Supporting Information

Functional chromatographic technique for natural product isolation

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A. Recombinant protein expression and purification. All proteins and used in this study were prepared by conventional recombinant protein expression followed by immobilized metal affinity chromatography (IMAC) or native purification in the case of p97.

p97. E. coli BL21(DE3) cells containing pET14b-p97 were grown in Luria Broth (LB) medium containing 100 µg/mL ampicillin at 37°C to an OD₆₀₀ of 0.8, followed by induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 4 h at 37°C. Cells were collected by centrifugation (3000 × g for 10 min), resuspended in 40 mL lysis buffer (50 mM HEPES pH 7.4, 150 mM KCl, 5 mM MgCl₂, 5% glycerol, 2 mM β-mercaptoethanol (BME), one complete EDTA-free protease inhibitor cocktail (Roche) per 50 mL of buffer), and lysed by single passage through an M110-T microfluidizer (Microfluidics). The lysate was clarified by centrifugation (118,834 × g, 1 h, 4°C) and the resulting supernatant was incubated for 1 h at 4°C with Talon metal affinity resin (Clontech) equilibrated in 50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 5% glycerol. The resin and supernatant were then loaded into a 25 mL disposable column (BioRad), washed with 10 column volumes of wash buffer (50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 5% glycerol, 2 mM BME, 5 mM imidazole), followed by 10 column volumes of stringent wash buffer (50 mM HEPES, pH 7.4, 1 M KCl, 5 mM MgCl₂, 5% glycerol, 2 mM BME, 20 mM imidazole), and eluted with elution buffer (50 mM HEPES, pH 7.4, 1 M KCl, 5 mM MgCl₂, 5% glycerol, 2 mM BME, 250 mM imidazole). Fractions were analyzed by 12% SDS PAGE and those containing p97 were pooled and dialyzed into storage buffer (20 mM HEPES, pH 7.4, concentrated using a 30 kDa Ultra-15 centrifugal filter (Amicon) to yield a solution of p97 at 12 mg/mL, which was aliquoted, frozen in liquid N₂, and stored at -80°C until needed.

HSPA1A and HSC70. E. coli BL21(DE3) cells containing pSpeedET-HSPA1A or pSpeedET-HSC70 were grown in Luria Broth (LB) medium containing 50 µg/mL kanamycin at 37°C to an OD₆₀₀ of 0.5, cooled in 16°C for 1 h, followed by induction with 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 48 h at 16°C. Cells were collected by centrifugation (3000 × g for 10 min), resuspended in 40 mL lysis buffer (50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 5% glycerol, 2 mM BME, one complete EDTA-free protease inhibitor cocktail (Roche) per 50 mL of buffer), and lysed by single passage through an M110-T microfluidizer (Microfluidics). The lysate was clarified by centrifugation (118,834 × g, 1 h, 4°C) and the resulting supernatant was incubated for 1 h at 4°C with Talon metal affinity resin (Clontech) equilibrated in 50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 5% glycerol, and 5% glycerol. The resin and supernatant were then loaded into a 25 mL disposable column (BioRad), washed with 10 column volumes of wash buffer (50 mM HEPES, pH 7.4, 150 mM KCl, 5 mM MgCl₂, 5% glycerol, 2 mM BME, 20 mM imidazole), and eluted with elution buffer (50 mM HEPES, pH 7.4, 1 M KCl, 5 mM MgCl₂, 5% glycerol, 2 mM BME, 250 mM imidazole). Fractions were analyzed by 12% SDS PAGE and those containing HSPA1A or HSC70 were pooled and dialyzed into storage buffer (20 mM HEPES, pH 7.4, concentrated using a 30 kDa Ultra-15 centrifugal filter (Amicon) to yield a solution of HSPA1A at 2.0 mg/mL or HSC70 at 9.8 mg/mL, which was aliquoted, frozen in liquid N₂, and stored at -80°C until needed.

B. Protein resin preparation. An aliquot of a slurry of Affigel-15 resin (Bio-Rad) was transferred to a microcentrifuge tube, collected by centrifugation on a minicentrifuge at 2000xg, and the aqueous phase was removed. Next, the resin was washed three times with an equal volume of buffer (20 mM HEPES pH 7.4, 150 mM KCl, 1 mM MgCl₂, 2 mM BME). The protein was added in an equal volume of buffer (comparable volumes of resin and aqueous
phase are used) and the resin was incubated by inverted shaking at 4°C. Aliquots were taken every 10-15 min for 5 h to monitor coupling. After protein has finished coupling, the resin bound protein is washed three times with two volumes of buffer (twice the volume of resin). The activity of each batch of resin was checked using either the ATPase activity assay (p97 and HSC70) as described in Section G or a L-malate dehydrogenase assay (MDH) as described in Section H. HSPA1A could not be evaluated by the ATPase assay as described in the manuscript text.

C. Functional chromatography. The following provides a general procedure for the functional chromatographic purification: a 200 µL aliquot of solution of Parmotrema tinctorium extract (2 mg/mL) in buffer (20 mM HEPES pH 7.4, 150 mM KCl, 1 mM MgCl₂, 2 mM BME) containing 2% DMSO was added to 200 µL of resin bearing 5 mg/mL of protein in a 2 mL Eppendorf tube. The tube shaken on an inversion rotor for 12 h at 4°C. After, the supernatant was discarded and the resin washed three times with 500 µL of buffer. After the second wash, the resin was suspended in 500 µL of buffer and transferred to a ½ dram glass vial. After removal of the supernatant, 200 µL of 95% EtOH was added and the mixture was incubated for at rt for 10 min. The EtOH was collected and dried by airflow.

