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Small molecule-induced DNA hydrogel with encapsulation and release properties

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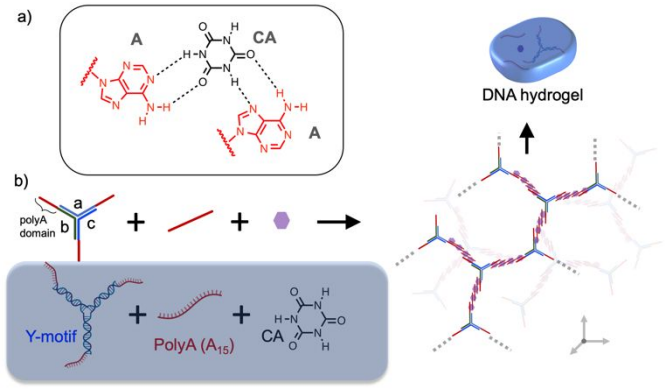
Hydrogels are networks of polymers that can be used for packaging different payload types. They are proven to be versatile materials for various biomedical applications. Implanted hydrogels with encapsulated drugs have been shown to release the therapeutic payloads at disease sites. Hydrogels are usually made through chemical polymerization reactions. Whereas, DNA is a naturally occurring biopolymer which can assemble into highly ordered structures through noncovalent interactions. Here, we have employed a small molecule, cyanuric acid (CA), to assemble polyA-tailed DNA motif into a hydrogel. Encapsulation of a small molecule chemotherapeutic drug, a fluorescent molecule, two proteins and several nanoparticle formulations has been studied. Release of doxorubicin, small fluorescent molecule and fluorescently-labeled antibodies has been demonstrated.

DNA has long been used as a building block or a template for engineering highly ordered biomaterials.[1, 2] Owing to its remarkable programmable nature DNA is no longer considered only as a genetic material.[3-6] The highly predictable/adaptable binding and folding ability of DNA enabled sophisticated designs of two- and three-dimensional soft materials.[7-9] It is fundamentally interesting to observe DNA-templated engineering however it is equally important to construct functional materials using this versatile biopolymer.[10-12]

Watson-Crick base-pairings is the main driving force for hybridization of DNA strands. However, the binding between DNA strands is not limited to A-T and G-C base-pairings. For example, Hg^{2+} could link two thymine nucleobases to form T-Hg^{2+}-T bridges between DNA strands, whereas Ag^{+} could make C-Ag^{+}-C linkages with cytosine nucleobases.[13, 14] Recently, it has been discovered that a small molecule cyanuric acid (CA) can assemble unmodified polyA strands into a non-canonical motif through A:CA binding (**Scheme 1a**).[15] CA has three complementary thymine-like faces **(Scheme 1a)** which promote assembly of unmodified polyA strands into high-density fibrils. Though this discovery is remarkable from the fundamental standpoint further investigations could demonstrate its potential in biomaterials science.[16]



Scheme 1. (a) Small molecule cyanuric acid (CA) has three complementary thymine-like faces which assemble unmodified polyA strands into a non-canonical motif through A:CA binding. (b) A three-armed DNA motif with a "Y" shape is assembled using equimolar DNA strands (a, b, c). The polyA domains in the Y-motif and free polyA strands (A_{15}) assemble with each other in the presence of CA to form a DNA hydrogel.

Here, we have employed polyA:CA interaction to form a DNA hydrogel and illustrated its ability to encapsulate a number of different compounds within its body. We have forced a Y"-shaped DNA motif and polyA (A_{15}) DNA strands into a hydrogel form using CA. Encapsulation of a fluorescent molecule, doxorubicin, fluorescently-labeled (Red and Green) Streptavidin and several nanoparticle formulations has been demonstrated. Release of doxorubicin, a fluorescent compound and Red-Avidin from hydrogel body has been studied over time.

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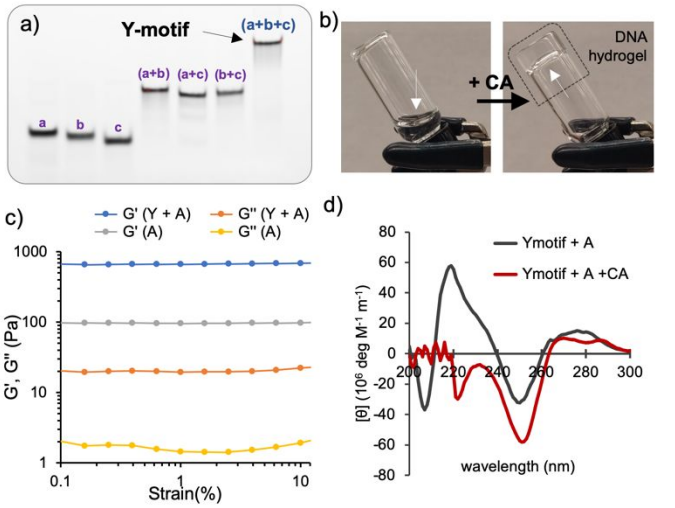

