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Supporting Information

Rational design of self-quenched rhodamine dimers as fluorogenic aptamer probes for live-cell RNA imaging

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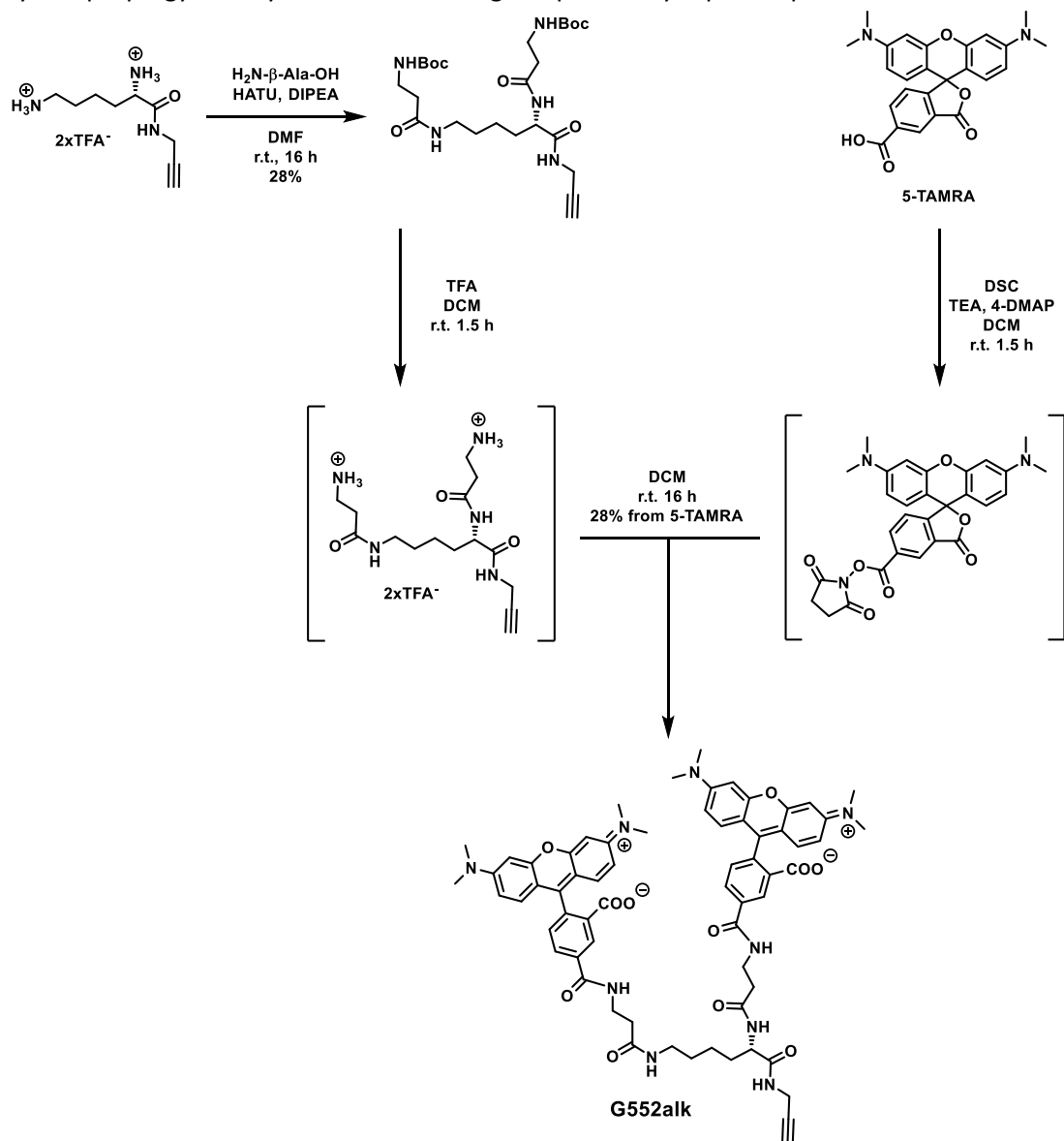
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Synthesis protocols, characterizations and $^1\text{H}/^{13}\text{C}$ NMR/HRMS spectra

5-Carboxytetramethylrhodamine (5-TAMRA) was synthesized and the single 5-isomer was isolated following reported protocol.²

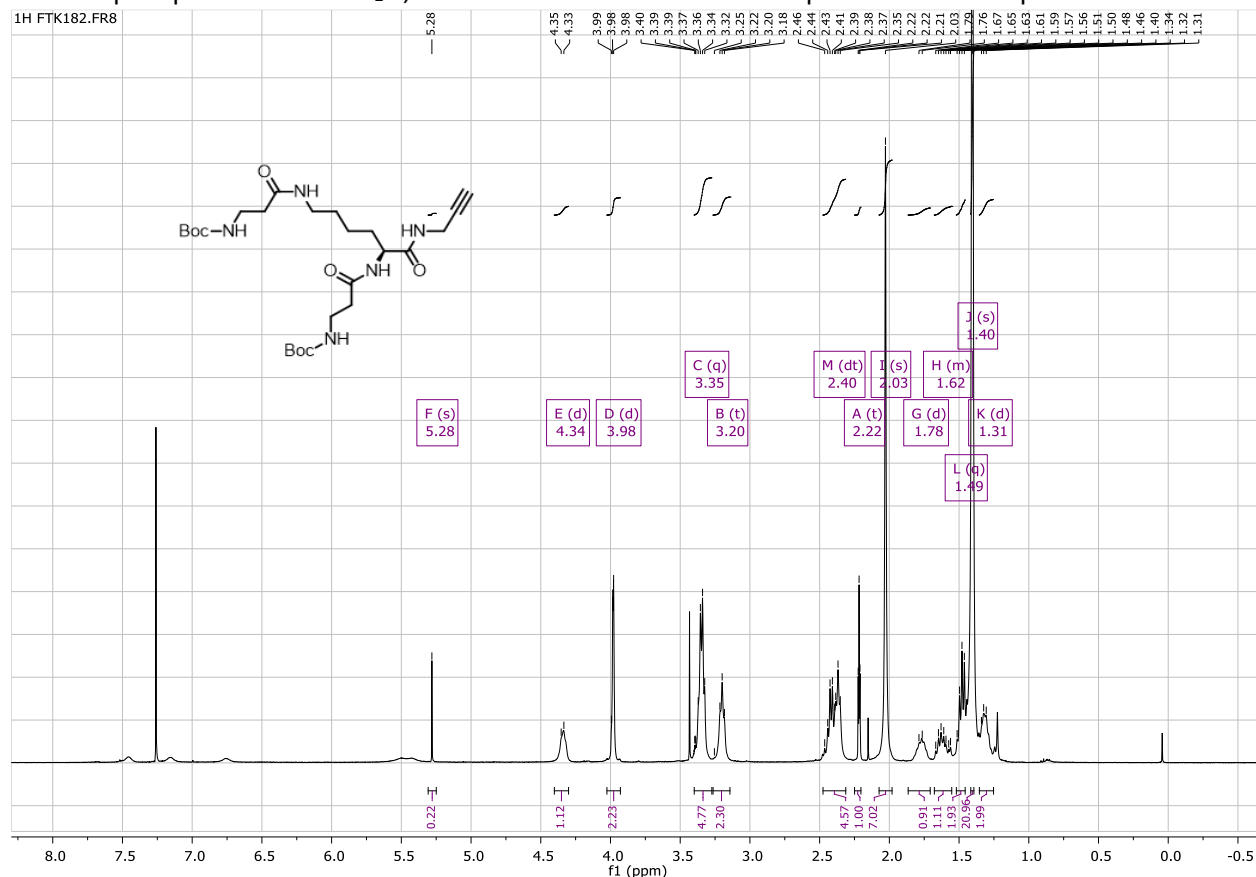
Lysine-propargyl was synthesized following our previously reported protocol.³



