Russian Chemical Reviews **80** (12) 1179 – 1190 (2011) © 2011 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2011v080n12ABEH004246

Molecular rotors as luminescence sensors of local viscosity and viscous flow in solutions and organized systems

B M Uzhinov, V L Ivanov, M Ya Melnikov

Contents

I.	Introduction	1179
II.	Structure and functioning of molecular rotors	1179
III.	Application of molecular rotors in chemistry and biology	1185
IV.	Conclusion	1188

Abstract. The results of studies of various classes of molecular rotors are generalized. The compounds in question are fluorophores whose molecules can pass to a twisted state upon charge transfer on excitation, this state being characterized by non-radiative deactivation. The structures of the main classes of molecular rotors and principles of their functioning are analyzed. In the discussion of applications of molecular rotors, the attention is focused on their use as sensors of polymerization, aggregation and conformational changes in protein molecules, as local viscosity probes in biological systems and flow sensors and also for viscosity measurements in liquids. The bibliography includes 104 references.

I. Introduction

The term 'molecular rotors' refers to compounds whose molecules are composed of two or more moieties able to rotate relative to one another. In most cases, one moiety with a greater moment of inertia is considered as being fixed and is called the stator and the other moiety having a smaller moment of inertia is called the rotator or rotor.[1] Molecular rotors are classified according to various criteria. In particular, molecular rotors in solutions, vapours or solids and on a surface are distinguished. The intramolecular rotation is induced by an external action (light, electric field, liquid flow, and so on).

Among the diversity of molecular rotors, there is a group of compounds whose molecules can undergo twisted intramolecular charge transfer (TICT) upon electronic excitation. This means that the charge transfer is followed by rotation of one moiety of the molecule relative to the other. The fluorescence quantum yield of these compounds depends considerably on the viscosity of the medium. This dependence is used to measure local viscosity changes in chemical and biological systems, in particular, in the cells of living organisms.

The review literature on this topic is rather scarce. Among the recent works, quite a lot of information is provided in the paper by Haidekker and Theodorakis,[2] who considered the functioning mechanisms of molecular rotors and their possible applications as fluorescence biosensors of local viscosity and viscous flow. This review is devoted to the molecular rotors used as luminescence sensors of local viscosity, viscous flow in solutions, organized systems and biological objects and as sensors of dynamic processes that accompany polymerization and of conformational changes of protein molecules.

II. Structure and functioning of molecular rotors

Diffusion is of prime importance in chemical and biological processes. A key factor determining the rate of diffusion in condensed media is the viscosity. The viscosity is classified into macroviscosity (averaged viscosity over the whole volume) and microviscosity (the viscosity of particular local areas). The macroviscosity of solutions is measured by mechanical techniques using a capillary viscometer, falling-ball viscometer or rotary viscometer.[2] The operation of these devices is based on measurement of the internal friction, *i.e.*, resistance of the liquid to the external force applied. The internal friction of a liquid is proportional to the dynamic viscosity and the gradient of the velocity of mutual migration of layers moving at different velocities.

The local viscosity in solutions and in biological fluids in living cells is measured by sensors comparable in size with the microenvironment (*i.e.*, several micrometres). For this purpose, optical methods using various fluorescence markers are employed. Microscopic objects are not destroyed during optical studies; therefore, they are successfully used to study biological systems. The most widely used fluorescence methods for determining the local viscosity in

B M Uzhinov, V L Ivanov, M Ya Melnikov Department of Chemistry, M V Lomonosov Moscow State University, Leninskie Gory 1, 119991 Moscow, Russian Federation. Fax (7-495) 932 88 46, tel. (7-495) 939 26 85, e-mail: uzhinov@light.chem.msu.ru (B M Uzhinov),
tel. (7-495) 939 26 33, e-mail: ivanov@photo.chem.msu.ru (V L Ivanov)
tel. (7-495) 939 18 14, e-mail: melnikov46@mail.ru (M Ya Melnikov)

Received 29 June 2011
Uspekhi Khimii **80** (12) 1231 – 1243 (2011); translated by Z P Svitanko

solutions are fluorescence anisotropy (fluorescence is excited by polarized light)[3] and fluorescence recovery after photobleaching (FRAP).[4,5]

Typical anisotropic fluorophores are excited by the polarized light the electric vector of which is parallel to the vector of the dipole moment of the molecule. Light emission occurs in the dipole plane. During the lifetime of the excited state, the molecule rotates thus changing the polarization plane. The rotation depends on the viscosity of the medium; therefore, the local viscosity can be estimated from fluorescence measurements with polarized light.

The FRAP method is based on fluorophore diffusion after the photobleaching by a strong focused laser pulse. Diffusion leads to exponential recovery of fluorescence intensity. The local viscosity can be determined from the diffusion constants.[6–8] A drawback of FRAP is limitation of the temporal resolution of the signal.[7] The small size of the spot hampers the fluorescence recovery measurements and limits the spatial resolution. Furthermore, the energy density of the bleaching pulse reaches 1 MW cm^{-2}, which is sufficient for the formation of free radicals upon photolysis and for local heating, resulting in the destruction of the object, for example, protein.

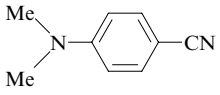
In recent years, molecular rotors have been finding increasing use, as their fluorescence ability depends on the viscosity of the medium.[2] Molecular rotors have a number of advantages over the considered methods (especially when they are used as local viscosity probes in biological systems): the use of molecular rotors requires simple equipment, and the emission intensity can be observed on a real-time basis with spatial resolution being limited only by the optical system resolution. Local concentration changes, non-uniform illumination and changes in the optical properties (scattering, absorption) are taken into account by means of reference fluorophores and measurement of the fluorescence lifetimes.

Yet another important advantage of molecular rotors is the possibility of monitoring the viscous flow of liquids. The flow velocity usually varies across the section perpendicular to the liquid flow direction. The spatial velocity gradient is called the shear rate. The product of this quantity by the viscosity gives the shear stress — the primary determinant of the flow-related forces. Apart from numerical methods, the viscous flow velocity can be determined using the ultrasonic Doppler effect, laser Doppler technique or magnetic resonance.[9] Particle tracking is also often used.[10]

As applied to biological objects, fluorescent labels provide mainly qualitative information, while the use of molecular rotors is suitable for quantitative characterization of the fluorescence response. Rotation of one moiety relative to another is the predominant factor in the molecular rotor photophysics. This moiety (dimethylamino or cyano group) is usually rather small and has more freedom to rotate than typical markers for anisotropy. The presence of a relationship between the viscosity, rotational diffusion and intramolecular rotation makes molecular rotors attractive markers for the microenvironment, as determination of the local viscosity can be reduced to fast spectroscopic measurements of the fluorescence intensity.[11]

After light absorption, the molecular rotor can return to the ground state from either the locally excited (LE) or TICT state. If both routes of radiative deactivation are effective, two-band fluorescence is observed. The energies of the LE and TICT states are substantially different, and the emission maximum from the TICT state is either shifted to longer wavelengths or absent from the spectrum. The rate of deactivation of the TICT state depends on the local environment of the fluorophore molecule, in particular, on the local viscosity and the solvent polarity.

The two-band fluorescence of 4-dimethylaminobenzonitrile (**1**) in polar solvents was first discovered by Lippert *et al.*[12]



1

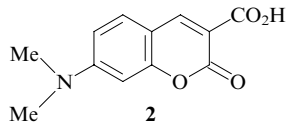
The emission spectra of this compound in solvents of various polarity have two bands and the intensity of long-wavelength fluorescence increases with increase in the solvent polarity. Molecular rotors typically undergo intramolecular charge transfer (ICT) upon excitation. The ICT gives rise to high-polarity excited state, which is transformed into the TICT state upon intramolecular rotation around a single bond. The TICT state model was proposed by Grabowski and co-workers.[13] The key issues concerning the TICT state were reviewed.[14] The formation of the twisted state considerably depends on the environment (steric hindrance hampers the appearance of this state). The radiative transition competes with the intramolecular rotation, which induces non-radiative deactivation of the excited state. The rate constant for the non-radiative deactivation decreases in viscous media; therefore, the fluorescence quantum yield and lifetime are rather great in a viscous microenvironment and are relatively small at low local viscosity. The physical mechanism that is responsible for the viscosity dependence of the quantum yield and the fluorescence lifetime is brought about by steric hindrance to the intramolecular rotation. The intramolecular rotation is a function of the free volume of the fluorophore microenvironment. If rotation is hampered by the high viscosity of the microenvironment (small free volume), the probability of radiative deactivation increases and, hence, the fluorescence intensity also increases.

