Supplementary Information

Action-Self Quenching: Dimer-induced Fluorescence Quenching of Chromophores as a Probe for Biomolecular Structure.

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Figure S1: The structure of Atto 520 maleimide.

Figure S2: (a) UV absorption spectrum of Atto 520 in 1:1 H_2O:CH_3OH v/v at 1 µM concentration. (b) Fluorescence emission spectrum of Atto 520 in 1:1 H_2O:CH_3OH v/v at 1 µM concentration following excitation at 520 nm.

Figure S3: Figure S3. Fluorescence intensity (solid black line) at 543 nm of a 2:1 ratio of Atto 520 and the tripeptide CAC in H_2O:CH_3OH following photoexcitation at 520 nm. Also shown is the relative intensity of singly (blue) and doubly tagged (red) CAC following ESI of an identical solution. The red dashed lined indicates where the relative intensity of doubly tagged CAC becomes higher than the singly tagged CAC.

Figure S4: Figure S4. Action spectra for mass selected $[A520]^+$ (a) and $[A520\text{-}CAC]^+$ cations, showing the same data as Figure 2(a) and (b) with a zoomed y-scale.

Figure S5: Jablonski diagrams showing the photophysical processes occurring in monomers (a) and dimers (b) of rhodamine derivatives. For the monomer, absorption of a photon takes the system to the first excited singlet state S_1. From here it is possible to relax via fluorescence, or to absorb a second photon to a higher lying singlet state S_n, leading to fragmentation. For the dimer, absorption of a photon does not take place to the S_1, which is optically dark, but to a close lying bright state S_b. This optically excited state is expected to quickly convert to the S_1 state by internal conversion. Since fluorescence is not possible from a dark state, an alternative relaxation to the ground state must occur, which may lead to photofragmentation.

Figure S6: Illustration of the cleavages observed in either LID or CID of doubly tagged CAC. See Figure 4 and Tables S1 and S2 for further details. Note that in the case of cleavages associated with chromophore linker chain, they can occur equally for both chromophores and are only illustrated for one of the two equivalent bonds for clarity.

Table S1: Assignment of the CID spectrum of $[A520\text{-}CAC\text{-}A520]^{2+}$ for fragments with greater than 1% relative intensity. See also Figure 4 and Figure S3.

Table S2: Assignment of the LID spectrum of $[A520\text{-}CAC\text{-}A520]^{2+}$ at 495 nm for fragments with greater than 1% relative intensity. See also Figure 4 and Figure S3.

