Tailored Bioorthogonal and Bioconjugate Chemistry: A Source of Inspiration for Developing Kinetic Target-Guided Synthesis Strategies

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ABSTRACT: Kinetic target-guided synthesis (KGTS) is a promising tool for the discovery of biologically active compounds. It relies on the identification of potent ligands that are covalently assembled by the biological targets themselves from a pool of reagents. Significant effort is devoted to develop new KTGS strategies, however, only a handful of biocompatible reactions are available, which may be insufficient to meet the specificities (stability, dynamics, active site topology, etc…) of a wide range of biological targets with therapeutic potential. This Review proposes a retrospective analysis of existing KTGS ligation tools, in terms of their kinetics and analogy with other biocompatible reactions, and provides new clues to expand the KTGS toolkit. By way of examples, a non-exhaustive selection of such chemical ligation tools belonging to different classes of reactions as promising candidate reactions for KTGS are suggested.

INTRODUCTION

The search for ligands that bind relevant biological targets through non-covalent interactions with high affinity and specificity is a key feature in the fields of molecular imaging,[1] proteomics,[2] molecular diagnostics, and therapeutics.[3] Beyond the high-throughput screening paradigm that uses drug-like molecules for drug discovery, fragment-based drug discovery (FBDD) techniques offer the advantage of a better coverage of the chemical space by identifying high-affinity ligands from small-size and low-affinity fragments that bind target's adjacent binding-sites.[4] Importantly, the affinity (*i.e.* binding affinity, K_D) of optimally linked fragments can be dramatically enhanced, up to multiple orders of magnitude as compared to the affinity of parent fragments. Such a super-additivity or "linking effect", is ascribed to the fact that the sum of free energy of binding ΔG (defined by $\Delta G = -RT \ln K_D$) for two parent fragments includes two unfavourable rigid-body entropy barriers (translational and rotational) upon their complexation to the biological target, while the assembled ligand includes only one unfavourable term.[5-6]

Already 40 molecules discovered by FBDD are in various stages of clinical trials in 2020, three of which have been approved by the FDA. The recently introduced kinetic target-guided synthesis (KTGS) strategy brings the biological target to the forefront of the FBDD process. In fact, the biological target is not only used to determine the potency of ligands though biological assays, but also to chemically assemble them through the formation of covalent and irreversible bonds between complementary reactive fragments (Figure 1A). Indeed, fragments that bind the biological target will benefit from their spatial proximity to react together preferentially and form the corresponding multi-site ligand. This methodology has been applied with success for the discovery and the design of numerous ligands and with different classes of biological targets including proteins, and nucleic acids. The term "fragment" will be used throughout the

review to refer to chemical precursors for KTGS, although it should be stressed that this term was originally associated with low affinity small molecules that obey a “Rule of Three” (in particular molecular weight <300; number of hydrogen bond donors ≤3; number of hydrogen bond acceptors ≤3; and ClogP ≤3).[7] This was recently clarified by Deprez-Poulain et al.[8]

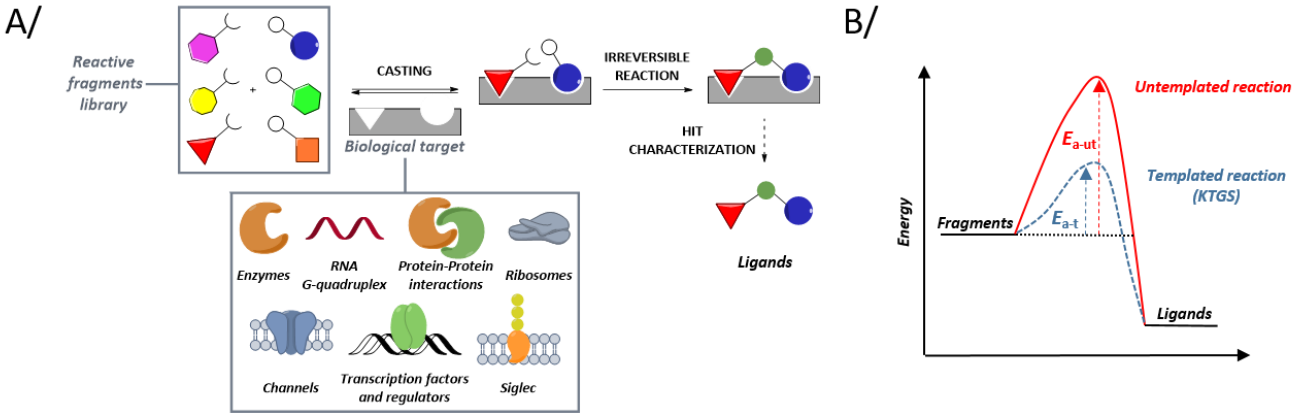


Figure 1. Principle of kinetic target-guided synthesis (KTGS) with different classes of biological targets (A); and energy of activation of template & untemplated reactions (B).

