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Supporting informations

Combining sol-gel and microfluidics process for the synthesis of protein-containing hybrid microgels

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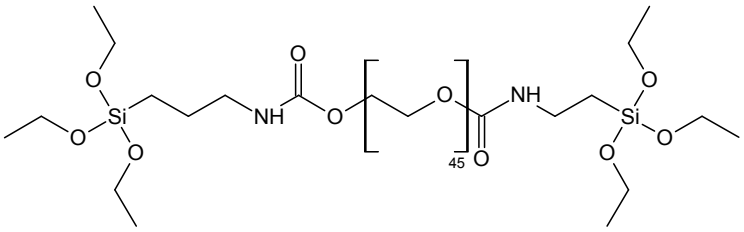
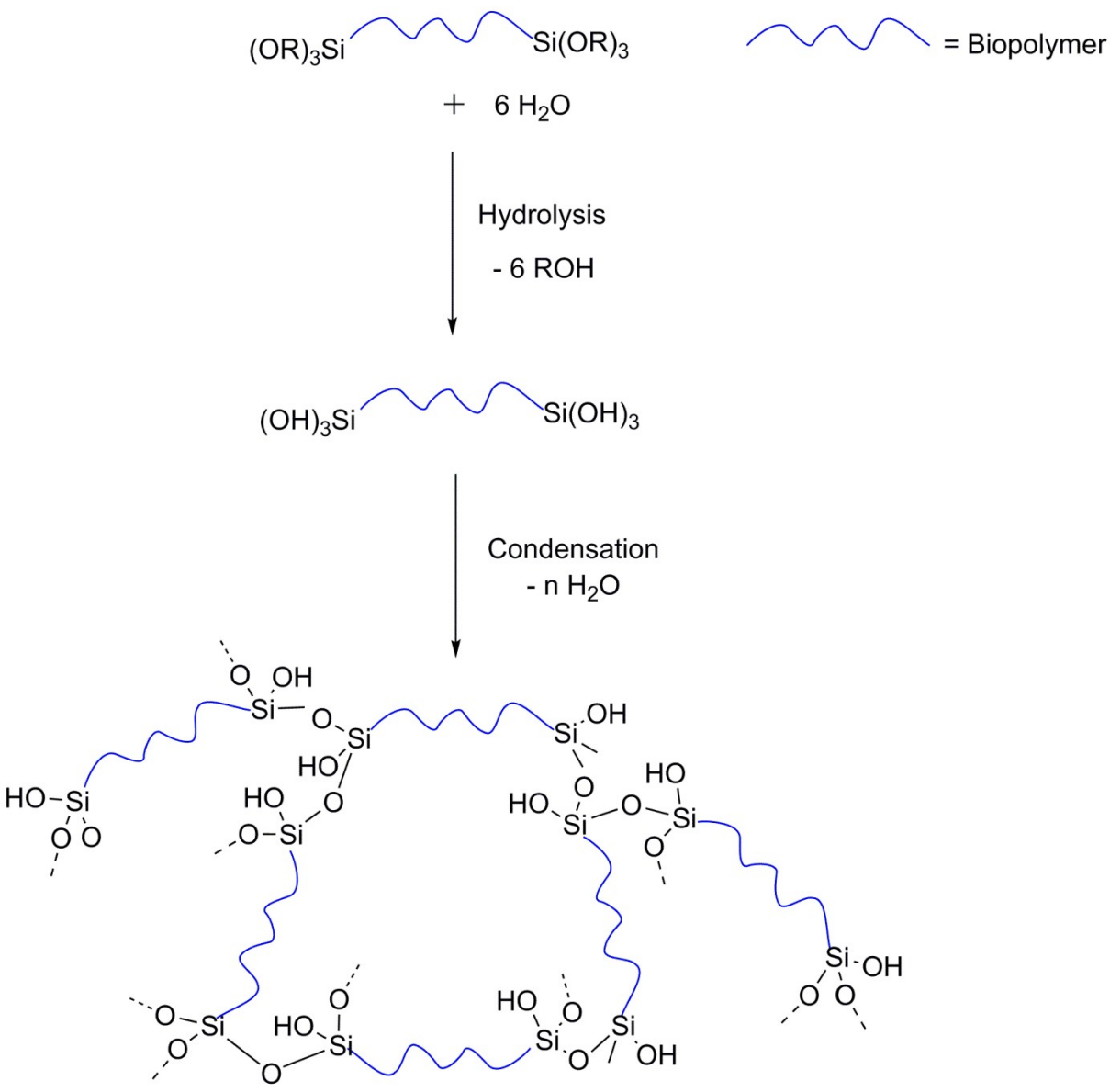
SI 1: Bis-silylated PEG (Si-PEG) synthesis.



