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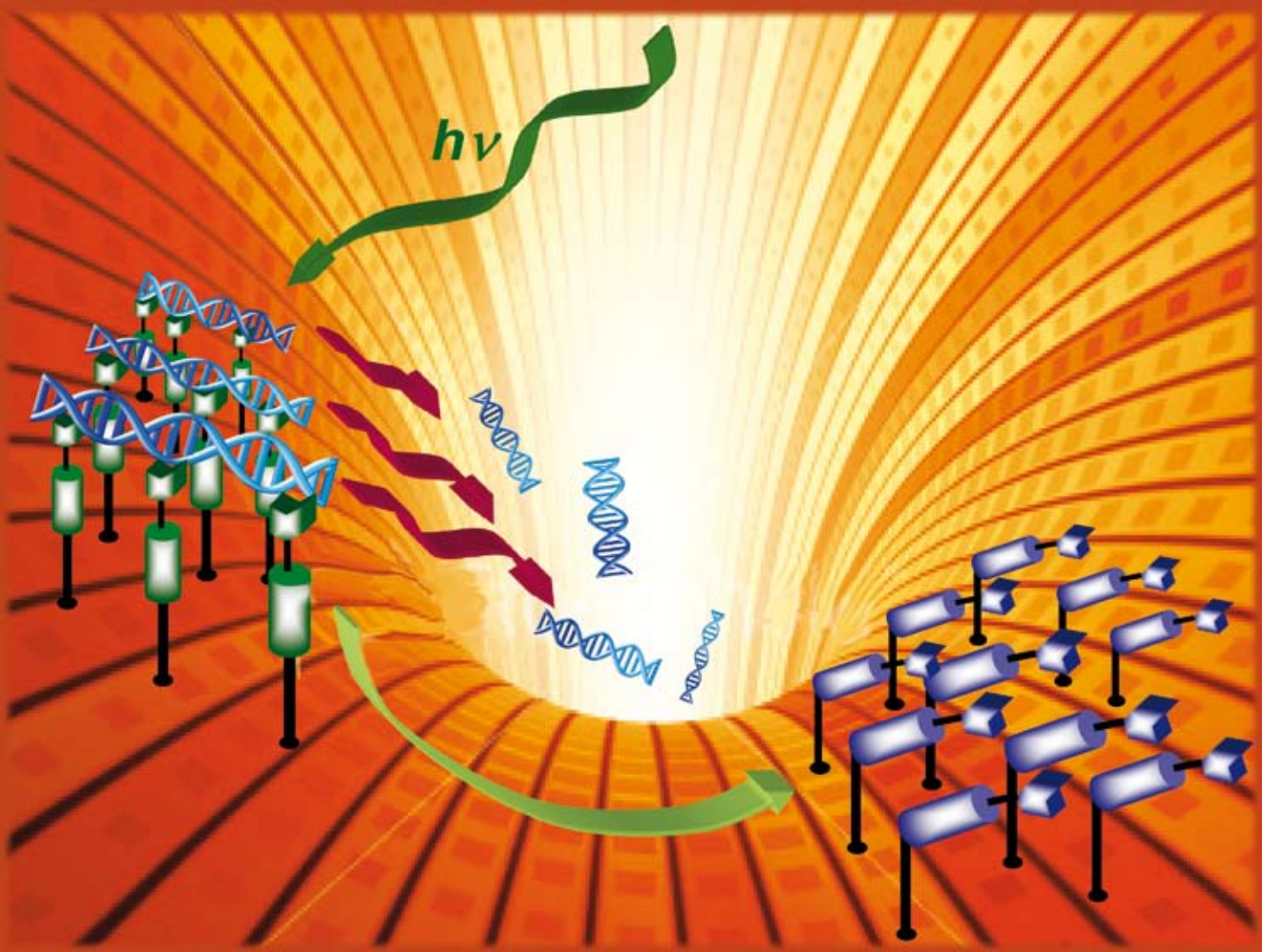

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Light-triggered DNA release by dynamic monolayer films

