Functionalization of fluorous thin films *via* "click" chemistry†

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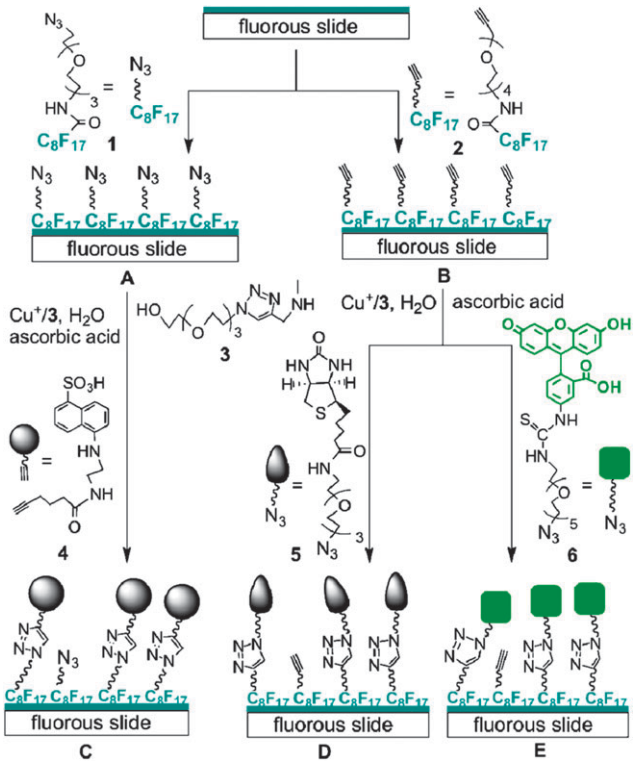
The first covalent modification of thin films non-covalently immobilized *via* fluorous interactions was demonstrated with "click" reactions in 70–80% yields.

Surface functionalization based on "click" chemistry[1] with copper-catalyzed azide–alkyne cycloaddition (CuAAC) reactions has attracted great interest.[1–3] Although CuAAC on a variety of substrates has previously been demonstrated,[2,4–11] introducing suitable handles to the substrate surface to render it "clickable" has been achieved through covalent immobilization, which can be cumbersome. For example, glass surfaces presenting ethynyl or azido groups were prepared by lengthy reactions.[5,7] Since many applications involving optical detection are performed on glass substrates, a practical method for generating "clickable" glass surfaces is valuable. Herein, we show such a method based on the fluorous immobilization of "clickable" thin films on commercially available fluorous glass slides. We demonstrate that the fluorous immobilized films are sufficiently stable to allow functionalization *via* CuAAC in aqueous or suitable organic solvents, and in a microarray format. This method is complimentary to the direct fluorous immobilization of tagged molecules,[12–17] and extends the application of fluorous thin films.

The efficiency of direct fluorous immobilization has been demonstrated by the preparation of high quality microarrays of fluorous-tagged carbohydrates and small molecules on fluorous surfaces.[12–16] Despite the exciting development of this practical immobilization method, its scope and limitations await to be established. For example, for systems where non-fluorous interactions are strong, a sufficiently large perfluorocarbon tag, *e.g.*, C_8F_{17}, is required to immobilize the molecules.[13] However, large fluorous tags greatly decrease the solubility of molecules in aqueous solution. In fact, to date all fluorous thin films were deposited from a solution containing organic solvents that may possibly denature the molecules. These concerns can be circumvented by our click chemistry-based approach on fluorous thin films. Furthermore, click chemistry is specific and compatible with a wide variety of functional groups, and can be performed in both aqueous and organic solvents.[1,2]

As shown in Scheme 1, "clickable" surfaces **A** and **B**, presenting azido and ethynyl groups, respectively, were readily prepared simply by immersion of a fluorous glass slide (Fluorous Tech., Inc.) in a 1 mM solution of the fluorous azide **1** or alkyne **2** in methanol, followed by washing with methanol. The CuAAC reaction on the surfaces were catalyzed by a complex of Cu^+ with ligand **3** containing a secondary amine and a triazole ring. The amine is as an electron donor to Cu^+, which accelerates the reaction, while the triazole ring readily dissociates to allow formation of the Cu(I)-acetylide–ligand complex.[18] The Cu^+–ligand **3** complex is similar to those reported by Fokin and coworkers for CuAAC,[18] but is modified with oligo(ethylene glycol) chains to increase water solubility. Thus, using a general procedure,[19] the sulfur-containing alkyne **4**, the azido-tagged biotin derivative **5**, or the FITC-labeled azide **6** was readily "clicked" onto surface **A** or **B**, leading to surface **C**, **D** or **E**.



Scheme 1 The preparation of "clickable" fluorous films and demonstration of their covalent functionalization *via* the CuAAC reaction.