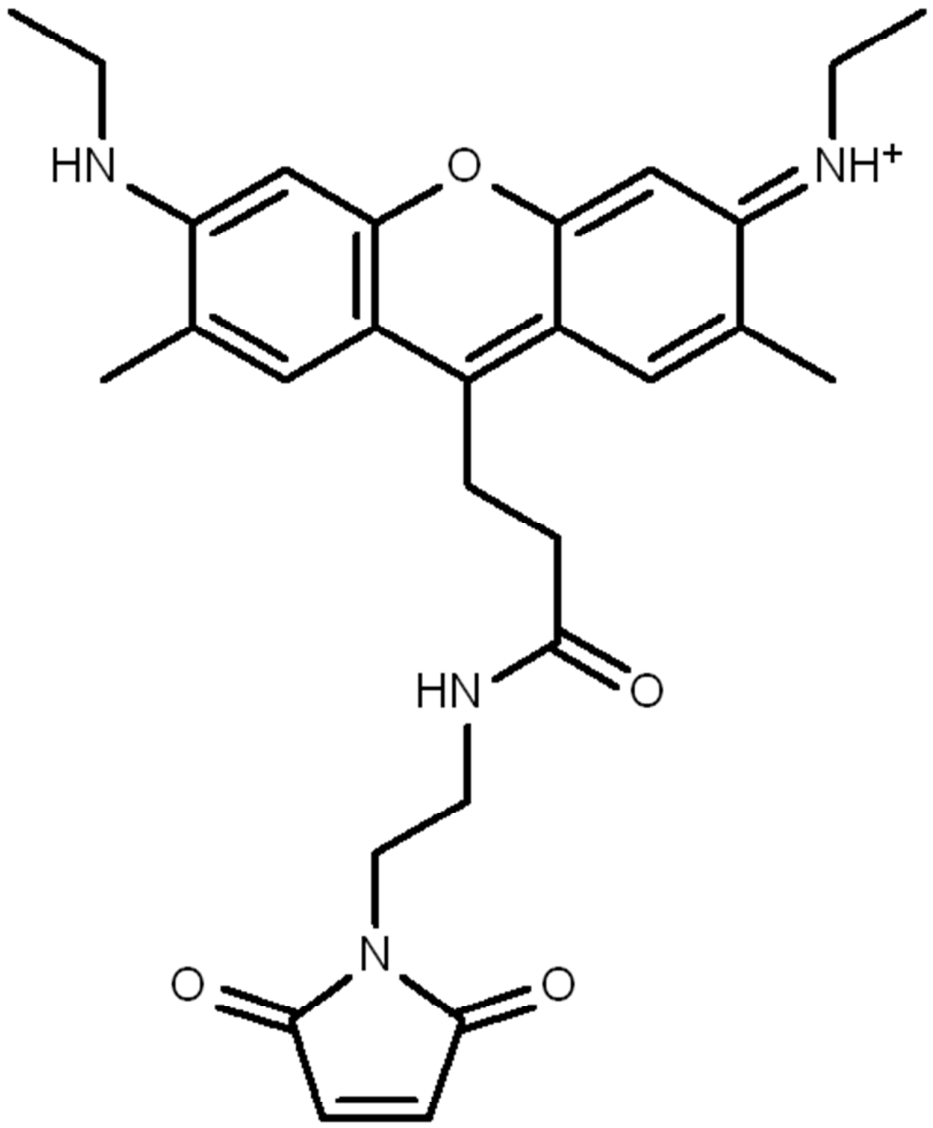
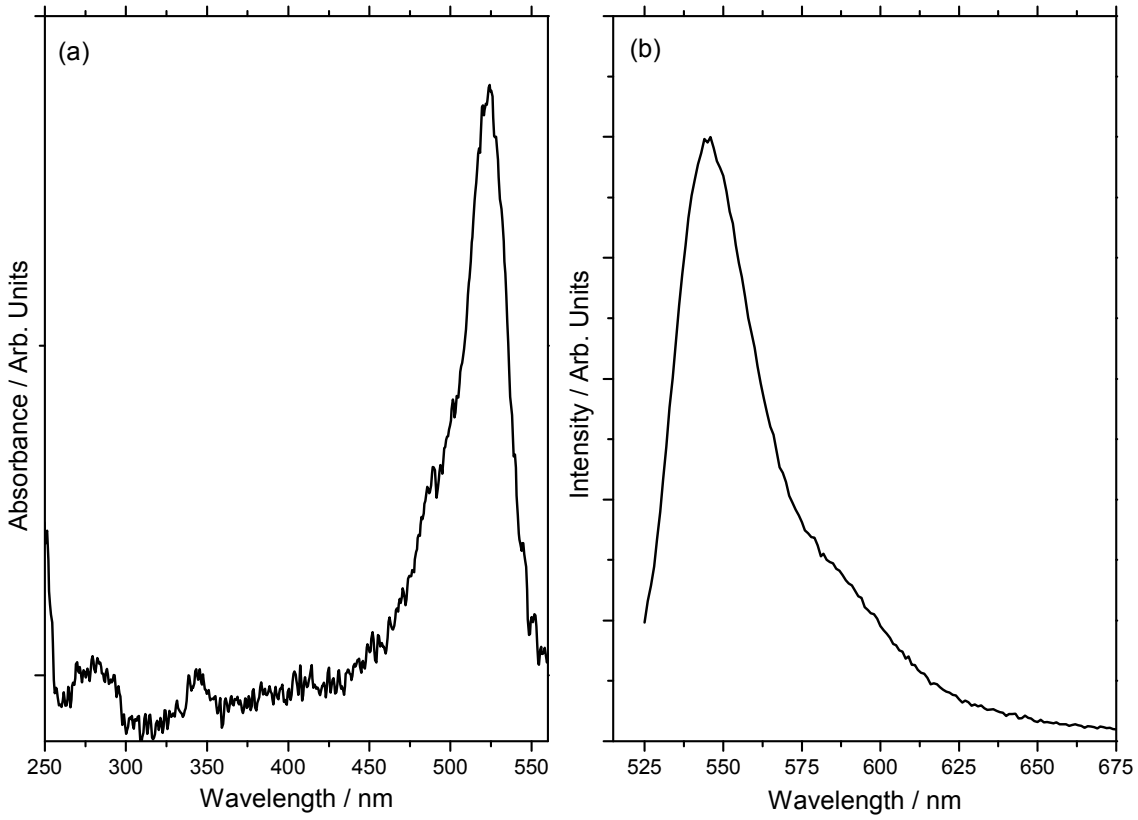


