Electronic Supplementary Material (ESI) for Faraday Discussions.

Kinetic analysis of bioorthogonal reaction mechanisms using Raman microscopy

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Supplementary Information

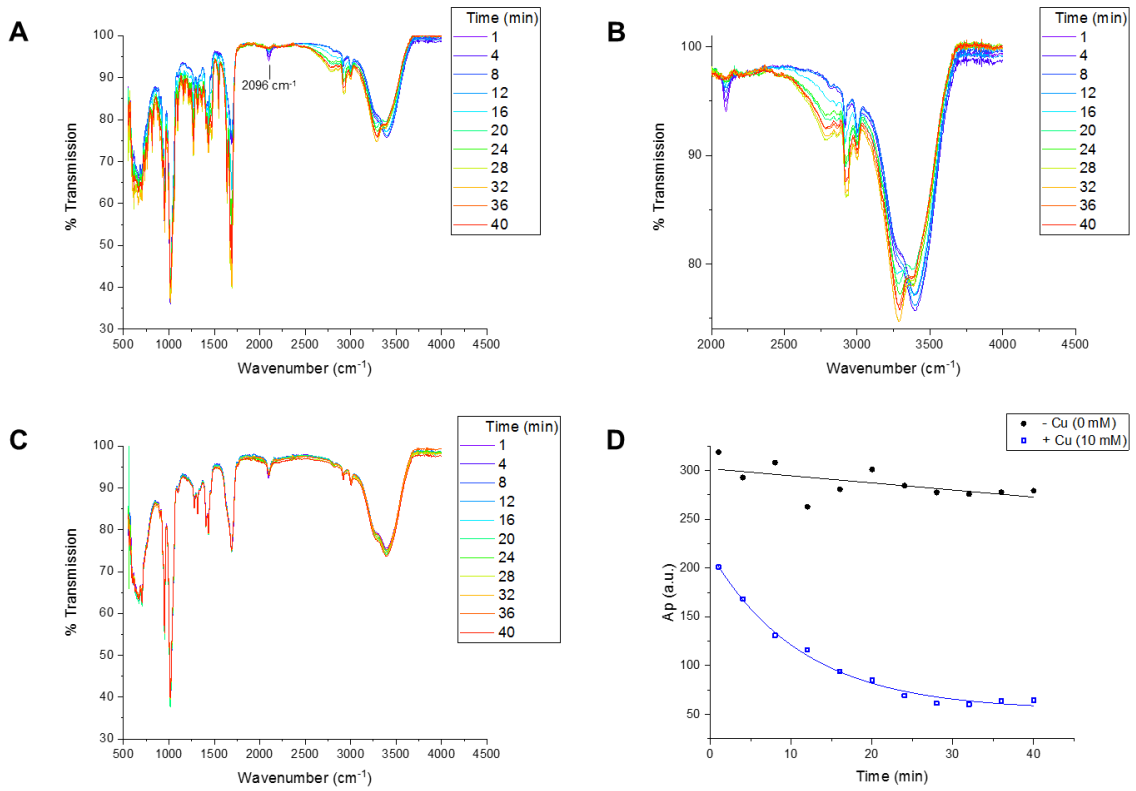


Figure S1 Reaction monitoring of the CuAAC reaction between EdU (**1**) and biotin-PEG3-azide (**2**) using infrared (IR) spectroscopy. **A** IR spectra of reaction mixture comprising EdU (450 mM), biotin-PEG3-azide (450 mM), $CuSO_4$ (10 mM) and Na ascorbate (10 mM) in DMSO/TBS 3:2 v/v. An aliquot of the reaction mixture was removed at each timepoint and the individual IR spectra presented between 0-40 min. The IR spectra were normalised to the baseline at 2220 cm^{-1}. Peak annotation: 2096 cm^{-1} (N_3, azide) **B** Expanded view of the spectra provided in **A** scaled between 2000-4500 cm^{-1}. **C** Control CuAAC reaction where [Cu] = 0 mM (using the same concentrations for the other reagents as in **A**, and consistent acquisition & normalisation procedures). **D** Integration analysis of the normalised IR peak at 2096 cm^{-1} (N_3, biotin-PEG3-azide) for the reaction presented in **A** (blue squares, +Cu (10 mM) with exponential fitting applied) and a control reaction (black circles, -Cu, linear fitting) where [Cu] = 0 mM. A_p = peak area @ 2096 cm^{-1}.

