Electronic Supplementary Information

Cereblon covalent modulation through structure-based design of histidine targeting chemical probes

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General synthetic methods

Unless otherwise indicated, reagents and solvents were used as received from commercial suppliers. All reactions were monitored using a Shimadzu® LC-20AD high performance liquid chromatography/mass spectrometry (HPLC/MS) system using Kinetex® BEH C18 column (2.1 x 30 mm, 5 μm particle size). Detection methods were diode array (DAD). MS mode was positive electrospray ionization. MS range was 100-1000. HPLC method A: the gradient was 5-95% B in 1.50 min. 5% B in 0.01 min, 5-95% B (0.01 - 0.70 min), 95% B (0.70 - 1.15 min), 5% B in 1.16 min with a hold at 5% B for 0.34 min; solvent A = 0.04% trifluoroacetic acid in H₂O; solvent B = 0.02% trifluoroacetic acid in acetonitrile; flow rate: 1.5 mL/min. Purification of reaction products was carried out by flash chromatography using CombiFlash®Rf with Biotage - Isolera® normal-phase silica flash columns; or Waters® high performance liquid chromatography (HPLC) system using Phenomenex Luna C18 (80*30mm*3μm): solvent gradient 0% to 99% acetonitrile in H₂O (0.1% trifluoroacetic acid (TFA) as additive); flow rate: 25 mL/min, or Phenomenex Luna C18 (100*30mm*5μm): solvent gradient 0% to 99% acetonitrile in H₂O (0.2% formic acid (FA) as additive); flow rate: 25 mL/min, or Phenomenex Luna C18 (80*30mm*3μm): solvent gradient 0% to 99% acetonitrile in H₂O (0.04% hydrochloric acid (HCl) as additive); flow rate: 25 mL/min. The purity of all compounds was over 95% and was analyzed with Shimadzu® HPLC system. ¹H NMR and ¹³C NMR spectra were obtained using Bruker Avance III spectrometers (400 MHz for ¹H, and 100 MHz for ¹³C). Chemical shifts are reported relative to deuterated methanol (δ = 3.31) or dimethyl sulfoxide (DMSO) (δ = 2.50) for ¹H NMR. Spectra are given in ppm (δ) and as br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and coupling constants (J) are reported in Hertz.
Compound synthesis

Synthesis of 2-(2,6-dioxopiperidin-3-yl)-3-oxoisooindoline-5-sulfonyl fluoride (1, EM12-SO$_2$F)

Synthesis of 3-(6-(benzylthio)-1-oxoisooindolin-2-yl)piperidine-2,6-dione
To a solution of 3-(6-bromo-1-oxo-isooindolin-2-yl)piperidine-2,6-dione (0.8 g, 2.48 mmol) and phenylmethanethiol (308 mg, 2.48 mmol) in dioxane (12 mL) was added DIEA (640 mg, 4.95 mmol). The mixture was degassed and purged with N$_2$ three times. Then Pd$_2$(dba)$_3$ (136 mg, 148.54 µmol) and xantphos (172 mg, 297.08 µmol) were added, and the mixture was degassed and purged with N$_2$ three times and then stirred at 120 °C for 5 h under N$_2$. The reaction was cooled to rt, filtered and the solid collected was washed with H$_2$O (50 mL) and EtOAc (50 mL). The filter cake was further dried under vacuum to yield the crude title compound (900 mg) as a gray solid, which was used without further purification. MS (M+H$^+$): 367.1.

Synthesis of 2-(2,6-dioxopiperidin-3-yl)-3-oxoisooindoline-5-sulfonyl chloride
To a solution of 3-(6-benzylsulfanyl-1-oxo-isooindolin-2-yl)piperidine-2,6-dione (0.4 g, 1.09 mmol) in H$_2$O (2 mL) and AcOH (18 mL) was added N-chlorosuccinimide (NCS, 437 mg, 3.27 mmol). The reaction was stirred at 20 °C for 2 h, then diluted with H$_2$O (20 mL) and filtered. The filter cake was washed with water (20 mL) and dried further under reduced pressure to yield the crude title compound (0.2 g) as a yellow solid, which was used without further purification. MS (M+H$^+$): 343.0.

Synthesis of 2-(2,6-dioxopiperidin-3-yl)-3-oxoisooindoline-5-sulfonyl fluoride (1)
To a solution of 2-(2,6-dioxo-3-piperidyl)-3-oxo-isooindoline-5-sulfonyl chloride (0.2 g, 583.51 µmol) in acetone (10 mL) and H$_2$O (10 mL) was added KF (509 mg, 8.75 mmol). The reaction was stirred at 20 °C for 1 h. The reaction mixture was diluted with H$_2$O (50 mL) and filtered. The
filter cake was washed with water (20 mL) and dried under reduced pressure to give a residue. The residue was purified by prep-HPLC to yield the title compound (90 mg, 272 µmol, 47% yield) as a white solid. MS (M+H⁺): 326.9; HRMS (M+H⁺) calculated 327.0456, observed 327.0447; ¹H NMR: (400 MHz, DMSO-­d₆) δ = 11.06 (s, 1 H), 8.41 (dd, J = 8.13, 1.63 Hz, 1 H), 8.33 (s, 1 H), 8.07 (d, J = 8.13 Hz, 1 H), 5.19 (dd, J = 13.32, 5.07 Hz, 1 H), 4.65 - 4.74 (m, 1 H), 4.53 - 4.61 (m, 1 H), 2.82 - 3.00 (m, 1 H), 2.62 (br d, J = 17.76 Hz, 1 H), 2.37 - 2.47 (m, 1 H), 2.00 - 2.08 (m, 1 H).

Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1-oxoisooindoline-5-sulfonyl fluoride (2)

Synthesis of 3-(5-(benzylthio)-1-oxoisooindolin-2-yl)piperidine-2,6-dione
To a solution of 3-(5-bromo-1-oxo-isooindolin-2-yl)piperidine-2,6-dione (1 g, 3.09 mmol) and phenylmethanethiol (385 mg, 3.09 mmol) in dioxane (20 mL) was added DIEA (800 mg, 6.19 mmol). The reaction was degassed and purged with N₂ three times. Then Pd₂(dba)₃ (170 mg, 185.68 µmol) and xantphos (215 mg, 371.35 µmol) was added. The mixture was degassed and purged with N₂ three times and stirred at 120 °C for 5 h under N₂. The reaction was then cooled to rt, filtered and the solid collected washed with H₂O (20 mL) and EtOAC (20 mL). The filter cake was dried under reduced pressure to yield the crude title compound (1 g) as a white solid, that was used without further purification. MS (M+H⁺): 367.2.

Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1-oxoisooindoline-5-sulfonyl chloride
To a solution of 3-(5-benzylsulfanyl-1-oxo-isooindolin-2-yl)piperidine-2,6-dione (0.1 g, 272.90 µmol) in H₂O (0.5 mL) and AcOH (4.5 mL) was added NCS (109 mg, 819 µmol). The reaction was stirred at 20 °C for 2 h, then diluted with H₂O (20 mL) and filtered. The filter cake was washed with water (20 mL) and dried further under reduced pressure to yield the crude title compound as a yellow solid, which was used without further purification. MS (M+H⁺): 343.3.
Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoline-5-sulfonyl fluoride (2)

To a solution of 2-(2,6-dioxo-3-piperidyl)-1-oxoisindoline-5-sulfonyl chloride (90 mg, 262.58 µmol) in acetone (5 mL) and H₂O (5 mL) was added KF (229 mg, 3.94 mmol). The mixture was stirred at 20 °C for 1 h and then purified by prep-HPLC to yield the title compound (43 mg, 131 µmol, 50% yield) as a white solid. MS (M+H⁺): 326.8; HRMS (M+H⁺) calculated 327.0456, observed 327.0447; ¹H NMR (400 MHz, DMSO-d₆) δ = 2.01 - 2.13 (m, 1 H) 2.45 (br dd, J =13.13, 4.38 Hz, 1 H) 2.63 (br d, J =18.14 Hz, 1 H) 2.87 - 2.99 (m, 1 H) 4.50 - 4.58 (m, 1 H) 4.62 - 4.70 (m, 1 H) 5.20 (dd, J =13.26, 5.00 Hz, 1 H) 8.11 (d, J =8.13 Hz, 1 H) 8.28 (d, J =8.00 Hz, 1 H) 8.51 (s, 1 H) 11.07 (s, 1 H).

Synthesis of 3-(6-((2H-1,2,3-triazol-2-yl)sulfonyl)-1-oxoisindolin-2-yl)piperidine-2,6-dione (3)

To a 0 °C solution of 2-(2,6-dioxo-3-piperidyl)-3-oxoisindoline-5-sulfonyl chloride (0.2 g, 583.51 µmol) in methylene chloride (DCM, 5 mL) were added DIEA (151 mg, 1.17 mmol) and 2H-triazole (202 mg, 2.92 mmol). The mixture was then stirred at 20 °C for 12 h, concentrated under reduced pressure. The residue was purified by prep-HPLC to afford the title compound (5 mg, 13.25 µmol, 50% yield) as a white solid. MS (M+H⁺): 376.0; HRMS (M+H⁺) calculated 376.0721, observed 376.0708; ¹H NMR (400 MHz, DMSO-d₆) δ = 11.03 (s, 1H), 8.31 (s, 2H), 8.27 (dd, J =1.9, 8.1 Hz, 1H), 8.15 (d, J =1.5 Hz, 1H), 7.96 (d, J =8.1 Hz, 1H), 5.14 (dd, J =5.1, 13.3 Hz, 1H), 4.67 - 4.58 (m, 1H), 4.54 - 4.45 (m, 1H), 2.98 - 2.84 (m, 1H), 2.60 (br d, J =17.6 Hz, 1H), 2.45 - 2.36 (m, 1H), 2.06 - 1.97 (m, 1H).

Synthesis of 3-(6-((1H-1,2,4-triazol-1-yl)sulfonyl)-1-oxoisindolin-2-yl)piperidine-2,6-dione (4)

The title compound was prepared from 2-(2,6-dioxo-3-piperidyl)-3-oxoisindoline-5-sulfonyl chloride and 1,2,4-triazole using the method described for compound 3. MS (M+H⁺): 376.0; HRMS (M+H⁺) calculated 376.0721, observed 376.0707.
Synthesis of methyl 5-acetoxy-2-methyl-benzoate
To a solution of methyl 5-hydroxy-2-methyl-benzoate (4.5 g, 27.08 mmol) in THF (100 mL) were added triethylamine (8.2 g, 81.24 mmol), dimethylaminopyridine (331 mg, 2.71 mmol) and Ac₂O (4.15 g, 40.62 mmol). The reaction was stirred at 20 °C for 12 hr. The reaction was then quenched with aq. 6 N HCl until pH reached 1~3 and then further diluted with H₂O (100 mL). The reaction was then extracted with EtOAc (100 mL x 3). The layers were separated, and the combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give the crude title compound (6.2 g) as a light-yellow liquid, which was used without further purification. MS (M+H⁺): 209.1.

Synthesis of methyl 5-acetoxy-2-(bromomethyl)benzoate
To a solution of methyl 5-acetoxy-2-methyl-benzoate (5.7 g, 27.38 mmol) in CHCl₃ (110 mL) were added N-bromosuccinimide (5.4 g, 30.11 mmol) and AIBN (450 mg, 2.74 mmol). The mixture was stirred at 70 °C for 2 hr under N₂. The reaction was quenched by addition of H₂O (150 mL). The layers were separated, and the aq. layer was extracted with EtOAc (150 mL x 3). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue that was purified by column chromatography (petroleum ether/ethyl acetate = 3/1 to 2/1) to afford the title compound (6.5 g, 22.64 mmol, 82.70% yield) as a light-yellow oil. MS (M+H⁺): 288.9.

