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Macroscopic self-assembly through molecular recognition

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Molecular recognition plays an important role in nature, with perhaps the best known example being the complementarity exhibited by pairs of nucleobases in DNA. Studies of self-assembling and self-organizing systems based on molecular recognition are often performed at the molecular level, however, and any macroscopic implications of these processes are usually far removed from the specific molecular interactions. Here, we demonstrate that well-defined molecular-recognition events can be used to direct the assembly of macroscopic objects into larger aggregated structures. Acrylamide-based gels functionalized with either host (cyclodextrin) rings or small hydrocarbon-group guest moieties were synthesized. Pieces of host and guest gels are shown to adhere to one another through the mutual molecular recognition of the cyclodextrins and hydrocarbon groups on their surfaces. By changing the size and shape of the host and guest units, different gels can be selectively assembled and sorted into distinct macroscopic structures that are on the order of millimetres to centimetres in size.

Over the last three decades, a large body of research has been amassed on the topics of molecular recognition[1], supramolecular complexes[2,3] and the self-organization of molecules[4–8]. Recently, much more attention has been directed towards supramolecular polymers[9–12] and materials[13]. Although there have been numerous studies on the self-assembly and self-organization of molecules[14–17] and cells[18,19], there are few that describe macroscopic-scale self-assembly. Self-assembly with macroscopic dimensions has been reported using magnetic interactions[20–22], electrostatic interactions[23,24], hydrophile–lipophile balance[25–28] and capillary effects[29–32]. However, to the best of our knowledge there have been no reports on the self-assembly of macroscopic materials through molecular recognition.

If molecular recognition can be shown to work in a predictable fashion on the macroscopic scale, then macroscopic self-assembly based on molecular recognition should allow a variety of architectures and functions to be realized—and offer new opportunities for materials science[33]. Herein, we demonstrated that macroscopic soft materials, which are on the millimetre or centimetre scale, are differentiated through molecular recognition to give macroscopic association structures. This enables specific molecular recognition events to be visualized on a macroscopic scale. The findings in this study can be applied to instantly connect various soft materials as well as to construct macroscopic architectures using various host and guest combinations, thereby enhancing the concept of supramolecular science as a means to produce practical materials.

Results and discussion

Adhesion of host gels to guest gels. In this study, acrylamide-based gels bearing host (that is, cyclodextrin, CD) or guest moieties were used owing to their relative ease of preparation and the lack of or weak interaction between polyacrylamide and CDs. We selected adamantyl (Ad), *n*-butyl (*n*-Bu) and *t*-butyl (*t*-Bu) groups as the guest moieties (Fig. 1). All the gels were prepared by radical copolymerization under conventional conditions (see Supplementary Information). Additionally, an acrylamide gel bearing neither CD nor a guest moiety (blank gel) was prepared in a similar manner. Most of the gels were stained by dyes for visualization: α-CD-gel (blue), β-CD-gel (red), Ad-gel (light green), *n*-Bu-gel (yellow) and *t*-Bu-gel (dark green).

β-CD-gel was found to bind Ad-gel strongly through molecular recognition. In addition, a mixture of pieces of α-CD-gel, β-CD-gel, *n*-Bu-gel and *t*-Bu-gel exhibited excellent fidelity only by mixing and shaking in water; α-CD-gel specifically adhered to *n*-Bu-gel, and β-CD-gel selectively adhered to *t*-Bu-gel to form macroscopic self-assemblies.

When a piece of β-CD-gel (a host gel) was brought into contact with a piece of Ad-gel (a guest gel) in water, the β-CD-gel adhered firmly to the Ad-gel to form a combined gel (Fig. 2a). When pieces of β-CD-gel and Ad-gel were mixed and shaken in water, β-CD-gel and Ad-gel stuck to each other to form an aggregate (Fig. 2b, Supplementary Movie S2). Closer examination of the aggregate revealed that pieces of β-CD-gel are only in contact with Ad-gel pieces and vice versa (Fig. 2b). In contrast, pairs of β-CD-gel/β-CD-gel or Ad-gel/Ad-gel did not stick together. Moreover in control experiments, pieces of blank gel did not stick together or form aggregates with pieces of β-CD-gel or Ad-gel. These observations indicate that molecular recognition plays an important role not only on the molecular level, but also on the macroscopic level.

