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Surface functionalisation with viscosity-sensitive BODIPY molecular rotor

Aurimas Vyšniauskas[1,2], Ismael Lopez-Duarte[2], Alex J Thompson[2,3], James A Bull and Marina K Kuimova[4]

Chemistry Department, Imperial College London, Exhibition Road, SW7 2AZ, United Kingdom

[1] Present Address: Center for Physical Science and Technology, Sauletekio av. 3, Vilnius, LT-10257, Lithuania.

[2] These authors contributed equally to this work.

[3] Present address: Surgical Innovation Centre (Paterson Building), St Mary's Hospital, Department of Medicine, Imperial College London, South Wharf Road, London, W2 1NY, United Kingdom.

[4] Author to whom any correspondence should be addressed.

E-mail: m.kuimova@imperial.ac.uk

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Abstract

Surface functionalisation with viscosity sensitive dyes termed 'molecular rotors' can potentially open up new opportunities in sensing, for example for non-invasive biological viscosity imaging, in studying the effect of shear stress on lipid membranes and in cells, and in imaging contacts between surfaces upon applied pressure. We have functionalised microscope slides with BODIPY-based molecular rotor capable of viscosity sensing via its fluorescence lifetime. We have optimised functionalisation conditions and prepared the slides with the BODIPY rotor attached directly to the surface of glass slides and through polymer linkers of 5 kDa and 40 kDa in mass. The slides were characterised for their sensitivity to viscosity, and used to measure viscosity of supported lipid bilayers during photooxidation, and of giant unilamellar vesicles lying on the surface of the slide. We conclude that our functionalised slides show promise for a variety of viscosity sensing applications.

Introduction

Fluorescent viscosity probes—molecular rotors—are an established class of fluorophores used for micro-viscosity sensing [1–3]. The small size of the rotors (few nm or less), as well as the nature of sample interrogation (fluorescence microscopy) allow this method to be used for local viscosity measurements in microscopic samples, where 'bulk' rheological methods are unsuitable. Additionally, the signal from rotors can be detected by conventional fluorescence imaging or fluorescence lifetime imaging (FLIM) microscopies [4], which provide a convenient way of obtaining viscosity *maps* of samples, instead of providing only an average value or a single point measurement of viscosity. This gives the rotor-based imaging a significant advantage over alternative optical techniques, such as fluorescence recovery after photobleaching (FRAP) [5] or fluorescence correlation spectroscopy (FCS) [6, 7], often used for measuring viscosity or diffusion coefficients in heterogeneous media.

The viscosity sensitivity exhibited by molecular rotors arises from the conformational change that occurs following excitation. The rate or efficiency of this conformational change is often dependent on the local viscosity of the rotor's environment or, alternatively, on the solvent free volume. E.g. the intramolecular rotation may drive the transition of the rotor from the fluorescent state to a non-fluorescent one, and this transition becomes impeded at high viscosity. Thus, in a medium of higher viscosity, a rotor is forced to stay longer in the fluorescent state, which leads to a higher quantum yield of fluorescence and to a lower rate constant of its fluorescence decay. While technically more demanding, it is often beneficial to use the fluorescence lifetime to sense viscosity. Barring aggregation and self-quenching, the lifetime is not dependent on a local concentration of fluorophores, which can vary significantly in heterogeneous samples. Thus, using fluorescence lifetime allows viscosity measurements in a quantitative manner [8–10].

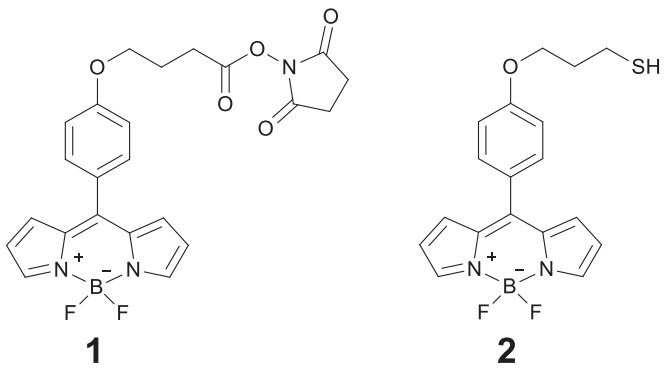


Figure 1. BODIPY rotors **1** and **2** with different end groups used for preparing functionalised slides with a short (<1 nm) linker (**1**) and with 5 kDa or 40 kDa linker (**2**).

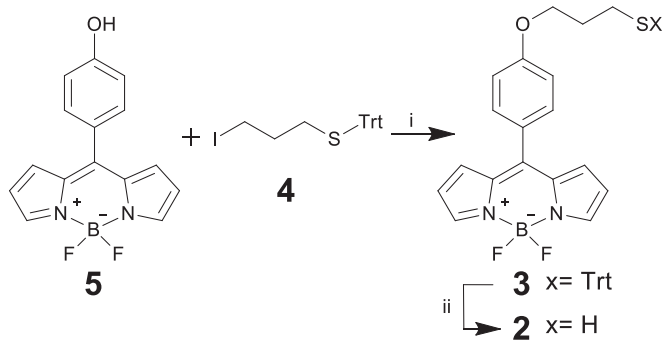
Molecular rotors have been employed in measuring viscosity in a wide range of different samples, such as atmospheric aerosols [11–13], polymers [14], model lipid membranes [15, 16] and live cells [4, 10, 17–21]. For many of these applications it might be beneficial to create reusable and less invasive sensors, whereby a rotor is not simply added to a sample of interest, but covalently attached to an optically transparent surface. The rotor can then be automatically incorporated into a sample upon contact with such a surface and can be recovered from the sample after measurements.

