Electronic Supplementary Information (ESI)

Programmable 3D Rigid Clathrate Hydrogels Based on Self-assemble of Tetrahedral DNA and Linker PCR Products

Xu Chen,[a,b] Yinxia Xie,[b] Yangzi Zhang,[a,b] Chenwei Li,[a,b] Wentao Xu,[a,b,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China.

[b] Key Laboratory of Safety Assessment of Genetically Modified Organism (Food Safety), Ministry of Agriculture, P. R. China, Beijing, 100083, China.

[*] Corresponding author: Wentao Xu; E-mail: xuwentao@cau.edu.cn.
Fax: +8610 62738793; Tel: +8610 62738793

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1. Experimental section

1.1 Materials and methods.

The Salmonella spp. was preserved in 20% (v/v) glycerol solution at -80 °C until it was used. Strain was grown in Luria-Bertani broth medium (NaCl 5 g, yeast extract 5 g, tryptone 10 g and H2O to 1000 mL) at 37 °C for 16 h. This process was followed by the extraction of genomic DNA templates with a Bacteria DNA Kit (TianGen, Beijing, China) according to the method described by TIANamp Bacteria DNA Kit Handbook.[1] The different sequences of oligonucleotides with spacer (Table S1) were designed to construct a TDN-p. The core unit of TDN was assembled using four designed DNA strands. All single-stranded DNAs were purchased from Invitrogen TM (Shanghai, China). The TDN was assembled with single-stranded Ta-p, Tb-p, Tc-p, or Td-p; A certain amount of each single-stranded DNA was added to Tris-MgCl2 solution [10 mM Tris and 20 mM MgCl2(pH 8)]. The TDN-p was assembled at 95 °C for 3 min and cooled rapidly to 4 °C for 5 min in the Life Express Thermal Cycler (Hangzhou BIOER Technology Co., Ltd.). F1-primer and R1-primer including the forward primer and reverse primer of *salmonella* genome, respectively, which partially complementary to TDN-p connection arm. Linker PCR products was assembled consists of two parts. The first part is the amplification reaction of *salmonella* genome. The process including heated at 95 °C for 30s, cooled to 53 °C for 30s and maintained at 72 °C for 45s repeated for 50 times in the Life Express Thermal Cycler (Hangzhou BIOER Technology Co., Ltd.). The second part is the amplification reaction for introducing complementary linker. This process was same with the first part except different template was used. Note that linker PCR products of salmonella genome can be obtained by one round of amplification in real-world applications. All other chemicals were of analytical grade, and all chemicals were used without further purification. All solutions were prepared with Milli-Q water (resistivity = 18 MΩ cm) from a Millipore system.

1.2 UV-vis spectroscopy

UV-vis spectroscopy was used to record the absorbance of each solution before and after the addition of NaCl.[2]

1.3 SEM characterization of TDN-PCR hydrogels.

The TDN-p and TDN-PCR products were quick-frozen in the liquid nitrogen and then fully dried in a vacuum-freeze dryer overnight. The dried samples were Pt-coated with 20 mA for 6 minutes and characterized by using scanning electron microscope at the voltage of 5 kV.

1.4 Agarose gel electrophoresis.

The *Salmonella* genome used in this study and TDN-PCR products were analyzed by 2.0% agarose gel electrophoresis.

1.5 Rheological characterization of TDN-PCR hydrogels.

Rheological tests of TDN-PCR hydrogels proceeded by rheometer with a gap of 0.15 mm, which was consistent previous study.[3] Frequency sweep test was carried out from 0.1 to 10 Hz with a fixed strain of 1 % and a fixed temperature of 25°C. Temperature sweep test was carried out from 25°C to 75°C at a heating rate of 1 °C/min with a fixed strain of 1 % and a fixed frequency of 1 Hz.

References

1. Y. Zhang, S. Li, J. Tian, K Li, Z. Du, W. Xu, Universal linker Polymerase Chain Reaction-triggered Strand Displacement Amplification visual biosensor for ultra-sensitive detection of Salmonella. Talanta, 2021, 222, 121575.
2. Z. Wang, J. Zhang, J. Ekman, P. Kenis, Y. Lu, DNA-Mediated Control of Metal Nanoparticle Shape: One-Pot Synthesis and Cellular Uptake of Highly Stable and Functional Gold Nanoflowers. Nano Lett., 2010, 10, 1886-1891.
3. H. Song, Y. Zhang, P. Cheng, X. Chen, Y. Luo, W. Xu, Rapidly self-assembling soft-brush DNA hydrogel based on RCA Products. Chem. Commun., 2019, 55, 5375-5378.

