Enzymatic Degradation of Biopolymers: A combined Light Scattering - Fluorescence study

Vincenzo Ruzzi, Carole Antoine Assor, Claire Dumont, Antoine Bouchoux, Lucas Cipelletti, Laurence Ramos

ENZYMATIC DEGRADATION OF BIOPOLYMERS: A COMBINED LIGHT SCATTERING – FLUORESCENCE STUDY

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22/05/2024

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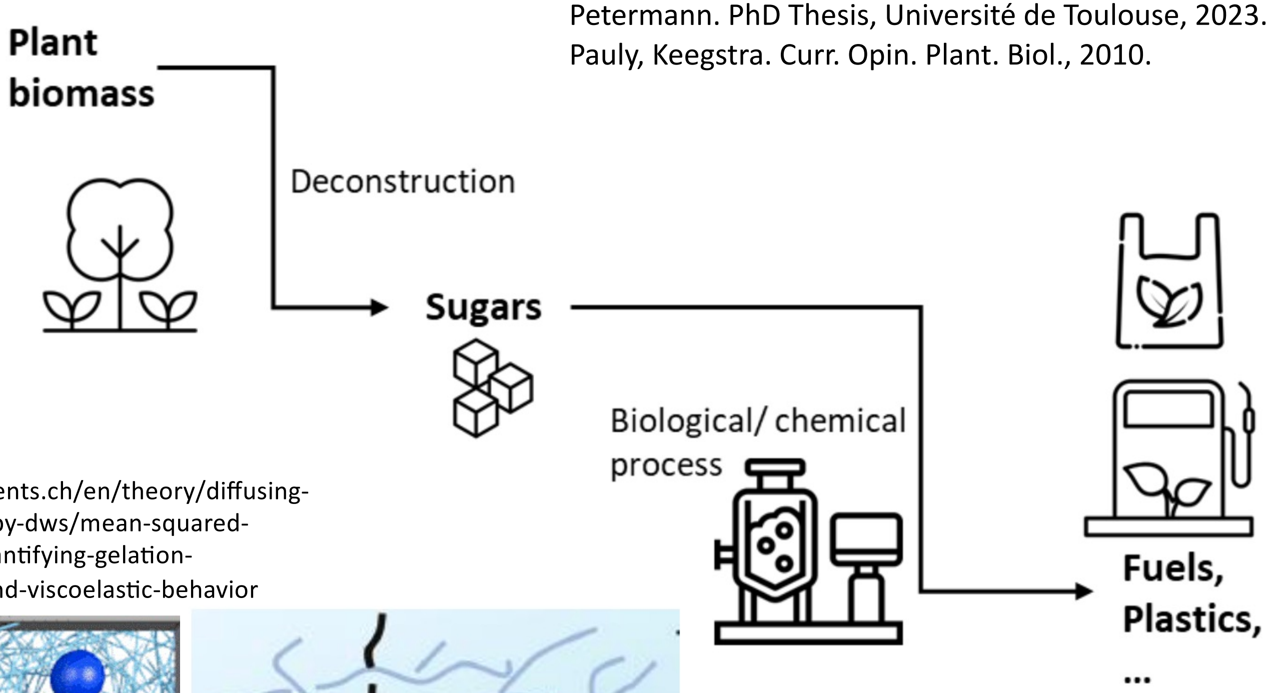
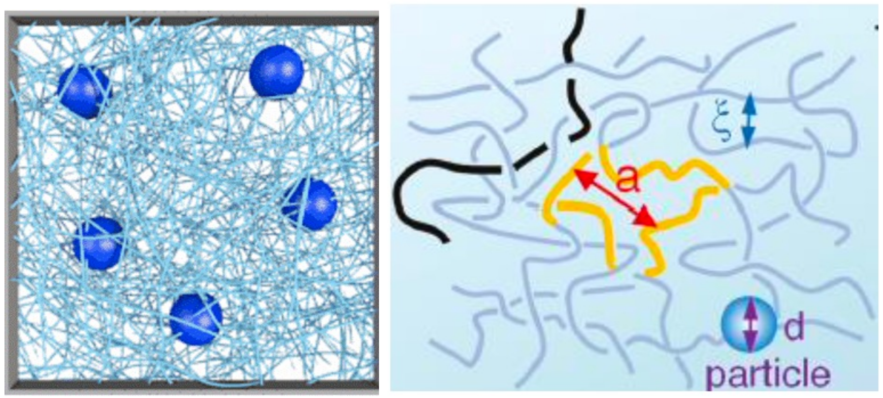
Why Enzymatic Degradation (and Gelation) of (Bio)Polymers?

Petermann. PhD Thesis, Université de Toulouse, 2023.
Pauly, Keegstra. Curr. Opin. Plant. Biol., 2010.



- Valorization of plant biomass

https://lsinstruments.ch/en/theory/diffusing-wave-spectroscopy-dws/mean-squared-displacement-quantifying-gelation-microstructure-and-viscoelastic-behavior



Cai, Panyukov, Rubinstein. Macromolecules, 2011.

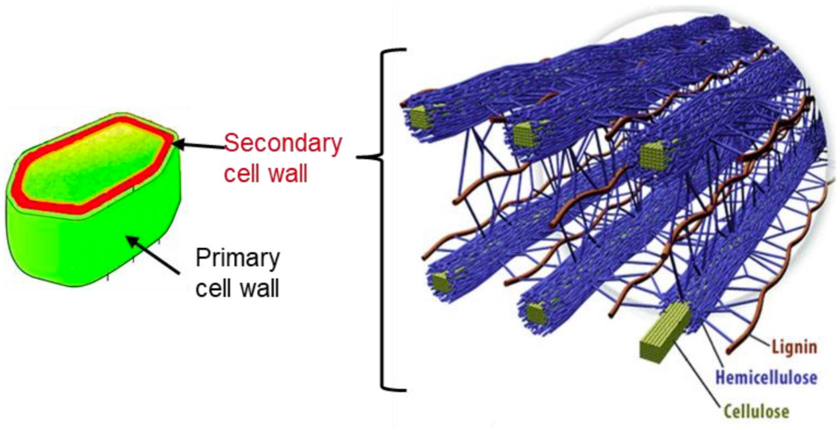
- How do enzymes move while reacting with their own substrate / what are the diffusive mechanisms of particles in polymer networks?

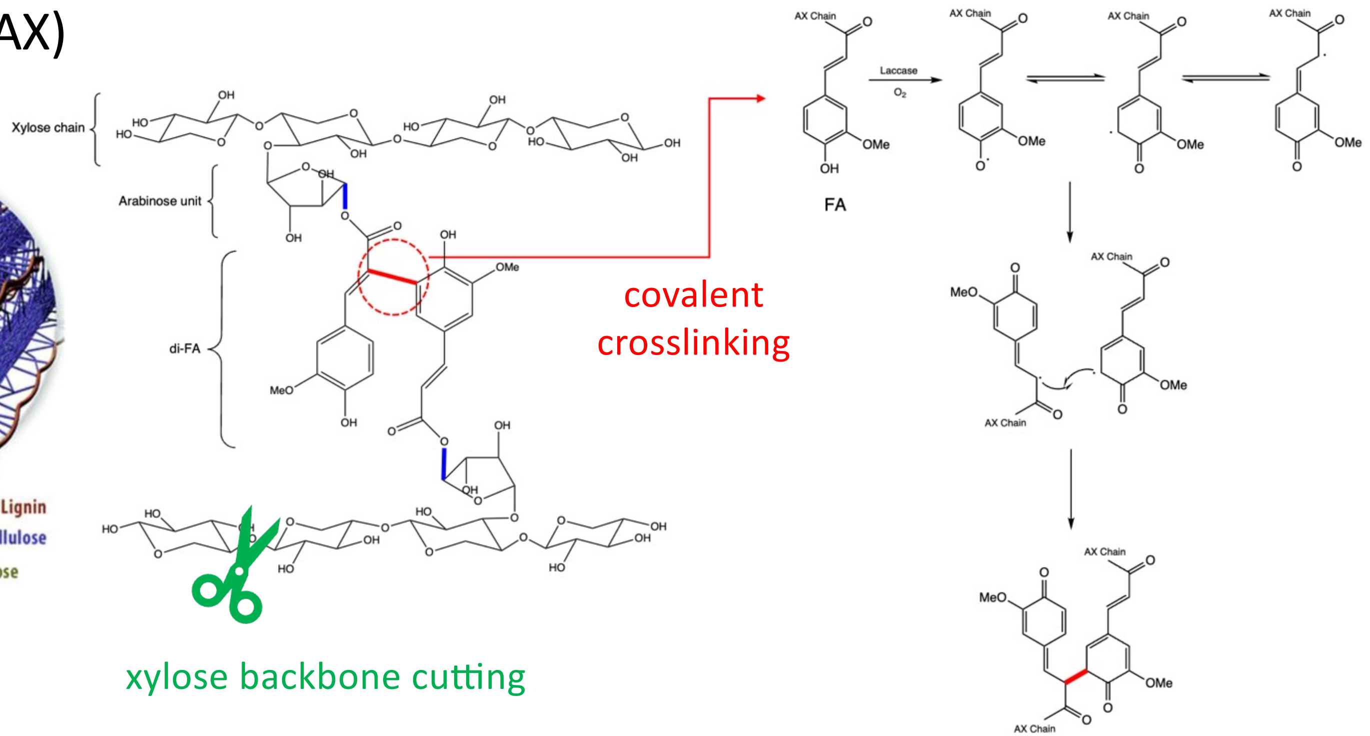
$z \sim t^{0.5}$ **?**

The system under investigation

Aqueous solutions of a model biopolymer and enzymes to induce gelation and/or degradation

- The polymer: ARABINOXYLAN (AX)





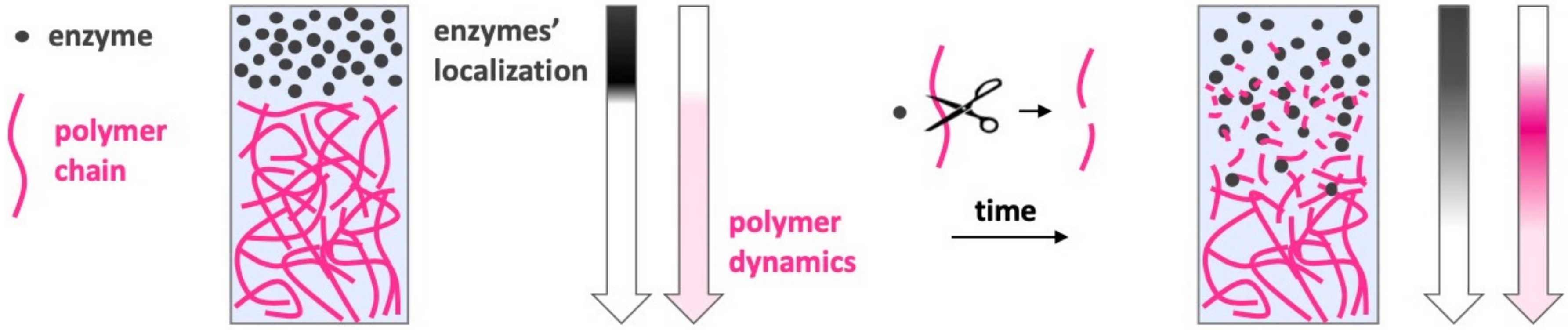
- The enzymes: LACCASE (*gelation*) and XYLANASE (*degradation*)

Petermann. PhD Thesis, Université de Toulouse, 2023.

Assor-Antoine, Cassan, Carvajal-Millan, Bouchoux, Micard. ACS Appl. Polym. Mater., 2021.