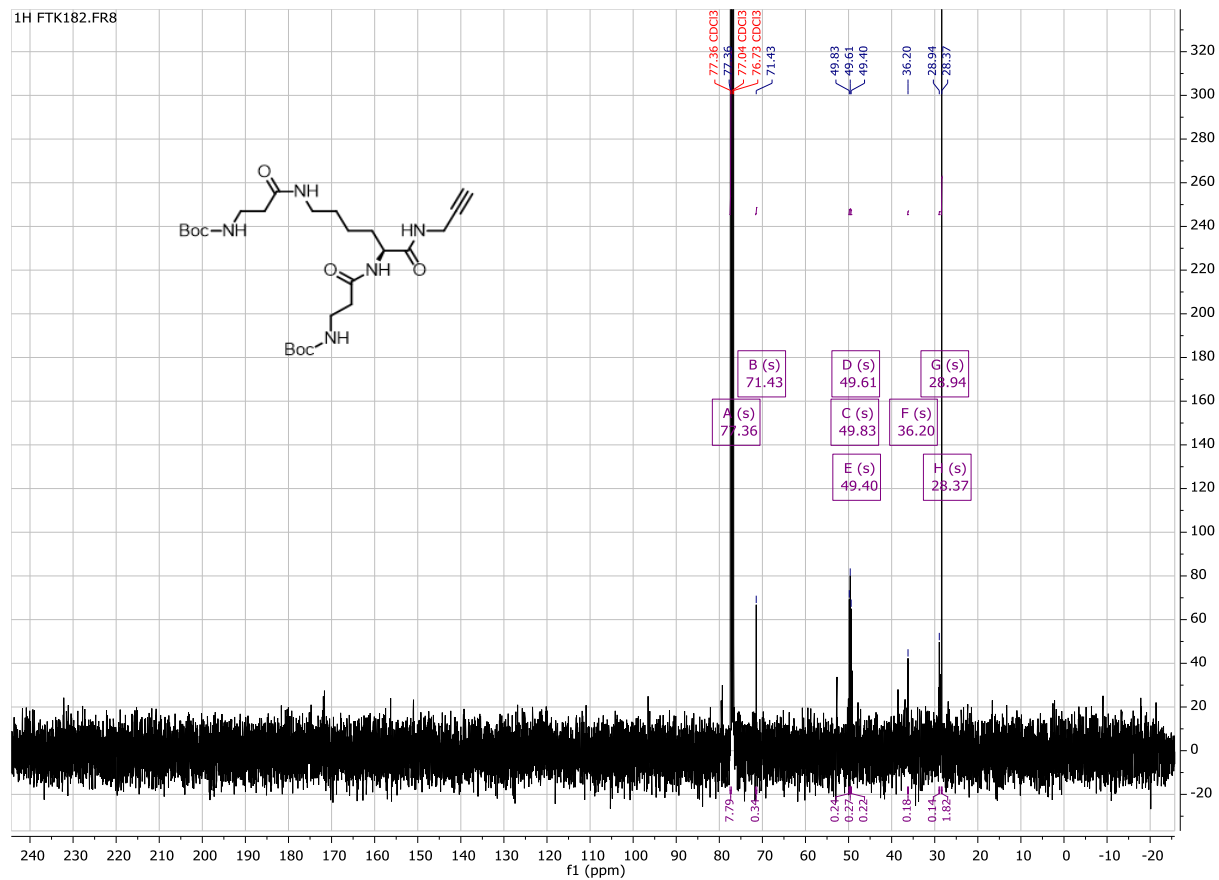
Scheme S1. Synthesis of G552alk.

diAla-Lys-propargyl. Lysine-propargyl (TFA salt) (1.68 g, 4.08 mmol, 1 eq.) was dissolved in 50 mL of anhydrous DMF, followed by addition of HATU (4.04 g, 10.6 mmol, 2.6 eq.), DIPEA (8.54 mL, 49 mmol, 12 eq.) and Boc-beta-Ala-OH (1.55 g, 8.16 mmol, 2 eq.). The reaction mixture was flushed with Ar and left stirring for 16 at room temperature. Solvent was evaporated under vacuum and the residue was purified on silica column (DCM:MeOH=9:1) to obtain white foam (676 mg, 28%). HRMS (ESI+), calc. for $\text{C}_{25}\text{H}_{43}\text{N}_5\text{O}_7$ $[\text{M}+\text{H}]^+$ 526.3162, found 526.3289. ^1H NMR (400 MHz, Chloroform-d) δ 5.28 (s, 1H), 4.34 (d, $J = 7.7$ Hz, 1H), 3.98 (d, $J = 2.7$ Hz, 2H), 3.35 (q, $J = 6.3$ Hz, 4H), 3.20 (t, $J = 6.5$ Hz, 2H), 2.40 (dt, $J = 22.7, 6.6$ Hz, 5H), 2.22 (t, $J = 2.5$ Hz, 1H), 1.78 (d, $J = 9.4$ Hz, 1H), 1.68 – 1.55 (m, 1H), 1.49 (q, $J = 7.0$ Hz, 2H), 1.40 (s, 21H),

1.31 (d, J = 7.4 Hz, 2H). ¹³C NMR (101 MHz, Chloroform-d) δ 77.36, 71.43, 49.83, 49.61, 49.40, 36.20, 28.94, 28.37. **diAla-Lys-propargyl** was further deprotected with TFA in DCM. TFA have been exchanged to HCl salt by dissolving in 0.1 M aqueous HCl/MeCN (1:3 v/v) and re-evaporating three times. Finally the di-HCl salt was precipitated in cold Et₂O, filtrated and used in the next step without further purification.

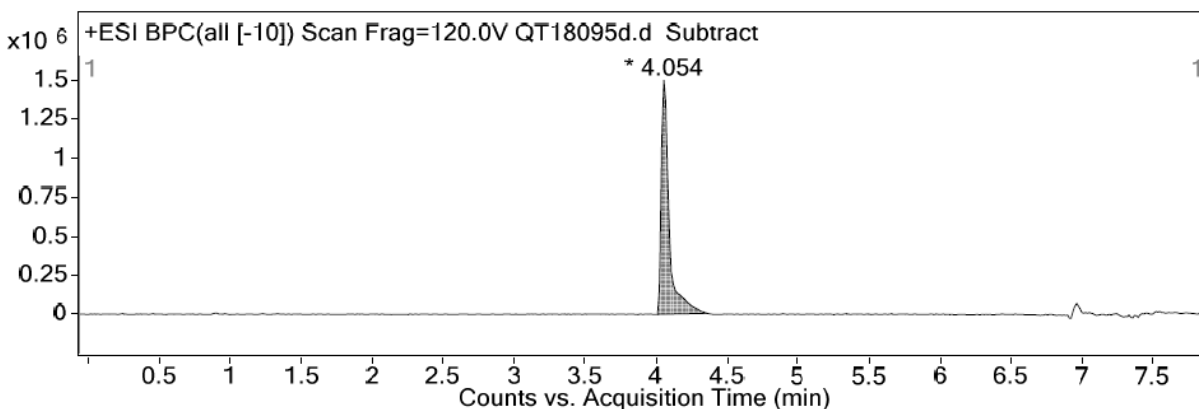


¹H NMR of Boc-diAla-Lys-propargyl.

