Electronic Supplementary Material (ESI) for Molecular BioSystems.

SUPPLEMENTARY INFORMATION

Probing the effect of an inhibitor of an ATPase domain of Hsc70 on clathrin-mediated endocytosis

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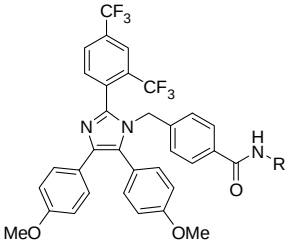
Synthesis of Az derivatives



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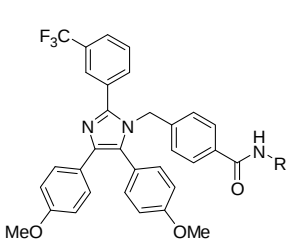

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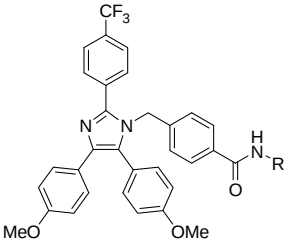

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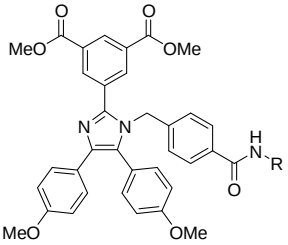

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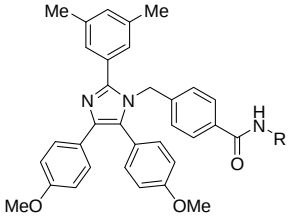

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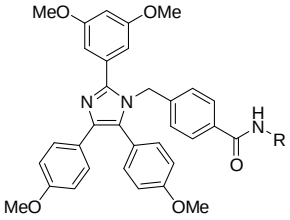

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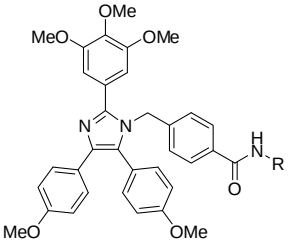

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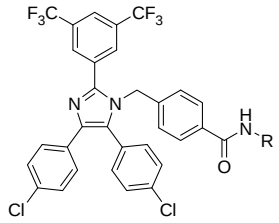

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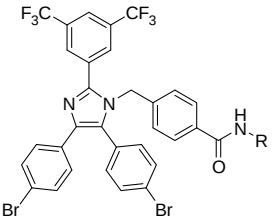

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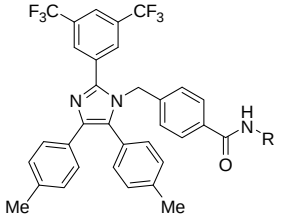

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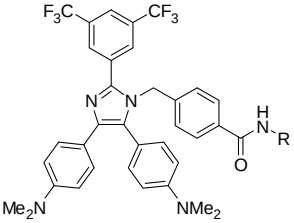

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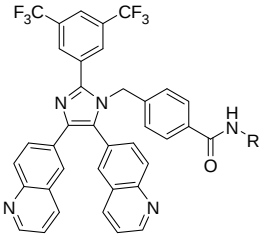

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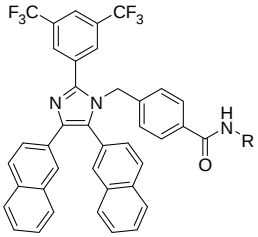

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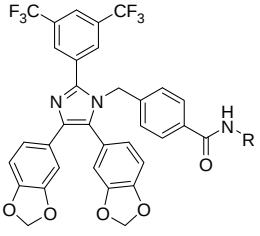