On the other hand, the viscosity sensitive dye 2-dicyanomethylene-3-cyano-2,5-dihydrofuran (DCDHF) was recently used for mechanosensing, in order to measure the area of contact between surfaces upon applied force/pressure [22]. For the latter application it was required that the rotor is covalently attached to one of the surfaces in contact, namely, to a glass coverslip. Apart from mechanosensing mentioned above, only a limited number of covalently attached molecular rotors were reported in the literature, e.g. N-9-(2-carboxy-2-cyanovinyl)-julolidine (CCVJ) [23, 24]. In all these cases fluorescence intensity of the dyes was used as a marker of contact/viscosity, offering only qualitative viscosity measurements. Furthermore, sensitivity to viscosity (where investigated), was significantly reduced compared to the parent rotor, allowed to rotate freely in solution without the constraints of surface attachment, presumably due to the relative ease of changing conformation in solution. Here we investigate an optimal strategy for covalent incorporation of BODIPY-based molecular rotors to an optically transparent surface of a microscope glass slides.

Boron dipyrromethene (BODIPY) rotors are perhaps one of the most widely used class of lifetime-based molecular rotors to date. The use of the fluorescence lifetime of BODIPY allows quantitative measurements of microscopic viscosity, with an extremely wide dynamic range of lifetimes, from 100 s ps to 6 ns, in the viscosity range between 1 to 10 000 cP [4, 15]. As a result, BODIPY rotors have been applied to a variety of viscosity sensing tasks, including measurements in atmospheric aerosols [11, 13], polymers [14], lipid-based systems [15, 25], hippocampal membrane [26] and in live biological cells [4, 9, 10, 20, 27, 28]. They are easily accessible synthetically and suitable for multiple modifications which do not affect their rotor function [20, 28, 29].

The purpose of the current work was two-fold: (i) to engineer an optimal surface modification strategy to allow BODIPY viscosity probes to be attached to surfaces with a minimal loss of their sensing ability and (ii) to investigate the viscoelastic behaviour of lipids in contact with such surfaces.

To achieve our first aim, we found optimal reaction conditions and surface density of the BODIPY rotors to minimise aggregation. Aggregation is a process detrimental for sensing, since it directly affects the photophysical properties of the rotors and reduces (or even removes) its sensitivity to viscosity. In addition, we used neutral 'stopper' molecules to further 'dilute' the sensor on the surface and thus prevent short intermolecular distances between rotors leading to fluorescence quenching. We tested multiple strategies in functionalising the slides, using NHS-ester or thiol-functionalised BODIPYs, figure 1. We also varied the distance between the rotor and the surface by using a NHS-PEGn-Maleimide linker (<1 nm linker, 5 kDa linker and 40 kDa linker) in order to investigate the effect of this parameter on the rotor's viscosity-sensitive photophysics. The linker might be necessary in future biological studies, since during cell adhesion to surfaces the average distance of a cell to the surface can be up to 200 nm [30] and hence the rotors in immediate proximity to the surface might not reach the cell plasma membranes. Finally, we have tested new functionalised slides in sensing viscosity of supported lipid bilayers (SLBs) and giant unilamellar vesicles (GUVs). Overall, we demonstrate the optimal strategy in preparing functionalised slides with BODIPY molecular rotors and show that our slides with the BODIPY rotor show promise as a useful tool for future viscosity sensing applications.



Scheme 1. Synthesis of BODIPY **2**: (i) K_2CO_3, CH_3CN, 50 °C, 5 h, yield: 86%; (ii) TFA, iPr_3SiH, CH_2Cl_2, 0 °C to rt, 1 h, yield: 79%.

Results and discussion

Synthesis

We have chosen to attach BODIPY rotors to commercially available amine-modified glass slides, due to the perceived ease of a coupling reaction that completes rapidly at ambient conditions, as well as the fact that amine-modified slides were accessible from the manufacturer in several 'surface densities', that is, with several orders of magnitude difference in concentration of surface amine groups. Several strategies were employed in parallel, scheme 2, to enable a range of coupling schemes and conditions to be tested. In each case, the obtained slides were characterised by fluorescence confocal imaging and FLIM, in order to establish the uniformity of coverage, the presence of aggregated species that could impede imaging and the dynamic range of viscosity sensing.

