***Experimental Procedures***

**Ag:DNA Synthesis** DNA strands were used as received from IDT (standard desalting). Ag:DNA solutions were synthesized by mixing hydrated DNA (ammonium acetate buffer, pH 7) with $AgNO_3$, then reducing with $NaBH_4$. The Ag/strand ratio was optimized for fluorescence brightness for each strand (Table S1). For each HPLC injection, samples were concentrated ~5x by centrifugal filtration using Amicon Ultra 0.5 mL, regenerated cellulose, 3kDa cutoff centrifugal filters (Millipore).

| Strand | Sequence (5'-3') | DNA ($\mu$M) | $NH_4OAc$ (mM) | $AgNO_3$ ($\mu$M) | $NaBH_4$ ($\mu$M) |
|---|---|---|---|---|---|
| Green1 | CGCCCCCCTTGGCGT | 25 | 10 | 250 | 60 |
| Green2 | CGCCCCCCTCGGCGT | 25 | 40 | 150 | 75 |
| Green3 | TGCCTTTTGGGGACGGATA | 15 | 10 | 187.5 | 94 |
| Orange | TTCCCCACCACCCAGGCCCCGTT | 25 | 10 | 300 | 150 |
| Red | TTCGCCCCCCGCCCCAGGCGTT | 25 | 10 | 300 | 150 |
| IR | CCCACCCACCCTCCCA | 15 | 10 | 120 | 60 |

***Table S1.*** **Strand composition and the final concentrations used in Ag:DNA synthesis.**

**HPLC** All HPLC runs used a Waters 2695 Separations Module with auto-injector and a Waters 2487 Dual Wavelength absorbance detector (10 $\mu$L volume), set to monitor 260nm. Pre-concentrated samples were injected in 100 - 200 $\mu$L volumes into a 50mm x 4.6mm Kinetex C18 core-shell column with 2.6$\mu$m particle size and 100Å pore size (Phenomenex). All samples were run at room temperature at 1mL/min.

In the first stage of HPLC purification, the mobile phase consisted of 35 mM triethylammonium acetate (TEAA), water and methanol (pH 7). A 500mM stock of TEAA was prepared by adding 17.4 mL triethylamine (TEA) to 7.15 mL glacial acetic acid in 100 mL HPLC-grade water. The total volume was brought up to 500 mL and the pH adjusted to 7. Solvents "A" and "B" were prepared by diluting the stock solution to 35mM TEAA in water for the "A" component; and in MeOH for the "B" component. This initial purification of Ag:DNAs used 1% per minute, linear methanol gradients starting at 5%. Final methanol concentration was 38% for the "Orange" and "Red" strands; and otherwise, 50%. Each gradient was preceded by 10 minutes equilibration at 5% methanol, and followed by 10 minutes at 95% methanol.

In the second stage of HPLC purification, the mobile phase consisted of 400mM 1,1,1,3,3,3-Hexafluoro-2-propanol, water and methanol (pH 7). Two 800mM stocks of HFIP were prepared separately in A, water and B, MeOH from 99.5+% HFIP (Acros) and adjusted to pH 7 with TEA. Solvents "A" and "B" were prepared by diluting the stock solutions to 400mM in water for the "A" component and in MeOH for the "B" component. Ag:DNAs were purified using linear gradients, for "Green1" and "Red", from 10% to 50% methanol over 40 minutes; for "Green2", 10%-50% over 30 minutes; for "Orange", 10%-40% over 30 minutes; for "Green3", 15%-55% over 30 minutes and for "IR", 17-47% over 30 minutes. Each gradient was preceded by a 10 minute equilibration at the initial methanol percentage and followed by a 10 minute wash at 95% methanol.

**Fluorescence** Full emission spectra were collected every second by a thermoelectrically cooled array detector (QE65000, Ocean Optics) which was fiber coupled to an 11$\mu$L, in-line fluorescence flow cell. Excitation of all Ag:DNA species was accomplished using a 270nm LED.

**Mass Spectrometry** Tandem HPLC-MS was performed in negative ion mode using a Micromass QTOF2 set to 2.0 kV capillary voltage and 35 V cone voltage. The source and desolvation temperatures were set to 120 and 150°C, respectively. A splitter was put in the HPLC eluent line, yielding a 10$\mu$L/min flow rate into the mass spectrometer. Mass spectra were collected every second over the range 500-3000 m/z.

***Supplementary data***

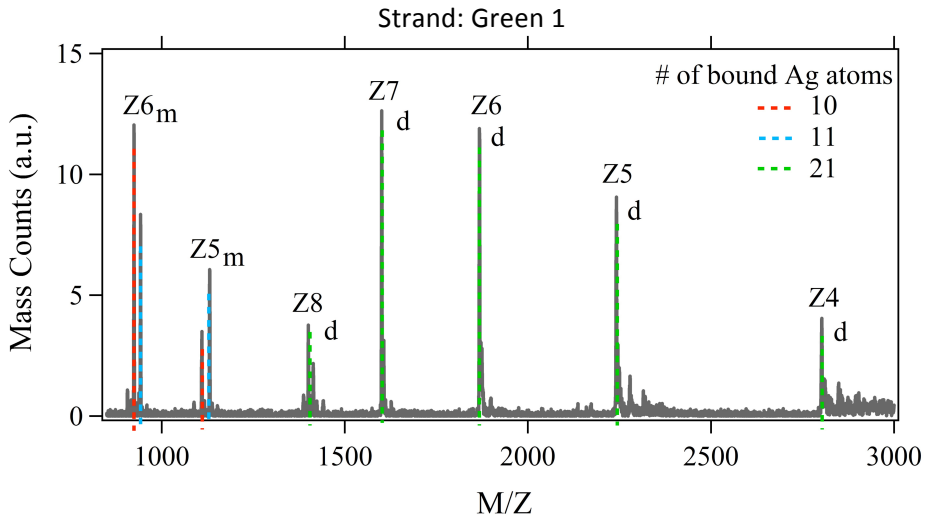


***Figure S1a.*** **Mass Spectrum of the twice-purified, 558 nm "Green1" emitter.**

Mass spectrum of the twice-purified "Green1" emitter exhibits a charge ladder of peaks that correspond to a $N_{Ag}$ = 21, strand dimer complex. In addition, at lower M/Z there are peaks corresponding to $N_{Ag}$ = 10 and 11 silver atoms bound to a single "Green1" strand. These are fragments generated within the mass spectrometer: Given the large difference in base numbers (15 for strand monomers, versus 30 for strand dimers), it is implausible that these species would have precisely equal retention times. We note that loosely bound complexes are expected to undergo fragmentation by the same Coulomb explosion process responsible for desolvation in the electrospray ionization process, consistent with the rapid cut-off in the dimer charge state ladder beyond Z = -7.

