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Unver, M. Yagiz; Gierse, Robin M.; Ritchie, Harry; Hirsch, Anna K. H.

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Druggability Assessment of Targets Used in Kinetic Target-Guided Synthesis

Miniperspective

M. Yagiz Unver,^{†,‡,||,⊥} Robin M. Gierse,^{†,‡,⊥} Harry Ritchie,[‡] and Anna K. Hirsch^{*,†,‡,§,Ⓜ}

[†]Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

[‡]Helmholtz Institute for Pharmaceutical Research (HIPS) – Helmholtz Centre for Infection Research (HZI), Department for Drug Design and Optimization, Campus Building E 8.1, 66123 Saarbrücken, Germany

[§]Department of Pharmacy, Saarland University, Campus Building E8.1, 66123 Saarbrücken, Germany

Supporting Information

■ INTRODUCTION

The drug-discovery process, from identification of a new active chemical entity to regulatory approval, is a long and expensive path. This process takes approximately 10–15 years and sometimes even longer due to the complexity of drug development (Figure 1).¹ There are no simple solutions to shorten this lengthy process; however, methods used in each phase can improve its efficiency and result in a significant acceleration of the overall process.

Target-guided synthesis (TGS) is a powerful strategy in which the biological target selects its own inhibitors by assembling them from building blocks in a biocompatible reaction.² There are two main strategies in this approach: dynamic combinatorial chemistry (DCC), in which the target selects and amplifies its own ligands from a library of products formed from reversibly connected building blocks, and kinetic target-guided synthesis (KTGS) in which the assembly takes place in an irreversible manner. Both techniques hold great potential, yet they are still unconventional and remain relatively unexplored.² The distinction between these two techniques is artificial in terms of application. In this review, we will categorize the methods according to reversibility of the

reaction and focus on targets that facilitated irreversible assembly of the inhibitors.

DCC has emerged as a powerful tool to identify binders for biological targets. It facilitates the reversible combination of building blocks by forming dynamic combinatorial libraries (DCLs) of potential binders in an efficient manner. Since the reaction facilitating DCLs is reversible, upon binding of the library members with the strongest affinity to the biological target, the product composition re-equilibrates, resulting in a shift in the equilibrium. Ultimately, the best binders are amplified, which circumvents the need for the synthesis and biochemical evaluation of each library member (Scheme 1).^{3–8}

A number of issues should be taken into consideration in the selection of a compatible reaction for DCC: (i) the reaction must be carried out in aqueous medium; (ii) equilibration of the DCL should be fast enough at the desired pH and the temperature where the protein is stable; (iii) the reaction should be chemoselective such that cross-reactivity with functional groups in the library or with the target is avoided.

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Figure 1. Drug-discovery timeline.

Scheme 1. Schematic Representation of the Fundamental Concept of DCC^a

^aAlternatively, in fragment ligation, the protein can be introduced prior to equilibrium formation.⁹ Adapted from Mondal et. al.⁷ (CC BY-NC 3.0).

Scheme 2. Schematic Representation of the Fundamental Concept of KTGS

The other critical points for a DCC setup are (i) selection of building blocks with comparable reactivity, (ii) in case mass spectroscopy is the analytical technique, avoiding the use of building blocks with identical molecular weights, (iii) high solubility of individual building blocks and the products to prevent precipitation in the reaction mixture, (iv) freezing of the equilibrium prior to analysis, and (v) selection of a proper analytical technique depending on the availability of the biological target.

In order to overcome the stringent requirements for DCC, dynamic ligation methods, in which the fragment combinations are screened one by one coupled with bioactivity-based detection, have been developed successfully.⁹

KTGS represents another type of target-guided approach, which enables the assembly of the inhibitors in situ via an irreversible process. In this approach, the biological target accelerates the reaction between complementary building blocks by bringing them in close proximity and proper orientation (Scheme 2).¹⁰

The first step in KTGS is the selective binding of building blocks from the library mixture to the specific pocket, which is mostly the active site/targeted site of the protein. Once the building blocks are bound to adjacent pockets of the target in the proper orientation and in close proximity, an irreversible reaction takes place, assembling the best binder and leading to a stable complex with the protein. This technique does not

require prior synthesis, purification, and biochemical evaluation of the library members, enabling rapid and cheaper screening of large numbers of compounds. Therefore, it has a great potential to decrease the costs and the time needed to discover hits in the early phases of drug discovery.²

The irreversible reactions used to connect fragments in KTGS should also be biocompatible; products formed as well as individual building blocks in the mixture should be stable and soluble under physiological conditions and of comparable reactivity. In addition to this, a substantial rate difference between biomolecule-templated and blank reactions is required. Because the protein-templated reactions are only used for analytical purposes and the compounds are formed in the reaction mixtures in trace amounts, follow-up synthesis is required to confirm their activity. Therefore, the synthetic protocols to get the desired compounds should be readily available.¹⁰

As the criteria for the reactions in this field are very stringent, only a couple of reactions have been reported (Scheme 3).

The most widely used reaction in KTGS is the 1,3-dipolar cyclo-addition of azides and alkynes. Both azides and alkynes are bio-orthogonal reagents, which makes this irreversible reaction suitable for KTGS. It is the first reaction ever reported in this context^{11,12} and allows for the formation of *syn*- and

Scheme 3. Chemical Reactions Reported for KTGS in the Literature

1,3-Dipolar cycloaddition between azides and alkynes

Amidation reaction between sulfonyl azides and thioacids (sulfo-click reaction)

Amidation reaction between amines and activated acids

Alkylation reaction of thiols or amines with alkyl halides

Addition of thiols to alkenes

Ring opening of epoxides by thiols or amines

anti-triazoles, expanding the number of compounds screened.^{13–16}

Sharpless¹⁷ and co-workers coined the term “in situ click reaction”. The principle of this reaction is that the 1,3-dipolar cycloaddition can be templated by the protein under metal-free conditions by the protein. The target binds to the initial blocks with the strongest affinity and holds them in the proper orientation and in close proximity to finally “click” them together, furnishing the corresponding triazoles.

The second reaction reported in this field is the sulfo-click reaction between electron-deficient sulfonyl azides or electron-rich azides such as alkyl or aryl azides and thioacids to form acyl sulfonamides.^{18,19}

Very recently Rademann and co-workers²⁰ reported the amidation reaction by using a set of activated carboxylic acid derivatives.

Protein-templated alkylation of thiols by halides was described by Chase et al.,²¹ demonstrating C–S bond formation between bromoacteylcarnitine and coenzyme A (CoA).

