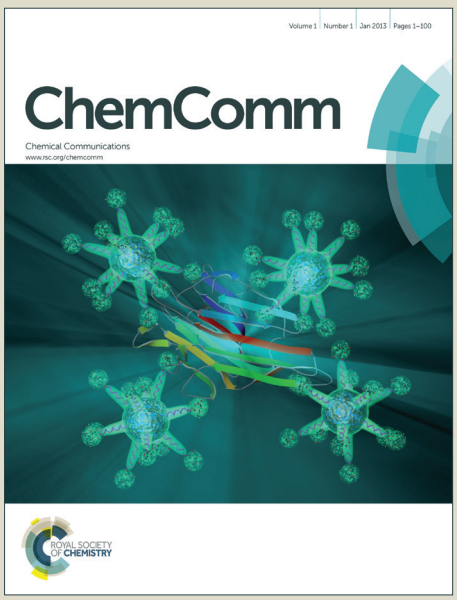
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

UV-mediated tuning of surface biorepulsivity in aqueous environment

Theresa Weber[a], Nikolaus Meyerbröker[a,b], Nuruzzaman Khan Hira[b], Michael Zharnikov[b]*, and Andreas Terfort[a]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

While it is well-known that oligoethylene glycol (OEG) terminated self-assembled monolayers (SAMs) can be deteriorated by UV irradiation in air, we now report that the analogous modification can also be performed in water, opening the opportunity for *in situ* tuning of biorepulsive properties. Surprisingly, this deterioration also takes place even in the absence of molecular oxygen, resulting in a very selective process.

Control of surface properties in terms of biocompatibility and biorepulsivity are primary challenges for a variety of frontier technologies, including fabrication of biosensors, medicine, marine biofouling, etc.[1] A popular approach in this regard is surface functionalisation with oligoethylene (OEG) based materials, which can either be deposited as OEG brushes by so-called "grafting to"[3] or "grafting from"[4] methods or as OEG terminated self-assembled monolayers (SAMs).[2] The biorepulsive properties of the latter systems depend on the amount of the OEG material so that they can be adjusted to some extent by molecular design.[2] An alternative way for such an adjustment is the exposure of OEG terminated surfaces to electrons[5] or UV light[6], resulting in a predominant damage of the OEG segments. This approach is particularly attractive since the irradiation treatment can be performed in a lithographic way, resulting in complex bioaffinity patterns imbedded into biorepulsive backgrounds.[7] The bioaffinity, which is initially non-specific, can then be easily turned into a specific one by a subsequent exchange reaction of the electron/UV damaged SAM constituents with the moieties bearing specific receptors for target biomolecules.[6] This approach represents a promising alternative to the standard methodology, viz. the preparation of bioaffinity patterns on bare substrates with subsequent backfilling by suitable OEG terminated SAMs.[8]

In comparison, the tuning of biorepulsive properties by UV light is preferable over the use of electrons because the former is much simpler and requires less expensive experimental setups, in particular because no vacuum is needed. Moreover, implementation of advanced patterning approaches[9] and the possibility to modify OEG materials at relatively long wavelengths (up to 390 nm)[6] enable the use of well-developed standard optics and commercial patterning strategies.

So far the modification of OEG terminated SAMs by UV light was only performed at ambient conditions since it is generally accepted that the involved chemical reactions require oxygen as co-reactant or mediator.[10] It would be, however, of advantage, to perform this process in aqueous environment, which is the natural medium for biomolecules. A combination of suitable microfluidic setup and UV-based lithographic equipment would then enable the preparation and characterisation of complex biological assemblies and patterns within a single experimental platform. Here we provide a basis for such future developments, reporting that the terminal OEG segments of the SAM constituents can be modified by UV light in an aqueous environment. We also show that this photo-process is even possible in the absence of oxygen, which, as mentioned above, was up to now always considered as the main reactant in the photolysis of OEG materials.[10]