The Bis-silylated PEG was synthesised according to the protocol previously described by Echalier et al.. [1] Polyethylene glycol 2000 g/mol (N°CAS 25322-68-3, Alfa Aesar, France) (2.00 g, 1.00 mmol) was vacuum-dried overnight at 80°C and dissolved in anhydrous THF (12 mL) under argon. Triethylamine (≥99.5%, SIGMA-ALDRICH, France) (1.66 mL, 12 mmol, 12 eq) and 3-isocyanatopropyltriethoxysilane (>95.0%(GC), TCI Chemicals, Europe) (744 µL, 3 mmol, 3 eq) were added. The mixture was heated under reflux for 48 h. Then the solvents were removed under reduced pressure and the reaction mixture was precipitated in hexane. The white solid was washed 3 times with hexane and vacuum-dried. Hybrid PEG was then stored at 4°C under argon.

[1] C. Echalier, C. Pinese, X. Garric, H. Van Den Berghe, E. Jumas Bilak, J. Martinez, A. Mehdi and G. Subra, *Chem. Mater.*, 2016, **28**, 1261–1265.

SI 2: Reactions involved on the hydrogel elaboration. In order to facilitate the representation, we considered that hydrolysis is total before the polycondensation reaction.

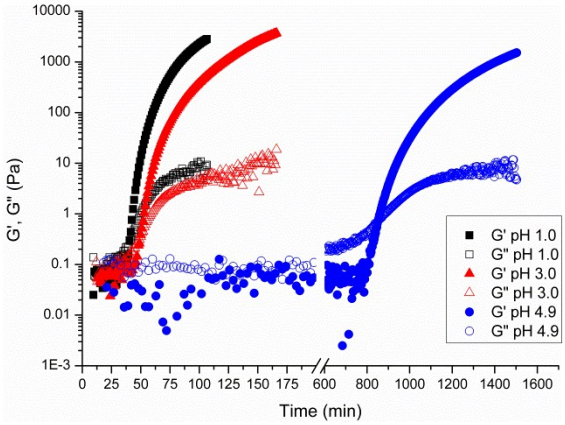


SI 3: Determination of the gelation times, T_{gel}, as a function of a) the pH during hydrolysis, and b) the aging time. Tables give G' (Pa) and the calculated mesh sizes.

Variable parameters : sol aging time and initial pH. All experiments were done on solutions at [Si-PEG] = 20 %wt., in HCl/citrate/Na_2HPO_4 buffers at pH 1.0, 3.0, 4.0, 4.9 and 7.4, prepared by mixing the appropriate volumes of HCl (37%, SIGMA-ALDRICH) at 5 M, monohydrated citric acid ($C_6H_8O_7$: H_2O, ≥99.0%, CAS: 5949-29-1, SIGMA-ALDRICH) solution at 0.1 M, and disodium hydrogen phosphate (Na_2HPO_4:H_2O, ≥99.0%, CAS: 10049-21-5, SIGMA-ALDRICH) at 0.2 M.

