ACS Macro Letters

Letter

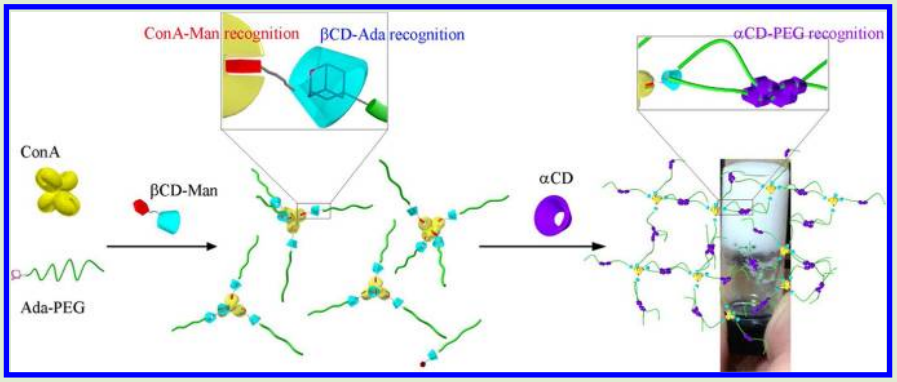
pubs.acs.org/macroletters

Dual Molecular Recognition Leading to a Protein–Polymer Conjugate and Further Self-Assembly

Kongchang Wei, Jun Li, Guosong Chen,* and Ming Jiang

State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science, Fudan University, Shanghai 200433, China

Supporting Information



ABSTRACT: Supramolecular conjugation between native protein concanavalin A (ConA) and synthetic polymer PEG (polyethylene glycol) was achieved by dual molecular recognition interactions via a linker, βCD-Man, of which β-cyclodextrin (βCD) and α-mannopyranoside (Man) recognized the adamantane (Ada) end of PEG and lectin ConA orthogonally. Further self-assembly of the resultant supra-conjugates of ConA-PEG was induced by the addition of αCD, which was selectively threaded by PEG chains, leading to nanoparticles in dilute solution or hydrogel at a higher concentration. The moduli of the obtained hydrogel were three magnitudes higher than those of the control sample without ConA, showing the dramatic cross-linking effect of ConA achieved by its rather weak interaction with α-D-mannopyranoside.

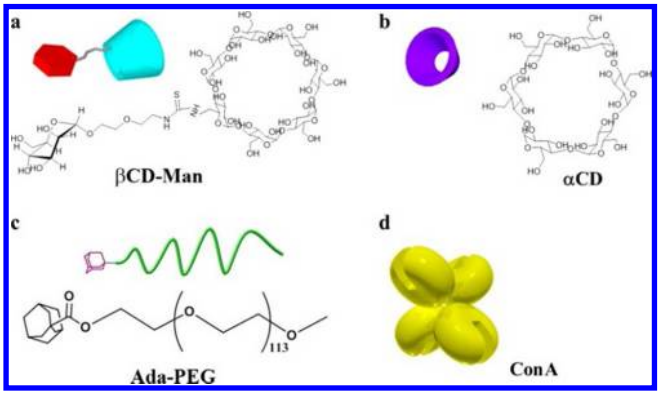
Molecular recognition in supramolecular chemistry originally stemmed from the lock-and-key concept, which was achieved for biological interaction systems of sugars and enzymes by Fischer about 100 years ago.[1] Although more and more recognition pairs with crucial roles for thousands of important biological processes with widespread existence in nature have been revealed,[2] such success had a little effect to the ever-increasing researches of molecular recognition in supramolecular chemistry[3−6] until about the end of the last century.[7,8] Since then there has been an ever-growing realization that transferring the achievements in molecular recognition found in biological systems into the synthetic supramolecular chemistry is imperative, because without such efforts, gaining a deep understanding of the biological processes and impelling the applications of supramolecular entities into biological systems are almost impossible.[9,10] Among the widespread interest based on such combinations of synthetic and biological interactions, protein–polymer conjugates[11−13] have attracted special attention as such complex structures, which merge the biological activity of proteins with desirable properties of synthetic polymers[14,15] with broad potential applications in nanomedicine and biorelated technologies.[14,16−20] Such conjugates can be produced by (a) polymerization initiated by an activated protein,[21,22] (b) linking protein to the end-functionalized polymer,[15] and (c) covalently or noncovalently attaching of protein to nanoparticles or micelles of a polymer.[23] In these preparation strategies of the conjugates, a few using molecular recognition interactions from supramolecular chemistry,[24] or biology[25] have been reported. Meanwhile, in self-assembly studies, only few strong binding pairs originated from biology, for example, streptavidin and biotin with an association constant as high as 10^{13} M^{-1} have been used because of their versatility and stability.[26,27] However, life in fact is a symphony of multiple recognition pairs with various affinities from strong to weak; in other words, each performs its own functions, and in many circumstances, the relatively weak binding pairs play even more important roles than the stronger ones. For example, rolling of lymphocytes on endothelial cells are elegantly controlled by weak binding at first while the stronger binding will be activated afterward, which prevents further rolling and induces migration of lymphocytes to infection sites.[28] Therefore, in the self-assembly studies of combining the chemical and biological interactions, more attention should be paid on the weak biological interactions.