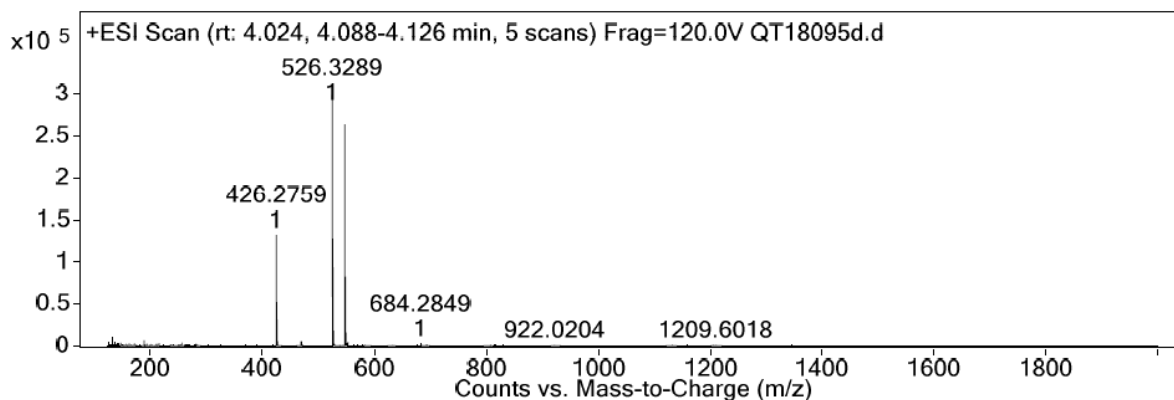


¹³C NMR of diAla-Lys-propargyl.

Fragmentor Voltage 120 Collision Energy 0 Ionization Mode ESI

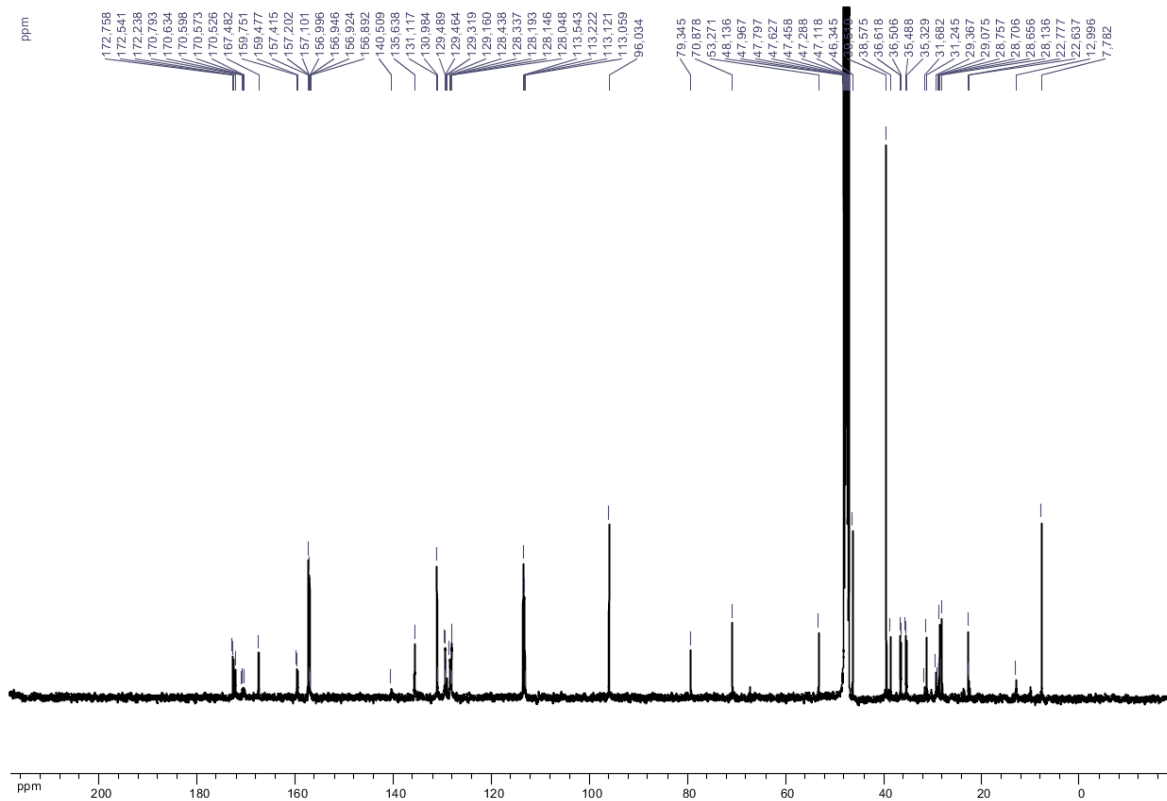


Spectrum Source Peak (1) in "+ BPC(all [-10]) Scan Sub" Fragmentor Voltage 120 Collision Energy 0 Ionization Mode ESI



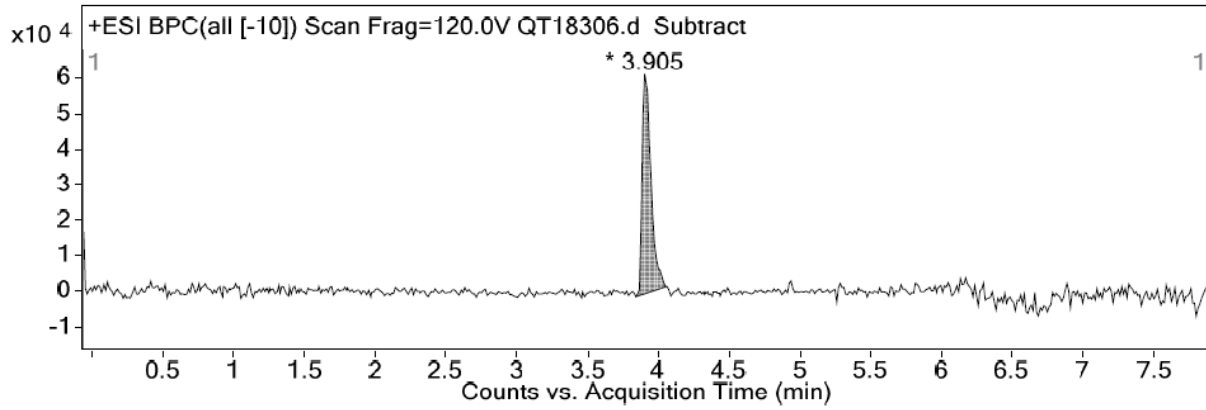
LC and HRMS of diAla-Lys-propargyl.