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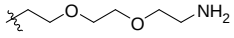
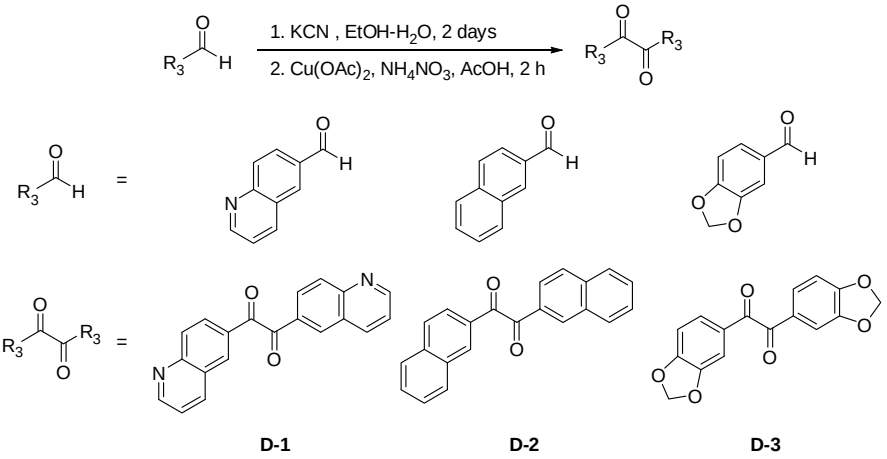
R = 

General procedure for synthesis of diketones



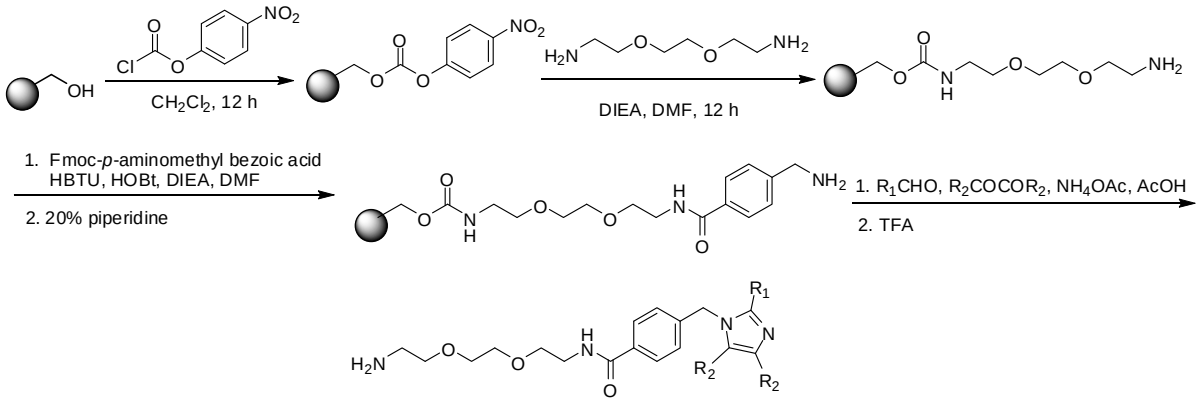
A stirred solution of aldehyde (10 mmol, 1 equiv) and KCN (0.5 equiv) in EtOH and H_2O (5:1, 25 mL) was heated under reflux for 2 days. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. A stirred solution of the crude product, $Cu(OAc)_2$ (0.3 equiv) and NH_4NO_3 (1.1 equiv) in acetic acid and H_2O (2:1, 25 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature, diluted with EtOAc, and washed with water, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (EtOAc : hexane = 1:10) to give diketone in 15–20% yield.

D-1: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.01 (d, *J* = 3.2 Hz, 2 H), 8.59 (s, 2 H), 8.46 (d, *J* = 8.0 Hz, 2 H), 8.41 (d, *J* = 9.2 Hz, 2 H), 8.22 (d, *J* = 8.8 Hz, 2 H), 7.64-7.60 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.2, 154.2, 151.0, 139.8, 139.7, 134.4, 131.8, 130.6, 128.7, 128.7, 123.5; MALDI-TOF-MS calcd for $C_{20}H_{13}N_2O_2$ $[M+H]^+$ 313.09, found 313.35.

