HHS Public Access

Author manuscript

Langmuir. Author manuscript; available in PMC 2017 December 05.

Published in final edited form as:

Langmuir. 2015 November 03; 31(43): 11769–11773. doi:10.1021/acs.langmuir.5b02818.

Modulating the Substrate Selectivity of DNA Aptamers Using Surfactants

Amberlyn M. Peterson[†], **Frank M. Jahnke**[‡], and **Jennifer M. Heemstra**[†]

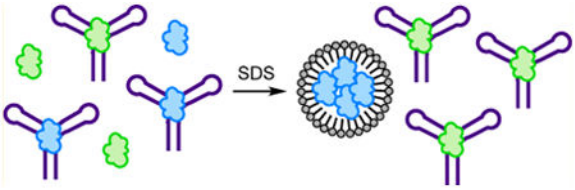
[†]Department of Chemistry and the Center for Cell and Genome Science, University of Utah, Salt Lake City, Utah 84112, United States

[‡]Sonata Biosciences, Inc., Auburn, California 95603, United States

Abstract

Nucleic acid aptamers have a number of advantages compared to antibodies, including greater ease of production and increased thermal stability. We hypothesized that aptamers may also be capable of functioning in the presence of high concentrations of surfactants, which readily denature antibodies and other protein-based affinity reagents. Here we report the first systematic investigation into the compatibility of DNA aptamers with surfactants. We find that neutral and anionic surfactants have only a minor impact on the ability of aptamers to fold and bind hydrophilic target molecules. Additionally, we demonstrate that surfactants can be utilized to modulate the substrate binding preferences of aptamers, likely due to the sequestration of hydrophobic target molecules within micelles. The compatibility of aptamers with commonly used surfactants is anticipated to expand their scope of potential applications, and the ability to modulate the substrate binding preferences of aptamers using a simple additive provides a novel route to increasing their selectivity in analytical applications.

Graphical abstract



Introduction

Nucleic acid aptamers[1–3] hold significant promise for replacing antibodies in analytical applications, as aptamers are capable of binding to a wide variety of small-molecule and

Correspondence to: Jennifer M. Heemstra.

Supporting Information: The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.5b02818.

Author Contributions: The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes: The authors declare the following competing financial interest(s): F.M.J. has an equity interest in Sonata Biosciences, Inc., which holds certain IP rights to the contents of this manuscript.

protein targets.[4–8] The most commonly cited benefits of aptamers relative to antibodies include their ability to retain function after thermal denaturation and the fact that they are chemically synthesized, which reduces both cost and batch-to-batch variation.[8,9] We were curious as to whether aptamers might also have the advantage of functioning in the presence of chemical denaturants such as surfactants, but we found no reports in the literature exploring this intriguing question. Antibodies and other proteins are readily denatured by surfactants, as the hydrophobic portion of the surfactant can interact with hydrophobic surfaces on the protein, reducing the enthalpic cost of protein unfolding in an aqueous medium.[10] However, unlike proteins, nucleic acids do not possess large surfaces composed of aliphatic side chains, and thus we hypothesized that they would be less likely to be disrupted by surfactants.

In addition to exploring the ability of aptamers to function in the presence of surfactants, we envisioned that the surfactants could provide a unique dimension of control over the substrate binding preferences of aptamers. At low concentrations, amphiphilic surfactant molecules are dispersed in solution and form a monolayer at the air–water interface. However, at concentrations above the critical micelle concentration (CMC) of the surfactant, self-assembly occurs to form micelles.[11] These spherical or ellipsoidal structures possess a hydrophobic core that is capable of sequestering nonpolar molecules. As a result, surfactants are commonly used for applications such as purification and reaction catalysis.[12,13] In the context of aptamer-target binding, we hypothesized that analytes would show variable partitioning into the micelle core depending upon their hydrophobicity, effectively increasing the selectivity of aptamers toward hydrophilic analytes. Substrate binding selectivity is critical to many applications of aptamers, and previous studies have explored approaches to modulating selectivity through sequence mutation, the incorporation of unnatural bases, or the addition of hydrophobic groups near the binding pocket of the aptamer.[14–17] Due to the nature of these chemical modifications, they typically increase the binding affinity for hydrophobic targets. Thus, the use of surfactants offers a complementary approach to modulating the substrate binding selectivity of aptamers.