Polymer dynamics: **DYNAMIC LIGHT SCATTERING**

Fluorescent enzyme propagation in the polymer matrix: **FLUORESCENCE**

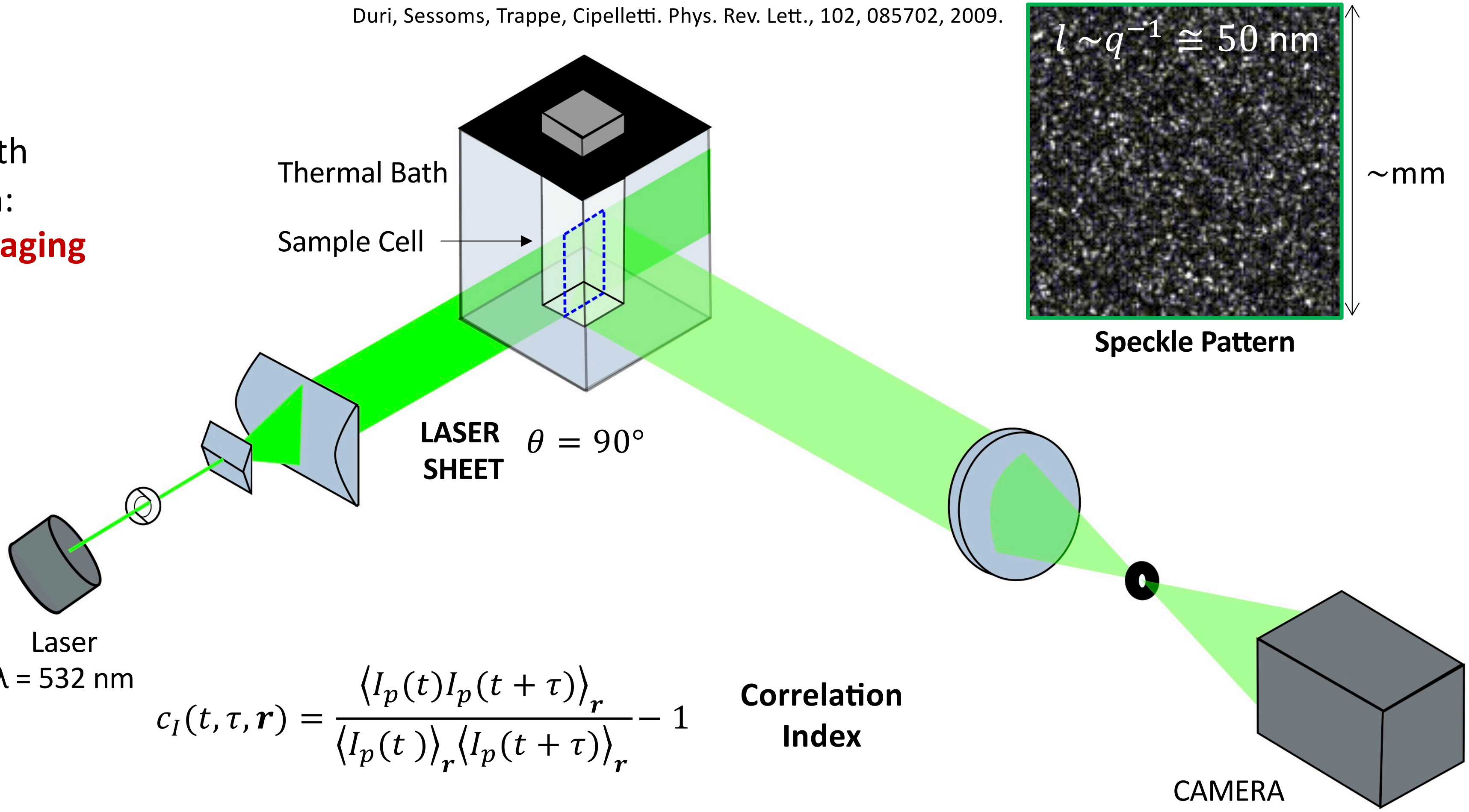


1. Photon Correlation Imaging - Fluorescence setup;
2. Enzymatic degradation of Arabinoxylan Gels;
3. Conclusions and Perspectives;

MultiSpeckle Light Scattering and Photon Correlation Imaging (PCI)

Duri, Sessoms, Trappe, Cipelletti. Phys. Rev. Lett., 102, 085702, 2009.

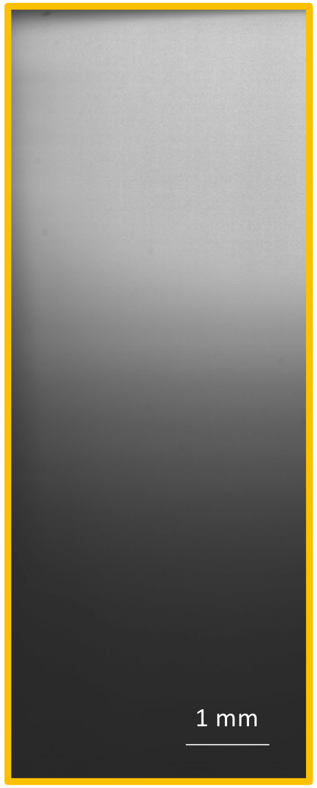
SLOW dynamics with SPATIAL resolution:
Photon Correlation Imaging



$$c_I(t,\tau,\boldsymbol{r}) = \frac{\langle I_p(t) I_p(t+\tau)\rangle_{\boldsymbol{r}}}{\langle I_p(t)\rangle_{\boldsymbol{r}} \langle I_p(t+\tau)\rangle_{\boldsymbol{r}}} - 1$$ **Correlation Index**

$$g_2(\tau,\boldsymbol{r}) = \langle c_I(t,\tau,\boldsymbol{r}) + 1\rangle_{\delta t}$$ **Intensity Correlation Function**

Photon Correlation Imaging (PCI) *and* Fluorescence Microscopy

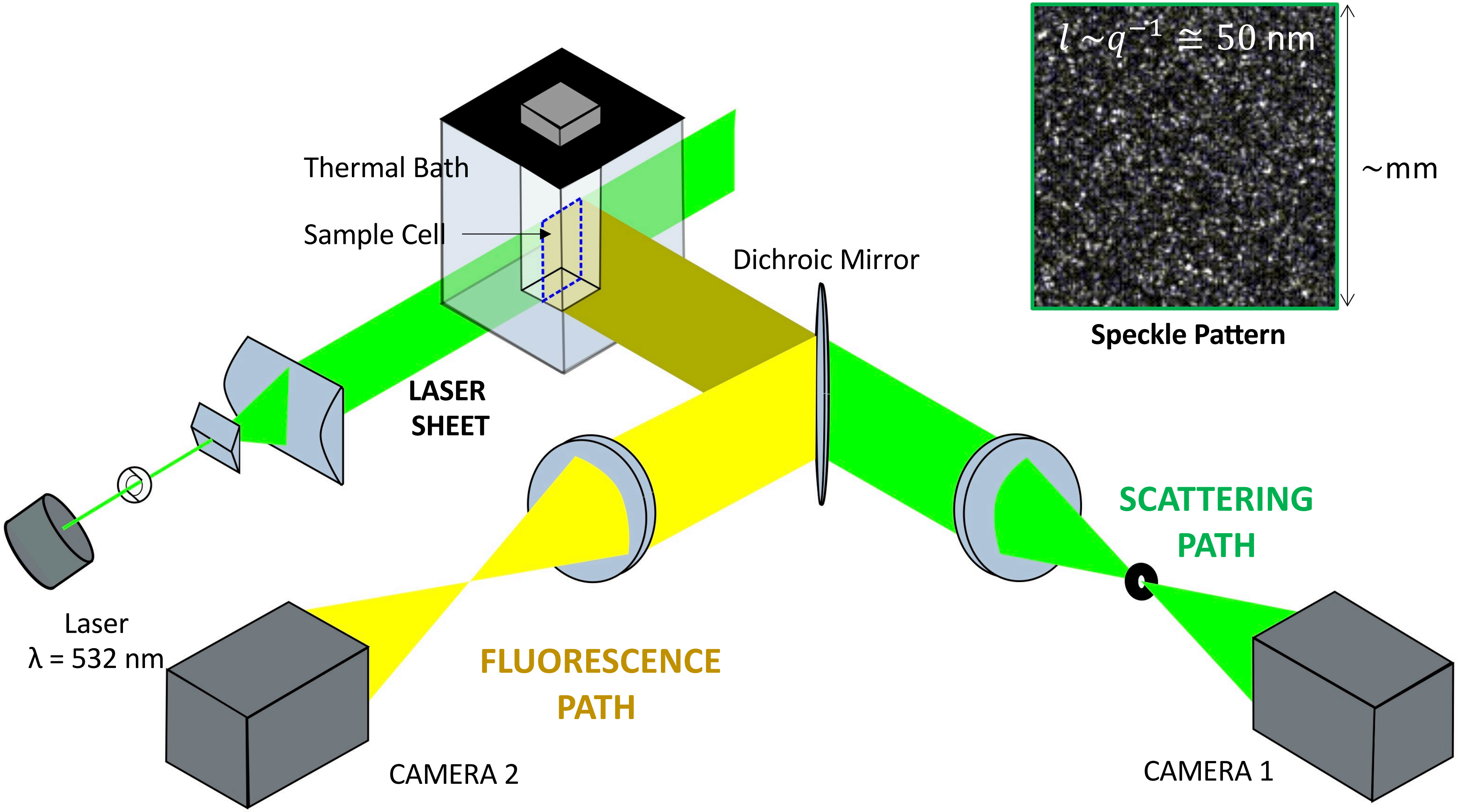


Fluorescence Image

Fluorophore: **TRITC**

λ_{exc} = **532 nm**

λ_{em} = **580 nm**



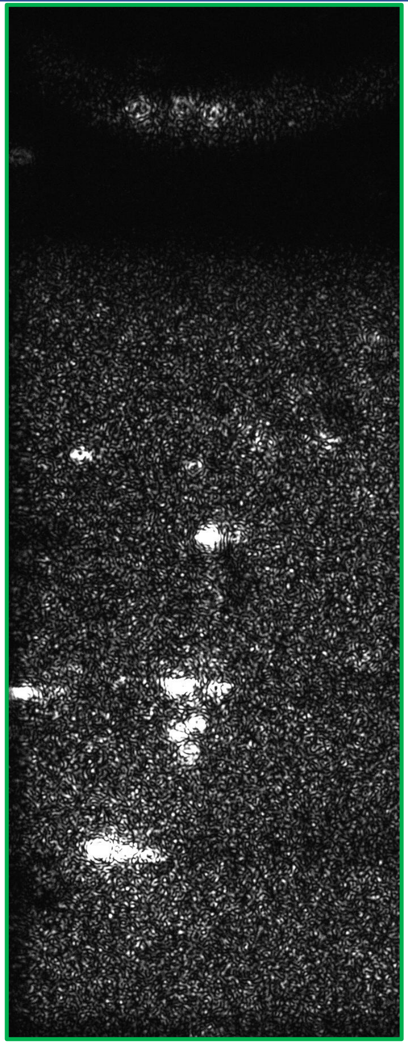
Speckle Pattern

Degradation of Feruloylated AX c = 10 g/l

fungal endo **Xylanase**
*Np*Xyn 11A in
phosphate-citrate
buffer 50 mM, pH = 5

Degree of Labelling: 2.8
final concentration 2.2 µM

SCATTERING



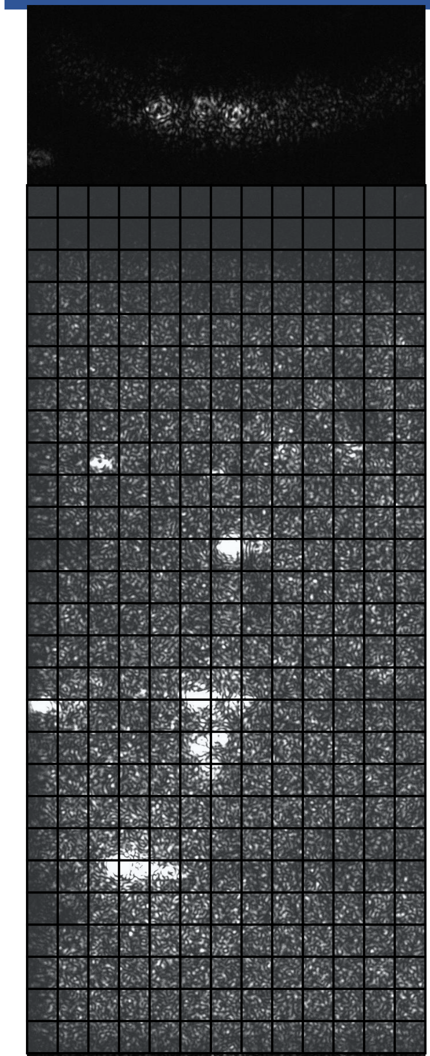
FLUORESCENT XYLANASE INJECTION

FLUORESCENCE

Acquisition started 5
minutes after
xylase injection

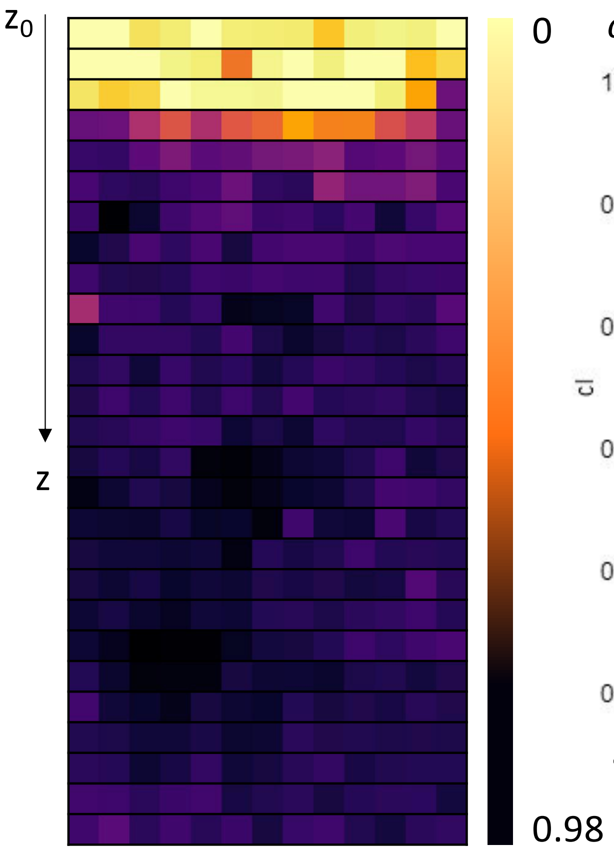
Duration: 13.8 hours

Evolution of degradation front (I)