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We illustrate a simple strategy to immobilize single and double strand DNA on a two-dimensional surface and to trigger their release under physiological conditions, under the exclusive control of light stimuli. A tailored azobenzene derivative has been self-assembled on transparent platinum electrodes to form cationic-terminated monolayer films. These monolayers encourage the binding of DNA with the metal surface through effective electrostatic interactions with the negatively charged polynucleotide backbone. Irradiation of the film with UVA light induces *trans* to *cis* isomerization of the photoresponsive azobenzene units leading to significant changes of surface hydrophilicity and decreasing the binding affinity for DNA, which is consequently released into the solution. It is shown that the amount of DNA released can be precisely tuned by controlling the illumination conditions and is strictly related to the photoinduced structural modifications at the film surface. After the release of DNA the functional monolayers can be recycled through illumination with visible light which causes the *cis* form of the azo-chromophore to revert to the *trans* form, restoring the initial conditions. Given the non-specific nature of the Coulombic interactions the approach presented herein may, in principle, also be extended from polynucleotides to other negatively charged biomolecules, making these dynamic monolayers appealing model systems from the perspective of nanoscaled devices for biomedical applications where spatiotemporal control of biological material is required.

Introduction

Immobilization of DNA on two-dimensional (2D) surfaces and its controlled release without damaging its structure are topics of enormous interest in the development of a variety of systems ranging from gene delivery therapy,[1] biosensors[2] and biochips[3] to studies of DNA itself.[4] For these applications, where the length scales are in the submicrometres domain, the surface properties become increasingly significant since the chemistry can be almost exclusively dominated by surface interactions.[5] Therefore, the fabrication of substrates whose surface properties and, as a consequence, the binding affinity to DNA can be dynamically changed upon application of external inputs represents one of the most exciting challenges in the wide arena of biointerfacial science.[6] Several protocols focused on the immobilization of DNA on 2D surfaces are known in the literature, ranging from covalently linked DNA[7] to multilayered polymer–DNA thin films.[8] Of these, the use of tailored surfaces bearing cationic terminations is one of the simplest and most tested strategies to successfully anchor the DNA through electrostatic interactions, without impairing its intrinsic high order.[9–13] In contrast, the controlled delivery of DNA from surfaces is a relatively unexplored issue. Some of the instances reported to date have demonstrated DNA release from tailored surfaces upon electrochemical,[14] thermal,[15] pH[16] and ionic strength stimuli.[17] Light is a very appealing on/off trigger. Its easy availability and manipulation associated with the instantaneous initiation/stopping of the photochemical reactions make light-regulated systems, in principle, particularly suited to accurately control the DNA release kinetics, which is of key importance for clinical applications. Furthermore, using photons as external triggers offers the great advantage of not affecting important parameters such as temperature, pH, ionic strength *etc*. Nevertheless, to the best of our knowledge, no reports on light-controlled DNA delivery from 2D surfaces without damaging its structure are known to date.† Self-assembled monolayers (SAMs) on noble metal substrates are easily prepared by the spontaneous chemisorption of alkanethiols, alkyl disulfides and thioethers, and form quite uniform surfaces of great interest in the development of biointerfacial surfaces.[18,19] The introduction of photoresponsive units into the SAMs structure offers the opportunity to obtain "intelligent" monolayer films whose functions and properties may be easily regulated by appropriate light stimuli.[19,20] In our recent work we have demonstrated that the tailored azobenzene derivative **1** (Scheme 1) forms photoswitchable self-assembled monolayers (SAM-**1**) on platinum substrates.[21] Alternate UVA–Vis irradiation of SAM-**1** triggers the reversible *trans* ⇆ *cis* interconversion of the photoactive azobenzene moiety inducing considerable changes in surface hydrophilicity. We have shown that "catch-and-release" of anionic porphyrins at the surface of these monolayers can be achieved by exploiting the different

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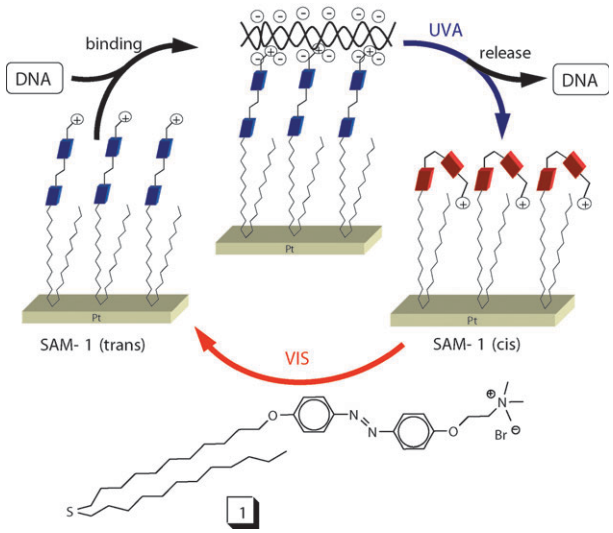
† We and others (see refs. 11–13) have reported model systems addressing the induction of photocleavage of DNA assembled on a 2D surface. This topic is, of course, different from the aim of the present study which is focused on the release of DNA without altering its chemical structure.