The last two reactions used in this field are the thio-Michael addition reaction, which is used for both DCC and KTGS¹⁶ and S_N2 ring opening of epoxides.²²

Pioneering works in KTGS were published using acetylcholine esterase (AChE) as the biological target.¹⁷ Until recently, KTGS found application mainly in the discovery of enzyme inhibitors. Nevertheless, recent studies revealed that KTGS is also applicable for the discovery of ligands for neurotransmitter-gated ion channel (ACh-binding protein), EthR, a receptor of the TetR family as well as for protein–protein interactions (PPIs; e.g., 14-3-3) (Figure 2).²

In this review, we will examine the druggability of the targets that have been used in KTGS (Table 1). The term druggability was defined by Hopkins and Groom as the ability of a target to be modulated by a potent, small, “drug-like” molecule.²³

Figure 2. Classes of proteins used in KTGS.

Table 1. Targets Used in KTGS, Adapted from Deprez and Co-Workers²

species	individual protein	number of KTGS examples
<i>Electrophorus electricus</i>		
<i>Torpedo californica</i>	Acetylcholine esterase	11
<i>Mus musculus</i>		
<i>Homo sapiens</i>		
HIV-1	Aspartic protease	3
<i>Endothia parasitica</i>	Endothiapepsin	
<i>H. sapiens</i>	Insulin-degrading enzyme	1
<i>Serratia marcescens</i>	Chitinase	2
<i>Staphylococcus aureus</i>	Biotin-protein ligase	2
<i>Plasmodium falciparum</i>	Tryptophanyl tRNA synthetase	1
<i>H. sapiens</i>	Bcr-Abl	1
<i>Bos taurus</i>	Carbonic anhydrase II	2
<i>H. sapiens</i>	Cyclooxygenase-2	2
<i>Lymnaea stagnalis</i>	Acetylcholine binding protein	1
<i>Aplysia californica</i>		
<i>Mycobacterium tuberculosis</i>	EthR	1
<i>H. sapiens</i>	Bcl-XL	2
<i>H. sapiens</i>	14-3-3 protein	1
<i>H. sapiens</i>	Factor Xa, serine protease	1

The software-based evaluation of the druggability of the target's pockets not only provides a numerical value (DScore) but also calculates the pocket properties, which we used to search for possible discriminating factors. For the prediction, a representative protein structure from each target protein was chosen. For all KTGS studies, in which crystallographic validation of the results was performed, the reported crystal structures were used. Otherwise, the protein model on which docking studies were performed or the model that the KTGS warhead was designed with was chosen. If the KTGS study did not rely on a protein model, a representative, high-resolution structure of the protein was selected from the RCSB Protein Data Bank.²⁴ In this case, a structure with a bound ligand was selected to identify the active site and take into account a possible induced fit mechanism, which might occur during binding of KTGS fragments.

The set of proteins obtained in this way was treated as a positive sample set for successful KTGS experiments. Because negative results are rarely published, we could not obtain a “negative” set of structures, for which KTGS failed. To circumvent this, a set of 20 “classical” protein targets with an approved drug was chosen randomly from the RCSB PDB Web page Drug and Drug Target Mapping.^{24,25}

We performed a druggability assessment, using the known structural information, for all of the collected protein targets.

DogSiteScorer was used to search for druggable pockets and calculate their geometrical as well as their physicochemical properties.²⁸ The identified pockets were scored in descending order of their volume and named P0, P1, etc. If they were divided, the label was expanded with an additional number; e.g., P_0_0 and P_0_1 are two subpockets of pocket P0. Typical properties of druggable and undruggable pockets, as described by Volkamer et al., are shown in Table 2. In general, pockets with a Dscore higher than 0.7 were considered druggable, although no strict cutoff was applied.²⁷

Table 2. Mean Values for Druggable and Undruggable Pockets as Defined by Volkamer et al.^{26,27}

	volume (Å ³)	depth ^a (Å)	enclosure ^b	fraction of apolar amino acids
druggable pocket	900	21	0.08	0.57
undruggable pocket	300	13	0.17	0.37

^aDefined as the farthest distance between solvent and buried part of the pocket. If the pocket is completely buried, the farthest distance between two hull grid points (grid points at the pocket border that have fewer than 25 neighbors) is calculated. ^bDefined as the portion of the lid of the pocket compared to the hull.

We carried out a comparison of the mean values for the classical drug targets and the targets successfully addressed with KTGS as well as the values of the positive and negative set of the groups undruggable and druggable, which were used to train the machine learning algorithms of the program DogSiteScorer by Volkamer et al.²⁶

Finally, we compared the targets used in KTGS and those used in “classical” drug development with the help of different methods of data analysis and visualization to check if certain properties of a protein pocket may be indicative of a successful KTGS experiment.

■ ACETYLCHOLINE ESTERASE (ACHE)

AChE, a protein that plays a key role in nerve impulse propagation, is the most widely used protein in KTGS. This enzyme was validated as a drug target to cure Alzheimer's disease. It has a deep catalytic active and large peripheral site on its surface, making it a convenient enzyme for KTGS.

The first series of inhibitors feature tacrine (**1**) (active site inhibitor) and propidinium (**2**) (peripheral site binder) moieties in their scaffolds (Figure 3).

Tacrine strongly binds to the active site of AChE with a K_d of 16 nM, and propidinium is binding specifically to the peripheral site with moderate K_d value. Keeping those moieties constant, the authors designed a library of compounds bearing azide and alkyne functional groups. By using in situ click chemistry, they could screen more than 300 compounds and

Figure 3. Structures of tacrine (**1**) and propidinium (**2**) used for the first KTGS experiment with acetylcholine esterase.

identified a series of less favored 1,5 triazoles **3–10** with femtomolar activity (Scheme 4).²⁹

To assess the druggability of AChE, a protein structure (PDB code 1ACJ) was used, which contains a bound tacrine ligand in its active site and was also used by Sharpless et al. for their docking studies.³⁰ The active site of AChE is predicted to be a subpocket (P_0_0) of the most druggable pocket P0 which extends to the surface of the protein. It has a volume of 704 Å³ and a Dscore of 0.79. The second-ranked pocket P1 is located at the back of the protein and features a small volume of 416 Å³, which is close to undruggable pockets. The properties of all other pockets identified display small volumes and low Dscores and are consequently in the category of undruggable pockets (Table S1). The combination of a deeply buried and well-defined catalytic site with no other druggable pockets might be a reason why AChE was successfully used as a model enzyme for KTGS; a third of all published studies were performed on this enzyme.²

■ HIV-1 PROTEASE

HIV-1 protease is another target used in KTGS and is a retroviral aspartic protease, which plays a crucial role in the life cycle of HIV.³¹ It is an important target for inhibition of viral replication. There are several inhibitors already available on the market such as indinavir. Being inspired by this inhibitor, Fokin and co-workers³² performed the in situ click reaction using HIV-1-Pr, SF-2-WTQ7K-Pr (SF-2-Pr) to illustrate the assembly of inhibitor **13** ($IC_{50} = 6 \text{ nM}$) from alkyne **11** and azide **12** in the presence of the target (Scheme 5).