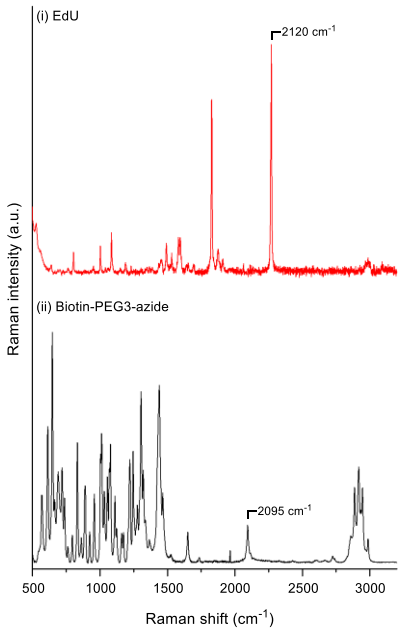


Figure S2 Raman spectroscopy of alkyne- and azide-labelled biomolecular reagents. Raman spectrum of (i) EdU (**1**) and (ii) biotin-PEG3- azide (**2**) acquired using λ_{ex} = 532 nm for 10 s, using a 20× objective lens and 1% laser power for (i) and 10% laser power for (ii). Raman spectra are scaled between (i) 0 – 38,000 and (ii) 0 – 60,000 counts and offset for clarity. The following peaks have been annotated: 2120 cm^{-1} (EdU; C≡C); 2095 cm^{-1} (biotin-PEG3-azide; N=N=N asymmetric stretch).

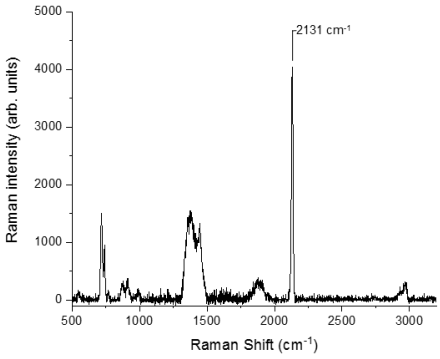


Figure S3 Raman spectrum of propargyl choline (bromide salt). Peak annotation 2131 cm^{-1} (propargyl choline bromide; C≡C). Raman spectrum was acquired using λ_{ex} = 785 nm, 30 s integration time and a 50× objective lens.

