Surface Chemistry

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Rapid and Complete Surface Modification with Strain-Promoted Oxidation-Controlled Cyclooctyne-1,2-Quinone Cycloaddition (SPOCQ)

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Abstract: *Strain-promoted oxidation-controlled cyclooctyne-1,2-quinone cycloaddition (SPOCQ) between functionalized bicyclo[6.1.0]non-4-yne (BCN) and surface-bound quinones revealed an unprecedented 100 % conjugation efficiency. In addition, monitoring by direct analysis in real time mass spectrometry (DART-MS) revealed the underlying kinetics and activation parameters of this immobilization process in dependence on its microenvironment.*

Since the term "click" chemistry was coined by Sharpless and co-workers in 2001,[1] this class of transformations has become of crucial importance in organic synthesis and materials science.[2] In the last decade, this field has focused more on novel click reactions that have an improved kinetic profile without the necessity of toxic metal catalysts like copper.[3] Among the reactions that emerged from this endeavor, the strain-promoted alkyne-azide cycloaddition (SPAAC) proved a particularly versatile alternative owing to its bioorthogonality.[4] Recently, van Delft and co-workers reported the strain-promoted oxidation-controlled cyclooctyne-1,2-quinone cycloaddition (SPOCQ) for selective protein conjugation[5] and hydrogel formation[6] with a reaction rate around three orders of magnitude higher than those observed for SPAAC. This combination of high yield and high reaction rates prompted us to study the analogous surface reaction, since SPAAC on a surface typically stalls at 15–80 % efficiency,[7] thereby limiting its practical applicability as unreacted surface sites cannot be removed by purification as in solution-based chemistry. Importantly, solving the issue of variable "clicking" efficiency on surfaces is severely hampered by a poor understanding of the kinetics of the solution-to-surface conjugation reactions. Apart from a handful of cases in which electrochemical methods have been used[8] and the recent measurement of SPAAC reactivity[7c] no rigorously measured kinetics on interfacial reactions involving self-assembled monolayers are available.

Herein, we report the first example of a surface-bound metal-free click reaction with complete conversion of immobilized groups. We show that SPOCQ is a far superior surface click-type reaction that yields fast surface modification with 100 % efficiency. In particular, we studied the application of SPOCQ in the reaction of a bicyclo[6.1.0]non-4-yne (BCN) derivative bearing an MS tag with a monolayer presenting 1,2-quinone groups. In addition, we determined the activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) by DART-HRMS to clarify, for the first time, the roles of enthalpy and entropy in this surface-bound reaction.

The surface chemistry described in this work is based on the use of phosphonic acids (PA) as anchoring groups for hydroxylated aluminum surfaces (Figure 1, Table 1).[9] Monolayer-coated aluminum surfaces were obtained from a reac-

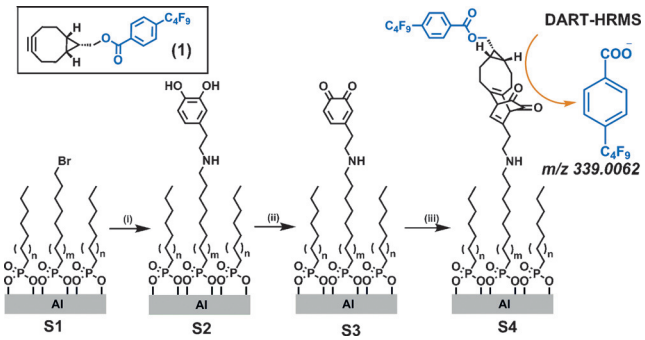

Figure 1. Schematic of SPOCQ on quinone-functionalized aluminum substrates. (i) Nucleophilic substitution with dopamine, (ii) oxidation with $NaIO_4$, and (iii) SPOCQ with BCN derivative **1**.

Table 1: Atomic percentages (%) of the elements on Al surface functionalized with Br (**S1**), dopamine (**S2**), 1,2-quinone (**S3**), and BCN derivative **1** (**S4**).

