Three-dimensional visualization of contact networks in granular material
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For the visualization of the force networks in granular material (chapter 1), fluorescent probes are needed that are highly sensitive for small changes in their environment on the molecular level. In this chapter, an overview is given of such probes. First, an introduction is given on absorption and fluorescence and an overview of the possible working principles that can be applied to the ideal fluorescent probe. Both solvatochromic probes, which are sensitive to changes in polarity, and rigidochromic probes, which are sensitive to changes in viscosity, are discussed. Also some attention is given to the possibility to visualize forces employing intermolecular interactions; especially the transfer of energy between two differently functionalized particles.
2.1. Absorption and fluorescence

The basic principle of absorbance and fluorescence is depicted in figure 2.1. Absorbance (striped arrow up) occurs when photons with the appropriate energy meet a molecule in the ground state ($S_0$). The energy of a photon is described by Planck’s law:

$$E = h \nu = hc/\lambda$$  \hspace{1cm} (Eq. 2.1)

Herein, $h$ is Planck’s constant and $c$ is the speed of light. $\nu$ and $\lambda$ represent the frequency and the wavelength of the photon respectively. Absorption of a photon by a molecule will only occur when the energy of that photon matches exactly with the energy difference between the ground state of that molecule and one of its excited states ($S_n; n = 1, 2, \ldots$). The molecule absorbs the energy of the photon to become excited.

The lowest energy electronic transition is mostly observed in the range of UV or visible light and is associated with the $S_0 \rightarrow S_1$ transition. In this respect, an electron is excited from the occupied molecular orbital which is highest in energy (HOMO) to the lowest unoccupied molecular orbital (LUMO).

Both the ground state and the excited state contain closely-spaced but distinct energy levels, associated with different vibrational states, depicted as different bands in figure 2.1. Thus, a molecule in the $S_0$ state may reside in one of the vibrational levels ($\Psi_m; m = 0, 1, 2, \ldots$). At room temperature, the great majority of the molecules in a sample will reside in the vibrational ground state ($S_0, \Psi_0$). The Boltzmann distribution allows for a small fraction of the molecules in vibrationally excited states ($S_0, \Psi_1$).

Upon excitation to the $S_1$ state, the molecule may end up in any of the vibrational levels associated with the $S_1$ state, depending on the specific energy of the incoming photon. Excess vibrational excitation energy is rapidly lost due to collisions with the solvent, a process called relaxation (solid arrows down in figure 2.1). The excited molecules will again adopt a Boltzmann distribution, in which the large majority occupies the lowest vibrational level of the $S_1$ state. So, any process occurring from the excited state, such as fluorescence (dotted arrow down), occurs almost exclusively from the $S_1, \Psi_0$ state. Similarly, when the energy of the incoming photon is large enough to excite the molecule to a state higher in energy than the $S_1$ state, the molecule will rapidly lose its energy to its surroundings, and fluorescence can normally only be observed from the $S_1, \Psi_0$ state. This phenomenon is referred to as Kasha’s rule. According to the Franck-Condon principle, only the electrons reorient during excitation, while the nuclei remain in the optimal configuration of the ground state.
Figure 2.1. Electronic and vibrational energy levels involved in absorption and fluorescence.

(geometry A in figure 2.1). In the excited state, this optimal configuration of the nuclei is shifted somewhat (geometry B), and release of vibrational energy during relaxation is accompanied by reorientation of the nuclei of the molecule.\[^1\]

The excited molecule may release its energy via re-emitting a photon. This process of radiative decay is called fluorescence, and is represented as a dotted arrow in figure 2.1. Similar as for absorption, the nuclei of the molecule do not shift during fluorescence, resulting in the occupation of a higher vibrational level in the $S_0$ state. This additional energy is again lost to the surroundings by relaxation and reorientation of the nuclei.

Due to these energy losses during relaxation processes, the energy of the emitted photon is lower than the energy of the absorbed photon. This difference in energy is called the Stokes shift, and its value is an indication of the difference in geometries in the different states. Experimentally, the Stokes shift is obtained from the difference in the positions of absorption and emission maxima.

As not all molecules exhibit fluorescence, decay may also occur non-radiatively. Examples of non-radiative decay include intersystem crossing to a triplet state, internal conversion, photochemical reactions and electron transfer. Further elaboration of these processes is beyond the scope of this introduction.

2.2. Possible working principles for fluorescent probes

Fluorescence spectroscopy is ideally suited for the analysis of many dynamic systems, because it is neither invasive nor destructive and can thus be used \textit{in situ} without
disrupting the processes under investigation. Furthermore, the technique is extremely sensitive and selective. It works on a short time scale (~$10^{-9}$ s). The key requirement for a successful fluorescence experiment is the incorporation of a suitable fluorescent probe into the system. These probes must be sensitive for the desired change in the microenvironment, in order to analyze the behavior of the system. This means that at least one of the fluorescent parameters has to respond to a change, such as emission wavelength, fluorescence lifetime or quantum yield. When a probe meets these requirements and can be incorporated into the system (for example on a solid support), a lot of valuable information can be obtained from fluorescence spectroscopy.\[^{3}\]

The best choice of a fluorescent probe is the one that represents the investigated variation in the environment best. With respect to polymeric materials, variations that can be easily studied by fluorescence are mainly depending on the changes in polarity or rigidity of the system. Fluorescence probes suitable for these experiments can be divided into the following two subgroups.