As test system we used SAMs of hexaethyleneglycol terminated undecanethiol (abbreviated as EG6, see Figure 1a) on gold. These films are well known to degrade if exposed to ambient light[11] and can also be modified in a controlled manner by UV light in air.[6] Under these conditions, the degradation of the OEG chain closely follows the mechanism observed for the analogous bulk materials, including chain scission and formation of esters and aldehydes.[12] In addition, a partial deterioration of the alkane chain as well as of the anchoring group has been found, including the formation of sulfone and sulfoxide species,[6] in agreement with the behaviour of non-substituted alkanethiol (NSAT) SAMs.[9] Such oxidised species are only weakly bound to the substrate and can be easily washed away or exchanged for other moieties.[5,13] To test the possibility to modify OEG terminated monolayers under aqueous conditions and to clarify the role of oxygen in related reactions and processes, we performed the irradiation treatment of the EG6 monolayers in a custom designed cell with a quartz window, filling the gap (~ 0.2 mm) between the sample and window with either air-containing or oxygen-free water. The irradiation was carried out with a conventional, mercury-vapor based laboratory fluorescence lamp (254 nm, 1.1 mW cm^{-2} at 5 cm distance). In the case of air-containing water, the thickness of the EG6 SAM, starting at ~ 2.9 nm as determined by ellipsometry, became nearly as quickly reduced as upon irradiation in air (Figure 1b, blue vs. red). X-ray photoelectron spectroscopy (XPS) data (Supporting Information) show that this reduction occurred predominantly on the expense of the OEG part, but also the normalized signals of the sulphur atoms (Figure 1d) and the alkyl part (C–C at 284.6 eV, Figure 1e) exhibited progressive decrease with UV dose. The material loss was accompanied by extensive oxidation of the residual OEG segments, as follows from the infrared reflection absorption

