Tutor/s

Dr. Jaume García Amorós
Departament de Química Inorgànica i Orgànica

Dra. María Dolores Velasco Castrillo
Departament de Química Inorgànica i Orgànica

Grau de Química

Treball Final de Grau

Analysis of the effect of viscosity in the design of fluorescent molecular rotors.

Anàlisi de l'efecte de la viscositat en el disseny de rotors moleculars fluorescents.

Albert Gambín Sáez

January 2024

Universitat de Barcelona

B:KC Barcelona Knowledge Campus
Campus d'Excel·lència Internacional

Aquesta obra està subjecta a la llicència de:
Reconeixement–NoComercial-SenseObraDerivada

http://creativecommons.org/licenses/by-nc-nd/3.0/es/

Hi ha una força motriu més poderosa que el vapor, l'electricitat i l'energia atòmica: la voluntat.

Albert Einstein

En primer lloc, voldria donar les gràcies als meus tutors, el Dr. Jaume García Amorós i la Dra. María Dolores Velasco Castrillo, per la seva implicació, confiança i ajuda durant tot aquest projecte, ja que sempre hi han estat quan els he necessitat.

Voldria agrair també a la meva família i parella per tot el suport incondicional que m'han donat durant aquest temps. També voldria agrair als meus amics que m'han recolzat sempre i que em fan riure i gaudir de cada moment.

Finalment, donar les gràcies a les meves companyes i companys del laboratori del Consorci Besòs-Tordera per haver fet de cada dia de feina una experiència inoblidable més i per haver-me tractat tan bé.

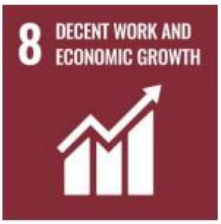
REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDG)

This work supports the Sustainable Development Goals (SDG) due to its allegiance to scientific advancements and aims for sustainability, also calling for responsibility towards a better future for everyone.

Fluorescent molecular rotors can be used as probes for a wide range of spectroscopic methods and for imaging the local viscosity of an environment. They can also be used to measure local microviscosity in individual compartments of living cells. New methods and dyes need to be discovered as this area of research continues to expand and gains interest worldwide, aiming at a better future.

It aligns with the 5Ps (People, Prosperity, Planet, Peace and Partnership), concretely with Prosperity P as this work follows **Goal 8: Decent Work and Economic Growth** by developing new methods that contribute to the advancement and improvement on this area, and promotes investment in sustainable chemistry too. It also tackles to **Goal 9: Industry, Innovation and Infrastructure** as there is the need to ensure that chemical-intensive industries that could possibly use this dyes rely on best available techniques and best environmental practices, also aiming to reduce raw material use and waste generation.



CONTENTS

1. SUMMARY

Fluorescent molecular rotors have gained popularity over the last 15 years. They are compounds with unique properties based on two moieties linked by a π-conjugated bridge. One moiety is called the stator and has a larger moment of inertia, while the other moiety is called the rotor or rotator, a fluorophore that can form a twisted internal charge transfer state (TICT) after photoexcitation, as it exhibits two different competitive pathways of energy loss towards the ground state: fluorescence emission and non-radiative deexcitation.

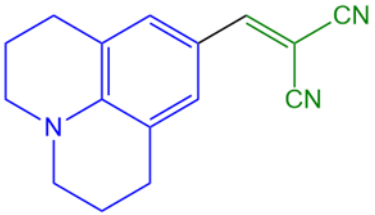


Figure 1. Structure of a julolidine-based FMR. Stator (blue) and rotor (green).

FMRs are widely used as viscosity probes due to their viscosity-sensitive fluorescence behaviour. In a low viscosity medium, the rotation of the rotor is not hindered and induces the non-radiative relaxation of the excitation energy, resulting in the quenching of the fluorescence intensity. In a higher viscosity medium, the rotation is more hindered and fluorescence intensity is enhanced.

The sensitivity to solvent viscosity can be quantified using the established relationship between the viscosity (η) of the medium and the fluorescence intensity contrast (I/I_0) or fluorescence quantum yield (ϕ_{FL}), known as the Förster-Hoffmann equation:

$$\log \frac{I}{I_0} = C + x \cdot \log \eta \qquad (1)$$

where x is the slope of the plot and is related to the viscosity sensitivity of the FMR, and C is an experimental constant related to the dye.

The structure of a fluorescent molecular rotor directly influences its ability to sense local viscosity. A classification of the main families of fluorescent molecular rotors that are going to be studied has been made: julolidine-based, BODIPY-based, cyanine-based and rhodamine-based FMRs.

Their structural design and sensitivity to viscosity will be reviewed to determine the best sensors of each dye family, and a comparison will be made to find out the best options as viscosity sensors.

Keywords: Fluorescent molecular rotor (FMRs), TICT, fluorescence, viscosity, steric hindrance, Förster-Hoffmann equation, structural design, julolidines, BODIPY, cyanines, rhodamines.

2. RESUM

Els rotors moleculars fluorescents han guanyat popularitat en els últims 15 anys. Es tracta de compostos amb propietats úniques basats en dues fraccions enllaçades per un pont π-conjugat amb. Una fracció s'anomena estator i té un moment més gran d'inèrcia, mentre que l'altra part s'anomena rotor o rotador i es basa en un fluoròfor que pot formar un estat de transferència de càrrega intramolecular torçat (TICT) després de la fotoexcitació, i mostra dues vies competitives diferents de pèrdua d'energia cap a l'estat fonamental: l'emissió de fluorescència i la desexcitació no radiativa.

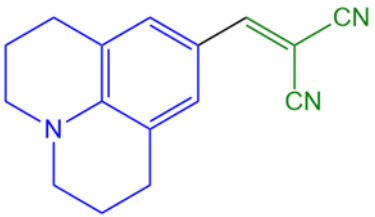


Figura 1. Estructura d'un FMR basat en una julolidina. Estator (blau) i rotor (verd).

Els FMR s'utilitzen àmpliament com a sondes de viscositat ja que presenten un comportament de fluorescència sensible a la viscositat. En un medi de baixa viscositat, la rotació del rotor no és obstruïda i s'indueix la relaxació no radiativa de l'energia d'excitació, resultant en la disminució de la intensitat de fluorescència. En un medi de viscositat més alt, la rotació es veu més obstaculitzada i la intensitat de fluorescència es potencia.

La sensibilitat a la viscositat del dissolvent es pot quantificar utilitzant la relació establerta entre la viscositat (η) del medi i el quocient de contrast d'intensitat de fluorescència (I/I_0) o el rendiment quàntic de fluorescència (Φ_{FL}), coneguda com a equació de Förster-Hoffmann:

$$\log \frac{I}{I_0} = C + \mathrm{x} \cdot \log \eta \qquad (1)$$

on *x* és el pendent de la recta i està relacionat amb la sensibilitat a la viscositat del FMR, i C és una constant experimental relacionada amb el compost.

L'estructura d'un rotor molecular fluorescent influeix directament en la seva capacitat de detectar la viscositat local. S'ha fet una classificació de les principals famílies de rotors moleculars fluorescents que s'estudiaran: julolidines, BODIPYs, cianines i rodamines.

Es revisarà el seu disseny estructural i la seva sensibilitat a la viscositat per determinar els millors sensors de cada família de rotors moleculars, i es farà una comparació per conèixer les millors opcions com a sensors de viscositat.

Paraules clau: Rotors moleculars fluorescents, TICT, fluorescència, viscositat, impediment estèric, equació de Förster-Hoffmann, julolidines, BODIPY, cianines, rodamines, disseny estructural.

3. INTRODUCTION

Luminescence refers to the emission of light (*lumen* = light in Latin) from any substance that occurs from electronically excited states [1,2]. There are several types of luminescence depending on the mode of excitation, such as electroluminescence (excitation mode by an electric field), chemiluminescence (by a chemical process (e.g. oxidation)), thermoluminescence (by heating after prior energy storage (e.g. radioactive irradiation)), etc, and photoluminescence.

Fluorescence and phosphorescence are particular categories of luminescence, in which excitation is induced by the absorption of a photon, placing the species in an electronically excited state. When deexcitation occurs, the emission of photons usually results in photoluminescence (fluorescence, phosphorescence or delayed fluorescence) as an effect from the interaction of matter with light.

The processes that take place between the absorption and emission of light can be illustrated by the Jablonski diagram, which will be used as the starting point for discussion. They represent and illustrate different molecular processes that can occur in excited states.

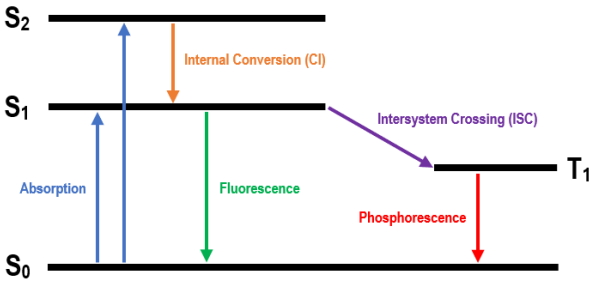


Figure 2. Typical Jablonski diagram.

The Jablonski diagram, as mentioned above, contains the electronic energy levels and is very useful to illustrate the processes that occur between the fundamental and excited states. S0 is the fundamental singlet electronic state, S_1 and S_2 are the excited singlet states, and T_1 is the triplet electronic state. At each electronic energy level, the fluorophore can contain several

vibrational energy levels, named as 0, 1, 2, etc [1], but to simplify, they are not shown. This diagram does not include other possible interactions such as quenching, energy transfer or solvent interactions.

The absorption of a photon (which usually takes about 10^{-15} s) causes the fluorophore to be excited from its lowest state (ground state S_0) to a higher (excited) level S_1 or S_2. When the fluorophore is excited, there are several deexcitation processes that can occur depending on which excited state the molecule is and will now be described. Note that the lifetime in the excited state S_1 is about 10^{-7} s.

Internal Conversion is a non-radiative process based on the transition between two electronic states, always towards the lowest vibrational level of the S_1 state, both sharing the same spin multiplicity. It occurs when a molecule is excited to an energy higher than the lowest vibrational level of S_1 (first excited state), usually within 10^{-11} or less. Looking at the Jablonski diagram (Figure 2), it involves the transition between excited states $S_2 \rightarrow S_1$.

Fluorescence is based on the $S_1 \rightarrow S_0$ relaxation that occurs with the emission of photons. The emission generally results from a thermally equilibrated excited state, usually the lowest energy vibrational state of S_1. Fluorescence has many applications such as spectroscopy, labelling, medicine, etc.

The intersystem crossing is a non-radiative conversion from S_1 towards the T_1 triplet state, $S_1 \rightarrow T_1$. The transition from T_1 to the ground state S_0 is prohibited (different multiplicity) but spin-orbit coupling can be large enough to make it possible. Note that heavy atoms (e.g. Br and I) favour intersystem crossing, enhancing phosphorescence quantum yield.

The emission from T_1 is called phosphorescence. It generally shifts to longer wavelengths (lower energy) in comparison to the fluorescence. At room temperature, the non-radiative deexcitation process from the triplet state is predominant over the radiative one (phosphorescence), but at lower temperatures, it can be observed as the lifetime of the state T_1 may be long enough.

Molecular rotors base their structure on one part that presents rotational motion and one fluorophore that can form a twisted internal charge transfer (TICT) state after the photoexcitation as they can exhibit two different competitive routes of energy loss towards the fundamental state, which are fluorescence emission and the non-radiative deexcitation.

Since excitation induces the movement of an electron to another orbital, when they are separated in space, the electronic transition occurs with a change in the dipole moment of the molecule accompanied by some structural changes.

The TICT phenomenon has recently been studied extensively and is considered to be a process that occurs in excited molecules containing a donor and acceptor moiety linked by a single bond [3]. Some structural changes can occur after excitation due to their structural flexibility.