Gemini-552-alkyne (G552alk-). 5-Carboxy-tetramethylrhodamine (215 mg, 600 μmol , 3 eq) was combined with TSTU (193 mg, 640 μmol , 3.2 eq) in dry DMF (2 mL) in presence of DIPEA (82 mg, 111 μL , 640 μmol , 3.2 eq). The reaction was stirred at room temperature for 2 h, protected from light. After that, deprotected diamine Boc-diAla-Lys-propargyl as 2xHCl salt (80 mg, 20 μmol , 1 eq) was added. The reaction was stirred 16 h at room temperature, then solvent was removed by concentrating in vacuo. The crude material was purified first by flash chromatography on virgin silica gel (25:5:1_{v/v} CHCl₃:MeOH:NH₄OH_{aq.} (28%)) then by automated reverse phase flash chromatography (Interchim Puriflash XS530, Interchim 15 μm C18 prepacked column, gradient 10 to 50% MeCN in water with 0.1%TFA). Finally, isolated di-TFA salt have been neutralized with Et₃N and refined by size exclusion chromatography (LH20 beads, DCM:MeOH 50/50 v/v) to provide 60 mg (10%) of as a dark violet solid. $\epsilon_{\text{Methanol}}$ (545 nm) = 232 900 L mol⁻¹ cm⁻¹. HRMS (ESI+), calc. for C₆₉H₆₇N₉O₁₁ [M+2H]⁺ 1149.51, found 1149.51. ¹H NMR (400 MHz, Methanol-d₄) δ 8.65 – 8.57 (m, 2H), 8.06 (ddd, *J* = 10.1, 7.9, 1.8 Hz, 2H), 7.34 (dd, *J* = 7.9, 1.3 Hz, 2H), 7.27 – 7.20 (m, 4H), 7.01 (dtd, *J* = 9.5, 2.4, 1.2 Hz, 3H), 6.92 (dd, *J* = 4.0, 2.4 Hz, 4H), 4.39 (dd, *J* = 9.3, 4.8 Hz, 1H), 3.99 (t, *J* = 2.6 Hz, 2H), 3.75 (ddt, *J* = 13.7, 9.5, 6.7 Hz, 4H), 3.37 (p, *J* = 1.6 Hz, 6H), 3.23 (t, *J* = 6.6 Hz, 2H), 2.78 – 2.66 (m, 2H), 2.67 – 2.55 (m, 3H), 1.97 (t, *J* = 7.4 Hz, 0H), 1.92 – 1.30 (m, 3H), 1.22 (d, *J* = 6.2 Hz, 1H). ¹³C NMR

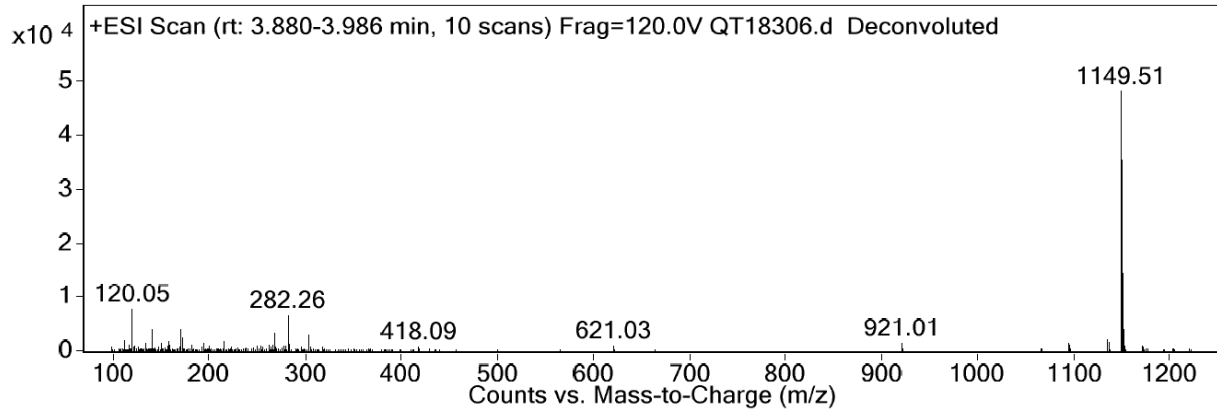


^{13}C NMR of G552alk.

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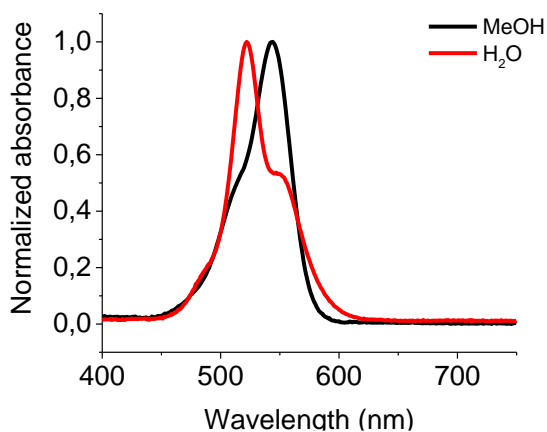
Fragmentor Voltage 120 Collision Energy 0 Ionization Mode ESI



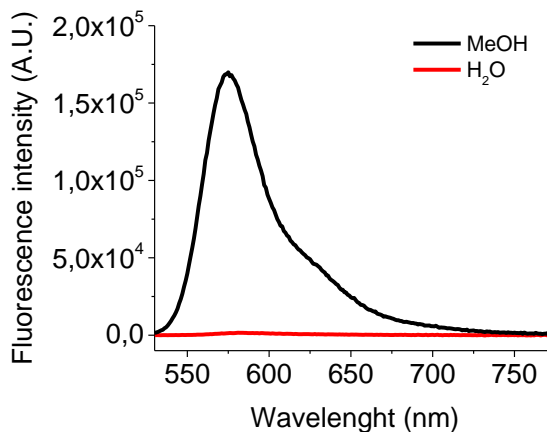
LC and HRMS of G552alk.

Additional spectral data

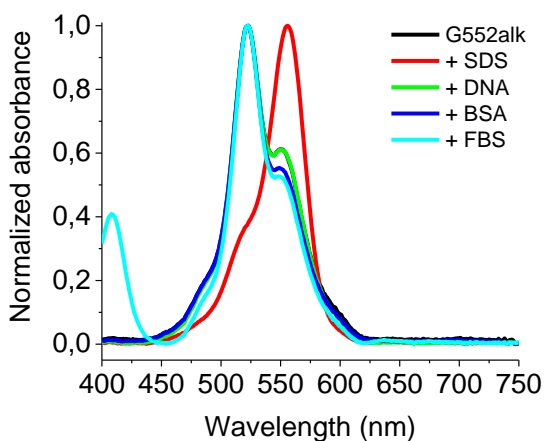
A



B



C



D

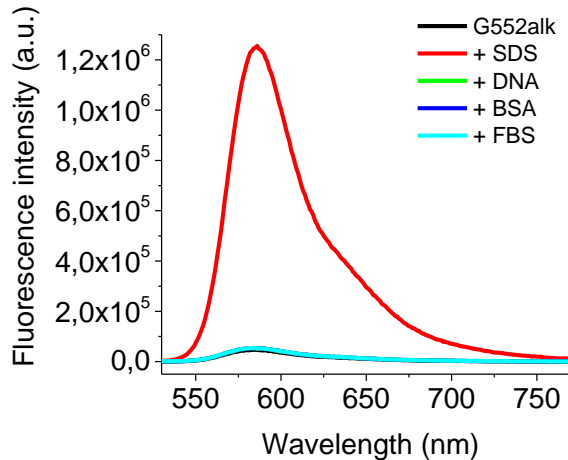


Figure S1. Spectral properties of G552alk (0.2 μ M). (A) Absorption spectra of G552alk in MeOH or water. (B) Fluorescence spectra of G552alk in MeOH or water. (C) Absorption spectra of G552alk in buffer (black line), in the presence of solubilizing detergent sodium dodecyl sulfate (SDS, 5 mg/mL, red line) or non-targeted biomolecule (DNA T20 0.6 μ M, BSA 0.1 mg/mL, FBS 10 mg/mL respectively). (D) Fluorescence spectra of G552alk in buffer (black line), in the presence of solubilizing detergent sodium dodecyl sulfate (SDS, 5 mg/mL, red line) or non-targeted biomolecule (DNA T20 0.6 μ M, BSA 0.1 mg/mL, FBS 10 mg/mL respectively). Excitation at 520 nm.

