Supplementary Information for

In silico design and validation of high-affinity RNA aptamers targeting epithelial cellular adhesion molecule dimers

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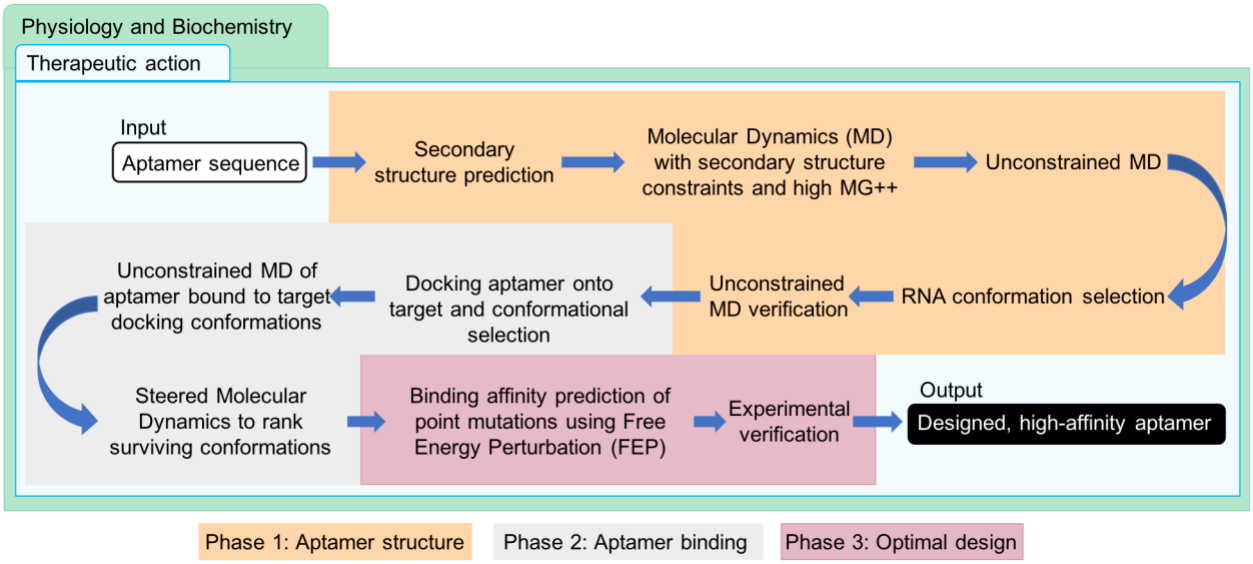

Figure S1. Aptamer design framework. Procedure followed herein to design high affinity RNA aptamers with physical simulations. Three phases were defined including constructing the aptamer structures, predicting aptamer binding conformations, and carrying the aptamer through mutations to optimize binding. In all steps of the pathway, knowledge of system physiology and biochemistry as well as the desired therapeutic action were considered for appropriate aptamer design.

A

Parameters	Ep23	A5U	G15U
Kd (nM)	39.89 ± 3.37	10.78 ± 1.37	11.91 ± 0.95
ΔG (kcal/mol)	-10.10 ± 0.04	-10.87 ± 0.07	-10.81 ± 0.05
ΔH (kcal/mol)	-17.15 ± 0.23	-17.28 ± 0.74	-17.52 ± 0.74
-TΔS (kcal/mol)	7.05 ± 0.24	6.40 ± 0.76	6.71 ± 0.78
N (sites)	0.469 ± 0.02	0.474 ± 0.02	0.458 ± 0.02

