

Automated Design of Protein-binding Riboswitches for Sensing Human Biomarkers in a Cell-free Expression System

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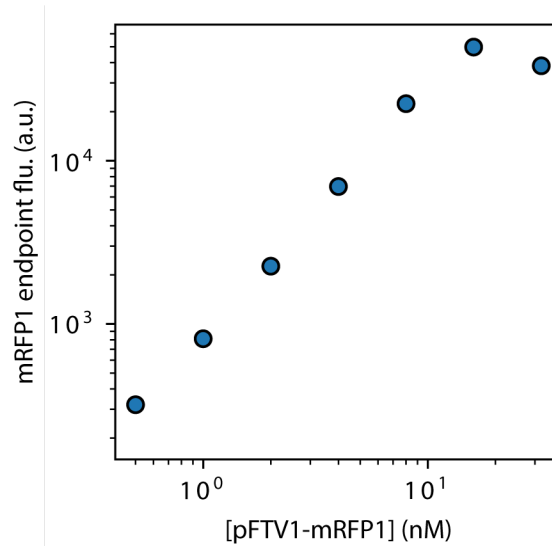
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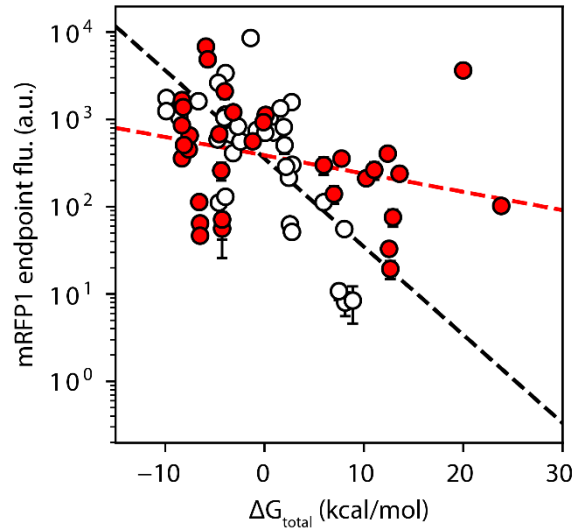
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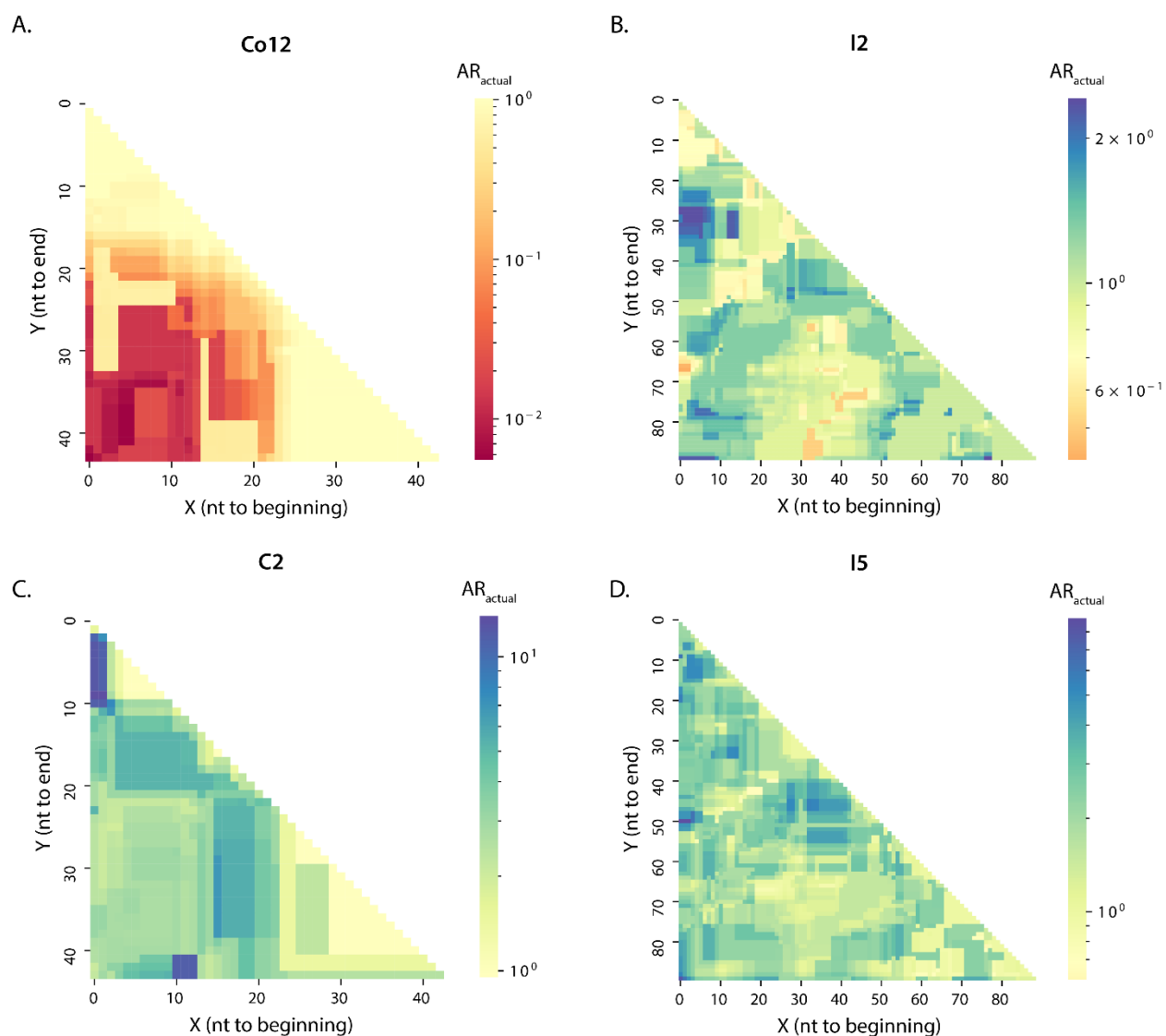
Supplementary Figures 1 to 3.