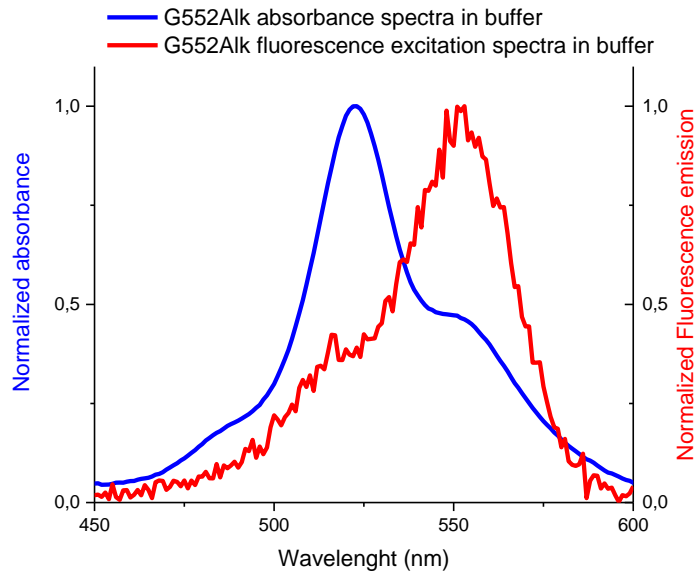


Figure S2: Normalized absorbance (blue) and fluorescence excitation (red) spectra of G552Alk at 200 nM in buffer. Fluorescence emission measured at 620 nm.

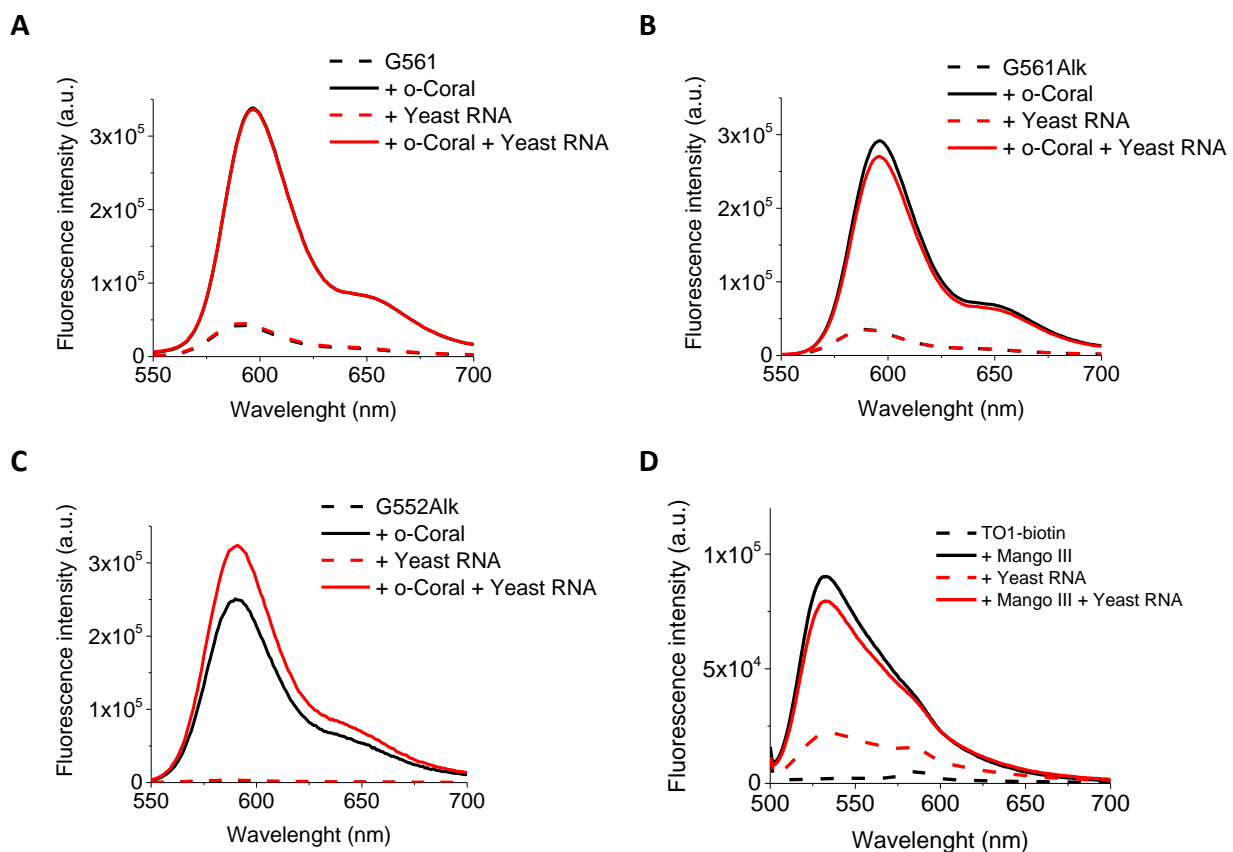


Figure S3. Fluorescence spectra of Gemini probes G561 (A), G561Alk (B), G552Alk (C) and TO1-biotin (D) at 10 nM concentration in presence of their cognate aptamer (respectively 1 μ M of o-Coral and Mango-III), total yeast RNA extract (x1000 excess of RNA by mass) or both. Excitation at 530 nm for Geminis and 488 nm for TO1-biotin.

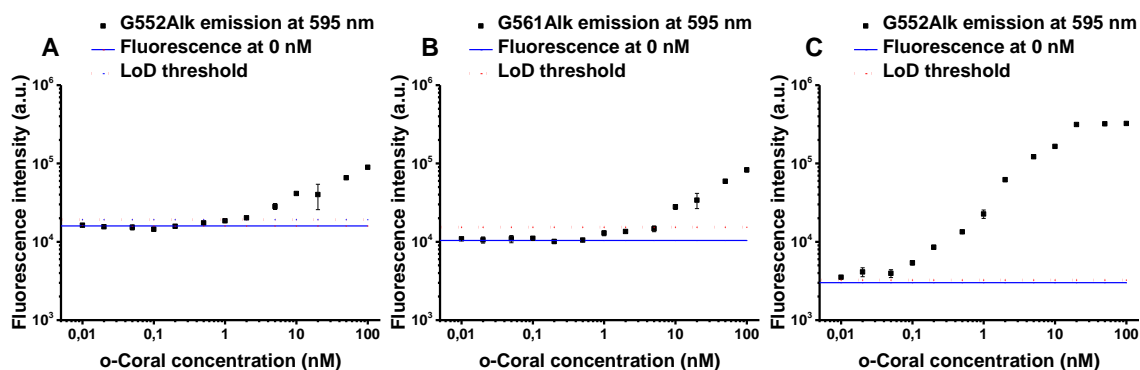


Figure S4. Limit of detection (LoD) measurements of Geminis at 10 nM. LoD threshold have been defined as the minimal concentration of aptamer providing a fluorescence signal significantly higher than the fluorescence signal + 3 times the standard deviation of the corresponding dye without RNA (red dotted line). Fluorescence signal with no aptamer (blue line) have been measured for $n = 8$ and titrations with RNA have been measured for $n = 3$. **(A)** Fluorescence signal at 595 nm of a solution of G561 at 10 nM according to the o-Coral concentration in buffer. **(B)** Fluorescence signal at 595 nm of a solution of G561Alk at 10 nM according to the o-Coral concentration in buffer. **(C)** Fluorescence signal at 595 nm of a solution of G552Alk at 10 nM according to the o-Coral concentration in buffer. Excitation at 530 nm.

Table S1. Fluorescence correlation spectroscopy of G552Alk and G561 with and without o-Coral in buffer.^a

	avg. Signal [kHz]	n	τ_{diff} [ms]	C _{pp} [kHz]	Concentration in fluorescent particles (nM)	Size (nm)	Brightness
TMR (Ref)	34.79	12.70	0.03	2.75	50.00	1.00	1.00
G552Alk + o-Coral	143.70	37.82	0.21	3.80	148.84	6.50	1.38
G561 + o- Coral	130.06	33.33	0.22	3.90	131.18	6.8	1.42
G552Alk	8.73	4.38	0.04	2.00	17.24	1.31	0.73
G561	9.57	8.31	0.03	1.16	32.72	0.89	0.42

^aTetramethylrhodamine (TMR) solution at 50 nM in water have been used as reference. Excitation with a 532 nm laser. n – number of emissive species per excitation volume; τ_{diff} – diffusion time; size – hydrodynamic diameter of fluorescent specie; brightness was measured with respect to one molecule of TMR (standard).