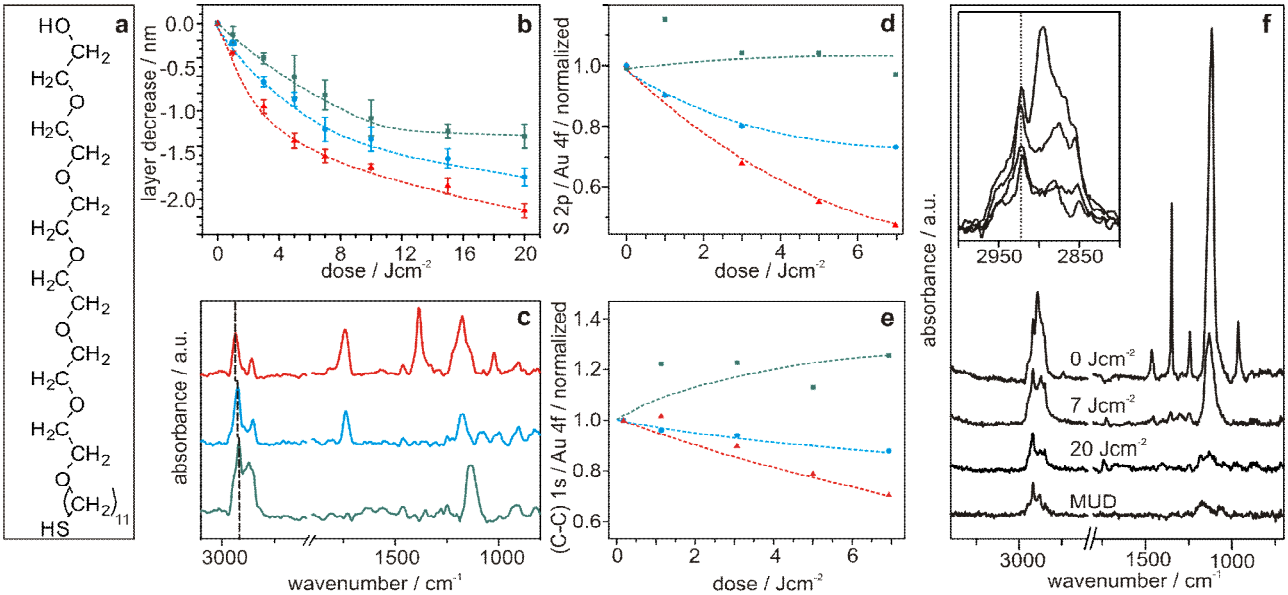


Figure 1. Data obtained for the irradiation of EG6 SAMs with UV light of 254 nm wavelength under different conditions: In air (red), in air-containing water (blue), and in oxygen-free water (green). (a) Structure of the EG6 molecule. (b) Decrease of the monolayer thickness with increasing irradiation dose. In deoxygenated water, the process stops after a loss of about 1.3 nm. The lines are guides to the eye. (c) IRRA spectra of the EG6 SAMs after irradiation with 20 J cm^{-2}. In the absence of oxygen, no signal for carbonyl groups (1730 cm^{-1}) could be found. The shift in the asymmetric CH_2 stretch vibration at around 2920 cm^{-1} indicates the different degrees of (dis)order in the systems. (d) S 2p/Au 4f XPS intensity ratio and (e) C 1s/Au 4f XPS intensity ratio, with only the signal of the alkane segments included, indicating a partial damage of the alkanethiolate linkers during irradiation in the presence of oxygen. (f) IRRA spectra of EG6 SAMs irradiated with different doses in oxygen-free water in comparison with the spectrum of a MUD SAM (lowest trace). The insert demonstrates that the order in the alkane part of the SAMs is not altered, as indicated by the CH_2 vibration at 2919 cm^{-1}.

(IRRA) spectra (Figure 1c), which exhibited a significant C=O signal at 1730 cm^{-1} for both air and air-containing water samples. The main difference was the appearance of an additional band at 1380 cm^{-1} in the former sample, assigned to formic esters, which, presumably, became hydrolysed in water. The results suggest that the oxygen content of the water, which under ambient conditions amounts to 8-9 mg/dm^3,[14] is sufficient to mediate a comparable extent of photo-induced degradation as in air.

However, to our great surprise, the reduction of the EG6 thickness occurred even under exclusion of molecular oxygen (Figure 1b, green), suggesting that the latter is not necessary for the photolysis of the OEG substituted SAMs. Interestingly, the thickness was not reduced continuously with increasing dose as in the presence of oxygen but exhibited a levelling-off behaviour at doses above 12 J cm^{-2}. Also, spectroscopy revealed significant differences between the UV effect in air-containing and oxygen-free environment: In the latter case, only tiny amounts of oxidised carbon species, which are mostly expected to show up in the EG part,[10] could be detected in the IRRA and XP spectra (Figure 1c and Supporting Information) of EG6 even after high UV doses. Moreover, neither the XP signal related to the sulphur atoms nor that associated with the alkyl chain became decreased upon irradiation (Figure 1d,e, green, the slight increase is caused by the noticeably different kinetic energies of the C 1s and Au 4f photoelectrons), indicating that the alkane thiolate part of the monolayer remains basically unaffected. This is in pronounced contrast to the above observations in air or in air-containing water, where the modification of the OEG segments is accompanied by a partial loss of the alkane thiolate linkers by oxidation of the anchoring group. Thus, under exclusion of molecular oxygen, the sulphur-gold bond stays intact, leaving the alkane chains in place resulting in a layer with a final thickness of about 1.6 nm after extensive UV exposure. Such a value would be expected for an ordered undecane thiolate SAM possibly terminated by hydroxyl groups. This assumption is supported by the IRRA spectra of EG6 SAM in oxygen-free water upon exposure to UV light (Figure 1f): Whereas the band assigned to the symmetric stretching mode of the alkyl segments at 2919 cm^{-1} does not shift upon irradiation (see insert in Figure 1f) indicating that these segments are not altered and stay well ordered, the band characteristic of the helical conformation of the OEG segments (at 2892 cm^{-1})[2] disappears already at small doses (1 J cm^{-2}), manifesting a disturbance of this segment. All other bands typical of the OEG segments (964, 1244, 1348, 1463, and in particular 1120 cm^{-1})[2] disappear gradually during the irradiation process (Figure 1f and Supporting Information). At a dose of 20 J cm^{-2} the IRRA spectrum of the irradiated EG6 monolayer resembles that of a highly ordered SAM of 11-mercaptoundecanol (MUD, in Figure 1f), with only about 4% of the intensity of the OEG signal at 1120 cm^{-1} remaining. This indicates a clean scission of the OEG part, while the rest of the SAM stays basically unaltered.

The loss of the OEG part should result in a loss of biorepulsivity. Several studies report that proteins adhere to MUD SAMs, although not as strong as to NSAT SAMs (CH_3 termination).[15] As test protein we used bovine serum albumin (BSA), which forms layers of about 1.3 nm thickness on MUD SAMs. We therefore exposed EG6 SAMs, which have been irradiated with different UV doses, to BSA and determined the amount of adsorbed proteins by ellipsometry and XPS (see Supporting Information).