BODIPY sensors

BODIPY rotor **1** was prepared as described elsewhere [31]. The synthesis of BODIPY rotor **2** was carried out in two synthetic steps (Scheme 1). First, BODIPY derivative **5** [32, 33] was allowed to react with an excess of compound **4** [34] to afford BODIPY derivative **3** in a yield of 86%. Then, the trityl protecting group was removed smoothly in the presence of TFA and triisopropylsilane to give desired BODIPY rotor **2** in 79% yield. The detailed information on synthesis and characterisation of all compounds is provided in the ESI.

Microscope slide modification and optimisation

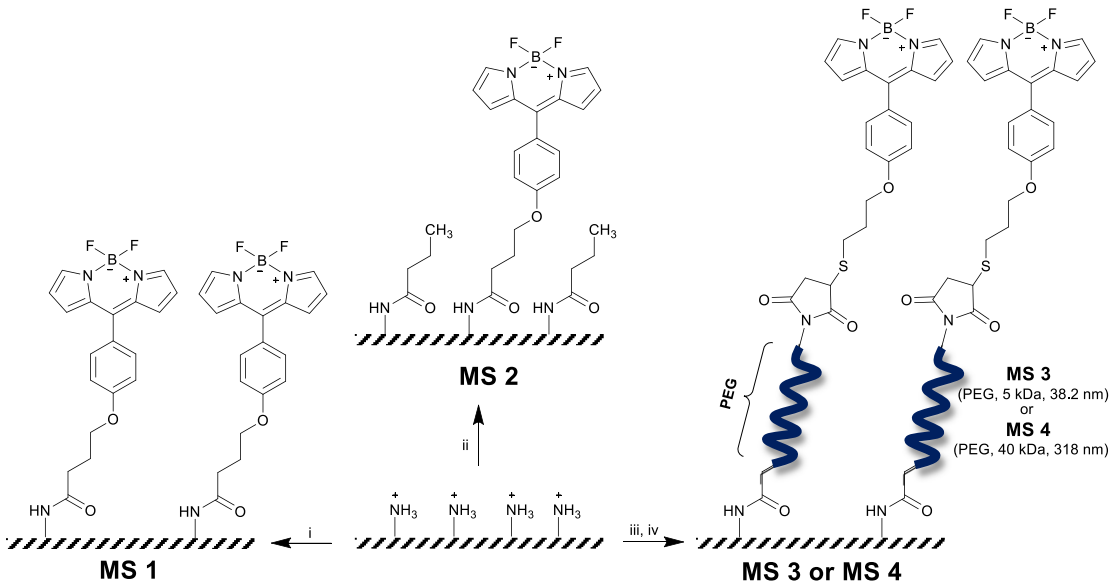
Commercially available positively-charged amine surface microscope slides *SuperAmine* 2 (2×10^{13} reactive groups per mm^2) and *SuperAmine* 2 Low Density (1×10^{12} reactive groups per mm^2) were used to test the immobilization of BODIPY rotor **1**. The microscope slides were immersed in a solution of rotor **1** and Et_3N in DMF in a TLC chamber for the reaction to take place (condition **MS1**, Scheme 2). First, we tested the fluorescence slides for the presence of any aggregates of the BODIPY rotor, since aggregation could negatively impact on the viscosity sensitivity of the bound dye. Fluorescence spectra (figure 2(A)) revealed that BODIPY rotor attached on the

SuperAmine 2 slides suffered from severe aggregation as indicated by significant fluorescence in the range of 600–700 nm, typical of BODIPY aggregated species [15].

A monomeric non-aggregated BODIPY rotor is expected to have a single emission peak with a maximum at 515 nm. Further evidence for the presence of aggregated species of BODIPY came from fluorescence lifetime detection (figure S1 is available online at stacks.iop.org/MAF/6/034001/mmedia, ESI) [15]. Namely, in the case of BODIPY aggregates being present, we expect to see a strong spectral dependence of the observed fluorescence lifetimes, with red detection wavelengths (those corresponding to aggregates) characterised by longer average fluorescence lifetimes. This kind of spectroscopic behaviour is evident in figure S1, ESI. This is due to the presence of longer-lived aggregated species, which emit predominantly at the red edge of the spectrum (>650 nm) [15].

In the case of *SuperAmine* 2 Low Density slides, the fluorescence spectra showed that the aggregation was significantly suppressed, figure 2(A), red spectrum, and figure S2A, ESI. However, the fluorescence decays still showed significant dependence of the fitted lifetimes on the emission wavelength. Furthermore, the fluorescence decays were strongly biexponential, which is another indication of BODIPY aggregation [15]. The sample data obtained from such slides are shown in figure 2(B) (in methanol) and figure S2B (in toluene), and both datasets clearly demonstrate the presence of aggregates.

To diminish aggregation, we tested different reaction times for the attachment of BODIPY rotor **1** on *SuperAmine* 2 Low Density slides, ranging from five days to 15 min. It was found that shorter reaction times below 1 h actually led to stronger fluorescence intensity of the slides (figure S3, ESI). This suggests that reaction times longer than 1 h might have resulted in ever increasing aggregation of the BODIPY molecules, which negated the advantages of having higher surface densities of BODIPY dyes on the slides.



