Liquid Crystal Re-orientation Induced by Aptamer Conformational Changes

Supporting Information

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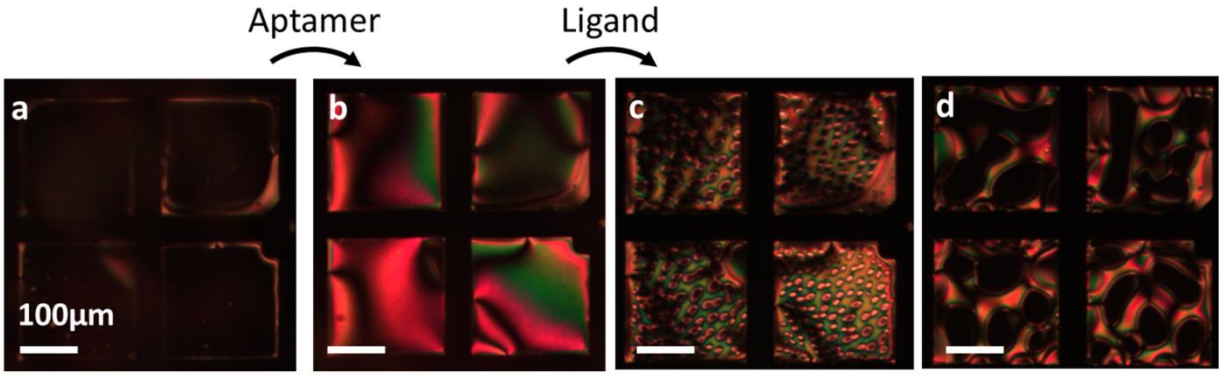

Figure S1 – Polarized light microscopy images of the aqueous/LC interface (a) laden with OTAB, (b) after adsorption of the arginine aptamer (2.5µM), (c) ≈20sec after addition of arginine (≈1mM), and (d) ≈5min after addition of arginine

