Revealing the nature of thio-click reactions on the solid phase†

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Thiol- and yne-functionalized beads were manufactured in a simple microfluidic setup. While CuAAC and thiol–yne reactions were performed on yne-functionalized beads, 9 different thiol–X reactions were compared, in terms of kinetics and conversion, on thiol-functionalized beads.

The introduction of the “click” chemistry concept, focusing on constructing carbon–heteroatom linkages in a modular and highly efficient manner, revolutionized the way that research is being conducted in numerous fields. Long time enduring synthetic challenges are being overcome, supramolecules met novel building blocks, two huge homopolymers or polymers with peptides can be coupled, macromolecular rings can be closed, and DNA molecules are getting accustomed to ground-breaking functionalization approaches, just to mention some of the breakthroughs. Cu catalyzed azide–alkyne cyclo-addition (CuAAC) had been dominating the field of click chemistry till the exploration of the click characteristics of radical mediated addition of thiols to olefins (thiol–ene). Moreover, the click potential of several other thiol-based conjugations (thio-click) has been recently highlighted, thiol–yne being the main example. The click repertoire is broader than ever and an efficiency comparison of these reactions is of prime interest within this evolving field, which has been the starting point of this research.

Solid phase synthesis (SPS) was defined by Sharpless et al., as a process where “ordinary” reactions are employed under click conditions, due to the large excess of reagents used in the mobile phase and to the possibility of using washing as a simple work-up procedure. Since working with 5 to 10 equivalents is a common practice in SPS, making it very expensive by wasting a large amount of reagents, we believe that it is reasonable to compare a series of click reactions on the solid state by utilizing a small excess of reagents in the mobile phase. We therefore decided to start with a thiol-functionalized resin and screen the click efficiency of a large variety of reaction partners. With this approach, we aim for the introduction of novel ligation reactions for different applications such as catalyst or enzyme immobilization.

Fig. 1 (A) Making thiol and yne beads via thiol–yne chemistry and fluorogenic click functionalizations. (B) Light microscopy image of monodisperse thiol beads. (C) Fluorescent yne beads after clicking with (4).

We report an easy, straightforward approach to construct not only thiol- but also yne-functionalized monodisperse beads (thiol and yne beads), which will undergo several click-type reactions. As we reported, thiol–ene/ynel chemistry is a good alternative for making functional resins, both porous and nonporous. With only a tetra-thiol (1) and a di-ynel (2), we can make either a thiol or an yne resin (see Fig. 1A).

Desired functionalities were proven by IR spectra (see ESI†, Fig. S3), i.e. S–H stretch at 2566 cm⁻¹, C≡C stretch at 2115 cm⁻¹ and C–H stretch at 3280 cm⁻¹. It is important to note that the IR spectrum of thiol beads does not possess any of the alkyne peaks and vice versa. A simple tubing-needle based microfluidic setup is used to obtain monodisperse particles with sizes around 400 μm (see Fig. 1B). Monodispersity is important for reproducible functionalization, hence comparison. Initially, the reactivity of the beads was attempted to be visualized by making use of fluorogenic reactions. Fluorogenic molecules are indeed effective tools for reaction monitoring since they only become fluorescent after the desired reaction occurs, i.e. triazole formation. Whereas the yne beads reacted with 3-azido-7-hydroxycoumarin (4) (Fig. 1A) to give fluorescent beads (Fig. 1C), thiol beads did not react with another fluorogenic thiol probe (3).

After demonstrating the reactivity towards the fluorogenic azide, the yne-functionalized beads were exposed to another CuAAC reaction as well as to a thiol–yne reaction with similar compounds (see Fig. 2). An intrinsic difficulty of this comparison was the double addition of thiols to the alkynes while the azide reacts only once (Fig. 2). The long C12-chain of the
chosen reagents avoided the explosive character and unpleasant smell of small azides and thiols, respectively. Commonly practiced literature conditions have been employed for both reactions (Fig. 2), including continuous UV-irradiation for the thiol–yne setup. For each reaction, several batches of the yne beads have been prepared and the reactions have been stopped by washing the beads after the predetermined time intervals (30 min, 1 h, 2 h and 4 h).

The kinetics of both reactions were followed by IR. Both C≡C and C–H peaks were clearly degrading as a function of time as a result of both CuAAC and thiol–yne reactions (see Fig. S4 and S5, ESI†). The larger C–H (3280 cm⁻¹) signal was used for reaction monitoring after normalization by using the carbonyl peak at 1740 cm⁻¹. While the area ratio is generally the most appropriate choice for kinetic studies, the height ratio has been used in this case to have a comparable set of data with the results on thiol beads (see Table 1).