Historically, the covalent bond formation relies on the use of 1,3-dipolar cycloaddition of azides with alkynes leading to the formation of 1,4- and/or 1,5-disubstituted 1,2,3-triazoles. This strategy, also termed “*in situ* click chemistry”, reported by Sharpless and coll. in 2002, has enabled the synthesis of the most potent non-covalent acetylcholinesterase (AChE) inhibitor.[9] Beyond drug discovery, this approach has led to impressive discoveries such as the identification of unknown protein conformations, which were not predicted from reported ligand-protein crystallographic complexes,[10] or the identification of previously hidden binding pockets of enzymes.[11] KTGS has also been leveraged for biomedical imaging, in particular for positron emission tomography (PET) tracer discovery,[12] as well as for identifying selective fluorescent probes for a specific topology of G-quadruplex nucleic acids.[13]

Since then, other chemical ligation strategies were successfully applied,[8, 14-17] such as the recently reported Ugi four-component reaction,[18] Mannich ligation reaction,[19] or aldol condensation.[20] In fact, continuous extension of the repertoire of chemical ligations is needed in particular to face the stability constraints of biological targets, as well as the wide diversity of their active site topologies. Indeed, long *in vitro* incubation times (from days to weeks) in particular for reactions with high energy barriers, can induce the aggregation of proteins, or impact their folding through the lack of important intermolecular interactions, which are ubiquitous in the cellular environment. Such deleterious events would lead to the premature loss of templating ability of unstable proteins, and thus generate false negative data. It is thus necessary to know, or determine in advance, their stability under the incubation conditions (incubation time, pH and temperature) used in KTGS experiments. In this context, faster ligation tools, characterized by lower energies of activation ($E_{a\text{-}ut}$) would be highly valuable, keeping in mind that a key for the success of KTGS strategy relies on a noticeable difference in kinetics between templated and untemplated reaction, which results from a marked difference in their activation energy

$E_{a\text{-}t}$ and $E_{a\text{-}ut}$, respectively (Figure 1B). It is important to note that the success of KTGS is also dependent on the ability of the two complementary reactive fragments to bind simultaneously the biological target, and conserve an optimal binding mode upon their ligation, in order to favor the aforementioned "linking effect" or super-additivity of fragment binding energies.[21] Finally, the nature of the linker itself, termed as "linkage effect", can significantly contribute to the overall affinity of the ligand through establishment of additional stabilizing H-bonding, π–π stacking, and dipole-dipole interactions, or through its intrinsic physicochemical properties (*e.g.* lipophilicity).[5, 19] The triazole ring system has proven to be such a notable example of linker that actively participates in binding to biological targets, which may explained why this *in situ* click reaction is involved in most of the successful KTGS described to date.[10]

In this Review, we have drawn a parallel between reported KTGS ligations and their counterpart transformations belonging to the bioorthogonal or bioconjugation toolkit (Figure 2A). However, these biocompatible reactions which have been optimized in order to exhibit fast rate constants (low activation energy E_a), cannot be used in KTGS without appropriate adjustments of their reactivity in order to get slower untemplated reactions kinetics (higher activation energy $E_{a\text{-}ut}$, Figure 2B)., and thus sufficient differences between template and untemplated reactions. This retrospective analysis may guide the development of new ligation reactions amenable for future KTGS applications, and some of these emerging reactions that might be suitable for this concept are proposed in the second part of this Review. Bioorthogonal chemistry and bioconjugation share some features such as fast rate constants, high chemoselectivity and the ability to proceed under physiological conditions. However, these are two distinct classes of reactions. Bioorthogonal reactions are defined as chemical reactions that can proceed in biological media without interfering with natural chemical functions, while bioconjugation reactions involve chemical functions (generally electrophilic systems) that react with naturally occurring functional groups (such as amines or sulfhydryl moieties).[22-23] With the advent of bioorthogonal[24] and bioconjugate chemistry,[25] in recent years, an increasing number of experimental[26-28] and theoretical studies,[29-30] computational analysis[31] on rate constants of such chemical reactions have been made available to the scientific community. Accordingly, in the light of reported ligation reactions for biorthogonal ligation or bioconjugation, a non-exhaustive list of new reactions amenable to KTGS strategies will be proposed (Figure 2A), as well as clues to tune the kinetics of these reaction to increase their potential to meet the requirements for use in KTGS strategies.

