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## COMMUNICATION

# Visualized Discrimination of ATP from ADP and AMP through Collapse of Supramolecular Gels

**Dong Yang, Changxia Liu, Li Zhang[*] and Minghua Liu[*]**

**A supramolecular gel was fabricated through mixing of a cationic gelator with methyl orange.  The addition of  ATP into the gel caused distinct gel-collapse, while ADP and AMP preserved the gel formation, which provided a simple visualized way to discriminate ATP from AMP and ADP.**

Supramolecular gel, in which solvents are immobilized by three dimensional entanglement networks self-assembled from low-molecular-weight gelators via non-covalent interactions, has obtained great interest.[1] While the design of the gelator and the gel formation is still interesting, the paradigm shifted to their functions.  With the advantage of the stimuli responsiveness and reversibility, various functional gels have been developed.[2] One of the important properties of the gel is its gel-sol transition with the heating or interacting with the specific chemical species.  Thus, the gels could be used to recognize or detect various species ranging from chiral molecules,[3] biomolecules[4] and anion[5] through such transition.  Besides, the color changes during the gels react with other species could also be used as the sensor.  A typical example is the visualized sensing of the fluoride anion using the gel systems.[5a]  On the other hand, although supramolecular gels have been extensively used, its application to detect the biological species is still fewer.[6]  In this communication, taking advantage of gel-sol transition, we proposed a supramolecular gel for the discrimination of the ATP from ADP and AMP.

Adenosine-phosphate (ATP, ADP and AMP) plays a key role in energy transduction and several major metabolic processes.[7]  Since ATP is made from ADP, and it converts back into ADP and phosphate(Pi) in metabolic process, it is very important to distinguish ATP from ADP and AMP.[8]  While a great deal of efforts has been devoted to the design and the development of the sensors for detecting ATP, most of these are based on the fluorescence changes, there is no report on the detection of ATP based on the supramolecular gels.

The designed gelator molecule is shown in Figure 1, which contains pyridinium covalently linked to amphiphilic glutamide (abbreviated as PUDG).  Although PUDG is not a good gelator in pure organic solvents or water, when a slight amount of water was added into organic solvents, PUDG could form good gels, similar to our previously reported PULG, which has the same structure but with the opposite chirality.[9] When more water was added, generally more than 30%, it failed to form gel again.  However, when an anionic dye, MO, was added into this mixed solvents with higher water content (more water in necessity to detect the water-soluble species), a binary yellow organogels could be easily formed.  This gel showed a visualized discrimination ability of ATP from ADP and AMP.
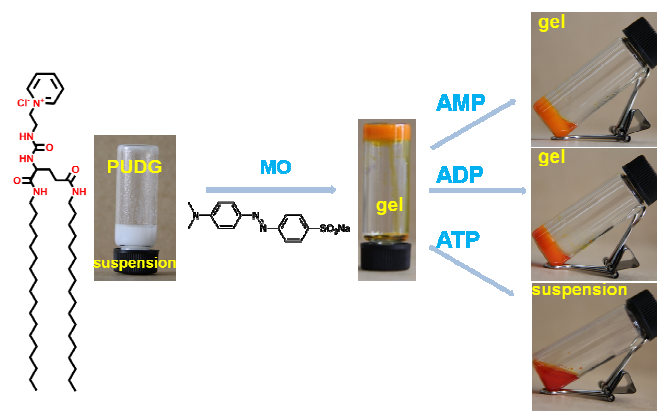


**Fig. 1** The molecular structure of PUDG and MO; the photo images of PUDG, PUDG/MO=1/1, PUDG/MO/AMP=1/1/0.5, PUDG/MO/ADP=1/1/0.5, PUDG/MO/ATP=1/1/0.5 in the mixture of ethanol and water. $C_2H_5OH/H_2O$=6/4 (V %), the concentrations of PUDG and MO are constant at $1.25\times10^{-2}$M respectively; the AMP (ADP or ATP) concentration is $6.25\times10^{-3}$M.

