Supporting Information for

From the Molecule to the Mole: Improving Heterogeneous Copper Catalyzed Click Chemistry using Single Molecule Spectroscopy

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Reagents

All chemicals were purchased from Sigma-Aldrich or Fisher Scientific with the exception of AlexaFluor^{TM} 488 alkyne and AlexaFluor^{TM} 594 azide, which were purchased from Molecular Probes (Life Technologies). Nanoparticulate 3 wt% copper in charcoal (Cu@Charcoal) was purchased from Sigma-Aldrich. All chemicals were used as received without further purification.

Instrumentation

TIRFM Image acquisition Fluorescence Imaging was performed with an Olympus FV1000 TIRF system. The Instrument is equipped with a CW Laser (488 nm Ar laser) and an EM-CCD Rolera EM-C2, QImaging) Controlled using QCapture Pro 7 software (QImaging) with exposure time set at 100 ms per frame. The laser beam was collimated and focused through a fiber-coupling unit. A beam splitter cube with a 488 nm dichroic filter, a 482/18 nm excitation filter and a 575 nm long pass emission filter (Semrock) was used to reflect the excitation light into the oil immersion TIR (total internal reflection) objective (100X, NA1.45, Olympus, PLAPO). NMR spectra were recorded at room temperature with a Bruker Avance 400 equipped with the Bruker Automatic Sample Changer (B-ACS) 60 and chemical shifts were referenced to tetramethylsilane (TMS), the internal standard for calibration in deuterated chloroform. Scanning Electron Microscopy (SEM) images were acquired at the University of Ottawa's Centre for Catalysis Research and Innovation (CCRI) with a Jeol JSM-1600 SE microscope. SEM samples were prepared by drop casting a water suspension of catalysts onto 400 square mesh carbon coated copper grids (Electron Microscopy Sciences). Particle sizes were determined with ImageJ analysis of SEM images. X-ray photoelectron spectroscopy (XPS) was recorded using Kratos analytical model Axis Ultra DLD instrument, using monochromatic aluminum Ka X-rays at 140 W. XPS samples were prepared by placing the solid samples on a 1 cm x 1 cm silicon wafer. XPS data were analyzed using CasaXPS software (Version 2.3.15). The binding energies in the XPS spectra presented in Figure 1 were calibrated by referring that of C 1s (284.8 eV). All fittings were obtained using a Gaussian/30% Laurentian analysis with a Shirley baseline.

Catalyst pretreatment

The commercial nanoparticulate 3 wt% Cu@Charcoal was weighted (200 mg), suspended in suspended in 50 mL of ethanol HPLC grade and subjected to sonication for 2 h (Method 1). Method 2 was performed suspending the catalyst in 50 mL of an aqueous $NaBH_4$ solution (10 eq.). The materials were filtered and dried under vacuum overnight prior to further use.

Bench scale experiments

The study of the catalytic efficiency of each of the three catalysts was performed using different amounts of catalyst (Cu: ~ 0.01 to 0.8 wt%). In general, 1.4 mg of Cu@Charcoal were dispersed in 1.5 mL of THF in a test tube and then the correspondent amount of each reactant (azide (0.45 mmol), alkyne (0.45 mmol), triethylamine (0.45 mmol)) was added. The reaction mixture was stirred at room temperature and under air for up to 12 h. The solid catalyst was separated by filtration and the pure product was obtained after vacuum evaporation. Caffeine was added after reaction and prior to analysis to be used as external standard (∂: 3.58 ppm). The yields were calculated by ^1H NMR in $CDCl_3$ using the signal related to the H in the 1,2,3-triazole ring. The same protocol was followed for every different catalyst concentration.

TIRFM sample preparation

Glass coverslips (25 mm circular, Fisher Scientific) were cleaned by soaking in piranha solution for 30 minutes followed by thorough washing with MilliQ H_2O, then dried with argon and baked at 120°C for 5 minutes. The clean, dry coverslips were then placed in a solution (4% in toluene) of 3-aminopropyltriethoxysilane (APTES) and agitated on an orbit shaker (Lab-line Instruments) for 90 min, followed by successive washing with toluene, acetone, ethanol and MilliQ water. APTES treated coverslips were stored in MilliQ water until use.