A common feature of molecular rotors is the presence of an electron-donating group, a conjugation chain and an electron-withdrawing group in the molecular structure. An alkylated nitrogen atom is an example of the versatile electron-donating group, and nitrile and ester groups or aromatic rings are usual electron-withdrawing groups. The conjugation chain facilitates the electron transfer from the donor to the acceptor upon photoexcitation.

There are several classes of chemical compounds the structures of which correspond to the above principle. The first class comprises 4-dimethylaminobenzonitrile (**1**) derivatives, which can be modified by replacing both electron-donating and electron-withdrawing groups. The dimethylamino group can be replaced by aliphatic or alicyclic amino groups. As the size of the aromatic fragment in the donor increases, the intensity of the charge transfer band increases.[15] An increase in the alkyl chain length in the electron-donating group results in a higher emission intensity from the LE state.[16] Nitrogen-containing heteroaromatic rings can also function as electron-donating groups.

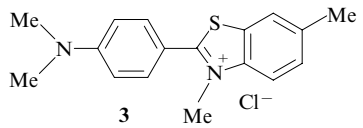
The π-conjugation chain can also be modified. The introduction of electron-donating groups into the benzene ring of 4-dimethylaminobenzonitrile does not affect much

the fluorescence properties until these groups start to affect rotation of the dimethylamino groups.[17,18] In the case of polysubstituted benzene rings, the steric effects prevail over electronic effects.[19] When the benzene ring is replaced by a naphthalene ring, the energy difference between the LE and TICT states decreases. If the electron-donating and -withdrawing groups are arranged along the longer axis of the naphthalene moiety, the Stokes shift and the fluorescence intensity considerably increase. In this case, this is attributable to the decrease in the energy of the lower singlet excited level, resulting in an increase in the energy difference between the singlet excited and TICT states. The replacement of the benzene moiety by a coumarin one affords a new subclass of molecular rotors, which have found use in viscosity and polarity measurements.[20] A typical representative of this subclass is 7-dimethylaminocoumarin-3-carboxylic acid (**2**).



2

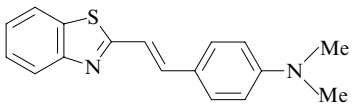
Modification of the electron-withdrawing moiety by replacing the nitrile group by a carboxy group does not affect substantially the fluorescence. The replacement of the nitrile group by a benzothiazole group gives rise to an important subclass of fluorescent compounds exemplified by a known molecular rotor, 3,6-dimethyl-2-(4-dimethylaminophenyl)benzothiazolium chloride (**3**) (or thioflavin T).



3

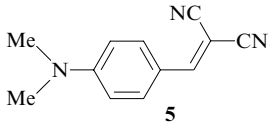
The absorption and emission properties of thioflavin T are determined by its ability to form micelles,[21,22] dimers[23] and excimers[24] and by deactivation upon intramolecular rotation.[25] The rate constant for the non-radiative deactivation of thioflavin T in n-propanol decreases by three orders of magnitude upon cooling from room temperature to 88 K. In this temperature range, the rate constant for non-radiative deactivation varies in parallel with the dielectric relaxation constant of the solvent.[26] A femtosecond fluorescence spectroscopy study of the effect of viscosity on the relaxation dynamics of the excited thioflavin T demonstrated that in the excited state, a new radiative state is formed upon rotation about the central C–C single bond. A good correlation is observed between the rate of formation of the radiative TICT state from the locally excited state and the viscosity of the medium in the range from 0 to 17.6 cP.[27]

An interesting compound is *trans*-2-(4-dimethylaminostyryl)benzothiazole (**4**). Its fluorescence characteristics depend not only on the viscosity of the medium but also on the polarity and pH of the solution.[28]



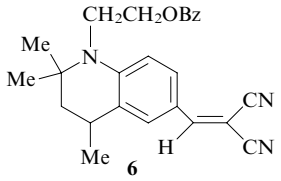
4

4-Dialkylaminobenzylidenemalononitrile derivatives form one more class of molecular rotors having intramolecular donor–acceptor interaction.

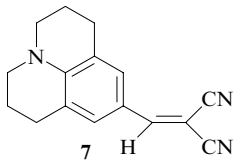


5

2-(4-Dimethylaminobenzylidene)malononitrile (**5**) is an example of fluorophores in which the TICT state is nonradiatively deactivated if the $S_1 - S_0$ energy difference in the TICT state becomes much smaller than in the LE state. The term 'molecular rotor' was first applied to this class of compounds by Loutfy and Law,[11] who used compound **5** and its derivatives — 1-(2-benzoyloxyethyl)-6-(2,2-dicyanovinyl)-2,2,4-trimethyl-3,4-dihydro-2*H*-quinoline (**6**) and 2-(2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-ylmethylidene)malononitrile [9-(2,2-dicyanovinyl)julolidine, **7**).



6

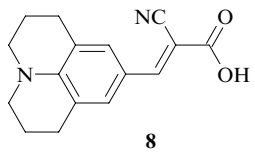


7

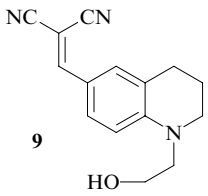
The molecule of **7** has an extended system of π-conjugation. The fluorescence spectrum exhibits one red-shifted band. Upon photoexcitation of the molecule of **7**, an electron migrates from the julolidine nitrogen atom to one of the CN groups.

The excited molecule switches to the ground state either through fluorescence emission or through non-radiative deactivation from the TICT state induced by intramolecular rotation about the double bond. At high viscosity of the medium, this rotation is hampered; hence, deactivation occurs through radiation. Conversely, in low-viscosity solvents, relaxation is mainly non-radiative and occurs from the TICT state. Thus, the fluorescence quantum yield depends on the viscosity of the medium. The rotation of molecular fragments of compounds **5** and **7** was confirmed in high-pressure experiments.[29] The fluorescence intensity from the TICT state increased as pressure was built-up to 20 kbar, and on further pressure growth it remained constant.

Among this class of compounds, (*E*)-2-cyano-3-(2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-9-yl)acrylic acid (**8**) and its derivatives are hydrophilic molecular rotors. They can be used to determine the viscosity of blood plasma or lymph.[30,31]



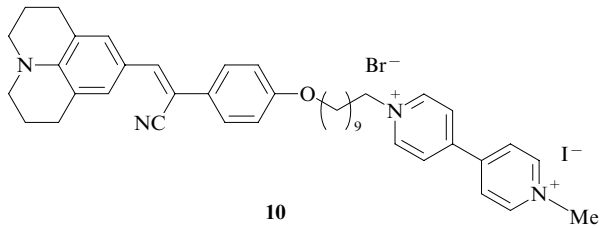
8



9

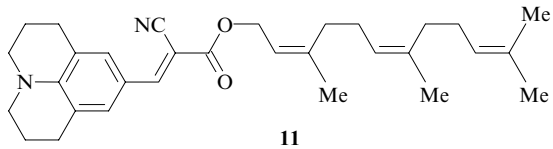
Other hydrophilic derivatives of benzylidenemalononitrile were also prepared.[32] 2-[1-(2-Hydroxyethyl)-1,2,3,4-tetrahydroquinolin-6-ylmethylidene]malononitrile (**9**), which was successfully used to study G–F transformations of actin (see below), proved to be the most efficient among them.

The cyano-containing julolidine moiety served as the basis for the synthesis of a molecular rotor, 1-methyl-1′-(10-{4-[1-cyano-2-(julolidin-9-yl)ethenyl]phenoxy}decyl)-4,4′-bipyridinium iodide bromide (**10**), which behaves in different ways depending on the viscosity. In aqueous solutions, this rotor can be blocked or activated as a result of reversible *trans–cis* photoisomerization of the cyanostilbene moiety or aggregation/deaggregation in β-cyclodextrin. This adjustable sensitivity to viscosity can be detected in the fluorescence spectra.[33]



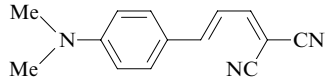
10

9-(2-Farnesyloxycarbonyl-2-cyanovinyl)julolidine (**11**) was utilized [34] to determine the dependence of the viscosity on the polymer molecular mass.