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 (Figure 1).[18] Each structure-switching biosensor is composed of an aptamer and a short complementary strand, which are functionalized with a fluorophore and quencher, respectively. In the absence of the target molecule, the complementary strand binds to the aptamer and fluorescence is quenched. However, in the presence of a target that binds to the aptamer, the complementary strand is displaced, resulting in a dose-dependent increase in fluorescence signal. Here we show that the aptamers maintain their secondary structure and substrate binding capability in the presence of neutral and anionic surfactants and that the presence of surfactant can be used to modulate the substrate binding preference to favor more hydrophilic ligands. The demonstrated ability of aptamers to function in the presence of surfactants is anticipated to expand their scope of potential applications. Additionally, the ability to modulate the substrate binding preferences of aptamers using a simple additive provides a novel route to increasing their selectivity in analytical applications.

Experimental Section

General

All DNA was purchased from the University of Utah DNA/Peptide Synthesis Core Facility, where it was synthesized using phosphoramidites and CPG cartridges from Glen Research. All other materials were purchased from commercial suppliers and used without further purification. Absorbance and fluorescence measurements were recorded using a Biotek Synergy Mx microplate reader.

Preparation of Stock Solutions

All samples were prepared in a buffer containing 20 mM Tris and 150 mM NaCl at pH 7.4. This salt concentration was chosen to avoid SDS precipitation. The aptamer and complementary strand were annealed by incubating at 90 °C for 5 min, followed by rapid cooling. The following DNA concentrations were used for each biosensor: DIS, 1 μM aptamer and 2 μM displacement strand; BE, 0.15 μM aptamer and 0.30 μM displacement strand; DCA, 1 μM aptamer and 2 μM displacement strand. DNA structure-switching biosensors rely on competing equilibria to bind the aptamer to the displacement strand or the target. Thus, the DNA concentration impacts the response of the sensor to the target ligand. The DNA concentrations used in our experiments were chosen empirically to maximize the signal-to-background ratio.

Stock solutions of surfactants were prepared by dissolving sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), Triton X-100, or Tween 20 in Tris buffer at 5 or 10% (w/ v). Ligand solutions were prepared by dissolving each steroid in DMSO (DCA, BE, DIS) or 2:1 $CHCl_3$/DMSO (DOA) at 500 mM and then performing a 3-fold dilution series in DMSO to maintain the concentration of organic solvent in all samples constant at 2%.

Fluorescence Measurements

For initial testing of the surfactant scope, solutions were prepared having 0 or 1% (w/v) surfactant. To monitor the effects of increasing SDS, solutions were prepared having 0, 0.01, 1, or 4% SDS. The DNA stock solution and surfactant were combined in Tris buffer and allowed to equilibrate for 5 min. The ligand was then added, and the solutions were incubated for 20 min at 25 °C. Fluorescence measurements were then acquired with λ_{ex} = 495 nm and λ_{em} = 525 nm at 25.0 ± 0.2 °C. The percent displacement (% D) for each biosensor was calculated using eq 1

$$\%D = \left(\frac{F - F_0}{F_m - F_0}\right) \times 100 \quad (1)$$

in which F is the measured fluorescence, F_0 is the fluorescence of the biosensor in the absence of ligand, and F_m is the fluorescence of the aptamer alone[19].

Circular Dichroism (CD) Analysis

CD spectra were acquired using a Jasco J815 CD spectrometer. The CD spectra were collected using unlabeled aptamers (10 μM) prepared in Tris buffer containing 0, 0.01, 1, or 4% SDS. As a positive control for denaturation, we also acquired CD spectra for each aptamer in Tris buffer with 8 M urea. Following heating and cooling, the aptamer strands were incubated at 25 °C for 2 h. All CD spectra were recorded at 23 °C, scanning from 220 to 320 at 100 nm/min (cell path length = 2.00 mm). Final spectra are an average of six scans.

Results and Discussion

Choice of Aptamer Sequences

To investigate the effect of surfactants on aptamer—ligand recognition, we realized that it was necessary to use aptamers that bind to small-molecule, rather than protein, targets. This is because nearly all protein-binding aptamers have been selected to recognize folded proteins, and thus even if the aptamer retained its structure and function, the addition of surfactant would compromise the protein target in such a way as to preclude binding. We also strategically sought to utilize aptamers that had been reported in a structure-switching biosensor format,[20] as this enables convenient fluorescence-based monitoring of target binding. Thus, we chose three aptamer biosensors previously reported by Stojanovic and co-workers that bind to small-molecule steroid targets[18]. These aptamers were selected using steroid targets DCA, DIS, and BE (Figure 1b) and were intentionally selected to have a broad substrate scope, with each aptamer sequence having an affinity for multiple steroid targets.

