while in other cases, the enrichment ratio of the target protein changed as a function of the saturation of the protein with each enantioprobe.

The combination of multiplexed quantification enables the use of concentration gradients with the enantioprobes, thus providing a high-resolution map of the fragment–protein interactions in the global proteome. These data enable higher confidence in the selection of fragment–protein interactions for further structure–activity relationship studies for the development of new probes and therapeutics. Future developments that could help with the discovery of new ligands include the expansion of enantioprobes to encompass more complex molecules and isomers (that is, diastereomers, atropisomers) that would enable the discovery of additional small molecule–protein interactions. In addition, enantioprobes could potentially be used to evaluate function at the protein binding site, or deliver new ligands for molecular chimeras for use in new therapeutic strategies such as protein degradation technologies. Further application of enantiomers in mechanistic studies would likewise improve the identification of protein targets that may have been previously overlooked with a racemic probe — particularly in situations in which the measured proteomic interaction would be artificially reduced if the protein target possessed enantioselective interactions.

Despite the clear advantages of enantioprobes, some limitations still persist. Although the triage of selective fragment–protein interactions is more readily obtained using the enantioprobe pairs, the measured fragment–protein enrichment may arise from several factors in addition to the enantioselective interaction with the protein target. Perhaps most importantly, the photochemical conjugation event is dependent on the local amino acid environment, and while the chemical preferences of the diazirine functional group with biomolecules has not been concretely defined, the differential orientation of the photochemical group on binding of the enantioprobe to the protein target may affect the efficiency of covalent bond formation — although alternative cross-linking technologies may help to address this. Separately, the chiral recognition of each enantioprobe in the proteome propagates throughout the cell, potentially affecting the uptake, trafficking and thus availability of each enantiomer for the protein target at the time of photolysis.

Furthermore, interpretation of the enriched proteome would be more complex in instances where an enantioprobe interacts with a protein or complex at one or more binding sites. Careful interpretation and validation by complementary experiments including binding site measurement and competitive displacement are necessary to confirm the stereoselective interaction and achieve higher structural resolution from these data. Nonetheless, through incorporation of principles of chirality, Cravatt and co-workers expedite a powerful method to translate screening of small molecules into their more relevant biological systems, and open a path to future identification of a small-molecule probe for every protein target. ❐

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SYNTHETIC METHODOLOGY

Alkenyl boost for Catellani

The Catellani reaction is a multi-component cascade sequence, catalysed by palladium and norbornene, which typically uses aromatic starting materials. Now, through the use of a modified norbornene co-catalyst, the scope of this reaction has been extended to alkenyl reagents, enabling the preparation of all-carbon tetrasubstituted olefins.

Giovanni Maestri and Etienne Derat

Functionalizing C=C bonds is a challenging reaction due to the strength of the Csp^2–H bond (around 460 kJ mol^{-1}). The energy requirement for breaking this bond means there are far fewer reports on vinyl C–H activation compared to the activation of alkyl- and aryl- groups[1,2]. In particular, access to tetrasubstituted alkenes in a regiochemical fashion from an unfunctionalized precursor remains very challenging[3]. One route to form tetrasubstituted alkenes is by performing the difunctionalization of an alkyne instead. Alternatively, a more straightforward route would involve an alkene carbometallation that would install a reactive metal, which could later be used in various organometallic couplings. Now, writing in *Nature Chemistry*, an alkenyl version of the Catellani reaction has been demonstrated by Guangbin Dong and co-workers, which overcomes some of the limitations of the classical Catellani reaction[4] and enables the modular and regioselective synthesis of all-carbon tetrasubstituted olefins.

