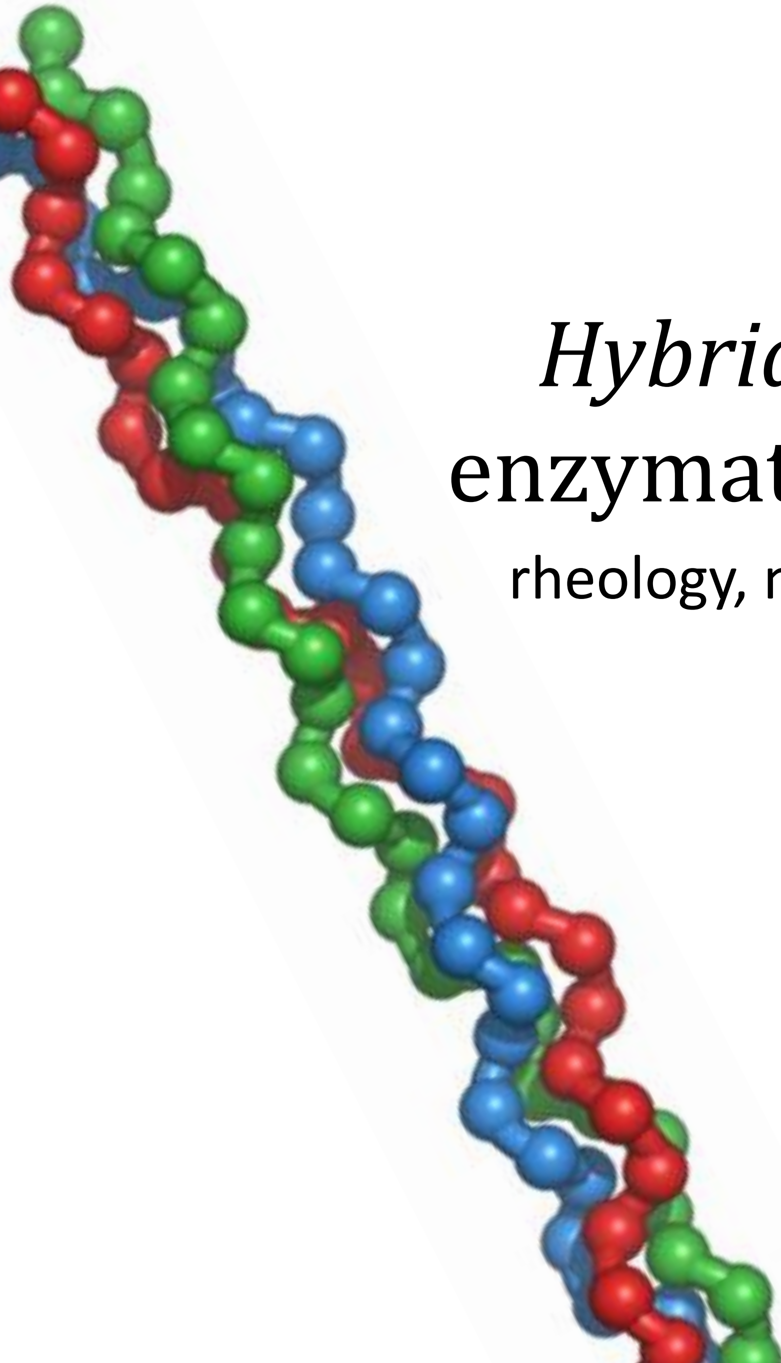


Hybrid gelation processes in enzymatically gelled fish gelatin:

rheology, nanostructure, and cellular response

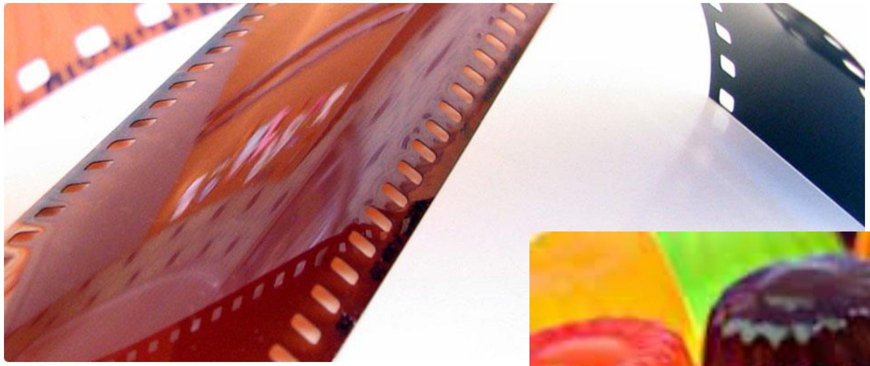
Cécile A. Dreiss

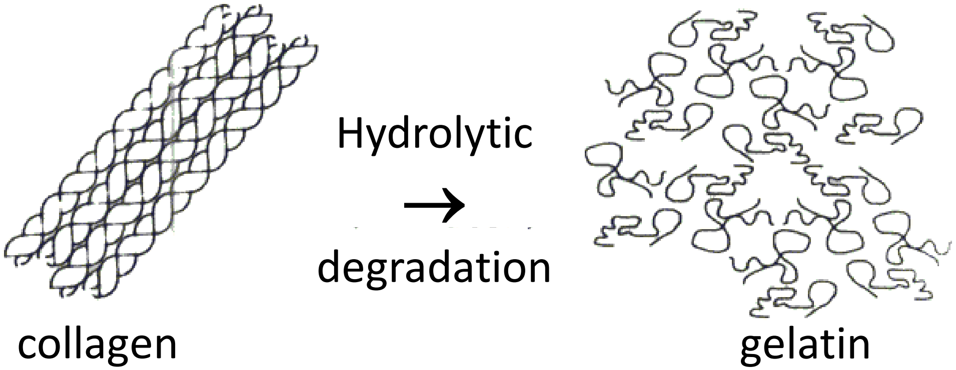
KING'S College LONDON

Objectives

- Design gels from simple biopolymers (gelatin, chitosan…) using an enzymatic cross-linking process
- Assess the influence of *hybrid* gelation processes on final gel properties (rheology and structure) and gelation kinetics
- Evaluate impact on cell response

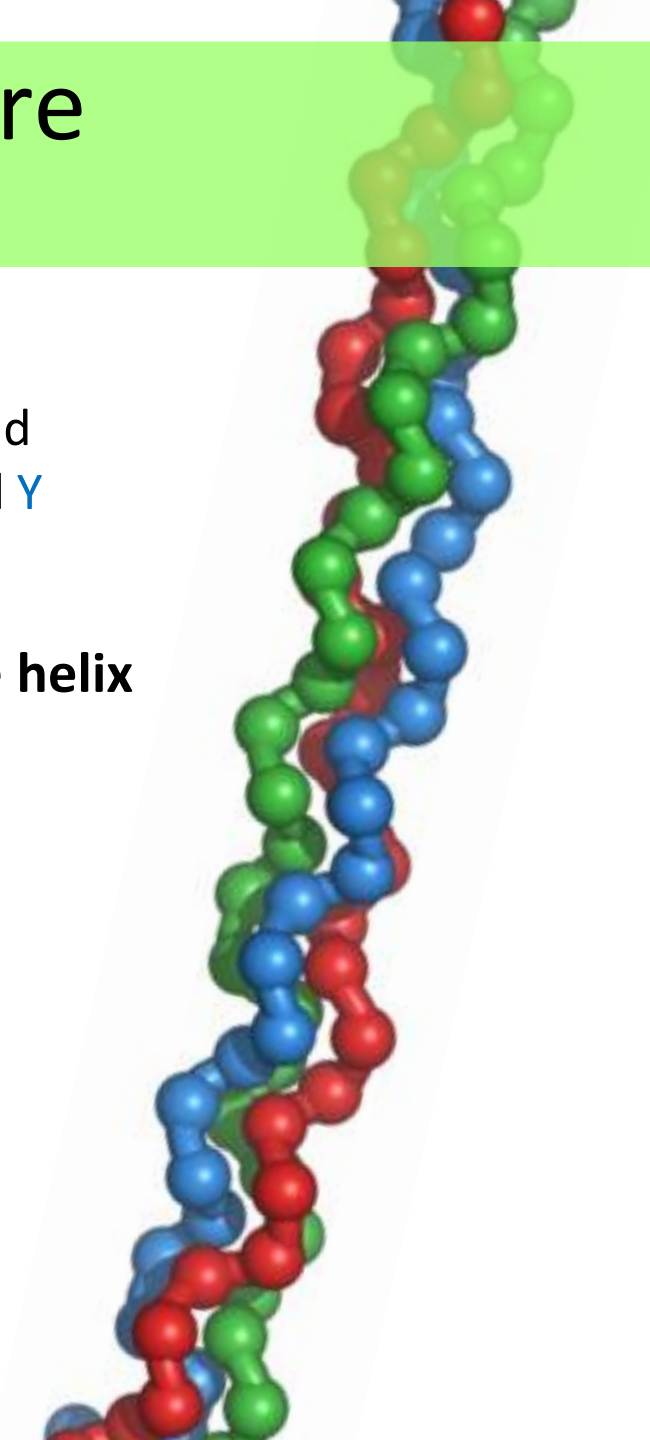
Gelatin



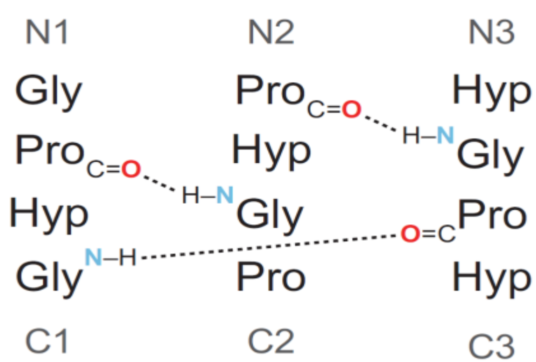


- Forms transparent gels below T_g
- Applications: food (improve stability/consistency), cosmetics, photographic industry, biomedicine (plasma expanders, microspheres, drug formulations, implants)…

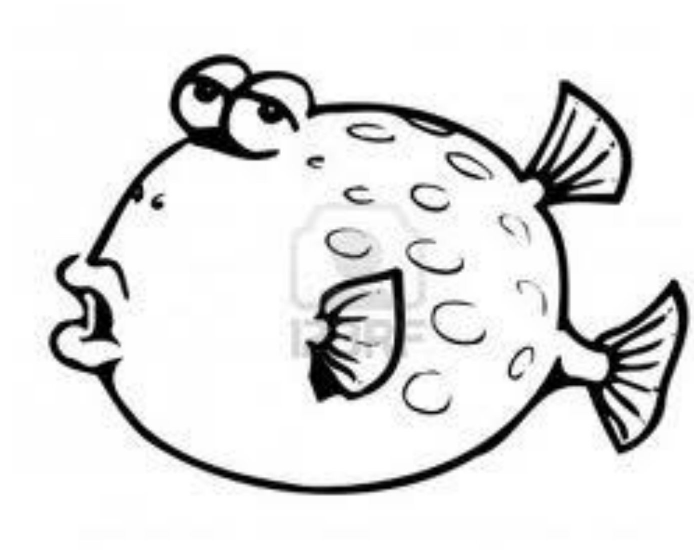
Collagen structure
the triple helix



- Tripeptide sequences: GLY-X-Y
  - GLY: every third position, smallest amino acid
  - (Hydroxy-)/Proline residues: mostly in X and Y
- Single strands form a P_{II} left-handed helix
- 3 chains assemble to form a **right-handed triple helix**
  - Steric restriction from PRO/HYPRO
  - PRO in *trans* configuration
  - GLY allows P_{II} chains to come close together
  - Stabilisation by H bonds



Gelatin: species variation
Amino acids composition



Mammalian:

33% GLY, 20% PRO/ HYP

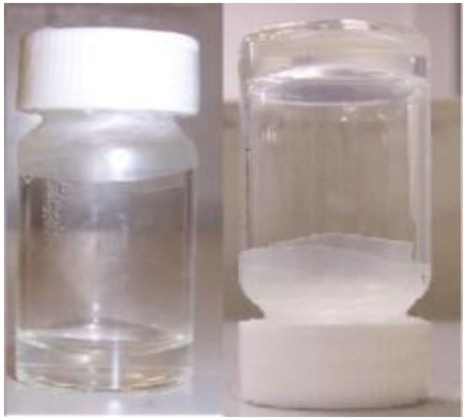
Fish:

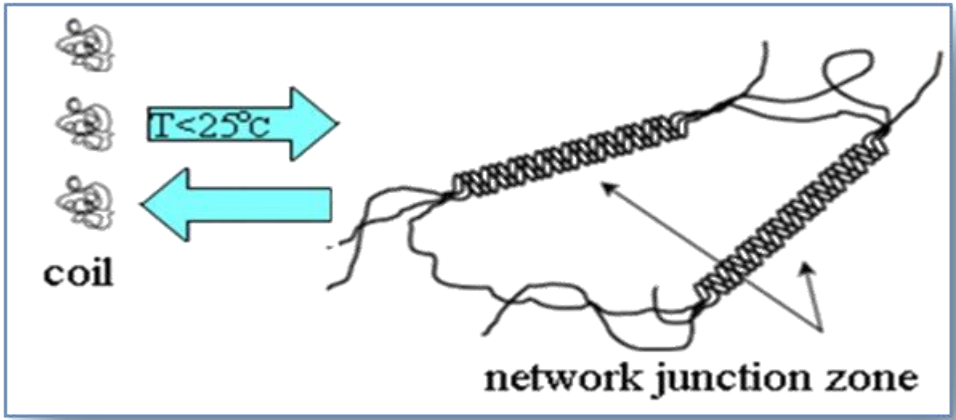
- wider variation in composition
- lower PRO/HYP content $\Rightarrow$ **lower T_g**
- fish skin/bones
  = 75% of total fish weight caught

We use Tilapia gelatin (fresh water fish /Middle East and Africa) T_g ~ 23°C

1. Physical networks

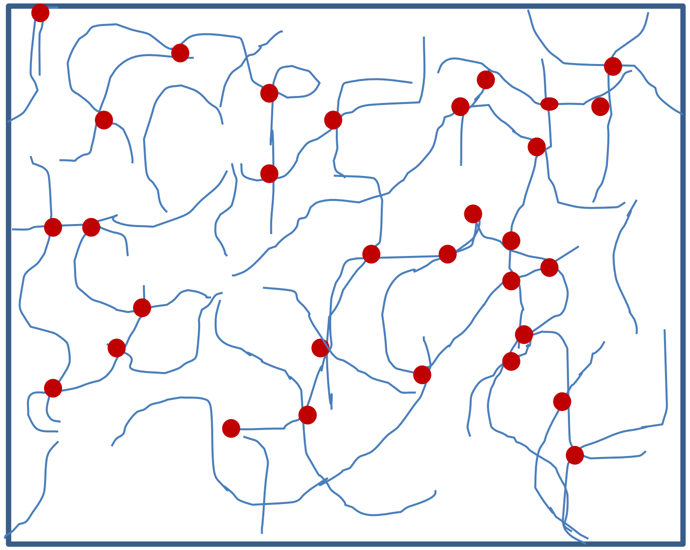
- Gelation by cooling: $T_g < 23°C$
- Occurs by formation of triple-helices = junction zones
- Thermally reversible process - ‘*melt-in-the-mouth*’ behaviour

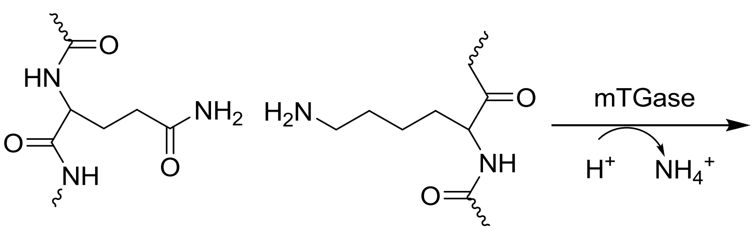




2. Chemical networks

- Covalent bonds/irreversible
- Cross-linker: the enzyme microbial Transglutaminase (TGase)
  (blood clotting, liver detoxification agent…/used in industry)





Binding sites availability:

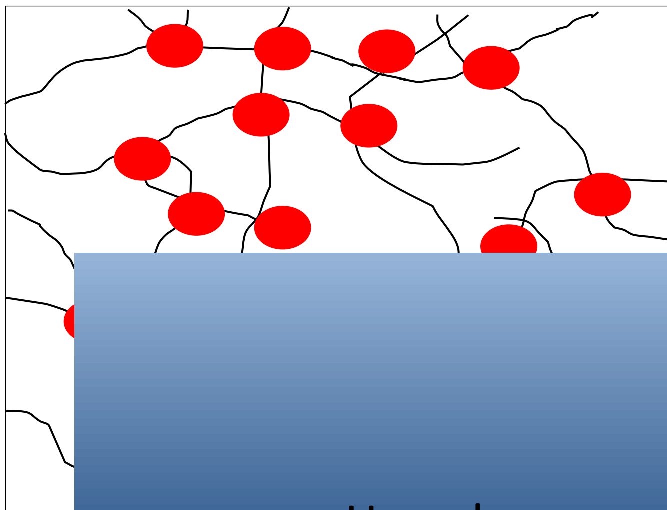
glutamine ~ 12%

lysine ~7%

Hybrid networks: combining chemical and physical

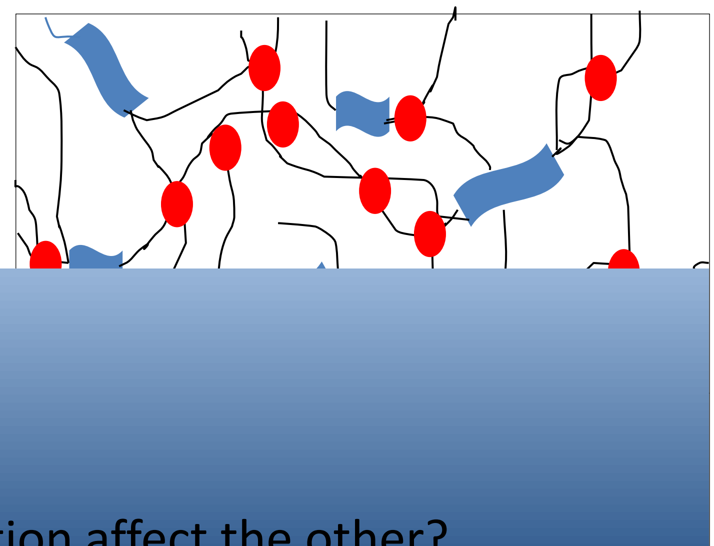
Chemical gels

Enzymatic cross-linking > Tg



Physical-*co*-chemical gels

Enzymatic cross-linking < Tg

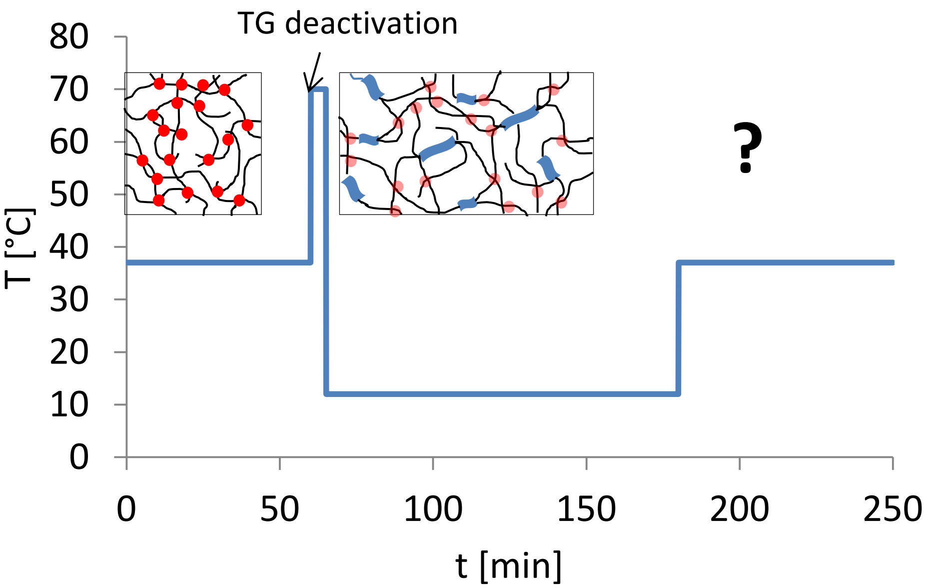


- How does one type of junction affect the other?
- Can we tune gel properties?
- Can helices be thermally stabilised by cross-links?
- How does gelation protocol impact cell behaviour?