Scheme 2. A schematic representation of the modification of the SuperAmine 2 Low Density microscope slides: (i) BODIPY rotor 1 for MS 1, Et_3N, DMF, rt; (ii) BODIPY rotor 1 and butyric acid N-hydroxysuccinimide ester (1:100) for MS 2, Et_3N, DMF, rt; (iii) NHS-PEGn-Maleimide (5 kDa, 38.2 nm) for MS 3 or NHS-PEGn-Maleimide (40 kDa, 318 nm) for MS 4, Et_3N, DMSO, rt; (iv) BODIPY rotor 2, Et_3N, DMF, rt.

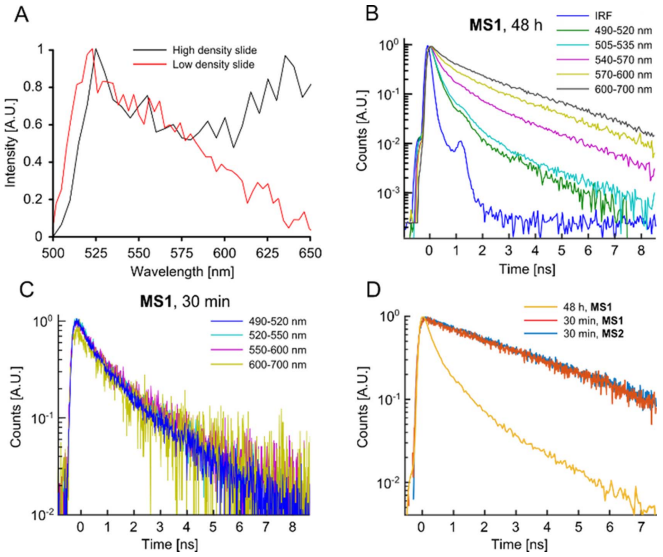


Figure 2. Spectroscopic characterisation of functionalised slides. (A) Fluorescence spectra of **MS1** slides in methanol prepared using high density (black) and low density (red) glass slides. (B), (C) Normalised fluorescence decays of **MS1** slides prepared using 48 h ((B), solvent—methanol) and 30 min ((C), solvent—DMSO) reaction times. The decays are shown at multiple emission wavelengths as indicated in the inserts. (D) Normalised fluorescence decays of **MS1** (red, yellow) and **MS2** (blue) slides in glycerol. The reaction times were 48 h (yellow) and 30 min (blue). Low density slides were used for functionalization in (B) and (C), fluorescence was recorded in a microscope setup over 490–520 nm range (monomer emission range) using two-photon excitation at 850 nm.

Therefore, only the reaction times of 1 h or less were further explored.

The reaction times limited to 1 h or less led to no significant variation of fluorescence decays with the detection wavelength (figure 2(C)). Furthermore, the decays obtained in highly viscous solvents could be fitted using a monoexponential function (figure 2(D) in red and blue). Again, this strongly contrasts the shapes of the decay obtained when longer reaction times were used (e.g. 48 h reaction time is shown in figure 2(D), in yellow). We further varied the reaction times from 15 min to 1 h but in all cases the variation in reaction times did not have any significant effect on fluorescence spectra (figure S4, ESI) or fluorescence decays (figure S5, ESI). For all future experiments we chose 30 min as the optimal duration of the conjugation reaction. We must note that even at these conditions fluorescence decays remain biexponential at most viscosities, which may result from a fraction of the rotor molecules binding to the surface of the slide,

similar to what was previously observed upon covalent attachment of rotors to aggregating proteins [31].

In parallel with varying the duration of the conjugation reaction, we have also explored whether the introduction of a large concentration of competing binder, a neutral 'stopper' molecule, (condition **MS2**, Scheme 2), will affect the observed sensor properties. Interestingly, introducing stopper molecules in order to 'dilute' the rotors on the surface and to prevent possible aggregation had no effect on the time resolved fluorescence decays whatsoever.

The example decays for two slides that were obtained by using stopper/not using stopper conditions at 30 min reaction time are shown in figure 2(D) in blue and red, respectively. Further data showing fluorescence kinetics and spectra of **MS2** slides is displayed in figures S4 and S5, ESI. Based on the data, we concluded that the presence of a stopper in the reaction solution—even with a stopper-to-BODIPY ratio of 100:1—does not affect the outcome of the manufacturing of the sensor. This result agrees with our previous hypothesis that biexponential fluorescence decays may result not only from aggregation of the rotors but also from binding to the surface.

