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From the molecule to the mole: improving heterogeneous copper catalyzed click chemistry using single molecule spectroscopy†

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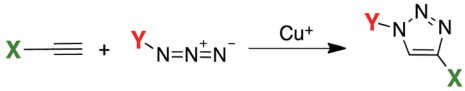
Single molecule spectroscopy (SMS) inspired the optimization of a heterogeneous 'click' catalyst leading to enhanced yields of the Cu-catalyzed reaction of azides with terminal alkynes. Changes in SMS data after optimization confirm the improvements in catalyst performance.

Advanced microscopy techniques, including single molecule spectroscopy and super-resolution techniques have led to spectacular advances in our ability to monitor reactions under conditions where the most intimate mechanistic details can be revealed.[1–3] 'Seeing' a single molecule, particularly as it undergoes chemical reaction, may have belonged in the realm of science fiction as we moved into the new millennium, yet, today this can be done in carefully designed systems. Biologists were first in recognizing the power of these tools in the understanding of cellular processes, where the sensitivity and spatial resolution of modern techniques offered an unprecedented level of information.[4–7] Chemists have been somewhat slower[8] in discovering how these microscopy tools can help them, although a number of groups have made excellent progress in this direction.[9–18]

In our group we have published several contributions, sometimes labelled as '*from the mole to the molecule*' where we take advantage of advanced microscopy to visualize single molecules as they reach and react at single catalytic sites and depart once the process is complete.[19–24] Yet, it is really '*from the molecule to the mole*' where the real advantages in catalysis and organic chemistry can be expected. In other words: can we use knowledge acquired using single molecule techniques ('*the molecule*') to improve processes at the bench and manufacturing level (the mole)? In this communication we report one example as a proof of concept where single molecule techniques inspired laboratory work that led to a dramatic ($>\times10$) improvement of the click reaction at the bench level.

The formation of 1,2,3-triazole by copper(I)-catalyzed cyclization between azides and terminal alkynes (CuAAC) is among all the click chemistry reactions the best known and widely used (Scheme 1).[25,26] Few examples of Cu-based heterogeneous catalysts have been reported in the past,[27,28] in order to avoid incorporation of toxic copper complexes in the end products. We have recently published the first example on a heterogeneous Cu photocatalyst that can generate Cu(I) species on demand.[29] Previously in our group, we were able to demonstrate, using Total Internal Reflection Fluorescence Microscopy (TIRFM) and a system suited for single molecule spectroscopy (*vide infra*), that click chemistry catalysed by copper nanoparticles is a truly heterogeneous process.[30] Later work at the single-molecule level, performed with a commercial copper-on-charcoal (Cu@charcoal) catalyst with 3% of Cu loading and particle sizes in the tens of micrometers indicated that only 0.003% of the catalyst surface was active in click chemistry.[23] Cu@charcoal is known as a very good catalyst, although these results suggest there is plenty of room for improvement. Here we present the first attempt to use catalysis studies at the single molecule-single catalytic site level to guide work at the bench scale.

Inspired by the previous results about the low efficiency of Cu@charcoal at the catalytic site level, we decided to try to improve the catalyst efficacy by using different catalyst pre-treatments. XPS analysis of this catalyst (Fig. 1) reveals the presence of CuO species, as confirmed by the characteristic satellite peaks of Cu(II) between 945–940 eV.[31] Thus, the reason of the low catalytic efficiency found at single molecule level could lay on the deficit of Cu(I) centres and



Scheme 1 Formation of 1,2,3-triazole by copper(I)-catalysed cycloaddition between azides and terminal alkynes (CuAAC).

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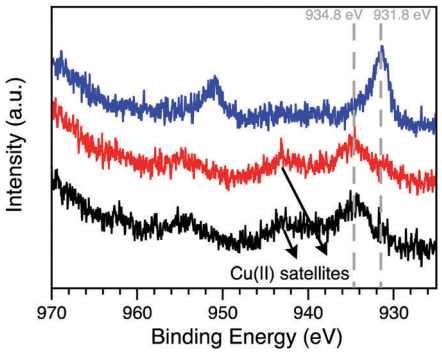


Fig. 1 Comparison between Cu 2p HR-XPS spectra for Cu@charcoal untreated (black) and treated by method 1 (red) and method 2 (blue).

