SUPPORTING INFORMATION

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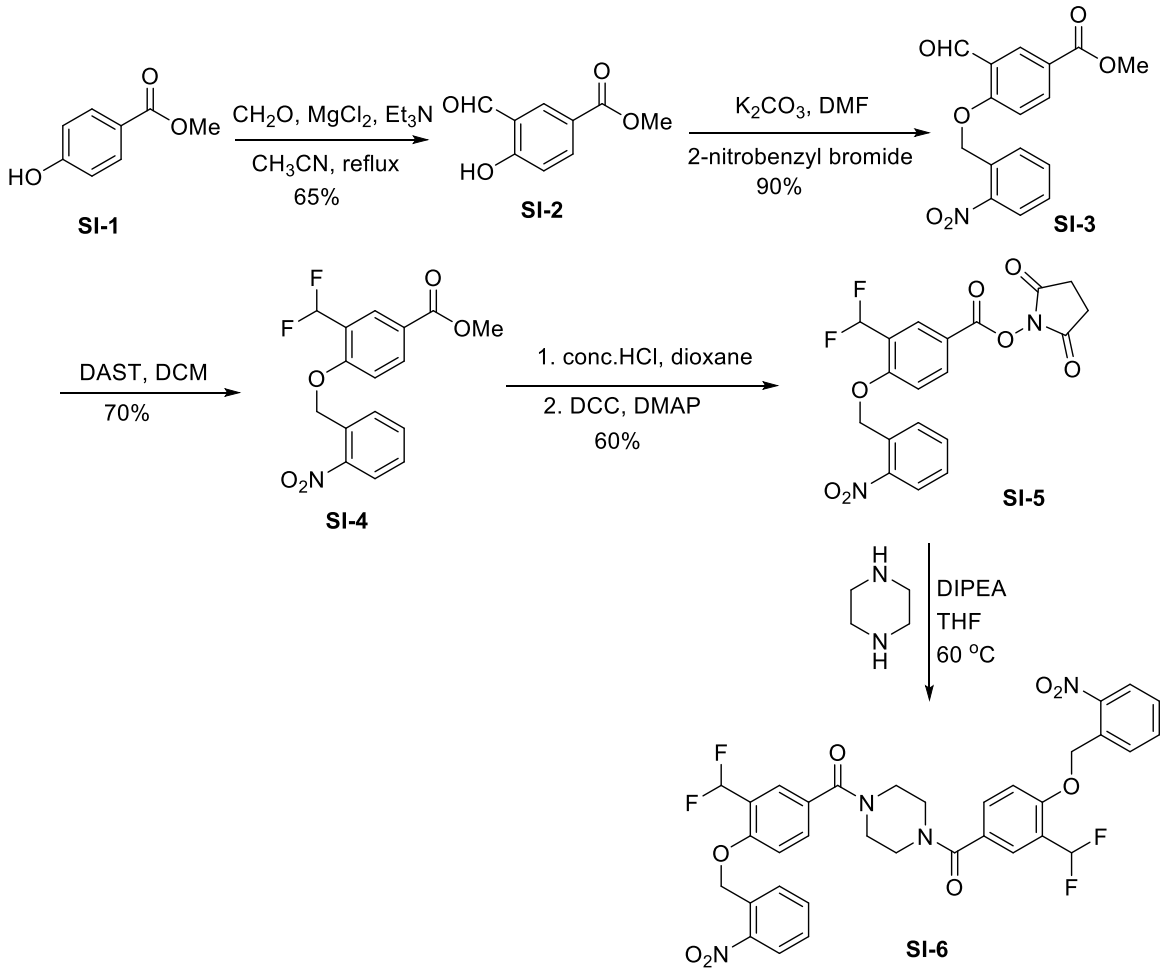
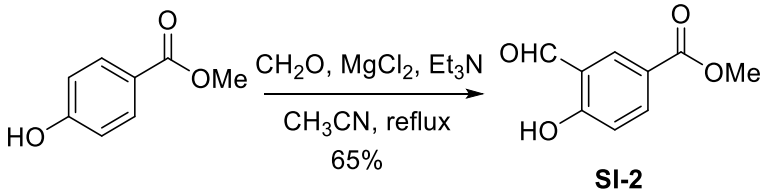
Experimental Procedures:

Molecular cloning

Primers were synthesized and purified by Integrated DNA Technologies (IDT), and plasmids were sequenced by GENEWIZ. All molecular biology reagents were obtained from New England Biolabs. 14-3-3 gene were codon optimized for *E. coli* expression, and synthesized by GENEWIZ. EGFR-GFP was a gift from Alexander Sorkin (addgene plasmid # 32751)[1]. Primers 14-3-3 NdeI for (GTTGTTCATATGGATAAAAATGAACTAGTACAAAAGGCTAAGTTG), 14-3-3 HindIII rev (GTTGTTAAGCTTTTAGTGATGGTGATGGTGATGGTTTTCACCACCCTCACCCGCCTC) were used to clone 14-3-3 into pBAD vector; primers 14-3-3 QQR NdeI for (CATATGGATAAAAATGAACTAGTACAAAAGGCTAAGCAGCAGCGTCAAGCTGAGCGCTAC) and 14-3-3 HindIII rev were used to obtain pBAD-14-3-3 QQR mutant; primers 14-3-3 HindIII for (GTTGTTAAGCTTGCCACCATGGATAAAAATGAACTAGTAC), 14-3-3 XhoI rev(GGTGGTCTCGAGTTAGTGATGGTGATGGTGATGGTTTTCAC) were used to subclone 14-3-3 or 14-3-3 QQR from pBAD into pCDNA 3.1.

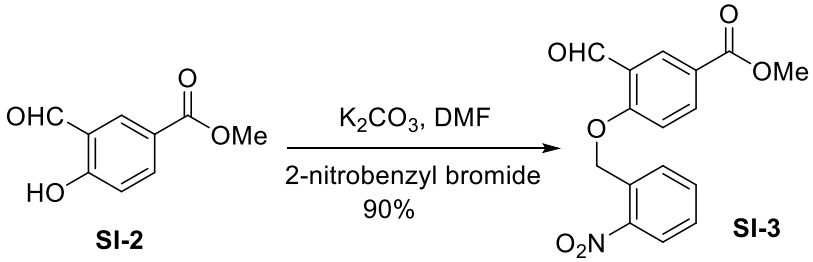
NHQM and HoQM chemical syntheses





SI-2

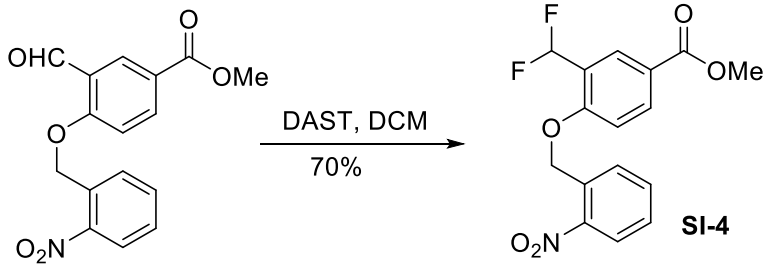
Parafomaldehyde (2.1 g, 67.5 mmol, 6.75 equiv) was added to a mixture of the methyl 4-hydroxybenzoate (1.5 g, 10 mmol, 1.0 equiv), anhydrous $MgCl_2$ (1.4 g, 15 mmol, 1.5 equiv) and Et_3N (5.3 mL, 37.5 mmol, 3.7 equiv) in CH_3CN (50 mL), and the mixture was heated under reflux until consumption of the starting material as determined by TLC. After the reaction mixture was cooled to rt, the reaction was quenched with 1 M HCl and the product was extracted with EtOAc (50 mL x 3). The organic layers were combined, washed with brine, dried over Na_2SO_4 and filtered. All volatiles were removed under reduced pressure and the product was isolated by flash chromatography (EtOAc/Hex) on silica gel (1.2 g, 65%). The NMR spectrum is the same as reported.



SI-2 **SI-3**

Aldehyde **SI-2** (2.00 g, 11.1 mmol, 1.0 equiv) from previous step in DMF was added K_2CO_3 (3.06 g, 22.2 mmol, 2.0 equiv). The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution and the product was extracted with EtOAc (50 mL x 3). The organic layers were combined, washed with brine, dried over Na_2SO_4 and filtered. All volatiles were removed under reduced pressure and the product was isolated by flash chromatography (EtOAc/Hex) on silica gel (3.1 g, 90%).

