Supporting Information

Zhongyu Cai,[a] Linda A. Luck,[b] David Punihaole,[a] Jeffry D. Madura[c] and Sanford A. Asher[a*]

[a] Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States.

[b] Department of Chemistry, State University of New York at Plattsburgh, Plattsburgh, NY 12901, United States.

[c] Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, Pennsylvania 15282, United States.

*Corresponding author. E-mail: asher@pitt.edu.

1. GGBP molecular weight and sequence

There are 309 residues in a glucose/galactose binding protein (GGBP) molecule. The molecular weight of GGBP is 33370 Da by mass spectrometry.

The protein sequence is shown below:

1-60

ADTRIGVTIYKYDDNFMSVVRKAIEQDAKAAPDVQLLMNDSQNDQSKQNDQID

VLLAKGV

61-120

KALKAINLVDPAAAGTVIEKARGQNVPVVFFNKEPSRKALDAYDKAYYVGTDS

KESIIQG

121-180

DLIAKHWAANQGWDLNKDGQIQFVLLKGEPGHPDAEARTTYVIKELNDKGIKTE

QLQLDT

181-240

AMWDTAQAKMDAMLSGPNAKIEVVIANNDAMAMGAVEALKAHNKSSIPVFGV

DALPE

241-300

ALALVKSGALAGTVLNDANNQAKATFDLAKNLADGKGAADGTNWKIDNKVVR

VPYVGVDK

301-309 DNLAEFSKK

2. Mechanism of Synthesis of GGBP Sensing Hydrogels

The GGBP cross-linking by glutaraldehyde occurs through reaction of the aldehyde functional groups with the free non-protonated ε-amino groups ($-NH_2$) of lysine or hydroxylysine of GGBP through a nucleophilic addition-type reaction. Although such reactions normally require weak acid conditions, neutral to slightly alkaline pH values are more favorable for GGBP cross-linking. More specifically, the first step of the reaction involves the nucleophilic addition of the ε-NH_2 groups to the carbonyl groups (C=O) of the aldehyde to form a tetrahedral unstable carbinolamine intermediate. In a second step, protonation of the −OH group followed by loss of a water yields the conjugated Schiff base. The scheme of the reaction is shown in Fig. S1.

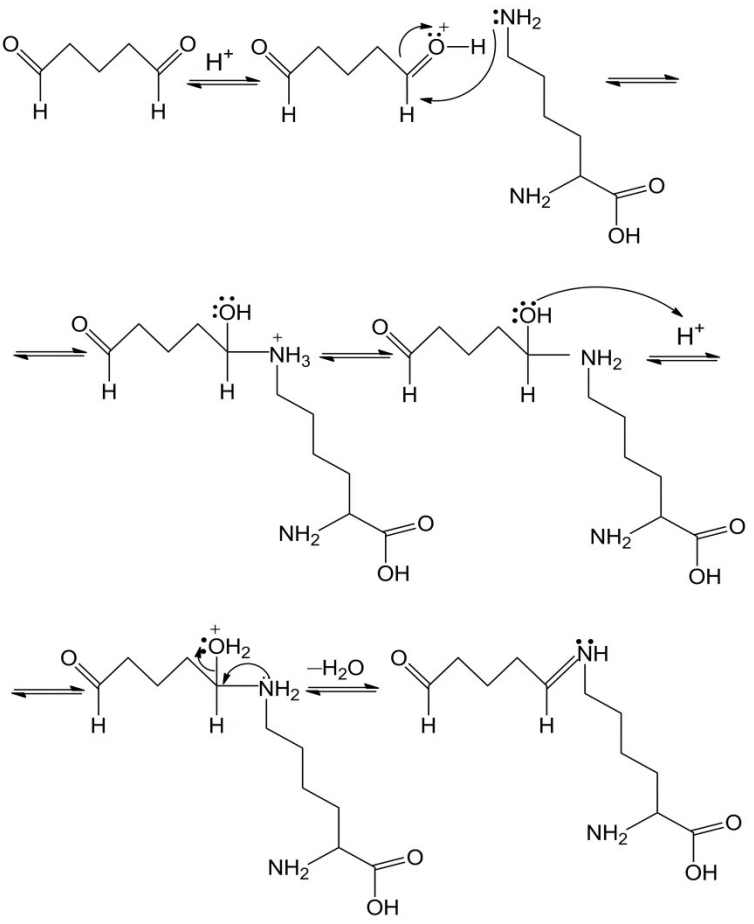

Fig. S1 Reaction mechanism between amino group of lysine and carbonyl groups of glutaraldehyde form Schiff base linkages.[1]

