

Figure 1: Background. A. Depiction of histone octamer/nucleosome core. Each nucleosome is composed of two tetramers, each with an H2A, H2B, H3, and H4 histone. There are approximately 147 bp of DNA wrapped about 1.7 times around each octamer. B. Depiction of organization of broccoli DNA construct.

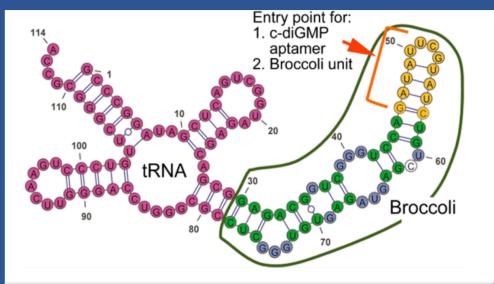


Figure 2: Broccoli structure as reported by Filonov, et. al. Here, broccoli is depicted attached to a tRNA scaffold for increased stability. For our purposes this scaffold would be unnecessary.

Aptamer:	Mango	Broccoli	Spinach-2
Thermostability:	-	More heat stable than spinach 2 at temperatures below 100°C	Begins degradation at 25°C
Kd:	-	360	560
Fluorophore:	TO (thiazole orange)	DFHBI	DFHBI
Fluorescence:	High	Higher fluorescence than spinach for low Mg concentrations	-
Salt Dependence:	No	No	Yes- high potassium dependence
Mg Dependence:	1 mM MgCl ₂ required in spectroscopy	Max fluorescence at 300 microM, but not required	Max fluorescence at 1 mM
In vitro/in vivo:	In vitro	Both	In vitro
Wavelength Excitation/Emission	510/535 nm	472/507 nm	482/505 nm

Figure 3: Comparison of properties of various viable aptamers.

REFERENCE

- Filonov, G. S., Moon, J. D., Svensen, N., & Jaffrey, S. R. (2014). Broccoli: rapid selection of an RNA mimic of green fluorescent protein by fluorescence-based selection and directed evolution. *Journal of the American Chemical Society*, 136(46), 16299–16308. https://doi.org/10.1021/ja508478x
- Okuda, M., Fourmy, D., & Yoshizawa, S. (2017). Use of Baby Spinach and Broccoli for imaging of structured cellular RNAs. Nucleic acids research, 45(3), 1404–1415. https://doi.org/10.1093/nar/gkw794
- Trachman, R. J., 3rd, Demeshkina, N. A., Lau, M., Panchapakesan, S., Jeng, S., Unrau, P. J., & Ferré-D'Amaré, A. R. (2017). Structural basis for high-affinity fluorophore binding and activation by RNA Mango. Nature chemical biology, 13(7), 807–813. https://doi.org/10.1038/nchembio.2392