

Optimization of the split-Spinach aptamer for monitoring nanoparticle assembly involving multiple contiguous RNAs

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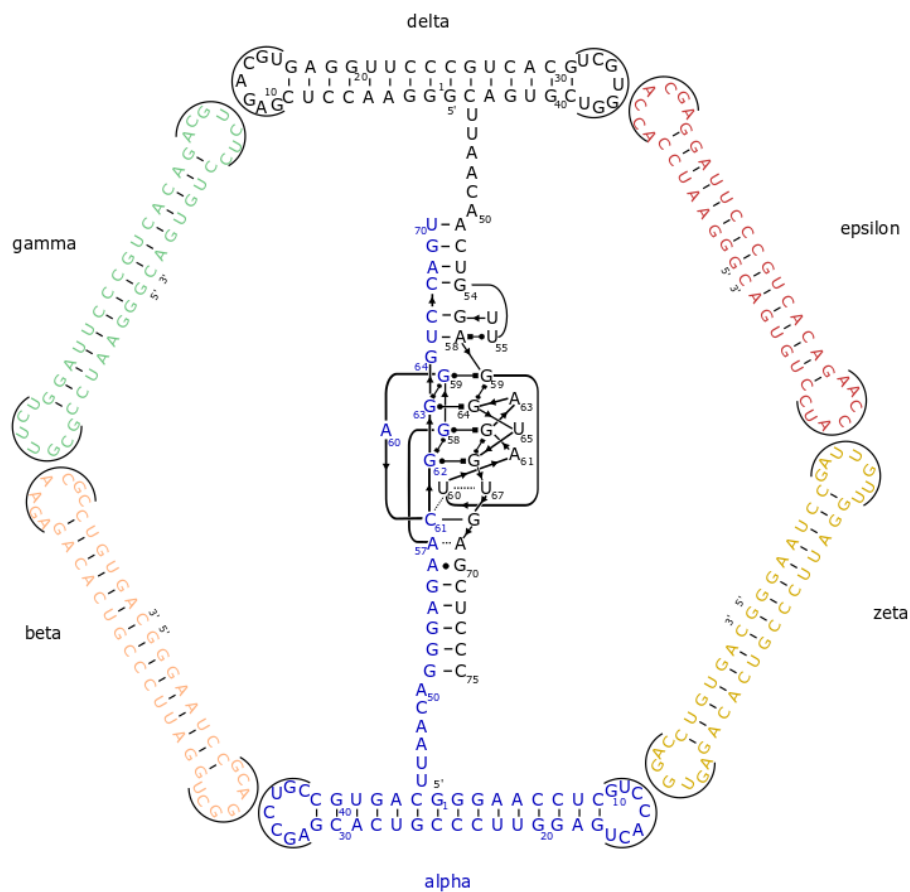


Figure S1. 2D sequences associated with the 5bp/6bp/6nt version of the split-aptamer integrated nanoring system.

Full-length Spinach control

5' - GGGAGAAGGACGGGUCCAGUGCCAAACA CGCACUGUUGAGUAGAGUGAGUCUCCC - 3'

Ring Struts

beta

5' - GGGAAcCuC GCGAGCU GaGgUUCcCGUCACG AGAACGC CGUGAC - 3'

gamma

5' - GGGAAcCuC GCGUUCU GaGgUUCcCGUCACG ACGUCUC CGUGAC - 3'

epsilon

5' - GGGAAcCuC ACCACGA GaGgUUCcCGUCACG AACCAUC CGUGAC - 3'

zeta

5' - GGGAAcCuC GAUGGUU GaGgUUCcCGUCACG AGUGGAC CGUGAC - 3'

Stem/linker exploration

5bp/6bp/6nt version of the split-aptamer

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGACUUAACA GGGAGAAGGACGGGUCCAGU 3'
5' GGGAAcCuC GAGACGU GAGGUUCCCGUCACGUCGUGGUCGUGACUUAACA ACUGUUGAGUAGAGUGAGUCUCCC 3'

5bp/7bp/6nt version of the split-aptamer

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGACUUAACA GGGAGAAGGACGGGUCCAGU 3'
5' GGGAAcCuC GAGACGU GAGGUUCCCGUCACGUCGUGGUCGUGACUUAACA CACUGUUGAGUAGAGUGAGUCUCCC 3'