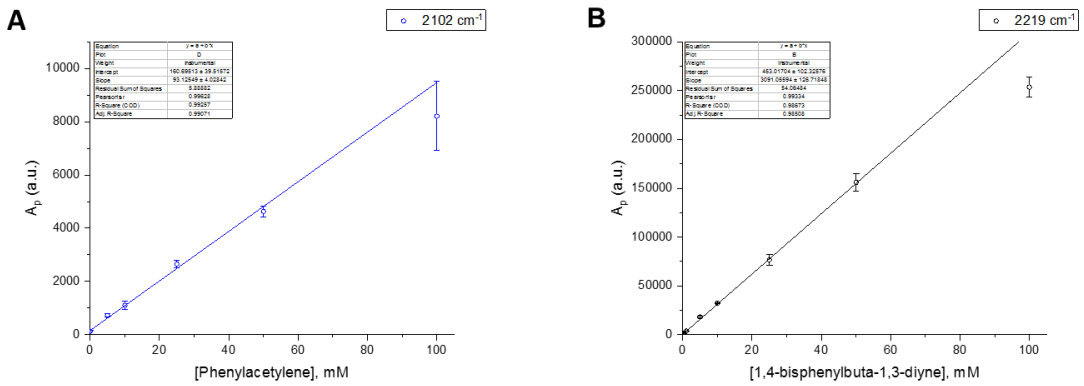


Figure S4 Calibration curves of phenylacetylene (PA) and 1,4-bisphenylbuta-1,3-diyne (**5**) by spontaneous Raman spectroscopy. Calibration curve of **A** phenylacetylene in DMSO (0 – 100 mM) and **B** 1,4-bisphenylbuta-1,3-diyne analysed by Raman spectroscopy. The spectra were normalised to the intensity peak at 1418 cm^{-1} (DMSO CH def.) and the area of the peak at 2102 cm^{-1} or 2219 cm^{-1} was determined in each case. The data points represent the mean of 6 replicate spectra, with error bars ± S.D. A linear fitting is applied to each data set. Raman spectra were acquired using λ_{ex} = 532 nm, 20 s integration time, 20× objective lens. A_p = peak area @ 2102 cm^{-1} / 2219 cm^{-1}.

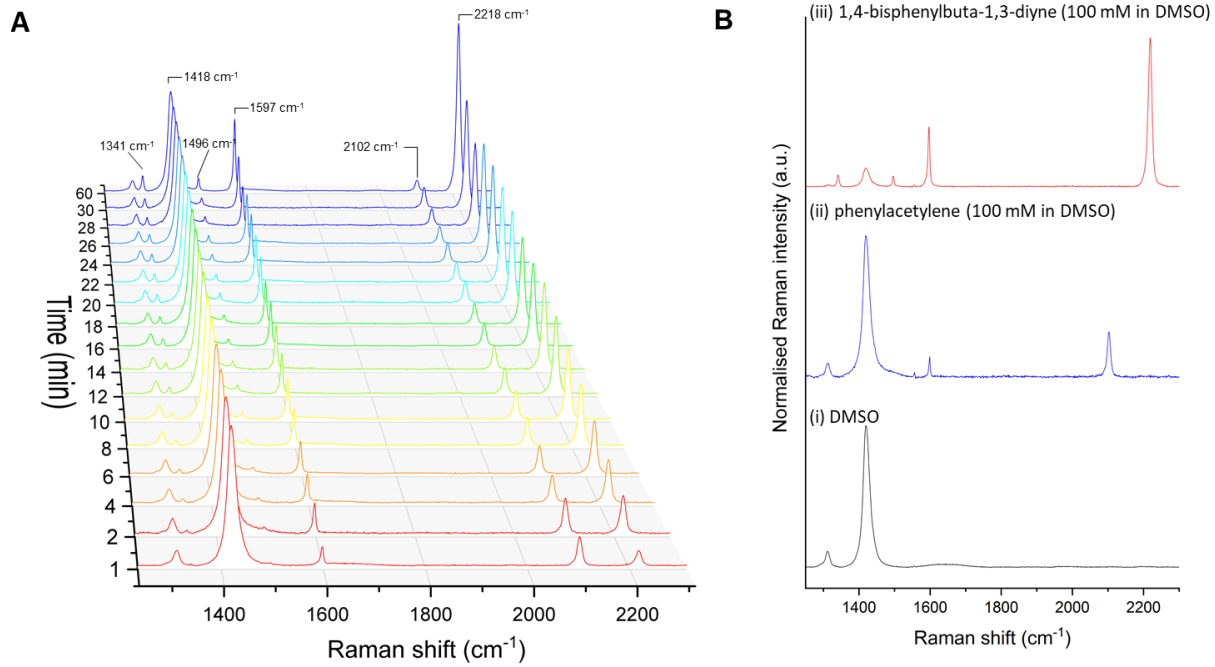
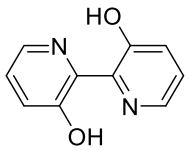
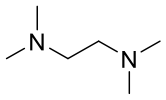