D-2: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, 2 H), 8.16 (d, *J* = 8.0 Hz, 2 H), 7.98 (d, *J* = 8.4 Hz, 2 H), 7.91-7.88 (m, 4 H), 7.63 (t, *J* = 7.2 Hz, 2 H), 7.54 (t, *J* = 7.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.9, 136.6, 133.8, 132.5, 130.6, 130.1, 129.7, 129.3, 128.1, 127.3, 123.9; MALDI-TOF-MS calcd for $C_{22}H_{15}O_2$ $[M+H]^+$ 311.10, found 311.15.

D-3: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39-7.36 (m, 4 H), 7.07 (d, *J* = 8.0 Hz, 2 H), 6.19 (s, 4 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 193.1, 153.6, 148.7, 128.1, 127.2, 108.8, 107.2, 102.8; MALDI-TOF-MS calcd for $C_{16}H_{11}O_6$ $[M+H]^+$ 299.05, found 299.12.

Solid-phase synthesis of Az derivatives



A solution of 4-nitrophenyl chloroformate (0.81 g, 4 mmol) in anhydrous CH_2Cl_2 was added to a Wang resin (1 mmol) in CH_2Cl_2 (9 mL) and lutidine (0.5 mL, 6 mmol) at 0 ^oC. After shaking for 12 h, the resin was washed with DMF and CH_2Cl_2 several times. A solution of 2,2'-(ethylenedioxy)bisethylenediamine (0.6 mL, 5 mmol) and diisopropylethylamine (DIEA, 2 mL, 10 mmol) in DMF was added to the resin. After shaking for 12 h, the resin was washed with DMF and CH_2Cl_2 several times. A solution of Fmoc-*p*-aminomethyl benzoic acid (1.2 g, 3 equiv), HBTU (1.3g, 3 equiv), HOBt (0.48 g, 3 equiv) and DIEA (1.2 ml, 6 equiv) in DMF was added to the amine conjugated resin (1 mmol). After shaking for 6 h, the resin was washed with DMF and CH_2Cl_2 several times. Fmoc group on the resin was removed by treatment with 20% piperidine in DMF.

The amino-containing resin (30 μmol), aldehyde (10 equiv), diketone (10 equiv) and ammonium acetate (40 equiv) was placed in a reaction vial (2 mL) and suspended in acetic acid (400 μL). The reaction vial was placed in a heat block on a shaker at 100 ^oC. After shaking for 8 h, the resin was filtered and washed with DMF, MeOH and CH_2Cl_2 several times. The product was cleaved from a solid support by treatment with TFA for 1.5 h. The crude product was analyzed by LC-MS with a gradient of 35–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 10 min.

Apoptozole: ESI-MS calcd for $C_{33}H_{25}F_6N_2O_4$ $[M+H]^+$ 627.17, found 627.12.

Compound 1: ESI-MS calcd for $C_{39}H_{39}F_6N_4O_5$ $[M+H]^+$ 757.36, found 757.53.

Compound 2: ESI-MS calcd for $C_{38}H_{40}F_3N_4O_5$ $[M+H]^+$ 689.29, found 689.52.

Compound 3: ESI-MS calcd for $C_{38}H_{40}F_3N_4O_5$ $[M+H]^+$ 689.29, found 689.45.

Compound 4: ESI-MS calcd for $C_{39}H_{45}N_4O_7$ $[M+H]^+$ 681.32, found 681.49.

Compound 5: ESI-MS calcd for $C_{39}H_{45}N_4O_5$ $[M+H]^+$ 649.33, found 649.53.

Compound 6: ESI-MS calcd for $C_{41}H_{45}N_4O_9$ $[M+H]^+$ 737.31, found 737.60.

Compound 7: ESI-MS calcd for $C_{40}H_{47}N_4O_8$ $[M+H]^+$ 711.33, found 711.56.

Compound 8: ESI-MS calcd for $C_{37}H_{33}Cl_2F_6N_4O_3$ $[M+H]^+$ 765.18, found 765.49.