Synthesis of methyl 2-(bromomethyl)-5-hydroxy-benzoate
To a solution of methyl 5-acetoxy-2-(bromomethyl) benzoate (3.5 g, 12.19 mmol) in MeOH (32 mL) and H₂O (8 mL) was added NH₄OAc (21.6 g, 280.38 mmol). The reaction was stirred at 20 °C for 2 hr. The reaction mixture was then quenched by addition of aq. sat. NaHCO₃ (50 mL)
solution, and then the aq. phase was extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (20 mL), dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (petroleum ether/ethyl acetate=3/1 to 2/1) to afford the title compound (1.3 g, 5.30 mmol, 44% yield) as a white solid. $^1$H NMR: (400 MHz, DMSO-$d_6$) $\delta$ = 9.91 (s, 1H), 7.32 (d, $J$ = 8.8 Hz, 1H), 7.28 (d, $J$ = 4.4 Hz, 1H), 6.98 (dd, $J$ = 2.4, 8.4 Hz, 1H), 5.24 (s, 2H), 3.80 (s, 3H).

Synthesis of 3-(6-hydroxy-1-oxo-isoindolin-2-yl)piperidine-2,6-dione

To a solution of methyl 2-(bromomethyl)-5-hydroxy-benzoate (1.2 g, 4.90 mmol) and 3-aminopiperidine-2,6-dione (806 mg, 4.90 mmol) in dimethylformamide (30 mL) was added N,N-diisopropylethylamine (DIEA, 1.90 g, 14.69 mmol). The mixture was then stirred at 90 °C for 5 hr. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (ethyl acetate/methanol = 1/0 to 10/1) to afford the title compound (500 mg, 1.56 mmol, 33% yield) as a light yellow solid. MS (M+H$^+$): 261.1.

Synthesis of 2-(2,6-dioxo-3-piperidyl)-6-fluorosulfonyloxy-1-oxo-isoindoline (5)

To a solution of 3-(6-hydroxy-1-oxo-isoindolin-2-yl)piperidine-2,6-dione (65 mg, 249.76 µmol) in THF (2 mL) were added N-(4-acetamidophenyl)-N-fluorosulfonyl-sulfamoyl fluoride$^1$ (94 mg, 300 µmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 84 mg, 550 µmol). The reaction was stirred at 20 °C for 10 min. The reaction was then quenched by addition of H$_2$O (5 mL), and then the layers were separated and the aq. layer extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine (10 mL), dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC to afford the title compound (5 mg, 14.0 µmol, 5.6% yield) as a white solid. MS (M+H$^+$): 342.9; HRMS (M+H$^+$) calculated 343.0406, observed 343.0396; $^1$H NMR: (400 MHz, DMSO-$d_6$) $\delta$ = 11.04 (s, 1H), 7.98 (d, $J$ = 2.2 Hz, 1H), 7.91-7.83 (m, 2H), 5.15 (dd, $J$ = 4.8, 13.4 Hz, 1H), 4.59-4.38 (m, 2H), 2.97-2.85 (m, 1H), 2.69-2.56 (m, 1H), 2.45-2.31 (m, 1H), 2.09-1.98 (m, 1H).

Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1-oxoisooindolin-5-yl sulfurofluoridate (6).
To a solution of 3-(5-hydroxy-1-oxo-isoindolin-2-yl)piperidine-2,6-dione (40 mg, 0.15 mmol) and N-(4-acetamidophenyl)-N-fluorosulfonyl-sulfamoyl fluoride (51 mg, 0.16 mmol) in THF (1 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 23 mg, 0.15 mmol). The mixture was stirred at 20 °C for one hr. After completion of the reaction, the reaction was concentrated under reduced pressure to give a residue, which was purified by prep-HPLC to afford the title compound (1.4 mg, 0.004 mmol, 3% yield) as a white solid. MS (M+H\(^+\)): 343.0; HRMS (M+H\(^+\)) calculated 343.0406, observed 343.0395; \(^1\)H NMR (400MHz, DMSO-\(d_6\)) \(\delta =11.03\) (s, 1H), 7.99 - 7.92 (m, 2H), 7.81 - 7.71 (m, 1H), 5.14 (dd, \(J = 5.1, 13.4\) Hz, 1H), 4.60 - 4.54 (m, 1H), 4.47 - 4.40 (m, 1H), 2.98 - 2.86 (m, 1H), 2.65 - 2.58 (m, 1H), 2.39 - 2.38 (m, 1H), 2.09 - 1.99 (m, 1H).

**Synthesis of 2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl sulfurofluoridate (7)**

![Chemical structure of compound 7]

The title compound was prepared similarly as described for compound 6 (34% yield) as a white solid. MS (M+H\(^+\)): 343.1; HRMS (M+H\(^+\)) calculated 343.0406, observed 343.0394; \(^1\)H NMR (400MHz, DMSO-\(d_6\)) \(\delta =11.00\) (s, 1H), 7.93 - 7.86 (m, 2H), 7.80 - 7.72 (m, 1H), 5.13 (dd, \(J = 5.1, 13.1\) Hz, 1H), 4.70 - 4.43 (m, 2H), 2.96 - 2.83 (m, 1H), 2.63 - 2.53 (m, 2H), 2.06 - 1.95 (m, 1H).

**Synthesis of 3-(2-((2,6-dioxopiperidin-3-yl)amino)-2-oxoethyl)benzenesulfonyl fluoride (8)**

![Chemical structure of compound 8]

The title compound was prepared using 2-(3-(fluorosulfonyl)phenyl)acetic acid following the general procedure described for compound 9 (50% yield) as a yellow solid. MS (M+H\(^+\)): 329.1; HRMS (M+H\(^+\)) calculated 329.0613, observed 329.0604; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta 10.85\) (s, 1H), 8.60 (d, \(J = 8.1\) Hz, 1H), 8.08 (br, 1H), 8.02 (d, \(J = 7.9\) Hz, 1H), 7.86 (d, \(J = 7.7\) Hz, 1H), 7.74 (t, \(J = 7.8\) Hz, 1H), 4.62 - 4.49 (m, 1H), 3.72 (s, 2H), 2.70 (dd, \(J = 10.8, 7.8\) Hz, 1H), 2.46 (d, \(J = 3.5\) Hz, 1H), 2.02 - 1.88 (m, 2H).
Synthesis of 4-(2-((2,6-dioxopiperidin-3-yl)amino)-2-oxoethyl)benzenesulfonyl fluoride (9)

