



	<b>Experiment title:</b> Structure formation in artificial extracellular matrix materials under strain	<b>Experiment number:</b> 26-02-728
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## Report:

### Goals

The final goal of the experiment was to investigate the impact of strain on the structure of a strain-stiffening synthetic hydrogels. To reach our goals, we studied hydrogel scattering in a controlled strain-environment, which was realised in a rheometer with custom-designed Couette geometry. In addition, we measured network morphology in the presence of different salts in a simple temperature-controlled capillary setup. Addition of salts changes the sol-gel transition, but not the network morphology, which makes it a strong tool to modify mechanical properties in series of identical gels.

### Motivation

Cells interact with their environment both chemically and mechanically. Over the last years increasing evidence indicates that the mechanical communication takes place in the nonlinear regime, i.e. the regime where the elastic response is a function of the applied strain. Nearly all biological materials are designed such that they access this nonlinear regime at relatively low strains. When higher strains are applied to such systems, they stiffen up, protecting the cells inside.<sup>1</sup>

For the design of artificial extracellular matrix (ECM) materials, many many gels have been proposed and tested. All besides one, have in common that they do not show such strain-stiffening behaviour, which nearly excludes them as realistic artificial matrix. Recently, we published a new synthetic polymer that shows exactly the same strain-stiffening behaviour as many biopolymers;<sup>2</sup> in fact, it is in structure, dimensions and mechanical properties nearly identical to intermediate filaments, a class of mechanical-sensitive proteins of the cytoskeleton. We also found that, by using an appropriate model, we were able to correlate the macroscopic mechanical properties of the gels to the molecular structure of the polymer.

Recently, we joined forces with the theory and simulations group of Prof. Fred MacKintosh (VU Amsterdam) who found that when tuned at the appropriate conditions, such gels can become critical and thus extremely responsive. This regime, called the marginal regime was recently experimentally observed for the

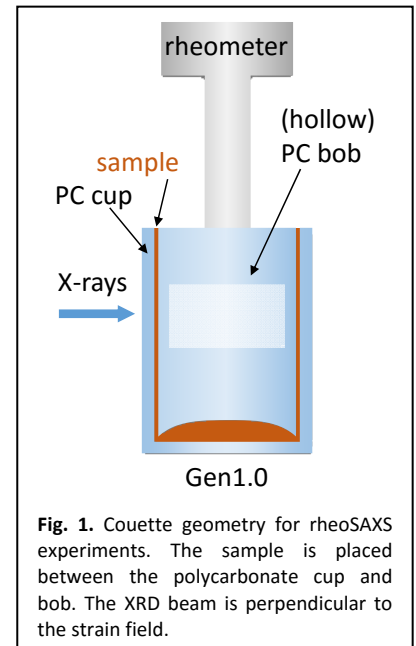
first time in such networks.<sup>3,4</sup> Although the models describe the macroscopic properties of the gels surprisingly quantitative, the microstructure of the gel is fully mispredicted. For instance, the model does not include bundle formation, which is a crucial process in our gel.

Earlier SAXS experiments (exp. BM26-02-685) showed the presence of bundles in our hydrogel<sup>5</sup> (which are extremely difficult to see in other techniques as a result of their low concentration and low order). Preliminary strain experiments did show some effect, but mechanical feedback in these experiments was missing. In these experiments, we will mount a rheometer with custom-designed Couette geometry to simultaneously collect mechanical and scattering data of our sample and study the change in network structure as a function of strain.

## Setup

Scattering patterns were collected on beam line BM26B with a 2D Pilatus1M detector placed at 3.5m from the sample. In the first part of our experiment we study the scattering patterns of the gel (in salt solutions) in capillaries. Temperature control between 5 and 50 °C gives the opportunity to follow the transition from a (low viscous) polymer solution (at low temperature) to an elastic gel (at elevated temperature). In second (major) stage of the experimental time, we performed measurements under stress using an Anton Paar rheometer with polycarbonate (PC) mini-Couette geometry (Figure 1). The mini-Couette exists in of a 0.5 mm thick PC cup with a hollow PC bob that is attached to the rheometer. The sample is placed between the cup and bob and after rotation of the bob, the generated strain-field is perpendicular to the incident X-ray beam. For temperature control, the entire geometry is place in an air-heated/cooled furnace

The rheometer is stress-controlled. To measure mechanical properties while probing the network structure, one cannot use common large amplitude oscillatory stress (LAOS) experiments. Rather we use the pre-stress protocol where a small oscillatory stress is superposed on a (more than ten times) larger pre-stress.

