Supporting Information

Mechanistic investigation and further optimization of the aqueous Glaser-Hay bioconjugation

Christopher R. Travis, Lauren E. Mazur, Emily M. Peairs, Gillian H. Gaunt, and Douglas D. Young *

FIGURES

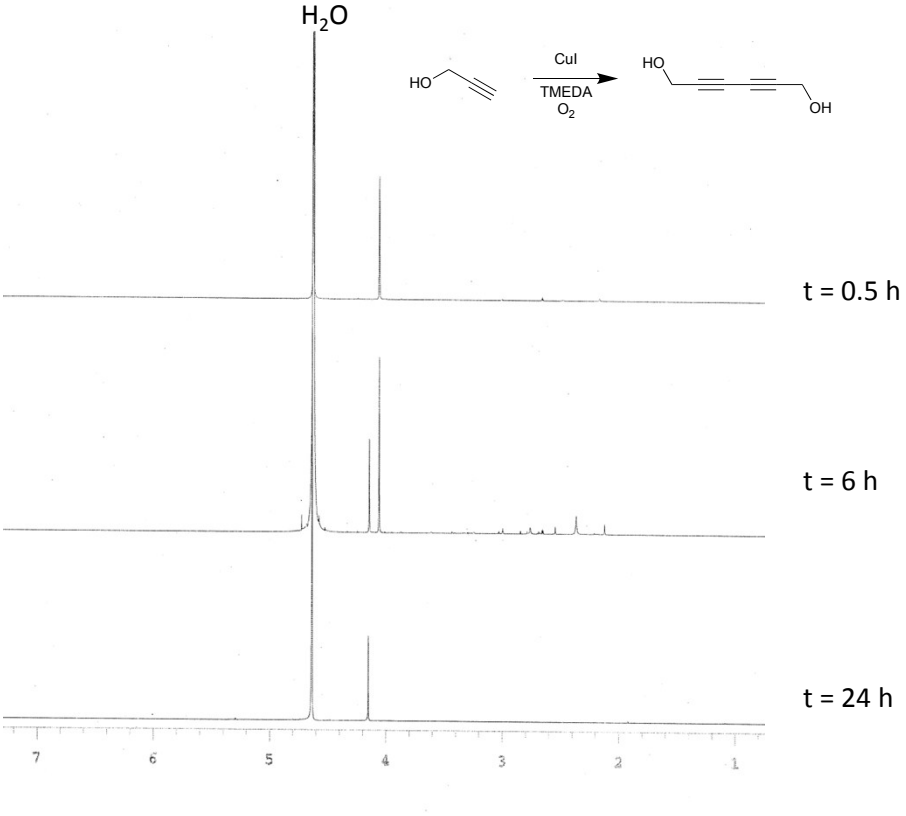

Figure S1. Representative ^{1}H NMR spectra during the time course to monitor the dimerization of propargyl alcohol. Immediately upon addition of the CuI, the disappearance of the terminal alkyne resonance was observed, indicating the formation of a Cu acetylide intermediate.

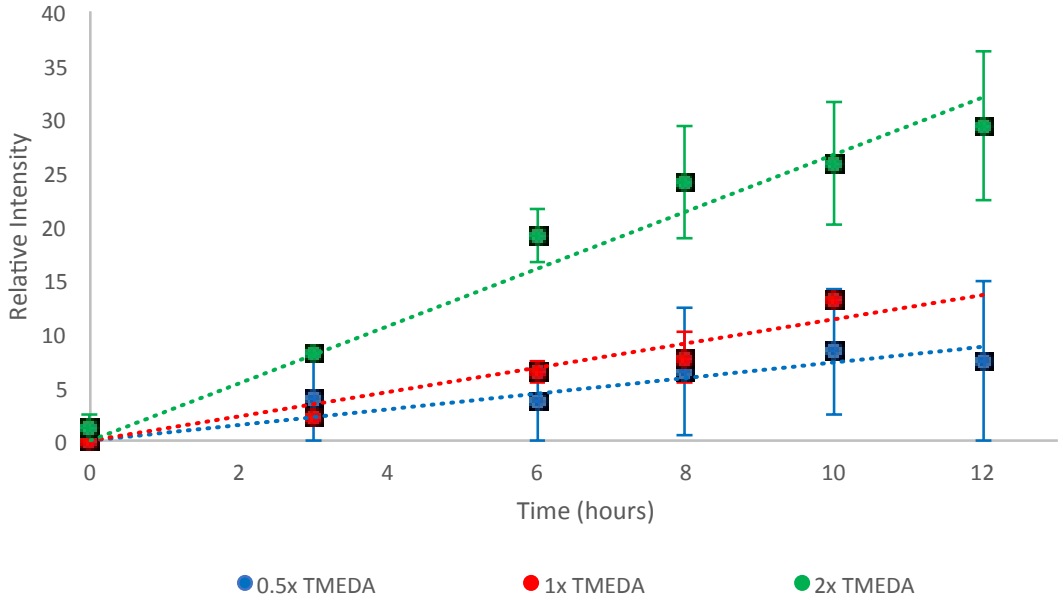

Figure S2. Effects of varying concentrations (2.0, 4.0 and 8.0 mol %) of TMEDA in the aqueous Glaser-Hay reaction.

