▌Surface Chemistry

Quantitative and Orthogonal Formation and Reactivity of SuFEx Platforms

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Abstract: The constraints of minute reactant amounts and the impossibility to remove any undesired surface-bound products during monolayer functionalization of a surface necessitate the selection of efficient, modular and orthogonal reactions that lead to quantitative conversions. Herein, we explore the character of sulfur–fluoride exchange (SuFEx) reactions on a surface, and explore the applicability for quantitative and orthogonal surface functionalization. To this end, we demonstrate the use of ethenesulfonyl fluoride (ESF) as an efficient SuFEx linker for creating "SuFEx-able" monolayer surfaces, enabling three distinct approaches to utilize SuFEx chemistry on a surface. The first approach relies on a di-SuFEx loading allowing dual functionalization with a nucleophile, while the two latter approaches focus on dual (CuAAC–SuFEx/SPOCQ–SuFEx) click platforms. The resultant strategies allow facile attachment of two different substrates sequentially on the same platform. Along the way we also demonstrate the Michael addition of ethenesulfonyl fluoride to be a quantitative surface-bound reaction, indicating significant promise in materials science for this reaction.

Introduction

The facile and robust attachment of molecular functionality to surfaces is receiving increasing scientific scrutiny.[1] It is of interest for a wide range of applications, including: the preparation of protein-repelling surfaces,[2] the attachment of biomolecules such as DNA for biosensor fabrication,[3] dynamic surface functionalization,[4] and nanoparticle immobilization.[5] Generally, surface modification is achieved through the formation of stable self-assembled monolayers (SAMs) or polymer brushes on a solid substrate, followed by subsequent functionalization.[6] In this regard, functionalization by click chemistry has proven to be the most efficient and kinetically superior method.[7] However, the stringent criteria that a transformation should meet to deserve the click label [modular, high yielding, wide in scope, generate minimal side-products and mild reaction conditions], inevitably limits the number of available reactions.[8] This acquires an even higher relevance in the context of polymer modification[9] or surface functionalization, where a post-reaction purification is rarely possible. In order to acquire optimal control over surface properties, a reaction efficiency of 100% is thus desirable. For example, the surface-bound Cu^I-catalyzed azide–alkyne cycloaddition (CuAAC)[10] has been shown to possess such characteristics by Chidsey et al.[11] However, the cytotoxic nature of copper catalysts along with the steric demands of the most effective Cu-ligands are limiting factors, and there is a growing demand for new interfacial relevant click reactions that offer the prospect of orthogonal reac-

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tivity.[12] This is not always trivial; some reactions that have been shown to proceed efficiently in solution (e.g. the strain-promoted alkyne–azide cycloaddition),[13] do not necessarily proceed with (near-) quantitative yields within an organic monolayer.[14]

The SuFEx (sulfur–fluoride exchange) family of click reactions reported recently by Sharpless and co-workers,[15] are practical metal-free transformations with wide application and scope.[16] SuFEx reactions involve the exchange of an S—F bond in a substitution reaction, typically with aryl silyl ethers, in the presence of a catalyst such as diazabicycloundec-7-ene (DBU) or tri-azobicyclodecene (TBD)[17] or HF_2^- anion.[18] The reactions take place in solution and in polymer synthesis, with complete selectivity and very high efficiency.[19] The newly formed S—O bonds yield stable connections, and SuFEx has proven extremely reliable in polymer synthesis[19] and post-polymerization modification.[20] For example, Locklin and co-workers recently demonstrated the utility of SuFEx for orthogonal post-polymerization functionalization of polymer brushes at a surface.[21]

However, while the SuFEx click chemistry of silyl ethers is well-documented,[19,22] also for surface modification, the use of silyl ethers typically requires additional synthetic steps. Therefore, we thought it to be of interest to explore the S—N forming SuFEx reaction, which has received much less attention. Given the abundance of available natural and synthetic amines, we envisioned that the development of a SuFEx platform for surface immobilization of amines would be highly advantageous. Moreover, since the resulting sulfur-linked amide bond is typically more stable than a corresponding ester, this would present an extra advantage in terms of stability, providing that the surface-bound SuFEx reaction can be shown with real click efficiency.

