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Supporting Information

Small molecule-induced DNA hydrogel with encapsulation and release properties

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Materials and Methods.

All oligonucleotides were purchased from Integrated DNA Technologies (IDT), USA with the following sequence information,

a: 5'-AAAAAAAAAAAAAAATGGATCCGCATGACATTCGCCGTAAG-3'

b: 5'-AAAAAAAAAAAAAAACTTACGGCGAATGACCGAATCAGCCT-3'

c: 5'-AAAAAAAAAAAAAAAAGGCTGATTCGGTTCATGCGGATCCA-3'

PolyA (A15), 5'-AAAAAAAAAAAAAAA-3'

Cyanuric acid and all other reagents were purchased from Sigma-Aldrich, St. Louis, MO 63103, USA and used without further purification. 10X Tris/Borate/EDTA (TBE) buffer was purchased from Fisher Scientific (Waltham, MA). 30% Arcylamide/Bis-arcylamide solution (29:1) was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). Alexa Fluor® 594-conjugated Streptavidin and Alexa Fluor® 488-conjugated Streptavidin were purchased from Jackson ImmunoResearch (West Grove, PA) and Molecular Probes (Eugene, OR), respectively.

Preparation of Y-motif. 20 μM stock solutions of Y-motif DNA components (a, b and c) were prepared in distilled water. Equimolar amount (10 μL) of each building strand (a, b and c) were combined and thermally annealed (95°C for 5 min, followed by slow cooling to 25°C) in 1x TAMg buffer (pH 6.8, 40mM Tris, 7.6mM $MgCl_2 \cdot 6H_2O$, 50mM NaCl: working buffer). All samples were incubated at 4°C overnight before gel electrophoresis. Three partially assembled units (duplexes formed with two out of the three building strands: (a+b), (a+c) and b+c) were also prepared under the same thermal annealing condition. The three single strands, three partially assembled units and Y unit were characterized by native PAGE gel electrophoresis (15%wt, Acrylamide:Bis-acrylamine=29:1, 1x TBE buffer).

Preparation of CA-mediated Y-motif and poly(A) DNA hydrogel and its analysis by CD. A mixture containing CA (10 mM), Y unit (50 μM) and poly(A) DNA (250 μM) in 1x TAMg buffer (pH 6.8) was prepared. Briefly, the Y unit and poly(A) DNA were thermally annealed (50 °C for 1 h, followed by slow cooling to 25 °C). The solution was incubated at 4 °C overnight prior to analysis. Digital pictures of each sample in glass vials or clear microtubes were taken to present the solidity of the hydrogel. CD measurements were performed on the experimental and control samples using Jasco J-815 CD spectrometer. As a negative control, the measurements were taken with a mixture without CA in it. Experiments were repeated with 250 μM of polyA, polyC (C_{15}) and

polyT(T_{15}) with and without CA (10 mM), as a positive and negative controls. All CD measurements were obtained at room temperature.

Rheology measurements. Rheology measurements were performed on experimental and control samples using ARES Rheometer. Experiments were performed using Y:A15 [CA (10 mM), Y unit (50 μM) and poly(A) DNA (250 μM)], A_{15} [CA (10 mM) and poly(A) DNA (250 μM)] and Y [CA (10 mM) and Y unit (50 μM)]. Experiments were carried out at five different temperatures at six different temperatures (0, 5, 10, 15, 20 and 25 °C). Both the storage modulus (G') and loss modulus (G'') of each sample were recorded.

Encapsulation and release of different payloads from the hydrogel. Different payloads were added to the 200 μl of hydrogel solution prior to its gelation. The CA-mediated Y unit and poly(A) DNA hydrogel was formed as described above. The payloads included: Two small molecules including a fluorescent molecule TAMRA (N,N'-tetramethylrhodamine), (~60 ng/mL (20 μl of 500 μM stock) and chemotherapeutic drug doxorubicin[1, 2] (~54 ng/mL – 20 μl of 1 mM stock); and two fluorescently labeled proteins Green-Avidin (Alexa Fluor® 488-conjugated Streptavidin: 2 μl of 2 mg/ml stock**)** and Red-Avidin (Alexa Fluor® 594-conjugated Streptavidin: 2 μl of 1.8 mg/ml stock). 20 μl of 13 nm-sized gold nanoparticles (AuNP, 20 μl of ~10 nM), 5 μl of 3.5 μM cy5.5- and 10 μl of 3.0 μM Dox-labeled superparamagnetic iron oxide nanoparticles (MNP-cy5.5, cy5.5/MNP =~5) and (MNP-dox, dox/MNP =~60) were encapsulated.[3, 4]