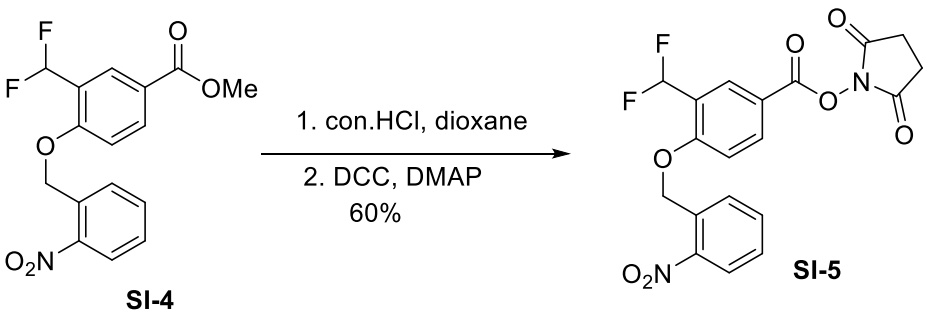
1H NMR (300 MHz, $CDCl_3$) δ 10.57 (s, 1H), 8.58 (d, J = 2.1 Hz, 1H), 8.27 (t, J = 7.7 Hz 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.79 (t, J = 7.4 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 5.72 (s, 2H), 3.95 (s, 3H); 13C NMR (75 MHz, $CDCl_3$): δ 188.5, 165.8, 162.9, 146.8, 137.2, 134.5, 132.1, 131.8, 129.0, 128.4, 125.4, 124.8, 123.8, 112.8, 67.7, 52.3.



SI-3 **SI-4**

Ester **SI-3** (3.00 g, 9.52 mmol, 1.0 equiv) from previous step in DCM was slowly added DAST at 0 ℃ (3.37 g, 20.9 mmol, 2.2 equiv). The reaction mixture was slowly warmed to the room temperature and stirred for 2 h. The reaction was quenched with saturated aqueous NH_4Cl solution and the product was extracted with EtOAc (50 mL x 3). The organic layers were combined, washed with brine, dried over Na_2SO_4 and filtered. All volatiles were removed under reduced pressure and the product was isolated by flash chromatography (EtOAc/Hex) on silica gel (2.2 g, 90%).

1H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.17 (td, J = 1.2, 8.5 Hz 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.75 (t, J = 8.5 Hz, 1H), 7.57 (t, J = 8.5 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 7.03 (d, J = 55.2 Hz, 1H), 5.66 (s, 2H), 3.94 (s, 3H); 13C NMR (75 MHz, $CDCl_3$): δ 165.9, 159.1, 146.7, 134.4, 134.1, 132.3, 128.8, 128.6 (t, J = 6.8 Hz), 128.1, 125.2, 123.2 (t, J = 43.9 Hz), 111.8, 111.4 (t, J = 237.1 Hz), 67.3, 52.2.

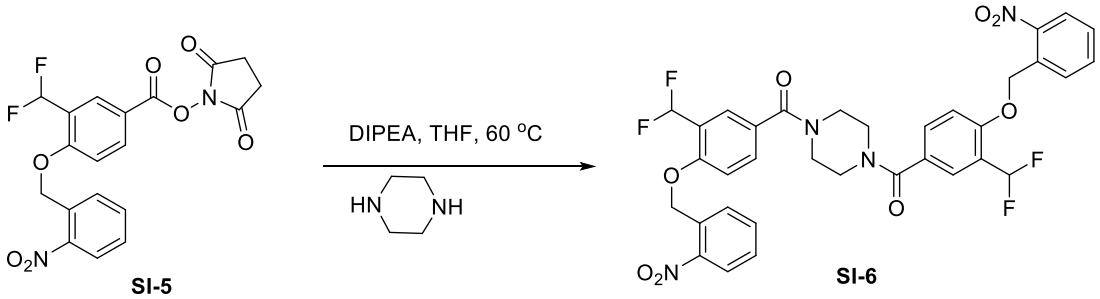


SI-4 **SI-5**

The difluo **SI-4** (100 mg, 0.3 mmol, 1.0 equiv) in 1,4-dioxane (5.00 mL) was added 0.5 mL of conc. HCl solution. The reaction mixture was stirred at 90 ℃ and monitored by TLC. After the starting material had been completely consumed by TLC, the mixture was cooled to room temperature. The white solid product was collected by filtration. The product was dried under high vacuum and then used directly for the next step.

The acid from previous step was dissolved in THF and added DCC (63.8 mg, 0.31 mmol, 1.01 equiv), N-hydroxysuccinimide (42.7 mg. 0.37 mmol, 1.2 equiv) and catalytic amount DMAP (1.84 mg, 0.015 mmol, 0.05 equiv). The reaction was stirred at room temperature for 12 h. The reaction was diluted with hexanes and remove the solid by filtration. The filtrate was concentrated under reduced pressure and the product was isolated by flash chromatography as white solid (78 mg, 60%).

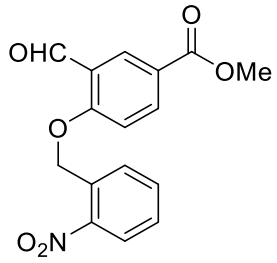
1H NMR (300 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.26 (d, J = 7.7 Hz, 2H), 7.82 (t, J = 7.7 Hz 1H), 7.74 (d, J = 7.7 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 7.03 (d, J = 55.2 Hz, 1H), 5.71 (s, 2H), 2.94 (s, 4H); 13C NMR (75 MHz, $CDCl_3$): δ 169.2, 160.7, 146.7, 135.3, 134.5, 131.8, 129.6 (t, J = 6.8 Hz), 129.0, 128.1, 125.3, 118.1 (t, J = 237.2 Hz), 112.3, 111.0, 67.6, 25.6.



SI-5 **SI-6**

The NHS ester **SI-5** (38.0 mg, 90.4 μmol, 2.0 equiv.) in THF (5.00 mL) was added piperazine (3.8 mg, 45.2 μmol, 1.0 equiv.) and DIPEA (8.76 mg, 67.8 μmol, 1.5 equiv.). The reaction mixture was stirred overnight under reflux. After starting material was consumed, the reaction was cooled to the room temperature. The reaction was concentrated under reduced pressure and the product was isolated by flash chromatography as white solid (12.00 mg, 38.1%).