Scheme 1 Schematic illustration of the immobilization and release of DNA by the dynamic, photochemically controlled SAM-**1**.

light-controlled electrostatic interactions with the two isomeric forms of SAM-**1**.[21] In view of the non-specific nature of the Coulombic interactions, the above approach may, in principle, also be extended to biomolecules rich in negative charges. With this in mind, the objective we pursue in the present study is to assess the possibility of using these dynamic monolayers to immobilize single and double strand DNA, and to trigger its spatial and temporal release exclusively by light inputs. As illustrated in Scheme 1, initial entangling of DNA by SAM-**1** in the *trans* form is expected to be highly favored by charge pairing. We envisage that the changes in surface properties associated with the photoisomerization of the azobenzene chromophore occurring upon UVA irradiation can be reflected in a lower binding affinity for DNA, which can consequently be released from the film surface.

Experimental

The cationic azobenzene derivative **1** was synthesized and purified according to our recently reported procedure.[21] Cy5-labeled 25-mer DNA from MWG Biotech, Germany and sonicated ct-DNA (phenol extracted, lyophilized, average size 2000 bases, range 200–6000 bases) from Pharmacia were used without further purification. Phosphate buffer (10 mM, pH 7.4) was prepared with biological grade reagents and all solutions were prepared with nanopure water (grade 18 MΩ). All other solvents used were analytical grade.

Platinum substrates were obtained by a cool sputtering system in argon gas on glass slides according to the procedure reported in detail in our recent paper. Before preparation of SAM-**1**, these metal substrates were extensively washed with CH_2Cl_2 and dried at room temperature. SAMs-**1** were obtained by immersion of the Pt substrates in a CH_2Cl_2 solution of **1** (0.5×10^{-3} M) for 20 h, at room temperature in the dark. Longer and shorter reaction times led to a lower degree of chemisorption of **1**. The modified substrates were then rinsed several times, first with CH_2Cl_2 and then with methanol to remove any physisorbed material, and dried at room temperature.

Aqueous contact angles were measured using a goniometer (KERNCO) under ambient conditions. UV–Vis absorption spectra were recorded with a Jasco *V-560* spectrophotometer. UV–Vis irradiation was performed, under ambient conditions, by using the monochromatic radiation of a Horiba Jobin Yvon Fluorolog-2 (mod. F-111) spectrofluorimeter. The incident photon flux on the samples was on the order of 10^{15} quanta s^{-1}. Fluorescence images from the substrates were recorded with a Perkin-Elmer ScanArray Express (resolution 10 μm) equipped with a 633 nm laser excitation source and a 670 nm cut-off filter.

Results and discussion

SAMs of the tailored azobenzene derivative **1** have been achieved by using our recently developed ultrathin Pt electrodes as suitable platforms.[22] These metal substrates (thickness *ca.* 20 nm) exhibit a unique combination of excellent optical transparency, homogeneity, robustness, and conductivity features.[22c] One of the main advantages of these ultrathin films consists in the easy probing of adsorbed chromophores simply by conventional spectrophotometers in transmission mode, without the use of sophisticated analytic techniques. Fig. 1 shows a representative spectrum of SAM-**1** which exhibits the typical absorption band with a maximum at *ca.* 360 nm safely attributable to the π–π* electronic transition of the *trans*-azobenzene moiety.‡ The estimated surface coverage was *ca.* 1 molecule/100 $Å^2$,§ a value basically the same as we have obtained for a similar azo-derivative not bearing the quaternary ammonium group and self-assembled on the same Pt substrates.[22c,d] Aqueous contact angle (θ_a) measurements are in good agreement with the structure of SAM-**1** which incorporates both hydrophobic and hydrophilic end groups in

