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14. ABSTRACT

The overarching goal of this research is to develop DNA amphiphiles as stimuli-responsive materials, capable of releasing guest molecules in response to specific chemical or biological stimuli.

Research during the project period focused on evaluating two DNA amphiphile architectures. Dendrimeric amphiphiles having three DNA strands on each monomer were explored, as the DNA strands are capable of forming noncovalent crosslinks to stabilize the micelle architecture. We did observe a reduced CMC for

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Report Title

Final Report: DNA-Crosslinked micelles as programmable materials for biosensing and responsive drug delivery

ABSTRACT

The overarching goal of this research is to develop DNA amphiphiles as stimuli-responsive materials, capable of releasing guest molecules in response to specific chemical or biological stimuli.

Research during the project period focused on evaluating two DNA amphiphile architectures. Dendrimeric amphiphiles having three DNA strands on each monomer were explored, as the DNA strands are capable of forming noncovalent crosslinks to stabilize the micelle architecture. We did observe a reduced CMC for crosslinked micelles; however, the change compared with non-crosslinked micelles was not as large as desired. Thus, we shifted our research plan to focus on controlling guest release by altering the DNA:polymer ratio of our monomers. This was accomplished by hybridizing or removing a complementary DNA strand on monomeric DNA amphiphiles. We have shown guest diffusion is 63-fold faster when the complementary DNA strand is not attached. We were also curious to explore the effect of amphiphiles on the function of nucleic acid aptamers. We found that small-molecule-binding aptamers were able to function in the presence of non-ionic or anionic surfactants at concentrations above the CMC value with only a small change in binding affinity for the specified target molecule.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
01/14/2016	9.00	Jennifer M Heemstra. Learning from the unexpected in life and DNA self-assembly, Beilstein Journal of Organic Chemistry, (12 2015): 2713. doi: 10.3762/bjoc.11.292
01/14/2016	11.00	Amberlyn M. Peterson, Zhesen Tan, Evelyn M. Kimbrough, Jennifer M. Heemstra. 3,3?-Dioctadecyloxacarbocyanine perchlorate (DiO) as a fluorogenic probe for measurement of critical micelle concentration, Anal. Methods, (2015): 0. doi: 10.1039/C5AY01444A
01/14/2016	10.00	Amberlyn M. Peterson, Frank M. Jahnke, Jennifer M. Heemstra. Modulating the Substrate Selectivity of DNA Aptamers Using Surfactants, Langmuir, (11 2015): 11769. doi: 10.1021/acs.langmuir.5b02818
04/23/2015	6.00	Amberlyn M. Peterson, Jennifer M. Heemstra. Controlling self-assemblyof DNA-polymer conjugatesfor applications in imagingand drug delivery, WIRES Nanomedicine and Nanobiotechnology, (05 2015): 282. doi:
04/23/2015	7.00	Trevor A. Feagin, David P. V. Olsen, Zachary C. Headman, Jennifer M. Heemstra. High-Throughput Enantiopurity Analysis using Enantiomeric DNA-Based Sensors, Journal of the American Chemical Society, (03 2015): 4198. doi:
09/17/2014	4.00	Alexandra D. Kent, Nicholas G. Spiropulos, Jennifer M. Heemstra. General Approach for Engineering Small-Molecule-Binding DNA Split Aptamers, Anal. Chemistry, (09 2013): 9916. doi:
TOTAL:		**6**

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Pacifichem 2015, “Small-molecule detection and enantiopurity measurement using DNA-based sensors,” Honolulu, HI, December 2015.

Nucleosides, Nucleotides & Oligonucleotides Gordon Research Conference, “Small-Molecule Detection and Enantiopurity Measurement Using DNA-Based Sensors,” Newport, RI, June 2015.

IUPAC 10th International Conference on Bio-Organic Chemistry, “Small-Molecule Detection and Enantiopurity Measurement using DNA-Based Sensors,” Pune, India, January 2015.

The Future of Chemistry in Chemical Ecology, “Harnessing Nucleic Acid Molecular Recognition and Self-Assembly for Biosensing and Bioimaging,” Max Planck Institute, Jena, Germany, December 2014.

Telluride Workshop on Nucleic Acid Chemistry, “Small molecule detection and characterization using DNA-based sensors,” Telluride, CO, July 2014.