In this paper, we report such a development, and demonstrate an efficient and quantitative interfacial SuFEx protocol between primary amines and a surface-tethered sulfonyl fluoride in the presence of TBD to give sulfonamide-terminated surfaces (Scheme 1a). To demonstrate the quantitative nature of the SuFEx reaction, we use, among other approaches, the sulfonamide linkage as a labile internal tag in direct analysis in real time-high resolution mass spectrometry (DART-HRMS),[23] and investigate three distinct approaches towards our goal (Scheme 1b). The first approach involves the preparation of a dual "SuFEx-able" platform, while the other two approaches we investigate the orthogonality of surface-bound SuFEx reaction with both CuAAC and the strain-promoted oxidation-controlled cyclooctyne quinone cycloaddition (SPOCQ).[24] In the first example, we also provide the first evidence for the "click" character of Michael addition of the SuFEx linker, ethenesulfonyl fluoride (ESF), with amines at an interface. Finally, we elucidate and rationalize the kinetics of the SuFEx reaction at the solution to solid interface, and demonstrate its efficiency using X-ray photoelectron spectroscopy (XPS) and ambient desorption/ionization mass spectrometry. In this way we aim to provide a quantitative click strategy for the surface attachment of ESF and of amine-functionalized molecules.
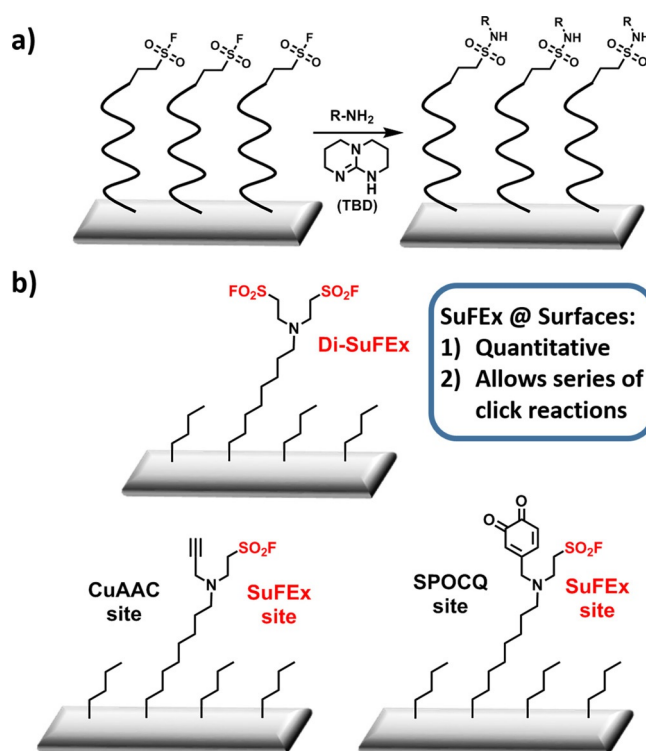
Scheme 1. a) Surface-bound SuFEx reaction with amines. b) Multiple or sequential orthogonal interfacial SuFEx click reactions as used in this study.

Results and Discussion

Fragmentation of SuFEx products in solution DART-HRMS

DART-HRMS is an ambient desorption ionization-mass spectrometry technique[25] that uses electronically excited metastable He species (23S, 19.8 eV) to generate a wide range of atmosphere-related reactive species (e.g. $O_2^{\bullet-}$, protonated water clusters, etc.). This broad set of ions can be used to obtain MS-detectable ionized fragments[26] from a wide range of functional groups in both solution phase[27] and on surfaces,[28] which are carried into the mass spectrometer by heated He gas. We have recently demonstrated the utility of this technique for qualitative and quantitative surface analysis of several surface-bound click reactions[14,29] and surface-bound hydrogen-bond formation and exchange.[30]

In order to investigate interfacial SuFEx reactions using DART-HRMS, it was first necessary to understand the solution phase behavior of SuFEx products under DART conditions. The objective thus was to outline the fragmentation patterns of SuFEx products under DART analysis conditions, and thereby learn about the fragmentation sites in these molecules. It would then also allow us to put a finger on whether positive or negative mode was to be preferred for surface analysis.

To achieve this objective, we performed solution DART fragmentation experiments with compounds **1–3**,[31] via dipping of a glass capillary in a methanolic solution of the respective compound, placing the capillary in front of the mass spectrometer, and observing the fragments formed. Interestingly, we found that for all three compounds, negative mode ionization

showed many different fragments obtained by cleavage around the S—X (X=N, O) bond (Figure 1). For example, compound **1** showed the fragmented sulfonate with the loss of either the morpholino (m/z 315.9604) or phenolate (m/z 308.9871) group along with a fragmentation product formed
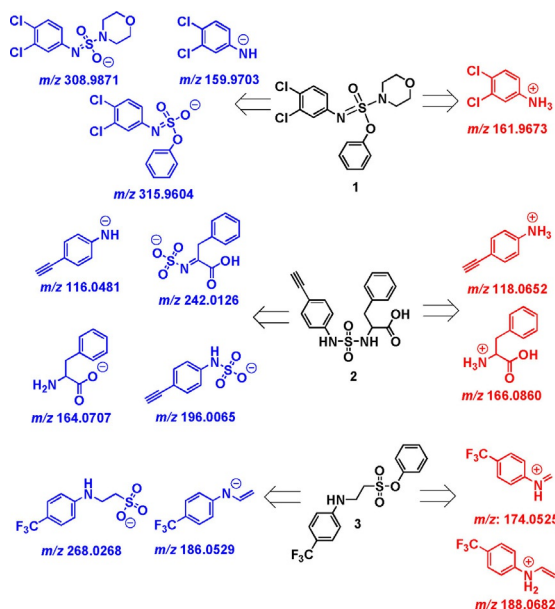
Figure 1. Fragments obtained in negative (blue) and positive (red) ion mode upon DART analysis of a selection of SuFEx products indicating cleavage of a S—N or S—OAr bond.

by cleavage at the S=N site (m/z 159.9703).[32] Interestingly, for compound **2**, we observed four different fragments that corresponded to cleavages which also occurred via bond ruptures of the various S—X links to the S-core except for the parent oxide (S=O) around the S—N bond. Most prominently, we observed the [M−H]− fragments for ethynylaniline (m/z 116.0481) and phenylalanine (m/z 164.0707).