All films were characterized by X-ray photoelectron spectroscopy (XPS), and selected results are presented in Fig. 1. An XPS survey of azido-terminated surface **A** (Fig. 1(a)) shows the presence of the expected C, F, N and O signals. The broad N 1s signal in the high resolution scan (Fig. 1(b)) was fitted and deconvoluted into three peaks: 400.1 eV and 403.2 eV attributed to the azido group (**N**═N═**N** and N═**N**═N,

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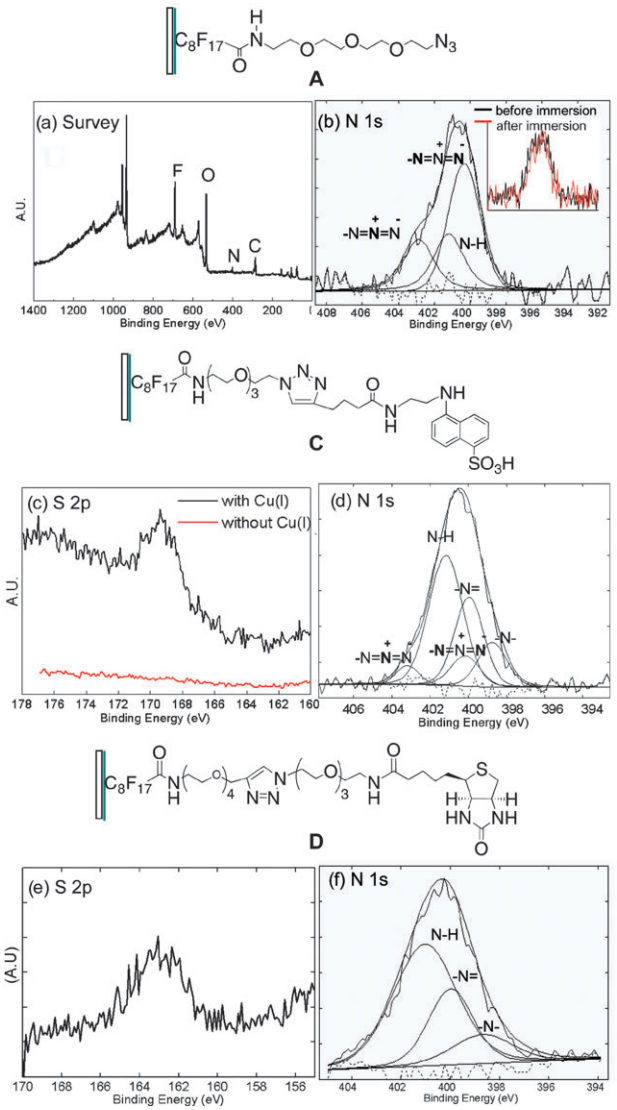

Fig. 1 XPS spectra of the fluorous thin films before and after the CuAAC reactions. (a) Survey and (b) N 1s narrow scan of azido-modified surface **A**. The inset of (b) is the overlay of the spectra obtained before and after 4 h immersion of surface **A** in methanol. (c) S 2p and (d) N 1s narrow scan of surface **C** upon CuAAC reaction of surface **A** and **4**. The red curve in (c) is the negative control without the copper catalyst. (e) S 2p and (f) N 1s narrow scan of biotin-presenting surface **D**. The dotted lines under the deconvoluted N 1s curves correspond to the difference between the original and fitted curves.

respectively),[4,20] and 401.3 eV assigned to the amide nitrogen.[6,21] The calculated ratio of the areas under the three peaks is 1.9 : 1 : 1.1, close to the expected ratio (2 : 1 : 1) for film **A**. To address the stability of the films during the reaction, a sample of **A** was immersed in methanol for 4 h. XPS showed that the intensities of the N signal remained unchanged (inset of Fig. 1(b)). Furthermore, only a small change in the F : C ratio (1.19 *vs.* 1.21) before and after the immersion was observed. The films were also stable in PBS buffer for 4 h (data not shown).

Upon CuAAC reaction between surface **A** and alkyne **4** to form surface **C**, XPS showed the presence of an S 2p peak at 169.8 eV, attributable to the sulfonic acid group[22] (Fig. 1(c)). The broad N 1s signal was centered at 400.4 eV (Fig. 1(d)). The S : N ratio provides a rough estimation of the reaction yield of ~80%.[23] The broad N 1s peak can be fitted and deconvoluted into five peaks with the following tentative assignments: 398.7 eV (**N**–N═N),[6] 400.0 (N═N),[6] 400.2 (**N**═N═**N**), 401.2 (O═C–N) and 403.2 eV (N═**N**═N). The ratio of the peak areas is 0.7 : 1.5 : 0.5 : 2.5 : 0.2, consistent with the resulting surface upon the CuAAC reaction in ~80% yield. In a control experiment, surface **A** was subjected to the same reaction conditions but in the absence of copper. No peak was observed in the high resolution scan of the S 2p region (Fig. 1(c), red curve), thus, no attachment of alkyne **4** to azido surface **A** was observed in the absence of the catalyst for the CuAAC reaction.