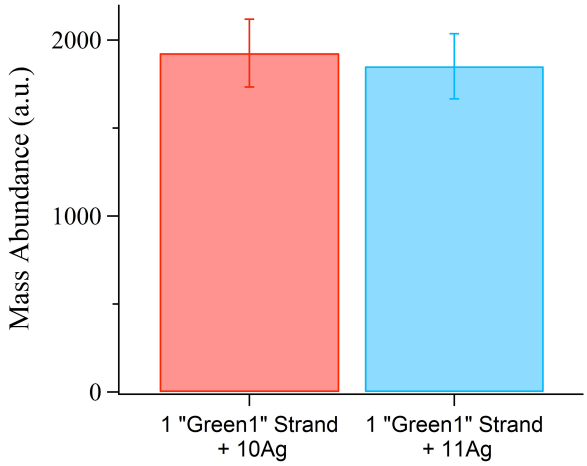


***Figure S1b.*** **Relative abundances of "Green1" monomer species with $N_{Ag}$ = 10 (red) and $N_{Ag}$ = 11 (blue).**

Integrated mass counts of the two monomer species show that they are present in equal abundances, as expected for fragments of the same complex.

Strand: Green 2

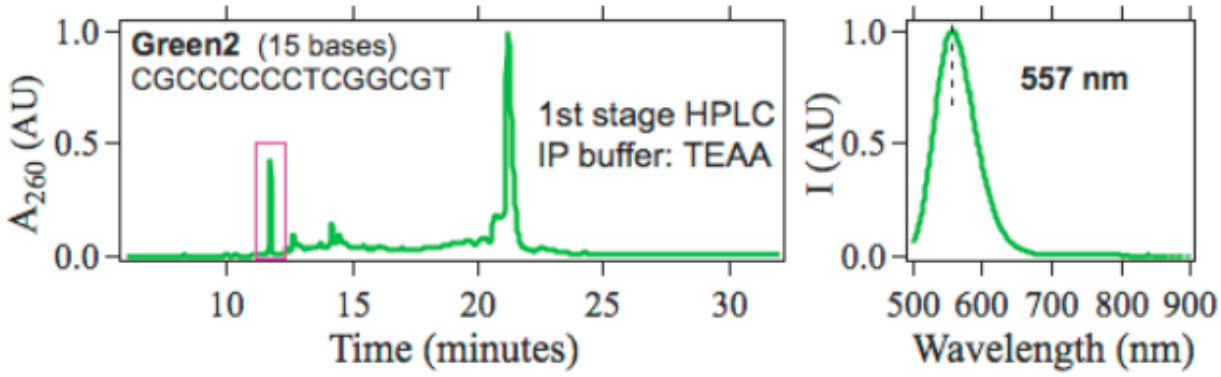


**Fig. S2a. Preliminary separation of the "green2" fluorescent Ag:DNA in TEAA, pH7.**

**Left panel:** $A_{260}$ chromatogram exhibits multiple Ag:DNA products formed on the "green2" strand. *Pink box:* absorbance peak corresponding to the bright, identified Ag:DNA species. **Right panel:** Emission spectrum corresponding the boxed A260 peak. Excitation at 270 nm.

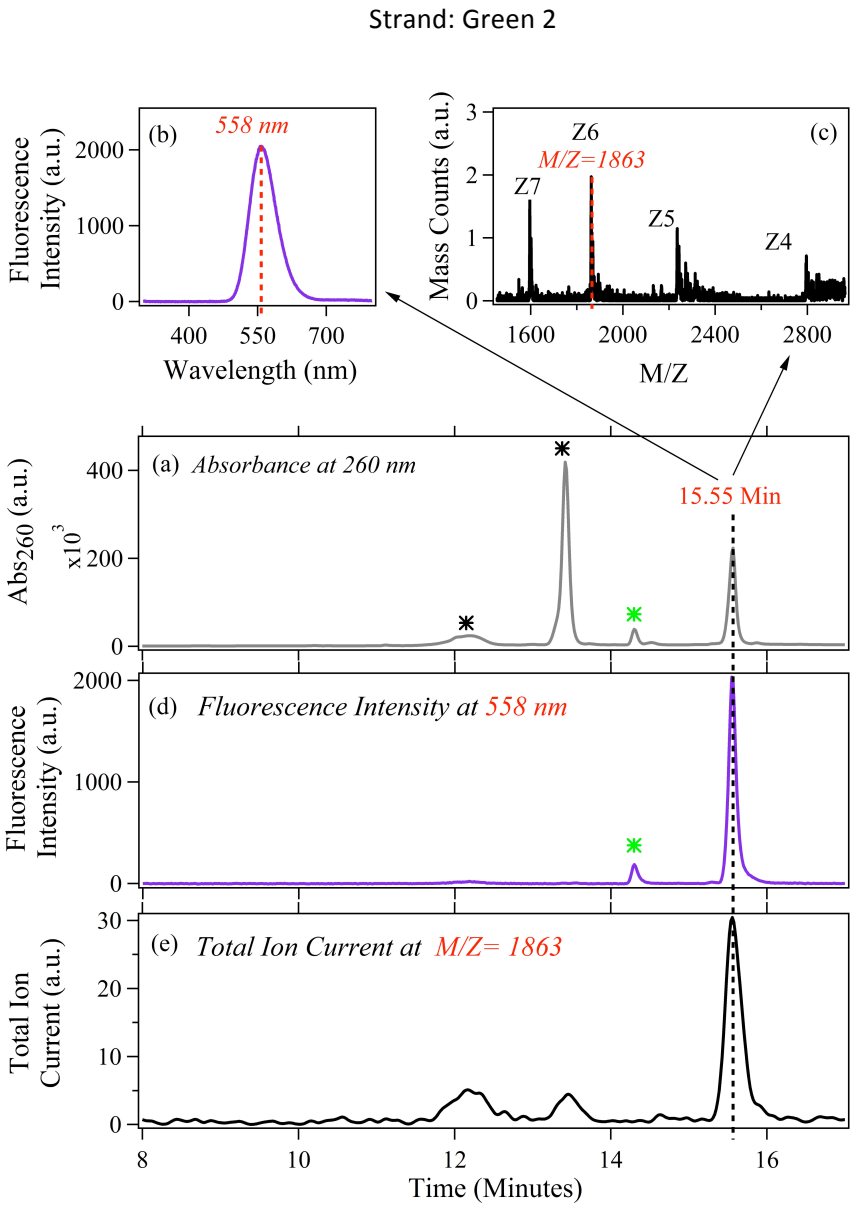


***Figure S2b.*** **Identification of the 558 nm "Green2" emitter from the second HPLC purification stage (HFIP/TEA IP buffer, pH 7), with tandem HPLC-MS and in-line spectroscopy**: $N_{Ag} = 21$, $n_s = 2$.

(a) Absorbance chromatogram. The green star marks elution of the T5-FAM marker. Black stars mark elution of *dark* Ag:DNA complexes. The fluorescent complex elutes at 15.55 minutes. (b) Emission spectrum and (c) MS corresponding to the 15.55 minute peak in the absorbance chromatogram. The charge Z= -4 to -7 peaks identify $N_{Ag} = 21$, $n_s = 2$. (d) Fluorescence chromatogram of 558 nm emission and (e) mass chromatogram of the fluorescent $N_{Ag} = 21$, $n_s = 2$ complex (Z = -6). The broadening of the mass chromatogram (e) relative to absorbance (a) and fluorescence (d) chromatograms is seen in all cases, and results from extra-column volume introduced by the flow splitter that is required to reduce flow rates into the mass spectrometer.