In contrast, positive ion analysis (Figure 1) revealed much simpler and specific modes of fragmentation to the observed ions. For example, compound **1** provided an intense [M+H]+ fragment (m/z 161.9673) attributable to cleavage at the S=N bond. Similarly, the fragmentation of otherwise very stable sulfonamide linkage in **2** provided protonated ethynylaniline (m/z 118.0652) and phenylalanine (m/z 166.0860) in high intensities. This was fortuitous, as formation of exclusive fragments in high intensities is quite advantageous for real-time kinetic analysis of low product amounts on surfaces, especially in the early part during the course of a reaction. [For a more detailed overview and fragmentation spectra, see section 5 in the Supporting Information.] Based on these experiments, we could conclude that the fragmentation of SuFEx products at an interface could be anticipated around S—N or S—OAr bonds. Most importantly, we observed that for sulfonamide linkages in SuFEx products, positive mode fragmentation was more useful than negative as it exclusively yielded amine fragments in high

intensities. Since we intended to use amines as nucleophiles for interfacial SuFEx, this knowledge was incorporated in the design of our surface experiments.

Surface aminolysis of S—F by RNH₂ and kinetics determination

Our experimental design for the three SuFEx approaches involved preparation of R-SO₂F-terminated surfaces that could then be reacted with an amine that would yield easily detectable product fragments in DART-HRMS (Scheme 2). The disappearance of the F1s signal (686.0 eV) in XPS simultaneously provides an indication of the degree of conversion via disappearance of the surface reactant (S—F). Based on our previous experience,[29c] we prepared C₁₂-amine (**M₁**) and C₁₂-bromo terminated (**M₄**) phosphonic acid (PA) monolayers on aluminum oxide surfaces in a 3:1 (C₁₂ amine PA:C₈ alkyl PA and C₁₂ bromo PA:C₈ alkyl PA respectively) dilution ratio. The monolayer composition for **M₁** and **M₄** surfaces was confirmed by N/P (1:4) and Br/P (1:4) ratios in XPS wide scans (Figure S4.3, S4.4 and S4.5). The stability of the monolayer attachment to the surface in all following conversions was shown by an XPS-based N/P ratio that was in agreement with the theoretically expected ratio within experimental error. Amine-terminated surfaces (**M₁**) were then successfully derivatized to their Michael adducts with commercially available ESF, to quantitatively yield N(CH₂CH₂SO₂—F)₂ terminated "SuFEx-able" surfaces (**M₂**).

The appearance of a strong F1s signal (686.0 eV) in the XPS spectra (Figure 2a and S4.6) along with observed F/P ratios (2:4) confirmed completion of the reaction (Figure S4.7). This ratio and its corresponding error (100±2%) was derived from
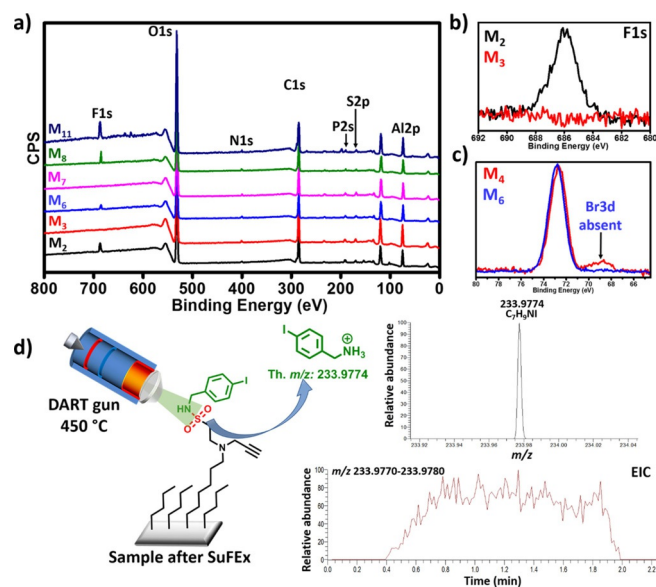
Figure 2. a) Stacked XPS wide spectra of **M₂-M₁₁** surfaces. b) Stacked F1s narrow spectra for **M₂** and **M₃** surfaces showing the disappearance of F1s peak upon complete reaction. c) Stacked Br3d narrow spectra for the **M₄** and **M₅** surfaces showing the disappearance of the Br3d signal upon complete propargylation. d) Schematic impression of the S—N bond fragmentation and subsequent ionization of protonated 4-iodobenzylamine (m/z 233.9774) by DART-HRMS.
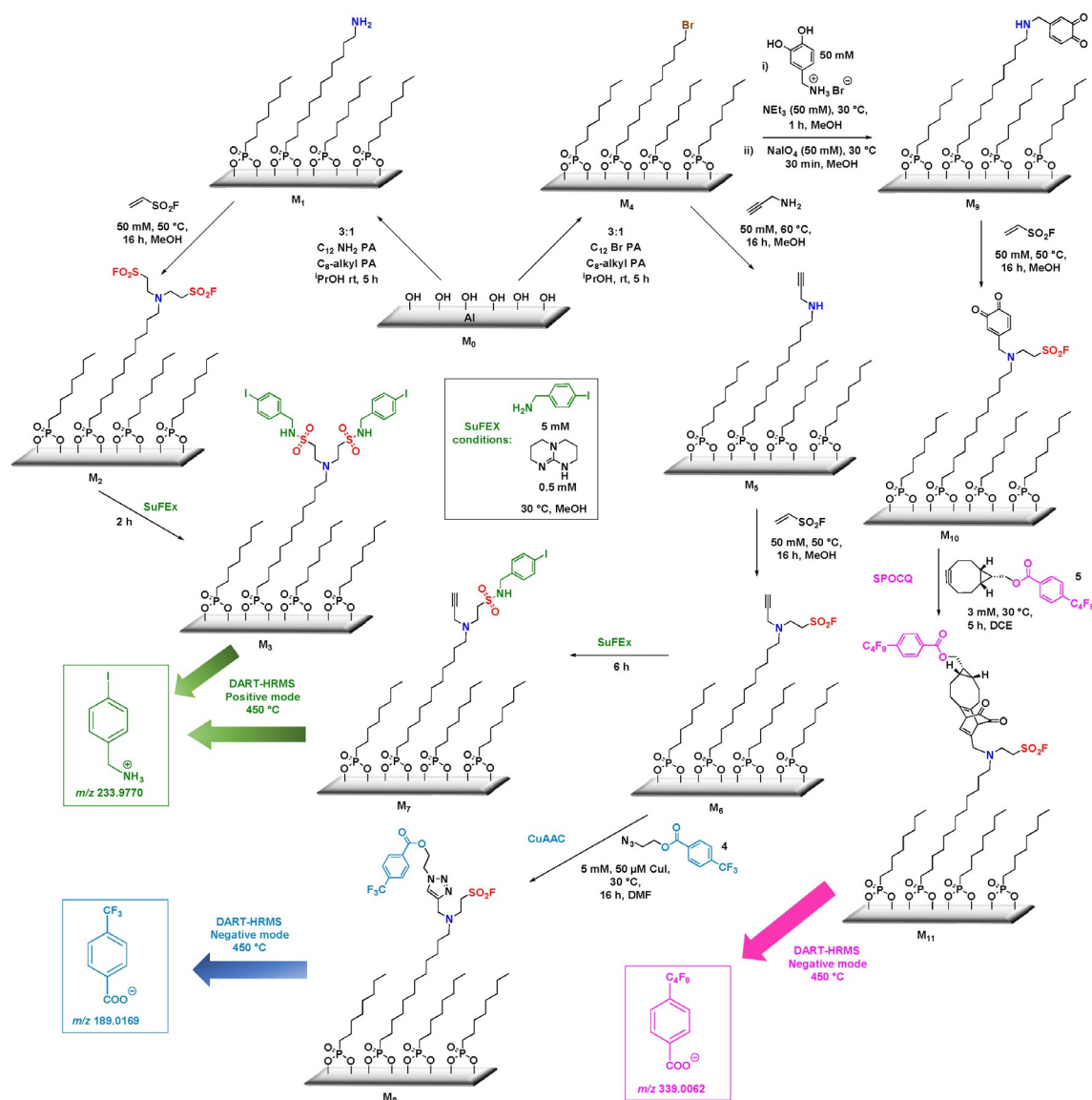
Scheme 2. General scheme showing the design of the interfacial SuFEx, CuAAC and SPOCQ reactions under study.