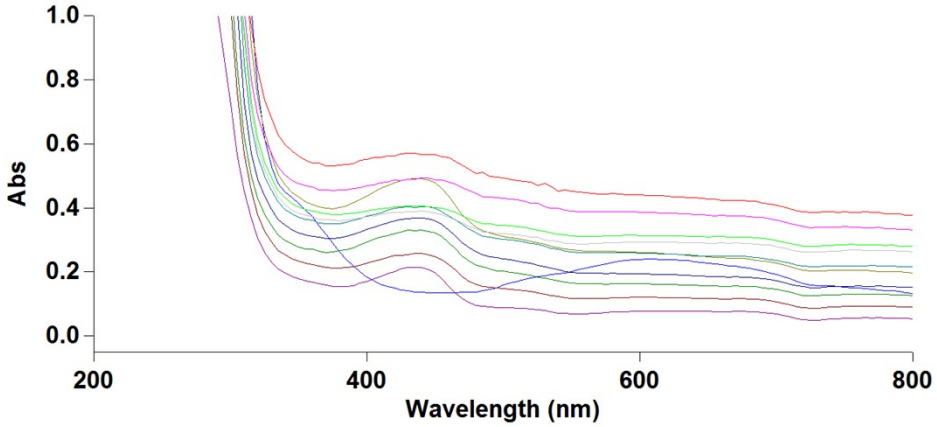

Figure S3. Absorbance spectra of the time course of the dimerization of propargyl alcohol through the Glaser-Hay coupling. The dark blue peak just above 600 nm corresponds to the absorbance of the Cu(I)/TMEDA catalyst mixture, prior to the addition of propargyl alcohol. The other peaks seen just above 400 nm correspond to different timepoints during the reaction, with intensity increasing with time.

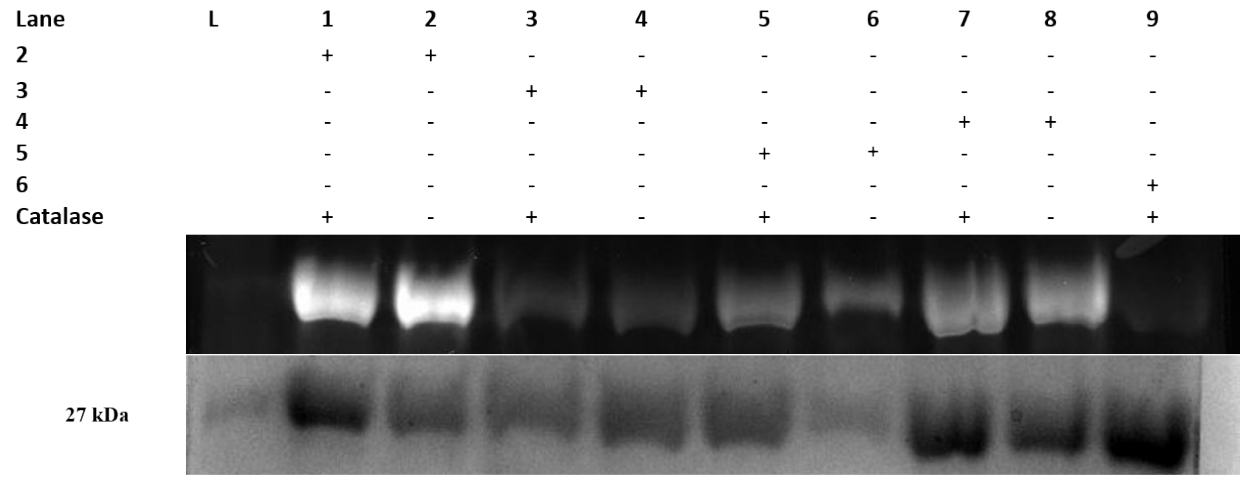

Figure S4. Glaser-Hay bioconjugations between GFP-151-*p*PrF and Fluor-488 alkyne were performed to test the effects of various nitrogenous ligands on protein degradation and coupling efficiency in the presence and absence of catalase. Reactions employing the traditionally used TMEDA (**2**) demonstrated reduced protein degradation and improved coupling efficiency when catalase was present. Reactions employing the previously reported dicarboxylated biphenyl ligand (**3**), a biquinoline ligand (**4**), or a dimethylated biphenyl ligand (**5**) demonstrate the same trends. Reactions employing a terpyridine ligand (**6**) demonstrate very low coupling efficiency. Overall, we demonstrate that the addition of catalase reduces protein degradation and improves coupling efficiency in the Glaser-Hay bioconjugation. Further, we report that ligand choice appears to be a key factor in coupling efficiency of the reactions, and we report 2,2'-biquinoline (**4**) as a promising new ligand worthy of further investigation.