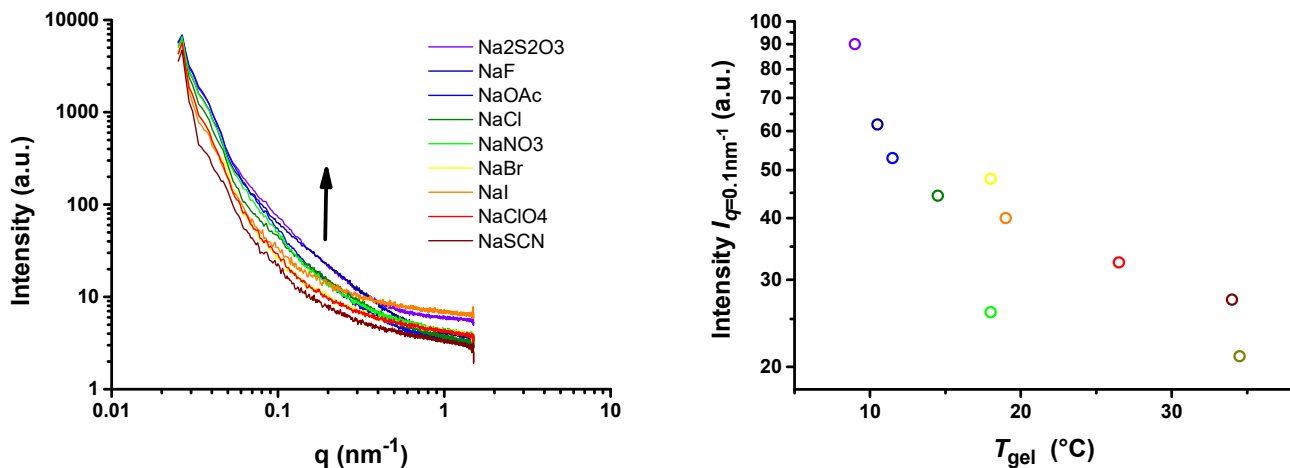


**Fig. 1.** Couette geometry for rheoSAXS experiments. The sample is placed between the polycarbonate cup and bob. The XRD beam is perpendicular to the strain field.