Compound 9: ESI-MS calcd for $C_{37}H_{33}Br_2F_6N_4O_3$ $[M+H]^+$ 853.07, found 853.33.

Compound 10: ESI-MS calcd for $C_{39}H_{39}F_6N_4O_3$ $[M+H]^+$ 725.28, found 725.57.

Compound 11: ESI-MS calcd for $C_{41}H_{45}F_6N_6O_3$ $[M+H]^+$ 783.34, found 783.85.

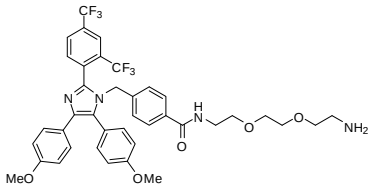
Compound 12: ESI-MS calcd for $C_{43}H_{37}F_6N_6O_3$ $[M+H]^+$ 799.28, found 799.52.

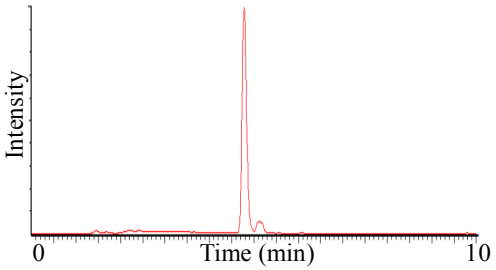
Compound 13: ESI-MS calcd for $C_{45}H_{39}F_6N_4O_3$ $[M+H]^+$ 797.28, found 797.46.

Compound 14: ESI-MS calcd for $C_{39}H_{35}F_6N_4O_7$ $[M+H]^+$ 785.23, found 785.35.

HPLC profiles of Az derivatives

Compound 1





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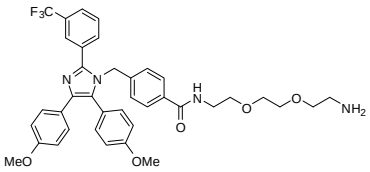

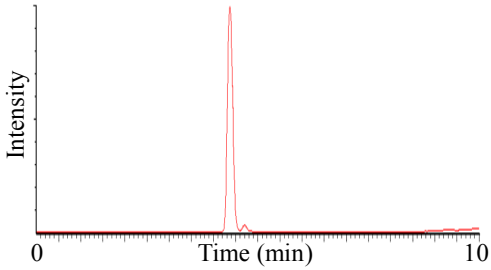

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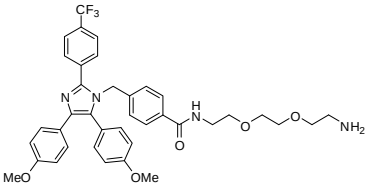

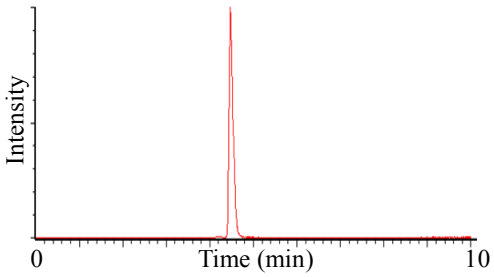

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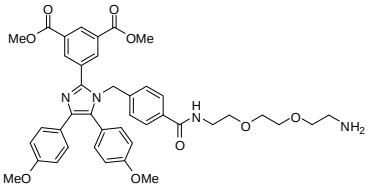

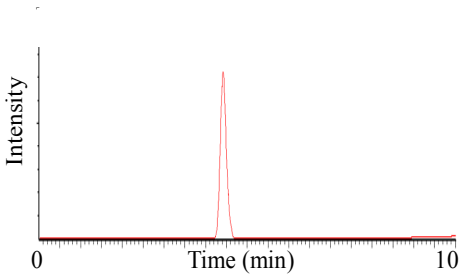

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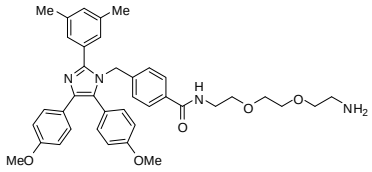