the reactions on six samples, prepared on different days and measured at multiple random spots on the samples using XPS. This click character of the Michael addition of ESF with surface-bound amines is in line with recent findings on dendrimer functionalization that show ESF-amine adducts as the most reliable embodiment of the Michael reaction known (yield >99.7%, likely more than >99.9%).[33,34] Our findings thus indicate that the Michael addition of ESF with amines can be characterized as a true click reaction, thereby demonstrating the reliability and selectivity of this bi-functional reagent.

To study the amine-based surface-bound SuFEx reaction, we chose 4-iodobenzylamine (IBZ) as a nucleophile since the iodo-phenyl motif aids detection in DART-HRMS.[30] TBD, which was found to be kinetically superior to DBU and triethylamine by Locklin and co-workers,[21a] was chosen as the non-nucleophilic catalyst. Upon stirring M_2 surfaces with IBZ (5 mM) at 30 °C, IBZ-terminated surfaces (M_3) were formed in a 100±3% yield in 2 h, as indicated by the N/P ratios (3:4) observed in the XPS

wide scan spectrum (Figure 2b and S4.9). The corresponding full disappearance of the F1s signal was also confirmed on a hexaplet of samples to within 2%. Furthermore, the absence of any carryover standard error (2–3% throughout) in the N/P ratio (changes 1:4 to 3:4 from M_2 to M_3) in XPS wide scan, which would have arisen in case of any incomplete reaction (either ESF attachment or subsequent aminolysis by IBZ), confirmed the quantitative nature of both these reactions (Michael addition with ESF and SuFEx). XPS C1s narrow scan analysis (Figure S4.10) of M_3 surfaces showed the presence of carbon atoms attributable to C–S, C–N and C–I regions, and the experimental C1s spectra correlated well with simulated spectra obtained using DFT calculations (see section 6 in the Supporting Information).[35] Upon analyzing these SuFEx-derived samples by DART-HRMS, we observed a strong signal for protonated **IBZ** (m/z 233.9774) with a characteristic trace in the extracted ion chronogram (Figure 2d and S4.11). This fragmentation pattern is akin to the S–N bond fragmentation observed for

compound **2** in solution DART experiments. This further confirmed that SuFEx with IBZ had indeed taken place and strengthened our hypothesis that S—N bond cleavage product could be used as an "internal tag" for reaction kinetics determination.