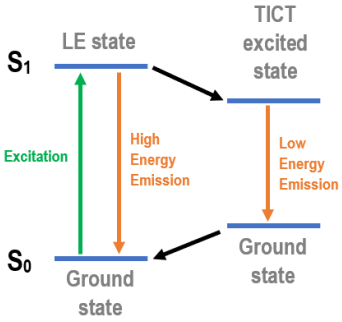


Figure 3. Jablonski diagram of the process involving the TICT state.

Figure 3 shows the mechanism based on TICT dynamics. Given a molecular rotor in a viscous medium, as excitation occurs via absorption of incident photons, electrons are lifted from the ground state S_0 to the first excited state S_1. There, the molecular rotor assumes the LE (locally excited) state. An energy loss of the excited electrons through a transition results in fluorescence emission with a lower energy than the incident photons, also known as the Stokes shift. The S_1 – S_0 energy gap in the TICT excited state is reduced, as is the relaxation energy compared to the other state.

3.1. FLUORESCENT MOLECULAR ROTORS

Molecular rotors (MRs) are compounds with unique properties based on two moieties that are linked by a specific bond. This bond consists of a π-conjugated bridge, usually with an electron donor and acceptor group [3,4], connected to two moieties that are able to rotate relative to each other.

One moiety, considered as the stator, has a bigger moment of inertia while the other moiety with a smaller moment of inertia is called the rotor or rotator [5,6]. The following figure shows the basic structure of fluorescent molecular rotors and differentiates between the two parts mentioned. This rotor belongs to the family of julolidine-based fluorescent molecular rotors (FMRs). The stator moiety is coloured in blue and the rotor is coloured in green.

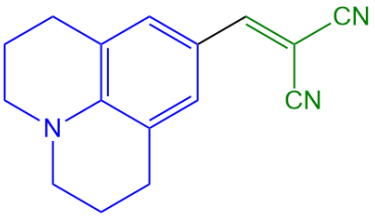


Figure 4. Basic structure of molecular rotors.

In a low viscosity medium the rotation of the rotor is greater than in a high viscosity medium, as it can twist freely and the rotation is not strained, inducing a non-radiative relaxation of the excitation energy, resulting in quenching of the fluorescence intensity. However, in a higher viscosity medium, as the rotation is more constrained, the fluorescence (FL) intensity and lifetime are enhanced [6]. So, when viscosity is increased, steric hindrance of intramolecular rotation becomes notable, and the fluorescence of the molecule is influenced by the media.

MRs have become very popular and useful probes for measuring the microviscosity of environments as they have a viscosity-sensitive FL behaviour. Usually, when the medium viscosity increases, the FL intensity also increases due to the slower rotation or the rotor.

The fluorescence quantum yield (Φ_F) is the ratio of the number of photons emitted to the number of photons absorbed (2). It is also defined by the fraction of excited state fluorophores that decay through fluorescence versus radiative and all non-radiative processes. It is given by equation (3), where $0 < \Phi_F < 1$. A larger value of Φ_F means that fluorescence is enhanced.

$$\phi_F = \frac{number\ photons\ emitted}{number\ photons\ absorbed} \quad (2)$$

$$\phi_F = \frac{k_F}{k_F + \sum k_{NR}} \quad (3)$$

where k_R and k_{NR} are the rate constant for radiative relaxation (fluorescence) and rate constant for all non-radiative relaxation processes.

A relationship has been established between the viscosity (η) of the medium or solvent and the FL intensity contrast (I/I_0) or fluorescence quantum yield (Φ_F), based on the Förster-Hoffmann equation:

$$\phi_F = C \cdot n^x \quad (4)$$

$$\log\left(\frac{I}{I_0}\right) = C + x \cdot \log \eta \quad (5)$$

where *C* is an experimental constant related to the dye, *x* is the slope of the plot and is related to dye-solvent interactions and the viscosity sensitivity of the MR. A small variation of this power law establishes the relationship, likewise, between the fluorescence quantum yield (Φ_F) and the viscosity of the medium (η).

Thus, the MR sensitivity to the viscosity of the medium can be quantified by the parameter *x*, as a higher value means that the fluorescent molecular rotor (FMR) is more sensitive to a change in viscosity.

In addition to viscosity, the temperature and polarity are also critical parameters affecting intramolecular dye rotation [4,7,8]. The polarity of the solvent does not primarily affect the quantum yield of MRs, but polarity does shift the wavelength of the emission peak [9]. A study published in 2012 [10] indicates that DCVJ and CCVJ (see **3.2.1. Julolidine-based FMRs**) have a big difference of viscosity sensitivity in polar protic and polar aprotic solvents, as the solvent can form hydrogen bonds.

Temperature also affects intramolecular motion, as an increase of temperature makes it more intense and causes fluorescence quenching. In order to minimise this effect or to control it, some molecular fluorescent thermometers have been developed. However, there are only a few articles on this topic, so more research needs to be done.

3.2. MAIN FAMILIES OF FLUORESCENT MOLECULAR ROTORS

Fluorescent molecular rotors can be classified in different types based on the differences presented on their structure.

3.2.1. Julolidine-based FMRs

Simple push-pull type FMRs have been reported as julolidine derivatives, which usually have short excitation and emission wavelengths and also small Stokes shifts, and the photophysical properties can be modified by replacing the electron withdrawing groups [4]. The DCVJ and the CCVJ rotors are the most popular ones and will be mentioned in the discussion.

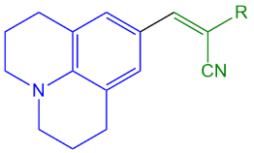


Figure 5. Core structure of a julolidine-based FMR.

The stator moiety of the dye is painted in blue, while the rotor is painted in green to distinguish the two parts. Julolidine-based FMRs rotate through the single bond that connects both moieties. Rotation is hindered by the nature and steric hindrance of the R group that is added on the rotor moiety.

Julolidine-based rotors have a good relationship between structure and photophysical properties and can be modified by changing the carboxyl group of CCVJ and adding different functional groups [4,11]. A general overview of their structure has been given. More information related to their specific structure can be found in the discussion.

3.2.2. Cyanine-based FMRs

Cyanine-based FMRs consist of a π-conjugated polymethine chain with a tunable length. The length of the chain is key to understanding the photophysical properties of this type of dye. The FL intensity is enhanced as the viscosity increases due to the hindrance of the chain rotation [3]. More information about their structure can be found in the discussion.

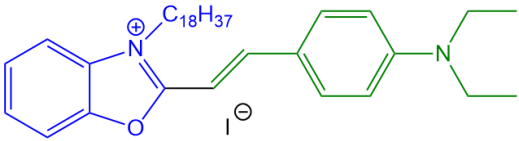


Figure 6. General structure of cyanine-based FMRs. Rotor (green) and stator (blue).

3.2.3. BODIPY-based FMRs

Boron-dipyrromethene (BODIPY) fluorescent molecular rotors have become very important and used lately due to their high fluorescence sensitivity and quantum yields and high rotability. A restricted rotation of the *meso*-phenyl group significantly enhances the fluorescence yield [6] (increasing viscosity), but a free rotation causes energy dissipations as non-radiative processes.

They are very tunable and easily modified and have become excellent viscosity probes. Moieties are mainly added at the *meso*- and the 2- and 6- positions. The rotation of the phenyl group around the C-C bond can be restricted by adding substituents at positions 1 and 7 in the BODIPY core or at the *ortho*-positions of the phenyl group [3]. As the viscosity increases, these FMRs enhance the fluorescence quantum yields and lifetimes. The basic structure of all BODIPY molecular rotors is shown below.

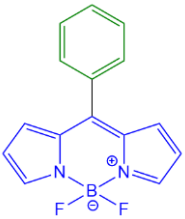


Figure 7. Basic structure from BODIPY-based FMRs. Rotor (green) and stator (blue).

3.2.4. Rhodamine-based FMRs

Rhodamine dyes are widely used due to their good spectroscopic properties (e.g. long absorption and emission wavelengths, high FL quantum yield).

However, most of the dyes have absorption/emission wavelengths which are below 560/580 nm that may result in self-absorption originating FL detection errors [12].

They basically consist of the rhodamine core structure (a subset of the triarylmethane dyes and xanthene) that has been substituted to increase the fluorescence. More information on their structure can be found in the discussion.

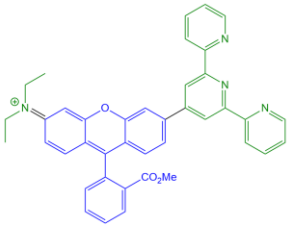


Figure 8. Rhodamine-based FMR. Rotor moiety coloured in green and stator moiety in blue.

3.2.5. Other classes of FMRs

Other popular classes of FMRs can be Dioxaborine-based FMRs and Porphyrin-based FMRs. Dioxaborine-based FMRs are very similar to BODIPY-based FMRs as they consist of a boron bridge with enol oxygen and ketone atoms. Porphyrin derivatives can be easily modified by chemical substitution so functional groups can be added simply.

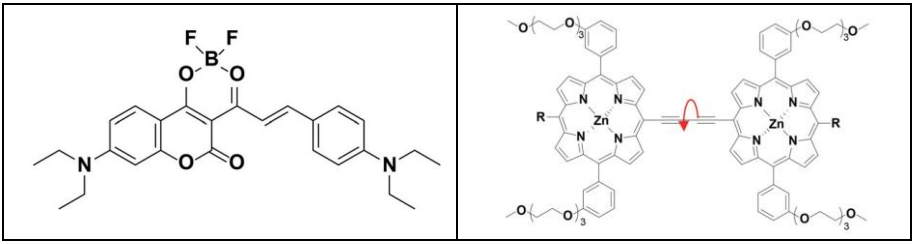


Figure 9. Dioxaborine-based (left) and porphyrin-based dimer FMRs (right); extracted from [3].

3.3. OTHER COMMON APPLICATIONS

3.3.1. Applications of FMRs in biological fields

Viscosity is closely related with fundamental cell functions. Membrane viscosity controls the activity of membrane proteins and the diffusion of molecules across the cell membrane, and cytoplasmic viscosity affects some metabolic reactions [3]. Accurate techniques for measuring microviscosity are needed. Some applications are listed below.

Cellular microviscosity: FMRs whose fluorescence intensities and lifetimes are very sensitive to molecular twisting motion, influenced by environmental viscosity, are used as probes to organelle-specific subcellular microenvironments, as they can provide more accurate information than classical techniques like the usage of capillary viscometers [3].

Organelle targeting: Organelle targeting requires chemical moieties that are similar to the target are needed [3]. For example, *Lee Seung-Chul et. al.* (2018) explain that organelle-specific anchoring motifs have been included in some studies: morpholine for lysosome targeting, N-methyl benzothiazolium for nuclear DNA or triphenylphosphonium (TPP) for mitochondrial targeting.

In vivo studies: *Lee Seung-Chul et. al.* (2018) commented that multi-photon fluorescence imaging provides deeper light penetration into the tissue with higher spatial resolution and reduced tissue damage. A two-photon FMR was used as a probe to image viscosity in vitro and in vivo in mouse liver tissue and living zebrafish using two-photon excitation.

3.3.1. Potential applications of MRs in food science

The versatility of MRs offers the possibility of direct measurements of physical properties related to food quality [4]. *F. M. Alhassawi et al*. (2018) comment that MR probes can contribute to understanding interactions between food components and the effect of microstructure on food properties, for example, in polymerization processes, protein degradation, colloidal stability, and phase states and transitions. However, their limited solubility or the fact that some of them are expensive limits their application on this field.

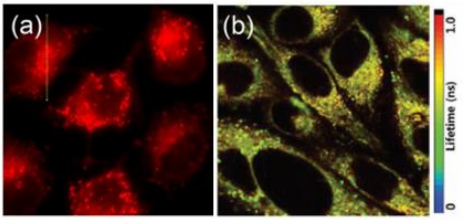


Figure 10. Cellular microviscosity imaging, a) FL and b) FLIM images of HeLa cells stained with the rotor for 30 min at 378ºC (λ_{ex}=470 nm). Extracted from [3].

