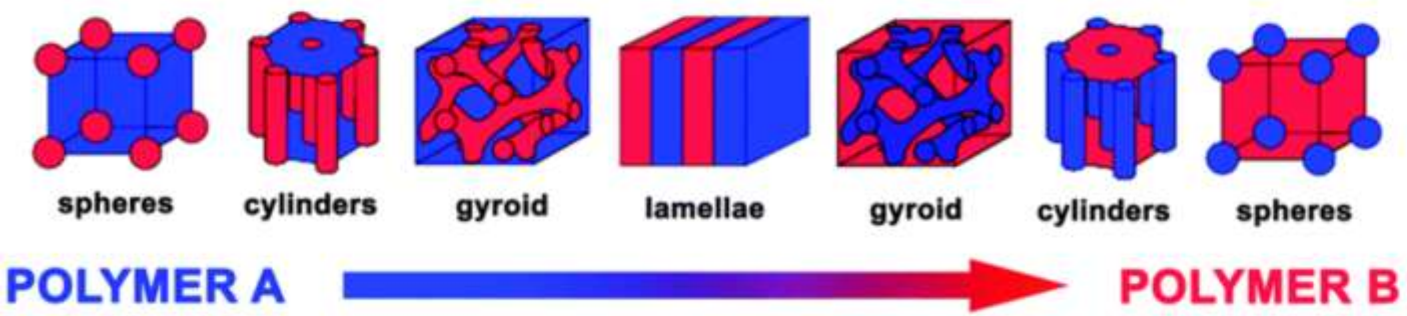
Salt partitioning is not universal

Quo vadis? I. Electrostatically stabilized microphases

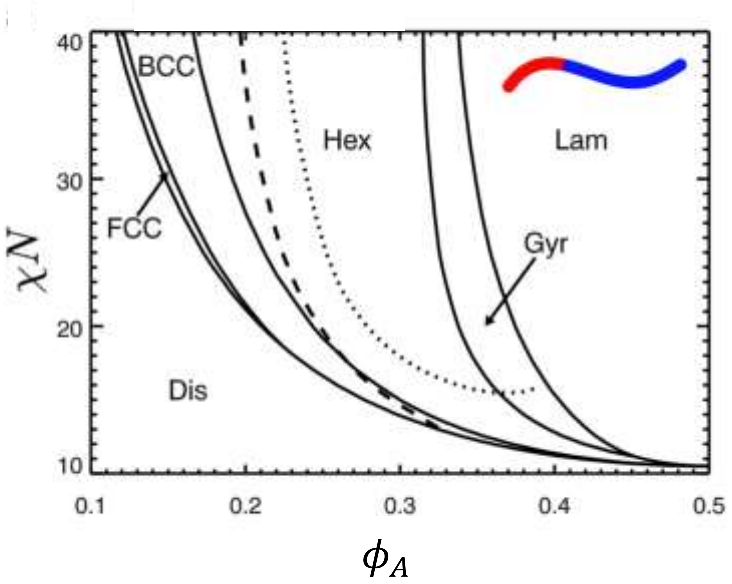
Block copolymers (BCPs) with incompatible A/B blocks assemble into ordered morphologies (microphase separation)







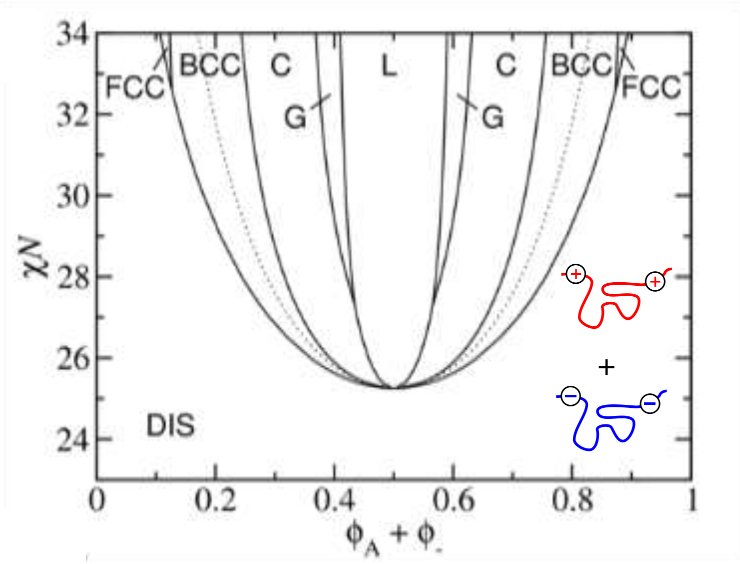
Important in development of battery electrolytes, mesoporous filtration membranes, and drug delivery vehicles



Morphologies are a function of length N, 'incompatibility' χ, and volume fraction ϕ

Phase behavior known for neutral BCPs

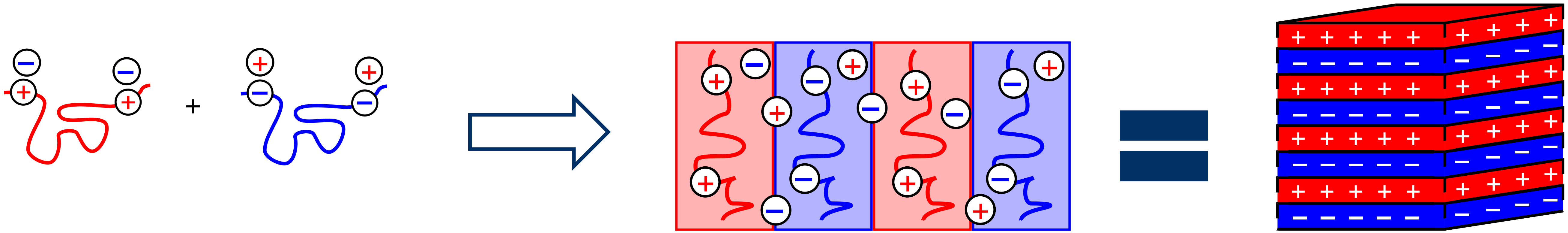
Similar phase diagram predicted for oppositely charged "homopolymers"



This theory has never been tested experimentally!