To a solution of 2-(4-(fluorosulfonyl)phenyl)acetic acid (100 mg, 0.40 mmol), 3-aminopiperidine-2,6-dione (120 mg, 0.68 mmol), EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 170 mg, 0.90 mmol), HOBT (hydroxybenzotriazole, 90 mg, 0.68 mmol) in anhydrous DMF (N, N-dimethylformamide, 3 mL), was added DIEA (N,N-diisopropylethylamine, 300 mg, 2.25 mmol), and the reaction was stirred at rt for 16 h. To the reaction was added water, and the resulted suspension was filtered. The solid collected was washed further with Et₂O (1 x 20 mL) to afford the title compound (84 mg, 56%) as a white solid. MS (M+H⁺): 329.10; HRMS (M+H⁺) calculated 329.0613, observed 329.0603; ¹H NMR (400 MHz, DMSO-d₆) δ = 10.86 (s, 1H), 8.62 (d, J = 8.2 Hz, 1H), 8.09 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 4.56 (q, J = 8.5 Hz, 1H), 3.72 (s, 2H), 2.79 – 2.66 (m, 1H), 2.52 – 2.40 (m, 1H), 1.96 – 1.90 (m, 2H).

Cellular CRBN NanoBRET Engagement Assay

HEK293T cells were transduced with lentivirus and put under puromycin selection (5 μg/mL) for two weeks to produce a cell line stably expressing CRBN with N-terminally fused NanoLuc luciferase (NanoLuc-CRBN). After antibiotic selection, cells were cultured in DMEM (Gibco, Life Technologies) supplemented with 10% FBS and 1 μg/mL puromycin to maintain stable NanoLuc-CRBN expression. To run the assay, cells were cultured to confluency in 10 cm² tissue culture treated plates (Corning, 430165), washed with PBS and trypsinized at room temperature to detach from the cell culture plate. After 3-4 min, the trypsin was quenched with 5x volume DMEM media (Gibco, Life Technologies) with 10% FBS and cells were collected by centrifugation (1000 rpm, 5 min). The supernatant was removed by vacuum aspiration and the pellet was then resuspended in Opti-MEM without phenol red. The density of this cell suspension was determined by diluting the cells 1:1 with trypan blue and counting using a Countess II (Thermo Fischer Scientific). The required volume of the cell suspension was prepared at 2 x 10⁵ viable cells/mL in Opti-MEM I
(Gibco, Life Technologies). To this suspension was added the CRBN engagement tracer (stock at 10 μM in 31.25% PEG-400, 12.5 mM HEPES, pH 7.5, filtered using a 0.22 μm nitrocellulose membrane; final concentration in cell suspension for assay at 250 nM). Cells were then plated in a white/opaque cell culture treated 384-well plate (Corning, 3570) at volume of 50 μL/well. After plating, the assay plate was centrifuged (500 x g, 5 min) and covered in aluminum foil. Compounds for testing were added to the plate using a D300e Digital Dispenser (HP) in duplicate 12-pt titrations from a 10 mM stock in DMSO, with DMSO normalized to 1% total volume. The plate was then placed in an incubator at 37 °C, 5% CO₂ for two hours. After incubation, the plate was removed and set on the bench to cool to room temperature (~10-15 min). The NanoLuc substrate (500X solution) and extracellular inhibitor (1500X solution) were diluted in Opti-MEM I (Gibco, Life Technologies) to prepare a 3X solution. This was then added to each well (25 μL/well). The plate was read on a Pherastar FSX microplate reader with simultaneous dual emission capabilities to read 384-well plates at 450 and 520 nm. The NanoBRET ratio was calculated by dividing the signal at 520 nm by the signal at 450 nm for each sample. Duplicate points were averaged and plotted against [compound, M] to generate an EC₅₀ curve. The Nluc substrate and extracellular inhibitor were purchased as a kit from Promega Corporation and used as is from the box - Promega NanoBRET Nano-Glo Substrate/Inhibitor; Promega Catalog number N2161 for 10,000 assay kit.

Biochemical TR-FRET CRBN binding assay

Human CRBN and DDB1 were cloned into pAC-derived vectors and recombinant protein complex was co-expressed as StreptagII™-Avitag™-CRBN and His₆-Spy-DDB1ΔB fusions in Trichoplusia ni High-Five insect cells using the baculovirus expression system (Invitrogen). Cells were lysed by sonication in 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP, 1 mM PMSF and 1x protease inhibitor cocktail (Sigma). Following ultracentrifugation and filtration, the soluble fraction was incubated with Streptactin XT 4Flow resin for 1 hr at 4°C and eluted with buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP) containing 100 mM biotin. The protein was biotinylated overnight at 4°C by adding 150 ug of BirA, ATP and MgCl₂ to a final concentration of 10 mM. The complex was further purified via anion exchange chromatography (Poros 50HQ) and size exclusion chromatography in 25 mM HEPES pH 7.4, 200 mM NaCl and 1 mM TCEP. Fractions containing the purified CRBN-DDB1 complex were concentrated using ultrafiltration (Millipore), flash frozen in liquid nitrogen, and stored at −80°C.
Assay mix containing 100 nM biotinylated StreptagII™-Avitag™-CRBN-DDB1ΔB, 20 nM BODIPY-lenalidomide probe, and 2 nM terbium-coupled streptavidin (Invitrogen) in a buffer composed of 50 mM Tris pH 7.5, 200 mM NaCl, 1 mM TCEP, and 0.1% Pluronic F-68 solution (Sigma) was dispensed into a 384-well microplate (Corning, 4514). EM12-SO₂F was dispensed into the microplate containing assay mix using the D300e Digital Dispenser (HP) normalized to 1% DMSO. TR-FRET measurements were conducted at the timepoints indicated (Figure S1).

After excitation of europium fluorescence at 337 nm, emission at 490 nm (europium) and 520 nm (Alexa Fluor 647) were recorded, with a 70 µs delay over 600 µs to reduce background fluorescence, using a PHERAstar FS microplate reader (BMG Labtech). The TR-FRET signal was calculated as the 490/520 nm ratio for each time point. Data were calculated as the average of 4 technical replicates per concentration of compound for each time point, and plotted and fit using nonlinear fit variable slope equation in GraphPad Prism 8 to yield IC₅₀ values that were plotted in Figure S1.