The druggability assessment was performed using a protein structure of HIV-1 protease with the bound inhibitor atazanavir (PDB code 2O4K). The active site, with a volume of 828 Å³ and a Dscore of 0.68, was confirmed to be druggable. It has the shape of a tunnel and was correctly identified as subpocket P_0_0. The main pocket P0 offers the subpockets P_0_1 and P_0_3 as space for growing of the ligand out of the tunnel (Figure S2) and has an overall Dscore of 0.81, which is higher than that of the ligand binding subpocket. The HIV-1 protease has a molecular weight of 22.3 kDa, representing a rather small protein with no further identified druggable pockets (Table S2).

■ INSULIN-DEGRADING ENZYME

Insulin-degrading enzyme (IDE) is a protease, which plays a role in the cleavage of insulin or other bioactive peptides. It is a member of the M16 metalloenzyme family and has a large zinc binding site. Deprez and co-workers used KTGS to discover IDE inhibitors for the first time.³³ They used in situ click chemistry and designed a library of compounds comprising azide-bearing hydroxamic acids **14** and **15** for the zinc binding site and various alkynes. They performed SAR studies and their best hit identified as **16** displays an IC_{50} value of 56 nM (Scheme 6).

The authors were able to obtain crystal structures with bound ligands for three hits. We evaluated the pockets in terms of their druggability for the crystal structure with the most potent hit **16** (PDB code 4NXO) (Table S3).³³ IDE is an atypical enzyme with a large cavity. The zinc coordination site, which is addressed by the chelating hydroxamate group, is at the N terminal domain and buried inside the protein. The cavity is formed starting from an open state following an induced fit mechanism after a substrate is bound to the

Scheme 4. Example of KTGS Using Acetylcholine Esterase, Affording Inhibitors Binding to the Active and Peripheral Sites²⁹

enzyme. Only in the closed state is the catalytic site formed by the N- and C-terminal domains of the IDE. The model of the IDE protein is in its closed state, and the predicted binding pockets are in good agreement with the experimental results. The highest-ranked pocket P0 has a volume of 1700 Å³ and overlaps with the binding site of ligand 16. After its formation by induced fit, the apolar residues of the C-terminus can form a lot of hydrophobic and π - π interactions with the alkyne part of the ligand. Consequently, the authors of this study designed hydrophobic alkynes and used KTGS to screen for the most suitable residue to react with the chelating azide warhead.

P1 was identified and predicted by DogSiteScorer to be a second druggable pocket. With a volume of 1000 Å³, it is slightly smaller than the active site and is located at the opposite side of P0, at the hinge region, which connects the C- and N-terminal domains of IDE. Its Dscore is with 0.8 as high as that of P0, which was successfully targeted. If a warhead for this site could be designed, it might be worth exploring. By inhibiting the conformational change of the protein between the open and closed cavity, it could act as an allosteric binding site.

Scheme 5. HIV-I-Protease-Templated in Situ Click Chemistry³² Scheme 6. Insulin-Degrading Enzyme-Templated in Situ Click Chemistry³³ Scheme 7. *Serratia marcescens* Chitinase-Templated in Situ Click Chemistry³⁴

■ CHITINASE

Chitinases are enzymes responsible for the hydrolysis of chitin, the second most abundant polysaccharide in nature. They are biological targets for antifungal, antibacterial, and antiparasitic agents. Omura and co-workers discovered an inhibitor with low-nanomolar activity by using *Serratia marcescens* chitinase (SmChi, a chitinase from *S. marcescens*)-templated click chemistry (Scheme 7).³⁴

To identify an anchor point for the KTGS experiments, the authors used the protein structure in complex with the natural inhibitor argifin published by van Aalten and co-workers in 2002.³⁵ The X-ray structure shows that at least four conserved hydrogen bonds between the $N\gamma$ -methylcarbamoyl-L-arginine residue of the cyclic peptide and the hydrolytic pocket of the chitinase are important for binding. This modified amino acid,

which is deeply buried in the hydrolytic pocket of the enzyme, was therefore chosen as a starting point for the KTGS experiment. Its N- and C-functions were linked via a peptide bond to an azide-bearing amino acid and hydrophobic residues. The designed compounds were screened for activity against chitinase, and the hit with the highest activity was chosen as starting reagent for their KTGS reaction.

The prediction of druggable binding sites was performed using the same protein structure (PDB code 1H0G) and identified the argifin pocket as the most druggable pocket P0 with a volume of 990 Å³ and a Dscore of 0.81, although not to its full extent. In close proximity to the end of the $N\gamma$ -methylcarbamoyl-L-arginine bearing the azide functionality, two additional binding pockets are located, P1 and P4, which are shown in Figure 4. Pocket P4, on the one hand, is on its

Figure 4. Active site of the enzyme chitinase (PDB code 1H0G) with bound ligand arginin in the predicted pocket P0 (orange) and predicted druggable pockets P1 (purple) and P4 (blue).

own not druggable as reflected by a small volume of only 191 Å³ and a low-ranking Dscore of 0.46, but the ligand may be grown in this direction. Pocket P1 with a volume of 647 Å³ and a Dscore of 0.76 is, on the other hand, druggable and might accommodate larger molecules that could be linked to the azide. It is likely that both pockets were addressed by the alkynes during the KTGS experiments and a cocrystal structure of the resulting hit **19** with chitinase would be of considerable interest.

BIOTIN-PROTEIN LIGASE

Since there is continuous need for novel antibiotics nowadays due to the increase in the emergence of drug-resistant pathogenic bacteria, biotin-protein ligase represents an important and promising drug target for new antibacterial research. Tieu et al. demonstrated the applicability of KTGS by using a previously identified triazole inhibitor as a starting point for its optimization on the target *Staphylococcus aureus* biotin-protein ligase (BPL) (Scheme 8).³⁶

We used the crystal structure of BPL with bound inhibitor **23** (PDB code 3V7R) for the prediction of druggable pockets. The two well-defined and conserved binding pockets for biotin

and ATP were identified by DogSiteScorer as subpockets of pocket P0. The pocket has a total volume of 1356 Å³ and is predicted to be druggable. The binding sites for biotin and ATP are on their own difficult to address in a selective manner, but in this case KTGS proved to be an ideal tool to search for an optimal linker length and structure. The linking part is contributing to the selectivity of the inhibitor, and the formed triazole seems to act as a phosphate bioisostere. Overall, a more than 1000-fold selectivity of the inhibitor identified against *S. aureus* BPL, compared with the human BPL, could be achieved.³⁶ Pocket P1 was calculated to have a high Dscore of 0.74 but has a volume of 483 Å³, which means it belongs to the category of poorly druggable pockets. The pocket is separated from the active site but might show allosteric effects, if occupied. All other predicted pockets for BPL are less likely to be druggable (Table S5).