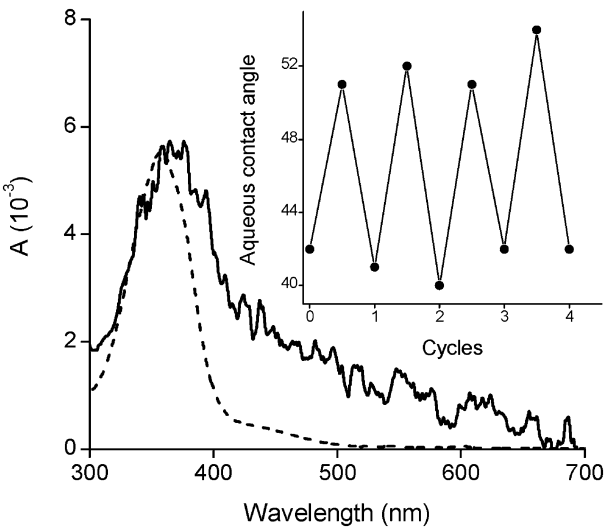


Fig. 1 Absorption spectrum of SAM-**1** (solid) recorded using as a reference sample the same ultrathin Pt substrate before chemisorption of **1**. The absorption spectrum of **1** in CH_2Cl_2 solution (dashed) is also shown, for the sake of comparison. The inset shows the switching of the aqueous contact angle observed upon alternate cycles of UVA (350 nm, 5 min) and Vis (450 nm, 20 min) light irradiation.

equivalent amounts. The obtained value of $\theta_a \approx 42°$ is in fact the average of those expected for typically hydrophobic ($>100°$) and hydrophilic ($<10°$) monolayers.[18]

The interfacial properties of SAM-**1** are modified by light excitation. UVA irradiation of the film, besides inducing the bleaching of the main absorption band according to the *trans* to *cis* isomerization of the photoresponsive azobenzene units,[21] leads to an increase of *ca.* 10° in the θ_a value, accounting for a reduced hydrophilicity of the *cis* isomer of **1** in the film, probably due to a partial hindering of the quaternary amino group to the water surface. In view of the photoreversibility of the *trans* ⇆ *cis* process, the interfacial properties of the SAM can be dynamically controlled by light of different wavelength, as demonstrated by the increasing and decreasing of the θ_a values upon alternate cycles of UVA and Vis irradiation (inset Fig. 1).

The suitability of SAM-**1** to entangle and photorelease single strand DNA (ss-DNA) was proven by using a Cy5-labeled 25-mer DNA. The Cy5 probe presents ideal spectroscopic prerequisites to assess either the presence or absence of ss-DNA at the SAM-**1** surface by simple spectrophotometric measurements. In fact, its absorption spectrum is characterized by an intense band at *ca.* 650 nm and a negligible absorption in the whole region of the azobenzene (Fig. 2A). In addition, Cy5 exhibits an intense fluorescence emission at 670 nm in contrast to the nonfluorescent azobenzene units. SAM-**1** was soaked in a buffered aqueous solution of ss-DNA for 3 h.¶ The substrate was then withdrawn, washed with phosphate buffer and dried at room temperature. The absorption spectrum of the resulting sample (***a*** in Fig. 2B) shows clearly the presence of the typical Cy5 absorption at *ca.* 650 nm along with the absorption band of the azobenzene chromophore at 360 nm, clearly indicating the entangling of ss-DNA into the SAM-**1** structure. By assuming no significant differences in the molar absorptivity of both **1** and Cy5 upon binding,‖ we can roughly estimate the ratio **1** : Cy5 in the film to be *ca.* 12 : 1, a reasonable value on the basis of (i) the length of the 25-mer ss-DNA, (ii) the presence of only one Cy5 probe in the DNA strand and (iii) a quite packed structure of the azobenzene-derivative SAM-**1**.