Nyrkova et al. *Macromolecules* **1994**, *27*, 4220. Grzetic et al. *Macromolecules* **2021.** Fodor et al. *RSC Adv.* **2017**, *7*, 6827. Dorfman, K. D. *Macromolecules* **2021**, *54*, 10251.

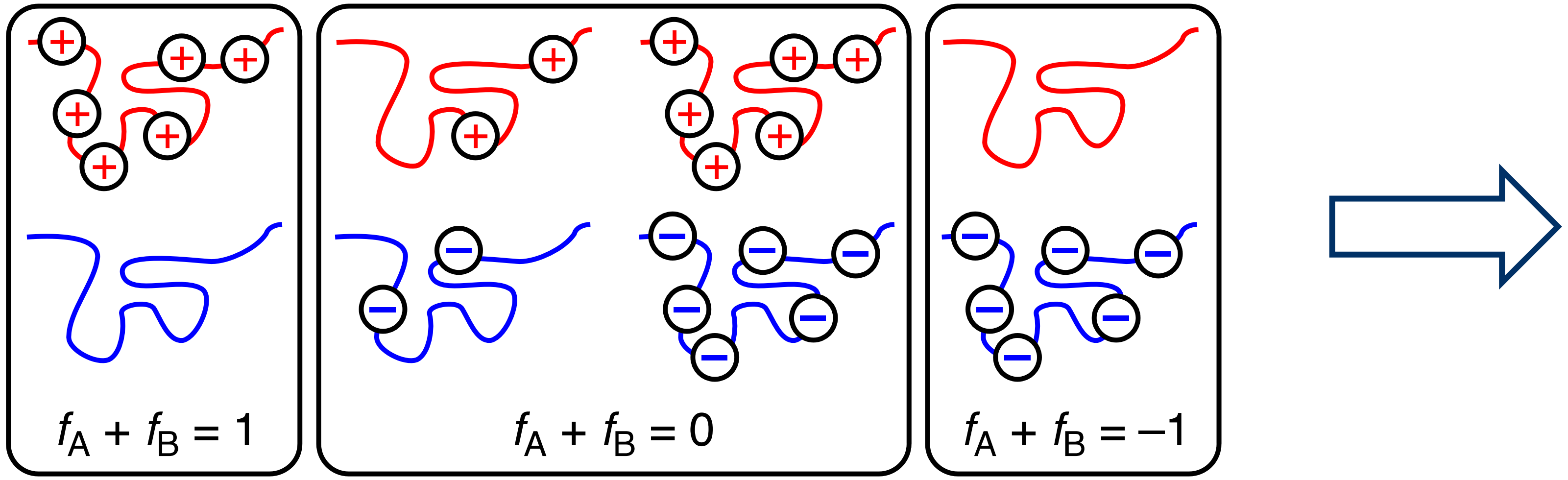
Quo vadis? I. Electrostatically stabilized microphases

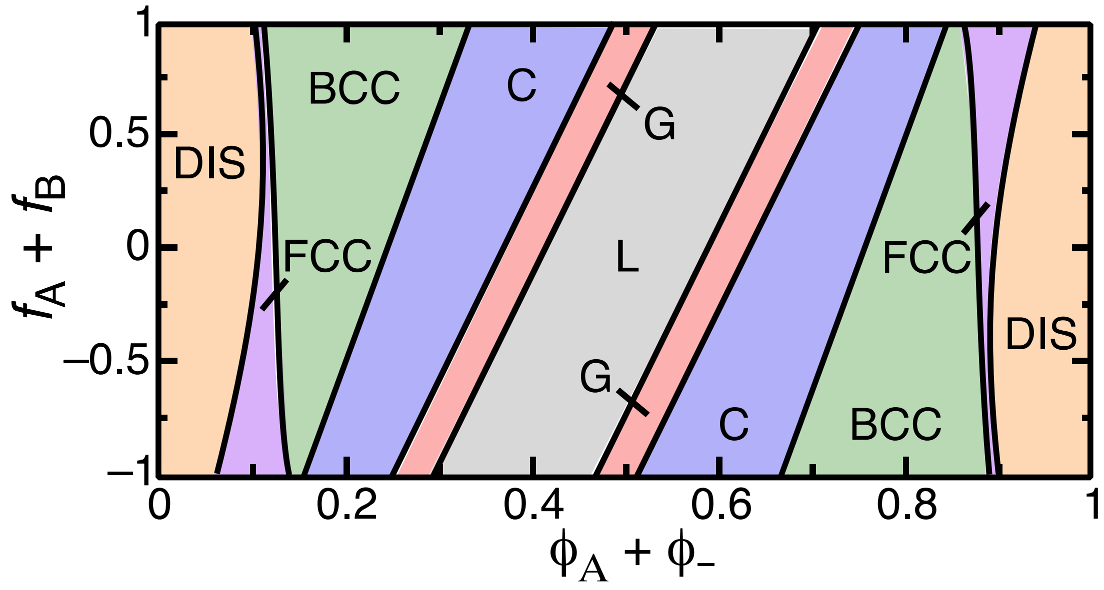


weakly charged, incompatible PEs

electrostatically stabilized microphases

charged microdomains





Quo vadis? II. Self-patterning Layer-by-Layer polyelectrolyte assemblies

Gero Decher

Université de Strasbourg/CNRS

Tailormade polyelectrolytes

“liquid-former”

low charge density
short (low N)
strongly hydrated ions

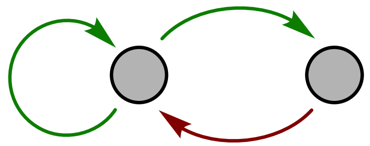
+

“solid-former”

