Fluorescent molecular rotors
From working principles to visualization of mechanical contacts
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Visualizing Contacts Between Objects*

Abstract

The contact area between two objects was detected and measured by using strong fluorescence enhancement of fluorescent molecular rotors that were attached to one of the surfaces in contact. Contact induced local confinement hinders the intramolecular motion that leads to the excited-state deactivation of the molecular rotor, and results in a dramatic fluorescence enhancement. We demonstrate this approach by imaging the contact area of a round PMMA sphere that is pressed onto a flat glass cover slip with covalently attached fluorescent molecular rotors. The measured contact area values show excellent agreement with those predicted by Hertz classical theory based on elastic deformation.

*This chapter is adapted from:
3.1 Introduction

For virtually any mechanical system, the understanding of contacts between its constituents is essential. Friction, for example, is responsible for $\sim 30\%$ of the world energy consumption,\textsuperscript{1} and results from molecular interactions that take place within the contact.\textsuperscript{2,3} The study of contact mechanics dates back to 1882 and Heinrich Hertz,\textsuperscript{4} yet surprisingly little is known about how the physical contacts between objects arise, although this is essential for the understanding of their mechanics.\textsuperscript{2,5} The main challenge comes from the fact that since most (if not all) surfaces possess a certain roughness, the actual contacts may occur on microscopic length scales, even for large macroscopic bodies. Bowden and Tabor were the first to emphasize the importance of surface roughness for bodies in contact.\textsuperscript{6}

Herein we describe the first direct visualization of mechanical contacts at the microscale by means of fluorescence microscopy, using specifically developed probe molecules that fluoresce when confined in a contact. To achieve this goal we synthesized rigidochromic fluorescent molecules that fluoresce only very weakly in (low-viscosity) solutions owing to the presence of rapid nonradiative relaxation pathways for the excited state.\textsuperscript{7–9} This fast nonradiative decay is triggered by the rotation around a specific bond in the molecule. When the rotation of the bond is hindered, the nonradiative decay is suppressed, and the excited-state decays by emitting a photon. When rigidochromic molecules are incorporated in a very viscous medium, such as a glassy polymer matrix, a strong fluorescence is observed. This effect has been used to measure local viscosities in polymer films and study their free volume and glass transition\textsuperscript{10–13} and to investigate the viscosity of membranes and intracellular media.\textsuperscript{8,14} We show that the confinement between two surfaces also impedes the nonradiative relaxation of the probe molecule 1 that starts fluorescing strongly when confined. This effect then allows the detection of the physical contacts between surfaces on a molecular scale.

Scheme 3.1: Molecular probes used in this work.
3.2 Experimental

Compound 1 was prepared following the procedures described by Twieg, Moerner, and coworkers for related compounds.\textsuperscript{15,16} We used 4-piperidine acetic acid as the amine in the last coupling step (Scheme 3.2). Subsequently, the dye was attached to amino-functionalized glass surfaces (see below).

\begin{center}
\textbf{Scheme 3.2:} Synthesis of probe 1.
\end{center}

Synthesis of 2-(1-(4-(4-cyano-5-(dicyanomethylene)-2,2-dimethyl-2,5-dihydrofuran-3-yl)phenyl)-piperidin-4-yl) acetic acid (1)

2-(3-Cyano-4-(4-fluorophenyl)-5,5-dimethylfuran-2(5H)-ylidene) malononitrile (P3)\textsuperscript{15} (0.98 g, 3.5 mmol, 1 eq) and 4-piperidine acetic acid (1.5 g, 10.5 mmol, 3 eq) were dissolved in pyridine (10 mL). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture turned red during this time. It was poured into 200 mL of cold water and left standing in the refrigerator overnight.
The precipitate was filtered and purified via flash chromatography (gradient of dichloromethane (DCM) to DCM/MeOH 10:1). Compound 1 was isolated in 26% yield (0.36 g, 0.89 mmol).

$^1$H NMR (400 MHz, THF-d8): δ (ppm) = 10.77 (s, 1H), 8.07 (d, J = 9.4 Hz, 2H), 7.07 (d, J = 9.4 Hz, 2H), 4.15 (d, J = 13 Hz, 2H), 3.07 (m, 2H), 2.21 (d, J = 6.9 Hz, 2H), 2.07 (m, 1H), 1.89 (d, J = 11.6 Hz, 2H), 1.83 (s, 6H), 1.31 (m, 2H).