1.6 SEM-EDS analysis.

Imaging in energy dispersive X-ray spectroscopy (EDS) analysis using an S-4800 Scanning Electron Microscope (Hitachi, Japan) equipped with Energy-Dispersive spectrometer (Oxfordshire, United Kingdom) for elemental analysis (punctual and imaging). The samples were prepared by quick-frozen in the liquid nitrogen and then fully dried in a vacuum-freeze dryer overnight. The dried samples were Pt-coated at vacuum for 6 minutes. Measurements over three times per sample were carried out.

2. Table S1. Experimental DNA Sequences.

Table S1 DNA sequence used in our experiments

Name	Sequence（5'→3'）
Ta-p	ACCTAAGCTCCAGTGGCTTTTTTTACATTCCTAAGTCTGAA**A**CATTACA GCTTGCTACAC**GA**GAAGAGCCGCCATAGTA
Tb-p	ACCTAAGCTCCAGTGGCTTTTTTTTATCACCAGGCAGTTGA**CA**GTGTAG CAAGCTGTAAT**AG**ATGCGAGGGTCCAATAC
Tc-p	ACCTAAGCTCCAGTGGCTTTTTTTTCAACTGCCTGGTGATA**AA**ACGACA CTACGTGGGAA**TC**TACTATGGCGGCTCTTC
Td-p	ACCTAAGCTCCAGTGGCTTTTTTTTTCAGACTTAGGAATGT**GC**TTCCCA CGTAGTGTCGT**TT**GTATTGGACCCTCGCAT
F	TCACC GTGGT CCAGT TTATC
R	CATCA ATACT CATCT GTTTA CCG
F1-primer	AGCC ACTGG AGCTT AGGT TTTTTT**X**TCACC GTGGT CCAGT TTATC
R1-primer	AGCC ACTGG AGCTT AGGTTTTTTT**X**CATCA ATACT CATCT GTTTA CCG
P12	TTAGGTTGGATT
P24	TGGAGCTTAGGTTGGATTCGAGGT
P36	AGCCACTGGAGCTTAGGTTGGATTCGAGGTCACCGA

"X" indicates SpacerC18. F and R represent forward primer and reverse primer of salmonella genome, respectively. Forward primer was same with segmental DNA sequence of salmonella genome, while reverse primer was reverse complement with segmental DNA sequence of salmonella genome. Thus, this method displays excellent selectivity for Salmonella detection and it can be used for detection of virtually any target based on the change of forward primer and reverse primer.

3. Double stranded DNA show lower binding affinity toward the gold nanoparticle surface

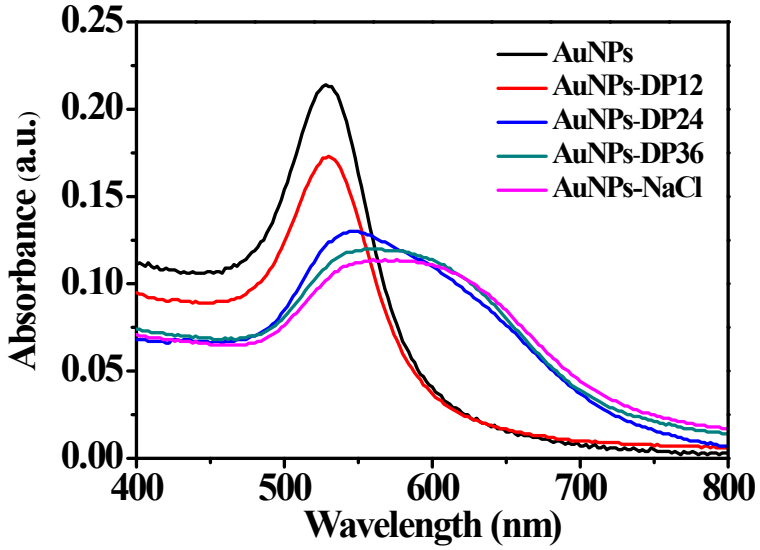

Fig. S1 UV-vis spectra of the gold nanoparticle solutions treated with 0.1 M NaCl after the addition of double-strand P12 (DP12), double-strand P24 (DP24), double-strand P36 (DP36), or without adding DNA.