BCR-ABL

Bcr-Abl (tyrosine kinase) is an important anticancer target (leukemia). Passarella and co-workers confirmed the capacity of this target in KTGS,³⁷ assembling its own inhibitor from a pool of complementary building blocks derived from a known inhibitor reported with an IC₅₀ value of 0.9 μM (Scheme 9).

We used the protein structure in complex with a bound ligand (PDB code 1IEP), which was used by Passarella et al. to design the KTGS library, to assess the druggability of this enzyme. DogSiteScorer identified seven pockets, of which two were predicted to be druggable. Pocket P0 is forming a tunnel through the protein and is occupied by the inhibitor PD173955. It has a volume of 1250 Å³ and a Dscore of 0.80. The second-ranked pocket P1 forms a long groove at the protein surface and its volume of 500 Å³ is on the edge of druggable pockets. With a Dscore of 0.85 it is predicted to be highly druggable. Pocket P2 is scored lower in druggability but might be interesting due to its close proximity to P0 and the binding site of inhibitor PD173955. All other pockets identified have volumes of less than 250 Å³ and are therefore probably undruggable.

CARBONIC ANHYDRASE II

Carbonic anhydrase II (CA II) is a protein that assists the interconversion between CO₂ and HCO₃[−]; therefore it is responsible for the regulation of pH in the blood and renal reabsorption of NaCl and bicarbonate in the proximal tubule.²

Scheme 8. Assembly of **23** from **22** and **21** with *Streptococcus aureus* Biotin-Protein Ligase-Templated in Situ Click Chemistry³⁶

Scheme 9. Bcr-Abl-Templated Synthesis of Inhibitor 35³⁷ Scheme 10. First Example of Carbonic Anhydrase II-Templated Synthesis of 43³⁸

Huc and co-workers disclosed the first example of KTGS using CA II, showing the rate enhancement of the reaction in the presence of the target³⁸ (Scheme 10), while Kolb and co-workers published the same strategy to discover more potent inhibitors with improved affinity.³⁹

Druggability assessment of carbonic anhydrase was done on the apo structure of CA II (PDB code 1V9E). The most druggable pocket P0 has a volume of $519 \text{ \AA}^3$ and a Dscore of 0.79. The second-ranked cavity P1 is much smaller with a volume of $307 \text{ \AA}^3$ and a Dscore of 0.49. All other pockets identified are predicted to be undruggable with volumes of less than $300 \text{ \AA}^3$ and low Dscores. Even though there is a significant difference in Dscore between P0 and P1, pocket P1 is the active site of the protein and targeted in this KTGS study. The binding site of CA II contains a zinc binding motif. Known inhibitors interact with the Zn^{2+} cation, which is also the case for compound 37, in which the nitrogen atom of the sulfonamide functionality coordinates with the zinc atom. This was used as a warhead to selectively address the active site. For CA II, the zinc cation in the active site is the main contributor for binding. As it is now, the program DogSiteScorer does not recognize chelating properties of pockets. Judging only from the computed properties of pocket P0, it might be possible to design binders for this pocket, but it is questionable if they would show any biological activity on their own. However, the pockets are only separated by a short N-terminal part of the protein. This tail has a high *B*-factor and a low electron density (Figure S14), which are indicative of flexible regions of a protein. All this gives hints that further growing of an inhibitor into the direction of P0 and a displacement of the N-terminal residues might be possible.

■ ACETYLCHOLINE BINDING PROTEIN

Acetylcholine binding protein (AChBP) is the homologous protein of nicotinic acetylcholine receptors (nAChRs), a ligand-gated ion channel, which is an important target to

cure Alzheimer's disease. It is homologous to the extracellular domain of nAChR.⁹ For the proof-of-principle work, the authors first screened a library of triazoles by traditional synthesis against AChBP from *Lymnaea* (Ls), *Aplysia californica* (Ac), Y55W *Aplysia* mutant (AcY55W), and the best hit 46 was identified. In order to prove the methodology, the fragments of the best hit were incubated in the presence of Ls, Ac, AcY55W AChBP and they observed that Ls selectively templated the formation of 46 from the corresponding azide 44 and alkyne 45 (Scheme 11).⁴⁰

Scheme 11. Acetylcholine-Binding Protein-Templated Synthesis of Inhibitor 46⁴⁰

The AChBP is a large homopentameric protein with five similar binding sites for the identified inhibitor 46 at the interfaces between the subunits. Because of small conformational differences between the subunits in the protein model (PDB code 4DBM), the five binding sites are calculated and scored differently. The highest-ranked binding site of the inhibitor 46 is identified by DogSiteScorer as subpocket P_0_0 with a volume of $760 \text{ \AA}^3$, which is the central part of the $2300 \text{ \AA}^3$ large, branched pocket P0. The pockets P1 up to P4 are, with decreasing volumes down to $1300 \text{ \AA}^3$, also binding sites of inhibitor 46. In spite of the large differences in the assigned volume of the pockets, all five pockets are ranked with the same Dscore of 0.81 or 0.80. The different calculated sizes and additional subpockets of the ligand binding pockets P0 to

Scheme 12. *Mycobacterium tuberculosis* EthR-Templated Formation of 49⁴¹ Scheme 13. Bcl-XL-Templated Amide Formation To Afford Compound 59⁴²

P4 (Table S9, Figure S9) influence the identification of other pockets, which is why we abstain from interpretation of those.

■ EthR

EthR, a *Mycobacterium tuberculosis* transcription repressor (Rv3855) and member of the family of TetR transcriptional regulators, is regulating the expression of EthA, which is a mycobacterial monooxygenase that plays a causative role in the treatment of multidrug-resistant tuberculosis. Ethionamide is a prodrug activated by EthA, and its active form inhibits InhA, which is actively involved in the biosynthesis of mycolic acid in the bacteria cell wall. Accordingly, Deprez and co-workers proposed that inhibition of EthR would improve the efficacy of ethionamide by increasing the transcription of EthA.⁴¹ Upon screening of the azide 47 with a library of alkynes; they observed the protein-templated formation of hit 49 (Scheme 12).

EthR was crystallized in complex with the ligand 49 (PDB code 3O8G) and, by comparison with a structure in complex with the precursor 47 (PDB code 3O8H), enables us to observe an important advantage of KTGS.⁴¹ The binding site for the azide 47 is similar in both structures, whereas the binding pocket of the alkyne undergoes a rearrangement. The flipping of two phenylalanines could not be taken into consideration in classical docking calculations. Computation of druggable pockets resulted in the identification of the crystallographically validated binding site of 49 as pocket P0

with a volume of 913 Å³ and a Dscore of 0.84. The small transcription factor has no other druggable pockets; the second-ranked pocket P1 already has a volume of only 342 Å³.

