Mechanisms by which Organic Solvent Exchange Transforms Responsive Pure Protein Hydrogels into Responsive Organogels

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1. Sample Holder for Cryo-SEM Frozen Hydrated Samples

Figure SI 1. Cryo-SEM sample holder. The sample cavity on the right contains one of the Ted Pella brass planchets that is used to freeze and fracture the hydrogel or organogel samples. The sample cavity on the left is empty.

2. UVRR Spectra of BSA Hydrogels and BSA Protein Monomers in Aqueous Solutions
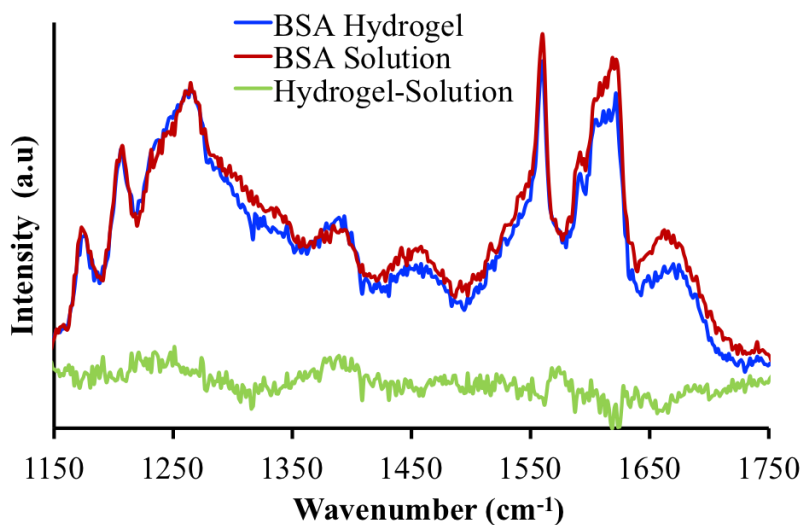

Figure SI 2. UVRR spectra of BSA hydrogel (blue), BSA protein monomer solution (red), and BSA hydrogel-monomer difference spectrum (green).

3. UVRR Measurements and Protein Secondary Structure Determination

Fitting the BSA UVRR Spectra

We used Grams software (version 8.0, Thermo Fisher Scientific, Inc. Waltham, Mass., USA) to model our UVRR spectra as a sum of Gaussian and Lorentzian bands:

$$f(x) = \sum_i (1-M)(He^{-(\frac{x-x_0}{w})^2(4\ln(2))}) + (M)\left(\frac{H}{4(\frac{x-x_0}{w})^2+1}\right)$$

where H is the peak height, X_0 is the peak frequency, w is the full width at half height, M=1 if the band is Lorentzian, and M=0 if the band is Gaussian for the ith UVRR band. The AmIII$_3^S$ fitted bands were modeled as Gaussian bands because they are inhomogenously broadened due to the peptide Ψ angle distribution.[1]

Ψ Ramachandran Angle Calculation

Details describing the methodologies for calculating Ψ angles are reported by Mikhonin et al.[1-2] We use the following equation to calculate the Ψ angle for the α-helix AmIII$_3^S$ bands:

$$v_i = 1244\ cm^{-1} - 54\ cm^{-1}\sin(\Psi + 26)$$

where v_i is the AmIII$_3^S$ frequency and Ψ is the backbone Ramachandran Ψ angle. As discussed by Mikhonin et al.,[1] this equation is used to calculate the Ψ angles for the peptide backbones involved in backbone-backbone hydrogen bonding.[1]

Estimation of the Change in BSA Organogel α-Helix Populations

To estimate the change in protein secondary structure in the BSA organogel, we divided the total intensity of the AmIII$_3$ bands in BSA by the intensity of the hydrogel-organogel difference spectrum. We find that the intensity of the hydrogel-organogel difference spectrum is ~4% that of the total intensity of the AmIII$_3$ band. Therefore, we estimate that the organogel contains ~4% more α-helical peptide bonds compared to that of the hydrogel.

