

Understanding gelation of gluten proteins thanks to neutron and X-ray scattering

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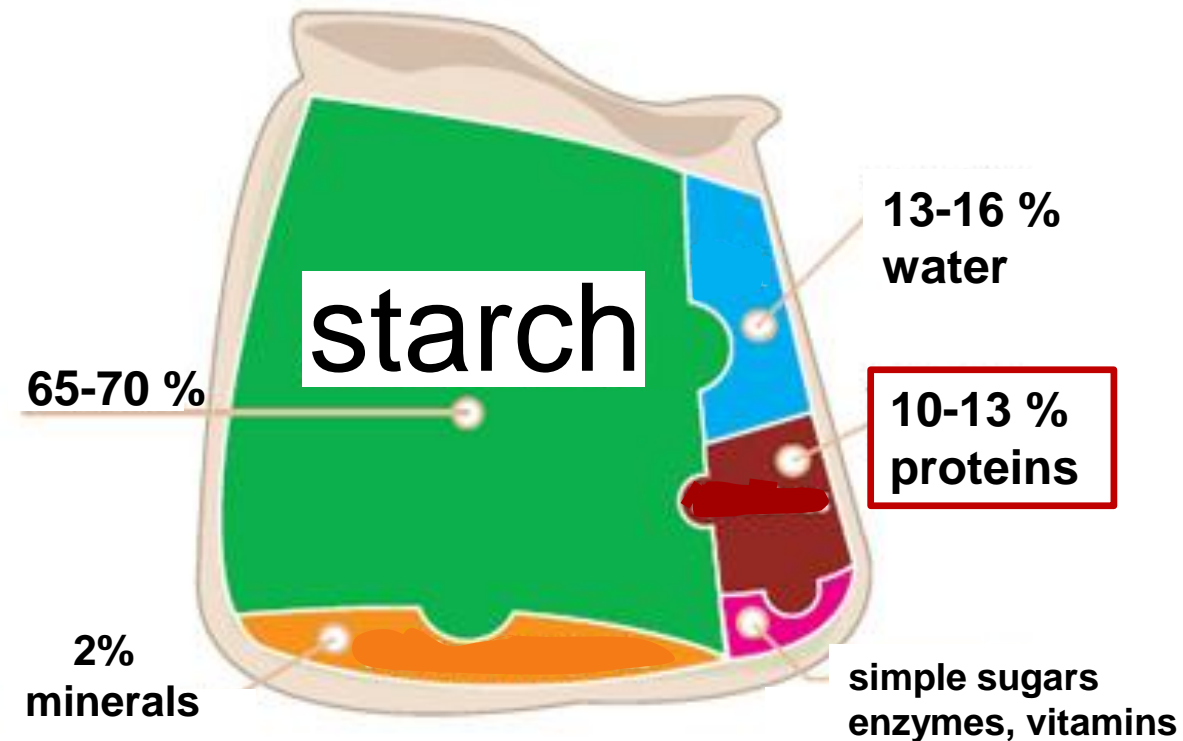


Gluten



PROTEIN FROM WHEAT GRAIN

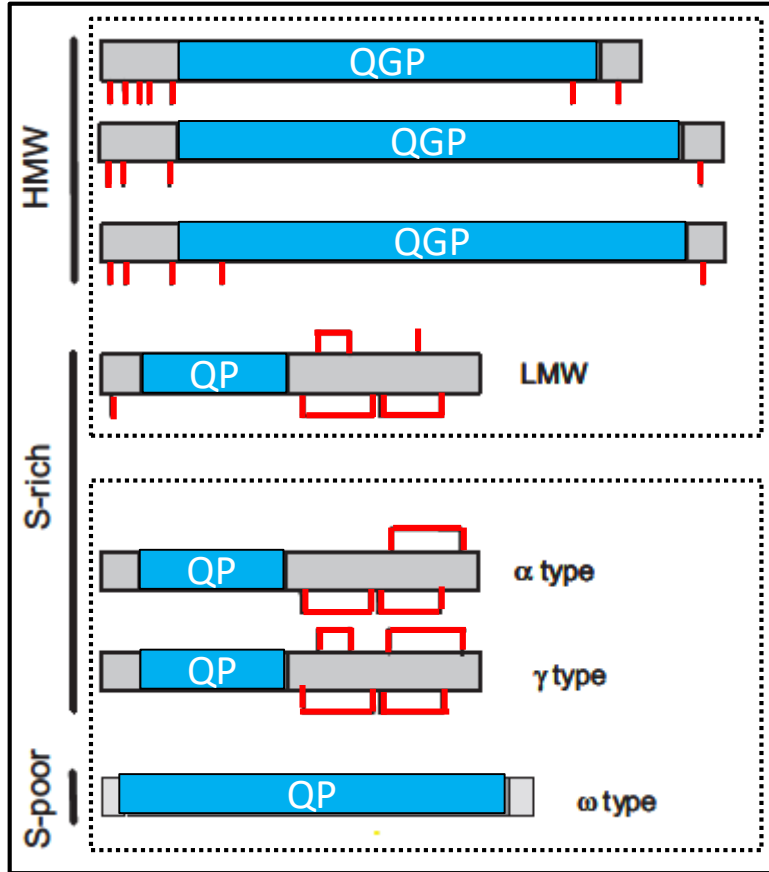
A VISCOELASTIC NETWORK



Unique
but very complicated

Gluten Proteins

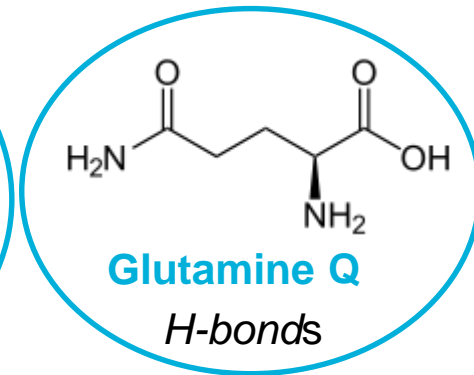
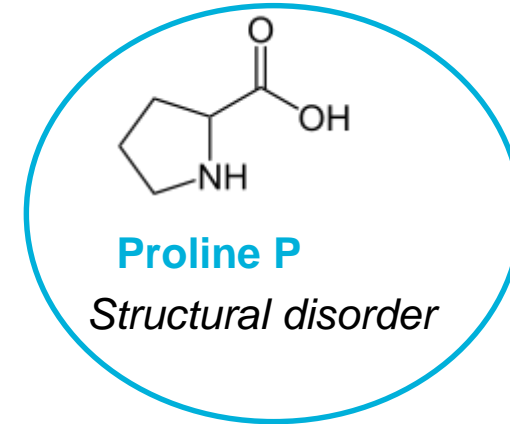
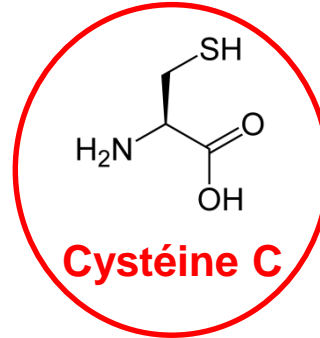
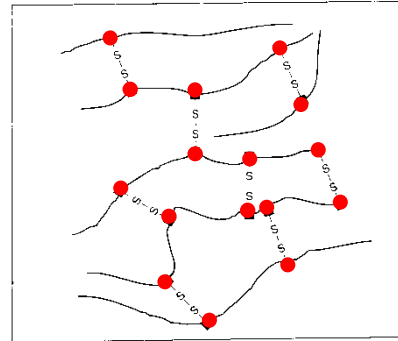
More than 60 polypeptides ...



