Supporting Information

Activity-Linked Labeling of Enzymes by Self-Immolative Polymers

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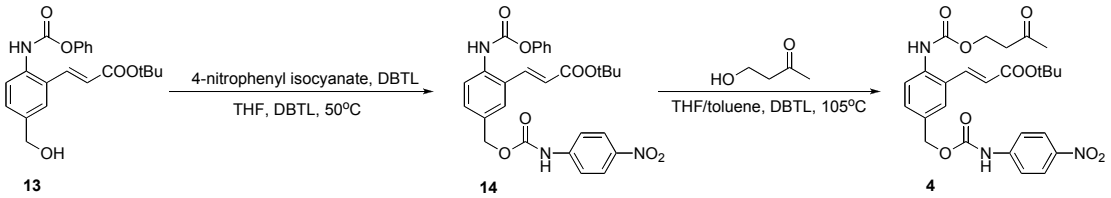
Synthesis of monomeric labeling probe 13



Compound 13. Compound **13** was synthesized and characterized according to published procedure.(25)

Compound 14. To a solution of compound **13** (400 mg, 1.08 mmol, 1 eq) in THF (5 mL), 4-nitrophenyl isocyanate (178 mg, 1.08 mmol, 1 eq) and DBTL (32 μL, 0.054 mmol, 0.05 eq) were added. The reaction was heated to 50°C and monitored to completion (15 minutes) by TLC (EtOAc:Hex 3:7). The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (EtOAc:Hex 1:3) to give compound **14** (510 mg, 88%) as a white solid.

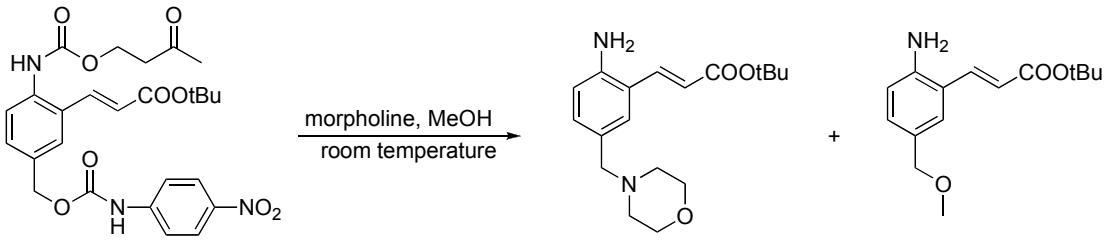
^{1}H NMR (400 MHz, DMSO-d^{6}): δ = 10.36 (1H, s), 9.27 (1H, s), 8.17 (2H, d, *J* = 7.0 Hz), 7.74 (1H, d, *J* = 15.7 Hz), 7.63-7.70 (3H, m), 7.52 (1H, s), 7.09-7.13 (5H, m), 6.66 (1H, d, *J* = 8.3 Hz), 6.24 (1H, d, *J* = 15.7 Hz), 4.98 (2H, s), 1.44 (9H, s). ^{13}C NMR (100 MHz, DMSO-d^{6}): δ = 166.4, 157.7, 153.7, 153.4, 148.7, 146.2, 146.0, 142.2, 142.0, 130.1, 129.8, 129.0, 123.7, 119.1, 118.4, 117.8, 116.7, 115.1, 80.4, 67.2, 28.4. MS (ESI): calculated for [M^{+}] $C_{28}H_{27}N_3O_8$ 533.2; found 533.2.

Compound 4. Compound **14** (240 mg, 0.45 mmol, 1 eq) was dissolved in a mixture of THF:toluene (10 mL, ratio 3:7), preheated to 105°C. 4-Hydroxy-2-butanoe (240 μL, 2.72 mmol, 6 eq) was added, followed by DBTL (15 μL, 0.022 mmol, 0.05 eq) and the reaction was monitored to completion (45 minutes) by TLC (EtOAc:Hex 4:6). The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 4:6) to give compound **4** (205 mg, 85%) as a yellow solid.

^{1}H NMR (200 MHz, DMSO-d^{6}): δ = 10.50 (1H, s), 9.45 (1H, s), 8.20 (2H, d, *J* = 7.2 Hz), 7.89 (1H, s), 7.66-7.74 (3H, m), 7.37-7.48 (2H, m), 6.45 (1H, d, *J* = 15.8 Hz),

5.17 (2H, s), 4.25 (2H, t, *J* = 6.2 Hz), 2.82 (2H, t, *J* = 6.2 Hz), 2.14 (3H, s), 1.48 (9H, s). ^{13}C NMR (50 MHz, DMSO-d^6): δ = 206.8, 166.3, 154.8, 153.6, 148.7, 146.1, 142.0, 139.8, 133.6, 128.9, 126.7, 121.0, 118.0, 117.6, 116.7, 80.4, 67.2, 60.2, 42.5, 30.4, 28.3. MS (ESI): calculated for [M$^+$] $C_{26}H_{29}N_3O_9$ 527.2; found 527.2.

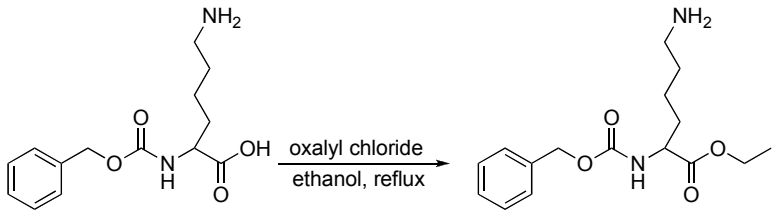
Competition behavior between a triggering nucleophile and polar solvent



4 **4c** **4b**

Compound 4b. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.72 (1H, d, *J* = 15.8 Hz), 7.31 (1H, s), 7.14 (1H, d, *J* = 8.3 Hz), 6.69 (1H, d, *J* = 8.3 Hz), 6.32 (1H, d, *J* = 15.8 Hz), 4.34 (2H, s), 3.36 (3H, s), 1.44 (9H, s). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 166.5, 147.0, 144.9, 130.3, 128.4, 124.4, 123.9, 120.3, 116.6, 80.4, 74.3, 57.7, 29.6. MS (FAB$^+$): calculated for $C_{15}H_{21}NO_3$ [M$^+$]: 263.1; found: 263.1.

Compound 4c. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.71 (1H, d, *J* = 15.8 Hz), 7.30 (1H, s), 7.11 (1H, d, *J* = 8.2 Hz), 6.65 (1H, d, *J* = 8.2 Hz), 6.30 (1H, d, *J* = 15.8 Hz), 3.92 (2H, s), 3.69 (4H, t, *J* = 4.7 Hz), 3.38 (2H, s), 2.42 (4H, t, *J* = 4.7 Hz), 1.47 (9H, s). ^{13}C NMR (50 MHz, $CDCl_3$): 166.8, 144.5, 138.8, 132.1, 130.3, 128.9, 126.8, 120.1, 116.5. MS (FAB$^+$): calculated for [M+H$^+$] $C_{18}H_{26}N_2O_3$: 319.2; found: 319.2.