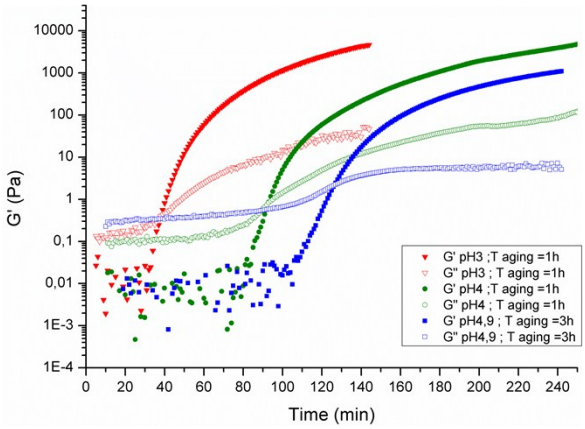
The gelation time T_{gel} of our material was determined at the intercept of the curves representing G' and G" overtime. The viscoelastic behaviour of a system is characterized by the storage (G') and loss (G") moduli which respectively quantify the solid-like and fluid-like contributions to the measured response when a sinusoidal shear deformation is applied to the system. Oscillatory shear measurements were performed on the Si-PEG solutions at 25°C using an AR 2000 rheometer (TA Instruments, Inc) with a 20 mm diameter parallel geometry. The solutions were deposited on the Peltier plate and the gap was set at 500 mm. The dependence of G' and G" moduli was measured as a function of time within the linear viscoelastic regime of the hydrogel (25% strain, 1 Hz). Initially, G" was greater than G' which indicated a viscous behaviour of the sample. During the experiment, G' increased quickly due to the formation of elastic intermolecular cross-links upon polycondensation while G" increased negligibly. Thus a crossover of G' and G" was observed at 90 min corresponding to the sol gel polymer transition onset. Afterwards, the sample presented solid-like properties. Frequency sweep experiment was also carried out and showed no dependence of the G' modulus as a function of the frequency at 25% strain.

a)



pH	G' (Pa)	ξ (nm)
1.0	2840	11.3
3.0	3850	10.2
4.9	1520	13.9

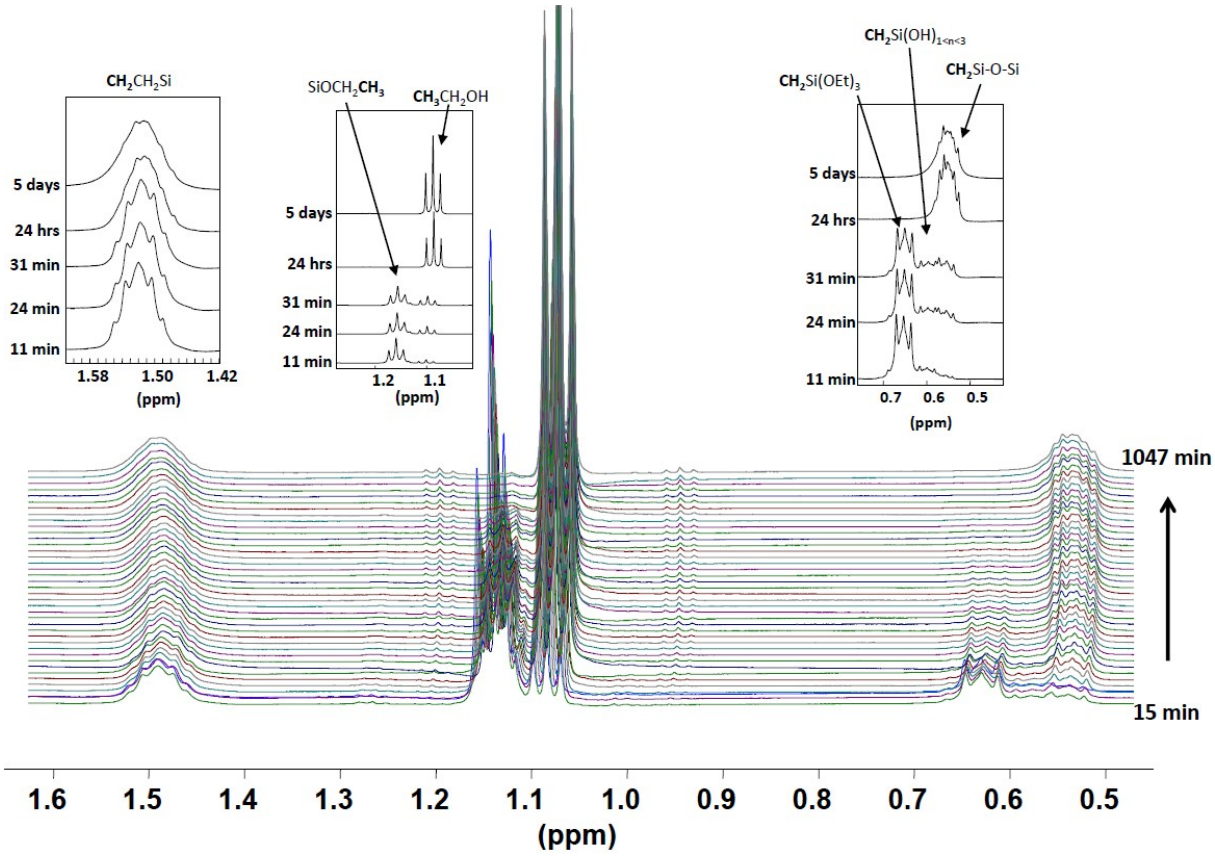
b)



