Characterization of Self-Assembling Quinoline-Based Foldamers by Fluorescence Anisotropy

by

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AUTHOR’S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.
Abstract

Foldamers represent a family of synthetic macromolecules which, like their biological counterparts, are able to adopt a well-defined conformation in solution. Oligoquinoline-carboxamides (Qn) are a group of foldamers that adopt a helical conformation in solution. A series of Qn foldamers were prepared by chromatography-free large-scale synthesis and segment-doubling strategy. The C-terminal ester group of the Qn foldamers could be hydrolyzed to yield acid-functionalized foldamers (QnA) which could self-assemble into larger ((QnA)_2-Na) complexes by metal coordination with a sodium cation. Moreover, the addition of a bis-acid functionalized tetramer (AQ_2PQ_2A) to a solution of (QnA)_2-Na complexes resulted in insertion oligomeric products. To characterize these complexes in solution, both Qn and QnA were end-labeled with an oligo(phenylene vinylene) dye (OPV) at their N-terminus via a rigid amide bond to yield the OPV-Qn and OPV-QnA fluorescent equivalents. OPV was used to conduct time-resolved fluorescence anisotropy (TRFA) measurements on the OPV-Qn and OPV-QnA foldamers, the (OPV-QnA)_2-Na complexes, and the OPV-Qn-Na-(AQ_2PQ_2A)_n oligomers. Analysis of the TRFA of the OPV-Qn foldamers yielded the rotational time (\(\phi\)) of the fluorescent species, which was found to reflect the hydrodynamic volume (\(V_h\)) of the foldamers. The straight line obtained by plotting \(\phi\) as a function of the number of (quinoline) units (NUs) demonstrated that the foldamers behaved in solution as rigid cylinders for all lengths examined. The linearity of the \(\phi\)-vs-NU plot was employed as a calibration curve against which the rotational time of the QnA-complexes could be compared. Within experimental error, the rotational time of a Q_{n+m} complex was found to equal the sum of the rotational times obtained for Q_n and Q_m. This result suggests that the complexation of two acid-functionalized oligoquinoline foldamers in solution generated a fully stacked foldamer with a NU equal to the sum of the NUs of its constituting elements. Hetero-complexes between OPV-Q_8A
and Q_{16}A were also produced by adding a 10-fold excess of Q_{16}A to an OPV-Q_{8}A solution. Complexation was demonstrated by the $\phi$ value of the mixture, that equaled that of an OPV-Q_{24} foldamer. Dilution experiments on a solution of OPV-Q_{8}A-Na-Q_{16}A complexes led to the dissociation of the complexes into their OPV-Q_{8}A and Q_{16}A constituting elements, as evidenced by the progressive decrease in $\phi$ from the value obtained for OPV-Q_{24} to that of OPV-Q_{8} upon decreasing foldamer concentration. Similarly, the addition of increasing amounts of AQ_{2}PQ_{2}A to a solution of OPV-Q_{8}A in chloroform resulted in an increase in $\phi$, demonstrating the formation of complexes between OPV-Q_{8}A and AQ_{2}PQ_{2}A until $\phi$ reached a plateau for large OPV-Q_{8}A/AQ_{2}PQ_{2}A molar ratios. In the plateau region, the rotational time of the oligomeric complexes generated from OPV-Q_{8}A and AQ_{2}PQ_{2}A stabilized by isobutyl or hexyl side chains was equal to that of an OPV-Q_{n} foldamer with $n$ equal to 24 or 30, respectively. The apparent absence of further polymerization, evidenced by the constant $\phi$ value reached for high OPV-Q_{8}A/AQ_{2}PQ_{2}A molar ratios, was attributed to aggregation of longer complexes and their precipitation. This study represents the first example in the scientific literature where TRFA was applied to characterize the NU of helical self-assembling foldamers in solution.
I want to express my sincerest gratitude to several people who helped me and encouraged me to carry out this project.

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Abs
Absorption

$AQ_2PQ_2A$
Pentamer with a pyridine at its center, flanked by two quinoline dimers terminated at both ends with a carboxylic acid

$\beta_A$
Angle between the absorption dipole moment of the dye and the main axis of the helix

$\beta_E$
Angle between the emission dipole moment of the dye and the main axis of the helix

$D$
Diameter of helical foldamer

$D_{//}$
Diffusion coefficient for the tumbling of a symmetric top macromolecule around its main axis of symmetry

$D_{\perp}$
Diffusion coefficient for the tumbling of a symmetric top macromolecule around its secondary axis of symmetry

$D_{w}$
Diffusion coefficient for wobbling of OPV around the main axis of a helical foldamer

$DP$
Degree of polymerization

$\Delta h$
Helical rise per quinoline residue

$E$
Molar extinction coefficient

$F_{OPV}(\lambda)$
Normalized fluorescence intensity of OPVA

$\phi$
Rotational time

$\Phi$
Quantum yield

$G$
G-factor

$\eta$
Solution viscosity

$K$
Equilibrium constant

$I_o$
Fluorescence intensity at $t=0$

$I_F$
Fluorescence intensity

$I_{XY}(t)$
Fluorescence intensity acquired with an excitation light polarized in the X-orientation and an emission polarized in the Y-orientation where the X and Y-orientations can be horizontal (H), vertical (V), or at the magical angle (M).
$\kappa^2$ Orientation factor describing the relative orientation in space of the transition dipoles of the donor and the acceptor

$L$ Length of a symmetric top macromolecule along its main axis of symmetry

$\bar{\mu}_D$ Emission dipole moment of the donor

$\bar{\mu}_A$ Absorption dipole moment of the acceptor

$N$ Refractive index

$N_A$ Avogadro number

$NU$ Number of quinoline units in an oligoquinoline foldamer

$N$ Flory exponent

$OPV$ Oligo(phenylene vinylene)

$OPV-Q_nA$ OPV-labeled foldamer acid

$OPV-Q_n$ OPV-labeled foldamer ester

$P$ Aspect ratio ($p=L/d$) of symmetric top macromolecules

$Q_n$ Quinoline-based aromatic oligoamide foldamer with $n$ units

$Q_nA-Na-Q_mA$ Foldamer complex between $Q_nA$ and $Q_mA$ formed via Na$^+$ coordination

$Q_n-CO_2H$ Acid-functionalized $Q_n$

$Q_n-COCl$ Acid chloride-functionalized $Q_n$

$Q_n-NH_2$ Amine-functionalized $Q_n$

$\theta_D$ Angle between the emission dipole moment of the donor and the vector joining the donor to the acceptor

$\theta_a$ Angle between the absorption dipole moment of the acceptor and the vector joining the donor to the acceptor

$R$ Steady-state anisotropy

$r(t)$ Time-resolved fluorescence anisotropy

$r_0$ Anisotropy at time $t=0$

$R$ Universal gas constant

$R_o$ Förster radius
$R_h$  
Hydrodynamic radius

$SSF$  
Steady-state fluorescence

$T$  
Time

$T$  
Absolute temperature

$TRF$  
Time-resolved fluorescence

$\tau_0$  
Natural lifetime of OPV

$\tau_n$  
Decay times with $n=1, 2, \text{ and } 3$

$V_h$  
Hydrodynamic volume

$\xi$  
Angle between the projection of the absorption and emission dipole moments in the plane perpendicular to the main axis of the symmetric top macromolecule.
Chapter 1

Introduction

1.1 Background

Foldamers are artificial oligomers designed to fold in solution via non-covalent intramolecular interactions.\(^1,2\) Folding of these linear macromolecules is inspired from natural biomacromolecules such as peptides, oligonucleotides, or oligosaccharides and uses comparable types of interactions to lead to similar molecular architectures such as helices, sheets, or loops. The obvious parallels that exist between foldamers and biological macromolecules in terms of conformation suggest that foldamers could be designed to perform many of the functions conducted by biological macromolecules in nature.\(^3,4\) However and contrary to nature, chemists are not limited by the bioavailability of natural building blocks such as amino acids, nucleobases, or sugars but can expand the scope of monomers used in foldamer preparation to all kinds of chemically accessible units.\(^5\) This diversity may give access to new functions and usages remote from those commonly observed in nature, such as in molecular electronics\(^6–8\) or in the nanoengineering field\(^9–11\).