Hybrid networks

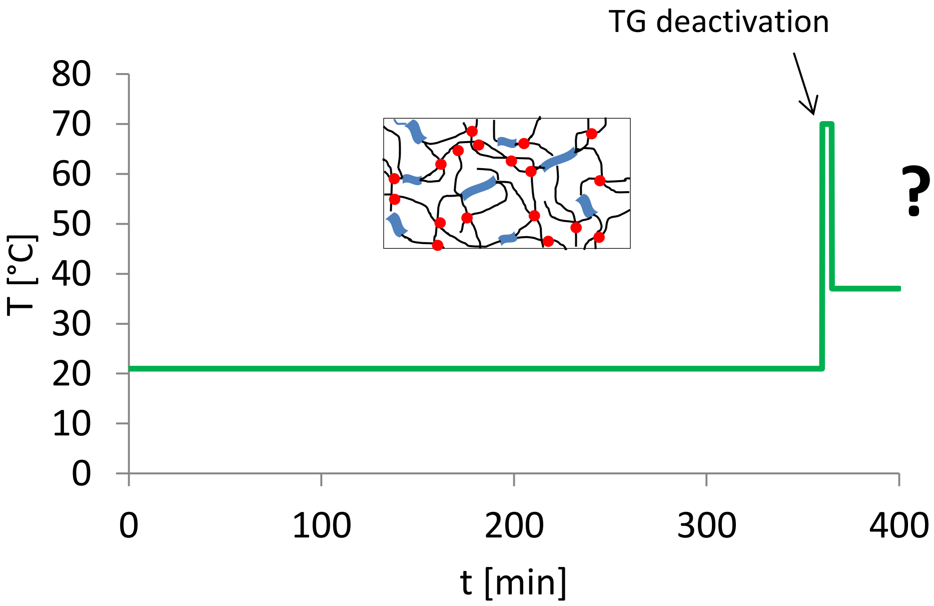
3. Chemico-physical

1. Cross-linking of the gel first
2. Cooling the gel



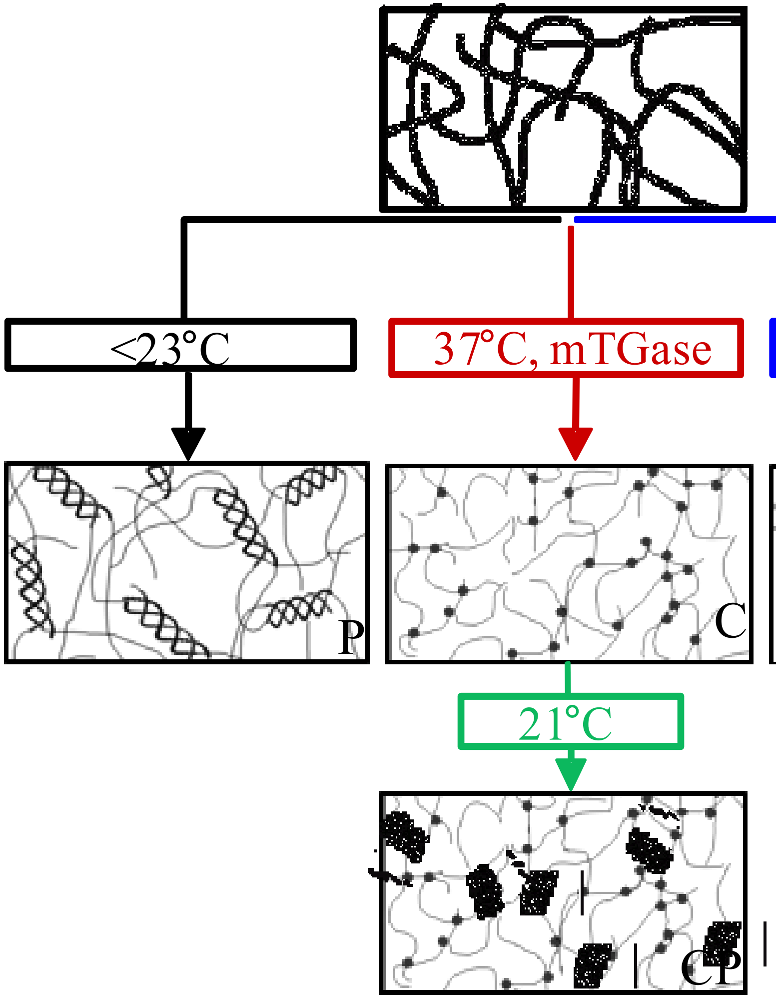
4. Physical-*co*-chemical

Simultaneous cross-linking and cooling



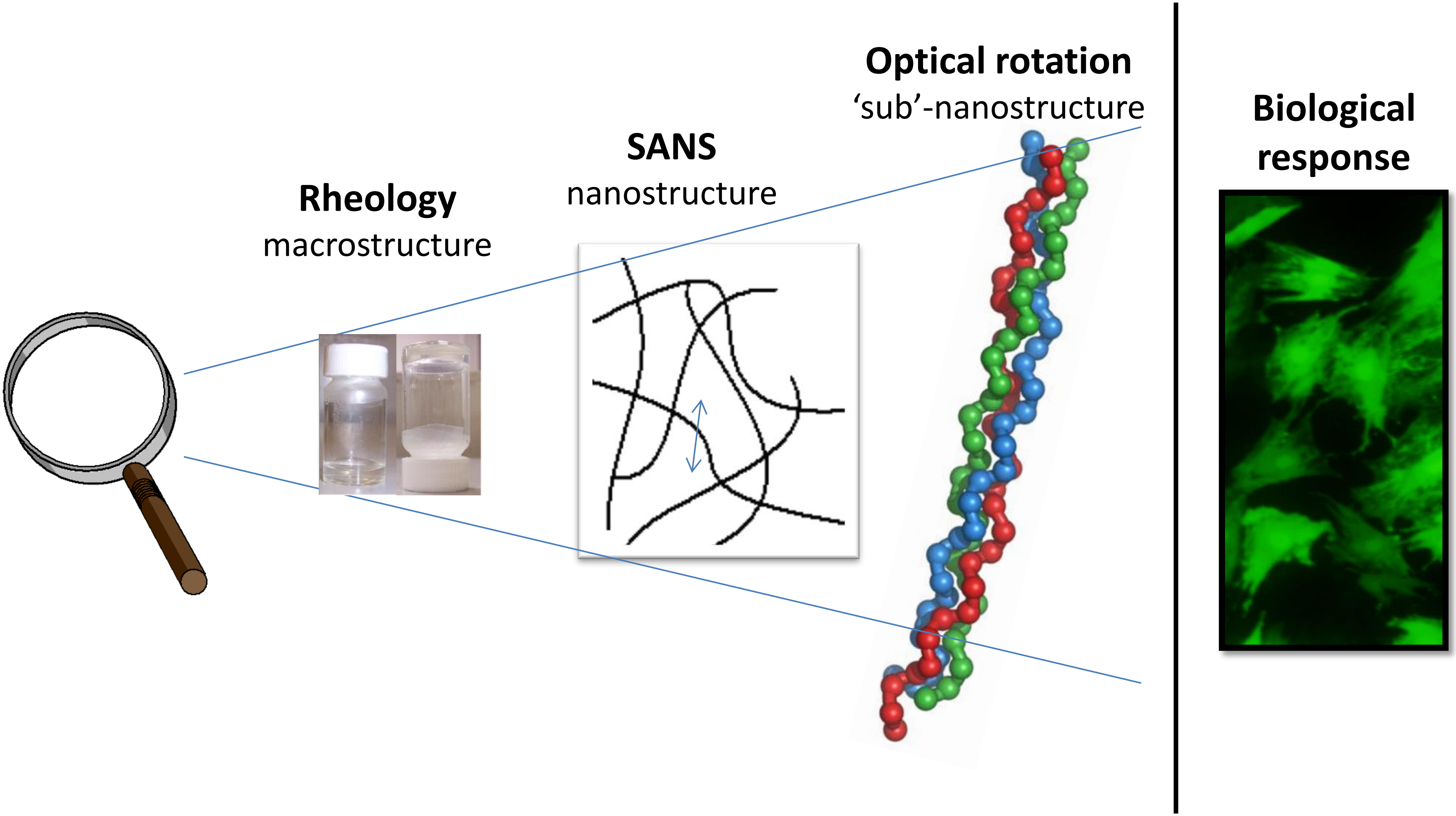
4 types of networks

Gelatin single strands

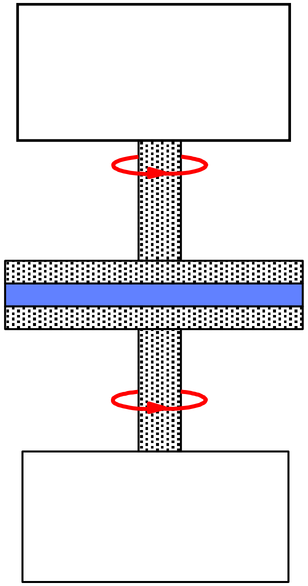


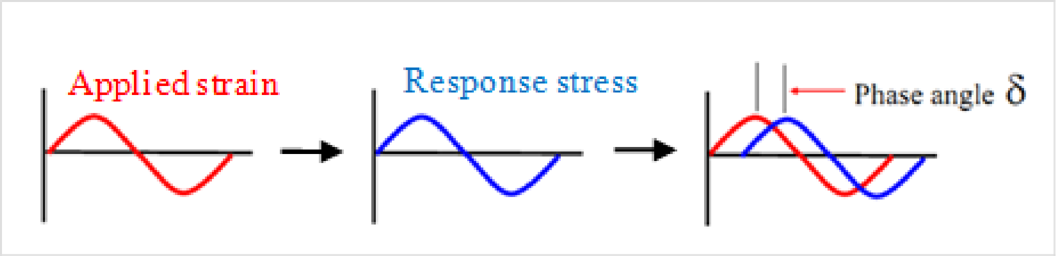
P: Physical
C: Chemical
CP: Chemico-physical
PC: Physical-co-chemical

Measuring gel properties

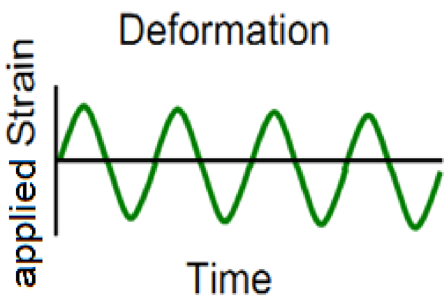


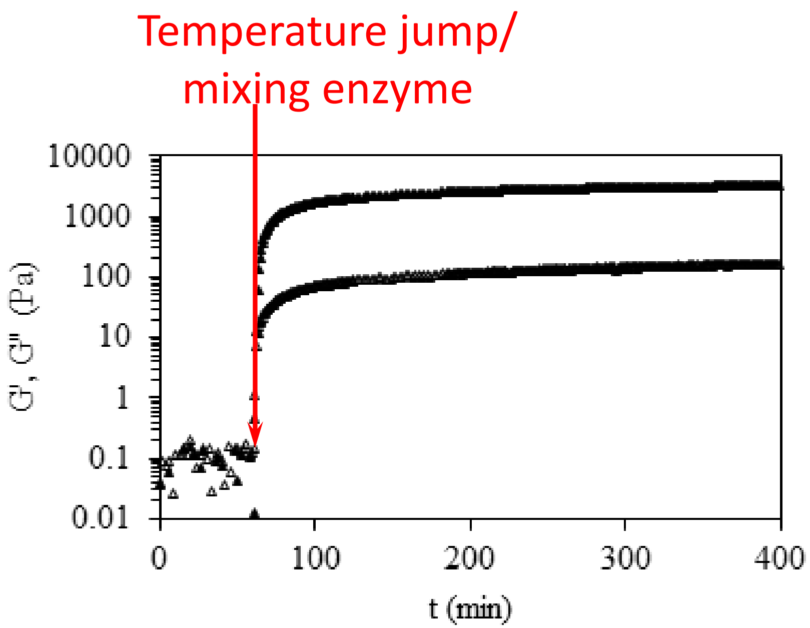
Nuts & Bolts of Rheology
Dynamic oscillatory experiments





Time sweep/cure experiments:



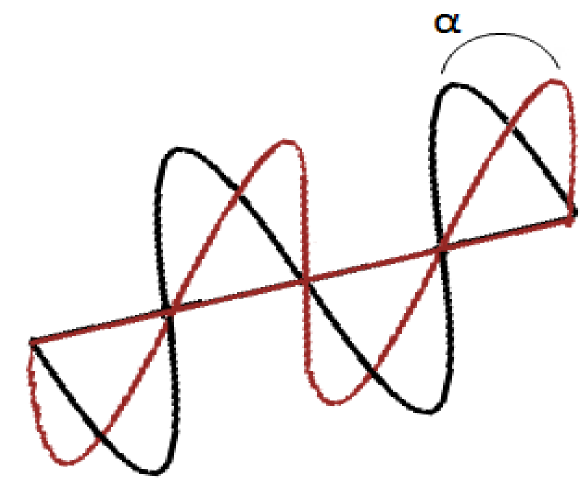


G': Elastic modulus

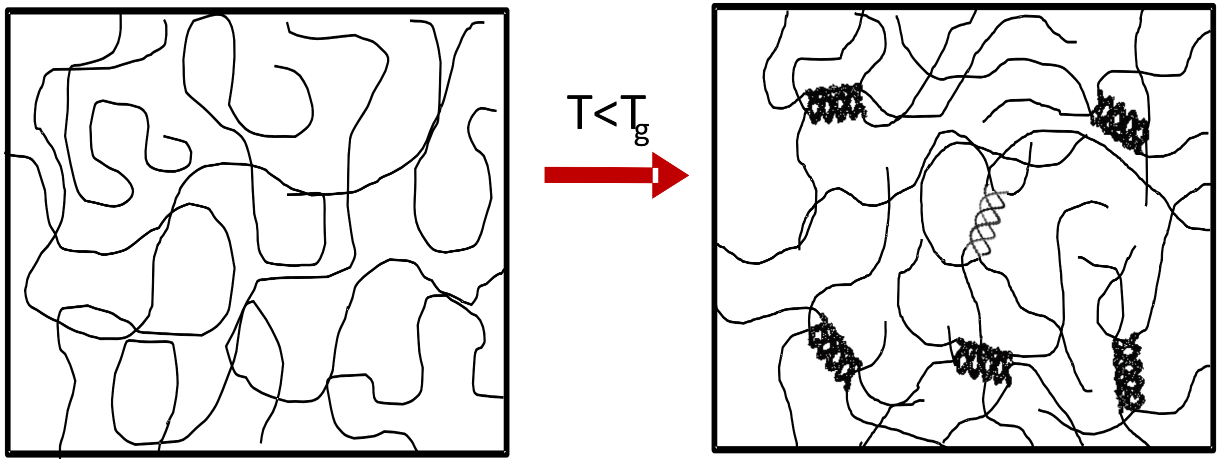
G'': Viscous modulus

Nuts & Bolts of Optical Rotation

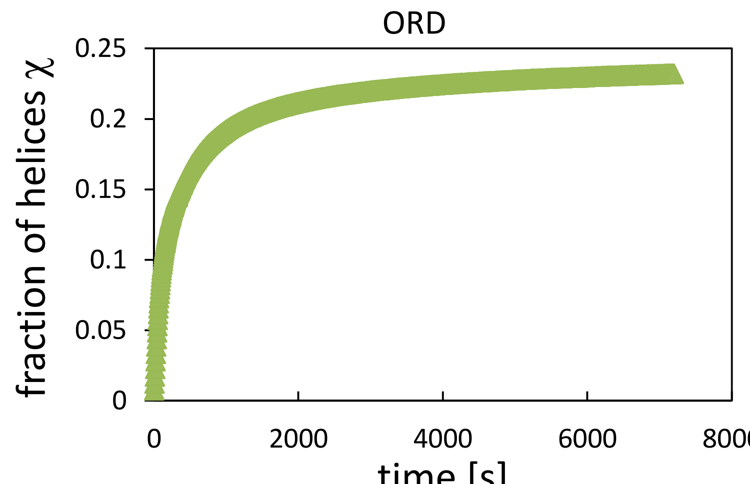
Helical content in gelatin gels



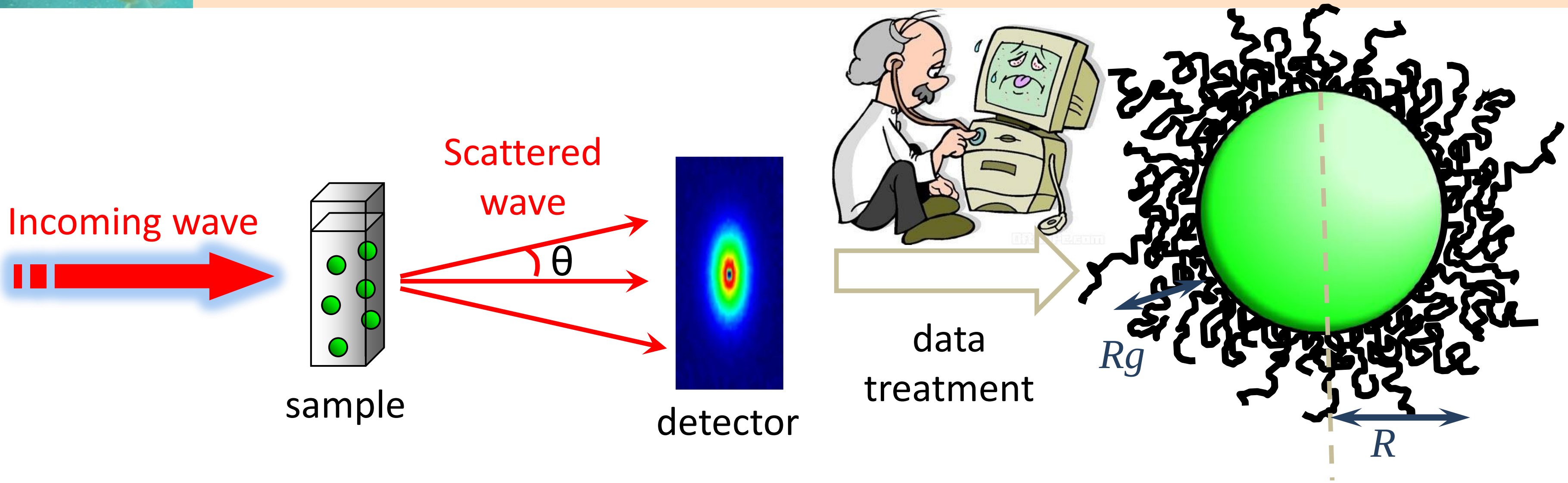
Linearly polarised light rotated by optically active sample.