pH (Aging)	T_{aging} (h)	T_{gel} (h)	G' (Pa)	ξ (nm)
3.0	1.0	0.62	4620	9.6
4.0	1.0	1.53	3790	10.3
4.9	3.0	2.10	1060	15.7

SI 4: ^{1}H NMR spectra of the Si-PEG solution at 20 %wt., during the hydrolysis reaction for 1047 min at pH 4.9.

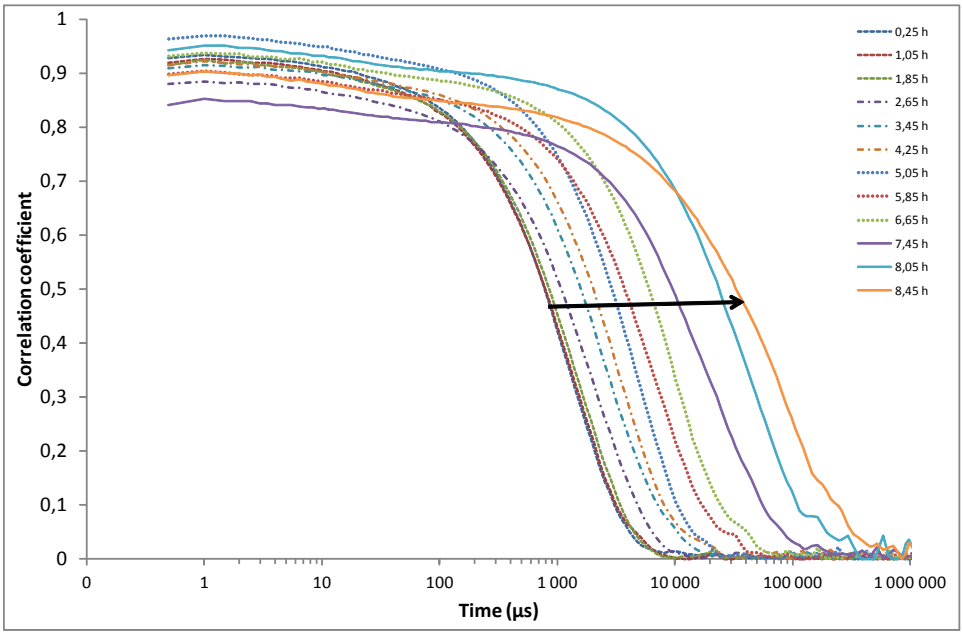
A 20 %wt. Si-PEG solution (with 20% v/v D_2O with purity of 99.90% and purchased from EURISCO-TOP) at pH 4.9 was transferred to the analysis tube. ^{1}H NMR spectra were recorded at steady sample times for 24 hours, then, once per day for 9 days, on spectrometer Avance III, 500 MHz (Brüker), at 25°C. The spectra were analysed by DMfit software[2] to determine the chemical shifts, the surface areas and peak height amplitudes.



