



Cite this: *RSC Med. Chem.*, 2024, 15, 2974

Synthetic modification of protein surfaces to mediate induced-proximity pharmacology

Lyn H. Jones ^{ab}

Received 26th May 2024,
Accepted 7th August 2024

DOI: 10.1039/d4md00388h

rsc.li/medchem

Molecular glues and bifunctional small molecules, such as targeted protein degraders, induce protein proximity to mediate gain-of-function pharmacology. Emerging technologies that synthetically manipulate protein surfaces to create neoproteins, and the development of covalent chemical probes for intra- and inter-protein surface labeling are described. Ligand-directed protein surface modification strategies have the potential to enhance the induced-proximity pharmacology toolkit and expand the druggable proteome, and this Opinion considers the opportunities and challenges that lie ahead.

Molecular glues – from serendipitous discoveries to rational drug development

Natural products have been a rich source of medicinal compounds, and many possess molecular modes-of-action that have inspired the development of novel therapeutic modalities and drug discovery technologies. For example, the antifungal metabolite and immunosuppressive drug rapamycin acts as a ‘molecular glue’¹ by engaging the immunophilin FKBP12 and forming a ternary complex with mTOR, allosterically inhibiting its kinase function (Fig. 1).² In 1994, just as the detailed mechanism of rapamycin was being elucidated,² I helped develop novel semi-synthetic ‘rapalogs’ with optimized pharmaceutical properties during a placement at Sandoz Pharma. We fortuitously discovered an oxidative decomposition synthetic by-product of the cyclohexanol subunit of rapamycin (Fig. 1) and decided to probe the structure–activity relationships (SARs) in this region in a systematic manner using sequential Swern and Baeyer–Villiger mediated oxidations.³ We discovered that oxidative cleavage of the cyclohexanol group reduced binding to FKBP12 only 6-fold, but the immunosuppressive activity was >380-fold weaker. These complex SARs, which are now appreciated as a common feature of molecular glue medicinal chemistry programs,^{4–6} were subsequently elucidated using structural biology studies that provided a molecular understanding of the disconnect between binding and function. As we anticipated, the cyclohexyl motif is located at

the interface of the FKBP12 and mTOR molecules and is thus a component of the so-called ‘effector domain’ of rapamycin (Fig. 1).⁷ A related natural product glue called FK506 possesses the same FKBP binding domain as rapamycin, but an alternative effector moiety that binds and inhibits the phosphatase calcineurin.² These findings suggested that novel rapalogs may be developed to glue different partners to FKBP12 beyond mTOR (and calcineurin) through changes to the effector domain. Inspired by this potential, the group of Jun Liu prepared a 45 000-member synthetic library of macrocyclic hybrid molecules by swapping the effector motif with oligopeptides.⁸ Screening the library in cells yielded a molecule called rapadocin that potently and selectively inhibited the nucleoside transporter SLC29A1 by forming a complex with FKBP12. Although there is a lack of structural biology information, presumably due to the complexities of generating a ternary complex structure with an integral membrane protein, it is likely that the binding domain of rapadocin engages FKBP12, while the peptidic effector element mediates simultaneous intracellular binding of SLC29A1 such that adenosine import is blocked by

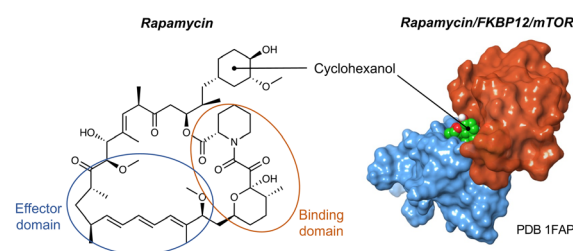


Fig. 1 Rapamycin structure showing the binding domain that engages FKBP12, and the effector domain that binds mTOR. The cyclohexanol motif binds at the interface of FKBP12 (orange surface) and mTOR (blue surface).