$^{13}$C NMR (100 MHz, THF-d8): δ (ppm) = 177.79, 174.66, 173.39, 155.11, 133.16, 115.34, 114.06, 113.62, 113.28, 112.24, 98.58, 93.39, 47.83, 40.83, 33.88, 32.44, 27.14.

MS (FAB+) MH+: 403.2 (calculated), 403.2 (observed).

Cover slip silanization

Glass cover slips were washed in 3% (v/v) Hellmanex III solution by sonication for 30 min at 40 °C and sonicated in deionized water for 10 min and in EtOH for 30 minutes. The cover slips were dried in an oven at 110 °C for 1 h and further cleaned in an ozone photoreactor for 2 hours. Cover slips were silanized with 2% (v/v) N-[3-(trimethoxysilyl)propyl]-ethylenediamine (AEAPTMS) in 96% EtOH in which 2% (v/v) of H$_2$O was added. The pH of this solution was adjusted to ∼5 by addition of acetic acid. A teflon rack with cover slips was kept for 30 minutes in this solution with stirring. The cover slips were afterwards sonicated three times in EtOH (20 min), washed with acetone and DCM, dried in air and put in an oven for 1 h at 110 °C. Some cover slips were silanized according to the procedure reported by Basabe-Desmonds *et al.*, but we did not observe any significant difference.

Immobilization of 1 on glass

Rigidochromic compound 1 (5 mg, 0.012 mmol, 1 eq), (benzotriazol-1-yloxy) tris(dimethyamino)-phosphonium hexafluorophosphate (BOP) (16 mg, 37 µmol, 3 eq), N-hydroxybenzotriazole (HOBt) (5.3 mg, 37 µmol, 3 eq), and diisopropyl-ethylamine (64 µl, 0.37 mmol, 10 eq) were added to the silanized cover slips in DMF (60 mL). The reaction mixture was stirred for 16 h at room temperature. After completion of the reaction, the glass cover slips were removed from the reaction mixture, sonicated in ethanol three times (60-120 min), and rinsed with DCM. The process is schematically shown in Scheme 3.3.
3.3 Results and discussion

3.3.1 Originally proposed model for fluorescence deactivation

The model for the excited-state deactivation of 2 was initially proposed by Willets et al. in ref. 18, and is schematically shown in Fig. 3.4. According to this model, the chromophore can, after initial photon absorption and Franck-Condon state formation, relax back to the ground state via two pathways. The first pathway (I) involves a small structural relaxation (mainly a small \( \beta \)-bond adjustment), upon completion of which the molecule assumes the fluorescent locally excited-state geometry. From this geometry, the molecule radiatively decays to the ground state structure, which subsequently relaxes towards the ground state minimum. The other pathway (II) is sterically much more demanding, and it mainly involves the torsional motion around the \( \gamma \) bond which leads to formation of the twisted dark state. This state is energetically very close to the ground state, which results in rapid nonradiative decay to the ground state, which is once again followed by a structural relaxation towards the optimized ground state structure.

While the proposed model,\(^{18}\) based on quantum-chemical calculations, explains the rigidochromism observed, the actual excited-state dynamics is more compli-
cated, as discussed in Chapters 4 and 5. In the following experiments we demonstrate that the molecules immobilized on a glass surface experience a significant confinement when pressed upon with another surface and make use of this effect in order to visualize the contact area between solid objects.

3.3.2 Basic photophysical characterization of 1

Representative steady state spectra of 1 and 2 in toluene, EtOAc and DMSO are shown in Fig. 3.1. As the solvent polarity increases, the spectra shift towards lower energies. The spectral shift is accompanied by the disappearance of vibrational structure. Both the spectral shifts, and the lack of vibrational structure in polar environment indicate a significant charge-transfer character of the first excited state. Steady-state spectra of 1 and 2 are, as expected, very similar. A difference between the relative intensities of the vibrational bands exhibited by 1 and 2 in non-polar toluene and slightly polar EtOAc (see Fig. 3.1 a) and b)) is noticeable. A direct comparison between the absorption spectra of the two molecules shows a slight broadening in case of 1, thus pointing towards the possibility of intermolecular interactions (self-association) due to the presence of a -COOH group. This, however, does not seem to significantly influence the photophysical properties of 1 in solutions, as the obtained fluorescence quantum yields, spectral peak positions, and average fluorescence lifetimes closely resemble those that were obtained for 2 (see Table 3.1).