In that context, foldamers based on aromatic quinoline oligoamide backbones were designed to fold into helical architectures stabilized by intramolecular hydrogen bonds and aromatic stacking.\(^12\) Recent synthetic developments have allowed the preparation of nanometer-sized objects, whose length scale and intrinsic conducting properties make them interesting as building blocks for materials science applications and for molecular electronics, respectively.\(^10\) Furthermore, the preparation and characterization of dimer and polymer complexes obtained through complexation by metal coordination of different oligoquinoline foldamers, terminated at one or both ends with a carboxylate anion, also provide a new method to prepare longer helical
strands. However, these synthetic developments also brought to the fore a dearth of analytical techniques to characterize the overall dimensions of such large macromolecular constructs in solution.

By and large, single crystal X-ray diffraction (SCXRD) is the technique of choice to characterize the conformation of foldamers in the solid state. Unfortunately, crystal packing forces are also known to induce conformations in the solid state that might not be observed in solution for a same macromolecule, and complementary solution studies by NMR or circular dichroism are necessary. For instance, a combination of NMR spectroscopy and molecular modeling may provide a measure of the helical twist of a foldamer that can be used to predict its conformation in solution. Enhanced or vibrational circular dichroism is able to assess the extent of folding of a foldamer in solution. Changes in the UV-Vis absorption spectrum can indicate an increase in foldamer length. However all these techniques characterize short range distances between residues in the foldamer and do not provide a sense of the overall dimensions of the foldamer in solution.

The common technique to probe the dimension of nm-scale macromolecules in solution is dynamic light scattering (DLS). In the case of oligoquinoline foldamers, according to SCXRD, these constructs adopt a helical conformation with a 2.0 nm diameter and a 0.136 nm raise per quinoline residue. Therefore, if quinoline based foldamers 4-to-66 units in length were to retain their conformation in a solvent, they would be expected to maintain a 2.0 nm diameter with a length ranging from 0.54 to 9.0 nm. However most standard DLS instruments are not able to detect objects whose dimensions are less than 2 nm, equivalent to a 15 quinoline-long foldamer. Moreover, the standard DLS instruments model macromolecular objects as spheres, which would lead to errors for large helical foldamers since such rigid symmetric top macromolecules might
require two diffusion coefficients, one to handle the rotation around the main axis and another for the tumbling around the secondary axis perpendicular to the main axis. Besides DLS, it was shown using Guinier plots obtained from X-ray scattering that the radius of gyration of an \(m\)-phenylene ethynylene (\(m\)PE) octadecamer in acetonitrile equaled 1.47 nm as expected.\(^{24}\) While SAXS can probe the foldamer length scale at the nanometer level, the synchrotron high energy source implied that this study cannot be viewed as a routine experiment, and that the relatively high foldamer concentrations required (\(10^{-4} - 10^{-3}\) M) could become an issue for less soluble foldamers.

By contrast, an earlier study on helical oligonucleotide duplexes and hairpins has established that time-resolved fluorescence anisotropy (TRFA) is ideally suited to study rigid symmetric top macromolecules with dimensions below 11 nm.\(^{25}\) Furthermore, these TRFA experiments took advantage of the high sensitivity of fluorescence and were conducted at concentrations ranging between \(10^{-5}\) and \(10^{-4}\) M of macromolecular constructs, one order of magnitude lower than those conducted by SAXS. This study reports on the first example of the use of TRFA to probe the size and dynamics of a series of oligoquinoline foldamers in solution.

1.2 Quinoline-based foldamers labeled with oligo(phenylene vinylene)

Aromatic amide oligomers have many advantages as compared to other types of foldamers. These include the stability of the folded structure, predictability of the folding modes, propensity to crystallize, and relative ease of synthesis.\(^{10,26}\) This thesis focuses on helical aromatic oligoamide foldamers synthesized from methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate (Q\(_1\)), one of the most popular families of aromatic amide foldamers. A number of synthetic protocols have been established to prepare quinoline-based aromatic amide helical foldamers (Q\(_n\)).\(^{10,26}\) The controlled addition of monomer Q\(_1\) via amide coupling can result in the synthesis of Q\(_n\) foldamers with a sequence of up to 64 quinolines, that automatically adopt the helical conformation depicted in
Figure 1.1. Unfortunately, further elongation of these oligomers is prevented due to their poor solubility.

![Chemical structure of A) methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate (Q1) and B) Q8, and C) 3-D structure of helically folded octamer (Q8).](image)

**Figure 1.1.** Chemical structure of A) methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate (Q1) and B) Q8, and C) 3-D structure of helically folded octamer (Q8).

The Q8 oligomer can be used as a building block to generate more elaborate macromolecular constructs such as the photoactive triad shown in Figure 2, which is comprised of a central helical oligoamide foldamer bridge flanked by two chromophores. The Q8 foldamer provided a spacer of well-defined length to separate the oligo(\(p\)-phenylenevinylene) (OPV) electron acceptor at one end of the foldamer from the perylene \(bis\)-imide electron donor at the other end. The fact that the OPV did not seem to interact photochemically with the oligoquinoline backbone led to its selection for the TRFA study conducted in this project. A series of Q\(_n\) foldamers were synthesized with \(n = 4 – 33\) and covalently labeled at their N-terminal with an OPV derivative bearing a carboxylic acid via a rigid amide bond to yield the series of OPV-Q\(_n\) constructs shown in Figure 1.2B. The angle between OPV and the main axis of the helical foldamer equaled 99.5 °, implying that OPV was almost perpendicular to the helix main axis.
Figure 1.2. A) Structure of a photoactive triad comprised of a rigid foldamer bridge flanked by an electron donor OPV at one end and an electron donor perylene bis-amide at the other end. B) Chemical structure of OPV-Qn.

1.3 Self-assembling foldamers via metal coordination

Besides the traditional synthetic protocols developed for the preparation of oligoquinoline foldamers, ligation of helical foldamers through their complexation by metal coordination represents an innovative method to generate longer strands. Furthermore, since complexation can take place at foldamer concentrations that are much lower than those required for foldamer synthesis, this method might circumvent the solubility issues plaguing the more traditional synthetic protocols. Oligoquinoline foldamers terminated with a carboxylic acid (QnA) offer a coordination site for metal cations such as Na⁺, that enables the complexation of another QnA foldamer to form a two-strand metal complex (QnA-Na-QnA) as shown in Figure 1.3. The upfield shift of the signals in the ¹H NMR spectra, corresponding to the backbone amide protons of the QnA constructs, indicated the formation of a longer compound, while SCXRD showed that two QnA helical strands were coordinated to a Na⁺ cation in an end-to-end fashion to form a continuous helix in the solid state. However, the conformation adopted by this complex in solution is still unknown.
As depicted in Figure 1.3, the metal complexation of QₙA foldamers with a single carboxylic acid per strand doubles the foldamer length through the formation of a QₙA-Na-QₙA complex. In contrast, the foldamer AQ₂PQ₂A bearing one acid group at both ends in Figure 1.4 could polymerize into much longer complexes. In fact, unpublished work from the University of Bordeaux showed that titration of a chloroform solution of a Q₈A-Na-Q₈A complex with increasing amounts of AQ₂PQ₂A can induce the formation of long insertion polymeric products of sequence Q₈A-Na-(Q₂PQ₂-Na)ₙ-Q₈A, where Q₈A acts as an end-capping agent.
1.4 Time-resolved fluorescence anisotropy