After thus showing that the surface-bound SuFEx reaction can be made quantitative, we next focused our attention on demonstrating the orthogonal nature of the SuFEx reaction at a surface, with two other transformations that have previously been shown to proceed in a quantitative manner, also at a surface.[29a] To this aim, we chose two routes: Br-terminated surfaces (**M_4**) were reacted with propargylamine to yield alkyne-terminated surfaces (**M_5**), or with 3,4-dihydroxybenzylamine to yield quinone-terminated surfaces (**M_9**) upon oxidation. The formation of **M_5** surfaces was evidenced by the disappearance of Br3d signal (69.0 eV) in the narrow scan spectra of **M_5** and **M_6** (see for example, Figure 2c and S4.13). Further confirmation of propargyl attachment was obtained by the slight lowering of static water contact angle (from $103 \pm 2°$ to $92 \pm 2°$; Figure S4.14). Following this, the **M_5** surfaces were reacted with ESF for 16 h to provide dual CuAAC–SuFEx-ready functionalities (**M_6**). The quantitative conversion to **M_6** was confirmed by the appearance of a F1s signal in the XPS wide and narrow spectrum (Figure 2a and S4.15) and an eventual F/P ratio of 1:4 in the XPS wide spectrum (Figure S4.16). Upon performing SuFEx with IBZ, we found that **M_6** surfaces achieved quantitative reaction within 6 h to yield the IBZ-alkyne-terminated surfaces (**M_7**), as evidenced by the complete disappearance of F1s signal and N/P ratios (2:4) in XPS wide spectra (Figure 2a and S4.18). C1s narrow scan analysis of **M_7** surfaces (Figure S4.19) also showed the presence of carbons in distinct chemical environments, arising from C—S, C—N and C—I linkages, the latter attributable to the iodobenzyl motif.

To test the dual click nature of our strategy, we also performed CuAAC on **M_6** surfaces using a fluorinated azide tag **4** that is labile under DART conditions.[29b] Upon stirring **M_6** surfaces with a 5 mM solution of **4** in DMF for 16 h, we observed a $80 \pm 2\%$ surface conversion to **M_8** as confirmed by the F/P (4:4) ratios in XPS wide scan spectra (Figure S4.20). Although the reaction occurred in excellent yield, we did not achieve a quantitative conversion for surface bound CuAAC under our conditions as has been reported in literature before.[11] Furthermore, DART-HRMS analysis of **M_8** surfaces also showed the presence of the fluorinated mass tag (m/z 189.0169) in the EIC (Figure S4.21).

The dual SPOCQ–SuFEx platform was prepared by reacting the Br-terminated surfaces (**M_4**) with 3,4-dihydroxybenzylamine followed by oxidation to quinone (**M_9**) as evidenced by the N/P ratios in XPS wide scan spectrum (Figure S4.22). Directly after preparation, the o-quinone-terminated surfaces (**M_9**) were reacted with ESF to install the SO_2F moiety (**M_10**). The appearance of an F1s signal in the XPS spectra with the corresponding F/P ratio (1:4) confirmed quantitative attachment (Figure S4.24) [The o-quinone surface **M_9** may be in equilibrium with the hydroquinone surface obtained after internal nucleophilic attack of the amine N-atom to yield an aziridine surface **M_13**, but upon reaction with ESF, the equilibrium should favor

the o-quinone, which is necessary to allow the SPOCQ reaction to proceed (near-)quantitatively; see Scheme 3]. A subsequent SPOCQ reaction with a fluorinated BCN MS tag (**5**) provided **M_11** surfaces as substantiated by a strong F1s signal in wide
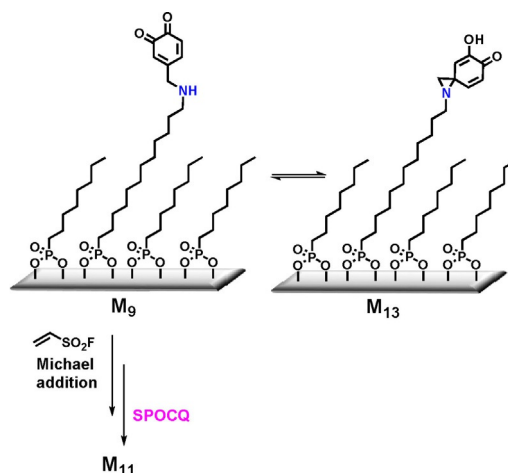

Scheme 3. Surface **M_9** might undergo equilibration with aziridine surface **M_13**, although the Michael addition and subsequently the SPOCQ reaction will pull the equilibrium to the left.

scan XPS spectra (Figure 2a). Furthermore, SPOCQ reaction on this platform occurred with excellent surface yield ($95 \pm 2\%$) as quantified using the F/P ratio (10:4) in XPS wide scan (Figure S4.25) within 5 h further displaying the modularity of our design. XPS C1s narrow scan analysis of **M_11** surfaces showed the different fluorinated carbons attributable to the C_4F_9- chain distinctly (Figure S4.26). Presence of the expected fluorinated MS fragment (m/z 339.0072) in negative mode DART-HRMS analysis of SPOCQ-modified **M_11** surfaces provided further proof of the reaction (Figure S4.27). In a previous paper the 100% efficiency of this SPOCQ reaction at a surface has been displayed—the high, but non-perfect yield (95%) obtained in the current reaction may be due to the intermittent Michael addition, where the quinones might undergo some slight reaction with for example, methanol.