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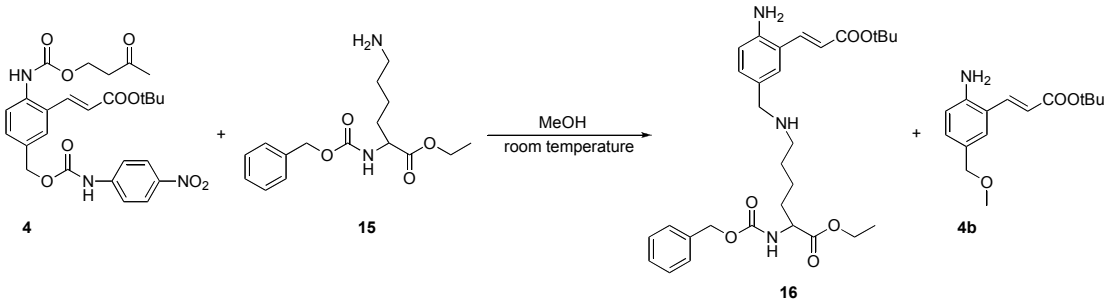
Compound 15. Z-Lys-OH (250 mg, 0.89 mmol, 1 eq) was suspended in ethanol (25 mL) and brought to reflux. Oxalyl-chloride (117 μL, 1.34 mmol, 1.5 eq) was added and the reaction was stirred for 45 minutes. After concentration to dryness, the residue was taken in EtOAc (100 mL) and washed with 1M NaOH. The organic phase was dried with $MgSO_4$, filtered and the solvent removed under reduced pressure to give compound **15** (257 mg, 93%) as yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29-7.35 (5H, m), 5.42 (1H, d, *J* = 7.1 Hz), 5.10 (2H, s), 4.34 (1H, q, *J* = 7.1 Hz), 4.18 (2H, q, *J* = 7.0 Hz), 2.66 (2H, t, *J* = 6.4 Hz), 1.79-1.88 (1H, m), 1.60-1.71 (1H, m), 1.30-1.56 (6H, m), 1.26 (3H, t, *J* = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 155.9, 136.3, 128.8, 128.7, 128.3, 127.9, 127.6, 66.9, 61.4, 53.8, 41.8, 33.1, 32.5, 22.5, 14.4. MS (FAB^+): calculated for [$M+H^+$] $C_{16}H_{24}N_2O_4$: 309.2; found: 309.1.



RP-HPLC-MS chromatogram and analysis of competition between Z-Lys(Boc)-OH and MeOH (ratio 75:1).