Glutenin

Gliadin

⇒ Disulfide bonds
(intra or inter-molecular)

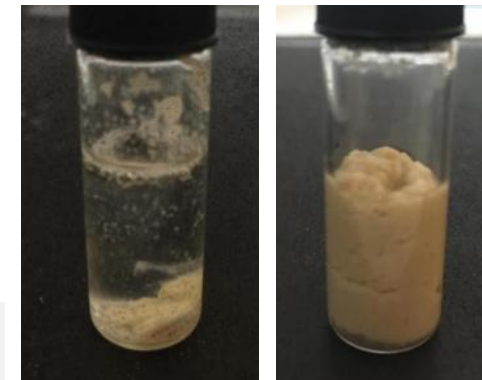


**INTRINSICALLY
DISORDERED**
repetitive domain

Subunits M_w 30 000 - 100 000g/mol

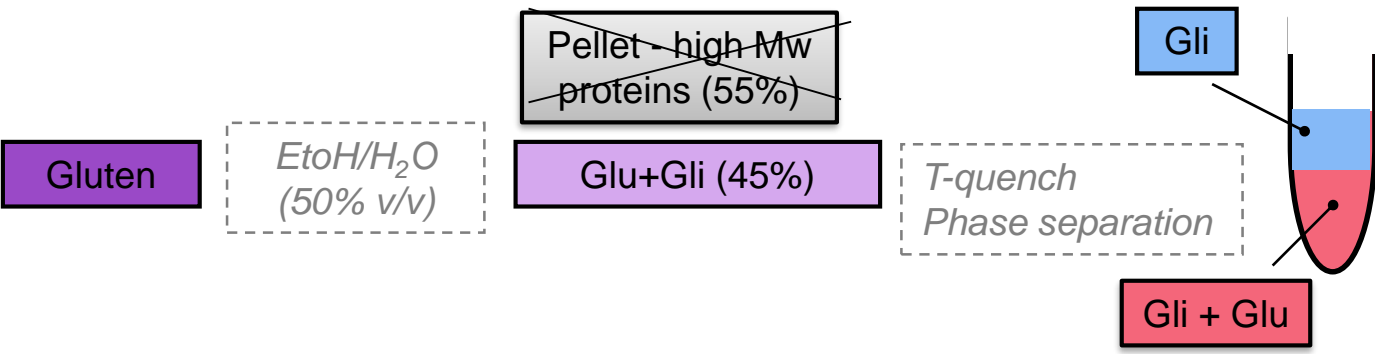
Polymer of glutenin (S-S bonds)
 M_w up to several 10^6 g/mol

NOT SOLUBLE IN WATER

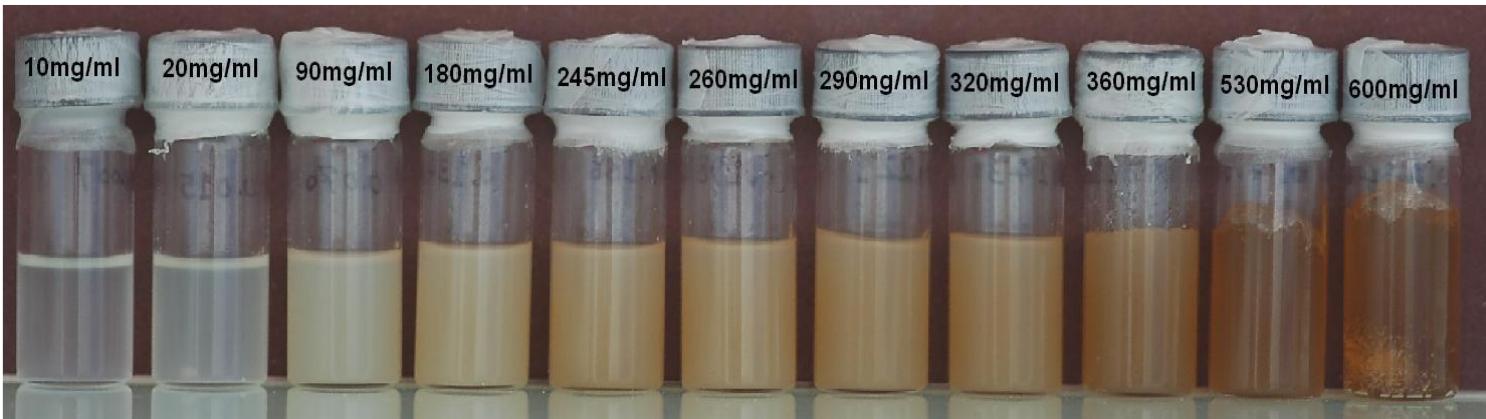


Model Systems

EXTRACTION

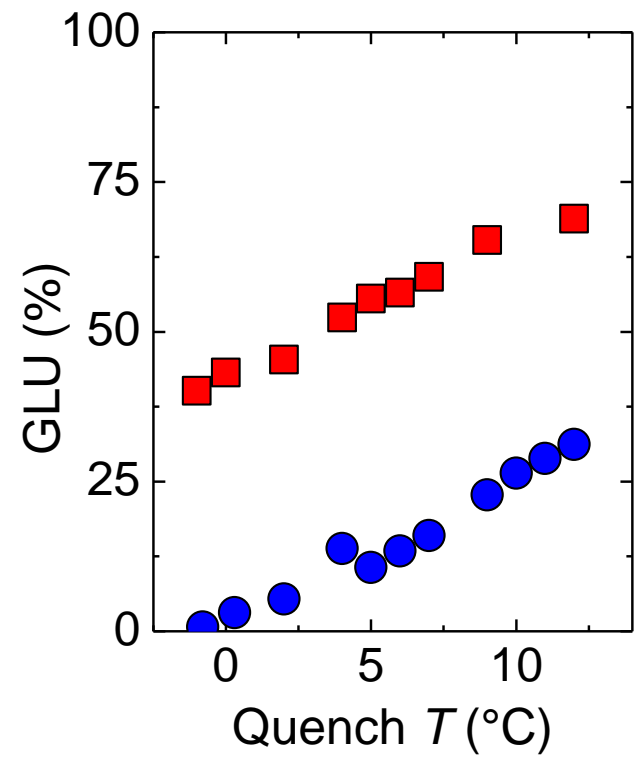


GLUTENIN / GLIADIN 50/50 FRACTION



in water/ethanol (1/1)
Glu=50%

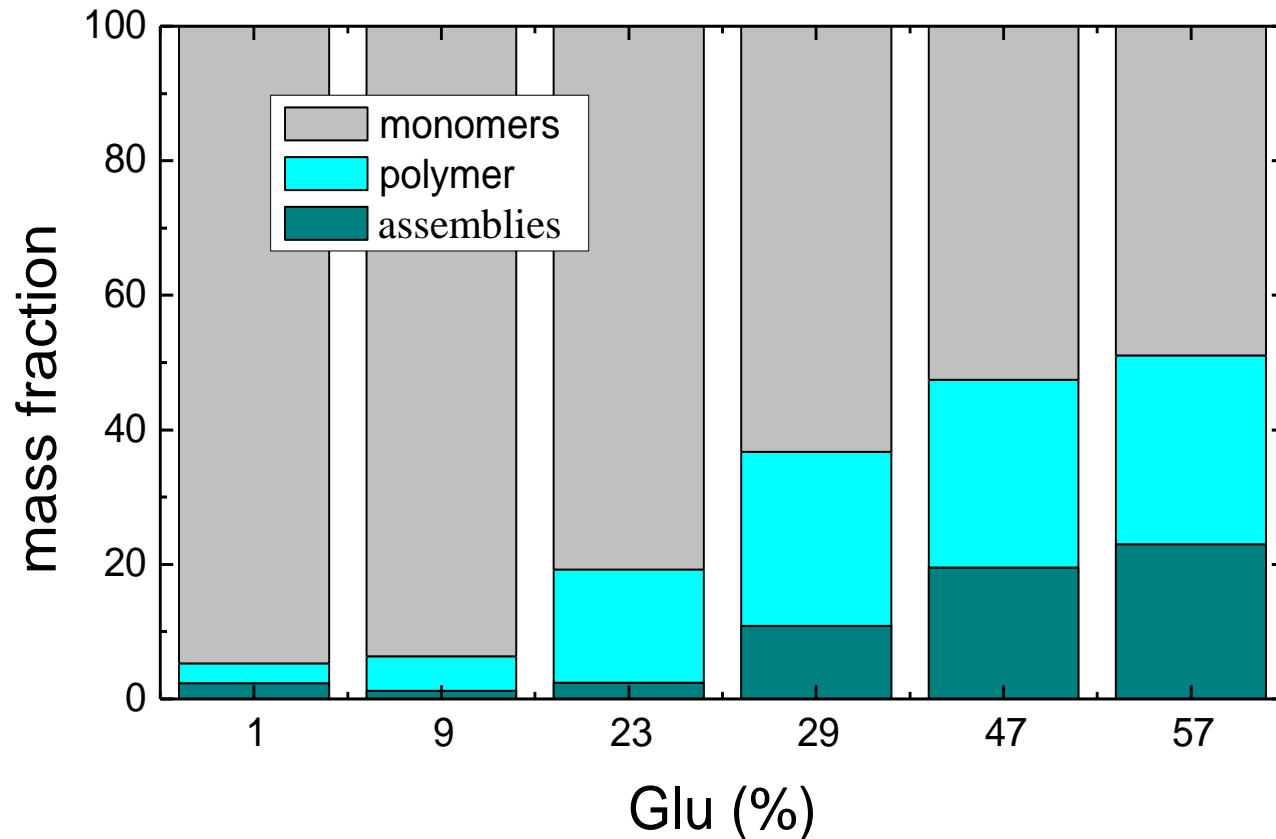
- Homogeneous samples in a broad range of concentrations
- Gels at high concentrations