■ Bcl-XL

KTGS has also been applied to PPIs. The first example is the Bcl-XL-templated sulfo-click reaction.^{42,43} PPIs are very important for many biological processes and represent an important target for therapeutics. Manetsch and co-workers performed a proof-of-principle work to demonstrate the compatibility of KTGS with PPIs. A library of sulfonamides 50–55 and thioacids 56–58, mimics of a Bcl-2 inhibitor, were designed and the library mixture was incubated with Bcl-XL (Scheme 13). Subsequently, they observed the assembly of compound 59, which was then proven to disturb the Bcl-XL/Bak-BH3 PPIs.⁴²

We used a model with a bound compound that mimics the BH3 domain to analyze the Bcl-XL protein (PDB code 2XYJ).⁴⁴ The H3 domain was identified as the most druggable pocket with a volume of 384 Å³ and a Dscore of 0.74. The second-ranked pocket P1 has a volume of 290 Å³, is adjacent to P0, and also accommodates a part of the bound ligand ABT-737. Both pockets on their own are in the volume range of undruggable pockets, but together they have a volume of more than 700 Å³. In this case, DogSiteScorer seems to have applied an unnecessary cutoff between those two pockets (Figure 5). All other identified pockets have a volume lower

Figure 5. Bcl-XL protein (PDB code 2XYJ). Shown is the H3 binding groove. Pockets P0 (orange) and P1 (purple) are occupied by the ligand ABT-737. Pocket P4 (blue) is also part of the hydrophobic binding groove.⁴⁵

Scheme 14. 14-3-3-Templated Epoxide Ring Opening Reaction for the Formation of **65**⁴⁶

than 260 Å³ and are, with a very low Dscore, predicted to be undruggable.

■ 14-3-3

14-3-3 is the other PPI target, which found an application in KTGS. It binds to key proteins involved in various processes such as apoptosis, transcription regulation, and intracellular signaling. Ohkanda and co-workers reported the template effect of recombinant human 14-3-3 protein for the in situ generation of inhibitors of PPIs.⁴⁶ Four epoxide derivatives

60–63 and the peptide **64** were incubated with 14-3-3. Although the background reaction was still present, they could see a rate enhancement by almost 200% for compound **61**. When the linker is short (compound **60**), the authors still observed the simultaneous binding of the building blocks to the adjacent pockets (37% inhibition), but they were unable to react (Scheme 14).⁴⁶

The 14-3-3 protein is often cocrystallized with a bound peptide in its peptide recognition groove. This peptide is not recognized by DogSiteScorer as a ligand, and therefore the

Scheme 15. Factor Xa-Templated Amide Formation of 68²⁰

largest binding pocket is not included in the predicted set of pockets. For our analysis, we chose the crystal structure of 14-3-3 in complex with the natural product fusicoccin A, which is a stabilizer of the PPI and bound in a hydrophobic pocket adjacent to the peptide-binding pocket (PDB code 5D3F). This fungal phytotoxin was used as the starting point for the design of this KTGS study.⁴⁶ Its binding pocket is recognized as the most druggable site P0 with a volume of 548 Å³ and a Dscore of 0.81. The second-ranked pocket P1 has a Dscore of 0.7 and is on the opposite site of the protein. With a volume of 313 Å³ it is already in the range of undruggable pockets.

■ FACTOR Xa

Factor Xa, a serine protease of the blood coagulation cascade and target of antithrombotic drugs, has been recently used in KTGS. Rademann and co-workers used factor Xa as a template for the formation of 68 from various activated carboxylic acid derivatives 66 and amine 67 by using fragment ligation screening (Scheme 15).²⁰

For this protein (PDB code 5K0H), the druggability assessment resulted in three pockets with similarly high Dscores between 0.76 and 0.82 and volumes of 400–500 Å³ (Table S13). The small volumes are between the typical values for druggable and undruggable pockets. In this case, the guidance of the KTGS reaction to the desired pocket P0 was achieved by selecting fragments known to bind to the pockets S2–S4 (66) and S1 (67) in the active site of the protease. The selective targeting of the active site was validated by enzyme-activity measurements immediately after the KTGS experiment. The aim of Rademann's work was not only to identify a good binding addition to a warhead but also to screen for an active ester suitable for protein-mediated amide bond formation.²⁰

■ COMPARISON OF THE DRUGGABILITY ASSESSMENT

Using DogSiteScorer, we evaluated the variables for the druggability assessment and found that many properties such as surface, depth, and number of atoms are proportional to the pocket volume as described by Volkamer et al. Therefore, in our analysis of the individual proteins, we focused on these variables and the predicted result from DogSite Scorer (DScore).²⁷

In order to find key features for KTGS experiments, i.e., variables that are indicative of a successful outcome, we compared a random set of 20 traditional drug targets (Table S13) and, to the best of our knowledge, all published KTGS targets (Tables S1–S12).

To compare the actual binding pockets, and not all pockets calculated by the druggability prediction, the active site was identified, and only this verified pocket was compared with the set of classical drug pockets. This was done by analyzing

published crystal structures of the protein in question with a bound ligand or an inhibitor. The identified binding pockets were compared with the predicted druggable pocket. The prediction proved to be very accurate and showed high accordance with the actual binding pockets (Figures S1–S12). Only in the case of unusual binding sites, such as zinc-coordinating CA II, the predicted most druggable pocket P0 was different from the actual active site of the enzyme.

Box plot visualizations of the most discriminative pocket properties, which are pocket volume, depth, enclosure, and the fraction of apolar amino acids, are shown in Figure 9.²⁷ For all other properties, see Figures S14–S18. A high variance can be observed for all four pocket properties shown. This might be due to the limited available data set of just 14 targets of successful KTGS experiments.

The mean depth of the KTGS pockets is 26 Å, nearly similar to the 26.5 Å of classical drug pockets. Looking at the apolar amino acid ratio, we can see that with 0.42 compared to 0.53 of traditional drug targets, KTGS pockets contain more polar amino acids. In addition, the volume of KTGS pockets is with 1445 Å³ slightly bigger than the 1338 Å³ of traditional drug pockets. The pocket enclosure seems to show a stronger difference between the KTGS and normal drug pockets (Figure 7). This value, which describes the ratio between solvent-exposed to hull grid points, is higher for more exposed pockets. KTGS pockets are with an enclosure of 0.114 more shallow and open than classical drug pockets, for which we calculated a mean of 0.077. The three included examples of PPI targets, which typically have a very shallow and large interaction site, might influence the set of KTGS pockets.

Comparing our sample set of classical drug targets with the mean values determined by Volkamer et al. on a larger sample set (Table 2) reveals that although the mean pocket volume is larger, the depth, fraction of apolar amino acids, and in particular the enclosure with 0.08 reflect the values of druggable pockets rather well.²⁷

To discover potential clustering of features on a volume-reduced data set, we performed a principal component analysis (PCA). For the volume restriction, all pockets with less than 50% of the maximum pocket volume were discarded. By application of this cutoff filter for small pockets, the bias from the high number of calculated but undruggable pockets (Figure 6) is reduced. In the PCA, the KTGS samples (Figure 8, green dots) show a difference in the component enclosure. This might be an additional hint that pocket enclosure is important for KTGS pockets. It is highly speculative, however, the more exposed pockets may enable a better diffusion of the two or more fragments of the KTGS experiment to the pocket.