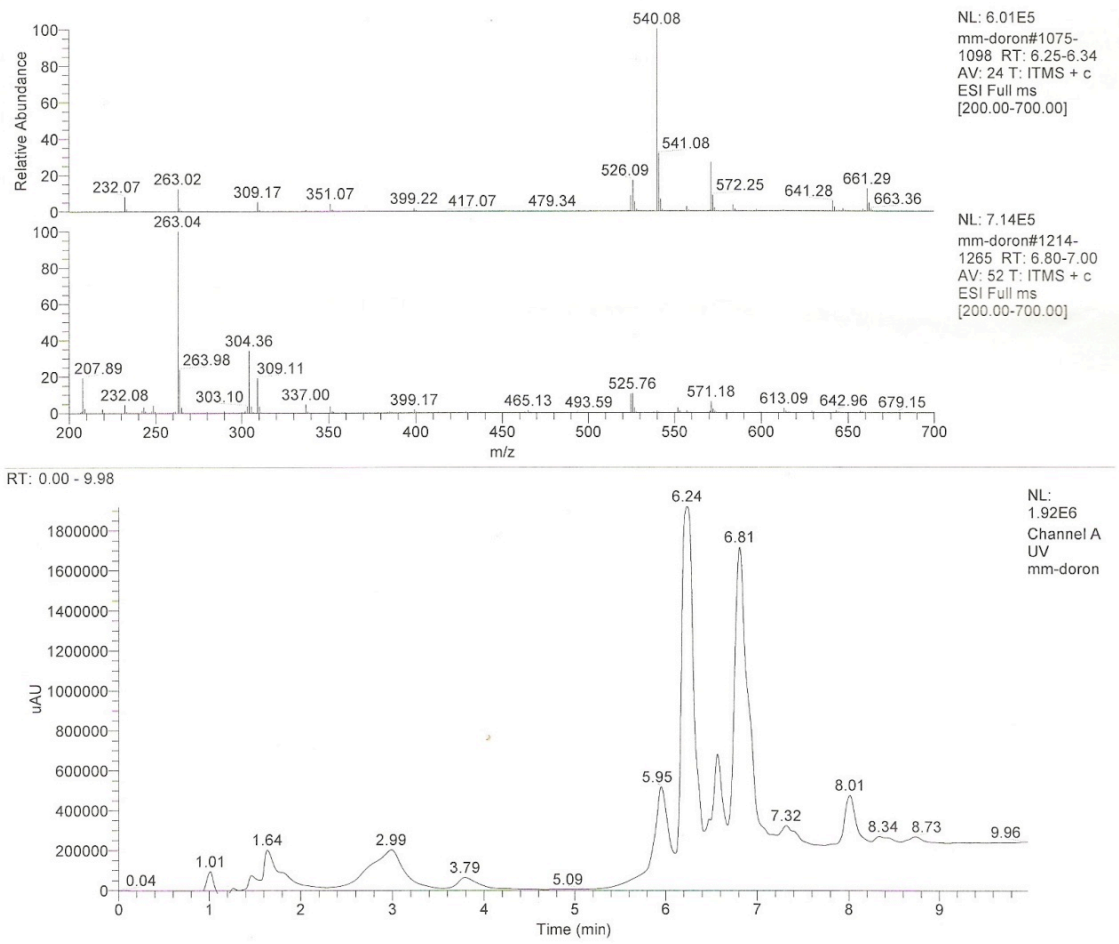


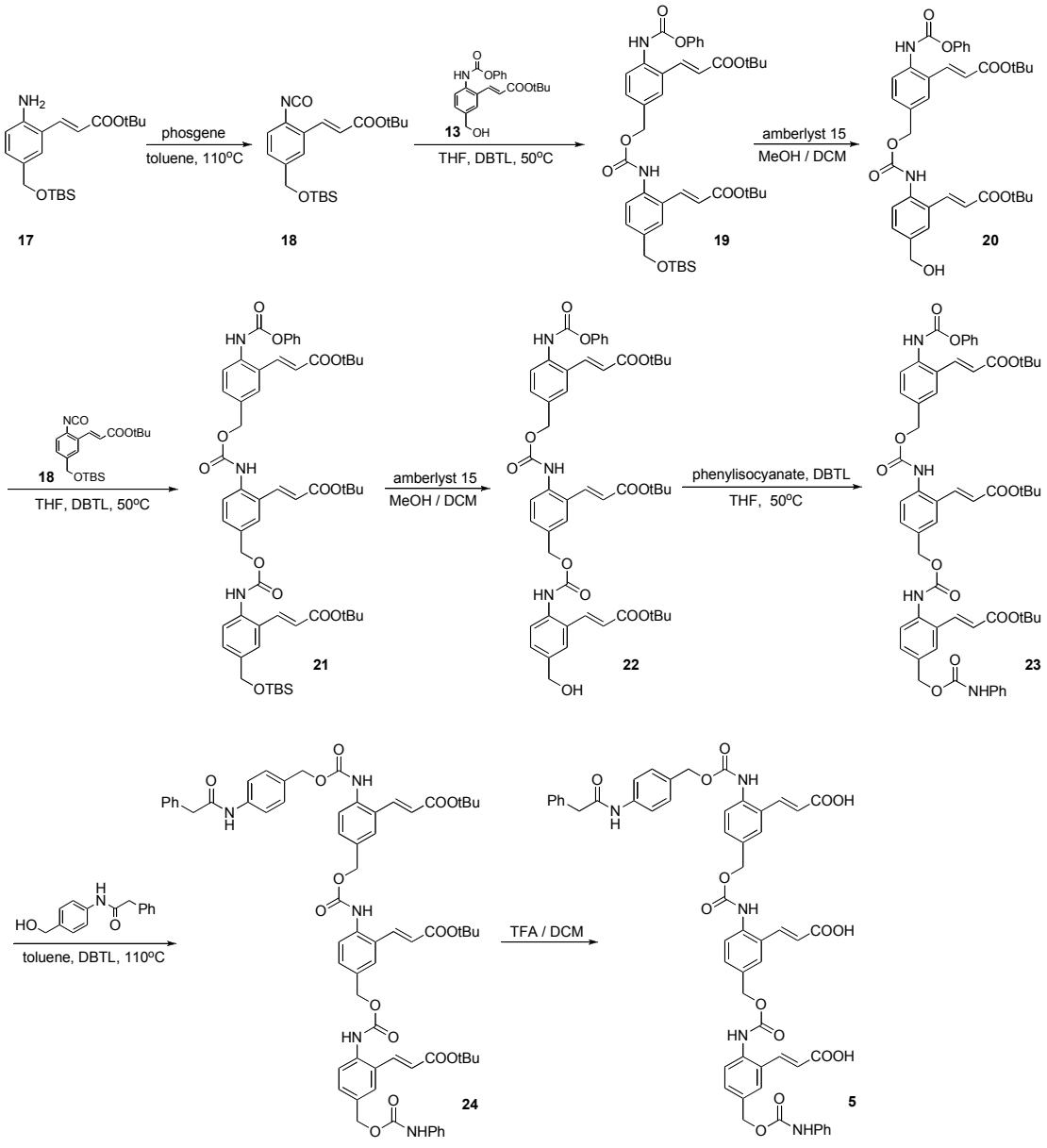
Figure 1S: RP-HPLC-MS chromatogram of compound **4** after incubated with Z-lys-OEt **15** in MeOH for 6 hours at Z-lys-OEt:MeOH ratio of 1:75. MS spectra of the peaks at 6.24 and 6.81 minutes are presented abouve.

	Retention time [minutes]	**Chemical formula**	**Calculated mass**	**Recorded mass**
Compound **16**	6.24	$C_{30}H_{41}N_3O_6$	539.30	540.08 [$M+H^+$]
Compound **4b**	6.81	$C_{15}H_{21}NO_3$	263.15	263.04 [M^+]

Table 1S: Analysis of RP-HPLC-MS chromatogram.

In order to validate these results, we have independently synthesized and fully characterized molecule **4b**. The retention time observed for the synthesized molecule **4b**, under the same separation condition in RP-HPLC, was 6.81 minutes. Furthermore, when Z-lys-OEt's initial concentration was increased or decreased, the peak at 6.24 minutes grew or shrunk accordingly.

Synthesis of trimeric labeling probe 5



Compound 17. Compound **17** was synthesized and characterized according to published procedure.(25)

Compound 19. Compound **17** (750 mg, 2.06 mmol, 1 eq), dissolved in toluene (5 mL), was added drop-wise into a solution of refluxed phosgene in toluene (10.3 mL, 10.30 mmol, 5 eq) over 10 minutes. The reaction was stirred for 15 minutes and the solvent removed under reduced pressure yielding the corresponding isocyanate as brownish oil (**18**). Isocyanate **18** was re-dissolved in dry THF (5 mL) at 50°C. Compound **13** (760 mg, 2.06 mmol, 1 eq) was added, followed by DBTL (61 μL, 0.103 mmol, 0.05 eq) and the reaction was monitored to completion (20 minutes) by TLC (EtOAc:Hex 15:85). The solvent was then removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 15:85) to give compound **19** (1.12 g, 72%) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d^{6}): δ = 9.32-9.38 (2H, m), 7.68-7.83 (5H, m), 7.35-7.50 (4H, m), 7.14-7.18 (2H, m), 6.68-6.76 (2H, m), 6.35-6.40 (2H, m), 5.14 (2H, s), 4.70 (2H, s), 1.46 (18H, s), 0.9 (9H, s), 0.08 (6H, s). ^{13}C NMR (100 MHz, DMSO-d^{6}): δ = 166.4, 165.9, 157.7, 150.2, 149.4, 148.0, 145.7, 141.9, 137.6, 137.5, 129.8, 128.8, 128.4, 127.9, 127.1, 122.5, 121.1, 119.9, 118.3, 116.5, 116.3, 115.3, 80.3, 79.8, 65.8, 62.7, 28.3, 28.2, 26.2, 18.4, -4.8. MS (MALDI-TOF): calculated for [M+Na^{+}] $C_{42}H_{54}N_2O_9Si$ 781.3; found 781.3.

Compound 20. Compound **19** (1.12 g, 1.47 mmol) was dissolved in MeOH:DCM mixture (3:1), amberlyst 15 was added and the reaction was monitored to completion (30 minutes) by TLC (EtOAc:Hex 4:6). The amberlyst 15 beads were filtered out, the solvent removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 4:6) to give compound **20** (935 mg, 98%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.32-9.36 (2H, m), 7.69-7.80 (5H, m), 7.34-7.51 (4H, m), 7.14-7.18 (2H, m), 6.69-6.78 (2H, m), 6.37-6.41 (2H, m), 5.13 (2H, s), 4.49 (2H, d, *J* = 4.8 Hz), 1.47 (18H, s). ^{13}C NMR (100 MHz, DMSO-d^{6}): δ = 166.4, 166.0, 157.8, 150.2, 149.4, 148.0, 145.7, 141.9, 137.6, 137.5, 129.8, 128.8, 128.4, 127.9, 127.1, 122.5, 121.1, 119.9, 118.3, 116.5, 116.3, 115.6, 80.3, 79.8, 65.8, 64.1, 28.3, 28.2. MS (MALDI-TOF): calculated for [M+Na^{+}] $C_{36}H_{40}N_2O_9$ 667.2; found 667.2.

Compound 21. Compound **17** (545 mg, 1.50 mmol, 1 eq), dissolved in toluene (5 mL), was added drop-wise into a solution of refluxed phosgene in toluene (7.5 mL, 7.50 mmol, 5 eq) over 10 minutes. The reaction was stirred for 15 minutes and the solvent removed under reduced pressure yielding the corresponding isocyanate as brownish oil (**18**). Isocyanate **18** was re-dissolved in dry THF (5 mL) at 50^{o}C. Compound **20** (905 mg, 1.4 mmol, 0.9 eq) was added, followed by DBTL (45 μL, 0.07 mmol, 0.05 eq) and the reaction was monitored to completion (20 minutes) by TLC (EtOAc:Hex 2:8). The solvent was then removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 2:8) to give compound **21** (908 mg, 63%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.48-9.51 (2H, m), 9.32 (1H, s), 7.70-7.84 (6H, m), 7.37-7.52 (6H, m), 7.14-7.18 (2H, m), 6.70-6.77 (3H, m), 6.37-6.41 (3H, m), 5.13 (4H, s), 4.70 (2H, s), 1.47 (27H, s), 0.91 (9H, s), 0.08 (6H, s). ^{13}C NMR (100 MHz, DMSO-d^{6}): δ =166.1, 165.9, 157.7, 155.0, 148.6, 147.5, 139.9, 139.6, 139.4, 139.1, 137.1, 136.0, 134.3, 134.1, 132.3, 130.0, 129.8, 128.8, 128.5, 128.3, 126.8, 126.6, 124.6, 124.3, 120.9, 120.7, 119.2, 117.8, 117.4, 117.0, 116.8 ,115.6, 80.4, 79.8, 66.7, 65.8, 64.2, 28.3, 28.2, 26.2, 18.4, -4.8. MS (MALDI-TOF): calculated for [M+Na^{+}] $C_{57}H_{71}N_3O_{13}Si$ 1056.4; found 1056.4.