Cu@Charcoal samples were prepared by spin coating at 3000 rpm 2x50 μL of a suspension of the supported catalyst in EtOH (1 mg/mL) onto piranha cleaned glass coverslips. Catalyst coated coverslips were placed in a flowcell (Live Cell Instrument, Chamlide Model CF-S25-B) above the objective of a TIRF enabled inverted-microscope for imaging. Reagent solutions used for imaging were prepared by diluting as received solutions of AF488 alkyne and AF594 azide with MilliQ purified water and adding triethylamine to a final concentration of 100 pM AF488, 100 pM AF594 and 10 nM Et_3N. A syringe pump was used to flow this solution at a rate of 0.5 mL per hour over the catalyst in the flow-cell positioned over the TIRF Microscope objective.

TIRF image analysis

Analysis of TIRFM image sequences (500 frames/image sequence, exposure time per frame: 100 ms) was carried out using a combination of ImageJ (NIH) and MATLAB (MathWorks) software. In brief, 3x3 px regions of interest (ROIs) were selected based on the automated identification of stochastic emission representing the formation of 1,2,3-triazol group. After background subtraction was performed with ImageJ (rolling ball algorithm) an in-house written MATLAB script developed in our group[1] was used to localize the bursting events in the image sequence. ImageJ was then used to measure the mean fluorescence intensity inside each ROI for every frame in a 50 s image sequence and the burst traces were examined graphically later on.

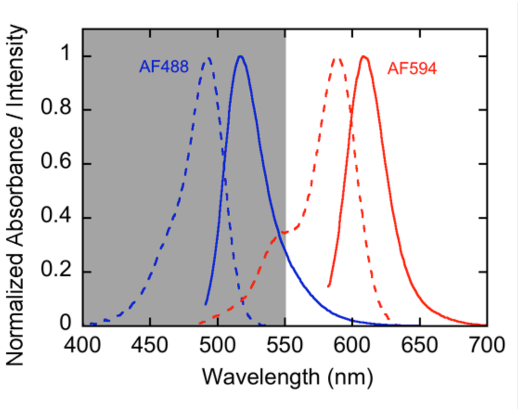

Figure S1. Normalized absorption (dashed) and emission (solid) spectra of AlexaFluor 488 alkyne and AlexaFluor 594 azide.

Scanning Electron Microscopy (SEM)

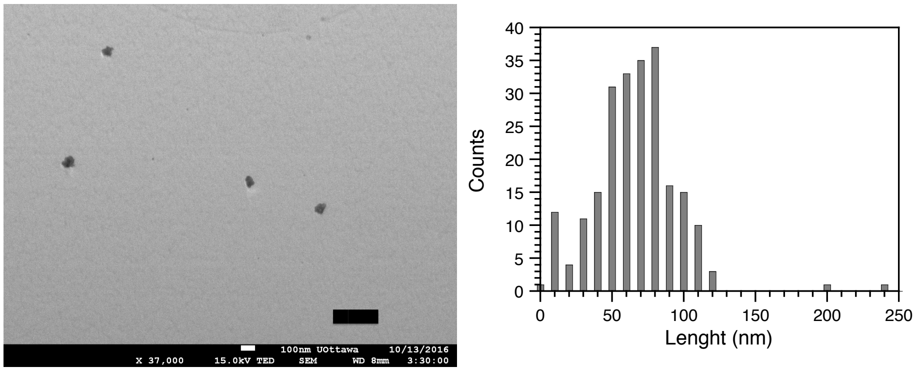

Figure S2: SEM image (left) reveals the presence of small particles in addition to the micrometer size particles found easily by optical microscopy. Scale bar: 300 nm. Right: particle size distribution measured by the length of particle major axis.

Poisson distribution analysis of catalytic events

The Poisson distribution following expression

$$P(k\ events) = \frac{\lambda^k e^{-\lambda}}{k!} \qquad (1)$$

Where λ is the average rate (average number of events per interval) and k is an integer number, the number of events in the interval. In our case 'k' represents the number of bursts that occur within the measurement time, 50 s in the trajectories we recorded. A value of k = 1 (also described as a success rate of 1.0) would signify that the number of bursts equal the number of catalytic sites, although some of these sites may not experience any catalytic events during the 50 s recording, and are in fact compensated by those sites showing multiple bursts so that for a success rate of 1.0 the total number of bursts equals the number of c. The equation also allows the calculation of the anticipated ratio of trajectories showing single, and multiple events, as illustrated in Figure S3.