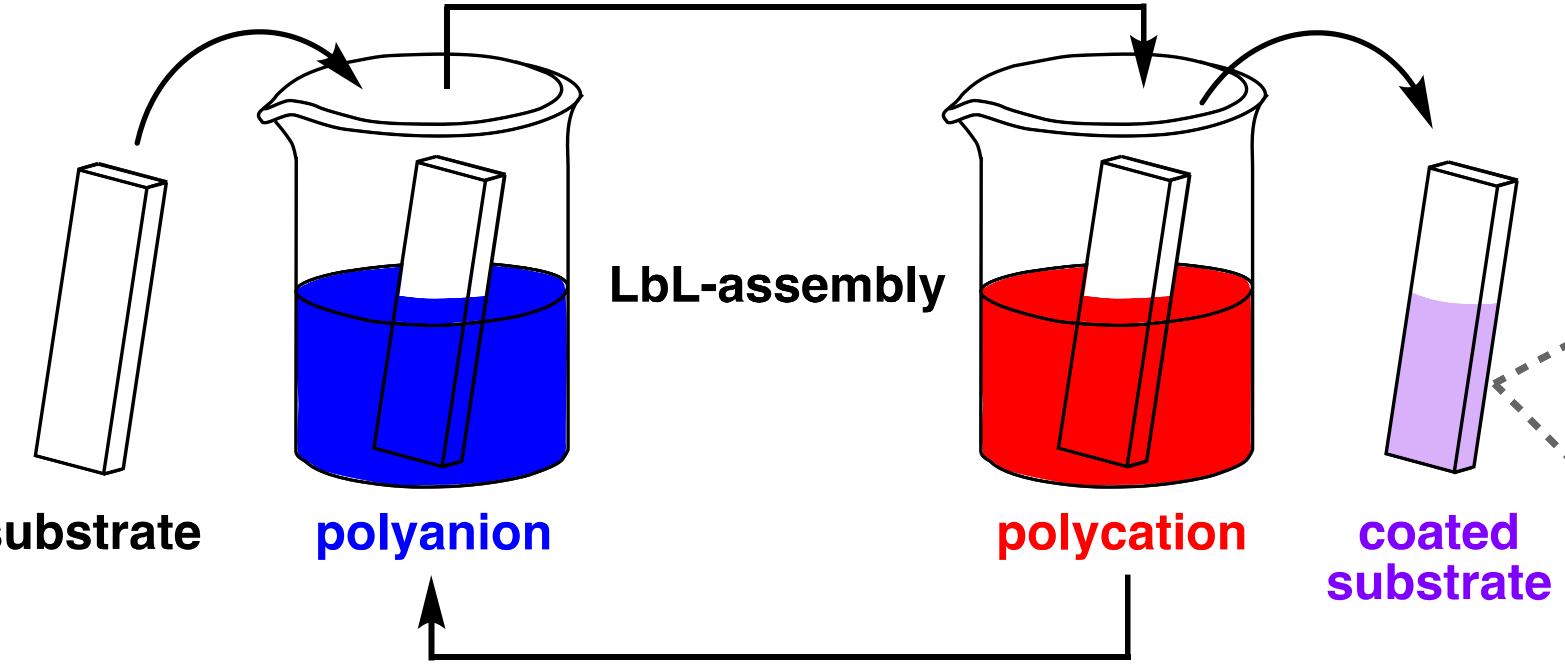
high charge density
long (high N)
weakly hydrated ions