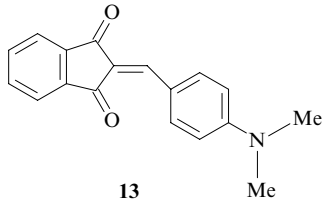
11

One more molecular rotor is 2-[(*E*)-(4-dimethylaminophenyl)allylidene]malononitrile (**12**).[35]



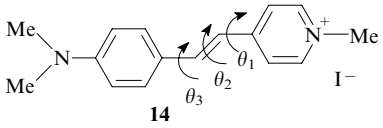
12

Rotation in the excited 2-(4-dimethylaminobenzylidene)indane-1,3-dione (**13**) molecule allows this compound to be classified as a molecular rotor.[36]



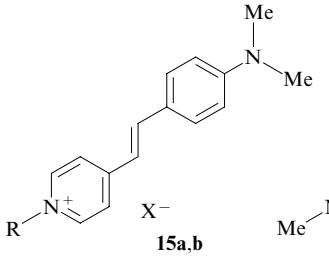
13

An increase in the length of the conjugation chain between the electron-donating and electron-withdrawing groups brings about changes in the fluorescence properties of fluorophores. An example is provided by (*E*)-4-(4-dimethylaminostyryl)-1-methylpyridinium iodide (**14**).

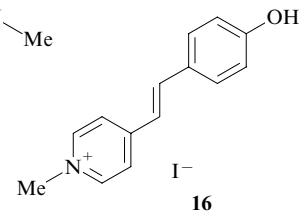


14

Molecular rotors based on the aniline moiety differ from the julolidine molecular rotors by a greater number of bonds about which rotation to give TICT states with different energies may occur. Initially it was assumed that rotation may occur about three C–C bonds of stilbene derivative **14** (θ_1, θ_2, θ_3).[37] However, subsequently it was shown that rotation about the stilbene double bond is impossible, so the non-radiative deactivation from the TICT state was assigned to the rotation about the single bond θ_3.[38]



15a,**b**

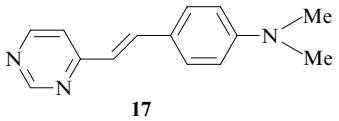


16

a: R = Bu^n, X = I;
b: R = n-C_8H_{17}, X = Br.

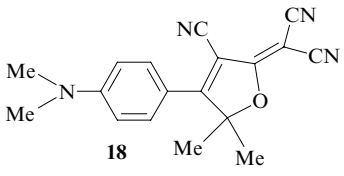
Other derivatives of cyanine dyes were also proposed as molecular rotors, for example, (*E*)-4-(4-dimethylaminostyryl)-1-n-butylpyridinium iodide (**15a**), (*E*)-4-(4-dimethylaminostyryl)-1-n-octylpyridinium bromide (**15b**) and (*E*)-4-(4-hydroxystyryl)-1-methylpyridinium iodide (**16**).[39]

The pyridinium and diazine stilbene derivatives such as (*E*)-4-(4-dimethylaminostyryl)pyrimidine (**17**) also behave as molecular rotors.[40]



17

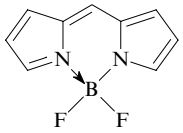
The chain of conjugation between the electron-donating and electron-withdrawing groups can be extended by introducing a dihydrofuran moiety.[41] This can be exemplified by 2-[3-cyano-4-(4-dimethylaminophenyl)-5,5-dimethyl-5*H*-furan-2-ylidene]malononitrile (**18**).



18

This fluorophore has two types of response to the local environment: solvatochromism as a result of charge transfer upon excitation and the viscosity dependence of the internal rotation, which promotes non-radiative deactivation.

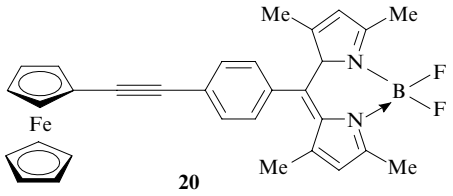
A separate class of molecular rotors is comprised of difluoroboraindacene dyes such as 4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene (**19**).



19

The involvement of TICT states upon excitation of these rotors is a debated issue.[42] As shown by quantum chemical calculations for 5-phenyl derivatives, the rotation of the benzene ring in position 5 is a barrier-free process.[43]

Yin *et al.*[44] attached the ferrocene moiety to **19** through an ethynyl bridge to obtain a typical D–π–A molecular rotor system (**20**); the fluorescence quantum yield of **20** depends substantially on the solvent viscosity.



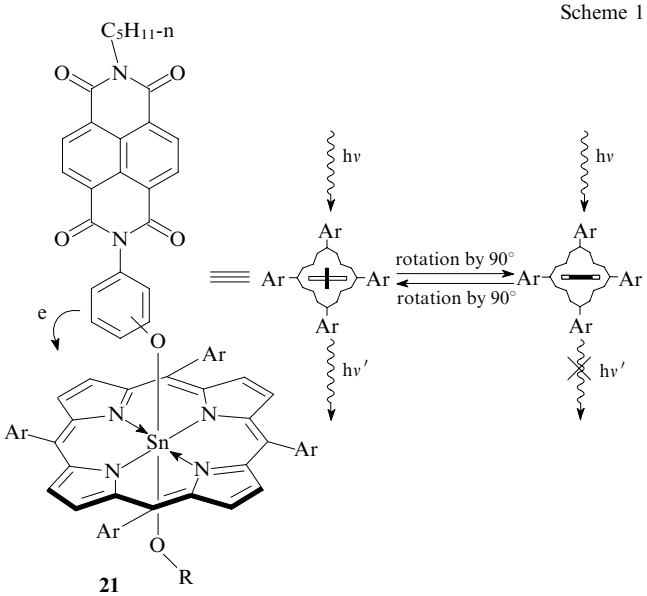
20

An especially valuable feature of this compound is the high sensitivity to the variation of the solvent viscosity for its values of < 2 cP, which is very important in the studies of biological systems.

An interesting class of molecular rotors is formed by Sn porphyrin derivatives (**21**).[45,46] The introduction of axial ligands, *m*- and *p*-oxyphenylnaphthalenediimides, into a Sn porphyrin molecule produces effective (solvent viscosity-dependent) quenching of the porphyrin fluorescence upon electron phototransfer from the phenoxide to porphyrin moiety (Scheme 1). The efficiency of electron transfer and, hence, of the quenching depends on the conformational dynamics of the ligand, *i.e.*, on the mutual positions of the naphthalenediimide and phenoxide moieties. The fluorescence quenching is considerably attenuated if the planes of the diimide and phenol moieties are mutually perpendicular. Thus, the fluorescence quantum yield of the porphyrin depends on the viscosity of the medium because it is affected by the rotational dynamics of the ligand moieties. The porphyrin molecular rotors cannot compete with the julolidine- or dimethylaminobenzonitrile-based rotors, as the quantum yield of their fluorescence does not exceed 0.1 but they may be functionalized when being located on the surface or inserted into more complex structures.

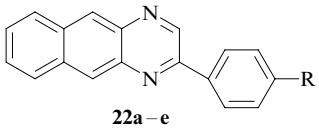
Five derivatives of 2-phenylbenzo[g]quinoxaline were prepared, namely, 2-(4-chlorophenyl)benzo[*g*]quinoxaline (**22a**), 2-phenylbenzo[*g*]quinoxaline (**22b**), 2-(4-hydroxyphenyl)benzo[*g*]quinoxaline (**22c**), 2-(4-methoxyphenyl)-benzo[*g*]quinoxaline (**22d**) and 2-(4-dimethylaminophenyl)-benzo[*g*]quinoxaline (**22e**). Only two compounds among

Scheme 1



21

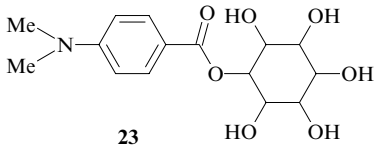
them (**22d** and **22e**) showed the behaviour of molecular rotors.[47]



22a–e

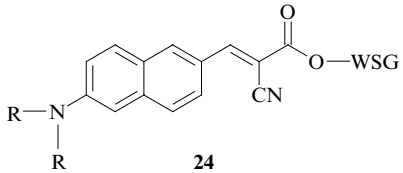
R = Cl (**a**), H (**b**), OH (**c**), OMe (**d**), NMe_2 (**e**).

Fluorophores with specific hydrophilic functions, namely, glucosamine and inositol derivatives were proposed as molecular rotors.[48] An example is the ester formed by 4-dimethylaminobenzoic acid and inositol, inosityl 4-dimethylaminobenzoate (**23**).



