recognized. As a consequence of these mild conditions, sensitive functional groups that are generally not tolerated in related methodologies, such as hydroxyl, amino and halogens were all compatible. Although terminal alkynes were unreactive in the system, the methodology is compatible with a wide array of symmetrical diaryl and dialkyacetylenes, and with unsymmetrical aryl–alkyl alkynes. In the latter cases, high regioselectivity in favour of the alkyl-branched stilbene derivative was observed. All of the reactions occurred with high stereoselectivity for *syn*-hydroarylation (the two substituents of the alkyne are appended on the same side of the alkene product). Ackermann and co-workers also reported that activated terminal olefins, in the presence of a suitable oxidant, are a valuable alternative to alkynes.

Another impressive feature of the ruthenium-catalysed decarboxylative hydroarylations of alkynes is the compatibility with *ortho*-, *para*- *and meta*-substituted benzoic acids, as well as the parent benzoic acid. Therefore, *meta*- and *para*-substituted alkenylarenes can be accessed (depending on the substitution pattern of the starting benzoic acid), whereas previous functionalization/decarboxylation tandem processes were mostly limited to *meta*- substitution. Indeed, *ortho*-substituted benzoic acids have much lower decarboxylation activation energy due to a combination of steric and electronic effects[10]. Hartwig and Zhao achieved the selective formation of *para*-substituted alkenylarenes by the installation of bulky *meta*-groups on the benzoic acid, thus preventing cyclometalation at the most hindered position.

Although these exciting new findings further highlight the multifaceted nature of ruthenium catalysis, the mechanistic factors controlling carbon dioxide extrusion still remain to be clarified. Understanding the basic principles governing this remarkable reactivity will be essential in order to further exploit carboxylates as traceless directing groups in other C–H transformations with different electrophiles. Moreover, in order to make this strategy more appealing to chemical industries, more efficient ruthenium catalysts have to be developed in order to reduce the currently high catalyst loadings while shortening reaction times. ❐

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ANTIBODY–DRUG CONJUGATES

The missing link

The targeted release of bioactive molecules to diseased tissues has the potential to improve therapeutic efficacy, but not all drugs contain a free functional group that can be easily attached to an antibody. Now, a linker technology has been developed to enable the traceless release of tertiary and heteroaryl amine-containing drugs.

Tiago Rodrigues and Gonçalo J. L. Bernardes

Arming antibodies with therapeutic agents is becoming increasingly important in the development of new therapies. This approach enables the selectivity and targeting of antibodies to be used to control the delivery and distribution of a drug. However, despite the advantages of creating such antibody–drug conjugates, there are a multitude of challenges that need to be overcome, ranging from chemical synthesis and stability to pharmacokinetics[1]. Typically, payloads are conjugated via oxygen atoms (for example, camptothecin), or primary or secondary amines (for example, monomethyl auristatins). However, many drugs do not contain a suitable functional group to engage in conjugation to antibodies using these linkages.

Nature typically favours using carbon and oxygen atoms to build intricate architectures[2]; nevertheless, many natural products (along with the derivatives and pharmaceuticals based on them) contain heavily decorated nitrogen atoms that are difficult to functionalize further. Therefore — and to great frustration — conjugation is usually limited to all-too-similar drugs, natural products and their modified 'me too' structures, which limits the chemical diversity of antibody–drug conjugates. The discovery of new linkers, and self-immolative spacers that can dock and conditionally release a broader range of payloads from an antibody (or another carrier) is therefore important in the development of modern therapeutics. Furthermore, attaching a drug via a linker to an antibody can affect the solubility and other physiochemical properties. This can be detrimental as aggregation of antibody–drug conjugates can elicit an immune response and hamper the therapeutic index — a design aspect that is often overlooked.

Now, writing in *Nature Chemistry*, Thomas H. Pillow and co-workers at Genentech and WuXi AppTec address both of these challenges by developing several new linkers that offer new opportunities for conjugating drugs to a carrier antibody[3].

The team envisioned that the connection of a payload through a *p*-aminobenzyl quaternary ammonium salt (PABQ) could lead to traceless release of the drug via the self-immolation of PABQ. However, to control drug release and provide stability, they needed a trigger event to start the reaction. To provide this, the team inserted a cleavable moiety into their linkers. Two different linkers were developed based on protease cleavage of a valine–citrulline peptide, or reductive cleavage of a disulfide bridge. Both of these systems were shown to trigger the self-immolation of PABQ, and consequently the release of the drug (Fig. 1). Importantly, by using a