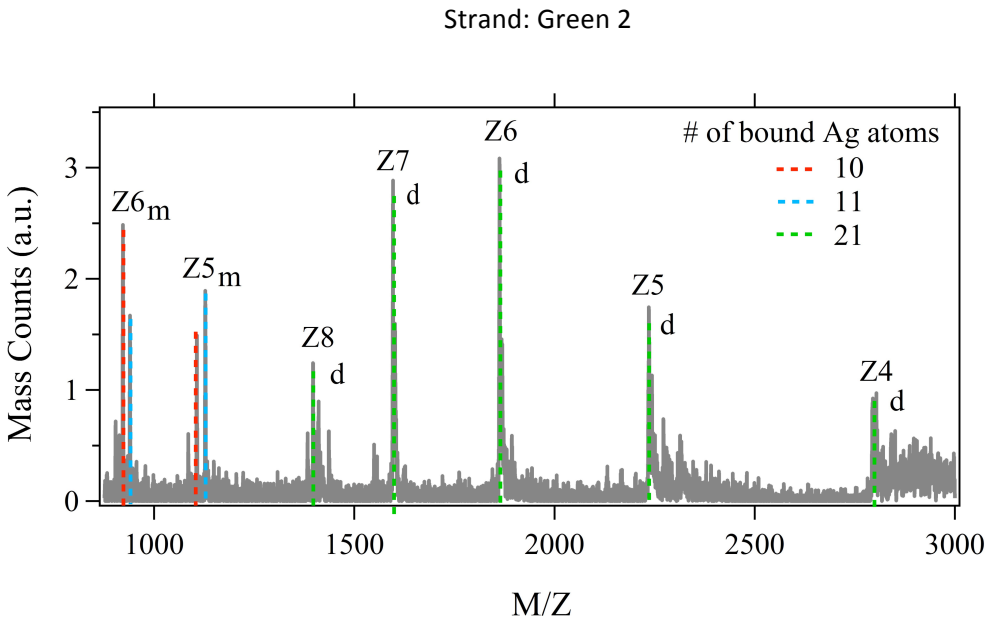


***Figure S3a.*** **Mass Spectrum of the twice-purified Green2 emitter shows a series of charge states belonging to a $N_{Ag}$ = 21, strand dimer complex (d) (with Na adducts to the right of main peak). In addition, there are fragment peaks (m) with $N_{Ag}$ = 10 and $N_{Ag}$ = 11 attached to individual "Green 2" strands.**

Mass spectra of the twice-purified "Green2" emitter exhibit a charge ladder of peaks that correspond to a $N_{Ag}$ = 21, strand dimer complex. In addition, at lower M/Z there are peaks corresponding to $N_{Ag}$ = 10 and 11 silver atoms bound to a single "Green2" strand. These are fragments generated within the mass spectrometer: Given the large difference in base numbers (15 for strand monomers, versus 30 for strand dimers), it is implausible that these species would have precisely equal retention times. We note that loosely bound complexes are expected to undergo fragmentation by the same Coulomb explosion process responsible for desolvation in the electrospray ionization process, consistent with the rapid cut-off in the dimer charge state ladder beyond Z = -7.

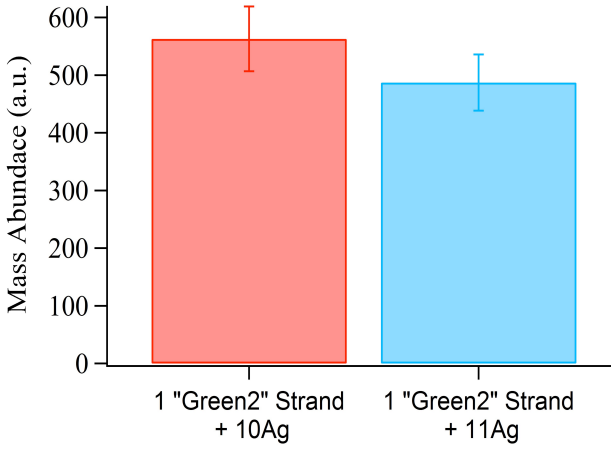


***Figure S3b.*** **Relative abundances of "Green2" monomer species with $N_{Ag}$ = 10 (red) and $N_{Ag}$ = 11 (blue).**

Integrated mass counts of the two monomer species show that they are present in equal abundances, as expected for fragments of the same complex.

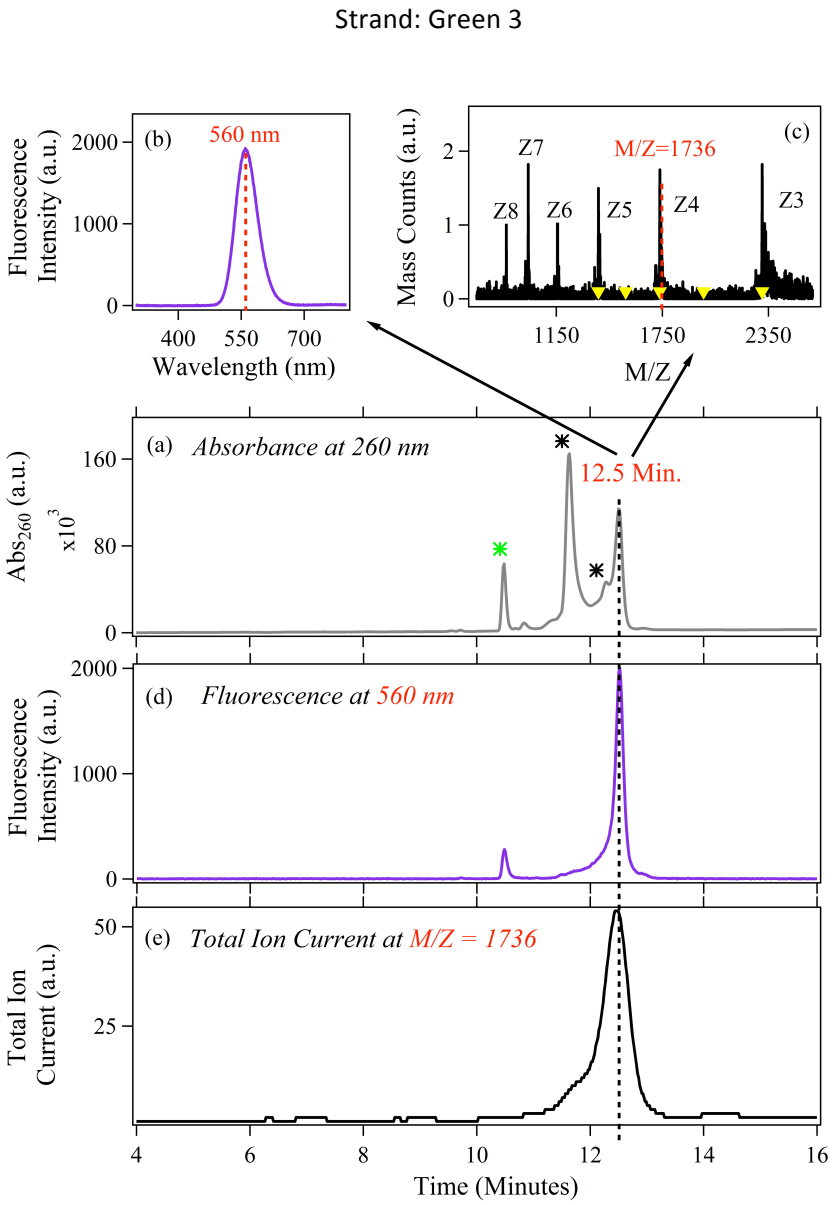


***Figure S4.*** **Identification of the 560 nm "Green3" emitter from the second HPLC purification stage (HFIP/TEA IP buffer, pH 7), with tandem HPLC-MS and in-line spectroscopy**: **$N_{Ag}$ = 10, $n_s$ =1.**