The Catellani reaction, discovered in the 1990's by Marta Catellani[5], is a catalytic reaction in which two key catalytic species are required: one is a low-valence palladium centre and the other is a norbornene. In the first step of the reaction, the palladium centre reacts with an aryl moiety through a classical oxidative addition pathway, but it is the second step that makes Catellani reaction so interesting. Norbornene inserts into the Pd-aryl bond and, thanks to its rigid geometry, hampers the usual β-hydrogen elimination reactivity that is a central feature of alkylpalladium complexes. This enables an aryl C–H activation step that generates a five-membered palladacycle that can undergo further reaction (Fig. 1a). In particular, it was demonstrated that this palladacycle can be involved in a second oxidative addition, leading to a Pd(IV) intermediate, even when employing weak oxidants such as aryl halides[6]. This Pd(IV) intermediate is very reactive towards

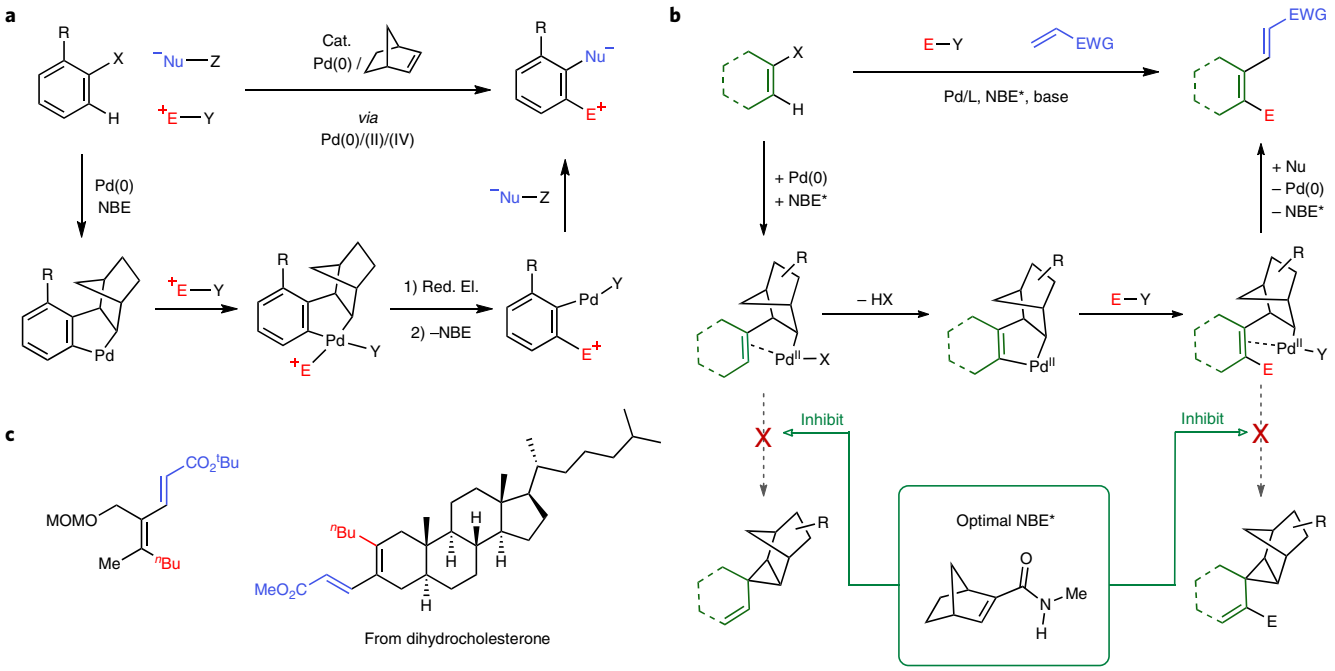

Fig. 1 | Variations on the Catellani reaction. a, The standard Catellani reaction, highlighting the passage through the various oxidation states that enable consecutive couplings. NBE norbornene, Red. El. reductive elimination. **b**, Alkenyl Catellani reaction reported by Dong and co-workers; the first step is identical to the standard Catellani reaction, but the modified NBE* inhibits β-hydrogen abstraction, allowing coupling with an electrophile. **c**, Selected examples of structures synthetized with the newly reported alkenyl Catellani reaction. Acyclic example on the left, and cyclic example derived from a ketone-containing natural product on the right. Credit: Panels b and c are adapted from ref. [4], Springer Nature Ltd

reductive elimination, thus enabling the rapid formation of C–C bonds, but also leaving a Pd(II) species after norbornene de-insertion that can be involved in a final reducing step to regenerate the metal catalyst (Fig. 1a).