Figure S5 Reaction monitoring of the Glaser-Hay homocoupling of phenylacetylene using Raman spectroscopy. **A** Glaser-Hay reaction of phenylacetylene (100 mM) using CuI (10 mM, 10 mol%) and TMEDA (10 mM, 10 mol%) in DMSO. Raman spectra of the reaction mixture acquired at the indicated timepoints following addition of the CuI/TMEDA catalyst. An aliquot of the reaction mixture were removed at each timepoint and the individual Raman spectra presented between 0-60 min. Spectra were acquired using λ_{ex} = 532 nm for 20 s using a 20× objective lens. The Raman spectra were normalised to the intensity of the peak at 1418 cm^{-1} (DMSO CH def.). **B** Raman spectroscopy of (i) DMSO, (ii) phenylacetylene (PA) (100 mM in DMSO) and (iii) 1,4-bisphenylbuta-1,3-diyne (**5**) (100 mM in DMSO). Spectra were acquired using λ_{ex} = 532 nm for 20 s using a 20× objective lens. The Raman spectra were normalised to the intensity of the peak at 1418 cm^{-1} (DMSO CH def.) and offset for clarity.



2,2'-bipyridine-3,3'-diol
bipy-diol



N,*N*,*N*',*N*'-tetramethylethylenediamine
TMEDA

Figure S6 Chemical structures of the copper co-ordinating ligands used in this study.

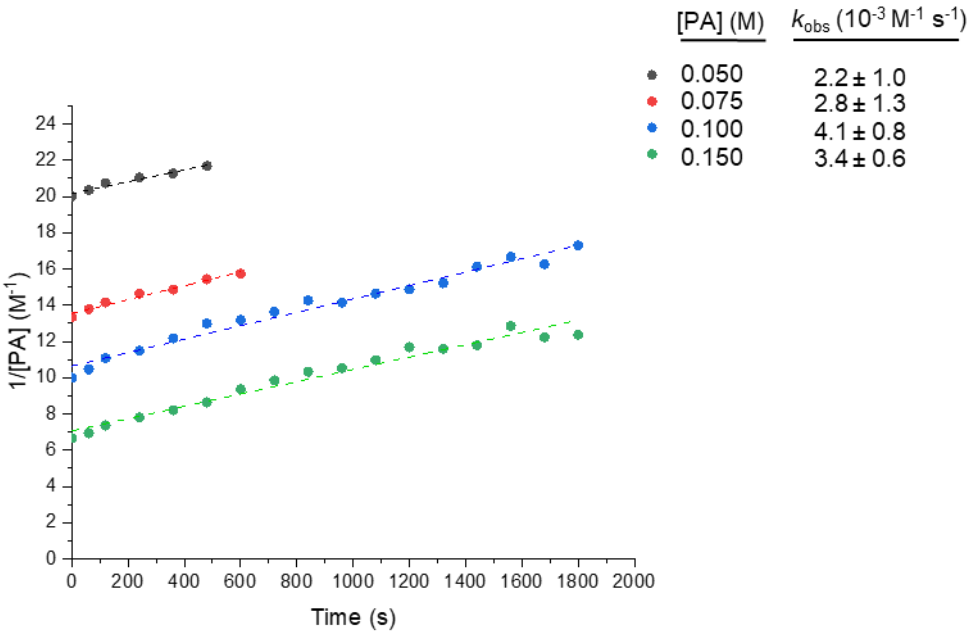


Figure S7 Second-order plot of initial phenylacetylene concentration in the Glaser-Hay reaction. Phenylacetylene (PA, 50-150 mM) was reacted with CuI (10 mM) and TMEDA (10 mM) by Raman spectroscopy using λ_{ex} = 532 nm for 20 s using a 20× objective lens. A linear fitting was applied to each dataset and rate constants are provided for each [PA] with k_{obs} ± 0.5 RMSE.