Exploring the Effect of Surfactant Type

We chose the DIS aptamer 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,[11] 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 2). 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. In fact, a 2% CTAB solution is often used for DNA precipitation.[21] We decided to utilize SDS for all further studies as the biosensor performed well in this surfactant and SDS is frequently used for protein denaturation.

Structural Analysis Using CD Spectroscopy

The ability of the DIS aptamer to bind its target molecule in the presence of 1% SDS suggests that this concentration of surfactant does not significantly disrupt DNA folding. To validate this idea and explore the tolerance of DNA folding to increased concentrations of

SDS, we acquired CD spectra for each of the three aptamers in the presence of 0, 0.01, 1, and 4% SDS. These SDS concentrations were chosen as they allow a comparison of DNA secondary structure at SDS concentrations below (0 and 0.01%) and above (1 and 4%) the CMC. As a positive control to ensure that a change in the CD spectrum would be observed upon DNA unfolding, we also acquired spectra for each aptamer in the presence of 8 M urea, which is well established to denature DNA secondary structure.[22]

As shown in Figure 3, the CD spectra for each aptamer remain constant as the SDS concentration is increased from 0 to 4% However, in the presence of 8 M urea, the CD signal undergoes a noticeable bathochromic shift and a slight decrease in intensity. Together, these data suggest that the aptamers are able to maintain their secondary structure in the presence of up to 4% SDS, which is impressive given that this concentration of SDS leads to the denaturation of most proteins.[10] Additionally, we were encouraged by these results in which all three aptamers were likely to maintain their target-binding ability in the presence of up to 4% SDS.

Modulating Target Selectivity

To test our hypothesis that surfactant could be used to increase the selectivity for hydrophilic ligands, we investigated the response of the DIS aptamer to both DIS and the more hydrophobic steroid, DOA (Table S1), in the presence of increasing concentrations of SDS (Figure 4). In buffer and 0.01% SDS, we observed that DOA binds to the DIS biosensor with a slightly higher affinity than the DIS ligand. In both of these solutions, the fluorescence signal from DOA unexpectedly decreases at high ligand concentrations, possibly due to aggregation of the hydrophobic steroid. Upon increasing the SDS concentration to 1 or 4% we were excited to observe that the biosensor shows no response to even millimolar concentrations of DOA but shows only a slightly attenuated binding to DIS. This switch in substrate binding preference presumably results from the sequestration of the hydrophobic DOA in the micelles, whereas the hydrophilic DIS remains solvated by the aqueous phase.

We also investigated the impact of increasing SDS concentration on the substrate specificity of the DCA and BE biosensors. As shown in Figure 5a–d, the DCA biosensor binds DCA with a slightly higher affinity than it does DIS in buffer or 0.01% SDS. However, in the presence of micelles at 1 or 4% SDS, the binding to DCA is dramatically attenuated, switching the preferred ligand to DIS. We were initially surprised to observe such a dramatic reduction in DCA binding in the presence of micelles as DCA has a charged carboxylate functional group and thus would be expected to have some ability to remain solvated by the aqueous phase. However, DCA does possess an additional aliphatic chain relative to DIS, and the sulfate group of DIS contains a greater number of polar heteroatoms than the carboxylate of DCA. Thus, it is reasonable that the micelles sequester DCA while leaving DIS free in solution.

In the case of the BE biosensor, the effect of SDS on substrate selectivity proved to be slightly more complex. At SDS concentrations below the CMC, the biosensor strongly favors BE, showing the highest affinity binding of all of the aptamer–ligand pairs (Figure 6a,b). Above the CMC, the biosensor shows nearly equal binding to both DIS and BE (Figure 6c,d). Increasing the concentration of SDS from 1 to 4% shows no appreciable effect

on the binding, which is somewhat surprising as we expected that BE would be strongly sequestered within the micelles due to its hydrophobicity. However, BE has been shown to bind to its cognate aptamer with a much higher affinity than any of the other aptamer–ligand pairs,[18] and the exchange of hydrophobic ligands between micelles is known to be a dynamic process.[23] Thus, we hypothesize that the anomalous behavior observed for BE reflects the ability of the aptamer to effectively compete with the micelles for binding to the BE that is transiently available in the solution. However, despite some unexpected results, we found that for each of the three biosensors, surfactant can be used to increase the relative affinity for hydrophilic over hydrophobic substrates.