Compound 22. Compound **21** (600 mg, 0.61 mmol) was dissolved in MeOH:DCM mixture (3:1), amberlyst 15 was added and the reaction was monitored to completion (45 minutes) by TLC (EtOAc:Hex 1:1). The amberlyst 15 beads were filtered out, the solvent removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 1:1) to give compound **22** (512 mg, 90%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.44-9.56 (2H, m), 9.28 (1H, s), 7.67-7.83 (6H, m), 7.32-7.47 (6H, m), 7.11-7.22 (2H, m), 6.68-6.73 (3H, m), 6.35-6.39 (3H, m), 5.12 (4H, s), 4.45 (2H, d, *J* = 5.6 Hz), 1.47 (27H, s). ^{13}C NMR (100 MHz, DMSO-d^{6}): δ = 166.4, 165.9, 157.7, 155.0, 148.6, 147.5, 139.9, 139.6, 139.5, 139.1, 137.1, 135.8, 134.3, 134.1, 132.3, 130.0, 129.8, 128.9, 128.5, 128.3, 126.8, 126.6, 124.6, 124.3, 121.0, 120.6, 119.2, 117.8, 117.6, 117.0, 116.8 ,115.6, 80.3, 79.9, 66.8, 65.8, 62.7,

28.3, 28.2. MS (MALDI-TOF): calculated for [M+Na^+] $C_{51}H_{57}N_3O_{13}$ 942.3; found 942.3.

Compound 23. Compound **22** (477 mg, 0.52 mmol, 1 eq) was dissolved in dry THF (5 mL), preheated to 50°C. Phenylisocyanate (57 μL, 0.52 mmol, 1 eq) was added, followed by DBTL (16 μL, 0.026 mmol, 0.05 eq) and the reaction was monitored to completion (10 minutes) by TLC (EtOAc:Hex 1:3). The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 2:8) to give compound **23** (416 mg, 77%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d^6): δ = 10.01 (1H, s), 9.71 (1H, s), 9.64 (1H, s), 9.28 (1H, s), 7.71-7.87 (6H, m), 7.39-7.47 (9H, m), 7.22-7.26 (5H, m), 6.95 (1H, t, *J* = 7.4 Hz), 6.70-6.74 (1H, m), 6.40-6.45 (3H, m), 5.10 (6H, s), 1.45 (27H, s). ^{13}C NMR (100 MHz, DMSO-d^6): δ = 166.4, 165.9, 157.7, 155.0, 154.9, 153.7, 148.6, 139.9, 137.0, 134.3, 134.1, 132.4, 130.6, 130.3, 130.0, 129.8, 129.5, 129.2, 128.9, 127.1, 127.0, 124.3, 122.8, 121.1, 121.0, 119.2, 118.7, 118.5, 117.6, 116.8, 115.7, 80.4, 79.9, 66.8, 65.9, 65.6, 28.3, 28.2. MS (MALDI-TOF): calculated for [M+Na^+] $C_{58}H_{62}N_4O_{14}$ 1061.4; found 1061.4.

Compound 24. Compound **23** (150 mg, 0.14 mmol, 1 eq) was dissolved in toluene (7 mL), preheated to 110°C. PGA substrate (155 mg, 0.64 mmol, 4.4 eq) was added, followed by DBTL (9.5 μL, 0.014 mmol, 0.1 eq) and the reaction was monitored to completion (1 hour) by TLC (EtOAc:Hex 6:4). The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 6:4) to give compound **24** (120 mg, 70%) as a white solid.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 10.20 (1H, s), 9.73 (1H, s), 9.56 (1H, s), 9.51 (1H, s), 9.46 (1H, s), 7.81 (2H, s), 7.75 (1H, t, *J* = 3.2 Hz), 7.71 (1H, t, *J* = 3.2 Hz), 7.68 (2H, s), 7.58-7.61 (4H, m), 7.41-7.42 (6H, m), 7.19-7.33 (9H, m), 6.36-6.43 (3H, m), 5.17 (1H, t, *J* = 5.6 Hz), 5.05-5.10 (8H, m), 3.62 (2H, s), 1.44 (27H, s). ^{13}C NMR (50 MHz, DMSO-d^6): δ = 169.6, 165.9, 155.0, 154.9, 140.5, 139.9, 139.7, 139.5, 137.0, 136.9, 136.4, 135.8, 134.4, 134.3, 131.6, 130.6, 130.2, 129.8, 129.6, 129.5, 129.2,

129.0, 128.9, 128.4, 127.2, 127.0, 126.8, 125.4, 125.1, 124.9, 124.6, 121.3, 121.0, 120.6, 119.8, 119.5, 119.3, 119.0, 80.4, 80.3, 66.3, 65.9, 65.8, 62.7, 60.2, 43.7, 28.3, 28.1. MS (MALDI-TOF): calculated for $[M+Na^+]$ $C_{60}H_{66}N_4O_{14}$ 1208.5; found 1028.5.

Compound 5. Compound **24** (5 mg) was dissolved in a 1:1 mixture of TFA and DCM, stirred for 5 minutes and the solvents removed under reduced pressure. The obtained compound **5** was used without further purification.

^{1}H NMR (200 MHz, DMSO-d^6): δ = 10.11 (1H, s), 9.74 (1H, s), 9.62 (1H, s), 9.53 (1H, s), 9.46 (1H, s), 7.80 (2H, s), 7.75 (1H, t, *J* = 3.0 Hz), 7.70 (1H, t, *J* = 3.0 Hz), 7.68 (2H, s), 7.55-7.62 (4H, m), 7.40-7.45 (6H, m), 7.19-7.36 (9H, m), 6.36-6.43 (3H, m), 5.16 (1H, t, *J* = 5.6 Hz), 5.06-5.13 (8H, m), 3.62 (2H, s).

Fluorescence emission of PGA upon labeling with trimeric probe 5

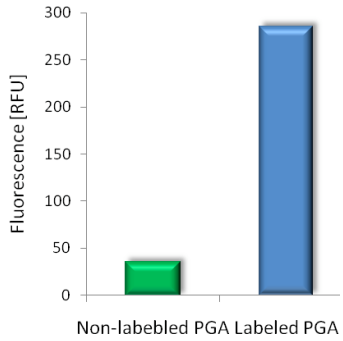
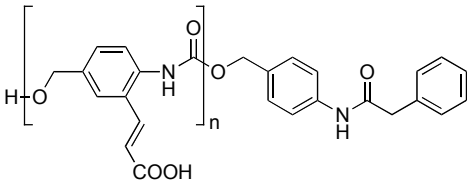


Figure 2S: Emitted fluorescence (λ_{ex} = 270 nm, λ_{em} = 510 nm) of PGA (28 μg) in PBS pH 7.4 (non-labeled PGA) and after treatment with trimer probe **5** [250 μM] (labeled PGA).