![EM12-SO₂F](image)

Figure S1. Time-dependent increase in potency of EM12-SO₂F in the TR-FRET biochemical assay

**Cereblon Labeling and Peptide Mapping MS**

The hsCRBN-DDB1 complex was treated with DMSO or an equimolar concentration of compound for 24 hrs at room temperature before being analyzed by LC−MS using an HPLC system (Shimadzu, Marlborough, MA) interfaced to an LTQ ion trap mass spectrometer (ThermoFisher Scientific, San Jose, CA). After injecting 5 µg, proteins were desalted for four minutes on column with 100% A and then eluted with an HPLC gradient (0–100% B in 1 min; A = 0.2 M acetic acid in water; B = 0.2 M acetic acid in acetonitrile). The mass spectrometer was
programmed to acquire full scan mass spectra (m/z 300–2000) in profile mode (spray voltage = 4.5 kV). Mass spectra were deconvoluted using MagTran software version 1.03b2. To identify the site of covalent modification, compound treated hsCRBN-DDB1 complex was reduced with 10 mM dithiothreitol for 30 min at 56 °C, alkylated with 25 mM 4-vinyl pyridine for 30 min at room temperature, and digested with trypsin (Promega, Madison, WI) overnight at 37 °C. Peptides were desalted using C18 (SOLA, ThermoFisher Scientific, Madison, WI), dried by vacuum centrifugation, reconstituted in 50% acetonitrile, 1% formic acid, 100 mM ammonium acetate, and analyzed by CE-MS using a ZipChip CE-MS instrument and autosampler (908 devices, Boston, MA) interfaced to a QExactive HF mass spectrometer (ThermoFisher Scientific). Peptides were resolved at 500 V/cm using an HR chip with a background electrolyte consisting of 50% acetonitrile with 1% formic acid. The mass spectrometer was operated in data dependent mode and the five most abundant ions in each MS scan (m/z 300–2000, 60K resolution, 3E6 target, 100 ms max fill time) were subjected to MS/MS (15K resolution, 1E5 target, 100 ms max fill time). Dynamic exclusion was enabled with a repeat count of 1 and an exclusion duration of five s. Raw mass spectrometry data files were converted to .mgf using Multiplierz software and searched against a custom database containing the hsCRBN-DDB1 sequences using Mascot version 2.6.2. Search parameters specified fixed vinylpyridine modification of cysteine, variable methionine oxidation, and variable modification of cysteine. Modified spectra were examined and figures prepared using mzStudio software.

Cereblon was exclusively labeled at His353 by the covalent probes. Figure S2a shows the mass spectra (left) and zero-charge mass spectra (right) of the CRBN-DDB1 complex treated with DMSO (top) or an equimolar concentration of compound 5 (EM12-FS) for 24 hrs at room temperature (bottom). CRBN exhibits an increase in mass consistent with modification by a single molecule of EM12-FS. Figure S2b shows the MS/MS spectrum of CRBN peptide 347AAYVNPH*GYVHETLTLYK364 [SEQ ID NO: 1] modified with EM12-FS indicating covalent labeling of His353. Ions of type b and y are indicated with blue or red glyphs, respectively. H*, EM12-FS modified residue. ++, doubly charged product ion. Related data for probes 2 and 3, 6 and 7, and 8 and 9 are shown in Figures S3, S4 and S5 respectively (15 μM CRBN, 15 μM probe).
Figure S2. MS spectra of CRBN-DDB1 complex labeled at His353 by EM12-FS (Δmass = 322).

Figure S3. MS spectra showing labeling of CRBN His353 by probes 2 and 3 after 4 hours treatment (Δmass = 307).
Figure S4. MS spectrum showing a) partial labeling of CRBN by probe 6 (Δmass = 322), and b) no labelling by probe 7 after 24 hours treatment.

Figure S5. Sulfonyl fluoride probes 8 and 9 label cereblon. CRBN-DDB1 complex treated with DMSO (top) or an equimolar concentration of probe 8 or 9 for 5 hours at room temperature (middle and bottom). CRBN exhibits an increase in mass consistent with modification by a single molecule.

Inhibition of IKZF1 degradation by lenalidomide using EM12-SO$_2$F
Experiments were carried out in a 6-well plate (Corning). 2 x 10$^6$ MOLT4 cells were dispensed into three wells in a total volume of 1.5 ml per well in RPMI 1640 medium and 10% FBS (both from Life Technologies). The DMSO-treated sample was initially treated with 1.5 μl DMSO. The lenalidomide-only treated sample was initially treated with 1.5 μl of DMSO (to balance overall DMSO content). The EM12-SO$_2$F-treated sample was treated with 1.5 μl of 1 mM EM12-SO$_2$F for a final concentration of 1 μM EM12-SO$_2$F. The samples were incubated for 2
hours at 37 °C and 5% CO₂. After 2 hours, 1.5 µl of DMSO were added to the DMSO treated sample. 1.5 µl of 1 mM lenalidomide were added to the lenalidomide-only treated sample for a final concentration of 1 µM lenalidomide, and 1.5 µl 1 mM lenalidomide were added to the EM12-SO₂F-treated sample for a final concentration of 1 µM lenalidomide. Cells were incubated at 37 °C and 5% CO₂ for 5 hours. After 5 hours, cells were removed from the 6-well plate and transferred to 1.5 ml microcentrifuge tubes. Cells were centrifuged for 5 minutes at 800 x g, the supernatant was removed, and the cells were washed with 1 ml of PBS. Pellets were flash-frozen in liquid nitrogen and left at -80 °C until further use. Cell pellets were lysed in RIPA buffer (Thermo Scientific) supplemented with 1x protease inhibitor cocktail (Roche cOmplete™). Briefly, cell pellets were resuspended in lysis buffer, briefly, vortexed, and incubated on ice for 10 minutes. After 10 minutes, lysates were centrifuged on a tabletop centrifuge for 10 minutes at maximum speed and 4 °C. Supernatants transferred to fresh 1.5 ml Eppendorf tubes and quantified via a BCA assay (Thermo Scientific). Lysates were separated on a Bolt™ 4-12% Bis-Tris Plus mini gel (Invitrogen), and transferred to a 0.2 µm nitrocellulose membrane using a TransBlot Turbo Transfer System from Bio-Rad. After transfer, the membrane was washed briefly with 1 x TBS-T and stained with Ponceau to be able to accurately divide the membrane for blotting. Membranes were blocked for 1 hour at room temperature in 10% milk (Lab Scientific bioKEMIX, Inc.) in TBS-T before adding IKZF1 (Cell Signaling Technologies) and TBP (Abcam, loading control) antibodies at dilutions of 1:1000 and 1:2000, respectively. Membranes were incubated overnight at 4 °C. The following morning, primary antibodies were discarded, and the membranes were washed 3 x 5 minutes with TBS-T, prior to adding secondary antibodies (anti-rabbit (Protein Simple) and anti-mouse (Invitrogen) HRP-conjugates for IKZF1 and TBP respectively) at a dilution of 1:5000 in 10% milk in TBS-T. Membranes were incubated at room temperature for 1 hour prior to discarding secondary antibodies and washing 3 x 5 minutes with TBS-T. Membranes were developed using a PicoWest detection kit (Thermo Scientific) and imaged on an Amersham Imager 600 (GE Healthcare) – see Figure S6.
Figure S6. a) Full blots of IKZF1 (upper) and TBP control (lower) for Figure 3b. b) Western blot quantification was performed using ImageJ (all samples were normalized to DMSO, which was set to a value of 1).