Surface	Al2p	O1s	P2p	C1s	Br3p	N1s	F1s
S1	28.1	45.0	3.1	23.0	0.8	–	–
S2	29.5	42.8	4.4	22.1	–	1.2	–
S3	30.8	44.0	3.0	21.2	–	1.0	–
S4[a]	27.6	39.8	4.0	24.1	–	1.0	3.5

[a] SPOCQ reaction for 1 h at 22 °C.

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tion with an octyl/12-bromododecyl PA mixture (3:1 ratio, in isopropanol) and confirmed by an increase in the static water contact angle (SCA) from 35° to 101°, in accordance with data reported for similar PA monolayers.[10] The presence of Br was confirmed by XPS, showing a Br/P ratio of 4.1 (theoretical ratio = 4), which also correlates with a mixed 3:1 monolayer. Next, dopamine was immobilized on the surface through nucleophilic substitution of the Br-terminated monolayer with dopamine (50 mM in methanol; 1 h). Dopamine attachment was reflected in the decrease of the SCA from 101° to 91° and in the complete disappearance and appearance of Br3d and N1s XPS peaks, respectively (Supporting Information, Figure S2). Finally, treatment with an aqueous sodium periodate solution for 30 min led to efficient and quantitative oxidation of catechol to 1,2-quinone as corroborated by a decrease in SCA (91° to 74°)[11] and by GATR-FTIR through the appearance of a peak at 1669 cm^{-1}, assigned to the carbonyl stretch of the 1,2-quinone. Atomic force microscopy imaging showed that the roughness as measured for the unmodified surface (rms ≈ 4.5 nm) remained constant upon monolayer formation. No evidence of granules was observed on the modified surfaces, supporting the presence of a fully formed monolayer.

C1s narrow scan XPS spectra provided chemical state information of the different functionalized surfaces (Supporting Information, Figure S7). **S3** surfaces showed three bands centered at 285.0 (C–C), 286.9 (C–O and C–N), and 289.0 eV (C=O), in accordance with the presence of an immobilized 1,2-quinone group on the surface (Figure 2A).[12a] After the SPOCQ reaction with derivative **1**, the peak deconvolution also showed components corresponding to the fluorinated tag (Figure 2B), such as a $-CF_3$ peak centered at 294.3 eV and a larger peak from $-CF_2-$ at 291.6 eV.[12b] M11/6-311 + G-(d,p)-derived simulated C1s XPS spectra agreed well with the experimental spectra (Supporting Information, Figure S6).[12c]

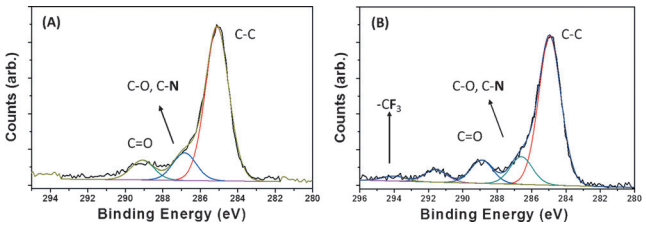

Figure 2. XPS C1s of a 3:1 mixture of octylphosphonic and 1,2-quinone-terminated surface A) before SPOCQ reaction (**S3**) and B) after SPOCQ reaction (**S4**) with BCN derivative **1** (3 mM in dichloroethane at 30°C, 8 h).