1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 8.0 Hz, 2H), 7.83 (d, J = 8.0 Hz 2H), 7.76 (d, J = 8.0 Hz, 2H), 7.70 (s, 2H), 7.58 (t, J = 8.0 Hz, 4H), 7.09 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 55.2 Hz, 2H), 5.63 (s, 4H), 3.69 (s, 8H); 13C NMR (75 MHz, $CDCl_3$): δ 169.4, 146.8, 134.3, 132.2, 131.7, 128.8 (t, J = 6.8 Hz), 128.2, 126.2, 125.2, 123.2 (t, J = 237.2 Hz), 112.3, 111.2, 67.3.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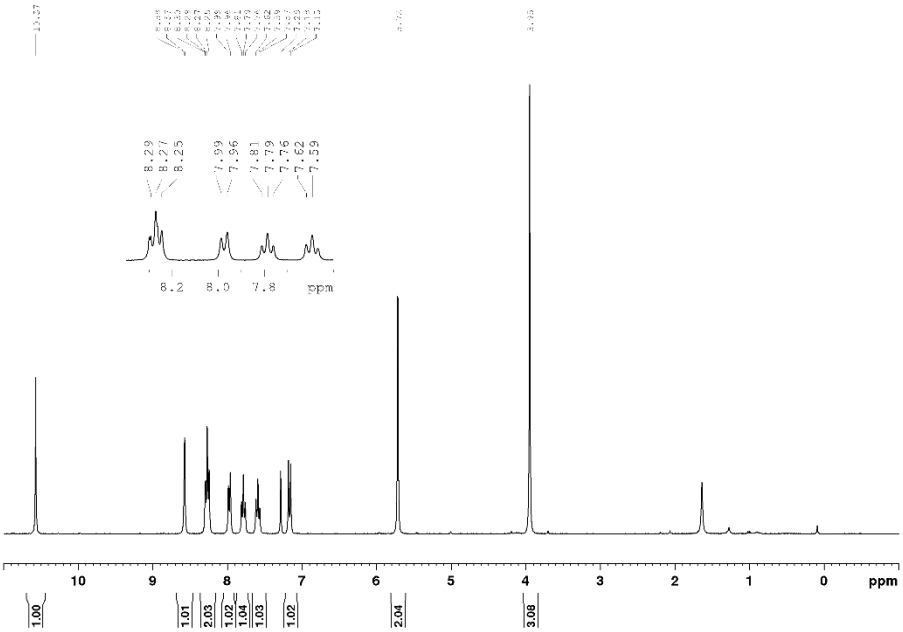
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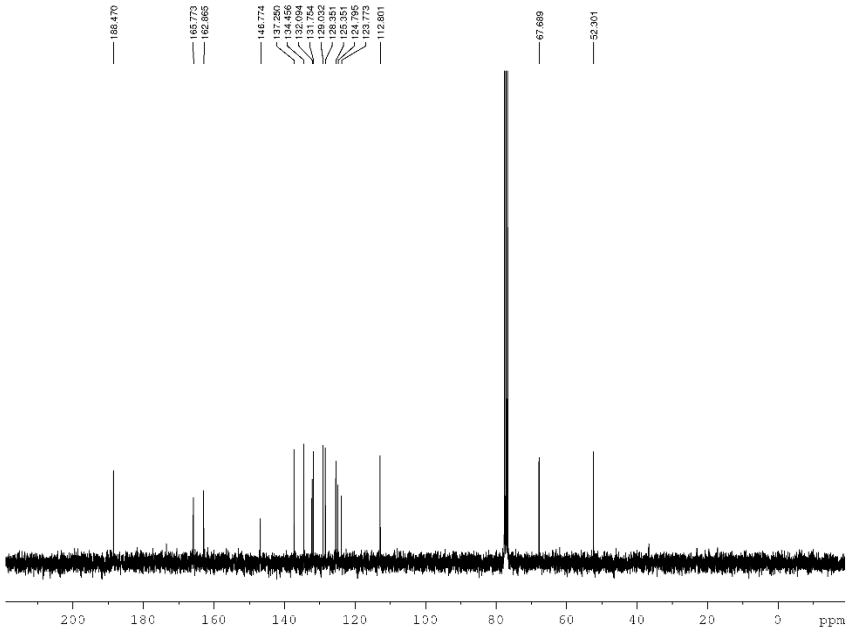
2.04
3.08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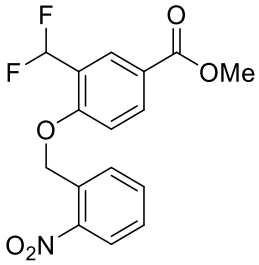
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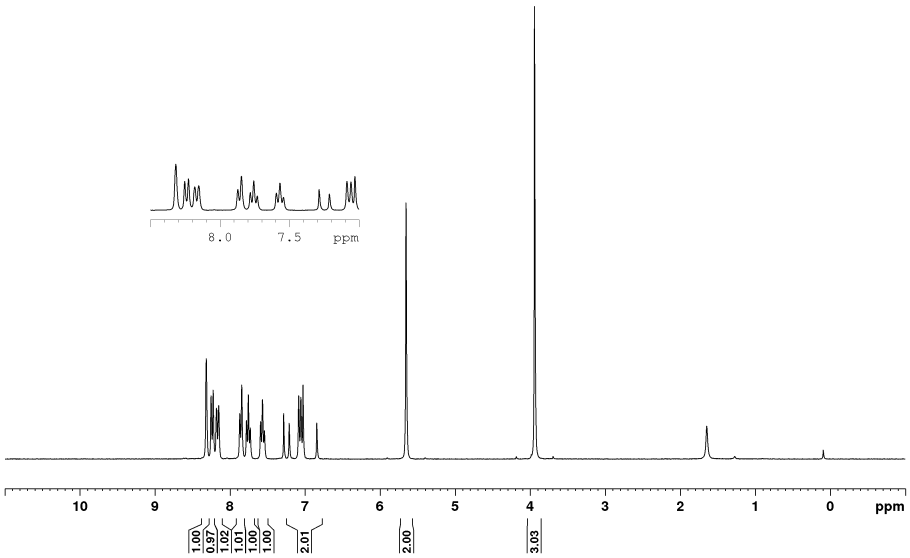
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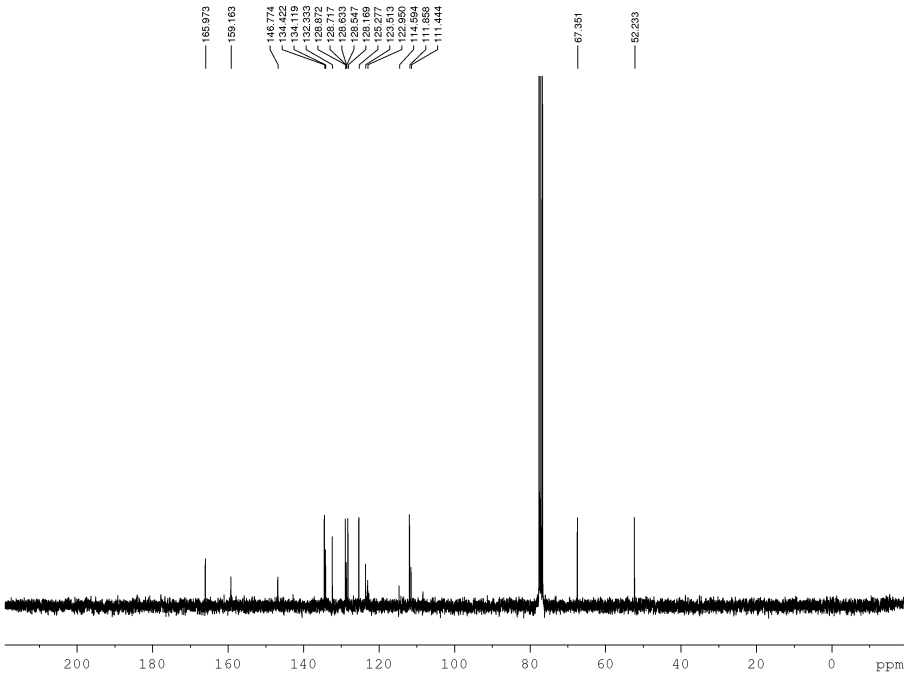
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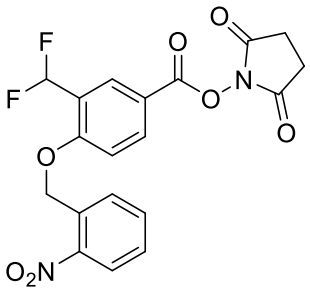
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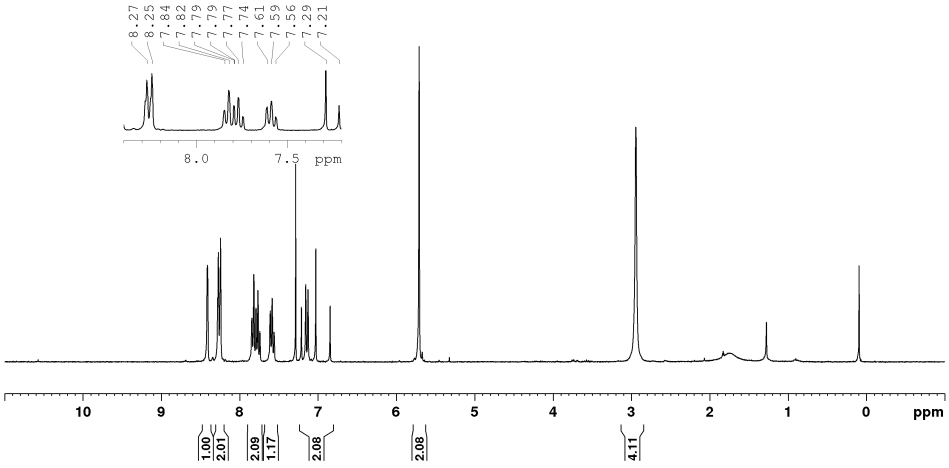
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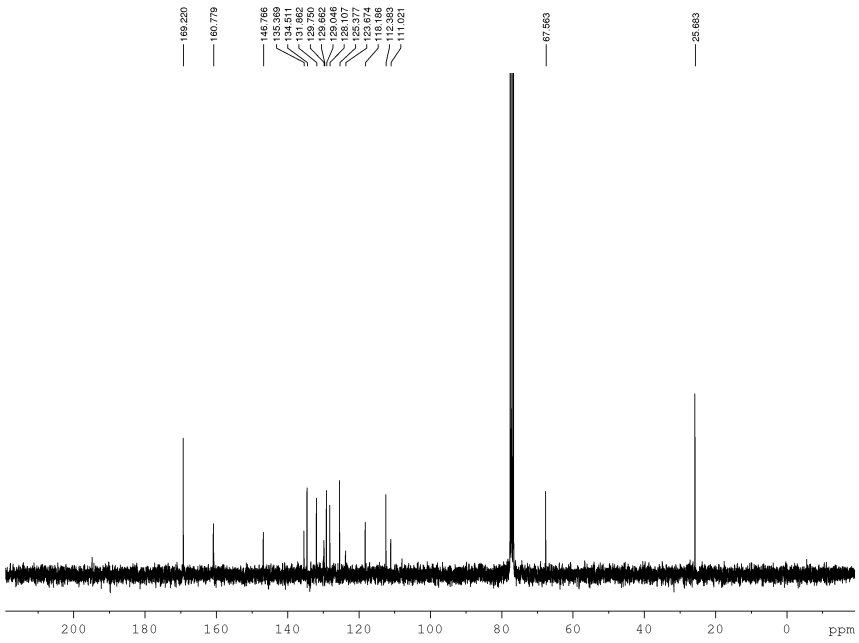
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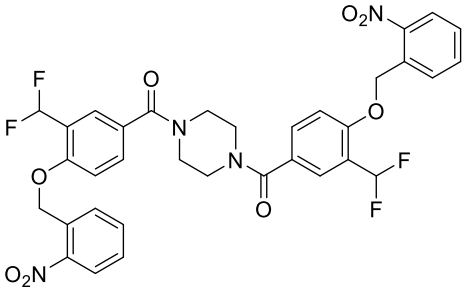
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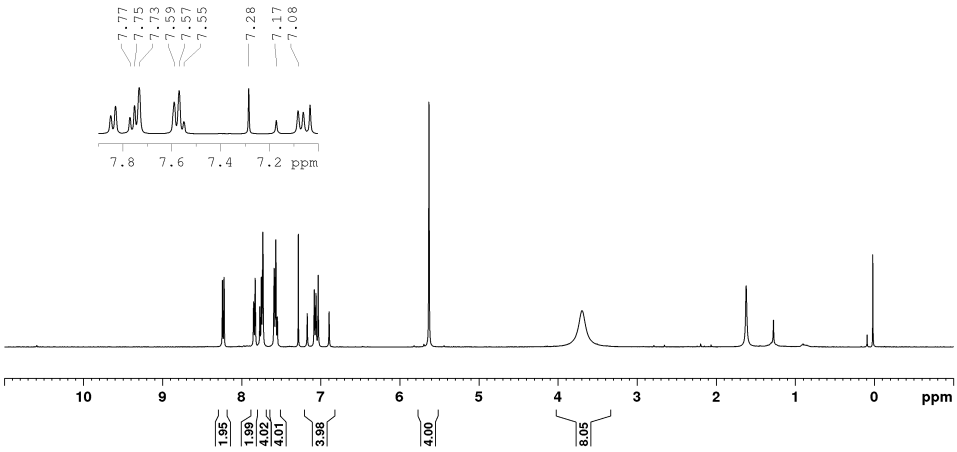
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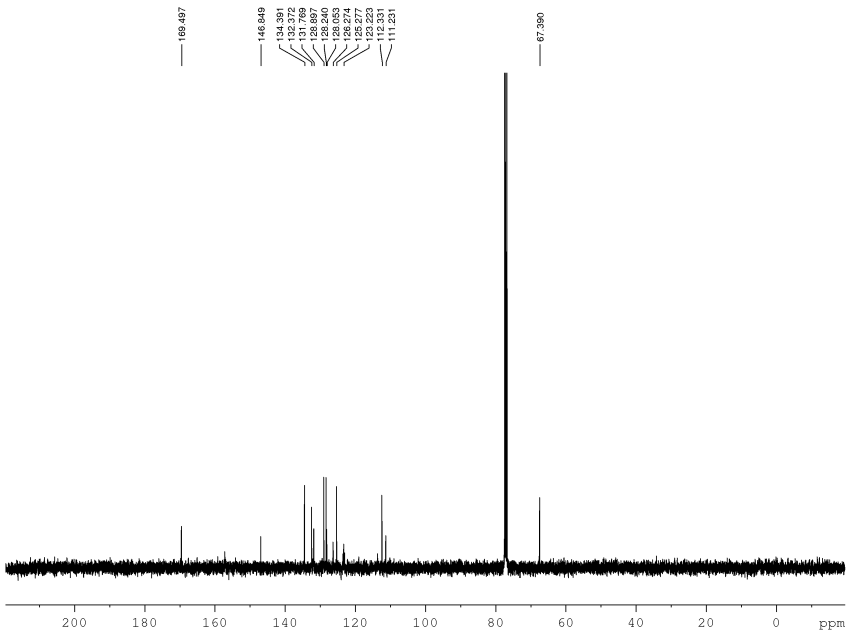
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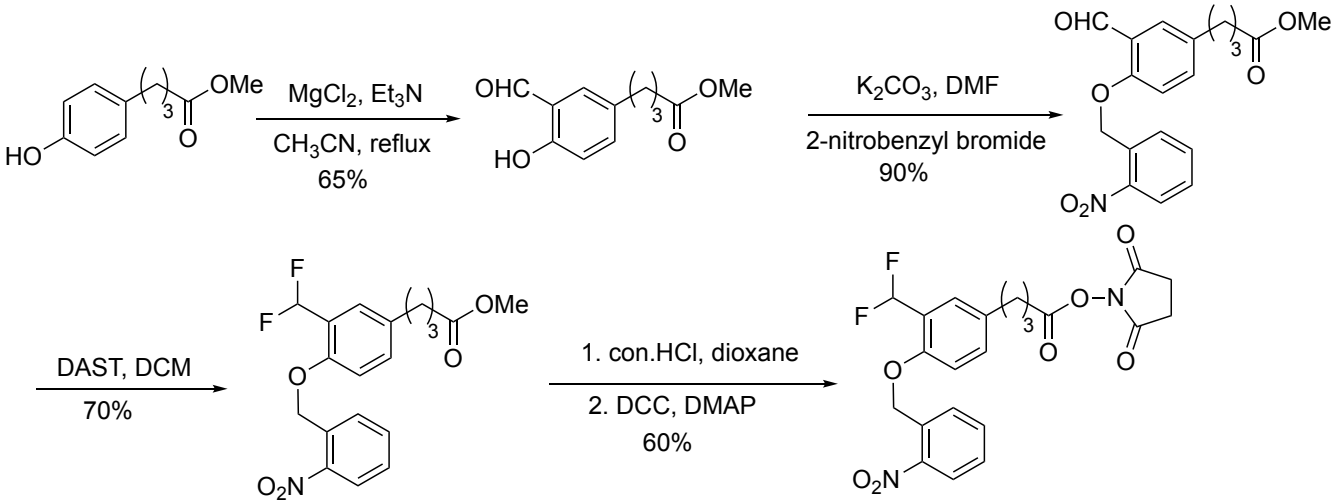
NHQM3C chemical synthesis



