

Supplementary Materials

Functional RNAs exhibit tolerance for non-heritable 2'-5' vs. 3'-5' backbone heterogeneity*

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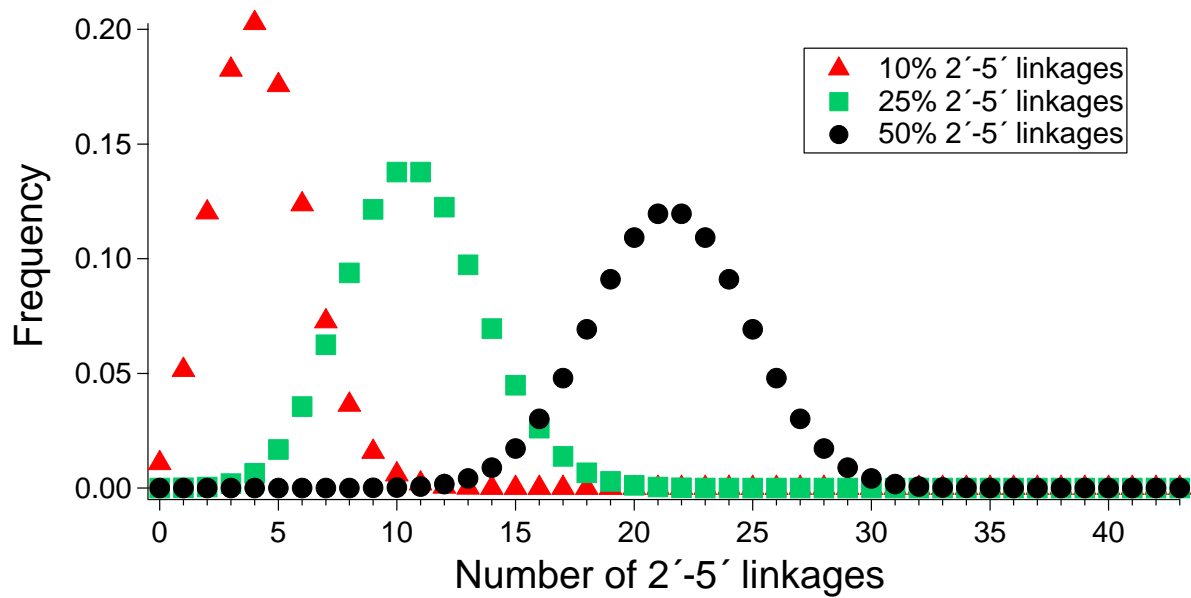
Supplementary Figure S1. Simulated binominal distributions of 2'-5' linkages in a) a 43-linkage half-functional RNA and b) a 5-linkage core of critical residues.

Supplementary Figure S2. ¹H-NMR spectrum (400MHz, D₂O) of H1' region of dinucleotides prepared from pure 2'-ODBTMS protected phosphoramidite, pure 3'-OTMBDS phosphoramidite, and a 1:1 mixture of the two phosphoramidites.