Both probes show a relatively weak solvatochromic effect in absorption and emission, and their fluorescence quantum yields ($\Phi_f$) are low and tend to decrease with increasing solvent polarity. Fluorescence decay times $\tau_f$ follow the same trend.
In some solvents, fluorescence decay times were shorter than time resolution of our instrument (∼10 ps). The quantum yields and fluorescence decay times are higher in solvents of higher viscosity. For example, in cyclohexanol $\tau_{\text{avg}} = 0.46$ ns, and $\Phi_f = 0.11$, while in 1-butanol $\tau_f = 0.056$ ns, and $\Phi_f = 0.02$ (see Table 3.1 and Fig. 3.2). This trend can be explained by the effect of viscosity on the rapid nonradiative deactivation of the excited states that occurs by twisting of the exocyclic $\text{C}=$C(CN)$_2$ bond, as reported previously for DCDHF chromophores.\textsuperscript{18} In the case of some solvents, we had to use biexponential decay function (Eq. 3.1) in order to obtain satisfactory fits. This indicates that the photophysical behavior of this chromophore is more complex than initially proposed in ref. 18 (see Chapter 4 and 5). For this reason, we report average fluorescence lifetime values $\tau_{\text{avg}}$ obtained according to the Eq. 3.2 (see Table 3.1). Using this definition of average lifetime we can relate it to the quantum yield of the fluorescent layer on the cover slip, with the commonly made assumption that the radiative rate constant $k_{\text{rad}}$ is the same for all dye molecules, regardless of their chemical environment.\textsuperscript{19–21} In the later work (Chapter 4 and 5), we show that excited-state deactivation of DCDHF occurs via two different pathways, and the dominant deactivation pathway strongly depends on the solvent polarity.
Table 3.1: Measured photophysical properties of 1 and 2 in various solvents. Last digit in a given number represents the estimated uncertainty of the measurement.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Compound 1</th>
<th></th>
<th>Compound 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>η&lt;sup&gt;a&lt;/sup&gt;</td>
<td>λ&lt;sub&gt;abs&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Φ&lt;sub&gt;f&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1,4-dioxane</td>
<td>1.37</td>
<td>476</td>
<td>517</td>
<td>0.08</td>
</tr>
<tr>
<td>toluene</td>
<td>0.59</td>
<td>283</td>
<td>510</td>
<td>0.06</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>0.45</td>
<td>483</td>
<td>526</td>
<td>0.013</td>
</tr>
<tr>
<td>cyclohexanol</td>
<td>41.1</td>
<td>497</td>
<td>530</td>
<td>0.11</td>
</tr>
<tr>
<td>DMSO</td>
<td>2.24</td>
<td>505</td>
<td>543</td>
<td>0.004</td>
</tr>
<tr>
<td>1-butanol</td>
<td>2.95</td>
<td>501</td>
<td>530</td>
<td>0.02</td>
</tr>
<tr>
<td>DMF</td>
<td>0.92</td>
<td>501</td>
<td>535</td>
<td>0.003</td>
</tr>
<tr>
<td>2-propanol</td>
<td>2.40</td>
<td>501</td>
<td>528</td>
<td>0.010</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>0.35</td>
<td>539</td>
<td>539</td>
<td>0.001</td>
</tr>
<tr>
<td>methanol</td>
<td>0.60</td>
<td>531</td>
<td>531</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Viscosity in mPa s.; <sup>b</sup>UV/VIS absorption maximum in nm.; <sup>c</sup>Emission maximum in nm.; <sup>d</sup>Fluorescence quantum yield (%) measured relative to C153.<sup>22</sup> Literature values are given in parentheses. For toluene, the initially published value from ref. 23 was corrected in ref. 24. Since the values in the other solvents published in ref. 18 were measured relative to the incorrect value of 0.10 in toluene we multiplied the published value by 0.44.; <sup>e</sup>Fluorescence decay times in ns and amplitudes (parentheses); <sup>f</sup>Average fluorescence decay times in ns.; <sup>g</sup>From ref. 18.
3.3 Results and discussion