Fluorescence anisotropy ($r$) measures the extent of depolarization of an excited chromophore after excitation by a photon obtained from a vertically polarized light source. Since the probability of exciting a photon depends on the squared cosine of the angle between the orientation of the excitation photons (i.e. vertical) and the absorption dipole moment of the chromophores randomly distributed in the solution, those chromophores whose absorption dipole moment is oriented vertically and parallel to the excitation polarization are most likely to become excited. This photoselection of the excited chromophores results in a difference between the photons emitted in the vertical and horizontal planes of detection. The anisotropy is determined by measuring the difference between the fluorescence intensity polarized in the vertical ($I_{VV}$) and horizontal ($I_{VH}$) directions following a vertically polarized excitation.\(^{27}\) As time elapses and the excited fluorophore tumbles randomly in solution, the photoselection of the chromophores disappears, the $I_{VV}$ and $I_{VH}$ intensities become equal, and the anisotropy reaches zero. The characteristic time taken by the anisotropy to reach zero is described by the rotational time ($\phi$) of the chromophore.\(^{27}\) As shown in Equation 1.1, $\phi$ depends on the solvent viscosity ($\eta$), the hydrodynamic volume ($V_h$) of the chromophore, and the solution temperature. If the chromophore is rigidly bound to a macromolecule, tumbling of the chromophore reflects the tumbling of the macromolecule, and $V_h$ represents the hydrodynamic volume of the macromolecule and the bonded chromophore. In turn, the hydrodynamic volume of a macromolecule can be determined from $\phi$ obtained from an anisotropy experiment, which is an interesting feature to probe the dimension of foldamers in solution.\(^{27}\)

\[
\phi = \frac{\eta V_h}{RT} \tag{1.1}
\]
A time-resolved fluorescence anisotropy (TRFA) experiment begins by acquiring the fluorescence decay \(I_{VM}(t)\) of the chromophore at the magic angle (54.7 °) upon excitation with vertically polarized photons. Setting the emission polarizer at the magic angle ensures that the chromophore is not subject to any polarization effect. The \(I_{VM}(t)\) decay is then fitted with an exponential according to Equation 1.2.

\[
I_{VM}(t) = I_o \exp(-t / \tau_o)
\]  

(1.2)

In Equation 1.2, \(I_o\) is the initial fluorescence intensity at \(t = 0\) and \(\tau_o\) is the natural lifetime of the chromophore. In this thesis, the chromophore is OPV bound to the foldamer. The vertically \((I_{VV}(t))\) and horizontally \((I_{VH}(t))\) polarized decays are then analyzed globally by fitting them to Equations 1.3 and 1.4, respectively.

\[
I_{VV}(t) = \frac{I_o}{3} \exp(-t / \tau_o) \times [1 + 2r(t)]
\]  

(1.3)

\[
I_{VH}(t) = \frac{I_o}{3G} \exp(-t / \tau_o) \times [1 - r(t)]
\]  

(1.4)

In Equations 1.3 and 1.4, \(G\) is used to normalize the \(I_{VV}(t)\) and \(I_{VH}(t)\) decays and is referred to as the G-factor, whereas \(r(t)\) represents the TRFA. \(r(t)\) can be approximated by a sum of exponentials as shown in Equation 1.5.
\[ r(t) = r_0 \sum_{i=1}^{n} a_i \times \exp\left(-t / \phi_i\right) \] (1.5)

In Equation 1.5, \( r_0 \) is the anisotropy at time \( t = 0 \) and \( a_i \) and \( \phi_i \) represent the \( i \)-th normalized pre-exponential factor and rotational time of the macromolecule, respectively. Fitting the polarized fluorescence decays \( I_{VV}(t) \) and \( I_{VH}(t) \) globally according to Equations 1.3 and 1.4 yields the rotational time \( \phi \). In turn, the rotational time provides information about the spherical or cylindrical geometry of the macromolecule, its dimensions, and its internal dynamics.

1.5 Outline

The primary goal of this project was to use TRFA to establish the relationship between the rotational time of quinoline-based foldamers and their chain length, and to apply this relationship to determine the dimensions of metal complexes formed between oligoamide foldamers in solution. The thesis describes how this goal was reached. It is divided in the following manner. The introduction chapter provides some background information on helical aromatic quinoline-based oligoamide foldamers (Q\(_n\)) and the principles of time-resolved fluorescence anisotropy (TRFA). The preparation of OPV labeled foldamer (OPV-Q\(_n\)) is described in Chapter 2. It closely follows the protocol published by the laboratory of Prof. Ivan Huc, formerly at the University of Bordeaux, France, and now at the University of Munich, Germany. A linear relationship was found in Chapter 3 between the rotational time of the OPV-Q\(_n\) foldamers and their number of units (NU), for foldamers with NU ranging between 4 and 33. The rotational times were obtained by global analysis of the polarized fluorescence decays of the OPV-Q\(_n\) foldamers using a monoexponential TRFA. The research described in Chapter 4 suggests that TRFA can be applied to measure the size
of foldamer complexes generated by the self-assembly of oligoquinoline foldamer acids. It also provides evidence that the metal coordination of foldamer acids can be used to form long and rigid helical structures equivalent to an OPV-Q₆₆ foldamer made of 66 quinoline units. Chapter 5 summarizes the main results obtained so far for this project and makes suggestions for future work.
Chapter 2

Preparation of OPV-labeled oligoquinoline foldamers

2.1 Overview

The protocols applied in the synthesis of helical nanosized foldamers can be divided into two categories. The chromatography-free large-scale synthesis is used for shorter oligomers\textsuperscript{26} such as the hexamer or octamer, whereas a segment doubling strategy is employed to prepare oligomers\textsuperscript{10} longer than the hexadecamer.

The synthesis of aromatic helical foldamers starts with methyl-4-isobutoxy-8-nitroquinoline-2-carboxylate (Q\textsubscript{1}), derived from dimethyl 2-(2-nitrophenylamino)-fumarate.\textsuperscript{26} In the convergent scheme (Scheme 2.1), the dimer (Q\textsubscript{2}) is obtained by coupling two monomers, the tetramer (Q\textsubscript{4}) by coupling two dimers, and the hexamer (Q\textsubscript{6}) is formed by coupling one tetramer and one dimer. Similarly, the octamer (Q\textsubscript{8}) was prepared by adding the dimer to the hexamer.\textsuperscript{26} Coupling of two oligomers requires that they be end-functionalized with either an amine, prepared by reduction of the N-terminal nitro group, or an acid chloride prepared by activating the terminal acid, obtained through the saponification of the C-terminal methyl ester with oxalyl chloride.\textsuperscript{26} Since only the short acid-functionalized Q\textsubscript{2} foldamer was added to the amino terminal of other foldamers in Scheme 2.1, the formation of the unwanted anhydride by-product between two Q\textsubscript{2} acid groups could be avoided, since their smaller size prevented them from adopting a helical conformation, known to stabilize anhydrides obtained with longer strands. Excess reagent could simply be removed by recrystallization. Therefore, the procedure of adding short oligomers was found to result in high coupling yields and could be scaled up to achieve multigram syntheses for the preparation of relatively short oligoamide foldamers.\textsuperscript{26} However this procedure failed to produce oligoquinolines much longer than the octamer, equivalent to a 1.1 nm-long foldamer.
One major challenge complicating the synthesis of longer nanosized foldamers is sterical hindrance, affecting the terminal reactive groups, that results from the stable conformation of the foldamer. Furthermore, both reactants and products have poor solubility, and the reaction is moisture sensitive. As it turns out, these limitations can be overcome by using pure chemicals at high concentrations, and allowing the coupling reaction between long oligoquinolines to proceed for longer times. The segment doubling strategy method developed by the Huc laboratory has allowed the gram-scale synthesis of nanosized helical aromatic foldamers, and the longest foldamer ever prepared and fully characterized was constituted of 64 quinoline units. This iterative strategy is described in Scheme 2.1 for the preparation of a 16-mer by coupling an octamer amine with an octamer acid chloride, or for the preparation of a 32-mer by coupling a 16-mer amine with a 16-mer acid chloride, etc… Theoretically and based on Scheme 2.1, long foldamers should be easily achieved by doubling the segment length. In practice, however, problems arise due to the low reactivity of the amine end-group and the formation of anhydrides between two acid end groups. This complicates the purification of the products as compared to the synthesis of shorter foldamers. In the protocol outlined in Scheme 2.1, chromatography was shown to be an effective method to separate the coupling products and unreacted starting materials, if some precautions were taken. For instance, the similar polarity of the product and the amine prevented their separation by column chromatography. Therefore, excess acid chloride was needed to consume all the amines before purification. Unfortunately, the anhydride was an unavoidable by-product, even if the system was maintained as anhydrous as possible. This impurity could be removed by refluxing the crude product in a pyridine/H2O mixture, to hydrolyze the anhydride back into the acid. After this, the acid could be separated and recovered from the reaction mixture by column chromatography on silica gel.
Earlier studies from the Duhamel Laboratory have established that oligo(phenylene vinylene) (OPV) would be a suitable dye to probe the hydrodynamic volume ($V_h$) of quinoline-based foldamers by fluorescence anisotropy. The OPV can be attached onto the foldamer by coupling the $Q_n$-amine foldamer with the OPV-acid chloride, prepared by chlorination of the OPV-acid with Ghosez reagent, in the presence of $N,N$-diisopropylethylamine DIPEA (Scheme 2.1).7

