Supplementary information

An aptamer-patterned hydrogel for the controlled capture and release of protein via biorthogonal click chemistry and DNA hybridization

Zheng Zhang[1], Jie Du[1,3], Yuli Li[2], Jincai Wu[3], Feng Yu*[1,3], Yong Chen*[1,3]

1. State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Haikou 570228, PR China.
2. Institution of Plastic Surgery, Weifang Medical University, Weifang 261042, PR China.
3. Hainan Provincial Key Laboratory of Research on Utilization of Si–Zr–Ti Resources, Hainan University, Haikou 570228, PR China.

1. Experiments and characterization

1.1 The gelation time of PEG hydrogel

The gelation time of the hydrogels was determined using a test tube inverting method reported by Jeong et al[1]. Freshly prepared 4-arm-PEG-Maleimide and 4-arm-PEG-SH with different concentration was mixed together at different temperature for observing the gelation time. When no fluidity was visually observed upon inverting the vial, the gel state was determined. The concentration of mixture solution was varied from 1% to 15% w/v and the reaction temperature was changed from 25 ^{o}C to 67 ^{o}C.

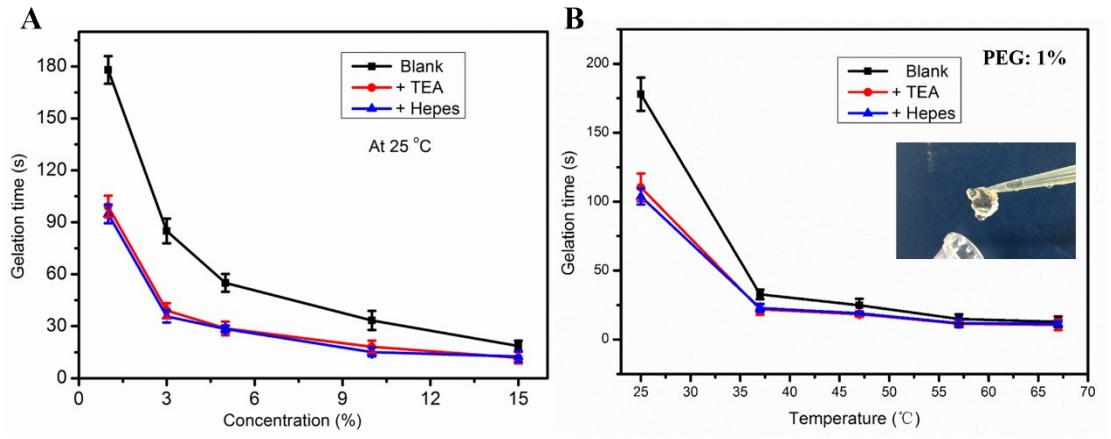


Figure S1. (A) The relationship of gelation time and PEG concentration at 25 ºC. (B) The influence of reaction temperature on gelation time (PEG concentration was constant at 1%). Triethanolamine (TEA) and Hepes were the catalytic agent.

As showed in Figure S1, the gelation time could be controlled by changing the PEG concentration and the temperature or by adding catalytic agent triethanolamine (TEA) or Hepes. Briefly, along with the increasing of PEG concentration from 1% to 15%, the gelation time was rapidly shortened from about 180 to 20 seconds. Similarly, the gelation time changed from 3 minutes to 30 seconds by increasing the temperature to 67 ºC when the PEG concentration was maintained at 1%. We believe that the controllable gelation time can provide convenience for the injectable application in biomedical area.

1.2The mechanical property of PEG hydrogel

The storage modulus and the loss modulus were obtained by changing the frequency from 0.1 to 10 Hz and keeping the amplitude at 25 mm by using a dynamic mechanical analyzer (DMA Q800, USA) in unconfined

compression mode. The hydrogel simples were cut into cylinders (of height about 5 mm and diameter 10 mm) for mechanical property.