4. OBJECTIVES

This work aims to carry out an extensive search and analysis of the effect of viscosity on fluorescent molecular rotors. Firstly, an introduction to what molecular rotors are and their properties will be made. Then, a classification of the different types of molecular rotors and their characteristics is going to be studied.

Each group of fluorophores will be analysed separately based on a comparison of the x values following the Förster-Hoffmann equation and their structure, and then some observations will be made from every group and rotors that share the same core structure. A general overview will be given at the end to weigh in the differences of each group.

Next, conclusions will be drawn for each group of FMRs and a comparison will be made to discuss the effect of viscosity on each. Finally, a general selection of the most suitable molecular rotors will be made and practical examples will be given.

5. METHODS

5.1. BIBLIOGRAPHIC RESEARCH

This bibliographic research has been mainly divided in three parts: the first research, the creation of the FMRs list and the research of viscosity-related parameters and features. All the articles have been found in either Web of Science or ScienceDirect databases. Depending on the goal of the research, different keywords have been used, and they are noted below on each part.

5.1.1. First research

At the beginning, after knowing the basics of the topic, the article research began. The initial search method is given below. The table summarises perfectly all the selections that have been made to get to the selected articles.

ENTRY	SEARCH IN	KEYWORDS	REFINED	NUMBER OF ARTICLES
1	Web of Science Core Collection	- "Fluorescent molecular rotor"	Review articles only	5
2	Web of Science Core Collection	- "Fluorescent molecular rotor" - "Viscosity"	Review articles only	5
3	Web of Science Core Collection	- "Molecular rotor" - "Viscosity" - "Fluorescence"	- "Quantum yield" or - "FL intensity"	4
4	ScienceDirect	- "Fluorescent molecular rotor" - "Viscosity"	-----	3

Table 1. First article research summary (topic-based). All articles found in Web of Science database have been searched on the SCI-Expanded (1900-present) edition.

A large number of articles were extracted from this search. Once the selection was made, the planning of the structure of this work began. It was decided that the different types of fluorescent molecular rotors would be studied separately, where structural information, viscosity-sensitive data and parameters would be important to differentiate between them, and also a final general overview to judge and decide the best FMRs and more suitable ones. Then, the creation of the rotors began.

5.1.2. Creation of the FMRs list

Based on the information extracted initially, four types of FMRs were going to be studied: Julolidine-based, BODIPY-based, Cyanine-based and Rhodamine-based molecular rotors. From each one, it was important to note the slope (x) on the Förster-Hoffmann equation and the graphic to see if it was fluorescence quantum yield (Φ_{FL}), fluorescence intensity (I_{FL}/I_0) or fluorescence

lifetime (τ) versus viscosity (η) (both parameters in logarithmic form). An additional search was needed to complement the information that was previously extracted from the initial research. This search was more specific and was focused on each group. Web of Science database was used here due to its enormous number of articles.

ENTRY	SEARCH IN	KEYWORDS	REFINED	NUMBER OF ARTICLES
1	Web of Science Core Collection	- "Fluorescent molecular rotor" - "Viscosity"	- "Julolidine"	5
2	Web of Science Core Collection	- "Fluorescent molecular rotor" - "Viscosity"	- "BODIPY"	8
3	Web of Science Core Collection	- "Fluorescent molecular rotor" - "Viscosity"	- "Cyanine"	9
4	Web of Science Core Collection	- "Fluorescent molecular rotor" - "Viscosity"	- "Rhodamine"	3

Table 2. FMRs list additional research summary (topic-based). All articles found in Web of Science database have been searched on the SCI-Expanded (1900-present) edition.

5.1.3. Viscosity-related research

The last step was meant to be for those molecular rotors who had a complex structure, whose parameters were not given or the graphic was not included, etc. Small details were corrected and additional information was found, such as viscosity-related behaviours of the rotors. This data was used to complement the information that was extracted previously. 5 articles were found on this step.

5.2. HISTORIC EVOLUTION OF FMRs

Fluorescent molecular rotors have been gaining attention and popularity and over the years. It seemed interesting to study the evolution of this topic over the years to see the articles published per year and compare it to notice the growth that has been made on this manner.

Web of Science database has been used to do so. There are 4193 articles that contain the topic "molecular rotor", while 651 articles result in the search of "fluorescent molecular rotor". Adding "viscosity" and clicking *refine results* drops the number of articles to 686 and 429, respectively (same as if we enter "viscosity" as a keyword and select MUST INCLUDE).

As this work studies the effect of viscosity in the design of fluorescent molecular rotors, the historic evolution will be shown of the number of articles that contain both "fluorescent molecular rotor" and "viscosity" to see the expansion that has occurred. Note that, as mentioned before, viscosity must be included on each article. The following graphic quantifies the number of articles that have been published per year.

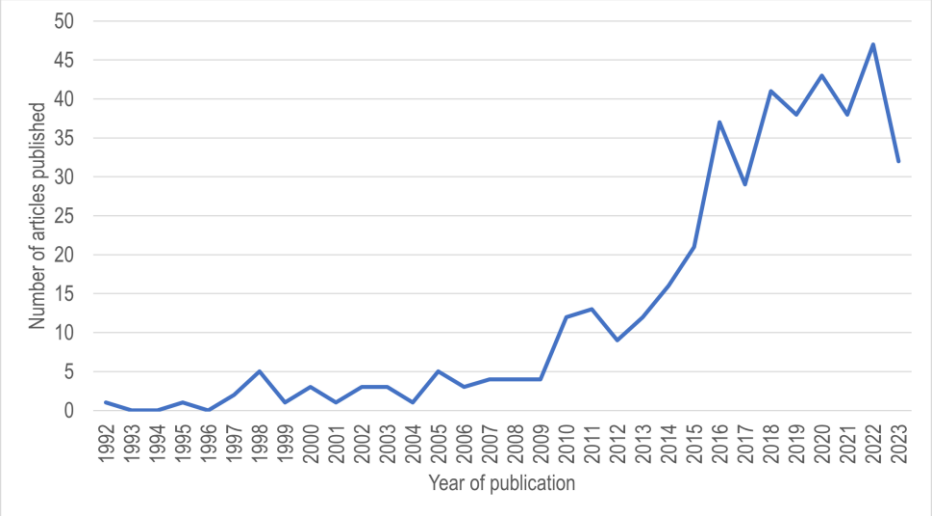


Figure 11. Number of publications per year about FMRs and viscosity.

It is clearly seen the growing tendency. 2010 was the first year in double digits, and from 2012 we can see the increase of articles published. As we are studying this, 2023 has not finished so the final number may be different than what it's showed here. The year with the most publications was 2022 with 47 articles published.

Also, it is interesting to know the top researchers of this field. Marina Kuimova (Imperial College London, Aarhus University, University of Nottingham) has a total of 54 articles published

on Web of Science, followed by Mark A. Haidekker (University of Georgia, University of Missouri Columbia, University of California San Diego, University of Bremen), who has 30 articles, and Aurimas Vysniauskas (Center for Physical Sciences & Technology – Lithuania, Imperial College London, Vilnius University) who has a total of 21 articles. Spain as a region has a total number of 17 articles published.

6. RESULTS AND DISCUSSION

All groups of FMRs studied show different viscosity behaviour due to differences in their core structure. It also strongly depends on the addition of substituents on key positions that affect the molecule's ability to rotate. A deep look into the structural changes must be taken in order to extract some conclusions about the rotor's ability to rotate as viscosity increases. FMRs will be divided in the four main families mentioned before and will be analysed separately. After, a general overview will be provided to compare them.

A careful choice must be made in order to have optimal conditions, as some aspects have direct influence over the results that can be extracted. Solvent properties such as the type of solvent, the dielectric constant or the solvent's viscosity as well are important when choosing. Fluorescent molecular rotors have different behaviours depending on the solvent, as they show different emission and absorption intensities.

For example, *M. A. Haidekker et al.* (2005) studied the influence of solvent polarity and viscosity on several compounds: three coumarin derivatives, DMABN (4,4-dimethylaminobenzonitrile) and the molecular rotor DCVJ (9-(dicyanovinyl)-julolidine). They said that peak intensity of the molecular rotor was influenced strongly by solvent viscosity. The solvent properties were also studied, as the next table resumes.

Solvent	Solvent type	Dielectric constant ϵ	Viscosity η (mPa s) (20 °C)
Water	Polar protic	80.1	1
Ethylene glycol	Polar protic	37.7	13.5
Glycerol	Polar protic	42.5	945
Ethanol	Polar protic	25	1.2
Methanol	Polar protic	33.6	0.59
Dimethylsulfoxide	Dipolar aprotic	48.9	2.47
Dimethylformamide	Dipolar aprotic	38.3	0.85
Acetone	Dipolar aprotic	20.7	0.32
Methylene chloride	Nonpolar	9.14	0.44
Benzene	Nonpolar	2.27	0.65
Toluene	Nonpolar	2.39	0.59
Hexane	Nonpolar	1.89	0.31

Figure 12. Solvent properties, extracted from [8].

It can be seen that ethylene glycol and glycerol are the top two solvents with higher viscosity. The following graphic shows the emission spectra of 9-(dicyanovinyl)-julolidine in different solvents. Emission intensity increases in ethylene glycol and mostly in glycerol, which shows high viscosity.

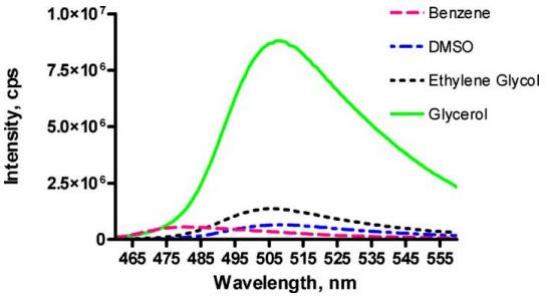


Figure 13. Emission spectra of DCVJ in different solvents, extracted from [8].

6.1. JULOLIDINE-BASED FMRs

Julolidine-based FMRs consist of two moieties, one being the julolidine (heterocyclic aromatic compound) and the other moiety being the one that can be derived, but always contains one cyanovinyl group. The julolidine part is the stator while the other moiety is the rotor. Depending on the group inserted, the rotor has different spectral properties and shows a unique behaviour to viscosity. FMRs will be studied in groups of similar structure to ease the understanding.

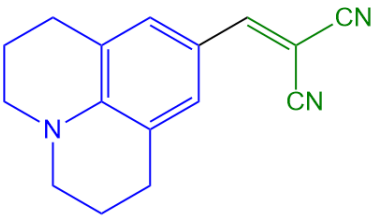


Figure 14. Structure of julolidine-based FMR **JL_1**. The stator (blue) and rotor (green) can be differentiated. All julolidines share the same core and the rotor moiety is the one that differentiates between them.

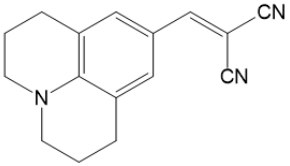
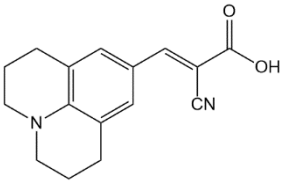
STRUCTURE	NAME	*x* VALUE	CONDITIONS	REF.
	**JL_1**	x = 0.40	Ethylene glycol - glycerol	[8]
		x = 0.58		[13]
	**JL_2**	x = 0.72	Dextran (77.8 kDa)	[14]
		x = 0.64	Dextran 249 kDa : 77.8 kDa (2:1)	[14]
		x = 0.49	Dextran (249 kDa)	[14]
		x = 0.52	Ethylene glycol - glycerol	[15]

Table 3. Parameters for julolidines **JL_1** and **JL_2** on different conditions.