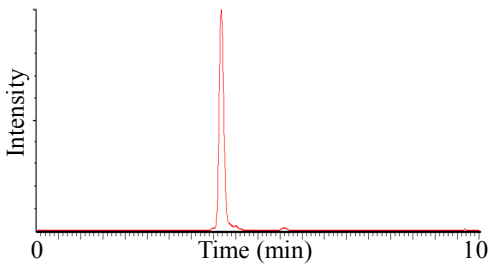

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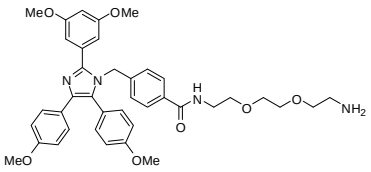

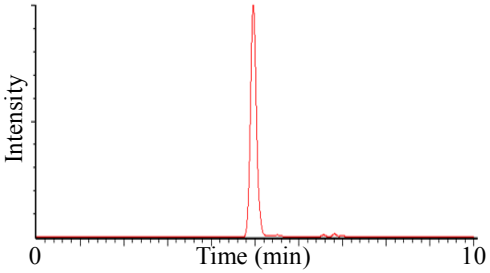

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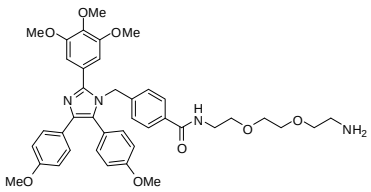

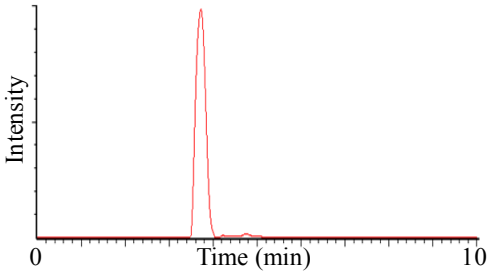

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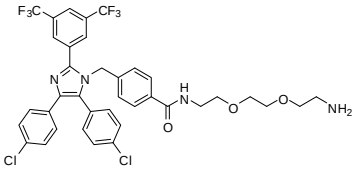

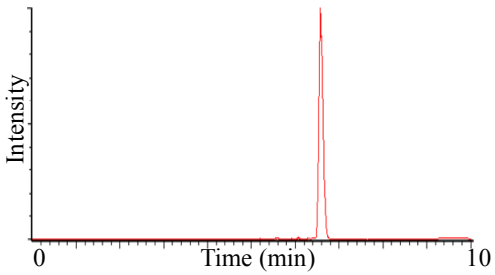

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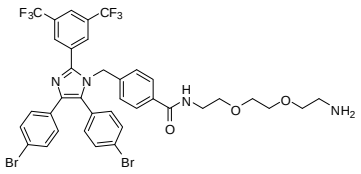

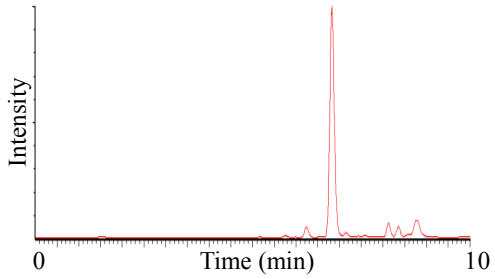

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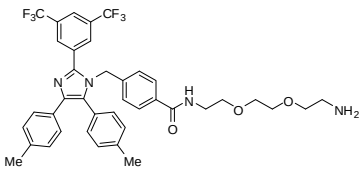