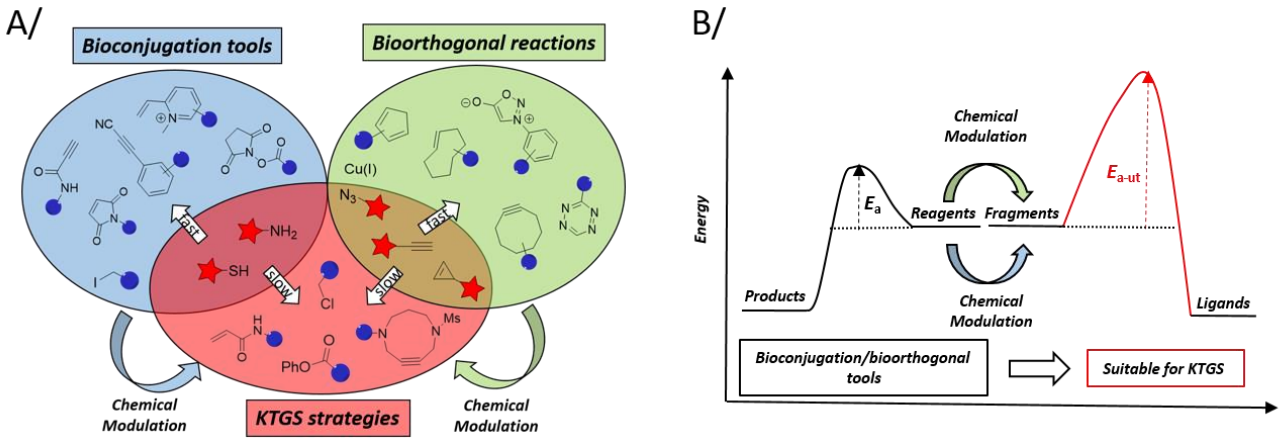
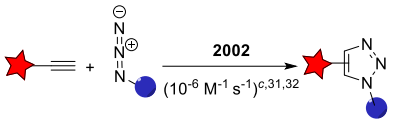
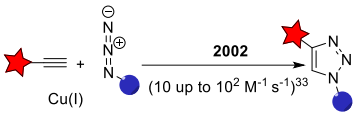
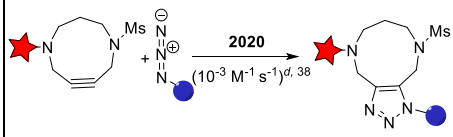
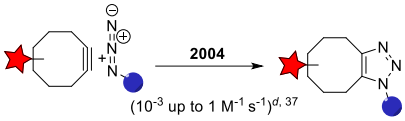
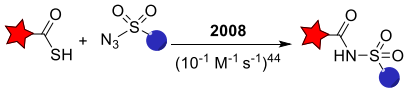
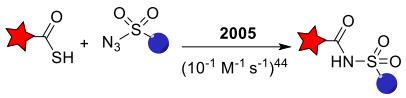
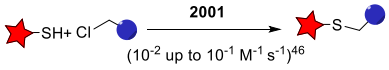
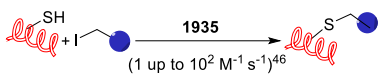
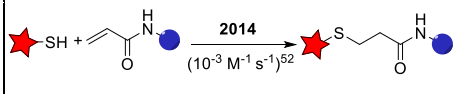
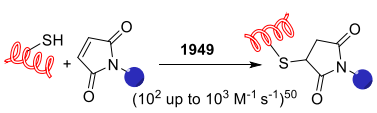


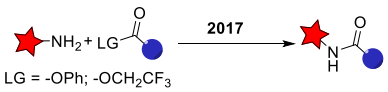
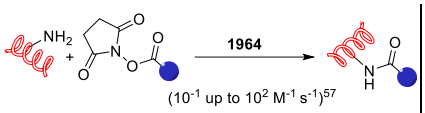
Figure 2. Bioorthogonal and bioconjugate reactions as a source of inspiration for KTGS ligations (A); Impact of chemical modifications of bioconjugation/bioorthogonal tools on their activation energy (E_a) in order to meet energy requirements ($E_{a\text{-}ut}$) of KTGS (B).