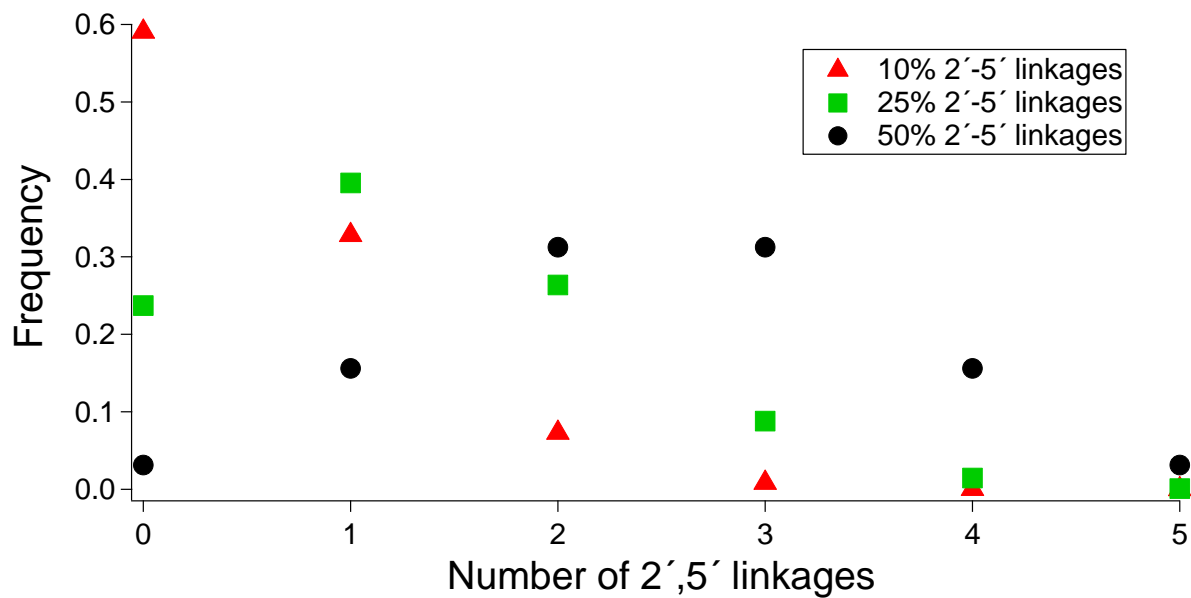
Supplementary Figure S3. Fluorescence titrations of 1 μM FMN with FMN aptamers.

Supplementary Table S1. HR-MS of synthesized RNAs.

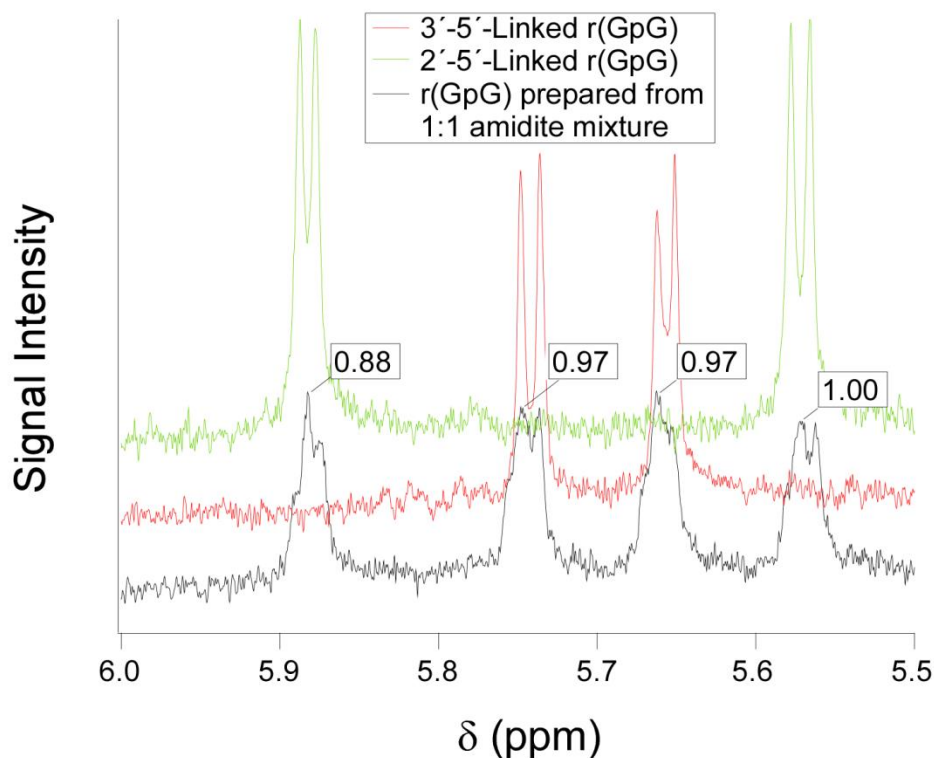
a)



b)