An interesting side-observation we made is that the volume of a pocket is highly correlated with the computed Dscore until a volume of ~800 Å³ is reached (Figure 9). After this threshold, an even bigger pocket size no longer contributes to

Figure 6. Volume of the KTGS and a random set of classical drug targets compared with the whole set of predicted pockets.

the predicted druggability of a pocket. On the other hand, we could observe no successful druggable pocket with a volume of less than 500 Å³. This finding might help in further drug-design studies to decide which pocket of a protein could be addressed.

■ ANALYSIS OF THE RELATIONSHIP BETWEEN THE ACTIVE SITE AND THE SITE OF THE KTGS REACTION

We further investigated if catalytically active amino acids from the active site of the target protein might also be involved in

Figure 8. Plot of two principal components from PCA. Red lines are the two highly significant components volume and enclosure, which explain most of the variability of the data set. Red dashed line indicates possible decision boundary between the two pocket types. Color code: dark purple, pockets; yellow, validated “classical” drug pockets; green, KTGS pockets.

the acceleration of the KTGS reaction by analyzing all available crystal structures with a bound product of a protein-templated reaction.

In the crystal structure of the zinc-dependent metalloprotease IDE obtained by Deprez and co-workers, compound **16** is chelated to the zinc cation.³³ The site of the protein-templated 1,3 alkyne–azide cycloaddition is 5.2 Å away from the zinc cation and might still be still affected by polarization effects. Arg824 and Asn139 are in proximity of the azide and alkyne with a distance of 3.7 and 3.2 Å, respectively.

Figure 7. Comparison of the most discriminative pocket properties from KTGS targets and classical drug targets: volume (top, left), enclosure (top, right), ratio of apolar amino acids (bottom, left), and depth (bottom, right).

Figure 9. Shown are all predicted pockets as a function of drug score against their volume. Color code: black, predicted pocket; red, predicted pocket with bound KTGS ligand; blue, predicted pocket with bound drug.

Both amino acids might help in stabilizing a particular resonance structure of the azide or intermediates of the reaction, in particular Arg824, which is directly interacting with the zinc-binding histidine residues. A similar arrangement and distances of an arginine (Arg125) aspartate (Asp180) can be observed in the crystal structure of the biotin-protein ligase in complex with compound 23.

In the crystal structure of AChBP with a bound compound 46 as well as EthR in complex with 29 the pocket characteristics are very different. The binding sites consist of aromatic and aliphatic amino acids, which are forming mostly hydrophobic interactions with the inhibitor.

Overall our observations are in agreement with the literature, in which the rate-accelerating effect of proteins is ascribed to their binding of the KTGS building blocks and facilitating the reaction by bringing their functional groups in close proximity and in a favorable orientation.^{9,17} The observation of an arginine and an acidic or very polar amino acid at the site of the 1,3- cycloaddition is probably a coincidence. However, it could also be a first hint of an arrangement of amino acids, which might be able to accelerate the Huisgen cycloaddition.

SUMMARY AND CONCLUSIONS

KTGS is a powerful technique that accelerates drug discovery by using a protein's pocket as a template and facilitates the selection of binders without prior synthesis and biochemical evaluation. The concept has gained great attention from scientists, and several successful examples to expand the number of reactions as well as the target scope have been demonstrated. In this review, we systematically analyzed the druggability and properties of the pockets in which the KTGS takes place to understand the nature of the template effect of the biological targets.

We could not find any physicochemical property predicted by DogSiteScorer with which we could clearly distinguish between KTGS targets and classical drug pockets. This is in agreement with current literature, in which no specific requirements for the addressed pocket are mentioned. The KTGS studies to date address proteins with active sites with

very different sizes, shapes, and properties, from large cavities and very enclosed tunnels to shallow, open active sites and PPIs.

The finding that we could not identify any special requirements on the protein structure in order to perform successful KTGS experiments should be encouraging for all who might want to try this technique on their protein target. For a successful KTGS experiment, additional general protein requirements need to be met, namely, having access to a stable, soluble, and pure protein in sufficient quantities. If these requirements are fulfilled, KTGS is a very versatile and powerful tool and has proven to be useful to screen many possible molecules at the same time and select the best binder(s).

Prior knowledge about the target that should be addressed to design a library of fragments and choice of a suitable reaction type are additional important aspects. If the KTGS reaction should be guided to a particular pocket of the protein, which is often the active site, prior information (structural information or known binders) to design a warhead is helpful for a successful outcome.

However, this strong guidance of the KTGS reaction to known pockets also affects the present study of the related pocket properties. In the future, if more “undruggable” proteins were investigated de novo by KTGS experiments, a more detailed picture of the protein pocket requirements for KTGS experiments might arise.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b00266.

Tables with results of the DogSiteScorer calculations; figures of the ligand binding sites; visualization of B-factor and electron density of CA II; list of drugs and targets used for the comparison set (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: Anna.Hirsch@helmholtz-hzi.de.

ORCID

Anna K. H. Hirsch: 0000-0001-8734-4663

Present Address

^{||}M.Y.U.: AGILEBiotics B.V., De Mudden 14, 9747 AW, Groningen, The Netherlands.

Author Contributions

[†]M.Y.U. and R.M.G. contributed equally.

Notes

The authors declare no competing financial interest.

Biographies

M. Yagiz Unver obtained his Master's degree at Middle East Technical University, Ankara, Turkey, in 2013. After gaining experience in asymmetric synthesis and organocatalysis, he pursued his Ph.D. at University of Groningen under the supervisions of Prof. A. K. H. Hirsch and Prof. B. L. Feringa. Throughout his Ph.D. program, he has been working on several drug-discovery projects in the early stage of drug development (hit identification and optimization). Thereby, he employed novel methodologies such as target-guided synthesis and structure/fragment-based drug design. During his Ph.D. studies, he was also working at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) in Saarbrücken, Germany. After receiving his Ph.D. in September 2017, he joined AGILEBiotics B.V. as a Research Scientist to support the development of novel antibiotic candidates against multidrug-resistant infections.

Robin M. Gierse studied Biochemistry at the Ernst-Moritz-Arndt University of Greifswald. His Bachelor's thesis was on the synthesis of 2'-O modified nucleoside triphosphates, and in 2015 he obtained his M.Sc. with a thesis on the synthesis and characterization of cross-link-active microRNAs in the bioorganic chemistry lab of Professor S. Müller. Subsequently, he worked at the company Enzymicals as a junior scientist. He joined the Hirsch group in the fall of 2016 as a Ph.D. student. His research focuses on the development of novel antibiotics and includes structural biology, computational drug design, and organic synthesis.