RETROSPECTIVE ANALYSIS OF LIGATION REACTIONS SUCCESSFULLY USED IN KTGS STRATEGIES AND THEIR CORRELATION WITH BIOORTHOGONAL AND BIOCONJUGATE LIGATION REACTIONS

A retrospective analysis of reactions developed for KTGS reveals that they all had their counterpart either in bioorthogonal chemistry or in the bioconjugate chemistry toolbox (Table 1). It should be stressed that all 2nd order rate constants given in Table 1 and 2 have been determined in the absence of the biological target mostly under aqueous-based conditions. These two sets of reactions are particularly attractive due to their high chemoselectivity and biocompatibility, as well as their ability to proceed under mild aqueous conditions.[32] However, KTGS relies on the use of reactions with dramatically slower reaction rates (up to 10^6 fold), with values generally in the range of ~10^{-2} to 10^{-4} M^{-1} s^{-1}. In order to reach this requirement, kinetics of bioorthogonal reactions or bioconjugation are altered either by the absence of catalysts (such as the Huisgen reaction), or by using akin less reactive chemical moieties, yielding less effective ligation processes, yet acceptable for KTGS applications through the proximity effect.

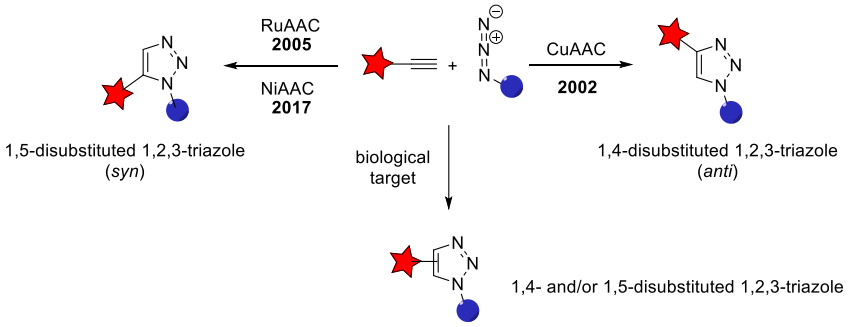
Table 1. Retrospective analysis of KTGS reactions and their related bioorthogonal or bioconjugation reactions. [a]

Entry	Reaction	Reaction used in KTGS (untemplated reaction 2nd order rate constant)[b]	Related bioorthogonal or bioconjugate reaction (2nd order rate constant)[b]	Chemical modification to tune the reactivity
1	Click chemistry			Removal of the catalyst
2	Strain-promoted 1,3-dioplar cycloaddition			Increase of the ring size and heteroatom-embedding of the cycloalkyne
3	Sulfo-click			No chemical modification
4	Alkylation			Decrease in the leaving group ability
5	Michael addition			Decrease in the electron density of the Michael acceptor

6	Amidation			Decrease in the leaving group (LG) ability of the activated carboxylic acid derivative

[a] The loop symbol is used with naturally occurring chemical functions, while the star and sphere symbols are used with synthetic functions. [b] Unless specified, rate constants were determined under aqueous-based conditions. [c] determined in aqueous formic acid at 40 °C. [d] Determined in CD_3CN at 25 °C.

KTGS related to bioorthogonal chemistry. It is worth noting that reactions in this section belong to the bioorthogonal toolbox and as such, are considered as universal KTGS reactions, as contrasted to bioconjugation tools that may induce side-reactions with biological targets. First, the copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of azides with alkynes (CuAAC) to yield corresponding 1,4-disubstituted (*anti*) 1,2,3-triazole products was developed independently by Meldal and Sharpless in 2002, as an easy-to-use ligation tool (Scheme 1).[33-35] Of note, 1,5-disubstituted (*syn*) analog scaffolds can regioselectively be formed in the presence of ruthenium (RuAAC),[36] or nickel (NiAAC)[37] catalyst. Accordingly, both regiosiomers are selectively synthetically accessible in high yields.



Scheme 1. Alkyne-azide 1,3-dipolar cycloaddition reactions.

In contrast, this reaction is slowed-down by several orders of magnitude in the absence of copper catalyst,[38-40] making it suitable for KTGS. This strategy was first introduced for KTGS by Sharpless and Finn in 2002 through the discovery of an acetylcholinesterase (AChE) noncovalent inhibitor having a dissociation constant in the femtomolar range.[9] In this case, the AChE generated predominantly the *syn* isomer in the sequestered region within the AChE gorge, while, our team observed a reversal of regioselectivity moving the reaction site toward the upper part of the gorge.[41] In the former example, the *anti* isomer not formed in the enzyme, was less active by more than one order of magnitude.[42] Importantly, triazole ring systems are also considered as amide bioisosteres, and as such, they may actively contribute to proteins binding.[10, 43]