Scheme 2.1. Procedures used to synthesize foldamers and label them with OPV.

2.2 Materials

All the chemicals were bought from Sigma-Aldrich and were reagent grade, if not specifically mentioned. They include 2-methyl propanol (Alfa Aesar), 2-nitroaniline, anhydrous MgSO$_4$, CaH$_2$, Celite (VWR international), chloroform, chloroform-$d$ (Euriso-top), dichloromethane, diisopropyl
azodicarboxylate, nitrogen, \textit{N,N}-diisopropylethylamine (DIPEA), dimethyl acetylene dicarboxylate, diphenyl ether, ethyl acetate, hydrochloric acid (aqueous solution, minimum 37\% (wt/wt)), hydrogen, isobutyl alcohol, methanol (MeOH), NaHCO$_3$, oxalyl chloride, Pd/C catalyst (10\%) (Alfa Aesar), Q1 and Q7-NH$_2$ (H-lab), silica gel, sodium chloride, tetrahydrofuran (THF), and triphenylphosphine (Alfa Aesar). Distilled in glass toluene and HPLC-grade chloroform were purchased from Aldrich and used for the fluorescence anisotropy experiments. All the chemicals were used as received. The preparation of OPV-CO$_2$H, used to label the amino-terminal of the foldamers, has been described earlier.$^6$

2.3 Equipment

Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60-F254 plates and observed under UV light. Column chromatography purifications were carried out on Merck GEDURAN Si60 (40-63 µm). ESI mass spectra were obtained at the Mass Spectrometry Laboratory of the European Institute of Chemistry and Biology (UMS 3033 - IECB), Pessac, France, and at the Mass Spectrometry facility of the University of Waterloo, Canada. NMR spectra were recorded on an Avance II NMR spectrometer (Bruker Biospin) with a vertical 7.05 T narrow-bore/ultrashield magnet operating at 300 MHz for proton. The chemical shifts of the foldamers synthesized are reported in Appendix A in parts per million (ppm, $\delta$) relatively to the $^1$H residual signal of the deuterated chloroform (7.3 ppm). Preparative recycling Gel Permeation Chromatography (GPC) was performed on a JAI LC-9130G NEXT using two JAIGEL 20×600 mm columns (Japan Analytical Industry) with 0.5 \% NEt$_3$ in chloroform (HPLC grade, ethanol-stabilized) as mobile phase, at a flow rate of 7 mL/min. After collection, the fractions were washed with a 5\% NH$_4$Cl solution and twice with water.
2.4 Reagent and equipment setup

The dry DCM and THF were obtained by filtration through activated alumina using a dedicated purification system and needed to be used immediately after collection. The dry chloroform and anhydrous DIEPA were obtained by distillation over CaH₂. All the glass equipment and stir bars were dried in an oven, and the plastic syringes and needles obtained after opening their packaging were considered to be dry. When they were reused, they were cleaned with acetone and dried under vacuum.

2.5 Preparation of foldamers

The synthetic work to prepare the foldamers was entirely done at the Université de Bordeaux, France, in the Huc laboratory (H-Lab) under the supervision of Dr. Victor Maurizot.¹² Some of the foldamers used in this thesis were prepared in the H-Lab. The synthesis of the foldamers prepared by the author of this thesis is described hereafter.

2.5.1 Preparation of the dimer (Q₂)

First, the nitro group of Q₁ needed to be reduced to obtain the corresponding amine. Q₁ (6.8 g, 22.4 mmol) was dissolved in 150 mL of ethyl acetate in a reaction flask. The solution was flushed with nitrogen to purge the air before adding the Pd/C catalyst (10%, 680 mg) under nitrogen atmosphere. Then the flask was filled with hydrogen using a balloon. The reaction was conducted at room temperature and stirred under hydrogen atmosphere for 12 h to obtain Q₁-NH₂. The purity of the product was verified by ¹H NMR. The reaction mixture was filtered through Celite, washed with water and brine, dried over Na₂SO₄, filtered again and the solvent was removed under reduced pressure. Q₁-NH₂ was obtained in a 6.10 g (yield 99.5%) yield as a yellow solid.

The methyl ester of Q₁ needed to be deprotected to regenerate the acid functionality. Q₁ (7.2 g, 23.7 mmol) was dissolved in 290 mL of 1,4-dioxane and 30 mL of distilled water and KOH
powder (3.30 g, 58.9 mmol) was added to the solution. The reaction was stirred at room
temperature and monitored by TLC until completion. The reaction was quenched with aqueous
citric acid (5%, wt/wt) up to a pH around 5. Volatile components were removed under reduced
pressure and the crude product was dissolved in DCM. The organic phase was washed with water
and brine, dried over MgSO₄ and the solvent was evaporated to yield Q₁-CO₂H as a yellow solid
(6.80 g, 23.4 mmol). Dry Q₁-CO₂H was dissolved in 45 mL anhydrous DCM and mixed with
oxalyl chloride (10 mL, 117 mmol) under nitrogen atmosphere. After stirring for two hours at
room temperature, the reaction was complete (as confirmed by NMR), and the solvent and
remaining oxalyl chloride were removed under vacuum for 5 h to yield the Q₁-COCl product (6.9
g, 23.4 mmol, yield 98%).

The coupling of Q₁-NH₂ and Q₁-COCl was carried out in the presence of DIEPA. Q₁-NH₂
(6.10 g, 22.3 mmol) was added to 55 mL of anhydrous DCM under nitrogen, and dry DIEPA (21.3
mL) was added to the flask. Q₁-COCl (6.9 g) was dissolved in 50 mL DCM and transferred into
the Q₁-NH₂ solution by syringe. After the reaction was stirred for 12 h, the Q₂ dimer was obtained.
The workup was finished by precipitating the reaction mixture with MeOH and washing the
product with water. The purity of Q₂ (7.5 g, yield 61%) was confirmed by NMR.

2.5.2 Preparation of the tetramer (Q₄)

The reduction of Q₂ (2.9 g, 5.31 mmol) proceeded in a manner similar to that of Q₁, except that it
was conducted with 340 mL ethyl acetate and 290 mg Pd/C catalyst at 50 °C under a nitrogen
atmosphere. The reduction yielded 2.70 g of Q₂-NH₂ (yield 98%), which could be used for the
coupling reactions.