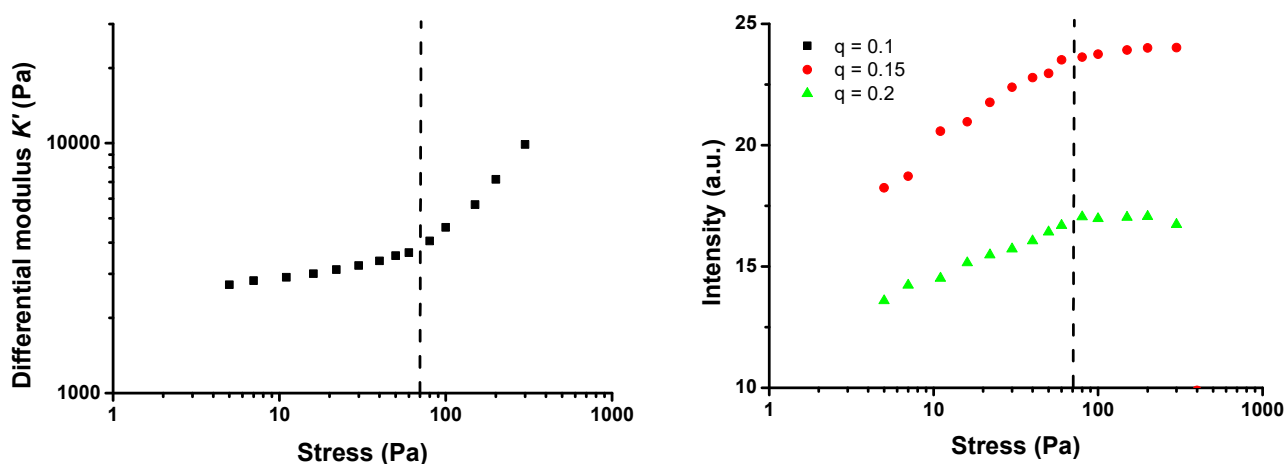
## Results and discussion

**Part A: capillary measurements.** In the capillary setup, we measured scattering profiles as a function of temperature in the presence of different salts. As the polymers exhibit a lower critical solution temperature (LCST) in water, one may expect a pronounced effect of salts on the transition temperature  $T_{gel}$ , the so-called Hofmeister effect. Indeed, we found that  $T_{gel}$  increases in the presence of chaotropic salts and decreases in solutions of kosmotropic ions.<sup>6</sup> The effect is tailored with the salt concentration and its hydrophilic or hydrophobic nature. As a result of the change in  $T_{gel}$ , also the mechanical properties at a set temperature ( $T_{measurement} = 37$  °C) change. We found that by using the appropriate model, we are able to accurately predict the mechanical properties of a gel in a salt environment, based on tabulated numbers, temperature and salt concentration. We assume in this model that the morphology of the gel is independent of the presence of the salt.

In order to test this hypothesis, we performed scattering experiments in the presence of different salts as a function of temperature. Figure 2a shows scattering curves at 37 °C of the gels in different 0.5 M salt solutions. The salts are given in the order of their Hofmeister effect. [NB: Data treatment and fitting has not been carried out as yet for this series, as we are still in the process of selecting an appropriate model for the gel.] Figure 2b, shows the scattering intensity at  $q = 0.1 \text{ nm}^{-1}$  plotted against  $T_{gel}$  of the different solutions. We know that the scattering intensity at these  $q$ -values is determined by the persistence length of the bundles, which in turn depends on how far the gel is heated in the gel phase, and thus directly on  $T_{gel}$ . Indeed, in these preliminary results, we see a clear trend in the data. However, one should consider that proper background subtraction needs to be carried out to validate the effect.



**Figure 2.** Crude scattering profiles (left) of hydrogels (5 mg/mL) in 0.5 M salt solutions at  $T = 37$  °C and corresponding scattering intensity (right) at  $q = 0.1$  nm<sup>-1</sup> (at 37 °C) as a function of the gelation temperature of the different solutions. The scattering intensity is correlated to the gel formation process, which in turn is correlated to the gel temperature. A lower gel temperature is a better developed gel at 37 °C.



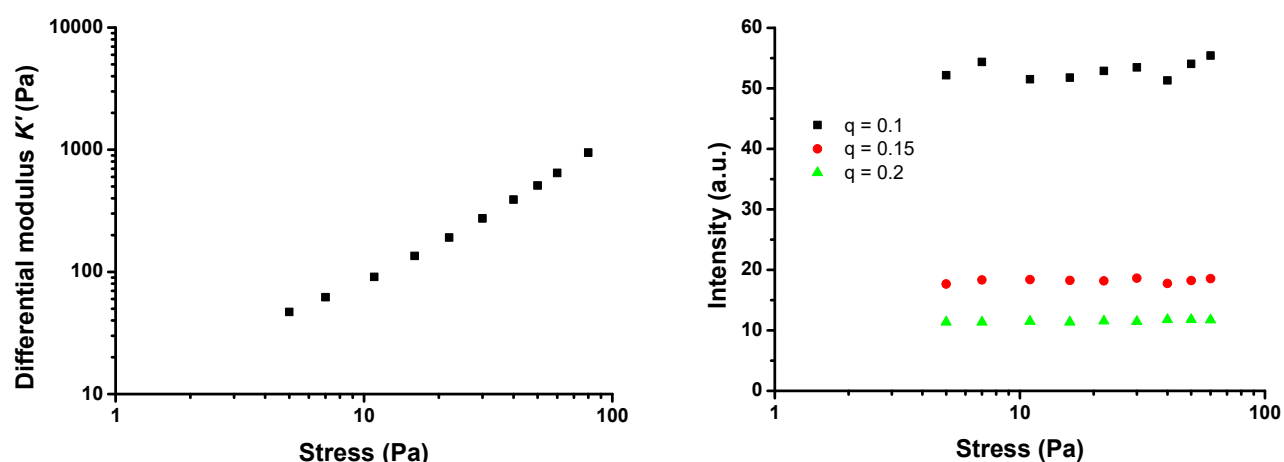
**Figure 3.** Combined rheology (left) and scattering (right) results of the PIC hydrogel (5 mg/mL) in water at  $T = 37$  °C. The dashed line at the critical stress marks the change in mechanical response from linear (low stress) to nonlinear (high stress). At the same stress levels, one see the scattering intensity at small angles becoming a constant.