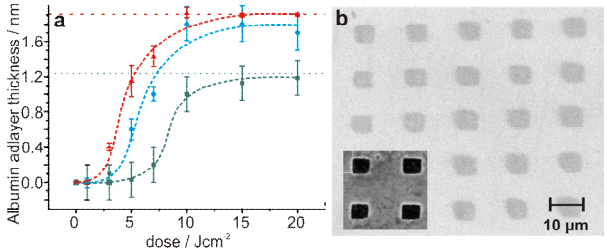


Figure 2. (a) Dose-dependent loss of biorepulsivity, as determined by the adhesion of BSA. In the case of the oxygen-free system (green) the protein adhesion is similar to that on a MUD monolayer (dotted line), while in the two other cases (air: red, oxygen-containing water: blue) the adhesivity becomes similar to that of a NSAT SAM. (b) SEM image of an EG6 surface patterned by irradiation through a mask followed by protein adhesion. The dark areas correspond to adherent BSA. For comparison, an image of the mask is provided in the insert.

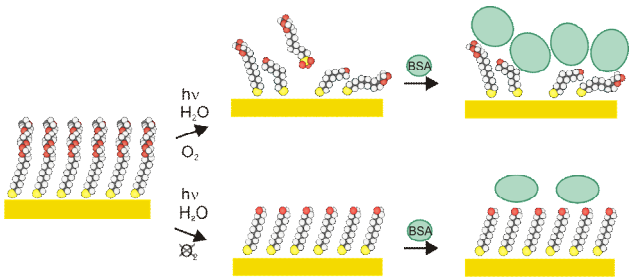


Figure 3. Outline of the observations: In the absence of oxygen, only the OEG part of the monolayer becomes decomposed, while in the presence of oxygen all parts of the SAM are affected. The former case allows for a better and finer control of the biorepulsivity.

As can be seen in Figure 2a (green), up to a dose of ~3 J cm^{-2} enough EG units remain in the layer to render it biorepulsive. At higher doses, the EG6 film progressively loses its biorepulsivity and more and more BSA molecules become deposited until the protein layer thickness levels off at doses above 15 J cm^{-2}. The respective value of 1.3 nm corresponds to the BSA layer thickness obtained on a MUD SAM (horizontal dotted line in Figure 2a). For comparison, also the data for the samples irradiated in air-containing water (blue) and in air (red) are depicted. In both cases the protein layer thickness increases up to 1.9 nm, a value that can be compared to the BSA thickness obtained on a NSAT SAM (horizontal dashed line in Figure 2a). It can then be assumed that the EG6 SAMs, exposed to high UV doses in air or air-containing water, are mostly comprised of distorted alkyl chains which act as effective adsorption sites for proteins as shown in Figure 3, addressing also the oxygen-free case.

As mentioned above, UV treatment of OEG terminated SAMs in aqueous environment can also be performed in a lithographic fashion, which is one of the major advantages of the approach. As a principal test, EG6 SAM was irradiated (10 J cm^{-2}) in deoxygenated water in a simple proximity printing geometry using a 2000 mesh metal grid as mask. After exposure to BSA solution, the selective deposition of the protein became visible in the scanning electron microscope as a contrast between the protein-covered (dark) and protein-free (light) areas (Figure 2b). Note that this result is only a principal proof of the approach: It can be expected that advanced lithographic tools will permit the formation of much more complex protein-affinity patterns.

Conclusions

The presented photo-chemically induced modification of the OEG terminated monolayers in water opens the opportunity for *in situ* adjustment of the layer properties, above all biorepulsivity. The observation that the presence/absence of oxygen results in different surface chemistries permits a further fine tuning. The exploitation of these observations as well as mechanistic studies are currently under way in our laboratories and will establish a new tool for performing *in situ* biochemical experiments at surfaces.

Notes and references

[a] *University of Frankfurt, Institute of Inorganic and Analytical Chemistry, Max-von-Laue-Str. 7, 60438 Frankfurt/Main, Germany.*
[b] *University of Heidelberg, Applied Physical Chemistry, Im Neuenheimer Feld 253, 69120 Heidelberg, Germany.*

† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

‡ This work has been financially supported by DFG (ZH 63/9-3 and TE 247/9-2).

