

High levels of molecular orientation of surface azo chromophores can be optically induced even in a wet biological environment

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We have developed polyelectrolyte multilayer bio-films containing azobenzene chromophores that enhance reversible photo-orientation upon irradiation with linearly polarized light, to effect surface photo-switching of adjacent biological systems. When conditions of film preparation and irradiation were optimized, we could observe the highest measured birefringence to date in amorphous systems ($\Delta n > 0.2$). This birefringence change to probe orientation was also for the first time measured and determined to be stable completely underwater, permitting optimization for *in situ* applications immersed in biological conditions.

Thin polymer coatings containing photo-isomerizable molecules, such as azobenzene dyes, have garnered great interest due to their capacity for reversible optical storage,^{1,2} optical switching,³ and most recently for photo-reversible bio-surfaces to control cell behaviour with visible light.⁴ The key to these applications is the material's ability to switch reversibly between distinct *trans* and *cis* geometries and their molecular orientations, readily measured as changes in birefringence. These molecular motions are mainly the result of the re-alignment and migration of azobenzene groups during repetitive *trans*-*cis* isomerization cycles with polarized light (Fig. 1). When using linearly polarized light, the azobenzene chromophores will continue to cycle and re-align until their dipole moments lie perpendicular to the polarization direction, depleting all other orientations and resulting in a build-up of molecular anisotropy. Most recently, by incorporating azo chromophores into soft bio-compatible films based on polyelectrolyte multilayers (PEMs) we have observed that significant and stable changes in surface energy are inducible, which can modulate cell behaviour. Chromophore re-orientation at the film surface is implicated as a mechanism, but has not yet been proven. With this

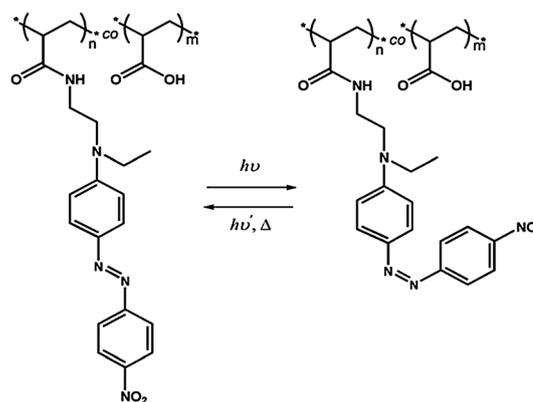


Fig. 1 Structure of p(DR2A-co-AA) in the *trans* (left) and *cis* (right) photo-isomerization states, interconverted by blue light at 488 nm.

recent interest in using photo-isomerizable materials in wet *in situ* applications such as bio-compatible films in cell media,⁴ it is especially important to understand how these molecular mechanisms work underwater, which can be markedly different than in the dry state where all previous measurements have been reported. There is also great interest in discovering conditions for both film assembly and irradiation that optimize this orientation.

The films for these bio-surfaces are constructed using the layer-by-layer (l-b-l) assembly method from two polyelectrolyte solutions.⁴⁻⁸ The build-up is initiated by submerging a negatively charged substrate into an aqueous solution of a positively charged polymer, allowing the polymer to self-assemble onto the surface, masking and reversing the charge to render the surface positive. The now positively charged film is then submerged into a solution with negatively charged polymers, resulting in a second layer being deposited reverting the surface charge back to negative. The process is then repeated to generate a coating that is held together strongly by many electrostatic interactions, is hydrophilic, and possesses physico-chemical properties that resemble biological tissue, and importantly can be fine-tuned *via* deposition parameters. This layering technique enables greater bio-compatibility

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and architectural control than for films made using traditional Langmuir–Blodgett or spin-coating due to the variable fabrication conditions, such as the number of layers, the pH/salt concentration during deposition, and the strength of the polyelectrolyte.^{5,6} These films, made using the l-b-l method, have sponge-like ability to soak up water and ions, properties recently discovered to be of paramount importance for good bio-compatibility.^{7,8}

The azobenzene chromophores incorporated into these l-b-l films we found previously to work best were based on a Disperse Red 1 (DR1) azo dye, and copolymerized with acrylic acid (AA) as the negatively charged layering polymer, so it could be subtly and controllably added to the multilayer film *via* the *n/m* ratio, ensuring quantitative and spatial control of azobenzene incorporation. Initially, p(DR1A-co-AA) was employed, due to its previously reported and facile synthesis. However, the labile ester linkage between the chromophore and the polymer backbone rendered the polymer susceptible to long-term hydrolysis with prolonged exposure to water at biological pH. To ensure stability of the azobenzene moiety, a variant of p(DR1A-co-AA) was synthesized (p(DR2A-co-AA)) (Fig. 1), which contained an amide linkage in place of the ester, and was then found to be completely bio-stable. The copolymerization enabled control of the ratio of azobenzene chromophores in the copolymer, which was varied from 0.5% to 40 mol% for various bio-applications. A 10 mol% p(DR2A-co-AA) polymer was used for the optical studies described here.

