Literature Report

Reporter: 许宁

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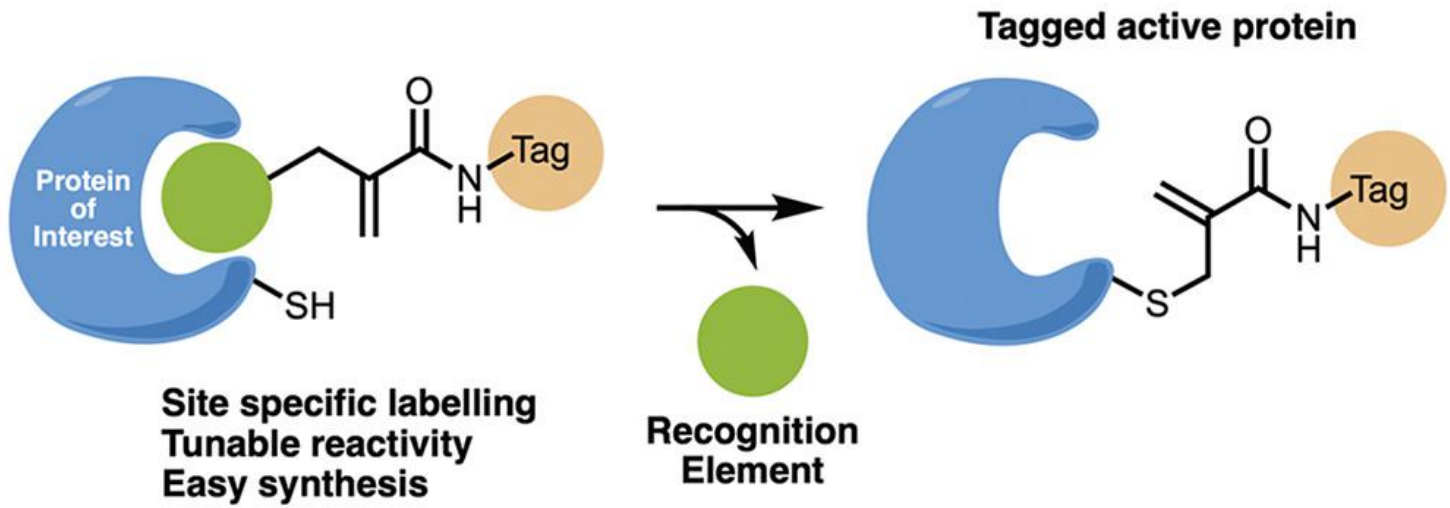
Article

Site-Specific Labeling of Endogenous Proteins Using CoLDR Chemistry

Rambabu N. Reddi,[#] Adi Rogel,[#] Efrat Resnick, Ronen Gabizon, Pragati Kishore Prasad, Neta Gurwicz, Haim Barr, Ziv Shulman, and Nir London*



2007, M.S., Computer sciences and computational biology, University of Jerusalem

2011, PhD, Microbiology and molecular genetics, Hadassah Medical School

2015, Postdoctoral Fellow, University of California, San Francisco

2019-present, The Alan and Laraine Fischer Career Development Chair, Department of Organic Chemistry, The Weizmann Institute of Science

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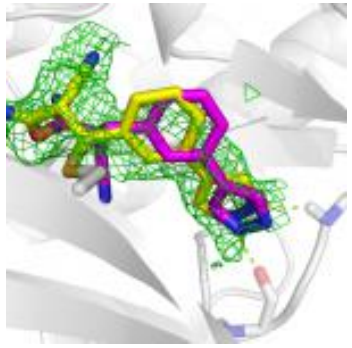
DOCKovalent

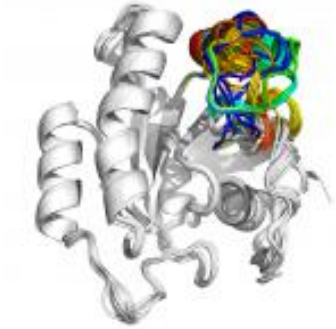
DOCKovalent is a covalent virtual screening software that was developed by Dr. London while at the Shoichet Lab at UCSF.
DOCKovalent is an adaptation of the DOCK virtual screening software to be able to **screen covalent inhibitors for a given protein structure**.

Highly specific kinase inhibition



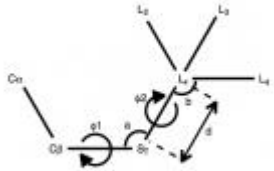
Kinases are extremely important for signaling and as potential drug targets, but are notoriously hard to inhibit specifically (without hitting off-targets). By targeting non conserved cysteine residues nearby kinase active sites we were able to **design very specific covalent kinase inhibitors.**

Covalent Allosteric Modulation



Through harnessing an irreversible covalent bond, a designed covalent probe could theoretically stabilize rare protein conformations. We are developing compounds that would bias signaling proteins to a specific activated conformation that would allow unprecedented control of signaling pathways.

Covalent docking - methods development



The lab continually strives to **advance our covalent docking technology**. This includes the implementation of new algorithms for improved sampling and scoring of covalent adducts as well as systematic analysis of available covalent complexes.

Publications

- Sulfopin is a covalent inhibitor of Pin1 that blocks Myc-driven tumors in vivo
  Dubiella C., Pinch B. J., Koikawa K. et al. (2021) Nature Chemical Biology. 17, 9, p. 954-963 Abstract [All authors]

- Intracellular protein-drug interactions probed by direct mass spectrometry of cell lysates
  Rogawski R., Rogel A., Bloch I., Gal M., Horovitz A., **London N.** & Sharon M. (2021) Angewandte Chemie (International ed.). 133, Abstract

- Proteolysis Targeting Chimeras for BTK Efficiently Inhibit B-Cell Receptor Signaling and Can Overcome Ibrutinib Resistance in CLL Cells
  Shorer Arbel Y., Katz B., Gabizon R. et al. (2021) Frontiers in Oncology. 11, 646971. Abstract [All authors]

- Tunable Methacrylamides for Covalent Ligand Directed Release Chemistry
  Reddi R. N., Resnick E., Rogel A. et al. (2021) Journal of the American Chemical Society. 143, 13, 4979-4992 Abstract [All authors]

- Crowdsourcing drug discovery for pandemics
  Chodera J., Lee A. A., **London N.** & Von Delft F. (2020) Nature Chemistry. 12, 7, p. 581-581 Abstract

- Efficient targeted degradation via reversible and irreversible covalent PROTACs
  Gabizon R., Shraga A., Gehrtz P. et al. (2020) Journal of the American Chemical Society. 142, 27, p. 11734-11742 Abstract [All authors]

- Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease
  Douangamath A., Fearon D., Gehrtz P. et al. (2020) Nature Communications. 11, 1, 5047. Abstract

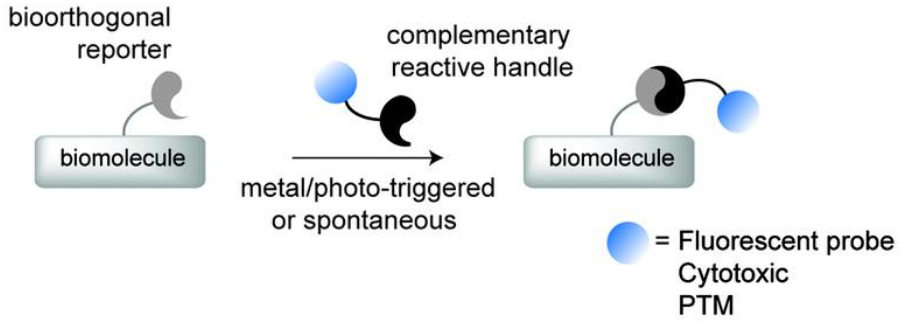
- A Fast and Clean BTK Inhibitor
  Gabizon R. & **London N.** (2020) Journal of Medicinal Chemistry. 63, 10, p. 5100-5101 Abstract

- Rapid Covalent-Probe Discovery by Electrophile-Fragment Screening
  Resnick E., Bradley A., Gan J. et al. (2019) Journal of the American Chemical Society. 141, 22, p. 8951-8968 Abstract [All authors]

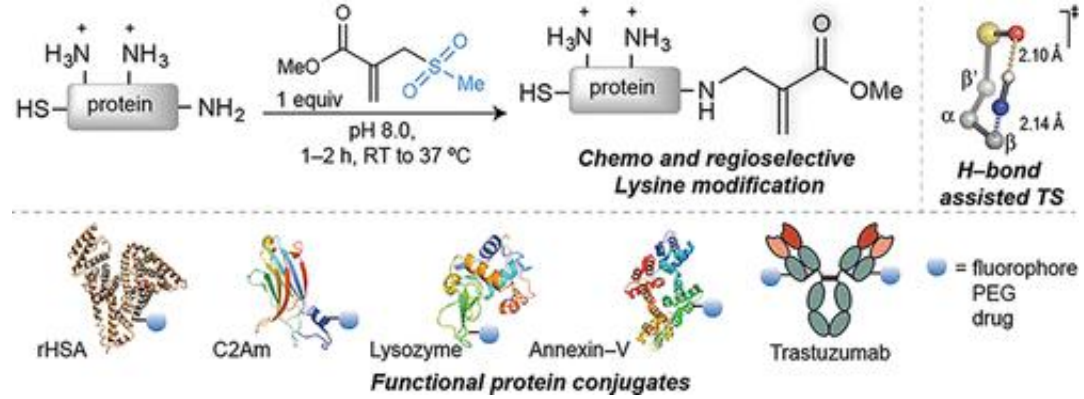
- Covalent Docking Identifies a Potent and Selective MKK7 Inhibitor
  Shraga A., Olshvang E., Davidzohn N. et al. (2019) Cell Chemical Biology. 26, 1, p. 98 - 108 Abstract [All authors]