Experimentally, PUDG and MO in molar ratio of 1:1 were mixed in ethanol/water (6/4 in v/v) and heated to a transparent solution.  Upon cooling to room temperature, a yellowish gel was formed, as shown in Figure 1.  It should be noted that without the addition of MO, individual PUDG could not form a gel but dispersion in this condition.  In order to clarify the gel formation, the xerogels were fabricated on silicon wafer and their SEM was observed, as shown in Figure 2.  The PUDG self-assembled into nanobelt structures in the dispersion, while the nanotwists with the right handed sense presented in the xerogels of PUDG/MO (Fig 2B). This result showed that the

chirality can be transferred from chiral center of glutamide to self-assembled nanostructure. In addition, the gel structures were uniform in the PUDG/MO gels, indicating that PUDG and MO formed a complex in the gel and self-assembled together. Thus, it can be suggested that the electrostatic interactions between cationic PUDG and anionic MO together with the π−π stacking of MO conjugated system are supposed to be the driving force for the binary gel formation.
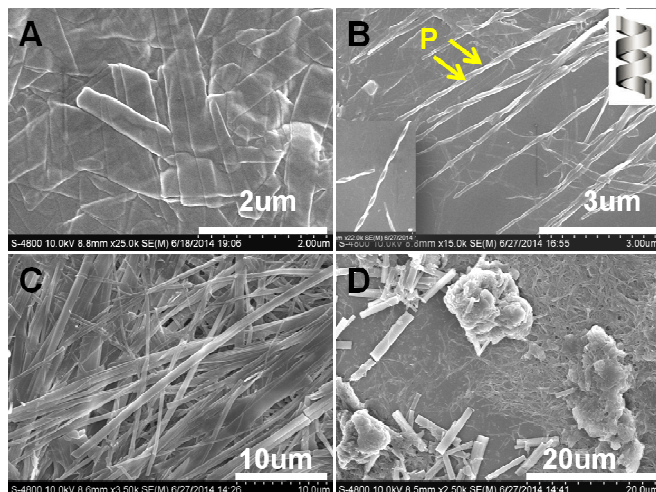


**Fig. 2** the SEM images of PUDG (A) and PUDG/MO=1/1 (molar ratio) (B) PUDG/MO/AMP=1/1/0.5(C), PUDG/MO/ATP=1/1/0.5 (D). In all experiments, the solvent is $C_2H_5OH/H_2O=6/4$ (V％), the concentrations of PUDG and MO are constant at $1.25 \times 10^{-2}$ M respectively.

Taken into account of electrostatic interaction between the PUDG and MO is the one of the driving force for gelation, the addition of anionic specie may influence the supramolecular gel state. Thus, various phosphate anions with 0.5 equivalent to MO were introduced into the PUDG/MO supramolecular gels. The different responses of the gel of PUDG/MO toward ATP, ADP and AMP were observed, as shown in Figure 1. When AMP or ADP was introduced into PUDG/MO system, the gel remained stable, only the color changed a little from yellow to saffron yellow. Remarkably, when ATP was added into the gel system, the gel turned into sol immediately with the gel-collapse. We have further observed the structural changes during such changes using the SEM. It was found that the architectures of PUDG/MO/AMP (Fig 2C) and PUDG/MO/ADP (Fig S2) changed from nanotwist to nanofiber structures. However, the system is still uniform without production of new structure. Obvious phase separation occurred in the complex of PUDG/MO/ATP, in which the nanorods, nanofibers and other aggregates coexisted (Figure 2D). These results confirmed the discrimination ability of the gel response toward the ATP.