Division in Regions of Interest: 300 x 300 µm

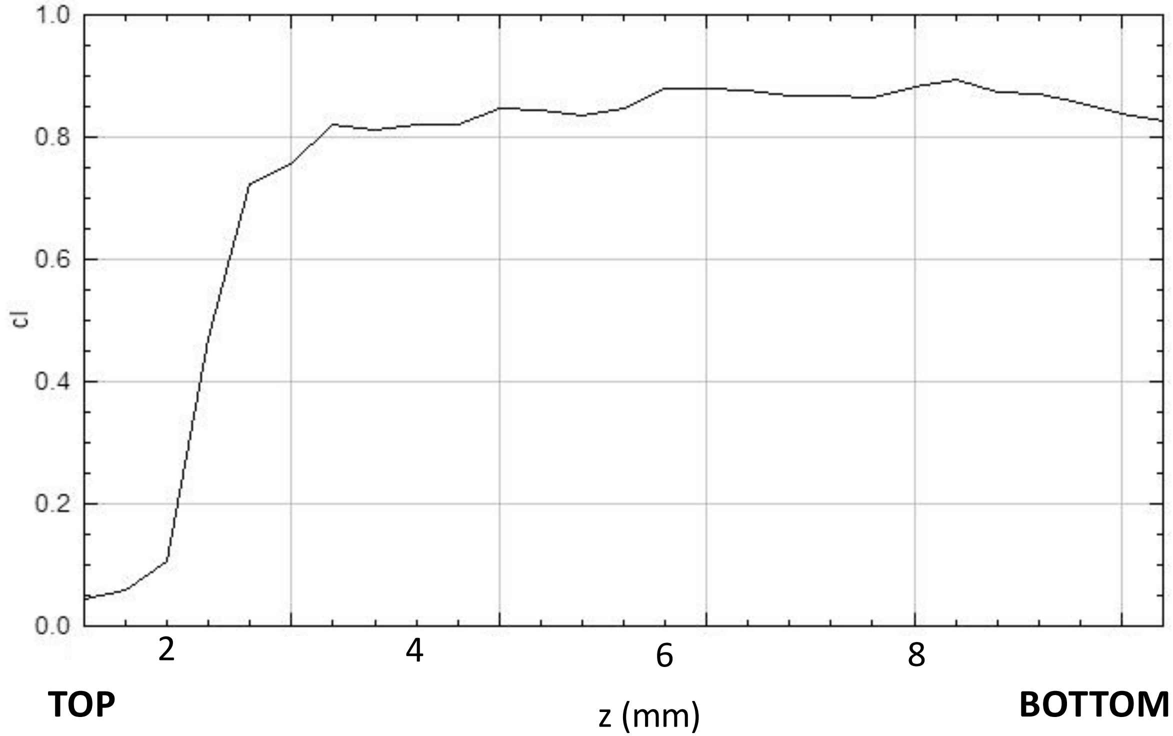
Dynamic Activity Map



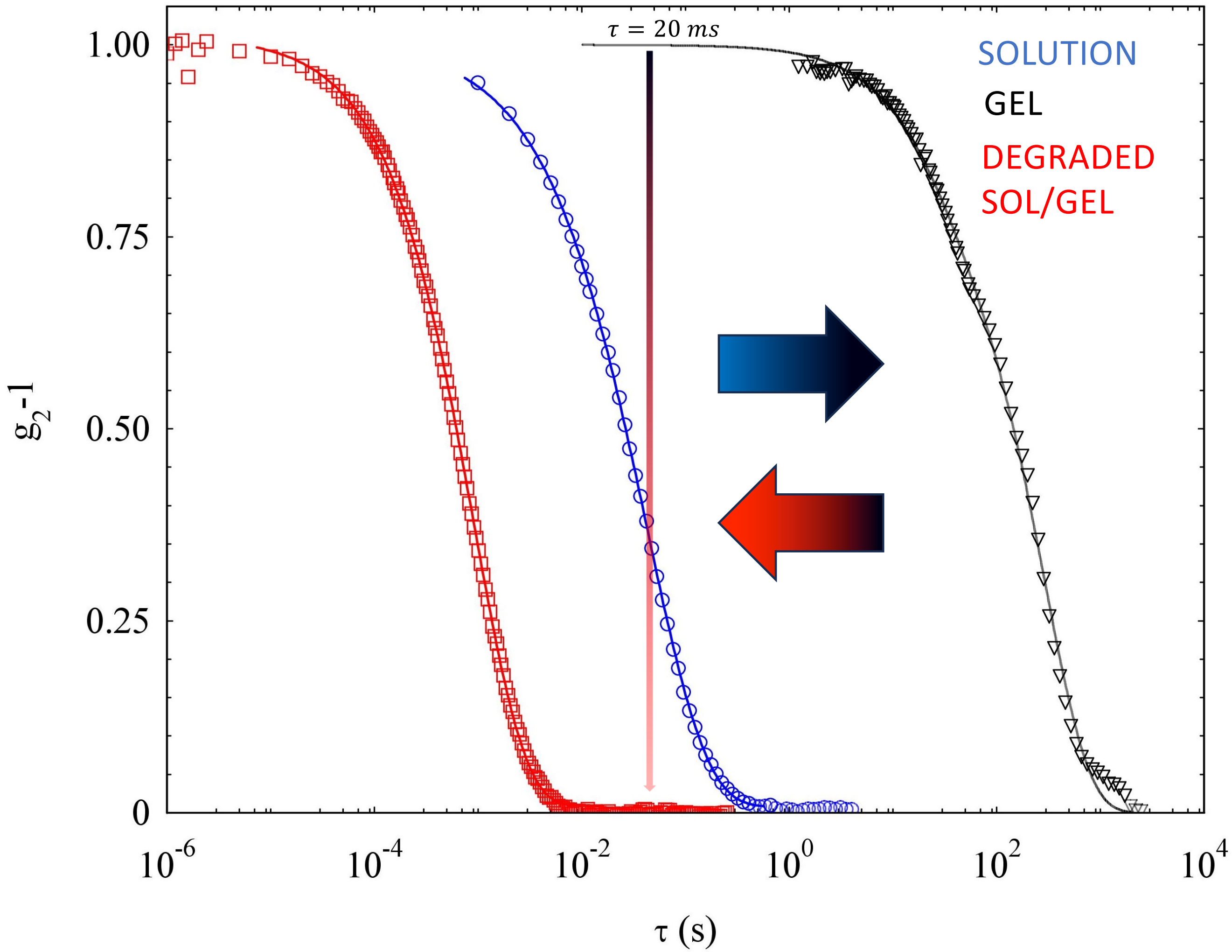
$c_I \rightarrow 0$: **FAST DYNAMICS**

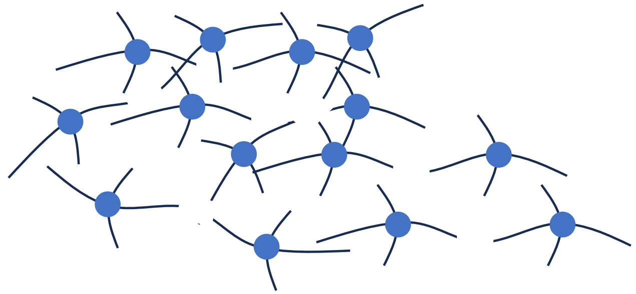
$c_I \rightarrow 1$: **SLOW DYNAMICS**

$c_I(t, \tau = 20\ ms, ROI)$



Polymer microscopic dynamics

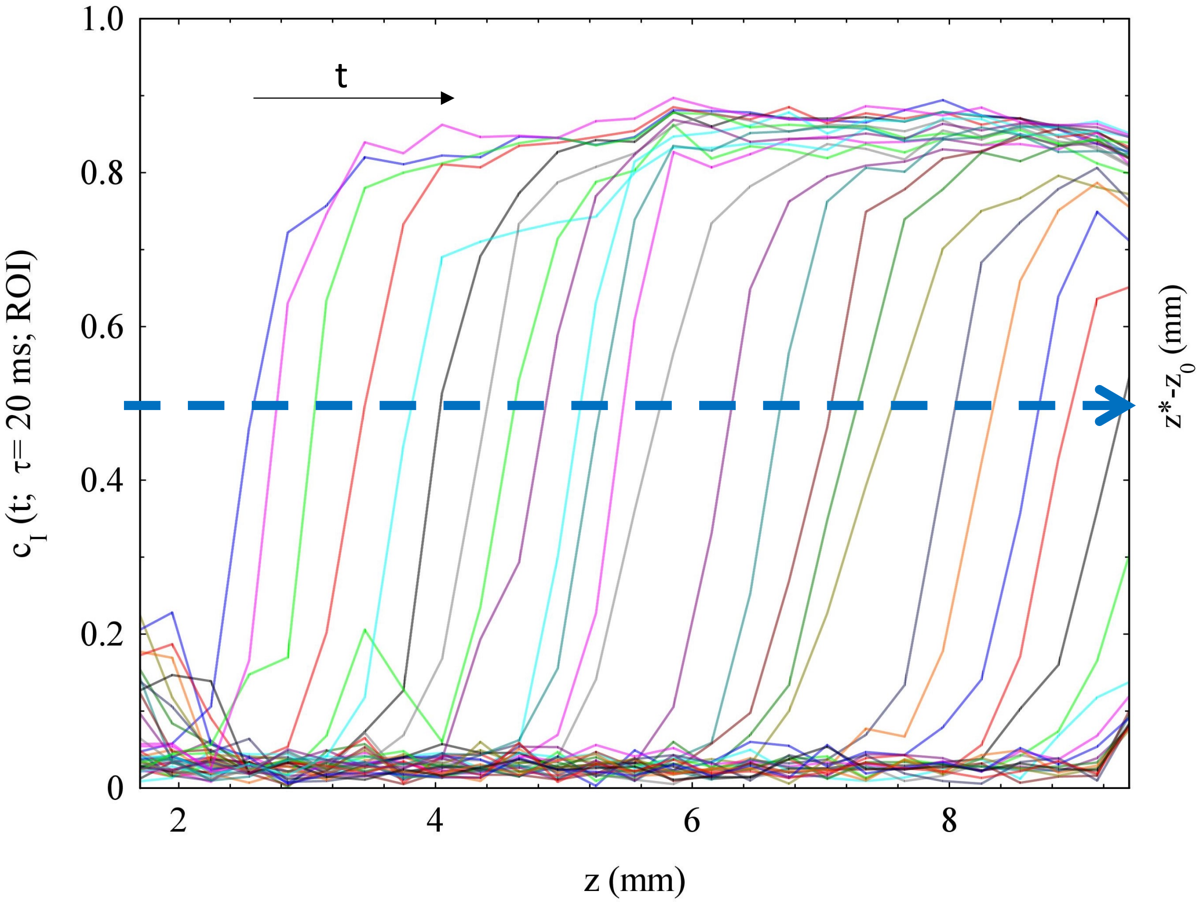




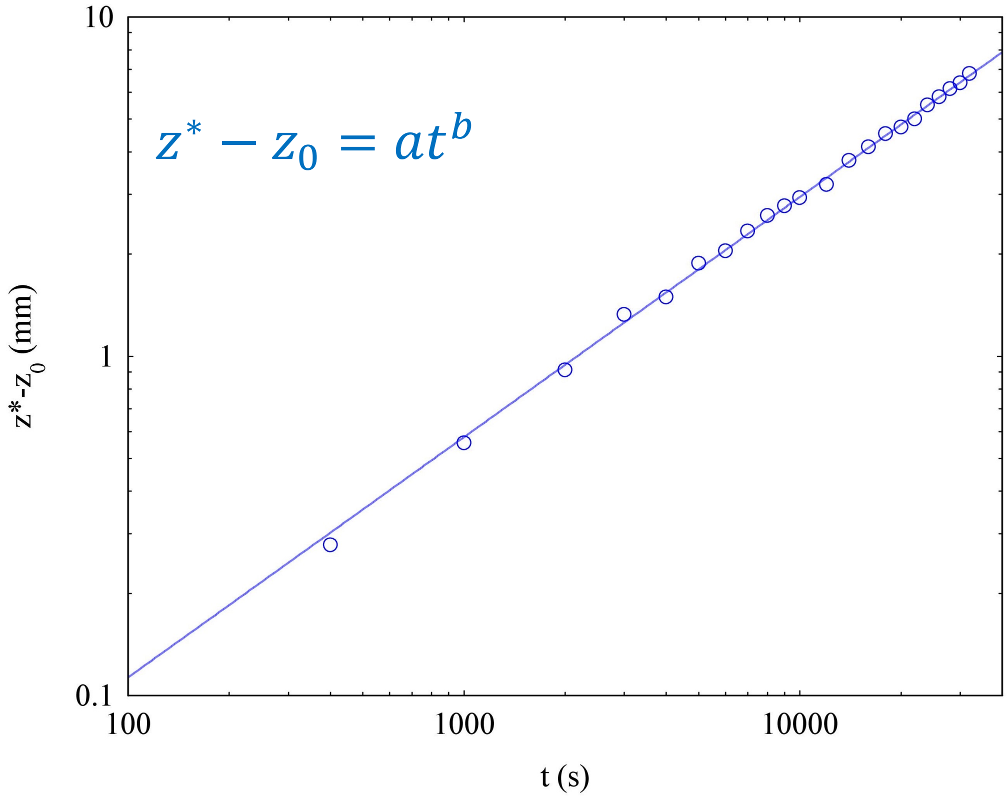
- Increase of the relaxation time during gelation;
- Decay time of the degraded gel lower than the initial solution;

diluted regime: $\tau = const, c \downarrow$

Evolution of degradation front (II)



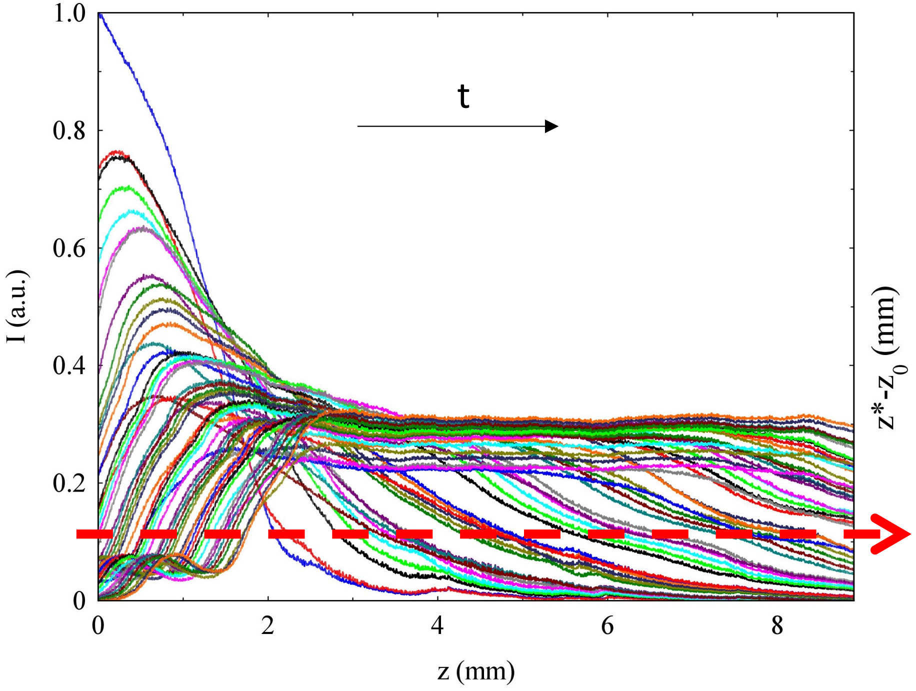
threshold: $c_I^* \approx 0.5$

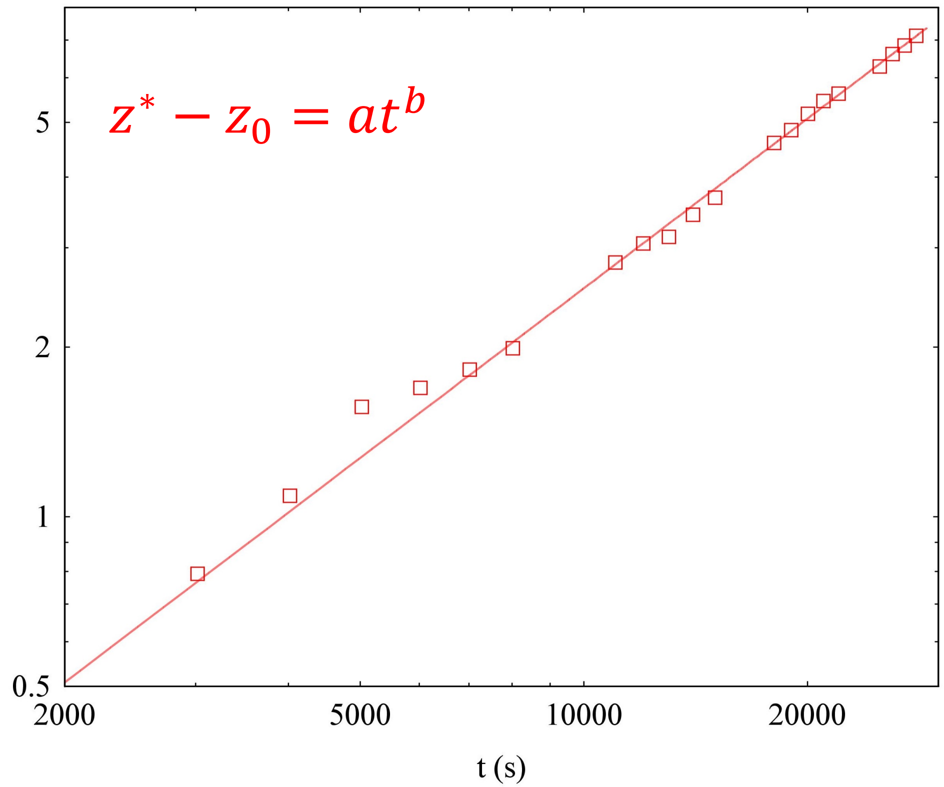


$a = 0.0043$ mm s^{-b}
$\boldsymbol{b = 0.71}$

Superdiffusive degradation front

Evolution of degradation front (III): enzyme kinetics





threshold: $I^* \approx 0.1 I_{MAX}$

$a = 0.00034$ mm s^{-b}