Figure S1. The structure of Atto 520 maleimide.



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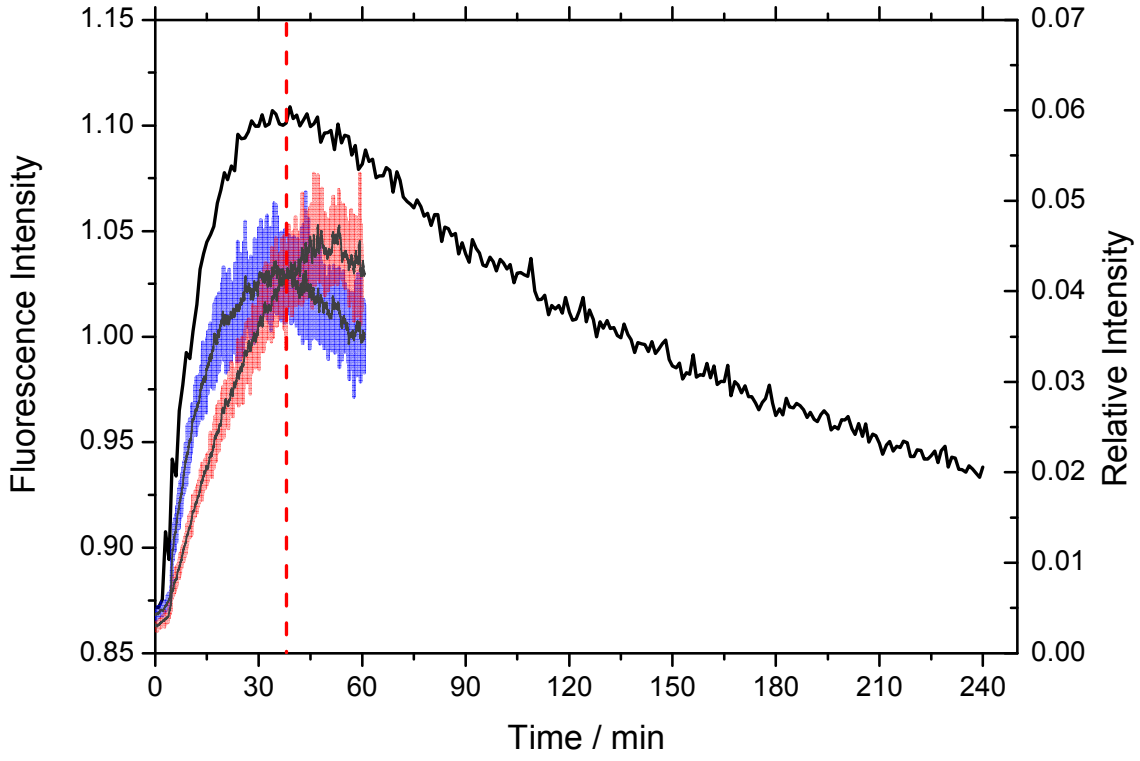