It is noteworthy that such a mechanism can result in the formation of new covalent bonds between gelatin molecules at either intramolecular (short-range) or intermolecular scale (long-range). The long distance crosslinking bridges form through the polymerization of glutaraldehyde in aqueous solution, or through aldol condensation reactions (Fig. S2). [2]

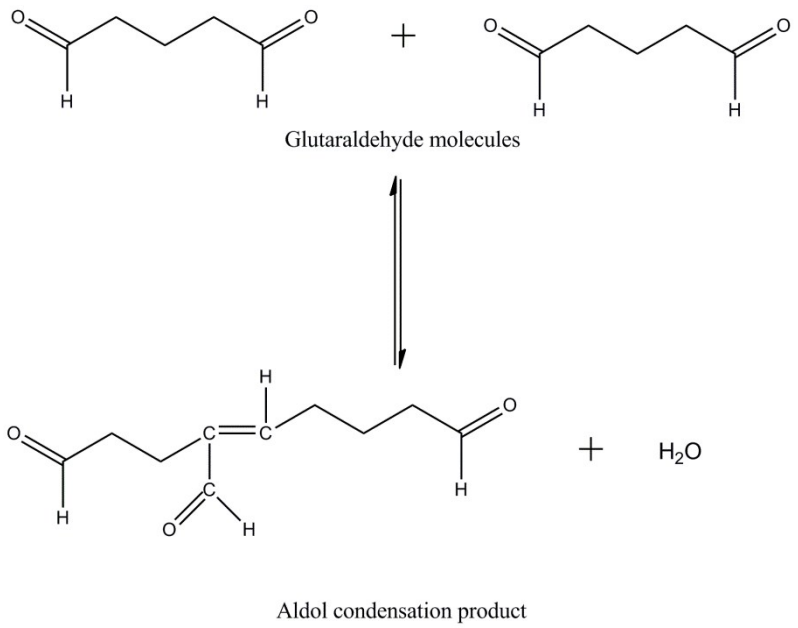

Fig. S2 Scheme of the aldol condensation reaction.

3. UV Resonance Raman (UVRR) spectra of GGBP monomer and cross-linked GGBP hydrogel

We utilized ~204 nm excitation UVRR to directly compare the GGBP hydrogel and monomer solution secondary and tertiary structures. The excitation wavelength is resonant with the peptide bond NV_1 $\pi \rightarrow \pi^*$ transition.[3] Thus, the UVRR spectra (Fig. S3) are dominated mainly by amide vibrations associated with the peptide backbone. The spectra also contains significant contributions from aromatic amino acid side chain vibrations (e.g. Trp, Tyr, and Phe) since the excitation wavelength is also resonant with their transitions.[4]

Fig. S3 shows the UVRR spectra of GGBP cross-linked in a hydrogel and in the monomeric solution state, with and without glucose. The bands between ~1645 cm^{-1} to

~1700 cm^{-1} derive from the Amide I vibrations, while the bands between ~1500 cm^{-1} to ~1600 cm^{-1} derive from Amide II vibrations. The sharp ~1560 cm^{-1} feature in the spectra derive from the air O_2 stretching band. The bands at ~1180 cm^{-1}, ~1210 cm^{-1}, and ~1620 cm^{-1} derive from the Y9a/F9a, Y7a/F7a, and Y8a bands, respectively, of Tyr, Phe, and Trp residues. These bands are useful probes of tertiary structure because they are sensitive to the local chemical environment of aromatic amino acids.[5, 6] The most conformationally sensitive bands in the UVRR spectra occur in the Amide III_3 region, which occurs between ~1200 to 1300 cm^{-1}. The Amide III_3 band of the glucose unbound GGBP monomer spectrum (shown in black in Fig. S3b) is broadly peaked between ~1235 cm^{-1} to 1270 cm^{-1}. The breadth of the Amide III_3 band indicates that GGBP consists of a mixture of α-helices and β-sheet/strand structures, which is consistent with the reported crystal structure.[7, 8]