In the spirit of further application of the dual click strategy for orthogonal functionalization, we performed a SuFEx microstamping experiment using aminoferrocene on **M_8** surfaces (Figure 3a). As already stated, these surfaces were CuAAC clicked with a fluorinated tag. Interestingly, after 16 h we observed a quantitative SuFEx reaction even on this sterically hindered substrate as confirmed by the N/P (5:4) and F/N (2.4:5) ratios in XPS wide scans upon aminoferrocene immobilization (Figure S4.28). The patterned surface could be easily visualized using scanning electron microscopy (SEM). SEM images (Figure 3b and S4.29) clearly showed regular patterns with a width of 5 μm. Moreover, XPS Fe2p narrow scan (705–725 eV) analysis clearly showed emergence of Fe2p signals (710.0 eV and 723.0 eV) characteristic of the ferrocene moiety (Figure S4.30).

Having established the reaction efficiency, orthogonality and applicability of SuFEx, we finally embarked on determination
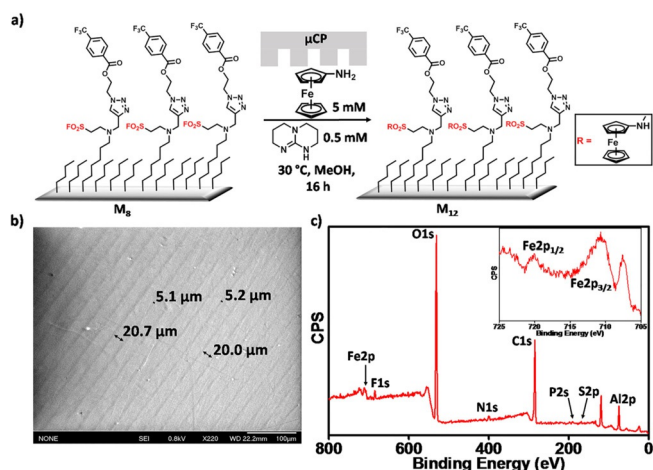
Figure 3. a) General schematic showing SuFEx reaction on M_8 surfaces by microcontact (μCP) stamping with aminoferrocene. b) SEM image obtained for M_{12} surfaces after μCP showing the 5 μm patterns (scale = 100 μm). c) XPS wide spectrum for M_{12} surfaces showing the Fe2p signal (inset: Fe2p narrow scan).

of the reaction kinetics by DART-HRMS. To this end, we reacted M_2 samples with IBZ (Figure 4a and 4b) for different time intervals (up to 4 h) and followed the signal intensity of protonated IBZ (m/z 233.9774) in DART-HRMS. The pseudo-first-order rate constant (k') was calculated as the slope of the plot of $\ln|(I_t - I_\infty)/(I_0 - I_\infty)|$ versus time (t), in which I_∞ corresponds to the asymptotic integrated extracted ion chronogram (EIC) intensity as obtained by exponential decay curve fitting of the data (Figure 4b). The pseudo first-order rate constant (k') for SuFEx on M_2 surfaces, at a concentration of 5.0 mM at 30 °C was $(9 \pm 1) \times 10^{-4}$ s^{-1}, yielding a second-order rate constant of 0.18 ± 0.02 M^{-1}s^{-1}. This rate constant refers to the initial well-behaved kinetic region as we observed two distinct kinetic regimes for this surface-bound SuFEx reaction: an initial fast regime followed by a slower, more complex one similar to that observed by us for surface-bound SPAAC and SPOCQ reactions previously.[14, 29a] However, in contrast to SPOCQ (reaction completion in 4 h), the SuFEx reaction was already quantitative on surfaces in 2 h under the conditions used in this study. These

findings unequivocally demonstrate that interfacial SuFEx is indeed an excellent candidate for orthogonal surface click functionalization.

Conclusions

In summary, we have developed a new platform for surface functionalization using SuFEx click chemistry and amine nucleophiles. The hypothesis in question was whether the click character of this reaction in solution could also reflect on a surface. After thorough XPS and DART-HRMS investigations, we indeed found this to be the case. In addition, we determined the second-order rate constant for this surface-bound reaction to be 0.18 ± 0.02 M^{-1}s^{-1}. We also explored the orthogonality of the SuFEx reaction by exploring a dual CuAAC/SPOCQ–SuFEx platform, where-by two click reactions could be conducted on a single chip in high yields. We found that even under sterically challenging environments, SuFEx maintained its click nature, thus providing quantitative conversion. Along the way, we also demonstrated the quantitative nature of the surface-bound Michael addition of the SuFEx linker, ethenesulfonyl fluoride with amines. Strong points of our SuFEx methodology when compared to other interfacial click reactions include the easy and efficient preparation of the sulfonyl fluoride motif, wide availability of amines, quantitative reaction yields and high modularity. Since interfacial reactions are typically displaying rather stringent steric limitations, this finding indicates that also this reaction has significant potential in materials sciences beyond that of surface modifications. This work therefore opens exciting prospects for further application of these reactions for other "SuFEx-able platforms" such as diverse solid surfaces, polymers and other complex organic materials.
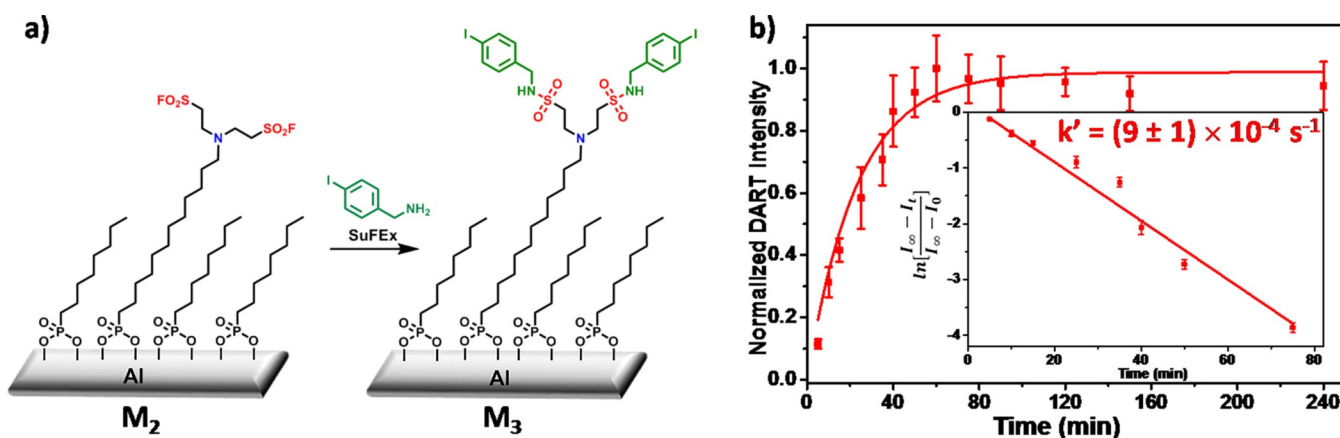
Figure 4. a) Schematic depiction of SuFEx reaction using IBZ on M_2, and b) normalized DART-HRMS intensity versus time (min) for di-SuFEx (M_2 to M_3) Inserts: Linear plots of $\ln[(I_\infty - I_t)/(I_\infty - I_0)]$ versus time (min) to obtain the pseudo-first order constants.