The interaction between β-CD-gel and Ad-gel was so strong that it was difficult to separate them from the gel assembly (Fig. 2c). Although the gel assembly did not dissociate at 80 °C, it did above 90 °C, indicative of reversible binding. When the gel assembly was pulled from both sides, one of the gel pieces broke without damaging the contact interfaces. It is noteworthy that the other host gel, α-CD-gel, adhered more weakly to Ad-gel than did β-CD-gel, consistent with the apparent association constants (K_a) estimated using homogeneous aqueous solutions of soluble guest

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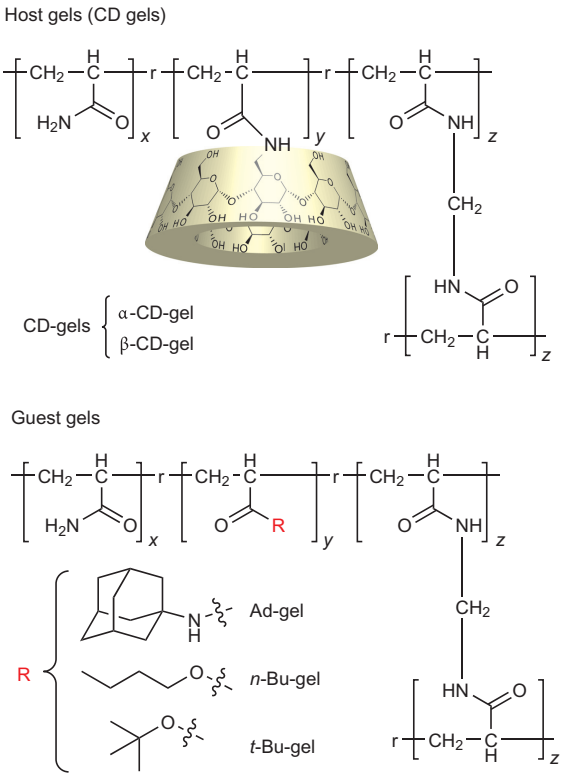

Figure 1 | Chemical structures of host and guest gels. Host gels: α-CD-gel and β-CD-gel; guest gels: adamantyl gel (Ad-gel), *n*-butyl-gel (*n*-Bu-gel) and *t*-butyl-gel (*t*-Bu-gel). α- and β-CDs are cyclic oligosaccharides consisting of six or seven glucopyranose units, respectively, attached by α-1,4-linkages. The character 'r' in the main chain of the polymers indicates that each monomer was copolymerized randomly. The molar ratio of acrylamide, host- or guest-modified acrylamide, and *N,N'*-methylenebis(acrylamide) is shown as *x*, *y* and *z*. In this study, host and guest gels with a molar ratio *x*/*y*/*z* of 0.948/0.047/0.005 were used.