The first group contains probes of which the fluorescence spectrum changes when the properties of the medium change. This group includes *solvatochromic* molecules, which exhibit variations in their emission spectra that depend on solvent polarity. For these molecules, the excited state typically has a significantly higher dipole moment than the ground state. Therefore, the excited state is more stabilized in polar solvents, giving rise to longer emission wavelengths.\[^{4}\]

Another interesting class of probes within this group is *rigidochromic*; that is, their emission is sensitive to medium viscosity. These molecules typically have a certain internal flexibility that allows an effective pathway for non-radiative decay from the excited state. When the viscosity of the medium rises, the internal movement is reduced by frictional resistance (internal rotations are less available) and thus radiative decay (fluorescence) is enhanced. This results in a higher quantum yield.\[^{4}\]

Thus, the term *rigidochromism* may also be used when there is no color change in response to the change in the medium viscosity, whereas *solvatochromism* always implies spectral changes in absorption or emission.

The second type of fluorescent probes that may be suitable in this project employs intermolecular interactions, in which quenching of the initially excited fluorophore plays an important role. During quenching, the fluorescence intensity of a substance is decreased by any of a variety of processes. Reactions in the excited state, energy transfer, complex-formation and collisions may all lead to quenching. As a consequence, quenching is often heavily dependent on pressure and temperature, and thus includes important candidates for the present project.

This group contains fluorophore pairs in which energy can be transferred from the donor in the excited state ($D^*$) to the ground state acceptor ($A$), producing the acceptor in the excited state ($A^*$). Thus, excitation of the donor can lead to emission of
the acceptor. This energy transfer can take place when the emission wavelengths of the donor overlap with the absorption wavelengths of the acceptor. This process of energy transfer could be either radiative or non-radiative. Energy transfer in the latter case can occur through long-range dipole-dipole interactions, and is dependent on the distance between the chromophores. This process is called Förster Resonance Energy Transfer (FRET).\textsuperscript{[5-7]} The acceptor is not necessarily emissive; in that case fluorescence is quenched when the donor and acceptor are in proximity (dark quenching). Non-radiative transfer may also happen through the Dexter Energy Transfer (DET), which occurs at short distances (15 – 20 Å). As the wavefunctions overlap, the excited electron may transfer from one molecule (the donor) to another (the acceptor), while a ground state electron simultaneously transfers the other way around. DET is rare while FRET is the more frequently occurring energy transfer mechanism.

### 2.3. Fluorescence depending on the environment

#### 2.3.1. Solvatochromism

As mentioned above, fluorescent probes can respond to their environment in several ways, one of which is that the color of the emitted light varies with the polarity of the solvent. This phenomenon is called solvatochromism.\textsuperscript{[4,8]}

According to the Franck-Condon principle, the nuclei of a molecule do not change position during the absorption of a photon. The following rearrangement of the nuclei into the vibrational ground state of the first excited state (relaxation) is the cause of the Stokes shift in fluorescence. The solvent molecules around the fluorophore also do not change position during photon absorption, and therefore the excited molecule keeps the solvation pattern of the ground state. Solvatochromism takes place when the fluorophore undergoes a significant change of dipole moment upon excitation. This is mostly due to intramolecular charge separation, which is caused by the fact that the HOMO and the LUMO are positioned at different sides of the molecule. Such an excited state is more stabilized in polar solvents, but some time is needed for the solvent molecules to orient themselves around the newly formed dipole and to reach the complete stabilization (figure 2.2). This can be viewed as the relaxation of the solvent. When this happens, longer emission wavelengths are observed in more polar solvents. This shift to longer wavelength is called a bathochromic shift (red shift), which is opposite to a hypsochromic shift towards shorter wavelengths (blue shift). The complete stabilization of the excited state in a polar solvent can only be reached when the solvent molecules have enough mobility. Thus, in highly viscous media, relaxation of the solvent takes more time than the lifetime of the excited state and therefore cannot be fully completed. Consequently, the emission wavelength is shorter than in a system with low viscosity.
Figure 2.2. Solvatochromic effect. Depicted are the fluorophore in green with dipole moment in the excited state (indicated with + and –) and the solvent molecules with dipole moment in blue.

The situation described above, when the excited state has a larger dipole moment than the ground state, is referred to as positive solvatochromism. However, some examples exist where the excited state has a small dipole moment, while the ground state has a larger dipole moment. This results in a hypsochromic shift as the solvent polarity rises. This phenomenon is called negative solvatochromism and is usually observed in absorption spectroscopy.\[8\]

The difference in dipole moments of the ground and excited states of a fluorophore is the origin of solvatochromism. The extent of solvatochromism, expressed in the change in Stokes shift, can be used to determine the difference in dipole moment of the ground state and the excited state. The dielectric continuum model, developed by Lippert and Mataga, can be used in this respect.\[9,10\] In this model, the interaction of the dipole moments in the ground (\(\vec{\mu}_g\)) and excited states (\(\vec{\mu}_e\)) of the solute with the dielectric continuum of the medium are regarded. The equations that describe changes in the absorption (\(E_{abs}\)) and emission energies (\(E_{em}\)), induced by the polarity of the solvent, relative to their respective vacuum values, are given in equations 2.2 and 2.3.\[11\]

\[
E_{abs} = E_{abs}^0 - \frac{1}{\rho^3} \left[ \vec{\mu}_g (\vec{\mu}_e - \vec{\mu}_g) f(\varepsilon) - f(n^2) + \frac{1}{2} (\mu^2_e - \mu^2_g) f(n^2) \right] \quad (Eq. 2.2)
\]

\[
E_{em} = E_{em}^0 - \frac{1}{\rho^3} \left[ \vec{\mu}_e (\vec{\mu}_e - \vec{\mu}_g) f(\varepsilon) - f(n^2) + \frac{1}{2} (\mu^2_e - \mu^2_g) f(n^2) \right] \quad (Eq. 2.3)
\]

In these equations, \(\rho\) is the radius of the molecular cavity in which the fluorophore resides. This cavity represents the boundary between the probe and the dielectric continuum, and is commonly approximated as spherical (Onsager theory).\[12\] The
molecular volume $\rho^3$ is generally derived from molecular computation or estimated empirically. The latter can be done by determining or assuming the density of the probe material and deriving the molecular volume $V$ therefrom. $\rho$ is then considered to be the radius of the corresponding sphere surrounding the molecule, via $V = 4\pi\rho^3/3$.