(a) Absorbance chromatogram. The green star marks elution of the T5-FAM marker. Black stars mark elution of *dark* Ag:DNA complexes. The fluorescent complex elutes at 12.5 minutes. (b) Emission spectrum and (c) MS corresponding to the 12.5 minute peak in the absorbance chromatogram. The charge Z= -3 to Z= -8 peaks identify $N_{Ag}$ = 10, $n_s$ =1. Yellow triangles mark expected M/Z values for dimer complexes with $N_{Ag}$ = 20, $n_s$ =2. The peaks corresponding to dimer complexes with odd Z are missing, confirming that the emitter is a strand monomer complex. (d) Fluorescence chromatogram of 560 nm emission and (e) mass chromatogram of the fluorescent $N_{Ag}$ = 10, $n_s$ =1 complex (Z = -4).

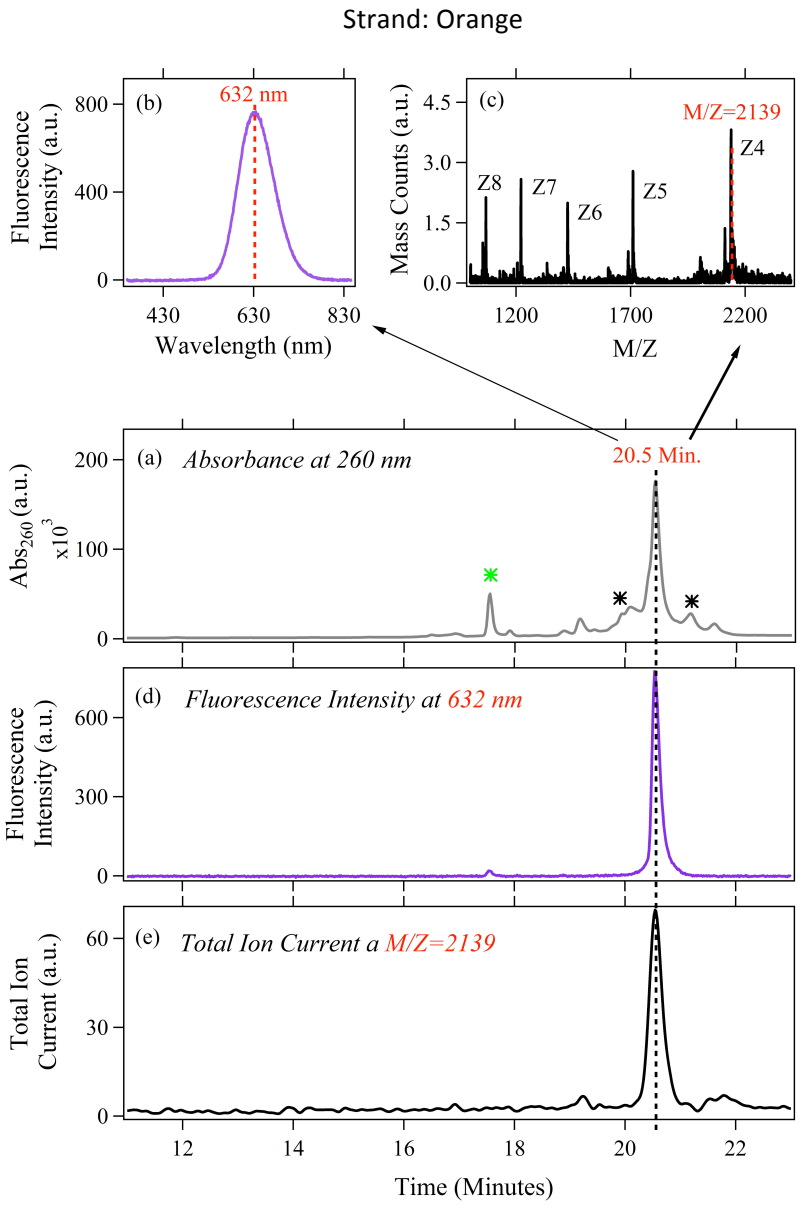


***Figure S5.*** **Identification of the 632 nm "Orange" emitter from the second HPLC purification stage (HFIP/TEA IP buffer, pH 7), with tandem HPLC-MS and in-line spectroscopy**: **$N_{Ag}$ = 16, $n_s$ =1 is more likely, but $N_{Ag}$ = 15, $n_s$ =1 cannot be definitively ruled out (Fig S6).**

(a) Absorbance chromatogram. The green star marks elution of the T5-FAM marker. Black stars mark elution of *dark* Ag:DNA complexes. The fluorescent complex elutes at 20.5 minutes. (b) Emission spectrum and (c) MS corresponding to the 20.5 minute peak in the absorbance chromatogram. The charge Z= -4 to -8 peaks identify $N_{Ag}$ = 16, $n_s$ =1. The mass spectrum additionally shows traces of a different Ag:DNA complex with $N_{Ag}$ = 15 and $n_s$ =1; however, its abundance is low compared to the $N_{Ag}$ =16 Ag complex (Fig. S6; next page), whose mass chromatographic profile better fits the fluorescence profile (Fig. S7; next page). (d) Fluorescence chromatogram of 632 nm emission and (e) mass chromatogram of the $N_{Ag}$ = 16, $n_s$ =1 complex (Z = -4).