Figure 1. (a) Gel electrophoresis showing the formation of three-armed Y-motif upon assembly of a, b and c DNA strands. (b) Hydrogel formation with Y-motif:A_{15} after addition of CA. (c) Rheology tests at 15°C showing higher storage modulus (G') and loss modulus (G''); stiffer hydrogel product; with Y-motif:A_{15}:CA when compared to A_{15}:CA. (d) Circular dichroism showing changes in CD spectrum upon addition of CA.

In this study, we utilized a three-armed DNA motif with a "Y" shape (Y-motif) as the backbone of the hydrogel. Liu and coworkers have used Y-motif to form a DNA hydrogel through interlocking i-motif domains and encapsulation of gold nanoparticles was demonstrated.[17] Whereas in this study we have demonstrated that a small molecule CA can induce DNA hydrogel assembly without a need for an i-motif domain and can result in a stiff hydrogel with lesser amount of Y-motif.

The Y-motif was assembled using three equimolar DNA strands which are known to spontaneously hybridize and form a three-armed double stranded Y motif, **Scheme 1b**. In order to induce the assembly of the Y-motif with CA, a polyA (A_{15}) domain was included into the 5'-end of each strand (a, b and c), as shown in **Scheme 1b**. The polyA domain sticks out of the motif upon hybridization of the three sequences.

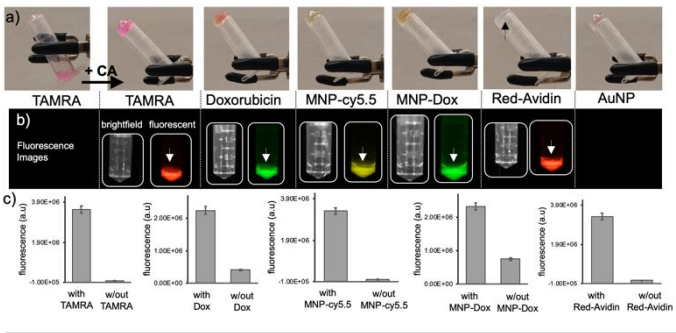

Figure 2. Encapsulation of a number of chemical, biological and nanoscale compounds was monitored by (a) naked eye, (b) fluorescence images and (c) fluorescence measurements.

We hypothesized that a three-armed motif would be sufficient for branching of the DNA network in the presence of CA. Additional free polyA (A_{15}) strands were incorporated into the mixture to support the branching, spacing and crosslinking in the DNA network, **Scheme 1**.

First, the Y-motif was prepared using its three building blocks and characterized using gel electrophoresis. Single bands in Lanes 1, 2 and 3 in **Fig 1a** show each DNA component used for the assembly. The hybridization between DNA components was first demonstrated using combinations of two strands in a 1:1 ratio, (a+b, a+c and b+c). As anticipated, the hybridization products displayed single bands with slower gel mobility **Fig 1a** (Lane 4, 5 and 6). Finally, the Y-motif was formed using equimolar concentrations of all three strands (a+b+c). After testing all possible seven combinations with the three DNA strands the Y-motif was imaged with a single band with the slowest gel mobility, **Fig 1a** (Lane 7).

After confirming the assembly of the Y-motif, we pursued hydrogel formation using all three components (Y-motif, A_{15} and CA). The mixture of 50 μM of Y-motif and 250 μM A_{15} (1:5 ratio) was mixed in a working buffer with 10 mM of CA. This ratio and the DNA concentrations were found to be optimum after several serial dilutions which enabled us to use as little DNA as possible for a stiff product. The solubility of the CA was taken in account when determining its final concentration. The mass ratio relative to water was calculated to be Y-motif: A_{15}: H_2O = 1.6: 1: 866. Nevertheless, the control sample without CA displayed liquid-like properties. However, the experimental DNA sample containing CA showed a stiff hydrogel product which retained at the bottom of glass vial when turned upside down (**Fig 1b**).

We performed a series of control studies to prove that all three components, the Y motif, polyA and CA are necessary for hydrogel formation. We have observed no hydrogel formation with Y-motif:CA mixture which could be due to the steric hindrance from the motif's arms and inability to branch out. We have observed a viscous solution with A_{15}:CA mixture however this mixture was not stiff. Partial gelation with A_{15}:CA is not surprising since CA is known to form long and stable fibrils with polyA.[15] However only the composition of Y:A_{15}:CA mixture resulted in a clear stiff hydrogel product, **Fig 1b**. All of these observations were further confirmed by rheology studies described below.