4. Preparation of rigid double-strand DNA.

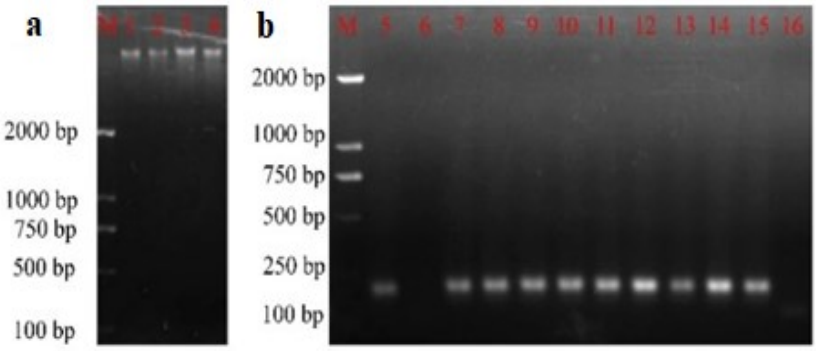

Fig. S2 Extraction of Salmonella genome (1-4: Salmonella DNA) and amplification results of PCR (5, 7-15 linker PCR products; 6 negative control without template; 16 PCR products).

5. Combination of different volume ratios of TDN-p system and linker PCR products.

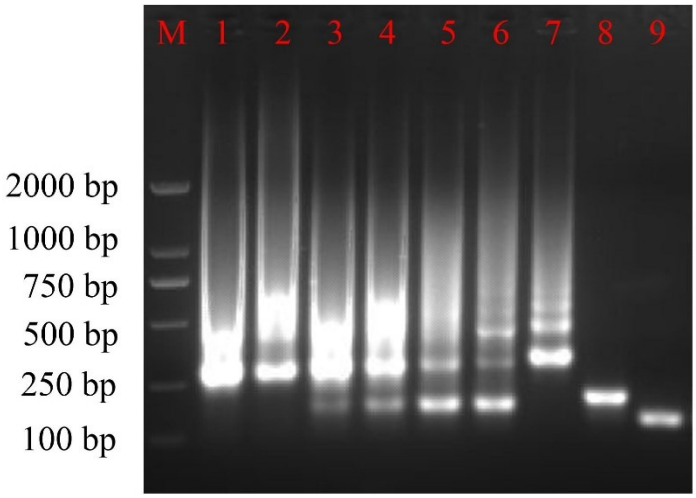

Fig. S3 Lanes 1-6 show TDN constructed with different ratios of linker PCR products (1：1, 1：2, 1：5, 1：10, 1：15, 1：20, v/v), respectively. Lane 7 shows TDN-p. Lanes 8 and 9 show linker PCR products and PCR products (constructed with TDN-p and its terminal complementary strand).