One of the possible applications of the microscope slides functionalised with the BODIPY rotor is measurements of the viscosity of the cellular membrane. However, the distance between the surface and the cell attached on the surface can be as high as 200 nm [30]. This relatively long distance will prevent rotors described in conditions **MS1** and **MS2** (Scheme 2) to effectively incorporate inside the plasma membrane of the surface-attached cells. Consequently, to increase the surface-sensor distance, we incorporated a heterobifunctional polyethylene glycol linker, using a strategy described previously for coupling adhesive arginylglycylaspartic acid ligands to the surface [35]. Polyethylene glycol is a hydrophilic molecule, which is well solvated by water molecules in the aqueous environment and it is not likely to incorporate into the hydrophobic lipid bilayers which are our samples of interest. We attached the rotor to the surface using 5 kDa or 40 kDa linkers (**MS3** and **MS4**, respectively, Scheme 2). Commercially available heterobifunctional polyethylene glycol tether molecules of varying length (5 kDa nm and 40 kDa) that contain an NHS ester at one end (to be connected to the glass surface) and a maleimide group at the other (to be linked to the BODIPY rotor **2**) were used. In a first step, the microscope slides were immersed in a solution of the corresponding NHS-PEGn-Maleimide crosslinker and Et_3N in DMSO in a TLC chamber at different reaction times, resulting in a maleimide-activated surface. This surface was further decorated with BODIPY rotor **2** in a second reaction step, following a similar protocol to that described above. The resulting slides did not display any significant fluorescence above 600 nm (figure S6, ESI) and did not show any other signatures of aggregation.

In summary, we have established that conditions **MS1**-**MS4** (Scheme 2) can produce microscope slides functionalised with BODIPY rotors. By keeping the reaction times shorter than 1 h, it was possible to avoid aggregation of the neighbouring BODIPY moieties on the slide, which could be detrimental to their viscosity-sensing properties.

Calibration of functionalised slides

In order to characterise the viscosity sensitivity of rotor-modified slides, we utilised room temperature methanol-glycerol solutions of varying viscosity. A wide range of viscosities from <1 cP to 1400 cP could be accessed in such a way. Previously, we have demonstrated for structurally similar BODIPY rotors free in solution that the high polarity of the mixture does not significantly affect the photophysics of the rotors, at least above 80 cP [25]. Hence, the results of viscosity calibration from methanol-glycerol mixtures can be translated to very non-polar systems such as castor oil, to lipid bilayers and, ultimately, to a cell plasma membrane [25].

The required solvent mixtures were placed on top of the slides and the fluorescence decays of immobilised BODIPY were measured using FLIM, figure 3. The decays could not be fitted using a mono-exponential function, however, our analysis of the decays in the green vs red edge of the spectrum showed no evidence for the presence of aggregates.

Therefore, the decays were fitted using biexponential function and the intensity-weighted mean lifetimes were calculated according to the following formula:

$$\tau_{mean} = \frac{\sum_i a_i \tau_i^2}{\sum_i a_i \tau_i} \quad (1)$$

where τ_i and a_i are lifetimes and amplitudes of the individual exponential components.

The calibration plot was constructed by plotting the intensity-weighted mean lifetimes against the viscosity of the mixtures, and the trend was fitted using the Förster-Hoffmann equation [36]:

$$\tau = C\eta^x \quad (2)$$

where τ is lifetime, η is viscosity, C and x are free parameters; and x defines the sensitivity of the rotor to viscosity.

The obtained fitting parameters are shown in figure S7, ESI. Fluorescence decays of BODIPYs on all three types of slides (figure 3) show clear viscosity dependence. However, the dependence is significantly greater for **MS3** and **MS4** slides with BODIPY rotor attached using a linker (figure 3(A)); the parameter x is 0.13 compared to 0.076 in the case of **MS1**. Most likely, close proximity of the surface in the case when

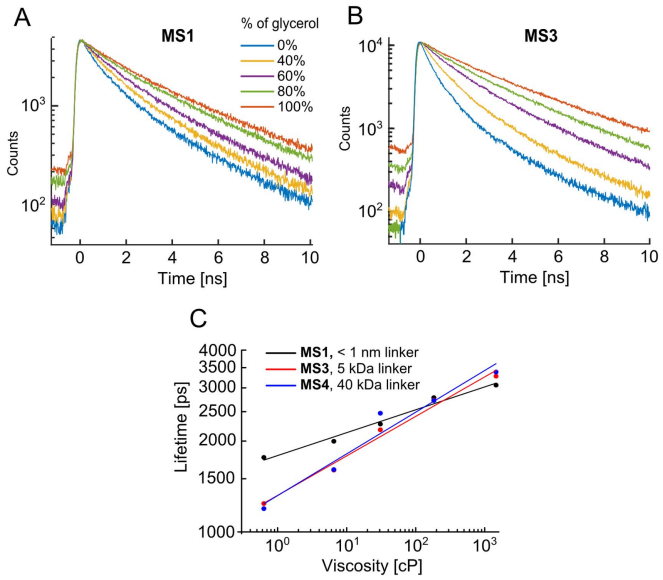


Figure 3. Fluorescence decays of BODIPY attached to the slides directly (**MS1**) (A) and using 5 kDa linker (**MS3**) (B). (C) Calibration lines for the **MS1** (black), **MS3** (red) and **MS4** (blue) slides. Intensity-weighted mean lifetimes are shown. Two-photon excitation at 850 nm in a microscope setup was used to excite fluorescence.