2 B. Alonso, A. Douy, E. Véron, J. Perez, M.-N. Rager and D. Massiot, *J. Mater. Chem.*, 2004, **14**, 2006–2016.

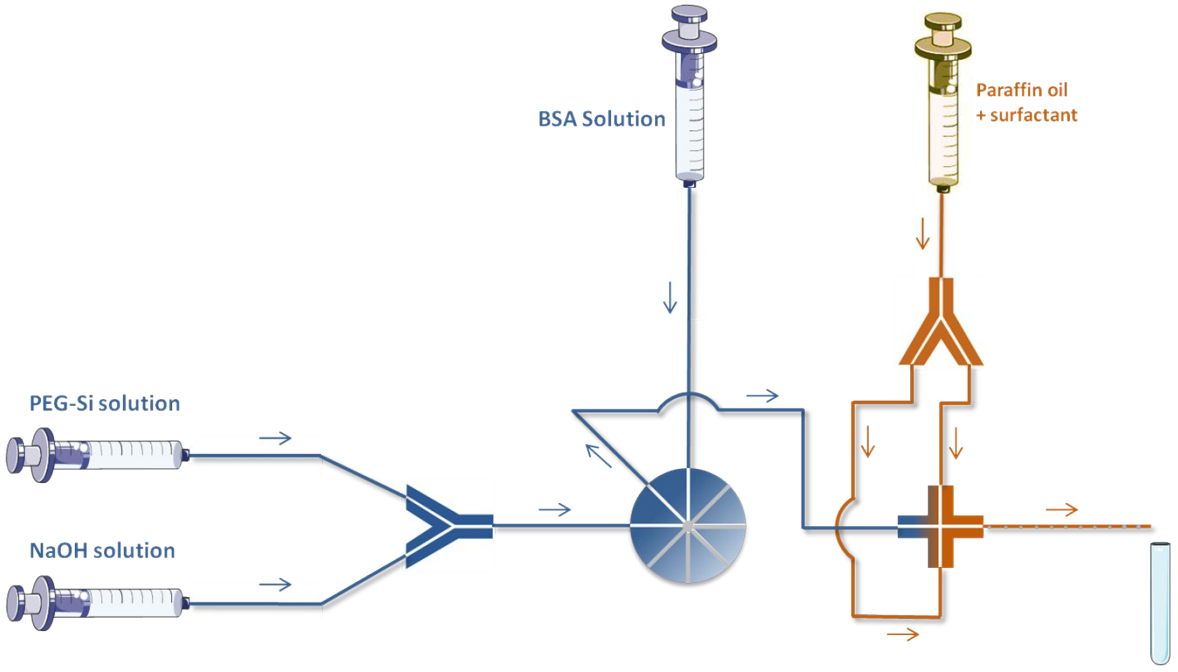
SI 5 : QELS experimental details and auto-correlation data recorded on a Si-PEG solution 20 %wt. at pH 4.9 (citrate/PBS buffer).

QELS measurements were done on a NanoZS zetasizer Malvern Instrument. A 20 %wt. solution of Si-PEG in citrate/Na_2HPO_4 buffer at pH 4.9 was left to stand in the zetasizer up to complete gelation. The data were acquired every 12 min, at a temperature of 25°C. The detection was done at an angle of 173°. The attenuator was fixed at position 7. Multimodal analysis was applied using the Non-Negative Least Squares (NNLS) algorithm. The data were selected for all the records giving 95% < % In Range < 98%. The mean size diameters were given on the intensity size distributions. Irregular ordinate values at 0.9 were ascribed to variable signal on noise, that was due to the presence of irregular and/or large siloxane units aggregates during there formation.