Conflict of interest

The authors declare no conflict of interest.

[1] a) S. Onclin, B. J. Ravoo, D. N. Reinhoudt, *Angew. Chem. Int. Ed.* **2005**, *44*, 6282–6304; *Angew. Chem.* **2005**, *117*, 6438–6462; b) R. Yan, L. Le Pleux, M. Mayor, M. Zharnikov, *J. Phys. Chem. C* **2016**, *120*, 25967–25976.

[2] Z. Wang, H. Zuilhof, *Langmuir* **2016**, *32*, 6310–6318.

[3] W.-W. Zhao, J.-J. Xu, H.-Y. Chen, *Chem. Rev.* **2014**, *114*, 7421–7441.

[4] C.-K. Liang, G. V. Dubacheva, T. Buffeteau, D. Cavagnat, P. Hapiot, B. Fabre, J. H. R. Tucker, D. M. Bassani, *Chem. Eur. J.* **2013**, *19*, 12748–12758.

[5] C. Tassa, M. Liong, S. Hilderbrand, J. E. Sandler, T. Reiner, E. J. Keliher, R. Weissleder, S. Y. Shaw, *Lab Chip* **2012**, *12*, 3103–3110.

[6] S. P. Pujari, L. Scheres, A. T. M. Marcelis, H. Zuilhof, *Angew. Chem. Int. Ed.* **2014**, *53*, 6322–6356; *Angew. Chem.* **2014**, *126*, 6438–6474.

[7] J. Escorihuela, A. T. M. Marcelis, H. Zuilhof, *Adv. Mater. Interfaces* **2015**, *2*, 1500135.

[8] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; *Angew. Chem.* **2001**, *113*, 2056–2075.

[9] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem. Int. Ed.* **2011**, *50*, 60–62; *Angew. Chem.* **2011**, *123*, 61–64.

[10] V. Castro, H. Rodríguez, F. Albericio, *ACS Comb. Sci.* **2016**, *18*, 1–14.

[11] J. P. Collman, N. K. Devaraj, T. P. A. Eberspacher, C. E. D. Chidsey, *Langmuir* **2006**, *22*, 2457–2464.

[12] S. V. Orski, A. A. Poloukhtine, S. Arumugam, L. Mao, V. V. Popik, J. Locklin, *J. Am. Chem. Soc.* **2010**, *132*, 11024–11026.

[13] N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047.

[14] R. Sen, J. Escorihuela, M. M. J. Smulders, H. Zuilhof, *Langmuir* **2016**, *32*, 3412–3419.

[15] J. Dong, L. Krasnova, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2014**, *53*, 9430–9448; *Angew. Chem.* **2014**, *126*, 9584–9603.

[16] a) C. J. Smedley, A. S. Barrow, C. Spiteri, M.-C. Giel, P. Sharma, J. E. Moses, *Chem. Eur. J.* **2017**, *23*, 9990–9995; b) A. Dondoni, A. Marra, *Org. Biomol. Chem.* **2017**, *15*, 1549–1553; c) W. Chen, J. Dong, L. Plate, D. E. Mortenson, G. J. Brighty, S. Li, Y. Liu, A. Galmozzi, P. S. Lee, J. J. Hulce, B. F. Cravatt, E. Saez, E. T. Powers, I. A. Wilson, K. B. Sharpless, J. W. Kelly, *J. Am. Chem. Soc.* **2016**, *138*, 7353–7364.

[17] J. Dong, K. B. Sharpless, L. Kwisnek, J. S. Oakdale, V. V. Fokin, *Angew. Chem. Int. Ed.* **2014**, *53*, 9466–9470; *Angew. Chem.* **2014**, *126*, 9620–9624.