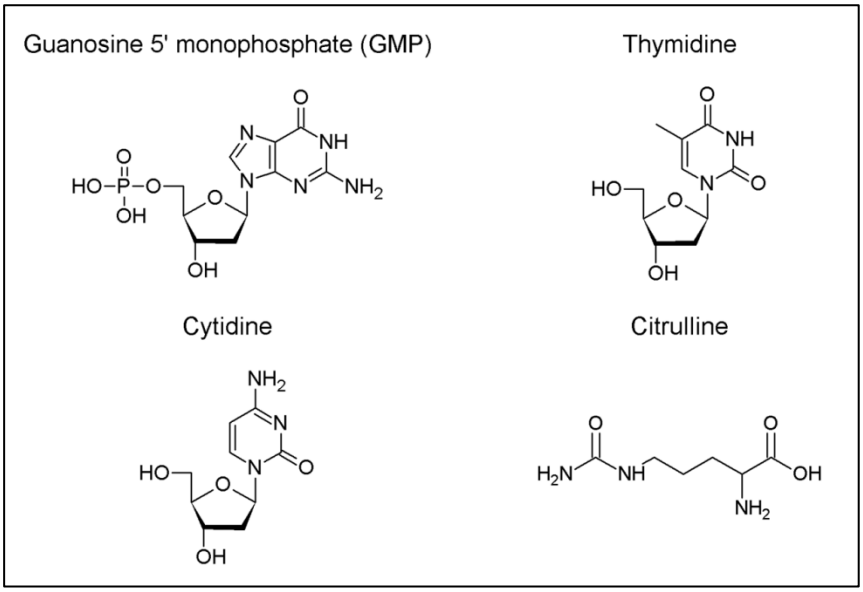

Figure S2 - Chemical structure of control species

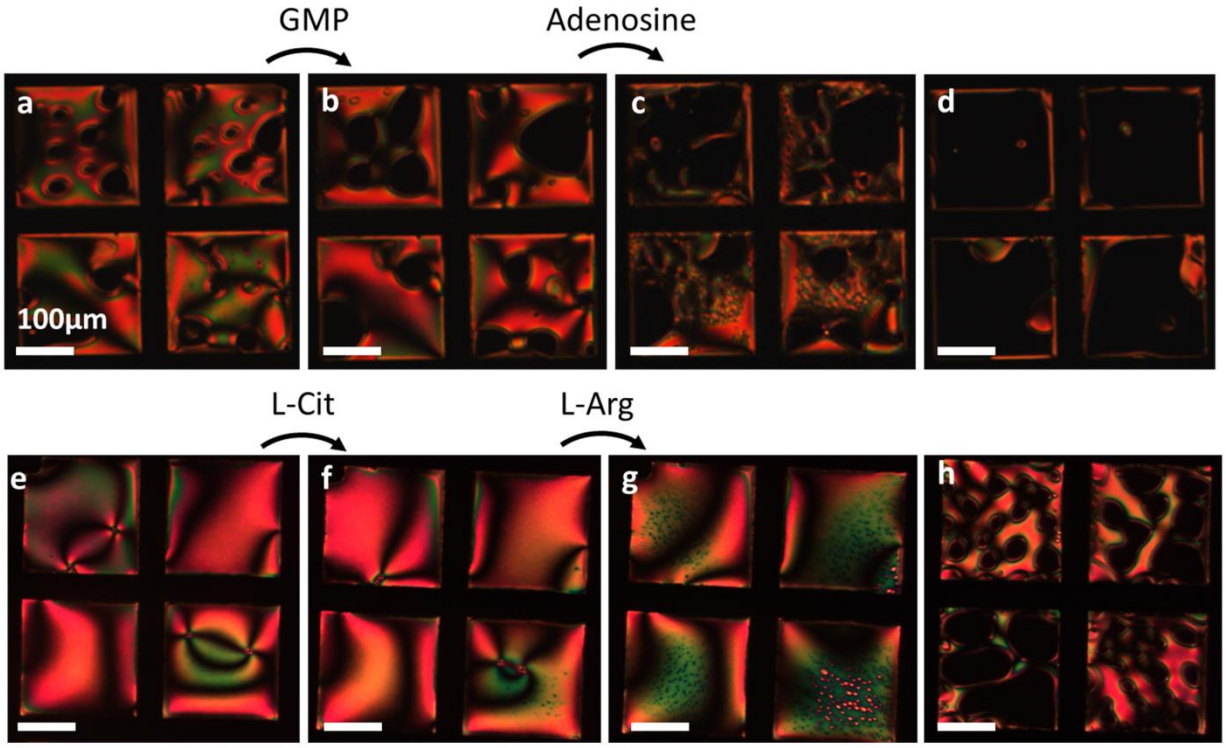

Figure S3 – Polarized light microscopy images of the aqueous/LC interface (a,e) laden with OTAB and after adsorption of either the adenosine (a) or arginine (e) aptamer (2.5µM), (b,f) ≈3min after addition of either GMP (≈300 µM) (b) or L-cit (≈1mM) (f), (c,g) ≈30 sec after addition of either adenosine (≈300µM) (≈300µM) (c) or Arginine (≈1mM) (g), and (g,h) ≈5min after addition of either adenosine or Arginine