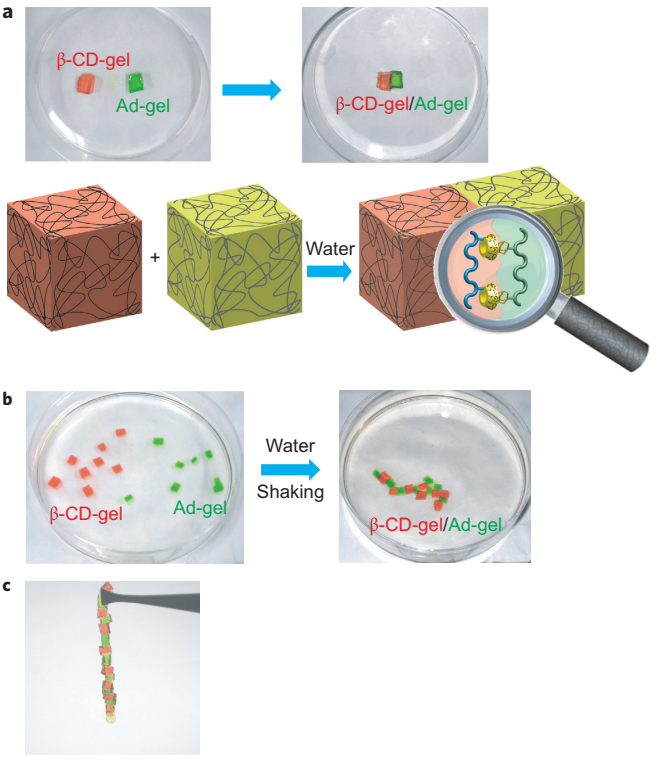

Figure 2 | Macroscopic self-assembly between CD host gels and guest gels. a, A β-CD-gel (red gel) was brought in contact with an Ad-gel (light green gel), as shown in Supplementary Movie S1. A schematic of the molecular recognition responsible for the two gels sticking together is shown below. **b**, Pieces of β-CD-gel (red) and Ad-gel (light green) were mixed and shaken in water, as shown in Supplementary Movie S2, resulting in a self-assembled structure. **c**, β-CD-gel adheres firmly to Ad-gel, and alternating self-assembled structures were formed in accordance with the high complementarity of the host and guest gels.

polymers (without crosslinking) and CDs (Table 1). The estimated K_a values were 9.8×10^1 and 1.5×10^3 M^{-1} for pairs of Ad/α-CD and Ad/β-CD, respectively.

Competitive experiments confirmed self-assembly based on molecular recognition. Ad-gel did not form a gel assembly with β-CD-gel in aqueous solutions containing excess β-CD, because the added β-CD masked the Ad groups in the gel. When pieces of β-CD-gel and Ad-gel were mixed and shaken in aqueous solutions of 1-adamantanamine hydrochloride (AdA, 0.6 w/v%), β-CD-gel adhered weakly to Ad-gel. Table 1 shows the strain and stress values when the combined gels separated from one another or broke at one of the component gels (measured by a creepmeter). A smaller stress or strain value was obtained in the assembly between β-CD-gel and Ad-gel prepared in the presence of 0.6% AdA than that without AdA. The addition of 1.2% of AdA to the gels as a soluble competitive guest caused no adhesion of gels. These results indicate that the addition of a soluble competitive host or guest inhibits the association of gels by blocking all of the available binding sites on the guest gel or host gel, respectively.

Selective assembly of gels. Figure 3a shows the results following mixing and shaking pieces of α-CD-gel, *n*-Bu-gel and *t*-Bu-gel in water. Only α-CD-gel and *n*-Bu-gel resulted in the formation of a

Table 1 | Properties of macroscopic self-assemblies between host and guest gels.

Host gel	Guest gel	Assembly*	Stress (Pa)†	Strain (%)†	K_a (M^{-1})‡
α-CD-gel	Ad-gel	A	900±150	75±10	98
α-CD-gel	*n*-Bu-gel	A	250±50	18±3	57
α-CD-gel	*t*-Bu-gel	N	—	—	~15
β-CD-gel	Ad-gel	sA	1,050±150§	87±2	1,500
β-CD-gel	Ad-gel + AdA‖	A	520±30	30±15	—
β-CD-gel	*n*-Bu-gel	N	—	—	<10
β-CD-gel	*t*-Bu-gel	A	800±150	22±4	170

*Interactions between host gels and guest gels; strongly aggregated (sA) difficult to separate, aggregated (A), no interactions (N) were observed in water or in air. Host/guest interactions were tested at least three times. †Physical properties of host gels and guest gels obtained by materials tension testing machines. The stress and strain were measured twice in each system. The data are shown as averages with standard deviations. ‡Association constants (K_a) were determined by Benesi–Hildebrand plots of the chemical shifts of the peaks in the ^{1}H NMR spectra of soluble guest polymers with CDs. §The part of Ad-gel in β-CD-gel/Ad-gel was broken without damaging the contact interface during the materials tension testing. ‖AdA, 1-adamantanamine hydrochloride. An assembled gel was obtained by mixing and shaking the pieces of β-CD-gel and Ad-gel in aqueous solutions of AdA (0.6 w/v%).