$1\% \leq Glu \leq 70\%$

Dilute samples

ASYMMETRICAL FLOW FIELD – FLOW FRACTIONATION

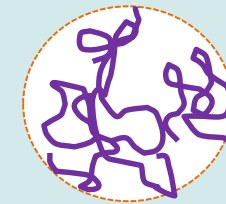


- 3 classes of objects [monomers, polymers and assemblies]
- Amount of assemblies correlated with % of Glu

 small « monomer » ~7 nm
($M_W \sim 8 \cdot 10^4$ Da)



Polymeric chains in good solvent conditions ~20 nm
($M_W \sim 3 \cdot 10^5$ Da)

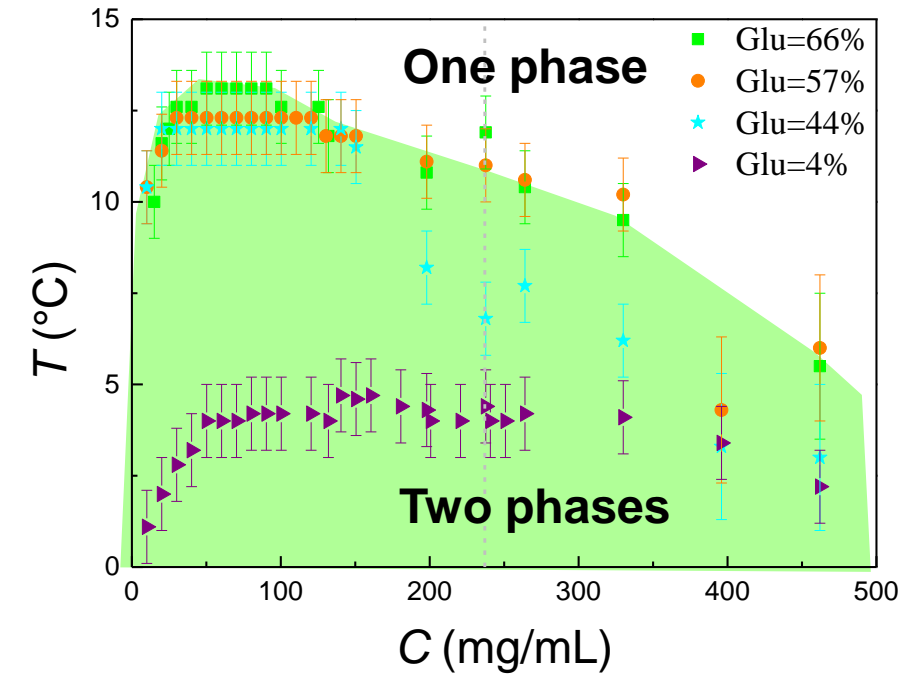


~90 nm

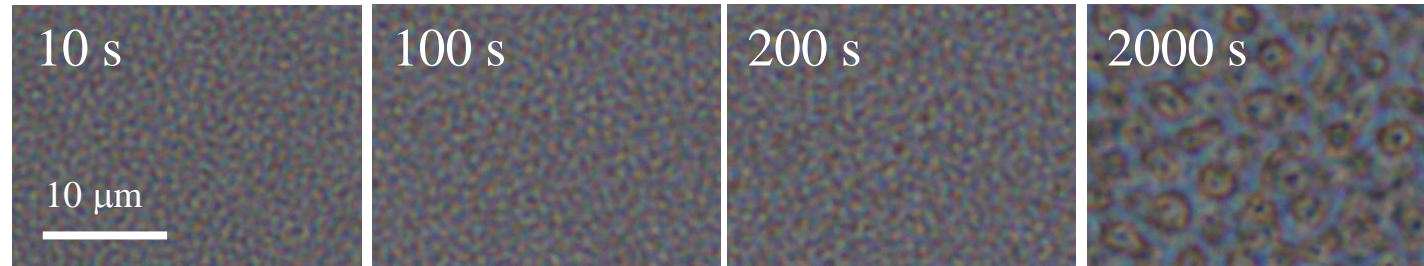
- Large objects comprising several proteins ($M_W \sim 30 \cdot 10^6$ Da)
- Branched polymeric loose structure

Signature of Assemblies in Semi-Dilute Regime?

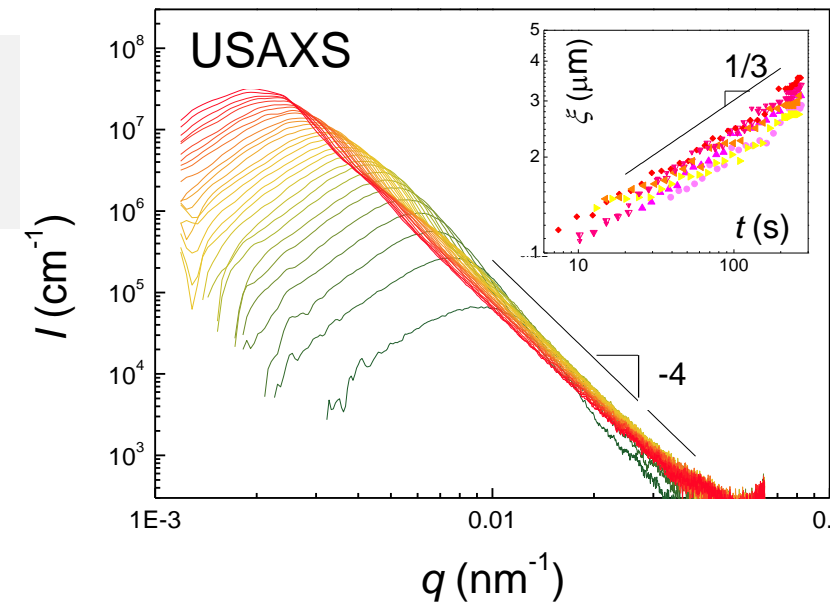
PHASE-DIAGRAMS



One-step quench

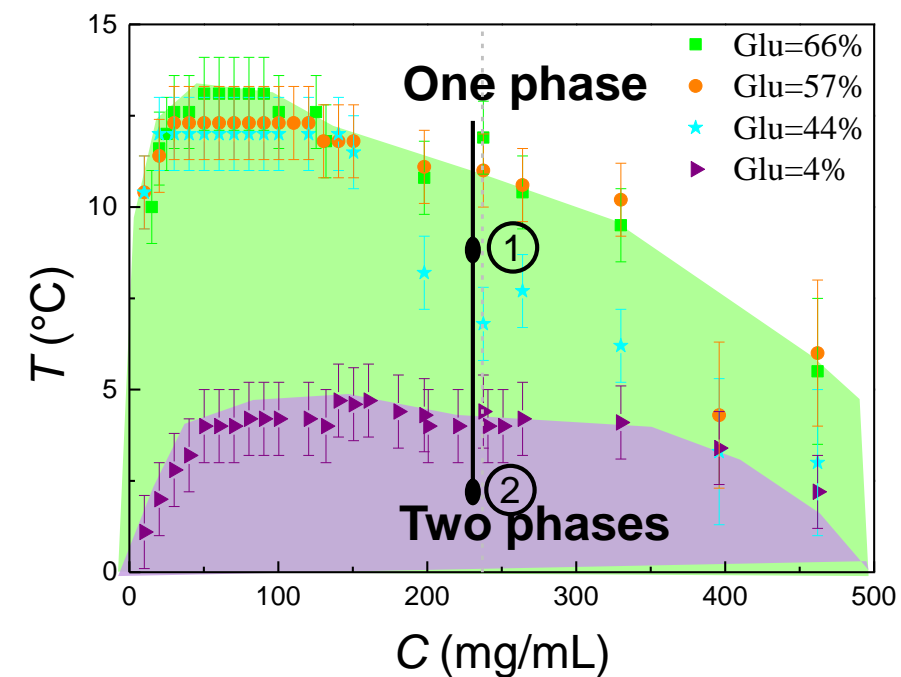


➤ STANDARD or ANOMALOUS spinodal decomposition depending on sample viscoelasticity