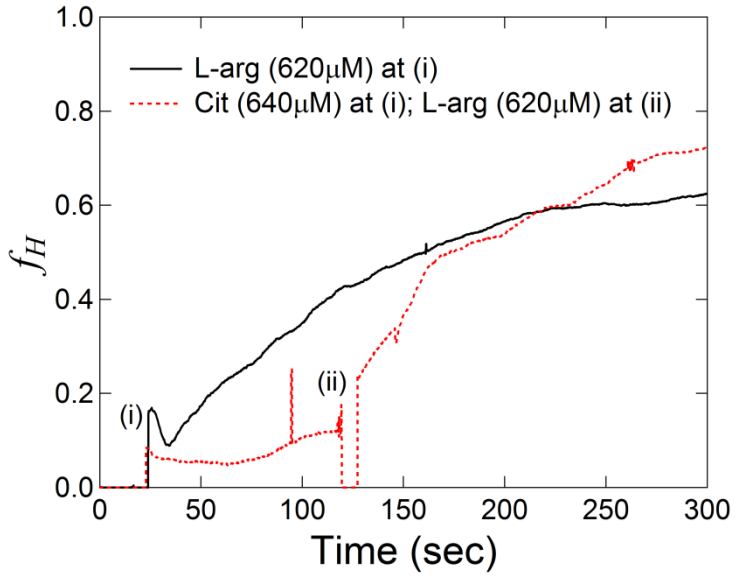

Figure S4 –Dynamic LC response upon addition of ligands

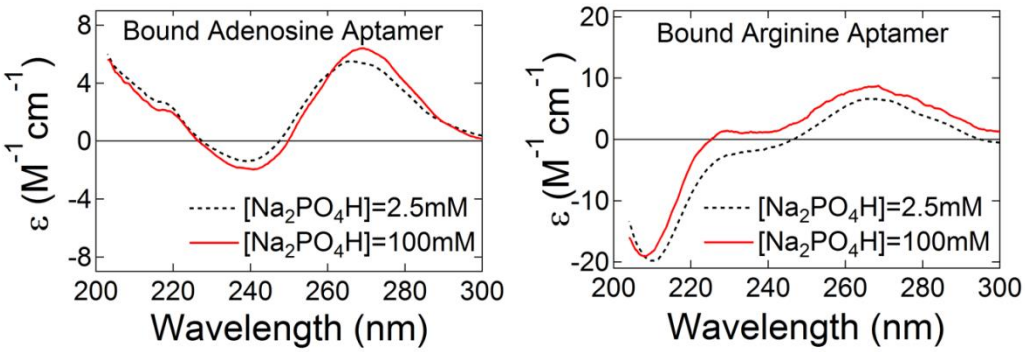

Figure S5 – CD spectra of aptamers bound to their appropriate target at varying ionic strength

Image Analysis

Polarized light microscopy images were analyzed using ImageJ (NIH Freeware) to determine the fractional increase in fractional homeotropic (f_H) (or planar [f_P]) area upon addition of target. The images were first binarized using a common threshold value that allowed for a qualitative distinction between birefringent (bright) and homeotropic (dark) regions. It is noted that this binarization did not provide a pure measure of the fractional homeotropic area, since azimuthal orientation of the LC around defects resulted in extinction. However, normalizing the fraction of dark pixels within a pore of the grid by the average fraction of dark pixels at c_o and at saturation accounted for the dark pixels that were not due to homeotropic LC orientation. This normalization was confirmed through qualitative inspection to verify that at c_o there were no homeotropic domains and at saturation the grids were in fact 100% homeotropic. Equation S1 was used to calculate the fractional homeotropic area at a given concentration (c), where f_x = fraction of dark pixels at c, f_o = fraction of dark pixels at c_o, and f_f= fraction of dark pixels at saturation. To calculate f_P we replaced all dark pixel fractions with bright pixel fractions in equation S1.

$$f_H = \frac{f_x - f_o}{f_f - f_o} \qquad (S1)$$

LC Response Specificity

The specificity of the LC response was tested qualitatively through visual inspection of polarized light microscopy images and quantitatively via measuring the time dependence of f_H. As stated in the main text the LC reorientation for the adenosine and arginine aptamer were found to be specific to their appropriate target. Figure S3 shows images that supplement the plots displaying the time dependence of f_H (Figure 1c, Figure S4). While we were able to consistently achieve a specific response for both aptamers under the appropriate conditions, the specificity of the adenosine aptamer was mildly sensitive to pH. At pH<7, a slight response to GMP was observed. However, this response observed to GMP was consistently less than that to adenosine. For example, in a sample that allowed for a transition to 100% homeotropic coverage upon addition of adenosine, the LC reorientation upon addition of GMP (at low pH) caused nucleation of homeotropic domains but the steady state homeotropic coverage was only ~20-40% of that observed for adenosine. This indicated a finite dissociation constant between GMP and the aptamer at pH<7 while at pH>7 the dissociation constant was so large that no LC reorientation was observed (due to minimal association of GMP-aptamer complexes). The dissociation constant likely varied with pH since guanosine is deprotonated at high pH (pKa≈9)[1]. This deprotonation may have induced an electrostatic repulsion between the negatively charged DNA and guanosine. In contrast, adenosine was not deprotonated at high pH and remained neutral, explaining the specificity of aptamer binding at pH>7.