4. NIR Absorption of Ethylene Glycol-Water Solutions and BSA Organogels

Small volumes of water were added to 1.6 mL aliquots of EG. The EG aliquoits were directly taken from the final EG solution in which the organogels were equilibrated. During the solvent exchange, the organogels were placed on a shaker to mix the solutions. The exchange to pure EG took place over 2 days, in which ~250 mL of pure EG was replaced 3 times daily to remove bulk water in the samples.

The 1400-2000 nm NIR absorption spectra of ethylene glycol (EG) solutions containing 0, 5, 10, 15, 20, and 25 µL water are shown in Figure SI 3a. EG-water solutions were measured in a 5 mm path length quartz cuvette. Water has strong absorption peaks at ~1400 and ~1900 nm that are used to monitor the samples water content.[3,4-5] These absorption bands derive from the first overtone of the OH-stretching band (~1400 nm) and the combination of the OH-stretching band and the O-H bending band (~1900 nm).[6]

We utilize the ~1900 nm peak to monitor the water content in the mobile phase because EG has stronger absorption around 1400 nm due to its O-H stretching mode. EG has minimal absorption at ~1900 nm.[7] Pure EG in a 5 mm pathlength cuvette shows an absorbance of 0.7 at 1915 nm.[7] We measured an absorbance of 0.74 at 1915 nm for the EG extracted from the bottle containing the organogels, corresponding to 0.08 M water in the bulk EG.

The difference spectrum of the EG/water solutions with the pure EG spectrum subtracted is shown in Figure SI 3b. The increasing absorbance from the increasing water concentration at ~1400 and ~1900 nm is clearly evident. The 1915 nm absorbance of the EG/water solutions from this difference spectrum (Figure SI 4) was used to calculate the molar absorptivity, ε, of water using the Beer-Lambert equation. The 1915 nm molar absorptivity coefficient of water was calculated to be $\varepsilon = 0.95 \ M^{-1} \ cm^{-1}$.
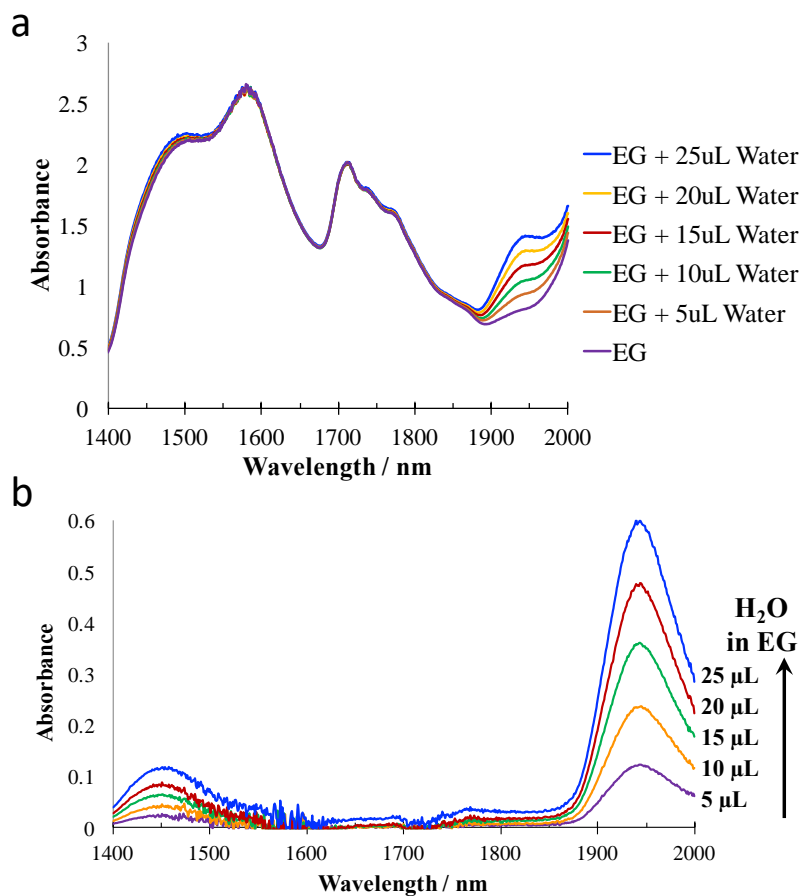
Figure SI 3. NIR absorbance of EG and its water solutions in a 5 mm path length quartz cuvette between 1400 nm to 2000 nm. (a) Spectra of pure EG with 0, 5, 10, 15, 20, and 25 µL water added (b) Difference Spectra of (EG/water solution) - EG spectra.
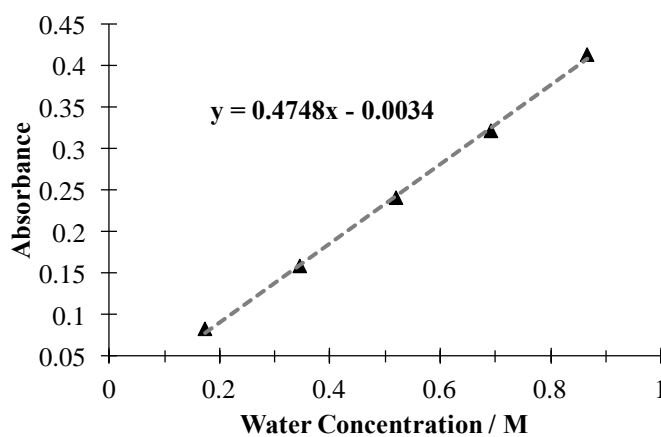