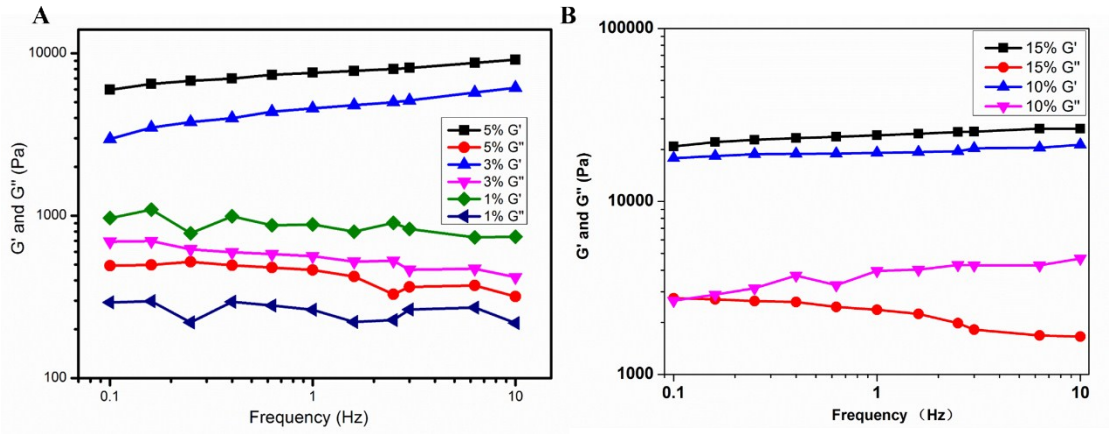


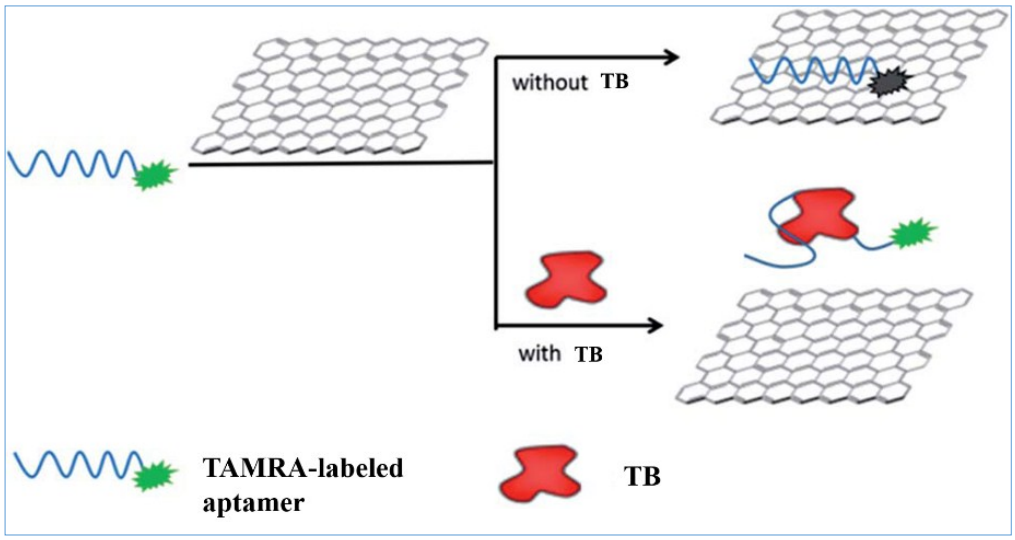
Figure S2. The storage and loss modulus of PEG hydrogel with different PEG concentration (1%, 3%, 5%, 10% and 15%).

As showed in Figure S2, along with the increasing of PEG concentration, the storage modulus of hydrogel was improved from about 1 kPa at the concentration of 1% to 25 kPa at the concentration of 15%. Loss factor which was calculated by G''/G' demonstrated the elasticity of hydrogel. The smaller the loss factor, the more elastic of hydrogel. From the data showed in Figure S2, we proved that 15% PEG hydrogel owned a smallest loss factor at 0.08. Considering the good mechanical property at concentration of 15%, it was chosen as the carrier for the protein capture and release.