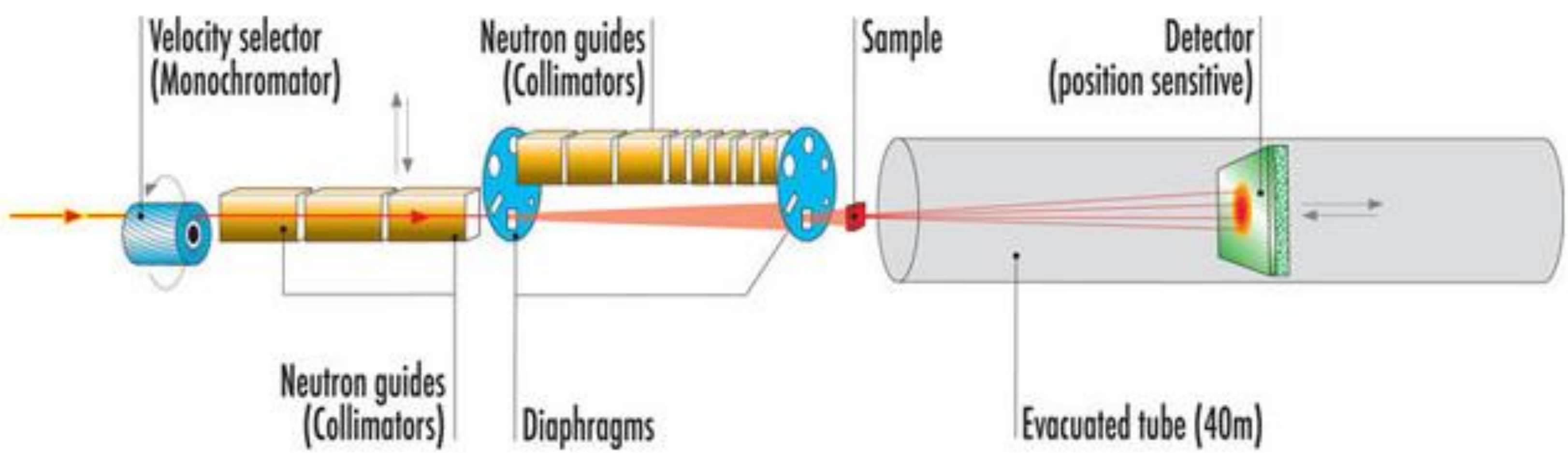


P_{II} make gelatin chiral:
Rotation of the plane of linearly polarised light

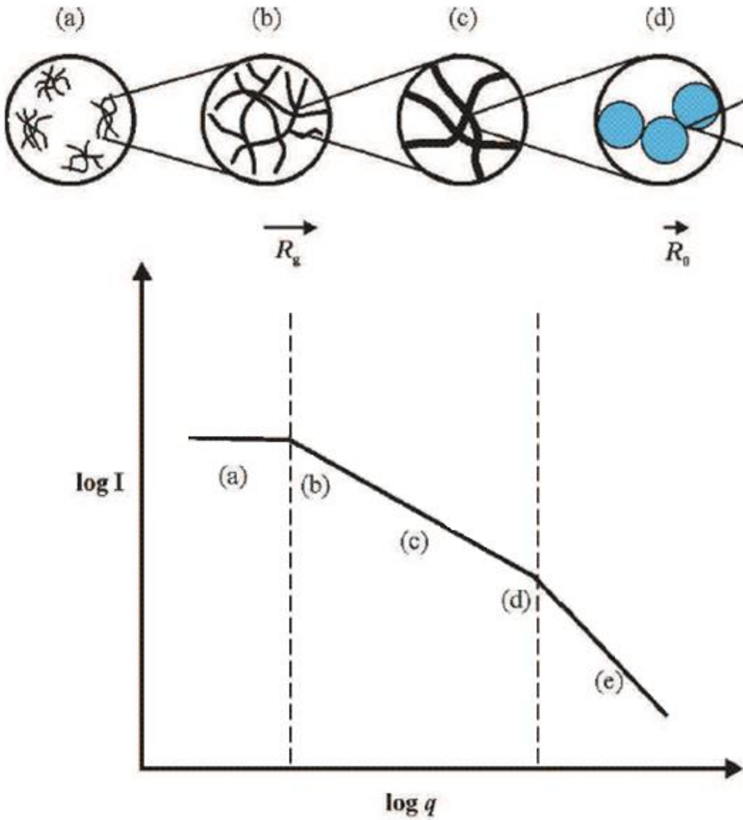


Nuts & Bolts of SANS
Principles & Instrument



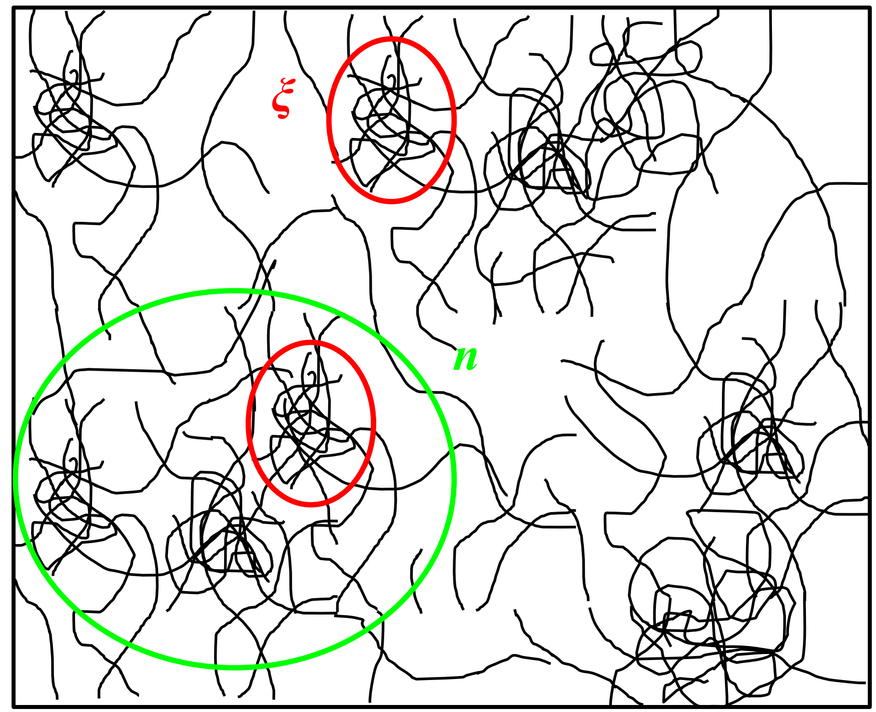


Nuts & Bolts of SANS
What do we 'see'?



The scattering vector:

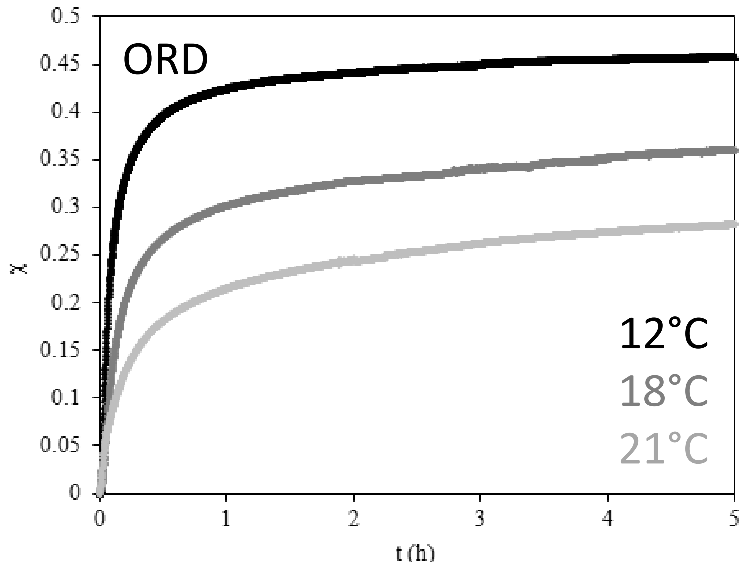
$$q = \frac{4\pi}{\lambda}\sin\frac{\theta}{2}$$

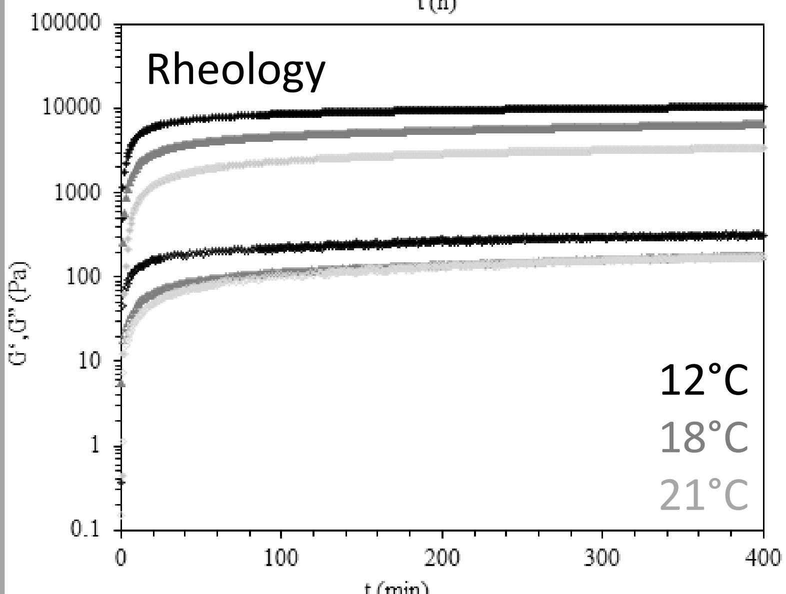


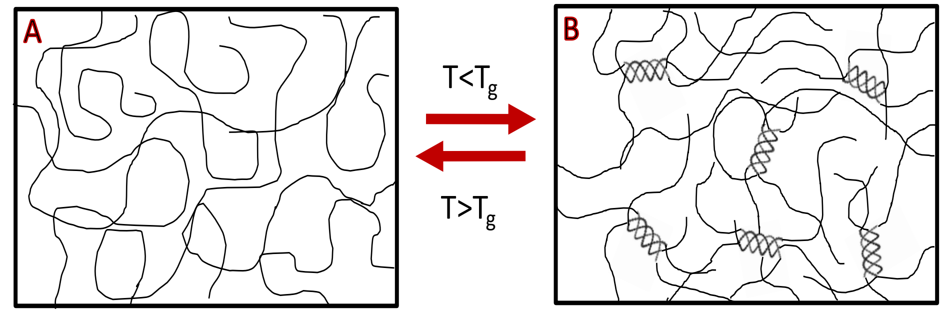
ξ – intermediate *q* region- 10-100s Å
n- low *q* region 1000s Å

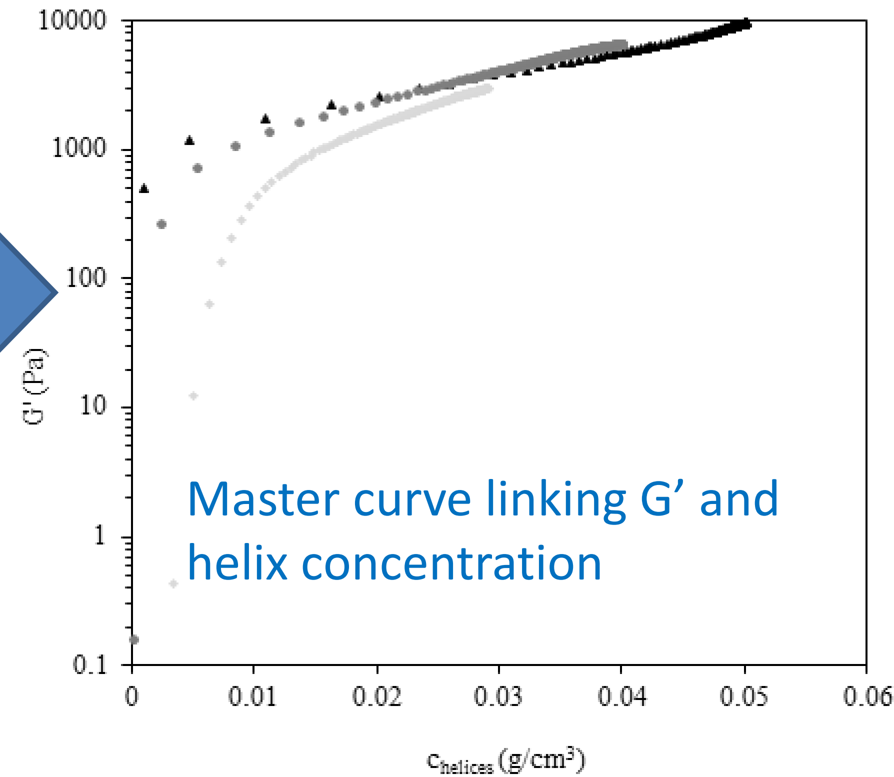
1. Physical networks

Linking helix content to elasticity









Joly-Duhamel et al, **2002**, *Langmuir*

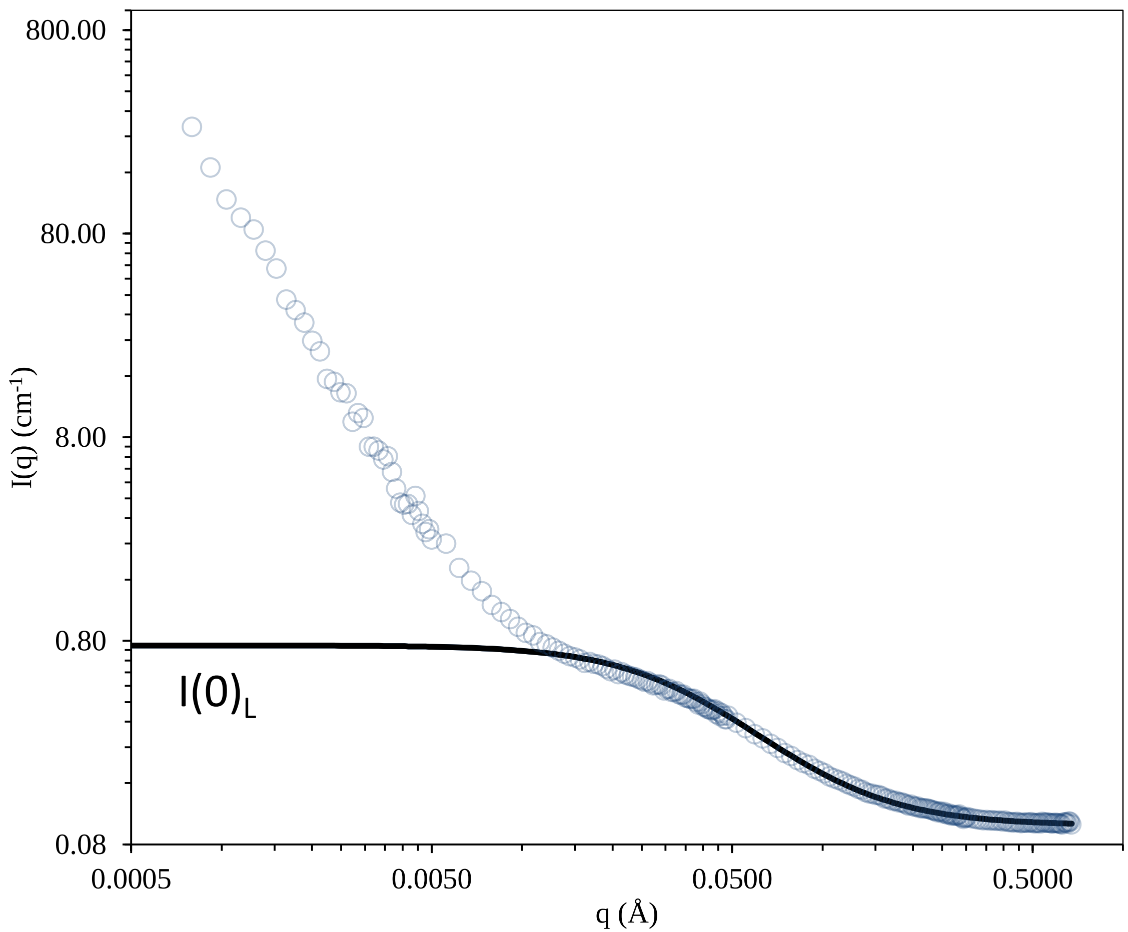
Biomacromolecules **2011**, *12*, 3741-52

1. Physical networks

SANS model for gelatin gels and coils

Orstein-Zernike: $$I(q) = \frac{I_L(0)}{1+q^2\xi^2}$$

Correlation length



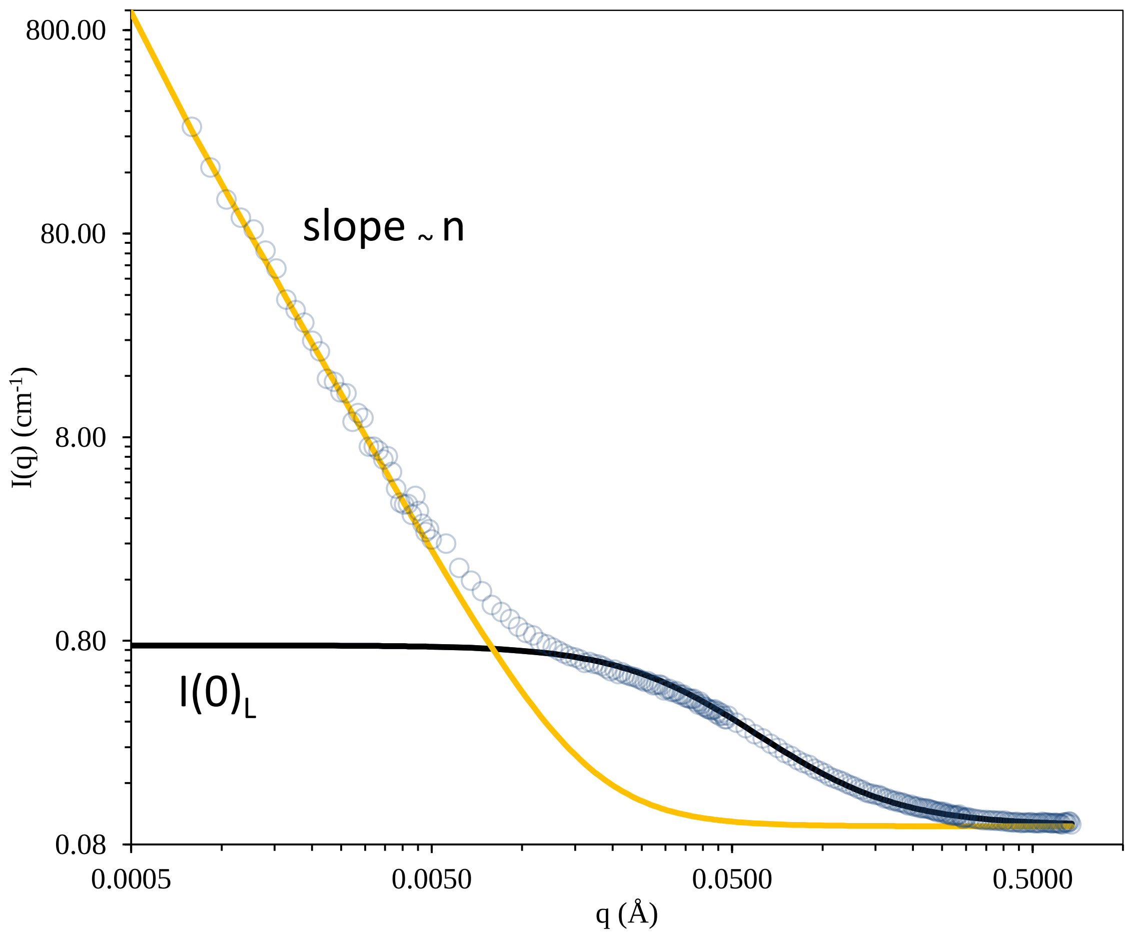
1. Physical networks

SANS model for gelatin gels and coils

Orstein-Zernike + power Law: $$I(q) = \frac{I_L(0)}{1+q^2\xi^2} + \frac{A}{q^n}$$

'Large' aggregates

Correlation length



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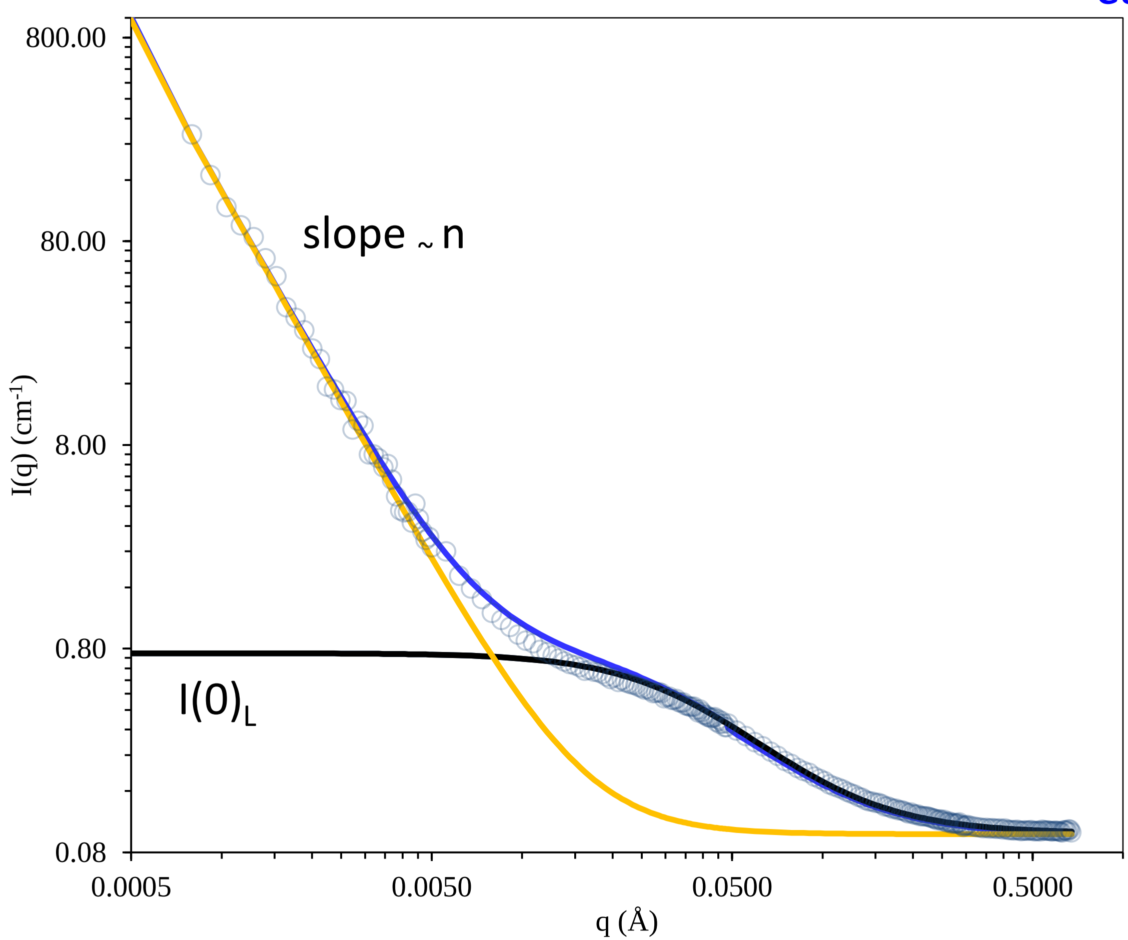