Next, the strain-promoted cycloaddition with BCN derivative **1** (3 mM in 1,2-dichloroethane) was investigated. As shown in previous studies, steric factors from the microenvironment surrounding the reacting site play an important role in interfacial reactions.[7,13] Therefore, SPOCQ was studied for a series of mixed monolayers with variable alkyl:quinone ratios (from 0:1 to 3:1). Not surprisingly, the surface coverage of the attached BCN groups was related to surface density of the quinone, with the highest conversion

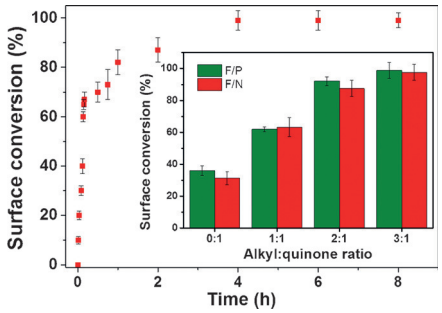

Figure 3. Reaction efficiency (determined by F/N XPS ratio) vs. time for the SPOCQ reaction for the 3:1 mixed monolayer. Inset: Surface conversion (determined by F/P (green) or F/N (red) XPS ratio) for the SPOCQ reaction of BCN derivative **1** with immobilized 1,2-quinones in different mixed monolayers.

observed for the 3:1 mixed (that is, the most diluted) monolayer (Figure 3, inset). For this monolayer, we found that the yield of incorporation of BCN was up to 82% after only 1 h, while after 4 h, 100% incorporation of BCN (according to both the F/P and F/N ratios by XPS) was achieved (Supporting Information, Figure S12 and Table S1). The reaction efficiency was determined by comparing the atomic ratio determined by XPS with the theoretical value of 9:1 (for F/N) or 9:4 (for F/P), which corresponds to the 100% surface conversion of the 1,2-quinone-functionalized monolayer. This quantitative conversion stands in contrast to surface functionalization with several other popular metal-free click chemistry reactions (Supporting Information, Table S2), including SPAAC,[7c] inverse electron-demand Diels–Alder,[14] and thiol-ene[15] and thiol-yne couplings,[3,16] in which surface analyses invariably indicate incomplete conversion (typically 15–90%) of the reactive groups.

The SPOCQ reaction was also studied (under exactly the same conditions as used for hydroxylated aluminum surfaces) using other surfaces (silicon and stainless steel), as well as other linkage reactions to attach dopamine to the surface (see Supporting Information). XPS measurements (F/N ratio) also showed a full conversion of quinone groups in all cases, although Si (111) and stainless steel surfaces took slightly longer (6–8 h).

The specificity of the SPOCQ reaction was demonstrated by generation of a pattern of 1,2-quinone moieties through oxidation by microcontact printing of a sodium periodate solution over a catechol-terminated surface. After reaction with a dye-labelled BCN solution (DY649p1), fluorescence was visualized exclusively on the oxidized regions, confirming the absence of SPOCQ in areas where no oxidation was induced (Supporting Information, Figure S19).

Intrigued by the efficiency of SPOCQ implemented on a surface, we performed an in-depth investigation of the kinetics of this cycloaddition by DART-HRMS, using the intensity of the MS tag as a measure for conversion. Among the different techniques that can be used to study the kinetics of interfacial reactions,[17] direct analysis in real time (DART) has been shown to be particularly useful due to its ability to detect small molecules ($m/z < 400$) without the use of a matrix. Furthermore, DART shows excellent compatibility

with robust covalent surface chemistry,[18] unlike self-assembled monolayers for matrix-assisted laser desorption ionization mass spectrometry (SAMDI) and desorption ionization electrospray (DESI). Recently, we have shown that DART-HRMS accurately and rapidly offers quantitative rate information for interfacial reactions.[7c]

The intensity of the MS tag is directly proportional to the extent of reaction, as this moiety can only be on the surface as a result of a successful reaction between surface-bound 1,2-quinone and BCN. To ensure pseudo-first order kinetics, a large excess of BCN reagent was used. As shown in Figure 4, the reaction proceeds extremely fast and the conversion reaches an asymptotic limit after only 5 min ($\approx 70\,\%$). After that time, the remaining 30% of unreacted surface sites is converted using more complex kinetics, over a 4 h period.