Nucleic Acid Summit, “Engineering DNA Split Aptamers for use in Small-Molecule Detection Assays,” San Diego, CA, June 2014.

University of Oregon Materials and Optical Symposium, “DNA-based biosensors using small-molecule-directed macromolecular assembly and disassembly,” Eugene, OR, September 2013.

246th ACS National Meeting, “Small-molecule detection using DNA assembly-driven reactions,” Indianapolis, IN, September 2013.

Reactive Chemical Systems Workshop, “DNA-Crosslinked Micelles as Programmable Materials for Biosensing and Responsive Drug Delivery,” Providence, RI, October 2012.

Number of Presentations: 9.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
02/07/2015	5.00	Trevor Feagin, David Olsen, Zachary Headman, Jennifer Heemstra. High-Throughput Enantiopurity Analysis using Enantiomeric DNA-Based Sensors, J Am Chem Soc (01 2015)
04/23/2015	8.00	Amberlyn M. Peterson, Zhesen Tan, Evelyn M. Kimbrough, Jennifer M. Heemstra. 3,3'-Dioctadecyloxacarbocyanine Perchlorate (DiO) as a Fluorogenic Probe for Measurement of Critical Micelle Concentration, Analytical Chemistry (04 2015)
08/26/2013	1.00	Alexandra D. Kent, Nicholas G. Spiropulos, Jennifer M. Heemstra. A General Approach for Engineering Small-Molecule-Binding DNA Split Aptamers, Anal. Chemistry (08 2013)
08/28/2014	3.00	Jennifer M. Heemstra, Amberlyn M. Peterson. Programming Self-Assembly of DNA-Polymer Conjugates for Applications in Drug Delivery and Cellular Imaging, WIRES Nanomedicine and Nanobiotechnology (05 2014)
TOTAL:		**4**

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Depletion of Abundant Serum Proteins to Facilitate Biomarker Discovery (provisional patent filed June 2015)

Patents Awarded

Awards

Cottrell Scholar Award (2015)
Myriad Award of Research Excellence (2015)
University of Utah College of Science Professorship (2014)

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Trevor Feagin	0.15	
Tilani De Costa	0.25	
Amberlyn Peterson	0.60	
FTE Equivalent:	**1.00**	
Total Number:	**3**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Jennifer Heemstra	0.08	
FTE Equivalent:	**0.08**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Annika Pecchia-Bekkum	0.20	Chemistry
Evelyn Kimbrough	0.20	Chemistry
FTE Equivalent:	**0.40**	
Total Number:	**2**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 1.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 1.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 1.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 1.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 1.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Tilani De Costa
Trevor Feagin
Amberlyn Peterson
Total Number: **3**

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

Collaboration with Sonata Biosciences to commercialize use of aptamers in the presence of surfactants.

Collaboration with BellBrook Labs to commercialize aptamer-based small-molecule detection assays.

Statement of the problem studied

The overarching goal of this research project is to develop DNA amphiphiles as stimuli-responsive materials, capable of releasing guest molecules in response to specific chemical or biological stimuli.

Summary of the most important results

I. Synthesis and analysis of dendrimeric DNA-containing amphiphiles

Dendrimeric DNA amphiphiles were synthesized having three DNA strands to enable non-covalent crosslinking between complementary DNA strands on adjacent monomers (Figure 1a), and we hypothesized that these interactions would stabilize the micelles and thus lower their CMC. To generate the dendrimeric DNA amphiphiles, we utilized trebler **1** and hydrophobic tocopherol monomer **2** (which was chosen for its ability to be easily incorporated into the monomers using a DNA synthesizer) and synthesized the sequences listed in Figure 1a. The DNA strands of sequences **3** and **4** are complementary to one another and thus can form cross-linked micelles. Sequence **5** serves as a control having four mismatches to prevent hybridization to **3**. We analyzed the dendrimeric DNA amphiphiles both alone and in mixtures using dynamic light scattering (DLS), and observed peaks that are suggestive of micelle structures with a hydrodynamic diameter of 20-50 nm (Figure 1b). We also investigated the morphology of the assemblies using TEM, and observed spherical structures having a diameter of 20-50 nm, consistent with the formation of micelles (Figure 1c).