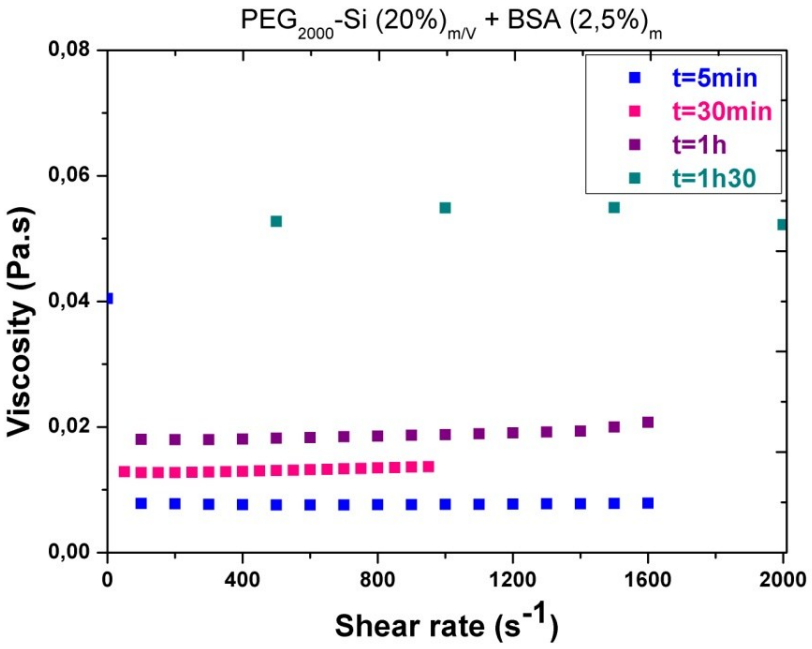


SI 6: Microfluidic assembly scheme and microgels conditions of production.

The microfluidic system was composed of two Twin Syringe Pump (model 33, Havard Apparatus). PEEK capillaries with ID = 500 µm were used for the liquid phases distribution. A PEEK Y junction (ID = 500 µm; swept volume = 1.7 µL) (Upchurch Scientific®), was used for the mixing of both aqueous phases, 20 %wt. Si-PEG solution at pH 4.9 after 3 hours of aging and 4M NaOH for neutralization. The flow rate of both aqueous solutions was adjusted to reach a final pH of 7.4, and was found to be Si-PEG:NaOH = 18. A PEEK cross junction (ID = 500 µm; swept volume = 3.8 µL), was used to deliver the oil phase (light liquid paraffin oil, INCI: Paraffinum liquidum N°CAS: 8042-47-5, Interchimie, France) with 2 %wt. of sorbitan oleate (Dehymuls® SMO, BASF, France) (determined after optimization using an emulsion bulk process). The relative flow rates of the aqueous phase and the oil phase were adjusted to reach the dripping regime, in the range of a water phase to oil phase ratio of 0.110. Bovine Serum Albumin (BSA, lyophilized powder, ≥96%, SIGMA Life Science, France) solution at 0.12 g/mL in PBS at pH 7.4 was added by mean of a PEEK 9-port manifold (ID = 1.00 mm µm; swept volume = 139 µL) (Upchurch Scientific®). The final loading ratio BSA/Si-PEG was 1%. After collection, the microgel's suspension was left stand under mild agitation at 25°C over night to complete the cross-linking.



SI 7: Viscosity measurements were performed on neutralized Si-PEG solutions containing BSA at 25°C using an AR 2000 rheometer (TA Instruments, Inc) equipped with a cone plate Acrylic. Samples were analysed at different time of the condensation process.