^a Center for Protein Degradation, Dana-Farber Cancer Institute, 360 Longwood Avenue, Boston, MA, USA

^b Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA. E-mail: lyn_jones@dfci.harvard.edu

sequestration of the FKBP. Other examples of natural product-inspired molecular glues in clinical development include sanglifehrin analogs that form a complex between cyclophilin and RAS proteins,⁶ and rocaglamide derivatives that glue eIF4A to mRNA polypurine sequences.⁹

These studies complement the phenotypic assessments of molecules not derived directly from natural products that have also unearthed diverse therapeutic modalities, including molecular glue activity. For instance, immunomodulatory imide drugs (IMiDs) thalidomide, lenalidomide and pomalidomide (Fig. 2a) are used to treat multiple myeloma, but their mechanism was determined following approval. IMiDs were found to bind cereblon (CRBN),¹⁰ the substrate adaptor subunit of the E3 ubiquitin ligase complex CRL4^{CRBN}, and remodel its surface to induce interactions with neosubstrates, that are subsequently polyubiquitinated and degraded.¹¹ Traditional neosubstrates possess the so-called 'G-loop' degron which is a β -hairpin loop containing a key glycine residue that enables binding to the IMiD-CRBN surface (Fig. 2b).^{12,13} Many CRBN neosubstrates, such as zinc-finger transcription factors, were previously deemed undruggable because they lack small molecule binding pockets.¹⁴ Consequently, there are considerable research efforts in the drug discovery community currently exploring the breadth of the degradable proteome through the development of novel molecular glue degraders that recruit CRBN.¹⁵

In another example of fortuitous discovery, mechanistic studies of aryl sulfonamide antiproliferative compounds discovered they bind the E3 ligase DCAF15 causing the recruitment and degradation of the RNA splicing factor

RBM39, and one such compound (E7820) is currently in a trial for myeloid cancers.^{16–18} Ligands of the VHL E3 ligase have been chemically linked to protein binders to create numerous bifunctional degraders (PROTACs),¹⁹ but a recent study revealed that a small molecule binder of VHL recruited the neosubstrate cysteine dioxygenase (CDO1).²⁰ These studies suggest that ligands of a variety of E3 ligases may act as molecular glue degraders.

Synthetic neofunctionalization of protein surfaces

Missense mutations and post-translational modifications (PTMs) play key roles in evolutionary biology through the formation of new protein interactions that drive phenotypic diversity.^{21,22} Taking this inspiration from Nature and applying the learning of molecular glue SARs such as those mentioned above, we hypothesized that synthetic re-engineering of protein surfaces in cells using covalent molecular glues has the potential to mimic PTMs and missense mutations by creating neo-associations in a site-specific manner. Ligand-directed covalent labelling of a protein surface may impose distinct conformations and present physicochemistry not available to reversible binding small molecules. Equally, covalent surface engagement may deliver more potent and longer duration neo-associations. As a proof-of-principle, we rationally developed an IMiD containing a sulfonyl exchange warhead, the fluorosulfate EM12-FS, to covalently modify His353 in the sensor loop of CRBN,²³ locking this motif in the closed conformation that is necessary for neosubstrate recruitment (Fig. 2b and c).²⁴ Based on computational modelling, we expected the modified His353 surface residue to assume a different conformation to that required for traditional neosubstrate binding and the sulfonyl oxygen atoms of the neoprotein were also expected to clash with the canonical G-loop degron.²⁵ Indeed, EM12-FS degraded exclusively a novel neosubstrate never previously observed for reversible binding IMiDs, namely N-terminal glutamine hydrolase 1 (NTAQ1), that is involved in the N-end rule pathway and formerly considered undruggable.²³ Further work includes structural confirmation of the alternative binding mode of NTAQ1, and the use of EM12-FS to probe NTAQ1 and N-end rule pathway biology. Notwithstanding the opportunities for further research, this case study clearly exemplifies the concept of synthetic neofunctionalization of proteins in cells using small molecule drugs.