Selective modifications of native proteins

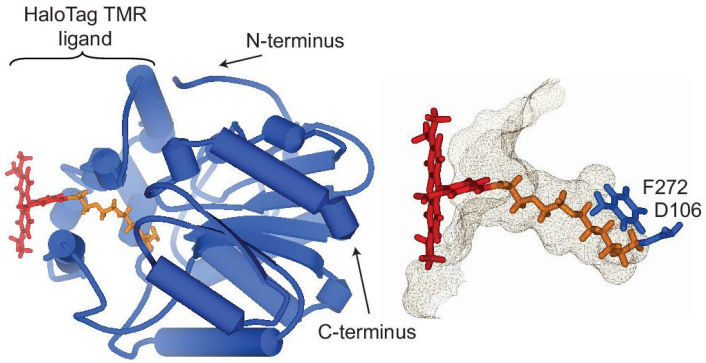
Genetic engineering → **Chemical bioconjugation**



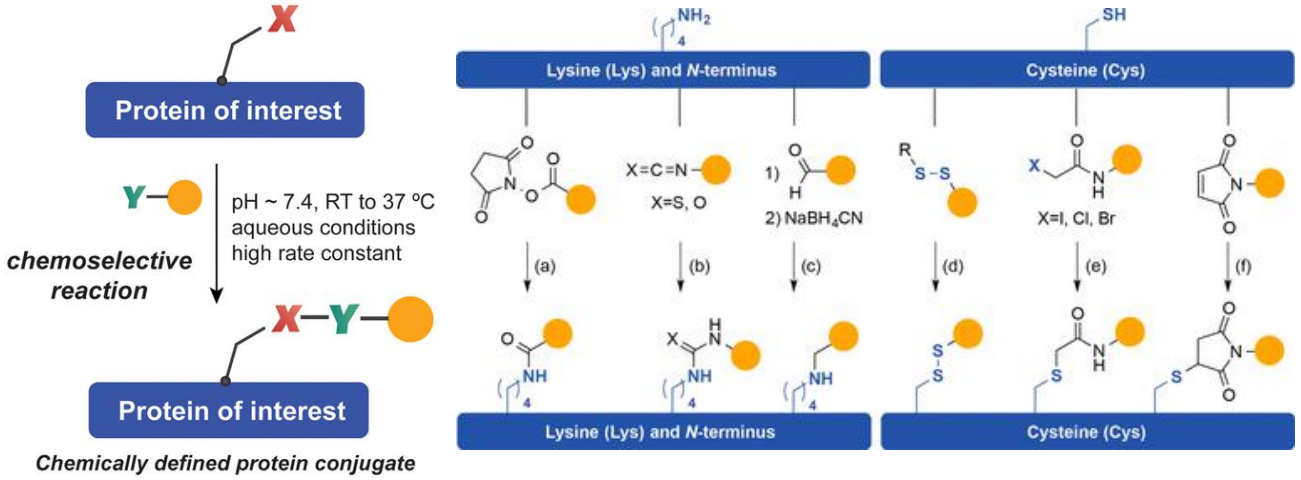
Chem. Soc. Rev. 2017,46, 4895.



J. Am. Chem. Soc. 2018, 140, 4004.

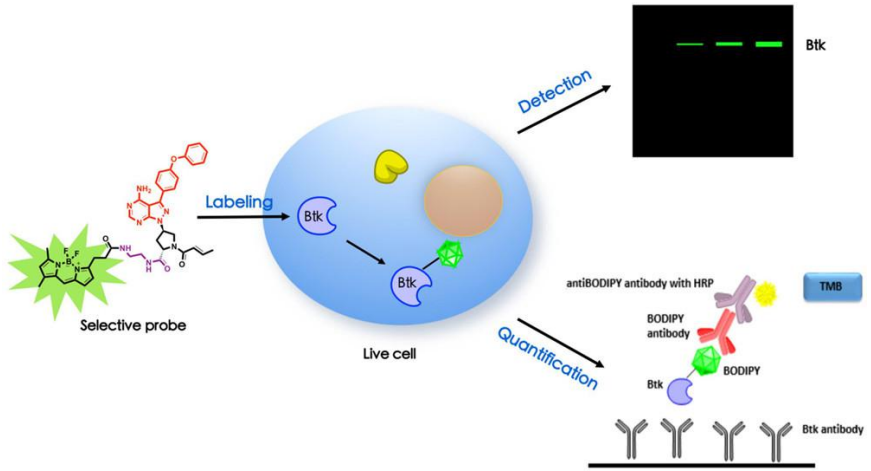


ACS Chem. Biol. 2008, 3, 373.

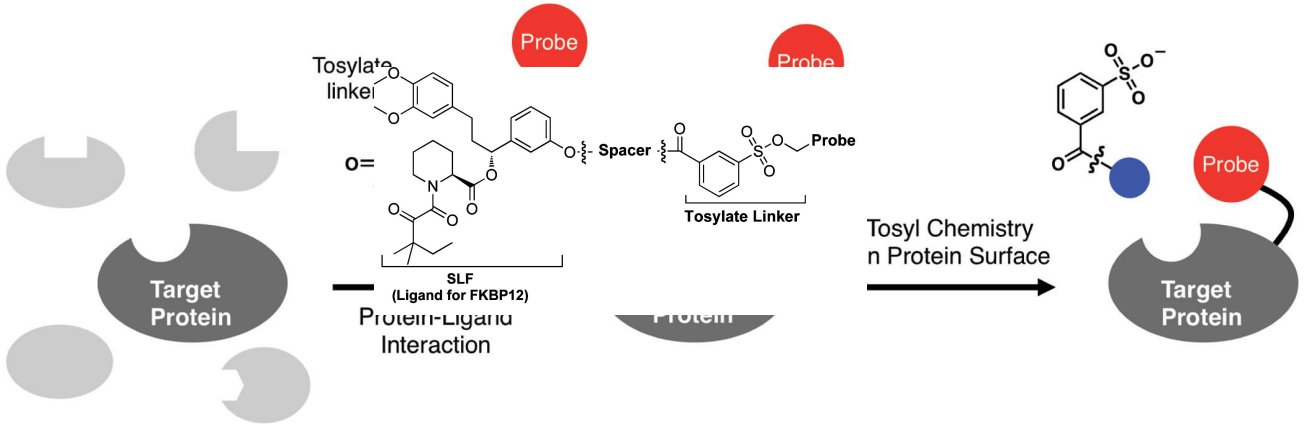


Chem. Rev. 2015, 115, 2174.

Selectively label endogenous proteins in live cells

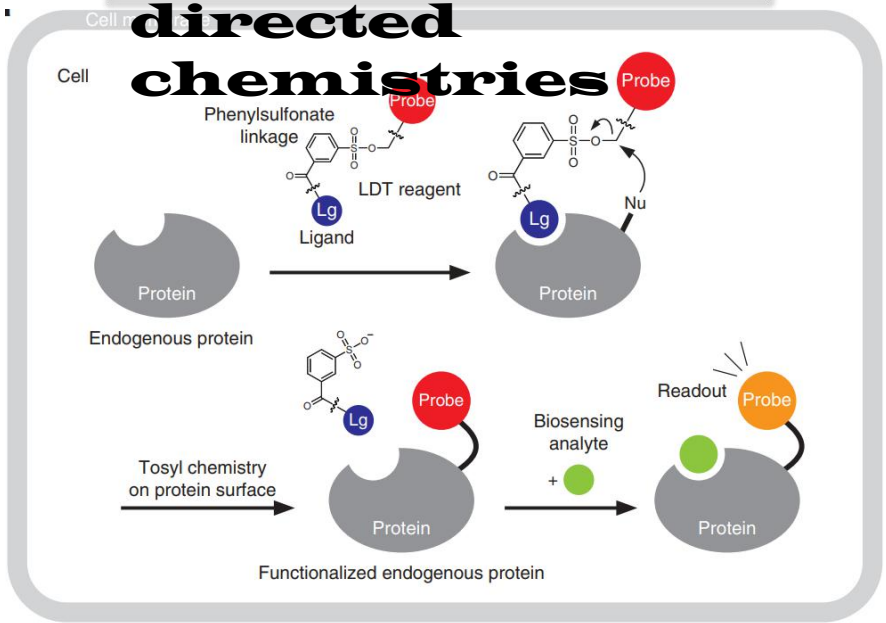


Bioconjugate Chem. 2018, 29, 1640.