The sample was then placed in a spectrophotometric cell (10 mm pathlength) containing 3 mL of phosphate buffer and irradiated with 360 nm light (which is not absorbed by DNA) at different time intervals. After each step of irradiation the substrate was withdrawn, dried and investigated by UV–Vis spectroscopy. A photostationary state was reached after *ca.* 20 min irradiation. As shown in Fig. 2B, the corresponding

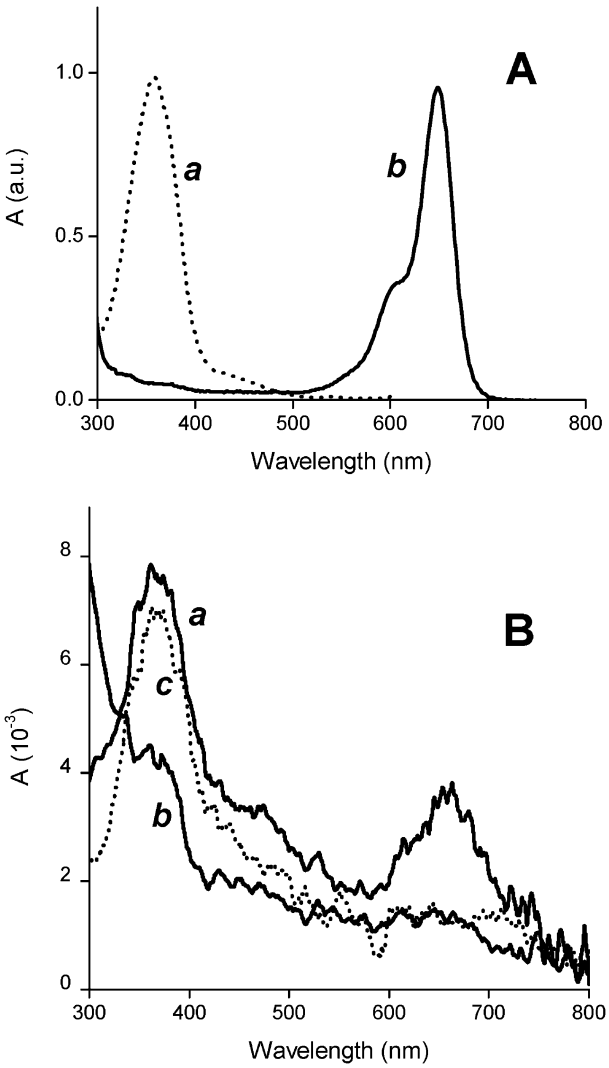


Fig. 2 (A) Absorption spectra of **1** (***a***), in CH_2Cl_2 solution, and Cy5-labeled 25-mer DNA (***b***), in phosphate buffer solution. (B) Absorption spectrum of SAM-**1** after immersion for 3 h in a 20 μM solution of Cy5-labeled 25-mer DNA and washing with phosphate buffer before (***a***) and after 20 min irradiation with UVA (360 nm) light (***b***) and subsequent 25 min irradiation with Vis (450 nm) light (***c***).

absorption spectrum (***b***) reveals the occurrence of the *trans–cis* photoisomerization of SAM-**1** accompanied by the almost complete disappearance of the Cy5 absorption.** This finding accounts for light-triggered release of ss-DNA from the film and is in line with the changes of the surface properties of the SAM occurring upon the UVA irradiation. In fact, the

‡ The presence of a tail extending into the Vis region in the absorption spectrum of the film is presumably due to the imperfect correction from the blank sample (Pt film before chemisorption of **1**).

§ The surface coverage (Γ_{surf}) was estimated by the relation $\Gamma_{surf} = A\varepsilon^{-1}$, where A is the absorbance of the SAM and ε is the molar extinction coefficient of the chemisorbed species. In our case, we assumed for **1** the ε_{max} (30 000 M^{-1} cm^{-1}) value determined in CH_2Cl_2 solution.

¶ Longer reaction times and higher DNA concentrations did not affect the amount of ss-DNA bound to the monolayer, as resulted from the absorbance values at 650 nm (*vide infra*).

‖ We assumed for Cy5 the ε_{max} (200 000 M^{-1} cm^{-1}) value determined in buffered aqueous solution.

** The reason for the lack of quenching of the azobenzene photoisomerization by Cy5 can be two-fold: (i) the value of the J-overlap is negligible; (ii) the photoisomerization is a process fast enough to strongly compete with the energy transfer process. Despite the visible band of the *cis* isomer not being well observed upon 360 nm light irradiation (probably due to the combination of the small molar absorptivity of the *cis* isomer and the absorption of the Cy5 at *ca.* 450 nm which would hide the absorption of the *cis* isomer), its formation is well confirmed by the bleaching at 360 nm and by the almost complete restoration of the absorption of the *trans* form of **1** observed upon 450 nm light irradiation.