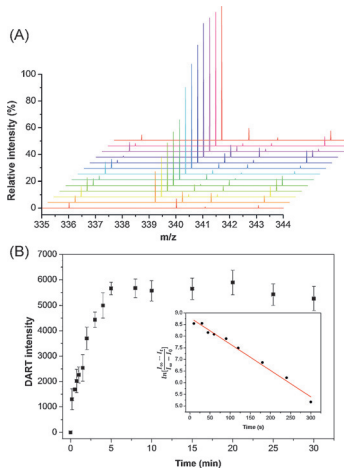

Figure 4. A) Relative intensity for the for MS tag at different reaction times (from 0 to 15 min). B) Normalized intensity for MS tag for the SPOCQ reaction. Inset: Plot of $\ln[(I_\infty - I_t)/(I_\infty - I_0)]$ vs. time, used to obtain the rate constant.

To determine pseudo-first-order rate constants, the rate of reaction was modeled as the change in MS intensity relative to the asymptotically obtained maximum value. For that, the pseudo-first order rate Equation (1) was used,

$$\ln\left(\frac{I_\infty - I_t}{I_\infty - I_0}\right) = -k' t \qquad (1)$$

where k' is the pseudo-first order rate constant, and I_0, I_t, and I_∞ are the MS intensities at $t = 0$, at time interval t, and at the end of reaction (average of 5–15 min). Based on this model, the rate constant for SPOCQ was found to be $(10.1 \pm 0.3) \times 10^{-3}\,s^{-1}$ (Figure 4 B, inset), two-fold faster than the interfacial SPAAC of BCN with azides and about 15-fold faster than other reported surface-SPAAC reactions.[17d] This finding differs dramatically from the 1000-fold larger difference in magnitude observed for SPOCQ and SPAAC in protic solutions (methanol/water 1:1).[5]

It was reasoned that the solvent plays a crucial role in the relatively small observed difference in surface-bound SPOCQ and SPAAC reaction rates (in 1,2-dichloroethane) compared to those reported in solution (protic solvents). Therefore, we measured pseudo-first order reaction rates (in 1,2-dichloroethane at 25 °C) for both SPAAC and SPOCQ of BCN with benzyl azide and 4-*tert*-butyl-1,2-quinone, respectively. A significant solvent effect was observed for the reaction rate of the SPOCQ reaction, as the rate constant in 1,2-dichloroethane was found to be only 10-fold higher ($0.098 \pm 0.002\,s^{-1}$) than that for SPAAC ($0.009 \pm 0.001\,s^{-1}$). A direct competition experiment between SPOCQ and SPAAC using ^1H NMR quantification in $CDCl_3$ yielded a similar rate difference (Supporting Information, Figures S25–27), close to the rate difference observed for the interfacial reaction.

Next, we studied the interfacial SPOCQ reaction at 22 °C in two additional microenvironments, in which the 1,2-quinone was either partially accessible (**M2**), or completely buried within the monolayer (**M3**). For that, the mixed monolayers (in a 3:1 ratio, containing hexadecyl and 12-bromododecyl PA or hexadecyl and 3-bromopropyl PA) were prepared and the quinone was introduced as described previously. Although the use of mixed monolayers is unlikely to be homogeneous if very short and long adsorbates are combined (for example, as in **M3**),[19] no evidence of the formation of microdomains was observed in the studied surfaces. Under the optimized conditions, the pseudo-first order reaction rate constants obtained by DART for the partially accessible (**M2**) and the buried 1,2-quinone (**M3**) monolayers were $(4.98 \pm 0.32) \times 10^{-3}\,s^{-1}$ and $(2.92 \pm 0.15) \times 10^{-3}\,s^{-1}$, respectively. In other words, the significant variation in steric hindrance (between for example, **M1** and **M3**) yields a difference in reaction rate of only a factor of three.