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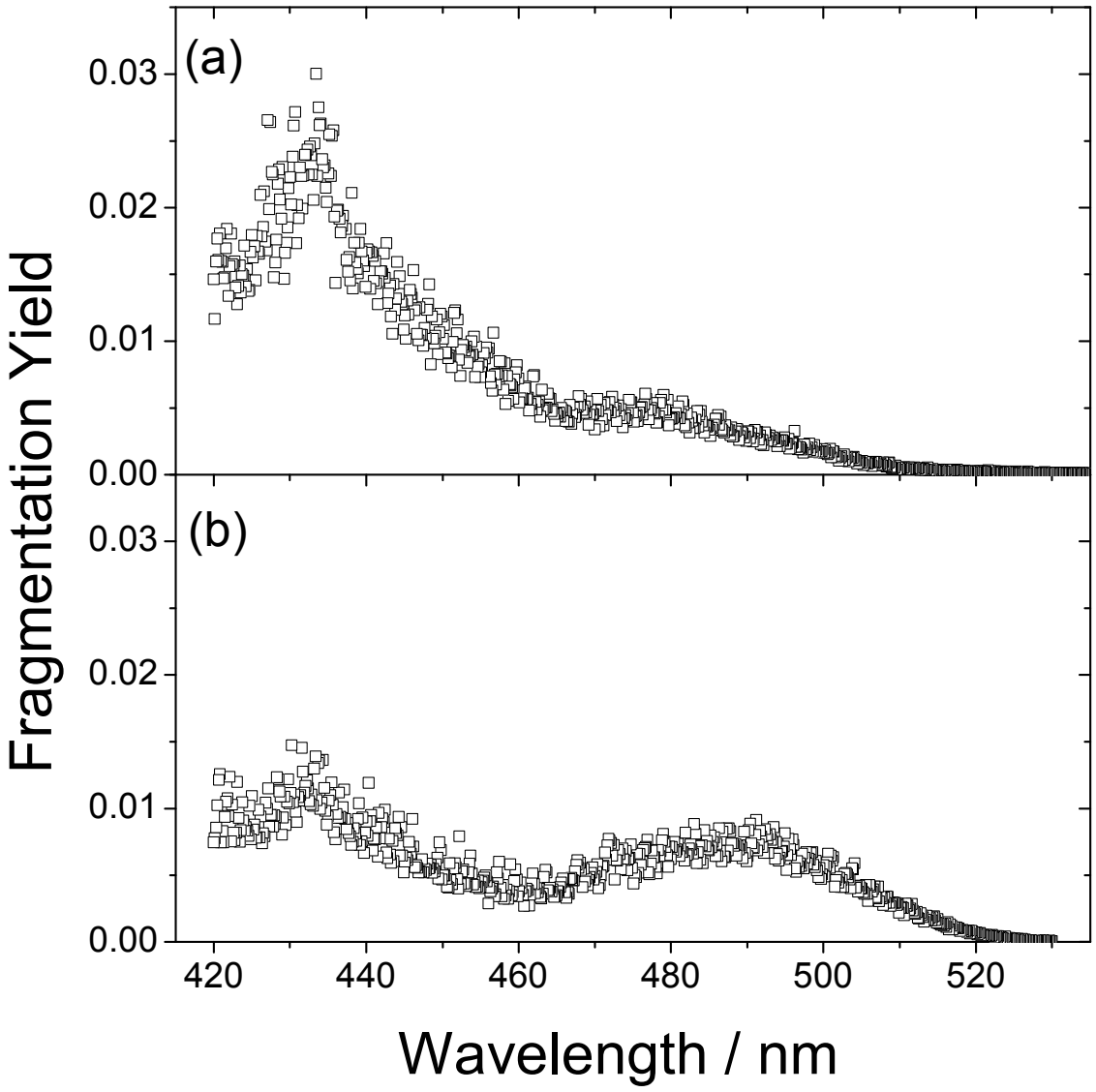