Covalent bifunctional molecules also hold significant promise by providing a modular approach to induced-proximity pharmacology. For instance, in a pilot study we developed a covalent PROTAC by linking EM12-FS to the BRD4 inhibitor JQ1 to create FS-ARV-825 that degraded BRD4 in cells (Fig. 2d).²⁶ FS-ARV-825 possessed a more durable pharmacodynamic effect through modification of His353 CRBN *versus* the reversible binding PROTAC ARV-825. Ligand-directed protein labelling can also be exploited to functionalize the surface of a target protein with a variety of

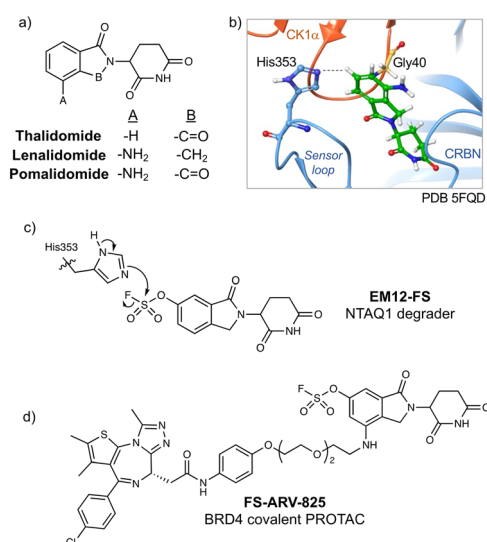


Fig. 2 a) Chemical structures of the IMiDs. b) Ternary complex crystal structure of lenalidomide/CRBN/CK1 α showing the proximity of His353 to the IMiD scaffold. Gly40 in the G-loop degron of neosubstrate CK1 α is highlighted in yellow. c) Schematic of the reaction of His353 in the CRBN sensor loop with the fluorosulfate electrophile of molecular glue degrader EM12-FS. d) Structure of covalent BRD4 degrader FS-ARV-825.

chemical motifs. A methodology developed by the Hamachi group leveraged ligand-directed bioconjugation strategies to enable proximity-induced protein surface labelling.²⁷ *N*-Acyl-*N*-alkylsulfonamide (NASA) warheads were appended to small molecule ligands that labelled lysine residues proximal to the binding site, delivering fluorescent dyes and biotin tags to the protein surface (Fig. 3).²⁷ Gray and co-workers attempted to co-opt this chemistry to deliver a tethered IMiD to the surface of CDK2 using NASA-mediated acylation of Lys89 in order to recruit CRL4^{CRBN}.²⁸ Although ligand-directed surface functionalization was successful, CDK2 degradation was not observed because the modified CDK2 protein was unable to form a productive complex with CRBN.

The same NASA labelling approach was used by Fang, Zhang, Li, and colleagues to deliver an adamantane hydrophobic tag, supposedly mimicking a protein misfolding state, to the surface of BRD4 that appeared to mediate targeted protein degradation.²⁹ Peptide mapping mass spectrometry revealed several modified lysine residues on BRD4, though more mechanistic work is required to validate which labelled sites may have triggered degradation.

A conceptually similar methodology called covalent ligand directed release (CoLDR) was developed recently by the London group. The technique employed a cleavable cysteine-reactive acrylamide warhead to install a CRBN binding ligand onto the BTK surface through site-specific modification of Cys481 (Fig. 3).³⁰ The heterobifunctional molecule utilized the BTK covalent inhibitor drug ibrutinib as the basis for the ligand-directed motif. Although these early studies show promise, further work is needed to categorically demonstrate that covalent tagging is necessary to drive the targeted degradation event.

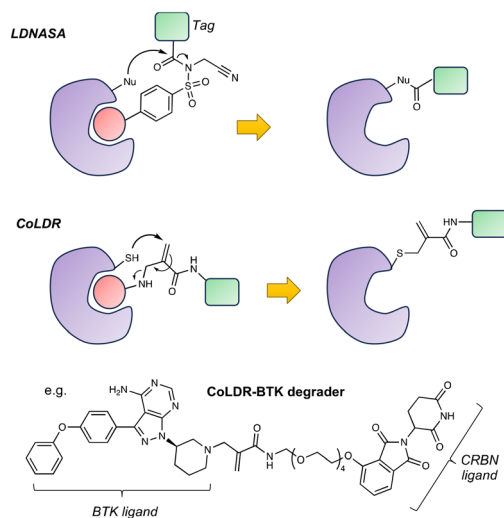


Fig. 3 Heterobifunctional ligand-directed protein surface labelling strategies using cleavable electrophilic warheads: ligand-directed *N*-acyl-*N*-alkylsulfonamide (LDNASA) and covalent ligand directed release (CoLDR). A BTK-directed CoLDR degrader probe based on the BTK inhibitor ibrutinib is shown.