The solvent polarity functions of the dielectric constant ($f(\varepsilon)$) and the refractive index ($f(n^2)$), the so-called Onsager polarity functions, are described by equations 2.4 and 2.5.\[^{[13]}\]

$$f(\varepsilon) = \frac{2(\varepsilon-1)}{2\varepsilon+1} \quad \text{(Eq. 2.4)}$$

$$f(n^2) = \frac{2(n^2-1)}{2n^2+1} \quad \text{(Eq. 2.5)}$$

$f(n^2)$ is related to the reorientation of the electrons in the solvent molecules as reaction to the newly formed exited state dipole. This reorientation happens instantaneously, together with the absorption of the photon. Because of this short timescale, $f(n^2)$ is called the high frequency polarizability function. $f(\varepsilon)$, on the other hand, relates to the reorientation of the solvent molecules themselves around the new dipole. The timescale that is associated with this is much longer, and hence $f(\varepsilon)$ is called the low frequency polarizability function.\[^{[4]}\] Together, $f(n^2)$ and $f(\varepsilon)$ describe the full dielectric response of the solvent.

The Stokes shift, i.e. the difference between emission and absorption energies, is related to the dipole moments $\mathbf{\mu}_g$ and $\mathbf{\mu}_e$ and the Onsager polarity functions as described by equation 2.6.

$$E_{abs} - E_{em} = E_{abs}^0 - E_{em}^0 + \frac{1}{\rho^3} \left[ (\mathbf{\mu}_e - \mathbf{\mu}_g)^2 (f(\varepsilon) - f(n^2)) \right] \quad \text{(Eq. 2.6)}$$

The difference between the dielectric constant polarity function and the refractive index one, is the orientation polarizability $\Delta f$; $\Delta f = f(\varepsilon) - f(n^2)$. In solvents with hardly any dipole moment, $\varepsilon$ is more or less equal to $n^2$, and $\Delta f$ approaches zero.

For a given fluorophore, the vacuum Stokes shift $E_{abs}^0 - E_{em}^0$ is a constant. In addition, the Stokes shift $E_{abs} - E_{em}$ can be expressed in wave numbers ($\tilde{\nu}$) as $h\cdot c\cdot(\tilde{\nu}_{abs} - \tilde{\nu}_{em})$, which simplifies equation 2.6 to:

$$\tilde{\nu}_{abs} - \tilde{\nu}_{em} = \frac{\Delta f}{h c \rho^3} (\mathbf{\mu}_e - \mathbf{\mu}_g)^2 + \text{constant} \quad \text{(Eq. 2.7)}$$

Herein, $h$ is Planck’s constant and $c$ is the speed of light.

In view of equation 2.7, the difference in dipole moment between the ground state and the excited state $(\mathbf{\mu}_e - \mathbf{\mu}_g)$ can easily be obtained by plotting the measured
Stokes shift ($\tilde{\nu}_{abs} - \tilde{\nu}_{em}$) versus the $\Delta f$ of a series of solvents, which should result in a linear relationship. The slope of the resulting line is determined by $(\tilde{\mu}_e - \tilde{\mu}_g)^2 / \rho^3$.

The above outlined dielectric continuum model by Lippert and Mataga has two major drawbacks. First of all, it does not account for specific solvent-solute interactions, such as hydrogen bonding or electrostatic interactions. These depend on the specific structure of the solvent in suit, and occur irrespective of their dielectric constants and refractive indices.

The second limitation originates from the approximation of a spherical molecular cavity, especially when elongated probes are used. Both these effects may lead to deviations from the linear relationship between the Stokes shift and $\Delta f$, and to difficulties in interpreting the comparative data.

A characteristic architecture of solvatochromic probes is the so-called push-pull system: an electron donating group and an electron withdrawing group connected via a conjugated linker. When an electron is excited, it is removed from the HOMO at the donor site and relocated in the LUMO at the acceptor site. This redistribution of the electron density results in an increased positive charge at the donor site of the molecule and a more negative charge at the acceptor site. Thus, an excited state dipole is formed, which is subject to the phenomenon described above.

2.3.2. Rigidochromism

In the case of rigidochromism, the fluorescence properties are also dependent on the viscosity of the system. The difference between the two phenomena is that for solvatochromism the wavelength of emission changes, while for rigidochromism the fluorescence quantum yield varies strongly. However, a small blue shift is also often observed for rigidochromic probes in more viscous media, because they typically have some push-pull character and are thus solvatochromic.

Probes suitable for rigidochromism experiments typically have a low fluorescence quantum yield at low viscosity, because non-radiative deactivation pathways are predominant. Mostly, this is because of the presence of an internal motion, such as the rotation around a bond. When the viscosity of the medium rises, these motions become more hampered, and thus the non-radiative decay processes become less available. Therefore, more molecules will deactivate via photon emission, resulting in an increase in quantum yield.