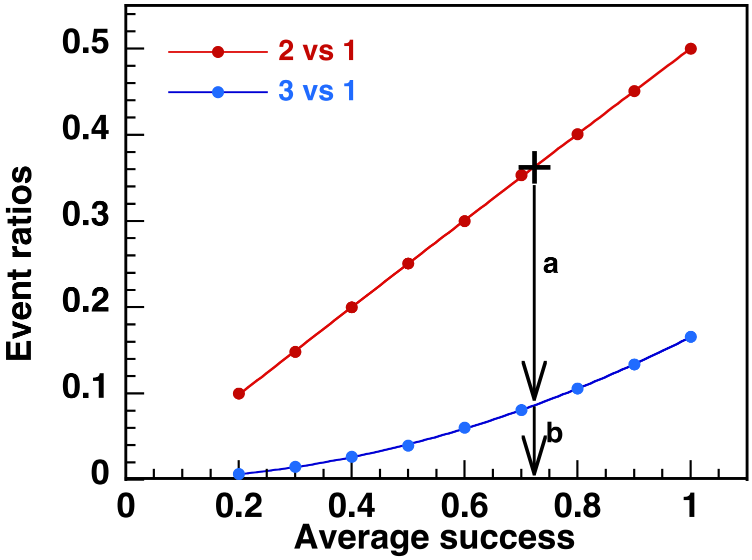

Figure S3. Calculated Poisson ratios of double and triple events relative to trajectories showing single fluorescence bursts. The '+' sign shows the experimental ratio of double to single events (0.36) for samples treated with method 2. A vertical projection (arrow **a**) gives the estimate of triple to single trajectories as 0.08 (from the blue curve), while extension to the axis (arrow **b**) gives the average success as 0.72.

Thus Figure S3 allows us to calculate the ratio of triple to single catalytic events as 0.08, that compares favorably with the experimental value of 0.07. The value is reassuring, but it should be kept in mind that triple events are sufficiently rare to lead to poor statistical significance. In any event, analysis of the data for samples treated with method 2 implies that within the 50 s window monitored, out of 100 sites potentially active, 28 show no activity during this time window, 50 show single bursts, while 18 and 4 show double and triple bursts, respectively.

Representative trajectories

Figure 3 illustrates a few trajectories, however several hundred were examined and a few more are illustrated in Figure S4 for catalyst samples treated by method 2.