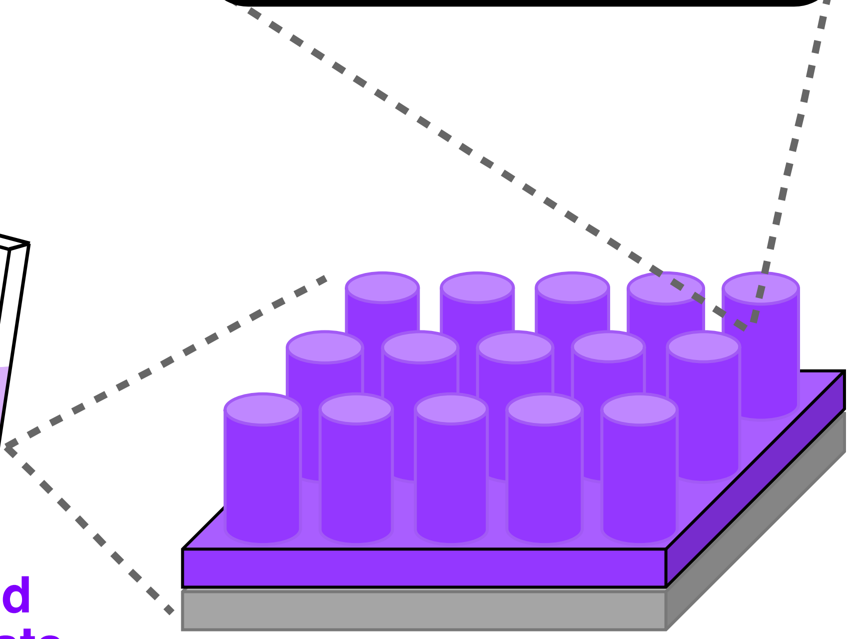
Self-patterning PEM films

Turing reaction-diffusion

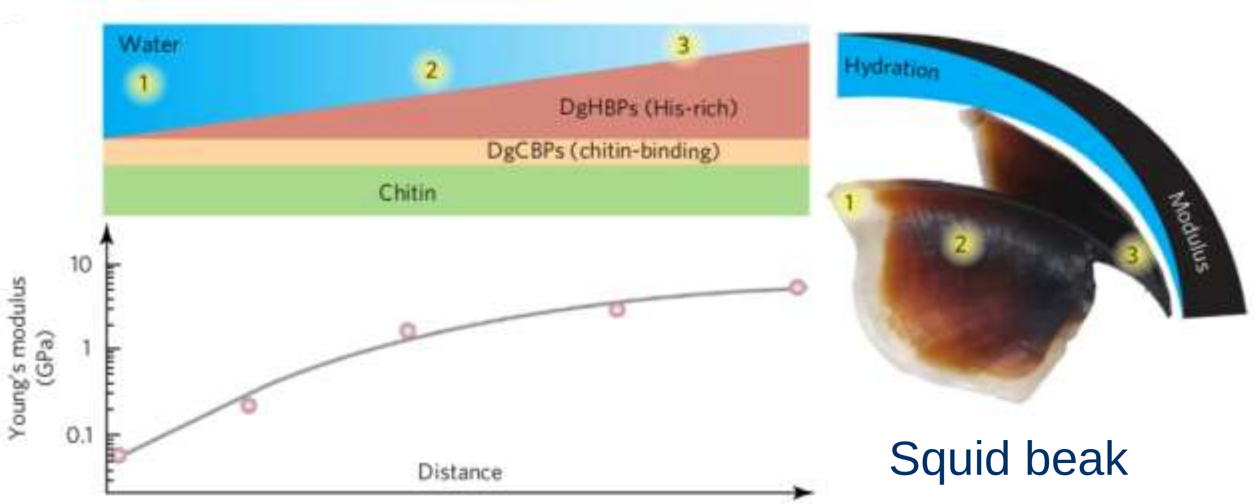


solid-liquid PEC interplay

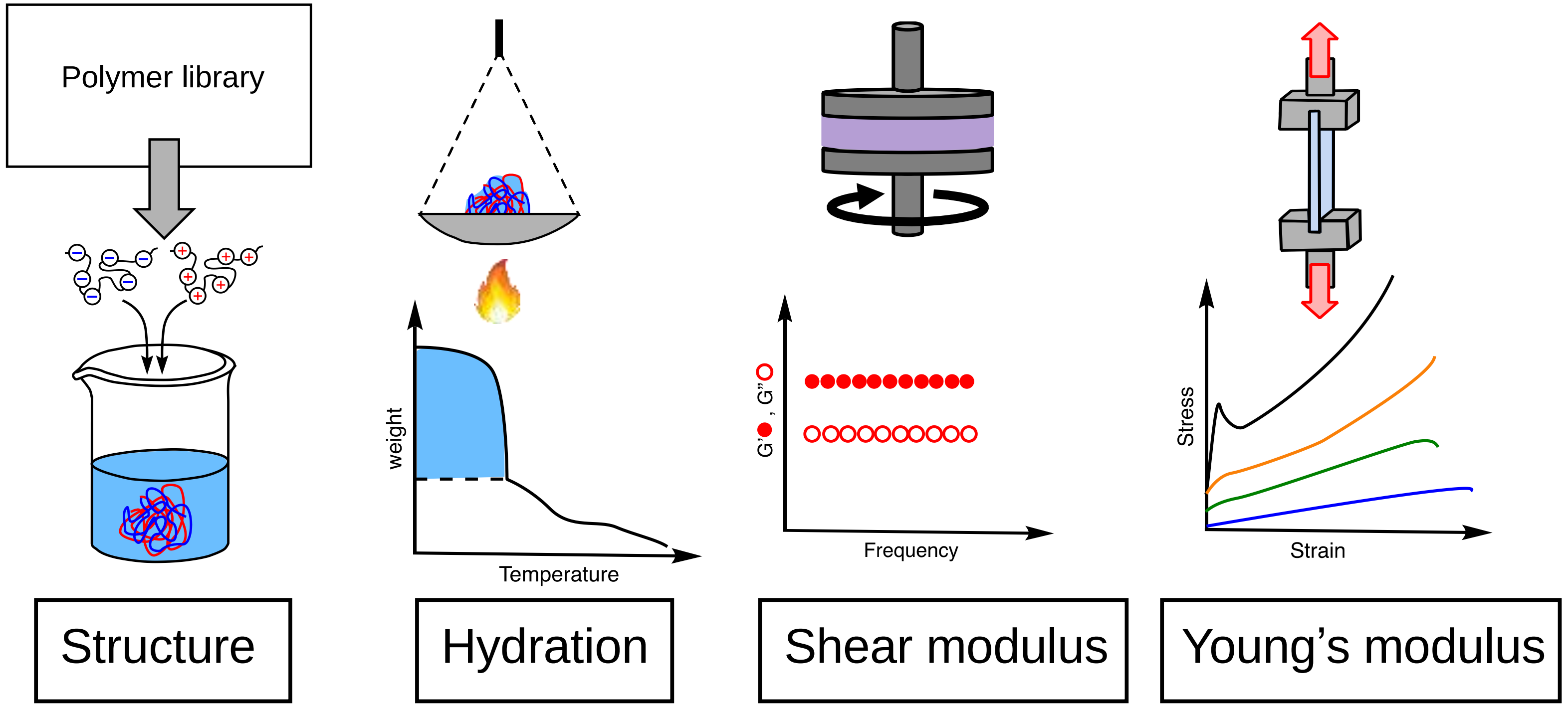