Figure SI 4. (EG+water)-EG difference spectra absorbance at 1915 nm as a function of water concentration. The slope of the best fit line is 0.47 A.U. M^{-1}.

The 1400 – 2000 nm NIR absorbance of our BSA organogels is shown in Figure SI 5. The organogel films are 420 µm thick such that the double film layer is 840 µm thick. The 1915 nm absorbance of these organogels are 0.32 for the single film layer and 0.63 for two film layers. These absorbance values yield organogel water concentrations of 8.1 M for a single organogel film and 7.9 M for two organogel films (average 8.0 ± 0.2 M water).
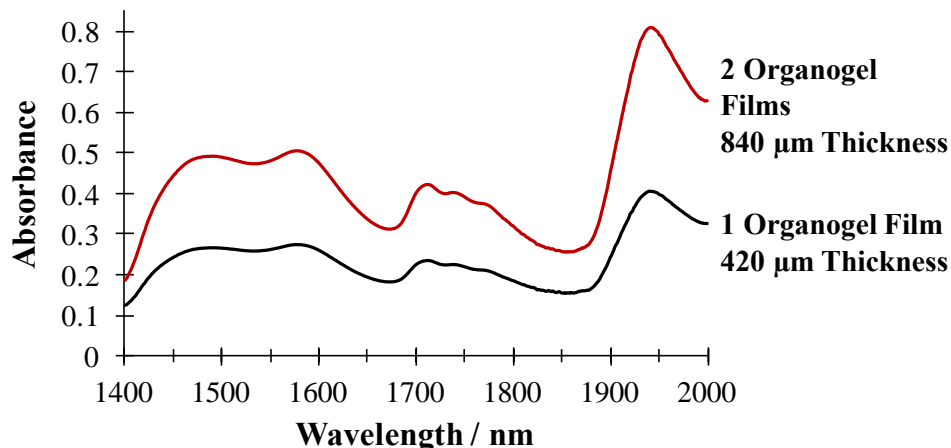

Figure SI 5. The 1400-2000 nm NIR absorbance of a single and double layer BSA organogel film.

To account for the small EG absorbance at 1915 nm in the organogel, we subtracted the absorbance of a pure EG film having a thickness of either 420 µm or 840 µm. A pure EG film of 420 µm thickness has a theoretical absorbance of 0.065 and an 840 µm thick EG film has an absorbance of 0.13. The 1915 nm absorbance of the organogel films decrease to 0.26 (420 µm) and 0.50 (840 µm) when the pure EG film absorbance is subtracted. The water concentration calculated from the EG subtracted organogel absorbance is now 6.5 M for a single organogel film and 6.3 M for two organogel films (average 6.4 ± 0.2 M water). This subtraction overestimates the absorbance from EG in the BSA organogels because the organogels contain less EG than a pure EG film. Thus, the water concentration calculated from the subtracted organogel absorbance underestimates the water content.