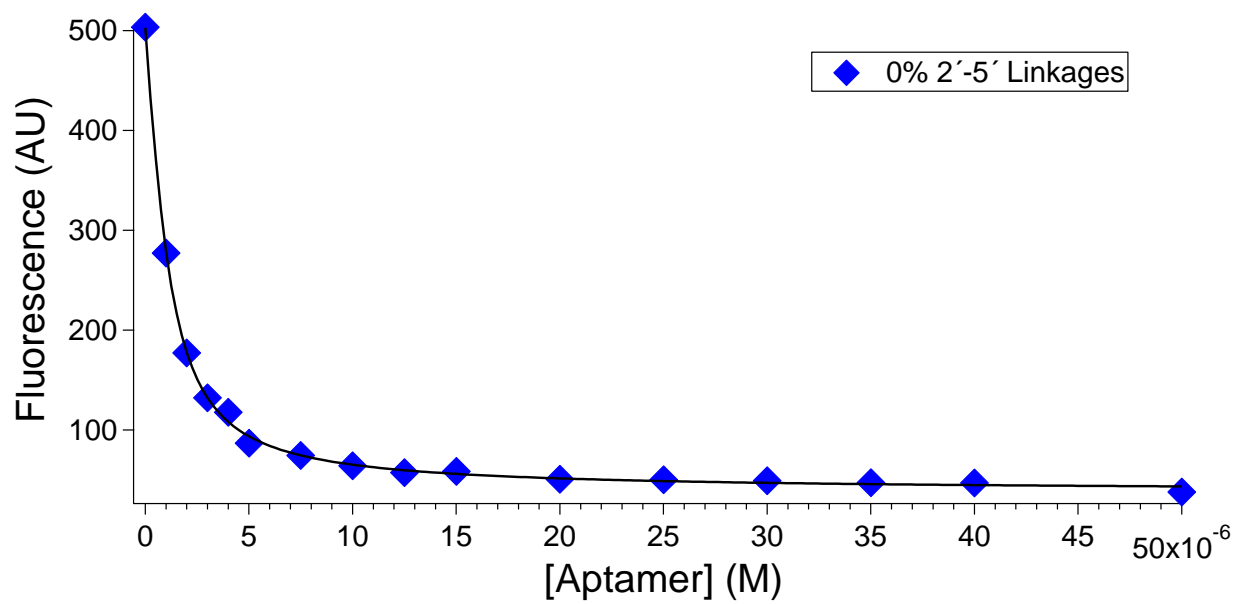


Supplementary Figure S1. Simulated binomial distributions of 2'-5' linkages in a) a 43-linkage functional RNA (i.e., the sum of the number of linkages found in **HH1** and **HH2**) and b) a 5-linkage core of critical residues (representative of a typical non-stem region, as found in, e.g., **FMN2**).