As explained above, the excited states of positive solvatochromic systems are stabilized by reorientation of the solvent. In rigid media, this reorientation is significantly slowed down or even completely halted. Therefore, the excited state cannot be stabilized completely, which result in a blue shift of the emission
wavelength.\cite{14} Thus, most solvatochromic probes also exhibit rigidochromism, in the sense that the solvent-induced spectral shift depends on viscosity.

Fluorescent solvatochromic and rigidochromic probes are sensitive to polarity and mobility, which forms the basis for their application in this project. At the points of pressure, the viscosity will be higher and therefore a change in fluorescence properties will be observed. Also, the solvent will be pushed away at the points of pressure and thus the polarity will be different locally. In the next section, possible probes for this project are discussed.

### 2.3.3. Potentially suitable fluorescent probes

For application in the present project, probe molecules must meet a number of requirements. Apart from a large response to changes of the medium, application of dyes in confocal microscopy requires strong absorption and emission in the visible range of the spectrum and good photostability. A large Stokes shift is advantageous for efficient detection of the emitted photons. Furthermore, the compounds should be synthetically accessible, and there should be a possibility to attach a linker for fixing the fluorophore to surfaces. In the next sections, some examples of solvatochromic and rigidochromic systems from the literature will be discussed.

#### 2.3.3.1. Solvatochromic probes

The vast majority of solvatochromic systems in the literature contain an electron-donating moiety connected to an electron-accepting moiety via a $\pi$-conjugated bridge, a so-called “push-pull” system. The most sensitive solvatochromic systems known, however, have a donor and an acceptor joined by a saturated bridge such as in 1 (figure 2.3).\cite{15} The electronically insulating bridge allows for an efficient charge separation in the excited state, affording a high dipole moment. Because of this large solvatochromic behavior, 1 has been coined FluoroProbe. This molecule has been used for the investigation of film formation in latex. For this purpose, the dye is embedded in the latex matrix via copolymerization. During evaporation of the apolar co-solvents from the polar latex formulation, a red shift was observed in the emission maximum.\cite{16,17} Emission ranges from 415 nm in hexane to 694 nm in acetonitrile, corresponding to a solvatochromic shift of 279 nm. Quantum yields were in general reasonably high, and ranged from $\Phi_F = 0.11$ to $\Phi_F = 0.60$, depending on the solvent. However, the absorption maxima of 1 is below 375 nm ($\lambda_{\text{max}} \sim 320$ nm). Adding an extra constraint onto the piperidine ring, resulting in bicyclic compound 2 (figure 2.3), shifted the absorption to the 350-420 nm range.\cite{18} Quantum yields were slightly higher ($0.25 \leq \Phi_F \leq 0.77$) and the solvatochromic behavior was maintained. Although...
the solvatochromic sensitivity is unrivalled, the short wavelength absorption and modest photostability render the FluoroProbes unsuitable for our purpose.

In literature, a plethora of solvatochromic push-pull compounds can be found. Because the systems are fully π-conjugated, the donor and acceptor units are not fully localized on the ends of the molecule, so the increase in dipole moment upon excitation is smaller. Compounds 3 and 4 gave multiple solvent-independent absorption bands with three broad bands centered around 306 nm, 386 nm, 508 nm for 3 and 306 nm, 400 nm and 550 nm for 4 (figure 2.4).\textsuperscript{[19]} B3LYP/6-31g* calculations showed that the HOMO was mainly located on the triphenylamine part of the molecule (donor) and the LUMO on the benzothiadiazole part (acceptor). Thus upon excitation, an intramolecular charge transfer takes place, resulting in the formation of an excited state dipole. In the donor-acceptor-donor system (D–A–D) 4, this implies that in the excited state the symmetry is broken; that is, only one of the donor groups participates in the charge transfer. The excited state can be roughly described as D^+–A^−–D. The Stokes shifts are relatively large, 172 nm for 3 and 149 nm for 4, both measured in acetone and were lower in apolar solvents (86 nm and 79 nm respectively in cyclohexane). The asymmetric compound 3 exhibited larger solvatochromic shift of 80 nm, which is consistent with the formation of a larger dipole moment in the excited state. The shift for 4 was only 63 nm. Quantum yields were highest in apolar solvents; $\Phi_\text{F} = 0.13$ for 3 and $\Phi_\text{F} = 0.038$ for 4 in cyclohexane.

A series of molecules was prepared by Spitler \textit{et al.},\textsuperscript{[20]} in which tetraethynylbenzene was functionalized with two donor and two acceptor moieties. Exemplary is compound 5 (figure 2.5), in which 2-pyridyl acceptors are combined with tertiary amine donors. Absorption of compound 5 ranged from 402 nm in toluene to 435 nm in dichloromethane. Also the emission maxima were red-shifted in dichloromethane with
Environment sensitive fluorescent probes

**Figure 2.5.** \(\pi\)-Conjugated frameworks synthesized by Spitler et al.\(^{[20,21]}\)

respect to toluene, but the effect was more pronounced (\(\lambda_{em} = 482\) nm in toluene, 547 nm in dichloromethane). The Stokes shifts for compound 5 were 80 nm in toluene and 112 nm in dichloromethane. Quantum yields were \(\Phi_F = 0.31\) and \(\Phi_F = 0.63\) in dichloromethane and toluene respectively. There are some marked differences in optical properties for the different isomers of 5. For example, the biggest Stokes shift is obtained when the nitrogen atom of the pyridine ring is placed at the \(para\) position with respect to the ethynyl group. The 4-pyridyl isomer of 5 exhibits the largest transition state dipole moment (\(\mu = 13.10\) D). However, this molecule has a lower quantum yield (\(\Phi_F = 0.20\)) than compound 5.

