Self-Immolative Linkers Literally Bridge Disulfide Chemistry and the Realm of Thiol-Free Drugs

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The ultimate goal of controlled, intracellulardrug delivery is to get the drug to the target cell without spilling the contents in transit and then release the entire payload upon cell entry. One of the most powerful platforms to achieve this relies on the intracellular disulfide reshuffling as a trigger for drug release form the engineered prodrugs. However, utility of disulfide reshuffling for drug release is naturally applicable only to the thiol containing molecules—ultimately leaving nearly all commercialized drugs beyond the scope of this platform. This is a drastic limitation. A cunning new tool of organic chemistry is fast entering the mainstream of prodrug design: the self-immolative linkers. This platform allows overcoming the natural chemical barrier and makes it possible to link virtually any drug to its carrier via a disulfide bond and engineer a specific intracellular release. It is a game-changing accomplishment of modern organic chemistry. The scope and limitations of this novel opportunity for medicinal chemistry and nanomedicine are outlined.

The tools of nanomedicine continue to mature and develop enhanced opportunities in drug delivery. The overall goal of controlled, intracellular drug delivery using injectable formulations is to see it that the drug gets to the nominated cell, and two challenges are equally important: the drug should not be spilled in transit, and the drug should be liberated in full once inside the cell. There is one unique platform to achieve this, namely drug release triggered via intracellular disulfide reshuffling.[1,2] It has been widely used for stabilization of supramolecular carriers, such as hydrogel particles and capsules,[3] polyplexes[4,5] and polymersomes[6,7] but could not be applied for the design of prodrugs and polymer conjugates. Until recently, utility of disulfide reshuffling for drug release was naturally kept within the borders of thiol-containing molecules, ultimately leaving nearly all commercialized drugs beyond the scope of this platform. This is a drastic limitation. Over the last few years a cunning new tool of organic chemistry emerged and was fast developed to overcome this chemical barrier: the self-immolative linkers (SIL).[1,8] It is now possible to link virtually any drug to its carrier via a disulfide bond, and achieve a specific intracellular release. It is a highly important, game-changing accomplishment of modern chemistry. Disulfide reshuffling is a unique, universal platform for drug release. Now it is coupled with a universal strategy to use disulfide linkages, even for thiol-free drugs and materials. Herein we wish to outline the scope and limitations of this novel opportunity for medicinal chemistry and nanomedicine.

Self-immolative linkers are a class of spacers that spontaneously disintegrate via end-to-end decomposition or cyclization mechanisms.[8,9] The drug is conjugated to the proximal end of the spacer, whereas the distal end—the one that initiates the decomposition—is protected to prevent the disintegration of the spacer. Removal of the protective group (often termed a trigger) initiates the decomposition process. Self-immolation results in the scission of bonds at the proximal end of the spacer, with an end result being the release of the conjugated cargo (Scheme 1A). This chemical technology is a powerful tool for development of prodrugs, and the nature of the trigger can be rationally chosen to respond to a particular nominated stimulus – chemical or physical.[9] In a specific example of enzyme-responsive protecting groups (responding to esterases, proteases, phosphoesterases), SIL are employed to increase accessibility of the scissile bond to the enzyme thus significantly enhancing the rates of bioconversion of the prodrug into its respective active therapeutic.[10,11] Popular SILs used in medicinal chemistry are the trimethyl lock,[12,13] the 1,6-benzyl elimination linker[11] and the oxymethylene spacer[10] (**Figure 1**B). Importantly, SIL can be found in existing, successfully marketed drugs and antibody-drug conjugates.[10]

From a less explored perspective, SIL technology also presents an incredible opportunity to engineer additional, temporary chemistry into the conjugated cargo. For example, a diazo linkage cannot be cleaved by mammalian enzymes, but is degraded by the bacterial counterparts—enabling bacteria-triggered decomposition of the prodrug for, for example, drug delivery to colon.[14] As a diazo linkage is between two nitrogen atoms it would not be applicable to the nitrogen-free drugs. Using aminobenzyl alcohol-based SIL, this incompatibility may be overcome, and bacteria-mediated degradation of the diazo linkage with ensuing drug release can be engineered into nitrogen-free molecules.