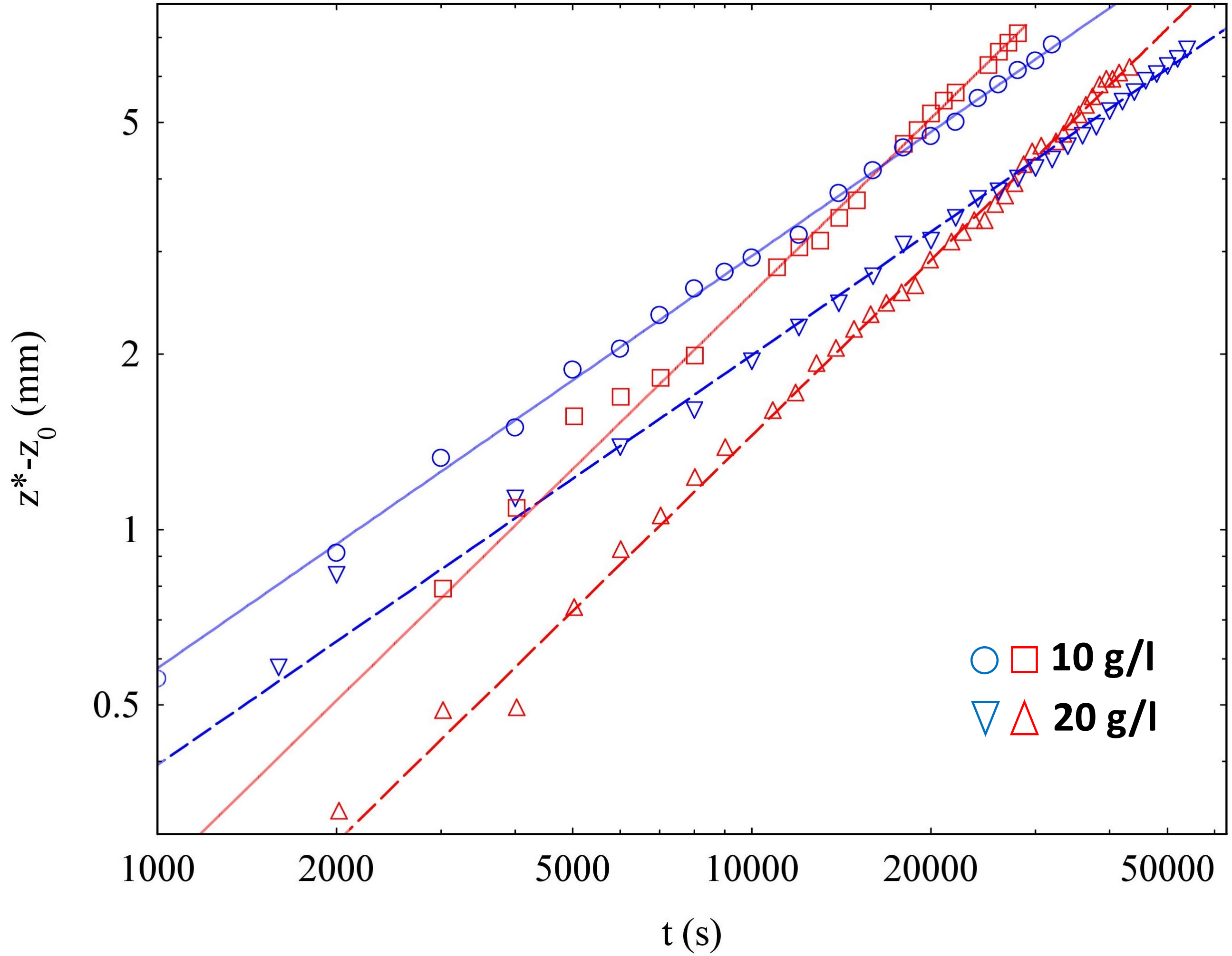
$\boldsymbol{b = 0.99}$

Diffusion of the enzyme in water:

68 hours vs 15 hours

Superdiffusive (linear) enzyme kinetics

10 g/l vs 20 g/l gels



Gel degradation

$$z^* - z_0 = at^b \begin{cases} \mathbf{b = 0.7} \\ a = 0.0043 \text{ mm/s}^b \text{, 10 g/l} \\ a = 0.0031 \text{ mm/s}^b \text{, 20 g/l} \end{cases}$$

Enzyme

$$z^* - z_0 = at \begin{cases} a = 3.4\ 10^{-4} \text{ mm/s, 10 g/l} \\ a = 1.5\ 10^{-4} \text{ mm/s, 20 g/l} \end{cases}$$

Slower degradation in more concentrated gels

Conclusions & Perspectives

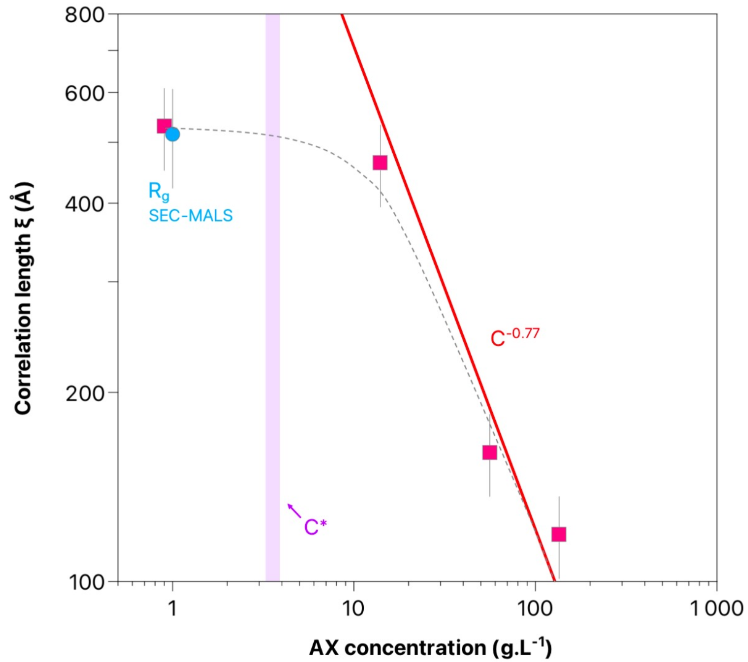
- Simultaneous detection of decomposer kinetics and degrading gel dynamics with a novel experimental setup;

- **Superdiffusive** kinetics of polymer degradation and enzyme motion:

$z^* = at^b, \mathrm{b} = 0.7$

$z^* = at$

- Slower degradation in the more concentrated gel;



Petermann et al. *Biomacromolecules* 24.8 (2023): 3619-3628.