23

A new class of molecular rotors based on (*E*)-3-(6-dialkylaminonaphthalen-2-yl)-2-cyanoacrylate (**24**) was proposed.[49]



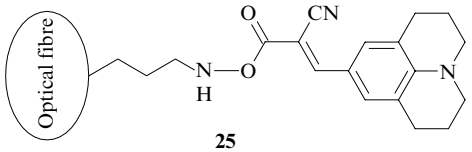
24

In this molecular rotor, the electron-donating moiety is connected to the electron-withdrawing moiety containing water-soluble groups (WSG). All of the synthesized rotors were bound to the aggregated β-amyloid protein present in the human brain tissue. The structural changes in the WSG did not affect the binding ability to the protein. However, a

change in the structure of the electron-donating moiety considerably affected the binding ability. An increase in the hydrophilicity of the electron-donating moiety enhanced the contrast between the amyloid deposits and the surrounding tissues in the histological experiments.

Attempts were made to immobilize molecular rotors on a solid surface.[50] Initially, this was done using the surface of optical fibre. The surface grafting was performed by covalent bonding *via* silane derivatives: 3-aminopropyltrimethoxysilane, *N*-(6-aminohexyl)aminopropyltrimethoxysilane, ureidopropyltrimethoxysilane.

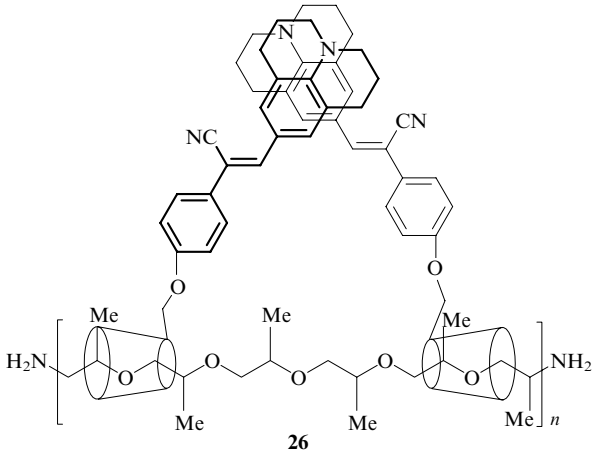
The *N*-hydroxysuccinimide ester of 9-(2-carboxy-2-cyanovinyl)julolidine (**25**) was used as a molecular rotor.



25

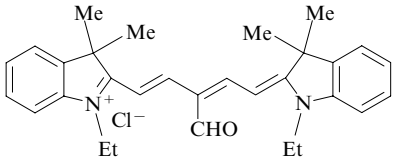
Covalent bonding of the molecular rotor to the optical fibre surface did not affect the viscosity-dependent fluorescence properties of the rotor. Subsequently, glass was used apart from the optical fibre as the substrate.[51] The results demonstrated good prospects of this approach.

The behaviour of a julolidine molecular rotor linked to β-cyclodextrin (**26**) was studied.† By means of reversible photoisomerization of the cyanostilbene moiety, the sensitivity of the molecular rotor to viscosity changes can be either switched off or activated, and these two states can be distinguished based on fluorescence. The cyclodextrin derivative forms a polypseudorotaxane. This supramolecular associate has two fluorescence channels: one arises upon the formation of intramolecular excimer and does not depend on the viscosity, and hence, it can be used to measure the compound concentration, while the other one reflects the presence of the molecular rotor, it is viscosity-dependent and can be switched off upon UV irradiation.[52]



26

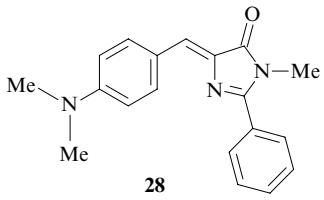
2-{(*1E*,*3Z*)-5-[1-Ethyl-3,3-dimethyl-1,3-dihydroindol-(2*E*)-ylidene]-3-formyl-1,3-dienyl}-3,3-dimethyl-1-ethyl-*3H*-indolium chloride (**27**) characterized by two-band fluorescence was offered as a molecular rotor.[44] One band (with a maximum at 456 nm, $S_2 \rightarrow S_0$ transition) is located in the short-wavelength region, while the other band (with a maximum at 650 nm, $S_1 \rightarrow S_0$ transition) is in the long-wavelength region of the spectrum.



27

In a low-viscosity solvent, the fluorescence intensity of the rotor is weak. As the solution viscosity increases, the fluorescence in the red region is considerably enhanced in parallel with the increase in the fluorescence lifetime, while the fluorescence in the short-wavelength region remains unchanged, *i.e.*, this parameter is insensitive to viscosity changes. The dependence of the fluorescence intensity on the viscosity is related to rotation of the aldehyde group. Thus, compound **27** can be used as an internal reference fluorophore; this is the first compound of this type.[53]

It was shown[54] that (Z)-4-(4-dimethylaminobenzylidene)-1-methyl-2-phenyl-1,4-dihydro-*5H*-imidazolin-5-one (**28**), an analogue of the green fluorescent protein, behaves as a molecular rotor.‡



28

The strong dependence of the fluorescence quantum yield of this compound on the viscosity attests to the existence of the excited TICT state, which is formed upon rotation about the single bond and is non-radiatively deactivated. Excitation is accompanied by charge transfer from the dimethylamino-substituted benzene ring (donor) to the imidazolin-5-one with the phenyl substituent at C(2) (acceptor).

The relationship between the fluorescence quantum yield (Φ) and the viscosity of the medium (η) is described by the Förster – Hoffmann equation

$$\log \Phi = C + x \log \eta ,$$

where C is a temperature-dependent constant, x is a constant depending on the nature of the fluorophore. This dependence was derived analytically[55–57] and confirmed experimentally.[55, 58]

Molecular rotors are considered as promising compounds for measuring the local viscosity based on the change in the fluorescence quantum yield. However, this

† In the formula of compound **26**, the frontal julolidine moiety is dark-coloured.

‡ The protein isolated from the jellyfish *Aequorea victoria* and fluorescing in the green spectral range when illuminated by blue light.

approach is faced with the problem of distinguishing between the effects of microviscosity and other factors that also affect the fluorescence quantum yield, *e.g.*, the local concentration of the fluorophore or local changes in the optical properties of the medium (*e.g.*, the refractive index). For solving the problem, the use of a reference compound was proposed.[59–61] In this method, the fluorescence intensity of one of two fluorophores does not depend on the viscosity and is used to determine the concentration, whereas the other fluorophore behaves as a molecular rotor. This enables precise calibration of viscosity against fluorescence intensity. Quite promising are systems in which the rotor molecule is covalently bonded to the reference fluorophore molecule.[53, 62, 63]

A method that involves recording the changes in the fluorescence lifetime of the molecular rotor as a function of viscosity was proposed.[64] The full local viscosity distribution map in a heterogeneous environment is obtained by the fluorescence lifetime imaging (FLIM), which is suitable for measuring the fluorescence lifetime (independent of the fluorophore concentration but fairly sensitive to parameters of the environment) with high spatial and temporal signal resolution. This is attained by using superfast excitation single photon counting fluorescence microscopy.[65] Measurement of the fluorescence kinetics of molecular rotors does not require their combination with another fluorescent label.

III. Application of molecular rotors in chemistry and biology

1. Sensors for polymerization

When molecular rotors are used as labels in the polymerizing medium, the intensity of their fluorescence increases.[56] Molecular rotors can be used to monitor aggregation and polymerization processes. It was found that the emission intensity of rotor **7** considerably depends on the tacticity of the poly(methyl methacrylate) molecule: the fluorescence quantum yields in syndiotactic and atactic poly(methyl methacrylate) films are 0.015–0.020 and 0.018–0.025, respectively.[66] In isotactic poly(methyl methacrylate), the fluorescence quantum yield markedly increases to reach 0.036–0.049, which is indicative of lesser flexibility of isotactic chains compared to atactic or syndiotactic chains. The fluorescence quantum yield of rotor **7** increases for polystyrene samples with increasing molecular mass.[67] The fluorescence emission of molecular rotor **11** depends appreciably on the interaction of macromolecular chains,[68] in particular, based on the fluorescence intensity one can judge whether poly(propylene oxide) exists in the melt as single untwisted or twisted chains.[34]

The relationship between the free volume and the fluorescence quantum yield of TICT fluorophores makes them suitable as sensors of the static and dynamic variation of the free volume in polymers.[69] There is an empirical relation between the viscosity and the free volume [70]

$$\eta = A \exp\left(B \frac{V_0}{V_f}\right),$$

where A and B are constants for the given solvent, V_0 is the van der Waals volume of the fluorophore, V_f is the polymer free volume.