The saponification of Q₂ (8.90 g, 16.3 mmol) was conducted in a manner similar to Q₁-
CO₂H, except that 270 mL of THF and 90 mL methanol were used to dissolve the starting material,
and NaOH (2.00 g, 50 mmol) was employed. After workup, 8.60 g (yield 98%) of Q2-CO2H was recovered. A fraction of the recovered Q2-CO2H (5.64 mmol) was dissolved in 45 mL of dry DCM, to which 2.4 mL oxalyl chloride (28.0 mmol) was added under nitrogen atmosphere to activate the acid. The same procedure as for Q1-COCl was applied for the workup of Q2-COCl.

Similarly, the coupling of Q2-NH2 (1.7 g, 3.29 mmol) and Q2-COCl (1.8 g) was conducted in the same manner as before. The amounts of DIEPA, DCM for the Q2-NH2 amine, and DCM for the acid chloride were 5, 10, and 40 mL, respectively. The product Q4 was recovered with 3.2 g yield (yield 91%).

2.5.3 Preparation of the hexamer (Q6)
The preparation of the hexamer started with the reduction of Q4 (3.1 g, 3 mmol) dissolved in 120 mL ethyl acetate and 30 mL ethanol. The solution was placed under nitrogen atmosphere before adding 300 mg of Pd/C (10%) catalyst and a catalytic amount of ammonium metavanadate at room temperature. An aqueous solution of ammonium formate (45-50 equiv) was slowly added to the reaction mixture in 3 to 5 portions at 95 °C. The reaction was allowed to react for 12 h and was monitored by NMR until it was complete. After workup, 2.95 g, equivalent to 2.94 mmol of Q4-NH2, was obtained as a yellow solid (yield 98%).

The reduction of Q4 was followed by the activation of Q2-CO2H (1.68 g, 3.18 mmol) with 1.6 mmol oxalyl chloride. Q6 was prepared by coupling Q2-COCl with Q4-NH2 in the presence of 3 mL DIEPA. After workup, 3.36 g (2.24 mmol) of Q6 was obtained (yield 76%).

2.5.4 Preparation of the octamer (Q8)
The reduction of Q6 (3.36 g, 2.24 mmol) was carried out in a mixture of 106 mL ethyl acetate and 26 mL EtOH with 0.336 g of Pd/C catalyst, 0.17 g ammonium metavanadate, and an aqueous solution of NH4HCO2 (40 to 50 equivalents relatively to Q6). The workup procedure was the same.
as for Q4-NH2, and 2.9 g (2 mmol) of Q6-NH2 was obtained (yield 89%). Q8 was obtained by coupling Q6-NH2 and Q2-COCl in equimolar amounts (2.1 mmol each) in the presence of DIEPA (1.93 mL). The workup yielded Q8 (3 g, 1.5 mmol, yield 75%).

2.5.5 Preparation of the 16-mer (Q16)

To 25 mL of a 4:1 mixture of EtOAc:EtOH was added Q8 (1.1 g, 0.55 mmol), Pd/C catalyst (10%, 0.1 g), and ammonium metavanadate (catalytic amount) at room temperature. The mixture was heated to 95 °C and 1.42 g of 1.7 mM ammonium formate in water was added in 5 portions. The reaction was stirred overnight at 95 °C. After applying the same workup as in the other reductions, Q8-NH2 (1.06 g, 0.5 mmol, and yield 90%) was recovered as a yellow powder (yield 90%). Q8 (2 g, 1 mmol) was dissolved in 70 mL THF and 7.7 mL MeOH before ground NaOH powder (0.8 g, 130 mL) was added. The mixture was stirred at room temperature for around 0.5 h and monitored by TLC until the reaction was complete. After workup, the reaction yielded 1.65 g (0.8 mmol) of Q8-COOH (yield 80%). Dry Q8-COOH (1.48 g, 0.75 mmol) was dissolved in 5.77 mL of CHCl3 to which was added 0.64 mL of oxalyl chloride. The mixture was stirred for 2 h. The liquid was removed under reduced pressure to yield Q8-COCl (1.49 g, yield 100%).

To a solution of Q8-NH2 (1.06 g, 0.5 mol) and DIEPA (0.25 mL) in CHCl3 (3.38 mL) was added a Q8-COCl (1.49 g, 0.75 mmol) solution in CHCl3 (5 mL). The mixture was stirred overnight at room temperature and monitored by NMR until the peak for the amine completely disappeared. The solution was evaporated under reduced pressure to remove the solvent and the product was refluxed in a mixture of pyridine/H2O (10 mL/2 mL) for 12 h to hydrolyze the anhydride by-product. Then pyridine and H2O were removed by azeotropic distillation with toluene, and the residue was purified by silica gel chromatography using a DCM/EtOAc mixture as eluent to yield Q16 (1 g, 250 µmol, yield 50%).
2.5.6 Preparation of the 24-mer (Q24)

The reduction of Q16 was conducted in a manner similar to that applied to reduce Q8. After conducting the reaction with Q16 (100 mg, 26 \( \mu \)mol), EtOAc (4 mL), EtOH (1 mL), ammonium metavanadate (2 mg), Pd/C catalyst (10 mg), ammonium formate (95 mg), and H2O (0.25 mL), 96 mg (25 \( \mu \)mol) of Q16-NH2 was obtained (yield 96%). The activation of Q8-CO2H (250 mg, 127 \( \mu \)mol) in 1.39 mL CHCl3 with 0.073 mL of oxalyl chloride yielded the activated Q8-COCl (127 \( \mu \)mol, yield 100%). Coupling Q16-NH2 (26 \( \mu \)mol) with Q8-COCl (127 \( \mu \)mol) overnight in the presence of 0.015 mmol DIEPA in 0.4 mL CHCl3 yielded Q24. The work up was the same as for Q16 resulting in 100 mg, 17 \( \mu \)mol of pure Q24 (yield 70%).

2.5.7 Preparation of the 32-mer (Q32)

The saponification of Q16 was conducted in the same manner as for Q8. Q16 (700 mg, 177 \( \mu \)mol) was dissolved in 25 mL THF and 3 mL MeOH. NaOH (140 mg) was added to the mixture to yield Q16-CO2H (664 mg, 170 \( \mu \)mol, yield 76%). The activation of Q16-CO2H was carried out in 3.65 mL CHCl3 with 0.073 mL oxalyl chloride (170 \( \mu \)mol, yield 100%). The coupling of Q16-NH2 (237 mg, 70 \( \mu \)mol) and Q16-COCl (170 \( \mu \)mol) was achieved with 0.04 mL of DIEPA in 5 mL CHCl3. The anhydride by-product was removed by refluxing in a pyridine/H2O (5 mL/0.5 mL) mixture before using silica gel chromatography to isolate the product. In the end, 130 mg (17.9 \( \mu \)mol) of Q32 was recovered (yield 26%).

2.5.8 Preparation of OPV-Q7

Q7-NH2 (20 mg, 11.5 \( \mu \)mol) and OPV-COCl (23 \( \mu \)mol), which had both been synthesized in the H-Lab,6 were reacted in the presence of DIEPA (3.7 \( \mu \)L). OPV-COCl was produced by the
activation OPV-CO\textsubscript{2}H (17.8 mg, 23 \(\mu\)mol) with 35 \(\mu\)mol of Ghosez reagent (1-chloro-N, N, 2-trimethyl-1-propenylamine) in CHCl\textsubscript{3}. Purification was completed by GPC.

**2.5.9 Preparation of OPV-Q\textsubscript{24}**

The reduction of Q\textsubscript{24} was carried out in the same manner as for the other foldamers. Q\textsubscript{24} (50 mg, 8.5 \(\mu\)mol) was dissolved in a mixture of 4 mL EtOAc and 1 mL EtOH in the presence of 0.8 mg ammonium metavanadate, 5 mg Pd/C (10\%) catalyst, 32 mg ammonium formate, and 0.085 mL H\textsubscript{2}O. The reduction was carried out under nitrogen atmosphere. The mass of Q\textsubscript{24}-NH\textsubscript{2} recovered after workup equaled 30 mg. Q\textsubscript{24}-NH\textsubscript{2} (30 mg, 5 \(\mu\)mol) was coupled with OPV-COC\textsubscript{1} (7.5 mg, 10 \(\mu\)mol) in the presence of DIEPA (3.7 \(\mu\)L). The final product was purified by GPC.