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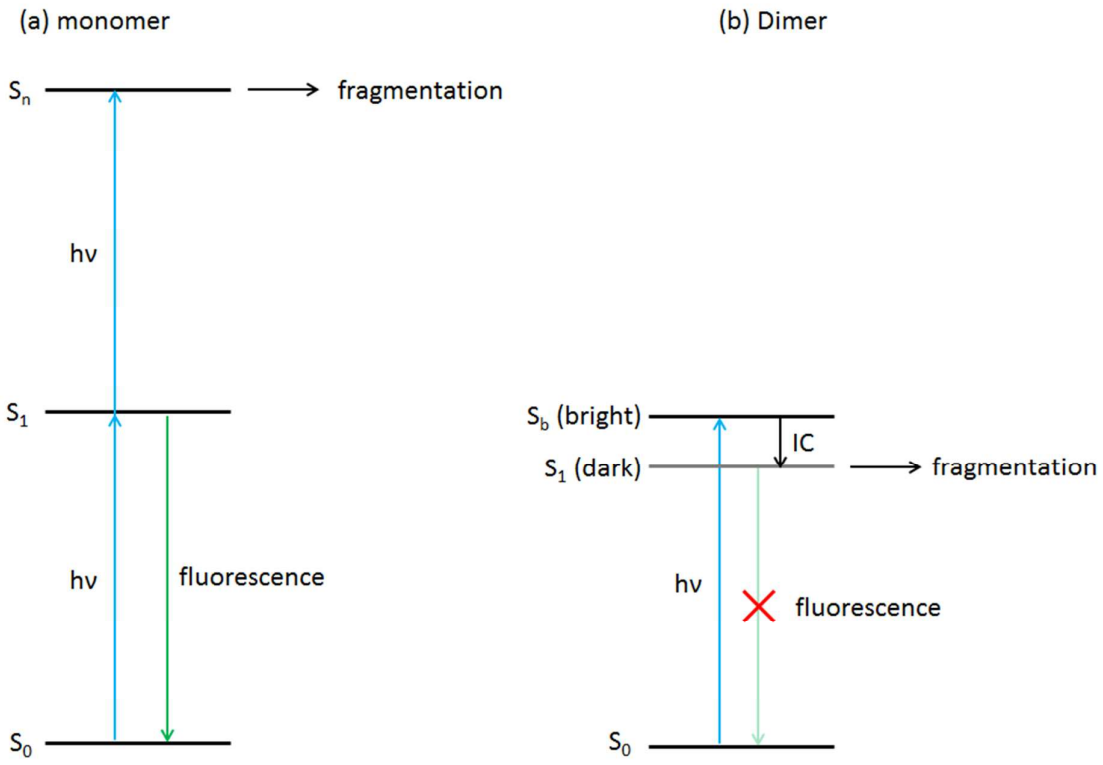