Toxicity assay

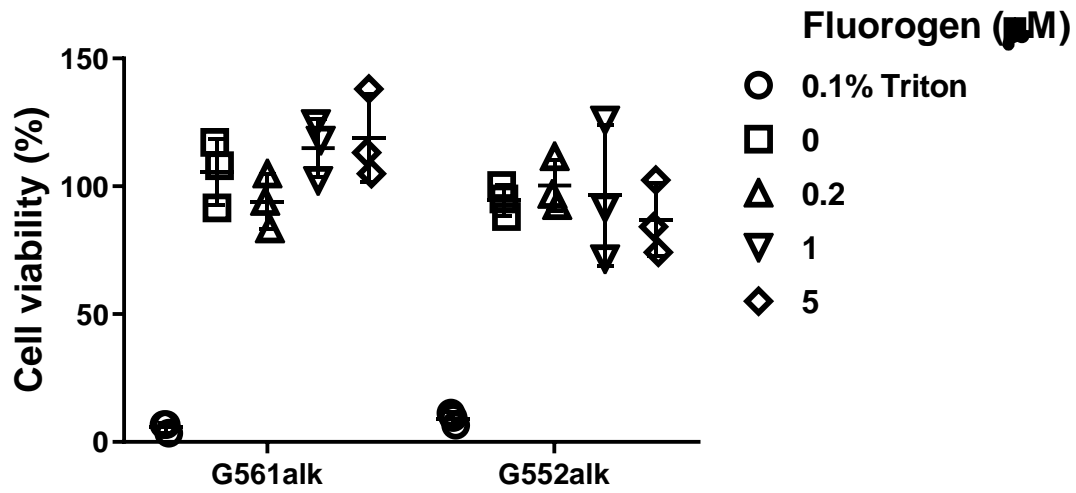


Figure S5. Cytotoxicity of o-Gemini assessed by MTT assay. HEK293T cells were treated with corresponding o-Gemini in a range of concentration for 24 h. 0.1% Triton X-100 was used as cytotoxic control. The values are average values \pm S.D. (n=3).

Sequences of o-Coral constructs

Table S2. Sequence of o-Coral constructs.

Name	Sequence
o-Coral ^a	5' GGGAGACAGCTAGAGTAC AGAACCCCGCTTCGGCGGTGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCCGGTGACGGGGCCTCGCTTCGGCGATGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCT GACACGAGCACAGTGTAC 3'
F30-o-Coral ^{a,b}	5' TTGCCATGTGTATGTGGGCCTGCAGG GGGAGACAGCTAGAGTAC AGAACCCCGCTTCGGCGGTGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCCGGTGACGGGGCCTCGCTTCGGCGATGATGGAGAGGCGCAAGGTTAACCGCCTCAGGTTCT GACACGAGCACAGTGTAC CCTGCAGGCCACATACTCTGATGATCCTTCGGGATCATTCATGGCAA3'
U6-F30-o-Coral ^{a,b,c}	5' <i>GTGCTCGCTTCGGCAGCACATATACTAGTCGACTTGCCATGTGTATGTGGGCCTGCAGGGGGAGA</i> CAGCTAGAGTAC AGAACCCCGCTTCGGCGGTGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCCGGTGACGGGGCCTCGCTTCGGCGATGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCT GACACGAGCACAGTGTAC CCTGCAGGCCACATACTCTGATGATCCTTCGGGATCATTCATGGCAATCTAGAGCGACTTCGGTCCGCTTTT3'
5S-F30-o-Coral ^{a,b,c}	5' <i>GTCTACGGCCATACCACCTGAACGCGCCGATCTCGTCTGATCTCGGAAGCTAAGCAGGGTCGGGCTGGTTAGTACTTGGATGGGAGACCGCTGGGAATACCGGGTGCTGTAGGGCTCGACTTGCCATGTATGTGGGCCTGCAGG</i> GGGAGACAGCTAGAGTAC AGAACCCCGCTTCGGCGGTGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCCGGTGACGGGGCCTCGCTTCGGCGATGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCT GACACGAGCACAGTGTAC CCTGCAGGCCACATACTCTGATGATCCTTCGGGATCATTCATGGCAATCTAGAGCGGACTTCGGTCCGCTTTT3'
eGFP mRNA-F30-o-Coral ^{a,b,c}	5' <i>ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGGCCCTGGCCACCCCTCGTGACCACCCCTGACCTACGGCGTGAGTGTTCAGCCGTACCCCGACCACATGAAGCAGCAGGACTTCTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGA</i> TTTCGGTACCGCCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGCCGCGACTCTAGATCATAATCAGCCATAACCACATTTGTAGAGGTTTTACTTTGCTTTAAAAAACCTCCACACCTCCCCTGAACCTGAAACATAAAAATGAATGCAATTGGTCGACTTGCCATGTGTATGTGGGCCTGCAGGAGAACCCCGCTTCGGCGGTGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCCGGTGACGGGGCCTCGCTTCGGCGATGATGGAGAGGGCGCAAGGTTAACCGCCTCAGGTTCTCCTGCAGGCCACATACTCTGATGATCCTTCGGGATCATTCATGGCAA3'

^a The sequence of 5' and 3' extensions are shown in bold. ^b The sequence of the F30 scaffold is underlined.

^c The sequences corresponding to U6 RNA, 5S rRNA or eGFP mRNA (5' end) and terminator (3' end) are italicized.