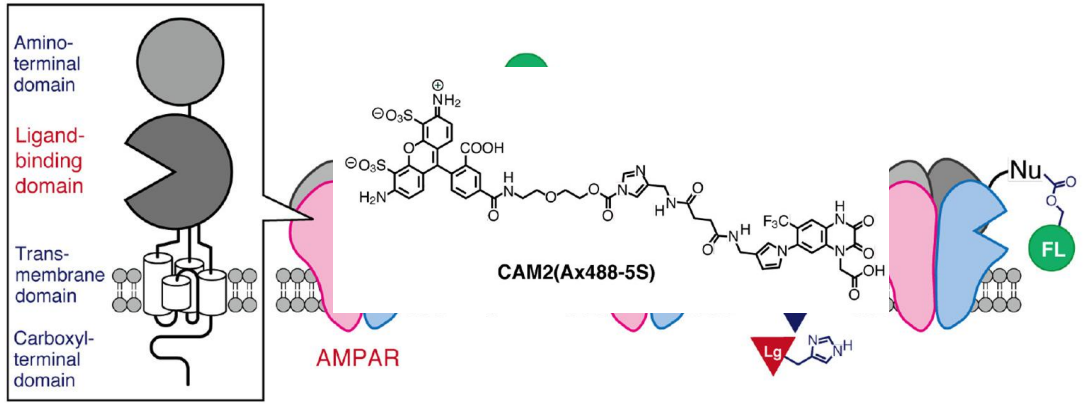


J. Am. Chem. Soc. 2012, 134, 2216.

Ligand-directed chemistries



Nat. Chem.



ACS Chem. Biol. 2018, 13,

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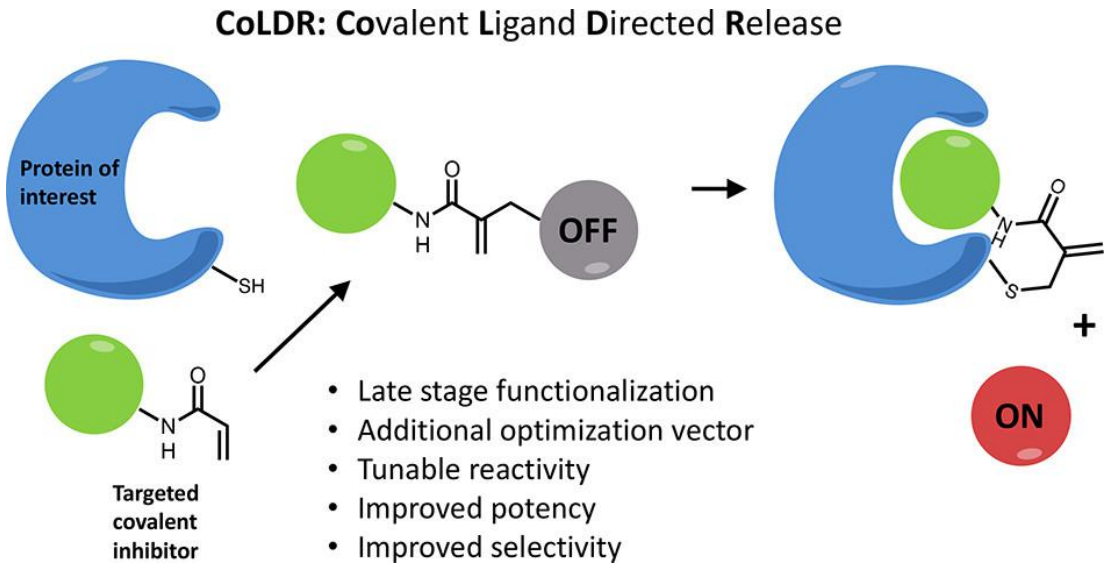
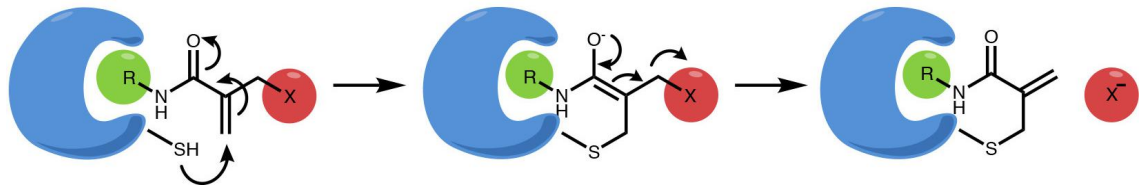
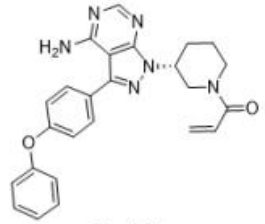
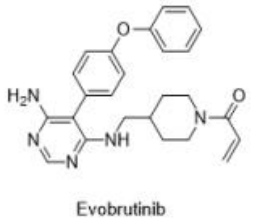
Tunable Methacrylamides for Covalent Ligand Directed Release Chemistry

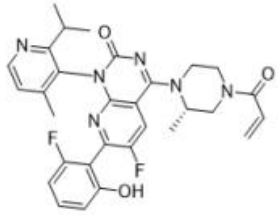
Rambabu N. Reddi,[#] Efrat Resnick,[#] Adi Rogel, Boddu Venkateswara Rao, Ronen Gabizon, Kim Goldenberg, Neta Gurwicz, Daniel Zaidman, Alexander Plotnikov, Haim Barr, Ziv Shulman, and Nir London*