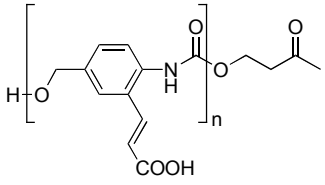
Synthesis of SIP labeling probe 7



7

Polymer 7. Polymer **7** was synthesized and characterized according to published procedure.(25) The formed polymer was constructed of 14 monomers in average (determined by ^{1}H-NMR).

Synthesis of SIP labeling probe 8



8

Polymer 8. Polymer **8** was synthesized and characterized according to published procedure.(25) The formed polymer was constructed of 15 monomers in average (determined by ^{1}H-NMR).

To exclude fluorescence increase due to flawed purification process, resulting in presence of free monomers in the purified PGA or Ab38C2 solution, we have incubated different amounts of protein with a fixed concentration of polymer **7** or **8**, correspondingly. Since in this experiment the same polymer concentration is used, if a certain percentage of monomers do pass through the purification process, a similar rise in fluorescence for all experiments would be expected. However, an increase in the fluorescence was observed upon growing amount of protein (Figure 3S), suggesting that presence of unattached monomers is not responsible for fluorescence change.

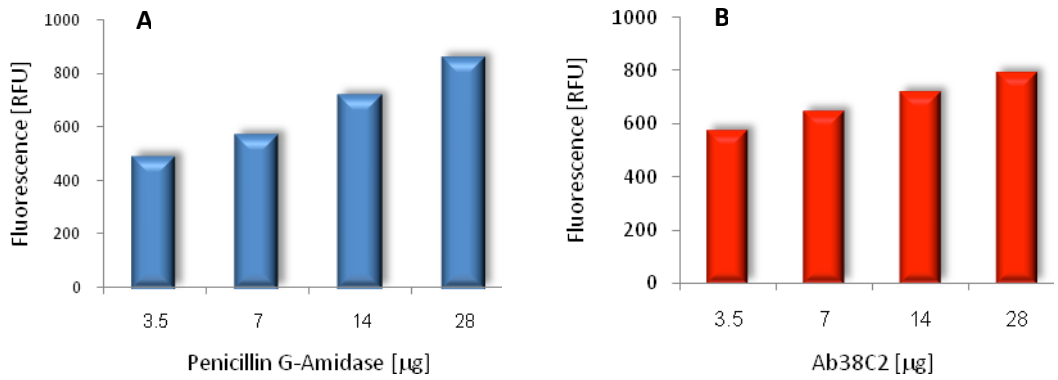
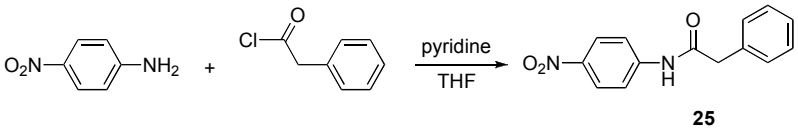


Figure 3S: Fluorescence measurements (λ_{ex} = 270 nm, λ_{em} = 510 nm) of increasing amounts of A) PGA after incubation with polymer **7** [250 μM], in 100 μL PBS pH 7.4. B) Ab38C2 after incubation with polymer **8** [250 μM], in 100 μL PBS pH 7.4.

Relative enzymatic activity of PGA

Synthesis of PGA probe 25

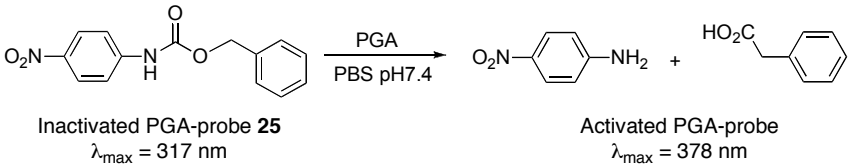


25

Compound 25. Pyridine (161 μL, 1.99 mmol, 1.1 eq) was added to a solution of 4-nitroaniline (250 mg, 1.81 mmol, 1 eq) dissolved in THF (5 mL) at 0°C and the mixture was stirred for 10 minutes. Then, phenacetylchloride (239 μL, 1.81 mmol, 1 eq) was added. The reaction was warmed to room temperature and monitored to completion (10 hours) by TLC (EtOAc:Hex 3:7). Upon completion, the reaction mixture was diluted with EtOAc (50 mL) and washed with saturated solution of NH_4Cl and brine. The solvents were removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 3:7) to give compound **25** (428 mg, 92%) as a yellow solid.

^{1}H NMR (200 MHz, DMSO-d^6): δ = 9.74 (1H, s), 8.16 (2H, d, *J* = 7.3 Hz), 7.79 (2H, d, *J* = 7.3 Hz), 7.22-7.30 (5H, m), 3.67 (2H, s). ^{13}C NMR (50 MHz, DMSO-d^6): δ = 170.5, 145.7, 142.6, 135.7, 129.6, 128.7, 127.1, 125.3, 119.2, 43.7. MS (FAB$^+$): calculated for $C_{14}H_{12}N_2O_3$ [M$^+$]: 256.1; found: 256.1.

Absorbance of activated and inactivated PGA-probe 25



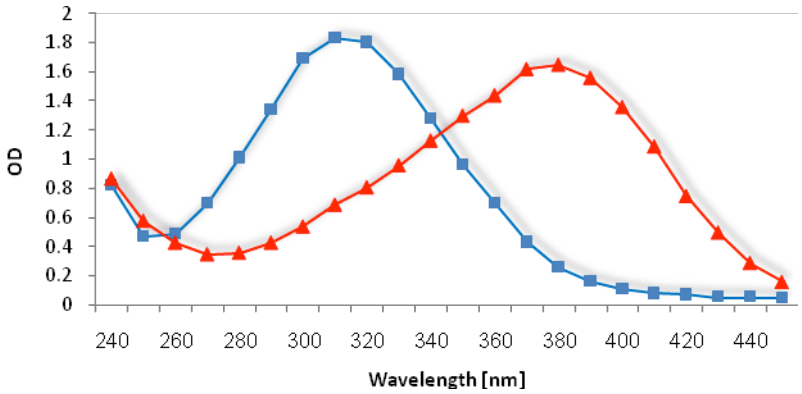
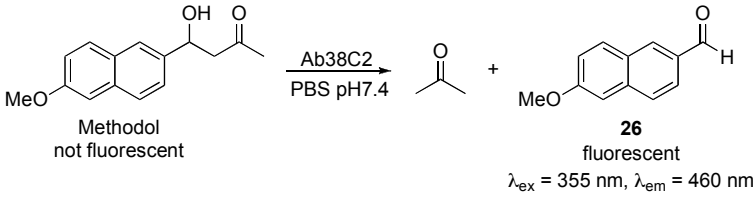


Figure 4S: UV absorbance spectra of (■) inactivated and (▲) activated PGA-probe **25** [250 μM].