Conclusions

Here we provide the first evidence that DNA aptamers can retain their secondary structure and substrate binding capability in the presence of up to 4% surfactant. We find that anionic and nonionic surfactants are well-tolerated, whereas cationic and zwitterionic surfactants do compromise substrate binding, likely because the positively charged functional groups on the surfactant interact with the negatively charged backbone of the DNA. However, SDS and Triton X-100 are among the most commonly used surfactants in biochemical applications, and SDS in particular is known to readily denature antibody reagents.[24] Thus, the ability of aptamers to maintain their function in the presence of both of these 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.

We also investigated the hypothesis that surfactant micelles could be used to modulate the substrate binding preferences of aptamers by selectively encapsulating more hydrophobic ligands. For all three aptamers tested, we observe that the presence of SDS at concentrations above the CMC greatly diminishes or completely eliminates the biosensor response to the more hydrophobic substrate. However, the biosensor response to the hydrophilic substrate is only slightly attenuated. Thus, the studies reported here establish surfactant addition as a novel, facile, and effective method for increasing the substrate selectivity of DNA aptamers. We anticipate that this will enable the use of aptamers having nonideal substrate selectivity for analytical applications where minimizing cross-reactivity is of critical importance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Science Foundation (CHE 1308364 to J.M.H.), the Army Research Office (59482CH to J.M.H.), and the National Institutes of Health (1R43GM108239-02 to F.M.J.).

References

1. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990; 249:505–510. [PubMed: 2200121]
2. Robertson DL, Joyce GF. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature. 1990; 344:467–468. [PubMed: 1690861]

3. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990; 346:818–822. [PubMed: 1697402]
4. O'Sullivan CK. Aptasensors–the future of biosensing? Anal Bioanal Chem. 2002; 372:44–48. [PubMed: 11939212]
5. Famulok M, Hartig JS, Mayer G. Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. Chem Rev. 2007; 107:3715–3743. [PubMed: 17715981]
6. Cho EJ, Lee JW, Ellington AD. Applications of aptamers as sensors. Annu Rev Anal Chem. 2009; 2:241–264.
7. Mascini M, Palchetti I, Tombelli S. Nucleic Acid and Peptide aptamers: fundamentals and bioanalytical aspects. Angew Chem, Int Ed. 2012; 51:1316–1332.
8. Liu J, Cao Z, Lu Y. Functional Nucleic Acid Sensors. Chem Rev. 2009; 109:1948–1998. [PubMed: 19301873]
9. Bradbury A, Pluckthun A. Reproducibility: Standardize antibodies used in research. Nature. 2015; 518:27–9. [PubMed: 25652980]