The vast majority of studies reported in the first decade of this chemistry are related to the development of methods varying this final step of the cascade by introducing different nucleophiles[7]. In particular, Heck-, Sonogashira- and Suzuki-type couplings can be performed, or various C-, N- and O-centred nucleophiles can be efficiently reacted. More recently, several elegant examples have introduced the possibility of varying the type of reagent that reacts with the palladacycle intermediate[8]. This has led to the development of sequences in which the electrophile that reacts on Pd(II) can be an oxidized nitrogen atom, the carbonyl of an anhydride or a strained three-membered heterocycle, thus greatly expanding the panel of structures accessible through this reaction. The Catellani reaction enables the formation of complex products through multiple catalytic events, with a good control, as long as the coupling partners are carefully selected.

Now, the studies from Dong and co-workers extend the capabilities of the Catellani reaction by developing conditions that enable the reaction of alkenyl reagents (Fig. 1b). This is in contrast to the commonly used aromatic moiety, and comes with the challenging task of breaking strong vinylic C–H bonds. Success was achieved by using a modified norbornene partner containing a secondary amide, which is shown to have key effects on the catalytic cycle. One of the major (and previously observed) by-products of the Catellani reaction is cyclopropane, built from the norbornene double bond through an *ipso* functionalization. This side reaction can take place on multiple intermediates involved in these cascades, thus severely hampering the overall selectivity of the reaction (Fig. 1b, lower part). The study by Dong and team now shows that suitable elaboration of the organic co-catalyst architecture can shut down these side reactions. A variety of norbonenes were investigated, and while non-functionalized norbonene gave no formation of the desired product (instead forming the cyclopropanation product), the introduction of functional groups at the C2 position successfully steered the course of the cascade. Through careful screening of candidates, the best results are obtained using norbonenes containing monosubstituted amides. Although the peculiar role of the amine moiety is not yet understood, the beneficial effects could be due to the potential chelation by the amide that may prevent the intramolecular cyclopropanation.

During the course of their experiments, Dong and team discovered that the phosphine needed to stabilize the reactive palladium species was transformed into phosphafluorene. This means that using the catalytic conditions reported, P–aryl bonds can be activated, and this can be exploited through a C–H activation followed by P–C coupling. This observation is potentially interesting for a wide range of researchers, such as those using phosphines in organometallic catalysis and potentially observing similar reactivity, as well as those trying to develop more elaborate phosphorus compounds for various applications. This result shows the virtues of carefully looking at the actual nature of minor by-products.

This new extension of the Catellani reaction represents a large and useful expansion of the scope, and even complex natural products can be functionalized using this new strategy (Fig. 1c). The use of several reagents that can terminate the sequence has been demonstrated, enabling

the formation of families of functionalized dienes, bi- and tricyclic styrenes and vinyl-substituted stilbenes through the combination of different classes of reagents in the three-component cascade. These findings will surely promote exciting further developments. Indeed, for each novel class of reagents that are shown to provide a Pd(II) metallacycle with norbornene, a huge variety of potential new combinations can be envisioned[7,8]. Of particular interest would be the possibility of introducing these alkenyl reagents in sequences designed to form fused polycyclic frameworks, based on the promising variety of complex heterocyclic cores that would be readily accessible. In addition, the preliminary results highlighting P–C activation suggest that the preparation of highly-functionalized tertiary phosphines might also be a challenging and rewarding future target for Catellani sequences. ❐

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