Ligand-directed inter-protein labelling

10 years prior to the development of fluorosulfate EM12-FS, we designed the first covalent chemical probes to site-specifically modify tyrosine, also using sulfonyl exchange chemistry.³¹ The aryl sulfonyl fluoride warhead was incorporated into small molecules discovered in a spinal muscular atrophy phenotypic screen, that were subsequently found to inhibit the mRNA decapping scavenger enzyme DcpS (a target more recently linked to leukaemia). *Para*-substituted sulfonyl fluoride SF-p1 was designed to label Tyr143 within the binding pocket that stabilized an asymmetric and inactive conformation of the homodimeric enzyme. However, in the first example of its kind, the *ortho*-substituted regioisomer SF-o1 was designed to crossover label Tyr113 on the surface of the partnering protomer, stapling the dimer shut and preventing conformational transitions required for enzymatic function.³¹ This work revealed that monovalent ligand-directed inter-protein labelling is a chemically feasible concept through the incorporation of reactive warheads into small molecule drugs and chemical probes without the need for chemical linkages.

Nomura and co-workers recently published proof-of-concept studies that applied a modular covalent molecular glue approach to the discovery of monovalent degraders.^{32,33} Phenyl-oxo-butenamide or vinyl sulfonamide covalent handles appended to a variety of enzyme inhibitors were found to induce degradation of their respective target proteins, mediated through serendipitous ligand-directed crosslinking to E3 ligases RNF126 or DCAF16 respectively, (e.g., BRD4 monovalent degrader ML 1-50, Fig. 4a). The BTK degrader example from this work is noteworthy because certain covalent BTK inhibitors were found previously to

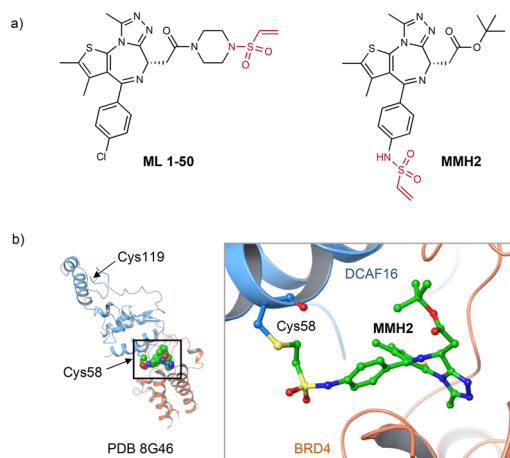


Fig. 4 a) Structures of BRD4 molecular glue degraders ML 1-50 (ref. 33) and MMH2 (ref. 35) which induce BRD4 proximity with the E3 ubiquitin ligase DCAF16 (vinyl sulfonamide electrophilic warheads in red). b) CryoEM structure of the BRD4/MMH2/DCAF16 complex showing crossover labelling of the Cys58 residue and the distal Cys119.³⁵

downregulate the kinase.³⁴ Interestingly, as mentioned above, the labelled cysteine residue (Cys481) sits at the surface of BTK, and it is thus possible that other covalent inhibitors may have, at least partially, induced proximity to DCAF16 or other E3 ligases through fortuitous crossover labelling.

In a recent study from Gray, Fischer, Ebert, and colleagues, JQ1 derivatives bearing covalent handles were also shown to crossover label DCAF16 (Fig. 4a).³⁵ CryoEM of the complex showed surface complementarity between the proteins and validated labelling of Cys58 on DCAF16 by vinyl sulfonamide MMH2 (Fig. 4b), which is a different residue to that modified by ML 1–50 (Cys119). Noticeably, Cys119 sits at the DDB1-binding domain of DCAF16, and so more mechanistic studies are required to explain these results. Meanwhile, Cys58 is located at a position where substrates are expected to bind and at the same interface of non-covalent ‘intramolecular bivalent glues’ that were discovered recently.³⁶

Outlook

Synthetic manipulation of protein surfaces, through inter- or intra-protein ligand-directed labelling strategies, promises to deliver a new paradigm in induced-proximity pharmacology. However, there are several hurdles that must be addressed for this field to realize its full potential. Some prospects and challenges are briefly described below:

- Considerable medicinal chemistry optimization of bifunctional molecules is required to deliver ‘beyond rule-of-five’ compounds with adequate pharmacokinetics for *in vivo* experimentation and drug discovery. The progress made thus far improving the pharmacokinetics of PROTACs is readily applicable to this field,³⁷ though with the added complication of ensuring the covalent warheads are drug compatible. Covalent molecular glues offer the advantage of being more amenable to traditional lead optimization strategies.