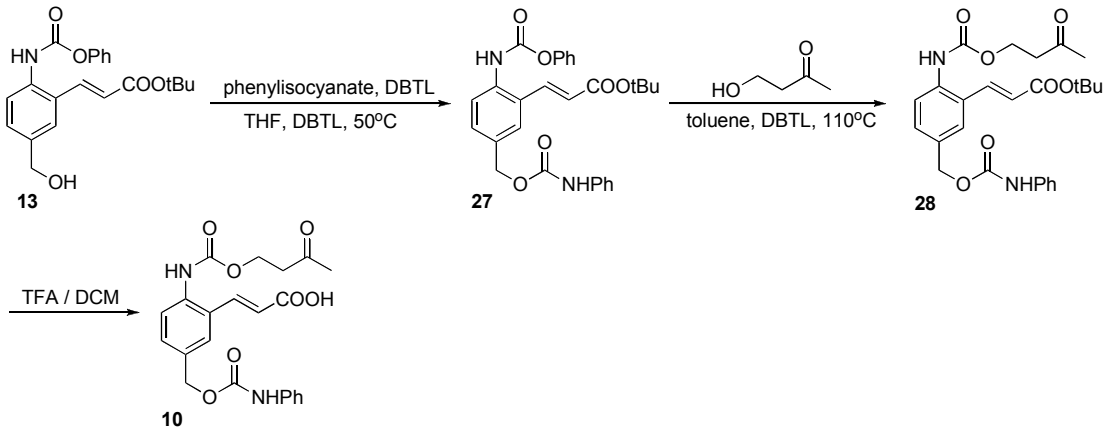
Relative enzymatic activity of Ab38C2



The relative enzymatic activity of labeled and unlabeled of Ab38C2 was tested for the *retro*-aldol reaction with methodol according to a published assay.(29) When used as a substrate for *retro*-aldol reaction, methodol disintegrates into acetone and the fluorescent aldehyde 6-methoxy-2-naphthaldehyde (**26**) (λ_{ex} = 355 nm, λ_{em} = 460 nm). Experiments were conducted in a 96-wells fluorescence measuring plate and fluorescence was monitored using a Molecular Devices SPECTRAmaxM2 plate reader.

Comparison of the labeling characterizations for monomeric (10), trimeric (11) and SIP (8) probes

Synthesis of monomeric labeling probe 10



Compound 27. Compound **13** (250 mg, 0.68 mmol, 1 eq) was dissolved in dry THF (5 mL), preheated to 50°C. Phenylisocyanate (78 μL, 0.71 mmol, 1.05 eq) was added, followed by DBTL (22 μL, 0.033 mmol, 0.05 eq) and the reaction was monitored to completion (10 minutes) by TLC (EtOAc:Hex 1:3). The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 2:8) to give compound **27** (345 mg, 95%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (1H, s), 7.89 (1H, d, *J* = 15.7 Hz), 7.56 (1H, s), 7.37-7.42 (5H, m), 7.27-7.32 (2H, m), 7.26 (1H, d, *J* = 1.6 Hz), 7.20-7.25 (2H, m), 6.80 (1H, s), 6.40 (2H, d, *J* = 15.7 Hz), 5.17 (2H, s), 1.56 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.8, 153.2, 152.0, 150.5, 137.6, 137.2, 235.5, 132.8, 130.8, 129.5, 129.4, 127.2, 123.8, 121.8, 121.6, 121.3, 119.5, 118.7, 81.2, 66.2, 28.4. MS (MALDI-TOF): calculated for [M+Na^+] $C_{28}H_{28}N_2O_6$ 511.1; found 511.1.

Compound 28. Compound **27** (200 mg, 0.41 mmol, 1 eq) was dissolved in toluene (4 mL), preheated to 110°C. 4-Hydroxy-2-butanoe (108 μL, 1.23 mmol, 3 eq) was added, followed by DBTL (25 μL, 0.041 mmol, 0.1 eq) and the reaction was monitored to completion (30 minutes) by TLC (EtOAc:Hex 4:6). The solvent was removed under reduced pressure and the crude product purified by column

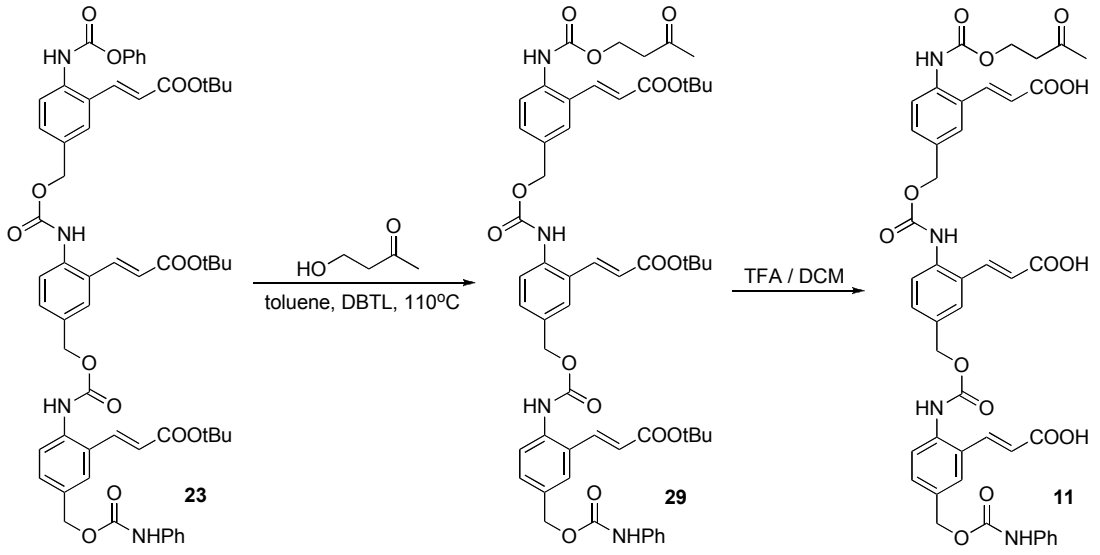
chromatography on silica gel (EtOAc:Hex 4:6) to give compound **28** (142 mg, 72%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d^6): δ = 9.69 (1H, s), 9.38 (1H, s), 7.79 (1H, d, *J* = 1.2 Hz), 7.65 (1H, d, *J* = 15.9 Hz), 7.33-7.42 (4H, m), 7.23 (2H, t, *J* = 7.4 Hz), 6.95 (1H, t, 7.4 Hz), 6.39 (1H, d, *J* = 15.9 Hz), 5.08 (2H, s), 4.21 (2H, t, *J* = 6.2 Hz), 2.77 (2H, t, *J* = 6.2 Hz), 2.09 (3H, s), 1.44 (9H, s). ^{13}C NMR (100 MHz, DMSO-d^6): δ = 206.8, 166.0, 154.9, 153.7, 139.6, 139.4, 136.8, 134.4, 129.5, 129.2, 129.0, 126.8, 123.1, 121.0, 118.8, 80.6, 65.6, 60.3, 42.4, 30.3, 28.4. MS (MALDI-TOF): calculated for [M+Na$^+$] $C_{26}H_{30}N_2O_7$ 505.2; found 505.2.

Compound 10. Compound **28** (5 mg) was dissolved in a 1:1 mixture of TFA and DCM, stirred for 5 minutes and the solvents removed under reduced pressure. The obtained compound **10** was used without further purification.

^{1}H NMR (400 MHz, DMSO-d^6): δ = 9.71 (1H, s), 9.38 (1H, s), 7.79 (1H, s), 7.65 (1H, d, *J* = 15.8 Hz), 7.33-7.42 (4H, m), 7.23 (2H, t, *J* = 7.4 Hz), 6.95 (1H, t, 7.4 Hz), 6.39 (1H, d, *J* = 15.8 Hz), 5.09 (2H, s), 4.20 (2H, t, *J* = 6.3 Hz), 2.75 (2H, t, *J* = 6.3 Hz), 2.10 (3H, s).

