Supplemental Data

Structural Basis for Specific, High-Affinity Tetracycline Binding by an In Vitro Evolved Aptamer and Artificial Riboswitch

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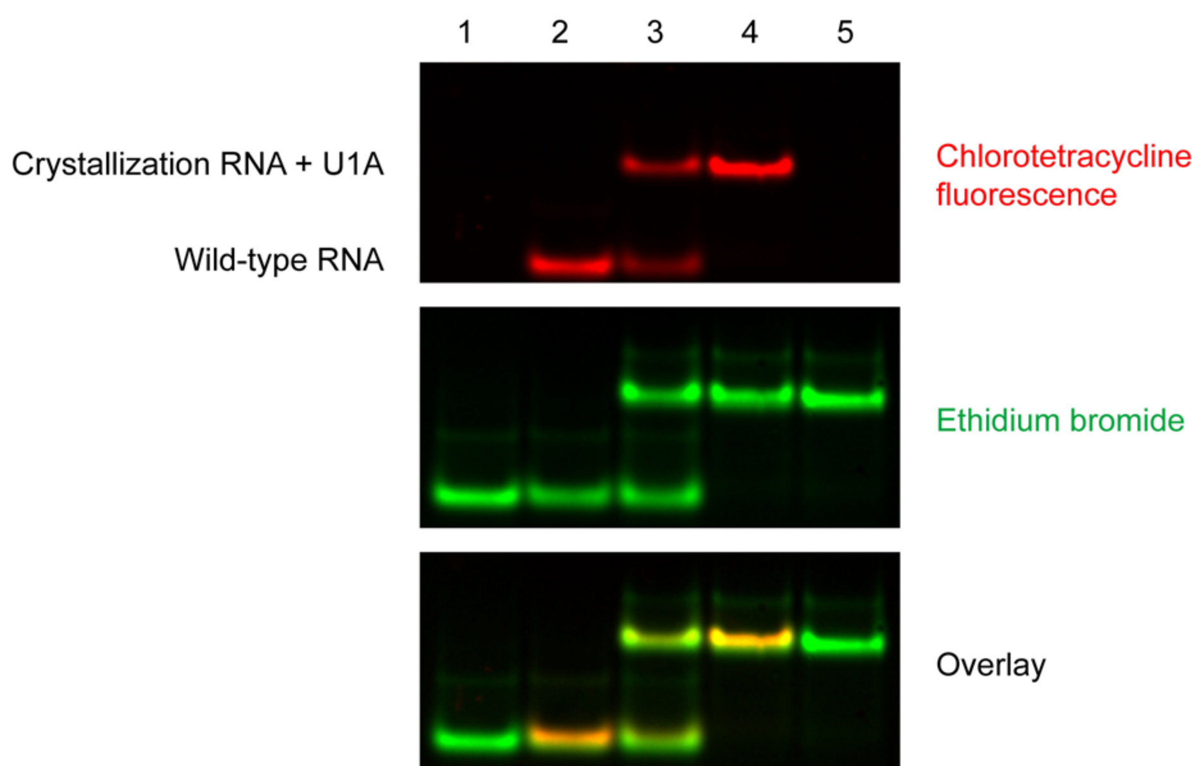

Figure S1. Comparison of Tetracycline Binding by Wild-Type (A) and Circularly-Permuted (B) Tetracycline Aptamer RNAs

Binding reactions contained, per lane, 20 µM RNA (20 µM of each of the two RNAs in lane 3). Lanes 2, 3, and 4 also contained 10 µM 7-chlorotetracycline. Lanes with the permuted crystallization RNA (lanes 3-5) also contained 20 µM U1A-RBD. Reactions were analyzed by native polyacrylamide gel electrophoresis (reaction and electrophoresis conditions are in Experimental Procedures). After electrophoresis, the gel was placed on an UV transilluminator (λ_{max} ~ 350 nM) and the intrinsic fluorescence of the bound chlorotetracycline in the visible spectrum recorded with a digital camera insensitive to UV light. The gel was then stained with aqueous ethidium bromide (0.01 % w/v) for 5 minutes, and the RNAs imaged in the same manner. Migration positions for the circularly permuted crystallization RNA in complex with U1A protein, and for the wild-type RNA are indicated on the left. For clarity, false colors are used in the three panels. The slight discrepancy in the positions of the lanes evident in the overlay results from swelling of the gel during ethidium bromide staining. The intensity of the two bands on lane 3 indicates that the affinity of the wild-type RNA and the crystallization RNA-protein complex for 7-chlorotetracycline are comparable.