To prepare p(DR2A-co-AA) PEM films, the l-b-l technique was used to sequentially adsorb poly(diallyl dimethyl ammonium chloride) (PDADMAC) and p(DR2A-co-AA) from 0.1 M solutions onto a glass slide. The stability of these films is attributable to strong electrostatic interactions and hydrogen bonding between the negatively charged carboxylic acid groups and the positively charged quaternary amine groups. Films of 20 bilayers were fabricated for this study, exhibiting an absorbance maximum at 462 nm in the stable *trans* conformation. The short half-life of the *cis* isomer (<1 s) precludes capturing an independent pure spectrum of the *cis* isomer; however the *cis* and *trans* absorption bands are known to strongly overlap,⁹ so the resultant films were suitable for irradiation with 488 nm light for fast inter-conversion between isomerization states needed for chromophore alignment, measured through crossed polarizers.¹

Both an advantage of and a challenge in studying PEMs is their large parameter space for fabrication, influencing material properties. This complexity can be exploited, however, for rational design of extremely highly oriented, birefringent films, based on previous studies demonstrating that increased mobility and freedom of motion can amplify the effect to extraordinary levels of birefringence, desired for optimal switching applications.^{10,11} Upon decreasing the pH of p(DR2A-co-AA) to the brink of water solubility, near its pK_a of 4.5, the persistence length of the polymer is decreased significantly as the chains become less strongly charged and thus more globular. The resultant adsorption captures large loops that are trapped on the surface, bound irreversibly by the electrostatic interactions with the charged segments.⁶ The weak thick ‘loopy’ films are

of relatively lower density and higher mobility, and have a larger free volume and higher water content, observed and measured *via* neutron reflectometry.¹² The films are then submerged into a solution of PDADMAC (M_w 100 000), an oppositely charged strong polyelectrolyte, highly charged at all pH values, with a long persistence length and linear conformation, resulting in a tight thin monolayer of relatively dense PDADMAC.^{13,14} This combination we found of weak and strong, ‘loose’ and ‘tight’ polyelectrolytes, assembled under these conditions, lessens interpenetration between layers, and the result is a polymer layer with high mobility in the local environment of the chromophores that is stabilized periodically to form a more stratified film.

In polymeric systems, the ability to induce chromophore alignment typically depends on light properties, dye quantum yields, local chromophore environment, and polymer chain mobility, whilst relaxation and loss of alignment in the dark are solely attributed to side-chain and polymer chain mobility, and available free volume.¹⁵ In general, to create a material with a maximal level of induced anisotropy, a balance must be found where the azo chromophores have enough space for isomerization and orientation, yet be in a polymer matrix that is firm and stable enough that subsequent thermal randomization is minimized: too ‘tight’ and not enough re-arrangement can be induced; too ‘loose’ and this induced order is unstable.

One of the most studied aspects of induced anisotropy is the effect of polymer chain mobility and free volume. Dall’Agnol *et al.* showed that birefringence in DR1-doped polystyrene films increased with increasing temperatures up to a maximum, but then decreased with additional heating.¹⁶ They positively correlated temperature with polymer chain mobility/free volume and suggested that an intermediate free volume is ideal for maximally inducing birefringence. Sekkat *et al.* tested this by applying pressure to polymeric systems to reduce the free volume, consequently noticing the reduction of induced birefringence.¹⁷ Conversely, Tawa *et al.* showed that polymer matrices with lower glass transition temperatures,¹⁸ and thus higher free volumes, had decreased induced birefringence.

In the few l-b-l azo-PEM materials previously reported, induced anisotropy was achieved in the dry state, yet was not unusually large, with a measured birefringence of <0.1.^{19–21} In these reports, there was no mention of varying deposition parameters to explore or find optimal conditions of mobility for increasing this birefringence. What we demonstrate here however is that with our azobenzene-containing PEMs built using the l-b-l method, under optimal assembly conditions for high orientation, we can observe extraordinary birefringence both in the ‘dry’ state, and also still significant stable birefringence when completely submerged underwater in the ‘wet’ state. To our knowledge, this is the first time that PEMs built with an azopolymer using the l-b-l method have been shown to exhibit any birefringence underwater, much less birefringence that is stable for long periods of time. Initially, the exploration of the dependence of polarization angle and incident angle on induced birefringence was conducted in order to determine the irradiation geometries for maximum birefringence that could be