Received: January 26, 2013
Accepted: March 6, 2013

Based on this background, it is desirable to develop simple chemical systems capable of instructing their own assembly through their mutual recognition, both chemically and biologically, leading to supramolecular assemblies toward the complexity and functionality of natural systems. To achieve this goal, the key species designed in this work is a low-molecular-weight dual-recognition linker composed of β-cyclodextrin (βCD) acting as a supramolecular host for a guest-ended polymer as well as α-mannopyranoside as a ligand for proteins. Specifically, here the strong recognition between βCD and the adamantane (Ada) end of poly(ethylene glycol) (PEG) with an association constant around 10^5 M^{-1} found in supramolecular chemistry, and the weak biological recognition, that is, α-mannopyranoside and its specific protein concanavalin A (ConA), with a binding constant of 8.0×10^3 M^{-1}, work together to form ConA-PEG conjugate with controllable compositions. This conjugate could further assemble into nanoparticles or hydrogels. Although effective noncovalent conjugations of proteins and polymers have been reported in literature,[24−27] it is very rare to achieve this goal via multiple molecular recognition interactions. Compared to the existing methods for protein−polymer conjugates, this approach of using a new dual linker implies some advantages, including modification-free for proteins, adaptivity for linking different polymers and proteins, and specificity for the used molecular recognition.

As illustrated in Scheme 1, for constructing the conjugate, the most common lectin ConA and synthetic polymer Ada-PEG

Scheme 1. Molecular Structures and Schematic Illustration of (a) βCD-Man, (b) αCD, (c) Ada-PEG, and (d) ConA



(MW = 5k Da, with functional Ada group at one end of PEG) are employed. Synthetic details and characterization of Ada-PEG are in Supporting Information (Scheme S1, Figures S1 and S2). To achieve the molecular recognition of the pairs of Mannose/ConA and Ada/βCD at the same time, a linker featuring the dual recognition property, βCD-Man (βCD monosubstituted by α-D-mannopyranoside) is designed and synthesized via convergent nine steps (Schemes S2−S4). Diethylene glycol is employed as the precursor of linker to ensure the independence of the two binding sites. 1-*O*-Trichloroacetimidate-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**M3** in Supporting Information) is an ideal glycosylation donor because of its high reactivity and regioselectivity for the α-glycosidic bond based on neighboring group participation. Success of the synthesis of βCD-Man was proved by ^{1}H NMR and MALDI-TOF MS as well as RP-TLC (reversed phase thin layer chromatography) before and after deacylation (Figures S3−S11). In the literature, other α-D-mannopyranoside modified CDs were reported for the study of multivalent binding between α-D-mannopyranoside and ConA.[29−35] As far as we know, this is the first time βCD-Man is employed as a linker for protein−polymer conjugation and the further self-assembly.

The dual molecular recognition behavior of the linker βCD-Man was demonstrated first by isothermal titration calorimetry (ITC). All the titration experiments were carried out in HEPES buffer solution containing Mn^{2+} and Ca^{2+}, and the pH value was fixed at 7.4, ensuring the tetrameric state and biological activity of ConA. The single injection mode (SIM) was first utilized to check the binding activity of the linker as shown in Figure 1a,b. Apparently, the binding of βCD-Man to ConA was