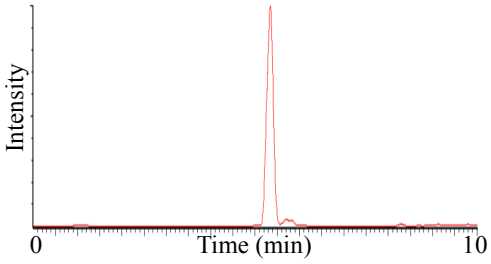

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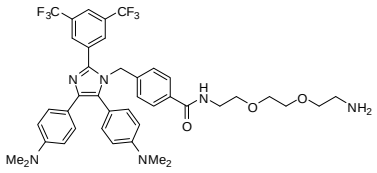

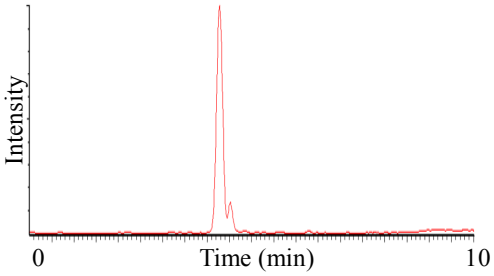

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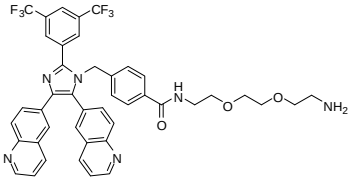

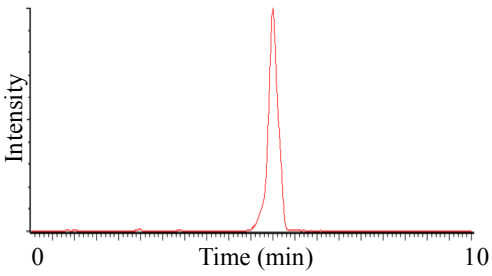

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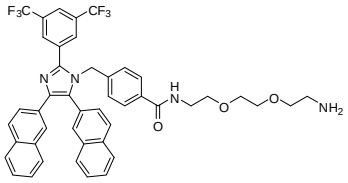

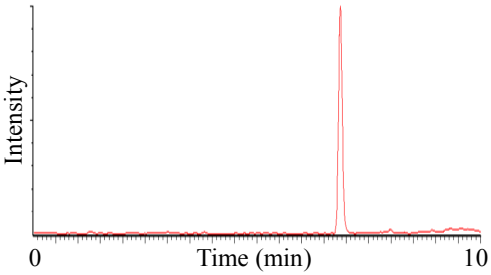

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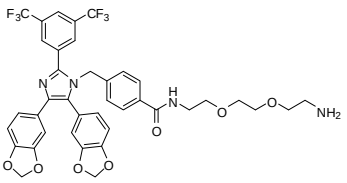

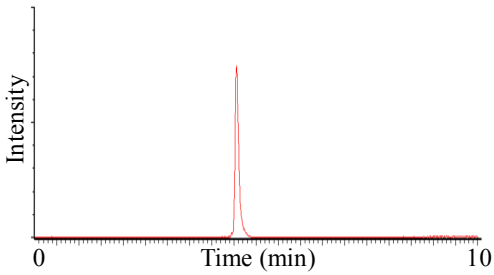

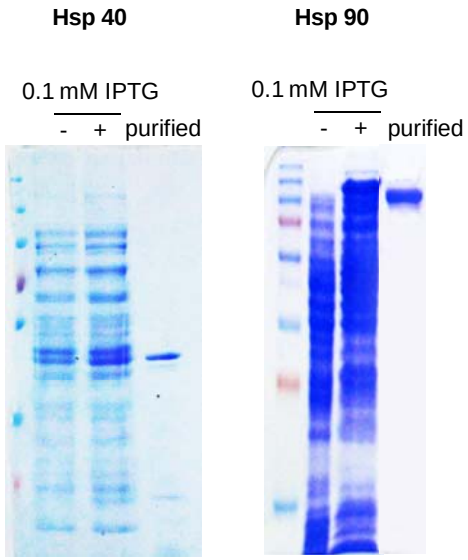

Fig. S1. Purified Hsp40 and Hsp90.