The rate of non-radiative deactivation can also be expressed in terms of the free volume [69]

$$k = k_0 \exp\left(-\frac{xV_0}{V_f}\right),$$

where k_0 is the specific relaxation rate independent of the solvent, x is the constant that depends on the solvent nature.

During the polymerization, the mobility of the molecular rotors is retarded as the monomers are converted to rigid polymeric structures. Simultaneously with increase in the solvent viscosity, the free volume decreases and the viscosity-dependent fluorescence quantum yield of molecular rotors increases.

The free volume of mixtures of polymers that undergo glass transition with the solvent varies with temperature [71]

$$V_f = \frac{\alpha_p V_p T_{gp} + \alpha_s (1 - V_p) T_{gs}}{\alpha_p V_p + \alpha_s (1 - V_p)},$$

where α_p is the thermal expansion coefficient of the polymer, V_p is the polymer volume, T_{gp} is the polymer glass transition temperature, α_s is the solvent expansion coefficient, T_{gs} is the solvent glass transition temperature. It can be seen from this relation that the reorientation velocity of molecular rotors depends on both the chemical process and the temperature.

The use of molecular TICT rotors as free volume sensors provides the possibility of monitoring polymerization, cross-linking, chain and thermal relaxation, thermal transitions, thermal, photochemical and physical degradation, transport of small molecules, microphase separation, crystallization, gelation and gel swelling.[72, 73] The fluorescence quantum yield of rotor **7** considerably increases on going from liquid to glassy state, which is attributable to the decrease in the polymer free volume during polymerization.[56] In some specific cases, an increase in the fluorescence quantum yield accompanied by a decrease in the non-radiative deactivation was observed for the formation of water-soluble copolymers of 1-methyl-3-vinylpyridinium salts,[74] methacrylate monomers [40] and polysaccharides.[20] The polymerization of acrylamide, type I collagen and tetramethoxysilane was monitored using rotor **8** whose fluorescence was enhanced in the latter two cases. Using rotor **8**, it was possible to distinguish between the hydrolysis and cross-linking dynamics of these macromolecules.[75] The formation of hydrophobic microdomains in amphiphilic polymers in water was monitored using a dimethylaminobenzonitrile derivative.[74] The LE band intensity increased at high concentrations of polymers and compounds with long alkyl chains, which was indicative of the formation of hydrophobic domains. Hydrophobic molecular rotor **11**, which is easily integrated into a hydrocarbon polymer, was used to determine the relationship between the viscosity and the molecular mass (M) of poly(propylene oxide) melts.[34] For low-molecular-mass melts (425–1000 g mol^{-1}), the viscosity is proportional to M to the first power, which is characteristic of the interaction dynamics of single chains. In high-molecular-mass melts (2.7–4 kg mol^{-1}), the dependence $\eta \sim M^{3.4}$ holds; this is in line with the reptation model in which more complex interaction of chains hampers their motion.

Molecular rotors based on cyanine dyes were used to determine the local viscosity in the micelles of the triblock copolymer Pluronic F127. The rotors had low fluorescence quantum yields at low temperatures but above the critical micelle temperature they increased. The molecular rotors were located in different parts of the micelle, which made it possible to determine the local viscosity. The viscosity of the micelle core was higher than the viscosity of the crown, and the crown viscosity increased toward the micelle centre.[39]

2. Sensors of aggregation and conformational changes of the proteins

The interaction of molecular rotors with the hydrophobic moieties of proteins or other macromolecules results in restriction of the intramolecular rotation of the rotors and thus increases the fluorescence quantum yields. Molecular rotors are convenient tools for the investigation of proteins: they provide information about the presence of a protein and the protein microenvironment.

Molecular rotors allow one to track the aggregation and conformational changes of protein molecules. It was shown[30] that heating of immunoglobulin-polysorbate with IgG inclusions in the presence of molecular rotors **7** and **8** induces the protein aggregation. This leads to an increase in the fluorescence quantum yield. Upon binding of rotor **7** to the protein tubulin, the fluorescence quantum yield was observed to increase.[76] Further increase in the fluorescence intensity of this rotor took place upon the aggregation of tubulin, while the fluorescence wavelength remained constant, as was later confirmed by other researchers.[77] Using benzylidenemalononitriles with hydrophilic groups as molecular rotors, Sawada *et al.*[32] observed pronounced growth of the fluorescence intensity of the molecular rotor on going from the protein G-actin (monomer) to F-actin (polymer). The sharpest effect was noted for rotor **9**. Lindgren *et al.*,[78] who studied the folding kinetics of transthyretin (a protein able to unfold to form amyloid deposits in the peripheral nerve tissue) in the presence of rotors **3** and **7**, found out that they bind mainly to the unfolded molecules and, hence, it is possible to follow specifically the dynamics of formation of the pathogenic transthyretin associates.

Using molecular rotors, it is possible to gain knowledge about the properties of the microenvironment in cyclodextrins[79,80] the hydrophobic core of which is used to deliver hydrophobic compounds to an aqueous medium. Molecular rotors having a two-band emission spectrum are very convenient for such studies: the TICT emission characterizes the hydrophobic core and the LE state emission characterizes the local environment of the core.

3. Local viscosity probes in the cells of living organisms

The studies dealing with the behaviour of molecular rotors in vesicles[81] and lipid bilayers[57,82] were the first to consider the application of molecular rotors as local viscosity probes in the cells of living organisms. Humphry-Baker *et al.*[81] showed that the indocyanine dye associates with micellar systems in aqueous suspensions. The dye migrates into the micelles; this is accompanied by an increase in the fluorescence quantum yield and a bathochromic shift of the fluorescence spectrum. The authors attributed the increase in the fluorescence quantum yield to the increase in the rigidity of the hydrophobic environment of micelles; however, no detailed interpretation of the formation of the TICT state was given. Kung and Reed[57] studied the fluorescence of rotor **7** in several solvents and liposomes and elucidated the relationship between the dielectric constant of the solvent and the maximum of the fluorescence spectrum on going from a non-polar (benzene) to a polar (ethanol) solvent. The bathochromic shift was 30 nm. The fluorescence quantum yield is related to the solvent viscosity by the expression

$$\Phi = C\eta^{0.6}.$$

For DPPC liposomes (DPPC is dipalmitoylphosphatidylcholine), the exponent sharply changes as the temperature is raised above the point of transition from one state to the other. It was shown experimentally[57] that a molecular rotor reflects the fast change in the free volume in phospholipids following transition from the gel to the liquid-crystalline state. Later, this effect was studied by another research group,[83] who confirmed the viscosity sensitivity of molecular rotors linked to the hydrophobic fragments of phospholipids.

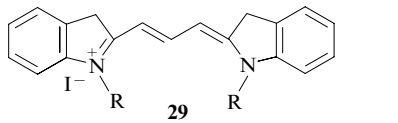
It was shown[82] that the temperature-dependent bathochromic shift of the fluorescence spectra of molecular rotor **5** in the DSPC vesicles (DSPC is the distearoylphosphatidylcholine) is greater that in the DPPC vesicles. This was attributed to a different localization of the molecular rotor in the DSPC vesicles with longer hydrocarbon (alkyl) chains. The study of Kung and Reed[57] demonstrated an important advantage of the julolidine molecular rotors, namely, different effects of the polarity and viscosity on the spectral maximum and the fluorescence quantum yield. Subsequently this was also confirmed by other researchers.[77,84] Noteworthy is the good linear correlation between the viscosity determined using molecular rotor **11** and the viscosity measured by FRAP.[85]

The cell biomechanics is mainly determined by its membrane, cytoskeleton and cytoplasm. Unlike the rigid cell cytoskeleton, the cytoplasm and cell membrane show viscoelastic properties, which change at various pathologies. For example, an increased viscosity of erythrocyte and thrombocyte membranes was observed in diabetes patients; a change in the viscosity decreases the tendency of the insulin receptor (membrane-attached protein) for aggregation.[86] An increase in the viscosity of the leukocyte membranes in Alzheimer's disease patients facilitates the aggregation of the amyloid precursor protein. The liver disease patients show high viscosity of erythrocyte membranes. The enhanced viscosity of leukocyte membranes is also related to ageing processes.