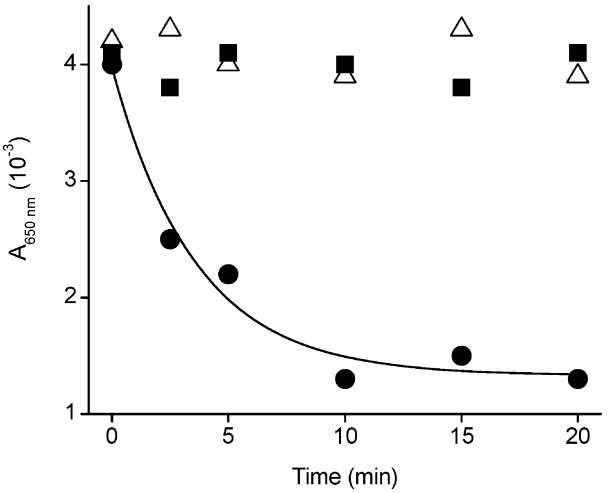


Fig. 3 Absorbance changes of the Cy5 absorption band in the case of the film irradiated at 360 nm (●), 650 nm (■) and non-irradiated (△).

reduced hydrophilicity observed in the case of the *cis* form of SAM-**1** (inset Fig. 1) suggests that the cationic end-groups switch in a conformation probably less favorable to effective electrostatic interactions with the polynucleotide which is consequently released into the solution. It should be stressed that the disappearance of the Cy5 absorption after UVA irradiation cannot be attributable to a trivial effect such as desorption of molecules of **1** from the SAM structure. Irradiation of the SAM-**1** with 450 nm light subsequent to the DNA release shows indeed the almost complete recovery of the original band of the *trans* form at *ca.* 360 nm (spectrum ***c*** in Fig. 2B), unequivocally indicating that the film structure reverts to the original state. The amount of DNA released is exclusively controlled by UVA light and, according to the photoisomerization process resembles a first-order kinetic reaction (Fig. 3). Furthermore, two key control experiments shown in Fig. 3 demonstrate that no significant changes in the absorption of Cy5 are observed when similar samples of SAM-**1** with entangled ss-DNA are either incubated in the dark or irradiated with visible light absorbed by Cy5, for the same time intervals, respectively.

Immobilization and photorelease of ss-DNA were also demonstrated by fluorescence measurements carried out under different experimental conditions. As displayed in Fig. 4, SAM-**1** is nonfluorescent (***a***) but exhibits the typical emission in the Cy5 region after binding with ss-DNA (***b***). Irradiation with 360 nm light induces a remarkable decrease of the

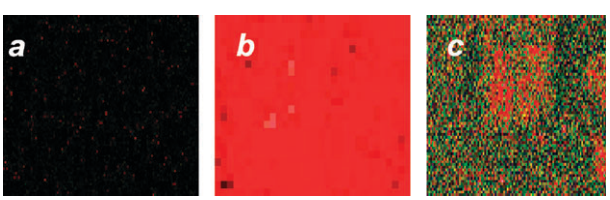


Fig. 4 Fluorescence images (λ_{exc} 650 nm, λ_{em} 670 nm) of SAM-**1** (***a***), SAM-**1** after immersion for 3 h in a 20 μM solution of Cy5-labeled ss-DNA and washing with phosphate buffer before (***b***) and after (***c***) 20 min irradiation with UVA (360 nm) light.