Figure 3.2: Fluorescence decays measured for 1 in low viscosity BuOH ($\eta = 2.95$ mPa s) and high viscosity cyclohexanol ($\eta = 41.1$ mPa s).

\[ I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \]  \hspace{1cm} (3.1)

\[ \tau_{\text{avg}} = \frac{A_1 \tau_1^2 + A_2 \tau_2^2}{A_1 \tau_1 + A_2 \tau_2} \]  \hspace{1cm} (3.2)

3.3.3 Sensitivity towards viscosity changes

We have examined the effect of solvent viscosity by monitoring the fluorescence response of 1 in a series of alcohols: methanol, ethanol, 1-propanol, 1-butanol, 1-hexanol, benzyl alcohol, 1-decanol and ethylene glycol. The results are shown in Figs. 3.3 a) and b). We find that the fluorescence intensity can be described well by the Förster-Hoffmann equation:

\[ \Phi_f = z\eta^\alpha, \]  \hspace{1cm} (3.3)

This way, we obtained a slope of $\alpha = 1.3$. Such a high value is not physically meaningful and indicates that polarity changes influence the response of the probe. This is also observable in the relatively low total intensity value obtained for polar ethylene glycol (Fig. 3.3 b)). The effect of solvent polarity on nonradiative decay of 1 will be examined and discussed in Chapters 4 and 5.

We systematically studied the effect of solvent viscosity with minimal effect on polarity by subjecting solutions of compound 1 in acetonitrile to different hydrostatic pressures. To convert the hydrostatic pressures to changes in viscosity, we used the relationship between viscosity of acetonitrile and pressure described by Martin et al.:

\[ \eta = \eta_0 [1 + \kappa(P - P_0)], \]  \hspace{1cm} (3.4)

where $\eta_0$ is the viscosity at the reference pressure ($P_0$), and $\kappa$ is the relative variation of the viscosity per unit pressure. The used viscosity data was obtained from Dymond et al.\cite{27}. The results are shown in Figure 3.3 c) and d). The obtained slope value of $\alpha = 0.66$ shows that fluorescence response of 1 is strongly dependent on micro-viscosity of the environment.\cite{25}
3.3.4 Contact confinement

To be able to look at the contact of an object with a flat surface, we covalently attached probe 1 to glass cover slips, as described in Chapter 3.2. Fluorescence emission and excitation spectra of surface-bound 1 (Fig. 3.4) were found to be very similar to those of 1 and 2 in solution. The absence of broadening of the bands shows that aggregation of surface-bound dye molecules does not occur or has no significant effect on the electronic structure of the chromophore.
On the other hand, the fluorescence lifetime of the bound molecules is quite different from the lifetimes obtained for 1 in liquid solvents. The fluorescence decay was measured at several locations on air-dried cover slips using the single photon timing unit of the confocal microscope (Chapter 2). The time profiles were fitted using a double exponential function (Eq. 3.1). A slow decay component ($\tau_1 = 1.4 \pm 0.2$ ns) was found to be present in addition to a faster one ($\tau_2 = 0.36 \pm 0.04$ ns). The deviation from single exponential decay can be attributed to spatial heterogeneity: the surface-bound probe may exist in different local environments, in which the molecules have different nonradiative decay rates. The quantitative measurement of fluorescence intensities of dye monolayers is difficult owing to the very weak absorption. Therefore we use the average lifetime to quantify the fluorescence intensity of the dye on the cover slip.$^{20,21}$ The quantum yield is expected to be linearly dependent on the lifetime according to Eq. 3.5, where $k_{rad}$ is the radiative decay constant of the chromophore. The values of $\Phi_f$ and $\tau_{avg}$ for compound 1 in several solvents give $k_{rad} = 0.24 \pm 0.06$ ns$^{-1}$. We do not observe a systematic dependence of $k_{rad}$ on solvent polarity and we assume that it does not change significantly when the dye is bound to the surface. Then, based on $\tau_{avg} = 0.7$ ns for cover slips functionalized with rigidochromic probe 1, we can estimate the fluorescence quantum yield to be about 0.17. Thus, on the cover slip, the emission of the probe is considerably stronger than in solution, but weaker than reported for 2 in the PMMA matrix.$^{18}$ This is because the surface-bound probe molecules interact strongly with the surface, reducing the freedom of intramolecular rotation. 