The NHQM3C chemical synthesis was carried out using the same procedures described in the above section for NHQM chemical synthesis, except for the different starting material shown in the scheme.

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Protein expression and purification

For protein expression and purification of 14-3-3 WT or 14-3-3 QQR, plasmid pBAD-14-3-3 or pBAD-14-3-3 QQR was transformed into *E. coli* BL21(DE3), and plated on LB argar plate supplemented with 100 μg/mL ampicillin. Several colonies were picked from above freshly transformed plate, and inoculated to 100 mL 2X YT (5 g/L NaCl, 16 g/L Tryptone, 10 g/L Yeast extract). The cells were grown at 37 °C, 220 rpm to an OD 0.5, with good aeration and the relevant antibiotic selection. Then the medium was added with only 0.2% L-Arabinose, and the expression were carried out at 18 °C, 220 rpm for 18-22 hr. The cells were harvested at 3000 g, 4 °C for 10 min. The cell pellet was washed with cold IMAC buffer (25 mM sodium phosphate, 20 mM imidazole, 500 mM NaCl, pH 7.5), and centrifuged again at 3000 g, 4 °C for 10 min, and resuspended in 15 mL IMAC buffer. The tube was then frozen on dry ice and stored in −80 °C. For protein purification, the frozen cells were thawed quickly and resuspended well, and supplemented with EDTA free protease inhibitor cocktail, 0.5 mg/mL lysozyme, 1 μg/mL DNase, and vortex for 2 min. The cells then were opened by sonification, after which the cell lysis solution was centrifuged at 25,000 g at 4 °C for 40 min. The supernatant was collected and incubated with 1 mL TALON® Metal Affinity resin. After excessive wash with IMAC buffer, the protein was eluted five times with 1 mL 25 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.5. The fractions containing the target protein were analyzed by running 10% Tris-tricine SDS−PAGE gel.

NHQM mediated 14-3-3 cross-linking in vitro

To test if NHQM could cross-link 14-3-3 protein in dimeric form, 8 μM WT 14-3-3 protein in PBS buffer, pH 7.4 were treated with or without 1 mM NHQM, with or without UV illumination for 15 mins at wavelength 365 nm. The reaction was then treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. After that, the reaction mixture was immediately treated with SDS loading dye with 100 mM DTT, and samples were boiled at 95 °C for 5 mins and run in 10% Tris-tricine SDS−PAGE gel.

Utilizing NHQM to differentiate 14-3-3 dimer versus monomer

To test if NHQM crossinking could differentiate dimer from monomer, 8 μM 14-3-3 WT or 14-3-3 QQR mutant in PBS buffer, pH 7.4 were treated with 0, 0.05, 0.1, 0.2, or 0.4 mM NHQM, and subjected to UV illumination for 15 min at wavelength 365 nm. The reaction was then treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The reaction mixture was immediately added with SDS loading dye containing 100 mM DTT, and the samples were boiled at 95 °C for 5 mins and run in 10% Tris-tricine SDS−PAGE gel.