Supplementary Figure 1. mRFP1 fluorescence vs. [pFTV1-mRFP1]. Mean fluorescence levels (blue circles) are proportional to mRFP1-expressing plasmid concentration up to 32 nM. N = 2 biological replicates.



Supplementary Figure 2. mRFP1 fluorescence levels vs. predicted binding free energies controlling translation initiation rates for all 35 riboswitches. The Riboswitch Calculator predicted the binding free energies (ΔG_{total}) of the ribosome-mRNA interactions controlling translation initiation rates for riboswitches in the uninduced and protein-induced states. White circles are mean uninduced mRFP1 endpoint fluorescence levels, while red circles are mean induced mRFP1 fluorescence levels. Dotted lines show the best-fit log-linear line, $\log(\text{mRFP1 endpoint flu.}) = \log(K) - \beta \Delta G_{\text{total}}$, where the parameter K is a proportionality constant and β is the Boltzmann constant relating free energies to state probabilities. The black dotted line corresponds to uninduced riboswitch fluorescence levels ($R^2 = 0.48$). The red dotted line corresponds to induced riboswitch fluorescence levels ($R^2 = 0.10$). The parameter β was previously determined to be 0.45 ± 0.05 in *in vivo* systems across diverse bacterial species (Salis et. al. 2009). In this cell-free, *in vitro* system, we found that β is 0.23, indicating a reduction in the dynamic range of cell-free translation. $R^2 = 0.48$ for uninduced riboswitches. Error bars represent 95% confidence interval ($N = 6$ biological replicates for MS2 ON-riboswitches, $N = 8$ biological replicates for all other riboswitches).



Supplementary Figure 3. The effect of varying the aptamer structural constraint region on mCRP and IL32 γ riboswitch predictions. The structural constraint of the aptamer region of riboswitches Co12, C2, I2, and I5 were varied, and the effects on the predicted translation rate ratios (AR_{actual}) were calculated. For all, X represents the distance from the start of the full aptamer sequence to the beginning of the sub-constrained region, and Y represents the distance from the start of the full aptamer sequence to the end of the sub-constrained region. **A.** The effect of varying the size of the mCRP aptamer structural constraint in riboswitch Co12. **B.** The effect of varying the size of the IL32 γ aptamer structural constraint in riboswitch I2. **C.** The effect of varying the size of the mCRP aptamer structural constraint in riboswitch C2. **D.** The effect of varying the size of the IL32 γ aptamer structural constraint in riboswitch I5.