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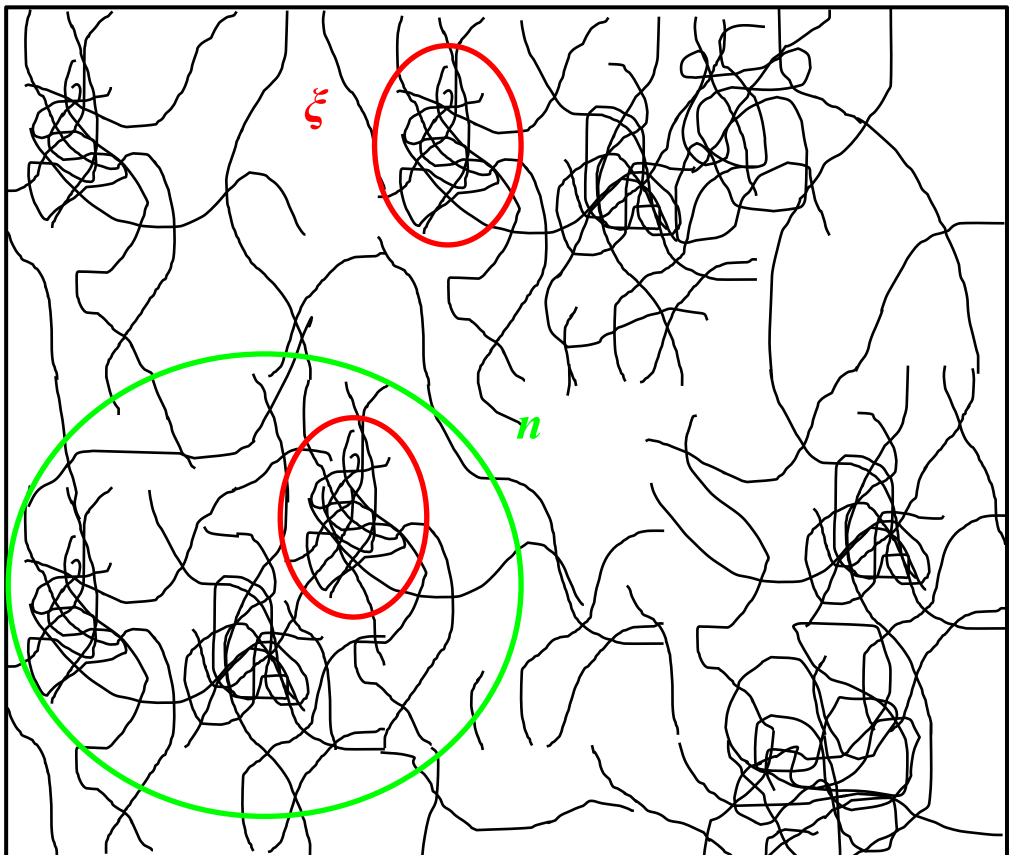


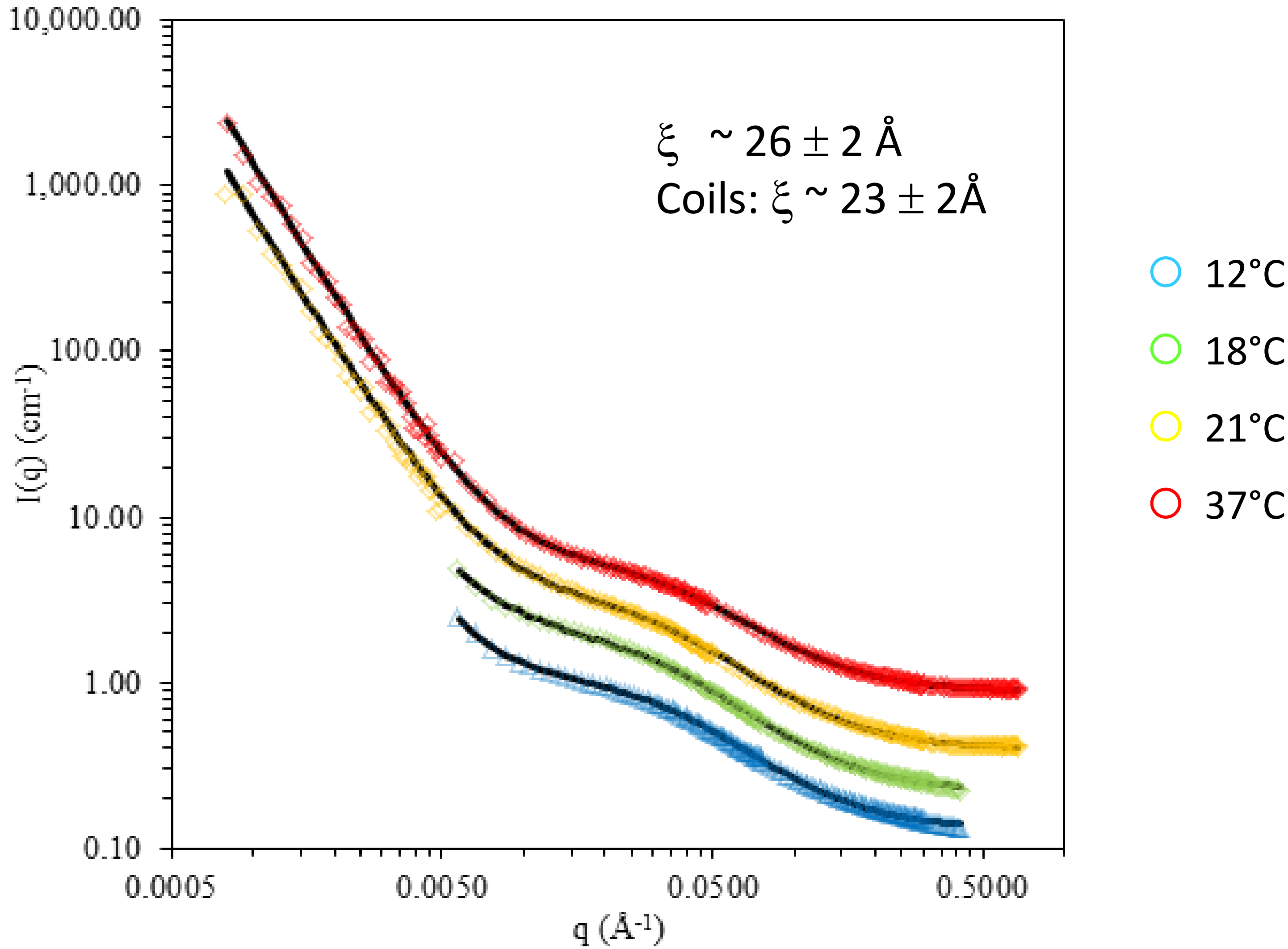
ξ – high q scaling- 10-100s Å
n- low q scaling 1000s Å

1. Physical networks

Structure of the networks

SANS

$$I(q) = \frac{I_L(0)}{1+q^2\xi^2} + \frac{A}{q^n}$$

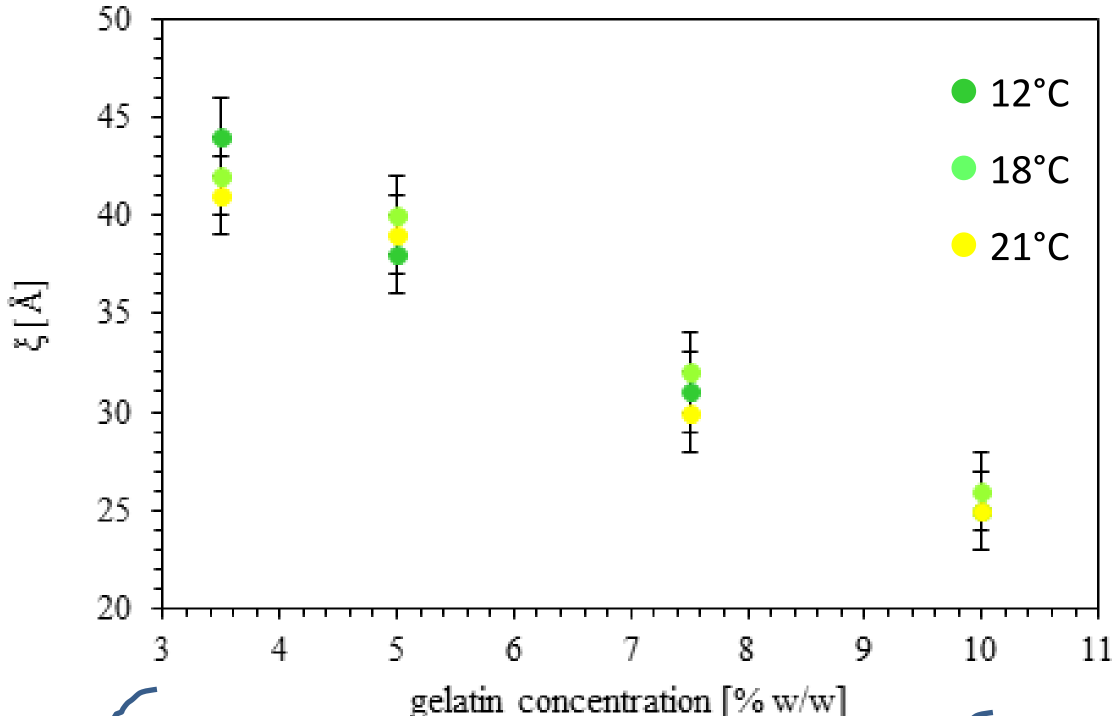


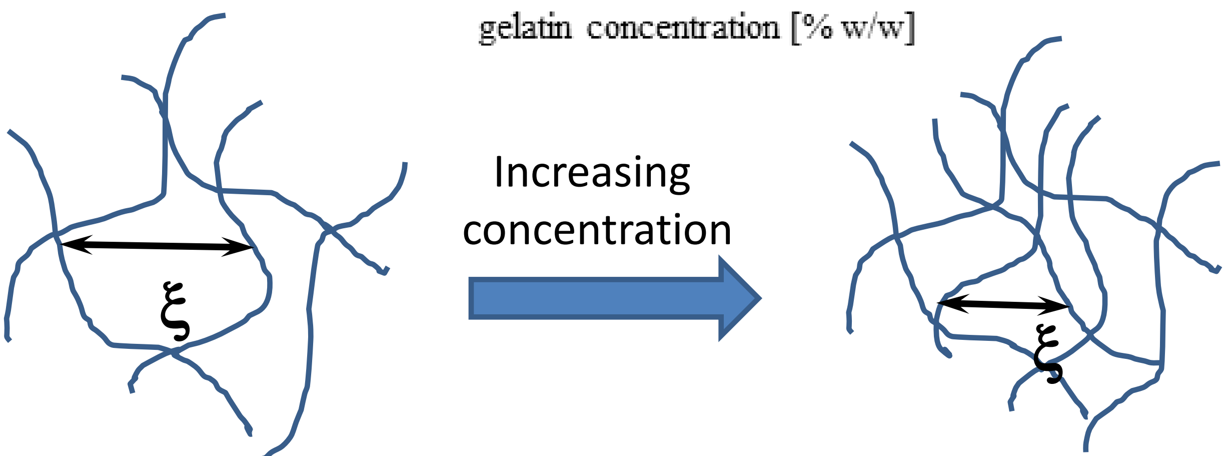
Soft Matter **2013**, *9*, 6986-99

1. Physical networks

Structure of the gels

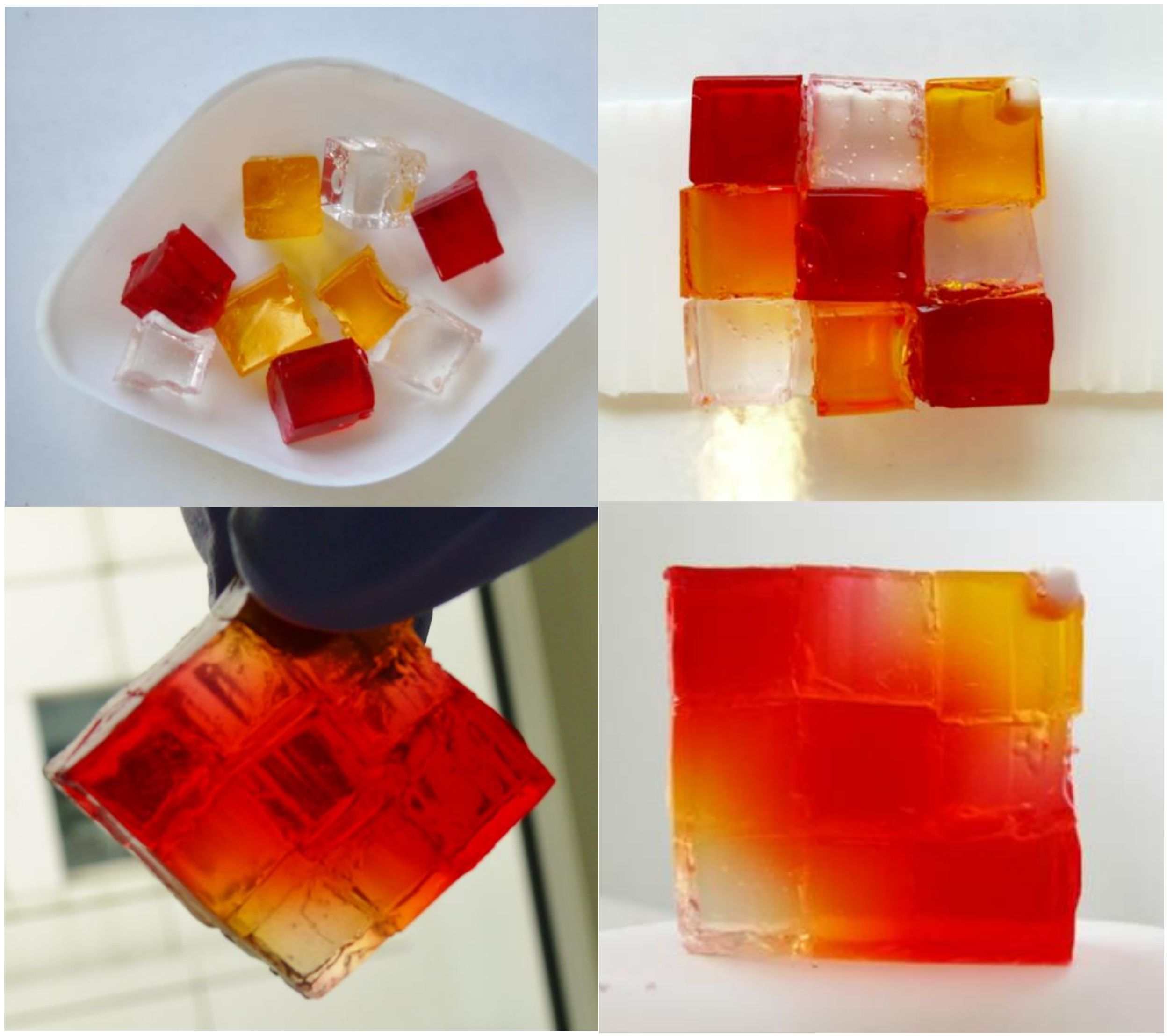
Correlation length vs. concentration





Enzymatic cross-linking:

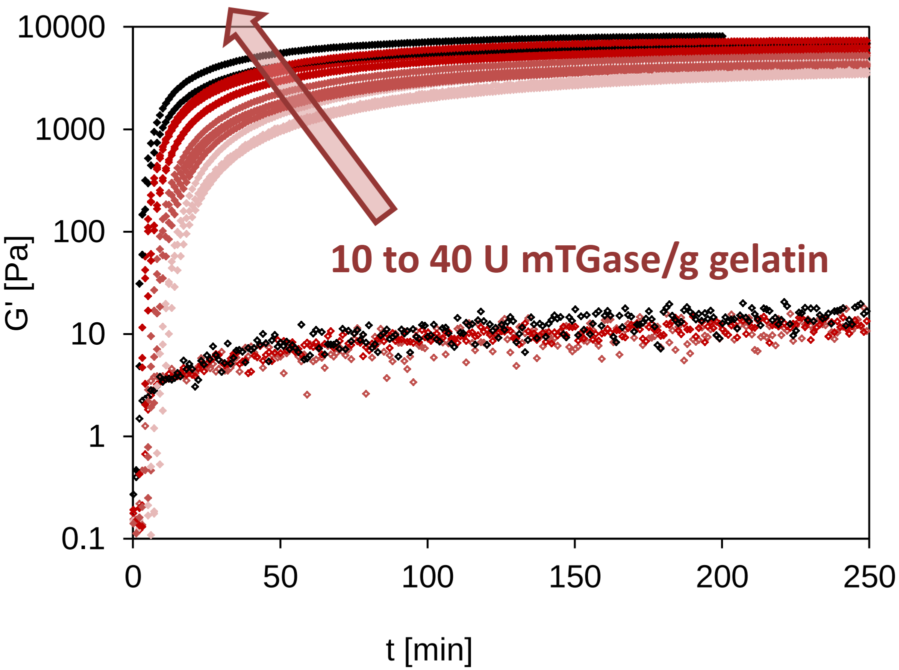
Gel healing



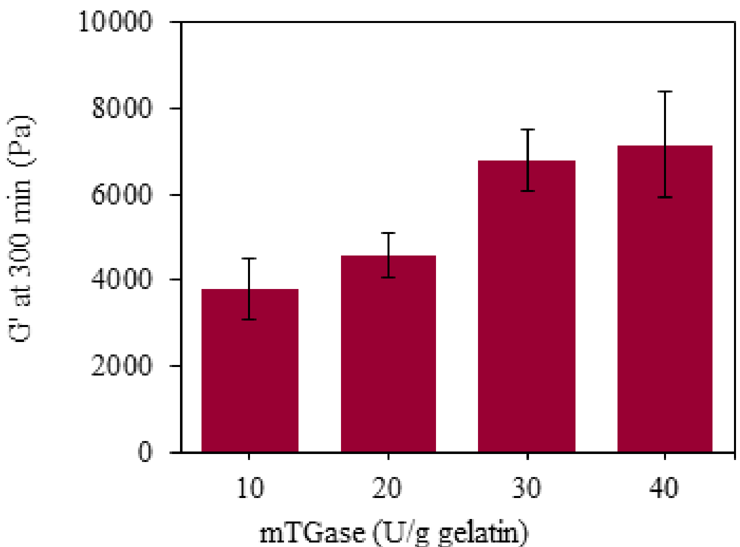
Biomacromolecules **2011**, *12*, 3741-52

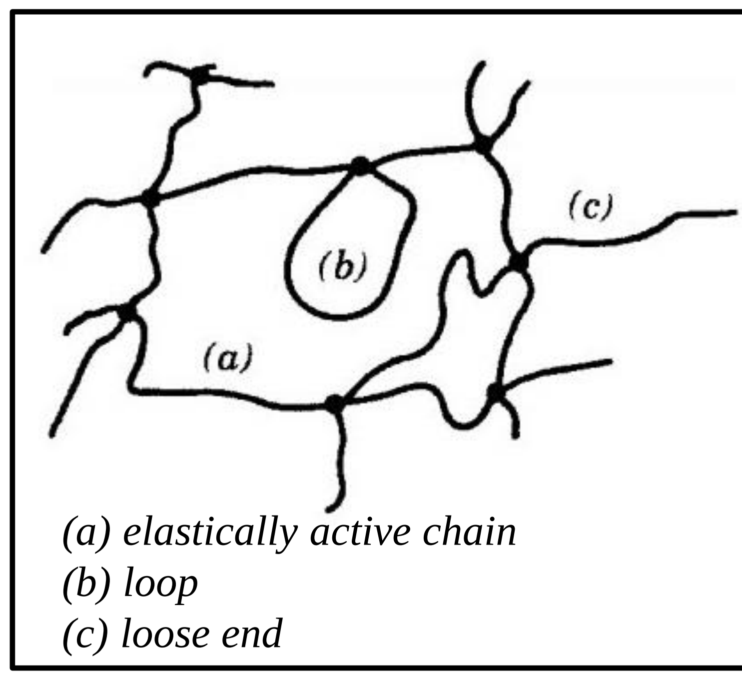
2. Chemical networks

Effect of enzyme concentration



- G’ increase with TGase concentration
- Faster kinetics with high TGase
- More loops at low enzyme?