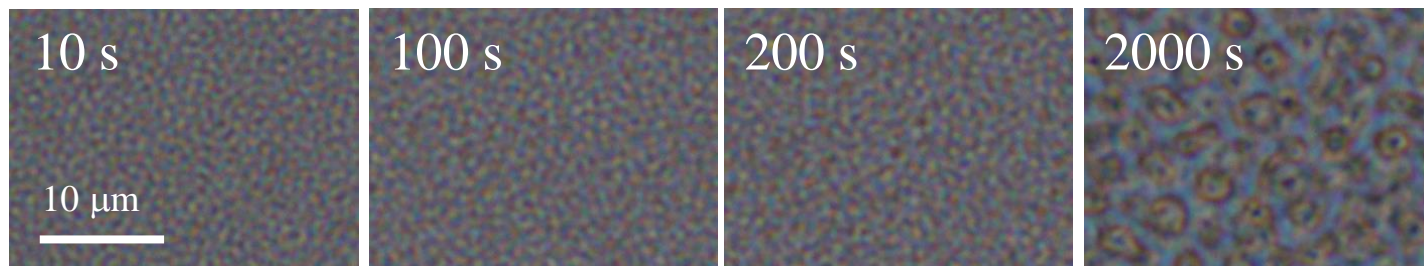


Signature of Assemblies in Semi-Dilute Regime?

PHASE-DIAGRAMS



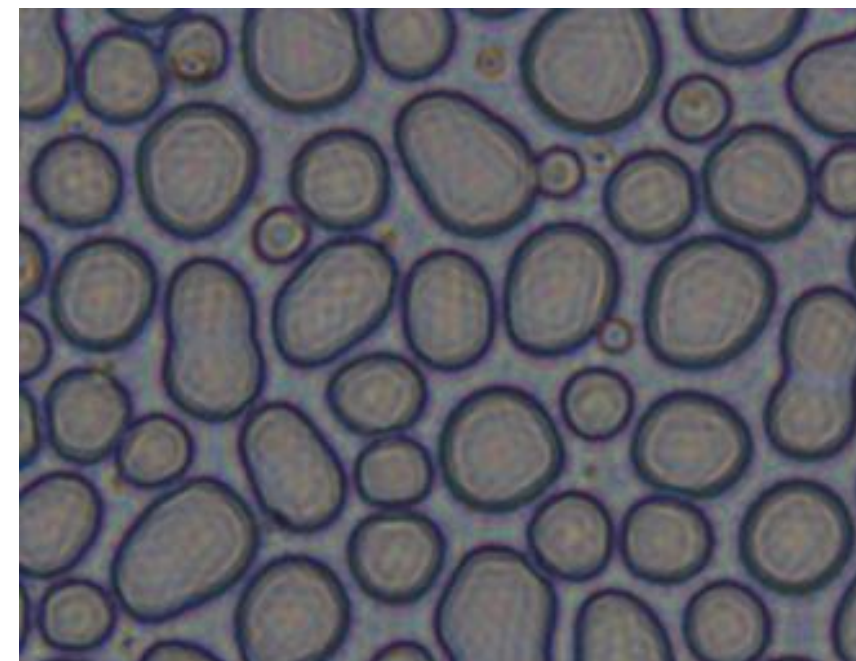
One-step quench



Two-step quench

"high" T : L/L phase separation of assemblies

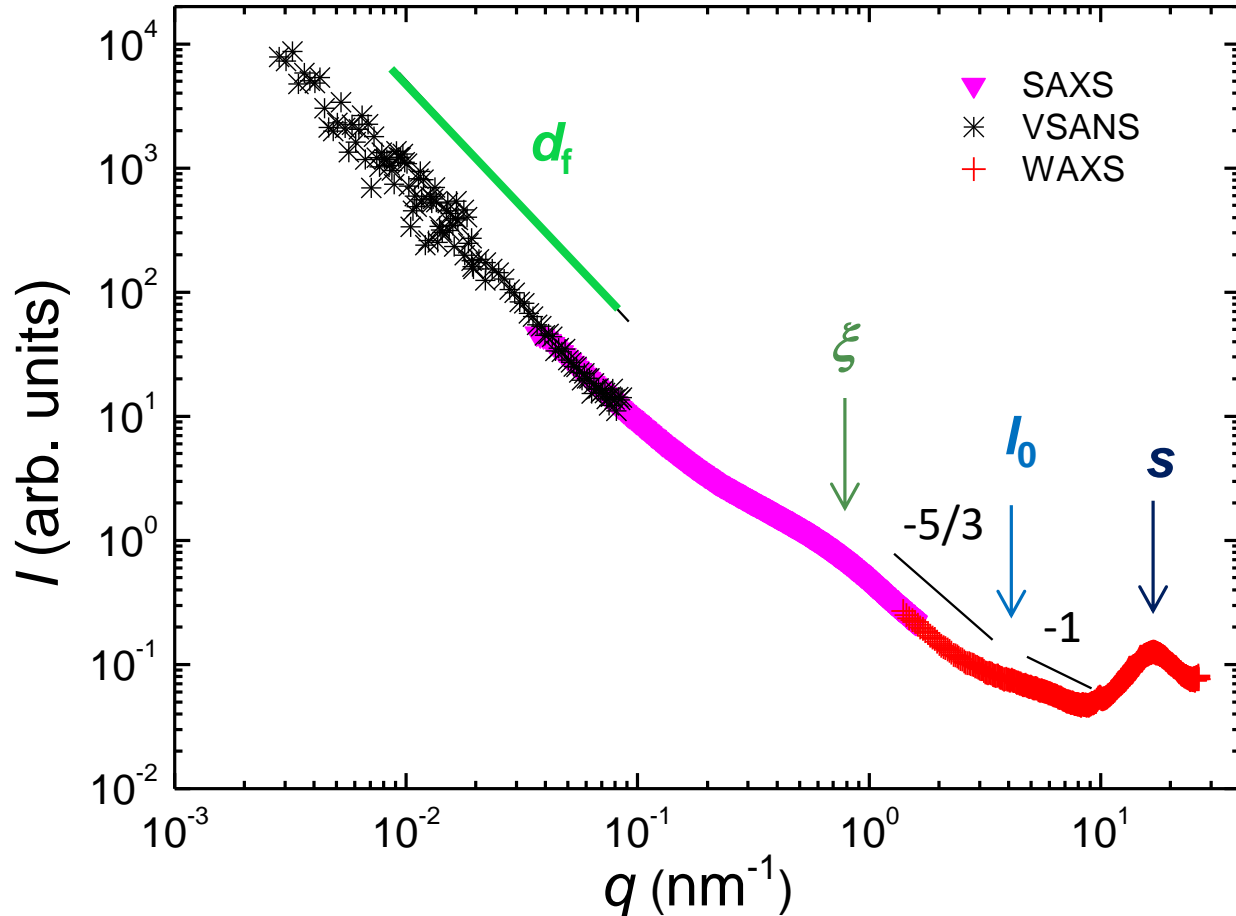
"low" T : L/L phase separation of individual species



➤ Independent phase-separation process for assemblies and gliadin?

Structure of Semi-Dilute Samples

WHOLE SCATTERING CURVE

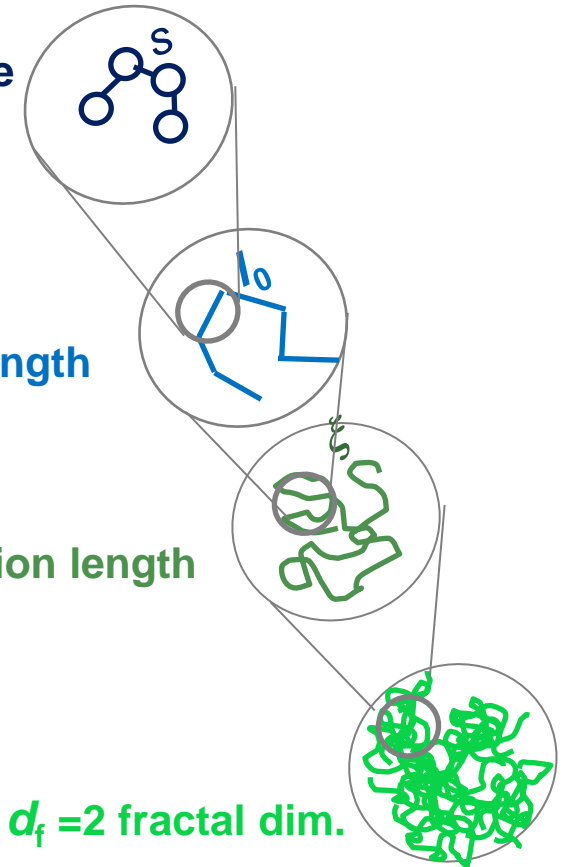


s : average distance between atoms

$l_0 = 0.6$ nm
persistence length

ξ : correlation length (blob size)

$d_f = 2$ fractal dim.