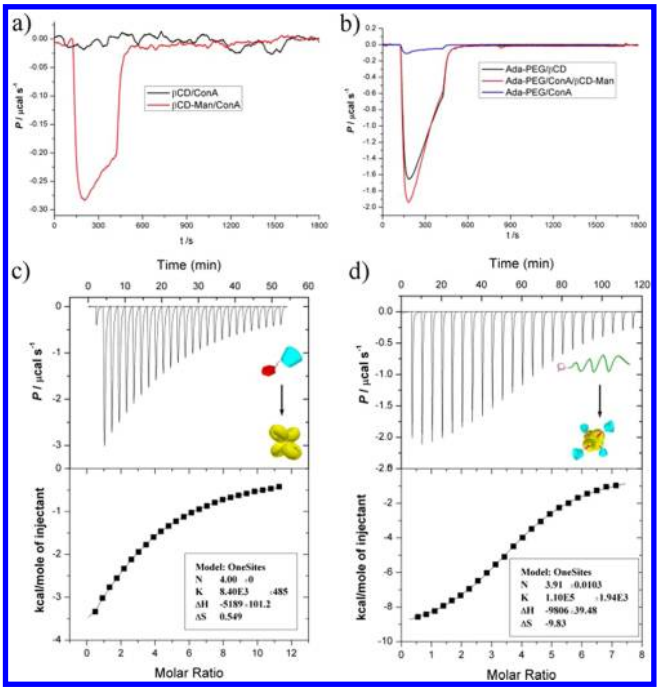


Figure 1. ITC results of (a) single injection mode titration of ConA (0.0125 mM) with βCD (0.485 mM) or βCD-Man (0.485 mM); (b) single injection mode titration of free βCD (0.05 mM), ConA/βCD-Man (ConA 0.0125 mM, βCD-Man 0.05 mM) mixture, or ConA (0.0125 mM) with Ada-PEG (0.485 mM). (c) Raw and integrated data for titration of ConA (0.05 mM) with βCD-Man (3.0 mM) and (d) titration of the mixture solution (0.02 mM ConA and 0.08 mM βCD-Man) with Ada-PEG (0.8 mM). A 20 mM HEPES buffer (pH 7.4) containing 300 mM NaCl, 5 mM $CaCl_2$, and 5 mM $MnCl_2$ at 25 °C was used for all solutions.

evidenced by the obvious exothermicity, while no thermal effect of free βCD with ConA was observed. As shown in Figure 1b, a further exothermicity was observed when the mixture of ConA and βCD-Man was titrated by Ada-PEG, which is equal to that from titration of free βCD with the same amount of Ada-PEG. Besides, in the control experiments where ConA was titrated with Ada-PEG, no greater heat than dilution was detected. By fitting the integrated curves of the titrations, quantitative results about the dual molecular recognition could be obtained. As shown in Figure 1c, the single site binding constant (K_1) between βCD-Man and ConA was measured to be around 8.40×10^3 M^{-1} by fitting the ITC results with OneSites Model.[36] Meanwhile, the association constant (K_2) between Ada-PEG and βCD of the linker attached to the ConA surface was around

dx.doi.org/10.1021/mz400036t | ACS Macro Lett. 2013, 2, 278−283

$1.10 \times 10^5\ M^{-1}$ (Figure 1d), similar to that between free βCD and Ada reported in the literature.[37] These results mean that the connection of βCD and mannose in the linker does not show any effect on the function of either βCD as a supramolecular host or that of mannose as a ligand in biological interactions.

The above ITC studies clearly demonstrated that the chemical and biological recognition interactions of the "sweet" linker βCD-Man performed orthogonally, that is, they do not interfere with each other. The formation of the protein−polymer supra-conjugate linked by βCD-Man could be monitored by DLS (dynamic light scattering) straightforwardly at a low concentration of ConA (0.05 mM) and equivalent molar ratios of ConA/βCD-Man/Ada-PEG = 1:4:4.

Compared to native ConA in solution (Figure 2a, black line), a longer relaxation time and increased hydrodynamic radius