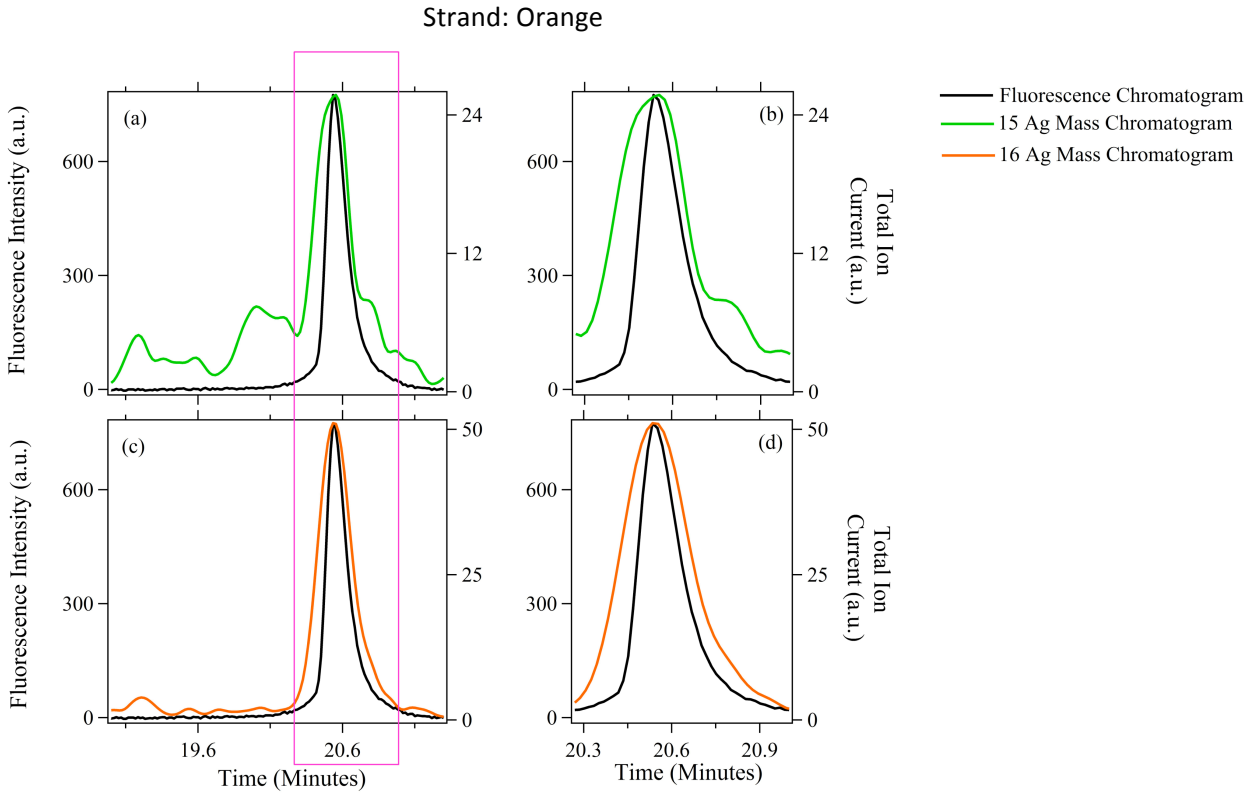


***Figure S6.*** **Comparison of the 632nm fluorescence chromatogram (black) and the $N_{Ag}$ = 15, $n_s$ =1, Z= -4 (top row) and $N_{Ag}$ = 16, $n_s$ =1, Z = - 4 (bottom row) mass chromatograms.**

(a) Overlay of the fluorescence chromatogram (black) and the $N_{Ag}$ = 15, $n_s$ =1 (green) mass chromatogram. The broadening of the mass chromatogram relative to the fluorescence chromatogram results from extra-column volume introduced by the flow splitter that is required to reduce flow rates into the mass spectrometer. (b) A zoom-in of (a) shows that the $N_{Ag}$ = 15, $n_s$ =1 mass chromatogram has a distorted peak shape and slight offset relative to the fluorescence chromatogram. (c) An overlay of the fluorescence chromatogram (black) and the $N_{Ag}$ = 16, $n_s$ =1 (orange) mass chromatogram. (d) A zoom-in of (c) shows that the $N_{Ag}$ = 16, $n_s$ =1 mass chromatograms and the fluorescence trace have similar peak shapes and equal retention times.

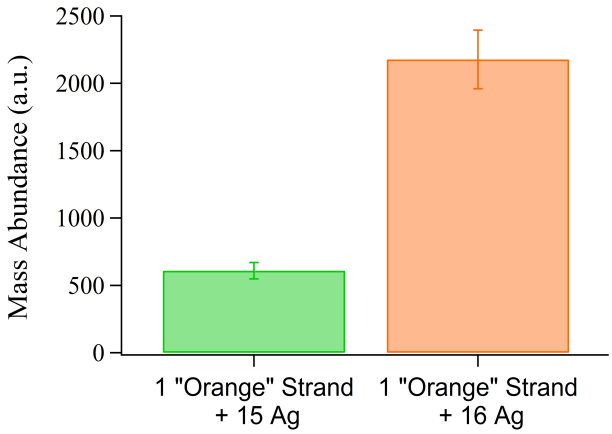


***Figure S7.*** **Relative abundances of the $N_{Ag}$ = 15, $n_s$ =1 complex (green) and the $N_{Ag}$ = 16, $n_s$ =1 complex (orange).**

Integrated mass counts over all charge states of the $N_{Ag}$ = 15, $n_s$ =1 Ag:DNA (green) and the $N_{Ag}$ = 16, $n_s$ =1 Ag:DNA (orange), show that the $N_{Ag}$ = 16 complex is ~4 times more abundant than the $N_{Ag}$ = 15 complex, after 2 separate stages of HPLC purification.