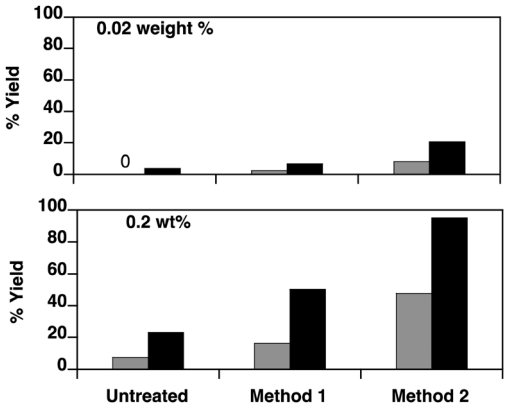


Fig. 2 Yields obtained for reaction in Scheme 2 using different concentration of catalysts: 0.02 wt% (top) or 0.2 wt% (bottom) of untreated and pre-treated Cu@charcoal catalyst after 3 h (grey) and 12 h (black) of reaction.

therefore pre-treatment strategies involving reduction of the material were studied.[32] Briefly, method 1 involves sonication of the material in ethanol for 2 h while method 2 uses a solution of $NaBH_4$ in ethanol during sonication. After treatment, the catalyst was tested in both bench and single molecule scale to evaluate its efficiency. The XPS analysis (Fig. 1) shows almost no changes in the nature of the Cu species after the catalyst is treated by method 1. Instead, more reduced Cu species are found when treated by method 2, as can be appreciated by the shift toward lower energies in the Cu $2p_{3/2}$ region, suggesting Cu(I) or Cu(0) species are now predominant in the material (differentiation between Cu(I) and Cu(0) is not accurate by this technique).[33]

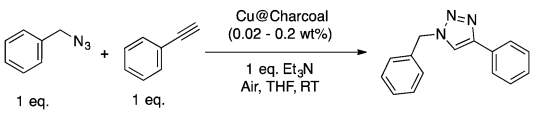
Once treated, the catalysts were tested at the bench level using the reaction shown in Scheme 2. Fig. 2 shows the yields of the reaction ran under the same conditions using untreated and treated Cu@charcoal by method 1 and 2. Clearly, the pre-treated catalysts have improved their efficiency over the untreated one. At least 10 times improvement in the catalytic activity is reached when the catalyst is pre-treated with $NaBH_4$, and is attributed to the presence of more reduced Cu species (Fig. 1).

These results illustrate how data from single molecule microscopy can be used to inspire and guide experiments in the laboratory that lead to dramatic improvements of the catalytic process with modest effort. The improvements observed at the bench scale could, in theory, be reproduced by single molecule microscopy. However, it is important to note that single molecule experiments are usually done in the 10^{-10} to 10^{-11} M concentration range, while bench experiments were typically around 0.3 M. In addition to the 9 or 10 orders of magnitude change in concentration, product formation always involves interaction of two organic molecules (azide and alkyne) and one catalytic site, albeit one of them (the alkyne) pre-associates with the catalyst.[30,34–36]

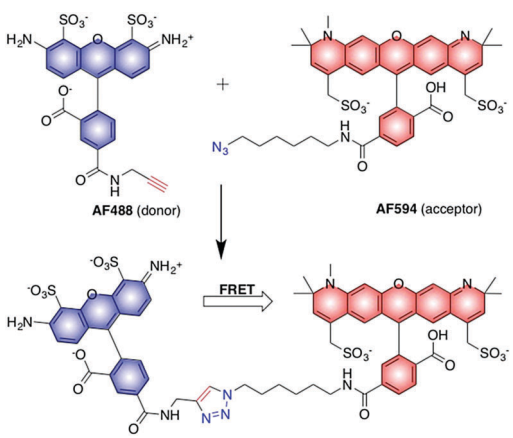
TIRFM studies would give more insights of the improvement at the catalytic site level but different reagents need to be chosen in order to follow the reaction by this fluorescence spectroscopy technique (Scheme 3). The reagents were carefully selected in order to use the Fluorescence Resonance Energy Transfer (FRET) as a probe for successful reaction, and are the same that proved suitable in an earlier publication.[23] Thus, both functional groups required for click chemistry (alkyne and azide) are attached to a dye, AlexaFluor488 (AF488) and AlexaFluor596 (AF596), respectively. AF488 acts as a donor chromophore, capable of transferring energy in a non-radiative process to the acceptor chromophore, AF596.