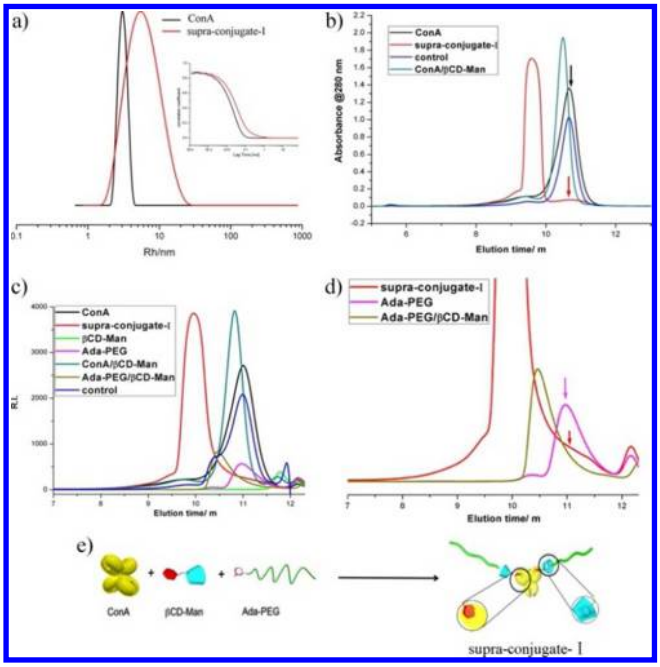


Figure 2. (a) Size distribution from DLS (inserted: the correlation function) for ConA (black) and the supra-conjugate-I (red), SEC traces from UV detector (b) and RI detector (c): ConA (black), supra-conjugate of ConA/βCD-Man/Ada-PEG (red), control sample of ConA/βCD/Ada-PEG (blue), ConA/βCD-Man (cyan), βCD-Man (green), Ada-PEG (purple), and Ada-PEG/βCD-Man (gold); (d) partially enlarged image of (c). (e) Schematic illustration of supra-conjugate-I formation. In all these samples, the concentrations were fixed as follows: tetrameric ConA (0.05 mM, 5 mg/mL), βCD-Man or βCD 0.2 mM, Ada-PEG 0.2 mM. All these samples were eluted by buffer (pH = 7.4) containing 20 mM HEPES, 5 mM $CaCl_2$, 5 mM $MnCl_2$, and 300 mM NaCl using water phase column.

were detected in the mixed solution of ConA/βCD-Man/Ada-PEG (Figure 2a, red line), indicating the formation of new species with a higher molecular weight as a result of the supramolecular conjugation of ConA and Ada-PEG. More evidence was given by SEC (size exclusion chromatography) with RI-MALLS-UV triple detectors at the same concentration of ConA (Figure 2b−d). Both UV (Figure 2b) and RI (Figure 2c) curves show that the mixture of ConA/βCD-Man/Ada-PEG was eluted much earlier than both ConA (black curve, M_w = 90 kDa by MALLS detector, indicating the tetrameric form of ConA) and ConA/βCD-Man (cyan curve), suggesting the successful formation of supra-conjugate between Ada-PEG and ConA via βCD-Man linkage. In the control sample (blue curve) where βCD was used instead of βCD-Man, no apparent size change was observed. In Figure 2b, where only ConA moieties presented signals, compared to native ConA, the signal of free ConA remaining in the mixture of ConA/βCD-Man/Ada-PEG was very weak, indicating that most ConA had been converted to supra-conjugate (Figure 2b, as the arrows indicate) under the given conditions. Furthermore, by RI detector, the peak areas of the species of βCD-Man (Figure 2c, green), Ada-PEG (purple), and Ada-PEG/βCD-man (gold) were also identified to be much smaller than the supra-conjugate. This result was quite remarkable considering the very limited success of labile noncovalent interactions characterized by GPC.[38] We suppose that, in this case, the random coil conformation formed by PEG might protect the dual molecular recognition interactions with retarded dissociation speed.

However, a closer examination of the SEC traces of supra-conjugate by RI detector revealed the existence of a substantial amount of free Ada-PEG and βCD-man/Ada-PEG in the mixture of ConA/βCD-Man/Ada-PEG (Figure 2d, enlarged vision of Figure 2c, as arrows indicate), indicating the insufficient conversion of PEG into the supra-conjugates. The high conversion of ConA but relatively low conversion of PEG in the equivalent mixture of ConA/βCD-Man/Ada-PEG implies that the majority of the conjugates may have one or two PEG chains only. Considering the low concentration of the constituents here and weak interactions between ConA and α-mannopyranoside, this result is understandable. The assembled entity obtained under this condition was named supra-conjugate-I, a scheme of which was shown in Figure 2e. Obviously, preparation at high concentrations of the constituents would increase the proportion of the conjugates with more, that is, 3 and 4 PEG chains. Our simple theoretical calculation pictures the dependence of the conjugate structure on concentration. As the four binding sites on ConA are far apart from each other (ca. 10 nm in distance), we could presume that all of the binding abilities of the four sites are the same. Then in regard to ConA, it may exist in five states in the solution (with only attached PEGs countable), that is, free protein (ConA-0PEG) and protein with different numbers of Ada-PEG chains attached (ConA-nPEG, n = 1−4). Meanwhile, there are also three states of Ada-PEG, that is, free Ada-PEG, Ada-PEG/βCD-Man, and Ada-PEG/βCD-Man/ConA. By using the binding constants of ConA/βCD-Man and βCD-Man (with ConA)/Ada-PEG measured from ITC, distribution diagrams of the five possible states of ConA and the three possible states of Ada-PEG as a function of concentration of ConA (ConA/βCD-Man/Ada-PEG = 1:4:4) were calculated (details in Supporting Information) and the results were shown in Figures 3a and S12. From the diagrams, the fractions of each species at a given concentration of ConA could be read out. Under the experimental condition of SEC, that is, [ConA] = 0.05 mM, only around 38% of Ada-PEG was attached to ConA, while more than 85% of ConA was conjugated to Ada-PEG (Table S1). This was consistent to the qualitative conclusion found from SEC mentioned above.