- Polymeric network in good solvent conditions with a fractal organisation at large length scale
- Any signature of the assemblies identified in the dilute regime?

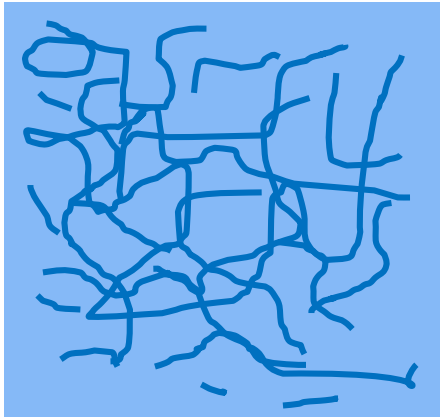
Signature of Assemblies in Semi-Dilute Regime?

SMALL-ANGLE NEUTRON SCATTERING

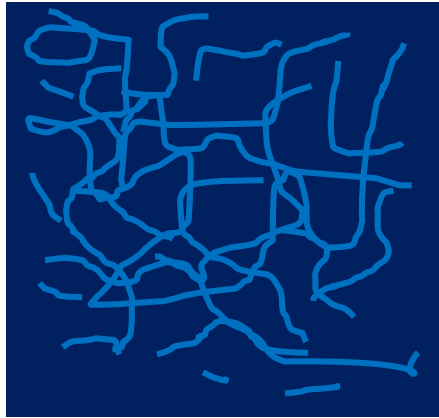
$$I(q) \propto (\Delta\rho)^2 P(q) S(q)$$

contrast between scattering objects and solvent

Hydrogenated solvent



Deuterated solvent



ρ (10^{-6} \AA^{-2})

3.6 $\text{D}_2\text{O}/\text{C}_2\text{D}_5\text{OD}$

2 Proteins

-0.5 $\text{H}_2\text{O}/\text{C}_2\text{H}_5\text{OH}$

Expectation

same scattering profile with
different intensity

Signature of Assemblies in Semi-Dilute Regime?

SMALL-ANGLE NEUTRON SCATTERING

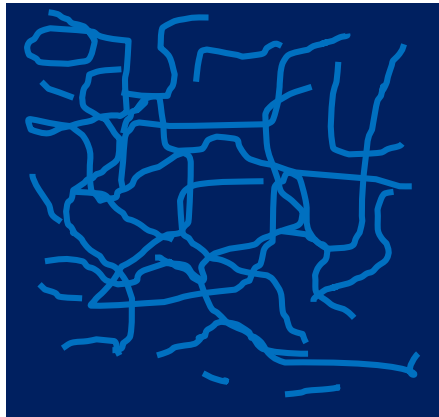
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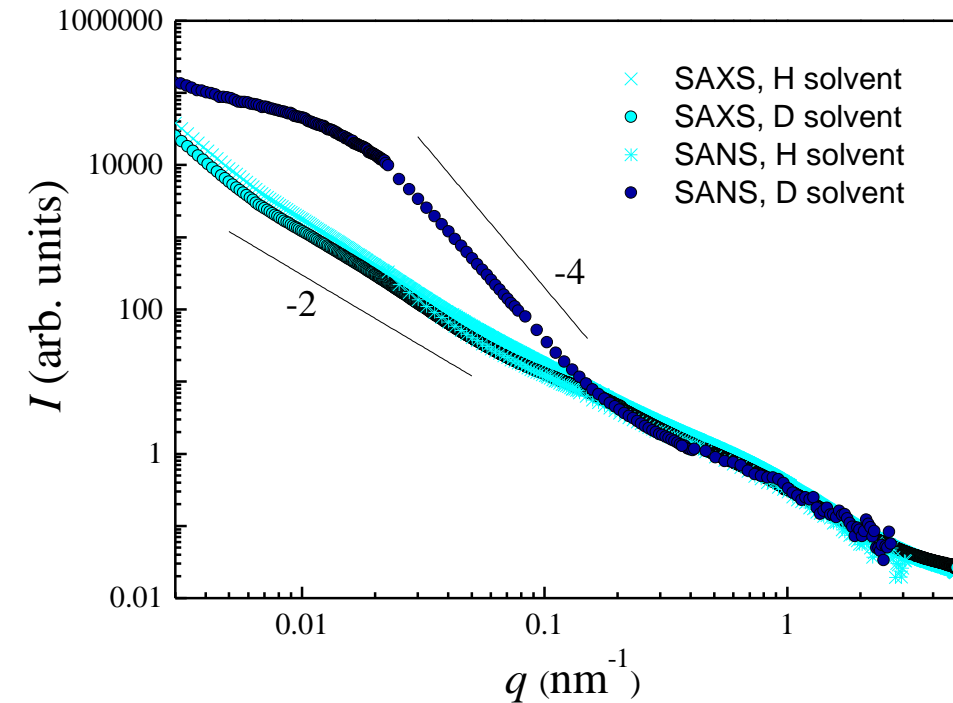
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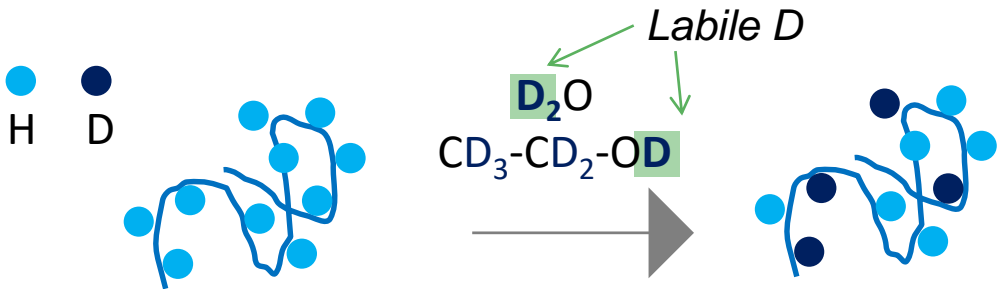


Observation

Strong evolution of SANS signal with solvent deuteration
(but no modification of SAXS signal)

Signature of Assemblies in Semi-Dilute Regime?

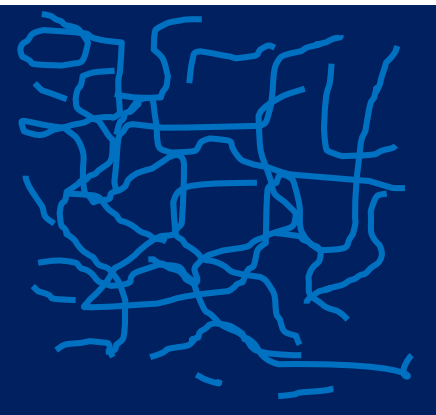
H/D EXCHANGE IN DEUTERATED SOLVENT



- H bonded to S, N, O atoms*
- *labile*
 - *potentially exchangeable*

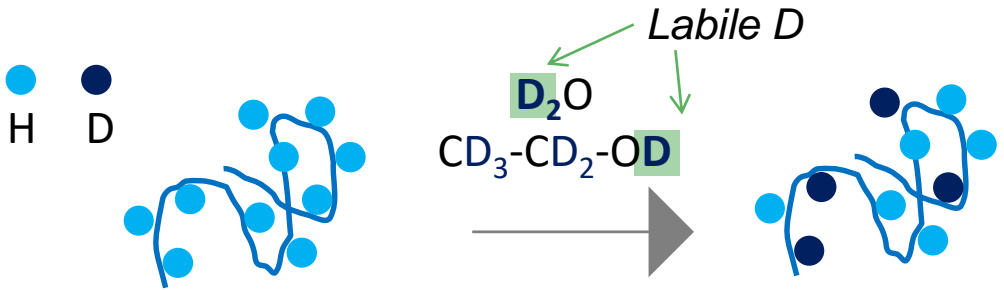
No exchange

Homogeneous exchange



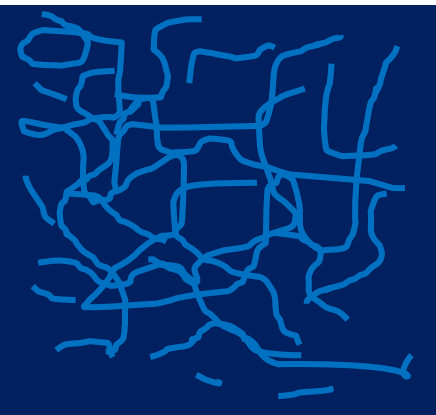
Signature of Assemblies in Semi-Dilute Regime?