Compounds of type 6,\(^{[21]}\) with two diethynyl bridges, dibutylamine donors and trifluoromethyl acceptors, also showed strong solvatochromic behavior. The absorption maxima for compound 6 in dichloromethane is 450 nm and a reasonable quantum yield was obtained (\(\Phi_F = 0.35\)). Emission was red shifted in benzene (\(\varepsilon = 2.3\)) with respect to dichloromethane (\(\varepsilon = 9.1\)), namely from 500 nm to 560 nm.

**Figure 2.6.** Compound 7 designed by Strehmel et al.\(^{[22,23]}\)

D–A–D compound 7 (figure 2.6), designed by Strehmel et al.,\(^{[22,23]}\) exhibits a large bathochromic shift in its emission spectrum with increasing solvent polarity. Upon excitation around 380 nm, emission was observed at 445 nm in toluene and 518 nm in acetonitrile. Quantum yields were very high, especially in toluene (\(\Phi_F = 0.99\)), but
diminished slightly in more polar solvents (acetonitrile: $\Phi_F = 0.69$). For our purpose, the excitation wavelength is a bit too short.

Compounds 8a, 8b and 9 have again a donor and an acceptor moiety linked with a $\pi$-system (figure 2.7). 8a has been investigated by many researchers, with slightly different results.$^{[24-27]}$

The absorption maximum of 8a in cyclohexane was 419 nm,$^{[25]}$ and all articles agreed on a large positive solvatochromic effect in the fluorescence spectra. Only the exact positions of the emission maxima varied. Elongation of the $\pi$-bridge in 8b resulted in a significant red shift in absorption and emission wavelengths. The absorption maximum was at 447 nm in THF and a solvatochromic effect was also observed in the emission spectra ($\lambda_{em} = 522$ nm in hexane; $\lambda_{em} = 641$ nm in ethyl acetate). Quantum yields of 8a and 8b were relatively low, especially in polar solvents ($\Phi_F (8a) = \Phi_F (8b) = 0.01$ in THF; $\Phi_F (8a) = 0.26$ in cyclohexane; $\Phi_F (8b) = 0.29$ in cyclohexane). Compound 9 absorbed at 459 nm in THF and emission ranged from 513 nm in hexane to 615 nm in ethylacetate. Quantum yields were low ($\Phi_F = 0.10$ in carbon tetrachloride; 0.01 in ethylacetate ).

The substituent pattern on the amine nitrogen (10a – 10c) was further investigated by Jager et al.$^{[26]}$ One or two methacryloyloxyethyl or isobutyryloxyethyl units are connected to nitrogen (figure 2.7), and this did not show any effect on the fluorescent properties. All compounds exhibited a bathochromic shift in the emission maxima when increasing the solvent polarity ($\lambda_{em} = 493 – 499$ nm in cyclohexane; $\lambda_{em} = 776 – 798$ nm in acetonitrile). Quantum yields were highest in diethylether ($\Phi_F = 0.26 – 0.27$).

The analogous compounds 11a – 11b also showed a positive solvatochromic effect, with slightly red shifted spectra for the longer bridge (figure 2.7)$^{[27]}$. For $n = 1$, emissions ranged from 439 nm in cyclohexane to 522 nm in acetonitrile. However, quantum yields were quite low in all tested solvents ($\Phi_F \leq 0.06$).

![Figure 2.7. Ethylene bridged push-pull systems 8 – 11.](25-27)
The donor and acceptor functionalized dithiophene 12 shows solvatochromic behavior\(^{[28]}\) (figure 2.8). Emissions ranged from 573 nm in cyclohexane to 678 nm in DMSO. Absorption in dichloromethane occurred at 600 nm (emission at 655 nm), which would be quite favorable for fluorescence microscopy applications, but unfortunately, no quantum yields were reported.

![Chemical structures of compounds 12, 13, and 14](image)

**Figure 2.8.** Dicyanoethylene containing solvatochromic probes 12, \(^{[28]}\) 13\(^{[29]}\) and 14\(^{[31]}\).

Compound 13 (figure 2.8), together with some derivatives, were synthesized by Bosch et al. and showed modest solvatochromism\(^{[29]}\). Absorbance maxima are at 488 nm in cyclohexane and at 522 nm in acetone. The fluorescence maximum of the compound is red-shifted on increasing solvent polarity (560 nm in diethyl ether, 584 nm in methanol). Stokes shifts are between 51 and 60 nm. The quantum yield of compound 13 is very low in all solvents tested (\(\Phi_F < 10^{-3}\)). This compound and analogues also show rigidochromic behavior, which is discussed later (see figure 2.13).

Intermediate cases also exist, in which both states have substantial charge transfer character. Thus, solvatochromic shifts occur in absorption as well as in emission. Merocyanine compounds 14 provide examples of such solvatochromic probes (figure 2.8).\(^{[30,31]}\) Both absorption and emission were red shifted with an increase in length of the conjugated bridge. The solvatochromic shift is the largest for the longest analogue; emission occurred at 625 nm in hexane and at 674 nm in DMSO, while the absorption maximum changed from 532 nm to 632 nm. Quantum yields were in general very low (0.0004 < \(\Phi_F < 0.15\)). When the quantum yields were measured in a polymer film or at 77 K (both highly viscous media), the quantum yield increased dramatically up to \(\Phi_F = 0.99\). Therefore, these probes are also rigidochromic.