Thus, among the conjugates, ConA-1PEG and ConA-2PEG are the majority. With concentration of ConA increasing, the fractions of ConA-0PEG and ConA-1PEG and free Ada-PEG decreased rapidly, while the fractions of conjugates of ConA-3PEG and ConA-4PEG increased accordingly (Figure 3a). Under the experimental conditions used below, the concen-

dx.doi.org/10.1021/mz400036t | ACS Macro Lett. 2013, 2, 278−283

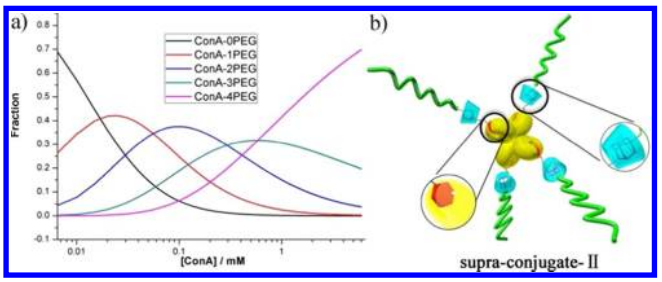


Figure 3. (a) Fractions of different conjugate species as a function of ConA concentration when the molar ratio was fixed as ConA/βCD-Man/Ada-PEG = 1:4:4. (b) A scheme for supra-conjugate-II.

tration of ConA increased to 0.5 mM and the ratios of ConA/βCD-Man/Ada-PEG were kept at 1:4:4; from Figure 3, we know that in resultant conjugates, named supra-conjugate-II, ConA-3PEG, and ConA-4PEG reached 70% (Figure 3b). The strong dependence of conjugate composition on the building block concentration is obviously resulted from the weak interaction between the ligand and the protein. This provides possibilities of constructing conjugates differing in protein–polymer ratio and, hence, characteristics. Supra-conjugate-I composed mostly of species with one or two attached PEG chains and supra-conjugate-II with majority species with 3 and 4 attached PEG chains could show very different assembly behavior.

It is well-known that αCD can be threaded by PEG chains, resulting in the supramolecular structure called pseudo-polyrotaxanes (PPR)[39−41] and PPR hydrogel when concentration is high.[42−44] This relatively weak molecular recognition between PEG and αCD from supramolecular chemistry was employed as the third one to drive further assembly of the conjugates into large objects. For the case of supra-conjugate-I, which was obtained at a low concentration, the process was monitored by DLS first. After addition of αCD into the solution of supra-conjugate-I, a gradual size increase was detected (Figure 4a) over a period of 4 h and it kept unchanged afterward. Meanwhile, the solution slowly turned turbid (Figure 4a, inset). The aggregation process became visible in situ by confocal laser scanning microscope (CLSM) observations. As shown in Figure 4b–d, where FITC-labeled ConA (ConA-FITC) was used instead of native ConA, in the absence of αCD, no fluorescence aggregate was detected (Figure 4b). A total of 1 h after the addition of αCD, fluorescent aggregates (Figure 4c) were observed and grew up (Figure 4d). The size increase of the fluorescent spots clearly proved the aggregation of the conjugates, as the ConA contained was the only component with fluorescence in the system. The aggregates were found to be spherical, with a diameter around 320 nm observed 1 h after addition of αCD by AFM (atomic force microscopy, Figure S13).