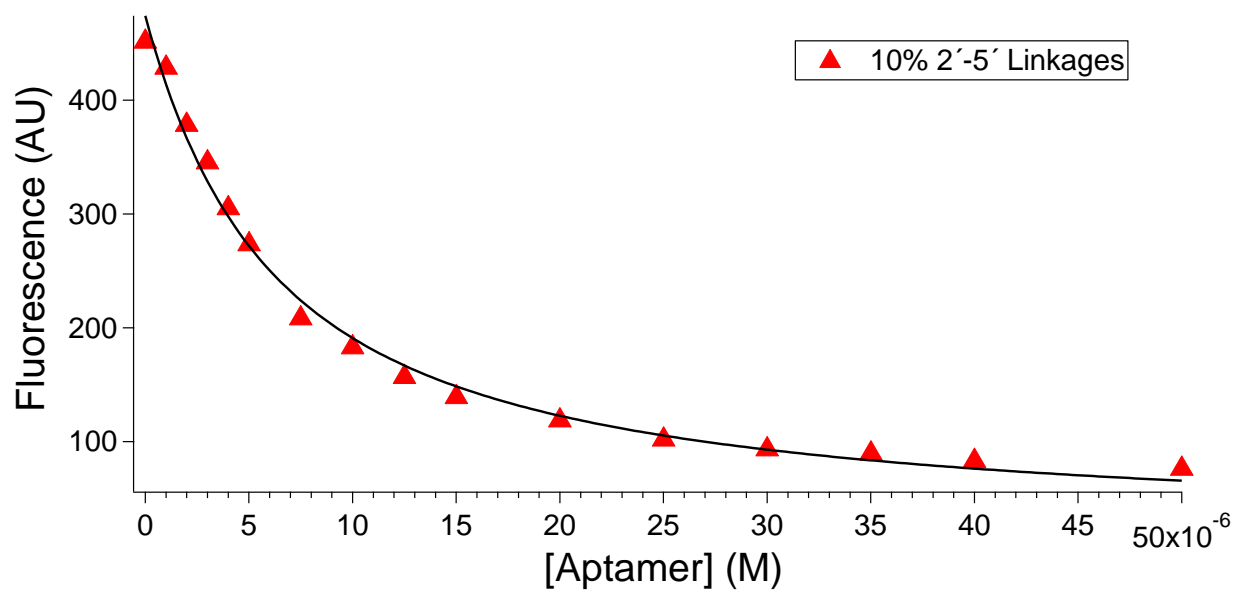


Supplementary Figure S2. ¹H-NMR spectrum (400MHz, D₂O) of H1' region of dinucleotides prepared from pure 2'-ODBTMS protected phosphoramidite, pure 3'-OTMBDS phosphoramidite, and a 1:1 mixture of the two phosphoramidites. Normalized, integrated peak intensities for the dinucleotide mixture prepared from mixed amidites are given as annotations.

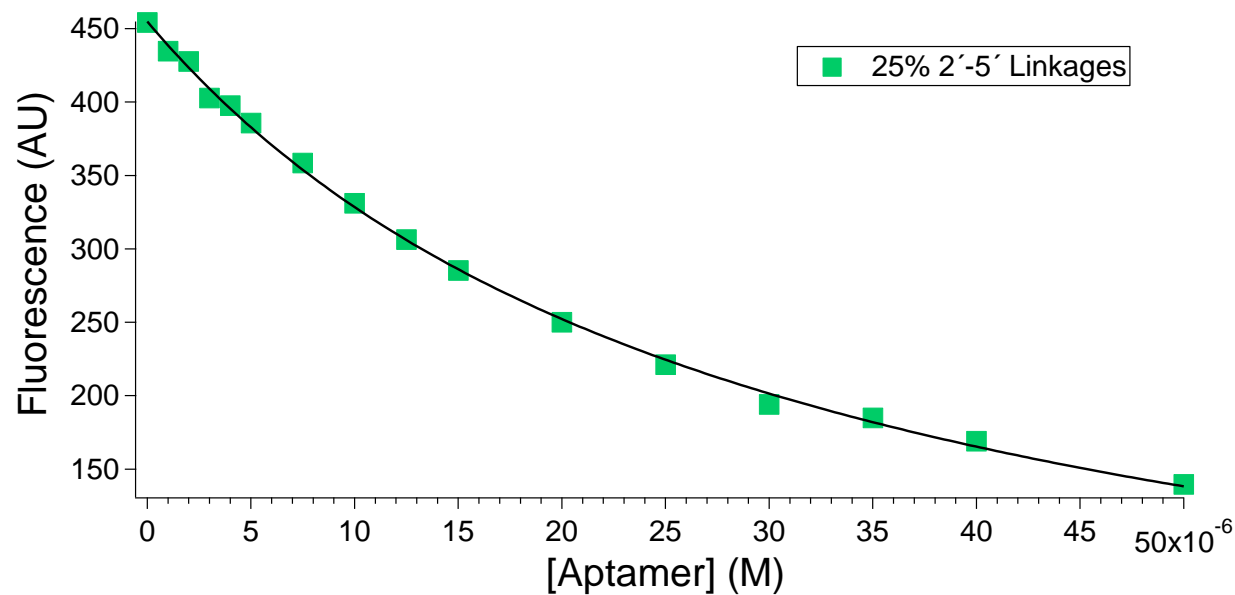
a)