**2.5.10 Preparation of OPV-Q\textsubscript{8A}**

Q\textsubscript{8}-NH\textsubscript{2} (150 mg, 77 \(\mu\)mol) was coupled with OPV-COC\textsubscript{1} (1.19 g, 1.5 mmol) in chloroform after stirring overnight in the presence of DIEPA (0.25 mL). The final product was purified by GPC to yield 150 mg OPV-Q\textsubscript{8}. Dry OPV-Q\textsubscript{8} (150 mg, 55 \(\mu\)mol) was dissolved in 38 mL THF and 0.5 mL MeOH before 60 mg ground NaOH powder was added. The mixture was stirred at room temperature for 30 min and monitored by TLC until the reaction was complete. This reaction yielded 123 mg, 45\(\mu\)mol OPV-Q\textsubscript{8A} (yield 58\%).

**2.5 Summary**

The work reported in this thesis describes the behavior in solution of a series of oligoquinoline (Q\textsubscript{n}) foldamers with a number of quinoline units ranging from 1 to 33, terminated by either ester (Q\textsubscript{n}) or carboxylic acid (Q\textsubscript{nA}) groups. The Q\textsubscript{n} or Q\textsubscript{nA} oligomers were linked to OPV-CO\textsubscript{2}H to form the OPV-labeled Q\textsubscript{n} esters (OPV-Q\textsubscript{n}) with \(n\) equal to 4, 7, 9, 17, 24, and 33 and the OPV-labeled acids (OPV-Q\textsubscript{nA}) with \(n\) equal to 4, 8, 17, and 33. Their purity was established by \(^1\text{H}\) NMR and MS. The chemical structure of the foldamers used in this study is provided in Table 2.1. Beside
the samples synthesized in Bordeaux by the author, OPV-Q₄, OPV-Q₄A, OPV-Q₉, OPV-Q₁₇A, OPV-Q₁₇, OPVOPV-Q₃₃A, OPV-Q₃₃, and AQ₂PQ₂A (with hexyl or isobutyl side chain) were also prepared in the H-Lab and were used as received.

**Table 2.1.** Chemical structure of the samples used in this study.

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<th>Samples</th>
<th>Number of Units</th>
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<tr>
<td><img src="image2.png" alt="Chemical structure" /></td>
<td>OPV-Q₇</td>
<td>7</td>
</tr>
<tr>
<td><img src="image3.png" alt="Chemical structure" /></td>
<td>OPV-Q₉</td>
<td>9</td>
</tr>
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<td>OPV-Q₂₄</td>
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</tr>
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<td>33</td>
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<td><img src="image10.png" alt="Chemical structure" /></td>
<td>Q₁₆A</td>
<td>16</td>
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</table>
Chapter 3

Application of TRFA to probe OPV-Qn

3.1 Introduction

The preparation of quinoline-based foldamers with up to 64 units has been published by the H-Lab. The conformation and dimension of the foldamers were investigated by SCXRD analysis. They were found to fold as helices in the solid state, with a 0.136 nm raise per quinoline unit. The relatively small size of the foldamers complicates their characterization in solution, as discussed in the Introduction. Therefore, TRFA was applied to probe the size and conformation of the foldamers in solution, as has been done earlier to study a series of well-defined DNA duplexes in solution.25

In this chapter, a series of quinoline-based foldamers (Qn with \( n = 1 - 33 \)) was prepared and they were labeled with an oligo(phenylene vinylene) (OPV) fluorophore. Time-resolved fluorescence anisotropy (TRFA) experiments were conducted by acquiring the fluorescence decays for OPV-Qn solutions in chloroform with a vertically polarized excitation, and an emission that was polarized either vertically or horizontally to yield the fluorescence decays \( I_{VV}(t) \) and \( I_{VH}(t) \), respectively. The rotational time (\( \phi \)) of the OPV-Qn molecules was retrieved from the global analysis of the \( I_{VV}(t) \) and \( I_{VH}(t) \) fluorescence decays. This chapter explains how the relationship between the rotational time and the oligoquinoline chain length was established to probe the helical conformation of foldamers in solution. Furthermore, the use of the diffusion coefficients (\( D_v \)) and (\( D_\perp \)), describing the rotation of the foldamers around and perpendicularly to the main helix axis, respectively, was also discussed to rationalize the anisotropy results.
3.2 Experimental

3.2.1 Materials

Chloroform (HPLC grade) was used as received for all the fluorescence experiments. The chemical structure of the OPV-Qₙ and QₙA oligomers used in this study is presented in Figure 3.1.

![Chemical structure of OPV-Qₙ and QₙA oligomers](image)

Figure 3.1. Chemical formula of the oligoquinoline foldamers and energy-minimized molecular models of the oligomers, with the OPV unit in red to illustrate its orientation perpendicular to the helical axis. *iso*-Butoxy side chains and protons are omitted for clarity.

3.2.2 Instrumentation

*Absorption measurements:* The absorption spectra were acquired on a Cary 100 UV-Vis spectrophotometer with quartz cells having a 1 cm path length.

*Steady-State Fluorescence (SSF):* Fluorescence spectra for OPV-Qₙ solutions in chloroform were acquired with a Photon Technology International LS-100 steady-state fluorometer using the right-
angle geometry. The OPV-Qₙ samples were excited at 408 nm and the fluorescence spectra were acquired from 420 to 700 nm.

Time-resolved fluorescence anisotropy (TRFA): TRFA measurements were conducted with a HORIBA Ultima Ultrafast Fluorescence Lifetime Spectrofluorometer equipped with a delta diode laser having a maximum emission intensity at 479 nm, to selectively excite the OPV fragment of the OPV-Qₙ foldamers (see Figure 3.2). The fluorescence decays were acquired with a Picosecond Photon Detection (PPD) Module 650, using a 495 nm cut-off filter to minimize scattered light from reaching the detector. The fluorescence intensity was controlled with neutral density filters and the width of the emission monochromator slits was set at 12 nm. The instrument response function was collected by monitoring the light reflected by a metal mirror at $\lambda_{em} = \lambda_{ex} = 479$ nm without the 495 nm cut-off filter. All the fluorescence decays were measured with a time-per-channel (TPC) of 12.8 ps/ch over 4,096 channels, except for the OPVA molecule whose shorter rotational time of 270 ps required a TPC of 1.37 ps/ch for more accurate analysis. For each OPV-Qₙ sample, a small amount of OPV-Qₙ was dissolved and diluted in chloroform to obtain an absorbance at 479 nm of 0.09 OD, corresponding to an OPV-Qₙ concentration of $1.4 \times 10^{-5}$ M assuming a molar extinction coefficient at 479 nm of 6,250 M$^{-1}$cm$^{-1}$ for OPV bound to the foldamers (see Results and Discussion section). The fluorescence decays were acquired using excitation light that was vertically polarized (V) and with the emission polarizer oriented at the magic angle ($I_{VM}(t)$), parallel ($I_{VV}(t)$), and perpendicular ($I_{VH}(t)$) to the vertically polarized excitation light. The analysis for a TRFA experiment began by fitting with a single exponential the fluorescence decay for the dye acquired with the excitation and emission polarizers set in the vertical and magic angle (54.7 °) orientations, respectively. The magic angle orientation used for the $I_{VM}(t)$ decay eliminates polarization effects.$^{27}$ All the fluorescence decays were acquired with
10,000 and 20,000 counts at the decay maximum. The fit of the \( I_{VM}(t) \) decays yielded a natural lifetime \( (\tau_o) \) of 1.6 ns for the OPV. The vertically \( (I_{VV}(t)) \) and horizontally \( (I_{VH}(t)) \) polarized fluorescence decays were fitted to Equations 3.1 and 3.2, respectively, which were presented as Equations 1.3 and 1.4 in the first chapter.