- It is important to confirm that induced-proximity pharmacology and the resulting phenotypic effects are a consequence of on-target neo-association and are not driven by off-target binding or labelling. Competition of functional effects with a reversible binding inhibitor in a dose-responsive manner, and site-directed mutagenesis of labelled residues may substantiate specificity. Clickable covalent probes will aid target occupancy measurements and selectivity determination through enrichment of labelled proteins followed by MS analysis. Confirmation of complex formation may be achieved biochemically using isolated proteins and through structural biology investigations.

- Covalent drug discovery is dominated by cysteine-targeting acrylamides, and the toolkit of drug compatible warheads is limited. To advance drug discovery, electrophiles must be developed that possess the desired on-target latent reactivity, where labelling only occurs in a ligand-directed and selective context, and where desirable properties such as metabolic stability and permeability are retained.

- Although some examples are described above, cysteine is rarely available at protein surfaces for site specific covalent modification. Advances in the development of next-generation covalent drug discovery are needed to enable targeting of residues beyond cysteine.³⁸ For example, sulfonyl exchange chemistry is particularly well-suited to ligand-directed protein surface modification, as shown by EM12-FS. Moreover, sulfonyl exchange electrophiles readily modify tyrosine residues, which are enriched at protein interfaces.³⁹

- Based on emerging studies, it would appear that many small molecule inhibitors could feasibly be converted into molecular glues through the incorporation of covalent motifs that label protein surfaces. A variety of electrophilic warheads may enable exploration of SARs where specific modifications of distinct residues may glue alternative proteins, leading to diverse functional effects.

- Ligand-directed technologies using cleavable covalent tethers such as NASA and CoLDR may enable induced-proximity pharmacologies beyond degradation by installing novel tags and PTM mimics onto protein surfaces.

- Site-specific installation of dehydroamino acids onto protein surfaces using ligand-directed covalent chemistries may yield new ways to crosslink proteins in cells.⁴⁰ This could be a way to mimic protein polymerization chemistry in nature and enable the construction of higher-order structures with applications in synthetic biology.

By harnessing the fields of covalent drug discovery and induced-proximity pharmacology I believe there will be a plethora of opportunities in the future to expand the druggable proteome. Specifically, medicinal chemistry may be on the verge of developing the tools to be able to rewire signalling pathways and correct aberrant biological processes through the creation of neoproteins in cells.

Data availability

As an opinion piece, there are no new data generated or shared.

Conflicts of interest

L. H. J. consults for Third Rock Ventures, Atlas Venture, Rapafusyn Pharmaceuticals, and Belharra Therapeutics, and holds equity in Jnana Therapeutics, Hyku Biosciences and Rapafusyn Pharmaceuticals. The Center for Protein Degradation at DFCI receives research funding from Deerfield.

References

- 1 S. L. Schreiber, *Cell*, 1992, **70**, 365–368.
- 2 S. L. Schreiber, *Cell*, 2021, **184**, 3–9.
- 3 R. Sedrani, L. H. Jones, A. M. Jutzi-Eme, W. Schuler and S. Cottens, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 459–462.
- 4 N. Kerres, S. Steurer, S. Schlager, G. Bader, H. Berger, M. Caligiuri, C. Dank, J. R. Engen, P. Ettmayer, B. Fischerauer, G. Flotzinger, D. Gerlach, T. Gerstberger, T. Gmaschitz, P.