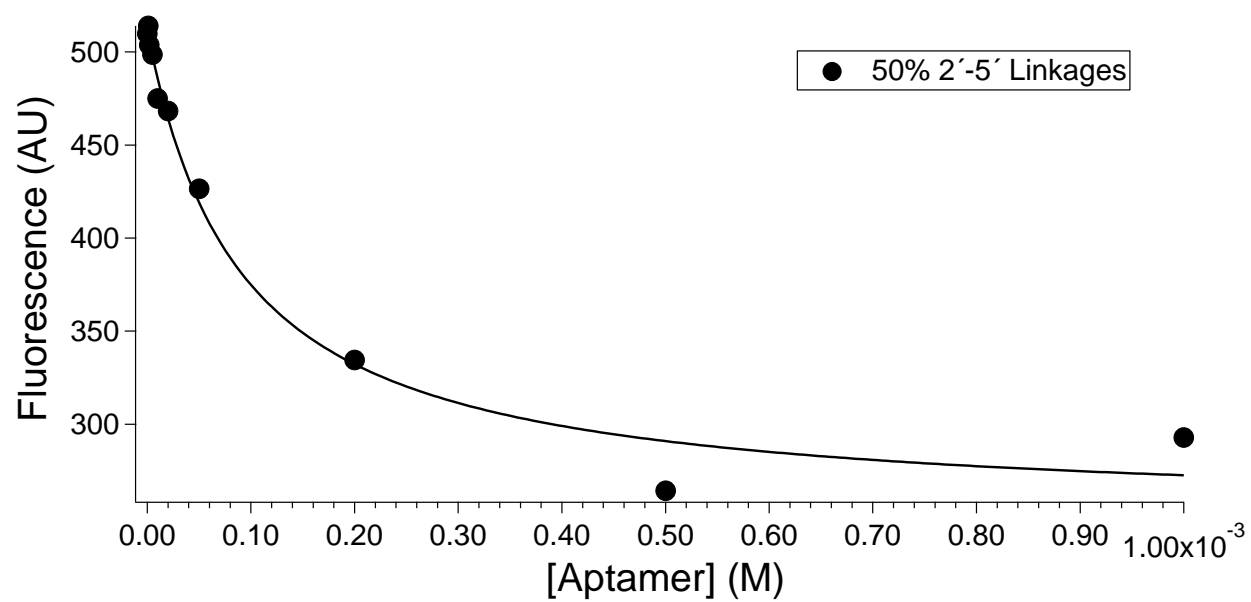
b)



c)



d)



Supplementary Figure S3. Fluorescence titrations of 1 μ M FMN with FMN aptamers with a) 0%, b) 10%, c) 25%, and d) 50% 2'-5' linkages. Curves represent fit to data.

Strand	Level of random 2'-5' substitution	Expected Mass	Actual Mass
FMN-1	0%	6482.906	6482.8861
FMN-1	10%	6482.906	6482.9050
FMN-1	25%	6482.906	6482.9087
FMN-1	50%	6482.906	6482.9081
FMN-2	0%	6095.884	6095.8724
FMN-2	10%	6095.884	6095.8903
FMN-2	25%	6095.884	6095.8894
FMN-2	50%	6095.884	6095.8930
HH-1	0%	8504.2571	8504.1738
HH-1-C19	n/a	8534.2571	8534.1095
HH-1	10%	8534.2571	8534.1656
HH-1	25%	8534.2571	8534.1615
HH-1	50%	8534.2571	8534.1815
HH-2	0%	6473.9111	6473.8903
HH-2-G13	n/a	6473.9111	6473.8738
HH-2	10%	6473.9111	6473.8947
HH-2	25%	6473.9111	6473.8919
HH-2	50%	6473.9111	6473.9566

Supplementary Table S1. HR-MS of synthesized RNAs. LC-MS was performed on an Agilent 1200 coupled to an Agilent 6520 Accurate-Mass Q-TOF LC/MS. Masses shown are the deconvoluted monoisotopic masses.