6. SEM imaging and SEM-EDS analysis

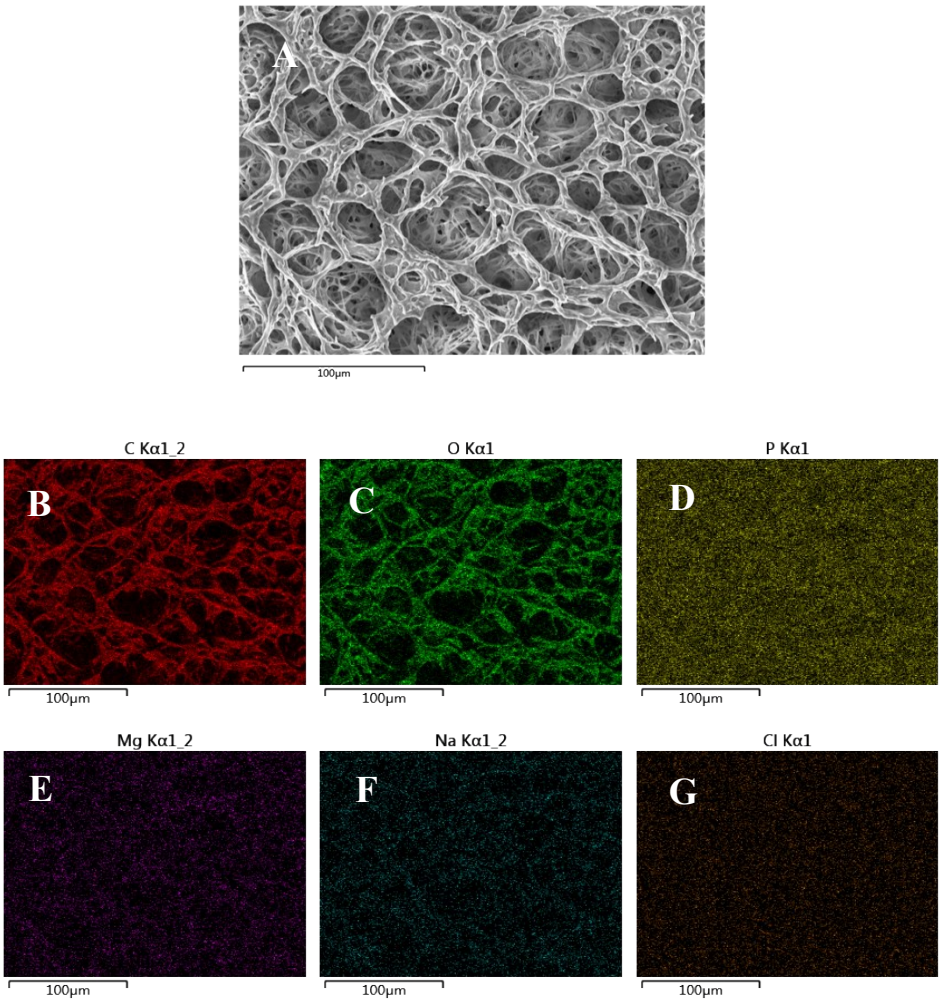

Fig. S4 Characterization of the TDN-p hydrogels by SEM imaging and SEM-EDS analysis. (A) Representative SEM image of TDN-p hydrogels, and (B-G) SEM-EDS elemental maps of C, O, P, Mg, Na and Cl of TDN-p hydrogels.