The difference spectra between glucose bound and unbound GGBP shown in Fig. S3 are relatively flat and featureless for both hydrogel and monomer UVRR spectra. This indicates that there are no substantial secondary or tertiary structure changes that occur upon binding glucose in GGBP for either the protein monomers or hydrogels. This observation is consistent with the reported crystal structures of GGBP with and without glucose.[7, 8] From the crystal structures (Fig. S5), it appears that the only major changes that occur in GGBP upon glucose binding are rigid body motions of the two domains relative to one another about the small hinge region in the protein.

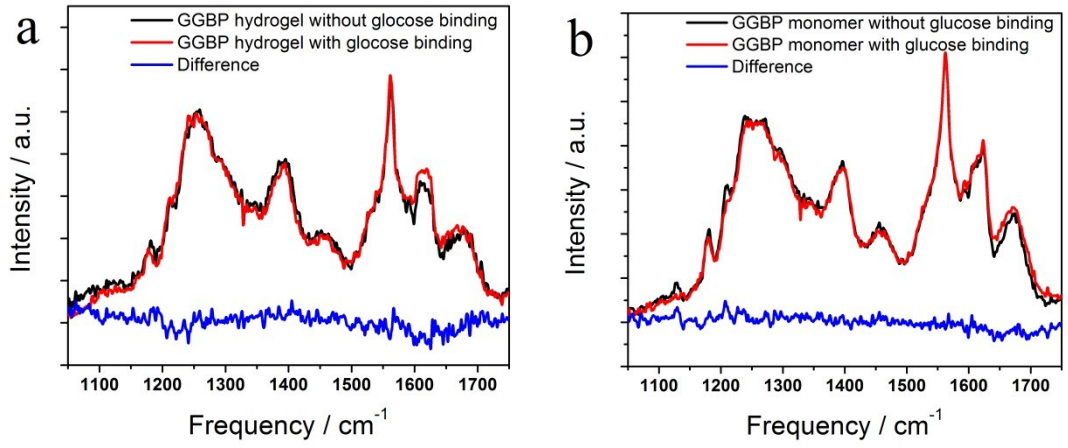

Fig. S3 UVRR spectra (excited at 204 nm) of GGBP hydrogel and monomer solution. (a), GGBP hydrogel and (b), monomer solution before and after glucose binding. The blue spectra are the glucose unbound – glucose bound difference spectra.

4. UV-Vis spectra of 2-D PC-GGBP to Glucose Solutions

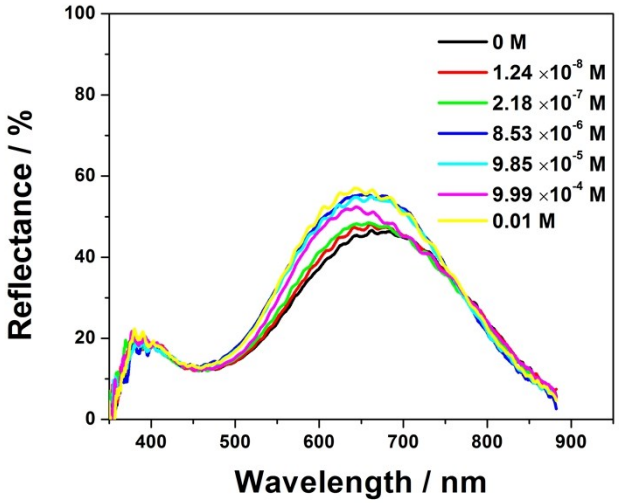

Fig. S4 Glucose concentration dependence of the hydrogel sensor reflectance measured with a spectrometer in the Littrow configuration, where the diffracted light diffracts back parallel to the direction of the incident light.