Since these rates can be measured rapidly using DART-HRMS, the effect of temperature on the reaction rate was studied at different temperatures (from −15 to 40 °C; Supporting Information, Table S2 and Figures S28–30), to extract the thermodynamic parameters of activation by Eyring plot analysis. Values for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated from the slope and the intercept, respectively, of the least-squares fit of $\ln(k'/T)$ versus $1/T$ (Figure 5). As shown in Table 2, the reaction of the BCN-derivative with the freely accessible 1,2-quinone (**M1**) had an enthalpy of activation of 9.6 kcal mol^{-1}. For the partially accessible 1,2-quinone (**M2**), $\Delta H^{\ddagger} = 12.8$ kcal mol^{-1}, while $\Delta H^{\ddagger}$ of the buried 1,2-quinone

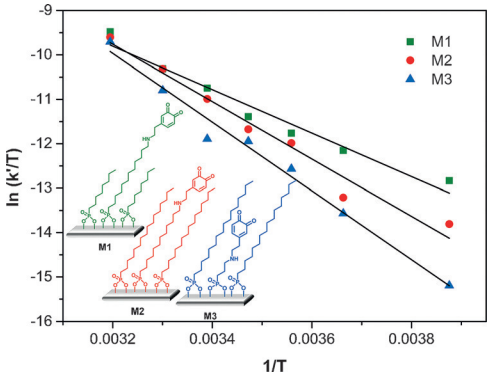

Figure 5. Plot of $\ln(k/T)$ vs. $1/T$ for the SPOCQ with monolayers presenting 1,2-quinone groups in either a freely accessible environment (**M1**), partially accessible (**M2**), or a buried environment (**M3**).

Table 2: Thermodynamic parameters of activation (determined by DART-HRMS) for the interfacial SPOCQ reaction with monolayers presenting 1,2-quinone groups in different microenvironment (**M1**, **M2**, and **M3**).

Microenvironment	$\Delta H^{\neq}$ [kcal mol^{-1}]	$\Delta S^{\neq}$ [cal K^{-1} mol^{-1}]
M1	9.7 ± 0.8	-35.9 ± 2.9
M2	12.8 ± 0.7	-25.5 ± 2.4
M3	15.4 ± 0.8	-18.8 ± 3.0

(**M3**) was 15.4 kcal mol^{-1}, which may be readily explained by the increased steric hindrance imposed by the surrounding alkane chains. In contrast, the loss of disorder ($\Delta S^{\neq}$) is smallest for the buried systems, suggesting some pre-organization prior to the rate-determining step. These trends in $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are clearly compensating one another, to give rather similar free energies of activation ($\Delta G^{\neq}$), explaining the modest three-fold difference in reaction rate around room temperature.

The observed activation parameters also shed light on the two-fold difference between the interfacial SPOCQ and SPAAC reactions. The activation enthalpy of the SPOCQ reaction is, for the sterically most accessible mixed monolayer (**M1**), about 6 kcal mol^{-1} higher than that for the SPAAC reaction.[7c] However, since SPOCQ is still faster than SPAAC, the reaction rate is determined by the entropy of activation, and indeed the loss of disorder is much less for the transition state of SPOCQ than that of SPAAC. More extensive studies of the activation parameters for a range of surface-bound organic reactions are underway to shed light onto the differences between different reactions and to explain in more detail the differences between solution-phase and surface-bound reactions.

In conclusion, we have demonstrated the superior behavior of SPOCQ for the functionalization of surfaces, as shown by an unprecedented complete reaction of immobilized quinones on the surface. The reaction rate of interfacial SPOCQ was successfully measured using DART-HRMS, and it was established that interfacial cycloaddition proceeds with high reaction rates. We additionally determined the activation parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$), which help to understand the mechanistic basis for the variation in rate constants observed within the series of monolayers. We expect this facile and quantitative surface attachment using SPOCQ chemistry to be of particular value for the immobilization of small and large (bioactive) molecules on a wide variety of surfaces, which may find useful application in materials science, life sciences, and health care (next-generation diagnostics).

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Conflict of interest

The authors declare no conflict of interest.

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