

# **iSpinach: a fluorogenic RNA aptamer optimized for *in vitro* applications**

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## Supplementary Tables

**Table S1.** Folding efficiencies of Spinach2, Broccoli and iSpinach as a function of temperature in the presence of sodium or potassium. Values are expressed in percentage of folded molecule and are the mean of three independent experiments and error bars correspond to  $\pm 1$  standard error.

	25 °C		37 °C		45 °C	
	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup>
<b>Spinach 2.0</b>	61.0 $\pm$ 12.2	n.m.	27.0 $\pm$ 4.7	n.m.	2.3 $\pm$ 1.4	n.m.
<b>Broccoli</b>	17.7 $\pm$ 3.2	n.m.	15.5 $\pm$ 4.8	n.m.	9.9 $\pm$ 2.6	n.m.
<b>iSpinach</b>	69.0 $\pm$ 4.0	59.6 $\pm$ 8.1	53.7 $\pm$ 3.7	23.6 $\pm$ 3.4	37.4 $\pm$ 3.2	6.4 $\pm$ 1.5

n.m. : not measurable

**Table S2.** Experimental parameters.

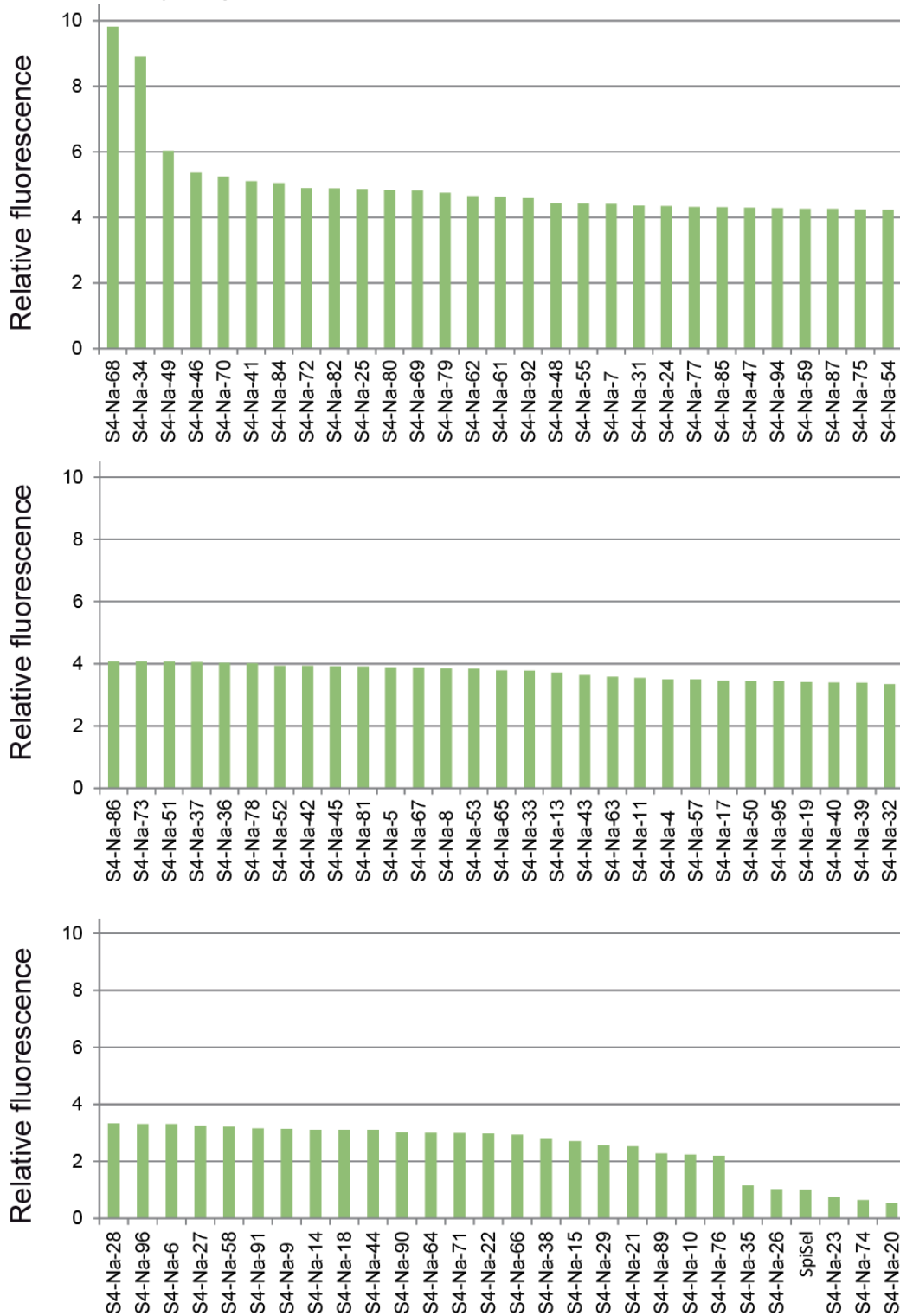
Salt	Round	PCR droplets occupancy (%)	$\lambda$ value <sup>a</sup>	Fusion efficiency (%)	Number of analyzed droplets	Number of sorted droplets
Sodium	S1	32	0.38	92	1,392,500	9,028
	S2	12	0.12	75	250,950	2,526
	S3	15	0.16	85	776,251	11,278
	S4	16	0.17	90	879,000	7,896
Potassium	S1	26	0.30	70	738,875	22,730
	S2	23	0.26	87	397,950	4,514
	S3	17	0.18	80	1,526,750	28,071
	S4	18	0.19	75	225,750	2,589