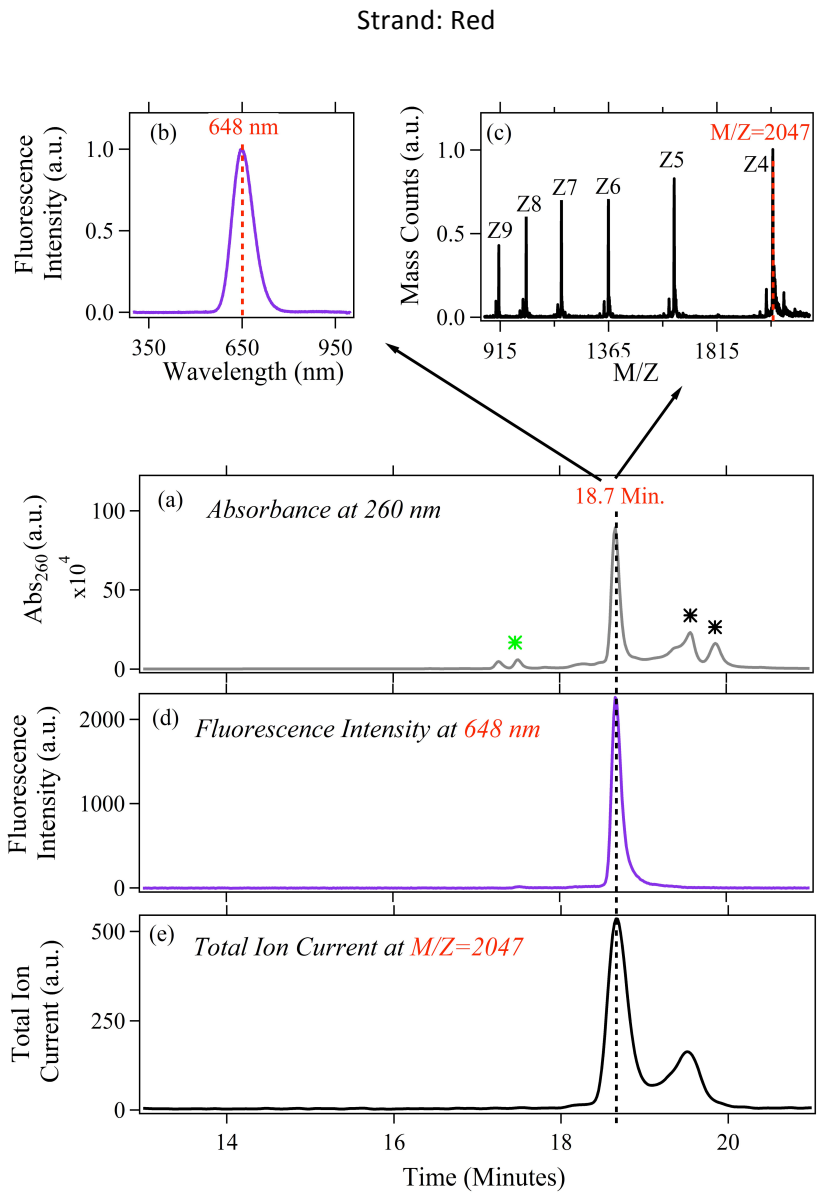


***Figure S8.*** **Identification of the 648 nm "Red" emitter from the second HPLC purification stage (HFIP/TEA IP buffer, pH 7), with tandem HPLC-MS and in-line spectroscopy**: **$N_{Ag}$ = 15, $n_s$ =1.**

(a) Absorbance chromatogram. The green star marks elution of the T5-FAM marker. Black stars mark elution of *dark* Ag:DNA complexes. The fluorescent complex elutes at 18.7 minutes. (b) Emission spectrum and (c) MS corresponding to the 18.7 minute peak in the absorbance chromatogram. The charge Z= -4 to -9 peaks identify $N_{Ag}$ = 15, $n_s$ =1. The mass spectrum additionally shows traces of a different Ag:DNA complex with $N_{Ag}$ = 14 and $n_s$ =1 (Figs S9 and S10, next page). (d) Fluorescence chromatogram of 648 nm emission and (e) mass chromatogram of the $N_{Ag}$ = 15, $n_s$ =1 complex (Z = -4).

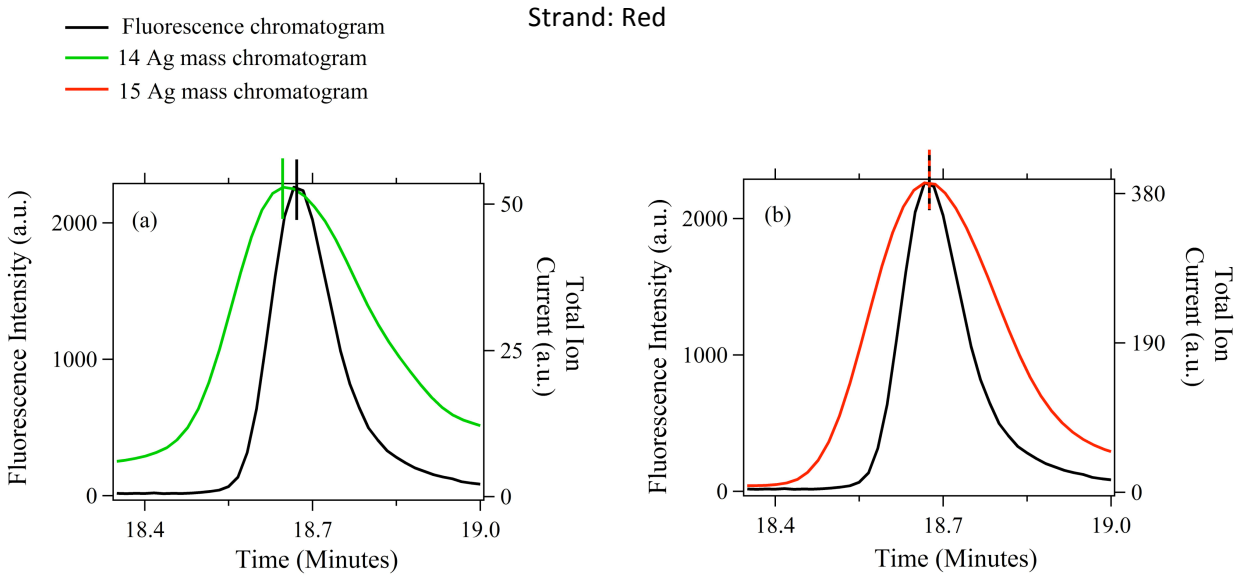


***Figure S9.*** **Comparison of the 648nm fluorescence chromatogram (black) and the $N_{Ag}$ = 14, $n_s$ =1, Z = -4 (green) and $N_{Ag}$ = 15, $n_s$ =1, Z = -4 (red) mass chromatograms.**

(a) An overlay of the fluorescence chromatogram (black) and the $N_{Ag}$ = 14, $n_s$ =1 (green) mass profile show that the mass chromatogram of the $N_{Ag}$ = 14 complex peaks before the emission at 648 reaches its maximum. (b) An overlay of the fluorescence chromatogram (black) and the $N_{Ag}$ = 15, $n_s$ =1 (red) mass chromatogram show that the M/Z counts of the $N_{Ag}$ = 15 complex and emission at 648 peak simultaneously. The broadening of the mass chromatogram relative to the fluorescence chromatogram results from extra-column volume introduced by the flow splitter that is required to reduce flow rates into the mass spectrometer.

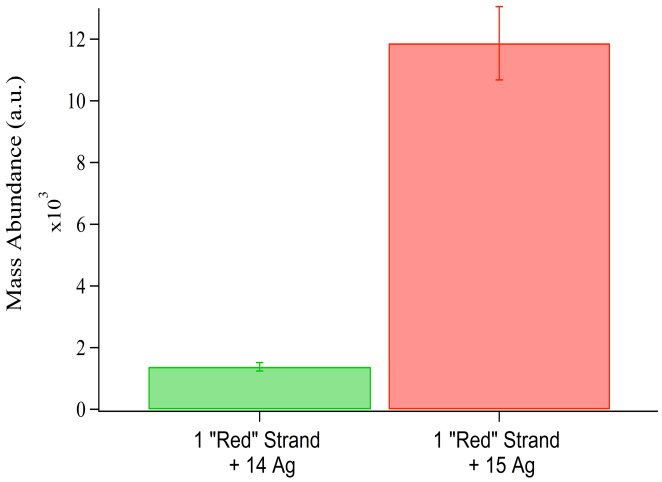


***Figure S10.*** **Relative abundances of the $N_{Ag}$ = 14, $n_s$ =1 complex (green) and the $N_{Ag}$ = 15, $n_s$ =1 complex (red).**

Integrated mass counts over all charge states show that the $N_{Ag}$ = 15 complex is ~10 times more abundant than the $N_{Ag}$ = 14 complex, after 2 separate stages of HPLC purification.