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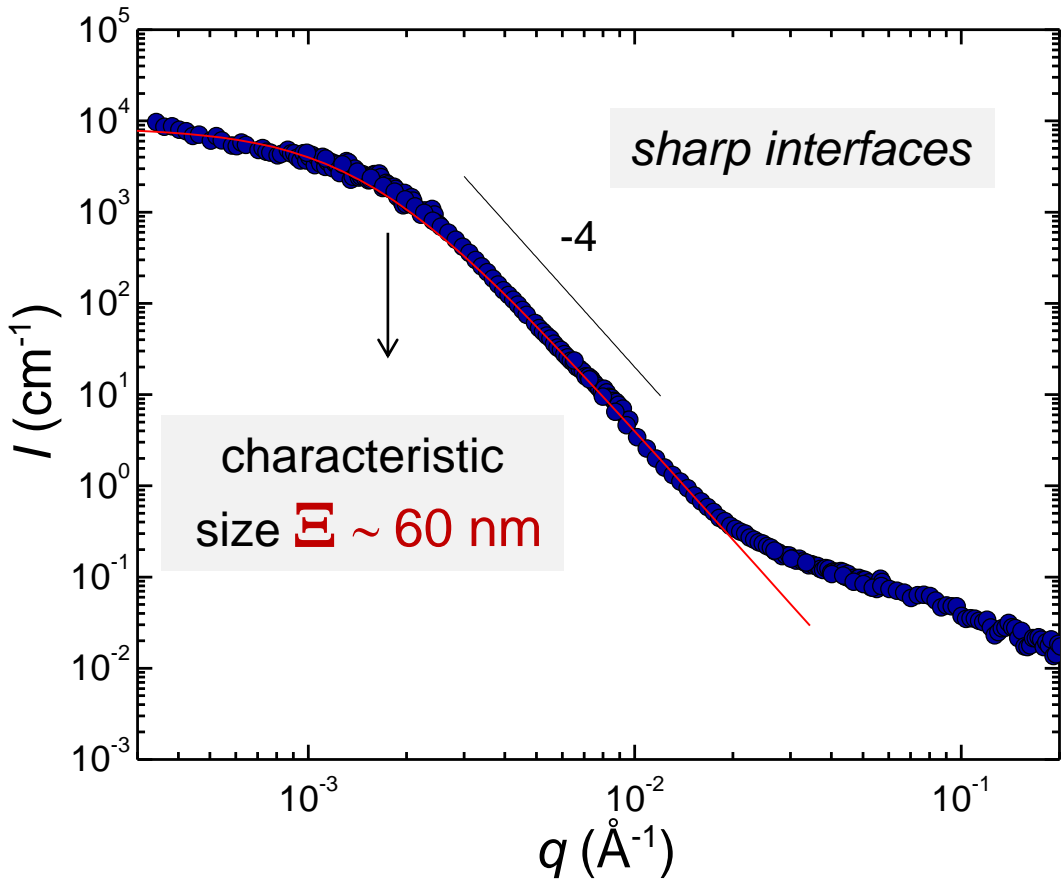


Inhomogeneous exchange



Signature of Assemblies in Semi-Dilute Regime?

PROBING H-BONDS



Debye Buëche model

$$I(q) = \frac{I_o}{(1 + (q\xi)^2)^2}$$

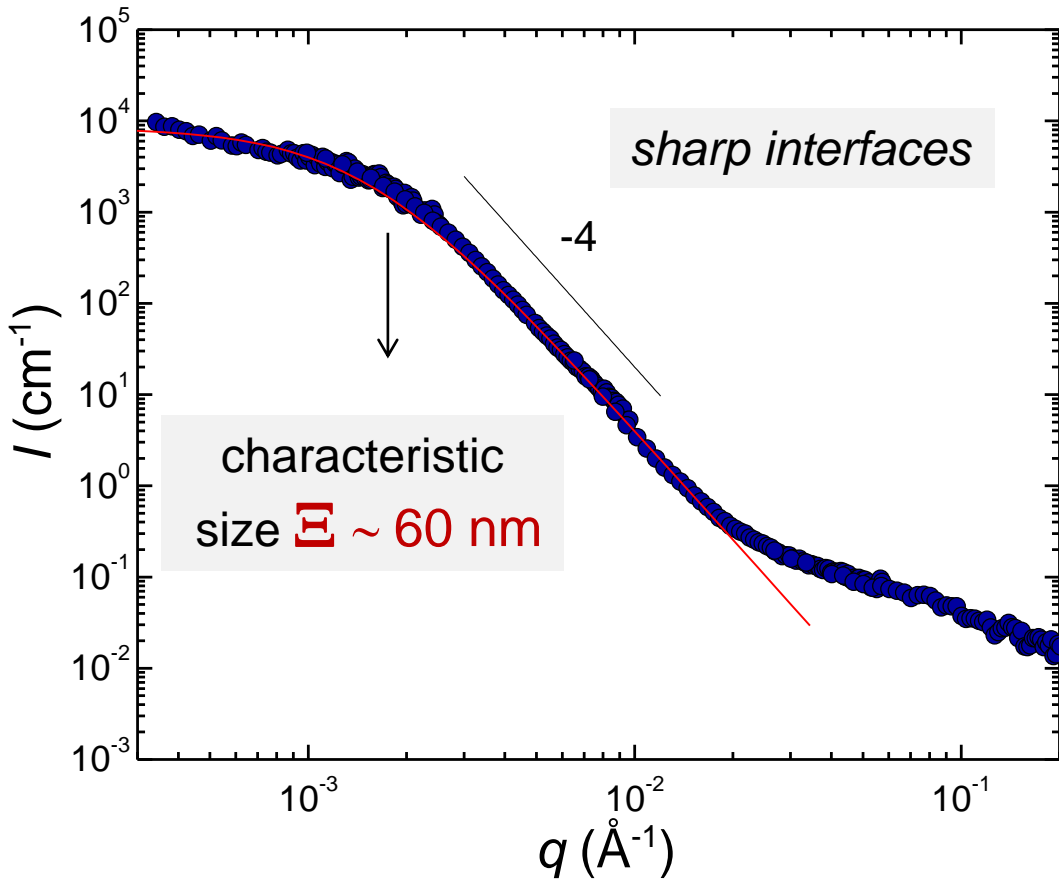
Correlation length in a two-phase sample

- No H/D exchange in the regions with many/strong H-bonds interactions between proteins
- Link with protein assemblies?