Harry Ritchie is a Master's student in Bioinformatics at the Saarland University. He has completed a Bachelor of Applied Science in Biochemistry and Mathematics at Queensland University of Technology. His interests lie in protein structures and applications of machine learning. He is currently doing his Master's research project in the Hirsch group.

Anna K. H. Hirsch read Natural Sciences at the University of Cambridge and did her Master's research project in the group of Prof. Steven V. Ley. She received her Ph.D. with Prof. François Diederich from ETH Zurich in 2008 on de novo design and synthesis of the first inhibitors of an anti-infective target. After a postdoc in the group of Prof. Jean-Marie Lehn in Strasbourg, she took up a position as Assistant Professor at the Stratingh Institute for Chemistry at the University of Groningen in 2010 and was promoted to Associate Professor in 2015. In 2017, she became head of the department for drug design and optimization at the HIPS. Her work focuses on rational approaches to drug design and innovative hit-identification strategies, in particular anti-infective targets.

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ABBREVIATIONS USED

ACHBP, acetylcholine binding protein; BPL, biotin-protein ligase; CA II, carbonic anhydrase II; DCC, dynamic combinatorial chemistry; DCL, dynamic combinatorial library; IDE, insulin degrading enzyme; K_d , dissociation constant; KTGS, kinetic target-guided synthesis; nAChR, nicotinic acetylcholine receptor; PCA, principal component analysis; PPI, protein-protein interaction; TGS, target guided synthesis

REFERENCES

- (1) Everts, M.; Cihlar, T.; Bostwick, J. R.; Whitley, R. J. Accelerating Drug Development: Antiviral Therapies for Emerging Viruses as a Model. *Annu. Rev. Pharmacol. Toxicol.* **2017**, *57*, 155–169.
- (2) Bosc, D.; Jakhlal, J.; Deprez, B.; Deprez-Poulain, R. Kinetic target-guided synthesis in drug discovery and chemical biology: a comprehensive facts and figures survey. *Future Med. Chem.* **2016**, *8*, 381–404.
- (3) Li, J.; Nowak, P.; Otto, S. Dynamic combinatorial libraries: From exploring molecular recognition to systems chemistry. *J. Am. Chem. Soc.* **2013**, *135*, 9222–9239.
- (4) Lehn, J.-M. From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. *Chem. Soc. Rev.* **2007**, *36*, 151–160.
- (5) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J. L.; Sanders, J. K.; Otto, S. Dynamic combinatorial chemistry. *Chem. Rev.* **2006**, *106*, 3652–3711.
- (6) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. Dynamic covalent chemistry. *Angew. Chem., Int. Ed.* **2002**, *41*, 898–952.
- (7) Mondal, M.; Hirsch, A. K. H. Dynamic combinatorial chemistry: a tool to facilitate the identification of inhibitors for protein targets. *Chem. Soc. Rev.* **2015**, *44*, 2455–2488.
- (8) van der Vlag, R.; Hirsch, A. K. H. Analytical Methods in Protein-Templated Dynamic Combinatorial Chemistry. In *Comprehensive Supramolecular Chemistry II*; Atwood, J. L., Ed.; Elsevier, 2017; Vol. 5, pp 487–509.
- (9) Jaegle, M.; Wong, E. L.; Tauber, C.; Nawrotsky, E.; Arkona, C.; Rademann, J. Protein-Templated Fragment Ligations—From Molecular Recognition to Drug Discovery. *Angew. Chem., Int. Ed.* **2017**, *56* (26), 7358–7378.
- (10) Oueis, E.; Sabot, C.; Renard, P.-Y. New insights into the kinetic target-guided synthesis of protein ligands. *Chem. Commun.* **2015**, *51*, 12158–12169.
- (11) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
- (12) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Manimaran, T. L. Cycloaddition induced by cucurbituril. A case of Pauling principle catalysis. *J. Org. Chem.* **1983**, *48*, 3619–3620.
- (13) Manetsch, R.; Krasinski, A.; Radić, Z.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. Enzyme inhibitors made to their own specifications. *J. Am. Chem. Soc.* **2004**, *126*, 12809–12818.
- (14) Sharpless, K. B.; Manetsch, R. Click Chemistry: a Powerful Means for Lead Discovery. *Expert Opin. Drug Discovery* **2006**, *1*, 525–538.
- (15) Kolb, H. C.; Sharpless, K. B. The growing impact of click chemistry on drug discovery. *Drug Discovery Today* **2003**, *8*, 1128–1137.
- (16) Millward, S. W.; Agnew, H. D.; Lai, B.; Lee, S. S.; Lim, J.; Nag, A.; Pitram, S.; Rohde, R.; Heath, J. R. In situ click chemistry: from small molecule discovery to synthetic antibodies. *Integr. Biol.* **2013**, *5*, 87–95.