Further assembly of supra-conjugate-II obtained at the high concentration with addition of αCD was studied together with a negative control, that is, mixing αCD, Ada-PEG, and βCD-Man at the same concentration without addition of ConA. After all of the components were mixed for a while, both of the solutions of supra-conjugate-II and the control turned to solid-like state, that is, both of the vials can be turned upside down (Figure 5). It is known that the formation of microcrystal domains between αCDs in PPRs may lead to a hydrogel at certain concentrations. However, a dramatic difference in the performance in rheological measurements between the supra-

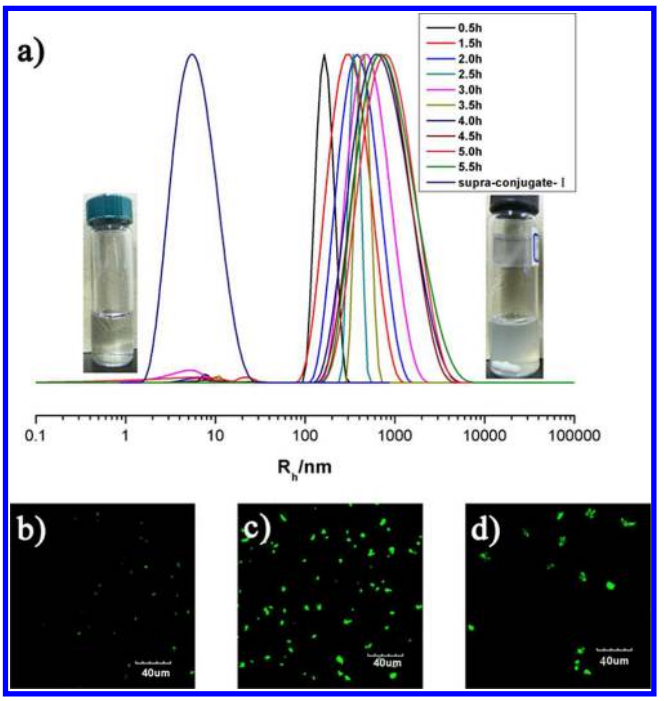


Figure 4. (a) Size distribution of supra-conjugate-I and its aggregation monitored by DLS, the inserted photos were taken 5.0 h after the supra-conjugates were in the presence (right) or absence (left) of αCD; aggregation of the supra-conjugates with FITC-labeled ConA observed by CLSM (b) before the addition of αCD, (c) 1 h after the addition of αCD, and (d) 5 h after the addition of αCD.

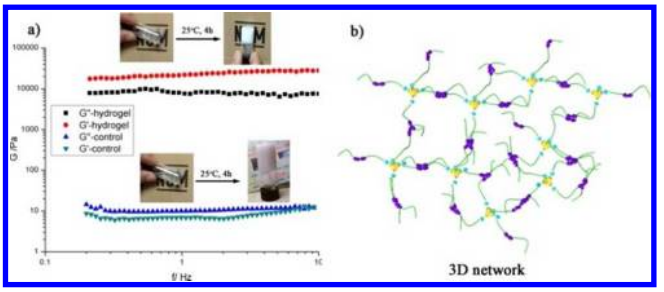


Figure 5. (a) Storage moduli (G') and viscous moduli (G'') of hydrogel containing 0.5 mM ConA, 2 mM βCD-Man, 2 mM Ada-PEG, and 0.1 M αCD, compared to a control sample containing 2 mM βCD-Man, 2 mM Ada-PEG, and 0.1 M αCD without ConA. (b) Cartoon illustration of the hydrogel.

conjugate-II and the control was found. As shown in Figure 5, 4 hours after addition of αCD into the solution of the preformed supra-conjugate-II, the resultant material gave storage modulus G' and loss G'' being as high as 10^4 Pa, and the former kept higher than that of the latter over the measured frequency range. This clearly indicates the formation of a hydrogel. More interestingly, moduli of supra-conjugate-II gel were three magnitudes higher than those of the control, which showed G'' larger than G'. This rheology study demonstrated the importance of ConA for gelation. It is known that the strength of PPR hydrogel is generally rather weak. However, the dynamic modulus of this supra-conjugate-II based PPR hydrogel is not only much higher than those of PPR reported in literature,[46] but also even two magnitudes higher than that of the enhanced PPR hydrogel with clay nanosheets we prepared at the similar experimental conditions.[45] It means that the strengthening effect of the "soft" protein ConA is even superior