The payloads were added into the Y unit and poly (A) DNA mixture prior to gelation process with CA. Encapsulation of the materials was confirmed by digital pictures of hydrogels and by fluorescence imaging using ChemDoc Imaging System. For fluorescence measurements the following emission wavelengths were used for each compound TAMRA (λ_{em} 576 nm), Dox (λ_{em} 595 nm), cy5.5 (λ_{em} 720 nm), Red-Avidin (Alexa-594; λ_{em} 594 nm) and Green-Avidin (Alexa-488; λ_{em} 488 nm). Quantitative analysis of fluorescence intensity was performed using Image J software. The synthesis of iron oxide nanoparticles and their chemical conjugation with TAMRA and Dox is described in detail in our previous reports.[3]

To assess release of the encapsulated materials, 1x TAMg buffer (300 μL) was added on top of the hydrogels containing the encapsulated fluorescent payloads. Aliquots of the supernatant buffer (20 μL) were collected at different timepoints (2, 4, 6, 8 and 24 h) and transferred to 384-well plate. Release of the fluorescent materials was then monitored by BioTek Synergy H1 Hybrid Multi-Mode Reader.

Layer-by-layer encapsulation: Two-layered hydrogel was prepared using DNA mixtures containing Red and Green Avidin. First layer was prepared using Red Avidin according to the aforementioned procedure. In a separate microtube, a solution containing CA (10 mM), Y unit (50 μM), poly(A) DNA (250 μM) and Green Avidin in 1x TAMg buffer (pH 6.8) was prepared. The solution was heated to 50 °C for 1 h, followed by slow cooling to 25 °C. The solution was added on top of the previously formed hydrogel containing Red Avidin and cooled to 4 °C overnight. Formation of the two-layered hydrogel was confirmed by fluorescence imaging using ChemDoc Imaging System.

References.

1. C. M. Alexander, J. C. Dabrowiak and M. M. Maye, *Bioconjug. Chem.*, 2012, **23**, 2061.

2. C. M. Alexander, K. L. Hamner, M. M. Maye and J. C. Dabrowiak, *Bioconjug. Chem.*, 2014, **25**, 1261.

3. N. M. Robertson, Y. Yang, I. Khan, V. E. LaMantia, M. Royzen and M. V. Yigit, *Nanoscale*, 2017, **9**, 10020.

4. M. V. Yigit, S. K. Ghosh, M. Kumar, V. Petkova, A. Kavishwar, A. Moore and Z. Medarova, *Oncogene*, 2013, **32**, 1530.

Figures.