A few years later, the development of the SPAAC (stain-promoted azide-alkyne cycloaddition), a catalyst-free version of this transformation involving more reactive cycloalkynes was reported by Bertozzi for uncatalyzed covalent modification of biomolecules in living systems (entry 2).[44] In fact, while both copper and copper-free strategies lead to the formation of triazole-containing products from bioorthogonal azides and alkynes, strained alkynes exhibit favorable second order reaction rate constants (up to 1 $M^{-1} s^{-1}$)[45] for bioorthogonal labelling, without the need for a cytotoxic copper catalyst. Very recently, a slower strain-promoted reaction, with a rate constant of 10^{-3} $M^{-1} s^{-1}$,[46] based on the use of a heteroatom-embedded cycloalkyne, 4,8-diazacyclononyne, proved amenable for KTGS. This approach enabled the templated synthesis of a recombinant 14-3-3ζ protein inhibitor.[47] Of note, the KTGS carried out with the

terminal alkyne analogue of the 4,8-diazacyclononyne derivative, proved unsuccessful. This comparative study illustrates the importance of extending the repertoire of ligation tools by tailoring the reaction rate constant.

The sulfo-click chemistry involves thio acids and sulfonyl azides yielding N-acyl sulfonamide linkers. It was first used as chemoselective ligation tool for peptide synthesis.[48-49,50] Shortly after, this amidation reaction was leveraged by Manetsch in KTGS for the *in situ* assembly of Bcl-X_L inhibitors (entry 3).[51] With a reaction rate constant recently determined of 10^{-1} M^{-1} s^{-1},[52] the sulfo-click reaction represents a rare example of KTGS reactions whose bioorthogonal counterpart was used without additional tuning of their reactivity. It is believed that this reaction is at the borderline in terms of kinetics to be used both in bioorthogonal chemistry (lower limit) and in KTGS applications (upper limit).

KTGS related to bioconjugate chemistry. Aside from bioorthogonal reactions, a greater number of KTGS reactions have their counterpart in bioconjugate chemistry. First, iodoacetamide is a well-known alkylating agent to block all free cysteine residues of proteins by reacting irreversibly with their sulfhydryl groups (entry 4).[53] While this reaction proceeds with high rate constants (10 M^{-1} s^{-1}), their chlorinated analogue reacts, in contrast, much more sluggishly (10^{-2}–10^{-1} M^{-1} s^{-1}).[54] The latter derivative was used by Huc in 2001 in the design of inhibitors of bovine carbonic anhydrase (CA II). However, due to the fast alkylation of thiols with alkyl chlorides, this strategy required competitive experiments involving one thiol and two different alkyl chlorides, in order to determine whether the biological target affects or not the relative proportion of the two formed thioethers.[55]

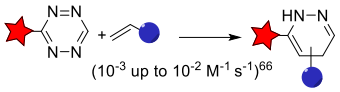
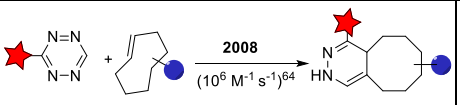
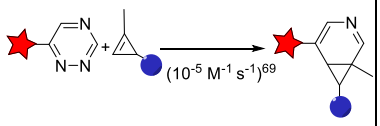
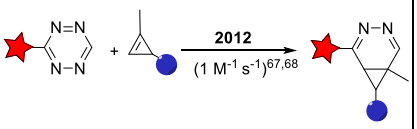
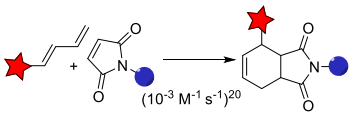
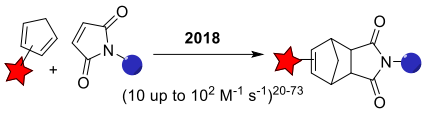
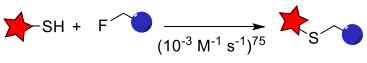
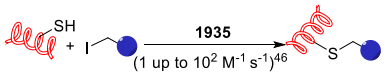
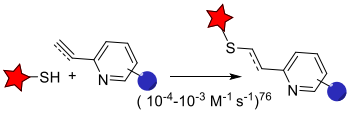
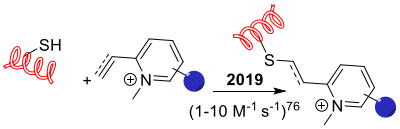
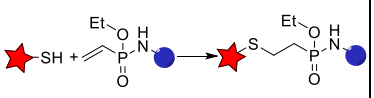
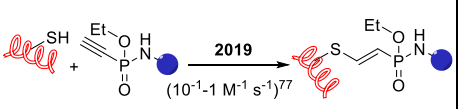
Thio-Michael addition of biothiols with maleimides is one of the most widely used bioconjugation ligation tool,[56] and prominent applications include the synthesis of marketed antibody-drug conjugates Adcetris (brentuximab vedotin) or Kadcyla (trastuzumab emtansine).[57] This reaction is characterized by high reaction speed[58,-59] and forms a thiosuccinimide adduct which has the propensity to undergo retro-Michael addition. However, by fine-tuning of the Michael acceptor, the reaction can be considerably slowed down (up to 10^5-fold) by using acrylamide partners,[60] forming an irreversible thioamide linker (entry 5). As a KTGS counterpart, an enzymatic hydrolysis/Michael addition cascade led to the formation of thioamide binders of m-AChE with IC_{50} values in the low and sub-nanomolar range.[61] This thio-Michael strategy was further illustrated by the discovery of bivalent kinase inhibitors by Soellner.[62] Interestingly, acrylamide moiety which was recently genetically encoded in *Escherichia coli*, has found to be stable in cells, despite the ubiquitous presence of free thiols.[60, 63] This important result opens new opportunities for the use of acrylamide moieties for KTGS in cells.