<1 nm linker was used prevents the solvent from fully surrounding the BODIPY rotor and, as a result, its sensitivity to solvent viscosity is diminished. By introducing a linker, the distance of the BODIPY rotor from the surface becomes greater, which boosts the sensitivity of the rotor. Our results indicate that the length of the linker does not seem to matter, as the calibration curves shown in figure 3(C) for **MS3** and **MS4** slides are nearly identical. In either case, the distance to the surface is many times greater than the size of the molecule, which leads to a similar environment for the BODIPY rotor in both cases. However, we must point out that even when the longer linker is used, BODIPY rotor shows much weaker sensitivity compared to free BODIPY in solution ($x = \sim 0.5$) [19].

Application of microscope slides with BODIPY rotor attached

We went on to test our modified microscope slides for measuring viscosity of lipid bilayers. In this simplest case of a lipid-based sample we do not expect large distance between the surface and the sample (as would be expected in the case of cells) and therefore, **MS1, MS3** and **MS4** slides were expected to be suitable for this experiment.

First, we prepared a supported lipid bilayer (SLB) from 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipids on an **MS1** slide with a short (<1 nm) linker. The maximum possible density of BODIPY rotors on **MS1** is 10^{12} molecules/mm^2, close to 1.4×10^{12} lipids/mm^2, (calculated from the area taken by a lipid molecule, ~0.7 nm^2 [37]). However, it is likely that our functionalization reaction does not proceed in 100% yield and, therefore, the lipid-to-dye ratio in our experiments is greater than 1.4:1. Our goal was to test the slide by (i) measuring the viscosity of the SLB via FLIM and comparing it with our previous results in model lipid bilayers [15, 25]; and (ii) monitoring a *dynamic* process using the surface bound rotor, namely to photo-oxidise the SLB and ascertain if we can monitor the corresponding increase in viscosity, as observed previously using the free BODIPY rotor [38].

In order to generate suitable ROS (singlet molecular oxygen) for photooxidation of lipids we utilised tetraphenylporphine (TPP). The use of TPP had an additional advantage, since TPP is a hydrophobic molecule and should effectively stain the lipid bilayer. Hence, its fluorescence in the red spectral region allowed us to verify the formation of the SLB over the surface of the functionalized microscope slide.

The fluorescence spectra of the bilayer (figure 4(A)) clearly show fluorescence spectra of both the BODIPY rotor and TPP confirming that the SLB formation was successful. Irradiation of TPP should produce singlet molecular oxygen $^1O_2$, in high quantum yield, which is expected to effectively oxidise the unsaturated DOPC bilayer [38]. We measured FLIM images of the BODIPY before and after irradiation at the Soret absorption peak of TPP (420 nm). The irradiation was performed by zooming in at the center of the image and continuously scanning the zoomed in area using the 420 nm laser. This approach should leave the borders of the image non-irradiated and non-affected by ROS production, at least not directly.

The results of the photooxidation experiment are shown in figure 4(B). Before irradiation, the average lifetime of BODIPY molecules was ~2550 ps and it

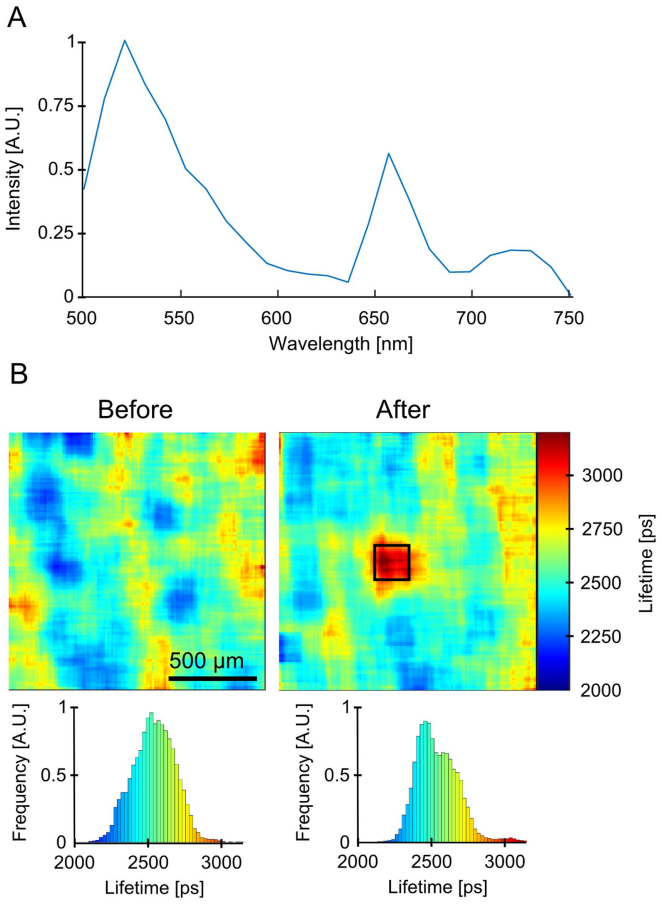


Figure 4. (A) Fluorescence spectrum of **MS1** slide incorporating the BODIPY rotor, with DOPC supported lipid bilayer on top, containing TPP porphyrin (lipid-to-dye ratio—1000:1). The spectrum clearly shows fluorescence peaks of the BODIPY rotor (at 520 nm) and of TPP (at 650 nm, 720 nm). (B) FLIM images of supported lipid bilayer on **MS1** slide. The images were taken before and after irradiation at the image centre. Irradiation was performed at 420 nm in order to produce a high quantity of potent ROS, singlet molecular oxygen $^1O_2$, in order to photo-oxidise DOPC. The lifetime histograms for the images are shown at the bottom.