CD Spectroscopy Analysis

CD spectroscopy reports how circularly polarized light interacts with chiral molecules. As such, conformational inferences of polymeric molecules can be made by comparing experimental CD spectra to the spectra of well-known structures. CD has been extensively used for measuring conformations of proteins[2] and nucleic acids[3] and models have been developed to try and correlate the observed CD spectra with a well-defined structure.[2,4,5] While these models for calculating the CD spectra of nucleic acids have been successful in some cases[4-6], a comprehensive strategy for extrapolating high resolution structures from CD spectra of nucleic acids has yet to be realized, especially when studying aptamer-ligand complexes. It is well known that base stacking, and consequently DNA sequence, is one of the major contributors to the CD spectra of nucleic acids.[4,5,7] While this area has been well studied, the contribution of non-Watson-Crick base pairing or interactions with ligands is not as well studied, making it difficult to apply a model for structures that involve these types of interactions (ie. aptamer-ligand complexes). For these reasons, the structural inferences we make here, involve comparing the spectra of known nucleic acid conformations to our experimental data.

In the main text we inferred from the CD spectra of the free adenosine and arginine aptamer at high and low ionic strength that the adenosine aptamer was in a random coil at low ionic strength and a weak hairpin at high ionic strength, while the arginine aptamer was in a random coil at low and high ionic strength. The CD spectra of the free adenosine aptamer upon increasing the ionic strength revealed the appearance of a shoulder at ≈210nm, a decrease in the negative peak at ≈240nm, and an increase and shift of the positive peak at ≈270nm. While the exact spectral

shifts are highly dependent on sequence, the appearance of a shoulder at ≈210nm and the shift of the peak at ≈270nm upon ligand binding were consistent with previous studies of the CD spectral changes that occur during DNA melting[3]. Thus, we concluded that the adenosine aptamer forms a weak hairpin at this increased ionic strength. The CD spectra of the free arginine aptamer at high and low ionic strength revealed no significant spectral differences. At low ionic strength (2.5mM [Na_2PO_4H]) we expected minimal electrostatic screening and consequently a random coil configuration. As we increased the ionic strength to 100mM, it was unclear what configuration to expect since there was a potential for significant electrostatic screening, but since we observed no spectral shifts at this ionic strength we conclude that the ssRNA remained in a random coil configuration even at increased ionic strength. Furthermore, the observed spectra is consistent with others previously reported for ssRNA.[7]

We also measured the CD spectra of these aptamers in the presence of ligand at ≈10K_D (Figure S5). For both aptamers we observed dramatic shifts in the CD spectra at high and low ionic strength, consistent with previously reported crystal structures. Upon binding, the adenosine aptamer is known to undergo Watson-Crick base-pairing at its tails and form a G-quadraplex structure at its head.[8] These types of conformational changes are known to induce large CD spectral shifts.[3-5] However, the CD spectrum expected from the aptamer-ligand complex will be an average of the contributions from the double helix structure and the G-quadraplex structure, thus a theoretical spectrum of this structure would be speculative and provide little value over an empirical comparison. The spectral shifts we observed upon addition of adenosine, at low and high ionic strength, involved an increase and shift to lower wavelength in the positive peak at ≈270nm, a decrease in the negative peak at ≈260nm, and an increased CD signal at λ<210nm.

Previous CD spectral studies of DNA melting[3] are consistent with the spectral shifts of the peak at ≈270nm, as mentioned above. The literature on the CD spectra of the G-quadraplex[4,5] known to form for the adenosine aptamer (antiparallel)[8] are consistent with our observation of a decrease in the negative peak at ≈240nm and an increase in the positive peak at ≈270nm. It is also noted that the CD spectral shifts that occur upon G-quadraplex formation are more dramatic than those that occur upon helix formation, explaining why we still see a dramatic change in the CD spectra at ionic strengths (≈100mM [Na_2PO_4H]) where we expect that ligand binding does not induce significant Watson-Crick base pairing. The arginine aptamer revealed a decrease in the negative peak at ≈205nm upon ligand binding. This shift occurred at high and low ionic strength, consistent with ligand binding under these conditions. CD studies of RNA have revealed that an increased intensity of the negative CD peak at ≈205nm is consistent with Watson-Crick base pairing.[7] The spectral shifts of the base pairing are highly dependent on sequence and usually also involve an increase in the CD peak at ≈265nm. However, the structural changes that are known to occur do not purely involve base pairing but rather involve hydrogen bonding of the bases in the binding pocket with arginine as well as base pairing in other parts of the RNA strand.[9] Nevertheless, the increased intensity of the negative CD peak at ≈205nm is consistent with calculations and observations from the literature and is a good indication of ligand binding to the arginine aptamer.

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