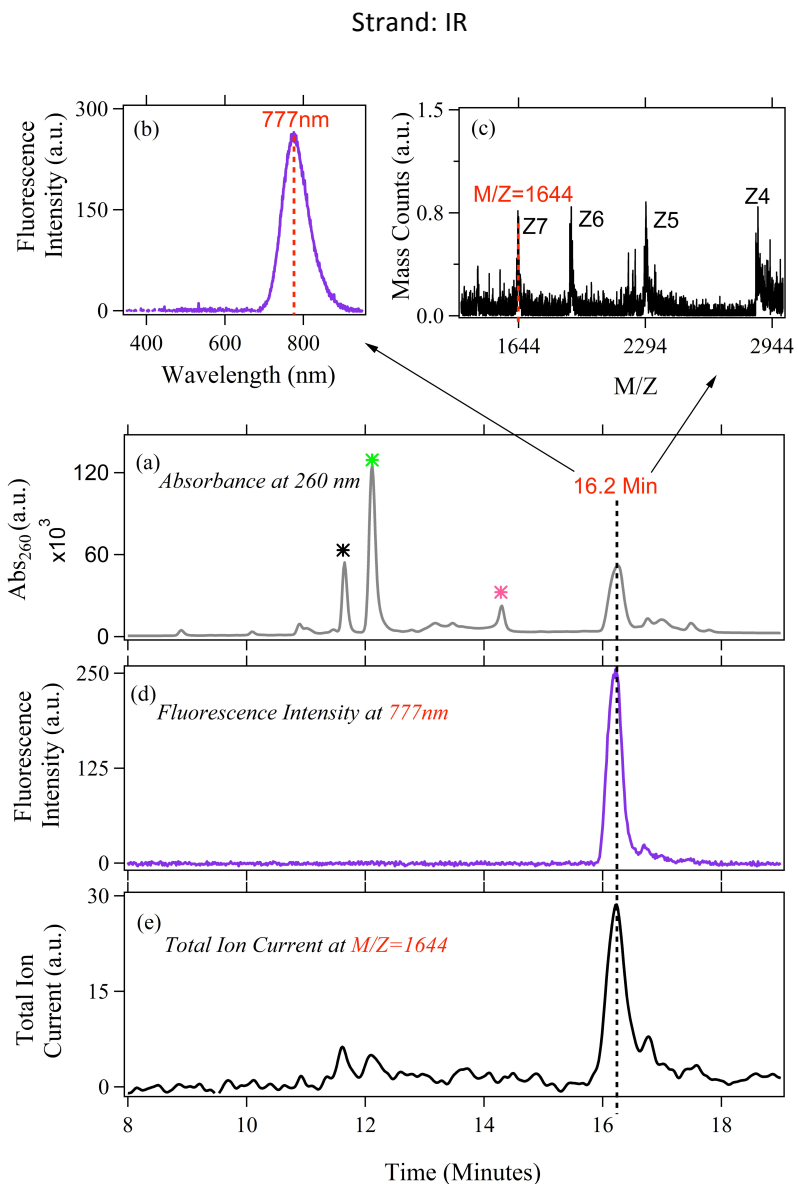


***Figure S11.*** **Identification of the "IR" emitter from the second HPLC purification stage (HFIP/TEA IP buffer, pH 7), with tandem HPLC-MS and in-line spectroscopy**: **$N_{Ag}$ = 20, $n_s$ =2.**

(a) Absorbance chromatogram. The green star marks elution of the T5-FAM marker. The black star marks elution of a dark Ag:DNA complex with $N_{Ag}$ = 10, $n_s$ =1 (Fig. S12; next page), and the pink star marks elution of the bare DNA strand. The IR fluorescent complex elutes at 16.2 minutes. (b) Emission spectrum and (c) MS corresponding to the 16.2 minute peak in the absorbance chromatogram. The charge Z= -4 to -7 peaks identify $N_{Ag}$ = 20, $n_s$ =2. The peaks at m/z=1644 and 2294 rule out the possibility of a $N_{Ag}$ = 10, $n_s$ =1 complex (which could only produce these peaks given impossible, half integer values of Z). (d) Fluorescence chromatogram of 777 nm emission and (e) mass chromatogram of the $N_{Ag}$ = 20, $n_s$ =2 complex (Z = -7). A separate data set, taken in ammonium acetate buffer (Fig. S13), indicates that the 31% methanol content at elution is responsible for the ~ 30 nm solvent-shift relative to a previous study [15] of "Strand IR".

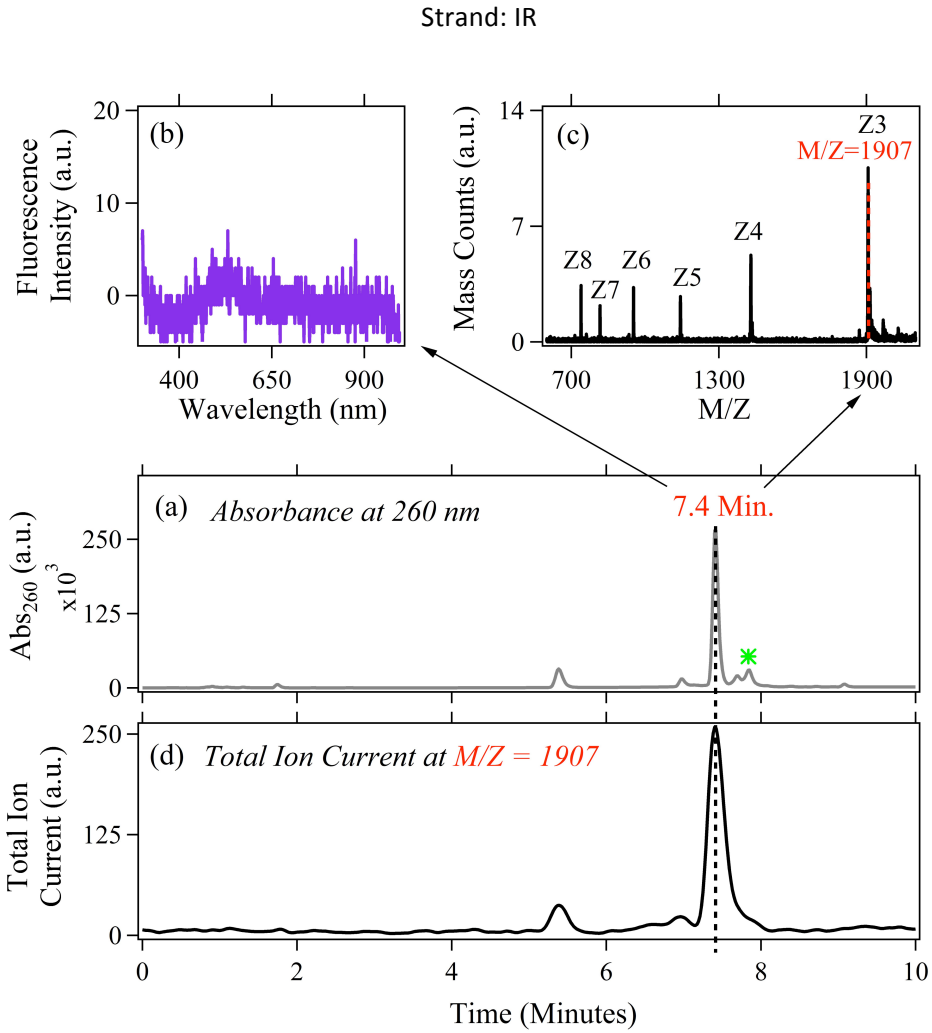


***Figure S12.*** **Identification a dark complex with $N_{Ag}$ = 10, $n_s$ =1, formed on "Strand IR" (Table S1). HFIP/TEA IP buffer, pH 7.**

(a) Absorbance chromatogram. The green star marks the T5-FAM marker. The dark $N_{Ag}$ = 10, $n_s$ = 1 complex elutes at 7.4 minutes (earlier than in the separation in Fig. S11, due to the 4% smaller initial methanol concentration of 13%). (b) Emission spectrum, showing negligible fluorescence; and (c) MS corresponding to the 7.4 minute peak in the absorbance chromatogram. The charge Z= -3 to -8 peaks identify $N_{Ag}$ = 10, $n_s$ =1. (d) Mass chromatogram of the dark complex (Z = -3).