Synthesis of trimeric labeling probe 11



Compound 29. Compound **23** (150 mg, 0.14 mmol, 1 eq) was dissolved in toluene (6 mL), preheated to 110°C. 4-Hydroxy-2-butanoe (80 μL, 0.86 mmol, 6 eq) was added, followed by DBTL (10 μL, 0.014 mmol, 0.1 eq) and the reaction was monitored to completion (45 minutes) by TLC (EtOAc:Hex 4:6). The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel (EtOAc:Hex 4:6) to give compound **29** (134 mg, 93%) as a white solid.

^{1}H NMR (200 MHz, DMSO-d^{6}): δ = 9.74 (1H, s), 9.54 (2H, s), 9.47 (1H, s), 7.67-7.87 (6H, m), 7.37-7.48 (8H, m), 7.27 (2H, t, *J* = 7.5 Hz), 7.00 (1H, t, *J* = 7.5 Hz), 6.39-6.50 (3H, m), 5.13 (6H, s), 4.25 (2H, t, *J* = 6.2 Hz), 2.82 (2H, t, *J* = 6.2 Hz), 2.14 (3H, s), 1.46 (27H, s). ^{13}C NMR (50 MHz, DMSO-d^{6}): δ = 206.8, 166.3, 165.8, 155.0, 154.8, 153.7, 148.5, 139.9, 139.4, 137.6, 137.1, 136.9, 134.3, 134.1, 132.2, 130.6, 130.3, 129.1, 128.9, 128.8, 128.4, 126.7, 124.3, 122.8, 121.1, 118.6, 117.7, 117.6, 116.7, 80.4, 79.8, 66.7, 65.9, 65.5, 28.2. MS (MALDI-TOF): calculated for [M+Na^{+}] $C_{56}H_{64}N_{4}O_{15}$ 1055.4; found 1055.4.

Compound 11. Compound **29** (5 mg) was dissolved in a 1:1 mixture of TFA and DCM, stirred for 5 minutes and the solvents removed under reduced pressure. The obtained compound **11** was used without further purification.

^{1}H NMR (400 MHz, DMSO-d^{6}): δ = 9.77 (1H, s), 9.52 (2H, s), 9.45 (1H, s), 7.65-7.89 (6H, m), 7.33-7.50 (8H, m), 7.26 (2H, t, *J* = 7.5 Hz), 7.00 (1H, t, *J* = 7.5 Hz), 6.36-6.49 (3H, m), 5.14 (6H, s), 4.21 (2H, t, *J* = 6.2 Hz), 2.83 (2H, t, *J* = 6.2 Hz), 2.14 (3H, s).

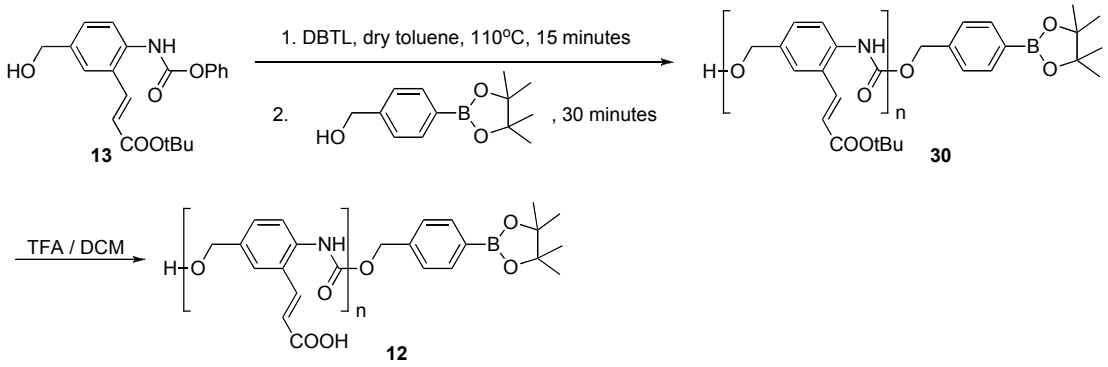
Labeling experiments

The labeling of Ab38C2 was performed for three final aza-quinone methide concentrations, as presented in Table 2S.

Final aza-quinone methide concentration	SIP 8[a] concentration	Trimer 11[b] concentration	Molecule 10[c] concentration
3.5 mM	250 μM	1.16 mM	3.5 mM
2.1 mM	150 μM	700 μM	2.1 mM
1.05 mM	75 μM	350 μM	1.05 mM

Table 2S: [a] SIP **8** is constructed of 15 monomers (on average), therefore, each polymer molecule is expected to release 14 aza-quinone methides. [b] Trimer **11** is constructed of 3 monomers, therefore, each trimer molecule is expected to release 3 aza-quinone methides. [c] Molecule **10** is constructed of one monomer, therefore, each molecule is expected to release one aza-quinone methide.

Synthesis of hydrogen-peroxide-activated SIP labeling probe 12



Polymer 30. Compound **13** (150 mg, 0.40 mmol, 1 eq) and DBTL (13 μL, 0.02 mmol, 0.05 eq) were dissolved in dry toluene (400 μL), preheated to 110°C, under

argon atmosphere. The reaction was stirred for 15 minutes and 4-(Hydroxymethyl)benzeneboronic acid pinacol ester (56 mg, 0.24 mmol, 0.6 eq) was then added. The reaction mixture was stirred for additional 30 minutes and then allowed to cool to room temperature. The polymer was precipitated from methanol, filtered and dried under reduced pressure for three hours. Polymer **30** (85 mg) was obtained as a white powder. The formed polymer was constructed of 15 monomers in average (determined by ^{1}H-NMR).

^{1}H NMR (400 MHz, DMSO-d^{6}): δ = 9.60 (15H, s), 7.86 (15H, s), 7.77 (17H, d, *J* = 15.6 Hz), 7.35 (30H, s), 7.30 (2H, s), 6.45 (15H, d, *J* = 15.6 Hz), 5.14 (30H, s), 4.49 (2H, s), 1.47 (135 H, s), 1.29 (12H, s).

Polymer 12. Polymer **30** (5 mg) was dissolved in a 1:1 mixture of TFA and DCM, stirred for 5 minutes and the solvents were then removed under reduced pressure. The obtained polymer **12** was used without further purifications.

^{1}H NMR (400 MHz, DMSO-d^{6}): δ = 9.59 (15H, s), 7.75-7.86 (32H, m), 7.29-7.36 (32H, m), 6.45 (15H, d, *J* = 15.7 Hz), 5.14 (30H, s), 4.47 (2H, s), 1.30 (12H, s).