Mass spectrometry proteomics

MOLT4 cells were treated with 1 μM compound for 5 hours and protein levels were determined using quantitative mass spectrometry proteomics (detailed protocol published previously). Probes 1, 2, 3, 4, 8 and 9 do not degrade any protein to a significant extent under these conditions (Figure S7).
Figure S7. Sulfonyl fluoride and triazole probes 1, 2, 3, 4, 8 and 9 do not appreciably degrade any proteins in MOLT4 cells. Dotted lines in all plots signify a 2-fold change in protein levels and a p value of 0.001.
THP1 cells were treated with 1 μM EM12-FS (probe 5) for 5 hours and protein levels were determined using quantitative mass spectrometry proteomics as above. NTAQ1 was the only protein degraded (Figure S8).

![Figure S8. Fluorosulfate probe 5 (EM12-FS) degrades NTAQ1 exclusively in THP1 cells. Dotted lines signify a 2-fold change in protein levels and a p value of 0.001.](image)

**Western blot verification of NTAQ1 degradation by EM12-FS**

HEK293T cells were seeded overnight at a density of 750,000 cells per well in a 6-well plate. Cells were cultured in DMEM supplemented with 10% FBS and kept in an incubator set to 37 °C (5% CO₂). 1 μg per transfection condition of NTAQ1 construct (pTwist-CMV-FLAG-NTAQ1, AMP resistance marker) was added to pre-warmed medium and mixed with FuGENE HD transfection reagent (Promega E2311) at a 3:1 ratio of FuGENE:DNA. The mixture was incubated for 10 minutes at room temperature and added to cells, which were further incubated for 24 hours. Cells were then treated at the indicated concentrations of test compound for 24 hours. To test degradation dependence, cells were pre-treated with 100 nM of MLN4924 for 2 hours. After treatments, cells were washed in cold 1X PBS, trypsinized for 5 minutes at room temperature, and collected by centrifugation (500 RCF for 5 minutes, 4°C). The cell pellet was lysed in 200 μL of cold lysis buffer (RIPA Lysis and Extraction Buffer, Thermo Fisher 89900) supplemented with 1X Pierce Protease and Phosphatase Inhibitor (Thermo Fisher A32961), for 10 minutes on ice and the soluble fraction was collected by centrifugation (20000 RCF for 5 minutes, 4°C). After protein normalization using BCA (Thermo Fisher 23227), protein lysates were mixed with LDS sample
buffer (Thermo Fisher B0007) and reducing agent (Thermo Fisher B0009). Samples were then denatured at 70 °C for 10 minutes. The denatured samples were resolved on a 4-12% gradient polyacrylamide gel (Thermo Fisher NW04125BOX), transferred onto a 0.2 μM nitrocellulose membrane (Bio-Rad 1704159), and the resulting blots were probed for the indicated targets using the appropriate antibodies (Figure S9). The following antibodies were used: anti-NTAQ1 at 1:500 (Thermo Fisher PA5-117702), anti-FLAG M2 at 1:1000 (Sigma Aldrich F1804), anti-β-actin at 1:2000 (R&D Systems MAB8929).

![Image of Figure S9](image)

**Figure S9.** a) Full blots of NTAQ1 (upper), anti-FLAG (middle) and β-actin control (lower) for Figure 4b. b) Western blot quantification was performed using ImageJ (all samples were normalized to DMSO, which was set to a value of 100%).