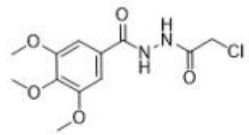


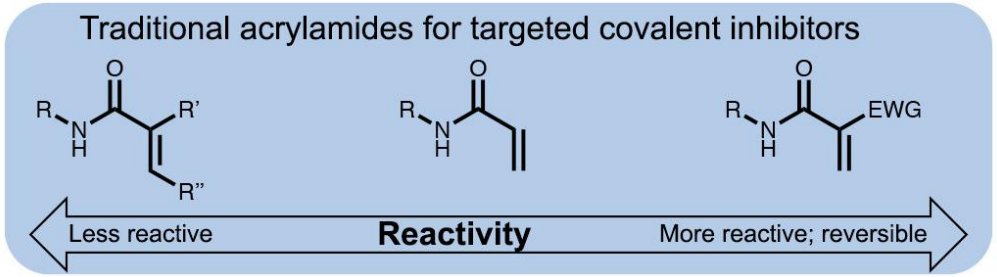


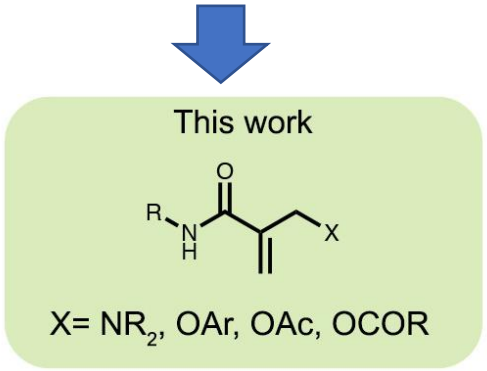

Ibrutinib


Evobrutinib


Amg-510


PL pro inhibitor





Development of ligand-directed cysteine labeling probes

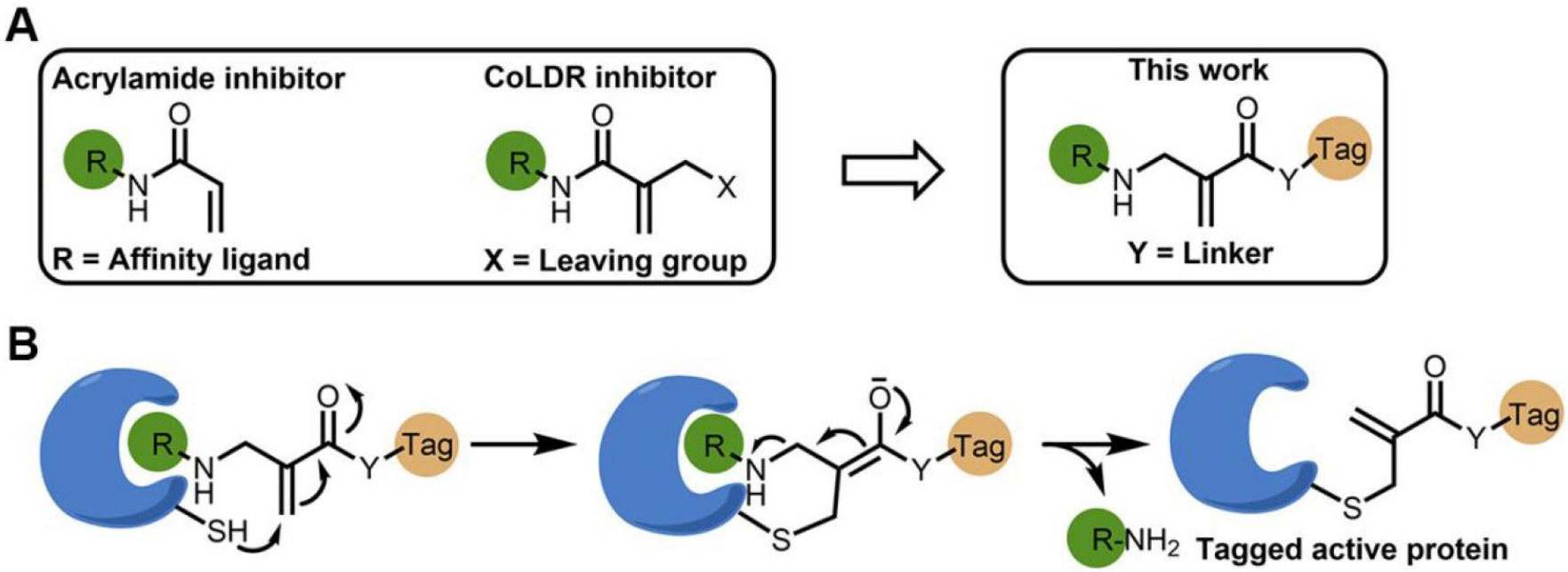
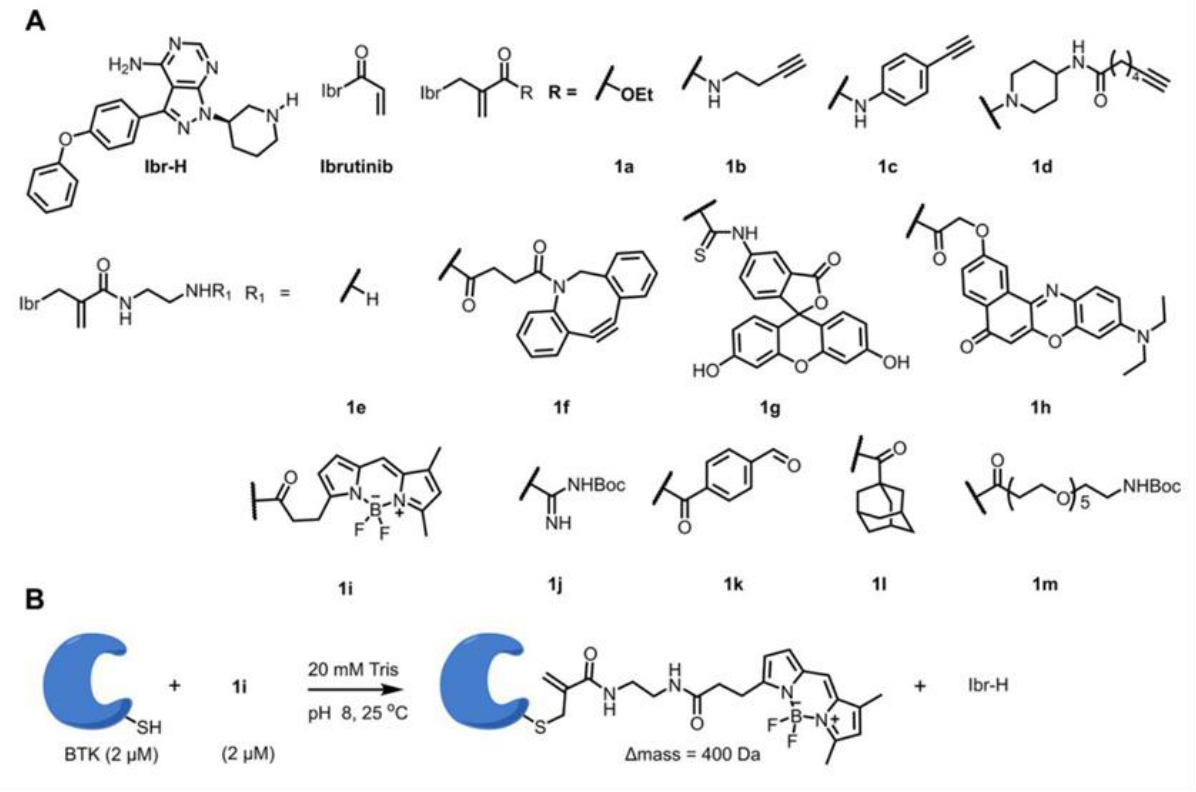
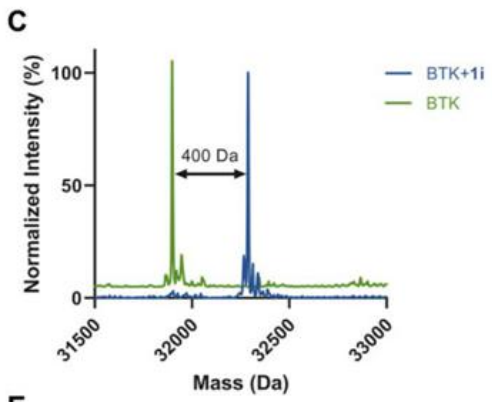


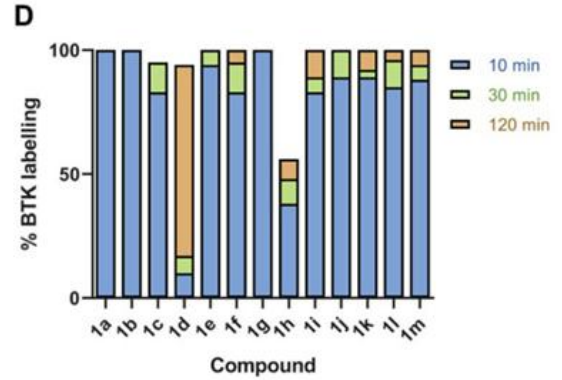
Figure 1. Development of ligand-directed cysteine labeling probes. (A) By reversing the directionality of our previously developed CoLDR chemistry, (37) we generate probes that place the electrophilic carbon in the exact same position but now release the protein recognition moiety (R; typically an inhibitor). (B) Schematic representation of the reaction of a target cysteine with a substituted α-methacrylamide through CoLDR chemistry.

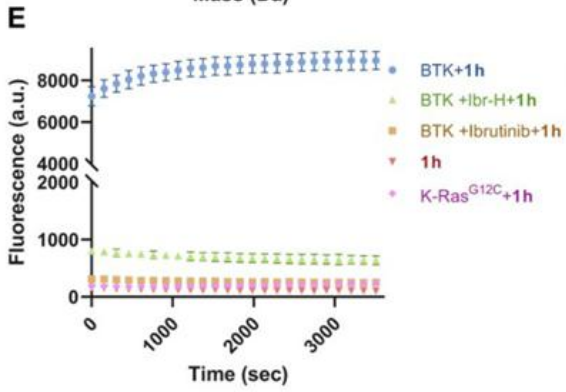
Site-selective labeling of BTK using CoLDR chemistry

BTK: **Bruton's tyrosine kinase**









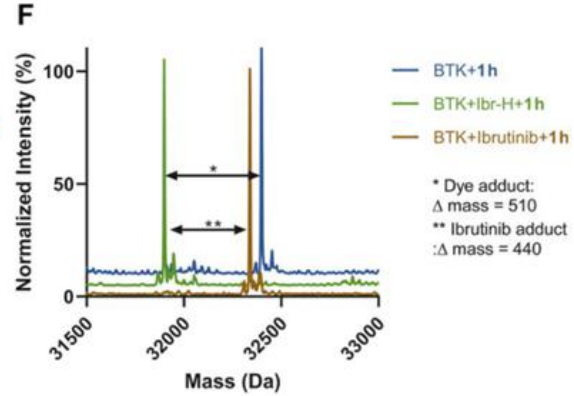
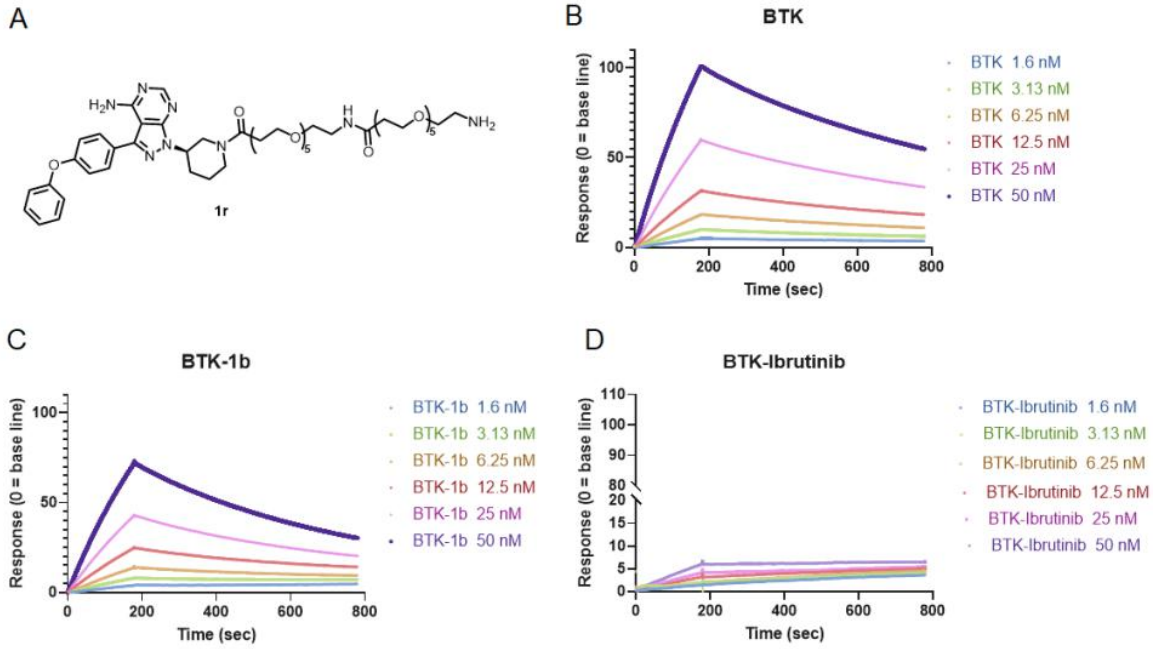


Figure 2. Site-selective labeling of BTK using CoLDR chemistry. (A) Chemical structures of ibrutinib-directed methacrylamides with various functional tags. (B) Typical example of the reaction of BTK (2 μM) with 1i (2 μM) in a 20 mM Tris buffer at pH 8, 25 °C. (C) Deconvoluted LC/MS spectrum shows the labeling of a BODIPY probe and demonstrates Ibr-H leaving. (D) Percent of labeling of BTK (2 μM) with the probes (1a–1m; 2 μM) at 10, 30, and 120 min in 20 mM Tris buffer at pH 8, 25 °C. (E) Kinetics of the increase in fluorescence intensity measured at Ex/Em = 550/620 nm (n = 4) upon addition of BTK (2 μM) to 1h (2 μM) in 20 mM Tris buffer at pH 8, 37 °C (blue). Control experiments without BTK (red), preincubation of ibrutinib (4 μM) and Ibr-H (4 μM) prior to adding 1h (green and orange, respectively), and incubation of K-RasG12C (pink) with 1h show no fluorescence. (F) Deconvoluted LC/MS spectra for BTK incubated with 1h at the end of the fluorescence measurement (shown in E). The adduct mass corresponds to a labeling event in which the Ibr-H moiety was released, validating the proposed mechanism.