dx.doi.org/10.1021/mz400036t | ACS Macro Lett. 2013, 2, 278−283

to the "hard" clay nanosheets. However, in the case of supra-conjugate-I, with only one or two bound sites to PEG, no gel formed. In addition, the SEM image (Figure S14a) of the PPR hydrogel after freeze-drying showed the porous structure, while the channel-type structure from PPR was evidenced by the XRD result (Figure S14b, red line) with the characteristic peak at $2\theta = 20.1°$, similar to that of the control sample (Figure S14b, black line). Combining all the results, we may conclude that the "optimum" cross-linking and strengthening effect of ConA is resulted by the sufficient attachment of Ada-PEG. In other words, the nature of the multiple binding of ConA is the determining factor in forming the strong gel, despite the inherently weak interaction between ConA and βCD-Man. Now the advantage of using weak molecular recognition became clear, that is, the conversion ratio of protein and ligand in the self-assembled system could be easily tuned, resulting in complexity as close as and more similar to the biological ones.

In conclusion, by using a dual recognition linker, βCD-Man, which can act as a host for chemical molecular recognition and a ligand for biological recognition simultaneously, self-assembled protein−polymer (ConA-PEG) conjugates were attained. The two different molecular recognition pairs were proved orthogonal and essential to the conjugation process. Importantly, due to the relatively weak biological recognition used, the composition of the formed conjugates can be adjusted by changing the experimental conditions. The conjugates with less and more attached polymer PEG chains show very different behavior in their further assembly with additional host αCD, that is, resulting in nanoparticles and high-strength hydrogel, respectively. Thus, in this study, the contributions of multiple molecular recognition interactions with different origins, including the rather weak biological one, to self-assembly have been highlighted.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, including the preparation and characterization of βCD-Man and Ada-PEG, as well as the theoretical calculation. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: guosong@fudan.edu.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Ministry of Science and Technology of China (2011CB932503 and 2009CB930402) and National Natural Science Foundation of China (No. 91227203) are acknowledged for their financial support.