NHQM mediated Trx cross-linking with interacting proteins in *E. coli* cell lysate

Plasmid pBAD-Trx was transformed into BL21(DE3) *E. coli* cells, and the Trx protein expression was carried out following procedures described above except that the cell culture volume was decreased to 20 mL. After protein expression, the cells were harvested and resuspended in 15 mL 50 mM sodium phosphate buffer containging 200 mM NaCl, pH 7.5. The cells were broken by sonification. 15 μL cell suspension were taken and added with 0, 1, or 10 mM NHQM and incubated at RT for 15 min, followed by UV illumination at wavelength 365 nm for another 15 min. The reaction was then treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The samples were then quickly treated with SDS loading dye containing 100 mM DTT, boiled at 95 ℃ for 5 min, and then analyzed with Western blot using anti-His antibody.

NHQM mediated 14-3-3, GST, or EGFR cross-linking in mammalian cells

Plasmid pCDNA3.1-14-3-3, pCDNA3.1-GST, or pCDNA3.1-EGFR (2 μg each) was transfected into one well of 6-well plate of HEK293T cells, respectively. After transfection, the cells were cultured at 37 °C for additional 24 hr. The cells were harvested and washed with PBS, pH 7.4 for one time, followed by resuspension in 50 μL PBS, pH 7.4. The cells were either added nothing or added with 4 mM NHQM and incubated at RT for 15 min. Then the cells were illuminated with or without UV at wavelength 365 nm for another 15 min. After that, the reaction was treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The samples were then quickly treated with 2X SDS loading dye containing 100 mM DTT, boiled at 95 °C for 5 mins, and analyzed by running Western blot using an anti-His antibody.

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NHQM mediated endogenous EGFR cross-linking in MCF10A cells

The MCF10A cells were cultured in Mammary Epithelial Cell Growth Medium (PromoCell, C-21110). When the cell population reached 80% confluence, cells were harvested and washed with PBS, pH 7.4 for one time, followed by resuspension in four equal aliquots of 30 µL PBS, pH 7.4. The cells were treated with or without 1 mM NHQM and incubated at RT for 30 mins. Then the cells were illuminated with or without UV at wavelength 365 nm for another 15 min. After that, the reaction was treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The samples were then quickly treated with 2X SDS loading dye containing 100 mM DTT, boiled at 95 °C for 5 min, and analyzed with Western blot using an anti-EGFR antibody.

HoQM mediated 14-3-3 cross-linking in vitro

To test if HoQM could cross-link 14-3-3 protein in dimeric form, 20 µM WT 14-3-3 in PBS buffer, pH 7.4 were treated with or without 1 mM HoQM, with or without UV illumination for 15 min at wavelength 365 nm. Then the reaction mixture was immediately treated with SDS loading dye containing 100 mM DTT, and the samples were boiled at 95 °C for 5 min followed by running in 10% Tris-tricine SDS−PAGE gel.

HoQM mediated 14-3-3 cross-linking in *E. coli* cells

To evaluate if HoQM could cross-link 14-3-3 directly in *E. coli* living cells, 100 µL *E. coli* BL21(DE3) cells expressing WT 14-3-3 protein with pBAD vector were spun down using a benchtop centrifuge. The cell pellet was then resuspended in 50 µL PBS, pH 7.4, and treated with or without 1 mM HoQM for 1 hr at RT, after which the samples were illuminated with or without UV at 365 nm for 15 min. Then the reaction mixture was immediately treated with 2x SDS loading dye containing 100 mM DTT, and the samples were vortexed for lysis, boiled at 95 °C for 5 min, and analyzed with Western blot using an anti-His antibody.

HoQM mediated 14-3-3 cross-linking in mammalian cells

Plasmid pCDNA3.1-14-3-3 (2 µg) was transfected into one well of 6-well plate of HEK293T cells. The media was changed to DMEM with 10% FBS after 15 hr. The cells were cultured at 37 °C for additional 24 hr. HoQM (0.6 mM) was directly added to the cell culture medium and incubated for an additional 4 hr or 8 hr. HoQM was removed by gently washing with PBS for one time. Then the cells in each time point were harvested, resuspended, and separated in 4 equals of 15 µL PBS, pH 7.4. The cells were subsequently illuminated with or without UV at wavelength 365 nm for another 10 min. The samples were then quickly treated with 2X SDS loading dye containing 100 mM DTT, boiled at 95 °C for 5 min, and analyzed with Western blot using an anti-His antibody.

NHQM mediated cross-linking of SSB protein with M13mp18 in vitro

To test cross-linking of M13mp18 DNA with the SSB protein using NHQM, in 10 µL reaction, 3 ng/µL M13mp18 was incubated with or without 0.7 mg/mL SSB protein at 37 °C for 30 min. The reaction mixture was then added with 1 mM NHQM and incubated at RT for 15 min, followed with or without UV illumination at wavelength 365 nm for another 15 min at RT. The reaction was treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The samples were then quickly treated with RNA loading dye containing 100 mM DTT, and were boiled at 95 ℃ for 5 min. The samples were quickly put on ice after boiling, and run in 5% TBE-Urea gel.

NHQM mediated cross-linking of SSB protein with 19(3x) or ATC(4x) in vitro

The sequence of 19(3x) is the following: TGTAGCTGTTGATCTAAGTTGTAGCTGTTGATCTAAGTTG TAGCTGTTGATCTAAGT. The sequence of ATC(4x) is the following: AATTCGCCAATGACAAGACG CTGGGCGGGGCCGGATCCATCATCATCATCTAGAAGCT. To test cross-linking of 19(3x) or ATC(4x) DNA with the SSB protein using NHQM, in 10 µL reaction, 1.5 µM 19(3x) or ATC(4x) was incubated with or without 0.2 mg/mL SSB protein at 37 °C for 30 min. The reaction mixture was then added with 1 mM NHQM and incubated at RT for 15 min, followed with or without UV illumination at wavelength 365 nm for another 15 min at RT. Then the reaction was treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The samples were then quickly treated with RNA loading dye containing 100

mM DTT, and were boiled at 95 ℃ for 5 min. The samples were quickly put on ice after boiling, and run in 5% TBE-Urea gel.

In-solution digestion of cross-linked proteins

Protein digestion was carried out by following a procedure described previously.[2] Briefly, after cross-linking protein samples were quenched by adding 100 mM Tris-HCl, pH 7.4 for 15 min. The protein samples were then precipitated by adding six volumes of acetone at −20 °C for 30 min. Protein was collected by centrifugation for 10 min at 15,000 g. Precipitated proteins were dried in air and resuspended in 8 M urea, 100 mM Tris, pH 8.5. After reduction with 2 mM DTT for 20 min and alkylation with 10 mM iodoacetamide for 15 min in the dark, samples were diluted to 1 M urea with 100 mM Tris, pH 8.5, and digested with trypsin (at 50:1 protein: enzyme ratio) at 37 °C for 16 h. Digestion was terminated by adding formic acid at final concentration 5% (v/v). Digested peptides were desalted with C18 ZipTip, and eluted peptides were dried down with SpeedVac.

In-gel digestion of cross-linked 14-3-3 monomer

NHQM cross-linked 14-3-3 protein in 50 mM phosphate buffer, 200 mM NaCl, pH 7.5 was quenched by adding 100 mM Tris-HCl, pH 7.4 for 15 min. Then the protein was reduced by 20 mM DTT at 95 °C for 20 min with 1X glycine loading dye. IAM (50 mM) was used to alkylate exposed cysteines after cooling down. Samples were subjected to 10% Tris-tricine gel for separation of cross-linked dimers from the monomers. After Coomassie staining and destaining, gel band containing the monomers was cropped and subject to in-gel trypsin digestion at 37 °C overnight.[3] Digested peptides were extracted in 5% formic acid/acetonitrile. After drying and re-dissolving in 1% TFA, the peptides were cleaned-up using Pierce C18 tips. Eluted peptides were dried down with SpeedVac and then subjected to tandem mass spectrometry.

Mass spectrometry

Digested peptides were dissolved in 200 mM NH_4HCO_3 after clean-up and then subject to tandem mass spectrometry on a Thermo Q-Exactive Orbitrap.Cross-linked peptides. Peptides were separated by nano-LC Ultimate 3000 high-performance liquid chromatography system using an Acclaim PepMap C18 column (Thermo Scientific). Samples were analyzed with a 145 min 2%-95% acetonitrile gradient with 0.1% formic acid at flow rate 200 nL/min. The Q-Exactive mass spectrometer was operated in data-dependent mode with one full MS scan at R = 70,000 (m/z = 200) followed by ten HCD MS/MS scans at R = 17,500 (m/z = 200) using a stepped normalized collision energy of 28, 30, 35 eV. The AGC targets for the MS1 and MS2 scans were 3×10^6 and 1×10^5, respectively, and the maximum injection time for MS1 was 250 ms, and for MS2 was 200 ms. Precursors of the + 1, + 6 or above, or unassigned charge states were rejected; exclusion of isotopes was enabled; dynamic exclusion was set to 30 s. The cross-linking mass spectra were analyzed with pLink 2.3.[4] For searching cross-linked peptides, parameters were used as follows: Flow type: Conventional Cross-linking (HCD); Process number: 4; precursor mass tolerance and fragment mass tolerance were set as 20 ppm; FDR≤ 5%; the peptide length was set to 3–60 with mass variation from 300-6000; Carbamidomethyl [C] and Oxidation [M,C,Y] as variable modifications.