\[
I_{VV}(t) = \frac{I_o}{3} e^{-t/\tau_o} \times [1 + 2r(t)] \tag{3.1}
\]

\[
I_{VH}(t) = \frac{I_o}{3G} e^{-t/\tau_o} \times [1 - r(t)] \tag{3.2}
\]

In Equation 3.1 and 3.2, \( G \) is the G-factor for TRFA measurements and \( r(t) \) represents the TRFA. Contrary to classic experimental set ups for TRFA measurements for which the intensity of the excitation source must be carefully monitored to determine the G-factor by flipping the emission polariser at set times between the vertical and horizontal positions, Equations 3.1 and 3.2 make it clear that the G-factor is only a scaling factor which needs to be optimized in the global analysis of the fluorescence decays. The advantage of this approach is that it does away with the tedious and time-consuming alternation of polarizer orientations, so that both decays can be acquired with the same number of counts at the decay maximum to maximize the signal-to-noise ratio, which is inherently impossible to achieve with the standard procedure. Additional experimental details about this procedure can be found in Appendix B. All the TRFA could be approximated by a single exponential, as shown in Equation 3.3. The program written in house to fit the \( I_{VV}(t) \) and \( I_{VH}(t) \) decays globally was aniso01c.

\[
r(t) = r_o \times e^{-t/\phi} \tag{3.3}
\]
The $I_{VV}(t)$ and $I_{VH}(t)$ decays acquired with 20,000 counts at the decay maximum were also fitted with a triexponential TRFA, and the program used for this analysis was aniso02n-3. Global analysis of the polarized fluorescence decays $I_{VV}(t)$ and $I_{VH}(t)$ according to Equations 3.1 and 3.2 yielded good fits, with a $\chi^2$ parameter smaller than 1.3 and the residuals and autocorrelation of the residuals evenly distributed around zero. All the parameters retrieved from the analysis of the fluorescence decays are listed in Appendix D. Each TRFA measurement was conducted in triplicate to assess the error in the rotational times.

The rotational time of the OPV-labeled foldamers was then related to the hydrodynamic volume of the macromolecules according to Equation 1.1. It must be stated that, strictly speaking, Equation 1.1 should only be used for spherical objects, which the OPV-labeled foldamers are not. Nevertheless, it was found to satisfyingly describe these constructs over the range of foldamer lengths studied in this chapter, and it was used as an empirical expression to describe the rotational time of the foldamers.

Approximating the shape of the foldamer to a cylinder implied that $V_h$ would be a function of the squared hydrodynamic radius ($R_h^2$) of the cylinder, which is constant, the height of the foldamers, which is related to the number of units ($NU$) in the foldamer, the helical rise per quinoline residue ($\Delta h$), and the volume ($V_o$) of the OPV label, as shown in Equation 3.4. Consequently, $\phi$ was expected to increase linearly with increasing number of quinoline units ($NU$) when the temperature and viscosity of the solution remained constant.

\[
\phi = \frac{\eta}{k_B T} \times \left( V_o + \Delta h \times NU \right) \quad (3.4)
\]
Since $\Delta h$ is known to equal 0.136 nm based on a pitch of 0.34 nm for an oligquinoline helix containing 2.5 quinolines per helical turn, Equation 3.4 implies that a plot of $\phi$-versus-$NU$ should yield a straight line if the OPV-labeled foldamers adopted a rigid helical conformation in solution.

3.3 Results and Discussion

This study aimed to apply TRFA to characterize the size of a series of oligoquinolines in solution. The chromophore OPV was rigidly attached onto the foldamers via an amide bond, so that its tumbling in solution probed by TRFA closely reflected that of the foldamers. Since the absorbance spectrum for the oligoquinoline backbone overlapped with that for the OPV, the excitation and emission wavelengths employed in the TRFA measurements needed to be carefully selected.

3.3.1 Quantum yield of OPV

The OPV-Q$_n$ samples were excited at 408 nm and the fluorescence spectra were acquired from 420 to 700 nm. The quantum yield ($\Phi_{OPV}$) of OPV-Q$_8$A in chloroform was calculated by applying Equation 3.5,

$$\Phi_{OPV} = \Phi_{PBA} \times \frac{I_F(\text{OPV})}{I_F(\text{PBA})} \times \frac{\text{Abs}(\text{PBA})}{\text{Abs}(\text{OPV})} \times \frac{n_{\text{Chl}}^2}{n_{\text{C}_{4}X}^2}$$  \hspace{1cm} (3.5)

where 9,10-bis(phenyl-ethynyl) anthracene (BPA) was used as a fluorescence standard ($\Phi_{PBA} = 1$ in cyclohexane)$^{28}$ $I_F(\text{OPV})$ and $I_F(\text{PBA})$ are the fluorescence intensity obtained from the integration from 440 to 680 nm of the fluorescence spectra for solutions of OPV in chloroform and PBA in cyclohexane, having absorptions $\text{Abs}(\text{OPV})$ and $\text{Abs}(\text{PBA})$ at 430 nm of 0.047 and
0.049, respectively. The refractive indices $n_{\text{chl}}$ and $n_{\text{CX}}$ of chloroform and cyclohexane were taken as 1.446 and 1.426, respectively.\textsuperscript{29} $\Phi_{\text{OPV}}$ for the OPV-Q\textsubscript{8}A foldamer in chloroform was determined to equal 0.62 ± 0.04.

3.3.2 Absorption and fluorescence spectra

The spectra for the molar extinction coefficient (MEC) of Q\textsubscript{8}A (dashline) and OPVA (solid line) and the fluorescence of OPVA are shown in Figure 3.2. The absorption and fluorescence spectra of oligoquinoline and OPVA match those reported in the literature.\textsuperscript{7} Although the molar extinction coefficient of OPVA passes through a maximum at 430 nm, the oligoquinoline backbone exhibits residual absorption at this wavelength. To avoid any complications, all the fluorescence decays were acquired with a 479 nm excitation wavelength and the fluorescence was collected at 510 nm. This wavelength selection ensured that the excitation photons would solely target the OPV fluorophore while yielding a strong enough fluorescence signal, regardless of the quantity of oligoquinoline foldamer present in the solution.
Based on their chemical composition shown in Figure 3.1, the absorption of the OPV-Q\(_n\) foldamers should show a linear increase in the relative absorption of the oligoquinoline backbone with respect to that of OPV when plotted as a function of the number of units (NU) of the foldamers. This is indeed observed in Figure 3.3A for the OPV-Q\(_n\) foldamers, where the absorption spectra were normalized to a value of unity at 450 nm where only OPVA absorbs (see Figure 3.2). The relative absorption of the oligoquinoline backbone was found to increase with increasing foldamer length. This trend was better illustrated by plotting the ratio of the absorbance at 326 nm over that at 450 nm, namely the \(\text{Abs(326nm)} / \text{Abs(450nm)}\) ratio as a function of the number of quinoline units in Figure 3.3B. The linear trend obtained in Figure 3.3B is predicted by Equation 3.6, where \(\varepsilon_{\text{OPV}}(326\,\text{nm})\), \(\varepsilon_{\text{OPV}}(326\,\text{nm})\), and \(\varepsilon_{\text{OPV}}(450\,\text{nm})\) are the molar extinction coefficient (MEC) of the OPV moiety at 326 nm, one quinoline moiety at 326 nm, and the OPV moiety at 450 nm,
respectively. The MEC of one quinoline moiety was determined experimentally by plotting in Figure 3.3C the MEC at 326 nm ($\varepsilon_{QnA}(326 \text{ nm})$) of the Q1A, Q8A, Q16A, and Q32A foldamers, which were available in larger quantities, as a function of the number of quinoline units. A linear relationship was obtained with a slope of $5,600 \pm 130 \text{ M}^{-1}\text{.cm}^{-1}$, a value consistent with $\varepsilon_Q(326 \text{ nm})$ of Q1 found to equal $5,840 (\pm 100) \text{ M}^{-1}\text{.cm}^{-1}$.