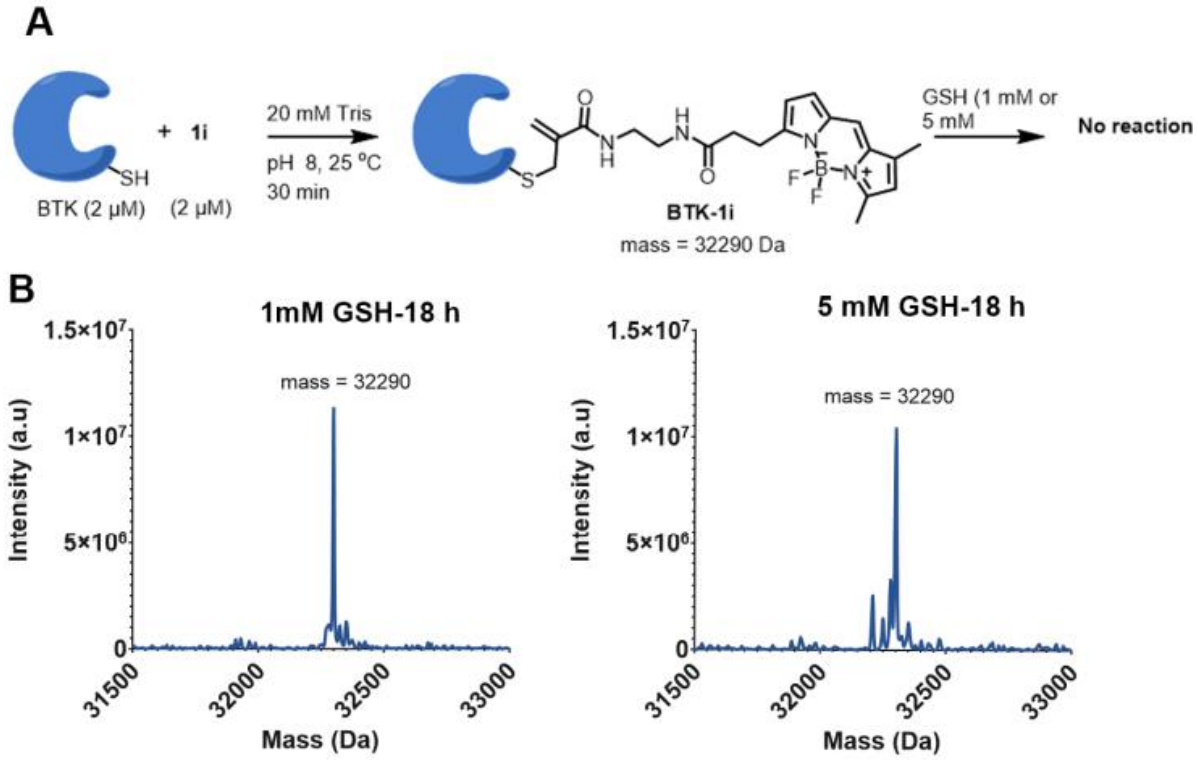
Site-Specific Labeling Probes for BTK

表面等离子共振（SPR）实验



E

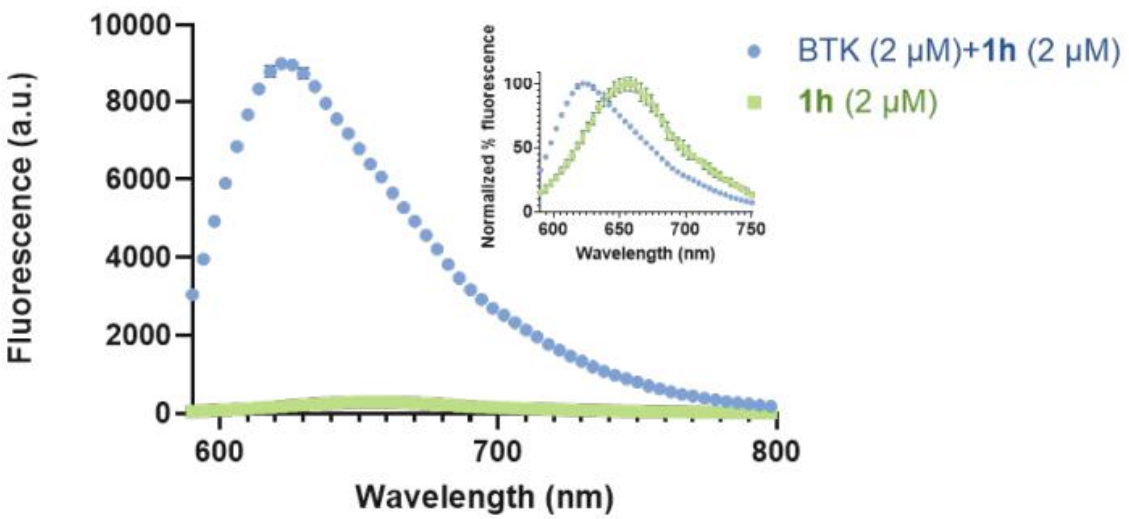
	k_a (1/Ms)	SE(k_a)	k_d (1/s)	SE(k_d)	K_D (M)	Chi² (RU²)
BTK	9.04E+04	27	0.001361	2.60E-07	1.51E-08	0.0612
BTK-1b	1.76E+05	4.70E+02	0.003287	2.20E-06	1.87E-08	0.0568