In order to further clarify such different gel changes response to the ATP, ADP and AMP, the UV-Vis and CD spectra of the systems have been measured since the system contained a chiral gelator. Figure 3 shows the UV-Vis and CD spectra of PUDG/MO gels upon addition ATP, ADP and AMP.
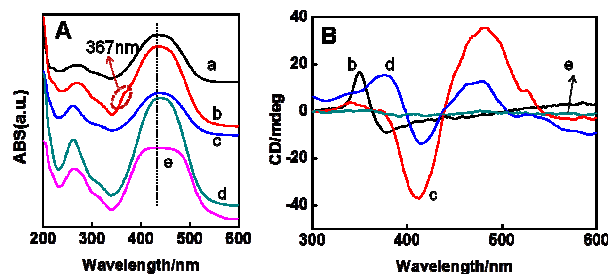


**Fig. 3** the UV (A) and CD (B) spectrum of a) MO solution b) PUDG/MO=1/1 gels, c) PUDG/MO/AMP=1/1/0.5 gels, d) PUDG/MO/ADP=1/1/0.5 gels, e) PUDG/MO/ATP=1/1/0.5 suspension. The solvent is $C_2H_5OH/H_2O=6/4$ (V%)

MO in ethanol/water solution shows an absorption band at 273 nm and 435nm, which is ascribed to the absorption of benzene ring in MO molecule and π-π* absorption of MO monomer.[10] After forming the co-gels with PUDG, except the peak at 435nm, a new weak adsorption band is observed at 367nm, which shows blue-shift comparing with 435nm, indicating that the MO packed as H-like aggregates in the gels.[11] Upon addition of AMP or ADP, the band at 435nm red-shifts to 442 nm, which in accordance with the gel color changing yellow to saffron yellow. But the complex system of PUDG/MO/ATP shows a very broad peak from 420 nm to 460 nm. All the systems containing phosphate anions, the peak at 367 nm becomes faint. In CD spectra, MO is CD silent due to the achiral molecule nature. When it forms co-gels with PUDG, an obvious bisignal Cotton effect with the crossover at 367nm is observed from CD spectrum, which is consistent with the adsorption band of MO H-like aggregates. This result proves that the chirality of PUDG is effectively transferred to MO H-like assemblies. Upon addition of ADP and AMP, the CD spectra showed remarkable change. As for the system containing AMP and ADP, the strong positive bisignal CD signal is obtained at around 440 nm, which is accordance with the monomer state of MO. This indicated that although the aggregation of MO was destroyed by the addition of ADP and AMP, the MO is still attached to the PUDG nanostructures and showed the CD signals. However, the PUDG/MO/ATP system becomes CD silent, indicating that the interaction between PUDG and MO became destroyed.

Combined the data obtained above, the discrimination mechanism can be proposed, as illustrated in Scheme 1. First, MO formed H-like aggregates as side-by-side packing in the co-gels with PUDG, as illustrated in Scheme 1. With such interaction, the chirality of PUDG was transferred to the MO H-like aggregates via electrostatic interactions and π−π stacking between PUDG and MO. Secondly, with the addition of AMP or ADP, the phosphate anions competed with MO to interact with PUDG. With such competition, the H-like aggregate of MO was destroyed and the strong CD siganls shifted to MO monomer. However, most of MO still kept interacting with PUDG, which led to the preservation of gel state as well as strong CD of the monomer. When the anion volume was increased to ATP, the ATP inserted to the complex of PUDG/MO and replaced the MO from PUDG assemblies (Scheme 1), thus destroyed the gel. Due to the collapse of the gel, MO released into the solution and thus the color changed from yellow to dark red. Since the interaction between PUDG and MO was disturbed by the addition of ATP, the induced CD of MO disappeared.

We have also tested a series of ratios of adenosine-phosphate (ATP, ADP and AMP) to the phase state of PUDG/MO supramolecular gels. In addition, the $Na_2HPO4$, disodium pytophosphate and tripolyphosphate were also used to investigate the effect on gel formation. As shown in Fig S4, different ratios of phosphate varying from 1/1/1.5 to 1/1/0.25 were added into the supramolecular gel of PUDG/MO. The gel could remain stable upon the addition of AMP, ADP, $Na_2HPO_4$, disodium pytophosphate and tripolyphosphate during the ratio of them to MO at 1.5 to 0.25. On the contrary, the introduction of ATP caused the distinct gel collapse, indicating that the gel collapse is an effective way to discriminate ATP from other phosphates. We speculated that phosphates ions had competition with MO to interact with PUDG. The addition of phosphates ions could weaken and even destroy the gel of PUDG/MO. However, the ability of destroying the gel among these phosphates was notably different. If we kept the concentration of phosphate the same as that of in ATP, for example, the concentration of AMP and ADP was at 1.5 and 1.0 equivalents to MO, respectively, the supramolecular gel could still be kept. This result indicated that besides the electrostatic competence, the big size of ATP is also an important factor to show such discrimination.