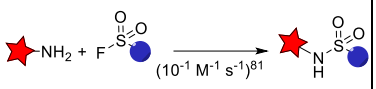
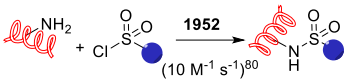
Finally, modification of proteins through the amine side chain of lysine residues is a popular method due to their high abundance on protein surfaces.[64] In this context, N-hydroxysuccinimide (NHS) esters are routinely used to form the corresponding amide bonds. As this chemical ligation may be too fast to be implemented in KTGS (10^{-1}–10^2 M^{-1} s^{-1}, entry 6),[65] protein-templated amidation reactions were carried out by Rademann with poorer leaving groups, displaying a lower electron withdrawing potency, namely phenol and trifluoroethanol, in order to suppress non-templated background reaction. Importantly, this approach enabled the identification of a superadditive inhibitor of factor Xa in the nanomolar range from fragments with millimolar affinities.[66] This was ascribed to the "linking effect" *i.e.*, to the minimization of the entropic penalty upon binding of fragments. Besides, this ligation also holds great promise since amide bonds are present in a large proportion of small-molecules therapeutics, partly due to their ability to be engaged in hydrogen-bonding interactions with biological targets.

CLUES FOR EXPANDING THE REPERTOIRE OF KTGS REACTIONS

The latter bibliographic analysis, which shows systematic correspondences between KTGS and bioorthogonal or bioconjugate chemistry, should open a new avenue for discovering a series of suitable reactions for KTGS. Accordingly, studies which report chemical modifications of existing biocompatible reactions that lead to a significant decrease of their reaction rate, should be thoroughly considered for KTGS. By way of examples, a selection of such chemical ligations belonging to different classes of reactions (cycloaddition, nucleophilic substitution, addition, etc…) have been identified from the literature. These reactions, proposed as eligible reactions for KTGS, are reported in Table 2.

Table 2. Perspective of KTGS reactions[a]

Entry	Reaction	Plausible reaction for KTGS (untemplated reaction 2nd order rate constant)[b]	Related bioorthogonal or bioconjugate reaction (2nd order rate constant)[b]	Potential chemical modification to tune the reactivity
1	Inverse electron-demand Diels–Alder			Use of unstrained alkene
2	Inverse electron-demand Diels–Alder			Decrease in the electron withdrawing character of the dienophile
3	Diels–Alder			Use of acyclic diene
4	Substitution			Decrease in the leaving group ability
5	Thiol-ene or thiol-yne			Use of non-quaternized pyridine
6	Michael addition			Use of less saturated electrophilic sytem

7	SuFEx			Decrease in the leaving group ability of the sulfonyl derivative

[a] [a] The loop symbol is used with naturally occurring chemical functions, while the star and sphere symbols are used with synthetic functions. [b] Rate constants were determined under aqueous-based conditions.

KTGS inspired from bioorthogonal and chemoselective ligation. Diels-Alder reactions possess many attractive features such as high chemoselectivities and biocompatibilites with several applications in bioorthogonal chemistry[67-68] and chemoselective ligations.[26, 57, 69-70] Besides, biological systems such as ribozymes, catalytic antibodies, and more recently natural enzymes were proved to be able to promote Diels-Alder reactions.[71] In particular, the inverse electron-demand 1,2,4,5-tetrazine-based reaction is a well-established bioorthogonal ligation reaction which proceeds with high second order kinetic rate constants (k ~ 1–10^6 M^{-1} s^{-1}) in the presence of strained alkynes/alkenes.[72-73] Although such extremely fast reactions are not suitable for KTGS, dramatic decreases in rate constants were observed with linear alkenes (k ~ 10^{-3} M^{-1} s^{-1}), thus reaching the chemical reactivity window of KTGS reactions (Table 2, entry 1).[74]