The stiffness of the hydrogel was evaluated by rheology measurements at different temperatures. The storage modulus (G') and loss modulus (G'') of the Y:A_{15}:CA hydrogel was measured and compared with control samples prepared with A_{15}:CA.[18] The viscous A_{15}:CA mixture showed smaller G' and G'' values than Y:A_{15}:CA, **Fig 1c**. The samples prepared with Y:CA only displayed the least elasticity measured by lowest G' and G'' values, **Fig S1a**. The data overall suggest that Y:A_{15}:CA: hydrogel displays the strongest elasticity among all samples. The studies were repeated at several temperatures ranging from 0 to 25 °C. A sharp decrease in the stiffness was observed beyond 20 °C, Fig S1h. Though the G' and G'' values were lower with higher temperatures, Y:polyA:CA hydrogel still displayed the stiffest

among all, **Fig S1b-f.** The G' and G'' values were measured to be about 800 and 20 Pa, respectively. Compared to a previous report[17] which used 2 mM of Y-motif for the assembly of a DNA hydrogel with comparable G' and G'' values we have used only 50 μM of Y-motif to achieve a similar stiffness.

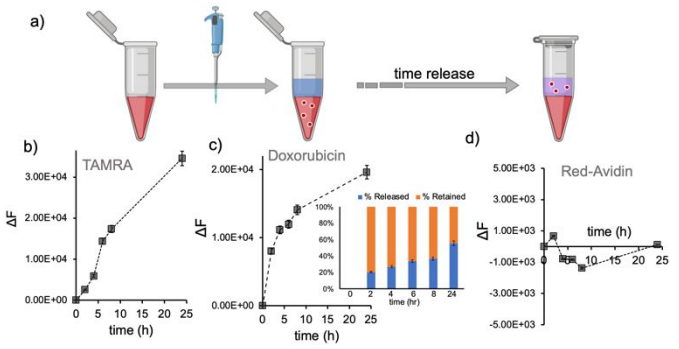

Figure 3. (a) Schematic representation of the release of encapsulated materials to the environment. Working buffer is placed on top of the hydrogel and, the diffusion of encapsulated compounds to the upper layer is studied. Time-dependent release of (b) TAMRA, (c) doxorubucin and (d) Red-Avidin measured by change in fluorescence at the upper layer buffered solution. (c) inset represents the percentage of released and retained doxorubucin.

After confirming the formation of the DNA hydrogel, we evaluated the changes in the CD spectrum after the addition of CA. We first confirmed that CA could induce assembly of A_{15} strands using CD spectroscopy. Addition of CA to A_{15} solution produced negative band at around 220 nm and 250 nm and, a positive band at 270 nm (**Fig S2a**) consistent with previous observations in the literature.[15] Control studies with polyT and PolyC strands produced insignificant changes in the CD spectra, **Fig S2b-c**. Later the CD spectrum of the Y:A_{15} DNA mixture was collected with and without CA. As predicated, similar spectral change (compared to A_{15}) is observed upon addition of the CA, **Fig 1d,** confirming CA-induced DNA assembly**.**

Later, we evaluated the hydrogel's ability to encapsulate different ligands. A diverse set of chemical, biological and nanoscale compounds were prepared as payloads for encapsulation studies, including a fluorescent dye TAMRA (N,N'-tetramethylrhodamine), a chemotherapeutic drug doxorubicin (Dox), gold nanoparticles (AuNP), superparamagnetic iron oxide nanoparticles conjugated with a cy5.5 fluorescent dye (MNP-cy5.5) and doxorubicin (MNP-Dox) and Alexa Fluor-594-conjugated (Red) Streptavidin, **Fig 2a**. All of these compounds were added to six separate mixtures of Y:A_{15} DNA. Before the CA addition the solutions were in a liquid form however after the addition of CA clear hydrogel form was observed. All of these compounds with different features were successfully packaged into the hydrogel's body, **Fig 2a**.

Encapsulation of each labeled compound was observed using fluorescence imaging and recorded by digital pictures. The hydrogels were stiff enough to retain at the bottom of microtubes when turned upside down. Later, the fluorescence image of each hydrogel was captured and quantified, **Fig 2b-c**. All of the encapsulated compounds, with the exception of AuNP, displayed detectable fluorescence which was recorded at their corresponding emission wavelengths.