10. Otzen D. Protein-surfactant interactions: A tale of many states. Biochim Biophys Acta, Proteins Proteomics. 2011; 1814:562–591.
11. Neugebauer, JM. [18] Detergents: An Overview. In: Murray, PD., editor. Methods in Enzymology. Vol. 182. Academic Press; 1990. p. 239-253.
12. Fendler, JH., Fendler, EJ. Catalysis in Micellar and Macromoleular Systems. Fendler, JH., Fendler, EJ., editors. Vol. Chapter 7. Academic Press; 1975. p. 230-253.
13. Paleologos EK, Giokas DL, Karayannis MI. Micelle-mediated separation and cloud-point extraction. TrAC, Trends Anal Chem. 2005; 24:426–436.
14. Green E, Olah MJ, Abramova T, Williams LR, Stefanovic D, Worgall T, Stojanovic MN. A Rational Approach to Minimal High-Resolution Cross-Reactive Arrays. J Am Chem Soc. 2006; 128:15278–15282. [PubMed: 17117880]
15. Stojanovic MN, Green EG, Semova S, Nikic DB, Landry DW. Cross-Reactive Arrays Based on Three-Way Junctions. J Am Chem Soc. 2003; 125:6085–6089. [PubMed: 12785838]
16. Reinstein O, Neves MA, Saad M, Boodram SN, Lombardo S, Beckham SA, Brouwer J, Audette GF, Groves P, Wilce MC, Johnson PE. Engineering a structure switching mechanism into a steroid-binding aptamer and hydrodynamic analysis of the ligand binding mechanism. Biochemistry. 2011; 50:9368–76. [PubMed: 21942676]
17. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, Carter J, Dalby AB, Eaton BE, Fitzwater T, Flather D, Forbes A, Foreman T, Fowler C, Gawande B, Goss M, Gunn M, Gupta S, Halladay D, Heil J, Heilig J, Hicke B, Husar G, Janjic N, Jarvis T, Jennings S, Katilius E, Keeney TR, Kim N, Koch TH, Kraemer S, Kroiss L, Le N, Levine D, Lindsey W, Lollo B, Mayfield W, Mehan M, Mehler R, Nelson SK, Nelson M, Nieuwlandt D, Nikrad M, Ochsner U, Ostroff RM, Otis M, Parker T, Pietrasiewicz S, Resnicow DI, Rohloff J, Sanders G, Sattin S, Schneider D, Singer B, Stanton M, Sterkel A, Stewart A, Stratford S, Vaught JD, Vrkljan M, Walker JJ, Watrobka M, Waugh S, Weiss A, Wilcox SK, Wolfson A, Wolk SK, Zhang C, Zichi D. Aptamer-Based Multiplexed Proteomic Technology for Biomarker Discovery. PLoS One. 2010; 5:e15004. [PubMed: 21165148]
18. Yang KA, Pei R, Stefanovic D, Stojanovic MN. Optimizing Cross-reactivity with Evolutionary Search for Sensors. J Am Chem Soc. 2012; 134:1642–1647. [PubMed: 22142383]
19. Feagin TA, Olsen DPV, Headman ZC, Heemstra JM. High-Throughput Enantiopurity Analysis Using Enantiomeric DNA-Based Sensors. J Am Chem Soc. 2015; 137:4198–4206. [PubMed: 25747268]
20. Nutiu R, Li Y. Structure-Switching Signaling Aptamers. J Am Chem Soc. 2003; 125:4771–4778. [PubMed: 12696895]
21. Del Sal G, Manfioletti G, Schneider C. The CTAB-DNA precipitation method: a common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. BioTechniques. 1989; 7:514–520. [PubMed: 2699240]
22. Albright, LM., Slatko, BE. Current Protocols in Nucleic Acid Chemistry. John Wiley and Sons, Inc.; Hoboken, NJ: 2001. Denaturing Polyacrylamide Gel Electrophoresis. Appendix 310.1002/0471142700.nca03bs00