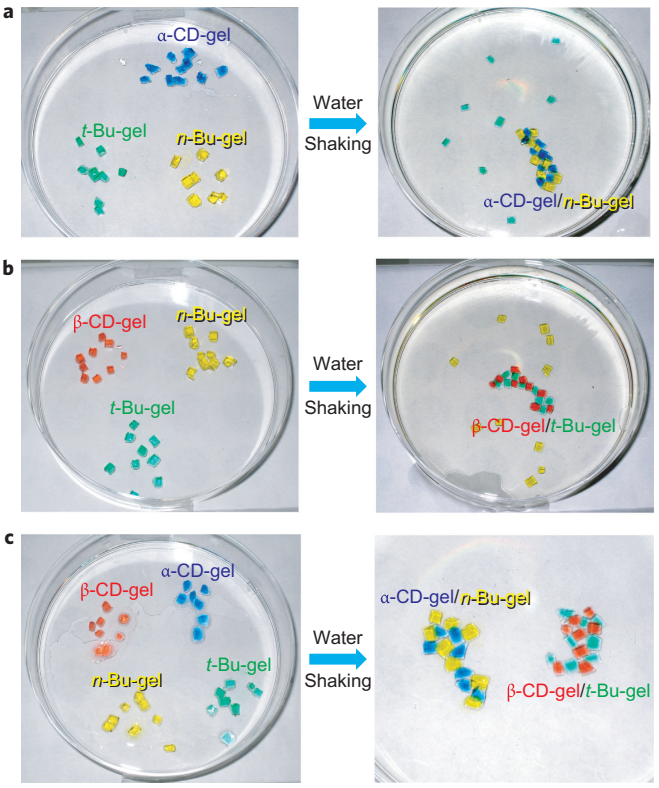

Figure 3 | Visualization of specific molecular recognition events on a macroscopic scale. a, α-CD-gel (blue) and guest gels (*n*-Bu-gel, yellow; *t*-Bu-gel, dark green) were placed in a petri dish. Adding 5 ml of water and shaking for a few minutes led to the selective formation of an alternating self-assembly of α-CD-gel (blue) and *n*-Bu-gel (yellow), as shown in Supplementary Movie S3. **b**, β-CD-gel (red) and guest gel (*n*-Bu-gel, yellow; *t*-Bu-gel, dark green) were placed in a petri dish. Adding 5 ml of water and shaking led to the selective formation of an alternating self-assembly between β-CD-gel (red) and *t*-Bu-gel (dark green), as shown in Supplementary Movie S4. **c**, Host gels (α-CD-gel, blue; β-CD-gel, red) and guest gels (*n*-Bu-gel, yellow; *t*-Bu-gel, dark green) were placed in a petri dish. Adding water and shaking for a few minutes led to the selective formation of alternating self-assemblies of α-CD-gel/*n*-Bu-gel and β-CD-gel/*t*-Bu-gel, as shown in Supplementary Movie S5.

gel assembly. In contrast, a mixture of pieces of β-CD-gel, *n*-Bu-gel and *t*-Bu-gel formed a gel assembly of β-CD-gel with *t*-Bu-gel (Fig. 3b). These results indicate that molecular recognition through host–guest interactions works on the macroscopic scale, which is consistent with the apparent K_a values estimated using aqueous solutions of soluble guest polymers and CDs (Table 1)[34]. A ^{1}H NMR study on the interactions between soluble guest polymers with CDs[35] showed that the *n*-Bu group was included in α-CD with a K_a of 5.7×10^1 M^{-1}, whereas the addition of β-CD did not shift the guest signals. In contrast, the *t*-Bu group was included in β-CD with a K_a of 1.7×10^2 M^{-1}, but the addition of α-CD showed little effect. These results confirm that the binding properties of CDs to the guest polymers in homogeneous solutions are reflected as the selective adhesion of the host gels to the guest gels.

When pieces of α-CD-gel, β-CD-gel, *n*-Bu-gel and *t*-Bu-gel were simultaneously mixed in water and shaken, only pieces of α-CD-gel stuck to pieces of *n*-Bu-gel, and only pieces of β-CD-gel stuck to pieces of *t*-Bu-gel, to give alternating or chequered structures (Fig. 3c). These results clearly indicate that a host gel recognizes the corresponding guest gel through molecular recognition to form macroscopic structures.