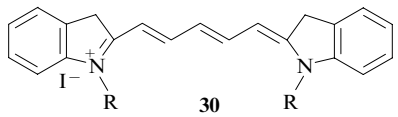
Molecular rotors were used for probing the living cell components, namely, the cell membrane, the cytoplasm and the cytoskeleton. A study involving rotor **7** showed a reversible decrease in the membrane viscosity upon application of shear stress.[87] This study provided the conclusion that the membrane is the most probable mechanoreceptor in the cell. Compound **7** has a high affinity for hydrophobic parts of the cell including the membrane and the cytoskeleton.[3] Phospholipid-bound molecular rotors **8** and **11** proved to be highly specific with respect to the membrane and also highly sensitive viscosity probes in liposomes and artificial endothelial cells.[8] However, they are cytotoxic, apparently, due to the fact that the introduction of phospholipids changes the natural phospholipid composition of the membrane. The hydrocarbon chains of the geranyl and farnesyl

residues improve the rotor location in the membrane. The accumulation of rotor **11** in the membrane is more efficient than that of rotor **7**; therefore, the fluorescence response was more pronounced in the former case.[68] It was proposed to use molecular rotors for monitoring the transport of lipids. Fluorescence microscopy, for example, with rotor **7**, may be complicated by fluctuations of its concentration in the caveolin-rich lipid tracks[88] but this difficulty can be overcome using the FLIM technique.

The cell cytoplasm is much less studied. The problems faced by investigation of the cytoplasm are related to its geometric inhomogeneity: the thickness and the local concentration gradient affect the measurable fluorescence intensity. It was proposed[61] to use indocyanine dyes Cy3 (**29**) and Cy5 (**30**) as a pair: Cy3 functions as a molecular rotor with a viscosity-dependent non-radiative deactivation channel, while the relatively rigid Cy5 serves as the reference fluorophore.



29



30

R = Alk.

The cytoplasm viscosity was reported[61] to be somewhat higher than the water viscosity and to vary insignificantly in the cell area under interest. However, Kuimova *et al.*[64] showed that the fluorescence lifetime (τ) of the indacene derivative introduced into the cell varies as a function of the viscosity of the medium according to the relation

$$\tau = z\,\eta^{\alpha},$$

where z and α are constants. The viscosity was found to be 140 ± 40 cP, which differs from the values obtained in the study cited above.[61] The dye distribution obtained by Kuimova *et al.*[64] may imply that the dye is either predominantly linked to the protein or forms micelles. Dyes Cy3 and Cy5 are water-soluble and indacene is hydrophobic. The discrepancy between the results of these two research groups was attributed to binding of indacene by the protein.

The cytoskeleton ensures the support of the cell structure and is responsible for its motility. The cytoskeleton is formed by three key structural components: filaments, intermediate filaments and microtubules. The filaments and the microtubules have been studied by molecular rotors. The main component of the filaments is the protein actin, *i.e.*, the molecule occurring in the cell as two forms: the monomeric globulin (G-actin) and the filament (F-actin). Actin is bound to adenosine triphosphate to form microfilaments, which are responsible for the cell shape and motility. The rate of polymerization of the monomeric form plays an important role in the cell motility. The actin filaments form the cell cortex, which is the base of the cytoskeleton. The cytoskeleton has the active connection with the plasmatic membrane.

The ability to track monomeric and polymeric substrates makes molecular rotors a powerful tool of kinetic measurements for reactions of proteins and protein assemblies. Molecular rotor **9** proved to be sensitive to G-actin polymerization to F-actin.[32,89] It is efficiently bound to actin, and this process is accompanied by enhancement of the fluorescence.[89] The interaction of actin with metal cations, for example, with Mg^{2+}, results in transition of G-actin to F-actin, which is accompanied by further enhancement of the fluorescence emission by rotor **9**. Hence, this rotor can be regarded as a sensor of the conformational changes undergone by the actin molecules on aggregation. It was shown[76] that molecular rotor **7** preferably binds to tubulin, the protein that forms the microtubules. The authors utilized this fact to study the mechanisms of binding of compound **7** to tubulin and tubulin aggregation. The binding of rotor **7** to tubulin is accompanied by enhancement of the fluorescence, which is indicative of hydrophobic non-covalent bonding that restricts the intramolecular rotation. The tubulin associate with this rotor induces conformational changes at the dye grafting site, which further hampers the rotational mobility of its molecule. The tubulin aggregation can be tracked by enhancement of the fluorescence emission by compound **7**. Moreover, the increase in the emission intensity attests to the difference between the microtubules and to the difference of the morphology of tubule aggregates at the molecular level. By measuring the dynamic changes in actin and tubulin, one can track the changes in the cytoskeleton on a real-time basis and correlate the cell functions.

A specific application of molecular rotors to studies of living cells is the measurement of the intracellular electric potentials. In conventional methods, this is done using microelectrodes. These methods do not ensure a satisfactory spatial resolution and have low signal-to-noise ratio. For a number of styryl dyes,[90] the potential sensitivity is due to the formation of the TICT state.[91] Ephardt and Fromherz[91] proposed two possible sensitivity mechanisms for one of these dyes (4-aminostyrylpyridinium) in phospholipid membranes. According to one mechanism, the electrostatic field directed parallel to the principal axis of the molecule interacts with the excited molecule. This should hamper the transition to the twisted state. The transition to the twisted state is retarded and fluorescence is enhanced. According to the other mechanism, the electrostatic field can displace the dye inside the heterogeneous membrane/water interface through, for example, rotation of the aniline group towards the aqueous phase. Hence, upon such displacement, the dye gets to a more polar environment with a lower viscosity, and the fluorescence intensity should decrease.

More recent studies of Jones and Bohn[92] revealed that the electrostatic field induced an increase in the fluorescence intensity, *i.e.*, this should have indicated that the dye sensitivity is controlled by charge redistribution, which increases the barrier to the TICT state, but the authors considered the phospholipid protonation and the dye dislocation to be the crucial factors for this behaviour.

4. Sensors of the viscous flow of liquids

In hydrophilic molecular rotors containing polar groups (carboxylic acid, ethanol or ethylene glycol residues), the fluorescence intensity in polar liquids (water, alcohols) increases upon application of shear stress.[93] This effect

was discovered using molecular rotor **8** and an ether (triethylene glycol). No such effect was found for other dyes such as fluorescein, which is not a molecular rotor, or compound **7**, which has no polar functional groups.

A study of systems where the liquid flow can be controlled more accurately using a peristaltic pump showed that the increase in the fluorescence intensity obeys a nonlinear dependence on the viscosity and on the liquid velocity. In particular, the saturation effect was observed where further increase in the flow did not induce measurable increase in the emission intensity. The saturation depends on the nature of the liquid used. The obtained empirical dependences are satisfactorily described by the equation

$$\Delta I = \Delta I_{\max}\left[1 - \exp\left(-\frac{\eta}{\eta_c}\right)\right]\left[1 - \exp\left(-\frac{v}{v_c}\right)\right],$$

where ΔI is the increase in the fluorescence intensity, η is the liquid viscosity, η_c is the intrinsic viscosity, v is the liquid velocity, v_c is the intrinsic rate constant.

Molecular rotors are highly sensitive flow sensors capable of measuring the liquid velocities to an accuracy of 0.5 mm s^{-1} with high spatial and temporal resolution. In particular, the Poiseuille flow velocity distribution in round tubes obeys the parabolic law. The fastest flow is observed at the tube centre and the slowest flow is near the tube walls. The emission intensity measured by fibre-glass optics follows the same parabolic law. In a narrow channel in the upper part of the flow chamber, a higher liquid velocity is observed. The flow velocity is reduced as soon as the liquid enters the wide part of the chamber. Thus, the area of the highest fluorescence intensity is located in a narrow entrance channel. In the proper volume of the chamber, the intensity in some regions does not exceed the background values. Since the spatial and temporal resolution of the molecular rotor sensors is limited only by the recording equipment, it is possible to obtain microscopic images with very high resolution. The sensitivity of molecular rotors as flow sensors is comparable with or even exceeds the sensitivity of any mechanical methods.

Molecular rotors **5** and **7** were used to study the intermolecular friction in conventional solvents and in ionic liquids.[94]

5. Determination of liquid viscosity

Molecular rotors are suitable for determining the viscosity of a liquid from the known viscosity of another liquid. The fluorescence intensity of fluorophore (I_{em}) is proportional to the intensity of the absorbed light (I_{ab}) and the fluorescence quantum yield

$$I_{em} \sim I_{ab}\,\Phi\,.$$

At low dye concentrations, the fluorescence intensity of the molecular rotor depends linearly on the concentration. According to the Förster–Hoffmann equation, for constant temperature and absorbed light intensity for the same molecular rotor, the following relation holds:

$$\frac{\eta_1}{\eta_2} = \left(\frac{\phi_1}{\phi_2}\right)^{1/2} = \left(\frac{I_1}{I_2}\right)^{1/2},$$

where the subscripts 1 and 2 refer to two different liquids. If the viscosity of one of the liquids is known, the viscosity of the other liquid can be determined without difficulty. Obviously, specific interactions between the fluorophore and the solvent should be excluded for such measurements.