Additional cellular experiments data

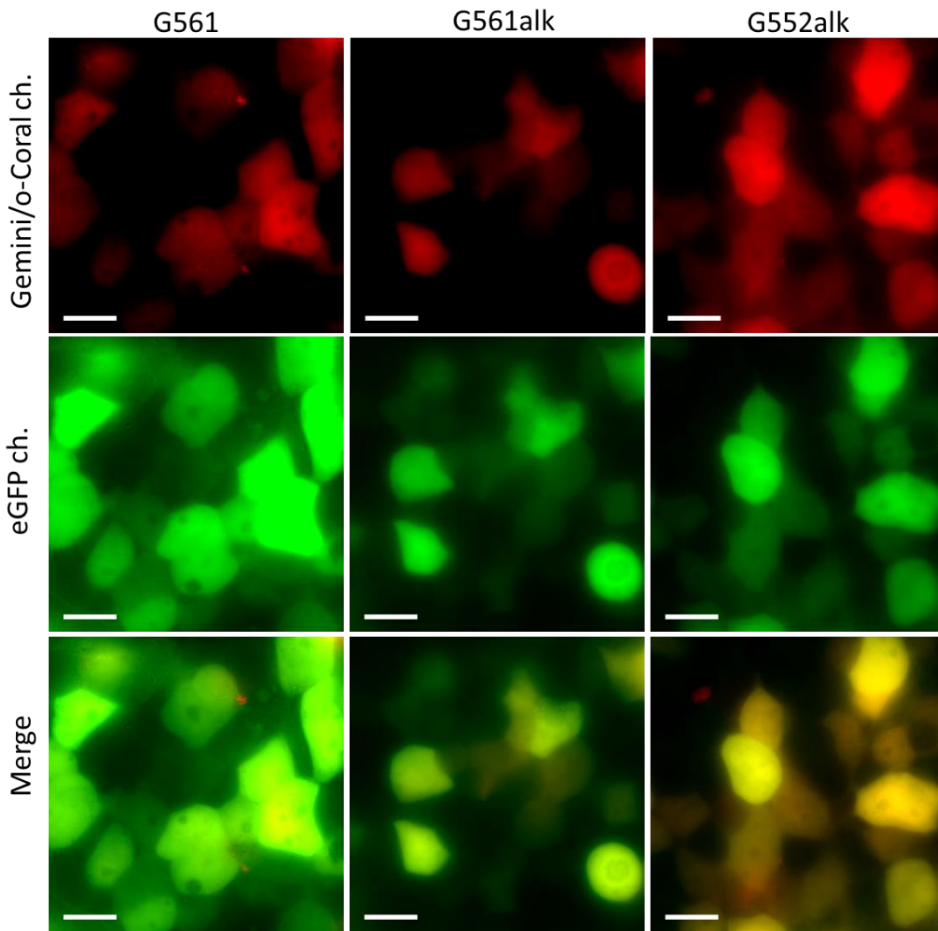


Figure S6. Fluorescence imaging of overexpressed o-Coral-tagged U6 RNA in live HEK293T cells upon treatment with o-Gemini fluorogens. Cells were incubated with respective o-Gemini (0.2 μ M) for 5 min and imaged without washing. The images were acquired using a 500 ms exposure time. The same fluorescence signal range was applied to all images. o-Gemini in red (ex: 550 nm, em: 595 \pm 40 nm) and eGFP in green (ex: 470 nm, em: 531 \pm 40 nm). Scale bar is 20 μ m.

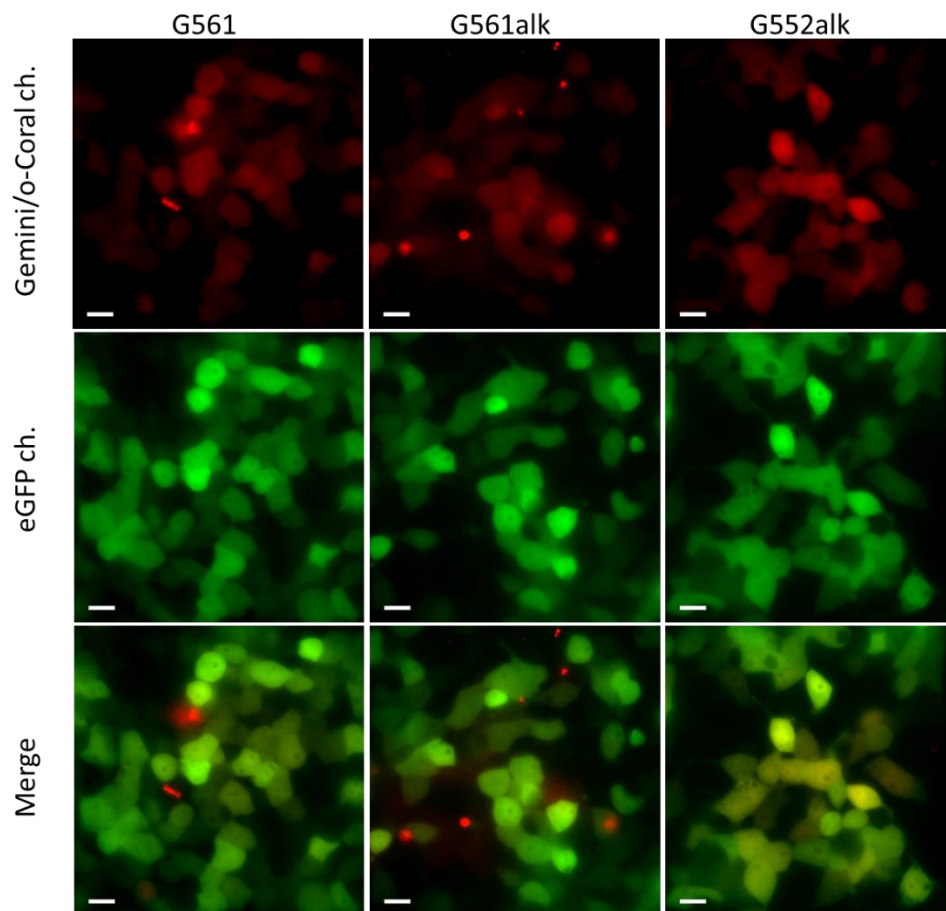


Figure S7. Fluorescence imaging of overexpressed o-Coral-tagged mRNAs of eGFP in live HEK293T cells upon treatment with o-Gemini fluorogens. Cells were incubated with respective o-Gemini (0.2 μM) for 5 min and imaged without washing. The images were acquired using a 500 ms exposure time. The same fluorescence signal range was applied to all. o-Gemini in red (ex: 550 nm, em: 595 \pm 40 nm) and eGFP in green (ex: 470 nm, em: 531 \pm 40 nm). Scale bar is 20 μm .

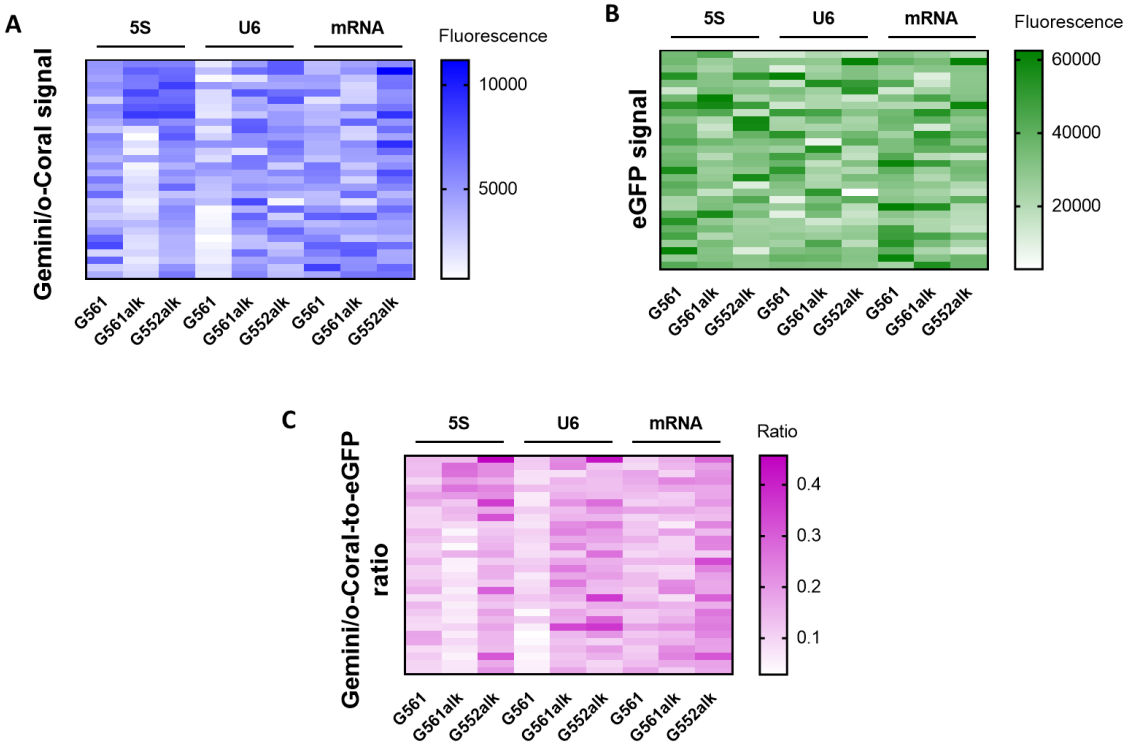


Figure S8. Comparative single-cell analysis of o-Geminis to detect o-Coral-tagged RNAs. **(A)** Heatmap showing single-cell Gemini/o-Coral fluorescence extracted from images (number of cells is 30). Background was subtracted. **(B)** Heatmap of eGFP fluorescence of the same population of cells (n = 30). **(C)** Heatmap of the intensity ratio of Gemini/o-Coral fluorescence to eGFP fluorescence of the same population of cells (n = 30).