Comparative quantum yield calculations

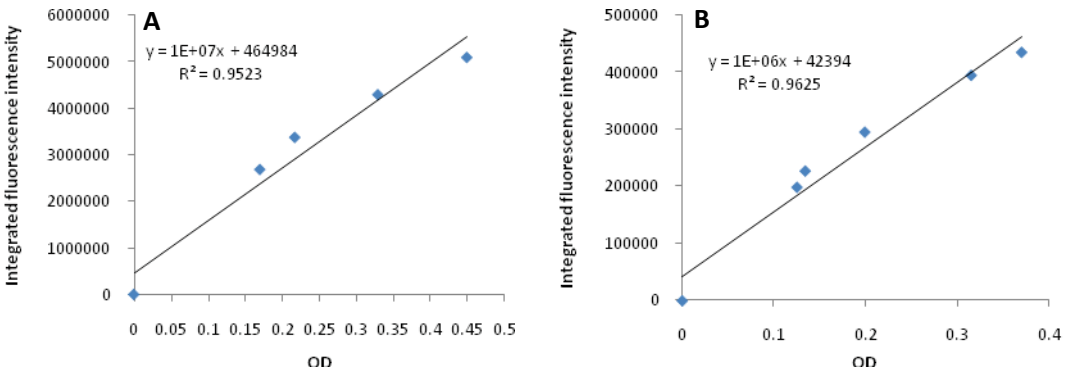


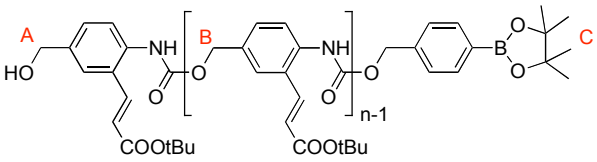
Figure 5S: Integrated fluorescence intensity *vs* absorbance at λ_{max} for: A) 1-naphtylamin and B) molecule **3**.

$$\Phi_X = \Phi_{ST} \cdot \left(\frac{Grad_X}{Grad_{ST}} \right) \left(\frac{\eta_X^2}{\eta_X^2} \right)$$

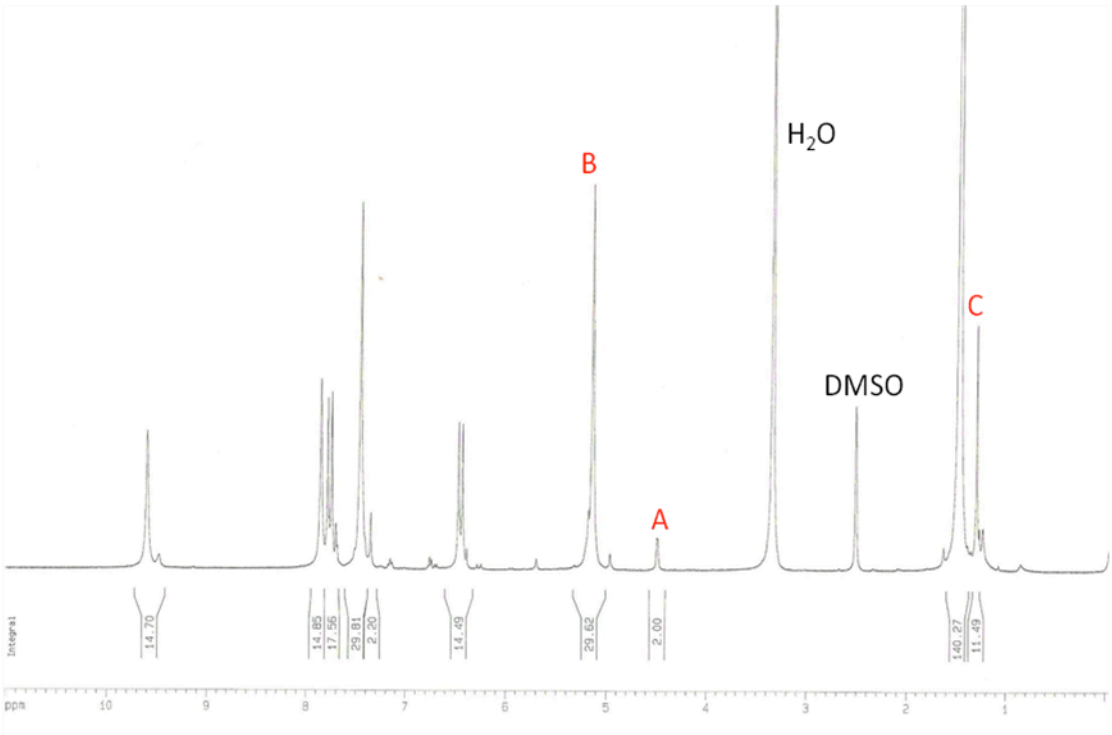
Equation 1: The subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, *Grad* the gradient from the plot of integrated fluorescence intensity *vs* absorbance at λ_{max}, and η the refractive index of the solvent.

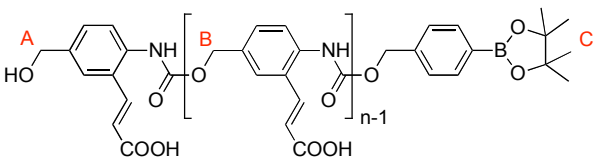
Since ethanol was used as solvent in all measurements, the influence of the refractive index of the solvent was dismissed and the quantum yield was calculated based on the results presented above using equation 1. The quantum yield found for molecule **3** in ethanol was $\Phi_F = 0.043 \pm 10\%$.

Spectral data of new SIPs



30

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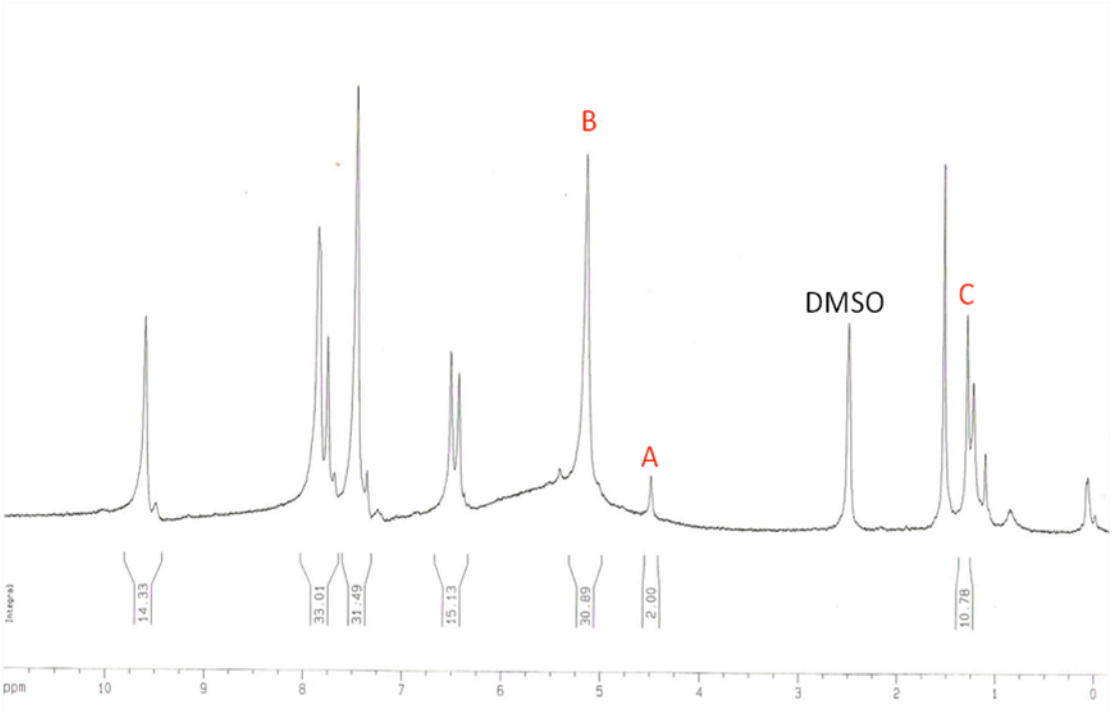
O
NH
O
O
n-1
COOH
COOH
B
O
O
C

12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DMSO
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15.13
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10.78
ppm
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6
5
4
3
2
1
0