\[
\Phi_f = k_{rad} \tau_{avg} \tag{3.5}
\]

Surface confinement is easily reduced by addition of a polar solvent. To obtain a suitable dynamic range for the rigidochromic effect, we immersed the slides in DMSO. This led to a clearly weaker emission, because the chromophore is solvated and free to undergo rotational motion in the excited state. The fluorescence lifetime of DMSO-wetted rigidochromic slips is reduced to $\tau_{avg} = 0.31$ ns, corresponding to a fluorescence quantum yield of 0.07. Thus, although the fluorescence is still stronger than in the solution, the nonradiative decay is faster than on the air-dried cover slips. The comparison of the air-dried and DMSO-wetted cover slips is shown in Fig. 3.5.

We generated contacts of spherical poly(methyl methacrylate) (PMMA) beads pressed onto the probe-functionalized cover slip, wetted with DMSO. A force transducer (rheometer) was used that exerts and records a well-defined force. Fluorescence was excited and detected through the cover slip, using an epifluorescence confocal microscope. The DMSO serves a dual purpose in these experiments: it not only reduces the fluorescence intensity before the contact is established but also provides a sufficient matching of the refractive indices of the glass and PMMA to avoid the effects of refraction of light at the interfaces. When the bead is pressed onto the cover slip, the confinement leads to a clear fluorescence increase owing to the rigidochromic effect: a roughly circular fluorescent spot appears and increases in size as the force is increased (Fig. 3.7). When the bead is retracted and placed again with the same load, the contact area is reproduced within 5%.
**Figure 3.5:** Fluorescence microscopy image of glass cover slip functionalized with 1 measured with the same excitation power: a) Dry cover slips; b) DMSO wetted cover slip; c) Corresponding fluorescence decay histograms.

**Figure 3.6:** Representative fluorescence intensity images with the focal plane positioned at the surface of a cover slip with covalently linked dye 1. A PMMA bead is pressed on the cover slip with the indicated loads, resulting in an increase in the contact area in which the fluorescence probe lights up. The size of the image is 200 µm × 200 µm. Exerted loads were a) 0.04 N, b) 0.20 N, c) 0.40 N, d) 0.55 N, e) 0.72 N and f) 1.01 N.
Figure 3.7: Radius of contact area observed in the fluorescence images (examples in Figure 3.6) as function of the normal force according to Equation 3.6.

To compare with the classical Hertz theory, which was devised exactly for this situation, we estimated the macroscopic contact area by fitting a circle to the fluorescent area. In Hertz theory, the radius $a$ of the contact area between a sphere (of a radius $R$) and a flat surface pressed against each other with force $F$ is described by:

$$a^3 = \frac{3R(1-\nu^2)}{4E} F,$$

(3.6)

where $E$ is the Young’s (shear elastic) modulus and $\nu$ is the Poisson ratio of the sphere material ($\nu = 0.37$ for PMMA). The modulus of glass can be ignored because it is much higher than that of PMMA. By relating the area to the load according to Eq. 3.6, we can derive the Young’s modulus of the PMMA sphere. The value found $E$(PMMA) = 2.0 GPa is a bit lower than the literature value for bulk PMMA, which is presumably due to a slight softening of the PMMA sphere by DMSO. Most importantly, we observe that the theory agrees remarkably well with the experiments, which strongly supports the validity of using immobilized compound 1 as a probe for mechanical contact.

We note that the fluorescent spot is not perfectly circular, and shows a significant amount of structure within it, implying that there are many small contacts at the microscopic scale, rather than one large homogeneous contact, as is commonly assumed in contact mechanics. At the same time, contact mechanics has been tested many times, and seems to be valid even when the microscopic structure of the contact is not taken into account. This presumably holds as long as the typical scale of the roughness is much smaller than both the radius of the bead and the contact area, which is the case for this experiment.
3.4 Conclusion

The present approach offers a unique method to directly observe the detailed structure of the contact area between two surfaces. We obtain diffraction-limited resolution in the imaging plane, but the resolution in the axial direction is determined by the thickness of the monolayer of dye molecules on the flat glass surface (roughness < 1 nm). Application of this new method towards understanding the relationship between friction and real contact area will be discussed in Chapter 8.

References

3. Young, H. D.; Freedman, R. A. Sears and Zemansky’s University Physics; Addison-Wesley, 2008.