■ REFERENCES

(1) Fischer, E. *Chem. Ber.* **1894**, *27*, 2985−2993.
(2) Pang, P. C.; Chiu, P. C. N.; Lee, C. L.; Chang, L. Y.; Panico, M.; Morris, H. R.; Haslam, S. M.; Khoo, K. H.; Clark, G. F.; Yeung, W. S. B.; Dell, A. *Science* **2011**, *333*, 1761−1764.
(3) Chen, G.; Jiang, M. *Chem. Soc. Rev.* **2011**, *40*, 2254−2266.
(4) Monika, M. *RSC Adv.* **2012**, *3*, 2630−2642.
(5) Uhlenheuer, D. A.; Milroy, L. G.; Neirynck, P.; Brunsveld, L. *J. Mater. Chem.* **2011**, *21*, 18919−18922.
(6) Dang, D. T.; Schill, J.; Brunsveld, L. *Chem. Sci.* **2012**, *3*, 2679−2684.
(7) Philp, D.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **1996**, *35*, 1154−1196.
(8) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2978−2996.
(9) Uhlenheuer, D. A.; Petkau, K.; Brunsveld, L. *Chem. Soc. Rev.* **2010**, *39*, 2817−2826.
(10) Liu, Y.; Wang, H.; Kamei, K.; Yan, M.; Chen, K. J.; Yuan, Q.; Shi, L.; Lu, Y.; Tseng, H. R. *Angew. Chem., Int. Ed.* **2011**, *50*, 3058−3062.
(11) Gauthier, M. A.; Klok, H. A. *Polym. Chem.* **2010**, *1*, 1352−1373.
(12) Heredia, K. L.; Maynard, H. D. *Org. Biomol. Chem.* **2007**, *5*, 45−53.
(13) Thordarson, P.; Droumaguet, B. L.; Velonia, K. *Appl. Microbiol. Biotechnol.* **2006**, *73*, 243−254.
(14) Jain, A.; Jain, S. K. *Crit. Rev. Ther. Drug Carrier Syst.* **2008**, *25*, 403−447.
(15) Broyer, R. M.; Grover, G. N.; Maynard, H. D. *Chem. Commun.* **2011**, *47*, 2212−2226.
(16) Abuchowski, A.; Davis, F. F. *Biochim. Biophys. Acta* **1979**, *578*, 41−46.
(17) Hadley, K. B.; Sato, P. H. *Enzyme* **1989**, *42*, 225−234.
(18) Savoca, K. V.; Abuchowski, A.; Vanes, T.; Davis, F. F.; Palczuk, N. C. *Biochim. Biophys. Acta* **1979**, *578*, 47−53.
(19) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347−360.
(20) Hoffman, A. S.; Stayton, P. S. *Macromol. Symp.* **2004**, *207*, 139−151.
(21) Sumerlin, B. S. *ACS Macro Lett.* **2012**, *1*, 141−145.
(22) Droumaguet, B. L.; Nicolas, J. *Polym. Chem.* **2010**, *1*, 563−589.
(23) Boyer, C.; Huang, X.; Whittaker, M. R.; Bulmus, V.; Davis, T. P. *Soft Matter* **2011**, *7*, 1599−1614.
(24) Biedermann, F.; Rauwald, U.; Zayed, J. M.; Scherman, O. A. *Chem. Sci.* **2011**, *2*, 279−286.
(25) Reynhout, I. C.; Cornelissen, J. J. L. M.; Nolte, R. J. M. *Acc. Chem. Res.* **2009**, *6*, 681−692.
(26) Kulkarni, S.; Schilli, C.; Müller, A. H. E.; Hoffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* **2004**, *15*, 747−753.
(27) Tao, L.; Geng, J.; Chen, G.; Xu, Y.; Ladmiral, V.; Mantovani, G.; Haddleton, D. M. *Chem. Commun.* **2007**, *33*, 3441−3443.
(28) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, 5th ed.; Garland Science: New York, 2007.
(29) Ortiz-Mellet, C.; Benito, J. M.; Garcla Fernández, J. M.; Law, H.; Chmurski, K.; Defaye, J.; O'Sullivan, M. L.; Caro, H. N. *Chem.—Eur. J.* **1998**, *4*, 2523−2531.
(30) Ortiz Mellet, C.; Defaye, J.; Garcla Fernández, J. M. *Chem.—Eur. J.* **2002**, *8*, 1982−1990.
(31) Benito, J. M.; Gomez-Garcia, M.; Ortiz, M. C.; Baussanne, I.; Defaye, J.; Garcla Fernández, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10355−10363.
(32) Gomez-Garcia, M.; Benito, J. M.; Rodriguez-Lucena, D.; Yu, J. X.; Chmurski, K.; Ortiz Mellet, C.; Gutlérrez-Gallego, R.; Maestre, A.; Defaye, J.; Garcla Fernández, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 7970−7971.
(33) Gomez-Garcia, M.; Benito, J. M.; Butera, A. P.; Ortiz Mellet, C.; Garcla Fernández, J. M.; Jiménez Blanco, J. L. *J. Org. Chem.* **2012**, *77*, 1273−1288.
(34) Nalluri, S. K. M.; Voskuhl, J.; Bultema, J. B.; Boekema, E. J.; Ravoo, B. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 9747−9751.
(35) Samanta, A.; Stuart, M. C. A.; Ravoo, B. J. *J. Am. Chem. Soc.* **2012**, *134*, 19909−19914.
(36) Dam, T. K.; Brewer, C. F. *Chem. Rev.* **2002**, *102*, 387−429.
(37) Palepu, R.; Reinsorough, V. C. *Aust. J. Chem.* **1990**, *43*, 2119−2123.
(38) Zimmerman, S. C.; Zeng, F.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095−1098.
(39) Harada, A.; Li, J.; Kitagawa, Y.; Katsube, Y. *Carbohydr. Res.* **1998**, *305*, 127−306.

(40) Harada, A.; Kamachi, M. *Macromolecules* **1990**, *23*, 2821−2823.
(41) Harada, A.; Li, J.; Kamachi, M. *Nature* **1992**, *356*, 325−327.
(42) Guo, M.; Jiang, M. *Prog. Chem.* **2007**, *19*, 557−566.
(43) Li, J.; Harada, A.; Kamachi, M. *Polym. J.* **1994**, *26*, 1019−1026.
(44) Liao, X.; Chen, G.; Liu, X.; Chen, F.; Chen, W.; Jiang, M. *Angew. Chem., Int. Ed.* **2010**, *49*, 4409−4413.
(45) Liao, X.; Chen, G.; Jiang, M. *Langmuir* **2011**, *27*, 12650−12656.
(46) Guo, M.; Jiang, M.; Pispas, S.; Yu, W.; Zhou, C. *Macromolecules* **2008**, *41*, 9744−9749.