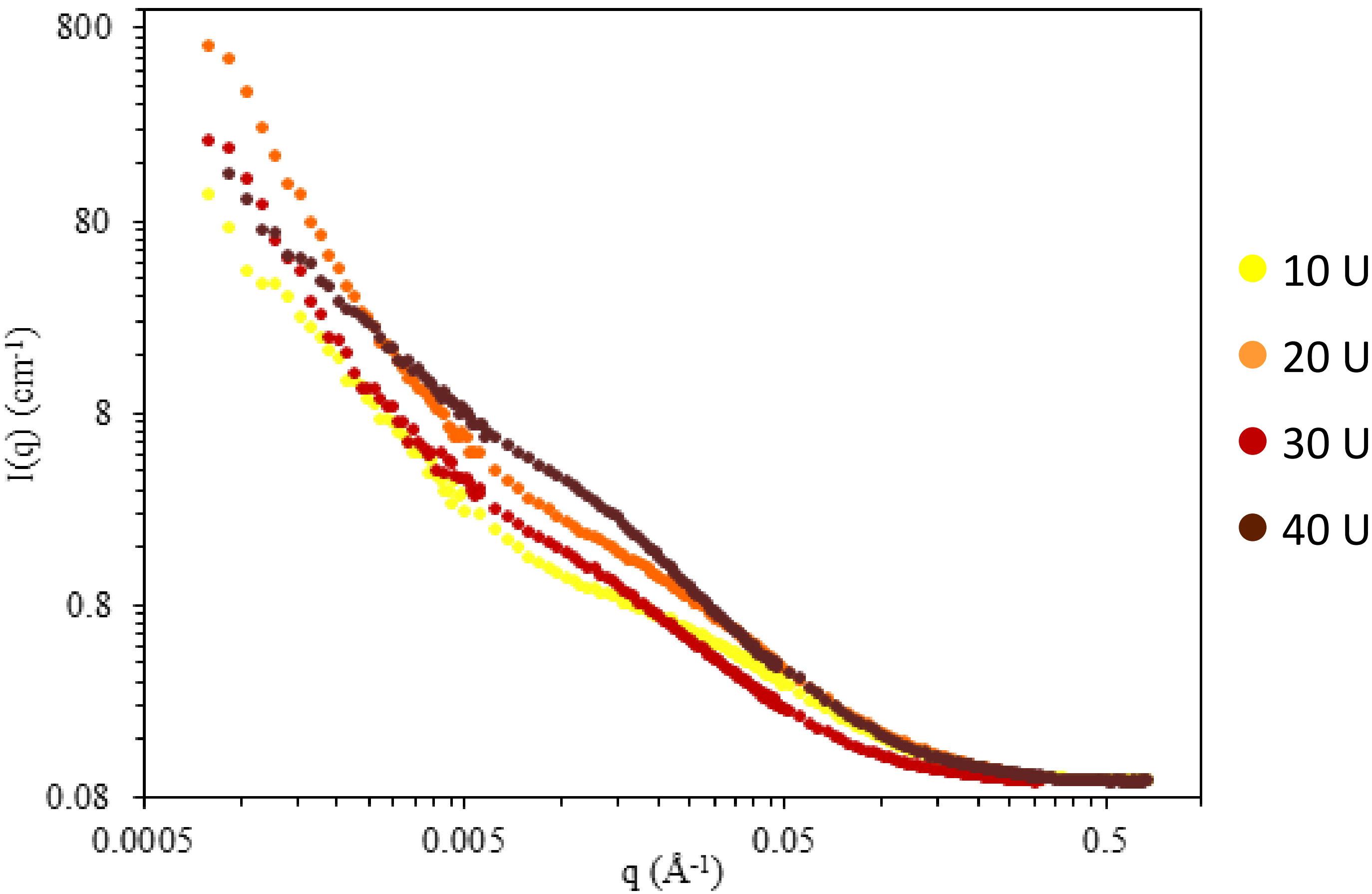




Biomacromolecules **2011**, *12*, 3741-52

2. Chemical networks

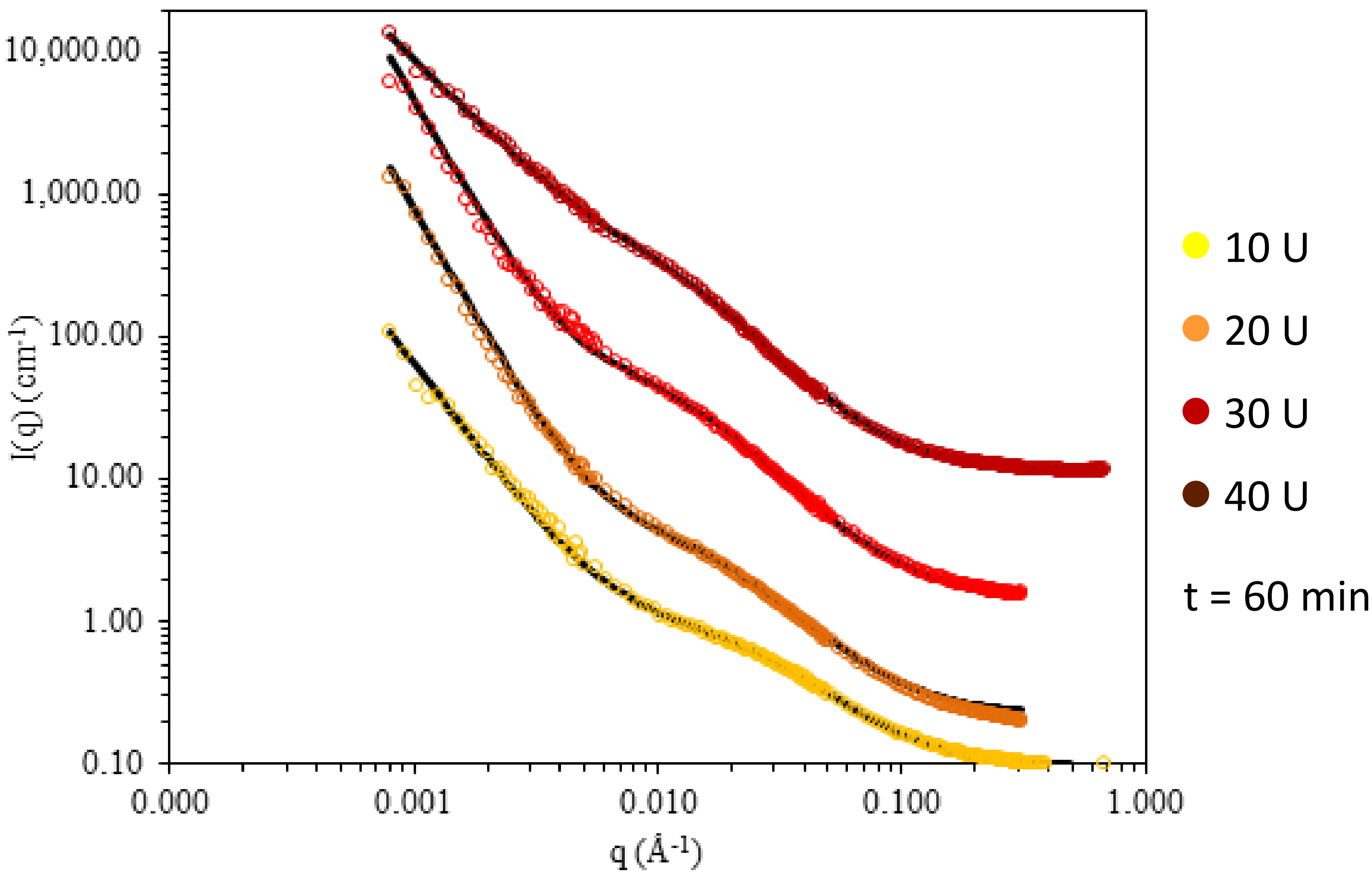
Structure of the networks
SANS



Structure of the networks

SANS

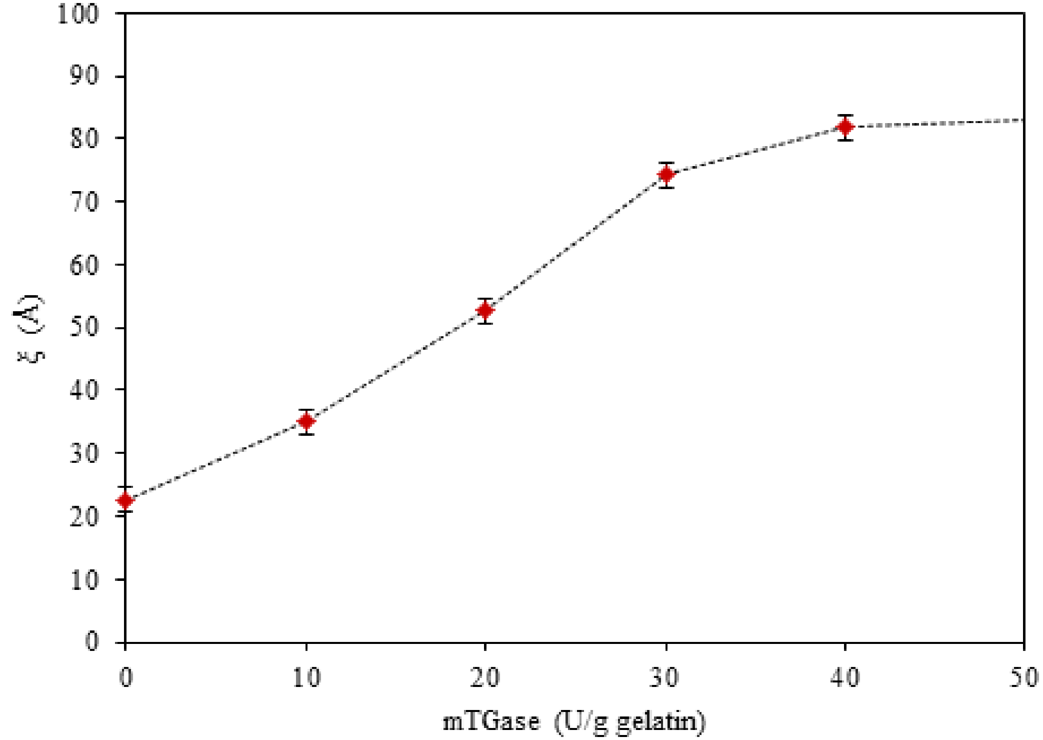
$$I(q) = \frac{I_L(0)}{1+q^2\xi^2} + \frac{A}{q^n}$$