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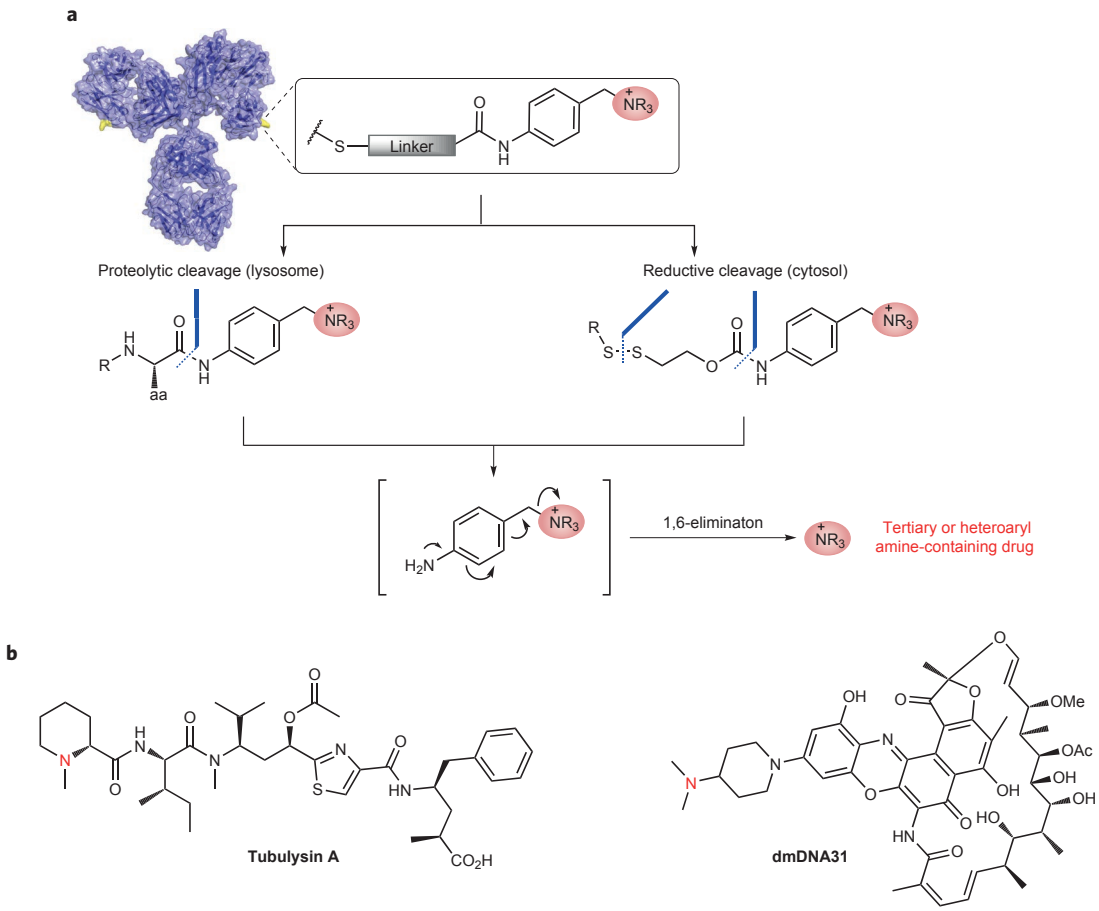

Figure 1 | Targeted delivery of tertiary and heteroaryl amine-containing drugs. **a**, Architecture of the antibody–drug constructs and enabling linker technology with self-immolation of the *p*-aminobenzyl moiety to the corresponding imine methide and free drug. **b**, Structures of the exemplary anticancer (tubulysin A) and antibiotic (dmDNA31) drugs used for proof-of-concept targeted release.

quaternary ammonium salt as the point of attachment, the new linker technology is capable of conjugating both tertiary and heteroaryl amine-containing bioactive molecules to antibodies. Furthermore, the incorporation of a positive charge into the designer linker also improved the physicochemical properties of the corresponding conjugates[3].

Drugs are attached to the linker via a benzylic ammonium species that is potentially labile; however, preliminary assays to test the stability of a model conjugate system under physiologically relevant conditions afforded remarkable results — no overnight cleavage of the benzylic linker was observed. In contrast, controlled drug release could be rapidly triggered from the construct via enzymatic cleavage of the linker by the protease cathepsin B. Optimization of the linker synthesis led to the valine–citrulline linker being produced using a protecting-group-free route that was compatible with payloads containing nitrogen atoms of varying nucleophilicities.

Next, the team demonstrated the synthetic robustness of the method through conjugation of several natural products and synthetic molecules, presenting either anticancer or antibacterial activities. Although conjugation of the drug auristatin E to antibodies through its monomethyl analogue is known to be a viable strategy, harnessing the anticancer potential of tubulysins has been an impossible task. This class of natural products is several-fold more potent than the compounds related to dolastatin 10 (including auristatins), but unfortunately chemical modification of tubulysins dramatically decreases their capacity to kill cells. For instance, abstraction of the piperidine-bound 'magic methyl' results in sharp loss of anticancer activity. Similar to another recent report[4], this new technology provides preliminary evidence that it may be used for the targeted delivery of tubulysins. Usefully, the positively charged nitrogen atom impacts tremendously on the solubility of the corresponding conjugate, decreasing the hydrophobicity by 2,000-fold, and opening new avenues to design conjugates with improved pharmacokinetics and therapeutic efficacy.