In **JL_1** , known as 9-(dicyanovinyl)-julolidine, intramolecular rotation occurs around the single C-C bond between the dicyanovinyl and the julolidine groups [3].

JL_1 has increased emission intensity in viscous solvents such as ethylene glycol and mostly glycerol compared to other options such as ethanol, water or any apolar solvent like benzene or toluene [8] and shows poor water solubility [13].

JL_1 is based on an electron-donating nitrogen, located inside the julolidine group, and two electron-accepting nitriles, and it follows strictly the power-law relationship of their emission intensity with the solvent's viscosity. This julolidine-based FMR shows a good behaviour and sensibility to viscosity changes, so it can be useful as a probe.

The rotor moiety consisting of the dicyanovinyl group rotates freely on low-viscous solvents, inducing the non-radiative thermal relaxation of the excitation energy, but the increase of viscosity hinders the rotation of the group, increasing the fluorescence emission intensity. 9-(dicyanovinyl)-julolidine is easily modified into other julolidine-based FMRs like 9-(2-Carboxy-2-cyanovinyl)julolidine, which happens to be **JL_2**, as one cyanide group is substituted by a carboxy.

It can clearly be seen that the dye's sensitivity decreases with increasing dextran's molecular weight (MW) comparing the x values, as it shows a slope value of 0.72 in dextran with a molecular weight of 77.8 kDa and 0.49 in dextran 249 kDa [14]. Their rotation becomes increasingly restricted as viscosity increases and steric hindrance on the rotor grows.

A mixture of dextran solutions with different MW and proportion produced a slope value between both, so the spherical nature of dextran must be important, as the rotation of the rotor itself would be more restricted in a solution with smaller spheres than bigger with the same viscosity, since higher particle density is needed to have the same viscosity.

This behaviour may be caused by direct non-covalent interaction of the dye with hydrophobic sections of the starch molecules, an effect that would withdraw some of the dye from the free-volume interaction [15].

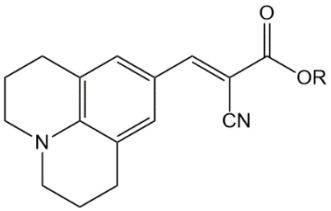
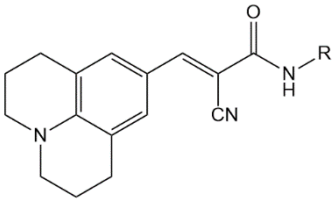
STRUCTURE	NAME	R GROUP	*x* [REF.]	COND.
	**JL_3**	R = CH_3	x = 0.67 [13]	A
	JL_4	R = C_2H_5	x = 0.60 [13]	A
	JL_5	R = ***n*** C_4H_9	x = 0.60 [13]	A
	JL_6	R = ***n*** $C_{20}H_{41}$	x = 0.49 [13]	A
	JL_7	R = $(CH_2CH_2O)_2CH_2CH_3$	x = 0.61 [15]	A
			x = 0.49 [8]	A
	JL_8	R = $(CH_2CH_2O)_3CH_3$	x = 0.59 [15]	A
			x = 0.44 [15]	B
	JL_9	R = farnesyl	x = 0.56 [13]	A
	**JL_{10}**	R = phospholipid 1	x = 0.52 [11]	A
	JL_{11}	R = phospholipid 2	x = 0.53 [11]	A
	JL_{12}	R = phospholipid 3	x = 0.48 [11]	A
	JL_{13}	R = phospholipid 4	x = 0.21 [11]	A

Table 4. Parameters for substituted julolidine-based FMRs on different conditions: **Conditions A** (Ethylene glycol – glycerol mixture) and **Conditions B** (water – dextran mixture). For julolidines **JL_{11}** and **JL_{10}** to **JL_{13}** see **Appendix 1: Complex Julolidines** for R GROUP structure information.

Julolidines **JL_3** to **JL_9** share the same core structure as they are based on the ester form of the julolidine-based rotor. It can be seen that an increase in the length of the R chain makes the rotor less sensible to viscosity changes as the slope value is reduced. Also, the intensity increases with increasing concentration of dextran. Furthermore, all compounds show similar x values [13], indicating that the photophysical properties have not changed by the chain attachment to the rotor, meaning that the modifications do not affect the molecule's ability to rotate.

A lower value was obtained for **JL_8** on a water – dextran solution. As mentioned before, the steric nature of dextran must affect the rotor's ability to rotate, hindering the molecule and impeding it, making globally the rotor less sensible. The same rotor on different conditions (ethylene glycol – glycerol mixture) had a different outcome, as the sensibility on a more polar solvent was way bigger (**JL_9**).

On the other hand, julolidines **JL_{10}** to **JL_{13}** contain an amide group of different size, where the R groups contain a phospholipid backbone, as *M. A. Haidekker et. al.* (2002) synthesized a new family of MR that would increase the membrane localization profile and be used as a membrane viscosity sensor in biological processes, and also be localized in the cell interior.

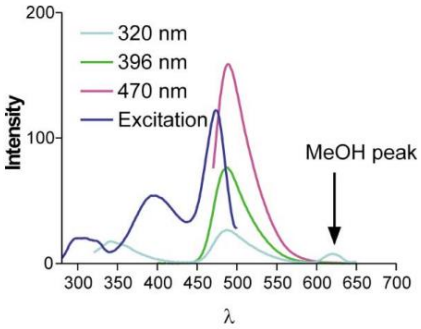


Figure 15. Excitation and emission spectra of the phospholipid-bound molecular rotor **JL_{10}**. Methanol is used as a solvent and shows a small peak at 620 nm, only visible for 320 nm excitation. Extracted from [11].

All (except **JL_{13}**) show a good sensitivity to viscosity changes, increasing the intensity of fluorescence emission as viscosity increases. They also have a decent slope value, meaning that the rotor–environment interaction does not change if the rotor is attached to the hydrophobic end of the phospholipid [11].

JL13 with a lower x value, has the rotor attached to the polar end of the phospholipid, so interactions within the molecule occur, which are responsible for the inhibition of free interaction with the environment, resulting in a decrease of viscosity sensitivity.

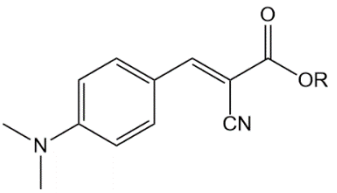
STRUCTURE	R GROUP	NAME	*x* [REF.]	COND.
	$R = CH_3$	**JL14**	x = 0.44 [15]	B
			x = 0.32 [15]	A
	$R = CH_2CHOH(CH_2OH)$	**JL15**	x = 0.54 [15]	A
			x = 0.37 [15]	B

Table 5. Parameters for open julolidine-based FMRs on different conditions: **A** (Ethylene glycol – glycerol mixture) and **B** (water – dextran mixture).

JL14 and **JL15** are based on the open form of a julolidine FMR. Both contain an ester, one having a methyl group and the other having a chain with two alcohol groups. It can be seen the choice of solvent's impact on both cases, as they have quite different values on each case. **JL14** is more sensible to viscosity changes in a water – dextran mixture (more apolar). A low value of x is caused by a saturation effect at high viscosities, where intensity no longer follows the power-law relationship at high viscosities [15].

JL15 is more sensible in the ethylene glycol – glycerol mixture, which is polar and protic, as the rotor itself also contains alcohol groups that can interact with the solvent and make hydrogen bonds that steady the molecule, decreasing its ability to rotate but increasing the fluorescence intensity and sensibility to viscosity changes.

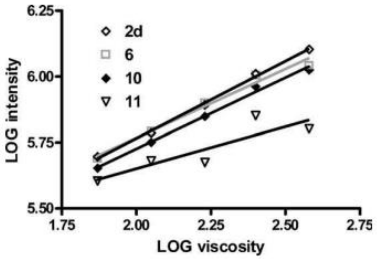


Figure 16. Double-logarithmic scale (emission intensity over the solvent viscosity) of dyes **JL$_8$** (2d), **JL$_1$** (6), **JL$_{15}$** (10) and **JL$_{14}$** (11), following the Förster-Hoffmann equation (4). Solvent is based on a mixture of ethylene glycol and glycerol. Extracted from [15].

6.2. BODIPY-BASED FMRs

The capacity to rotate for BODIPY-based FMRs depends on the solvent's viscosity and the ability of the benzene ring to rotate freely without being impeded. Any modifications on the benzene ring or nearby positions of the core may influence the rotation of the meso-phenyl group.

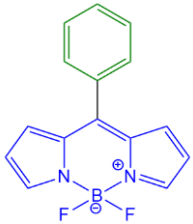


Figure 17. Structure of the BODIPY-based FMR **BDP$_1$**. The stator (blue) and rotor (green) can be differentiated.

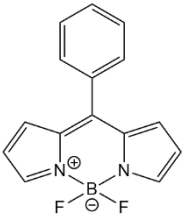
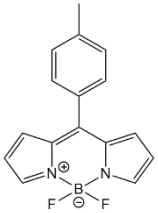
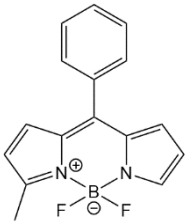
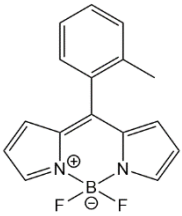
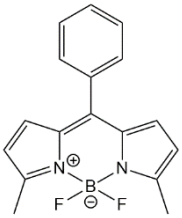
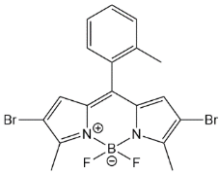
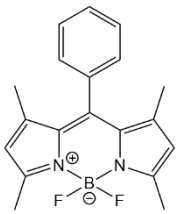
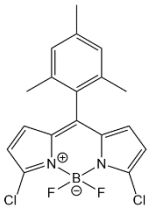
STRUCTURE	NAME	*x* [REF.]	STRUCTURE	NAME	*x* [REF.]
	**BDP$_1$**	x = 0.56 [16] [17] **1 ; 3** x = 0.76 [17] 2		**BDP$_5$**	x = 0.60 [7] **1**
	**BDP$_2$**	x = 0.49 [16] **1**		**BDP$_6$**	x = 0.06 [7] **1**
	**BDP$_3$**	x = 0.22 [16] **1**		**BDP$_7$**	x = 0.04 [7] **1**
	**BDP$_4$**	x = 0.06 [16] **1**		**BDP$_8$**	x = 0.02 [7] **1**

Table 6. Parameters for BODIPY-based FMRs. Study of the effect of the methyl group on the pyrrole and benzene rings. Results obtained in ethlyene glycol and glycerol ([1]), in methanol and glycerol ([2]) and in toluene and Castor oil ([3]). See **Appendix 2: Castor Oil structure** for structural information.

The previous table resumes the effect that the addition of methyl groups has on the pyrrole and benzene rings.

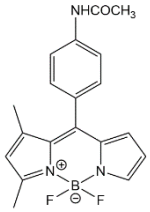
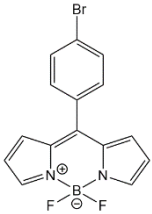
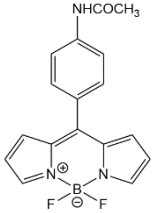
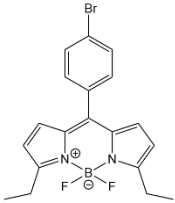
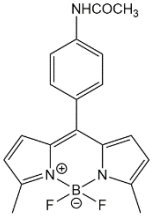
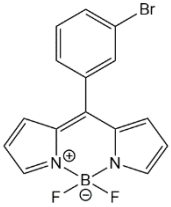
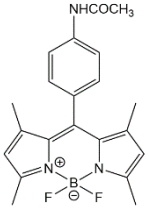
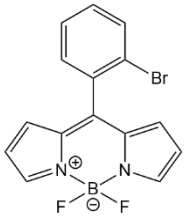
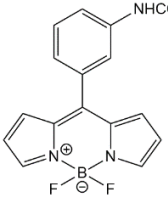
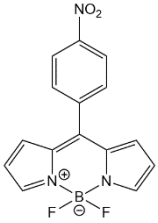
Molecule **BDP_1** is the unsubstituted BODIPY, which shows good sensibility to viscosity changes of the solution. The derivatives containing methyl groups show a lower slope value as they cannot rotate totally free like the first one.