5bp/7bp/5nt version of the split-aptamer

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGACUUAACA GGGAGAAGGACGGGUCCAGU 3'
5' GGGAAcCuC GAGACGU GAGGUUCCCGUCACGUCGUGGUCGUGACUUAACA CACUGUUGAGUAGAGUGAGUCUCCC 3'

alpha-strands tested

GGG-AGU

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGACUUAACA GGGAGAAGGACGGGUCCAGU 3'

GGA-AGU

5' GGGAAcGUC GUCCACU GACGUUCCCGUCACGAGCCUGCCGUGACUUAACA GGAAGAAGGACGGGUCCAGU 3'

GGG-GGU

5' GGGAAcGUC GUCCACU GACGUUCCCGACACCAGCCUGCGGUGUCUUAACA GGGAGAAGGACGGGUCCAGU 3'

GGG-AGU.1

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGAC AAUUUU GGGAGAAGGACGGGUCCAGU 3'

GGG-AG_.1

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGAC AAUUUU GGGAGAAGGACGGGUCCAG. 3'

GGG-AGC.2

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGACUUAACU GGGAGAAGGACGGGUCCAGC 3'

GGG-AGC.3

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGACUUAACU GGGAGAAGGACGGGUCCAGC 3'

GG-AGC.1

5' GGGAAcCuC GUCCACU GAGGUUCCCGUCACGAGCCUGCCGUGAC AAUUUU GGGAGAAGGACGGGUCCAGC 3'

CCU-AGC

5' GGGAAcGUC GUCCACU GACGUUCCCGUCACGAGCCUGCCGUGACUUAACA CCUAGAAGGACGGGUCCAGC 3'

CCC-GGU

5' GGGAAcGUC GUCCACU GACGUUCCCGUCACGAGCCUGCCGUGACUUAACA CCCAGAAGGACGGGUCCAGU 3'

CCC-AGU

5' GGAACGUCGUCCACUGACGUUCCCGUCACGAGCCUGCCGUGACUUAACACCUAGAAGGACGGGUCCAGU3'

CCU-AGU

5' GGAACGUCGUCCACUGACGUUCCCGUCACGAGCCUGCCGUGACUUAACACCUAGAAGGACGGGUCCAGU3'

CCU-AGU

5' GGAACGUCGUCCACUGACGUUCCCGUCACGAGCCUGCCGUGACUUAACACCUAGAAGGACGGGUCCAGU3'

delta-strands tested

ACU-CCC5'

GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACUUAACAACUGUUGAGUAGAGUGAGCUCCC3'

ACU-UCC5'

GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACUUAACAACUGUUGAGUAGAGUGAGCUUCC3'

ACU-CCC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACUGUUGAGUAGAGUGAGCUCCC -3'

ACU-UCC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACUGUUGAGUAGAGUGAGCUUCC -3'

ACC-UCC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACCGUUGAGUAGAGUGAGCUUCC -3'

ACC-CCC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACCGUUGAGUAGAGUGAGCUCCC -3'

GCU-CCC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUGCUGUUGAGUAGAGUGAGCUCCC -3'

GCU-CC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUGCUGUUGAGUAGAGUGAGCUCC. -3'

ACU-CC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACUGUUGAGUAGAGUGAGCUCC. -3'

ACU-CC.1

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACUGUUGAGUAGAGUGAGCUCC. -3'

ACU-GGG.2

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACUGUUGAGUAGAGUGAGCUGGG -3'

ACU-AGG.2

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUACUGUUGAGUAGAGUGAGCUAGG -3'

GCU-GGG.2

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUGCUGUUGAGUAGAGUGAGCUGGG -3'

GUU-GGG.2

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGCAAAUUUUGUUGUUGAGUAGAGUGAGCUGGG -3'

GUU-AGG.2

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGCAAAUUUUGUUGUUGAGUAGAGUGAGCUAGG -3'

GCU-GG.2

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUGCUGUUGAGUAGAGUGAGCUGG. -3'

GCU-AGG.3

5' GGAACCUCGAGACGUGAGGUUCCCGUCACGUCGUGGUCGUGACAAUUUUGCUGUUGAGUAGAGUGAGCUAGG -3'

Table S1. Complete list of sequences used in study. Green highlights represent the portion of the Spinach aptamer that was appended to the alpha strand of the nanoring. Yellow highlights represent the portion of the aptamer appended to the delta strand. Light blue highlights show the kissing loop sequences associated with the programmable nanoring.