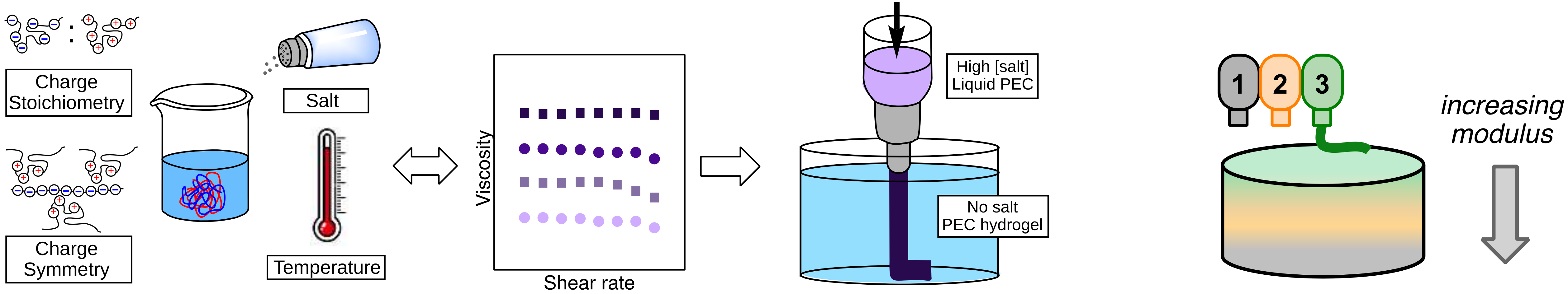


Quo vadis? III. Functionally graded materials synthesis

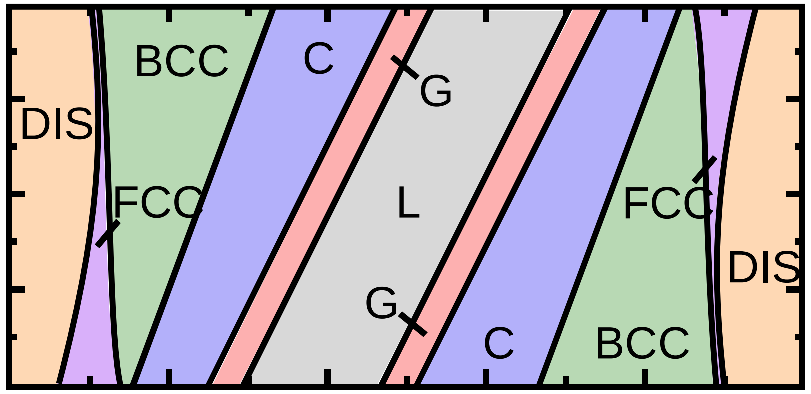


Squid beak

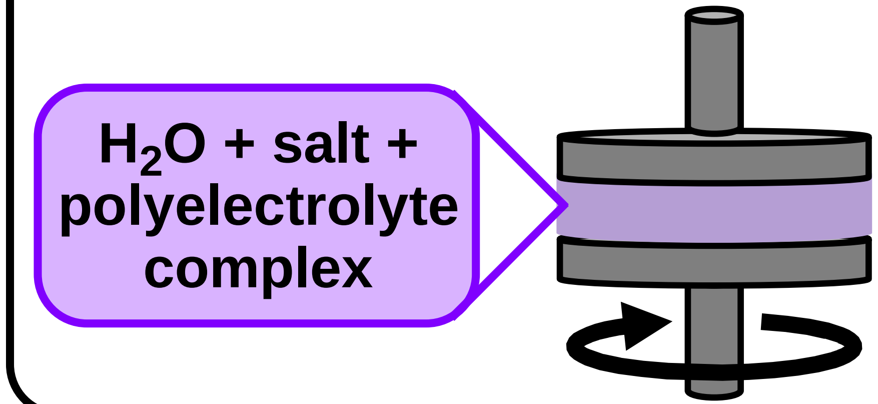