- (17) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radić, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. Click chemistry in situ: Acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053–1057.
- (18) Oueis, E.; Sabot, C.; Renard, P.-Y. New insights into the kinetic target-guided synthesis of protein ligands. *Chem. Commun.* **2015**, *51*, 12158–12169.
- (19) Hu, X.; Sun, J.; Wang, H. G.; Manetsch, R. Bcl-XL-templated assembly of its own protein-protein interaction modulator from fragments decorated with thio acids and sulfonyl azides. *J. Am. Chem. Soc.* **2008**, *130*, 13820–13821.
- (20) Jaegle, M.; Steinmetzer, T.; Rademann, J. Protein-Templated Formation of an Inhibitor of the Blood Coagulation Factor Xa through a Background-Free Amidation Reaction. *Angew. Chem., Int. Ed.* **2017**, *56*, 3718–3722.
- (21) Chase, J. F.; Tubbs, P. K. Conditions for the self-catalysed inactivation of carnitine acetyltransferase. A novel form of enzyme inhibition. *Biochem. J.* **1969**, *111*, 225–235.
- (22) Kulkarni, S. S.; Hu, X.; Doi, K.; Wang, H.-G.; Manetsch, R. Screening of protein-protein interaction modulators via sulfo-click kinetic target-guided synthesis. *ACS Chem. Biol.* **2011**, *6*, 724–732.
- (23) Hopkins, A. L.; Groom, C. R. Target Analysis: A Priori Assessment of Druggability. In *Small Molecule-Protein Interaction*; Waldmann, H., Koppitu, M., Eds.; Springer: Berlin, 2003; pp 11–17.
- (24) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28* (1), 235–242.
- (25) Wishart, D. S.; Feunang, Y. D.; Guo, A. C.; Lo, E. J.; Marcu, A.; Grant, J. R.; Sajed, T.; Johnson, D.; Li, C.; Sayeeda, Z.; et al. DrugBank 5.0: A Major Update to the DrugBank Database for 2018. *Nucleic Acids Res.* **2018**, *46*, 1074–1082.
- (26) Volkamer, A.; Kuhn, D.; Grombacher, T.; Rippmann, F.; Rarey, M. Combining Global and Local Measures for Structure-Based Druggability Predictions. *J. Chem. Inf. Model.* **2012**, *52*, 360–372.
- (27) Masini, T.; Kroezen, B. S.; Hirsch, A. K. H. Druggability of the Enzymes of the Non-Mevalonate-Pathway. *Drug Discovery Today* **2013**, *18*, 1256–1262.
- (28) Volkamer, A.; Kuhn, D.; Rippmann, F.; Rarey, M. Dogsitescorer: A Web Server for Automatic Binding Site Prediction, Analysis and Druggability Assessment. *Bioinformatics* **2012**, *28*, 2074–2075.
- (29) Krasinski, A.; Radić, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. In situ selection of lead compounds by click chemistry: Target-guided optimization of acetylcholinesterase inhibitors. *J. Am. Chem. Soc.* **2005**, *127*, 6686–6692.
- (30) Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. Quaternary Ligand Binding to Aromatic Residues in the Active-Site Gorge of Acetylcholinesterase. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 9031–9035.
- (31) Brik, A.; Wong, C. HIV-1 protease: mechanism and drug discovery. *Org. Biomol. Chem.* **2003**, *1*, 5–14.
- (32) Whiting, M.; Muldoon, J.; Lin, Y. C.; Silverman, S. M.; Lindstrom, W.; Olson, A. J.; Kolb, H. C.; Finn, M. G.; Sharpless, K. B.; Elder, J. H.; Fokin, V. V. Inhibitors of HIV-1 protease by using in situ click chemistry. *Angew. Chem., Int. Ed.* **2006**, *45*, 1435–1439.
- (33) Deprez-Poulain, R.; Hennuyer, N.; Bosc, D.; Liang, W. G.; Enée, E.; Marechal, X.; Charton, J.; Totobenazara, J.; Berte, G.; Jahklal, J.; Verdelet, T.; Dumont, J.; Dassonneville, S.; Woitrain, E.; Gauriot, M.; Paquet, C.; Duplan, I.; Hermant, P.; Cantrelle, F. X.; Sevin, E.; Culot, M.; Landry, V.; Herledan, A.; Piveteau, C.; Lippens, G.; Leroux, F.; Tang, W. J.; van Endert, P.; Staels, B.; Deprez, B. Catalytic site inhibition of insulin-degrading enzyme by a small molecule induces glucose intolerance in mice. *Nat. Commun.* **2015**, *6*, 8250.
- (34) Hirose, T.; Sunazuka, T.; Sugawara, A.; Endo, A.; Iguchi, K.; Yamamoto, T.; Ui, H.; Shiomi, K.; Watanabe, T.; Sharpless, K. B.; Omura, S. Chitinase inhibitors: extraction of the active framework from natural argifin and use of in situ click chemistry. *J. Antibiot.* **2009**, *62*, 277–282.
- (35) Houston, D. R.; Shiomi, K.; Arai, N.; Omura, S.; Peter, M. G.; Turberg, A.; Synstad, B.; Eijssink, V. G. H.; van Aalten, D. M. F. High-Resolution Structures of a Chitinase Complexed with Natural Product Cyclopentapeptide Inhibitors: Mimicry of Carbohydrate Substrate. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 9127–9132.
- (36) Tieu, W.; Soares da Costa, T. P.; Yap, M. Y.; Keeling, K. L.; Wilce, M. C. J.; Wallace, J. C.; Booker, G. W.; Polyak, S. W.; Abell, A. D. Optimising in situ click chemistry: the screening and identification of biotin protein ligase inhibitors. *Chem. Sci.* **2013**, *4*, 3533.
- (37) Peruzzotti, C.; Borrelli, S.; Ventura, M.; Pantano, R.; Fumagalli, G.; Christodoulou, M. S.; Monticelli, D.; Luzzani, M.; Fallacara, A. L.; Tintori, C.; Botta, M.; Passarella, D. Probing the binding site of Abl tyrosine kinase using in situ click chemistry. *ACS Med. Chem. Lett.* **2013**, *4*, 274–277.
- (38) Nguyen, A.; Huc, I. Using an Enzymes Active Site To Template Inhibitors. *Angew. Chem., Int. Ed.* **2001**, *40*, 1774–1776.
- (39) Mocharla, V. P.; Colasson, B.; Lee, L. V.; Röper, S.; Sharpless, K. B.; Wong, C. H.; Kolb, H. C. In situ click chemistry: Enzyme-generated inhibitors of carbonic anhydrase II. *Angew. Chem., Int. Ed.* **2005**, *44*, 116–120.
- (40) Grimster, N. P.; Stump, B.; Fotsing, J. R.; Weide, T.; Talley, T. T.; Yamauchi, J. G.; Nemecek, A.; Kim, C.; Ho, K. Y.; Sharpless, K. B.; Taylor, P.; Fokin, V. V. Generation of candidate ligands for nicotinic acetylcholine receptors via in situ click chemistry with a soluble acetylcholine binding protein template. *J. Am. Chem. Soc.* **2012**, *134*, 6732–6740.
- (41) Willand, N.; Desroses, M.; Toto, P.; Dirie, B.; Lens, Z.; Villeret, V.; Rucktooa, P.; Loch, C.; Baulard, A.; Deprez, B. Exploring drug target flexibility using in situ click chemistry: Application to a mycobacterial transcriptional regulator. *ACS Chem. Biol.* **2010**, *5*, 1007–1013.
- (42) Hu, X.; Manetsch, R. Kinetic target-guided synthesis. *Chem. Soc. Rev.* **2010**, *39*, 1316–1324.
- (43) Hu, X.; Sun, J.; Wang, H. G.; Manetsch, R. Bcl-XL-Templated Assembly of Its Own Protein-Protein Interaction Modulator from Fragments Decorated with Thio Acids and Sulfonyl Azides. *J. Am. Chem. Soc.* **2008**, *130*, 13820–13821.
- (44) Lee, E. F.; Czabotar, P. E.; Smith, B. J.; Deshayes, K.; Zobel, K.; Colman, P. M.; Fairlie, W. D. Crystal Structure of ABT-737 Complexed with Bcl-xL: Implications for Selectivity of Antagonists of the Bcl-2 Family. *Cell Death Differ.* **2007**, *14*, 1711–1713.
- (45) Yang, C. Y.; Wang, S. Hydrophobic Binding Hot Spots of Bcl-xL Protein-Protein Interfaces by Cosolvent Molecular Dynamics Simulation. *ACS Med. Chem. Lett.* **2011**, *2*, 280–284.
- (46) Maki, T.; Kawamura, A.; Kato, N.; Ohkanda, J. Chemical ligation of epoxide-containing fusicoccins and peptide fragments guided by 14-3-3 protein. *Mol. Biosyst.* **2013**, *9*, 940–943.