- Greb, B. Han, E. Heyes, R. E. Jacob, D. Kessler, H. Kölle, L. Lamarre, D. R. Lancia, S. Lucas, M. Mayer, K. Mayr, N. Mischerikow, K. Mück, C. Peinsipp, O. Petermann, U. Reiser, D. Rudolph, K. Rumpel, C. Salomon, D. Scharn, R. Schnitzer, A. Schrenk, N. Schweifer, D. Thompson, E. Traxler, R. Varecka, T. Voss, A. Weiss-Puxbaum, S. Winkler, X. Zheng, A. Zoephel, N. Kraut, D. McConnell, M. Pearson and M. Koegl, *Cell Rep.*, 2017, **20**, 2860–2875.
- 5 Z. Kozicka, D. J. Suchyta, V. Focht, G. Kempf, G. Petzold, M. Jentzsch, C. Zou, C. Di Genua, K. A. Donovan, S. Coomar, M. Cigler, C. Mayor-Ruiz, J. L. Schmid-Burgk, D. Häussinger, G. E. Winter, E. S. Fischer, M. Ślabicki, D. Gillingham, B. L. Ebert and N. H. Thomä, *Nat. Chem. Biol.*, 2024, **20**, 93–102.
 - 6 C. J. Schulze, K. J. Seamon, Y. Zhao, Y. C. Yang, J. Cregg, D. Kim, A. Tomlinson, T. J. Choy, Z. Wang, B. Sang, Y. Pourfarjam, J. Lucas, A. Cuevas-Navarro, C. Ayala-Santos, A. Vides, C. Li, A. Marquez, M. Zhong, V. Vemulapalli, C. Weller, A. Gould, D. M. Whalen, A. Salvador, A. Milin, M. Saldajeno-Concar, N. Dinglasan, A. Chen, J. Evans, J. E. Knox, E. S. Koltun, M. Singh, R. Nichols, D. Wildes, A. L. Gill, J. A. M. Smith and P. Lito, *Science*, 2023, **381**, 794–799.
 - 7 J. Choi, J. Chen, S. L. Schreiber and J. Clardy, *Science*, 1996, **273**, 239–242.
 - 8 Z. Guo, S. Y. Hong, J. Wang, S. Rehan, W. Liu, H. Peng, M. Das, W. Li, S. Bhat, B. Peiffer, B. R. Ullman, C. M. Tse, Z. Tarmakova, C. Schiene-Fischer, G. Fischer, I. Coe, V. O. Paavilainen, Z. Sun and J. O. Liu, *Nat. Chem.*, 2019, **11**, 254–263.
 - 9 J. T. Ernst, P. A. Thompson, C. Nilewski, P. A. Sprengeler, S. Sperry, G. Packard, T. Michels, A. Xiang, C. Tran, C. J. Wegerski, B. Eam, N. P. Young, S. Fish, J. Chen, H. Howard, J. Staunton, J. Molter, J. Clarine, A. Nevarez, G. G. Chiang, J. R. Appleman, K. R. Webster and S. H. Reich, *J. Med. Chem.*, 2020, **63**, 5879–5955.
 - 10 T. Ito, H. Ando, T. Suzuki, T. Ogura, K. Hotta, Y. Imamura, Y. Yamaguchi and H. Handa, *Science*, 2010, **327**, 1345–1350.
 - 11 P. P. Chamberlain and L. G. Hamann, *Nat. Chem. Biol.*, 2019, **15**, 937–944.
 - 12 E. S. Fischer, K. Böhm, J. R. Lydeard, H. Yang, M. B. Stadler, S. Cavadini, J. Nagel, F. Serluca, V. Acker, G. M. Lingaraju, R. B. Tichkule, M. Schebesta, W. C. Forrester, M. Schirle, U. Hassiepen, J. Ottl, M. Hild, R. E. Beckwith, J. W. Harper, J. L. Jenkins and N. H. Thomä, *Nature*, 2014, **512**, 49–53.
 - 13 P. P. Chamberlain, A. Lopez-Girona, K. Miller, G. Carmel, B. Pagarigan, B. Chie-Leon, E. Rychak, L. G. Corral, Y. J. Ren, M. Wang, M. Riley, S. L. Delker, T. Ito, H. Ando, T. Mori, Y. Hirano, H. Handa, T. Hakoshima, T. O. Daniel and B. E. Cathers, *Nat. Struct. Mol. Biol.*, 2014, **21**, 803–809.
 - 14 J. Krönke, N. D. Udeshi, A. Narla, P. Grauman, S. N. Hurst, M. McConkey, T. Svinkina, D. Heckl, E. Comer, X. Li, C. Ciarlo, E. Hartman, N. Munshi, M. Schenone, S. L. Schreiber, S. A. Carr and B. L. Ebert, *Science*, 2014, **343**, 301–305.
 - 15 N. R. Kong and L. H. Jones, *Clin. Pharmacol. Ther.*, 2023, **114**, 558–568.