- Systematic study in polymer solutions at different concentrations, to vary the r/ξ size ratio

Thank you for the attention!

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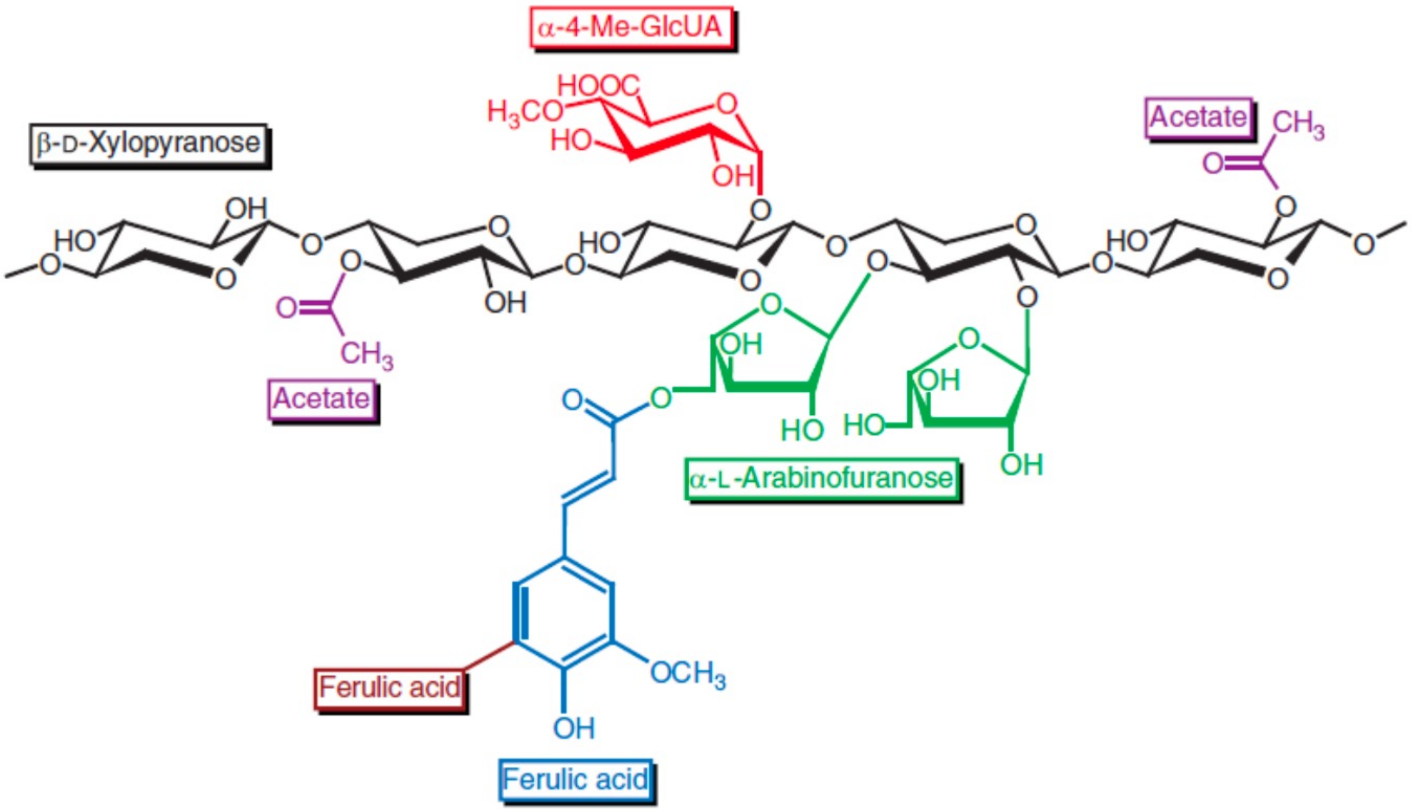
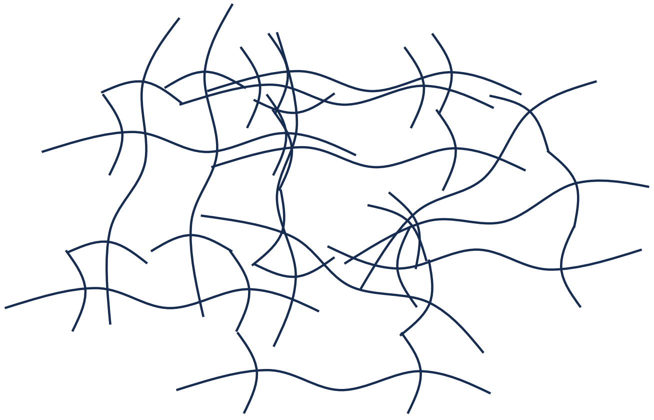
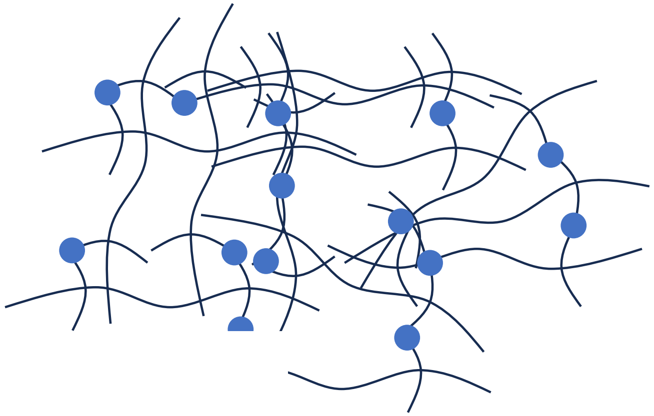
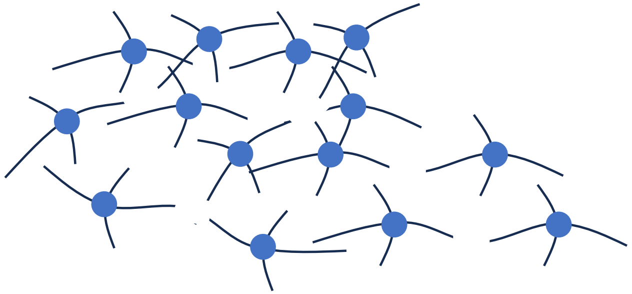
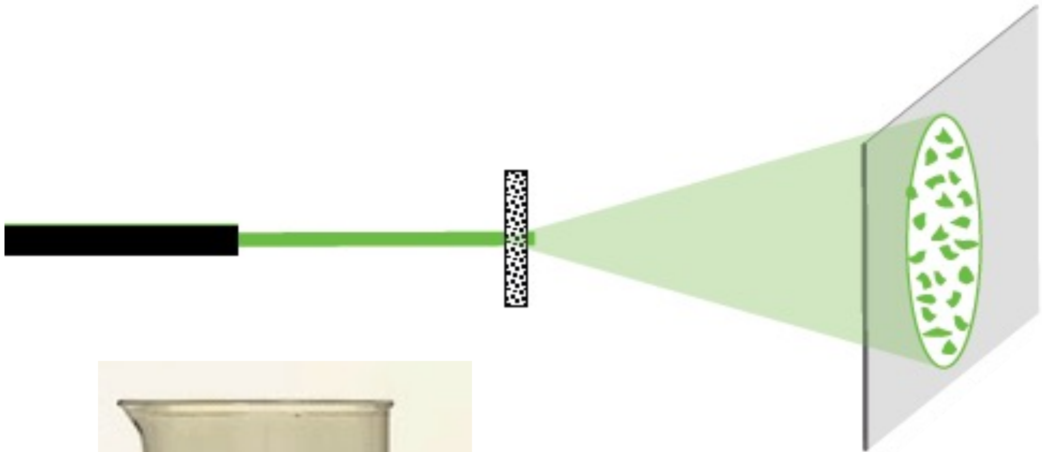
General structure of AX







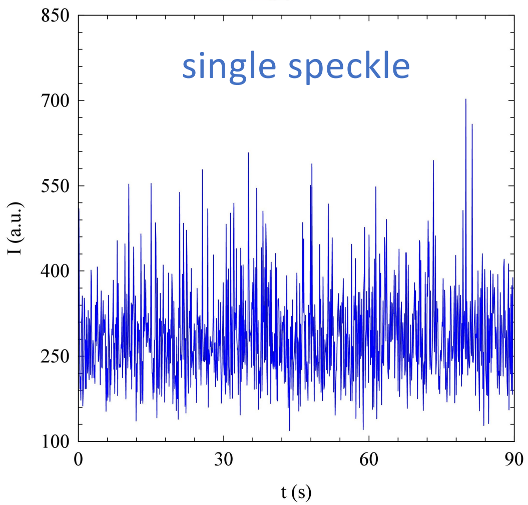


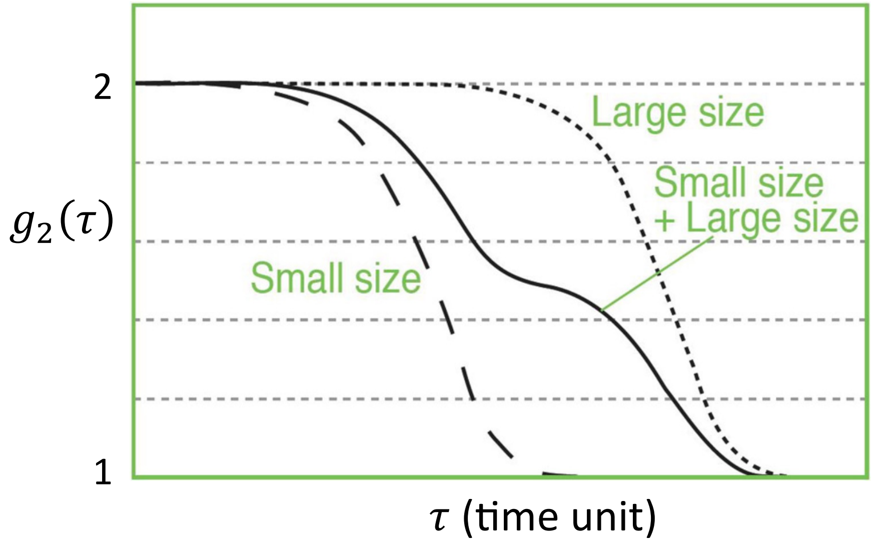


When a diffuser is illuminated by a laser, a "maculated" pattern forms on the screen "after" it:

