Published in final edited form as:

Langmuir. 2013 May 7; 29(18): 5383–5387. doi:10.1021/la400172w.

Squish and CuAAC: Additive-Free Covalent Monolayers of Discrete Molecules in Seconds

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Abstract

A terminal alkyne is immobilized rapidly into a full monolayer by squishing a small volume of a solution of the alkyne between an azide-modified surface and a copper plate. The monolayer is covalently attached to the surface through a copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction, and the coverages of the immobilized electroactive alkyne species are quantified by cyclic voltammetry. A reaction time of less than twenty seconds is possible with no other reagents required. The procedure is effective in aerobic conditions using either an aqueous or aprotic organic solution of the alkyne (1–100 mM).

Introduction

The ability to reproducibly immobilize small molecules to planar surfaces through covalent bonds is critical for a diverse range of applications and fundamental studies. Covalent surface immobilization enables mechanistic investigation of catalytic systems,^{1–3} biosensor development,⁴ protein function⁵ and enzyme activity⁶ studies, and advanced diagnostic tools^{5,7}. A variety of coupling methods are available for small-molecule covalent surface immobilization.^{6,8–10} Among these choices, the copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction is distinguished by the orthogonality of the azide and alkyne reagents to other functional groups, the reaction's high yield and rate, its regioselectivity for the 1,4-triazole product, the oxidative and hydrolytic stability of the resulting triazole linker and the electronic conjugation of the linker¹¹. For these reasons, the CuAAC reaction has been adopted for a wide variety of applications including surface immobilization.^{12–14} CuAAC has proven to be a useful immobilization strategy on silica,¹⁵ titania,^{12,16} gold,¹⁷ diamond,^{18,19} and graphitic materials^{3,20,21}. The CuAAC reaction is catalyzed by Cu(I) complexes, and therefore requires a reductant when carried out using Cu(II) catalyst precursors, a commonly used method. However, copper metal or Cu(I) oxides can also provide the Cu(I) cycloaddition catalyst.^{22–28}

Here we report a remarkably simple, fast and robust procedure to covalently immobilize a monolayer of discrete molecules on a flat metal-oxide surface using the CuAAC reaction. This "squish-and-CuAAC" procedure requires only a freshly cleaned copper plate, a solution of a terminal alkyne and an azide-terminated metal oxide surface. No stoichiometric reductants or supporting ligands^{12,17} are needed in the solution, and the reaction may be run aerobically using either an aqueous or acetonitrile solution of the alkyne. Electrochemical quantification demonstrates that this convenient, additive-free procedure can immobilize a complete monolayer in less than twenty seconds.

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Supporting Information

Experimental details, complete surface coverage data on concentration-dependent rate of immobilization, and fluorescence intensity data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Results and Discussion

Glass slides coated with fluorine-doped tin oxide (FTO) were used as electrodes. Indium-doped tin oxide (ITO) surfaces were equally effective. The electrodes were modified with organic azide groups using a version of a previously reported gas-phase silane deposition method.¹⁵ A copper plate was immersed in glacial acetic acid for 1 minute and then dried in a stream of dinitrogen gas. A 5 μL drop of the alkyne solution (typically 1 mM) was deposited on the copper plate, and a 1 cm^2 azide-modified electrode was placed immediately face down over the drop, squishing the solution between the electrode and the copper plate. This volume of alkyne solution wetted the entire electrode surface, indicating an average separation between the electrode and the copper plate of about 50 μm and, in the case of a 1 mM solution, providing approximately a ten-fold excess of alkyne relative to the maximum observed coverage. After a period of 10 to 180 seconds, the electrode was removed from the copper plate, rinsed with isopropanol and analyzed electrochemically.

An electrode modified by this method, using a 1 mM *aqueous* solution of iron terpyridyl complex **1**, shows a faradaic wave due to the oxidation and re-reduction of the Fe(II) center (Figure 1). The observed half-wave potential of 1.21 V vs. NHE agrees well with the potential observed in solution for the parent complex bearing no alkyne group.²⁹ The same procedure carried out with an acetonitrile solution of **1** provided similar results (Figure S1). No faradaic wave is observed on the electrode when the procedure is carried out using the parent complex lacking an alkyne group in place of **1**, or when a glass slide is used in place of the copper plate, regardless of the choice of solvent.

The anodic peak potentials vary linearly with scan rate (Figure S2), diagnostic of a redox species immobilized at the electrode.³⁰ The nearly symmetric peak shape of immobilized **1** is characteristic of identical, independent, non-interacting redox species. The width of the faradaic wave of immobilized **1** at half its maximum height is approximately 120 mV, slightly larger than the ideal value of 90 mV.³⁰ The splitting between the oxidative and reductive peaks is 35 mV at a scan rate of 0.1 V s^{-1} . The highest coverage of **1** observed in our experiments is 1.6×10^{14} molecules cm^{-2} , very close to the sterically limited coverage of 1.5×10^{14} molecules cm^{-2} calculated from the crystal structure dimensions³¹ of the parent complex.³² Based on the width of the undecyl chain linking the azide group to the oxide surface, the coverage of azide groups is estimated to be 5×10^{14} groups cm^{-2} .³³