D. HPLC-MS analyses. The following outlines the general conditions and methods used for the LC-MS analyses included in Figure 4.

Column Dimensions: Symmetry C18 4.6 × 75.0 mm Column. Particle Size = 3.5 µM
Solvent A: 25:925 CH₃CN:H₂O containing 0.1% formic acid
Solvent B: 95:5 CH₃CN:H₂O containing 0.1% formic acid
Flow rate: 0.2 mL/min
Injection Volume: 35 µL

Method: 5% - 95% Line B

0-12 min = 95% A and 5% B
12-48 min = gradient to 95% B
48-60 min = hold 95% B

E. ATPase activity assays. ATPase activity was evaluated using Malachite green as an indicator for each protein. Assay buffer (50 mM HEPES pH 7.4, 150 mM KCl, 10 mM MgCl₂, 2 mM BME) was prepared in 100 µL reaction volume and protein was added to 1 µM followed by incubation at 37°C for 10 min, ATP hydrolysis was initiated by adding 2 mM ATP to each microcentrifuge tube, followed by incubation at 37°C. Aliquots (20 µL) were taken at 2 min, 4 min, 6 min, 8 min, and 10 min and added immediately into 800 µL of malachite green solution (9.3 µM malachite green, 53 mM (NH₄)₂MoO₄, 1M HCl, 10% Tween 20). After 1 min, 10 µL of 34% sodium citrate was added and the OD₆₆₀ was read on a Genesys 10S Vis Spectrophotometer (Thermo Scientific).

F. His₆-p97 stability test. His6-p97 was coupled onto Affigel-15 resin and stored at 4°C. Three preparations were tested, uncoupled H6p97, ethanolamine capped H6p97, and uncapped H6p97. These were assayed over the course of 31 days using the ATPase activity assay.
G. MDH assay. L-Malate dehydrogenase (Roche) purchased as a 3.2 M ammonium sulfate suspension was dialyzed into (20 mM HEPES pH 7.4, 150 mM KCl, 1 mM MgCl₂, 2 mM BME) using a Slide-A-Lyzer Dialysis Cassette (Thermo Scientific). Uncoupled Malate Dehydrogenase, Affigel-10-MDH, Affigel-15-MDH were prepared and assayed in Methacrylate cuvettes. The MDH Assay buffer contains (50 mM HEPES pH 7.4, 2 mM ketomalonate, 180 µM NADH). Cuvette was blanked with assay solution without NADH. NADH was then added and a baseline of the assay buffer was taken. A 1 µL aliquot of each MDH preparation was added and read at 340 nM at 1 sec intervals for 60 sec using a Biomate3 Spectrophotometer (Thermo Scientific).

H. UbG76VGFP and TCRα-GFP assays. HEK293 cells stably expressing UbG76V-GFP or TCRα-GFP were kindly provided by Dr. Ron Kopito (Stanford University). For live cell imaging, HEK293 cells stably expressing UbG76VGFP and TCRα-GFP were seeded at 2 × 10⁶ cells in phenol-red free Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) on 35 mm glass-bottom dishes (Bio Express). After incubation at 37°C under a 5% CO₂ atmosphere for 18 h, each dish was treated with either 10 µM, 20 µM, or 50 µM of 1 or 2 from DMSO stock solutions (0.1% final DMSO concentration). DMSO (0.1%) was used as a negative control. The cells were imaged in phenol red free DMEM supplemented with 10% FBS. The fluorescence intensity was determined by flow cytometry on a FACScan flow cytometer (BD Biosciences) equipped with an air-cooled 15 mW Argon ion laser tuned to 488 nm. The emission fluorescence of GFP was detected and recorded through a 530/30bandpass filter. List mode data files consisting of 10,000 events gated on FSC (forward scatter) versus SSC (side scatter) were acquired and analyzed using CellQuest PROsoftware (BD Biosciences) at a rate of 200-400 events per second. Data from these studies are provided in Figures S3 and S4.
**Figure S1.** Resin loading efficiency. Affi-gel resins allows up to 25 mg/mL protein loading. In this study, we evaluated to loading of His$_6$-p97, His$_6$-HSPA1A, and MDH (malate dehydrogenase) on to either Affi-gel 10 or Affi-gel15 resin.

**Figure S2.** His$_6$-p97 resin stability. The ATPase activity of free His$_6$-p97 and Affi-gel 15 resin bearing 11.4 mg/mL of His$_6$-p97 was measured over the course of a month at 4 °C. ATPase activity was monitored by the Malachite green assay as described in the experimental procedures.
**Figure S3.** Ub^{G76V}GFP assay. Oxaspirol B (2) prevents degradation of Ub^{G76V}GFP in HEK-293 cells as compared to MG132, a proteasome inhibitor, while oxaspirol C (1) does not. Concentrations are provided in µM.

**Figure S4.** TCRα-GFP assay. Oxaspirol B (2) prevents degradation of TCRα-GFP in HEK-293 cells as compared to MG132, a proteasome inhibitor, while oxaspirol C (1) does not. Concentrations are provided in µM.
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