The accuracy of viscosity determination by optical methods based on the use of molecular rotors is comparable with the accuracy of determination by usual mechanical flow meters.[95,96] However, only a few publications report determination of the viscosity of liquids using molecular rotors. A probable reason is the availability of mechanical flow meters. The main advantage of fluorescence methods over the use of mechanical flow meters is quickness of the measurement.

Molecular rotors have been used to determine the viscosity of biological fluids. The viscosity of these fluids changes at various pathologies (infectious diseases, infarctions,[97] hypertension,[98] diabetes,[99] atherosclerosis[100]) and due to body ageing.[101] In particular, it was shown that the viscosity of blood plasma increases upon regular smoking[102] and the viscosity of the lymphatic fluid increases in breast cancer patients.[103]

Of obvious interest is the publication[104] in which the averaged viscosity was compared with the local viscosities of seven ionic liquids. The molecules of the liquids contained an imidazole moiety. For six liquids, the local viscosities determined using molecular rotor **7** were higher than their averaged viscosities. This was attributed to the microheterogeneous nature of the liquids: the viscosity is the average value of the viscosities of various microenvironments, while the local viscosity found using a molecular rotor is the viscosity of the particular local area where the rotor is located. In experiments carried out at different temperatures, the rotor was found to travel to another microenvironment at 28–30 °C.

IV. Conclusion

The molecular rotors considered in this review represent the subgroup of fluorophores the molecules of which can switch to the TICT state and be non-radiatively deactivated from this state. The high rate of formation of the TICT state results in a low quantum yield and weak fluorescence intensity.

The rate of formation of the TICT state is determined by the solvent viscosity; therefore, the molecular rotors can serve as fluorescent labels for viscosity determination with a real response time and high spatial resolution of the signal. Molecular rotors can be modified for particular chemical or biological applications. A common feature of the rotors is the presence of an electron-donating group, a conjugation chain and an electron-withdrawing group in the molecule. The alkylated nitrogen atom is a versatile electron-donating group, while electron-withdrawing groups include nitrile or ester groups or aromatic rings. Electron-donating and electron-withdrawing groups are connected *via* a conjugation chain, which facilitates the electron transfer upon photoexcitation. The solvent viscosity and the fluorescence intensity are related in a particular fashion, which allows for determination of the liquid viscosity by measuring the emission intensity provided that the solvent calibration is known. This relationship is valid for viscosity range of $10-10^4$ cP. By using fluorescence methods, the viscosity can be measured faster and more accurately than using the

conventional mechanical viscometers. Molecular rotors are successively used as sensors of polymerization and protein aggregation and for determining the local viscosity of cell components. Molecular rotors containing polar functional groups can be used as fast-response high-resolution viscous flow sensors. The sensitivity of these sensors is comparable to or higher than the sensitivity of any mechanical devices.

This work was supported by the Russian Foundation for Basic Research (Project No. 11-03-01026a).

References

1. G S Kottas, L I Clarke, D Horinek, J Michl *Chem. Rev*. **105** 1281 (2005)
2. M A Haidekker, E A Theodorakis *Org. Biomol. Chem*. **5** 1669 (2007)
3. M Shinitzky, Y Barenholz *Biochim. Biophys. Acta* **515** 367 (1978)
4. S Seiffert, W Oppermann *J. Microsc*. **220** 20 (2005)
5. M J Dayel, E F Y Hom, A S Verkman *Biophys. J*. **76** 2843 (1999)
6. D Axelrod, D E Koppel, J Schlessinger, E Elson, W W Webb *Biophys. J*. **16** 1055 (1976)
7. J C G Blonk, A Don, H Van Aalst, J J Birmingham *J. Microsc*. **169** 363 (1993)
8. E J van Zoelen, L G Tertoolen, S W de Laat *Biophys. J*. **42** 103 (1983)
9. D Liepsch *J. Biomech*. **35** 415 (2002)
10. M Sugii, S Nishio, K Okamoto *Ann. N.Y. Acad. Sci*. **972** 331 (2002)
11. R O Loutfy, K Y Law *J. Phys. Chem*. **84** 2803 (1980)
12. E Lippert, W Lüder, F Moll, W Nagele, H Boos, H Prigge, I Seibold-Blankenstein *Angew. Chem*. **73** 695 (1961)
13. K Rotkiewicz, K H Grellmann, Z R Grabowski *Chem. Phys. Lett*. **19** 315 (1973)
14. Z R Grabowski, K Rotkiewicz, W Rettig *Chem. Rev*. **103** 3899 (2003)
15. W Rettig *J. Luminesc*. **26** 21 (1980)
16. W Schuddeboom, S A Jonker, J M Warman, U Leinhos, W Kuehnle, K A Zachariasse *J. Phys. Chem*. **96** 10809 (1992)
17. K A Zachariasse, T von der Haar, A Hebecker, U Leinhos, W Kmhnle *Pure Appl. Chem*. **65** 1745 (1993)
18. K Rotkiewicz, W Rettig, N Detzer, A Rothe *Phys. Chem. Chem. Phys*. **5** 998 (2003)
19. Y Shinohara, T Arai *Bull. Chem. Soc. Jpn*. **81** 1500 (2008)
20. P Even, F Chaubet, D Letourneur, M L Viriot, M C Carre *Biorheology* **40** 261 (2003)
21. S Kumar, A K Singh, G Krishnamoorthy, R Swaminathan *J. Fluoresc*. **18** 1199 (2008)
22. R Khurana, C Coleman, C Ionescu-Zanetti, S A Carter, V Krishna, R K Grover, R Roy, S Singh *J. Struct. Biol*. **151** 229 (2005)
23. L R Naik, A B Naik, H Pal *J. Photochem. Photobiol. A*. **204** 161 (2009)
24. C Retna Raj, R Ramaraj *Chem. Phys. Lett*. **273** 285 (1997)
25. V I Stsiapura, A A Maskevich, V A Kuzmitsky, V N Uversky, I M Kuznetsova, K K Turoverov *J. Phys. Chem. B* **112** 15893 (2008)
26. N Amdursky, R Gepshtein, Y Erez, D Huppert *J. Phys. Chem. A* **115** 2540 (2011)
27. P K Singh, M Kumbhakar, H Pal, S Nath *J. Phys. Chem. B* **114** 5920 (2010)
28. S K Saha, P Purkayastha, A B Das, S Dhara *J. Photochem. Photobiol. A* **199** 179 (2008)
29. Z A Dreger, J O White, H G Drickamer *Chem. Phys. Lett*. **290** 399 (1998)
30. A Hawe, V Filipe, W Jiskoot *Pharm. Res*. **27** 314 (2010)
31. M A Haidekker, T P Brady, S H Chalian, W Akers, D Lichlyter, E A Theodorakis *Bioorg. Chem*. **32** 274 (2004)
32. S Sawada, T Iio, Y Hayashi, S Takahashi *Anal. Biochem*. **204** 110 (1992)
33. L-L Zhu, D-H Qu , D Zhang, Z-F Chen, Q-C Wang, H Tian *Tetrahedron* **66** 1254 (2010)
34. D Zhu, M A Haidekker, J-S Lee, Y-Y Won, J C-M Lee *Macromolecules* **40** 7730 (2007)
35. C Damas, M Adibnejad, A Benjelloun, A Brembilla, M C Carré, M L Viriot, P Lochon *Colloid Polym. Sci*. **275** 364 (1997)
36. V Gulbinas, G Kodis, S Jursenas, L Valkunas, A Gruodis, J-C Mialocq, S Pommeret, T Gustavsson *J. Phys. Chem. A* **103** 3969 (1999)
37. B Strehmel, H Seifert, W Rettig *J. Phys. Chem. B* **101** 2232 (1997)
38. X Cao, R W Tolbert, J L McHale, W D Edwards *J. Phys. Chem. A* **102** 2739 (1998)
39. Y Shiraishi, T Inoue, T Hirai *Langmuir* **26** 17505 (2010)
40. P Bosch, C Peinado, V Martin, F Catalina, T Corrales *J. Photochem. Photobiol. A* **180** 118 (2006)
41. S J Lord, N R Conley, H D Lee, S Y Nishimura, A K Pomerantz, K A Willets, Z Lu, H Wang, N Liu, R Samuel, R Weber, A Semyonov, M He, R J Twieg, W E Moerner *Chem. Phys. Chem*. **10** 55 (2009)
42. M A Haidekker, M Nipper, A Mustafic, D Lichlyter, M Dakanali, E A Theodorakis, in *Advanced Fluorescence Reporters in Chemistry and Biology I: Fundamentals and Molecular Design* Vol. 8 (Ed. A P Demchenko) (Berlin, Heidelberg: Springer, 2010) p. 267
43. H L Kee, C Kirmaier, L Yu, P Thamyongkit, W J Youngblood, M E Calder, L Ramos,B C Noll, D F Bocian, W R Scheidt, R R Birge, J S Lindsey, D Holten *J. Phys. Chem. B* **109** 20433 (2005)
44. X Yin, Y Li, Y Zhu, X Jing, Yu Li, D Zhu *Dalton Trans*. **39** 9929 (2010)
45. K P Ghiggino, J A Hutchison, S J Langford, M J Latter, M A P Lee, P R Lowenstern, C Scholes, M Takezaki, B E Wilman *Adv. Funct. Mater*. **17** 805 (2007)
46. M J Latter, S J Langford *Int. J. Mol. Sci*. **11** 1878 (2010)
47. K Wang, W Shi, J Jia, S Chen, H Ma *Talanta* **77** 1795 (2009)
48. M C Carré, C Geoffroy-Chapotot, M Adibnejad, P Berroy, J F Stoltz, M L Viriot *J. Fluoresc*. **8** 53 (1998)
49. W M Chang, M Dakanali, C C Capule, C J Sigurdson, J Yang, E A Theodorakis *ACS Chem. Neurosc*.; doi: org/10.1021/cn200018v (2011)
50. M A Haidekker, W J Akers, D Fischer, E A Theodorakis *Opt. Lett*. **31** 2529 (2006)
51. D J Lichlyter, M A Haidekker *Sens. Actuators, B: Chem*. **139** 648 (2009)
52. L-L Zhu, X Li, F-Y Ji, X Ma, Q-C Wang, H Tian *Langmuir* **25** 3482 (2009)
53. X Peng, Z Yang, J Wang, J Fan, Y He, F Song, B Wang, S Sun, J Qu, J Qi, M Yan *J. Am. Chem. Soc*. **113** 6626 (2011)
54. B K Rajbongshi, P Sen, G Ramanathan *Chem. Phys. Lett*. **494** 295 (2010)
55. T Förster, G Hoffmann *Z. Phys. Chem*. **75** 63 (1971)
56. R O Loutfy *Pure Appl. Chem*. **58** 1239 (1986)
57. C E Kung, J K Reed *Biochemistry* **25** 6114 (1986)
58. T Iwaki, C Torigoe, M Noji, M Nakanishi *Biochemistry* **32** 7589 (1993)
59. M K Kuimova, S W Botchway, A W Parker, M Balaz, H A Collins, H L Anderson, K Suhling, P R Ogilby *Nat. Chem*. **1** 69 (2009)
60. M A Haidekker, T P Brady, D Lichlyter, E A Theodorakis *J. Am. Chem. Soc*. **128** 398 (2006)
61. K Luby-Phelps, S Mujumdar, R B Mujumdar, L A Ernst, W Galbraith, A S Waggoner *Biophys. J*. **65** 236 (1993)