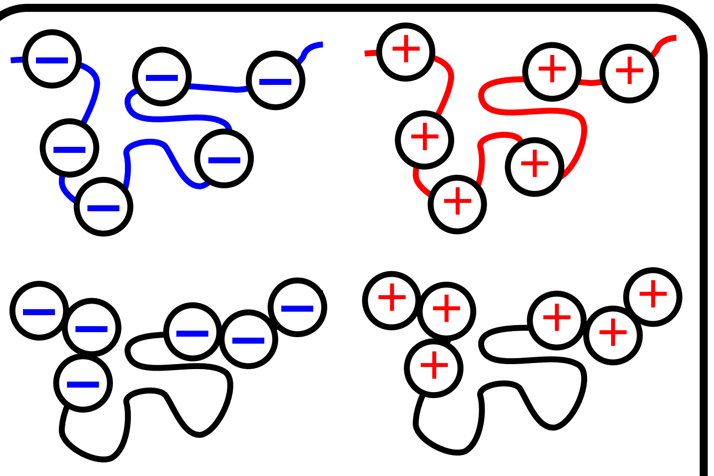


phase behavior and physics



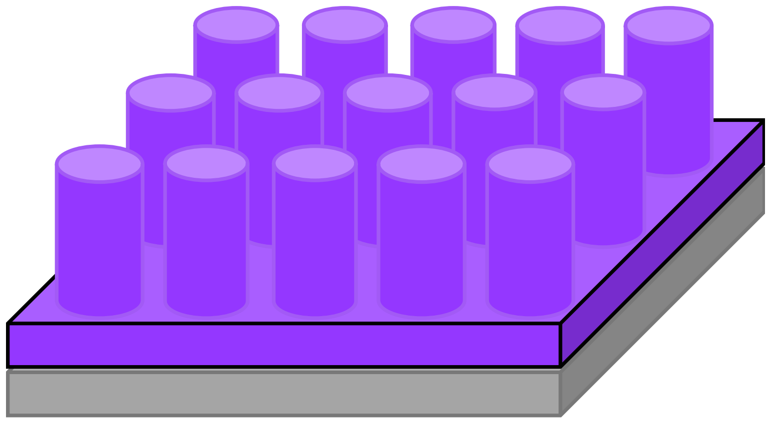
rheology and processing



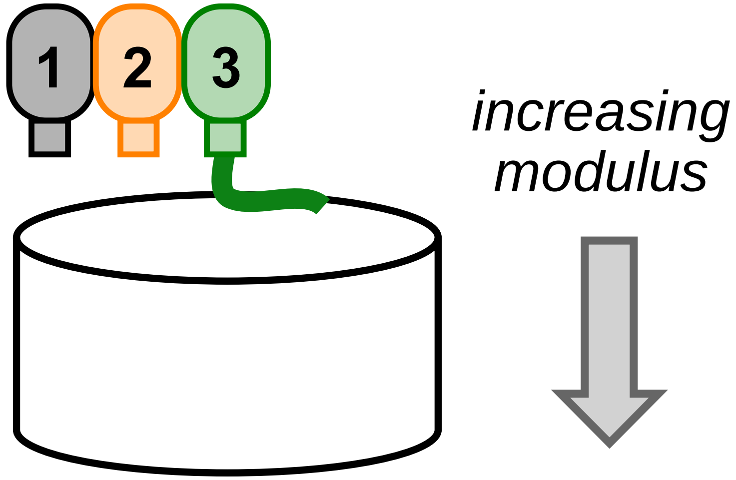


polyelectrolyte synthesis