Figure 4. Layer-by-layer encapsulation of Red- and Green Avidin. The brightfield

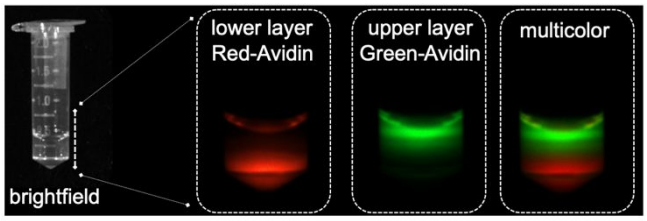

image shows the hydrogel at the bottom of the microtube wherease the fluorecence images show the encapsulation of each compound at different layers.

Later, we evaluated the release of encapsulated model payloads in the buffered environment over time. We hypothesized that the smaller molecules would be released with time whereas the large macromolecules would be trapped in the hydrogel body. The Red-Avidin hydrogel was chosen as a model system with large macromolecular structure and weight. TAMRA-hydrogel was chosen due to the relatively small size of TAMRA while Dox-hydrogel was chosen due to its potential medical relevance as a small chemotherapeutic agent.

Working buffer (300 μL) was added on top of TAMRA-hydrogel, Dox-hydrogel or Red-Avidin hydrogel (**Fig 3a)**. The release of each encapsulated compound was quantified by measuring the change in the fluorescence of the working buffer placed on top of the hydrogel body. A gradual release of small molecules (TAMRA and Dox) was observed over time, **Fig 3b-c**. However, Red-Avidin is a large macromolecule which didn't diffuse out of the hydrogel, **Fig 3d**. We believe that the large macromolecules are trapped in the crosslinked DNA structures which prevent them from diffusing out.

Finally, we have encapsulated two different compounds at different layers of the hydrogel to demonstrate its versatility. It is noteworthy to demonstrate the encapsulation of two or more different compounds at the different compartments/layers of a hydrogel. Such a capability could be useful for layer-by-layer encapsulation of different payloads with sequential release profiles.[19] For instance, studies have demonstrated that layer-by-layer encapsulation of drug molecules and their sequential release is more potent than simultaneous release profiles.[20]

To demonstrate this encapsulation feature, Red-Avidin was encapsulated at the lower layer. After the hydrogel formed a second (DNA) mixture with Green-Avidin (Alexa Fluor-488-conjugated Streptavidin) was placed at the upper layer. Encapsulation each protein and layer-by-layer hydrogel formation were confirmed by fluorescence imaging, **Fig 4**. Multicolored fluorescent images of the hydrogel showed a clear separation between each layer. Because the avidin is unable to diffuse out (**Fig 3d)**, the release of the encapsulated avidin from different layers was not demonstrated.

Conclusions

In conclusion, we have demonstrated that a small organic molecule, cyanuric acid, can induce formation of a hydrogel using polyA-tailed Y-motif DNA and free polyA stands. There have been several different approaches for fabrication of DNA hydrogels using DNA-polymer hybrids,[21, 22] enzymatic digestion/ligation[23] or hybridization.[24] All of these approaches are noteworthy, however, eliminating enzymatic reactions, chemical synthesis or any other biochemical/chemical steps is advantageous for scalability and simplicity standpoint.

The hydrogel was able to encapsulate different kinds of chemical, biological and nanoparticulate compounds in its body demonstrating the universality of the DNA hydrogel for encapsulation purposes. We have studied the release profile of the different encapsulated materials into the environment. The system was versatile enough to encapsulate different compounds at different layers of hydrogels.

The results overall suggest that cooperation between Watson-crick base pairing and a small molecule binding can induce formation of DNA hydrogel with potential applications in biomaterials science. The CA-forced DNA hydrogel reported here requires only 50 μM of Y-motif which is 45-fold less than a previous DNA hydrogel assembled using 2 mM of Y-motif for comparable stiffness.[17] Though there are still some challenges to address for real-world practice[25, 26], i.e., stability under physiological conditions, encapsulation capacity and release rate/degree for biomedical investigations, we believe that it is important to demonstrate the facile construction of functional DNA materials which can later be purposed for biomedical research.

Conflicts of interest

There are no conflicts to declare.