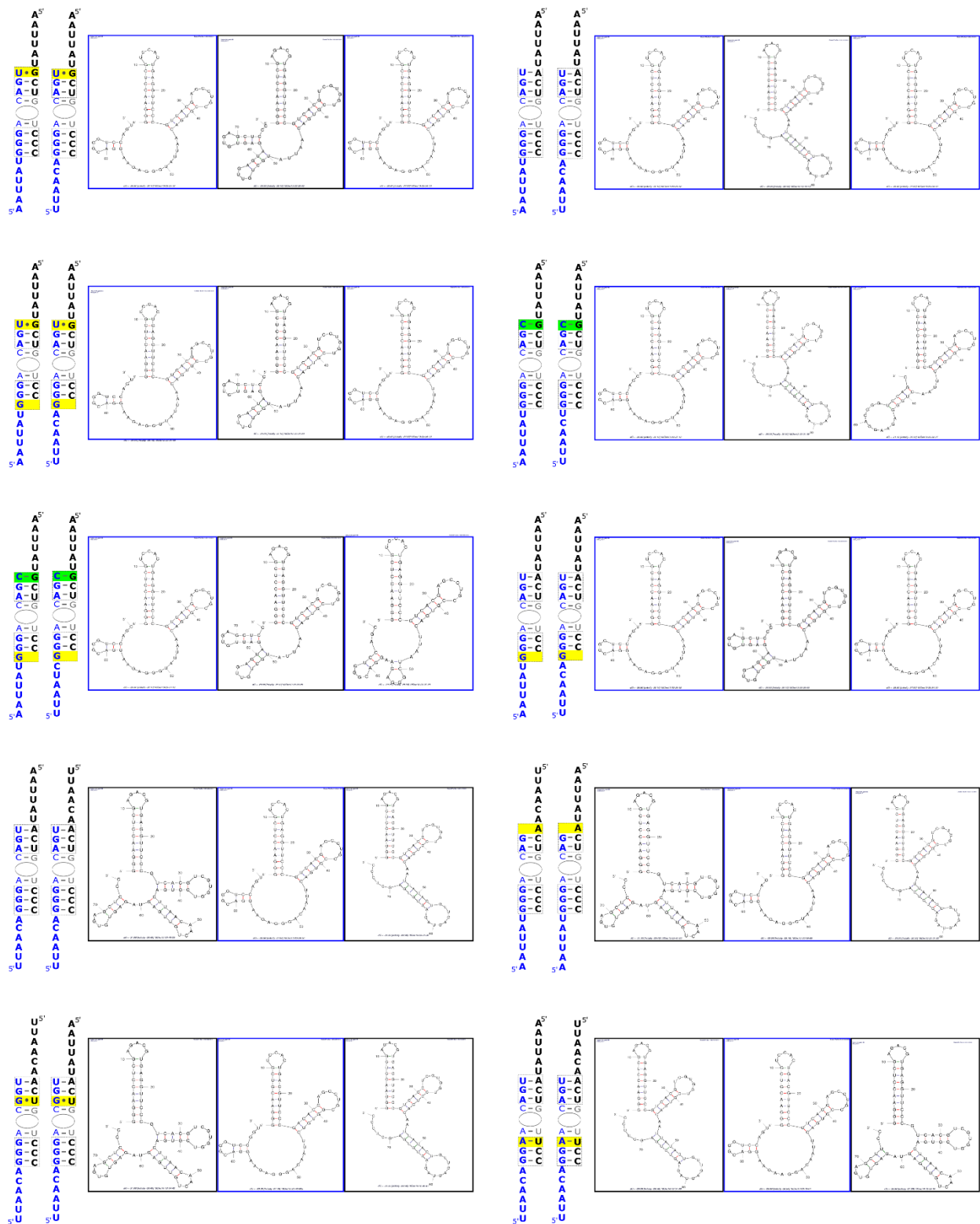


Figure S2. Predicted secondary structures for individual strands based on linker variants. The 2D structures were generated using the mfold server.¹ The two hairpins of 9- and 6-bp represent the struts of the nanoring where the blue boxes represent the alpha strand and black boxes represent the delta strand. The boxes in the middle of each grouping represent the strand that is shared between the two variants. The remaining portion represent the most stable predicted secondary structure of the linker and split-aptamer region. The predicted structures were evaluated to determine whether the linker prevented or facilitated additional secondary structure to sequester the aptamer strand.