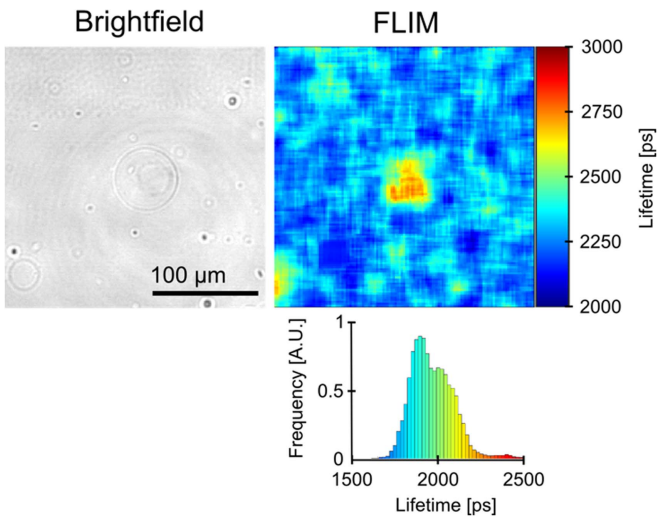


Figure 5. Brightfield and FLIM images of the **MS3** microscope slide with a 5 kDa linker. An aqueous solution containing GUVs was put on top of the slide. The lifetime of the BODIPY rotors is clearly higher in the location where the GUV is present, as can be seen on the brightfield image.

follows a normal distribution (figure 4(B), histogram on the left). This lifetime corresponds to ~100 cP viscosity, which is very similar to what we have observed in DOPC bilayers previously using free BODIPY (~90 cP) [25, 38]. Upon irradiation, the lifetime drastically increased in the center of the image to ~3000 ps,

corresponding to ~900 cP, while the lifetimes remained constant in the rest of the image, in agreement with our previously obtained results in photooxidated GUVs containing free BODIPY rotor [38].

Next, we tested **MS3** slides with a 5 kDa linker separating the rotor from the surface. The slide was covered with aqueous solution containing giant unilamellar vesicles (GUVs) and imaged using brightfield microscopy and FLIM. The resulting FLIM image (figure 5) clearly shows a higher lifetime of the BODIPY rotor (~2400 ps, corresponding to ~80 cP) in the location where the GUV is present, as seen in the brightfield image. The viscosity value obtained corresponds closely to the one measured using the functionalized slide with a <1 nm linker, figure 4(B). These results show that **MS3** slides are able to sense viscosity and provide reliable results. Overall, the results show the potential usefulness and applicability of microscope slides functionalized with BODIPY rotors for a variety of viscosity measurements.

Conclusions

To conclude, we have successfully prepared microscope slides with BODIPY-based molecular rotors attached on the surface. Three types of slides were prepared: with the BODIPY rotor attached directly to the glass surface via a short <1 nm linker and those attached using significantly longer heterobifunctional polyethylene glycol tether molecules of 5 kDa and 40 kDa in length. We have optimised the synthesis procedure to minimise the aggregation of the rotors on the surface and then calibrated the slides in methanol-glycerol mixtures showing that the introduction of the linker significantly enhances the viscosity sensitivity of the surface-bound BODIPY rotor. Then we have tested the slides for measuring the viscosity of a SLB before and after photooxidation by TPP porphyrin and for measuring the viscosity of a GUV placed on the surface of the slide. In summary, through an optimised procedure we have prepared slides functionalised with a molecular rotor for viscosity sensing suitable for further applications.

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ORCID iDs

Aurimas Vyšniauskas https://orcid.org/0000-0002-4750-8149
Ismael Lopez-Duarte https://orcid.org/0000-0002-9941-8305
James A Bull https://orcid.org/0000-0003-3993-5818