On the other hand, frequency rheological measurements of the PUDG suspension, PUDG/MO gels and PUDG/MO gels upon addition of adenosine-phosphate were carried out. (Fig S5) In the range of 0.1–100 rad $s^{-1}$, both the value of G'(storage elastic modulus) and G'' (loss elastic modulus) of PUDG itself were much lower than that of PUDG/MO, which was consistent with the state of PUDG suspension and the gel state of PUDG/MO. Upon addition of AMP and ADP, the value of G' and G'' decreased, but still were much higher than that of PUDG/MO/ATP system. The rheological measurements supported that the effect of ATP on PUDG/MO gels was obviously different from that of AMP and ADP.
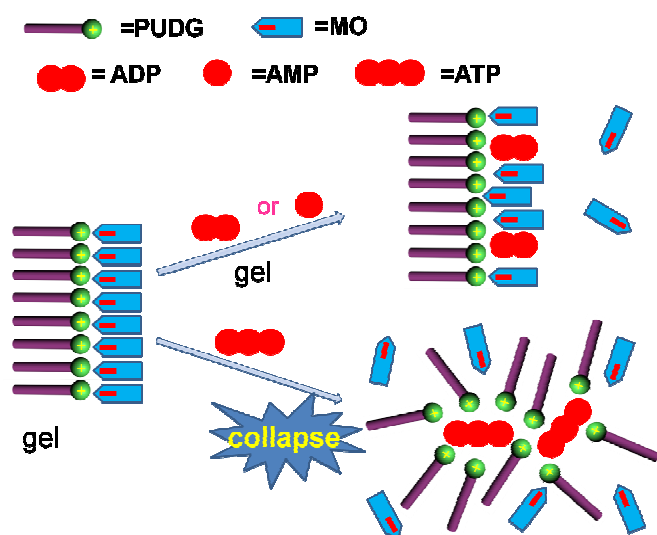


**Scheme 1** Illustration of supramolecular PUDG/MO gel for the discrimination of the ATP from ADP and AMP. PUDG and MO could form gel in $C_2H_5OH/H_2O=6/4$ (V%). When AMP or ADP was introduced into the system, the gel state still preserved. However, introduction of ATP into the gel, the system collapsed because of the large size of anion and strong electrostatic interaction between ATP and PUDG.

In conclusion, a two-component supramolecular gel has been designed by a commercially available anionic dye, MO, and a cationic gelator containing pyridium and amphiphilic glutamide (PUDG). Investigation of the gel response to a series of phosphate ion containing substances led to the development of the sensor to visualizedly discriminate ATP from ADP and AMP. That is, supramolecular gel collapsed toward ATP, while it kept for other phosphate anions, including $Na_2HPO_4$, disodium pytophosphate, tripolyphosphate, AMP and ADP can preserve the gel state. This work demonstrates that the phase transition in the supramolecular gels are potential to be used for sensing of important biological molecules.

**Notes and references**

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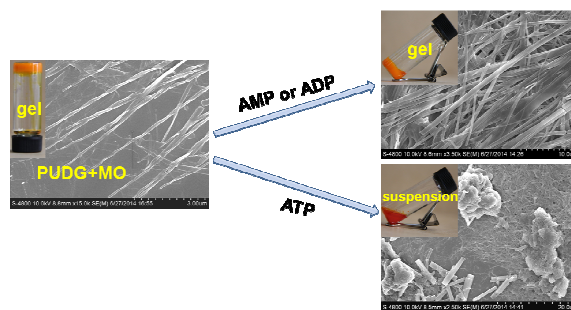


supramolecular PUDG/MO gel used for the visualized discrimination of the ATP from ADP and AMP