CuAAC could also be performed on ethynyl-terminated surface **B** (Scheme 1) with azido-labeled biotin **5** following the general procedure.[19] The XPS narrow scans of the S 2p and N 1s regions for the resulting biotinylated surface, **D**, are shown in Fig. 1(e) and (f), respectively. Based on the S : N ratio, the yield of the reaction was estimated to be ~70%.[23] This yield is among the highest of those reported for click reactions of azides with surfaces presenting ethynyl groups.[2] The close proximity of the ethynyl groups may lead to side reactions, such as copper-catalyzed homocoupling in the presence of adventitious O_2.[24] A broad peak at 162.7 eV in the S 2p region was attributed to the biotin.[25] The broad N 1s signal was fitted and deconvoluted into three peaks with the following tentative assignments: 398.7 (**N**–N═N), 400.0 (N═N) and 401.2 eV (O═C–N). The ratio of the peak areas is 0.7 : 1.4 : 3.2, which correlates well with the calculated ratio for a 70% yield reaction (0.7 : 1.4 : 3.1). Unreacted azides were not present after the CuAAC reaction, as no peak was observed near 403 eV.

The presence of biotin covalently attached to the surface upon CuAAC was confirmed by its specific binding with FITC-labeled avidin. Specifically, a solution of FITC–avidin (0.5 mg mL^{-1}) in PBS buffer was spotted on a biotinylated surface, **D**. As a control, a solution of biotin-saturated FITC–avidin (1 mg mL^{-1}) in PBS buffer was spotted on the adjacent area. The spotting was performed using a SpotBot® 2 microarrayer (Telechem Int. Inc., CA). The samples were incubated at 58% relative humidity for 30 minutes, and then washed twice with PBS buffer. Fluorescence images show that the FITC–avidin bound to surface **D** (Fig. 2(a)), while biotin-saturated FITC–avidin did not bind to the surface (Fig. 2(b)), thus proving that the surface was biotinylated and specifically bound to avidin.

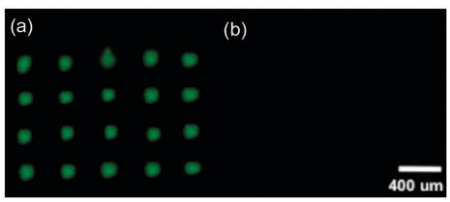

Fig. 2 Fluorescence image of biotinylated surface **D** after spotting a solution of (a) FITC–avidin and (b) biotin-saturated FITC–avidin.

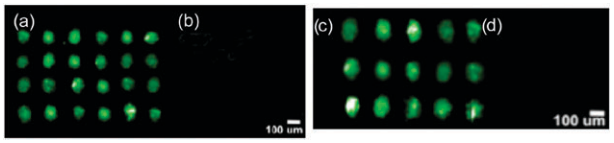

Fig. 3 Functional microarrays on fluorous slides *via* click chemistry. (a) Fluorescence image after incubation of arrays of azide **6** in the presence of the Cu^+ catalyst on ethynyl-terminated surface **B**. (b) Fluorescence image of the negative control with **6** on **B** without the Cu^+ catalyst. (c) Fluorescence image after incubation of arrays of FITC–BSA–azido on **B**. (d) Fluorescence image of the negative control with FITC–BSA–azido on **B** without the Cu^+ catalyst.

The surface CuAAC reaction on the fluorous immobilized thin films could also be performed in microarray format. Thus, a mixture of the azide-tagged FITC dye **6** (10 mM) with $Cu(MeCN)_4PF_6$ (2.5 mM), ligand **3** (25 mM) and ascorbic acid (50 mM) in a methanol–water (1 : 9 v/v) mixture was spotted on the ethynyl-terminated fluorous thin film **B** (Scheme 1). Similarly, click reactions on a microarray format were also performed to attach proteins, such as BSA (bovine serum albumin) modified with both FITC and azido groups (see the ESI†). The samples were incubated at 58% relative humidity for 6 h, and washed with water and methanol (see the ESI†). The fluorescence images in Fig. 3(a) and (c) show the intense green spots corresponding to the immobilized FITC dye in the resulting surface, **E**, and FITC-labelled BSA, respectively. As a negative control, the same reaction mixtures in the absence of the copper catalyst were spotted on adjacent areas. No fluorescence was observed on these areas (Fig. 3(b) and (d)) upon otherwise the same treatment, thus establishing that the molecules cannot be immobilized in the absence of the Cu^+ catalyst for the click reaction.

In conclusion, we have developed a practical method for surface immobilization on fluorous substrates *via* click chemistry. The thin films with ethynyl or azido handles are readily prepared on commercial fluorous glass slides, and such non-covalently immobilized thin films are surprisingly stable, allowing covalent functionalization in good yields (70–80%) *via* CuAAC reactions in aqueous or suitable organic solvents, and in microarray format. This method is complementary to the direct deposition of fluorous-tagged compounds. Furthermore, the same approach can be used for other "click" reactions on fluorous surfaces.

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