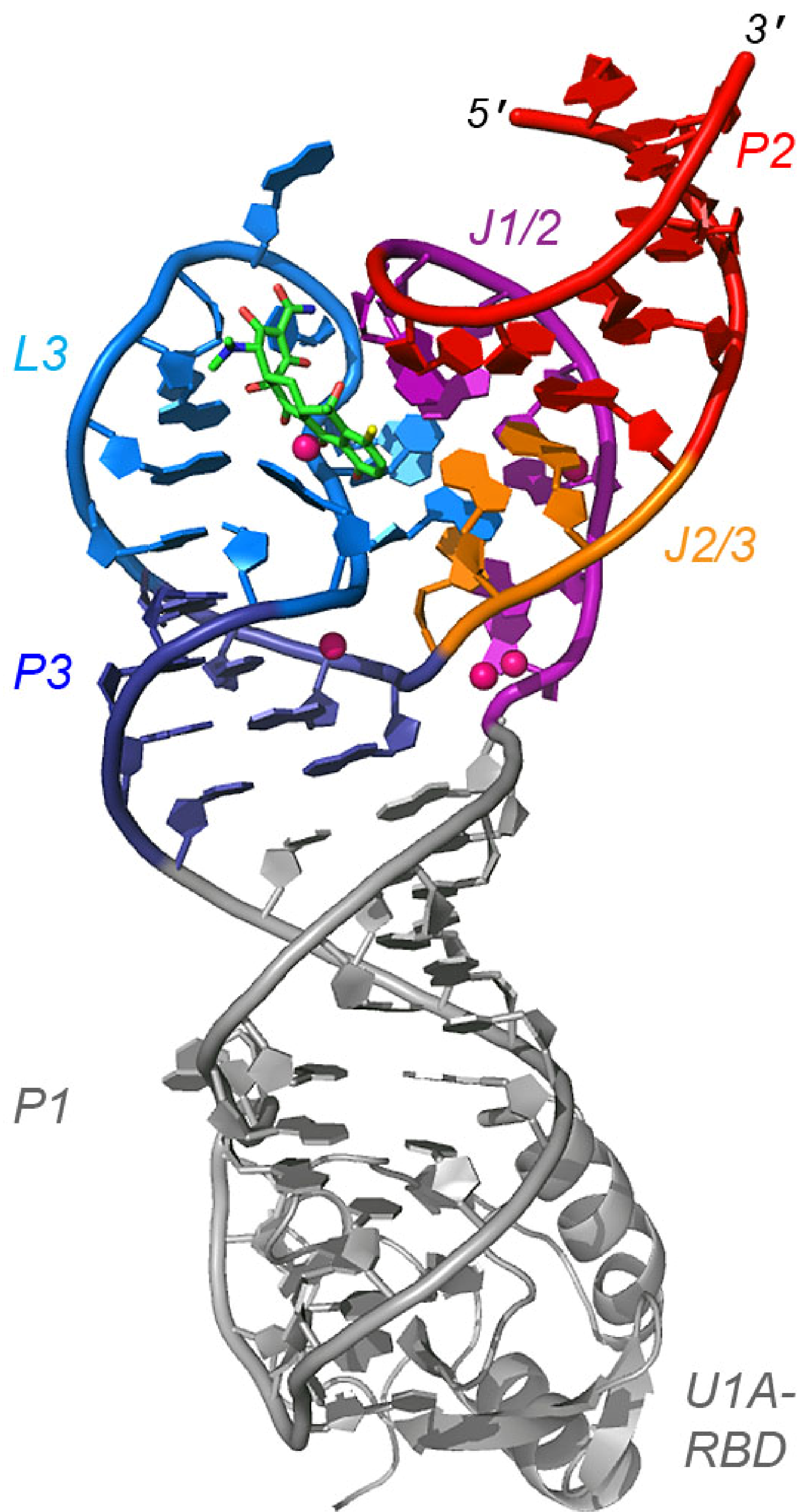
3′
5′
P2
J1/2
L3
J2/3
P3
P1
U1A-
RBD

Figure S2. Cartoon Representation (DeLano, 2002) of the Full Crystallographic Model, Excluding Bound Waters and Some Cations, of the Cocrystal Structure of the Tetracycline Aptamer, Depicting the U1A RNA Binding Site and the U1A-RBD Protein