BDP_1 shows different values of x obtained from different sources, who studied the viscosity-sensitivity in viscous solvents with different polarity, as some dyes are sensitive to their polarity or their ability to form hydrogen bonds [17]. Mixtures of ethylene glycol / glycerol (high polarity), methanol / glycerol (moderate polarity) and toluene / Castor oil (non-polar) were used. It can be seen that the rotor behaves well in all cases, especially in the polar and protic mixture of methanol and glycerol (x = 0.76).

Rotors **BDP_2** and **BDP_3** show a decreasing value as methyl groups are added to the pyrrole rings, meaning that the rotation of the benzene ring is slowly being obstructed, possibly due to steric hindrance [16], enhancing fluorescence. *Liu, Xiao, et al.* (2020) note that the rotation of the phenyl ring goes along with a considerable bending of the BODIPY scaffold (core) that raises the steric repulsion between the methyl substituents.

BODIPY **BDP_4** shows almost no sensitivity to viscosity changes, as both newly added methyl groups hinder effectively the motion of the phenyl group [16]. The decrease of sensitivity from **BDP_1** to **BDP_4** demonstrates that the modification of the pyrrole rings can affect the response of BODIPY molecular rotors to viscosity severely without modifying the benzene ring.

Rotors **BDP_5** to **BDP_8** study the same effect but on the benzene ring. The position of the methyl group directly affects the molecule's ability to rotate in a viscous media. **BDP_5** with the methyl group in *para*- position has an excellent sensitivity as it does not affect at all, while rotors **BDP_6** and **BDP_7** show a big insensitivity to viscosity as the methyl group in *ortho*- position and the addition of the bromide and methyl groups to the pyrrole rings hinders almost completely, which is maximized in **BDP_8** with two methyl groups in the *ortho*- positions.

STRUCTURE	NAME	*x* [REF.]	STRUCTURE	NAME	*x* [REF.]
	**BDP$_9$**	x = 0.73 [7] 1		**BDP$_{15}$**	x = 0.64 [7] 1
	**BDP$_{10}$**	x = 0.60 [7] 1 x = 0.59 [16] 1		**BDP$_{16}$**	x = 0.36 [7] 1
	**BDP$_{11}$**	x = 0.33 [7] 1		**BDP$_{17}$**	x = 0.63 [7] 1
	**BDP$_{12}$**	x = 0.14 [7] 1		**BDP$_{18}$**	x = 0.07 [7] 1
	**BDP$_{13}$**	x = 0.55 [16] 1		**BDP$_{19}$**	x = 0.65 [17] 2 x = 0.58 [17] 3

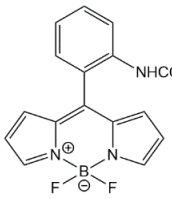
	**BDP$_{14}$**	x = 0.39 [16] 1			

Table 7. Parameters for BODIPY-based FMRs. Study of the effect of the amide, bromide and nitro groups on the benzene ring. Results obtained in ethylene glycol and glycerol (1), in toluene and Castor oil (2) and in methanol and glycerol (3). See **Appendix 2: Castor Oil structure** for structural information.

Dyes **BDP$_9$** to **BDP$_{14}$** present the effect that the amide group on the benzene ring has to the molecule's viscosity-sensitivity. **BDP$_9$** has the acetamide group in *para*- position and, interestingly, despite having two methyl groups on one pyrrole ring, exhibits the highest sensitivity of this group, followed by **BDP$_{10}$** which has the acetamide group in *para*- position as well but does not have methyl groups. It is thought that the transformation energy barrier of **BDP$_9$** between the excited states is lower than that of **BDP$_{10}$** [7].

BDP$_{11}$ has a relatively high quantum yield because both methyl groups inhibit the conformational conversion to the non-emissive state. **BDP$_{12}$** has a small response due to the effective motion hindering by the four methyl groups of the pyrrole rings [7].

BDP$_{13}$ and **BDP$_{14}$** contain the acetamide group in *meta*- and *ortho* positions, respectively. Comparing the *x* value of them with **BDP$_{10}$** (*para*- position) can be crucial to understand the effect that positioning and steric hindrance has. The presence of an acetamido group at the *ortho*-position of the *meso*-phenyl ring reduces its viscosity sensitivity , but functionalization of the *meta*- and *para*- positions leads to a more viscosity-sensitive molecular rotor [16].

Among these three compounds, **BDP$_{10}$** containing the amide group in *para*- position has the best sensitivity. These results suggest that the *ortho*- modification of the phenyl ring is not effective, while the *para*- and *meta*- substitutions were relatively good to obtain a viscosity-sensitive FMR [16].

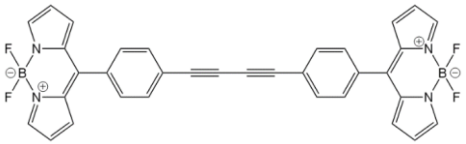
STRUCTURE	NAME	*x* [REF.]
	**BDP$_{20}$**	x = 0.46 [18]

Table 8. Parameters for the BODIPY-based dimer FMR, obtained in ethanol – glycerol mixture.

BDP$_{20}$ is an homodimeric BODIPY dye, consisting of two BODIPY cores linked by two conjugated triple C–C bonds. At low viscosities, the fluorescence intensity was minimally variant as the viscosity dependent rotational resistance in the dimer became insignificant [18]. This rotor shows a good sensitivity towards the increase of viscosity. The following figure exhibits the emission spectra of the BODIPY dimer in different viscosity mixtures of ethanol and glycerol. It can be seen that changing the viscosity of the media does not affect the peak emission wavelength. Increasing the viscosity of the media increased the emission intensity and fluorescence quantum yield [18].

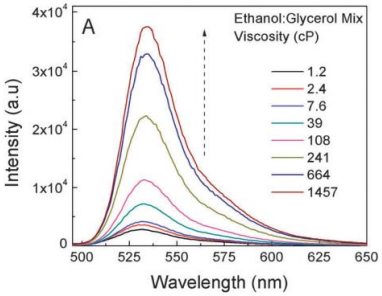


Figure 19. Emission spectra of the BODIPY dimer in different viscosity mixtures of ethanol and glycerol. Extracted from [18].

6.3. CYANINE-BASED FMRs

Cyanine-based FMRs have excellent properties and have been recently used for organelle viscosity imaging, mainly lysosomal and mitochondrial activity to target cell membrane viscosity [19].

Cyanine dyes are easily tunable by modifying the length of the π-conjugated polymethine chain [3].

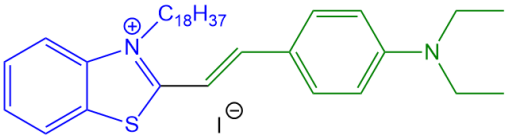


Figure 20. Structure of cyanine-based FMR **CY3**. Stator (blue) and rotor (green) moieties.

All cyanine-based FMRs share the same structural core, consisting of a conjugated bridge followed by the two moieties, usually containing N atoms that have a positive charge. This core has two contributing structures by resonance, depending on which N atom has the positive charge, which is delocalized through the conjugated system, and the rotor gains a little more.

Figure 21. Contributing structures of the cyanine-based core of FMRs.

Yiping Cai, et. al. (2022) designed and synthesized two novel fluorescence molecular rotors (dyes **CY10** and **CY11**) that exhibited excellent selectivity for staining intracellular lysosomes instead of mitochondria.

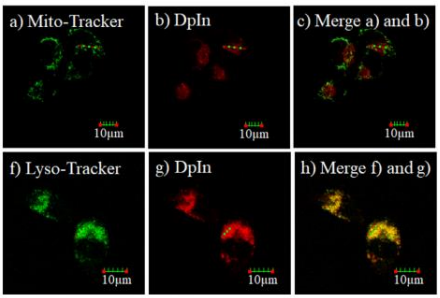
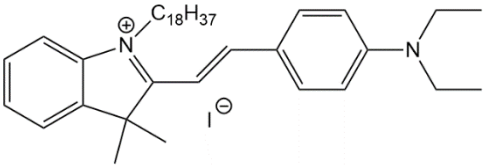
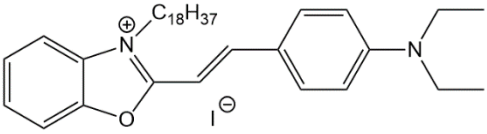
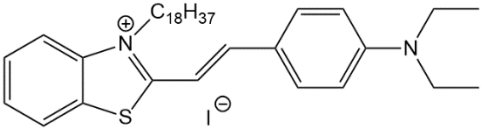
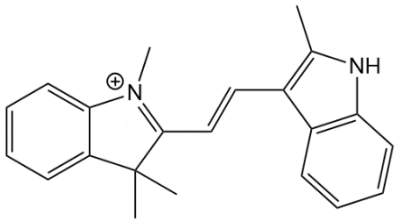


Figure 22. Colocalization fluorescence images of HeLa cells. Extracted from [20].

A and **F** show confocal images from Mito-Tracker Green (200 nM) and Lyso-Tracker Green (200 nM) in the green channel, respectively (λ_{ex} = 488 nm ; λ_{em} = 520 – 561 nm). **B** and **G** show confocal images from DpIn (1 μM) in the red channel (λ_{ex} = 635 nm ; λ_{em} = 668 – 768 nm). **C** and **H** are overlays of the green and red channels.

STRUCTURE	NAME	*x* [REF.]
	**CY_1**	x = 0.54 [19]
	**CY_2**	x = 0.54 [19]
	**CY_3**	x = 0.53 [19]
	**CY_4**	x = 0.61 [21]

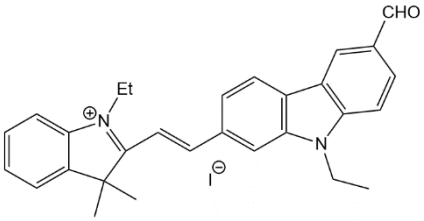
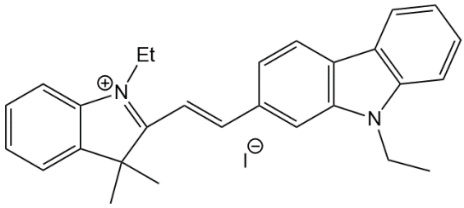
	**CY_5**	x = 0.54 [22]
	**CY_6**	x = 0.52 [23]

Table 9. Parameters for cyanine-based FMRs, studied on a water – glycerol mixture.

Dyes **CY_1** to **CY_3** adjust their sensitivity to viscosity by the rotation around the conjugated chain that links the heterocycles [19]. Thanks to their two stable resonance structures, they show improved photophysical properties and stability. They show excellent viscosity sensitivity with values of $x > 0.50$.

Dyes	Solvents	$\lambda^{a}_{Abs,max}$	$\lambda^{a}_{Em,max}$	Stokes shift [a]	ε^{b}	$\Phi^{c,d}$
1a	H_2O	497	–	–	4.23	0.2
1a	glycerol	523	563	40	16.1	77
1b	H_2O	520	–	–	19.5	0.3
1b	glycerol	561	599	38	26.4	67
1c	H_2O	501	–	–	3.34	0.7
1c	glycerol	551	601	50	8.37	54

[a]nm; [b]$\times 10^4$ M^{-1} cm^{-1}; [c]%; [d]Cresyl violet ($\Phi = 0.578$ in ethanol) was used as the standard, –: hardly detectable.