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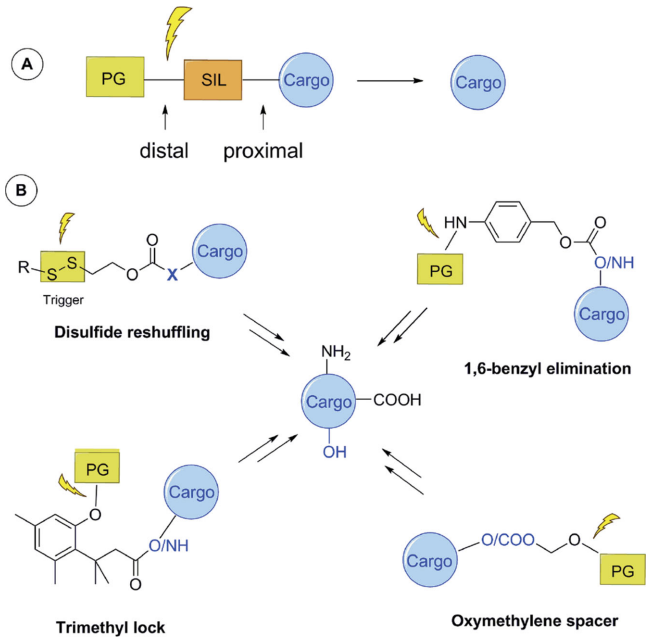

Figure 1. Schematic illustration of the concept of A) self-immolative linkers and B) examples of SIL. Removal of the protective group at the distal end of the SIL is followed by a fast removal of the SIL and release of the drug at the proximal end of the spacer. For details on disulfide reshuffling as a trigger for drug release, see Figure 2,3.

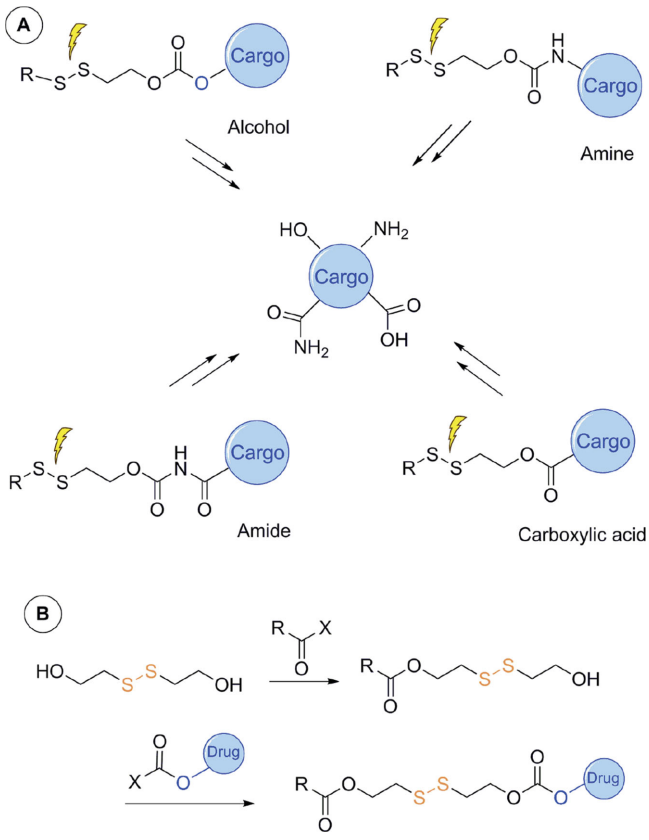

Figure 2. A) Generic chemical formulas for the SIL-containing prodrugs designed to release the drug upon cleavage of the disulfide linkage for diverse chemical functionalities (alcohol, amine, carboxylic acid, amide). B) Generic synthetic approach to the synthesis of the SIL-containing prodrugs which release the parent therapeutic upon disulfide reshuffling. X = Cl, *N*-hydroxysuccinimide, *p*-nitrophenol or other activating groups.