1.3Detection method for the concentration of thrombin

Method for detection the changed concentration of thrombin was based on graphene oxide-based fluorescent aptasensor.[2] The mechanism was showed in Scheme 3.

Scheme S1. The detection mechanism of graphene oxide-based fluorescent aptasensor for thrombin protein.



Briefly, 3 ml PBS solution containing 20 nM TAMRA-modified aptamer (5’ -TAMRA-AGTCCGTGGTAGGGCAGGTTGGGGTGACTGGTTGG TGTGGTTGG-3’) and 9μg/ml graphene oxide(GO) was firstly detected by RF-6000 fluorescence spectrometer (Shimadzu Co., Japan). TAMRA-modified aptamer was adsorbed on the surface of GO via π-π stacking interactions. So the fluorescence of TAMRA was efficiently quenched by GO via FRET between GO and TAMRA. Then, thrombin with different concentrations of (2, 4, 6, 8 and 10 nM) were added into above solution successively and incubated for 30 min. Based on the specific binding between aptamer and thrombin, the aptamer chain detached from GO surface. Therefore, the quenched fluorescence was recovered as showed in Scheme 3. Based on this mechanism, the standard curve was obtained by recording absorbance spectra at the wavelength of 508-600 nm with

excitation at 494 nm. By changing the concentration of TB, the fluorescence recovery degree was definitely different as showed in Figure S3. The standard curve showed a good linear relationship between TB concentration (nmol/L) and recovered relative fluorescence value ($\frac{F - F_0}{F_0}$). According to this method, the absorbing and releasing properties of aptamer-functionalized hydrogel could be characterized carefully.

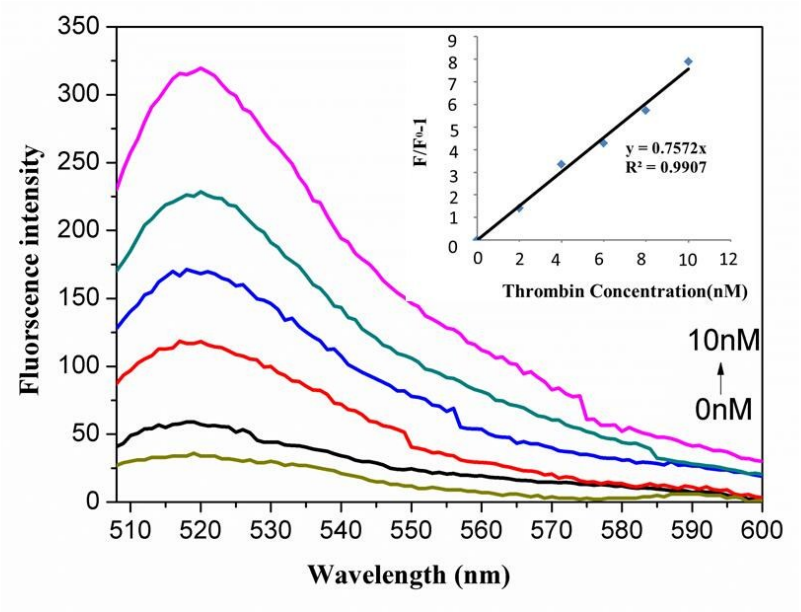


Figure S3. The recovered fluorescence intensity influenced by adding TB with concentrations of (2, 4, 6, 8 and 10 nM) and the linear relationship between TB concentration (nmol/L) and relative fluorescence value($\frac{F - F_0}{F_0}$).

References

1. B. Jeong, Y. H. Bae and S. W. Kim, *Macromolecules*, 1999, **32**, 7064-7069.
2. Y. Zhang, Y. Bai, F. Feng and S. Shuang, *Anal. Methods*, 2016, **8**, 6131-6134.