The efficiency of this FRET is inversely proportional to the sixth power of the distance between donor and acceptor and therefore at the concentrations of ~100 pM used FRET is expected when they are part of the same molecule after reaction as shown in Scheme 3. The selected dyes show absorption and emission spectra overlap that fit with the requirements of selective excitation of the donor and selective detection of the acceptor emission (see Fig. S1, ESI†).

During TIRFM experiments the FRET events can be detected as a burst in the intensity of the emission recorded *versus* time (bursting events), Fig. 3. Many fluorescence trajectories showed multiple bursting events, indicative of localized reaction, a typical indication of heterogeneous chemistry,[30] Fig. 3. However, we noted that most of these emission events did not occur where



Scheme 2 CuAAC used at bench top experiments.



Scheme 3 Click reagents employed for click chemistry with fluorescent reporters. The product likely undergoes to FRET.

the relatively large carbon particles were detectable using optical microscopy, as they had been in earlier work[23] with untreated catalyst, in fact, events shown are dominated by the population of small particles.

Examination of the treated and untreated catalyst samples using Scanning Electron Microscopy (SEM) revealed that in addition to the very large particles on which we reported single molecule studies earlier, there were numerous particles with sizes of 100 nm or less, as illustrated in Fig. S2 (ESI†) and noted in earlier work.[23] Particles of this size are not visible by optical microscopy, but they provide a straightforward rationalization for the observation of bursting at locations where the large (micrometer) particles are absent. It is likely that these particles are also responsible for increased light scattering compared with large or colloidal particles examined before. This is likely the reason for the increased background level noticeable in Fig. 3 compared to those in earlier reports.[23,30]

With treated catalysts there are several possible ways to analyse the data. The simplest, albeit rather qualitative approach consists in counting successful events, such as those illustrated in Fig. 3 and adding them up for each type of catalyst. For this purpose a trace that shows two events is counted as two instances of success, and similarly for any occurrence of multiple events. Here it is important to note that successful events are those that show fluorescence bursts within a 50 s recording of 500 images over an area of 34.5 μm × 34.5 μm. There can be other catalytic sites that while active showed no successful catalytic events (*i.e.*, fluorescence bursts) within the 50 s recording (see ESI† for Poisson type analysis). We note also that while identical procedures are used to prepare samples of different catalysts this does not ensure that the number of sub-diffraction particles deposited (and thus not observable by transmission optical microscopy) are identical in the area monitored. Fig. 4 summarizes these results. Note that with the untreated catalyst (and treated by method 1) triple events were rare, and their frequency was only ~3% among all the events detected,

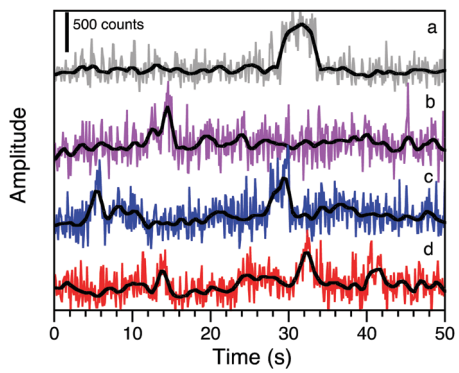


Fig. 3 Intensity trajectories showing multiple bursting events within the 50 s (500 frames) recording time of the video (excitation wavelength: 488 nm; emission filter: 575 nm long pass). Traces a (single event), b (single or double events) and c (double events) have been selected to be truly representative of the many traces analysed. In particular, b and c illustrate the challenges establishing the number of events. Trace d is one of the few rare cases where 3 events are clearly distinguishable. The black line is a 4-point smoothing function and is provided for visual guidance and is not strictly required to decide on the number of bursts per trajectory.