Treatment of an azide-terminated electrode with an acetonitrile solution of ethynylferrocene (**2**) results in two reversible faradaic waves, originating from the oxidation and re-reduction of the Fe(II) center (Figure 2). The lower-potential couple ($E_{1/2} = 0.62$ V) is similar to previous reports of immobilized ferrocene^{17,20}, and the splitting between the oxidative and reductive peaks for this couple is 10 mV at a scan rate of 0.1 V s^{-1} . The second peak ($E_{1/2} = 0.73$ V) indicates the presence of a second electrochemical population of ferrocene groups in a more oxidizing environment, a phenomenon previously observed when ferrocene is immobilized onto electrodes at high coverages.^{34–36}

The surface coverage of ferrocene groups approaches a maximum of approximately 4×10^{14} molecules cm^{-2} for alkyne concentrations of 10 mM and less. This exceeds the sterically limited closest-packing coverage of the ferrocene group by almost 50% (4×10^{14} molecules cm^{-2} vs. 2.7×10^{14} molecules cm^{-2})³⁴, but is less than the expected coverage of the azide (5×10^{14} molecules cm^{-2}). A reviewer offered one possible explanation of the higher than expected coverage: strong adsorption of bis(ferrocenyl)diyne produced by oxidative alkyne homocoupling, a side reaction known to occur under some CuAAC reaction conditions.³⁷ This product might however reasonably be expected to be extracted during the rinsing steps. Moreover, in a control procedure using rigorously anaerobic conditions, the resulting

coverage of surface ferrocene groups was comparable within the variance of the measurement. Another possible explanation consistent with both the higher-than-expected coverage and the electrochemical heterogeneity is a very dense layer of covalently attached ferrocene groups at the surface, in which some groups are buried under others, with a total coverage exceeding that of a densely packed ferrocene monolayer. A higher-than-unity roughness factor may also make a contribution to the anomalously high coverage.

The squish-and-CuAAC method is very rapid. At an alkyne concentration of 10 mM, a hundredfold excess relative to the maximum observed coverage, the immobilization of ethynylferrocene is complete in less than 20 seconds with a coverage of 4×10^{14} molecules cm^{-2} (Figure 3a). The rate of surface coverage growth depends strongly on the alkyne concentration between 0.5 and 10 mM. The squish-and-CuAAC method is also effective for immobilizing fluorescein alkyne **3** (Figure S4).

The strong dependence of the coverage growth rate on the concentration of the alkyne solution (Figure 3a) is consistent with the solution-phase CuAAC reaction^{38,40} and its prevailing mechanistic hypothesis^{12,38}. The growth rate observed with a 1 mM solution is described reasonably by the expression

$$\Gamma_t = \Gamma_\infty \{1 - \exp(-k[\text{alkyne}]t)\} \quad (1)$$

with $k = 25 \text{ M}^{-1} \text{ s}^{-1}$ and $\Gamma_\infty = 4.3 \times 10^{14} \text{ cm}^{-2}$ (Figure 3a). This expression describes a surface CuAAC reaction that is first-order in both alkyne and the surface azide. However, at very low alkyne concentrations (0.1 mM), where the alkyne is depleted,³⁹ and at very high alkyne concentrations (100 mM) other processes should become kinetically important.⁴⁰

Surface immobilization was carried out using a range of distances between the copper plate and the electrode. In the squish-and-CuAAC procedure, the thin layer of solution squished between the copper plate and the modified surface is approximately 50 microns thick.⁴¹ The reaction was also carried out with inter-surface distances of 250 and 500 microns, using spacers of known thickness. When the distance was widened from 50 to 500 microns, the time required to reach a coverage of 2×10^{14} molecules cm^{-2} increased more than ten-fold, from twenty to three hundred seconds (Figure 3b). Presumably this is because the rate of reaction becomes limited by the concentration of the copper catalyst at greater distances from the copper source.

The squish-and-CuAAC procedure exploits the convenience of using copper metal as a catalyst source for the CuAAC reaction. Copper metal is already known as a catalyst source for the CuAAC reaction from reports using copper turnings,²² copper particles,^{23,25,42} and flat copper surfaces^{26–28}. In previous reports using a copper surface to initiate CuAAC, generation of the Cu(I) catalyst was attributed to aerobic oxidation of the copper surface, which we presume occurs here.^{26,28} Rapid aerobic oxidation of a freshly etched Cu(0) surface, following the same acetic acid cleaning procedure employed in our experiments, has been characterized by XPS and Auger spectroscopy.^{43,44} Acetic acid is known to associate with copper surfaces in vacuum conditions,⁴⁵ and presumably remains adsorbed on the copper plate at the beginning of the squish-and-CuAAC procedure. This is unlikely to hinder the CuAAC reaction: Cu(I)(OAc) is a known source of catalytically active Cu(I) for CuAAC,⁴⁶ and the reaction may in fact be catalyzed by acetate-supported binuclear copper centers^{37,47}. Despite some claims to the contrary,^{48–50} the coupling of azides and terminal alkynes at ambient temperatures is generally accepted to require a Cu(I) catalyst. In the case of the squish-and-CuAAC procedure reported here, no surface coverage results when the procedure is carried out against a glass slide instead of a copper plate (Figure 1 and Figure 2). This result is consistent with the observation of Spruell *et al.* concerning the interfacial