Table S1 Kinetics of the CuAAC reaction of EdU and biotin-PEG3-azide with respect to varying $[Cu]_{tot}$. Tabulated data accompanying **Figure 5A**.

[EdU] (10^{-3} M)	[azide] (10^{-3} M)	[NaAsc] (10^{-3} M)	$[Cu]_{tot}$ (10^{-3} M)	[THPTA] (10^{-3} M)	k_{obs} (10^{-3} s^{-1})
100	100	100	0	0	≤0.2
100	100	100	1	1	0.1 ± 0.2
100	100	100	2	2	0.4 ± 0.3
100	100	100	5	5	3.4 ± 0.4
100	100	100	10	10	8.8 ± 1.2
100	100	100	15	15	13 ± 3

Table S2 Kinetics of the CuAAC reaction of EdU and biotin-PEG3-azide with respect to varying [THPTA/$[Cu]_{tot}$. Tabulated data accompanying **Figure 5B**.

[EdU] (10^{-3} M)	[azide] (10^{-3} M)	[NaAsc] (10^{-3} M)	$[Cu]_{tot}$ (10^{-3} M)	[THPTA] (10^{-3} M)	$[L]/[Cu]_{tot}$	k_{obs} (10^{-3} s^{-1})
100	100	100	5	0	0	0.3 ± 0.2
100	100	100	5	2.5	0.5	2.9 ± 0.8
100	100	100	5	5	1	3.4 ± 0.4
100	100	100	5	7.5	1.5	3.1 ± 0.7
100	100	100	5	10	2	3.6 ± 0.8
100	100	100	5	15	3	2.6 ± 0.8

Table S3 Experimental conditions for the Glaser-Hay homocoupling of phenyl acetylene.

Entry	[PA] (mM)	$[Cu]_{tot}$ (mM)	[Ligand] (mM)
1	100	0-20[a]	0-20
2	100	10	0-15[b]
3	100	10[c]	10
4	100	10	10[d]

[a] [CuI] = [TMEDA] for all combinations. [b] [TMEDA] varied with fixed [CuI] concentration. [c] Cu source varied between CuI and $Cu(OAc)_2$. [d] Ligand varied between none, TMEDA and bipy-diol.

Table S4 Kinetics of the Glaser-Hay homocoupling of phenyl acetylene with respect to varying $[Cu]_{tot}$. Tabulated data accompanying **Figure 7A**.

[PA] (10^{-3} M)	$[Cu]_{tot}$ (10^{-3} M)	[TMEDA] (10^{-3} M)	$[Cu]^2_{tot}$ (10^{-6} M^2)	k_{obs} (10^{-3} M^{-1} s^{-1})
100	0	0	0.0	≤0.2
100	2.5	2.5	6.2	0.5 ± 0.2
100	5.0	5.0	25	1.1 ± 0.5
100	7.5	7.5	56	2.5 ± 0.3
100	10.0	10.0	100	4.1 ± 0.8
100	15.0	15.0	225	11.2 ± 1.9
100	20.0	20.0	400	17.7 ± 2.2

Table S5 Kinetics of the Glaser-Hay homocoupling of phenyl acetylene with respect to varying $[TMEDA]/[Cu]_{tot}$. Tabulated data accompanying **Figure 7B**.

[PA] (10^{-3} M)	$[Cu]_{tot}$ (10^{-3} M)	[TMEDA] (10^{-3} M)	$[TMEDA]/[Cu]_{tot}$	k_{obs} (10^{-3} M^{-1} s^{-1})
100	10	0	0	0.07 ± 0.06
100	10	5	0.5	1.2 ± 0.4
100	10	10	1	4.1 ± 0.8
100	10	15	1.5	0.20 ± 0.07