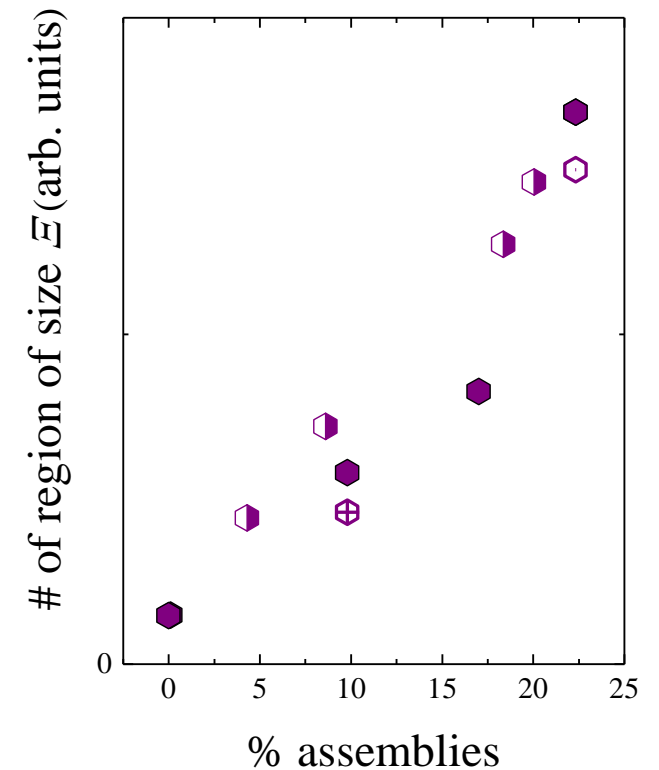
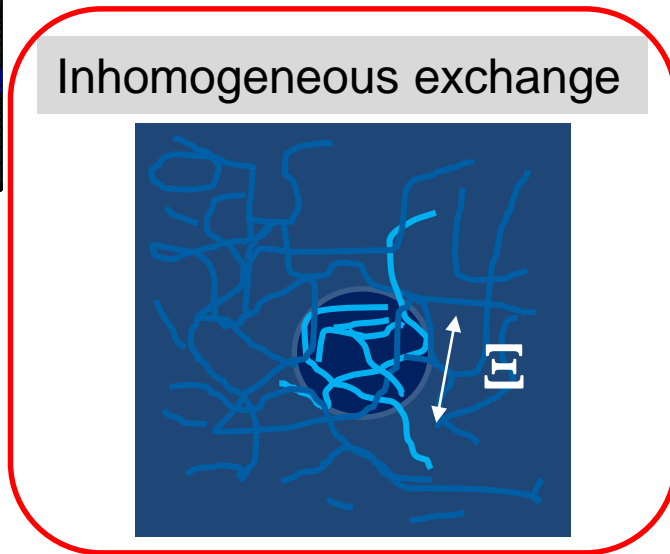
Inhomogeneous exchange



Correlation between Assemblies in Dilute Regime & Inhomogeneous Deuteration in Semi-Dilute Regime



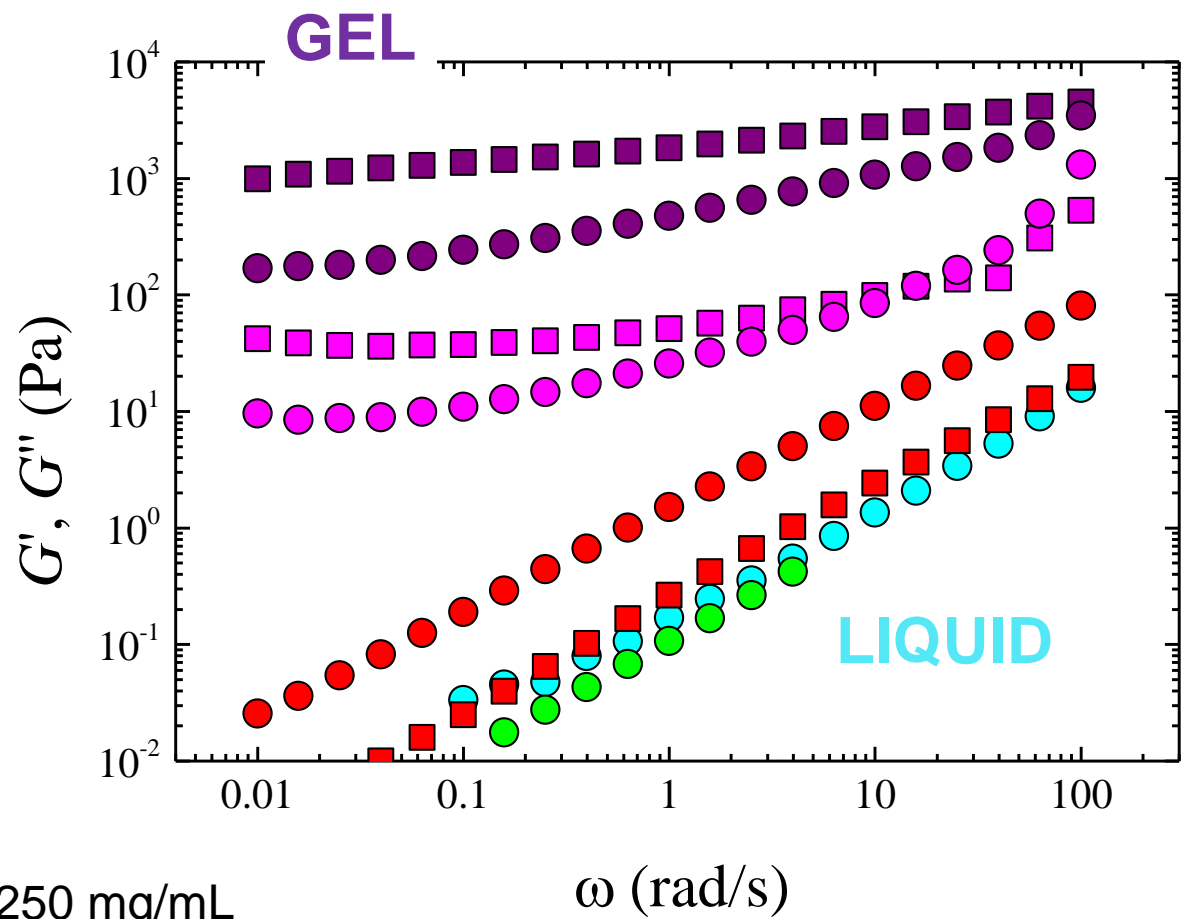
- ξ constant (with protein concentration and composition)
- Same size as protein assemblies
- Role of H-bonds
- Signature of protein assemblies thanks to contrast variation technique?