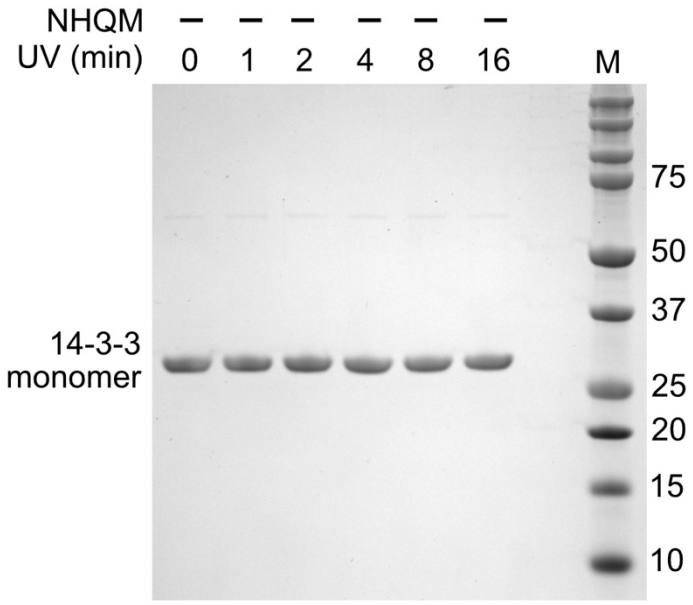

Figure S1. SDS-PAGE analysis of NHQM-mediated dimeric cross-linking of 14-3-3 with different time duration of UV exposure. 0.25 mg/mL 14-3-3 WT was incubated without 1 mM NHQM, followed UV illumination at 365 nm for 0, 1, 2, 4, 8, 16 min. At each time point, the reaction was treated by adding 100 mM Tris-HCl, pH 7.5 and incubated at RT for 15 min. The samples were then prepared with SDS loading dye containing 100 mM DTT, boiled at 95 ℃ for 5 min, and run in 10% Tris-tricine SDS−PAGE gel. Results for NHQM treated samples are shown in Figure 1D. This figure shows the negative control without adding NHQM.

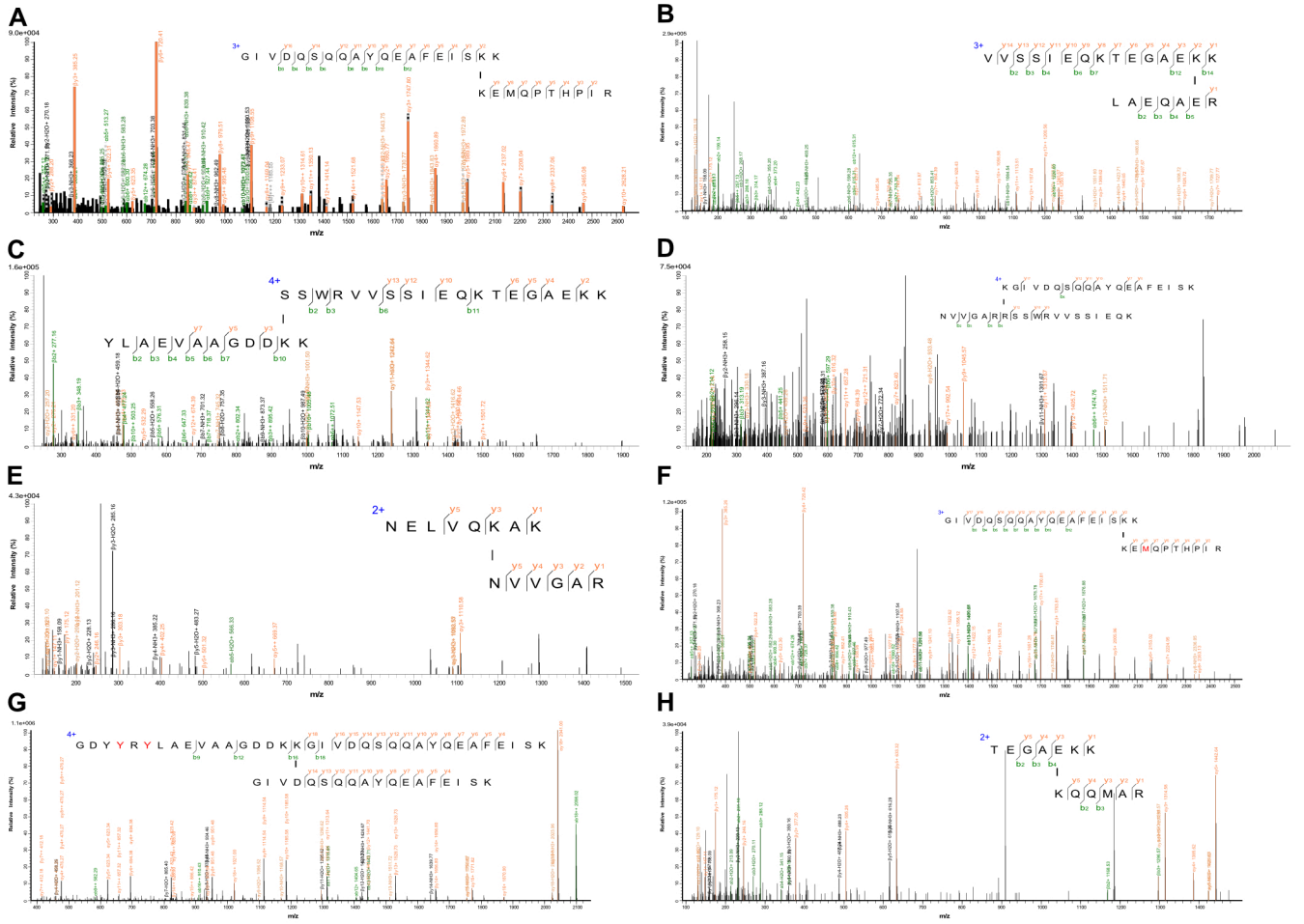

Figure S2. Tandem mass spectra of 14-3-3 cross-linked by NHQM. These spectra indicate that NHQM cross-linked Lys with Lys, Glu, Ser, Arg, Asn, and Asp, respectively. Spectrum of Lys-Gln cross-linking is shown in Figure 3B.

SUPPORTING INFORMATION

A

No.	Cross-linked site	Cross-linked peptides (Cross-linked residues in red)	Cα-Cα distance (Å)
1	14-3-3^{122}-14-3-3^{139}	MKGDYYR(2)-KGIVDQSQQAYQEAFEISK(1)	16.3
2	14-3-3^{87}-14-3-3^{138}	IETELR(2)-YLAEVAAGDDKK(11)	14
3	14-3-3^{74}-14-3-3^{75}	TEGAEKK(6)-KQQMAR(1)	3.8
4	14-3-3^{138}-14-3-3^{139}	YLAEVAAGDDKK(11)-KGIVDQSQQAYQEAFEISK(1)	3.8
5	14-3-3^{9}-14-3-3^{120}	MDKNELVQKAK(9)-VFYLKMK(5)	26.6