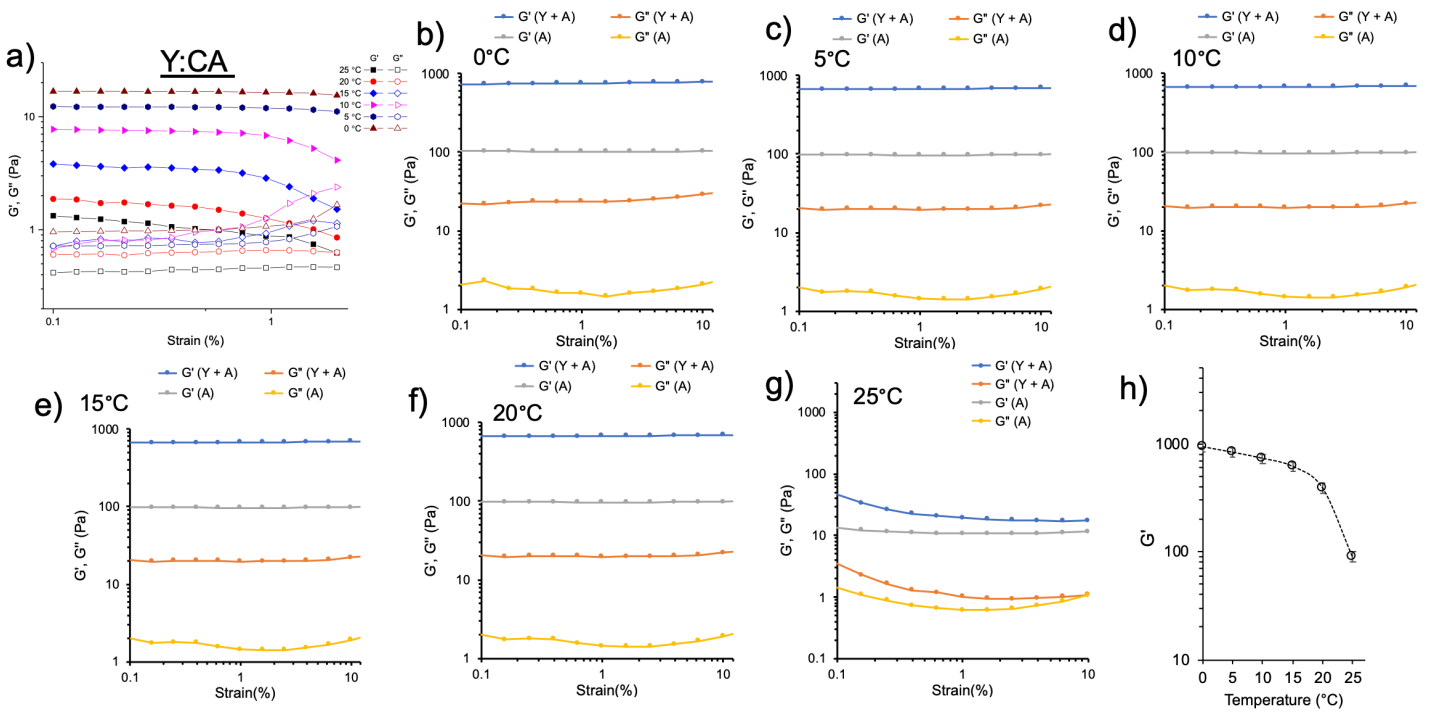

Figure S1. (a) Rheology tests of Y:CA at 15°C showing lowest storage modulus (G') and loss modulus (G'') compared to Y-motif:A_{15}:CA and A_{15}:CA at (b) 0°C, (c) 5°C, (d) 10°C, (e) 15°C (f) 20°C and (g) 25°C. (h) The storage modulus (G') of Y-motif:A_{15}:CA hydrogel at different temperatures.

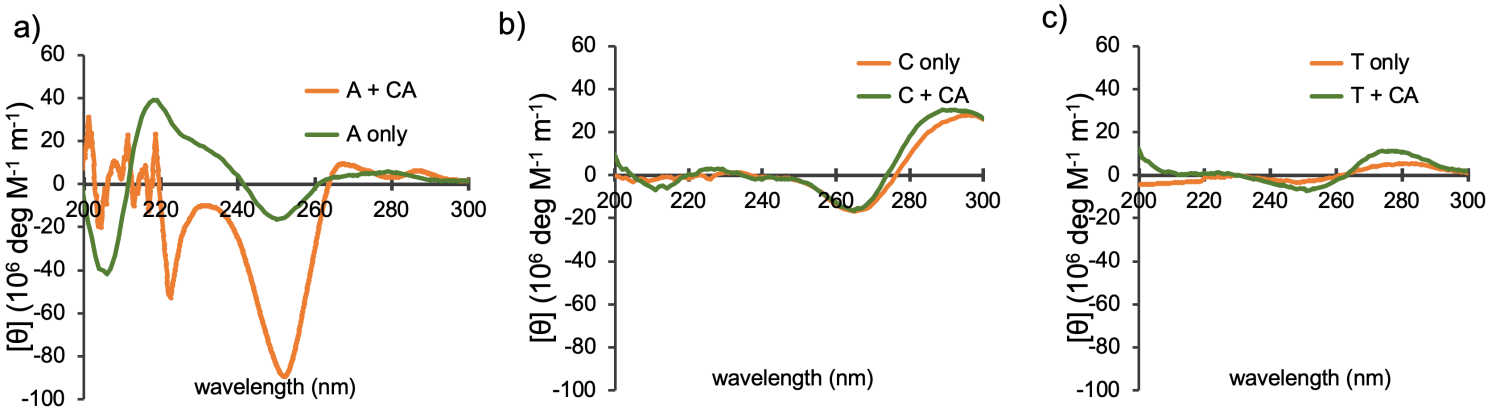

Figure S2. Circular dichroism showing changes in CD spectrum upon addition of CA in (a) A_{15}, (b) C_{15} and (c) T_{15}.