<sup>a</sup> Calculated as described in (1).



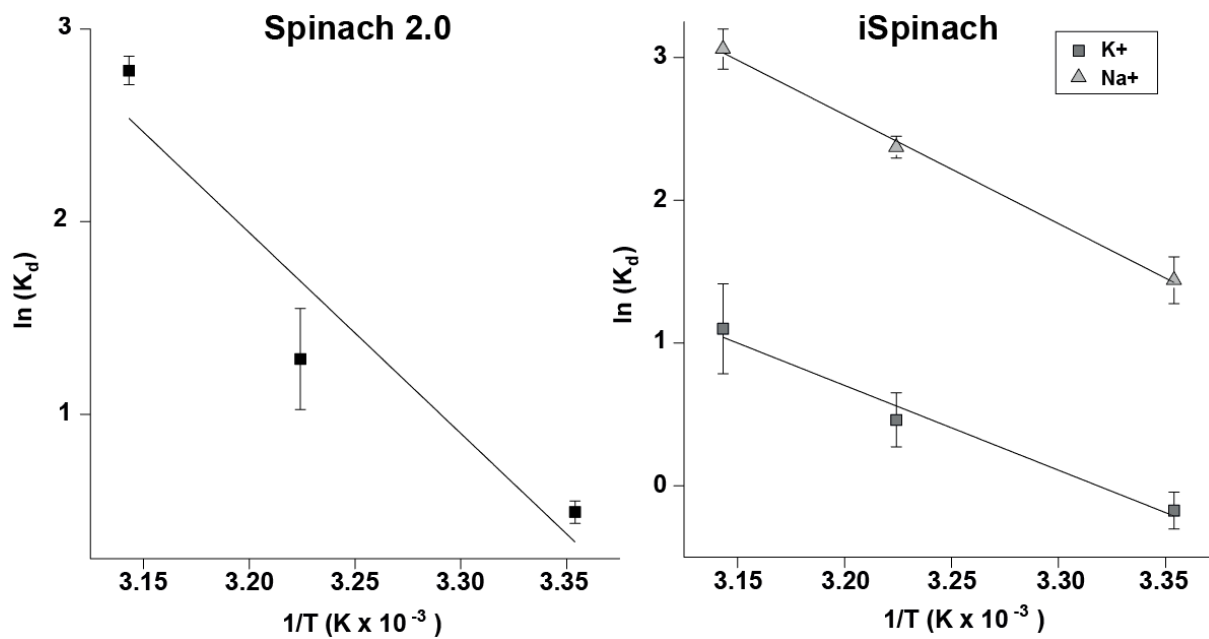


## Supplementary Figures



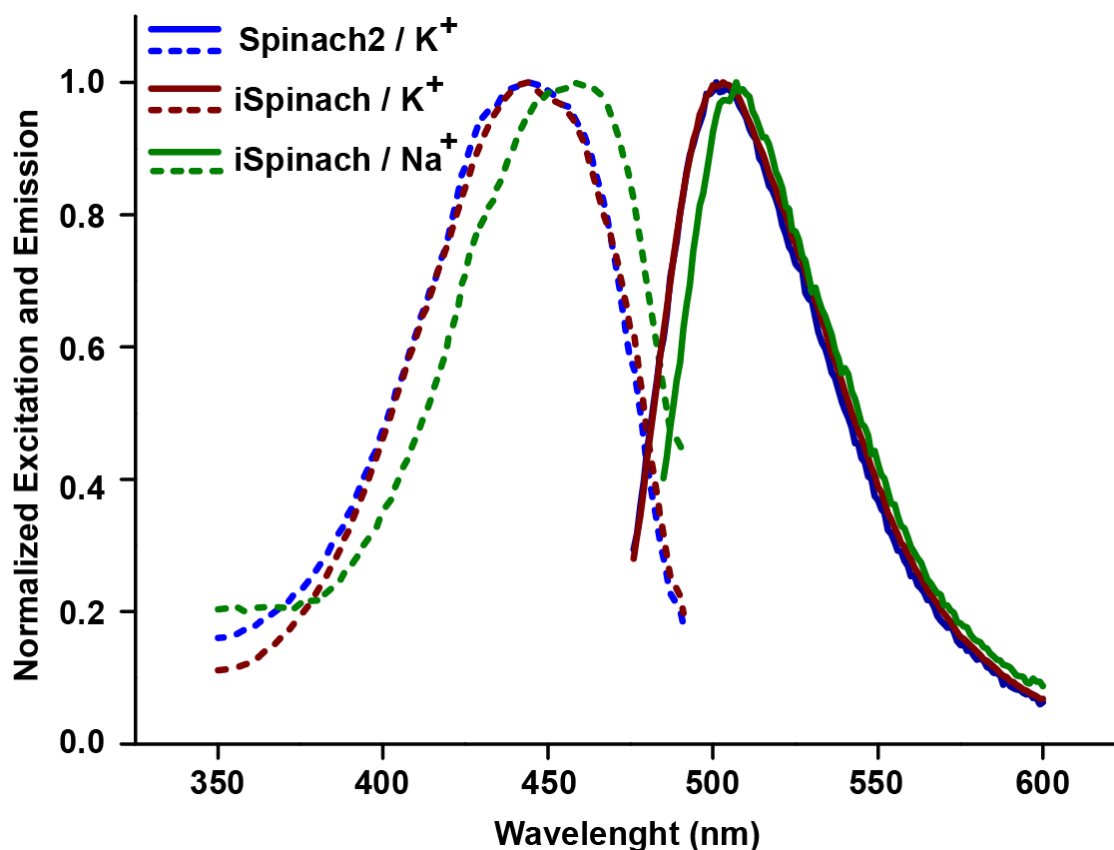
**Figure S1.** Fluorescence of isolated mutants. Aptamer-coding genes were PCR amplified and DNA *in vitro* transcribed in the presence of DFHBI. The co-transcriptional fluorescence increase was monitored at 37°C. Relative fluorescence was calculated by dividing the fluorescence increase rate of the analyzed clone by the one of SpiSel.





**Figure S3.** Effect of the temperature on RNA/DFHBI complex dissociation. Arrhenius plots are shown for DFHBI in complex with Spinach2 (left) or iSpinach (right) in the presence of potassium (squares) or sodium (triangles).





**Figure S4.** Excitation and emission spectra of DFHBI in complex with Spinach2 or iSpinach in the presence of sodium or potassium. Excitations spectra (dashed lines) were determined by recording the fluorescence emitted at 500 nm following excitation at a wavelength ranging from 350 nm to 400 nm with a step of 1 nm. Emission spectra (continuous line) were determined by exciting the sample at 400 nm and recording the fluorescence emitted from 475 nm to 600 nm with a step of 1 nm.

## References

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2. Warner, K.D., Chen, M.C., Song, W., Strack, R.L., Thorn, A., Jaffrey, S.R. and Ferre-D'Amare, A.R. (2014) Structural basis for activity of highly efficient RNA mimics of green fluorescent protein. *Nature structural & molecular biology*, **21**, 658-663.