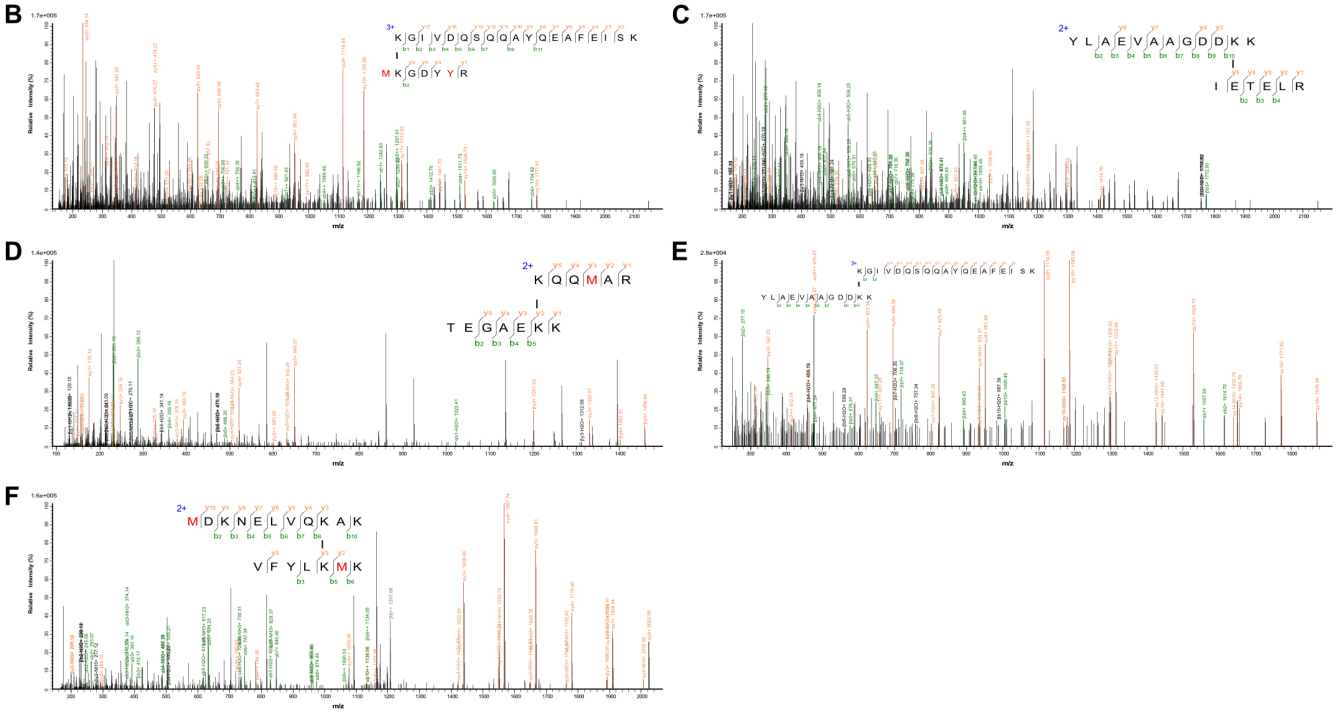

Figure S3. NHQM mediated 14-3-3 intramolecular cross-linking. To distinguish intermolecular cross-linking of the dimer from intramolecular cross-linking of the monomer, we separated the cross-linked 14-3-3 proteins by running SDS-PAGE and isolated the gel band containing the monomers. In-gel digestion was then performed followed by mass spectrometric analysis. **A**) CXMS analysis of the monomeric band of 14-3-3 protein cross-linked by NHQM, showing the intramolecularly cross-linked sites, residues, and their Cα-Cα distance. **B-F**) Tandem mass spectra for cross-linked peptides identified (red color indicates oxidation of the residue).

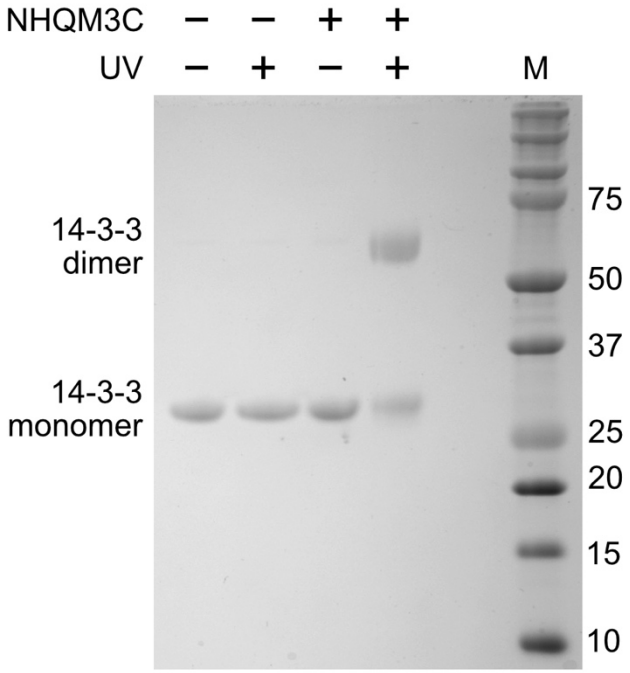

Figure S4. NHQM3C cross-linked WT 14-3-3 protein into dimeric form in vitro. The WT 14-3-3 was treated with or without UV illumination in the presence or absence of NHQM3C cross linker, and the cross-linking was detected by analyzing the mass shift observed by 10% Tris-tricine SDS−PAGE.

SUPPORTING INFORMATION

A

No.	Cross-linked site	Cross-linked peptides (Cross-linked residues in red)	Cα-Cα distance (Å)
1	14-3-3^{67}-14-3-3^{122}	VVSSIEQK(7)-MKGDYYR(2)	29.7
2	14-3-3^{73}-14-3-3^{122}	TEGAEK(5)-MKGDYYR(2)	37.7
3	14-3-3^{11}-14-3-3^{69}	AKLAEQAER(2)-TEGAEK(1)	14.6
4	14-3-3^{11}-14-3-3^{149}	AKLAEQAER(2)-GIVDQSQQAYQEAFEISK(10)	29.3
5	14-3-3^{69}-14-3-3^{85}	TEGAEK(1)-EKIETELR(2)	18.8
6	14-3-3^{11}-14-3-3^{14}	AKLAEQAER(2)-AKLAEQAER(5)	29.1

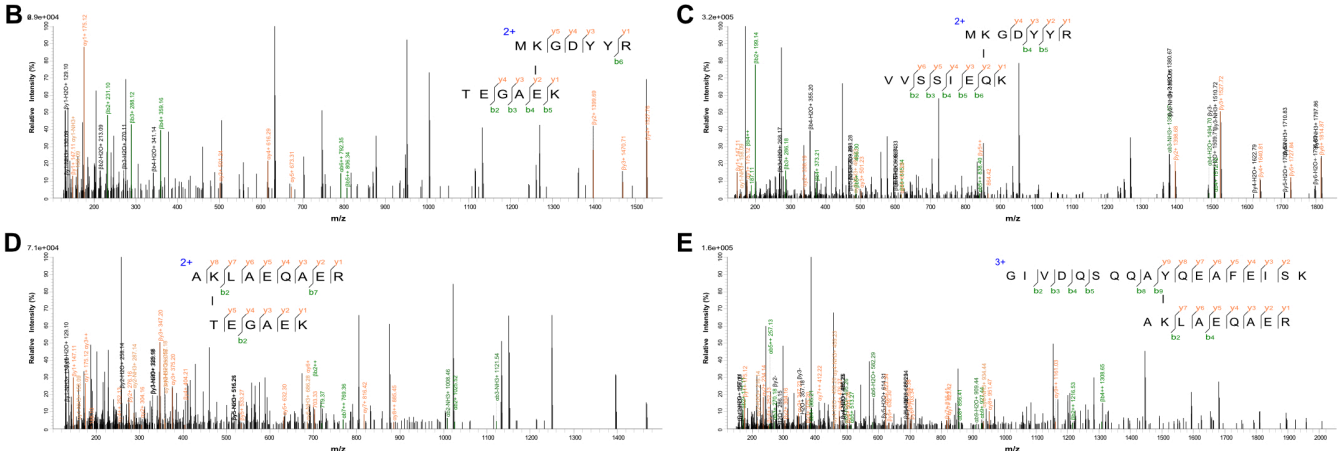

Figure S5. NHQM3C cross-linked multiple nucleophilic residues when cross-linking the WT 14-3-3 protein into dimeric form. A) CXMS analysis of WT 14-3-3 protein cross-linked by NHQM3C, showing the cross-linked sites, residues, and their Cα-Cα distance. **B-E**) Tandem mass spectra for cross-linked peptides identified from NHQM3C-cross-linked WT 14-3-3 protein, showing cross-linking of Lys with Glu, Gln, Thr, and Tyr, respectively.

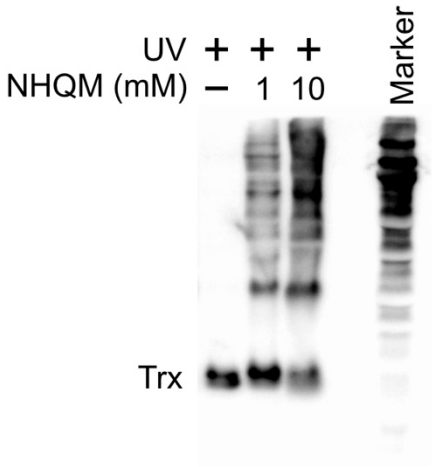

Figure S6. NHQM cross-linked Trx with interacting proteins in *E. coli* cell lysate. Cell lysate of *E. coli* expressing Trx was added with 0, 1 or 10 mM NHQM, and treated with UV at wavelength 365 nm for 15 min. The samples were then analyzed with Western blot using an anti-His antibody to detect this His6 tag appended at the C-terminus of Trx.