Figure 23. Optical properties of **CY_2** (1a), **CY_1** (1b) and **CY_3** (1c) in water and glycerol [19].

Their fluorescence intensity also grows with increased viscosity and not with polarity, as it was observed [19]. *Ya-Nan Wang et al.* (2021) detected the fluorescence responses of the three dyes to low-viscosity solvents, and it could be seen that the intensity did not change in solvents with different polarities.

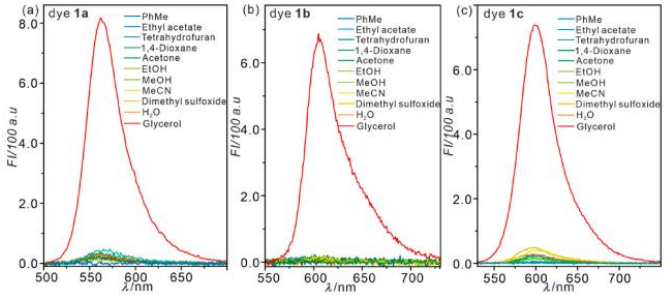


Figure 24. Fluorescence spectra of dyes **CY$_2$** (1a), **CY$_1$** (1b) and **CY$_3$** (1c) in various solvents with different polarities. Dye **CY$_2$** (λ_{ex} = 473 nm), dye **CY$_1$** (λ_{ex} = 514 nm) and **CY$_3$** (λ_{ex} = 520 nm). Extracted from [19].

CY$_4$ shows a greater sensitivity to viscosity than the previous dyes. The ethyl group is attached to the nitrogen atom instead of the large carbon chain of dyes **CY$_1$**, **CY$_2$** and **CY$_3$**, resulting in a lower steric hindrance meaning that the rotor can rotate more freely and fluorescence is enhanced. It also contains two rotational sites so it may influence in the fluorescence intensity as well.

Dye **CY$_5$** also has two rotating sites (aldehyde group and around the conjugated chain) which makes the rotor very sensitive towards viscosity [22]. It shows a strong fluorescence emission in a high viscous media. *Fei Liu et. al. (2018)* comment that the nonradiative deactivation was mainly accessed upon rotation of the bridge, and when the free rotation was restricted, an increase in the fluorescent quantum yield would be observed, and that with the aldehyde group it also resulted in great changes in the plotted slope.

Cyanine **CY$_6$** exhibits the same structure than **CY$_5$** without the aldehyde group. The emission of rotor **CY$_6$** was greatly enhanced in its fluorescence intensity in solvents of high viscosity [23]. The lower slope value than **CY$_5$** could be caused by the absence of the aldehyde group, which can rotate and enhance fluorescence.

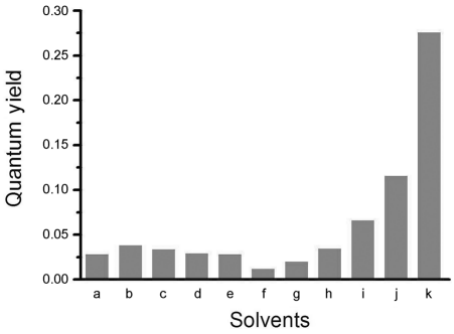
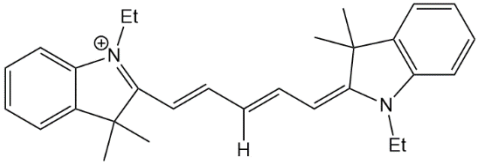
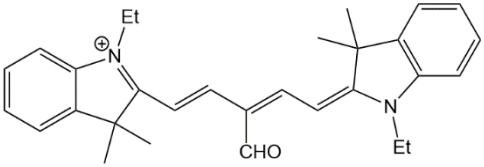


Figure 25. Histogram that shows the fluorescence quantum yield of dye **CY_6** (1 μM) in different solvents at λ_{em} = 580 nm. Solvents are a) dichloromethane, b) DMSO, c) ethanol, d) dioxane, e) methanol, f) water, g) water/glycerol (8:2 v/v), h) water/glycerol (6:4v/v), i) water/glycerol (4:6 v/v), j) water/glycerol (2:8 v/v), k) glycerol (99%). Extracted from [23].

STRUCTURE	NAME	*x* [REF.]	COND.
	**CY_7**	x = 0.04 [24]	D
	**CY_8**	x = 0.30 [24]	D

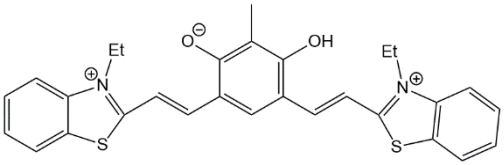
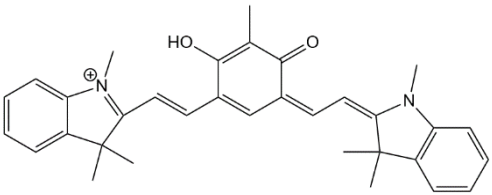
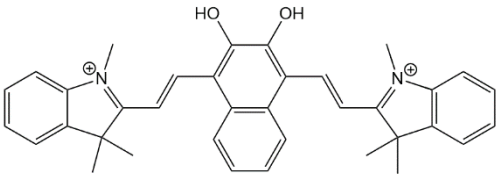
	**CY_9**	x = 0.57 [25]	C
	**CY_{10}**	x = 0.15 [20]	C
	**CY_{11}**	x = 0.32 [20]	C

Table 10. Parameters for cyanine-based FMRs. **Conditions C** (Water - glycerol), **Conditions D** (Ethanol - glycerol).

The big difference in sensitivity on dyes **CY_7** and **CY_8** containing an hydrogen atom and aldehyde group at the *meso*-position may be caused to the different substituent located at the central position of the conjugated chain, as with H, the dye has a high quantum yield but is not good for viscosity sensing, and with the aldehyde, the bulky substituent can rotate or vibrate and change the geometry of the molecule in the excited state [24]. Even though, they still show poor values of sensitivity ($x \leq 0.50$) so they cannot be contemplated as good or suitable options, but they have enhanced fluorescence emission intensity.

Molecular rotor **CY_9** shows a good sensitivity towards viscosity ($x = 0.57$). Because of the free rotation of a single bond between the cationic benzothiazole moiety and styryl unit, emission was observed in viscous medium [25].

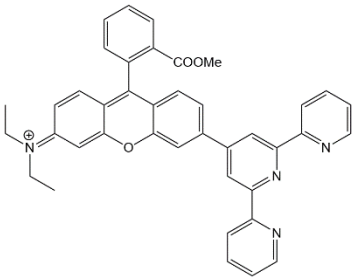
More importantly, this molecule shows a donor-two-acceptor system involving a phenolate donor unit and two benzothiazolium acceptor moieties, so it is influence by the pH as well.

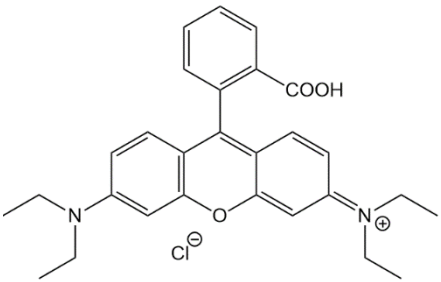
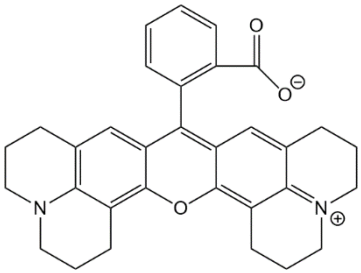
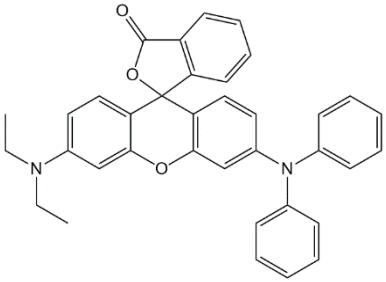
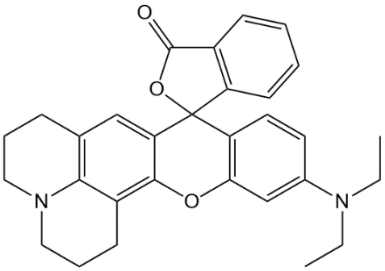
Xiaoxi Yin et al. (2020) comment that in an acid media (range of pH of 1.0 – 4.0), the molecule remained in a protonated dicationic diphenol form, and as the pH value was increasing (range of 5.0 – 9.0) it formed a monocationic cyanine-like keto form. The viscosity sensing property of the rotor demonstrated that the phenolate form underwent an ICT process and emitted a deep-red fluorescence when free rotation between cationic benzothiazene and the styryl unit was restricted by the viscous environment [25]. When the solution's pH was in range of pH > 10.0, both deprotonations had occurred and two donor moieties formed instead of the donor-acceptor pair fluorophore.

Cyanines **CY_{10}** and **CY_{11}** are modifications of the previous probe **CY_9**. **CY_{11}** exhibits more sensitivity towards viscosity changes, which can be attributed to the free rotation of the indolium and bridge moiety along the single bond of the vinyl linker [20]. *Yiping Cai et. al.* (2022) supposed that a lower viscosity solvent system, the rotation rate of *ortho*-dihydroxynaphthalene donor and indolium acceptor along the vinyl groups was faster than that in a higher viscosity solution.

6.4. RHODAMINE-BASED FMRs

Rhodamines are extensively used as fluorescent probes, chemosensors in the detection of small molecules, sensitizers in dye sensitized solar cells and as laser dyes [26], as their photophysical properties can be modified by modifying the amino groups of the xanthene moiety, the carboxyphenyl ring or the carboxylic acid group.

STRUCTURE	NAME	*x* [REF.]	COND.
	**RHO_1**	x = 0.41 [27]	Water - glycerol

	**RHO_2**	x = 0.04 [26]	Methanol - glycerol
	**RHO_3**	x = 0.04 [26]	Methanol - glycerol
	**RHO_4**	x = 0.42 [26]	Methanol - glycerol
	**RHO_5**	x = 0.09 [26]	Methanol - glycerol

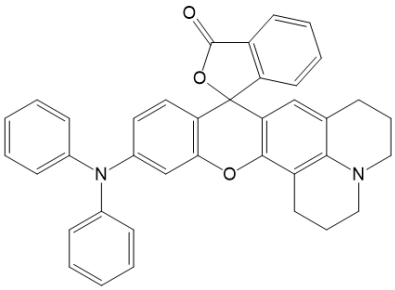
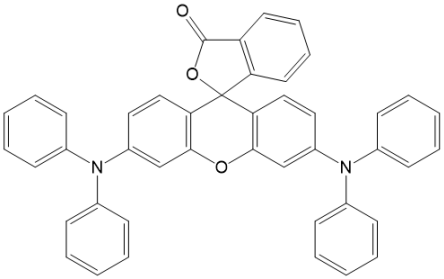
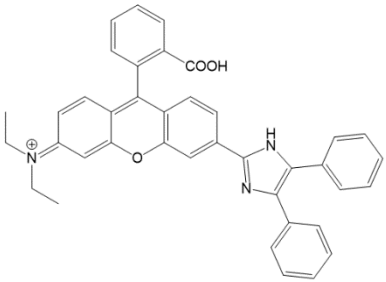
	**RHO$_6$**	x = 0.41 [26]	Methanol - glycerol
	**RHO$_7$**	x = 0.45 [26]	Methanol - glycerol
	**RHO$_8$**	x = 0.58 [12]	Methanol - glycerol

Table 11. Parameters for Rhodamine-based FMRs.