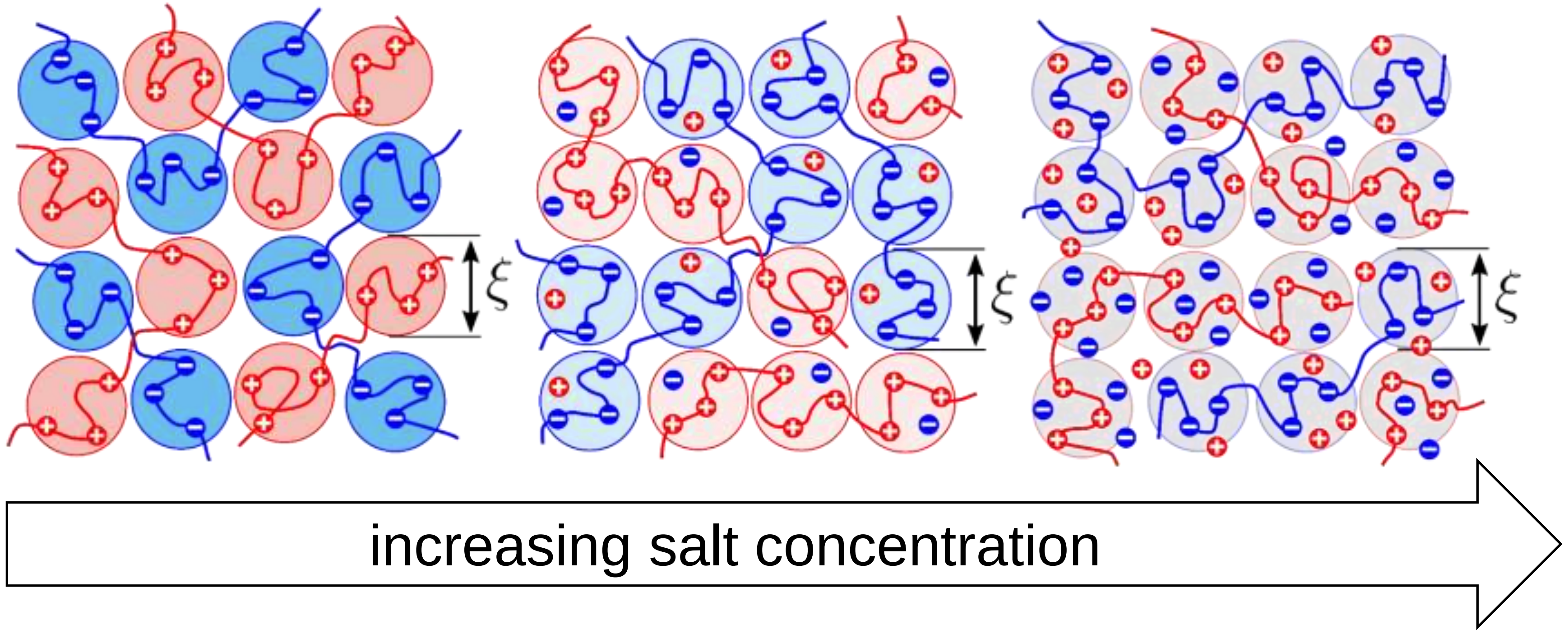
self-patterning PEM films

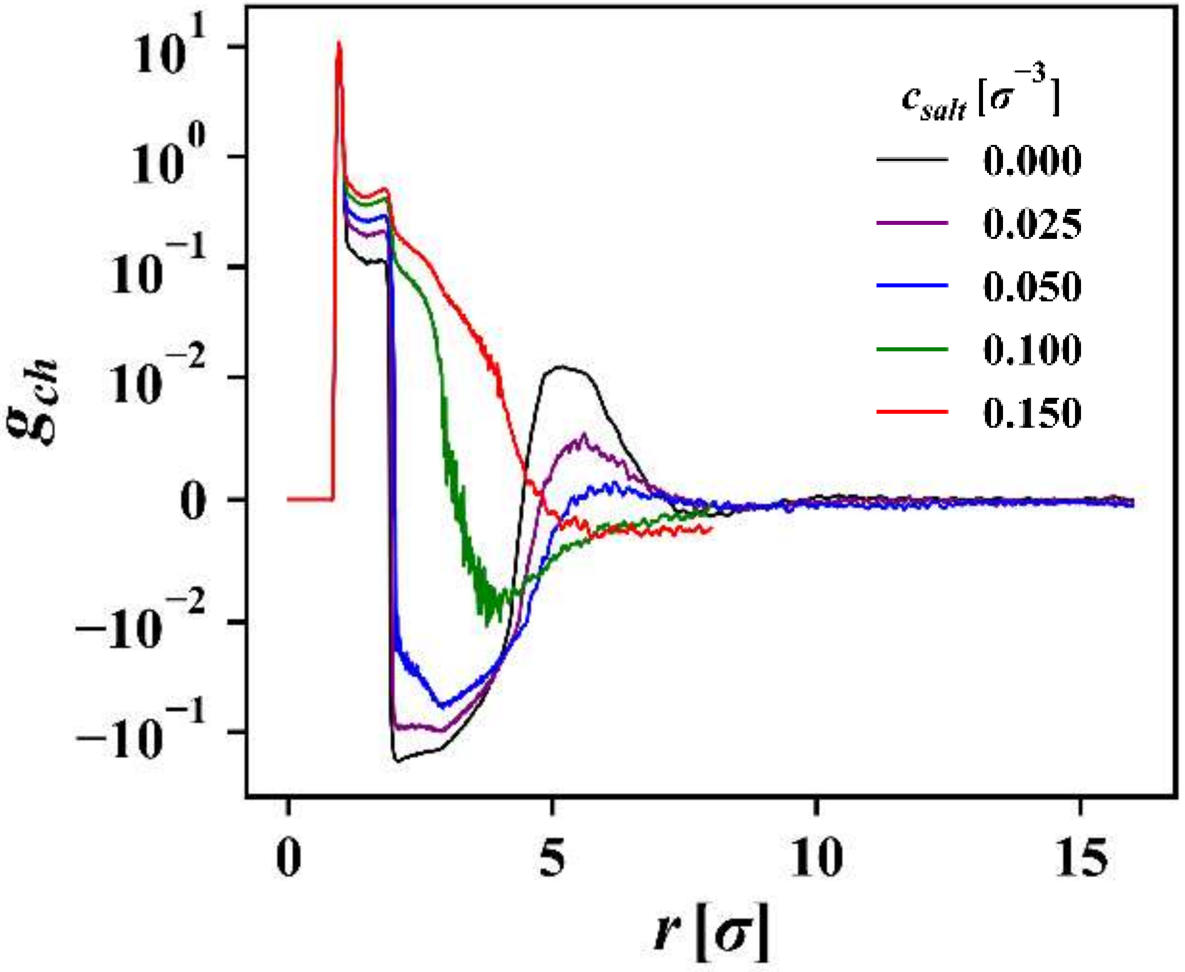


advanced materials with functional property gradients



Charge correlations between blobs decrease with ↑ [salt]