5. Crystal structures of GGBP

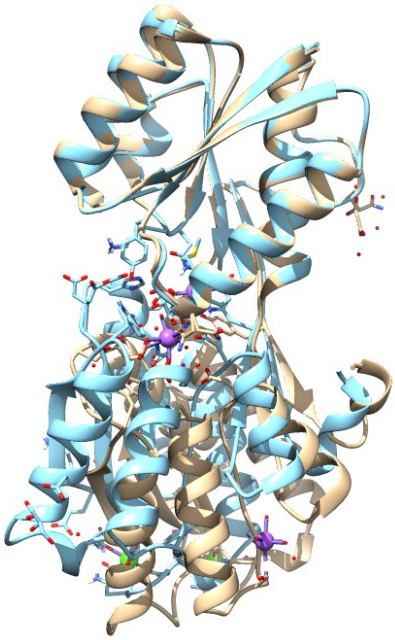

Fig. S5 Crystal structures of GGBP. Cyan coloring is the 2FVY structure, the glucose bound form. The tan coloring is the 2FW0 structure not containing glucose.[7] We overlaid them to show the rigid body motion of the two domains.

6. Calculation of the Effective Association Constant of Glucose to GGBP Hydrogel

The binding of GGBP to glucose is shown as follows:

[GGBP] + [Glucose] $\rightleftharpoons$ [GGBP-Glucose]

The effective association constant $K_a=\frac{[GGBP-Glucose]}{[GGBP][Glucose]}$. Therefore, K_a can be calculated when half of the GGBP is bound to glucose. We determine the free concentration of glucose [Glucose], for half of binding to be ~2.0×10^{-6} M from the concentration of glucose that gives rise to half of the 2-D PC-GGBP hydrogel response. For half of GGBP binding, the free concentrations of GGBP and GGBP-Glucose complex are equal, [GGBP]=[GGBP-Glucose].

Therefore, $K_a=1/[Glucose]=1/2.0 \times 10^{-6} =5.0 \times 10^5$ M^{-1}.

7. GGBP Hydrogels and Proteins Volume Change Calculations

GGBP hydrogel volume change upon glucose binding can be calculated from the 2-D array particle spacing change. The initial particle spacing is ~1028 nm, and the resulting particle spacing at 10 mM glucose is ~998 nm (Fig. 2). The volume ratio of glucose bound GGBP hydrogel to the original GGBP hydrogel is $(998/1028)^3$=91.3%. Therefore, the GGBP hydrogel volume decrease upon glucose binding is ~8.7%.

GGBP is calculated to undergo a 0.07% volume increase upon glucose binding by using the MSP program (www.biohedron.com) from the X-ray crystallographic data (unbound state GGBP with PDB ID: 2FW0, V= 38224Å^3; and bound state GGBP with PDB ID: 2FVY, V= 38198Å^3). This is consistent with our UVRR data of both GGBP monomer solution and hydrogel. By using this program, we calculated the solvent accessible surface areas. We found that the surface area decreases from 13644 to 13009 Å^2 upon glucose binding, indicating a 4.7% decrease in the solvent accessible surface area. This should give rise to less favorable free energy of mixing, and thus decrease the GGBP hydrogel volume.

References

1. S. Farris, J. Song and Q. Huang, *J. Agric. Food. Chem.*, 2009, **58**, 998-1003.
2. I. Migneault, C. Dartiguenave, M. J. Bertrand and K. C. Waldron, *Biotechniques*, 2004, **37**, 790-802.
3. S. A. Oladepo, K. Xiong, Z. Hong, S. A. Asher, J. Handen and I. K. Lednev, *Chem. Rev.*, 2012, **112**, 2604-2628.
4. S. A. Asher, M. Ludwig and C. R. Johnson, *J. Am. Chem. Soc.*, 1986, **108**, 3186-3197.
5. Z. Chi and S. A. Asher, *J. Phys. Chem. B*, 1998, **102**, 9595-9602.
6. M. Xu, V. V. Ermolenkov, V. N. Uversky and I. K. Lednev, *J. Biophotonics*, 2008, **1**, 215-229.
7. M. J. Borrok, L. L. Kiessling and K. T. Forest, *Protein Sci.*, 2007, **16**, 1032-1041.

8. N. Vyas, M. Vyas and F. Quiocho, *Science*, 1988, **242**, 1290-1295.