Role of the Assemblies in the Rheological Properties

Assemblies in dilute regime

Heterogeneous deuteration in semi-dilute regime



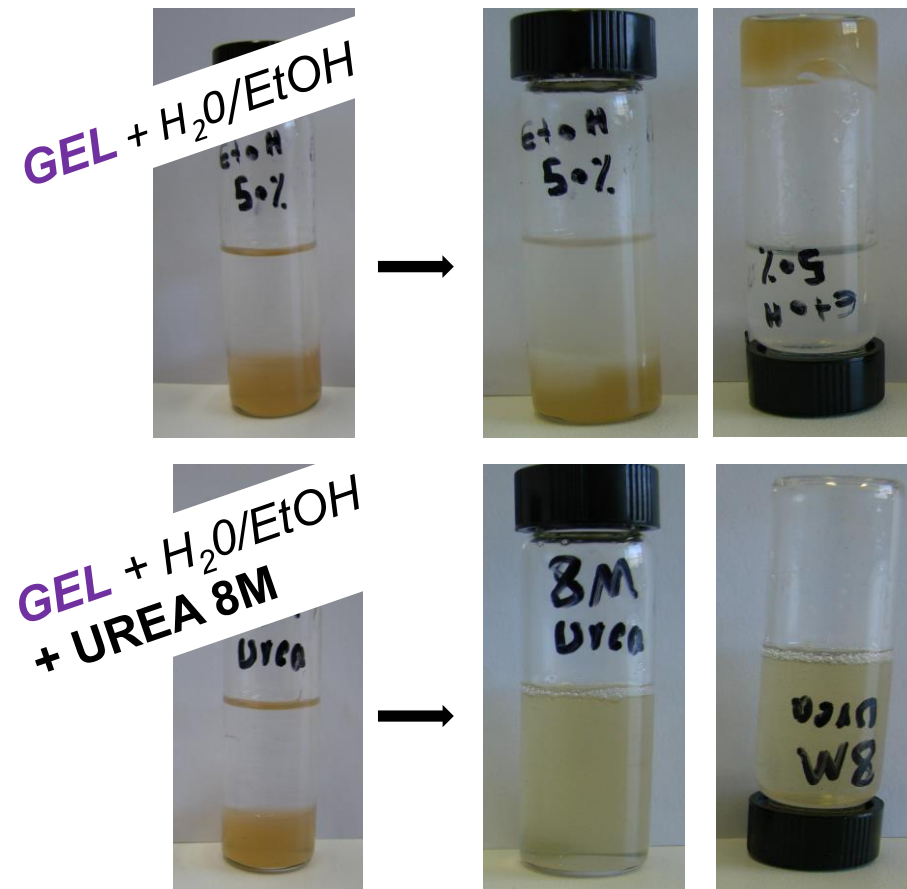
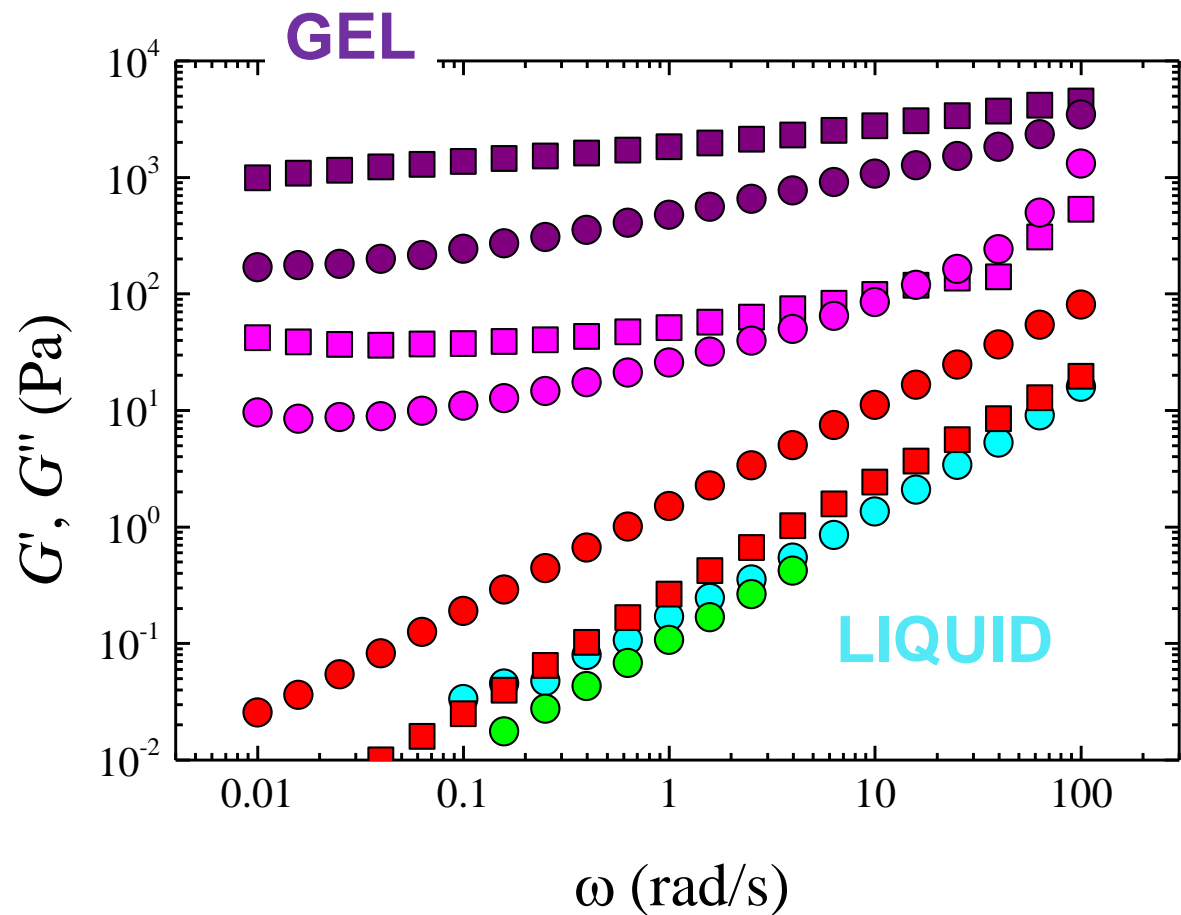
- Cyan circle: Glu=13%
- Green circle: Glu=19%
- Red square: Glu=44%
- Magenta square: Glu=56%
- Purple square: Glu=66%

No assemblies in dilute regime

No heterogeneous deuteration in semi-dilute regime

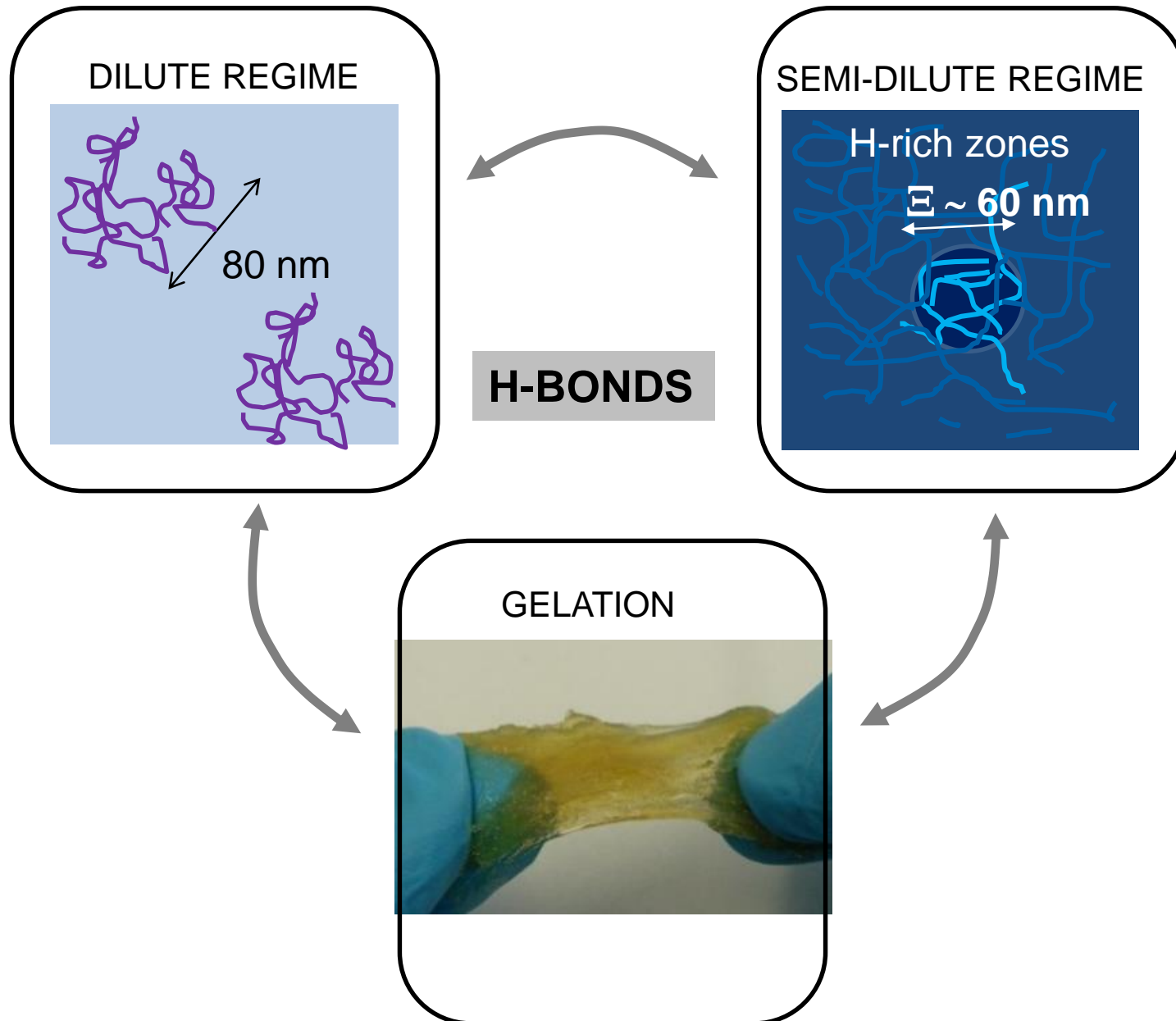
Role of the Assemblies in the Rheological Properties

Assemblies in dilute regime
Heterogeneous deuteration in semi-dilute regime



➤ Crucial role of H-bonds

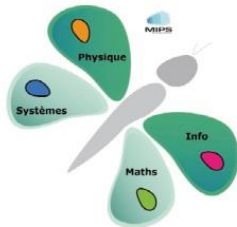
Take-home messages



Unique information from a combination of SAXS and contrast variation in SANS

Dahesh et al. J Phys Chem B 2014
Dahesh et al. Food Hydrocolloids 2016
Banc et al. Soft Matter 2016
Banc et al. J Cereal Science 2017
Banc et al. Soft Matter 2019
Morel et al. Food Hydrocolloids 2020
Costanzo et al. Macromolecules 2020
Ramos et al. J Physics: Condensed Matter 2021

Thanks to...



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A. Banc (L2C, Montpellier, France)
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F. Violleau (INP-Purpan, Toulouse, France)

... you all for your attention!