Salt screens Coulomb interactions between blobs

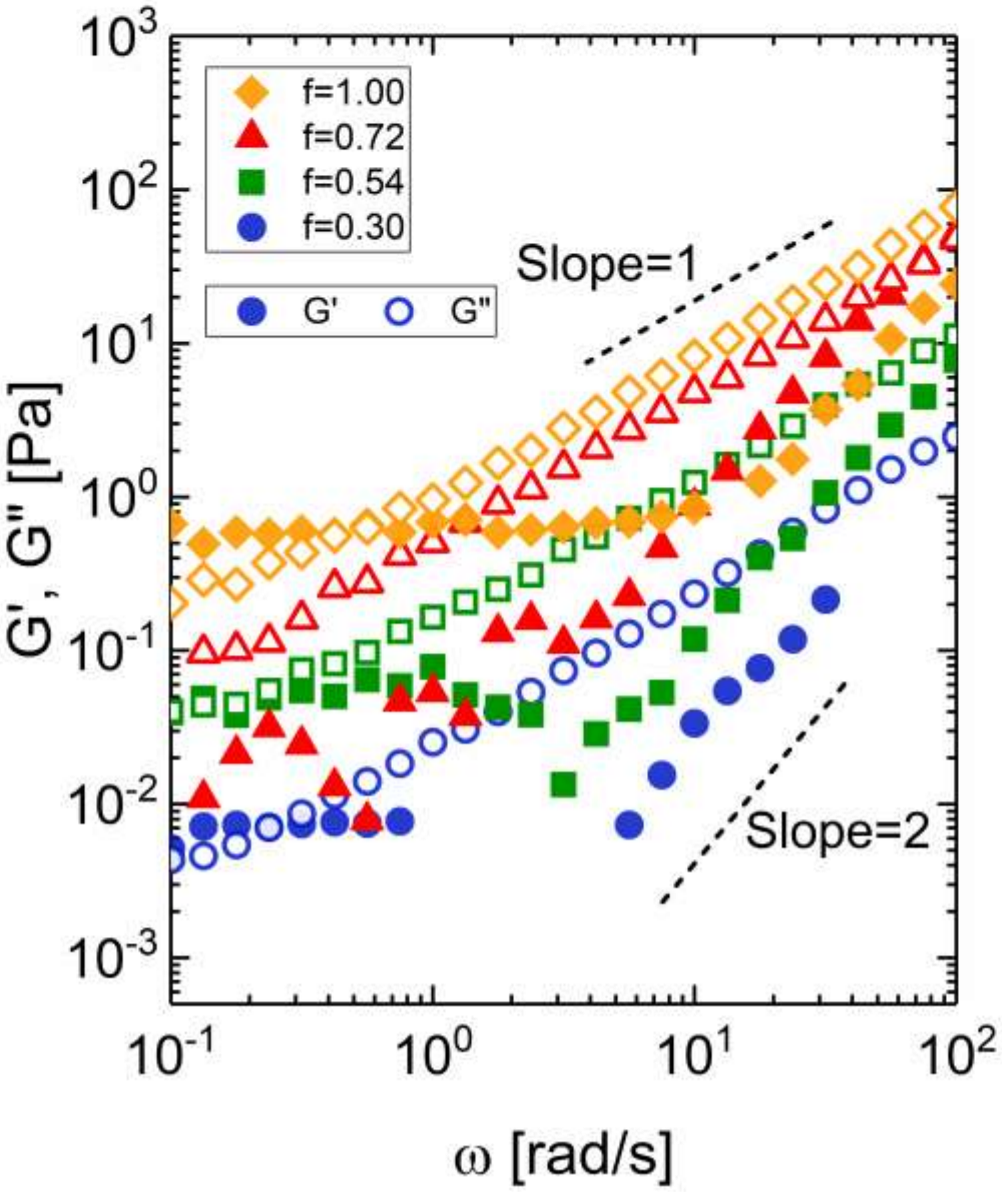
⇓

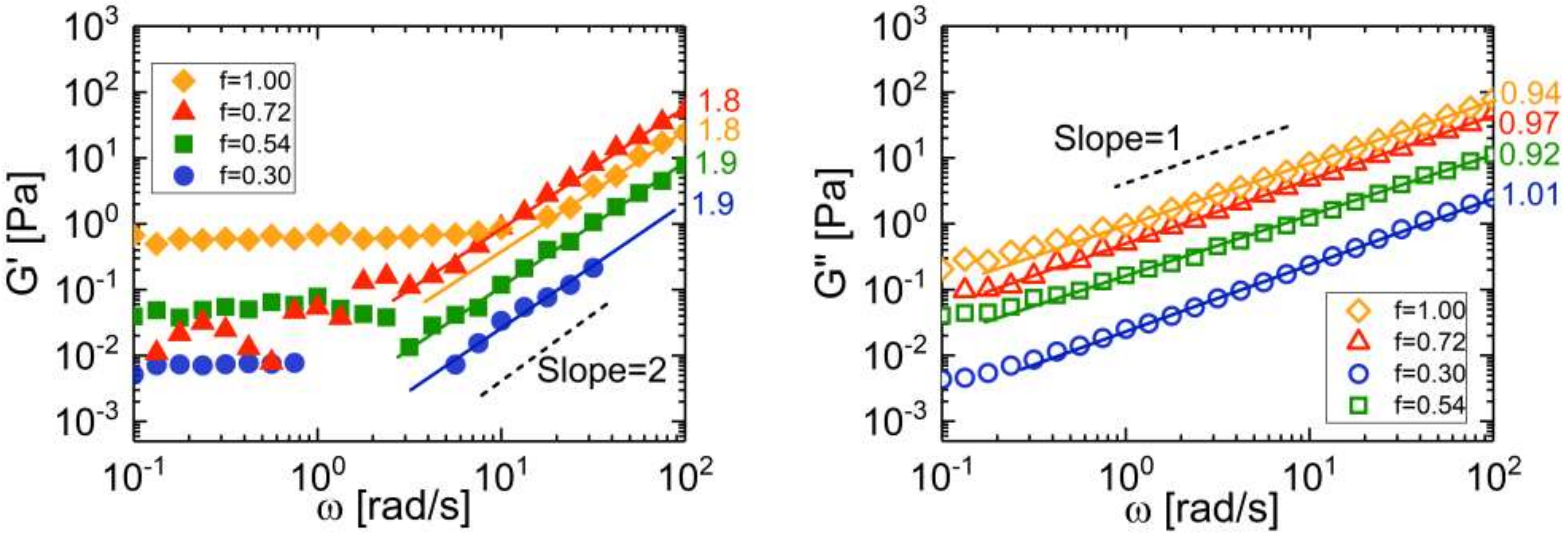
Positional correlations between polyanion and polycation blobs decrease & disappear

⇓

Scattering peak due to charge correlations decreases and disappears (OZ profile recovered)

PEC viscoelasticity as a function of charge density





Rouse dynamics for polymers in semidilute solution:

Storage Modulus: $G'(\omega) \equiv \frac{\rho RT}{M} \sum_{p=1}^{N} \frac{\omega^2 \lambda_p^2}{1+\omega^2 \lambda_p^2}$

Loss Modulus: $G''(\omega) \equiv \frac{\rho RT}{M} \sum_{p=1}^{N} \frac{\omega \lambda_p}{1+\omega^2 \lambda_p^2}$

Rouse dynamics are observed for all polyelectrolyte complexes in agreement with previous experiments