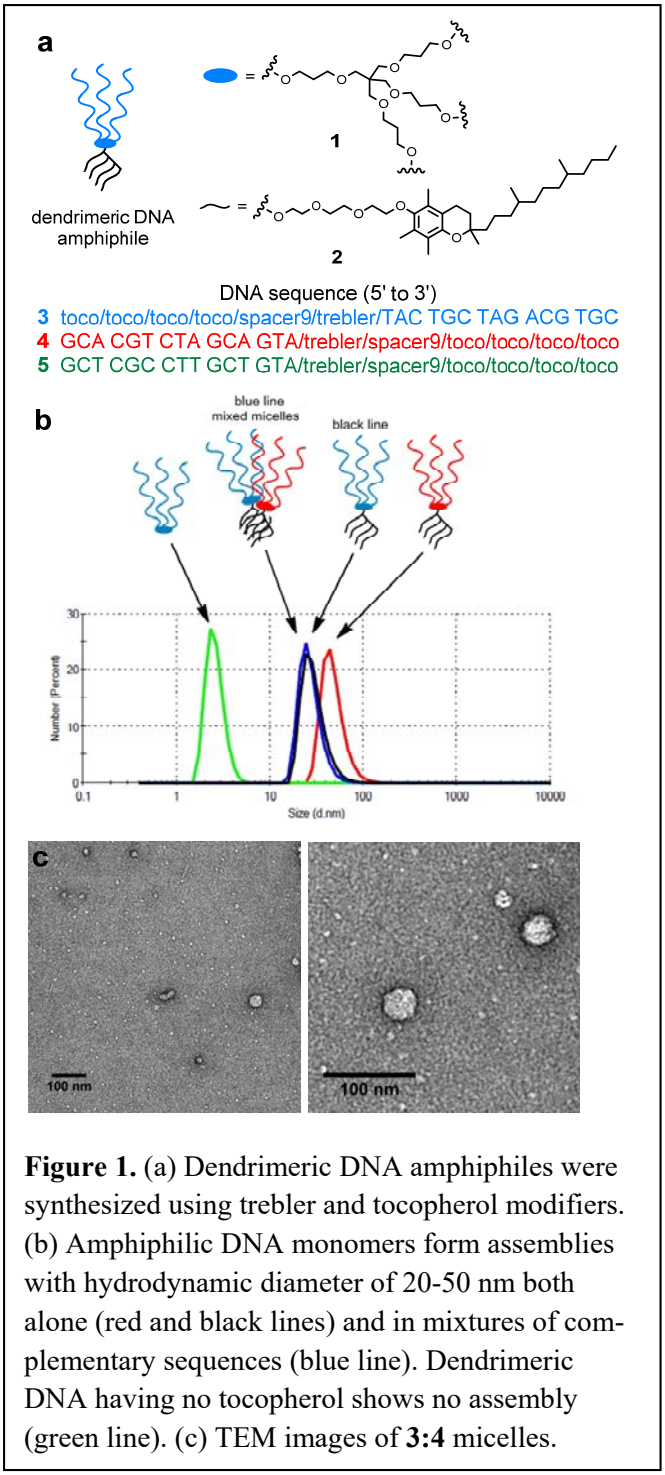

Figure 1. (a) Dendrimeric DNA amphiphiles were synthesized using trebler and tocopherol modifiers. (b) Amphiphilic DNA monomers form assemblies with hydrodynamic diameter of 20-50 nm both alone (red and black lines) and in mixtures of complementary sequences (blue line). Dendrimeric DNA having no tocopherol shows no assembly (green line). (c) TEM images of **3:4** micelles.