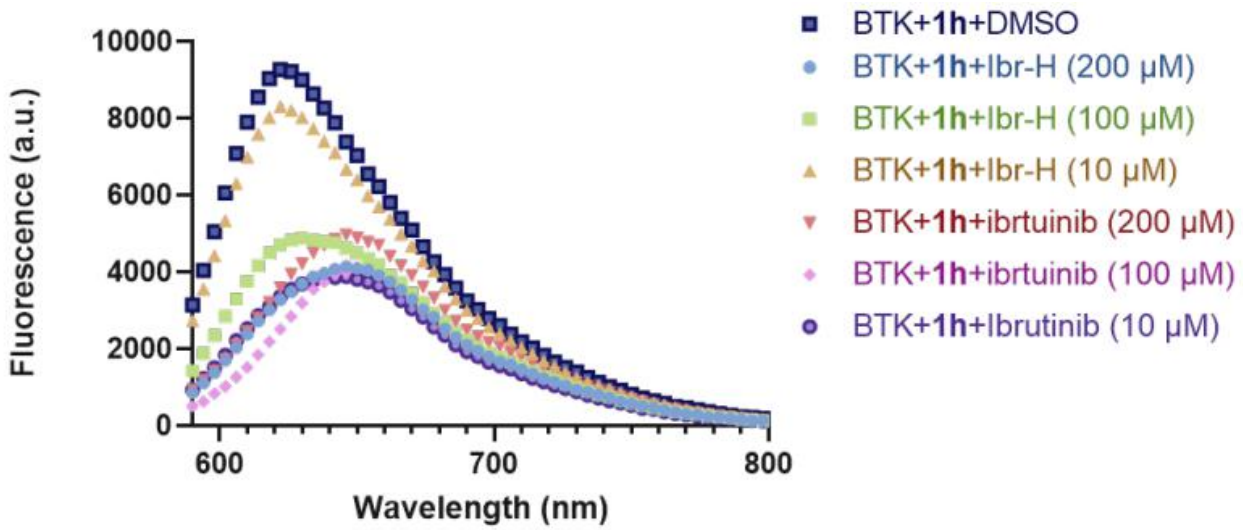


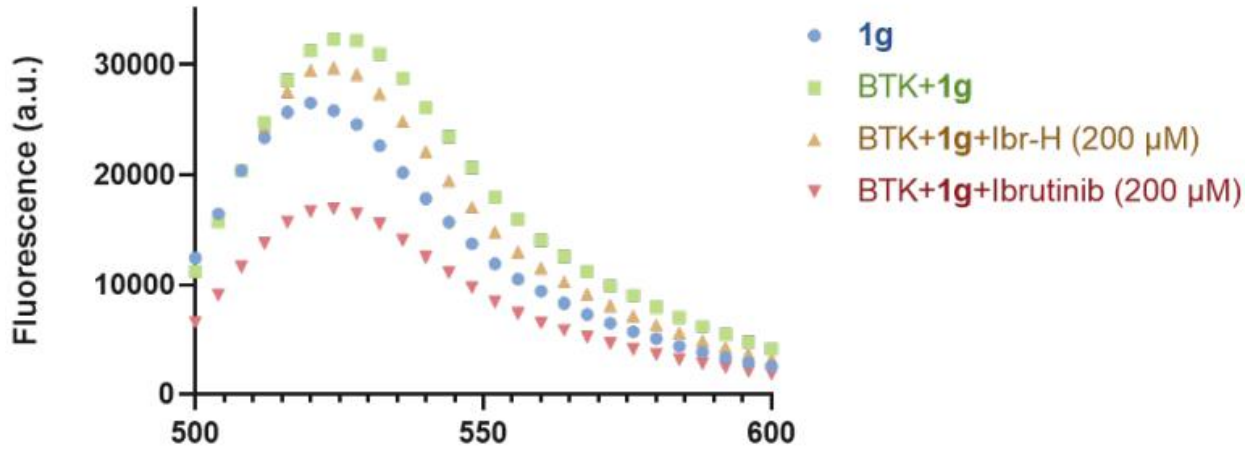
Stability of BTK labelled by 1i

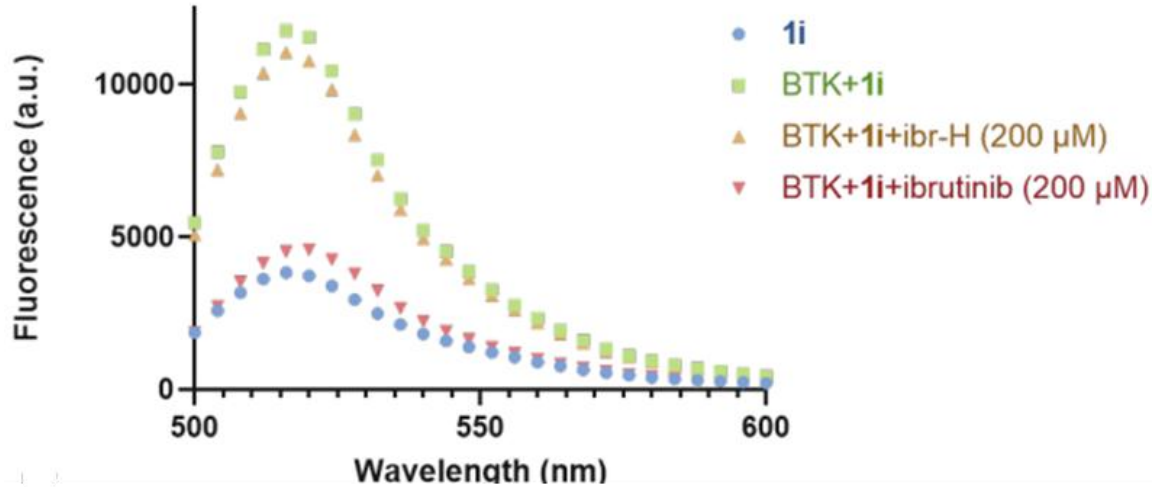
Labelling by CoLDR probes does not affect ligand binding.

Detecting binding events within the active site of BTK.









Turn-on fluorescent environmental sensitive probe can detect binding events to BTK.

Intrinsic Thiol Reactivity of BTK Probes

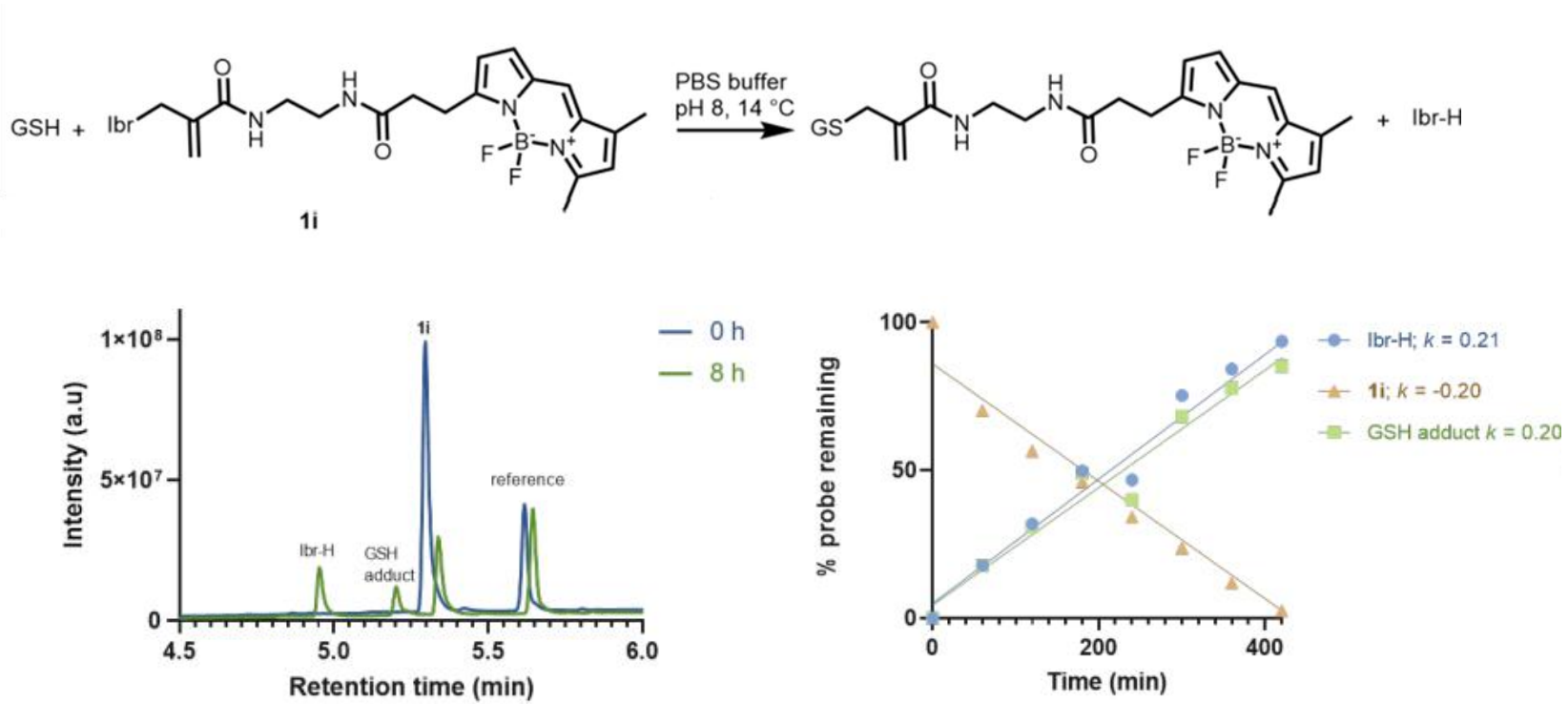


Figure S9. Reaction with reduced GSH validates the elimination of ligands and demonstrates their intrinsic thiol reactivity is tunable.