In conjugated D–π–A systems, some charge separation in the ground state cannot be avoided. In the cases discussed so far, however, the ground state has a small dipole moment and can be described as D–A, while the excited has a much larger dipole, and can be approximately described with the resonance structure D\(^+\)–A\(^-\). Negatively solvatochromic systems on the other hand, have opposite charge distributions; that is,
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\[ D^* - A^* \] is the ground state and \( D - A \) the excited state. An example is compound 15 (figure 2.9), which exhibits a large negative solvatochromism in its absorption spectra (613 nm in chloroform; 443 nm in water).\(^{[32]}\) This effect is much smaller in its fluorescence spectra (631 nm in chloroform; 582 nm in water), which means that the Stokes shifts increase dramatically (18 nm vs. 139 nm). Quantum yields were very low; \( \Phi_F = 0.0020 \) in methanol, \( \Phi_F = 0.0015 \) in water and even lower in apolar solvents.

![Resonance structure of 15](image)

**Figure 2.9.** Resonance structure of 15.\(^{[32]}\)

Perylenes 16 and 17 and naphthalene analogue compound 18, depicted in figure 2.10, were developed in our laboratory by Zoon and Brouwer, and show substantial solvatochromism.\(^{[33]}\) Their absorption and emission spectra were recorded in a series of solvents. Compound 17 has its absorption maximum between 521 nm (cyclohexane) and 556 nm (methanol, \( \varepsilon = 32.6 \)), and its emission maximum between 637 nm and 746 nm in the same solvents. For naphthalene analogue 18, the absorption maxima are between 420 nm and 457 nm and the emission maxima between 465 nm and 543 nm. The quantum yields of both compounds decrease with increasing solvent polarity; \( \Phi_F (17) = 0.48 \) (cyclohexane) and \( \Phi_F (17) = 0.22 \) (methanol); \( \Phi_F (18) = 0.80 \) (hexane); \( \Phi_F (18) = 0.03 \) (methanol).

![Perylene probes 16, 17 and naphthalene analogue 18](image)

**Figure 2.10.** Perylene probes 16, 17 and naphthalene analogue 18.\(^{[33]}\)
For **16**, both the absorption and emission maxima were highly dependent on the solvent polarity, but surprisingly the Stokes shift was not. Absorptions ranged from 552 nm to 645 nm and emissions from 645 nm to 741 nm. The quantum yield showed no clear dependence on the solvent polarity, and ranged from $\Phi_F = 0.30$ to $\Phi_F = 0.57$.

For the research described in this thesis, we considered compound **17** a suitable solvatochromic probe because of its high brightness in the visible spectral range, and proven photostability. The synthesis is well established, and modifications that allow anchoring to a surface are relatively straightforward, as described in *Chapter 4*.

### 2.3.3.2. Rigidochromic probes

As said before, rigidochromic probes are not applied in solvents with different polarities, but with different viscosities. Rhenium complexes are often encountered as rigidochromic probes. Wrighton and Morse$^{[34]}$ analyzed several $[\text{ReCl(CO)}_3(\text{phen})]$ analogs, depicted in figure 2.11. The dependence of the absorption and emission maxima on the ligand implied that these transitions had a metal to ligand charge transfer (MLCT) character. Absorptions for all complexes are between 377 nm and 397 nm, except for **20** having an absorption maximum at 438 nm (all in dichloromethane).

No emission was observed for **20, 22** and **26** at room temperature in dichloromethane. For the other complexes, emissions were observed between 577 nm and 588 nm, but with very low quantum yields ($\Phi_F < 0.04$). However, quantum yields were considerably enhanced when the measurements were performed in a highly viscous glass at 77K, even for the complexes that did not emit at room temperature. Emission wavelengths of all compounds ranged from 528 nm to 547 nm, except for **20** which emitted at 686 nm. Compound **21** has been used for monitoring the progress of acrylate and epoxy

![Figure 2.11. Phenantroline rhenium and tungsten complexes.](14,34,37,38)
Chapter 2

polymerizations.[35] As the polymerization proceeds, the quantum yield goes up. The lack of luminescence at room temperature is caused by rapid CO dissociation in the excited state. This process is suppressed in highly viscous media, enabling radiative decay.

Many derivatives of 19 have been prepared and analyzed on rigidochromism. For example, Pu et al.[36] functionalized the phenanthroline ligand with one or more dendrimers. However, excitation wavelengths in THF are slightly too small for our experiments (360 nm), but this might shift a little to the red in more polar media. Spectra recorded in more rigid thin films were compared with solution spectra, showing a blue shift in emission wavelength of 20 – 30 nm. Quantum yields increased from $\Phi_F = 0.005 – 0.03$ to $\Phi_F = 0.07 – 0.19$.

[ReCl(CO)$_3$(bpy)] (27) is an analog of 19 (figure 2.11), of which the rigidochromic behavior has been tested in gelation processes.[37,38] The probe was dissolved in Si(OEt)$_4$. This slowly hydrolyzes into a silicon dioxide gel and ethanol, which evaporates from the gel. As the rigidity of the gel increases, the emission maximum of 27 shifts from 605 nm to 530 nm. This was accompanied by an increase in quantum yield, as could be seen from the emission spectra. However, the authors did not comment on this phenomenon.

Rawlins et al.[14] investigated the spectroscopic aspects of W(CO)$_4$(4-Me-phen) (28), depicted in figure 2.11. The absorption spectrum shows an intense band around 500 nm, which is attributed to MLCT. Two emission bands (585 nm and 782 nm) were observed in benzene at room temperature (excitation at 400 nm). These bands underwent a blue shift in ethylphenylacetate at 80K (527 nm and 677 nm). A similar blue shift is observed when 28 is incorporated into polymerizing MMA. Again, a rise of the quantum yield is observed during this process, but was not discussed by the authors.