RHO$_1$ has an acceptable sensitivity towards viscosity (x = 0.41 [27]). It contains a big terpyridyl group that is very bulky, so an increase of viscosity should hinder the rotation of the group and enhance fluorescence emission. As *Peisheng Tang et. al.* (2023) report, the viscosity increase probably hinders the rotation of the terpyridyl group and reduces the non-radiation decay, and as the nonradiative decay rate decreases with increasing viscosity, it contributes to both emission intensity and lifetime sensitivity.

Rhodamines **RHO$_2$** and **RHO$_3$**, also known as Rhodamine B and Rhodamine 101, respectively, share the same core but are differently substituted. The two dyes show very poor sensitivity towards viscosity, as they clearly indicate the effect of phenyl substituent for rigidization of the system in viscous medium [26] that, given both cases, is not happening as it can rotate more freely than the other cases with no steric interactions, leading to a non-radiative relaxation that does not emit fluorescence.

Rhodamines **RHO$_2$** and **RHO$_3$** containing a positive charge on one N atom have two contributing structures each due to the conjugated system that they present, which results in a delocalization of the charge through it and the two twin N atoms.

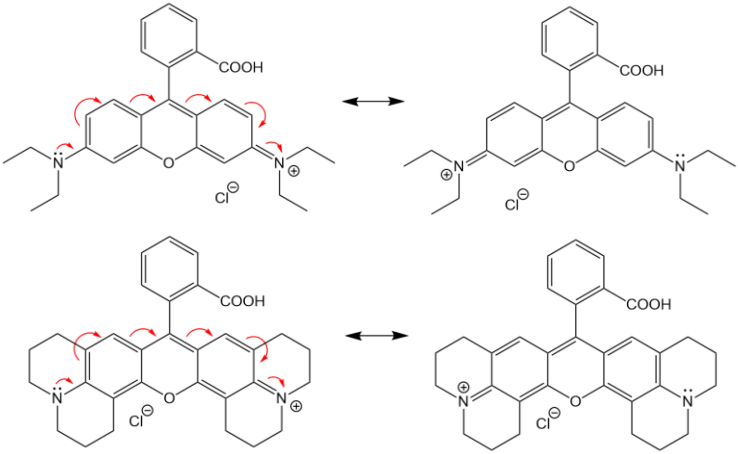


Figure 26. Contributing structures of Rhodamines **RHO$_2$** (up) and **RHO$_3$** (down).

Dye **RHO$_8$**, despite having the same core as the previous cases, shows a good sensitivity ($x > 0.50$). In a low-viscous media, the rotation between the imidazole ring and the xanthene core is not impeded, which leads to the non-radiative decay of the excited state, weakening the fluorescence emission of the probe. However, as viscosity increases, the free rotation of the imidazole moiety (very bulky as it contains two phenyl groups) is hindered, which reduces the non-radiative pathway and enhances the fluorescence emission [12].

On the other hand, dyes **RHO$_4$** to **RHO$_7$** sharing the same core clearly show different sensitivities to viscosity given the variation of the amino-substituents.

Rhodamine **RHO$_4$** has a good sensitivity (x = 0.42 [26]) due to the fact that it contains a diethylamine group that is not bulky and does not affect at all, and a diphenylamine group that hinders the rotation of the system, enhancing fluorescence emission as viscosity increases. Dye **RHO$_5$** containing a diethylamine and cyclic amine group shows a poor slope (x = 0.09 [26]) because the system is not rigid at all and it can rotate in the viscous medium, with the relaxation occurring in a non-radiative way.

Rhodamine **RHO$_6$** has a good sensitivity (x = 0.41 [26]) as it contains, like **RHO$_4$**, a diphenylamine group that hinders the rotation due to steric interactions with the system. From both dyes and x values given we can extract that the diethylamine and cyclic amine groups are not useful to enhance fluorescence intensity as they do not impede the rotation of the system. This hypothesis can be confirmed with **RHO$_7$**, incorporating two diphenylamine groups that provoke a major rigidization of the system, increasing with increased viscosity, as their steric hindrance is bigger than the other cases (x = 0.45 [26]).

6.5. GENERAL OVERVIEW

After reviewing all four types of fluorescent molecular rotors studied, a general overview of all four classes is needed in order to extract some global observations. A final classification of the dyes with the greater sensitivity of all families will be done to compare every class with the other ones.

Julolidine-based FMRs show a good response towards solvent's viscosity with great values of x obtained. They can be easily substituted by replacing one of the cyano groups of the dicyanovinyl moiety (electron withdrawing group). This chemical substitutions allow the introduction of some functional groups that modify the photo-physical properties of the rotors [3]. The rotation happens around the single bond between the vinyl and julolidine groups, the stator being the julolidine moiety and the rotor the vinyl group, so the substitution of one cyano group for another one becomes very important as it influences directly the rotors sensitivity towards viscosity changes.

By analysing the data collected, good examples of sensitive julolidine-based FMRs are 9-(dicyanovinyl)-julolidine and 9-(2-carboxy-2-cyano)vinyl julolidine, the two most popular options on this class. Moreover, substituted julolidines that are suitable rotors happen to be ester forms of the mentioned dyes.

For example, julolidines **JL$_3$**, **JL$_4$** and **JL$_5$** show excellent values of sensitivity towards viscosity (x = 0.67 [13] ; x = 0.60 [13] ; x = 0.60 [13], respectively) containing small alkyl chains. The addition of larger chains provokes an important decrease on the rotor's sensitivity to environmental viscosity.

Amide derivatives are good options as well. Considering the same criteria as the previous cases with julolidine ester derivatives, amide dyes show a better response when the added group is smaller, but the sensitivity does not change that much. The attachment of the rotor to the polar end of the phospholipid is found to have a reduced x value (**JL$_{13}$** [11]). Adding a bulky groups decreases the rotors sensitivity towards viscosity as well. Open julolidine-based FMRs have less sensitivity towards solvent's viscosity than the closed ones, and they show a lower enhancement on fluorescence emission intensity as viscosity increases.

In general, suitable choices for julolidine-based FMRs could be dyes **JL$_1$**, **JL$_2$**, **JL$_3$**, **JL$_4$**, **JL$_5$**, dyes **JL$_7$** and **JL$_8$** in ethylene glycol – glycerol mixture, **JL$_9$** and **JL$_{11}$** applied to practical and specific cases [11].

An adequate selection of a BODIPY-based FMR is necessary as well in order to have a good dye with a satisfactory behaviour towards environmental viscosity. After reviewing all experimental cases mentioned before, it can clearly be seen that there are some key positions on the BODIPY core that need to have small groups to minimize steric hindrance so that the molecule can rotate more freely.

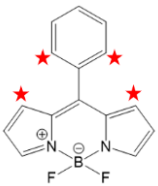


Figure 27. Key positions that have a bigger impact on the dye's ability to rotate.

The *ortho*- positions of the *meso*-phenyl ring and the marked positions of the pyrrole rings are the positions that have more influence on the rotor's ability to rotate through the viscous medium. Rotation occurs through the simple bond that connects the *meso*-phenyl ring with the core, so the addition of bulky groups at those positions hinders the rotation and enhances the fluorescence emission intensity.

Sensitive BODIPY-based FMRs with excellent *x* values are the BODIPY core itself and the dyes that have R groups in the *para*- or *meta*- positions of the *meso*-phenyl ring, or non-influential positions of the pyrrole rings. Sensitivity is lowered by adding bulky groups at the marked positions. A clear example of this observation are dyes **BDP$_1$** (x = 0.56 [16]), **BDP$_5$** (x = 0.60 [7]) and **BDP$_6$** (x = 0.06 [7]).

BDP$_1$ does not have any bulky group so the *meso*-phenyl group can rotate freely through the simple bond, becoming more sensitive towards environmental viscosity. **BDP$_5$** shows the same approximate sensitivity towards viscosity as **BDP$_1$**, and incorporates a methyl group in the *para*-position of the phenyl ring. By its value, it can be seen that it does not impede the rotation at all and the dye is sensitive to viscosity. Instead, dye **BDP$_6$** having the methyl group in *ortho*- position shows a very low sensitivity to viscosity, as this group hinders the rotation of the ring. A BODIPY-based dimer was also examined, and it shows a decent sensitivity towards viscosity.

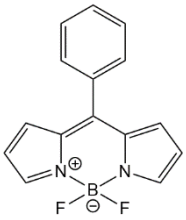
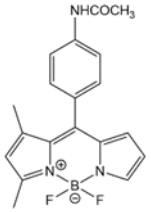
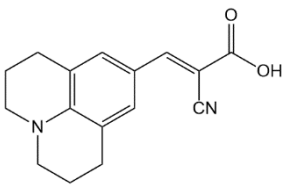
To resume, good fluorescent BODIPY-based dyes have the marked positions unsubstituted to avoid any steric hindering of the rotation. Suitable options could be dyes **BDP$_1$**, **BDP$_5$, BDP$_9$**, **BDP$_{10}$**, **BDP$_{13}$**, **BDP$_{17}$** and **BDP$_{19}$**, depending on the circumstances given, like the nature of the solvent, experimental conditions, etc.

Cyanines base their sensitivity towards viscosity to the rotation around the conjugated chain that links the heterocycles or moieties. A larger chain attached to the nitrogen atoms of the cycles leads to an increase of the sensitivity, but the presence of a bulky group in the middle of the conjugated bridge decreases the rotor sensitivity as the rotation of the bonds is more impeded. Some cyanine-based dyes have different behaviours depending on the pH of the medium, as seen with **CY$_9$** for example, so it should be considered as well. Good cyanine-based FMRs are those with large chains on the nitrogen atoms or bulky groups at the other end of the conjugated bridge, for example dyes **CY$_1$** to **CY$_6$** and **CY$_9$**.

Rhodamine dyes, as well as cyanines, have not been studied that much as the two first classes of rotors, but show promising results. Their sensitivity towards viscosity is based on the substitution of both amino groups of the xanthene core and the carboxyphenyl ring. Good sensors of environmental viscosity are those with two phenyl groups attached to each nitrogen atom or bulky groups as the terpyridyl or imidazole moiety because as the viscosity increases, the rotation of the phenyl ring becomes more impeded, enhancing fluorescence emission intensity and lifetime.

Suitable rhodamine-based options to quantify the microviscosity of specific environments can be rotors **RHO$_1$**, **RHO$_4$**, **RHO$_6$**, **RHO$_7$** and **RHO$_8$** as they show a good sensitivity towards viscosity changes.

In general, when a decision must be made, all factors need to be noted and studied as these dyes show more affinity to some solvents rather than other ones. The experimental environment is crucial as well as it is not the same to extract results on each case. For example, almost every BODIPY-based FMR noted is held on ethylene glycol – glycerol mixture. Comparing all values of sensitivity of all classes of FMRs, we can extract the top sensors to compare them.

POS.	STRUCTURE	NAME	*x* [REF.]	COND.
1		**BDP$_1$**	*x* = 0.76 [17]	Methanol – glycerol
2		**BDP$_9$**	*x* = 0.73 [7]	Ethylene glycol – glycerol
3		**JL$_2$**	*x* = 0.72 [14]	Dextran (77.8 kDa)

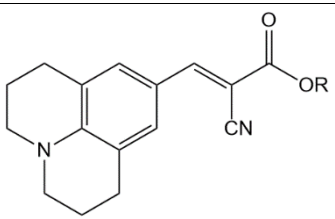
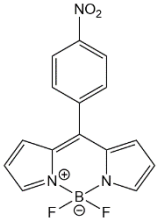
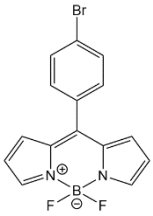
4		**JL_3** (R = CH_3)	x = 0.67 [13]	Ethylene glycol – glycerol
5		**BDP_{19}**	x = 0.65 [17]	Toluene – Castor oil
6		**BDP_{15}**	x = 0.64 [7]	Ethylene glycol – glycerol

Table 12. Dyes with the greater sensitivity of all classes of FMRs.