CoLDR Labeling is General across Protein Targets

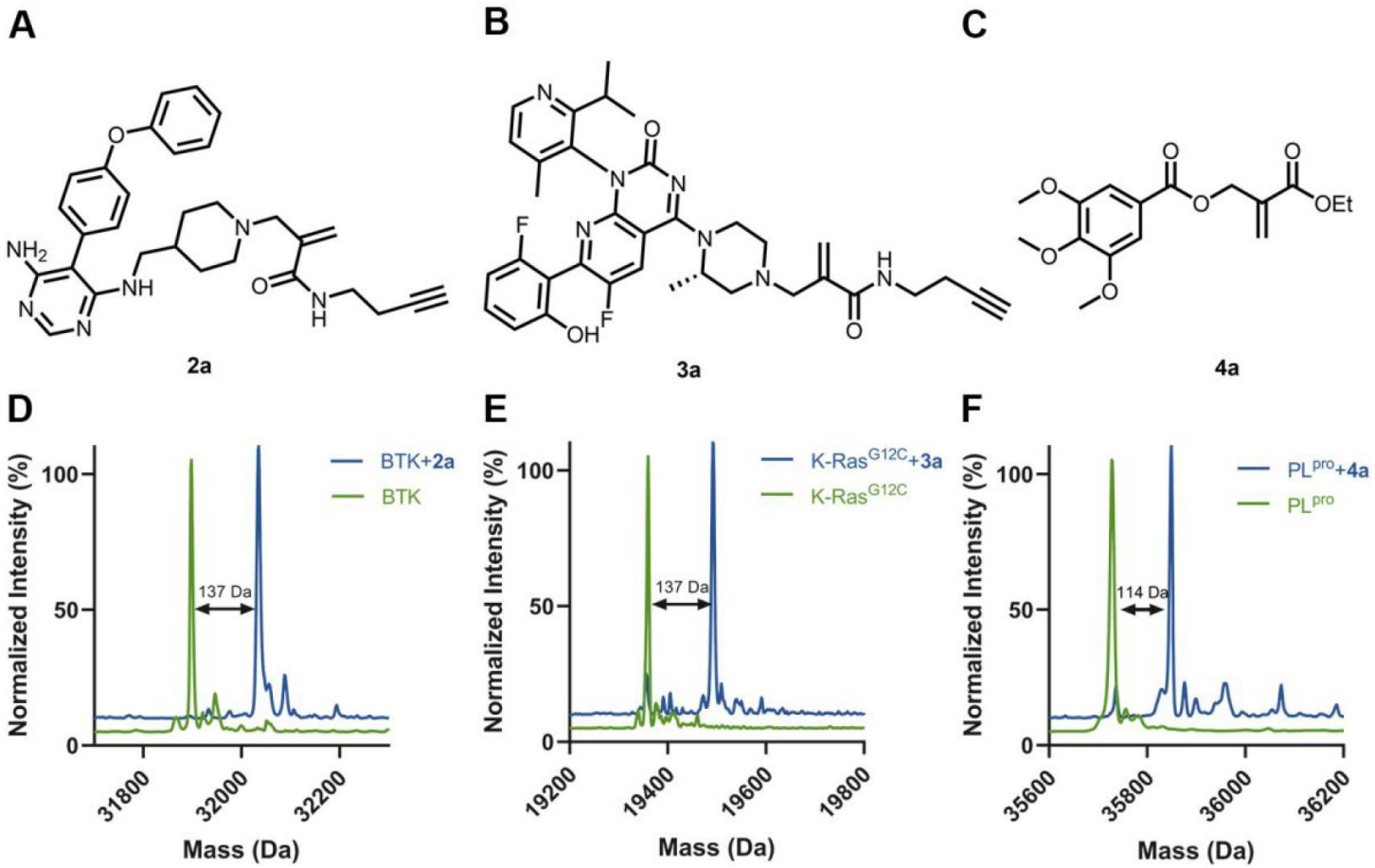
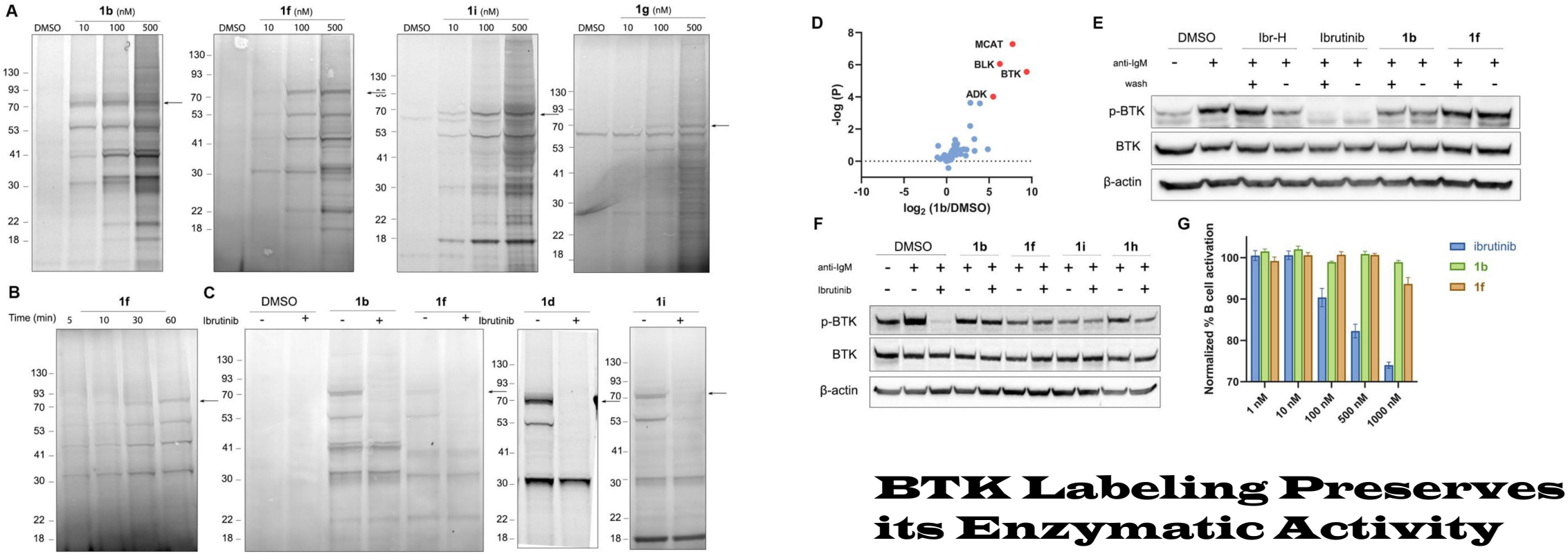


Figure 3. Selective labeling of various target proteins. Structures of alkyne/ester labeling probes for (A) BTK, (B) **K-RasG12C**, and (C) **SARS-CoV-2 PLpro**. Deconvoluted LC/MS spectra for (D) BTK (2 μM) incubated with 2a (2 μM) in 20 mM Tris buffer at pH 8, 25 °C, 10 min, (E) K-RasG12C (10 μM) incubated with 3a (100 μM) in 20 mM Tris at pH 8, 37 °C, 16 h, and (F) PLpro (2 μM) incubated with 4a (10 μM) in 50 mM Tris at pH 8, 25 °C, 16 h. The adduct masses correspond to a labeling event in which the ligand was released.

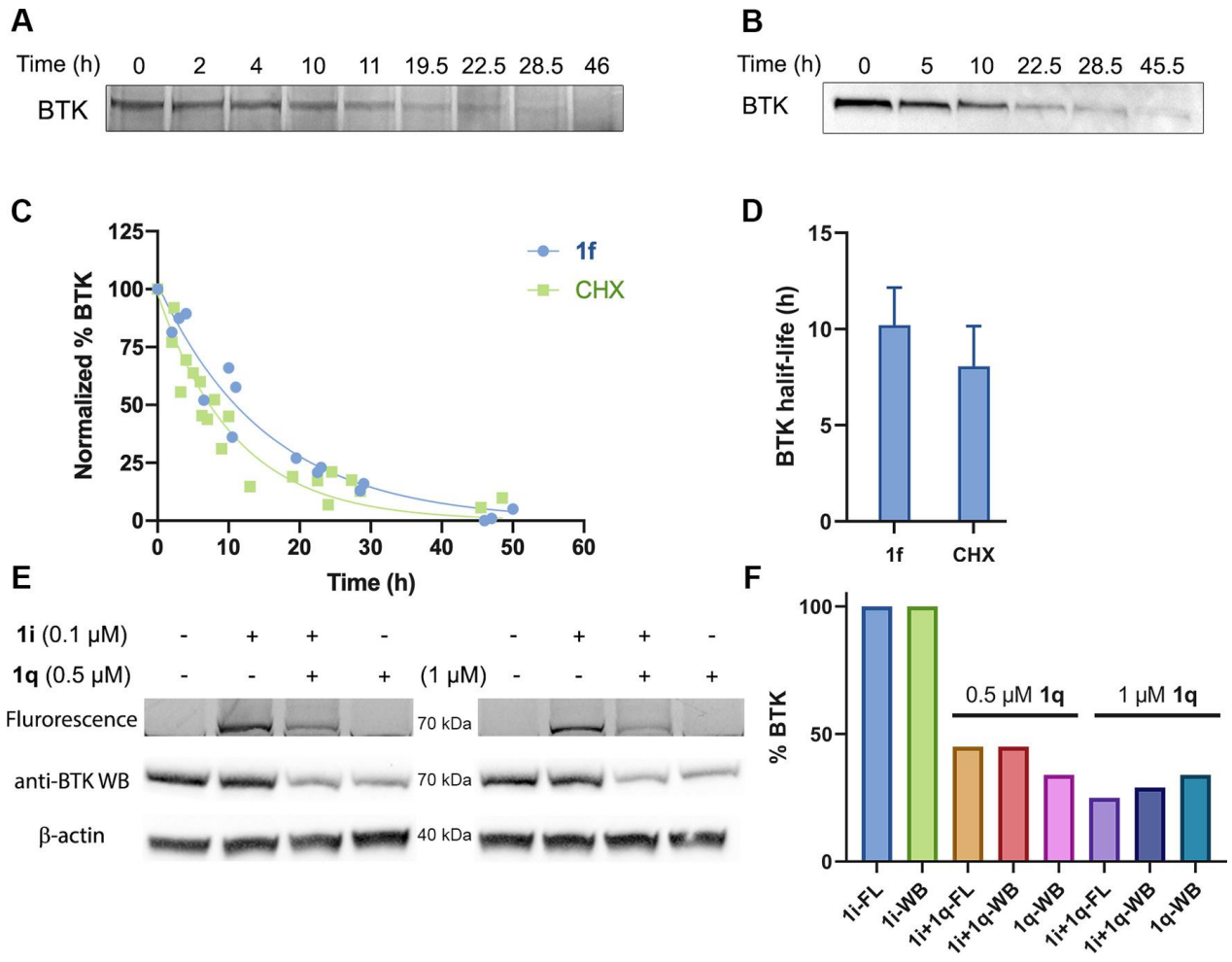
Ligand-Directed Site-Selective Labeling of BTK in Cells



BTK Labeling Preserves its Enzymatic Activity

Figure 4. Labeling BTK with CoLDR probes does not inhibit its activity in cells. (A) Cellular labeling profile of 1b, 1f, and 1i after 2 h of incubation with Mino cells and 1g in Mino cell lysate. 1b and 1f samples were further reacted with **TAMRA-azide in lysate before imaging**. An arrow indicates BTK's MW. (B) Time-dependent labeling profile of 1f with BTK after incubation of Mino cells with 100 nM probe followed by a click reaction with TAMRA-azide in lysate prior to imaging. (C) Competition experiment of 1b, 1d, 1f, and 1i with ibrutinib. The cells were preincubated for 30 min with either 0.1% DMSO or 1 μM ibrutinib, followed by 2 h of incubation with 200 nM 1b or 1f or 100 nM 1d or 1i. (D) Mino cells were incubated with 0.1% DMSO or 1b (100 nM). Samples were further reacted with biotin-azide in lysate, followed by enrichment, trypsin digestion, and peptide identification by LC/MS/MS. The log(fold-ratio) of proteins enriched by 1b over DMSO is plotted as a function of statistical significance. BTK is clearly identified as the most enriched target; additional prominent targets that correspond to bands identified by in-gel fluorescence (panel C) are indicated. (E) BTK activity assay in Mino cells as measured by autophosphorylation of BTK. The cells were incubated for 1 h with either 0.1% DMSO, 1 μM ibrutinib, 1 μM Ibr-H, or 100 nM 1b, 1f, 1h, or 1i. The cells were either washed or not before induction of BTK activity by anti-IgM. (F) BTK activity assay: Mino cells were incubated for 2 h with either DMSO or 1 μM 1b, 1f, 1i, and 1h, washed, and then incubated for 45 min with ibrutinib (100 nM). The cells were washed again before induction of BTK activity by anti-IgM. The CoLDR probes were able to rescue BTK activity from inhibition by ibrutinib. (G) Primary B cell activation induced by anti-IgM after 24 h of treatment with increasing doses of either ibrutinib, 1b, or 1f, showing no inhibition of the CoLDR probes.