Rigidochromic behavior of Cu$_4$I$_4$(pyridine)$_4$ cluster 29 (figure 2.12) was observed, when pressure was applied to a solution of it.[39] The emission maximum of 29 in benzene

![Figure 2.12. Copper cluster Cu$_4$I$_4$(pyridine)$_4$ 29.][39]
was 695 nm at ambient pressure, which did not change when the pressure was enhanced to 44 MPa. At this pressure, benzene is still a liquid, and only little rise in rigidity was achieved. Further raising the pressure resulted in solidification of benzene at room temperature; at 160 MPa, the emission wavelength had shifted to 575 nm. Similar behavior was observed in other solvents.

Analogs of the solvatochromic probe 13 (see figure 2.8) with low quantum yields have been tested for their rigidochromatic behavior. Compounds 30 – 32 (figure 2.13) were incorporated in polymerizing hexanedioldiacrylate and hexanedioldimethacrylate. When the polymerization proceeds, the emission intensities increased. Compound 31 absorbed at 428 nm in cyclohexane and emitted at 490 nm. These wavelengths exhibited a slight bathochromic shift when going to more polar solvents, so a small solvatochromic behavior was also observed. Compound 32 absorbed at 386 nm and emitted at 440 nm in cyclohexane. Compound 30 was already described by Loutfy in 1980, and showed strong rigidochromatic behavior; quantum yields increased from $\Phi_F = 0.0014$ at room temperature to $\Phi_F = 0.80$ at 77 K in 2-methyl-THF. Also a small bathochromic shift was observed in the emission maximum when going to more polar solvents.

![Figure 2.13. Dimethylaminoaryl rigidochromic probes.](image)

In the group of Moerner, strong rigidochromatic probes were designed, which also contain an amine donor moiety and an acceptor moiety with multiple cyano groups. This class of 2-dicyanomethylene-3-cyano-2,5-dihydrofuran (DCDHF) probes comprises numerous compounds. Exemplary are 33 – 35, as depicted in figure 2.14, which have a high quantum yield within a polymer matrix ($\Phi_F (33) = 0.92$; $\Phi_F (34) = 0.98$). Especially the phenyl-bridged 33 shows great rigidochromic behavior, with a quantum yield of only $\Phi_F = 0.044$ in toluene, while the naphthalene-bridged analogue 34 emits also intense fluorescence in low-viscous toluene ($\Phi_F = 0.85$). Strikingly, 34 hardly fluoresced in ethanol ($\Phi_F = 0.017$), which has approximately the same viscosity as toluene. These DCDHF probes, especially 33, are highly suitable as fluorescent labels, as they are able to emit millions of photons before photobleaching.
The favorable photophysical properties of the DCDHF probes led us to design a modified version of 33, which allows their covalent attachment to surfaces of glass or polymers, as described in chapters 4 and 5.

2.4. Intermolecular interactions

2.4.1. Theory

Förster resonance energy transfer (FRET)\(^{46-49}\) is a process in which the energy of an excited donor molecule is transferred to an acceptor. This transfer occurs non-radiatively, and may or may not result in fluorescence from the acceptor molecule. This phenomenon is named after the German scientist Theodor Förster, who described it for the first time in the 1940s\(^{5-7}\), but is often referred to as fluorescence resonance energy transfer. However, this inaccuracy may cause some confusion, since the energy is not transferred via fluorescence.

A schematic representation of a FRET process is given in figure 2.15. When the donor molecule D is excited (D\(^*\)), its energy is transferred to a nearby acceptor molecule A in the ground state. This causes the acceptor to be excited (A\(^*\)) and the donor to return to the ground state. This energy transfer happens through dipole-dipole interactions, and not by the emission and absorption of a photon. The excited acceptor can decay via fluorescence or via non-radiative processes (dark quenching). The rate of a FRET process \(k_f(r)\) is dependent on the spectral overlap \(J(\lambda)\) between the emission of the donor and the absorption of the acceptor, and on the sixth power of the distance \(r\) between the donor and acceptor molecules (equation 2.8).\(^{46}\)

\[
k_f(r) = \left(\frac{9000 (ln10)}{128\pi^5N_A}\right)\frac{J(\lambda)\Phi_Dk^2}{\tau_Dr^6n^4}
\]

(Eq. 2.8)

The part between brackets is constant, with \(N_A\) is Avogadro’s number. \(\Phi_D\) and \(\tau_D\) are the quantum yield and the lifetime of the donor, determined in absence of the
acceptor. \( n \) is the refractive index of the medium and \( \kappa^2 \) is an orientation factor. It describes the relative orientation of the dipole moments of both the donor and the acceptor in space. In isotropic solutions, its value averages out to 2/3. The formula for the spectral overlap (\( J(\lambda) \)) is given in equation 2.9. In equation 2.9, \( F_D(\lambda) \) is the fluorescence spectrum of the donor normalized to unity and \( \varepsilon_A \) is the molar absorption coefficient of the acceptor.

For any donor-acceptor pair a Förster distance \( R_0 \) exists, for which the efficiency of the energy transfer is 50%; half of the excited donor molecules transfers its energy to the acceptor and the other half decays via regular routes. FRET processes usually occur at donor-acceptor distances from 10 Å to 100 Å. \( R_0 \) is related to the FRET rate \( k_T(r) \) via equation 2.10.

\[
J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda
\]  
(Eq. 2.9)

\[
k_T(r) = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6
\]  
(Eq. 2.10)

![Förster resonance energy transfer (FRET) process](image)

**Figure 2.15.** Förster resonance energy transfer (FRET) process (solid spectra represents the absorbance (Abs) and emission (Em) of the donor; dotted spectra the absorbance (Abs) and emission (Em) of the acceptor; gray area is the spectral overlap \( J(\lambda) \)).