23. Jiwpanich S, Ryu JH, Bickerton S, Thayumanavan S. Noncovalent Encapsulation Stabilities in Supramolecular Nano-assemblies. J Am Chem Soc. 2010; 132:10683–10685. [PubMed: 20681699]

24. Brown D, Lydon J, McLaughlin M, Stuart-Tilley A, Tyszkowski R, Alper S. Antigen retrieval in cryostat tissue sections and cultured cells by treatment with sodium dodecyl sulfate (SDS). Histochem Cell Biol. 1996; 105:261–267. [PubMed: 9072183]

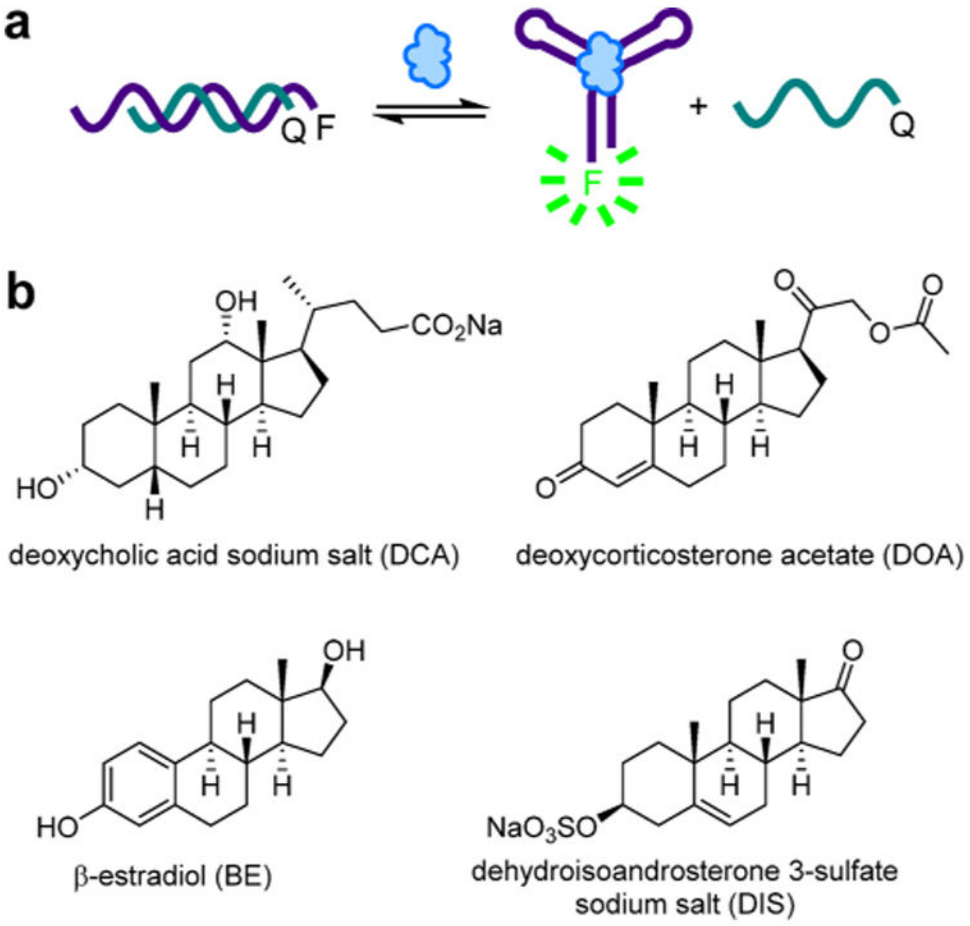


Figure 1.

(a) Structure-switching biosensors provide a dose-dependent fluorescence response to target analytes. (b) Chemical structures of steroid targets.

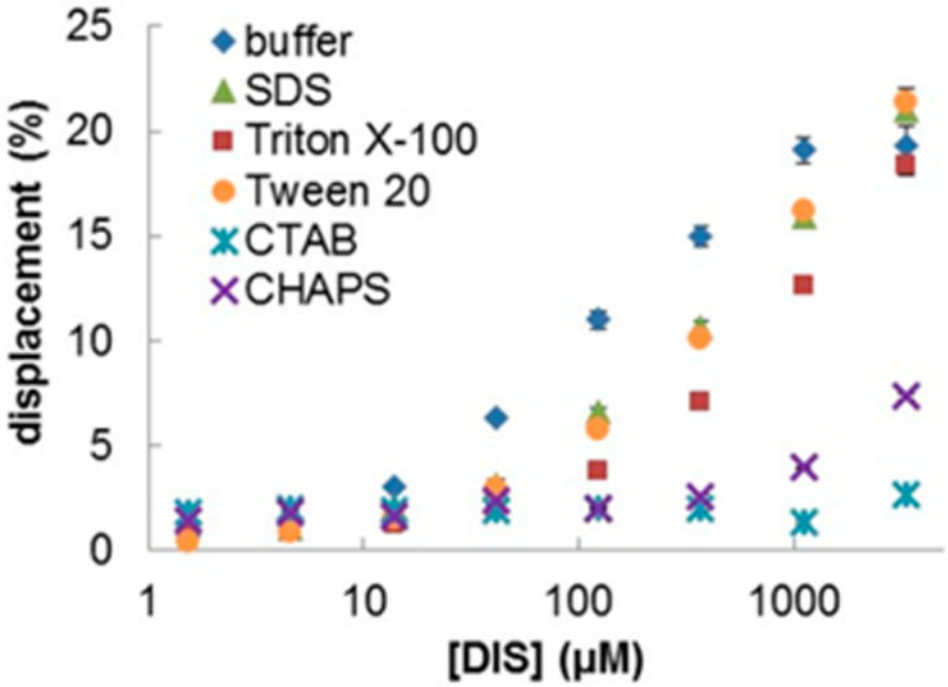


Figure 2.

Response of the DIS biosensor to increasing concentrations of DIS ligand in the presence of 1% of various commonly used surfactants. The error bars represent the standard deviation of three independent trials.