SPECKLE PATTERN

Colloidal suspensions
Polymer solutions





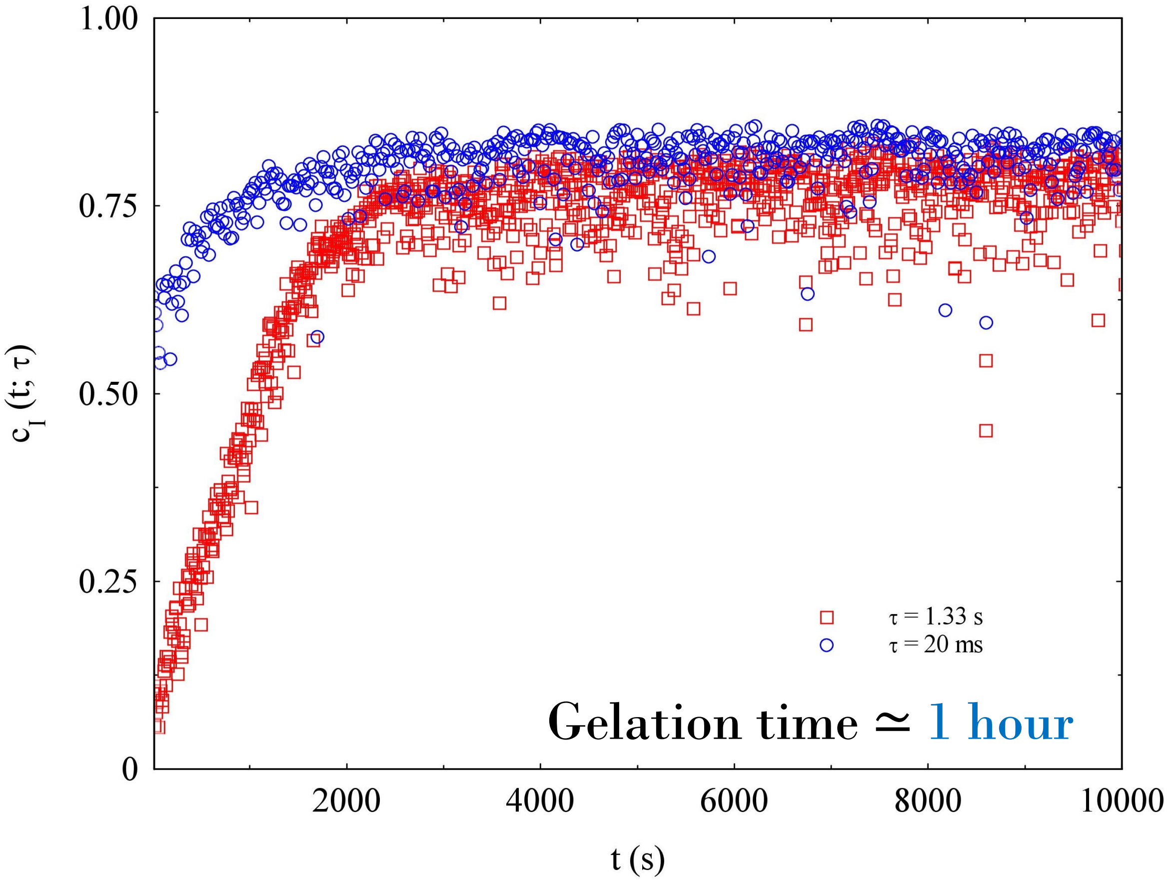
$$g_2(\tau) = \frac{\langle I(t) I(t+\tau) \rangle}{\langle I(t) \rangle^2}$$

Gelation of Feruloylated AX: c = 10 g/l

AX: 640 000 g/mol,
ferulic acid content: 2.32 µg/mg,
arabinose/xylose ratio: 0.58

Laccase from
Pycnoporus cinnabarinus
in phosphate-citrate
buffer 50 mM, pH = 5

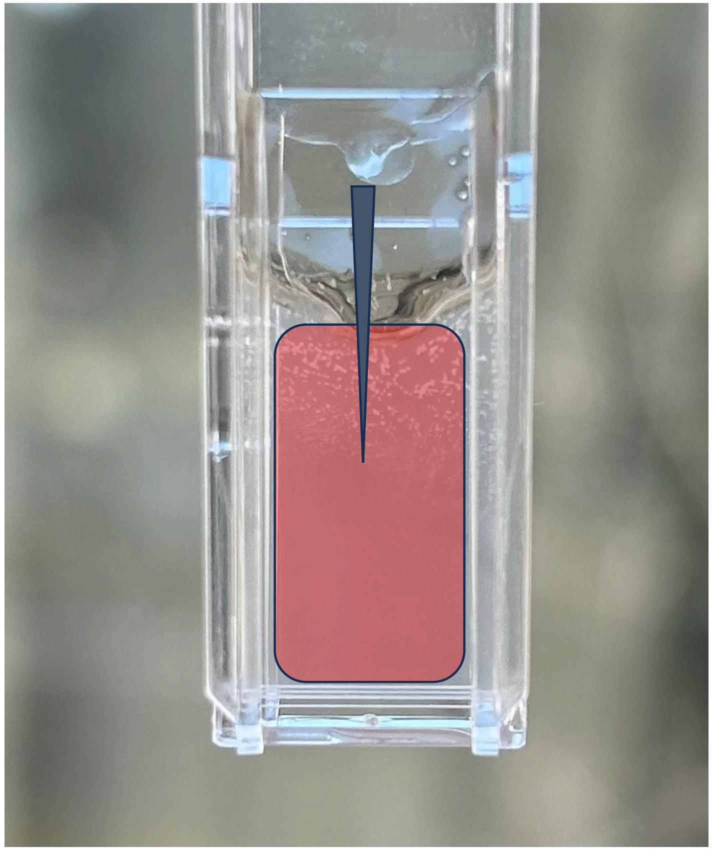
Duration: 3 hours



In good agreement with the rheological measurements in
Assor-Antoine, Cassan, Carvajal-Millan, Bouchoux, Micard. ACS Appl. Polym. Mater., 2021.

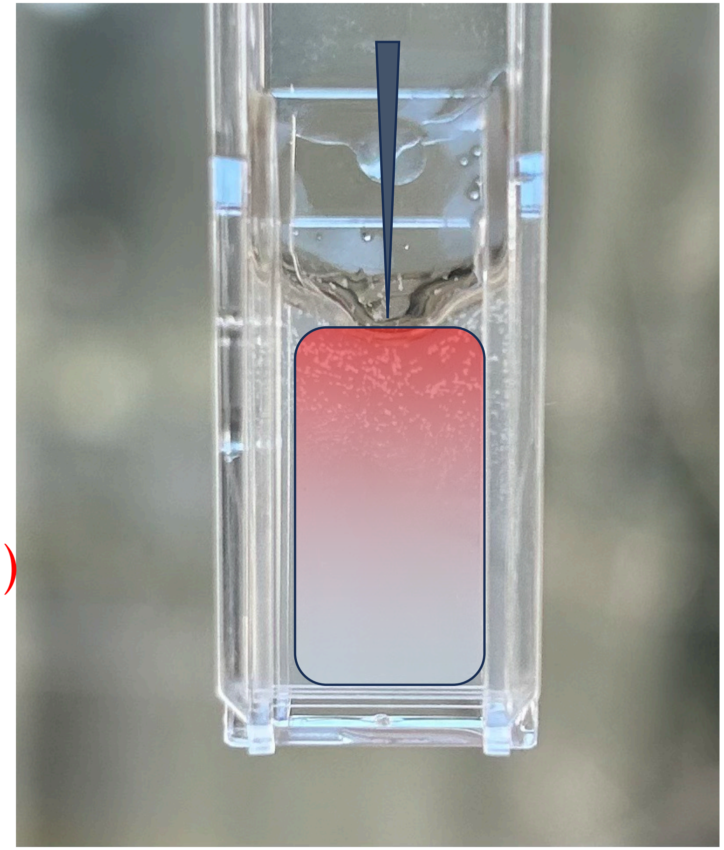
Homogeneous vs Heterogeneous Gelation

HOMOGENEOUS



enzyme
(LACCASE)

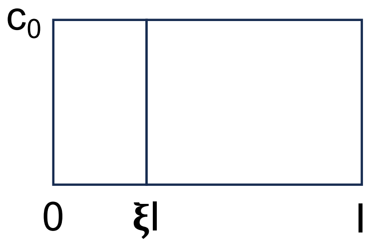
HETEROGENEOUS



Crank (1979), Hamada *et al.* (2020).

1D diffusion in a “confined” geometry: theory

$$\frac{\partial c}{\partial t} = D\,\frac{\partial^2 c}{\partial x^2}$$



$t = 0$: $c = c_0 \vartheta(\xi l - x)$

$t > 0$: $\dfrac{\partial c}{\partial x} = 0, x = 0, l$

change of variables

$\hat{x} = \dfrac{x}{l}, \hat{c} \to \dfrac{c}{c_0}, \hat{t} \to \dfrac{t}{\tau_D} = \dfrac{t}{l^2/D}$

$$\boldsymbol{\frac{\partial \hat{c}}{\partial \hat{t}} = \frac{\partial^2 \hat{c}}{\partial \hat{x}^2}}$$

$\boldsymbol{\hat{t} = 0: \hat{c} = \vartheta(\xi - \hat{x})}$

$\boldsymbol{\hat{t} > 0: \dfrac{\partial \hat{c}}{\partial \hat{x}} = 0, \hat{x} = 0, 1}$

(enzyme of radius ~ 2 nm)

$D = 1.64\ 10^{-10}$ m²/s

$t \simeq 0.4\ \tau_D = 0.4\ l^2/D$: equilibrium **68 hours vs 15 hours**

“Unconfined” case*:

$$c(x,t) = \frac{c_0}{2}\left[1 - \mathrm{erf}\left(\frac{x - \xi l}{\sqrt{4Dt}}\right)\right] = \frac{1}{2}\mathrm{erfc}\left(\frac{x - \xi l}{\sqrt{4Dt}}\right)$$

*Notice that if the initial condition is with the ‘opposite’ concentration step $c = c_0 \vartheta(x - \xi l)$ the solution would be:

$$c(x,t) = \frac{c_0}{2}\left[1 + \mathrm{erf}\left(\frac{x - \xi l}{\sqrt{4Dt}}\right)\right]$$

Confined case:

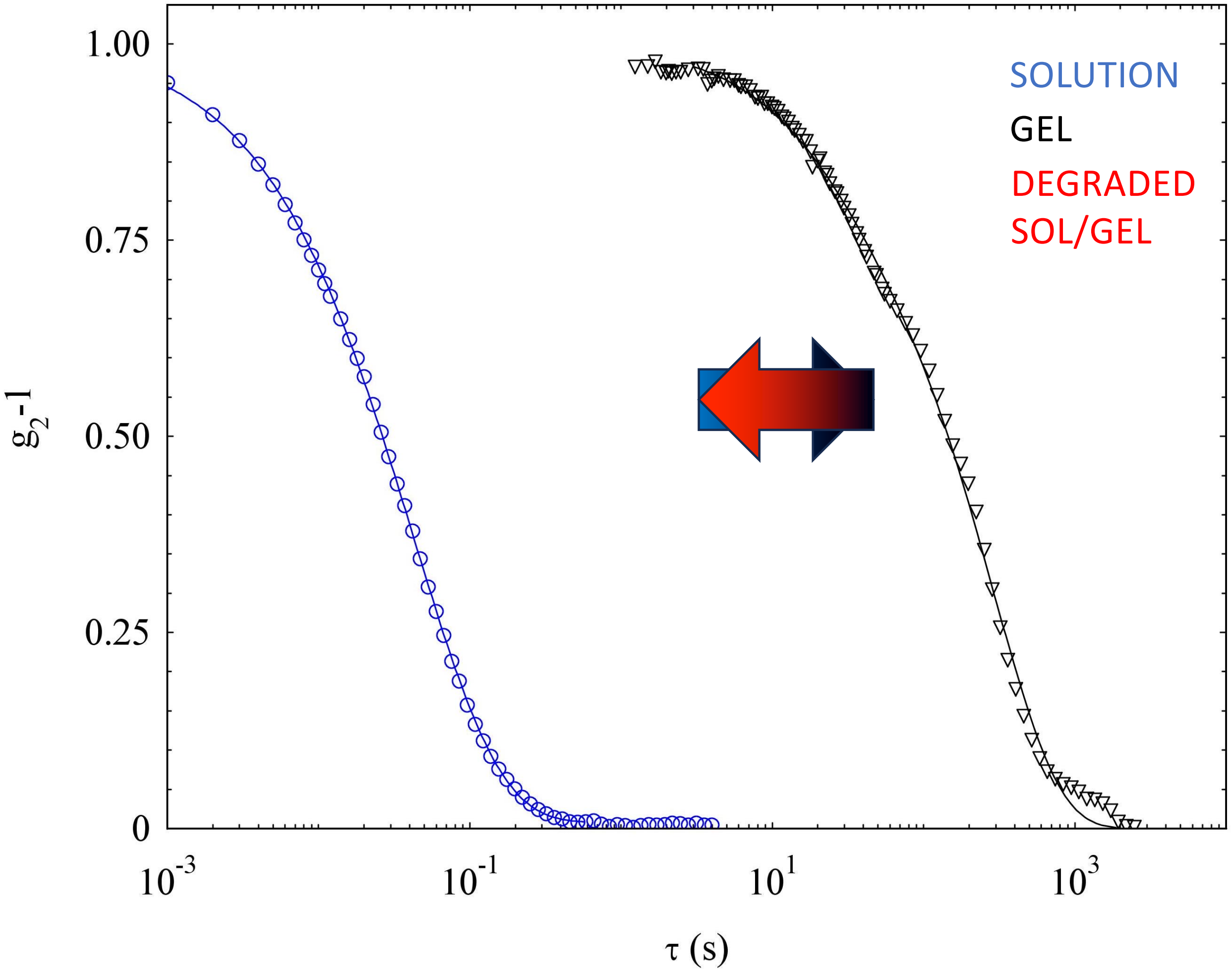
$$\hat{c}(\hat{x}, \hat{t}) = \sum_{m=1}^{\infty} 2\,\frac{\sin(\pi m \xi)}{\pi m}\cos(\pi m \hat{x})\,e^{-(\pi m)^2 \hat{t}} + c_f$$