Closely related to FRET is Dexter Energy Transfer (DET, see figure 2.16), in which excited state energy is transferred from a donor to an acceptor by exchange of two electrons. The electron which – after excitation – resides in the LUMO of the donor, hops over to the LUMO of the acceptor, while simultaneously an electron in the HOMO of the acceptor hops over to the HOMO of the donor.
Electron exchange can only occur in case of overlap between the wavefunctions of the donor and the acceptor, i.e. overlap of the respective electron clouds. Thus, DET will only occur at very short distances, typically below 10 Å. Such distances approach the sum of the Van der Waals radii of the donor and acceptor (L), and therefore DET is sometimes referred to as collision energy transfer. In contrast to FRET, DET does not depend on the transition dipole moments of the chromophores. The rate at which DET occurs is thus related to the distance \( r \) between donor and acceptor and the spectral overlap \( J \) as defined in equation 2.9. The rate of DET \( (k_T) \) can be calculated according to equation 2.11:

\[
 k_T(r) = J(\lambda)(-2r/L)^{2} 
\]

(Eq. 2.11)

The phenomena of FRET and DET may be applied in this project; 50% of the particles may be functionalized with donor probes and 50% with acceptor probes. At the points of contact between particles, donor and acceptor molecules will be in close proximity and will thus emit light of a longer wavelength than when only the donor is present. A drawback of this approach is that donor-donor and acceptor-acceptor combinations will also be obtained and therefore not all contacts can be directly observed.

### 2.4.2. Possible fluorescent FRET pairs

One of the standard donor-acceptor pairs is fluorescein (36, donor) and rhodamine (37, acceptor, of which the tetramethyl derivative is depicted in figure 2.17).\(^{[50]}\)

Fluorescein absorbs at 488 nm in DMSO and emits around 520 nm. When in proximity with rhodamine, its energy is transferred and rhodamine emission takes place above 600 nm. This donor-acceptor pair has a Förster distance of 55 Å.

---

**Figure 2.16.** Förster Resonance Energy Transfer vs. Dexter Energy Transfer.

---

**Figure 2.17.** Example of a donor-acceptor pair used in FRET experiments.
Fluorescein is also often encountered as acceptor, which is exemplified by the IAEDANS-fluorescein pair. IAEDANS is a widely used donor, but for our purposes has a too low absorption maximum of 336 nm.

Invitrogen developed and patented a family of fluorescent dyes, named Alexa Fluor. These dyes are synthesized through sulfonation of commonly encountered coumarin, rhodamine, xanthene and cyanine dyes. The presence of the sulfanyl group makes them negatively charged and thus hydrophilic. In general, these dyes are more photostable and brighter than their parent systems, but more expensive. Many dyes are available in this family, which together span the whole color spectrum. The absorption maximum of the dye is given in the number of the dye; Alexa Fluor 488 absorbs at 488 nm. These can be used as FRET donor or FRET acceptor in different combinations, where the $R_0$ ranges from 50 Å for Alexa Fluor 350 (D) and Alexa Fluor 488 (A) to 85 Å for Alexa Fluor 594 (D) and Alexa Fluor 647 (A).

The Alexa Fluor dyes can also be combined with non-emissive acceptors, such as Dabsyl (dimethylaminoazosulphonic acid) and derivatives of rhodamine.

Thomson et al. have imaged cellulose interfaces with the help of FRET and fluorescence microscopy. Donor 38 was used ($\lambda_{\text{exc}} = 440 \text{ nm}$, $\lambda_{\text{em}} = 480 \text{ nm}$) together with 39 as acceptor (figure 2.18), which absorbs at 500 nm and emits at 525 nm.
2.5. Conclusion

In this chapter, a number of possible approaches are outlined towards the visualization of force networks in granular systems and changes therein upon application of force, using fluorescence spectroscopy. Potentially suitable probes include polarity sensitive solvatochromic probes, viscosity sensitive rigidochromic probes and FRET and DET pairs, which are sensitive towards the proximity of neighboring probes. The photophysical properties of promising candidates to be used in this project exhibit dependence on e.g. the polarity or the viscosity of their surroundings renders them promising for the visualization of the force networks in granular materials.

The change in polarity in the contact points between two granules, especially when force is applied, is investigated with solvatochromic probe 17. Chapter 6 is devoted to this subject. Probe 17 has a large solvatochromic shift of over 100 nm, and is thus especially suited. This probe is designed in our laboratory and is synthetically well accessible. The viscosity change in granular material is explored with rigidochromic probe 33, which exhibits excellent rigidochromic behavior. The work in this respect is described in chapter 7.

Interestingly, the emission of rigidochromic probe 33 ($\lambda_{em} = 505 - 546$ nm) is more or less aligned with the absorption of solvatochromic probe 17 ($\lambda_{abs} = 521$ nm – 556 nm), which would make 33 and 17 a promising FRET pair. Although not investigated in this project, granular systems partly containing granules functionalized with 17 and partly granules functionalized with 33 may be suitable to visualize the force networks, as indicated in section 2.4.1.

Before the results obtained with the functionalized surfaces are described in chapters 6 – 8, this thesis sets out in chapters 3 – 5 to describe the synthesis of the probes and functionalized surfaces, and the methods used in the investigation of the granular materials.

2.6. References

5. Förster, T. “Energiewanderung und fluoreszenz” Naturwissenschaften 1946, 6, 166–175.
7. Förster, T. “Experimental and theoretical investigation of intermolecular transfer of electron activation energy” Naturforschung 1949, 4A, 321-327.