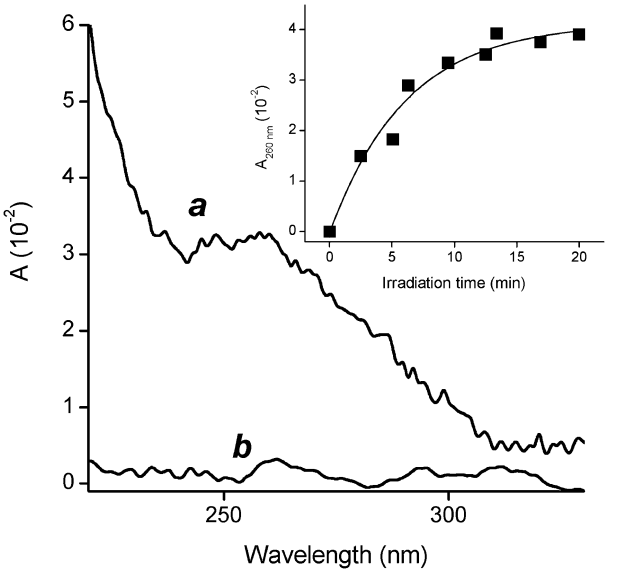


Fig. 5 UV spectra of aqueous solutions in which SAM-**1** containing immobilized ds-DNA were immersed and irradiated for 20 min with UVA (360 nm) light (***a***) and kept in the dark for 20 min (***b***). The inset shows the total absorbance monitored at 260 nm as a function of the irradiation time.

fluorescence intensity (***c***), accounting for photorelease of the ss-DNA from the film surface.

The suitability of the dynamic SAM-**1** to immobilize and release double strand DNA (ds-DNA) upon light excitation was verified by experiments carried out in the presence of calf thymus DNA. Analogously to the experiments performed with ss-DNA, SAM-**1** was first soaked in a buffered aqueous solution of ds-DNA for 3 h and then withdrawn, washed with phosphate buffer and dried at room temperature. The entangling of ds-DNA, which does not contain any spectroscopic probe, at the SAM-**1** surface was proven by contact angle measurements. The value of θ_a for SAM-**1** decreased significantly from 42° to *ca.* 31° after the treatment with ds-DNA, predictably due to the hydrophilicity of DNA and in excellent agreement with the values typically observed for DNA-terminated 2D surfaces.[23,24]

The sample was then placed in a spectrophotometric cell (10 mm pathlength) containing 3 mL of phosphate buffer and, similarly to the previous case, irradiated with 360 nm light at several time intervals. To reveal the ds-DNA photorelease from SAM-**1**, a convenient and simple procedure is to record the absorption spectrum of the bulk aqueous phase, after withdrawing the substrate following each step of irradiation. In fact, if SAM-**1** photoisomerization induces DNA release, this is expected to rapidly diffuse into the bulk solution, thus being easily detectable spectrophotometrically. Fig. 5 shows the spectrum of the aqueous phase obtained after SAM-**1** containing the immobilized ds-DNA has been irradiated for 20 min and subsequently withdrawn from the spectrophotometric cell. The presence of the typical absorption band around 260 nm due to the π,π* transition of DNA bases provides clear evidence that DNA has been released upon photoexcitation. On the other hand, no significant absorption of the aqueous phase was observed when ds-DNA

immobilized on SAM **1** was incubated in the dark for the same time interval. The absence of any significant absorption at 360 nm (absorption maximum of the azobenzene chromophore) in the aqueous phase suggests once again that no desorption of molecules of the azo derivative **1** takes place under irradiation. As illustrated in the inset of Fig. 5, similarly to the observations with ss-DNA, the amount of released ds-DNA increases mono-exponentially as a function of the irradiation time, reaching a plateau after *ca.* 20 min.

Conclusions

The present study demonstrates the *proof of concept* for immobilization and on-demand release of single and double strand DNA by exploiting the different electrostatic interactions with dynamic monolayer films whose surface features can be regulated by appropriate light stimuli. We have shown that the DNA release is strictly related to the photoisomerization of the photoactive unit of the film. This dependence makes it possible to exert a precise control on the DNA delivery kinetic profile under physiological conditions without affecting important parameters such as temperature, pH and ionic strength, offering a great advantage from the perspective of biomedical applications. A further remarkable point of interest of this work is the feasibility of recycling the dynamic monolayers. In fact, given their photochemical reversibility, the functional films are not decomposed during their working cycle and, after DNA release, can revert to the initial conditions on illumination with visible light. In view of the non-specific nature of the electrostatic interactions, this simple, light-mediated strategy might in principle also be extended for controlling the interfacial interactions with other biomolecules, making these dynamic monolayers appealing model systems from the perspective of nanodevices aimed at delivering therapeutic material with accurate spatiotemporal control.