**Purification of BirA**

*Escherichia coli* BirA (Uniprot ID: P06709) with a tandem N-terminal MBPc-His-S-PreScission sequence in T7 controlled Kanamycin resistant plasmid. The plasmid was transformed into BL-21 Rosetta 2 pLysS *Escherichia coli* cells (Novagen) and used to inoculate 5 ml of LB broth (Invitrogen) supplemented with 50 μg/ml kanamycin (Fisher Scientific). The culture was grown to saturation overnight at 37 °C on an orbital shaker. The following morning, the 5 ml of
overnight culture was used to inoculate 1 L of LB broth. IPTG (Goldbio) was added to a final concentration of 0.6 mM once the OD$_{600}$ of the culture reached a value of 0.6. Protein production was allowed to occur for o/n at 18 °C on an orbital shaker. The following morning, the culture was centrifuged for 10 minutes at 4,000 x g, and the supernatant was discarded. The resulting cell pellet was resuspended in 10 ml of PBS (Corning) and transferred to a 50 ml conical tube (Thermo Scientific). The tube was centrifuged for 10 minutes at 4,000 x g and the supernatant was removed. The pellet was washed once more with 10 ml of PBS and the cell pellet was flash frozen in liquid nitrogen and stored at -80 °C. For purification, the pellet was thawed and resuspended in 40 ml of cold lysis buffer (50 mM Tris, 200 mM NaCl, 20 mM imidazole, 1 mM TCEP, 1 mM PMSF, pH 8.0). The cell resuspension was lysed on ice water via sonication using a Fisherbrand Model 505 Sonic Dismembrator and 1/8" in. microtip probe; pulsing 20 seconds on/30 seconds off for 3 minutes of total on time. The lysate was cleared by centrifugation in a Beckman Coulter Optima XE-90 Ultracentrifuge in a Type 45 Ti rotor at 40,000 rpm for 1 hour. The supernatant was transferred to a 15 ml conical tube (Thermo Scientific) with 1.5 ml of Ni-NTA beads (3 ml 50% slurry) washed with lysis buffer without PMSF. The lysate and beads were incubated with end-over-end rotation (batch binding) for 1 hour at 4 °C. The sample was subsequently transferred to a 1.5 x 10 cm glass Econo-Column® from Bio-Rad. The beads were washed with 20 ml of lysis buffer without PMSF. 5 x 10 ml volumes of elution buffer (50 mM Tris, 200 mM NaCl, 300 mM imidazole, 1 mM TCEP, pH 8.0). A$_{280}$ measurements were made for each fraction, concentrated to 5 ml using a 3 kDa MWCO centrifugation filter (Milipore Sigma), and purified via size-exclusion chromatography (SEC) using a HiLoad 16/600 Superdex 200 pg column (Cytiva Lifesciences) on a Bio-Rad NGC Chromatography System. Integrity of the purified sample was confirmed via SDS-PAGE on a 4-20% Mini-PROTEAN TGX Stain-Free gel from Bio-Rad and imaged on a Bio-Rad GelDoc™ XR+ imager (data not shown).

Expression and purification of NTAQ1

Human wild-type NTAQ1 (Uniprot ID: Q96HA8 isoform 1) with a tandem N-terminal 6xHis-AviTag™ sequence followed by a TEV protease cleavage site was subcloned into a pET28a(+) vector and purchased from Twist Biosciences. The plasmid was transformed into BL-21 Rosetta 2 pLysS Escherichia coli cells (Novagen) and used to inoculate 100 ml of LB broth (Invitrogen)
supplemented with 50 mg/ml kanamycin (Fisher Scientific) and 35 µg/ml chloramphenicol (Sigma Aldrich). The culture was grown to saturation overnight at 37 °C on an orbital shaker. The following morning, 20 ml of overnight culture was used to inoculate 1 L of LB broth. IPTG (Goldbio) was added to a final concentration of 0.6 mM once the OD$_{600}$ of the culture reached a value of 0.6. Protein production was allowed to occur for 3 hours at 37 °C on an orbital shaker. After 3 hours, the culture was centrifuged for 10 minutes at 4,000 x g, and the supernatant was discarded. The resulting cell pellet was resuspended in 10 ml of PBS (Corning) and transferred to a 50 ml conical tube (Thermo Scientific). The tube was centrifuged for 10 minutes at 4,000 x g and the supernatant was removed. The pellet was washed once more with 10 ml of PBS and the cell pellet was flash frozen in liquid nitrogen and stored at -80 °C. For purification, the pellet was thawed and resuspended in 10 ml of cold lysis buffer (50 mM Tris, 200 mM NaCl, 20 mM imidazole, 1 mM TCEP, 1 mM PMSF, pH 8.0). The cell resuspension was lysed on ice water via sonication using a Fisherbrand Model 505 Sonic Dismembrator and 1/8th in. microtip probe; pulsing 20 seconds on/30 seconds off for 3 minutes of total on time. The lysate was cleared by centrifugation in a Beckman Coulter Optima XE-90 Ultracentrifuge in a Type 45 Ti rotor at 30,000 x g for 30 minutes. The supernatant was transferred to a 15 ml conical tube (Thermo Scientific) with 1 ml of Ni-NTA beads (2 ml 50% slurry) washed with lysis buffer without PMSF. The lysate and beads were incubated with end-over-end rotation (batch binding) for 1 hour at 4 °C. The sample was subsequently transferred to a 1.5 x 10 cm glass Econo-Column® from Bio-Rad. The beads were washed with 20 ml of lysis buffer without PMSF. 5 x 2 ml volumes of elution buffer (50 mM Tris, 200 mM NaCl, 300 mM imidazole, 1 mM TCEP, pH 8.0). A$_{280}$ measurements were made for each fraction to assess total protein content using elution buffer as a blank. All fractions were pooled. 10 ml of lysis buffer (without PMSF) were prepared with 100 mM biotin, 20 mM ATP, and 20 mM MgCl$_2$. This was added to the 10 ml combined elution. 150 mg of BirA enzyme were added and the sample was mixed and incubated overnight at 4 °C. The following morning, the sample was concentrated to 5 ml using a 3 kDa MWCO centrifugation filter (Milipore Sigma), and purified via size-exclusion chromatography (SEC) using a HiLoad 16/600 Superdex 75 pg column (Cytiva Lifesciences) on a Bio-Rad NGC Chromatography System. Integrity of pre-TEV cleavage, post-TEV cleavage, and post-SEC samples were analyzed via SDS-PAGE on a 4-20% Mini-PROTEAN TGX Stain-Free gel from Bio-Rad and imaged on a Bio-Rad GelDoc™ XR+ imager (Figure S10a). Confirmation of
biotinylation (Figure S10b) was by mass spectrometry performed using the same method as described above.