$\hat{t} \ll 1/\pi^2$: concentration profile as the unconfined case

$c_f = \hat{c}(\mathrm{t} \to \infty)$

$\hat{t} \simeq 0.4$: equilibrium

Polymer microscopic dynamics



$$g_2 - 1 = \exp\left(-2\left(\frac{\tau}{\tau_D}\right)^{\beta}\right)$$

$\boldsymbol{\beta = 0.752;\ q\xi\ ?> 1}$

concentrated regime: $\boldsymbol{\tau \uparrow, c \uparrow}$

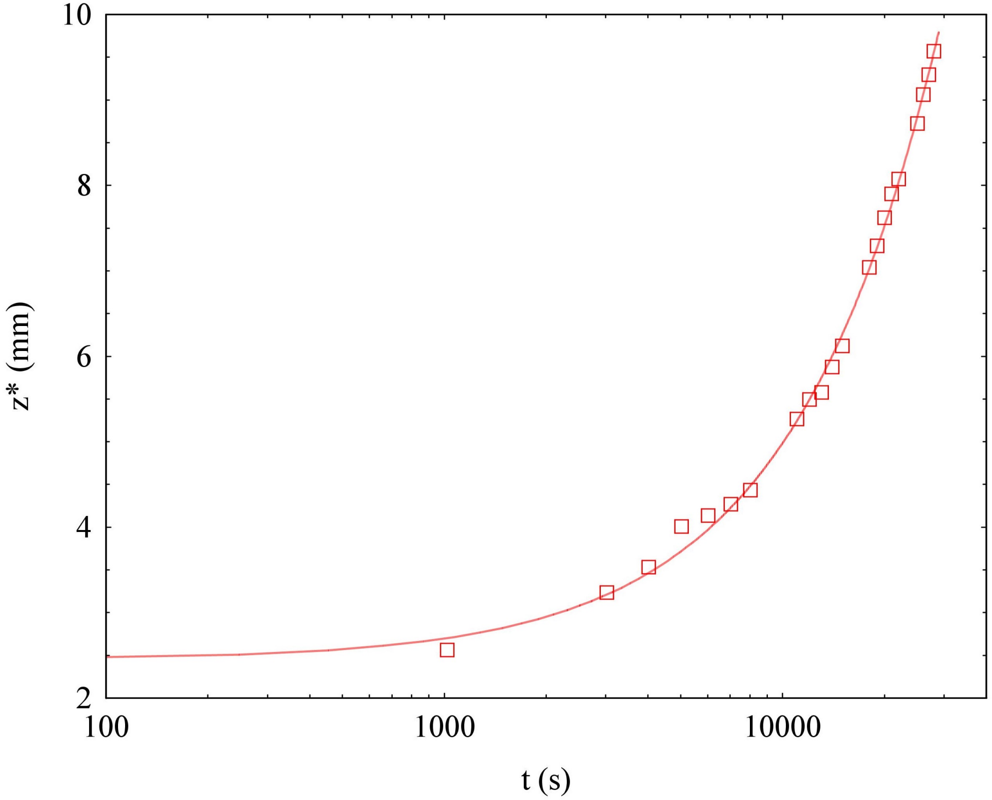
Kroy, Klaus, and Erwin Frey.
Physical Review E 55.3 (1997): 3092.

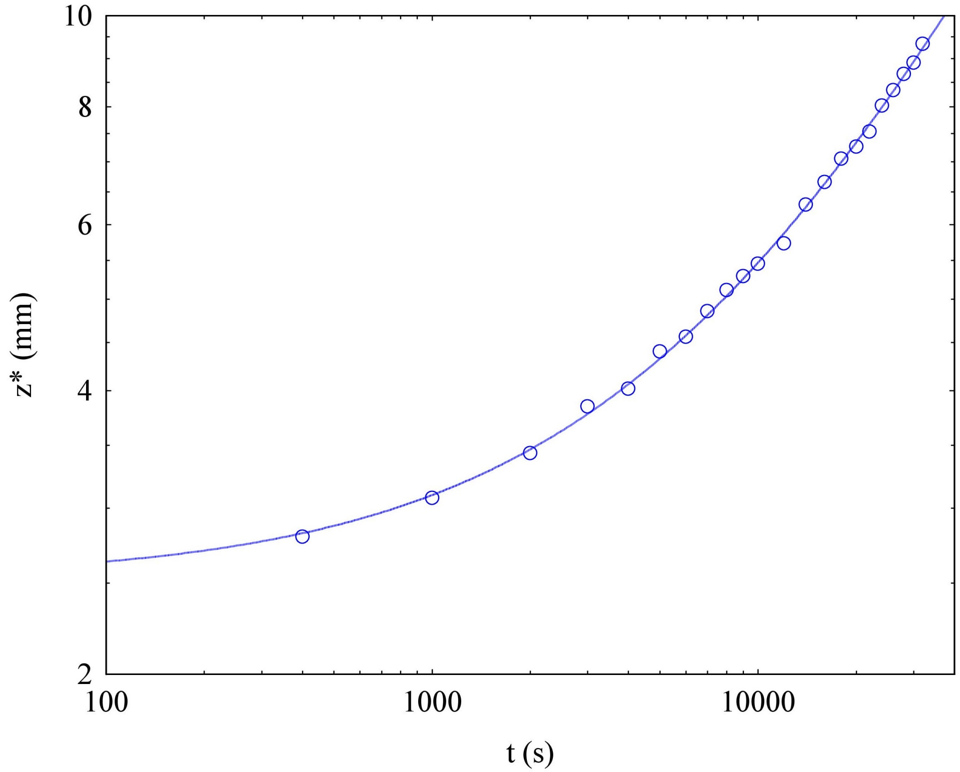
- Increase of the relaxation time during gelation
- Same relaxation time from degraded gel and degraded solution