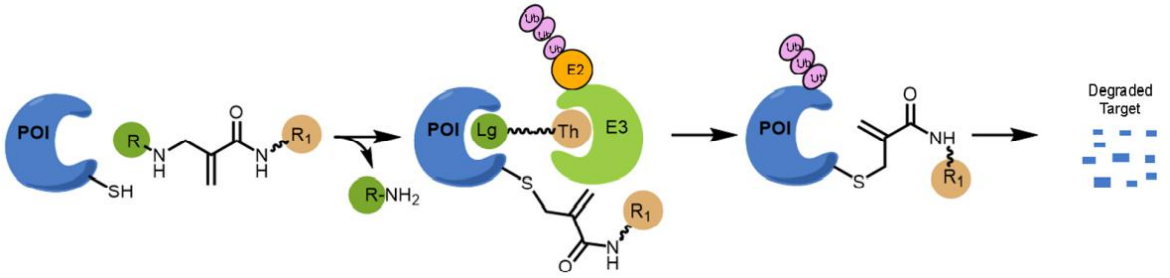
BTK half-life determination using CoLDR probes



BTK tagging don't Interfere with PROTAC binding

PROTAC : Proteolysis targeting chimeras

即蛋白水解靶向嵌合体，分子的一端与靶蛋白结合，另一端与E3泛素连接酶结合。



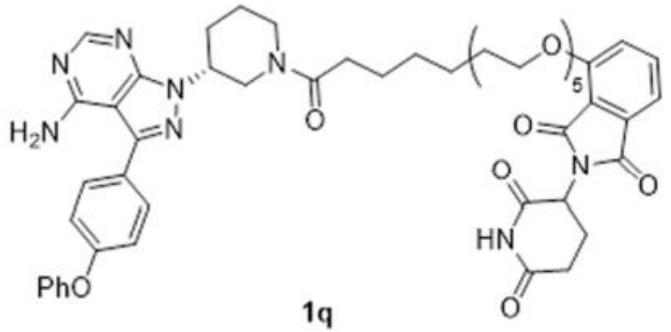


Figure 5. Measurement of BTK half-life. (A) Half-life measurement of BTK using 1f. Mino cells were pulse-labeled with 100 nM 1f for 1 h and were then washed to remove the excess probe. Cells were harvested at the indicated time points, and lysates were reacted with **TAMRA-azide.** The signal of BTK was quantified, and the half-life was calculated. (B) Half-life measurement of BTK with the cycloheximide (CHX) assay, using 20 μg/mL cycloheximide. (C) Quantification of BTK levels in A and B (by normalization to the protein concentration) in Mino cells (1f: n = 3, CHX: n = 4). (D) Calculated half-life by both methods, presented as mean ± SD. (E) Degradation of BTK labeled with 1i using PROTAC 1q. Mino cells were incubated with 1i (100 nM), then washed to remove the excess probe, again incubated with PROTAC 1q for 2 h at 0.5 and 1 μM, and then lysed. Samples are subjected to in-gel fluorescence (FL) and Western blot (WB). (F) Quantification of BTK levels in panel E (normalization to the β-actin has been done for Western blot).

CoLDR Chemistry Allows the Installation of a Degradation Handle

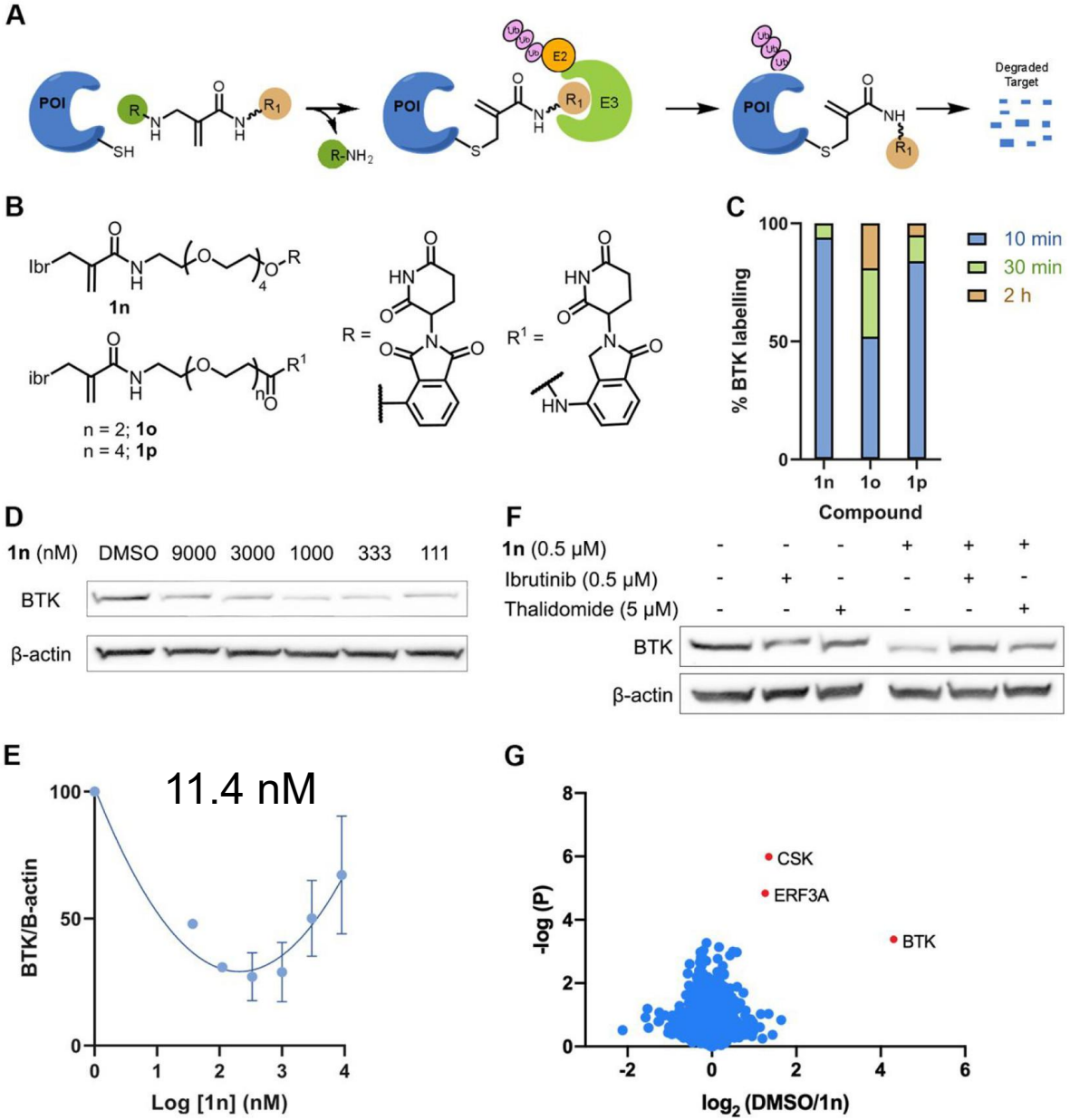


Figure 6. Measurement of induced degradation by CoLDR PROTACs. (A) Schematic representation of target degradation using CoLDR PROTACS. (B) Structure of CoLDR-based BTK PROTACS. (C) In vitro labeling of BTK (2 μM) with 1n–1p (2 μM) in 20 mM Tris buffer at pH 8, 25 °C. (D) Western blot evaluation of BTK levels in Mino cells in response to various concentrations of 1n after 24 h of incubation. (E) Quantification of BTK levels in D by normalization to the β-actin house-keeping gene in Mino cells. DC50 and Dmax were calculated by fitting the data to a second-order polynomial using the Prism software. (F) Mino cells were pretreated for 2 h with either ibrutinib/thalidomide-OH or DMSO before treatment with a BTK PROTAC for 24 h (n = 2). Subsequently, BTK levels were measured via Western blot. (G) Mino cells were treated for 24 h with either 0.1% DMSO or 1n (500 nM) in 4 replicates. Lysates were subjected to trypsin digestion and peptide identification by LC/MS/MS. The Log2(fold-ratio) of proteins enriched in the DMSO samples over 1n-treated samples is plotted as a function of statistical significance. Significantly degraded proteins are indicated in red and defined as Log2(DMSO/1n) > 1 and p-value < 0.01.