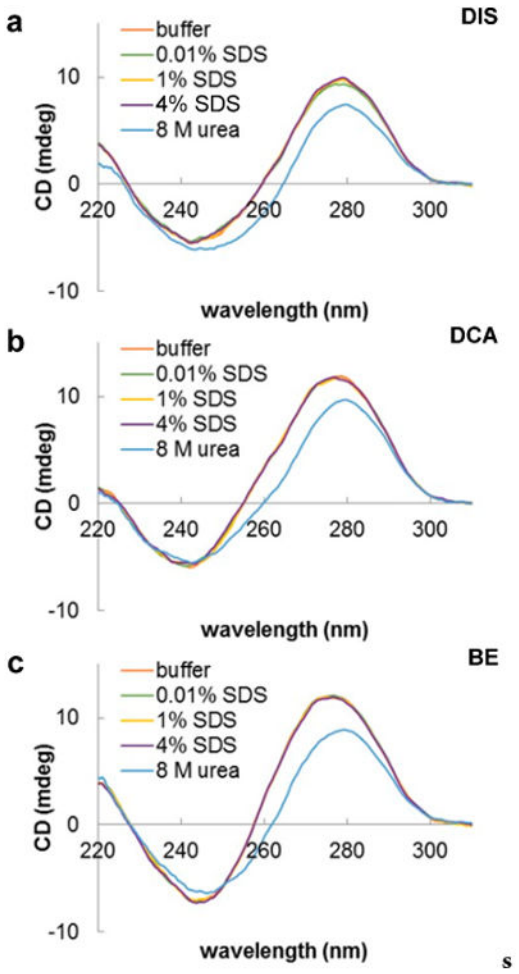


Figure 3.

CD spectra for the (a) DIS, (b) BE, and (c) DCA aptamers in the presence of 0, 0.01, 1, and 4% SDS or 8 M urea.

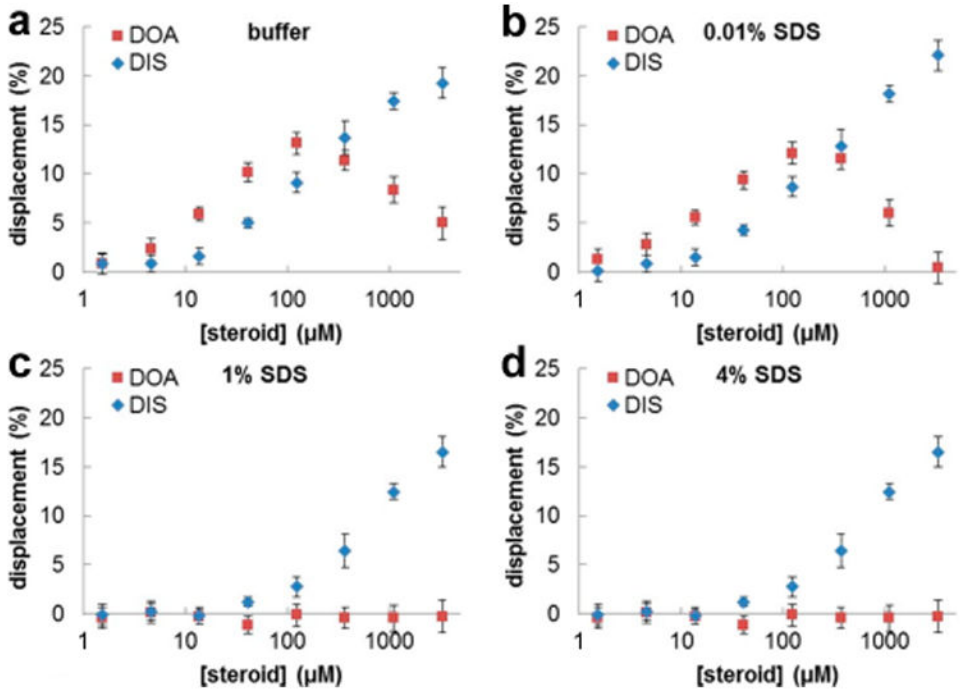


Figure 4.

Fluorescence response of the DIS biosensor to DOA (red) or DIS (blue) in (a) buffer, (b) 0.01% SDS, (c) 1% SDS, and (d) 4% SDS. The error bars represent the standard deviation of three independent trials.