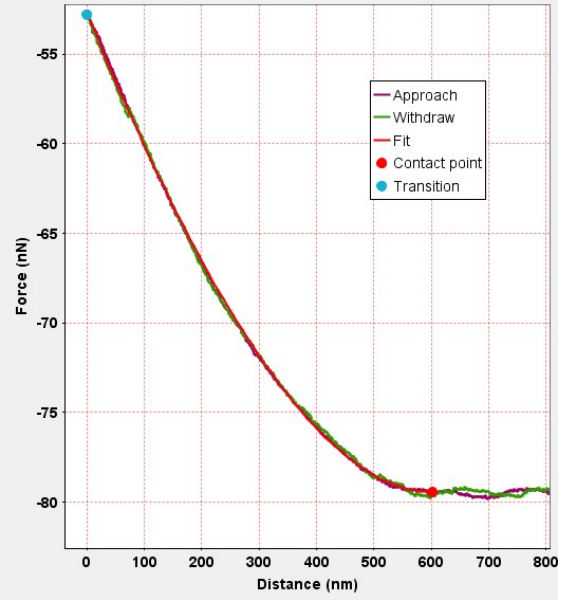


SI 8: Nano-Indentation Force to distance curve.

The nanomechanical properties of the microgels were studied by atomic force microscope (AFM D 3100 Brüker Instruments), driven by a Nanoscope 3A Quadrex , and using a PPP-ContPt cantilever (NanoSensors). This probe has a nominal spring constant of 0,4N/m (measured with thermal tune). A droplet of microgels suspension was deposited on a glass slide, the excess of water was carefully absorbed and the non-adherent microgels were removed, to prevent attachment of the microgels to the tip of the cantilever. The tip was immersed in citrate/Na_2HPO_4 buffer (pH = 7,5) to process the measurement in liquid environment. The contact mode was applied at fixed points to perform approach-retract curve. The measurement of the Force, F (N), to indentation distance, δ (m), were done on hydrated microgels[3]. The Young's elastic modulus, E (Pa), was calculated after linearization of the equation of the Sneddon model:

$$F = \frac{2}{\pi} \frac{E}{(1 - \upsilon^2)} \tan(\alpha)\, \delta^2$$

with the Poisson's ratio ν = 0.5, the half angle of the indenter α = 35°.



3 S. Dhahri, M. Ramonda and C. Marlière, *PLoS ONE*, 2013, **8**, e61663.

SI 9: Circular dichroism (CD) measures conditions.

Secondary structure of BSA was measured using a JASCO J-815 CD Spectrophotometer with a scanning speed of 100 nm/min. CD spectra in the far UV range (190–260 nm) were obtained using quartz cuvette with a 0.1 cm path length at 22°C. The gels were made of 20 %wt. Si-PEG and contained BSA at 4.4 µM. A BSA solution was prepared at the same concentration in PBS (KH_2PO_4/Na_2HPO_4) and in PEG 2000 g/mol at 20 %wt., both solutions got pH at 7.4. 30 µL of each preparation were deposited between both sides of the quartz cuvette, without forming air bubbles.

SI 10: Release tests experimental conditions.

The protein release test was done on a suspension of microgels in PBS at pH 7.4 and 37°C, in Sink conditions (volume = at least 3-10 times the saturation volume [35]) in a dissolution volume of 5 mL, under mild agitation. The dissolution medium was composed of 50 mM of phosphate KH_2PO_4 (9,073 g/l; Sigma-Aldrich ; Mw= 136 g/mol ; N°CAS 7778-77-0) and Na_2HPO_4 : 2 H_2O (11,87 g/l ; Sigma-Aldrich ; Mw= 177.98 g/mol ; N°CAS 10028-24-7), and 84 mM NaCl (Sigma-Aldrich ; Mw= 58,4 g/mol). Released BSA was determined by mean of microBCA kit assay (Thermo Fisher Scientific) on 0.150 mL sample, that was replaced by fresh dissolution medium.