




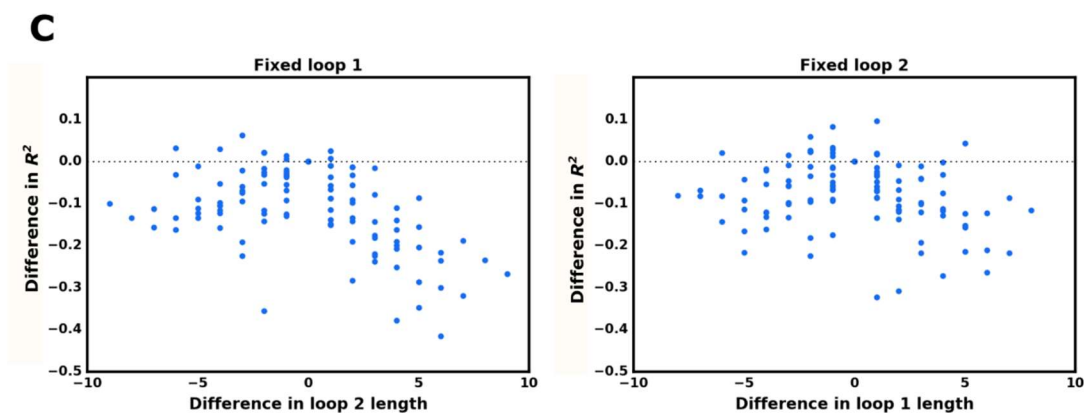


B

Predicting the activity of ribozymes in 

	Difference in loop 1 length	Difference in loop 2 length	$R^2$	Difference in $R^2$		
Training set		0	0	0.72	0.00	
		0	+1	0.32	-0.40	Fixed loop 1
		-4	+4	Not recorded		
		-1	0	0.66	-0.06	Fixed loop 2



**Figure 4-figure supplement 3. Model accuracy as a function of the similarity of structures in the test set to those in the training set.** A) Workflow for the evaluation of the model's accuracy when predicting the basal gene-regulatory value of ribozymes whose structure differs from the structures in the training set in one of the two loops. The sequences in the experimental dataset are first grouped into different segments based on the size of the loops and the stems in the computationally-predicted secondary structure such that each ribozyme in a segment shares the same structure. The gene-regulatory activity levels of the sequences in one segment are predicted using a model

trained on the sequences in another segment that shares both stem lengths and 1 loop length. This prediction is compared to the experimental data to determine the accuracy, as measured by the  $R^2$  value, of predicting that segment when the training set has one loop of different length. This process is repeated using each segment as the test segment, recording the accuracy and difference in loop length. B) Example for predicting a test segment using the individual training segments. First, the test segment (red) is sub-segmented into test and training sequences, and used to generate a baseline  $R^2$  of predicting the test segment when sequences in the training set have no difference in loop 1 or loop 2 length. The first training segment (teal), which fixes the length of loop 1 but not loop 2, is used to generate an  $R^2$  of predicting the test segment, which is compared to the  $R^2$  of the self-prediction to generate a difference in  $R^2$  when loop 2 length differs. The next training segment (light blue) is ignored because it fixes neither loop 1 or loop 2 lengths in relation to the test segment. The final training segment (dark blue) undergoes the same process as the teal training segment to measure how the accuracy changes when sequences in the training segment have a fixed loop 2 length, but loop 1 length differs. C) Plotting the model's accuracy as a function of the difference in the length of loops 1 or 2 between sequences in the training set and those in the test set. The individual points represent the difference in the  $R^2$  value for predicting ribozyme activity when ribozymes in the training set have a difference in loop length in one loop versus having the same loop size.