Similarly, tetrazines have found to react with “mini-tag” cyclopropanes very efficiently, illustrated by several biological applications (entry 2).[75-76] In contrast, less electron-deficient 1,2,4-triazines displayed theoretical rate constants with cyclopropanes that meet KTGS kinetics requirements (k ~ 10^{-5} M^{-1} s^{-1}).[77] It is worth noting that during the finalization of this article, Disney elegantly showed that tetrazine ligation was amenable to KTGS by using sterically congested cyclopropenes, which markedly slowed down the tetrazine-based chemical ligation process.[78] This study showed that r(CUG)^{exp}, a RNA repeat expansion that causes myotonic dystrophy type 1 (DM1), was able to template its own inhibitor with significant rate enhancement in comparison to the azide-alkyne strategy.[79]

Besides, the maleimide-based Diels-Alder reaction with cyclopentadienes,[80] is characterized by high reaction rates in aqueous systems (~ 10–10^2 M^{-1} s^{-1}).[26, 81] This chemoselective ligation was recently used for the production of robust antibody–drug conjugates.[82] In this context, recent studies from our group have shown that switching to linear diene such as *trans–trans*-2,4-hexadiene, the maleimide-based cycloaddition proceeded with a second-order kinetic rate constant in the range amenable for KTGS, namely 10^{-3} M^{-1} s^{-1}.[26] This experimental value shows that maleimide/hexadiene pair would be suitable for KTGS applications, in particular with biological targets that do not contain free cysteine residues, the latter being likely to react with maleimides.

KTGS inspired from bioconjugate chemistry. As shown previously, templated alkylation of thiols with alkyl chlorides requires competition assays since thiols readily react with such electrophiles in the absence of the protein target.[55] Alternatively, fluoroacetamides which have shown to react very smoothly with thiols (10^{-3} M^{-1} s^{-1}, entry 4),[83] would represent an effective background-free alternative that could be explored.

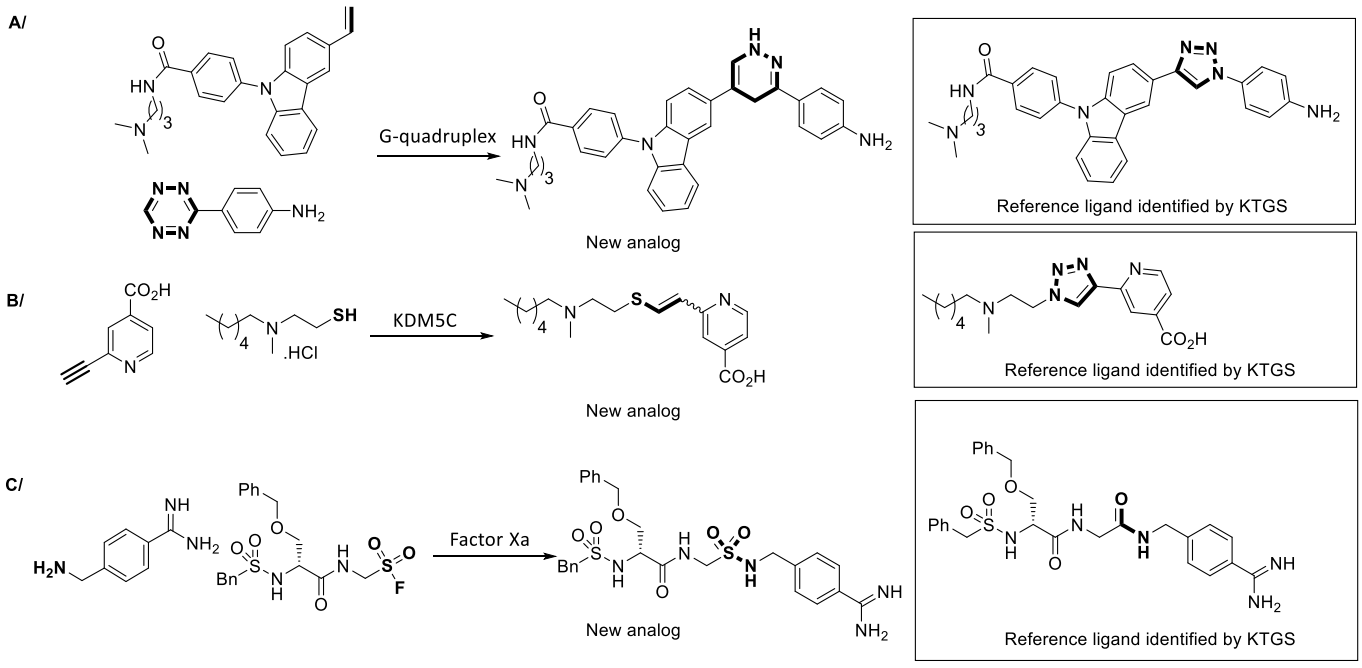
Besides, 2-vinyl/alkynyl pyridinium systems were recently identified as new bioconjugation tools by Bernardes for the fast and selective modification of cysteine-tagged proteins, with reaction rates in the range of 1–10 M^{-1} s^{-1} (entry 5).[84] This study also revealed that the addition of thiols was dramatically slower with 2-vinyl/alkynyl pyridine analogs, with reaction rates of 10^{-4}–10^{-3} M^{-1} s^{-1} that meet the requirements of KTGS.