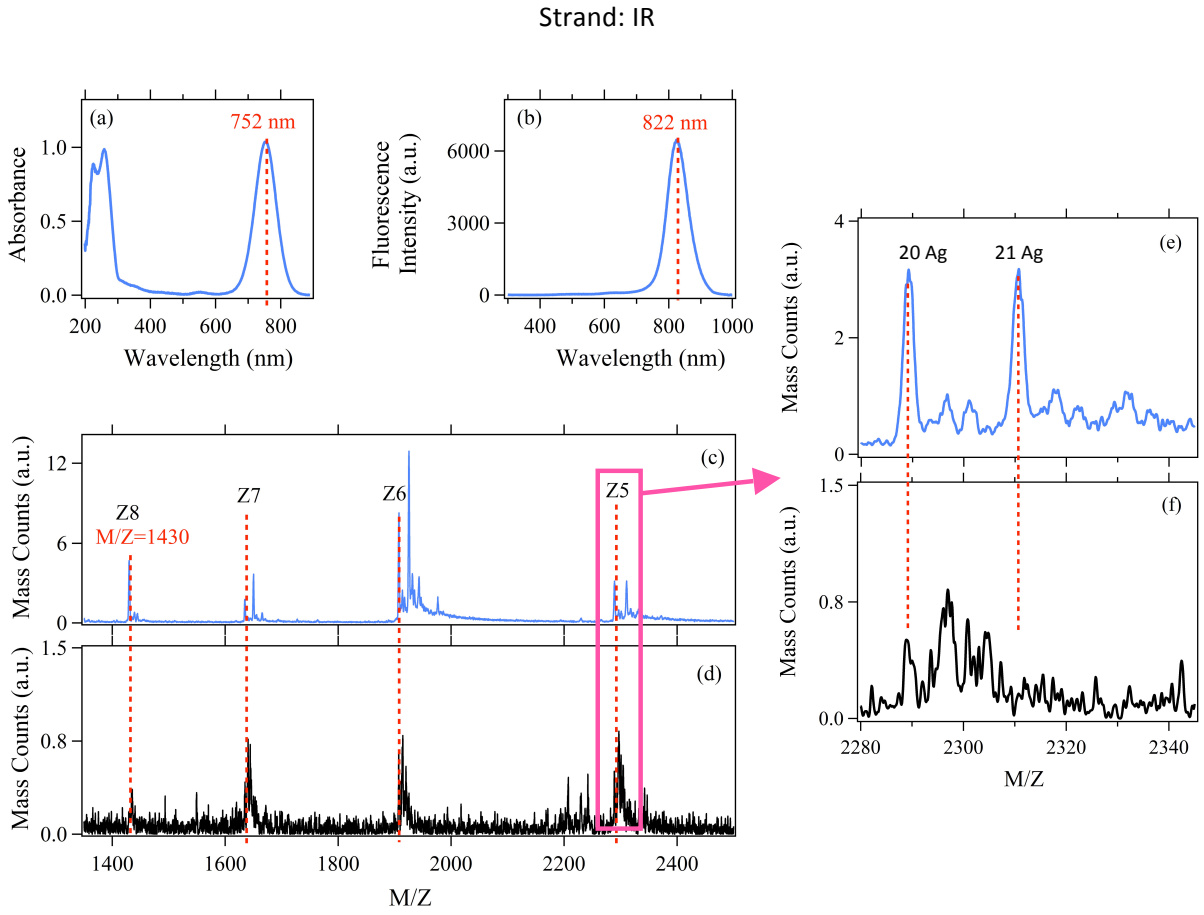


***Figure S13.*** **Solvent effects on optical properties of the IR emitter.**

(a) Absorbance and (b) fluorescence spectra of the partially purified, fluorescent "IR" solution prepared by combining several aliquots collected during TEAA- based HPLC, after concentration and methanol removal with multiple washes of 10mM $NH_4OAc$. This red-shifts the emission peak to 822 nm, relative to the 777 nm measured at 31% methanol concentration (Fig. 11b). (c) Manually-injected mass spectrum of the combined, concentrated sample in 10 mM $NH_4OAc$ shows a series of charge states (Z = -5 to -8) corresponding to $N_{Ag}$ = 20 and 21; $n_s$ =2. (d) The $N_{Ag}$ = 20, $n_s$ =2 peaks agree with those obtained using tandem HPLC-MS of the twice-purified IR emitter. (e,f) Zoomed-in image of the Z = -5 charge states obtained from manual injection MS of the once-purified, IR-emitting solution (e) and by tandem MS from the second HPLC purification stage (f). Red lines mark M/Z for $N_{Ag}$ = 20 and 21. Intervening peaks are salt adducts. Apparently the TEAA-based pre-purification (c,e) does not fully separate the $N_{Ag}$ = 21 complex from the $N_{Ag}$ = 20, IR-emitting complex.

| Strand Name | Peak emission wavelength (nm), TEAA-based HPLC | % methanol at elution, TEAA-based HPLC | Peak emission wavelength (nm), HFIP/TEA-based HPLC | % methanol at elution, HFIP/TEA-based HPLC |
|---|---|---|---|---|
| Green1 | 557 | 15.8 | 558 | 27.57 |
| Green2 | 557 | 14.7 | 558 | 28.13 |
| Green3 | 562 | 31.9 | 562 | 28.9 |
| Orange | 632 | 20.65 | 632 | 28.48 |
| Red | 644 | 19.45 | 648 | 26.67 |
| IR | 777 | 22.4 | 777 | 31.25 |

**Table S2. Peak emission wavelengths measured in-line during HPLC separation with both TEAA and HFIP/TEA buffers, and methanol percentage at elution, for all identified Ag:DNA emitters.**

Ag:DNA emission wavelengths in HFIP/TEA/MeOH are in good agreement with those in TEAA/MeOH, differing by 4nm or less, demonstrating HFIP/TEA to be a suitable mobile phase for the identification of emitter composition. We note that in the presence of methanol, the emission wavelength of the IR emitter is blue shifted relative to the 822 nm emission peak we find in ammonium acetate buffer (Fig. S13), and relative to previous reports of 810 nm emission in citrate buffer [15].

Retention in TEAA and HFIP/TEA differs due to the different nature of oligonucleotide-C18 interactions in these buffer systems. In HFIP/TEA, ion-pairing interactions dominate retention to C18. In TEAA, strongly base-dependent hydrophobic interactions (T > A > G > C) are also important [16].