References

[1] Haidekker M A and Theodorakis E A 2007 *Org. Biomol. Chem.* **5** 1669–78
[2] Kuimova M K 2012 *Phys. Chem. Chem. Phys.* **14** 12671–86
[3] Yang Z, Cao J, He Y, Yang J H, Kim T, Peng X and Kim J S 2014 *Chem. Soc. Rev.* **43** 4563–601
[4] Kuimova M K, Yahioglu G, Levitt J A and Suhling K 2008 *J. Am. Chem. Soc.* **130** 6672–3
[5] Dayel M J, Hom E F Y and Verkman A S S 1999 *Biophys. J.* **76** 2843–51
[6] Korlach J, Schwille P, Webb W and Feigenson G W 1999 *Proc. Natl Acad. Sci.* **96** 8461–6
[7] Iino R and Kusumi A 2001 *J. Fluoresc* **11** 187–95
[8] Kuimova M K *et al* 2007 *Photochem. Photobiol. Sci.* **6** 675–82
[9] Wang L, Xiao Y, Tian W and Deng L 2013 *J. Am. Chem. Soc.* **135** 2903–6
[10] Yang Z *et al* 2013 *J. Am. Chem. Soc.* **135** 9181–5
[11] Hosny N A *et al* 2016 *Chem. Sci.* **7** 1357–67
[12] Athanasiadis T, Fitzgerald C, Davidson N, Giorio C, Botchway S W, Ward A D, Kalberer M, Pope F D and Kuimova M K 2016 *Phys. Chem. Chem. Phys.* **18** 30385–93
[13] Hosny N A, Fitzgerald C, Tong C, Kalberer M, Kuimova M K and Pope F D 2013 *Faraday Discuss.* **165** 343–56
[14] Nölle J M, Jüngst C, Zumbusch A and Wöll D 2014 *Polym. Chem.* **5** 2700–3
[15] Wu Y, Stefl M, Olzyńska A, Hof M, Yahioglu G, Yip P, Casey D R, Ces O, Humpolíčková J and Kuimova M K 2013 *Phys. Chem. Chem. Phys.* **15** 14986–93
[16] Dent M R, López-Duarte I, Dickson C J, Chairatana P, Anderson H L, Gould I R, Wylie D, Vyšniauskas A, Brooks N J and Kuimova M K 2016 *Chem. Commun.* **52** 13269–72
[17] Kuimova M K, Botchway S W, Parker A W, Balaz M, Collins H A, Anderson H L, Suhling K and Ogilby P R 2009 *Nat. Chem.* **1** 69–73
[18] Peng X J *et al* 2011 *J. Am. Chem. Soc.* **133** 6626–35
[19] Levitt J A, Kuimova M K, Yahioglu G, Chung P H, Suhling K and Phillips D 2009 *J. Phys. Chem.* C **113** 11634–42
[20] López-Duarte I, Vu T T, Izquierdo M A, Bull J A and Kuimova M K 2014 *Chem. Commun.* **50** 5282–4
[21] Izquierdo M A, Vyšniauskas A, Lermontova S A, Grigoryev I S, Shilyagina N Y, Balalaeva I V, Klapshina L G and Kuimova M K 2015 *J. Mater. Chem.* B **3** 1089–96
[22] Suhina T, Weber B, Carpentier C E, Lorincz K, Schall P, Bonn D and Brouwer A M 2015 *Angew. Chemie—Int. Ed.* **54** 3688–91
[23] Lichlyter D J and Haidekker M A 2009 *Sensors Actuators, B Chem.* **139** 648–56
[24] Haidekker M A, Akers W J, Fischer D and Theodorakis E A 2006 *Opt. Lett.* **31** 2529–31
[25] Dent M R, Lopez Duarte I, Dickson C J, Geoghegan N D, Cooper J M, Gould I R, Krams R, Bull J A, Brooks N J and Kuimova M K 2015 *Phys. Chem. Chem. Phys.* **17** 18393–402
[26] Pal S, Chakraborty H, Bandari S, Yahioglu G, Suhling K and Chattopadhyay A 2016 *Chem. Phys. Lipids* **196** 69–75
[27] Dziuba D, Jurkiewicz P, Cebecauer M, Hof M and Hocek M 2016 *Angew. Chemie—Int. Ed.* **55** 174–8
[28] Sherin P S, Lopez Duarte I, Dent M R, Kubánková M, Vysniauskas A, Bull J A, Reshetnikova E S, Klymchenko A S, Tsentalovich Y P and Kuimova M K 2017 *Chem. Sci.* **8** 3523–8
[29] Vyšniauskas A, López-Duarte I, Duchemin N, Vu T-T, Wu Y, Budynina E M, Volkova Y A, Peña Cabrera E, Ramírez-Ornelas D E and Kuimova M K 2017 *Phys. Chem. Chem. Phys.* **19** 25252–9
[30] Toma K, Kano H and Offenhäusser A 2014 *ACS Nano* **8** 12612–9
[31] Kubánková M, López-Duarte I, Bull J A, Vadukul D M, Serpell L C, de Saint Victor M, Stride E and Kuimova M K 2017 *Biomaterials* **139** 195–201
[32] Kursunlu A N, Guler E, Ucan H I and Boyle R W 2012 *Dye. Pigment.* **94** 496–502

[33] Baruah M, Qin W, Basaric N, De Borggraeve W M and Boens N 2005 *J. Org. Chem.* **70** 4152–7
[34] Okubo H, Feng F, Nakano D, Hirata T, Yamaguchi M and Miyashita T 1999 *Tetrahedron* **55** 14855–64
[35] Attwood S J, Cortes E, Haining A W M, Robinson B, Li D, Gautrot J and del Río Hernández A 2016 *Sci. Rep.* **6** 34334
[36] Forster T and Hoffmann G 1971 *Zeitschrift Fur Phys. Chemie-Frankfurt* **75** 63–76
[37] White S H and King G I 1985 *Proc. Natl Acad. Sci.* **82** 6532–6
[38] Vyšniauskas A, Qurashi M and Kuimova M K 2016 *Chem.—A Eur. J.* **22** 13210–7