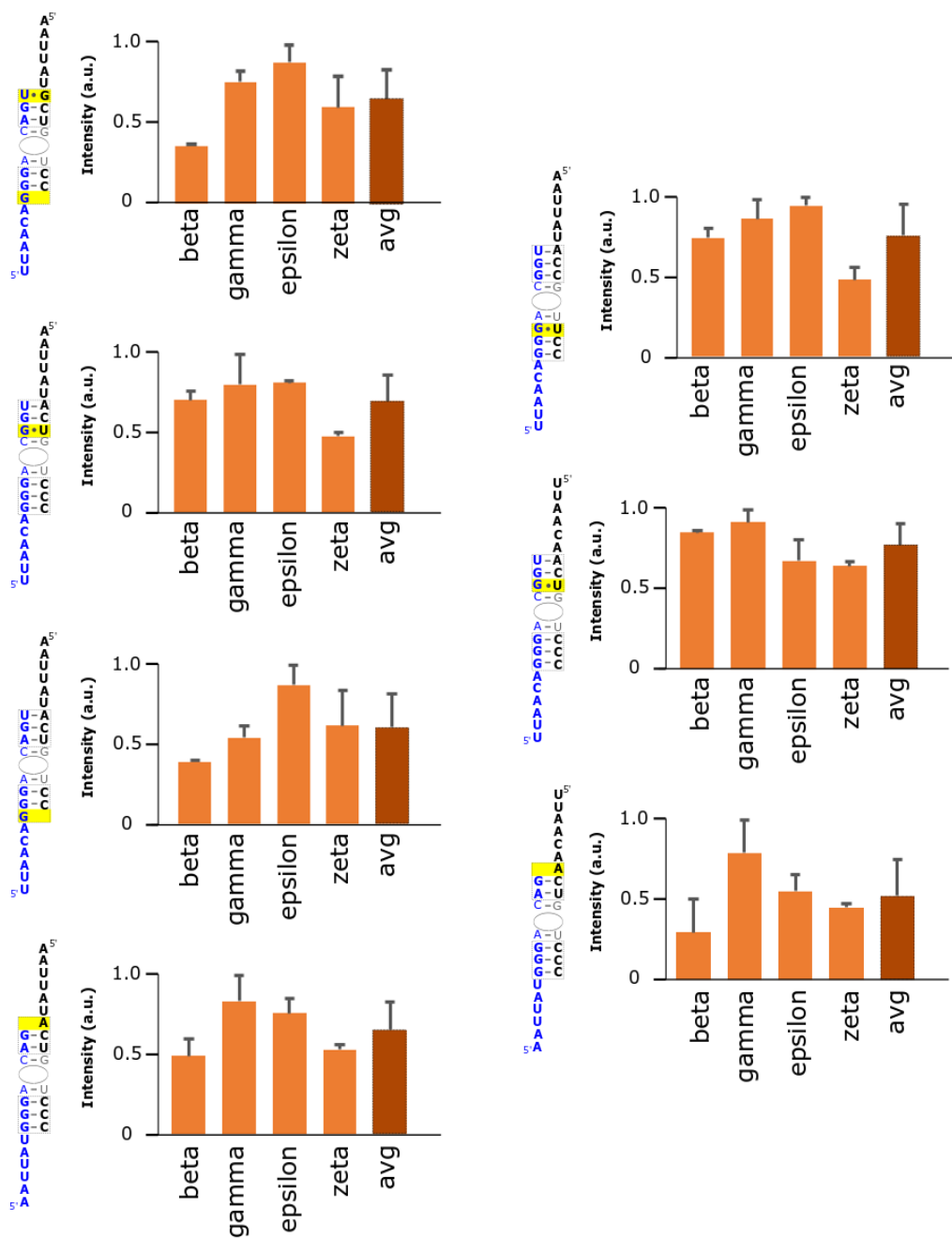


Figure S3. Fluorescent profiles of partially-assembled nanorings. The missing strut of each assembly is identified below each plot. The intensities of each the plots is normalized to the signal within the grouping that produced the highest fluorescence (orange bars). The average intensity when any one of the struts is missing (red bar).