The crystallization RNA construct is a circular permutation of the original cb28 aptamer of Berens et al. (2001). Cb28 comprises a loop (L2) closing the distal end of P2, and lacks a loop closing P1. Color coding as in Figure 1.

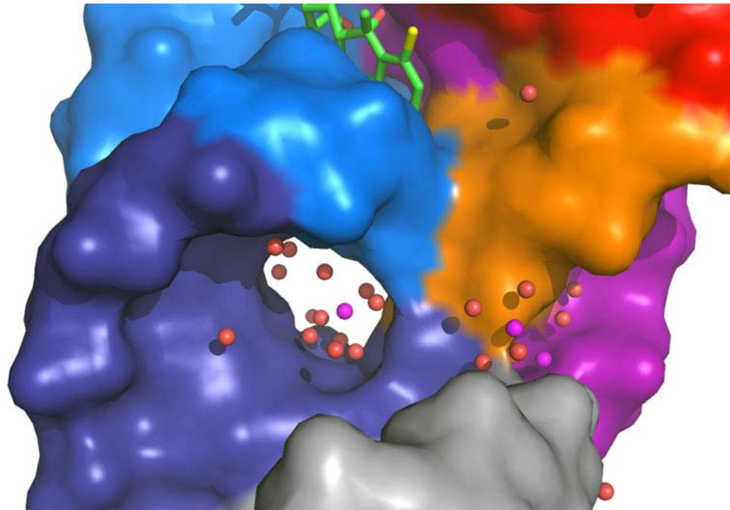

Figure S3. Molecular Surface Representation of the Tetracycline Aptamer and Artificial Riboswitch

Metal ions and water molecules are depicted as magenta and red non-bonded spheres, respectively. This figure emphasizes the hole at the center of the h-shaped RNA (the major groove of L3), which is occupied by waters and a hydrated magnesium ion.

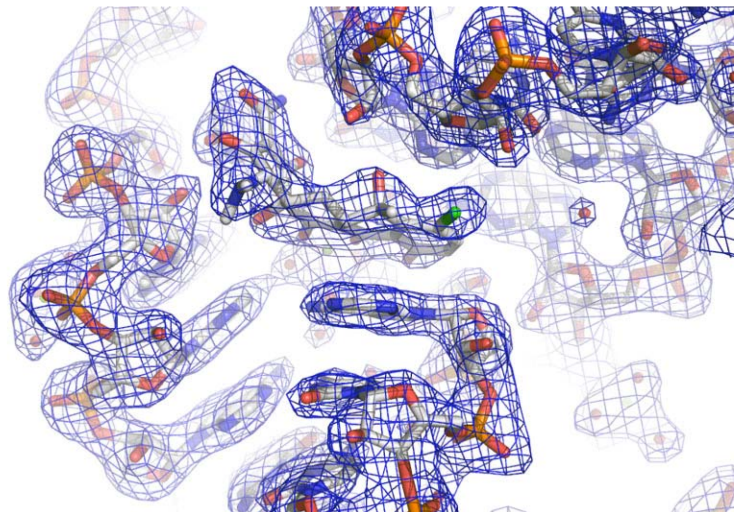

Figure S4. Portion of the Final Composite Anneal Omit $2|F_o|-|F_c|$ Electron Density Map Contoured at 1.0 S.D., Superimposed on the Final Crystallographic Model, Colored in CPK Convention

Supplemental References

Berens, C., Thain, A., and Schroeder, R. (2001). A tetracycline-binding RNA aptamer. Bioorg Med Chem *9*, 2549-2556.

DeLano, W.L. (2002). The PyMOL Molecular Graphics System (San Carlos: DeLano Scientific).