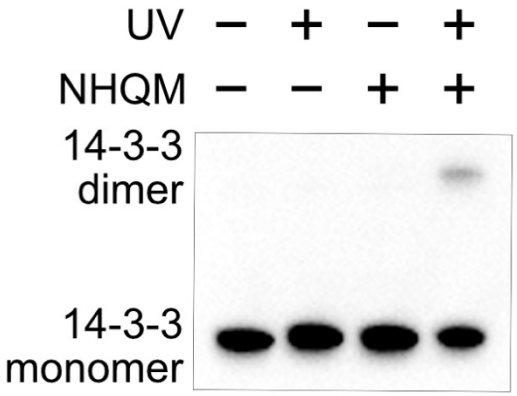

Figure S7. NHQM mediated 14-3-3 cross-linking in mammalian cells detected by Western blot. The HEK 293T cells expressing 14-3-3 were treated with or without 4 mM NHQM, and with or without UV at wavelength 365 for 15 min. The samples were analyzed with Western blot using an anti-His antibody to detect 14-3-3 monomer and dimer.

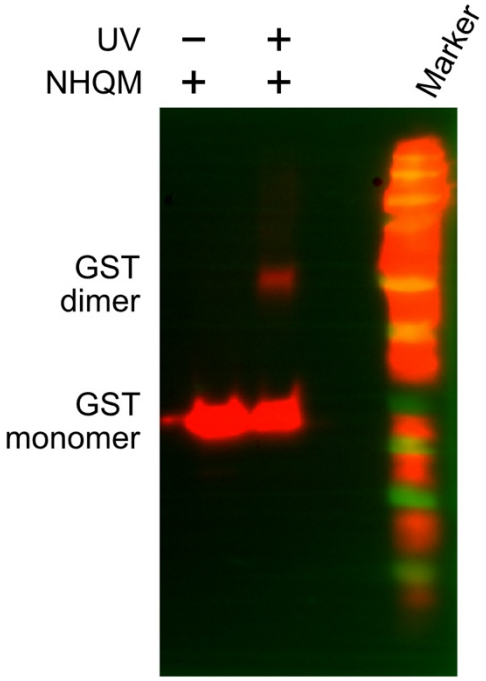

Figure S8. NHQM mediated GST cross-linking in mammalian cells detected by Western blot. The HEK 293T cells expressing GST were treated with or without 4 mM NHQM, and with or without UV at wavelength 365 nm for 15 min. The samples were analyzed with Western blot using an anti-His antibody to detect GST monomer and dimer.

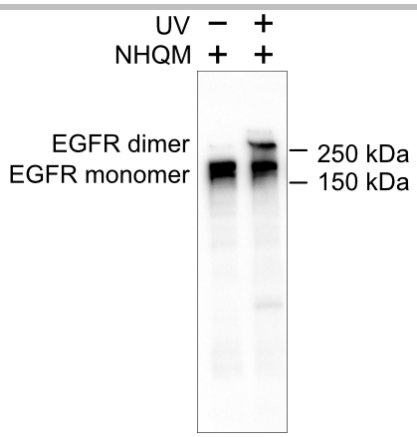

Figure S9. NHQM mediated EGFR cross-linking in mammalian cells detected by Western blot. HEK 293T cells expressing EGFR via plasmid transfection were treated with 4 mM NHQM and with or without UV illumination (λ = 365) for 15 min. The EGFR dimerization due to cross-linking was detected by Western blot using an anti-EGFR antibody.

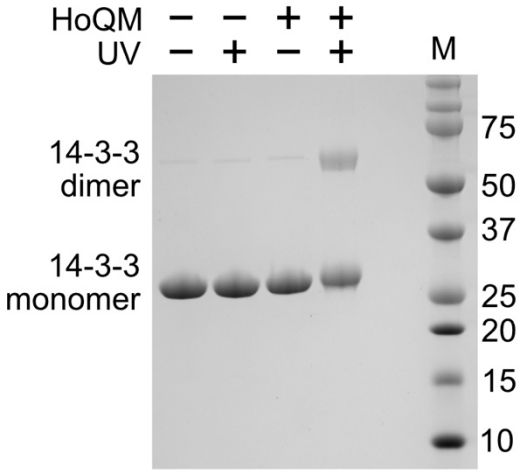

Figure S10. HoQM cross-linked WT 14-3-3 into dimeric form in vitro. The WT 14-3-3 protein was treated with or without UV in the presence or absence of HoQM cross-linker, and the cross-linking was detected by running 10% Tris-tricine SDS−PAGE.

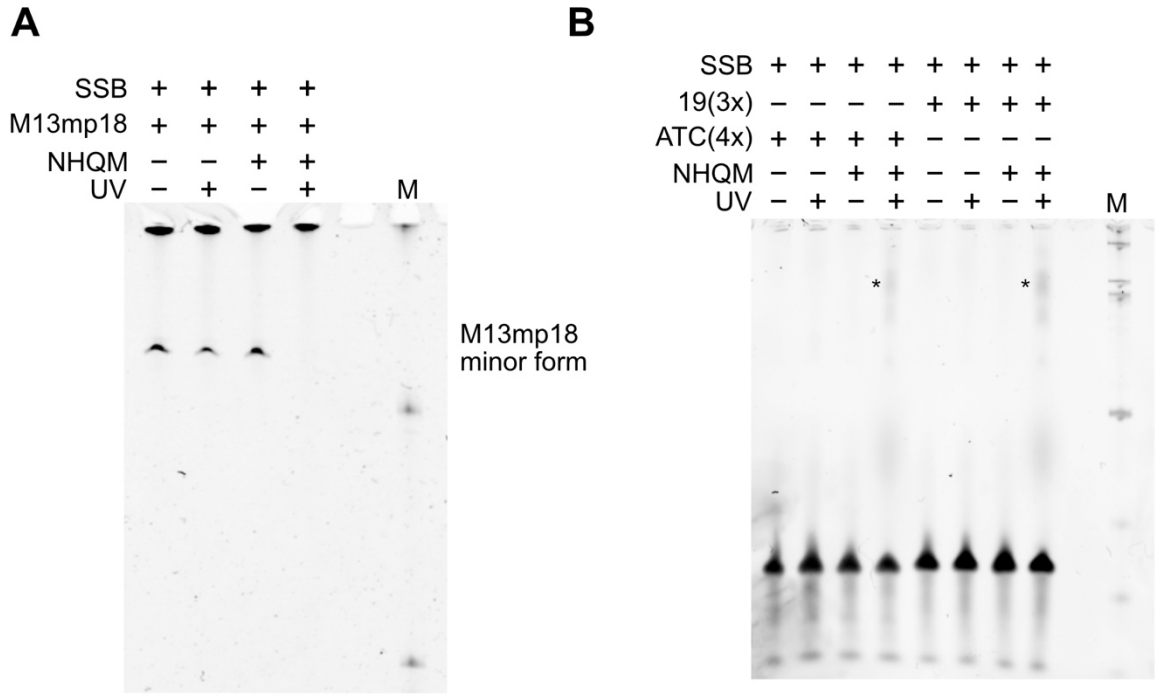

Figure S11. NHQM mediated protein-DNA cross-linking. **A**) TBE-urea gel shift assay of NHQM mediated cross-linking of the SSB protein with the viral M13mp18 DNA. The SSB protein was first reconstituted with M13mp18, and then they were treated with or without 1 mM NHQM, followed by with or without UV activation (λ = 365 nm) for 15 min. The samples were run in 5% TBE-urea gel. **B**) Same to A except the DNA used was 19(3x) or ATC(4x) for protein-DNA complex reconstitution (* indicates the cross-linked protein-DNA band). The results shown here are consistent with those shown in Figure 6, with more controls added: [SSB+/M13mp18+/UV+] in A; [SSB+/19(3x)+/UV+] and [SSB+/ATC(4x)+/UV+] in B, which indicate that UV (λ = 365 nm) alone without NHQM did not cross-link protein to DNA.

SUPPORTING INFORMATION

Supplementary references:

[1] R. E. Carter, A. Sorkin, *J. Biol. Chem.* **1998**, *273*, 35000-35007.

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[3] A. Shevchenko, H. Tomas, J. Havlis, J. V. Olsen, M. Mann, *Nat. Protocols* **2006**, *1*, 2856-2860.

[4] a) B. Yang, Y. J. Wu, M. Zhu, S. B. Fan, J. Lin, K. Zhang, S. Li, H. Chi, Y. X. Li, H. F. Chen, S. K. Luo, Y. H. Ding, L. H. Wang, Z. Hao, L. Y. Xiu, S. Chen, K. Ye, S. M. He, M. Q. Dong, *Nat. Methods* **2012**, *9*, 904-906; b) S. Lu, S.-B. Fan, B. Yang, Y.-X. Li, J.-M. Meng, L. Wu, P. Li, K. Zhang, M.-J. Zhang, Y. Fu, J. Luo, R.-X. Sun, S.-M. He, M.-Q. Dong, *Nat. Methods* **2015**, *12*, 329; c) Z. L. Chen, J. M. Meng, Y. Cao, J. L. Yin, R. Q. Fang, S. B. Fan, C. Liu, W. F. Zeng, Y. H. Ding, D. Tan, L. Wu, W. J. Zhou, H. Chi, R. X. Sun, M. Q. Dong, S. M. He, *Nat. Commun.* **2019**, *10*, 3404.