1 N. P. Desai, J. A. Hubbell, *Biomaterials,* 1991, **12**, 144; K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc*. 1993, **115**, 10714; A. Rosenhahn, S. Schilp, J. Kreuzer, M. Grunze, *Phys. Chem. Chem. Phys*. 2010, **12**, 4275.
2 P. Harder, M. Grunze, R. Dahint, G. M. Whitesides, P. Laibinis, *J. Phys. Chem. B,* 1998, **102**, 426; S. Herrwerth, W. Eck, S. Reinhardt, M. Grunze, *J. Am. Chem. Soc*. 2003, **125**, 9359.
3 N. Meyerbröker, Z. A. Li, W. Eck, M. Zharnikov, *Chem. Mater*. 2012, **24**, 2965.
4 H. W. Ma, M. Wells, T. P. Beebe, A. Chilkoti, *Adv. Funct. Mater*. 2006, **16**, 640.
5 N. Ballav, H. Thomas, T. Winkler, A. Terfort, M. Zharnikov, *Angew. Chem. Int. Ed*. 2009, **48**, 5833; C. M. Kolodziej, H. D. Maynard, *Chem. Mater*. 2012, **24**, 774; Y. L. Jeyachandran, M. Zharnikov, *J. Phys. Chem. C,* 2012, **116**, 14950.
6 M. Montague, R. E. Drucker, K. S. L. Chong, R. J. Manning, F. J. M. Rutten, M. C. Davies, G. J. Leggett, *Langmuir,* 2007, **23**, 7328; Y. L. Jeyachandran, A. Terfort, M. Zharnikov, *J. Phys. Chem. C,* 2012, **116**, 9019; Y. L. Jeyachandran, T. Weber, A. Terfort, M. Zharnikov, *J. Phys. Chem. C,* 2013, **117**, 5824.
7 Alternatively, zwitterionic polymer brushes can be used; see e.g. Y. Li, M. Giesbers, M. Gerth, H. Zuilhof, *Langmuir,* 2012, **28**, 12509.
8 R. K. Smith, P. A. Lewis, P. S. Weiss, Prog. Surf. Sci. 2004, **75**, 1. M. Mrkisch, C. S. Chen, Y. Xia, L. E. Dike, G. E. Ingber, G. M. Whitesides, Proc. Natl. Acad. Sci. USA 1996, **93**, 10775. A. S. Blawas, W. M. Reichert, Biomaterials 1998, **19**, 595. K. B. Lee, S. J. Park, C. A. Mirkin, J. C. Smith, M. Mrkisch, Science, 2002, **17**, 1702.
9 J. Adams, G. Tizazu, S. Janusz, S. R. J. Brueck, G. P. Lopez, G. J. Leggett, Langmuir 2010, **26**, 13600.
10 G. Qin, C. Cai, *Chem. Comm*. 2009, **105**, 5112; N. J. Brewer, R. E. Rawsterne, S. Kothari, G. J. Leggett, *J. Am. Chem. Soc*. 2001, **123**, 4089; N. J. Brewer, S. Janusz, K. Critchley, S. D. Evans, G. J. Leggett, *J. Phys. Chem. B,* 2005, **109**, 11247.
11 S. Zorn, U. Dettinger, M. W. A. Skoda, R. M. J. Jacobs, H. Preisert, A. Gerlach, T. Chassé, F. Schreiber, *Appl. Surf. Sci*. 2012, **258**, 7882.
12 R. E. Ducker, S. Janusz, S. Sun, G. J. Leggett, *J. Am. Chem. Soc.* 2007, **129**, 14842.
13 N. Ballav, T. Weidner, M. Zharnikov, *J. Phys. Chem. C,* 2007, **111**, 12002.
14 D. R. Lide (ed.) Handbook of Chemistry and Physics, 85th Edition CRC Press, Boca Raton, 2004, pp 8.
15 K. Wadu-Mesthrige, N. A. Amro, G. Liu, *Scanning,* 2000, **22**, 380; A. Sethuraman, M. Han, R. S. Kane, G. Belfort, *Langmuir,* 2004, **20**, 7779; T. Hayashi, Y. Tanaka, Y. Koide, M. Tanaka, M. Hara, *Phys. Chem. Chem. Phys*. 2012, **14**, 10196.