To test the ability of the micelles to respond to biological stimuli, we designed dendrimeric DNA amphiphiles **6** and **7** having the recognition sequence for the EcoRI restriction enzyme and functionalized the DNA sequences with FAM and Cy3 fluorophores, as these form an efficient FRET pair (Figure 2a). If the FAM- and Cy3-functionalized monomers form a mixed micelle, the ratio of fluorescence emission at 520/565 nm will decrease due to energy transfer from FAM to Cy3. However, if the DNA is cleaved by the EcoRI restriction endonuclease, the cleaved strands are too short to hybridize,

and thus the FAM and Cy3 diffuse away from one another, causing an increase in the 520/565 nm emission ratio (Figure 2b). The data in Figure 4c show the 520/565 nm emission ratio as a function of time for matched micelles (**6** + **7**), mismatched micelles (**6** + **8**), and control DNA (**9** + **10**) having the same sequence as the matched micelles, but unable to assemble into micelles. Each solution was reacted with EcoRI at 37 °C and monitored at 10 min intervals using a fluorescence plate reader. As anticipated, the matched micelles and control DNA both show a time-dependent increase in 520/565 nm emission ratio, presumably from enzymatic cleavage of the double-stranded DNA, whereas the mismatched micelles do not show a significant change. These data further established the formation of DNA-cross-linked micelles from hybridization of the matched monomers, and demonstrated the ability of restriction endonucleases to cleave DNA in the context of these cross-linked micelles.

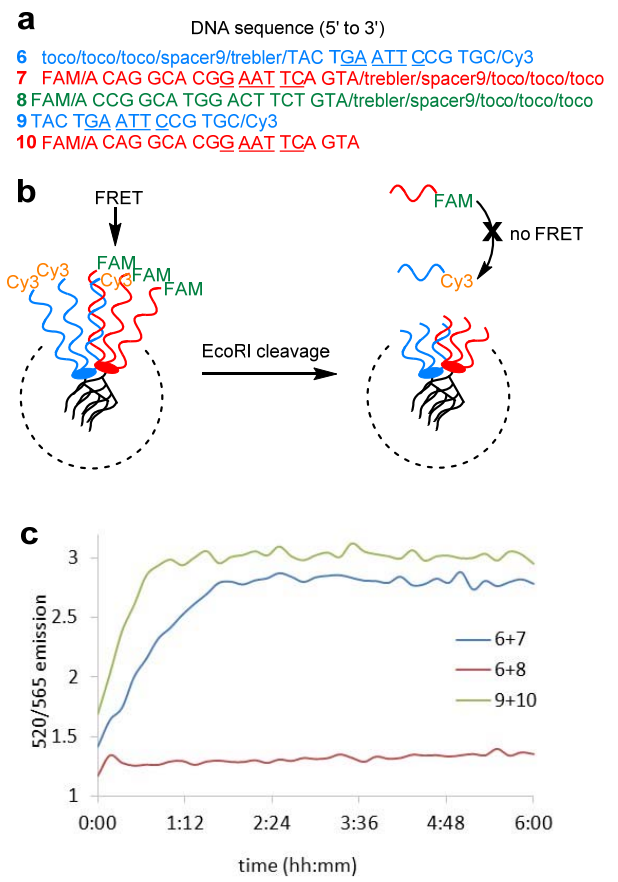

Figure 2. (a) Dendrimeric DNA sequences having the EcoRI cleavage site. (b) Matched monomers assemble to form micelles, resulting in FRET signal. FRET signal is lost upon EcoRI cleavage. (c) Data for EcoRI cleavage of matched micelles (blue), mismatched micelles (red), and control DNA (green).

In parallel with the studies described above, we sought to characterize the CMC values of the dendrimeric DNA amphiphiles. This proved to be a challenging task, as we attempted CMC measurements using DLS, tensiometry, Nile red, and FRET, but found that these methods did not offer sufficient sensitivity for measurement of the low CMC values of our micelles. However monitoring pyrene emission[1] did provide reproducible CMC values. We found that the CMC values for matched **3:4** micelles and mismatched **3:5** micelles were 470 and 590 nM, respectively. Similarly, an analogue of the **6:7** micelles having the truncated sequence resulting from digestion with EcoRI had an increased CMC value of 630 nM. While the un-cross-linked or nuclease digested micelles did have higher CMC values than the cross-linked micelles, the magnitude of this change was not as large as expected. However, we also investigated the rate of guest exchange between the micelles, and found that even without cross-links, hydrophobic guest molecules showed very slow diffusion in and out of the micelles. This is quite different from standard surfactants such as CTAB and Tween80, which show very rapid diffusion and guest exchange.[2]