$\boldsymbol{\beta = 0.876;\ qRg\ ?> 1}$

diluted regime: $\boldsymbol{\tau = const, c \downarrow}$

10 g/l



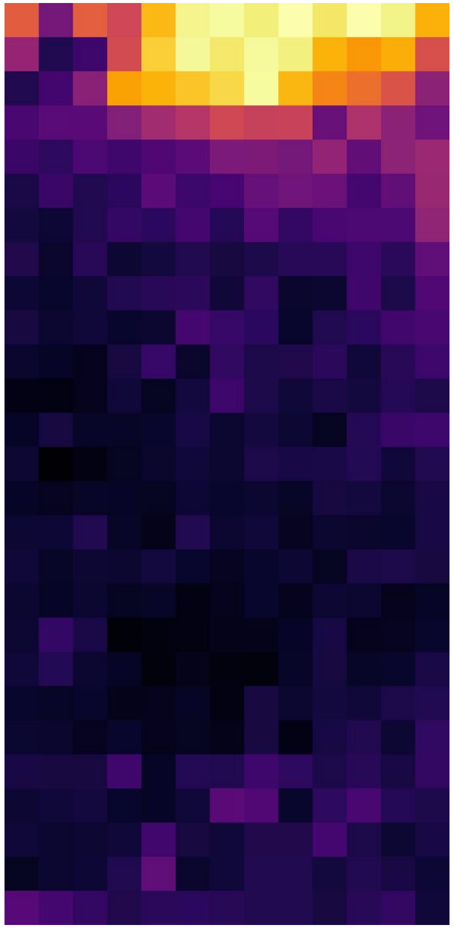


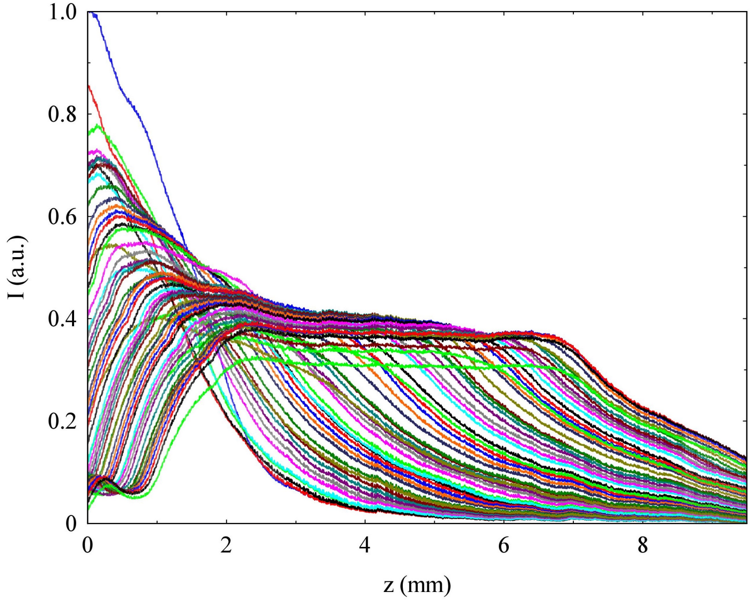
$z^* = at^b + c$

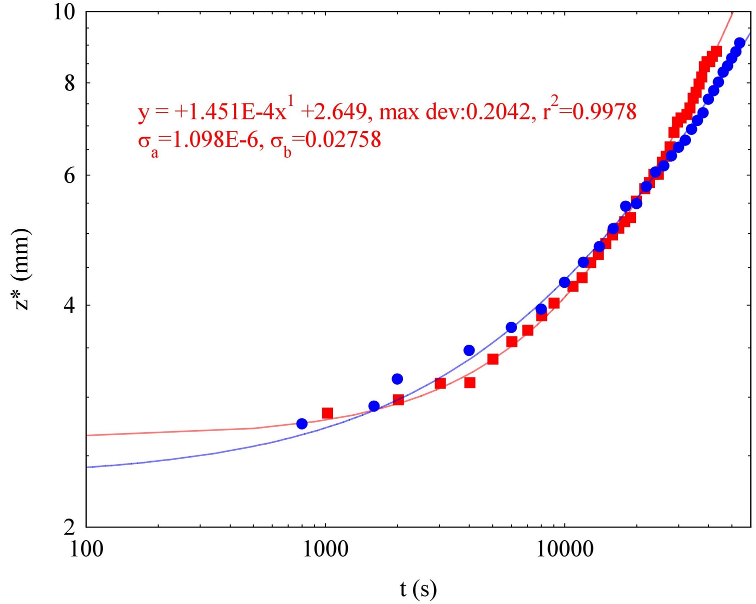
$a = 0.00034$ mm s^{-b}
$b = 0.99$

$z^* = at^b + c$

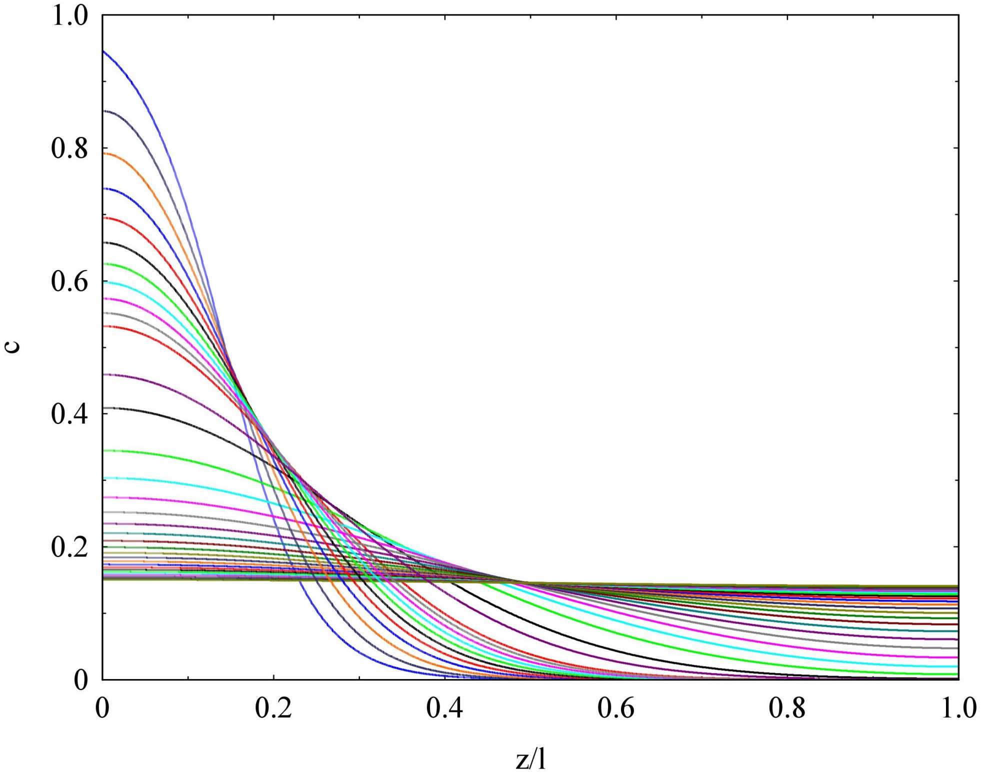
$a = 0.00434$ mm s^{-b}
$b = 0.708$

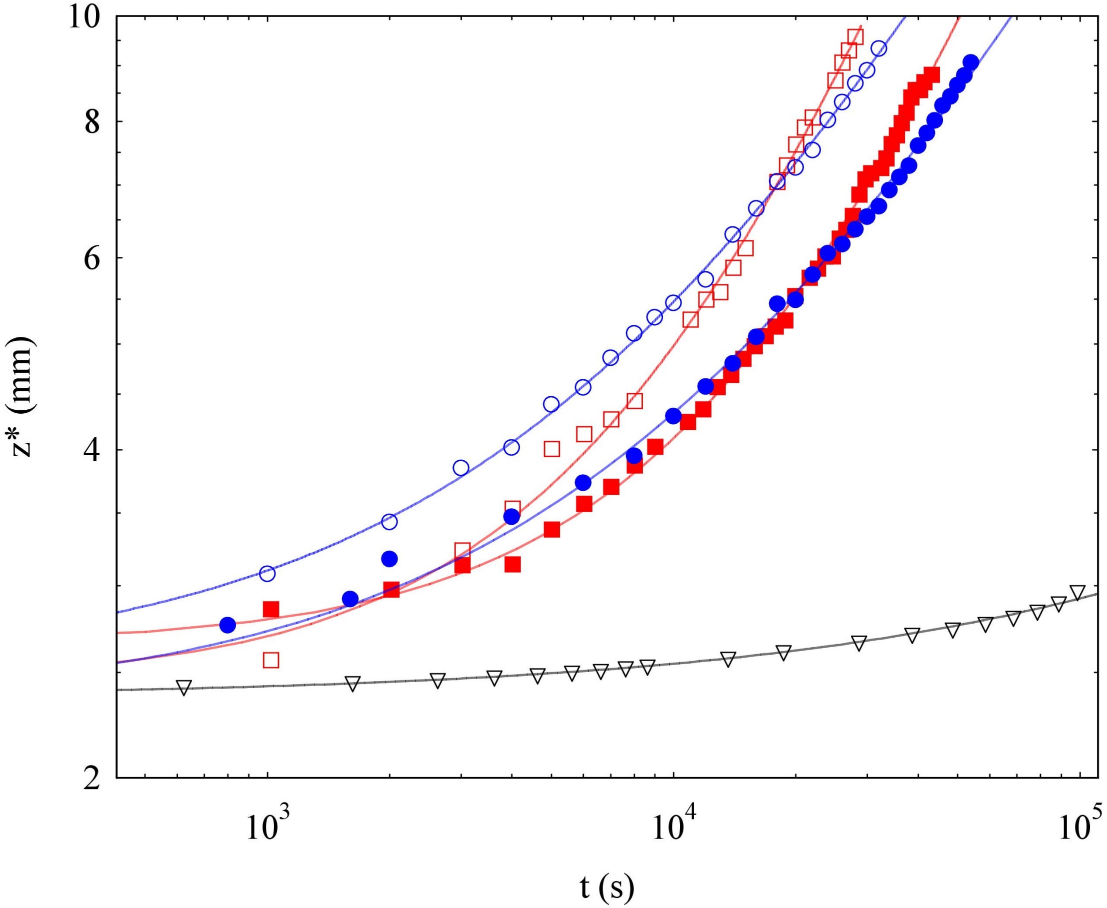



1.0
0.8
0.6
0.4
0.2
0
I (a.u.)
0
2
4
6
8
z (mm)

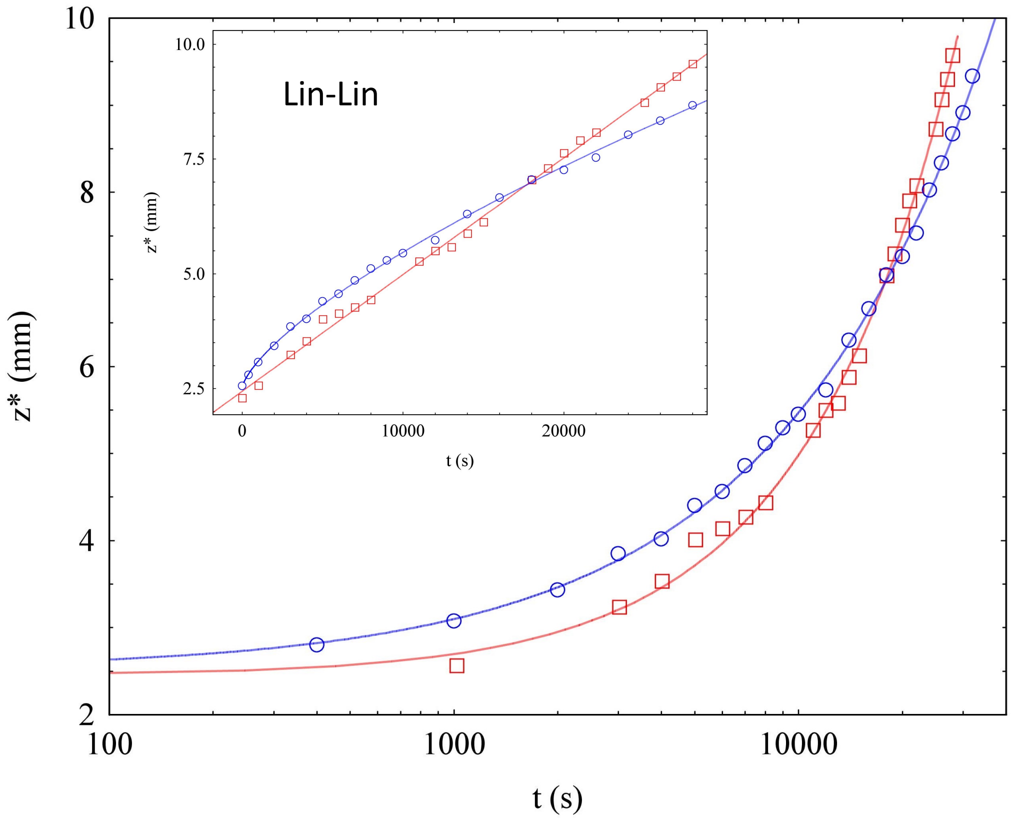

y = +1.451E-4x^1 +2.649, max dev:0.2042, r^2=0.9978
σ_a=1.098E-6, σ_b=0.02758
10
8
6
4
2
z* (mm)
100
1000
10000
t (s)

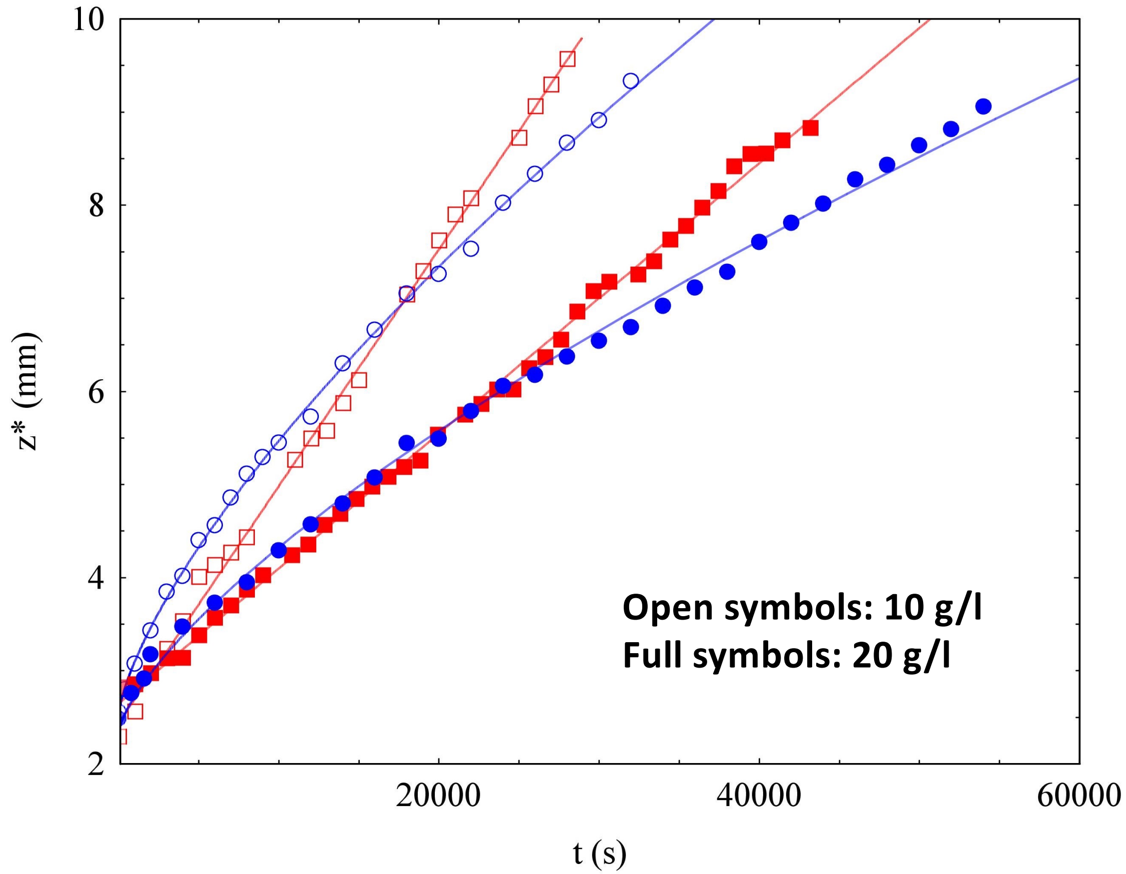
Diffusion simulations





Comparison: c = 10 g/l & c = 20 g/l





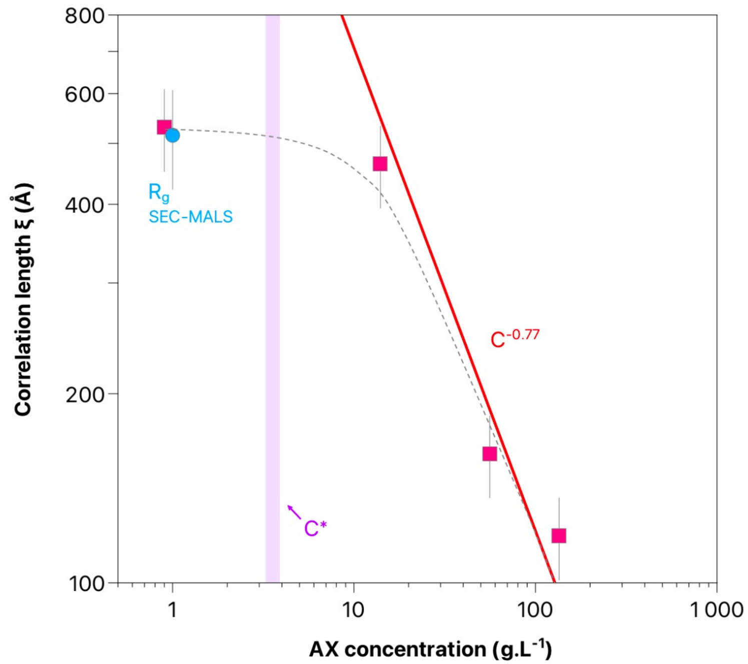
Gel degradation $z^* = at^b + c$

- $b = 0.7$
- $a = 0.00434$ mm/s^b , 10 g/l
- $a = 0.00305$ mm/s^b , 20 g/l

Enzyme $z^* = at + c$

- $a = 3.38\ 10^{-4}$ mm/s, 10 g/l
- $a = 1.45\ 10^{-4}$ mm/s, 20 g/l

- Experimental study of inactive enzyme diffusion in water;
- Systematic study in polymer solutions at different concentrations, to vary the r/ξ size ratio (also with inactive enzymes);
- SAXS/SANS to unravel the gel structure before/during/after degradation;



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