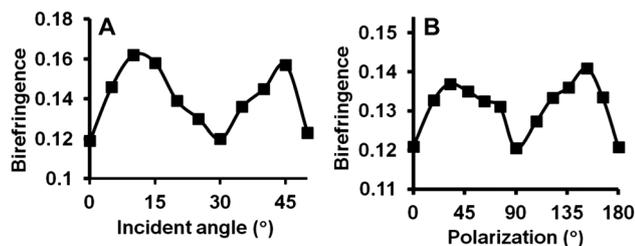


Fig. 2 Birefringence of a (p(DR2A-co-AA)/PDADMAC)₂₀ PEM film as a function of (A) beam angle (± 0.004) and (B) polarization angle (± 0.004).

induced in the sample. Interestingly, both parameters were observed to have a significant effect on the birefringence inducible in the PEM matrix with a similar sinusoidal pattern (Fig. 2). These maxima and minima appear to correlate strongly with shifts in surface energy, and a full study of these effects is currently underway.

The synthesis and full chemical and physical characterization of the new DR2-based copolymers and their resulting films are reported elsewhere,²² based on the previous preparation of DR1-based copolymers with AA, yielding water-soluble polymers with molecular weight in the range of 4000 to 12 500 g mol⁻¹.²³ The film thickness of 20 l-b-l PEMs ranges from approx. 120 nm when dry, to ~ 300 nm when saturated with water. The water content and physical film morphology have been characterized thoroughly previously for similar PEM films by *in situ* ellipsometry,⁷ solid state NMR spectroscopy,^{24–26} and *in situ* neutron reflectometry.^{12,27}

Under optimal film fabrication conditions, and with irradiation parameters optimized, an extraordinary birefringence of $\Delta n = 0.21$ could be repeatedly measured on replicate samples in the ‘dry’ state of ambient humidity, much higher than those in previous reports in dry amorphous systems. We then proceeded to test the effect of increasing water content on chromophore photo-alignment, as measured by birefringence levels at increasing absolute humidity (Fig. 3) using a variable humidity chamber and confirming water content *via* neutron reflectometry.¹² The birefringence then decreases as water content is increased, from $\Delta n = 0.21$ to levels of ~ 0.05 inducible completely submerged underwater. This behaviour can be rationalized as increased polymer chain mobility decreasing the net stable inducible chromophore alignment in more ‘wet’ PEM films.

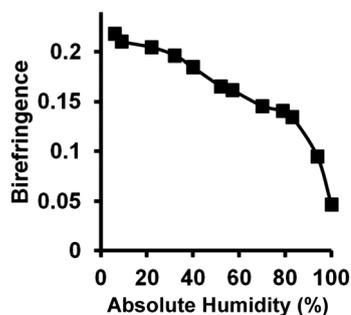


Fig. 3 Effect of humidity on the birefringence (± 0.005) of a (p(DR2A-co-AA)/PDADMAC)₂₀ film, at optimal angles of incidence and polarization.

Stronger PEMs are held together through many more electrostatic interactions, which would normally severely inhibit chromophore mobility; however, in this PEM system the DR2A chromophore is bound to the PAA backbone, which is itself highly constrained at the strata boundaries through electrostatic bonds, but retains volume for motion within the strata. Moreover, unlike the common commercially available pAZO chromophore, pDR2A is not electrostatically tethered within the multilayer as it lacks a charged functional group, significantly increasing its mobility. Therefore, polymer chain mobility may already be high enough that any further increase due to hydration would result only in an increase in thermal randomization, and thus a net decrease in inducible chromophore alignment. We suspect that the reason for significant birefringence seen for the first time in films completely submerged underwater is the unique internal architecture of our ‘weak/strong’ designed PEMs. Neither strong–strong nor weak–weak combinations we tried of the same PEMs produced such strong orientation, as these architectures containing chromophores attached to a highly mobile ‘loopy’ polymer chain backbone sandwiched by strata stabilize the polymer backbone through strong electrostatic interactions and hydrogen bonding between different strata.