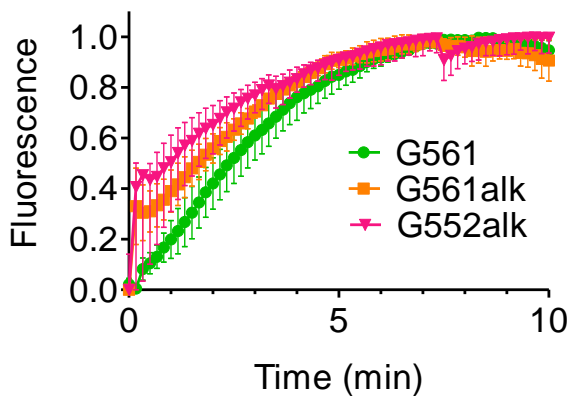


Figure S9. Kinetic analysis of the detection of o-Coral-U6 RNAs expressed in HEK293T by o-Geminis (G561, G561alk, and G552alk) immediately after addition to the cells. Fluorescence intensity was normalized to the maximum. Data represent average values of fluorescence extracted from images normalized to the

same regions of interest \pm S.D. ($n = 3$). The estimated halftimes of the intensity growth are 1.77, 1.13 and 1.11 min for G561, G561lk and G552alk, respectively.

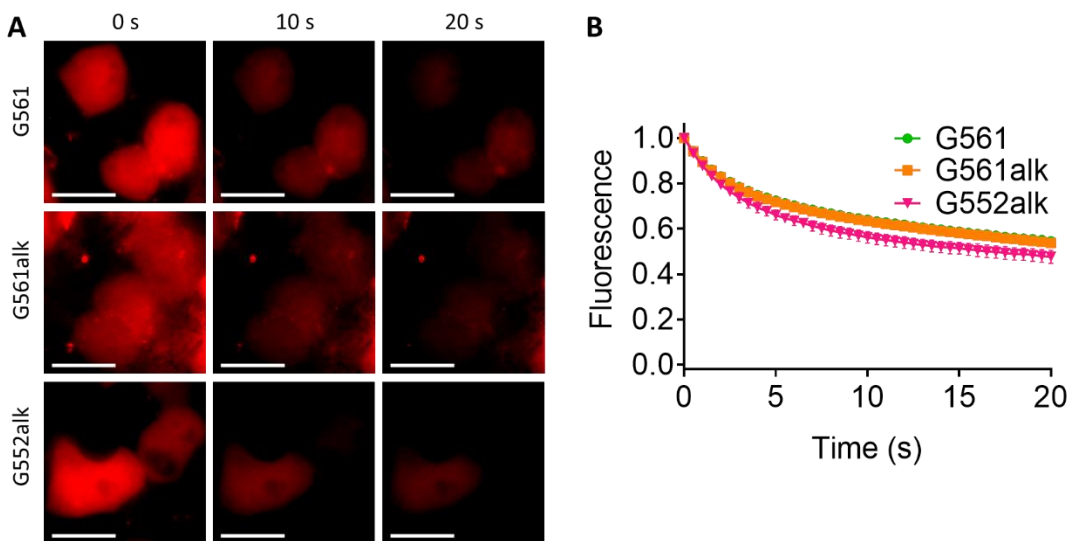


Figure S10. Photostability of o-Gemini/o-Coral-tagged 5S RNA complexes in HEK293T cells. **(A)** Fluorescence images of o-Coral-tagged 5S RNA after treatment with corresponding o-Gemini (0.2 μ M) for 5 min. Cells were exposed to continuous illumination after washing o-Gemini. Fluorescence was normalized to maximum and background was subtracted. The images were acquired using a 500 ms exposure time. Gemini channel (ex: 550 nm, em: 595 \pm 40 nm). Scale bar is 20 μ m. **(B)** Fluorescence decay curves over the time. Fluorescence was normalized to the maximum. Data represent average values of fluorescence extracted from images normalized to the same regions of interest \pm S.D. ($n = 3$).

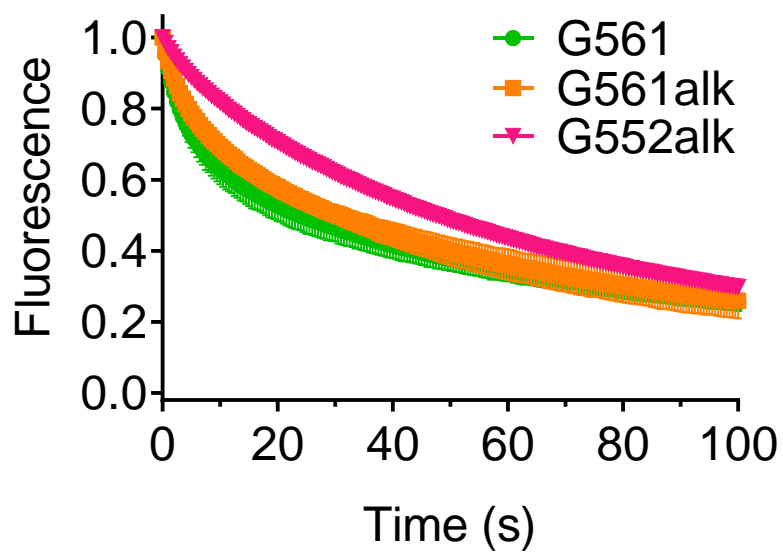


Figure S11. Photostability of o-Geminis bound to o-Coral-tagged 5S RNA in HEK293T cells. Fluorescence decay curves over the time. Fluorescence was normalized to the maximum. Data represent average values of fluorescence extracted from images normalized to the same regions of interest \pm S.D. (n = 3).

Additional References

- (1) Chevalier, A.; Renault, K.; Boschetti, F.; Renard, P.-Y.; Romieu, A. Rapid Synthesis of Unsymmetrical Sulforhodamines Through Nucleophilic Amination of a Monobrominated Sulfoxanthene Dye. *Eur. J. Org. Chem.* **2015**, *2015* (1), 152–165. <https://doi.org/10.1002/ejoc.201403165>.
- (2) Kvach, M. V.; Stepanova, I. A.; Prokhorenko, I. A.; Stupak, A. P.; Bolibrukh, D. A.; Korshun, V. A.; Shmanai, V. V. Practical Synthesis of Isomerically Pure 5- and 6-Carboxytetramethylrhodamines, Useful Dyes for DNA Probes. *Bioconjug. Chem.* **2009**, *20* (8), 1673–1682. <https://doi.org/10.1021/bc900037b>.
- (3) Bouhedda, F.; Fam, K. T.; Collot, M.; Autour, A.; Marzi, S.; Klymchenko, A.; Ryckelynck, M. A Dimerization-Based Fluorogenic Dye-Aptamer Module for RNA Imaging in Live Cells. *Nat. Chem. Biol.* **2020**, *16* (1), 69–76. <https://doi.org/10.1038/s41589-019-0381-8>.
- (4) Hatai, J.; Motiei, L.; Margulies, D. Analyzing Amyloid Beta Aggregates with a Combinatorial Fluorescent Molecular Sensor. *J. Am. Chem. Soc.* **2017**, *139* (6), 2136–2139. <https://doi.org/10.1021/jacs.6b10809>.