Intracellular disulfide reshuffling is a near-universal platform for intracellular drug release.[1,2] The overall red/ox potential in the human blood is oxidative, making disulfide linkages stable in circulation. In contrast, the intracellular milieu of mammalian cells is characterized by an overall reductive potential, and cytoplasm typically has millimolar concentration of glutathione (GSH), a natural tripeptide with a free thiol with reducing potential. Thiol-disulfide reshuffling is a spontaneous and a fast process, and through reshuffling with intracellular GSH the internalized disulfide-linked prodrugs undergo efficient intracellular decomposition.[1,2] However, the disulfide linkage is formed using two thiol groups, and this establishes a natural limit to the scope and utility of disulfide chemistry with regards to drug conjugation. Very few drugs have thiol groups. Yet with the use of SIL technology, the scope and utility of disulfide reshuffling for intracellular drug release becomes essentially universal, **Figure 2**A. Making it even more attractive, the synthesis of SIL linked to a disulfide bond is simple and in the majority of cases is accomplished in few synthetic steps, Figure 2B.

The main building block of the disulfide based SIL is mercaptoethanol—available commercially in its dimerized, disulfide-containing form. The two hydroxyls are both available for conjugation. To achieve SIL-assisted release of cargo, drugs are conjugated through the available functionalities. For the most established, hydroxyl containing drugs,[15–18] this can be achieved using a carbonate linkage using a suit of activated carbonates. While typically thiols cannot displace hydroxyls from a carbonate linkage, coupled to SIL, upon scission of the disulfide the thiol groups effectively release the conjugated drugs. The prodrugs may be symmetrical, that is, the two hydroxyls functionalized with identical drug molecules, or non-symmetrical in which case disulfide reshuffling releases two drugs, or a drug and an imaging reagent.[18,19] These opportunities were extensively reported by Jain et al. for a diverse range of therapeutic cargoes.[20] The approach is rather similar for amine-containing drugs, and the latter is contained within the prodrug via a carbamate linkage.[20,23] An interesting extension of this technology for materials design lies in that disulfide reshuffling can serve to recover the masked amine functionality, to create, for example, charge shifting/charge generating polymers.[21] Two less explored functionalities in the context of thiol-triggered drug release are carboxylic acids and amides, yet for these too, this platform allows the design of prodrugs which are degraded by the disulfide reshuffling.[20]

The mechanisms of drug release upon disulfide reshuffling are illustrated in **Figure 3**A. Reduction of the disulfide bond is a trigger, which generates a free thiol group with ensuing drug release. One mechanism includes the formation of a five-membered cyclic side-product and the free drug (mechanism 1). The other mechanism includes formation of the three membered cyclic side-product thiirane, carbon dioxide, and the free drug (mechanism 2). For carboxylic acid containing drugs, the latter mechanism is the only possibility. The formation of a three-membered thiirane is less favourable than the formation of a five-membered thiocarbonate due to ring strain, and drug