In addition, in the context of the development of bioconjugation techniques, ethynylphosphonamidates have very recently shown to undergo a cysteine-selective reaction with proteins or antibodies (entry 6).[85] On the other hands, vinyl analogues, vinylphosphonamidates, proved far less reactive with thiol groups.[86] Accordingly, as illustrated with the KTGS developed Michael addition of thiols with acrylamides, such an additional reactive moiety could be investigated for KTGS experiments.

Finally, sulfonyl chlorides, introduced first by Weber in 1952,[87] have shown to react readily with amino groups present in proteins, such as for example the fluorogenic reagent dansyl chloride,[88] widely used for the labelling of biomolecules (entry 7). In contrast, sulfonyl fluoride analogues are more stable in particular towards hydrolysis, and react much more slowly with amines,[89] which makes them well suitable for use as warheads for activity-based protein profiling. Importantly, the S-F bond may be activated by hydrogen-bonding between the leaving fluoride ion and proximal residues in the active site.[90] Sulfur(IV)–fluoride exchange (SuFEx) of sulfonyl fluorides has emerged as a new generation of click chemistry.

Importantly, for these latter two examples, both phosphonamidates and sulfonamides are protease transition-state isosteres, as they are non-hydrolyzable functional groups capable of mimicking the tetrahedral transition-state intermediate of enzyme-based peptide bond hydrolysis.[91-92] As a result, KTGS based on phosphonamide or sulfonamide moieties are expected to provide a substantial "linkage effect" on the overall affinity of protease templated ligands.

Finally, as a proof of concept, these reactions could first be tested on biological targets that have already proved successful for KTGS, such as G-quadruplex, lysine demethylase 5C (KDM5C), or Factor Xa, by targeting close analogs of ligands which were already assembled by these biological targets (Scheme 2). As examples, the tetrazine ligation and thiol-yne addition reaction could be investigated with G-quadruplex[93] and KDM5C[94] respectively, to furnish analogues of triazole-based ligands recently highlighted by *in situ* click chemistry (Scheme 2A and B). On the other hand, SuFEx chemistry could be investigated in place of the amidation reaction, which was successfully investigated with Factor Xa (Scheme 2C).[14]



Scheme 2. Potential ligands and targets for proof-of-concept experiments, in regard with the ligands already unveiled through successful KTGS strategies.

CONCLUSIONS

A bibliographic analysis showed that reactions successfully used in KTGS have strong connections with preexisting fast bioorthogonal or bioconjugate chemistry reactions. However, rate constants of KTGS reactions require to be dramatically slower, generally in the range 10^{-2}-10^{-4} M^{-1} s^{-1}. It comes out that reaction rate of fast biocompatible reactions can be significantly tuned in order to meet kinetics requirements of KTGS transformations. In particular, this can be achieved by playing on different factors such as removing the catalyst, switching strained unsaturated systems by linear alkene or alkyne functions, or using less electrophilic/nucleophilic analogous chemical moieties. Several reliable electrophilicity/nucleophilicity scales are now available, and supporting theoretical studies can now be routinely implemented to supplement reported studies. However, it should be kept in mind that, in contrast to bioorthogonal and bioconjugate chemistry, the size of reactive functions matters in KTGS and should be also taken into consideration. In fact, bulky chemical functions may drastically disturb the binding mode of the parent fragments, and therefore negatively impact the affinity of resulting ligands. Furthermore, as many as possible, effective chemical processes should also be associated to new KTGS strategies. They will be useful to provide readily obtained authentic standards readily obtained in order to assay the success of the KTGS process, as well as to upscale the synthesis of ligands unveiled by successful KTGS experiments, in order to further study, and tune their biological activity.

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Notes

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