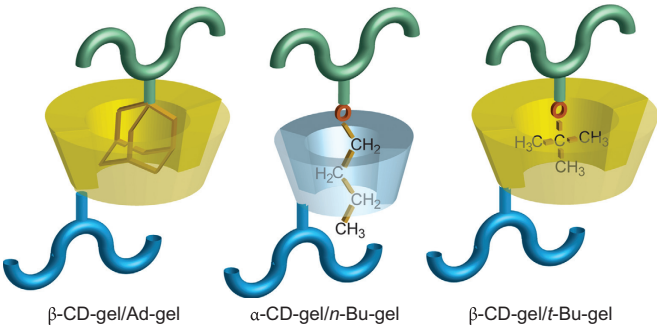

Figure 4 | Proposed structures of the complexes formed between CDs and guests attached to a gel. β-CD forms a complex with Ad with a high binding affinity. When the assembly of β-CD-gel with Ad-gel was pulled from both sides, one of the gel pieces broke without damaging the contact interfaces, indicating that the Ad group on the contact interface of the gels presumably forms an inclusion complex with β-CD in the β-CD-gel, in a similar fashion to the formation of complexes between soluble Ad-functionalized polymers with β-CD in homogeneous aqueous solutions. The linear shape of the *n*-Bu group is thought to fit well into the cavity of α-CD, and the bulky *t*-Bu group can be incorporated into the larger cavity of β-CD.

α-CD-gel strongly adhered to *n*-Bu-gel, but not to *t*-Bu-gel. In contrast, β-CD-gel strongly bonded to *t*-Bu-gel, but not to *n*-Bu-gel. As shown in Table 1, the strain and stress values are consistent with the association constants of the complexes of soluble guest polymers with CDs, except for the combined gel comprising β-CD-gel and Ad-gel, where the Ad-gel broke without damaging the interface between the gels. As for the β-CD-gel/Ad-gel system, the addition of aqueous solutions of soluble competitive guests such as *n*-butanol and *t*-butanol (2.0 v/v%) to the α-CD-gel/*n*-Bu-gel or β-CD-gel/*t*-Bu-gel system, respectively, caused no adhesion of gels. Figure 4 schematically represents the interactions between CD-gels and guest gels. The slim *n*-Bu group fits well in the α-CD cavity, and the bulky *t*-Bu group fits well in the larger β-CD cavity. Because the host gels are able to differentiate such a small difference in the guest parts, this system can be applied to differentiate macroscopic materials through molecular recognition of various guest parts.

Conclusions

We successfully constructed supramolecular architectures on a macroscopic scale. Selective molecular recognition of CDs to guest molecules led to the unique association of hydrogels. Host–guest interactions were visualized by means of the self-assembly of objects, which were on the scale of millimetres to centimetres. We expect this system to be applicable to numerous other types of gels and soft materials with components that have host–guest relationships. For example, biological macromolecules such as antibodies and avidin could be the host components in gels, and the corresponding antigens and biotin the guests, due to their specific molecular recognition ability with high affinity. Macroscopic-scale recognition by means of molecular recognition has potential use in self-assembly in the macroscopic-scale construction of new architectures by selective and reversible binding properties, and holds promise in the development of new medical applications.

Methods

A soluble host polymer containing β-CD was prepared by copolymerization of acrylamide (98 mol%) and 6-acrylamido-β-CD (2 mol%) using radical polymerization initiated by a redox pair of ammonium peroxodisulfate (APS) and *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) in water. Viscometry using formamide as a solvent estimated the molecular weight as 6.0×10^5. β-CD-gel was prepared by polymerization of acrylamide (94.8 mol%), 6-acrylamido-β-CD (4.7 mol%) and *N,N′*-methylenebis(acrylamide) (0.5 mol%) using APS and TMEDA

in water. α-CD-gel was prepared in a similar manner. Ad-gel was prepared by copolymerization of acrylamide, *N*-1-Ad-acrylamide, and *N,N′*-methylenebis (acrylamide) using 2,2′-azobis(isobutyronitrile) (AIBN) in DMSO at 60 °C. The gels were purified by exposure to water for several hours and then repeated washings with water, and were then cut into millimetre- to centimetre-sized pieces using a knife. Pieces of α-CD-gel, β-CD-gel, *n*-Bu-gel and *t*-Bu-gel were stained by soaking these gels in solutions of blue, red, yellow and green dyes, respectively. Ad-gel (light green) was also coloured in the same way. Each gel was placed in a petri dish, followed by the addition of 5 ml of water, then shaken for a few minutes. The rupture stress and strain of gels were measured by a creepmeter (Rheoner, RE-33005, Yamaden Ltd).

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Author contributions

A. Harada conceived the project and designed the experiments. R.K. contributed to the synthesis of the host and guest gels. A. Harada, Y.T., A. Hashidzume and H.Y. analysed the data and co-wrote the paper. All authors discussed the results and commented on the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/. Correspondence and requests for materials should be addressed to A. Harada.