Looking at Table 12, it can be seen that BODIPY-based and julolidine-based FMRs are dyes that present more sensitivity towards viscosity than the other classes studied. They are neutral dyes that are mainly studied in polar and protic solvents as ethylene glycol, glycerol and methanol.

7. CONCLUSIONS

The research involving fluorescent molecular rotors has increased significantly over the last fifteen years. The recent developments on new dyes has been very useful to study and apply them to new fields like measuring the viscosity of a local environment, organelle viscosity imaging or targeting cell membrane viscosity.

An extensive search and analysis of the effect of viscosity on fluorescent molecular rotors has been done. Thanks to the classification of all dyes into different families of rotors, their structure and viscosity sensitivity has been extracted and thoroughly studied. Their structural similarities and differences have been used to understand their viscosity-related parameters and compare them with the other dyes of the family of rotors and the other families as well. The structural design of a fluorescent molecular rotor determines its ability to sense local viscosity.

According to all the research that has been done and all the tables shown in this work, the type of solvent that is used most of the time is polar and protic, normally being alcohols mixed with glycerol. This is also a limitation as more research needs to be done to investigate if the type of solvent really affects the viscosity-sensitivity of the dye.

Julolidine-based FMRs generally show good sensitivity towards viscosity. The sensitivity is reduced when longer alkyl chains or bulky groups are added, as the steric hindrance inhibits the rotation of the single bond between the julolidine and vinyl moieties. Mostly, the open julolidine form shows less sensitivity than the closed form of the dye. Good julolidine-based sensors have small groups on the rotor moiety to lower steric effects and increase sensitivity.

Sensitive BODIPY-based FMRs are not substituted with voluminous groups on the ortho-positions of the meso- phenyl ring and on the closest positions (to the meso- phenyl ring) of the two pyrrole rings, as rotation occurs through the simple bond that connects the meso- phenyl ring with the core and a big group would hinder the rotation, lowering the sensitivity toward viscosity. An homodimeric BODIPY dye was found as well and shows good sensitivity towards the increase of viscosity. Although it contains two rotors, this is not directly related to whether it forms a more sensitive or unsensitive system.

Suitable cyanine-based fluorescent molecular rotors contain large chains on the nitrogen atoms and bulky groups on the rotor moiety. Their sensitivity is based on the rotation around the conjugated chain that links the heterocycles or moieties. Some dyes can change structurally due to variation of the pH of the medium.

Rhodamine-based FMRs base their sensitivity towards viscosity on the substitution of the carboxyphenyl ring and the amino groups of the xanthene core. Good rhodamine-based dyes are those with phenyl groups attached to each nitrogen atom and a carboxy or ester group on the ring.

Based on all the data collected in this work, BODIPY-based and julolidine based fluorescent molecular rotors are the most suitable options as viscosity sensors of all four families studied because they present the greater sensitivity values of all four families studied. The development of new experimental probes in combination with all the advances that have been made lately will allow to expand the knowledge and popularity of this subject.

8. REFERENCES

1. Lakowicz, Joseph R. *Principles of Fluorescence Spectroscopy*, Third Edition, Springer New York, **2006**, 1-5.
2. Valeur, Bernard. *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, **2001**, 34-41.
3. Lee, S.-C.; Heo, J.; Woo, H. C.; Lee, J.-A.; Seo, Y. H.; Lee, C.-L.; Kim, S.; Kwon, O.-P. Fluorescent molecular rotors for viscosity sensors. Chem. Eur. J. **2018**, 24, 13706 – 13718.
4. Fatemah M. Alhassawi, Maria G. Corradini, Michael A. Rogers & Richard D. Ludescher. Potential applications of luminescent molecular rotors in food science and engineering, Critical Reviews in Food Science and Nutrition, **2018**, 58:11, 1902-1916.
5. Uzhinov, B. M., Ivanov, V. L., and Melnikov, M. Y. Molecular rotors as luminescence sensors of local viscosity and viscous flow in solutions and organized systems. Russ. Chem. Rev. **2011**, 80, 1179 – 1190.
6. Miao W, Yu C, Hao E, Jiao L. Functionalized BODIPYs as Fluorescent Molecular Rotors for Viscosity Detection. Front. Chem, **2019**, 7:825.
7. Liu, Xiao, et al. A systematic study on the relationship between viscosity sensitivity and temperature dependency of BODIPY rotors. Bulletin of the Korean Chemical Society 42.1, **2021**, 91-94.
8. Haidekker, M. A., et al. Effects of solvent polarity and solvent viscosity on the fluorescent properties of molecular rotors and related probes. Bioorganic chemistry 33.6, **2005**, 415-425.
9. Loutfy, R. O. and Law, K. Y. Electrochemistry and spectroscopy of intramolecular charge-transfer complexes. p-N,N-Dialkylaminobenzylidenemalononitriles. J. Phys. Chem. **1980**, 84(21), 2803–2808.
10 Howell, S., Dakanali, M., Theodorakis, E. A. and Haidekker, M. A. Intrinsic and extrinsic temperature-dependency of viscosity-sensitive fluorescent molecular rotors. J. Fluorescence, **2012**, 22, 457–465.
11. Mark A. Haidekker, Thomas Brady, Ke Wen, Cliff Okada, Hazel Y. Stevens, Jeniffer M. Snell, John A. Frangos, Emmanuel A. Theodorakis. Phospholipid-Bound Molecular Rotors: Synthesis and Characterization, Bioorganic & Medicinal Chemistry, Volume 10, Issue 11, **2002**.
12. Guo, Rui, et al. A novel mitochondria-targeted rhodamine analogue for the detection of viscosity changes in living cells, zebra fish and living mice. Journal of Materials Chemistry B, 6.18, **2018**, 2894-2900.
13. Mark A. Haidekker, Taotao Ling, Michael Anglo, Hazel Y. Stevens, John A. Frangos, Emmanuel A. Theodorakis, New fluorescent probes for the measurement of cell membrane viscosity, Chemistry & Biology, Volume 8, Issue 2, **2001**, 123-131.
14. Akers W, Mark A. Haidekker, A molecular rotor as viscosity sensor in aqueous colloid solutions. J Biomech Eng. **2004**,126(3), 340-5.
15. Haidekker, M. A., Brady, T. P., Chalian, S. H., Akers, W., Lichlyter, D. and Theodorakis, E. A. Hydrophilic molecular rotor derivatives - synthesis and characterization. Bioorganic Chem. **2004**, 32(4), 274–289.
16. Liu, Xiao, et al. Molecular mechanism of viscosity sensitivity in BODIPY rotors and application to motion-based fluorescent sensors. ACS Sensors 5.3, **2020**, 731-739.
17. Toliautas, Stepas, et al. Enhancing the Viscosity-Sensitive Range of a BODIPY Molecular Rotor by Two Orders of Magnitude. Chemistry – A European Journal 25.44, **2019**, 10342-10349.
18. Raut, Sangram, et al. A homodimeric BODIPY rotor as a fluorescent viscosity sensor for membrane-mimicking and cellular environments. Physical Chemistry Chemical Physics. 16.48, **2014**, 27037-27042.

19. Ya-Nan Wang, Bing Xu, Li-Hua Qiu, Ru Sun, Yu-Jie Xu, Jian-Feng Ge. Viscosity sensitive fluorescent dyes with excellent photostability based on hemicyanine dyes for targeting cell membrane, Sensors and Actuators B: Chemical, Volume 337, **2021**, 129787.
20. Yiping Cai, Chang Liu, Zhaoxia Lei, Zhiming Wang, Yaye Bian, Song He, Xianshun Zeng. Novel lysosome-targeted fluorescent molecular rotors based on a cyanine-like modular system and their application in living cells. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Volume 265, **2022**, 120404.
21. Eizig Bar-On, Z., Iron, M.A., Kasdan, H.L. et al. The cationic dye basic orange 21 (BO21) as a potential fluorescent sensor. Photochem Photobiol Sci 17, **2018**,1417–1428.
22. Fei Liu, Yeshen Luo, Meiying Xu. Viscosity measurements using a two-photon ratiometric fluorescent sensor with two rotors, Tetrahedron Letters, Volume 59, Issue 52, **2018**, 4540-4544.
23. Liu, Fei, et al. Ratiometric detection of viscosity using a two‐photon fluorescent sensor. Chemistry–A European Journal 19.5, **2013**, 1548-1553.
24. Peng, Xiaojun, et al. Fluorescence ratiometry and fluorescence lifetime imaging: using a single molecular sensor for dual mode imaging of cellular viscosity. Journal of the American Chemical Society, 133.17, **2011**, 6626-6635.
25. Yin, Xiaoxi, et al. A deep-red fluorescent molecular rotor based on donor-two-acceptor modular system for imaging mitochondrial viscosity. RSC advances 10.51, **2020**, 30825-30831.
26. Amol G. Jadhav, Shantaram Kothavale, Nagaiyan Sekar. Red emitting triphenylamine based rhodamine analogous with enhanced Stokes shift and viscosity sensitive emission. Dyes and Pigments, Volume 138, **2017**, 56-67.
27. Peisheng Tang, Qing Wang, Qiuyan Tan, Kun Huang, Bingxin Du, Lijuan Liang, A terpyridyl functionalized rhodamine dye for viscosity sensing with fluorescence enhancement and mitochondria-targeting imaging, Microchemical Journal, Volume 194, **2023**, 109296.

APPENDICES

APPENDIX 1: COMPLEX JULOLIDINES

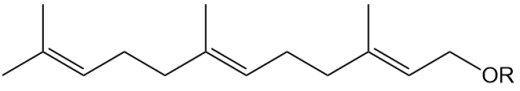
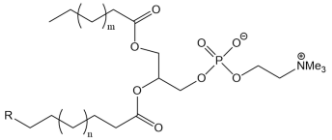
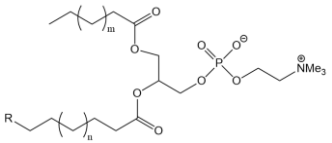
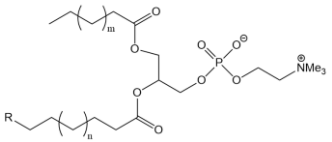
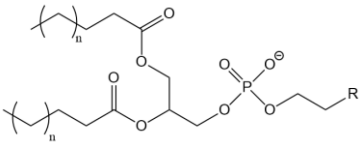
JL_x	REF.	R GROUP	STRUCTURE
JL_9	[13]	farnesyl	
JL_{10}	[11]	phospholipid 1 n = 1 m = 8	
JL_{11}	[11]	phospholipid 2 n = 1 m = 12	
JL_{12}	[11]	phospholipid 3 n = 6 m = 12	
JL_{13}	[11]	phospholipid 4 n = 10	

Table 13. R groups of some complex julolidine-based FMRs.

APPENDIX 2: CASTOR OIL STRUCTURE

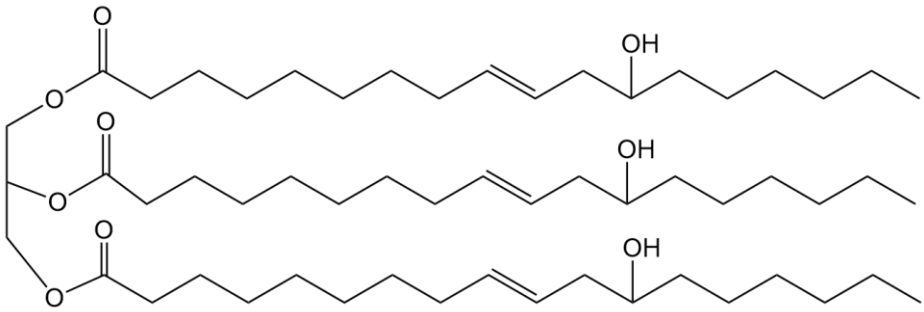


Figure 28. Castor Oil structure.