Based on these measurements, we calculate the number of water molecules per BSA protein in a 1 mL BSA organogel sample. The organogel has a BSA polymer volume fraction of $\phi = 0.27$. The organogel protein concentration is 360 mg/mL BSA (BSA MW: 66430 g/mol). Thus, a 1 mL sample of the BSA organogel contains 3.3 x 10^{18} molecules of BSA. A 1 mL BSA organogel sample that contains 6.5 – 8 M water has 3.9 x 10^{21} to 4.8 x 10^{21} molecules of water. Therefore, the ratio of water molecules per BSA protein in the organogel is calculated to be 1182-1477 water molecules per BSA protein.

5. Hydrophilic BSA Polymers Retain Much of Their Hydration Shell Waters in EG

Polar solvents, such as EG, more effectively strip water from a protein surface compared to non-polar solvents due to more favorable solvation enthalpies between the water and polar organic solvent.[8-11] Hydrated proteins exposed to non-polar organic solvents often retain more of their hydration layers than hydrated proteins exposed to polar organic solvents.[9,11] However, while these hydrophilic polar organic solvents can strip away water molecules that are loosely bound to the protein surface, the polar organic solvents have little effect on the tightly bound water molecules around ionic and polar amino acid residues on the protein surface.[9-11]

The protein hydration shell around the crosslinked BSA proteins in the pure EG organogel is mostly retained, at least in part due to the very hydrophilic nature of BSA. Gekko et al[12] experimentally demonstrated that BSA is preferentially hydrated in solutions containing 60% EG in water. As shown in Figure SI 6, the BSA surface exposed to solvent is predominately hydrophilic. The hydrophilic charged and polar amino acids are shown in blue in Figure SI 6, and the non-polar hydrophobic amino acids are shown in red. The hydration shell waters that are tightly bound to the

large surface area of hydrophilic domains in BSA likely remain intact when the aqueous mobile
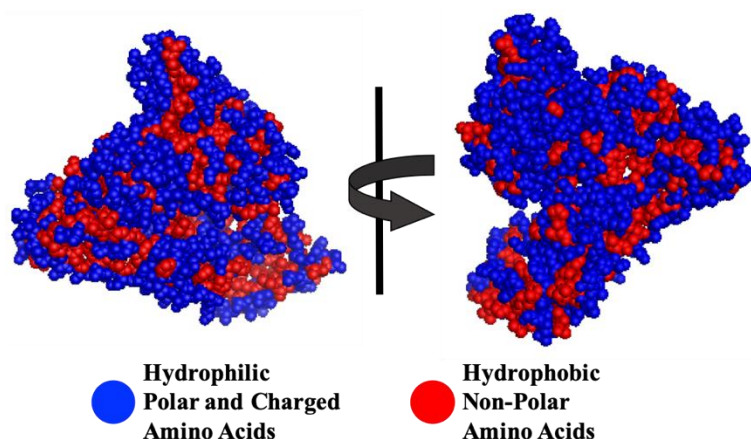
phase is exchange to pure EG.

Figure SI 6. BSA protein structure from X-ray crystallography (PDB: 3V03)[13]. Hydrophilic amino acid residues are colored blue (Lys, Arg, Asp, Glu, His, Ser, Thr, Asn, Gln, Cys); hydrophobic amino acid residues are colored red (Gly, Ala, Leu, Ile, Met, Val, Phe, Tyr, Trp, Pro).

Additionally, protein hydration shells have been protected by modifying the proteins or the

protein microenvironment to enhance protein activity in organic solvents. For example, attaching

highly hydrophilic groups to proteins is an effective method to protect the protein hydration shell

and increase enzyme activity.[14] These hydrophilic groups tightly bind numerous waters to form a

protective hydrating shell around the protein, thus decreasing the ability of the organic solvent to

strip waters from the protein surface. Improved protein hydration has also been demonstrated for

proteins immobilized on hydrophilic materials[15] and for proteins encapsulated in hydrophilic

polymer networks.[16]

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