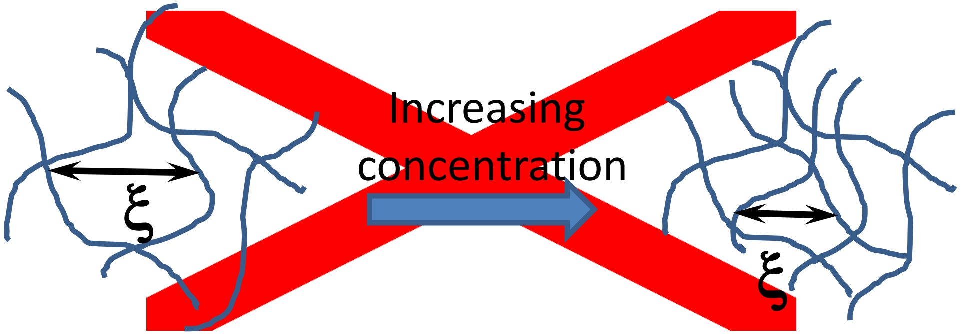
Soft Matter **2013**, *9*, 6986-99

Correlation length
vs. mTGase concentration

2. Chemical networks

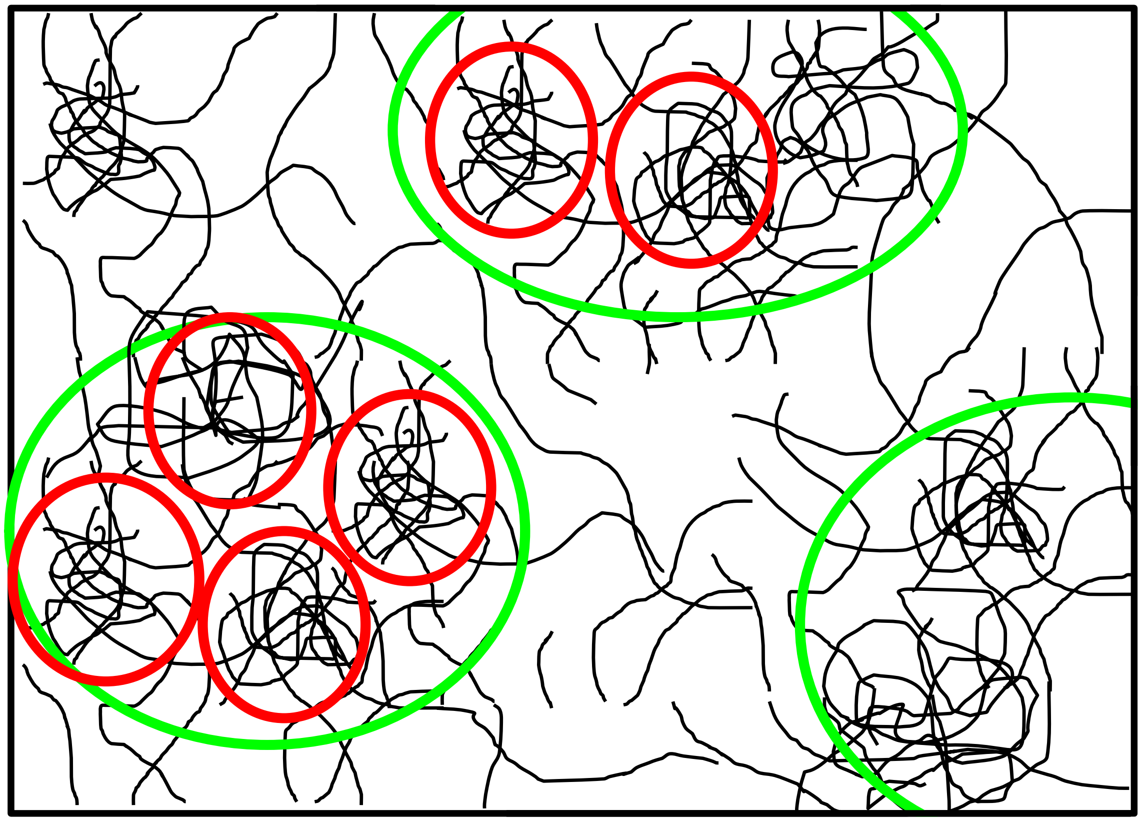


t = 60 min



Structure of the gels

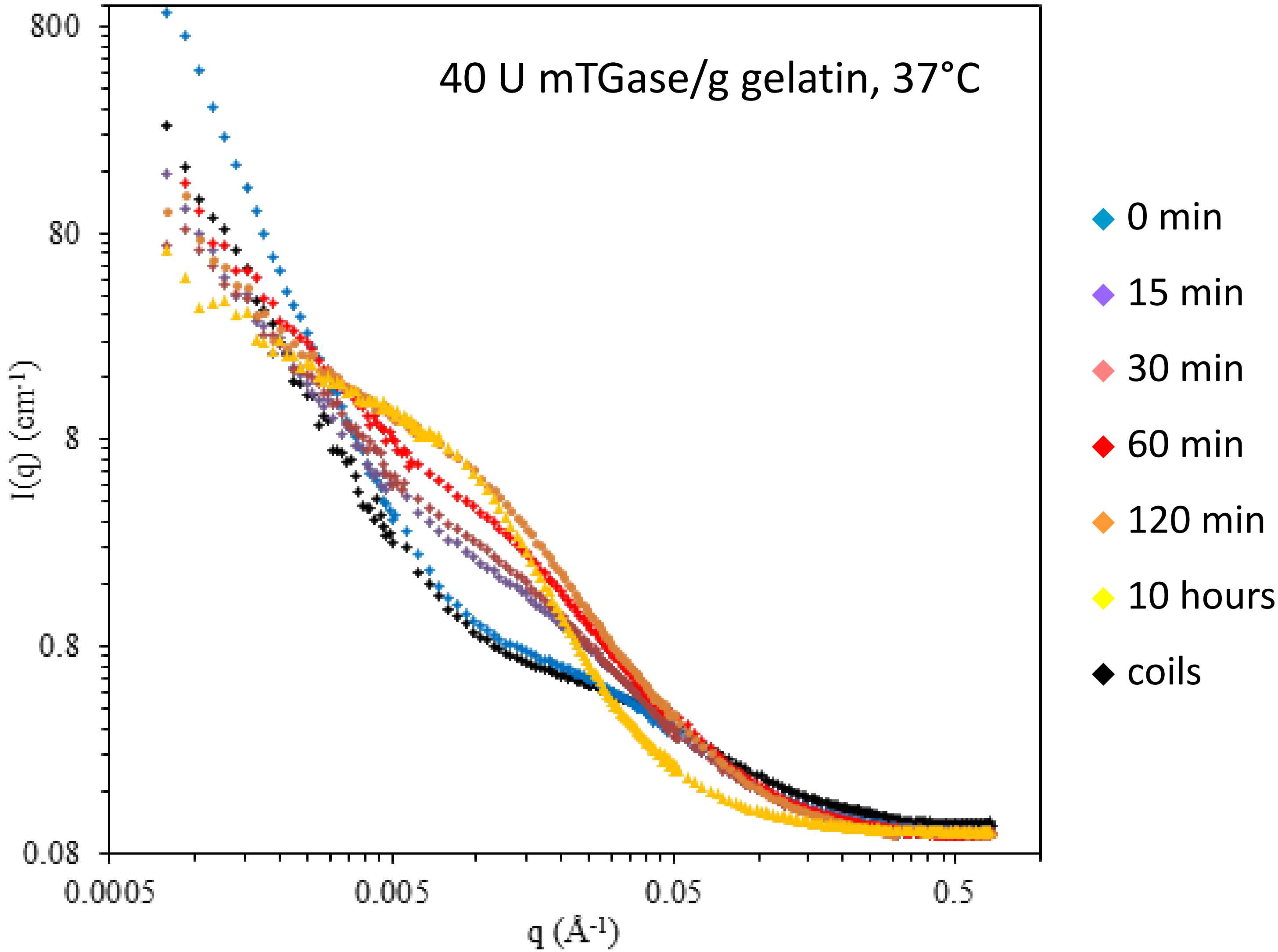
interpretation



SANS data reflect the size of growing clusters

Kinetics of gelation
SANS

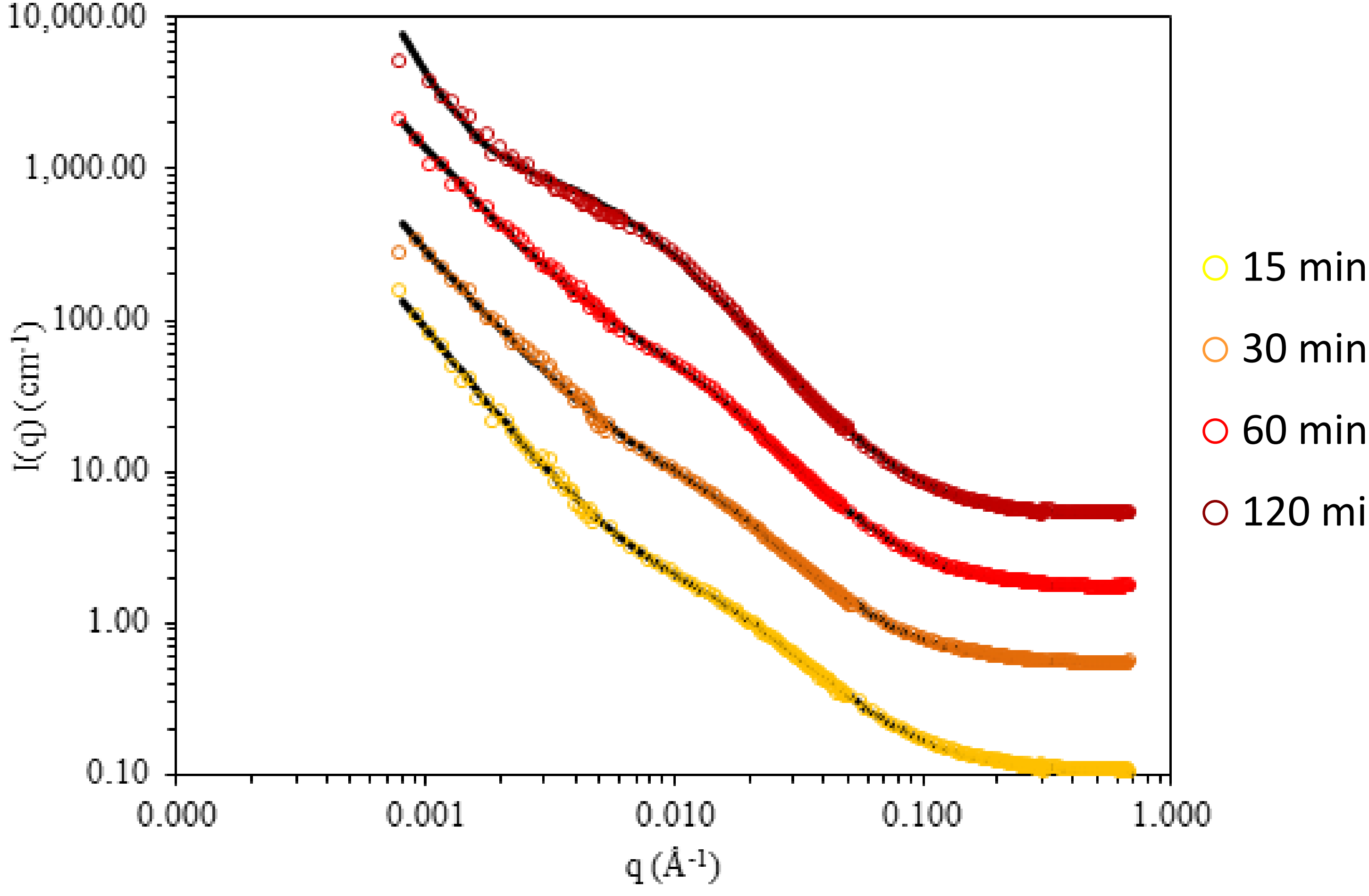
2. Chemical networks



Kinetics of gelation

SANS

$$I(q) = \frac{I_L(0)}{1+q^2\xi^2} + \frac{A}{q^n}$$



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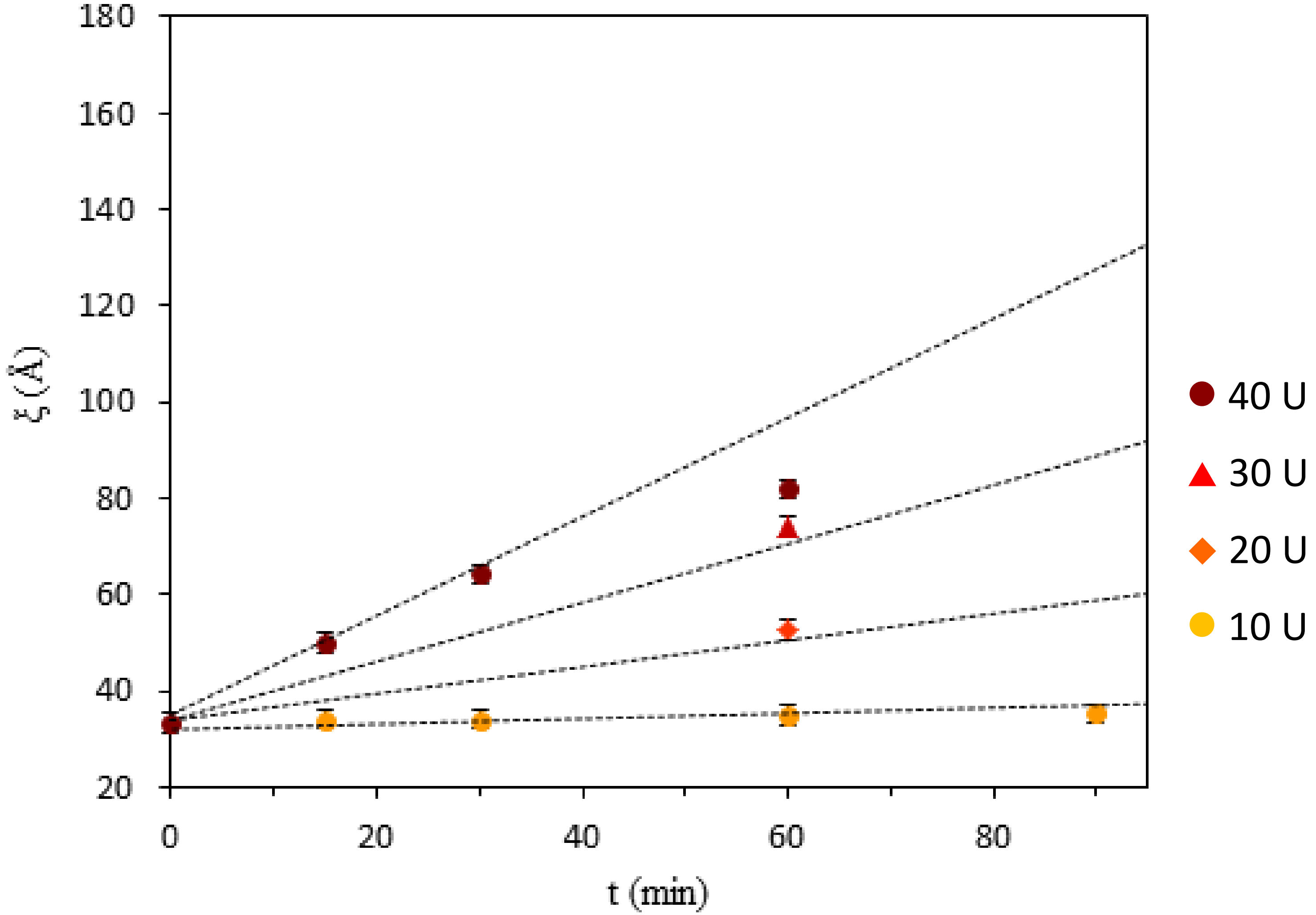
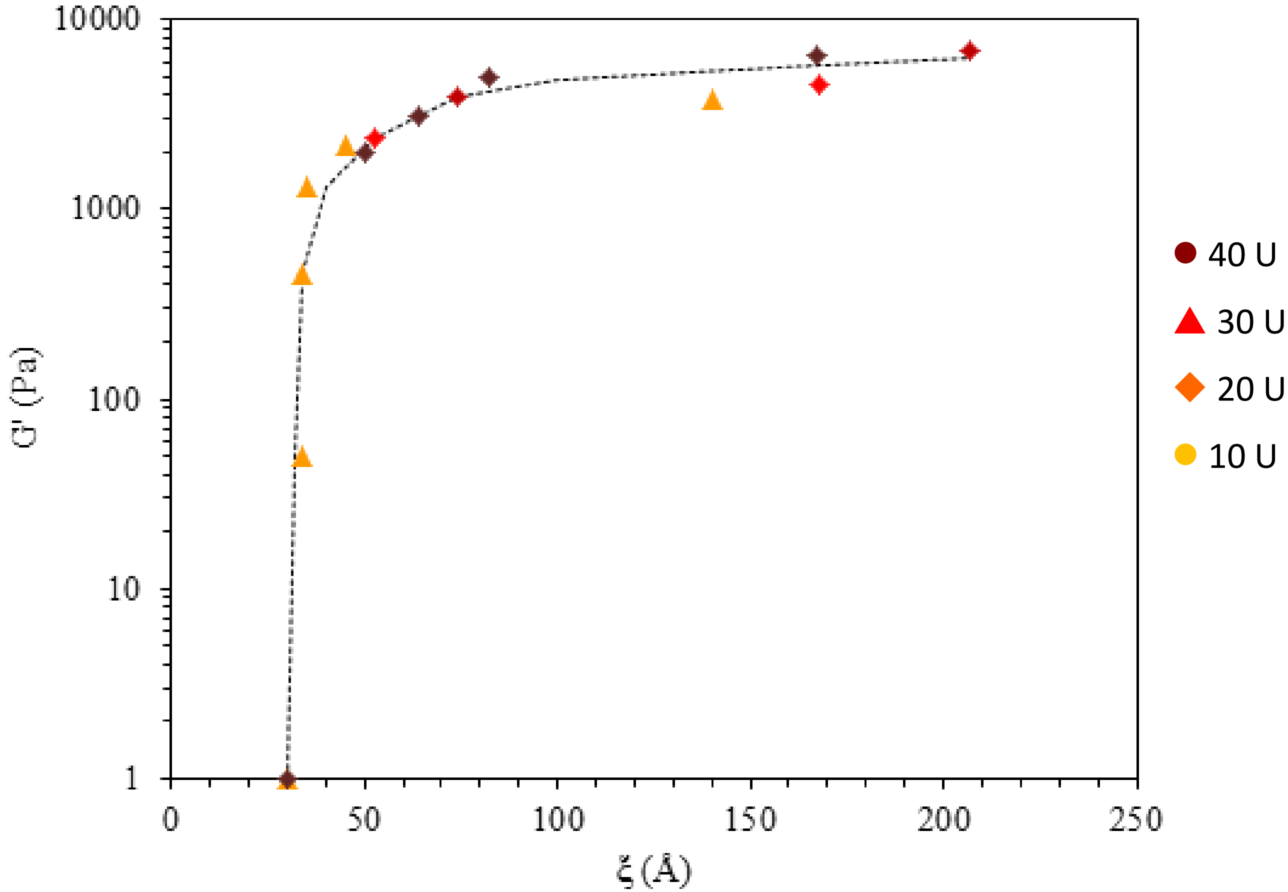
Kinetics of gelation
Evolution of the correlation length



ξ reflects the size of growing aggregates

2. Chemical networks

Linking the macro- and nano- scales



- all data points fall into a single master curve
- Critical value of ξ needed for gelation to occur = percolation threshold?
- G’ reaches a plateau while ξ keeps growing

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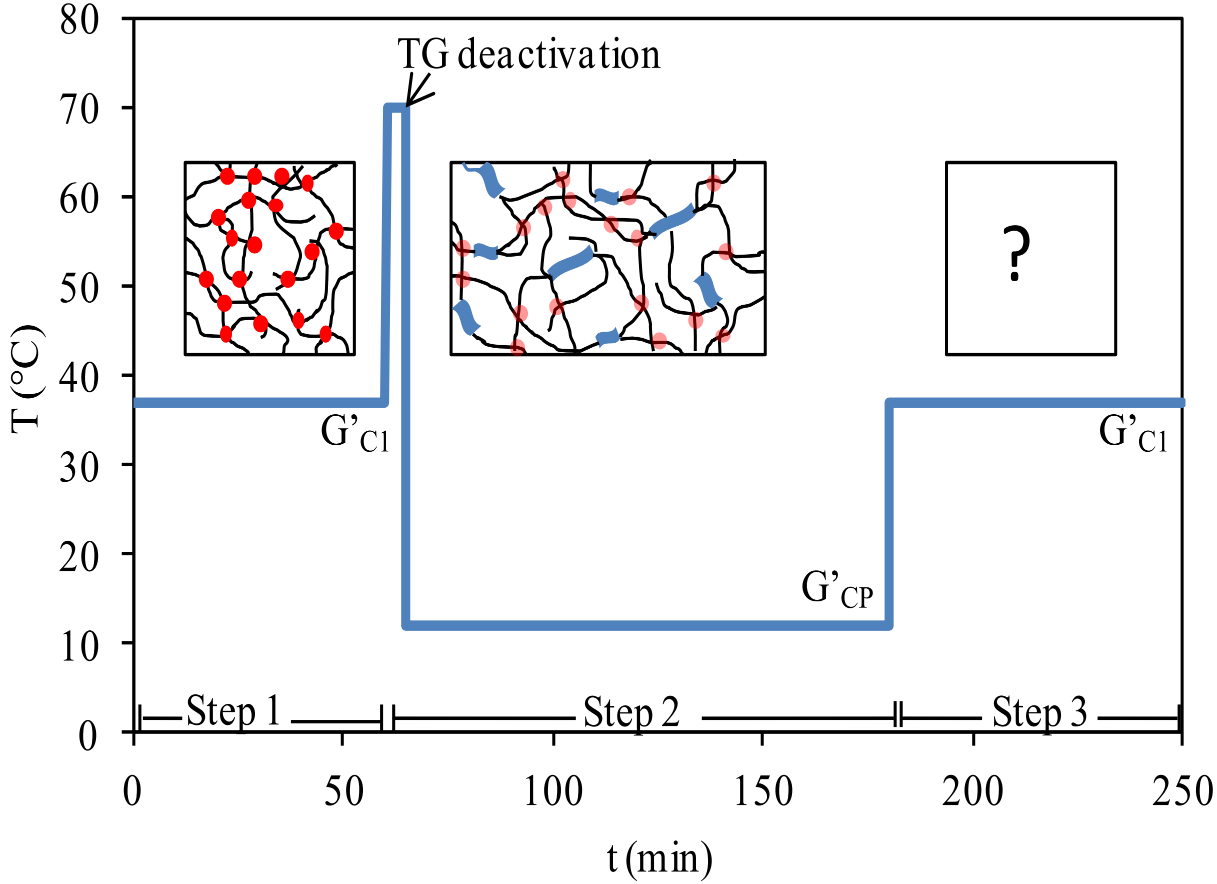
Chemico-physical networks

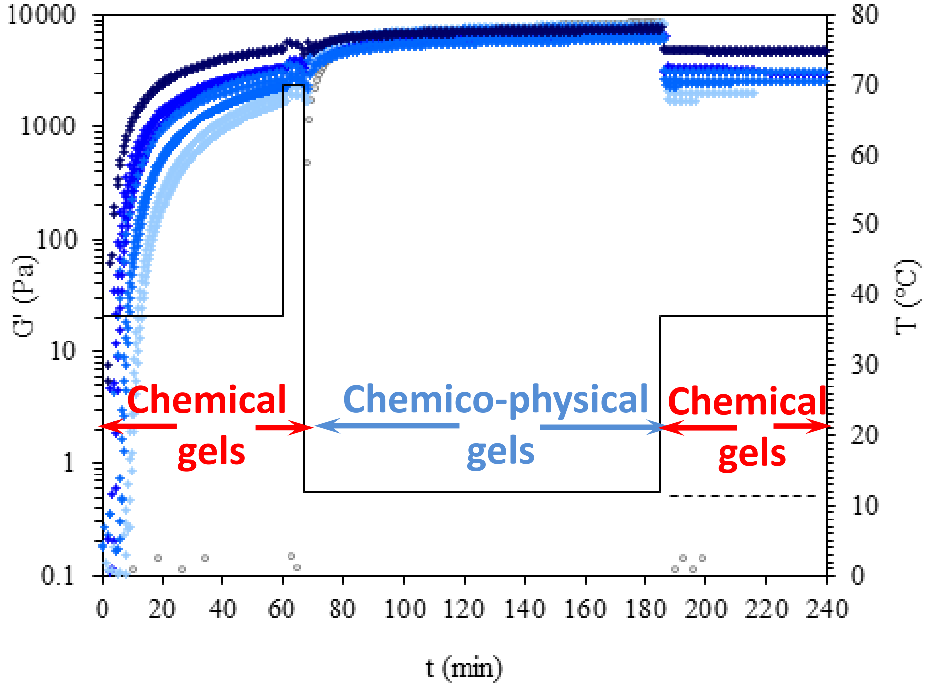
Protocol

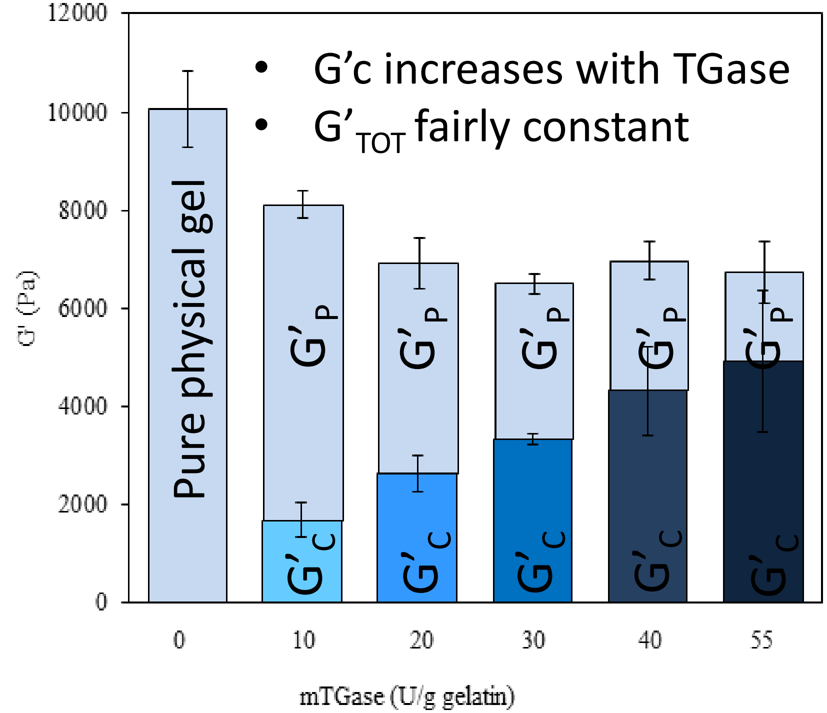
Sequential networks

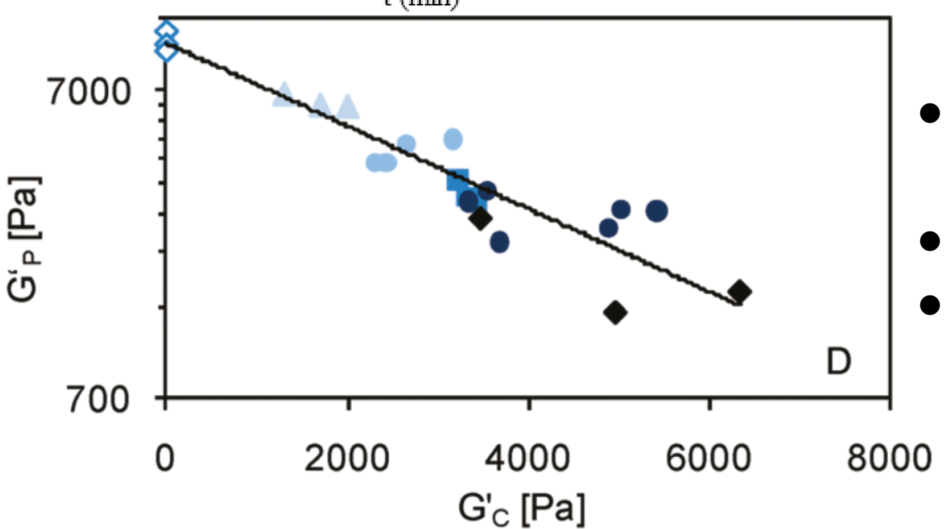


Physical vs. Chemical network

Rheology







- Exponential relationship between G'_P and G'_c
- Maximum G' for pure physical gels
- No benefit in building P over C

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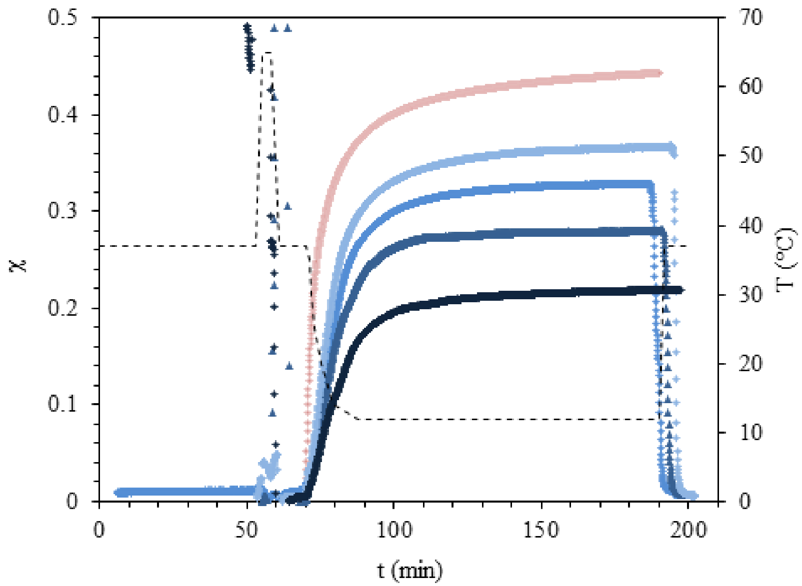
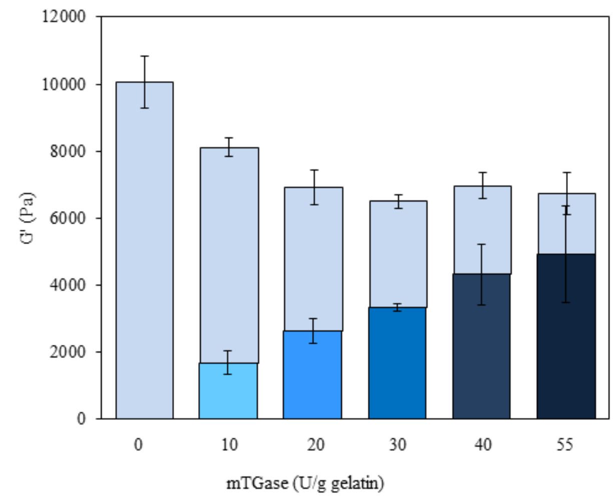
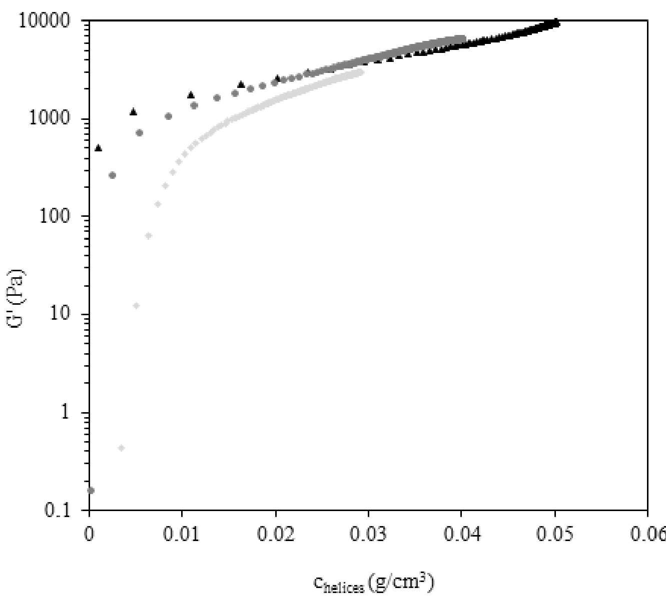
3. Chemico-physical networks