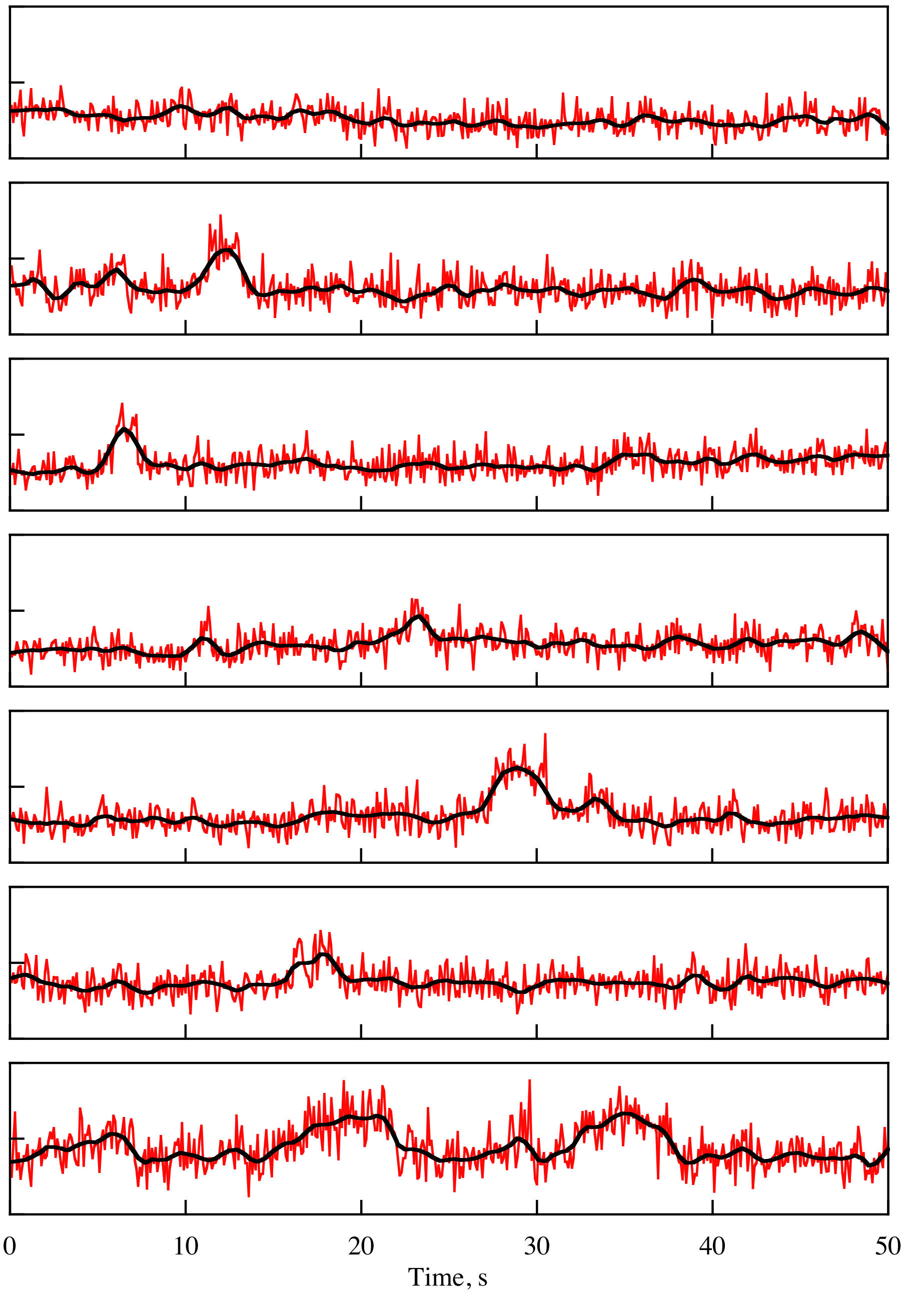

Figure S4: Representative trajectories selected at random, except for the top one, specifically selected to show background only and the bottom one to illustrate another of the rate examples showing three bursts. At vertical scales are 0-to-2000 counts

Catalyst re-usability

Catalyst reusability was tested on the early stages of this work as the conditions for reaction were optimized. The data in Figure S5 illustrates excellent reusability for a catalyst treated with $NaBH_4$ for an extended period of time. In all cases the catalyst was washed with THF, but best performance is observed when washing is accompanied by sonication. The reasons why yields never approach 100% are unclear, but presumed to be due to some retention of alkyne at the catalytic site.

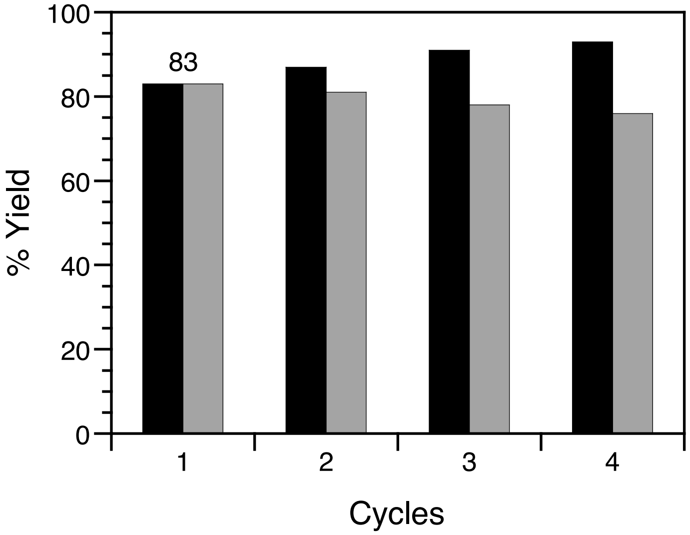

Figure S5. Catalyst reusability for various cycles of click reaction, after washing 3 times between cycles: rinse with THF (grey bars) or sonication in THF (black bars).

(1) Hodgson, G. K., Impellizzeri, S., Scaiano, J. C., *Chemical Science* **2016**, *7*, 1314-1321.