CuAAC reaction that "reaction without a [copper] catalyst results in very limited conversion, even over prolonged reaction times."²⁶

Interfacial CuAAC has been carried out with micron-scale spatial resolution by Spruell *et al.* using patterned stamps for microcontact printing,²⁶ and by Devaraj *et al.* using interdigitated electrodes⁵¹. Paxton *et al.* achieved 50 nm resolution when a copper-coated AFM tip was used to catalyze the interfacial CuAAC reaction.⁵² For those specific systems, the spatial resolution of the resulting pattern implies that the active Cu(I) catalyst does not diffuse over micron-scale distances. In contrast, surface CuAAC activity is observed in the squish-and-CuAAC system even when the catalyst source is separated from the reaction site by hundreds of microns, indicating that the active Cu(I) catalyst can diffuse across this distance under the squish-and-CuAAC conditions. The reason for this apparent difference in diffusion of the active catalyst is currently unknown.

Electrochemical analysis allows the use of quantified surface coverages when investigating the concentration-dependent kinetics of the surface CuAAC reaction. Recent work has probed the structure-dependent kinetics of the surface CuAAC reaction using fluorescence intensity as an indirect proxy for surface density.⁵⁰ The kinetics of different reaction conditions were examined by Spruell *et al.* using electrochemical surface quantification, but the concentration-dependent investigation of a single reaction system was not studied.²⁶ In this work, we have applied electrochemical quantification to the systematic concentration-dependent kinetic examination of a single reaction system.

Finally, the squish-and-CuAAC procedure provides the fastest CuAAC-based monolayer formation currently known – less than 20 seconds using a 10 mM alkyne solution. Several recent studies using potentially self-quenching and therefore nonlinear fluorescence detection report alkyne immobilization in minutes.^{26,48,53,54} Spruell *et al.* reported that an electrochemically quantified full monolayer was immobilized in 60 minutes using microcontact printing, a close-contact technique similar to the squish-and-CuAAC procedure.²⁶ Under comparable conditions of 1 mM alkyne, we observed complete monolayer formation of **2** in less than two minutes (Figure 3).

Conclusion

The squish-and-CuAAC immobilization procedure is convenient for rapid immobilization of dense monolayers on flat surfaces. The Cu(I) catalyst for the CuAAC reaction is presumably generated by spontaneous aerobic oxidation at the surface of bulk copper metal, consistent with control experiments and with previous analysis of surface copper oxidation. This procedure is effective using aqueous as well as aprotic organic solutions, extending its use to biologically important species. It tolerates ordinary aerobic conditions, and requires no accelerating ligand or chemical reductant. Electrochemical quantification of surface coverage indicates that a full monolayer is immobilized in seconds, and the rate of the reaction drops off slowly with increasing distance between the copper plate and the modified surface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Eric D. Stenejhem and Ali Hosseini for helpful discussions. We also thank Dr. Andrew Olson and the Stanford Neuroscience Microscopy Service, supported by NIH NS069375, for assistance with fluorescence

microscopy. This work was supported by the Global Climate and Energy Project at Stanford University and by NIH #GM050730.

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Figure 1.

Cyclic voltammogram of an azide-modified 1 cm² FTO electrode after squish-and-CuAAC with 5 μ L of 1 mM aqueous solution of **1** on a freshly etched copper plate (—), and after the same procedure on a glass slide (- - -) (180 second contact). (0.1 V s⁻¹, 0.1 M aqueous HClO₄.)

Figure 2.

Cyclic voltammogram of an azide-modified 1 cm² FTO electrode after squish-and-CuAAC with 5 μL of 1 mM acetonitrile solution of **2** on a freshly etched copper plate (—), and after the same procedure on a glass slide (- - -) (90 second contact). (0.1 V s⁻¹, 0.1 M aqueous HClO₄.)

Figure 3.

(a) Coverage of ferrocene groups on FTO electrodes, immobilized using the squish-and-CuAAC procedure, as a function of surface contact time for different concentrations of **2**: 0.1 mM (⊠); 0.5 mM (△); 1 mM (□); 10 mM (▽); 100 mM (○). Data points are averages of three to five individual measurements (see Figure S3 for complete data). The dotted line shows the coverage growth predicted by Equation 1 with $k = 25 \text{ M}^{-1} \text{ s}^{-1}$ and $[\text{alkyne}] = 1 \text{ mM}$. (b) Coverage growth of ferrocene groups on FTO electrodes at a freshly etched copper plate using the squish-and-CuAAC procedure with a 1 mM solution of **2** in acetonitrile, with 50 μm (□), 250 μm (●), and 500 μm (◆) separating the copper plate and the electrode.

Scheme 1.
Interfacial CuAAC reaction in the presence of a copper surface, and alkynes immobilized using the additive-free squish-and-CuAAC procedure.