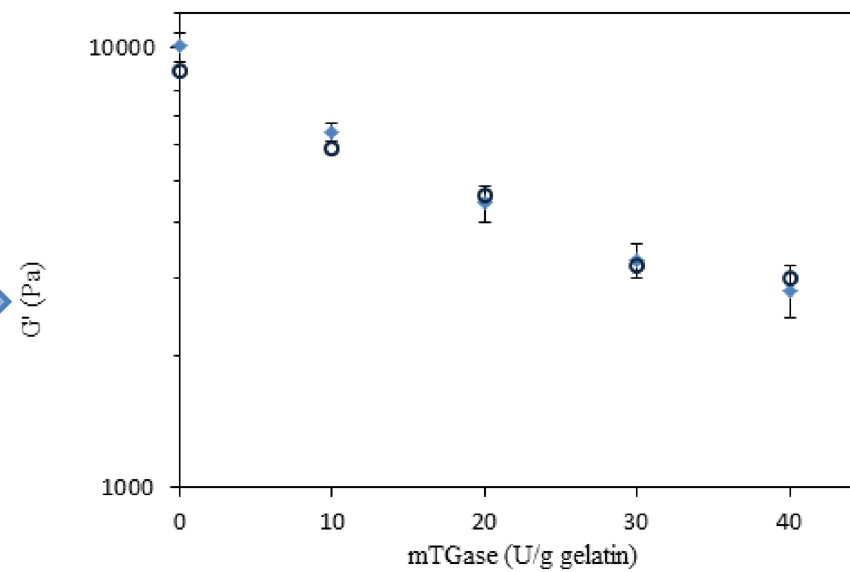
Effect of TGase concentration

ORD and rheology







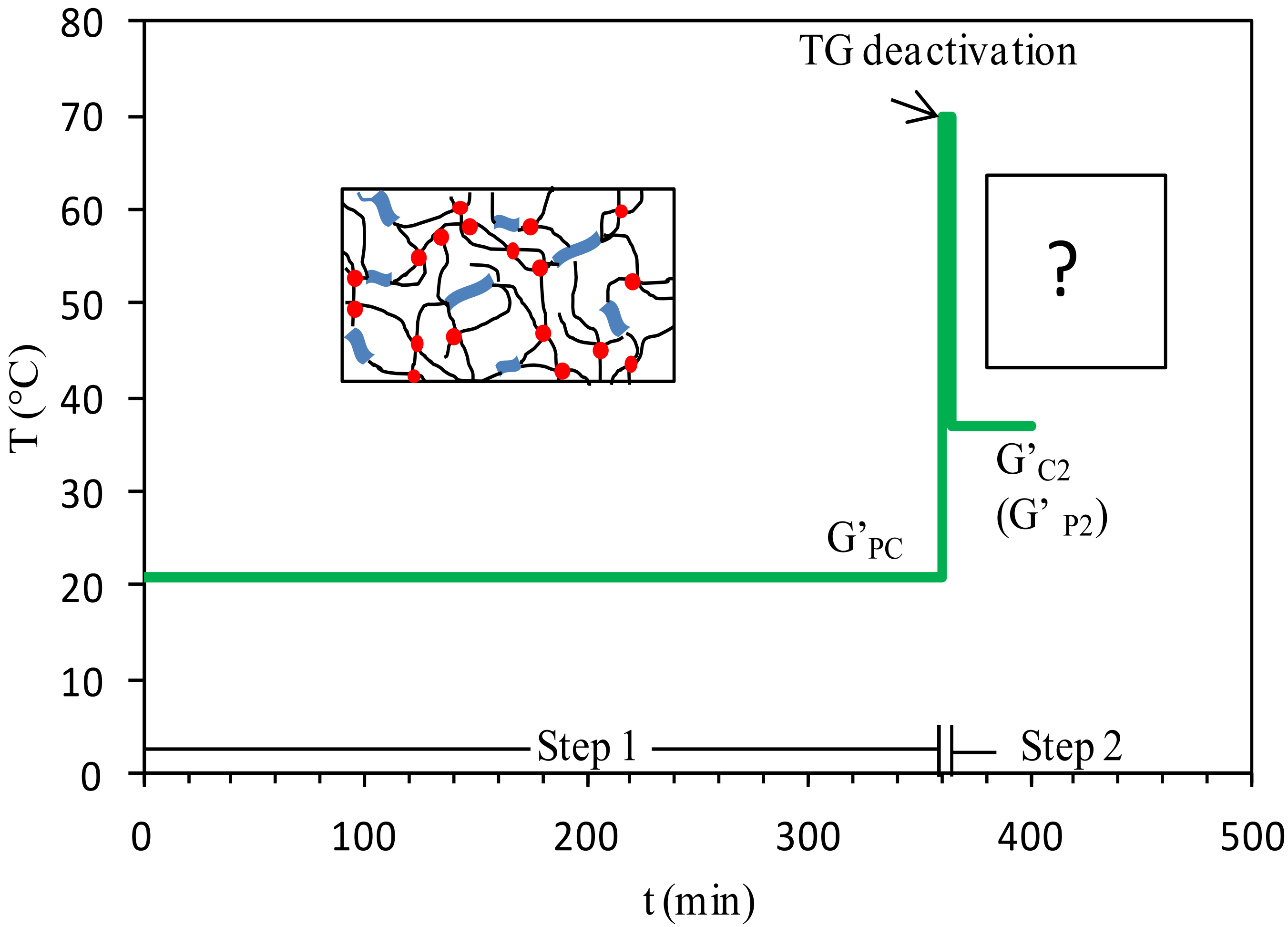


Additive networks

Physical-co-chemical networks

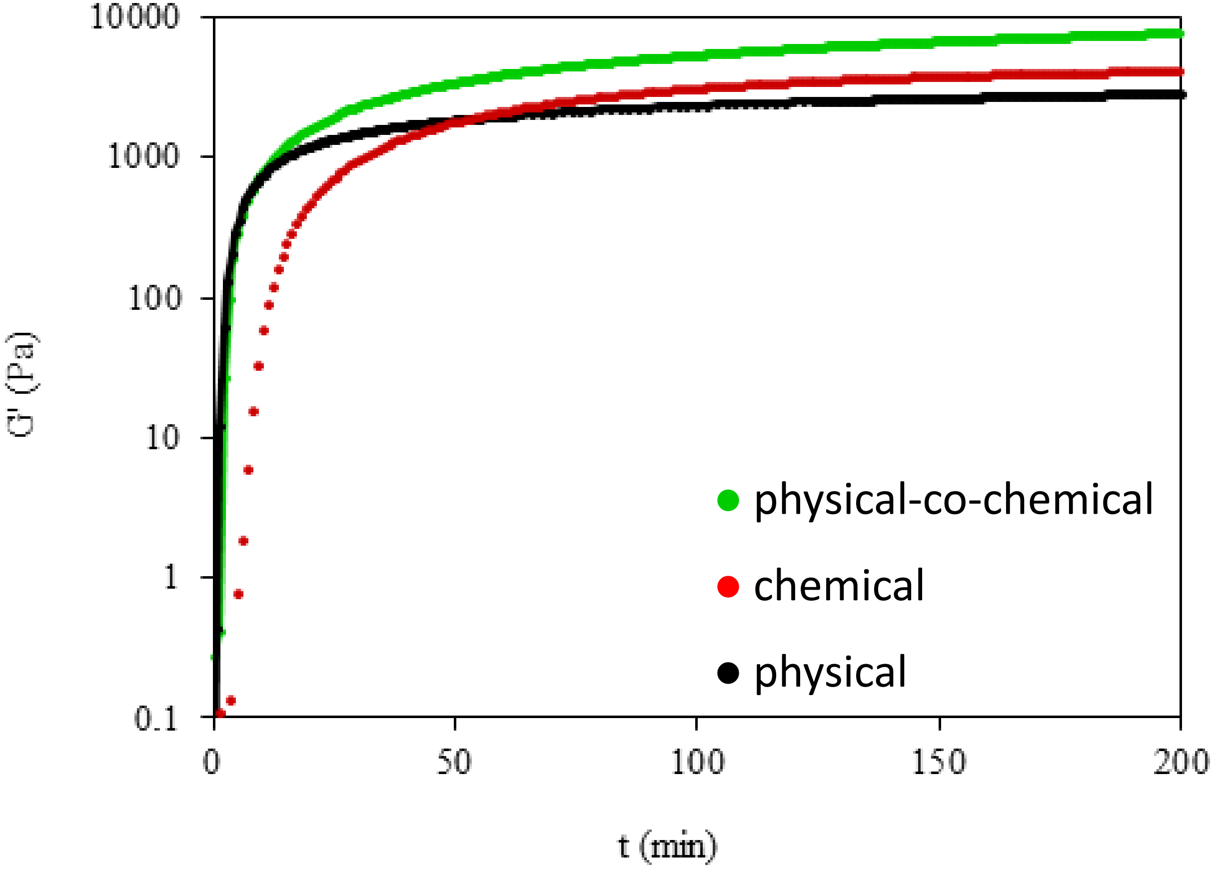
Protocol

Simultaneous networks



Physical-co-chemical networks

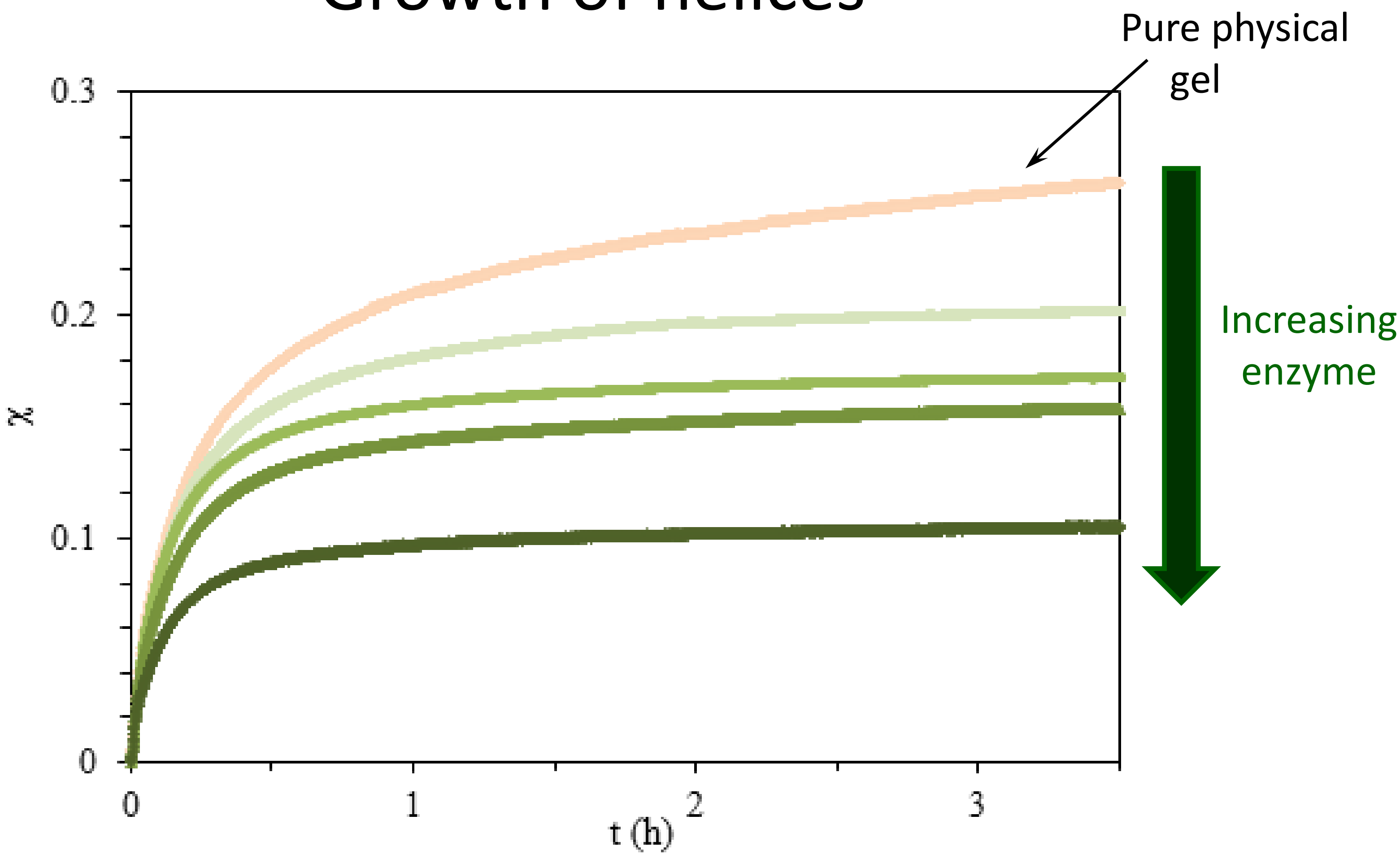
Rheology



Biomacromolecules **2011**, *12*, 3741-52

Physical-co-chemical networks

Growth of helices

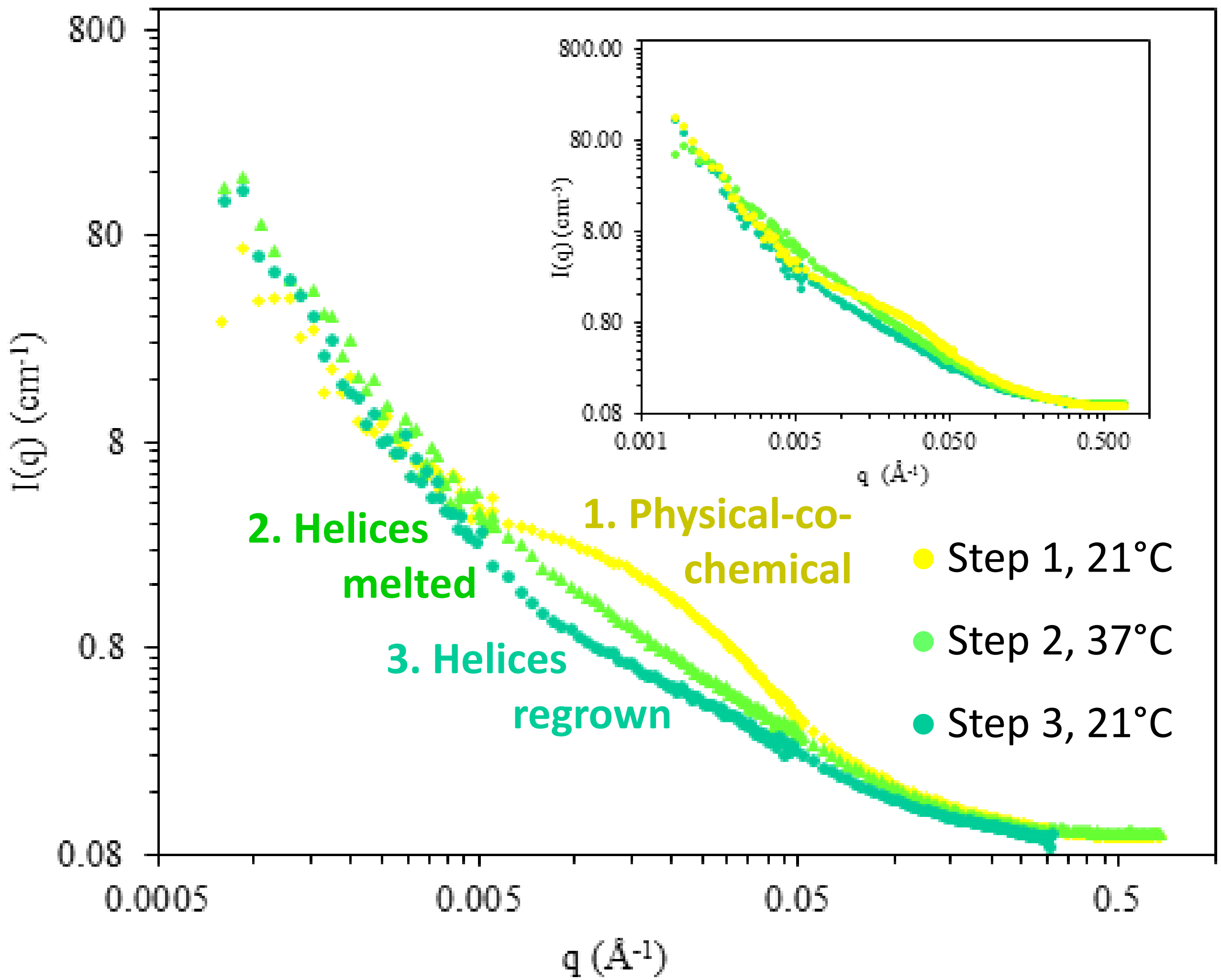


- Enzyme hinders helix concentration
- Helix concentration reaches a plateau, unlike physical gels

4. Physical-co-chemical networks

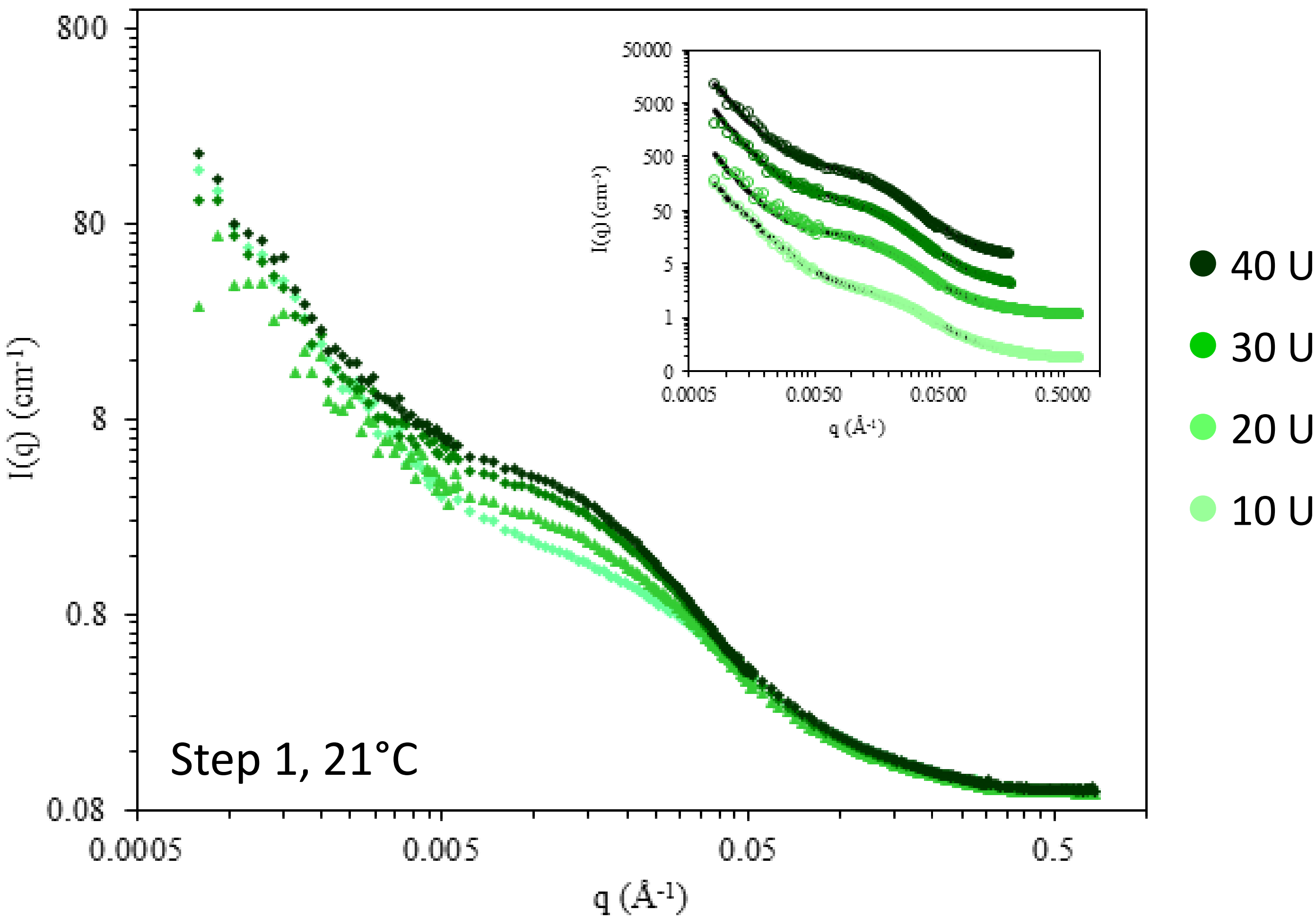
Structure of PC gels by SANS

3 time steps



Helices regrown after melting do not match the original physical network grown at the same time of the cross-linking