62. H-J Yoon, M Dakanali, D Lichlyter, W M Chang, K A Nguyen, M E Nipper, M A Haidekker, E A Theodorakis *Org. Biomol. Chem.* **9** 3530 (2011)
63. M E Nipper, M Dakanali, E A Theodorakis, M A Haidekker *Biochimie* **93** 988 (2011)
64. M K Kuimova, G Yahioglu, J A Levitt, K Suhling *J. Am. Chem. Soc.* **130** 6672 (2008)
65. F Festy, S M Ameer-Beg, T Ng, K Suhling *Mol. Biosyst.* **3** 381 (2007)
66. R O Loutfy, D M Teegarden *Macromolecules* **16** 452 (1983)
67. R O Loutfy *Macromolecules* **16** 678 (1983)
68. M A Haidekker, T Ling, M Anglo, H Y Stevens, J A Frangos, E A Theodorakis *Chem. Biol.* **8** 123 (2001)
69. R O Loutfy, B A Arnold *J. Phys. Chem.* **86** 4205 (1982)
70. A K Doolittle *J. Appl. Phys.* **23** 236 (1952)
71. F N Kelley, F Bueche *J. Polym. Sci.* **50** 549 (1961)
72. P Bosch, F Catalina, T Corrales, C Peinado *Chem. Eur. – J.* **11** 4314 (2005)
73. J Paczkowski, D C Neckers *Macromolecules* **24** 3013 (1991)
74. A Benjelloun, A Brembilla, P Lochon, M Adibnejad, M L Viriot, M C Carré *Polymer* **37** 879 (1996)
75. M A Haidekker, D Lichlyter, M B Johny, C A Grimes *Sensor Lett.* **4** 257 (2006)
76. C E Kung, J K Reed *Biochemistry* **28** 6678 (1989)
77. M A Haidekker, T P Brady, D Lichlyter, E A Theodorakis *Bioorg. Chem.* **33** 415 (2005)
78. M Lindgren, K Sörgjerd, P Hammarström *Biophys. J.* **88** 4200 (2005)
79. Y H Kim, D W Cho, M Yoon, D Kim *J. Phys. Chem.* **100** 15670 (1996)
80. K A Al-Hassan, M F Khanfer *J. Fluoresc.* **8** 139 (1998)
81. R Humphry-Baker, M Graetzel, R Steiger. *J. Am. Chem. Soc.* **102** 847 (1980)
82. S Luka *J. Am. Chem. Soc.* **106** 4386 (1984)
83. M A Haidekker, T Brady, K Wen, C Okada, H Y Stevens, J M Snell, J A Frangos, E A Theodorakis *Bioorg. Med. Chem.* **10** 3627 (2002)
84. B D Allen, A C Benniston, A Harriman, S A Rostron, C Yu *Phys. Chem. Chem. Phys.* **7** 3035 (2005)
85. M E Nipper, S Majd, M Mayer, J C-M Lee, E A Theodorakis, M A Haidekker *Biochim. Biophys. Acta* **1778** 1148 (2008)
86. O Nadiv, M Shinitzky, H Manu, D Hecht, C T Roberts Jr, D Le Roith, Y Zick *Biochem. J.* **298** (Pt 2) 443 (1994)
87. M A Haidekker, N L'Heureux, J A Frangos *Am. J. Physiol., Heart Circ. Physiol.* **278** H1401 (2000)
88. S Härtel, S Tykhonova, M Haas, H A Diehl *J. Fluoresc.* **12** 465 (2002)
89. T Iio, S Takahashi, S Sawada. *J. Biochem.* **113** 196 (1993)
90. A Grinvald, A Fine, I C Farber, R Hildesheim *Biophys. J.* **42** 195 (1983)
91. H Ephardt, P Fromherz *J. Phys. Chem.* **93** 7717 (1989)
92. M A Jones, P W Bohn *J. Phys. Chem. B* **105** 2197 (2001)
93. M A Haidekker, W Akers, D Lichlyter, T P Brady, E A Theodorakis *Sensor. Lett.* **3** 42 (2005)
94. H Jin, M Liang, S Arzhantsev, X Li, M Maroncelli *J. Phys. Chem. B* **114** 7565 (2010)
95. M A Haidekker, A G Tsai, T Brady, H Y Stevens, J A Frangos, E A Theodorakis, M Intaglietta *J. Physiol.* **282** H1609 (2002)
96. W J Akers, M A Haidekker *J. Biomech. Eng.* **127** 450 (2005)
97. J Harkness *Biorheology* **8** 171 (1971)
98. R L Letcher, S Chien, T G Pickering, J E Sealey, J H Laragh *Am. J. Med.* **70** 1195 (1981)
99. D E McMillan *Metabolism* **31** 274 (1982)
100. C Otto, M M Ritter, W O Richter, R Minkenberg, P Schwandt *Metabolism* **50** 166 (2001)
101. P F Roe, J Harkness *Gerontology* **17** 168 (1975)
102. E Ernst, A Matria, C Schmlltz, I Magyarosy *Brit. J. Haematol.* **65** 485 (1987)
103. D O Bates, J R Levick, P S Mortimer *Clin. Sci.* **85** 737 (1993)
104. A Paul, A Samanta. *J. Phys. Chem. B* **112** 16626 (2008)