To investigate the kinetics of chromophore alignment in ‘dry’ and ‘wet’ PEM films, the birefringence was monitored over time through three different irradiation regimes (Fig. 4): when the linearly polarized aligning beam is turned on (I), after it is turned off (II), and finally when irradiated with circularly polarized light to randomize (III). In the ‘dry’ state, 50% of maximum birefringence is reached in 175 ms, while when the same film is submerged underwater it takes 650 ms. However, the time required to reach $>95\%$ maximum birefringence is shorter in submerged films (60 s) than in ‘dry’ films (75 s). Birefringence kinetics are typically described using bi-exponential equations containing a ‘fast’ process, related to light-induced chromophore alignment, and a ‘slow’ process, related to polymer mobility.¹⁵ Since ‘dry’ films have a higher density than ‘wet’ films, the closer proximity of DR2A molecules can result in a cooperative chromophore interaction,²⁸ which leads to the

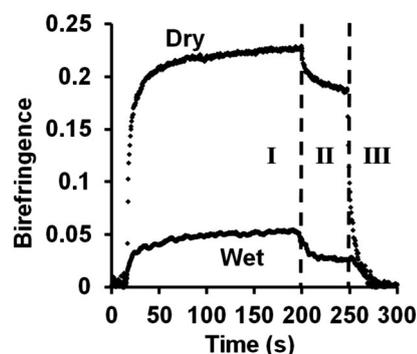


Fig. 4 Birefringence of a (p(DR2A-co-AA)/PDADMAC)₂₀ film as a function of time, measured both in a dry atmosphere (top) and completely underwater (bottom). Region I: irradiation with a linearly polarized beam at 488 nm; region II: relaxation after beam is turned off; region III: irradiation with circularly polarized light.

faster 'fast' process of chromophore alignment. Conversely, the 'dry' films have lower polymer mobility than 'wet' films, resulting in a slower 'slow' process.

In region II (Fig. 4) the linearly polarized writing beam is turned off and a small decrease in birefringence is observed in both the 'wet' and 'dry' PEM films. This decrease, according to Song *et al.*, is associated solely with polymer mobility.¹⁵ Therefore, due to higher thermal relaxation as a result of greater polymer mobility, the 'wet' films lose nearly 50% of their maximum birefringence in 20 s, while 'dry' films lose <20% in >50 s. In region III (Fig. 4) the films are exposed to circularly polarized light which addresses all chromophores, and quickly randomizes the induced order, as observed by the complete loss of birefringence. The time for complete loss of birefringence (<5% max) is similar for both conditions because this process is not influenced by polymer mobility.¹ Despite the somewhat reduced magnitude of birefringence, we have proved that photo-induced molecular orientation is achievable even completely underwater. This provides more confidence for application of some of the usual optical photo-reversible properties and features of azo films to biological fields, which would typically take place entirely in water or a similar aqueous environment.

In order to demonstrate that in principle the bio-compatibility of these new DR2-based azo films is similarly achievable to that previously reported for other azo-containing copolymers with acrylic acid,⁴ we carried out identical cell culture studies with neurons to assess cell adhesion and growth. Fig. 5 shows a photograph of cerebellar neurons from rat pups adhered on the same p(DR2A-co-AA) assembled PEM films on glass substrates. As is evident, the cerebella adhere well and grow significantly on the azo-modified surface after 8 days, which verifies that a basic level of bio-compatibility of the DR2-based self-assembled azo films suitable for neural cell growth control studies is achievable, as for that already reported for other azo-AA copolymer films.⁴ Desired optical patterns could thus be inscribed reversibly on these azo films by tuning the influential irradiation parameters (incoming beam direction, polarization, intensity, *etc.*), to explore the extent to which cell

growth could be directed with light. These photo-responsive azo films might thus be suitable applications in various bio-medical fields, including neural pathway regeneration, basic *in vitro* study of synapse formation, and controlled reversible on-off switching of specific pathways or connections in neural cell arrays.

Conclusions

We have demonstrated that by attaching azobenzene dyes to a copolymer backbone and sequentially multilayering with PDADMAC to make a stratified film, we can attain a PEM I-b-I film that can be induced with linearly polarized light to exhibit an extraordinarily high birefringence under optimized assembly and irradiation conditions. This orientation is stable over long times, even completely underwater, providing the first observation and measurement of surface re-orientation for bio-films that have been shown previously to exhibit reversible surface switching.

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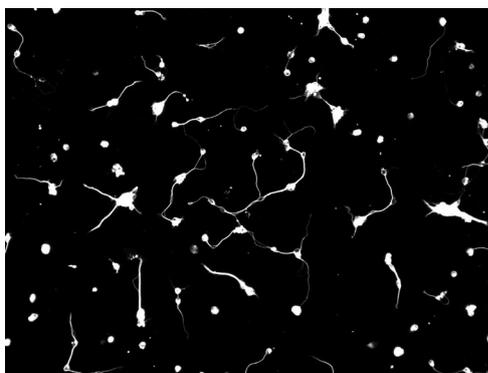


Fig. 5 Image of cerebellar neurons from post-natal day-8 rat pups showing adhesion and growth on p(DR2A-co-AA)-assembled surfaces, 20 PEMs on glass. Image frame is 600 × 450 microns.

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