[18] B. Gao, L. Zhang, Q. Zheng, F. Zhou, L. M. Klivansky, J. Lu, Y. Liu, J. Dong, P. Wu, K. B. Sharpless, *Nat. Chem.* **2017**, *9*, 1083–1088.

[19] J. Yatvin, K. Brooks, J. Locklin, *Chem. Eur. J.* **2016**, *22*, 16348–16354.

[20] S. Li, L. T. Beringer, S. Chen, S. Averick, *Polymer* **2015**, *78*, 37–41.

[21] a) J. Yatvin, K. Brooks, J. Locklin, *Angew. Chem. Int. Ed.* **2015**, *54*, 13370–13373; *Angew. Chem.* **2015**, *127*, 13568–13571; b) K. Brooks, J. Yatvin, C. D. McNitt, R. A. Reese, C. Jung, V. V. Popik, J. Locklin, *Langmuir* **2016**, *32*, 6600–6605.

[22] H. Zhu, D. Chen, N. Li, Q. Xu, H. Li, J. He, H. Wang, P. Wu, J. Lu, *Chem. Eur. J.* **2017**, *23*, 14712–14717.

[23] a) J. H. Gross, *Anal. Bioanal. Chem.* **2014**, *406*, 63–80; b) R. B. Cody, J. A. Laramee, H. D. Durst, *Anal. Chem.* **2005**, *77*, 2297–2302; c) S. C. Elena, G. E. Morlock, A. R. Igor, *Russ. Chem. Rev.* **2011**, *80*, 235.

[24] a) A. Borrmann, O. Fatunsin, J. Dommerholt, A. M. Jonker, D. W. P. M. Löwik, J. C. M. van Hest, F. L. van Delft, *Bioconjugate Chem.* **2015**, *26*, 257–261; b) J. J. Bruins, A. H. Westphal, B. Albada, K. Wagner, L. Bartels, H. Spits, W. J. H. van Berkel, F. L. van Delft, *Bioconjugate Chem.* **2017**, *28*, 1189–1193; c) J. J. Bruins, B. Albada, F. L. van Delft, *Chem. Eur. J.* **2018**, *24*, 4749–4756.

[25] M. E. Monge, G. A. Harris, P. Dwivedi, F. M. Fernández, *Chem. Rev.* **2013**, *113*, 2269–2308.

[26] a) L. Song, S. C. Gibson, D. Bhandari, K. D. Cook, J. E. Bartmess, *Anal. Chem.* **2009**, *81*, 10080–10088; b) L. Song, A. B. Dykstra, H. Yao, J. E. Bartmess, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 42–50.

[27] a) R. B. Cody, *Anal. Chem.* **2009**, *81*, 1101–1107; b) G. A. Gómez-Ríos, E. Gionfriddo, J. Poole, J. Pawliszyn, *Anal. Chem.* **2017**, *89*, 7240–7248.

[28] a) R. K. Manova, S. Joshi, A. Debrassi, N. S. Bhairamadgi, E. Roeven, J. Gagnon, M. N. Tahir, F. W. Claassen, L. M. W. Scheres, T. Wennekes, K. Schroën, T. A. van Beek, H. Zuilhof, M. W. F. Nielen, *Anal. Chem.* **2014**, *86*, 2403–2411; b) L. M. Sanchez, M. E. Curtis, B. E. Bracamonte, K. L. Kurita, G. Navarro, O. D. Sparkman, R. G. Linington, *Org. Lett.* **2011**, *13*, 3770–3773; c) S. Zhou, M. W. Forbes, J. P. D. Abbatt, *Anal. Chem.* **2015**, *87*, 4733–4740.

[29] a) R. Sen, J. Escorihuela, F. L. van Delft, H. Zuilhof, *Angew. Chem. Int. Ed.* **2017**, *56*, 3299–3303; *Angew. Chem.* **2017**, *129*, 3347–3351; b) R. Sen, D. Gahtory, R. R. Carvalho, B. Albada, F. L. van Delft, H. Zuilhof, *Angew. Chem. Int. Ed.* **2017**, *56*, 4130–4134; *Angew. Chem.* **2017**, *129*, 4194–4198; c) R. Sen, D. Gahtory, J. Escorihuela, J. Firet, S. P. Pujari, H. Zuilhof, *Chem. Eur. J.* **2017**, *23*, 13015; d) D. Gahtory, R. Sen, A. R. Kuzmyn, J. Escorihuela, H. Zuilhof, *Angew. Chem. Int. Ed.* **2018**, DOI: https://doi.org/10.1002/anie.201800937.

[30] D. Gahtory, R. Sen, M. M. J. Smulders, H. Zuilhof, *Faraday Discuss.* **2017**, *204*, 383–394.

[31] S. Li, P. Wu, J. E. Moses, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2017**, *56*, 2903–2908; *Angew. Chem.* **2017**, *129*, 2949–2954.

[32] M. Sun, W. Dai, D. Q. Liu, *J. Mass Spectrom.* **2008**, *43*, 383–393.

[33] Q. Zheng, J. Dong, K. B. Sharpless, *J. Org. Chem.* **2016**, *81*, 11360–11362.

[34] Q. Zheng, H. Wang, P. Wu, K. B. Sharpless, Unpublished results.

[35] M. Giesbers, A. T. M. Marcelis, H. Zuilhof, *Langmuir* **2013**, *29*, 4782–4788.