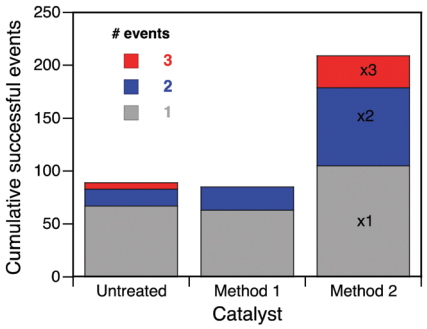
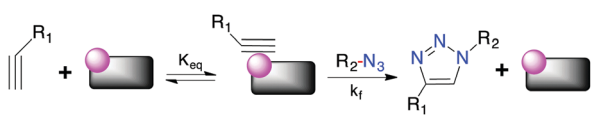


Fig. 4 Cumulative successful events from analysis of an average of 4 videos per catalyst. Different colours correspond to intensity trajectories showing single event (grey), double events (blue) and triple events (red).

and their numbers are too small for meaningful statistical analysis. It is evident that the number of successful events increased upon reductive treatment, in particular with $NaBH_4$ (triple events frequency ~7%). Although the improvement factor reached at the bench scale (10× or greater after treatment with $NaBH_4$) is clearly not the same at the single molecule level; these results are not surprising given the reaction order and the drastic decrease in the reactant concentrations used for regular single molecule studies (*vide supra*). No significant differences are found at the single molecule level when untreated catalyst is compared with the catalyst treated with ethanol (method 1), as can be noticed in Fig. 4. These results can account for the less drastic improvement (~2× or less) found at the bench scale (Fig. 2). A simple, semi-quantitative, approach to this issue is to consider the reaction as reminiscent of a Michaelis–Menten mechanism as illustrated in Scheme 4. The initial alkyne association equilibrium (K_{eq}) likely is displaced largely to the left under single molecule conditions (*i.e.*, sub-nanomolar concentrations), while at the high bench concentrations its likely totally converted to the alkyne–Cu@charcoal complex and thus at some point further increases in concentration are not reflected in the overall kinetics. Additionally, flow systems are a convenient tool to keep the local reactant concentrations as low as possible and minimize catalytic site poisoning.

The clearest indication of the improved catalyst is that in Fig. 4 about 50% of the bursts (*i.e.*, catalytic events) occur in trajectories showing multiple events, while in the untreated sample this fraction is reduced to about 20%. Such analysis is independent of possible deposition differences for different catalysts. Thus, not only the number of catalytic events changes, but their distribution of single and multiple events also changes. Looking at the fraction of single and double events observed with treated catalyst, a Poisson distribution analysis (see ESI†) predicts that triple events



Scheme 4 Schematic representation of the catalytic click reaction resembles the Michaelis–Menten mechanism, except for the need of the participation of a second reactant (the azide) in the final step leading to products.

will be rare, just as observed. Further, this statistical analysis suggests that for $NaBH_4$ treated samples about 28% of viable catalytic sites show no activity during any 50 s video recording.

It is worth noting that signal amplitude in this and previous work are similar, the background level is higher in this work, presumably due to significant increases in light scattering due to particles of $\sim$100 nm size. Some of these low signal-to-noise bursts were likely present but not observed in earlier work with large Cu@charcoal particles. Reductive treatment also made their presence evident. Exploratory studies included in the ESI† suggest that treated catalysts show excellent reusability.

The results presented in this communication demonstrate that single molecule techniques can be an exceptionally powerful technique to inspire and guide improvements in organic chemistry, in particular, as illustrated here for heterogeneous catalysis. Single molecule techniques also proved useful in verifying at the single molecule-single catalytic site level the origin of improvements at the bench. Combined they suggested imaging experiments, such as SEM (see Fig. 4) which assisted the rationalization of the data.

The same tools that mechanistically allow the transition '*from the mole to the molecule*' also inspire and guide changes at the bench that we refer to as '*from the molecule to the mole*'. The paradigms of organic chemistry need to view advanced microscopy as a practical, commercially available component of the organic chemistry toolkit.

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