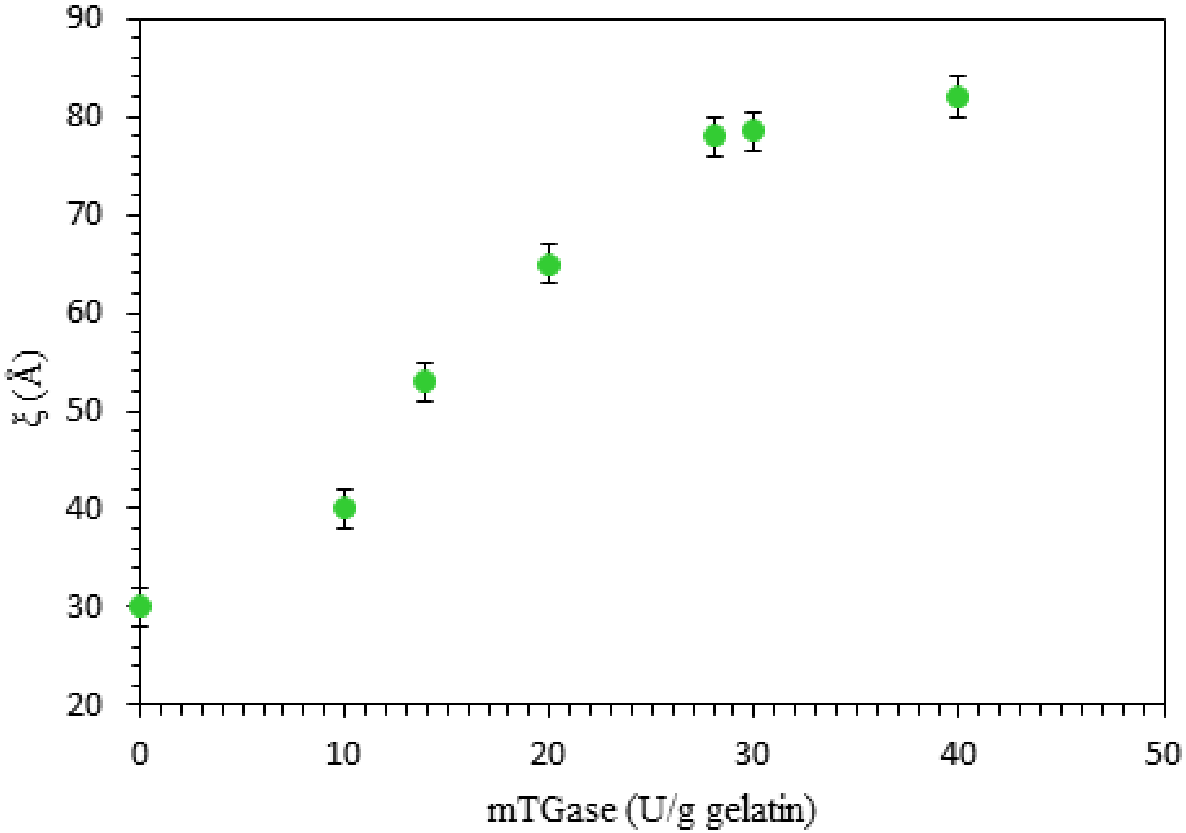
Structure of PC networks
Effect of enzyme concentration



Soft Matter **2013**, *9*, 6986-99

Structure of PC gels

SANS



At equivalent enzyme concentration:

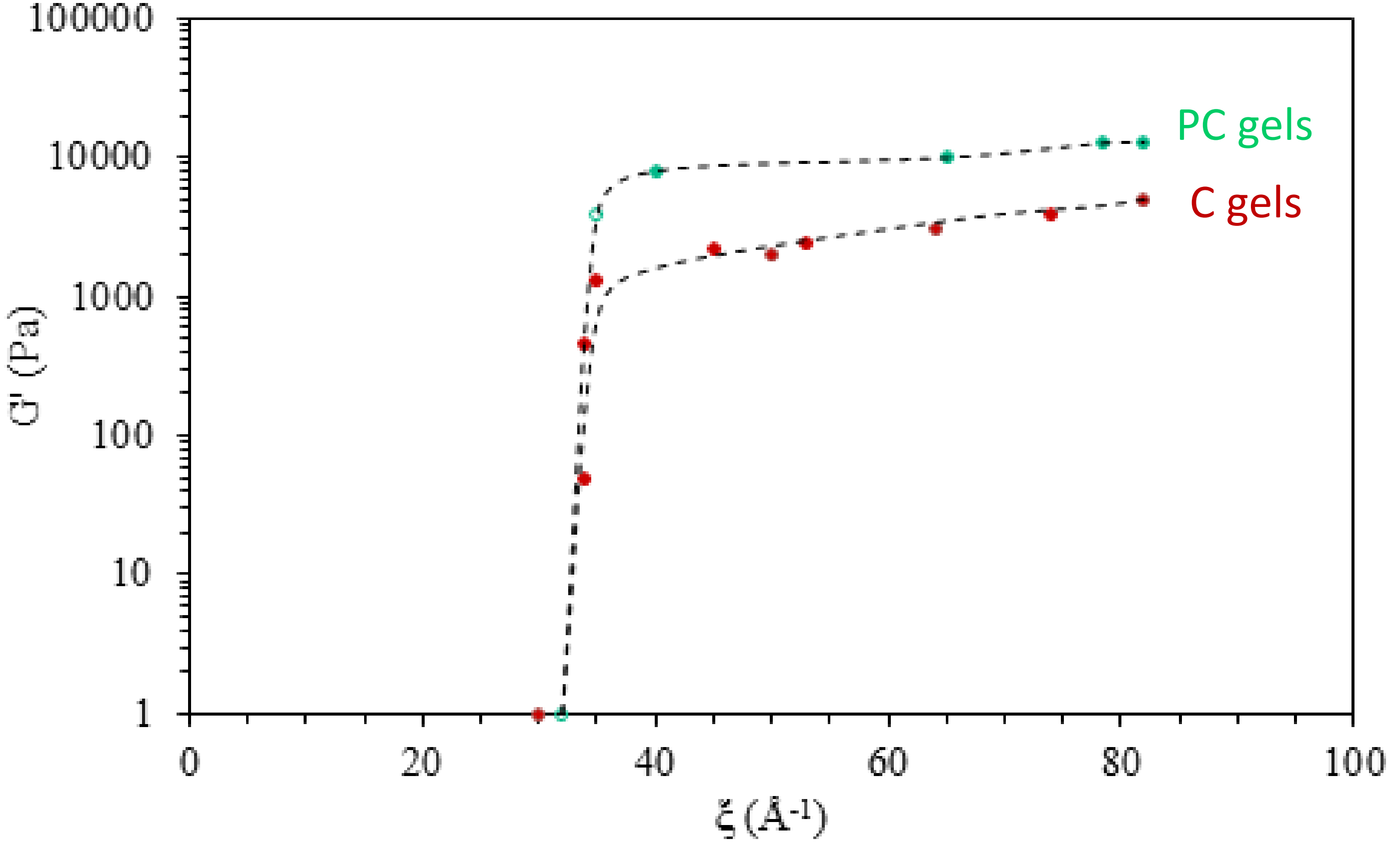
- correlation length is smaller than in pure chemical gels
- BUT contribution from chemical network to total G' is higher

$\Rightarrow$ the cross-links must be more efficiently distributed

Linking the macro- and nano- scales

Chemical vs. Physical-co-chemical

4. Physical-co-chemical networks



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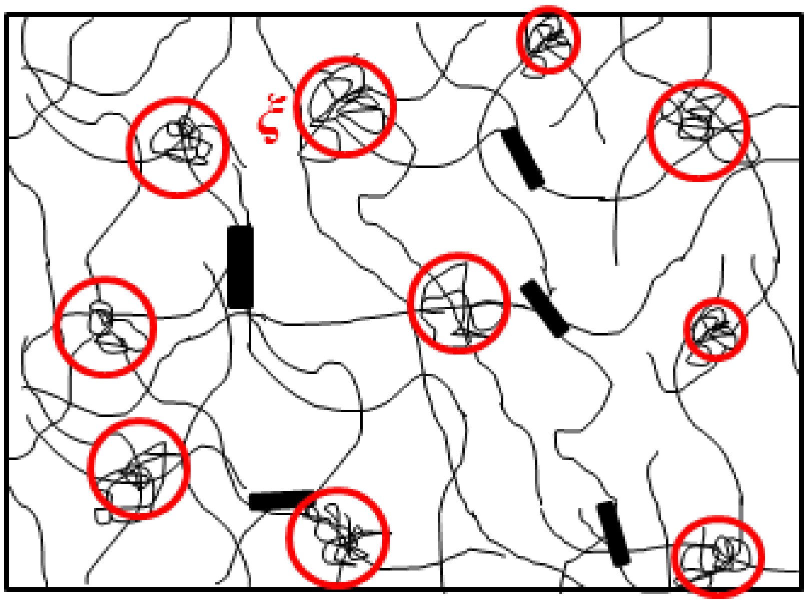
Structure of the gels

Chemical vs. Physical-co-chemical

Chemical gels



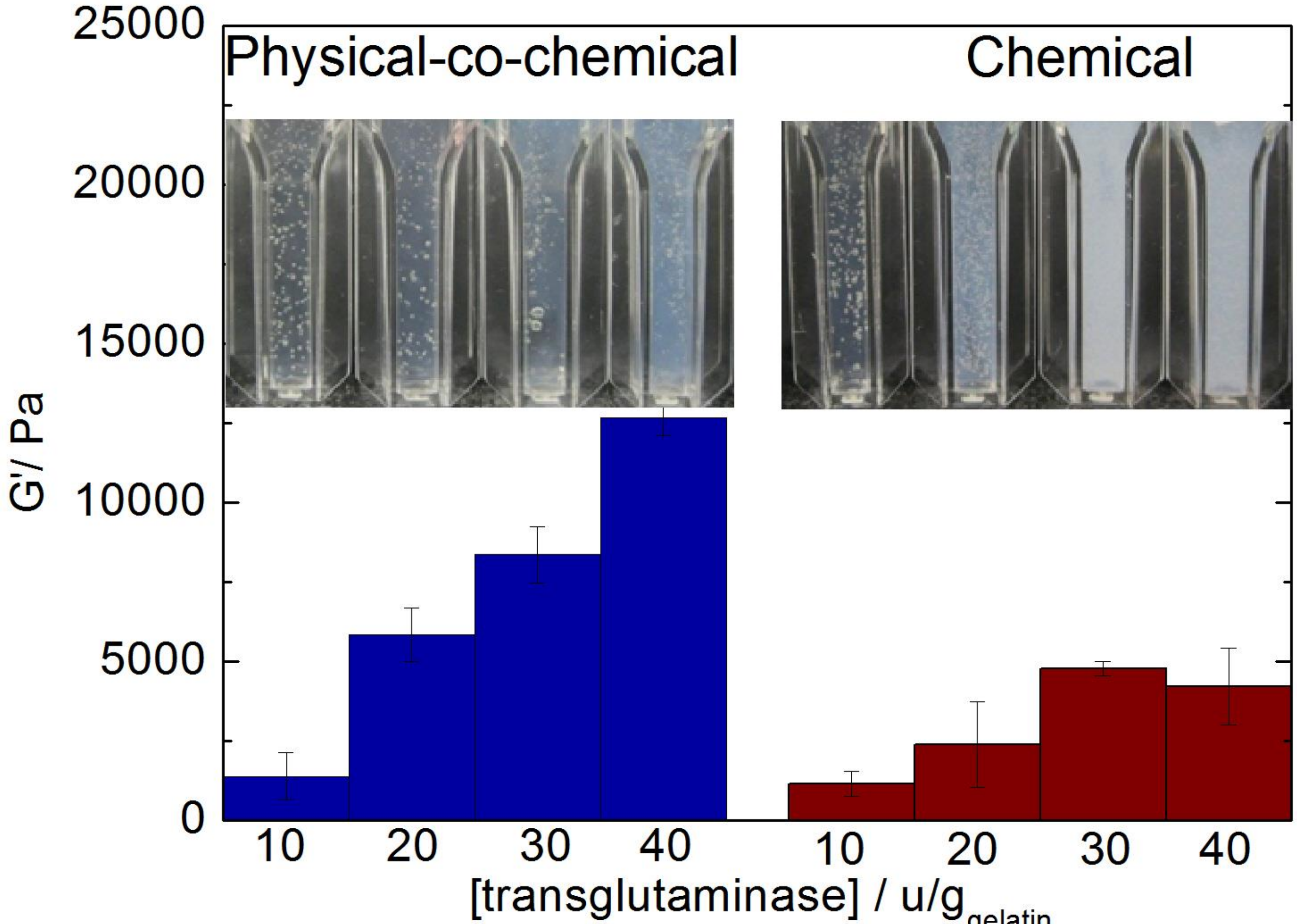
Physical-co-chemical gels:

Growth of clusters delimited by helices

More ordered network = more efficient

Synergy of *hybrid* networks

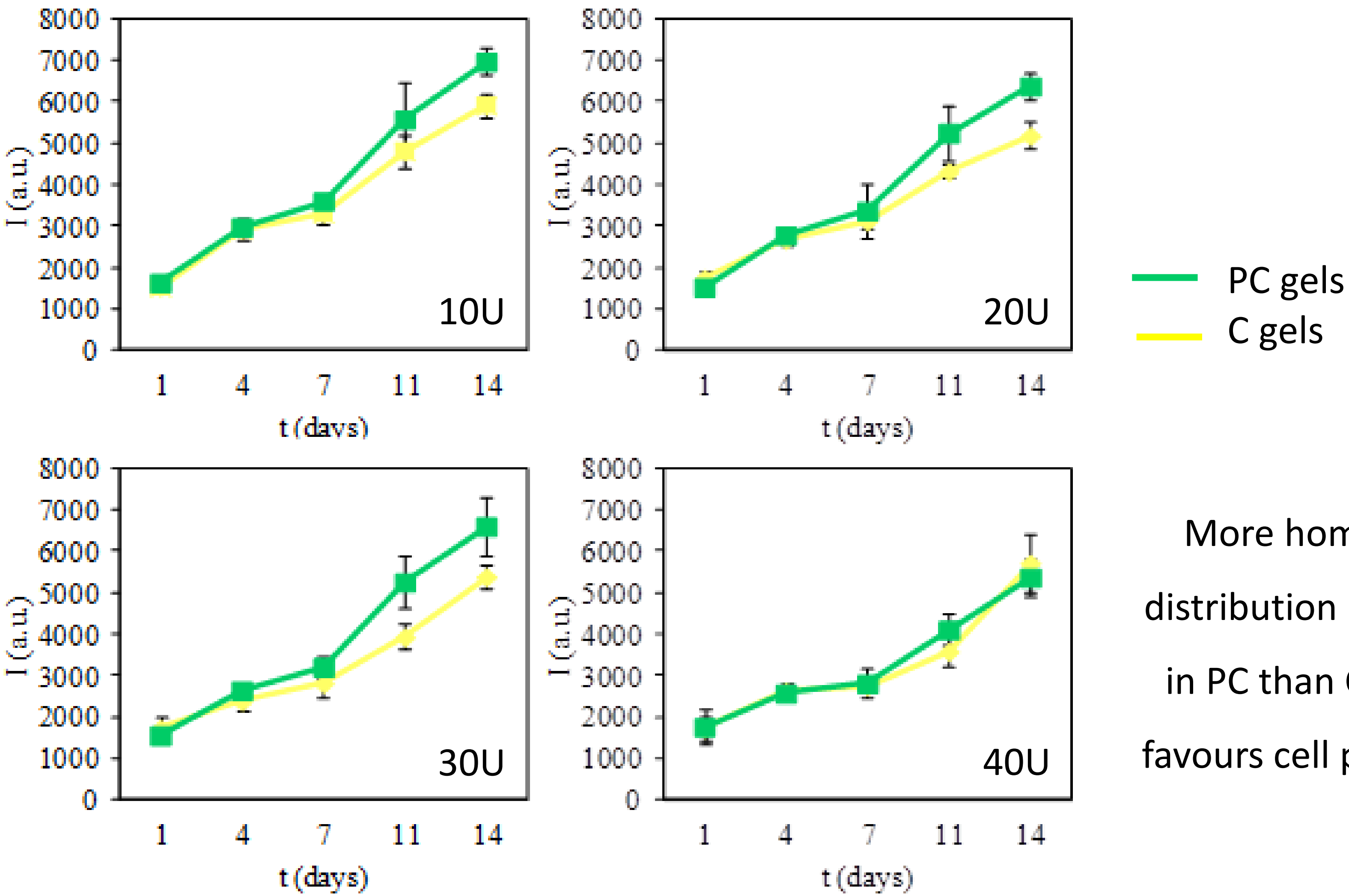
Chitosan/gelatin blends



Under review

Cell culture studies

Comparison C/PC gels



More homogeneous distribution of cross-links in PC than C networks: favours cell proliferation?

Cell viability of RBMS cells at 14 days

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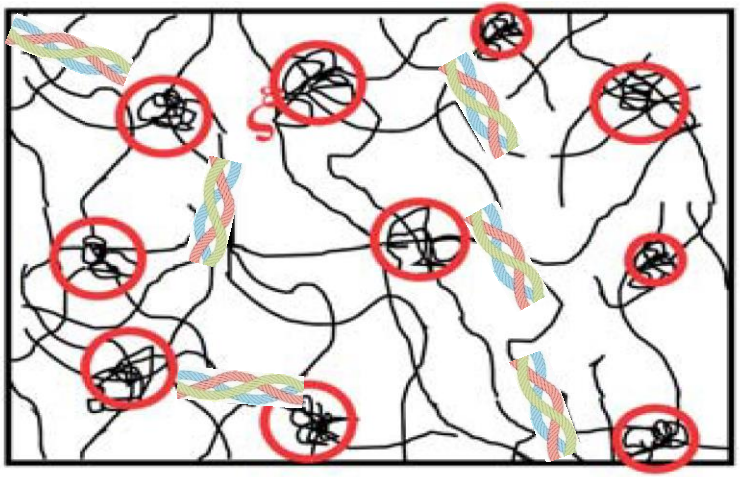
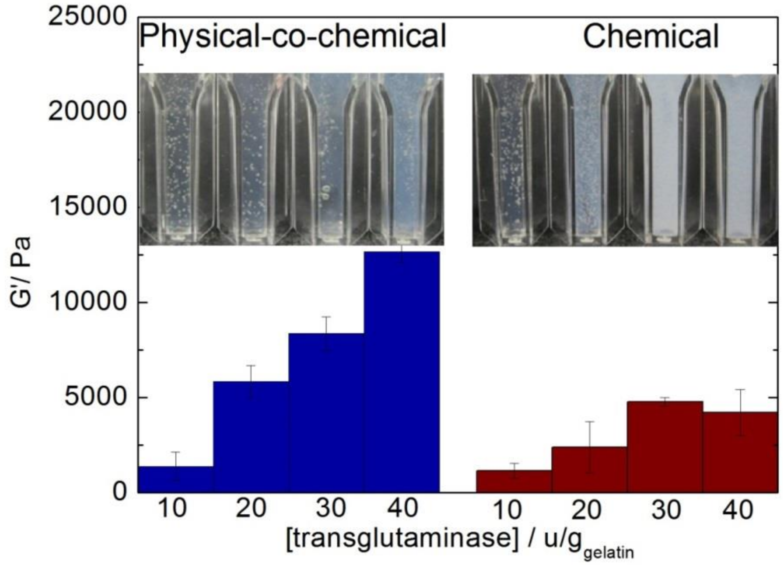
Conclusions
Hybrid gelation processes





- Cross-linking proceeds *via* the growth of clusters.
- When combined with physical gelation, these clusters are constrained by the triple-helices, leading to a **more ordered and efficient** network, which seems to favour cell proliferation.

- The cross-linking process is **guided** by the triple-helices, making gel **stronger** and **more homogeneous**.
- This **synergistic** effect is also seen in blends, and with other cross-linkers

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