In line with the preliminary stability data for small-molecule constructs, antibody–tertiary amine and –heteroaryl amine conjugates were fully stable over seven days during circulation in mice. These data provide further evidence of linker stability in whole blood, and that any drug bleaching — which would counteract the specific target recognition provided by using an antibody carrier — is limited. The team also showed that a targeted anticancer application developed using this technology led to either complete regression or stasis, depending on the dose, in a human lymphoma tumour xenograft in mice. Similarly, a suitable antibiotic (dmDNA31) could be delivered and was shown to clear intracellular reservoirs of methicillin-resistant *Staphylococcus aureus* with great efficacy. The pharmacodynamic effect was postulated to result from the fine

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balance between lipophilicity and charge distribution. In a previous application[5] by the same group of researchers, an antibody–rifabutin conjugate was shown to be superior to vancomycin *in vivo*, suggesting that antibody–antibiotic conjugates are viable for enriching the current therapeutic armamentarium.

As the team recognize, this is not a universal solution for drug delivery and a more comprehensive number of drugs and protein carriers will need to be assessed. Despite the potential to enrich the current chemistry toolbox in chemical biology, there are several important pending questions, including the suitability of the technology to expand the tractable drug space and address unmet medical needs in other disease areas, such as inflammation and immunomodulation. Moreover, the imine methide by-product resulting from the linker self-immolation deserves a comprehensive toxicological study given its similarity to quinone methides, which are members of the so-called pan assay interference compounds[6] — a group of molecules containing dreaded substructures that are notorious for their uncontrolled polypharmacology and associated toxicity. That said, despite the nucleophile acceptor potential of the imine methide motif, and any possible toxicity profile, it may still be compatible with the delivery of drugs for a disease — for instance, if the therapeutic benefit is high, or if this approach enables a lower dose of a cytotoxic drug to be used. Nonetheless, this report represents a disclosure that promises to enable the inclusion of new drugs in the realm of antibody– and carrier–drug conjugates in general, is highly welcomed and will certainly spur further studies in the future. ❐

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2016 NOBEL PRIZE IN CHEMISTRY

Molecular machines


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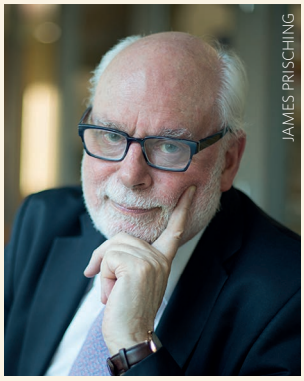
JAMES PRISCHING


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In what has been a giant leap forward for fundamental chemistry, researchers have spent the past two decades creating tiny machines that can perform tasks in response to external stimuli. These machines can synthesize or transport small molecules, and some have been shown to come together in large numbers to accomplish macroscopic work such as making objects bend, rotate or contract. In recognition of their pioneering efforts in this field, Jean-Pierre Sauvage, Fraser Stoddart and Ben Feringa (left to right) have been jointly awarded the 2016 Nobel Prize in Chemistry "for the design and synthesis of molecular machines".

The first real breakthrough came in 1983 when Jean-Pierre Sauvage, from the University of Strasbourg, devised a high-yielding metal-templated strategy to synthesize a catenane: an assembly of two molecular rings that are mechanically interlocked but can move freely with respect to one another. In 1991, Fraser Stoddart, now at Northwestern University, was responsible for the next major development: a rotaxane shuttle consisting of a macrocyclic ring that can move between two different 'stations' along the axle component on which it is threaded, trapped there by virtue of a bulky stopper at each end.

By creating entangled assemblies, Sauvage and Stoddart were armed with the tools they needed to build nanoscale machines — molecules with moveable parts that undergo reversible, positional displacements. The next step was to gain motional control, which they each achieved in 1994 when they introduced chemically distinct redox-active units into these systems and controlled the relative positions of catenane or rotaxane rings using electrochemistry. Between them they have since developed molecular muscles, logic gates, elevators and pumps, with Stoddart in particular having contributed significantly to the catalogue of available machinery.

Ben Feringa from the University of Groningen made significant contributions to the design of rotary molecular motors. In 1999 his team reported a molecule that possesses two blades that undergo 360° rotation in a single direction by photoisomerization of the double bond through which they are connected. Feringa has since introduced reversible directionality to his motor and increased the rotational frequency to over 12 MHz. His group also synthesized a molecule based on four rotors that can propel itself across a surface in a straight line in response to electronic excitation. The development of molecular motors has led to enormous progression in the field, with researchers now designing machines that function in high-energy states away from equilibrium — a state completely familiar to the biologist, but one that was relatively uncharted territory for the chemist.

VICTORIA RICHARDS

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