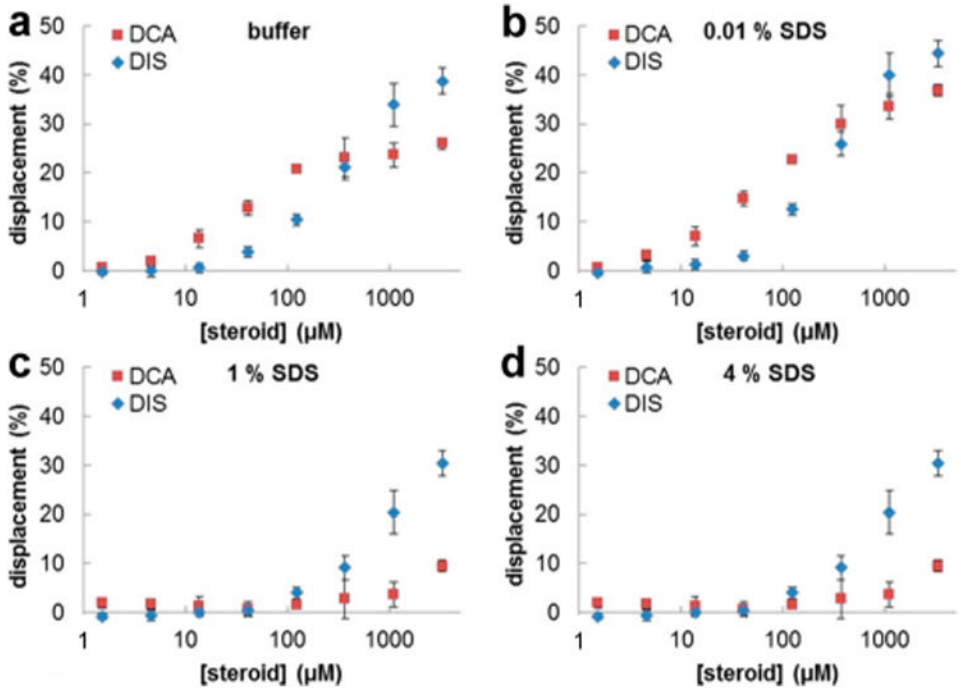


Figure 5.

Fluorescence response of the DCA biosensor to DCA (red) or DIS (blue) in (a) buffer, (b) 0.01% SDS, (c) 1% SDS, and (d) 4% SDS. The error bars represent the standard deviation of three independent trials.

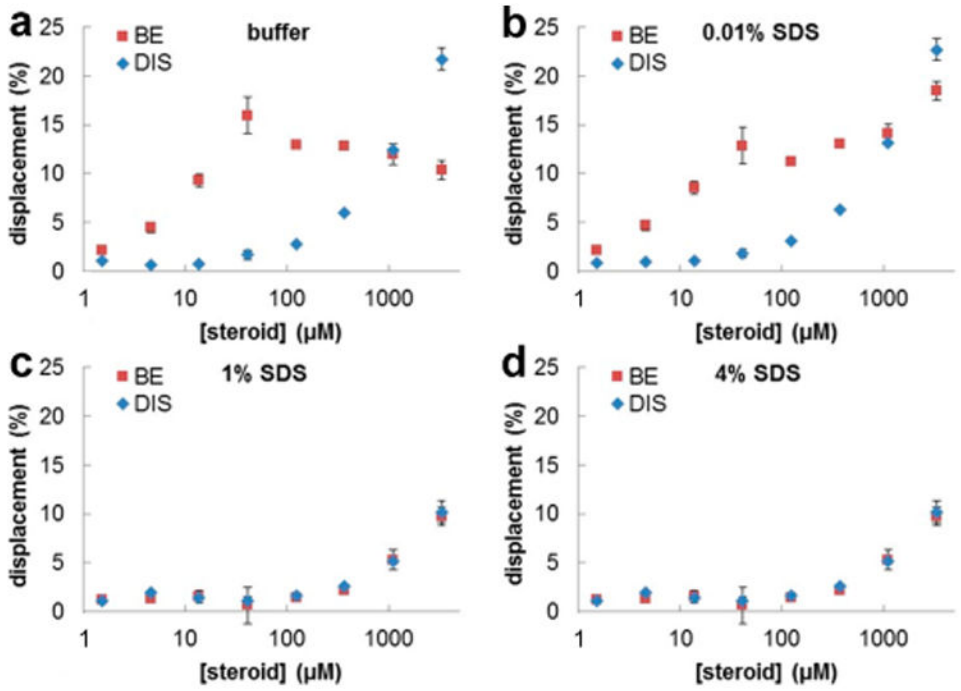


Figure 6.

Fluorescence response of the BE biosensor to BE (red) or DIS (blue) in (a) buffer, (b) 0.01% SDS, (c) 1% SDS, and (d) 4% SDS. The error bars represent the standard deviation of three independent trials.

Table 1

Sequences of DNA Aptamers (A) and Complementary Strands (CS)[a]

name	sequence (5′-3′)
BE-A	FAM-CTCTCGGGACGACATGGATTTTCCATCAACGAAGTGCGTCCGTCCCG
BE-CS	GTCGTCCCGAGAG-BHQ1
DCA-A	FAM-CTCTCTCGGGACGCTGGGTTTTCCCAGGACGAAGTCCGTCCCGA
DCA-CS	CGTCCCGAGAGA-BHQ1
DIS-A	FAM-CTGCTCTCGGGACGTGGATTTTCCGCATACGAAGTTGTCCCGAG
DIS-CS	GTCCCGAGAGCA-BHQ1

[a]FAM = fluorescein; BHQ1 = black hole quencher 1.