B

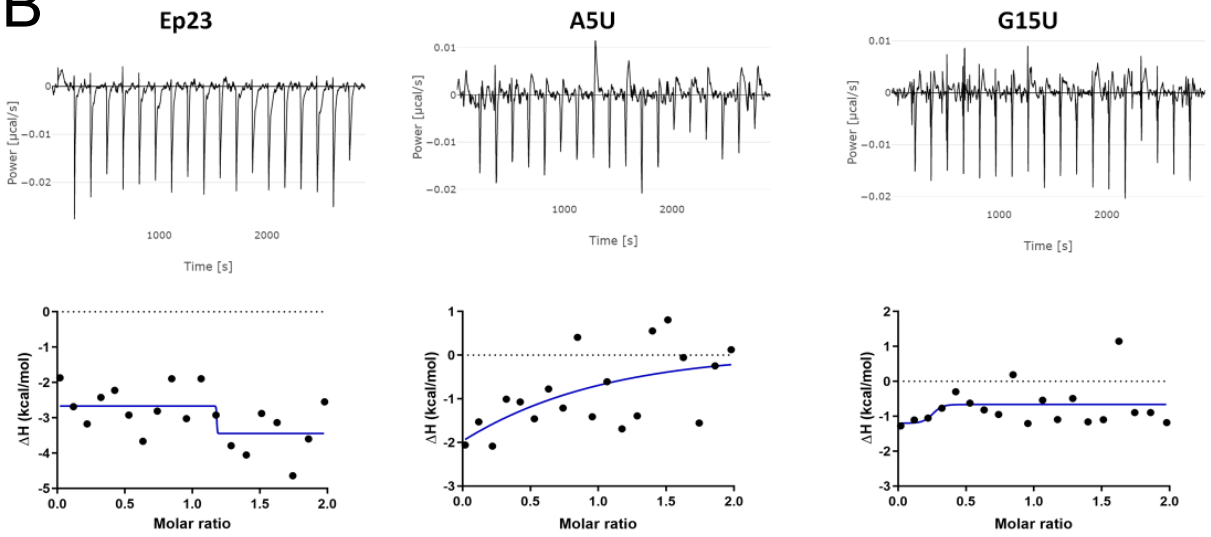

Figure S2. ITC thermodynamic parameters and experimental control. (A) Thermodynamic parameters determined from ITC experiments. Data shown are mean ± 95% CI. **(B)** Isothermal titration calorimetric analysis of the interaction of the 50 μM aptamer solutions from EP23, A5U or G15U with bovine serum albumin (5 μM) in ITC buffer at 25 ℃. The top and bottom panels show raw data and binding isotherm obtained over a series of injections of aptamer into EpCAM protein or BSA solution, respectively. The differential power (DP) (μcal/sec) versus time is presented in the form of integrated heat values in the top panels. The data were fitted using a one binding site model.

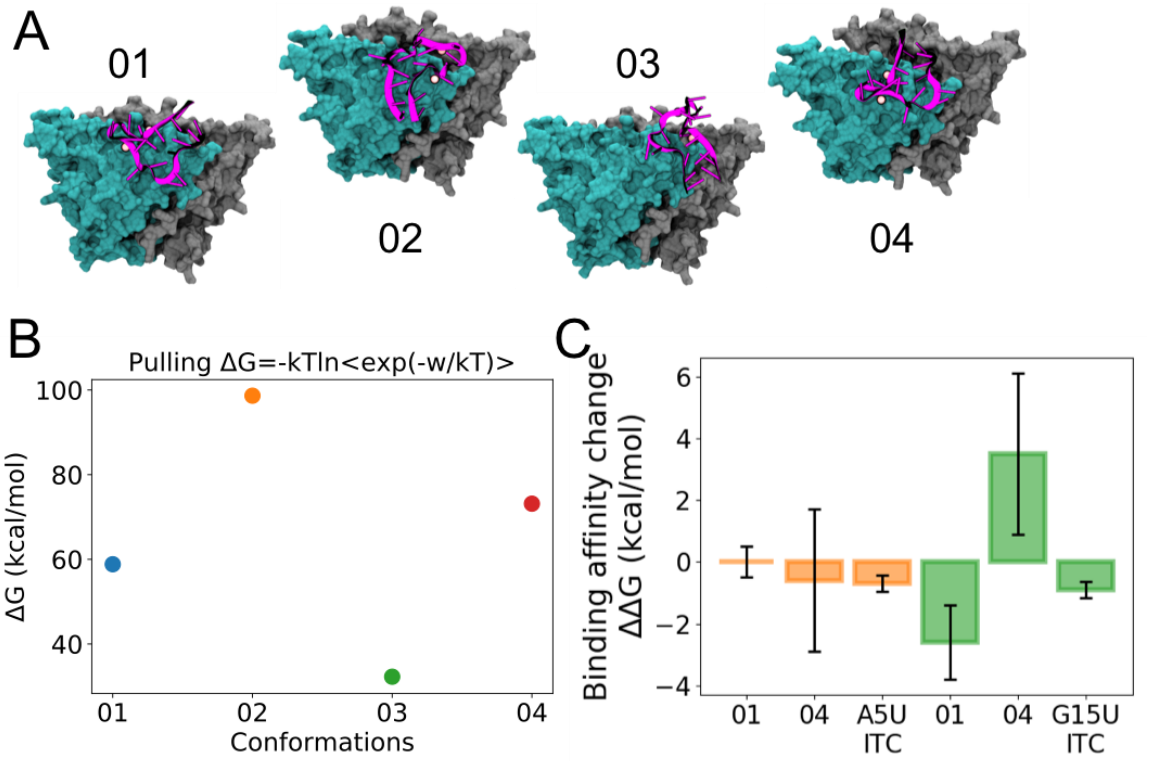

Figure S3. **Non-dominant EpCAM dimer binding conformations and mutational free energy. (A)** Four unique docking conformations from the top 10 docking conformations selected for further binding assessment. EpCAM dimer is shown in a surface representation and colored by protein monomer: cyan and gray, while the EP23 aptamer is illustrated as a cartoon in magenta with bound Mg^{2+} ions shown as pink spheres. **(B)** The work required to pull the EP23 conformation off of EpCAM dimer, with larger work showing stronger binding. Values shown are the mean, standard error bars are shown but within the data points. **(C)** Binding affinity values for conformations 01 and 04 compared to ITC experimental values. Orange is A5U and green is G15U mutations. Note that the 03 conformation unbound during MD simulations and was thus omitted from FEP affinity calculations. **(A-B)** are adapted from the manuscript Figure 3.