 - 16 D. E. Bussiere, L. Xie, H. Srinivas, W. Shu, A. Burke, C. Be, J. Zhao, A. Godbole, D. King, R. G. Karki, V. Hornak, F. Xu, J. Cobb, N. Carte, A. O. Frank, A. Frommlet, P. Graff, M. Knapp, A. Fazal, B. Okram, S. Jiang, P. Y. Michellys, R. Beckwith, H. Voshol, C. Wiesmann, J. M. Solomon and J. Paulk, *Nat. Chem. Biol.*, 2020, **16**, 15–23.
 - 17 T. B. Faust, H. Yoon, R. P. Nowak, K. A. Donovan, Z. Li, Q. Cai, N. A. Eleuteri, T. Zhang, N. S. Gray and E. S. Fischer, *Nat. Chem. Biol.*, 2020, **16**, 7–14.
 - 18 T. Han, M. Goralski, N. Gaskill, E. Capota, J. Kim, T. C. Ting, Y. Xie, N. S. Williams and D. Nijhawan, *Science*, 2017, 356.
 - 19 C. J. Diehl and A. Ciulli, *Chem. Soc. Rev.*, 2022, **51**, 8216–8257.
 - 20 A. Tutter, D. Buckley, A. A. Golosov, X. Ma, W. Shu, D. J. J. McKay, V. Darsigny, D. Dovala, R. Beckwith, J. Solomon, P. Rao, L. Xu, A. Fazal, A. Lingel, C. Wartchow, J. S. Cobb, A. Hachey, J. Tullai and G. A. Michaud, *bioRxiv*, 2024, preprint, DOI: [10.1101/2024.01.25.576086](https://doi.org/10.1101/2024.01.25.576086).
 - 21 D. Bradley, *Curr. Opin. Genet. Dev.*, 2022, **76**, 101956.
 - 22 X. Mo, Q. Niu, A. A. Ivanov, Y. H. Tsang, C. Tang, C. Shu, Q. Li, K. Qian, A. Wahafu, S. P. Doyle, D. Cicka, X. Yang, D. Fan, M. A. Reyna, L. A. D. Cooper, C. S. Moreno, W. Zhou, T. K. Owonikoko, S. Lonial, F. R. Khuri, Y. Du, S. S. Ramalingam, G. B. Mills and H. Fu, *Cell*, 2022, **185**, 1974–1985, e1912.
 - 23 J. T. Cruite, G. P. Dann, J. Che, K. A. Donovan, S. Ferrao, E. S. Fischer, N. S. Gray, F. Huerta, N. R. Kong, H. Liu, J. Marto, R. J. Metivier, R. P. Nowak, B. L. Zervas and L. H. Jones, *RSC Chem. Biol.*, 2022, **3**, 1105–1110.
 - 24 E. R. Watson, S. Novick, M. E. Matyskiela, P. P. Chamberlain, A. H. de la Peña, J. Zhu, E. Tran, P. R. Griffin, I. E. Wertz and G. C. Lander, *Science*, 2022, **378**, 549–553.
 - 25 J. Che and L. H. Jones, *RSC Med. Chem.*, 2022, **13**, 1121–1126.
 - 26 R. P. Nowak, L. Ragosta, F. Huerta, H. Liu, S. B. Ficarro, J. T. Cruite, R. J. Metivier, K. A. Donovan, J. A. Marto, E. S. Fischer, B. L. Zervas and L. H. Jones, *RSC Chem. Biol.*, 2023, **4**, 906–912.
 - 27 T. Tamura, T. Ueda, T. Goto, T. Tsukidate, Y. Shapira, Y. Nishikawa, A. Fujisawa and I. Hamachi, *Nat. Commun.*, 2018, **9**, 1870.
 - 28 M. Teng, J. Jiang, S. B. Ficarro, H. S. Seo, J. H. Bae, K. A. Donovan, E. S. Fischer, T. Zhang, S. Dhe-Paganon, J. A. Marto and N. S. Gray, *ACS Med. Chem. Lett.*, 2021, **12**, 1302–1307.
 - 29 Y. Wang, J. Zhang, J. Deng, C. Wang, L. Fang, Y. Zhang and J. Li, *CCS Chem.*, 2023, **5**, 2207–2214.
 - 30 R. N. Reddi, A. Rogel, E. Resnick, R. Gabizon, P. K. Prasad, N. Gurwicz, H. Barr, Z. Shulman and N. London, *J. Am. Chem. Soc.*, 2021, **143**, 20095–20108.
 - 31 E. C. Hett, H. Xu, K. F. Geoghegan, A. Gopalsamy, R. E. Kyne, C. A. Menard, A. Narayanan, M. D. Parikh, S. Liu, L. Roberts, R. P. Robinson, M. A. Tones and L. H. Jones, *ACS Chem. Biol.*, 2015, **10**, 1094–1098.
 - 32 E. S. Toriki, J. W. Papatzimas, K. Nishikawa, D. Dovala, A. O. Frank, M. J. Hesse, D. Dankova, J. G. Song, M. Bruce-Smythe, H. Struble, F. J. Garcia, S. M. Brittain, A. C. Kile,