Fig. 2 compares the addition of 1-dodecanethiol (5) and 1-dodecanethiol (6) to yne beads. While thiol–yne reaches to near completion in about 4 hours, CuAAC consumes 60% of the yne peak height in 2 hours without further improvement for longer reaction times. The thiol–yne reaction appears to be superior to CuAAC in these conditions, without neglecting the fact that the thiol concentration was two times higher and UV exposure was necessary. Of course, other ligands can be utilized to improve CuAAC. It should be noted that a further proof of the CuAAC was the appearance of a C≡C peak at 1660 cm⁻¹ originating from the triazole ring (see ESI†, Fig. S4). The vinyl sulfide C≡C peak appears for the thiol–yne reaction (see ESI†, Fig. S5), which was unexpected since the second thiol addition is reported to be faster than the first one. Furthermore, alkyne–nitrile oxide and alkyne–tetrazine conjugations were skipped in this study due to the instability of the nitrile oxides and the necessity of extensive heating for the latter.

More interestingly, the thiol beads allowed the comparison of 9 different thiol–X reactions, which are listed in Table 1 including reagents and anticipated products. Together with a non-activated ene (7), norborne (8) has been used since it receives thyl radicals, the fastest among enes. Two Michael additions were also selected: phosphine catalyzed thiol–acrylate and amine catalyzed thiol–maleimide reactions. Thiol–bromide exchange, which is also becoming accepted as a click reaction despite the evolving HBr, has also been performed. Furthermore, we focused on two types of three-membered heterocyclic rings. It is surprising that the thiol–epoxy conjugation is recently popularized as being a click reaction instead of the thiol–aziridine reaction, although the latter was highlighted in Sharpless’ review. Finally, amine catalyzed thiol–isocyanate and isothiocyanate conjugations were added to our comparison. Thiol–yne was deliberately avoided due to the double addition. Two equivalents of click reagents, 0.05 eq. initiator (for radical mediated click reactions) and 0.1 eq. of the base catalysts were always used, except for the thiol–bromo reaction where 1 eq. NEt₃ was necessary for the evolving HBr. The thiol beads had a quite high theoretical loading of 2.07 mmol g⁻¹ compared to the common resins used for peptide synthesis (experimental value: 1.75 mmol g⁻¹, see ESI†). With such a high thiol loading, the use of only 2 eq. of the click reagent and 0.1 eq. of the respective catalyst, the click status of these 9 reactions should be revealed, at least for the solid phase reactions. No heat, shaking or deoxygenation were applied, however UV light treatment was necessary for the radical-mediated thiol–ene reactions.

Fig. 3 compares the kinetics of the 9 thiol–X reactions, many of which being already accepted as click reactions. As mentioned above, we utilized the height depletion of the thiol peak (IR), normalized to the carbonyl peak of the resin, since area normalization was not possible anymore where a new carbonyl peak was being formed or carried by the clicking reagent. From this figure, it becomes clear that the thiol–isocyanate reaction is the fastest conjugation in our particular conditions, reaching up to 72% thiol peak height consumption in only an hour, which is extraordinary for such a highly loaded resin. Almost full conversion was reached in about 8 h. The UV-stimulated thiol–norborne reaction also reached high conversions in about 4 hours, although it started slower.

Table 1 Overview of thiol–X reactions performed on thiol beads
In conclusion, it was demonstrated in this communication that novel, high loading monodisperse alkyne or thiol functionalized beads can be easily manufactured by a tubing-needle based microfluidic setup via thiol–yne chemistry. Their ability to serve as ligation platforms has been demonstrated in a comparative manner. The yne beads react either with an azide or with two thiols. Thiol addition is found to be faster than the triazole formation with the continuous UV irradiation for the former being the penalty to pay. On the other hand, 9 different thiol–X reactions have been compared on the thiol beads. The thiol–isocyanate reaction is the fastest, being closely followed by the thiol–norbornene reaction. The full comparison is as follows: isocyanate > norbornene > acrylate ≈ isothiocyanate > maleimide ≈ isolated ene > α-bromo ester > epoxide ≈ aziridine. The kinetics of the reaction is found to be more dependent on the reactivity of a compound than on the class it belongs to or on the mechanism of the addition. This comparison may be the first step to guide researchers, especially in the field of SPS, to choose the most suitable ligation reaction according to their needs since a vast number of conjugations are recently being claimed to be part of the click family.

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Notes and references