Notes and references

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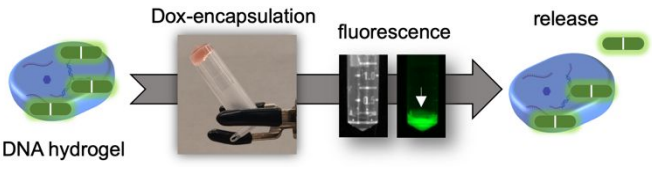
1. N. C. Seeman, *Annu Rev Biophys Biomol Struct*, 1998, **27**, 225.
2. J. H. Chen and N. C. Seeman, *Nature*, 1991, **350**, 631.
3. J. B. Lee, S. Peng, D. Yang, Y. H. Roh, H. Funabashi, N. Park, E. J. Rice, L. Chen, R. Long, M. Wu and D. Luo, *Nat Nanotechnol*, 2012, **7**, 816.
4. Y. Li, Y. D. Tseng, S. Y. Kwon, L. D'Espaux, J. S. Bunch, P. L. McEuen and D. Luo, *Nat Mater*, 2004, **3**, 38.
5. S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach and D. Luo, *Nat Mater*, 2006, **5**, 797.
6. D. Yang, M. R. Hartman, T. L. Derrien, S. Hamada, D. An, K. G. Yancey, R. Cheng, M. Ma and D. Luo, *Acc Chem Res*, 2014, **47**, 1902.
7. J. P. Sadowski, C. R. Calvert, D. Y. Zhang, N. A. Pierce and P. Yin, *ACS Nano*, 2014, **8**, 3251.
8. P. W. Rothemund, *Nature*, 2006, **440**, 297.
9. J. S. Kahn, A. Trifonov, A. Cecconello, W. Guo, C. Fan and I. Willner, *Nano Lett*, 2015, **15**, 7773.
10. E. E. Augspurger, M. Rana and M. V. Yigit, *ACS Sens*, 2018, **3**, 878.
11. I. Gallego, B. Manning, J. D. Prades, M. Mir, J. Samitier and R. Eritja, *Adv Mater*, 2017, **29**, 1603233.
12. R. Schreiber, I. Santiago, A. Ardavan and A. J. Turberfield, *ACS Nano*, 2016, **10**, 7303.
13. M. Rana, M. Balcioglu, N. M. Robertson, M. S. Hizir, S. Yumak and M. V. Yigit, *Chem Sci*, 2017, **8**, 1200.
14. A. Ono, H. Torigoe, Y. Tanaka and I. Okamoto, *Chem Soc Rev*, 2011, **40**, 5855.
15. N. Avakyan, A. A. Greschner, F. Aldaye, C. J. Serpell, V. Toader, A. Petitjean and H. F. Sleiman, *Nat Chem*, 2016, **8**, 368.
16. D. Luo, K. A. Carter and J. F. Lovell, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, 2015, **7**, 169.
17. E. Cheng, Y. Xing, P. Chen, Y. Yang, Y. Sun, D. Zhou, L. Xu, Q. Fan and D. Liu, *Angew Chem Int Ed Engl*, 2009, **48**, 7660.
18. H. Jiang, V. Pan, S. Vivek, E. R. Weeks and Y. Ke, *Chembiochem*, 2016, **17**, 1156.
19. N. M. Robertson, Y. Yang, I. Khan, V. E. LaMantia, M. Royzen and M. V. Yigit, *Nanoscale*, 2017, **9**, 10020.
20. S. W. Morton, M. J. Lee, Z. J. Deng, E. C. Dreaden, E. Siouve, K. E. Shopsowitz, N. J. Shah, M. B. Yaffe and P. T. Hammond, *Sci. Signal.*, 2014, **7**, ra44.
21. W. Guo, C. H. Lu, X. J. Qi, R. Orbach, M. Fadeev, H. H. Yang and I. Willner, *Angew Chem Int Ed Engl*, 2014, **53**, 10134.
22. Y. Hu, J. S. Kahn, W. Guo, F. Huang, M. Fadeev, D. Harries and I. Willner, *J Am Chem Soc*, 2016, **138**, 16112.
23. T. Noll, S. Wenderhold-Reeb, H. Schonherr and G. Noll, *Angew Chem Int Ed Engl*, 2017, **56**, 12004.
24. Y. Xing, E. Cheng, Y. Yang, P. Chen, T. Zhang, Y. Sun, Z. Yang and D. Liu, *Adv Mater*, 2011, **23**, 1117.
25. M. Czuban, S. Srinivasan, N. A. Yee, E. Agustin, A. Koliszak, E. Miller, I. Khan, I. Quinones, H. Noory, C. Motola, R. Volkmer, M. Di Luca, A. Trampuz, M. Royzen and J. M. Mejia Oneto, *ACS Cent Sci*, 2018, **4**, 1624.
26. J. M. Mejia Oneto, I. Khan, L. Seebald and M. Royzen, *ACS Cent Sci*, 2016, **2**, 476.



Small molecule cyanuric acid is used to assemble a novel DNA hydrogel which is programmed to encapsulate and release a variety of compounds including drug molecules