Acknowledgements

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References

1 (*a*) D. Luo and W. M. Saltzman, *Nat. Biotechnol.*, 2000, **18**, 33; (*b*) A. Meager, *Gene Therapy Technologies, Applications, and Regulations*, John Wiley and Sons Ltd, UK, 1999.
2 A. Sassolas, B. D. Leca-Bouvier and L. J. Blum, *Chem. Rev.*, 2008, **108**, 109.
3 J. H. Ng and L. L. Ilag, *Biotechnol. Annu. Rev.*, 2003, **9**, 1.
4 J. F. Brockman, A. G. Frutos and R. M. Corn, *J. Am. Chem. Soc.*, 1999, **121**, 8044.
5 D. T. Chiu, C. Wilson, A. Karlsson, A. Danielsson, A. Lundqvist, A. Strömberg, F. Ryttsén, M. Davidson, S. Norldholm, O. Oswar and R. N. Zare, *Chem. Phys.*, 1999, **247**, 133.
6 Y. Erkan, K. Halma, I. Czolkos, A. Jerorka, P. Dommersnes, R. Kumar, T. Brown and O. Orwar, *Nano Lett.*, 2008, **8**, 227.
7 E. J. Moore, M. Curtin, J. Ionita, A. R. Maguire, G. Ceccone and P. Galvin, *Anal. Chem.*, 2007, **79**, 2050.
8 C. M. Jewell, J. Zhang, N. J. Fredin, M. R. Wolff, T. A. Hacker and D. M. Lynn, *Biomacromolecules*, 2006, **7**, 2483.
9 F. Caruso, E. Rodda, D. F. Furlong, K. Niikura and Y. Okahata, *Anal. Chem.*, 1997, **69**, 2043.
10 K. Ren, J. Ji and J. Shen, *Bioconjugate Chem.*, 2006, **17**, 77.
11 S. Sortino, S. Petralia, G. G. Condorelli, S. Conoci and G. Condorelli, *Langmuir*, 2003, **19**, 536.
12 N. Higashi, T. Inoue and M. Niwa, *Chem. Commun.*, 1997, 1507.
13 Y. Zhang, S. Yang, C. Liu, X. Dai, W. Cao, J. Xu and Y. Li, *New J. Chem.*, 2002, **5**, 617.
14 J. Wang, G. Rivas, M. Jiang and X. Zhang, *Langmuir*, 1999, **15**, 6541.
15 T. Okano, N. Yamada, M. Okuhara, H. Sakai and Y. Sakuray, *Biomaterials*, 1995, **16**, 297.
16 T. Shutava, M. Prouty, D. Kommireddy and Y. Lvov, *Macromolecules*, 2005, **38**, 2850.
17 T. Radeva, K. Kamburova and I. Petkanchin, *J. Colloid Interface Sci.*, 2006, **298**, 59.
18 A. Ulman, *Chem. Rev.*, 1996, **96**, 1533.
19 X.-M. Li, J. Huskens and D. N. Reinhoudt, *J. Mater. Chem.*, 2004, **14**, 2954.
20 S. Sortino, S. Petralia, G. Compagnini, S. Conoci and G. Condorelli, *Angew. Chem., Int. Ed.*, 2002, **41**, 1914.
21 F. L. Callari and S. Sortino, *J. Mater. Chem.*, 2007, **17**, 4184.
22 (*a*) S. Sortino, S. Petralia, S. Conoci and S. Di Bella, *J. Am. Chem. Soc.*, 2003, **125**, 1122; (*b*) S. Sortino, S. Di Bella, S. Conoci, S. Petralia, M. Tomasulo, E. J. Pacsial and F. M. Raymo, *Adv. Mater.*, 2005, **17**, 1390; (*c*) S. Conoci, S. Petralia, P. Samorí, F. M. Raymo, S. Di Bella and S. Sortino, *Adv. Funct. Mater.*, 2006, **16**, 1425; (*d*) S. Sortino, S. Petralia, S. Conoci and S. Di Bella, *J. Mater. Chem.*, 2004, **14**, 811; (*e*) S. Sortino, S. Conoci, I. Yildiz, M. Tomasulo and F. M. Raymo, *J. Mater. Chem.*, 2006, **16**, 3171.
23 S. G. Ray, S. S. Daube and R. Naaman, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 15.
24 T. S. Seo, X. Bai, H. Ruparel, Z. Li, N. J. Turro and J. Ju, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 5488.