II. Controlling guest exchange by modulating DNA:polymer ratio

From this observation, we built a new hypothesis that the slow guest diffusion observed for our DNA amphiphiles results from the significant energy barrier of hydrophobic dyes crossing the DNA corona,

which has a high local ionic strength. According to this hypothesis, we should be able to control guest diffusion rate, and possibly CMC, by modulating the DNA:polymer ratio. Specifically, we envisioned that this could be accomplished in a stimuli-responsive manner by hybridizing a complementary DNA strand to monomeric DNA amphiphiles (Figure 3a). We chose to move from dendrimeric to monomeric DNA amphiphiles because the dendrimer unit was deemed unnecessary, and the monomeric structures offer increased synthetic flexibility. To test our hypothesis, we synthesized sequences **11** and **12**, in which **11** is functionalized with a C_{18} stearyl group to promote aggregation into micelles, and **12** is the cocaine aptamer which is complementary to **11**, but also has a long toehold that can be used for displacement by cocaine or a complementary nucleic acid target. Using a previously reported FRET-based method,[2] we measured the rate of guest exchange between micelles, as this is representative of the rate of diffusion of guest molecules in and out of the micelles. As shown in Figure 2b, the rate of guest exchange for **11** alone is 3.8 x 10^{-2} s^{-1}, compared with 6.0 x 10^{-4} s^{-1} for hybridized **11:12** micelles. We were very encouraged to observe that by modulating the DNA:polymer ratio, we could achieve a 63-fold difference in guest diffusion rate.

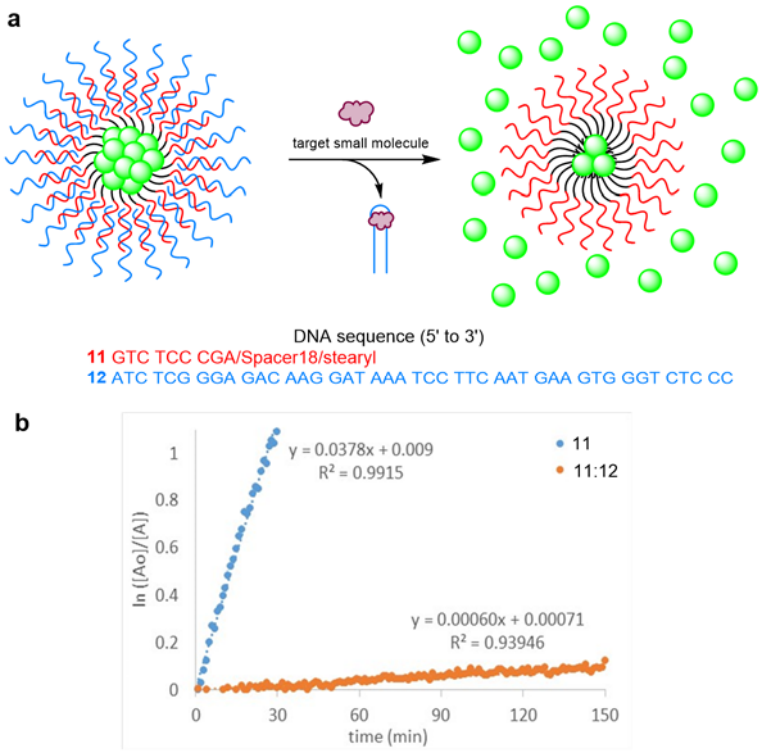

Figure 3. (a) Stimuli-responsive release of guest molecules by modulating DNA:polymer ratio. (b) Kinetic rate plots of guest exchange for hybridized (**11:12**) and un-hybridized (**11**) micelles.

III. Alternative solvatochromic fluorophores for measuring CMC values

In the process of measuring CMC values for our DNA amphiphiles, we recognized that a significant need exists for new solvatochromic fluorophores capable of use in CMC measurement. Nile red is frequently used to measure CMC values,[3] but in our hands, we have found that it adheres strongly to even non-stick plasticware (e.g., Eppendorf tubes), making its use inconvenient (Figure 4a). Additionally, while pyrene was successfully employed to measure CMC values for our