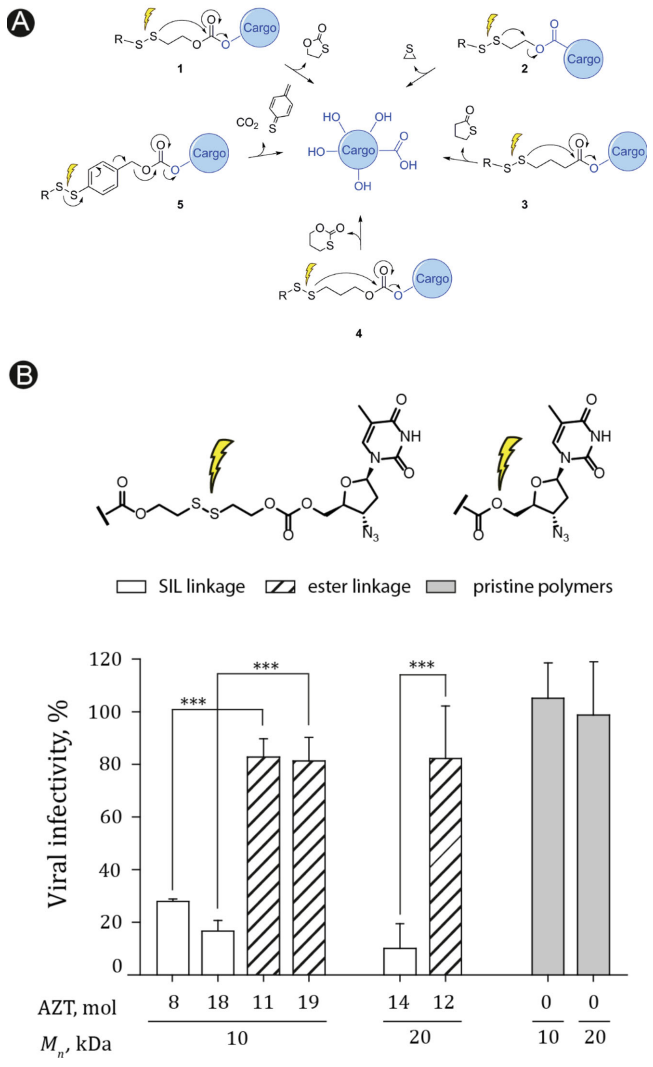

Figure 3. A) Schematic illustration of the mechanisms of SIL degradation and drug release triggered by disulfide reshuffling. B) Chemical formulas of AZT macromolecular prodrugs (MP) containing a disulfide / SIL linkage or an ester bond between the drug and the carrier polymer and experimental data on prevention of infectivity of human immunodeficiency virus by these MP. Carrier polymer: poly(*N*-2(hydroxypropyl methacrylamide)). Experimental data are reproduced with permission.[16] Copyright 2014, Royal Society of Chemistry.

release via this mechanism is therefore expected to be much slower. This has been proven in a release study where hydroxyl containing drugs are fully recovered faster than carboxylic acid containing drugs.[20] It was furthermore observed that carboxylic acid containing drugs release so slowly upon DTT exposure that metabolites containing a reformed disulfide between carboxylic acid prodrugs are seen. This has not been reported for hydroxyl containing drugs.

While the above designs are the most popular, variations of synthetic pathways are available and further broaden opportunities in the design of prodrugs responsive to the disulfide reshuffling. Thus, SILs can be designed such that the disulfide linkage is on the carboxylic acid side of an ester, and the SIL functions through intramolecular cyclization and formation of a 6-membered ring (Figure 3A, mechanism 3).[22] The disulfide trigger was also engineered onto *p*-mercaptobenzyl alcohol (much similar to *p*-aminobenzyl alcohol SIL shown in Figure 1) and applied for intestinal drug delivery—triggered by externally administered *N*-acetyl cysteine.[23]

To bring the SIL technology into the realm of nanomedicine, and specifically polymer-drug conjugates, one arm of the dimerized mercaptoethanol can be linked to a macromolecule. This can be achieved using a pre-synthesized polymer via a polymer-analogous reaction as shown by Yang et al. for camptothecin and hyaluronic acid.[24] Alternatively, SIL can be engineered into a monomer (e.g. acrylate or methacrylate) such that this can be further copolymerized using a suit of monomers with their own characteristics and properties.[16,17,25] The disulfide linkage proved to be stable under conditions commonly employed for controlled polymerization techniques, such as ring-opening polymerization,[25] RAFT,[15,16] and ATRP.[17] We applied this methodology to obtain macromolecular prodrugs of ribavirin (RBV) and azidothymidine (AZT) and obtained polymers in a range of molar mass and drug content up to 24% by weight.[15,16] Neither RBV nor AZT have thiols, yet using the SIL methodology, it became possible to engineer the release of drugs from their macromolecular prodrugs via intracellular thiol-disulfide reshuffling. We investigated drug release under a range of conditions, and showed that the SIL linkage is ultrasensitive to the presence of GSH: efficient and fast drug release was achieved even when the macromolecular prodrugs were mixed with diluted lysates of mammalian cells.[15,16] Owing to this, SIL-containing prodrugs were markedly more effective as antiviral agents as compared to their counterparts, based on an ester linkage between RBV or AZT and the carrier polymer.[15,16]

The above presentation aimed to illustrate a unique opportunity to install a universal trigger for intracellular drug release—a disulfide linkage—into thiol free drugs and materials. We strongly believe that this opens up incredible possibilities for biomedicine and drug delivery in particular. As a word of caution we do acknowledge that our private communication with multiple partners in the industrial drug development sector indicate a rather cold reception of the disulfide linkages as a platform for drug delivery—most commonly due to problems with materials aggregation and non-controlled disulfide reshuffling. Nevertheless, rectifying these problems is potentially highly rewarding and makes this research avenue highly deserving.

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