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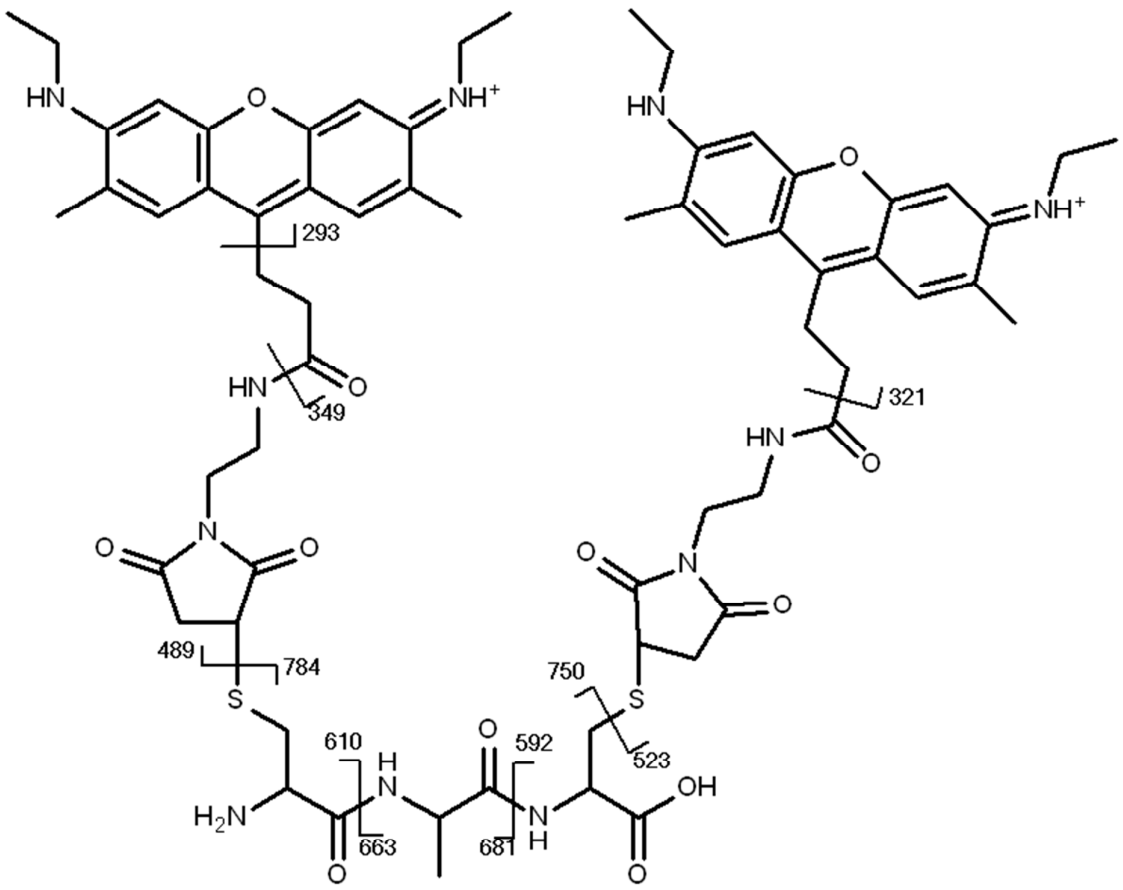

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m/z	z	MW	Loss	assignment
636.8	2	1273.6	0	parent
750.36	1	750.36	523.24	(A-CAC) minus SH (counter ion to m/z 523.2)
706.36	1	706.36	567.24	(A-CAC) minus SH + loss of CO_2 (m/z 750.36 + loss of CO_2)
663.32	1	663.32	610.28	a_2 peptide bond
627.8	2	1255.6	18	Loss of H_2O
614.8	2	1229.6	44	Loss of CO_2
610.28	1	610.28	663.32	b_1 peptide bond
605.8	2	1211.6	62	Loss of H_2O+CO_2
523.2	1	523.2	750.4	(A-SH)

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m/z	z	MW	Loss	assignment
636.8	2	1273.6	0	parent
750.35	1	750.35	523.19	(A520-CAC) minus SH (counter ion to m/z 523.2)
706	1	706	567.54	(A520-CAC) minus SH + loss of CO_2 (m/z 750.36 + loss of CO_2)
681.35	1	681.35	592.19	b_2
663.35	1	663.35	610.19	y_2
614.76	2	1229.52	44.02	Loss of CO_2
610.26	1	610.26	663.28	b_1
592.26	1	592.26	681.28	y_1
523.18	1	523.18	750.36	(A520-SH)
521.18	1	521.18	752.36	(A520-SH) + loss of H_2 (rearrangement of m/z 523.18?)
489.26	1	489.26	784.28	(A520)
475.29	1	475.29	798.25	??
349.26	1	349.26	924.28	A520 amide bond cleavage
321.26	1	321.26	952.28	A520 C-C cleavage
308.26	1	308.26	965.28	A520 amide bond cleavage + N-C side chain loss? or C-C cleavage?
293.17	1	293.17	980.37	A520 C-C break
278.17	1	278.17	995.37	A520 amide bond cleavage + two N-C side chain losses?

Table S2. Assignment of the LID spectrum of $[A520\text{-}CAC\text{-}A520]^{2+}$ at 495 nm for fragments with greater than 1% relative intensity. See also Figure 4 and Figure S3.