![Image](image_url)

**Figure S10.** a) SDS-PAGE and b) intact MS analysis of biotinylated recombinant NTAQ1

### CRBN-DDB1 purification

Human CRBN and DDB1 were cloned into pAC-derived vectors and recombinant protein complex was co-expressed as Flag-TEV-Spy-CRBN and His<sub>6</sub>-Spy-DDB1ΔB fusions in *Trichoplusia ni* High-Five insect cells using the baculovirus expression system (Invitrogen). Cells were lysed by sonication in 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP, 1 mM PMSF and 1x protease inhibitor cocktail (Sigma). Following ultracentrifugation and filtration, the soluble fraction was incubated with Flag-M2 sepharose for 1 hr at 4°C and eluted with buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP) containing 150 μg/ml 3X-Flag peptide. The complex was further purified via anion exchange chromatography (Poros 50HQ) and size exclusion chromatography in 25 mM HEPES pH 7.4, 200 mM NaCl and 1 mM TCEP. Fractions containing the purified CRBN-DDB1 complex were concentrated using ultrafiltration (Millipore), flash frozen in liquid nitrogen, and stored at −80°C.

### SpyCatcher S50C mutant purification

Spycatcher containing a Ser50Cys mutation was obtained as synthetic dsDNA fragment from IDT (Integrated DNA technologies) and subcloned as His<sub>6</sub>-TEV fusion protein in a pET-Duet derived vector. SpyCatcher S50C was expressed in BL21 (DE3) *E. coli*. The bacteria were lysed by
sonication in buffer containing 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP, 1 mM PMSF and 10 mM imidazole. Following ultracentrifugation and filtration, the soluble fraction was passed over Ni Sepharose 6 Fast Flow resin (GE Healthcare) and eluted with buffer containing 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP and 200 mM imidazole. The affinity-purified protein was subjected to size exclusion chromatography in 25 mM HEPES pH 7.4, 200 mM NaCl and 1 mM TCEP, concentrated, flash frozen in liquid nitrogen, and stored at −80°C.

**Labelling of SpyCatcher with Alexa Fluor 647-C2-maleimide**

Purified SpyCatcher<sub>S50C</sub> protein was incubated with DTT (8 mM) at 4°C for 1 h. DTT was removed using a S200 16/600 size exclusion column in a buffer containing 25 mM HEPES pH 7.4, 200 mM NaCl and 1 mM TCEP. Alexa Fluor 647-C2-maleimide (Thermo Fisher) was dissolved in 100% DMSO and added to SpyCatcher<sub>S50C</sub> to achieve 2.5 molar excess of Alexa Fluor 647-C2-maleimide. Labelling was performed at room temperature (RT) for 3 h before moving to 4°C overnight. Alexa Fluor 647-labelled Spycatcher<sub>S50C</sub> was purified on a S200 16/600 size exclusion column in a buffer containing 25 mM HEPES pH 7.4, 200 mM NaCl and 1 mM TCEP, concentrated by ultrafiltration (Millipore), flash frozen in liquid nitrogen, and stored at -80°C.

**Alexa Fluor 647-C2-SpyCatcher labelling of CRBN-DDB1ΔB**

Spy-tagged CRBN-DDB1 was incubated overnight at 4°C with Alexa Fluor 647-C2-labelled SpyCatcher<sub>S50C</sub> protein at 1:1.2 molar ratio of CRBN-DDB1 to SpyCatcher<sub>S50C</sub>. The protein was concentrated and purified using a S200 16/600 size exclusion column in a buffer containing 25 mM HEPES pH 7.4, 200 mM NaCl and 1 mM TCEP. Collected fractions corresponding to the labeled protein were pooled, concentrated by ultrafiltration (Millipore), flash frozen in liquid nitrogen, and stored at -80°C.

**TR-FRET dimerization assay**

Assay mix containing 100 nM CRBN-DDB1ΔB-SpyCatcher<sub>S50C</sub>-Alexa Fluor 647, 200 nM biotinylated strep-avi-NTAQ1, and 2 nM europium-coupled streptavidin (Invitrogen) in a buffer composed of 50 mM Tris pH 7.5, 200 mM NaCl, 1 mM TCEP, and 0.1% Pluronic F-68 solution (Sigma) was dispensed into a 384-well microplate (Corning, 4514). Compounds were dispensed into the microplate containing assay mix using the D300e Digital Dispenser (HP) normalized to 1% DMSO. The reactions were incubated at room temperature and TR-FRET measurements were conducted at the timepoints indicated. After excitation of europium fluorescence at 337 nm,
emission at 665 nm (europium) and 620 nm (Alexa Fluor 647) were recorded with a 70 μs delay over 600 μs to reduce background fluorescence, and the reaction was followed over 5×120 s cycles of each data point using a PHERAstar FS microplate reader (BMG Labtech). The TR-FRET signal was calculated as the average 665/620 nm ratio over 5 cycles for each data point. Data was calculated as an average of 2 technical replicates per concentration of compound, was plotted and fit using nonlinear fit variable slope equation in GraphPad Prism 8 (Figure S11).

![Graph](image_url)

Figure S11. TR-FRET CRBN-NTAQ1 dimerization dependence on EM12-SO₂F concentration

References
1. H. Zhou, P. Mukherjee, R. Liu, E. Evrard, D. Wang, J. M. Humphrey, T. W. Butler, L. R. Hoth, J. B. Sperry, S. K. Sakata, C. J. Helal and C. W. Am Ende, *Org Lett*, 2018, 20, 812-815.
2. K. A. Donovan, F. M. Ferguson, J. W. Bushman, N. A. Eleuteri, D. Bhunia, S. Ryu, L. Tan, K. Shi, H. Yue, X. Liu, D. Dobrovolsky, B. Jiang, J. Wang, M. Hao, I. You, M. Teng, Y. Liang, J. Hatcher, Z. Li, T. D. Manz, B. Groendyke, W. Hu, Y. Nam, S. Sengupta, H. Cho, I. Shin, M. P. Agius, I. M. Ghobrial, M. W. Ma, J. Che, S. J. Buhrlage, T. Sim, N. S. Gray and E. S. Fischer, *Cell*, 2020, 183, 1714-1731.e1710.
3. W. Abdulrahman, M. Uhring, I. Kolb-Cheynel, J. M. Garnier, D. Moras, N. Rochel, D. Busso and A. Poterszman, *Anal Biochem*, 2009, 385, 383-385.
4. B. Zakeri, J. O. Fierer, E. Celik, E. C. Chittock, U. Schwarz-Linek, V. T. Moy and M. Howarth, *Proc Natl Acad Sci U S A*, 2012, 109, E690-697.