- L. M. McGregor, J. M. McKenna, J. A. Tallarico, M. Schirle and D. K. Nomura, *ACS Cent. Sci.*, 2023, **9**, 915–926.
- 33 M. Lim, T. D. Cong, L. M. Orr, E. S. Toriki, A. C. Kile, J. W. Papatzimas, E. Lee, Y. Lin and D. K. Nomura, *ACS Cent. Sci.*, 2024, **10**, 1318–1331.
- 34 H. Wu, W. Wang, F. Liu, E. L. Weisberg, B. Tian, Y. Chen, B. Li, A. Wang, B. Wang, Z. Zhao, D. W. McMillin, C. Hu, H. Li, J. Wang, Y. Liang, S. J. Buhrlage, J. Liang, J. Liu, G. Yang, J. R. Brown, S. P. Treon, C. S. Mitsiades, J. D. Griffin, Q. Liu and N. S. Gray, *ACS Chem. Biol.*, 2014, **9**, 1086–1091.
- 35 Y. D. Li, M. W. Ma, M. M. Hassan, M. Hunkeler, M. Teng, K. Puvar, R. Lumpkin, B. Sandoval, C. Y. Jin, S. B. Ficarro, M. Y. Wang, S. Xu, B. J. Groendyke, L. H. Sigua, I. Tavares, C. Zou, J. M. Tsai, P. M. C. Park, H. Yoon, F. C. Majewski, J. A. Marto, J. Qi, R. P. Nowak, K. A. Donovan, M. Slabicki, N. S. Gray, E. S. Fischer and B. L. Ebert, *bioRxiv*, 2023, preprint, DOI: [10.1101/2023.02.14.528208](https://doi.org/10.1101/2023.02.14.528208).
- 36 O. Hsia, M. Hinterndorfer, A. D. Cowan, K. Iso, T. Ishida, R. Sundaramoorthy, M. A. Nakasone, H. Imrichova, C. Schätz, A. Rukavina, K. Husnjak, M. Wegner, A. Correa-Sáez, C. Craigon, R. Casement, C. Maniaci, A. Testa, M. Kaulich, I. Dikic, G. E. Winter and A. Ciulli, *Nature*, 2024, **627**, 204–211.
- 37 A. Pike, B. Williamson, S. Harlfinger, S. Martin and D. F. McGinnity, *Drug Discovery Today*, 2020, **25**, 1793–1800.
- 38 L. H. Jones, *Annu. Rep. Med. Chem.*, 2021, **56**, 95–134.
- 39 L. H. Jones, A. Narayanan and E. C. Hett, *Mol. Biosyst.*, 2014, **10**, 952–969.
- 40 L. H. Jones, *RSC Chem. Biol.*, 2020, **1**, 298–304.