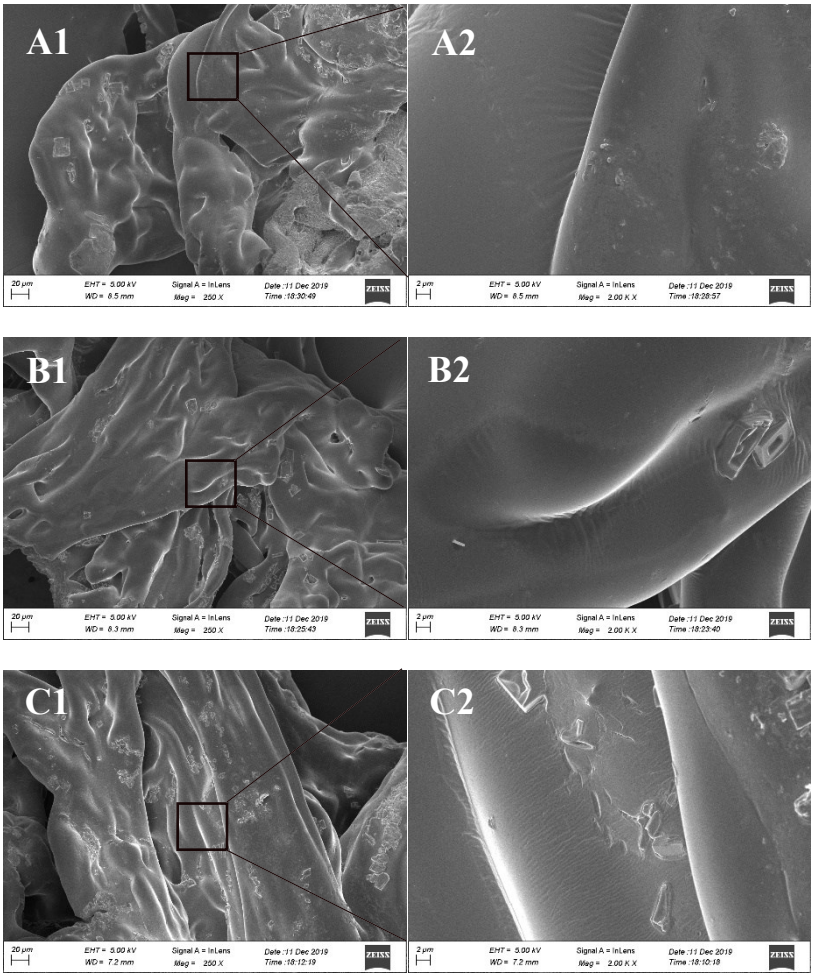

Fig. S5 SEM characterization of soft TDN hydrogels performed by single-strand DNA (A: P12, B: P24, C: P36) and TDN-p.

7. Dynamic rheological characteristics of TDN hydrogel.

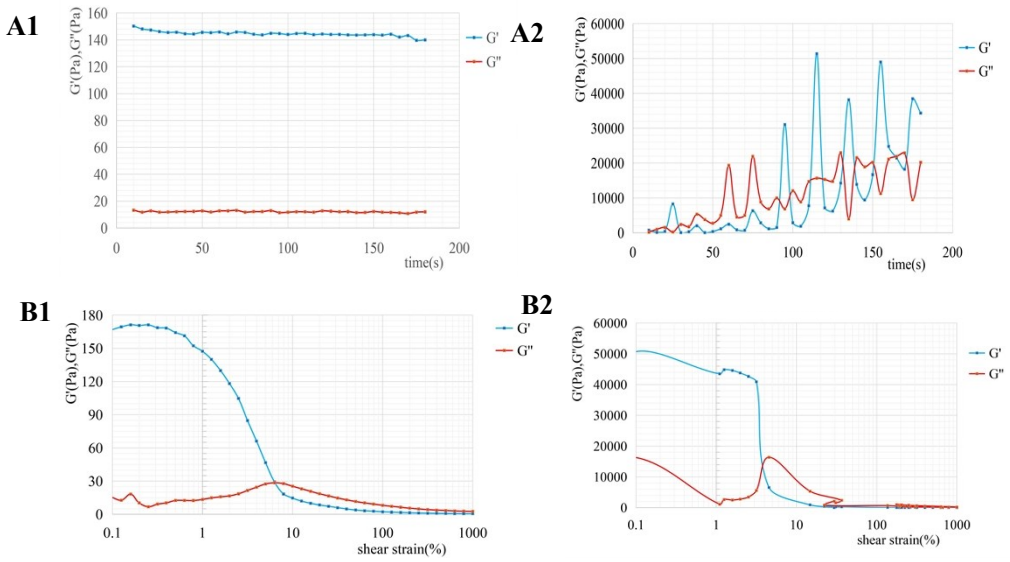

Fig. S6 Dynamic rheological characteristics of TDN hydrogel (A: Time scan test; B: Shear strain test. 1: Soft TDN-PCR hydrogels performed by single-strand DNA; 2: Rigid TDN-PCR hydrogels performed by double-strand DNA).

8. Results of selectivity verification.

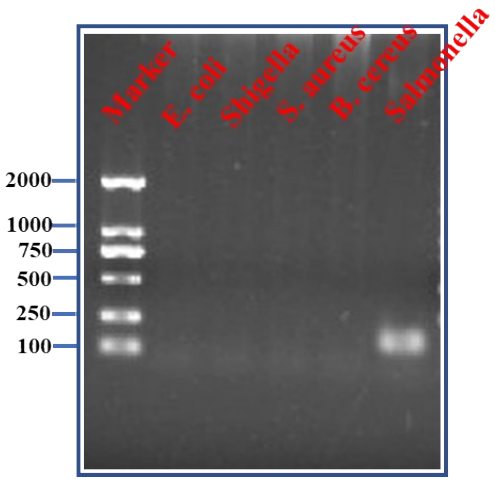

Fig. S7. Results of selectivity verification. F1-primer and R1-primer were used for preparing linker PCR products. E. coli, Shigella, S. aureus, B. cereus and salmonella genome was used as template, respectively.