**Part B: rheoSAXS measurements.** RheoSAXS experiments were carried out by applying a predetermined pre-stress on the sample and measure the (nonlinear) mechanical response with a superposed small oscillatory stress. Whilst applying the stress, scattering data is obtained. After applying the pre-stress and collecting the scattering patterns (5 minutes), the stress is removed (the sample relaxes) for 2 minutes and afterwards a higher stress is applied, and so on. Figure 3a shows the mechanical response of the sample (PIC polymer, 5 mg/mL,  $T = 37$  °) as a function of the applied pre-stress. At  $\sim 70$  Pa pre-stress (dashed line), one sees a change in the slope where the sample goes from a linear to a nonlinear response; the gel quickly stiffens upon applying more stress. This critical stress value  $\sigma_c$  is a function of concentration, temperature and persistence length, diameter and packing of the bundles.<sup>1,2</sup> Two potential mechanisms have been described in the literature that explain a two-stage mechanical response. The first assumes that in the semi-flexible bundles or fibrils, at low stress thermal fluctuations are straightened out (entropic effect) and that at the critical point one starts to strain the bundles themselves (enthalpic). The latter gives rise to the nonlinear response. The alternative explanation is that in the linear regime, the semi-flexible bundles bend in the shear direction and at the critical stress bending deformations change into stretching deformations.

The scattering intensities at small angles (Figure 3b) show an inflection point at the same stress. Here, the scattering intensity increases at low stress and levels off beyond the critical stress, in the nonlinear mechanical regime. We tentatively interpret these results using the second model: first bending, then

stretching. In this case, one would expect to see anisotropy in the scattering pattern. The data, however was very noisy and although, we seem to see an effect, we have to obtain better quality data to quantify it. Close to the gel temperature ( $T_{\text{gel}} = 15\text{ }^{\circ}\text{C}$ ), the gel becomes critical, in other words: extremely sensitive to deformation. The critical stress  $\sigma_c$  approaches zero and the gel only shows a nonlinear response. The experiment is shown in Figure 4. The Anton Paar rheometer is not very sensitive in the low stress regime and we are unable to probe the linear response. At the lowest stresses we apply, we directly see nonlinear mechanics. Analogous to the experiment at  $37\text{ }^{\circ}\text{C}$ , the scattering intensity at small angles in this regime is constant.

During the first experiments, we found that water evaporation at the Couette rim is a significant problem, as it causes small amounts of gel to precipitate, which has a giant impact on the mechanical properties and disqualifies the experiment. We were only able to record one good experiment at  $37\text{ }^{\circ}\text{C}$  and one around the critical point  $T = 15\text{ }^{\circ}\text{C}$ . In the next visit to ESRF, we will bring a different polycarbonate cup (with higher rims), which allows for a small excess of gel in the setup and, more importantly, a layer of mineral oil to prevent water evaporation. In this way, we will be able to measure all desired parameters to map this effect.



**Figure 4.** Combined rheology (left) and scattering (right) results of the PIC hydrogel (5 mg/mL) in water at  $T = 15\text{ }^{\circ}\text{C}$ . At such low temperatures, the gel is critical and has no linear regime. Consequently, we only see a plateau in the scattering intensity.

## Conclusions

In conclusion, rheoSAXS is a challenging technique, but once working, will provide valuable insight in network structure as a function of stress. The current rheology setup needs modification in the geometry to prevent water evaporation from the sample. Preliminary results already indicate that discrimination between different models that explain strain-stiffening in (biological) polymer gels is possible using SAXS.

## References and notes

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