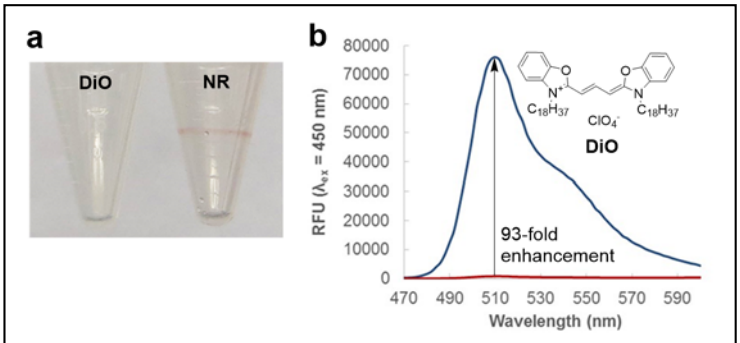

Figure 4. (a) Nile Red adheres to plasticware. (b) Structure and fluorescence enhancement for DiO.

DNA amphiphiles, the small changes in relative emission peak heights precludes use of a fluorescence plate reader for analysis. Rather, cuvette-based measurements are necessary, which is low throughput and thus very time consuming.

In response to this need for new solvatochromic fluorophores, we evaluated 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) as an alternative fluorogenic dye for the measurement of CMC values (Figure 4b). We investigated the utility of DiO for fluorescence-based CMC measurement, and directly compared its performance to that of NR. We found that DiO is compatible with a variety of surfactant types, and while NR and DiO both provide CMC measurements that agree with literature values, DiO did not suffer from failed measurements, as NR often did. Additionally, DiO was easier to handle than NR, as solubility and aggregation problems were not observed with DiO, but were frequent with NR.[4] Therefore, we concluded that DiO provides an accurate and reliable method for measuring CMC values without the need for specialized equipment. In future experiments aimed at characterizing DNA amphiphiles, we will utilize DiO for CMC measurement.

IV. Function of DNA aptamers in the presence of surfactants

Considering that the DNA amphiphiles we had synthesized are essentially large surfactants, we became interested in the question of whether aptamers are capable of functioning in the presence of surfactants. We envisioned that this study would be of use to our own project, but could also have far-reaching implications, as protein-based affinity reagents such as antibodies are readily denatured by most surfactants. To explore the effect of surfactants on aptamer function and substrate binding preference, we used a series of structure-switching DNA aptamer biosensors previously reported by Stojanovic and co-workers that bind to steroid targets.[5] We chose the aptamer for dehydroisoandrosterone 3-sulfate sodium salt dihydrate (DIS) as a model to survey the effect of varying surfactant types on substrate binding. Using five common surfactants that represent all four ionic states including cationic, anionic, nonionic, and zwitterionic, we measured the fluorescence response of the aptamer biosensor to DIS in the presence of 1% (w/v) of each surfactant. This concentration is above the CMC for each of the surfactants, ensuring the formation of micelles. We were very encouraged to observe that in the presence of SDS, Tween 20, or Triton X-100, the biosensor shows only a slightly attenuated response compared to its behavior in pure buffer (Figure 5).[6] However, the biosensor shows no detectible response in the presence of positively charged CTAB, and in zwitterionic CHAPS, the biosensor begins to show a response only at the highest DIS concentrations. This is not surprising, as surfactants having a positively charged functional group are more likely to interact with the negatively charged DNA backbone. We surveyed three additional small-molecule-binding aptamers and found that substrate binding for hydrophilic targets was only minimally perturbed even with SDS concentrations as high as 4% (w/v). The ability of aptamers to maintain their function in the presence of commonly used surfactants provides an additional competitive advantage relative to antibodies, and is likely to significantly increase the scope of analytical applications for which aptamers can be employed.

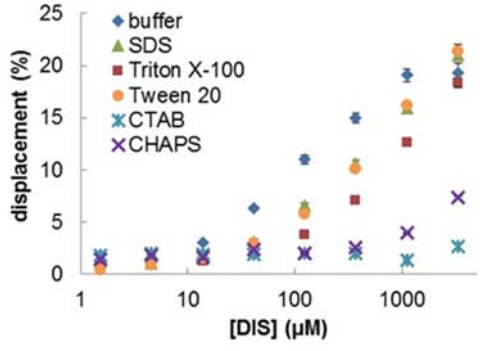

Figure 5. Response of the DIS biosensor to increasing concentrations of DIS ligand in the presence of 1% of various commonly used surfactants.

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