\[
\frac{Abs(326 \text{ nm})}{Abs(450 \text{ nm})} = \frac{\varepsilon_{OPV}(326 \text{ nm})}{\varepsilon_{OPV}(450 \text{ nm})} + NU \times \frac{\varepsilon_Q(326 \text{ nm})}{\varepsilon_{OPV}(450 \text{ nm})}
\] (3.6)

The intercept of the line in Figure 3.3B was found to equal $0.41 (\pm 0.09)$ which is comparable within experimental error with the $\varepsilon_{OPV}(326 \text{ nm})/\varepsilon_{OPV}(450 \text{ nm})$ ratio used in Equation 3.6, found to equal 0.52 for OPVA. The slope ($m$) of the straight line in Figure 3.3B equaled 0.173 ($\pm 0.005$). According to Equation 3.6, dividing $\varepsilon_Q(326 \text{ nm})$ by $m$ should yield $\varepsilon_{OPV}(450 \text{ nm})$. The ratio $\varepsilon_Q(326 \text{ nm})/m$ equaled $32,400 (\pm 750) \text{ M}^{-1}\text{.cm}^{-1}$, which is consistent with the MEC value of 29,300 M$^{-1}$cm$^{-1}$ at 450 nm for OPVA. However, since the MEC value of 32,400 (±750) M$^{-1}$cm$^{-1}$ was obtained for the OPV bound to the foldamers, it is this value and not that of 29,300 M$^{-1}$cm$^{-1}$ found for OPVA that was used to calculate the concentration of OPV-labeled foldamers by UV-Vis absorption.
Figure 3.3. A) Absorption spectra normalized at 450 nm. Bottom to top: OPVA (dashed line), and OPV-Q₄, OPV-Q₇, OPV-Q₉, OPV-Q₁₇, OPV-Q₂₄ and OPV-Q₃₃. Plots of B) the Abs(326 nm)/Abs(450 nm) ratio and C) the molar extinction coefficient at 326 nm for the Q₁₆A, Q₈A, Q₁₆A, and Q₃₂A foldamers as a function of the number of quinoline units.

3.3.3 Time-resolved fluorescence anisotropy

For each solution of $1.4 \times 10^{-5}$ M OPV-labeled foldamer in chloroform, a set of three fluorescence decays were acquired with the excitation light polarized vertically and the emission polarizer oriented at the magic angle, vertically, and horizontally to obtain the $I_{VM}(t)$, $I_{VV}(t)$, and $I_{VH}(t)$ fluorescence decays, respectively. Figure 3.4 presents the three decays for OPV-Q₃₃ as an example. The fits were good, yielding low $\chi^2$ values (< 1.3), and randomly distributed residuals and autocorrelation of the residuals.
Global analysis of the $I_{VM}(t)$, $I_{VV}(t)$, and $I_{VH}(t)$ fluorescence decays of OPV-Q$_{33}$ with Equations 1-2. $\lambda_{ex} = 479$ nm, $\lambda_{em} = 510$ nm, $\chi^2 = 1.16$.

Global analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ fluorescence decays according to Equations 3.1 and 3.2, respectively, yielded the rotational time ($\phi$) of the OPV-labeled foldamers. A plot of $\phi$ as a function of the number of units (NU) in the foldamer is shown in Figure 3.5. Within experimental error, all the $\phi$ values were clustered around a straight line as predicted by Equation 3.4. The intercept $\phi_0$ of the line with the y-axis equaled 0.32 (± 0.03) ns, which matches fairly closely the rotational time of 0.27 (± 0.02) ns for OPVA. Consequently, the trend shown in Figure 3.5 indicates that the hydrodynamic volume of an OPV-labeled foldamer increases linearly with the number of quinoline residues. This conclusion agrees with the notion that the oligoquinoline foldamers adopt a helical conformation in chloroform, whereby the addition of a single quinoline residue increases the volume of the foldamer by a set increment. A similar behavior has also been reported earlier, when monitoring the rotational time of a series of helical DNA duplexes as a function of the number of base pairs of the oligonucleotides.$^{25}$
The line in Figure 3.5 has a slope of 0.057 (± 0.001) ns. Based on the curvature of the helix, an oligoquinoline helix has 2.5 quinoline units per turn, and a pitch of 0.34 nm, and each quinoline unit contributes 0.136 nm to the helix. According to these parameters, Equation 3.4 suggests that the hydrodynamic radius of a Qₙ foldamer equals 1.01 (± 0.01) nm. The radius obtained by TRFA measurements is consistent with the 1.0 nm radius of Qₙ foldamers determined by SCXRD. Based on Equation 3.4, the straight line obtained in Figure 3.5A implies that the volume of a Qₙ foldamer is proportional to the number of its constituting quinoline units (NU). This behavior is very different from that expected for a flexible chain adopting a random coil conformation. In such a case the volume, and thus the rotational time of the macromolecule should increase as NU^{3ν}, where ν is the Flory exponent equal to 0.5 or 0.6 in a θ— or a good solvent, respectively. Regardless of whether ν equals 0.5 or 0.6, a dependency for ϕ as NU^{3ν} would result in a much steeper increase in rotational time than that shown in Figure 3.5B. Consequently, the linear increase observed for ϕ in Figure 3.5 rules out the possibility that the Qₙ foldamers adopt a random coil conformation in chloroform.

Since the molar concentration of the foldamers was fixed to equal 1.4×10⁻⁵ M in the experiments conducted to obtain the results shown in Figure 3.5, the mass concentration of foldamer increased with increasing foldamer length. To ensure that the results presented in Figure 3.5 were not due to the aggregation of OPV-Qₙ foldamers, that would increase with increasing foldamer length and poorer solubility, the OPV-Q₃₃ solution in chloroform was diluted 10-fold and the rotational time of the foldamer was monitored as a function of concentration. As can be seen in Appendix C, the rotational time of the OPV-Q₃₃ foldamer remained constant with the foldamer concentration, demonstrating the absence of aggregation for the solutions investigated.
Figure 3.5. A) Plot of the rotational times determined by TRFA as a function of the number of quinoline units constituting a foldamer. B) Log-log plot of $\phi - \phi_0$ as a function of the number of units. (___) $3\nu = 1.0$, (……..) $3\nu = 1.5$, (___) $3\nu = 1.8$. C) Plot of $r_o$ as a function of the number of quinoline units in the OPV-labeled foldamers. Results from the decays acquired with (○) 10,000 and (□) 20,000 counts; (×) average of all $\phi$ and $r_o$ values.

Finally, the $r_o$ values corresponding to the anisotropy at $t = 0$ were plotted as a function of $NU$ in Figure 3.5C for OPVA and the ester-terminated OPV-Qn foldamers. The largest $r_o$ value that can be obtained for any dye is 0.4, indicating that the absorption and emission dipole moments of the dye are parallel. The $r_o$ value of OPV equaled 0.39 (±0.01), a value close to 0.4, reflecting that the absorption and emission dipole moments of OPV are parallel, in agreement with earlier reports also stating that both dipole moments are aligned along the main OPV axis. As $NU$ increased, $r_o$ decreased slightly, indicating a loss of the initial orientation of the dipole moments that occurred on too short a time scale to be probed by the fluorometer. This rapid re-orientation was probably the result of wobbling of the OPV moiety with respect to the helical foldamer. For short $n$ values, tumbling of the OPV-Qn foldamer in solution is determined by the tumbling of the
OPV moiety. However, as \( n \) increases, it is the foldamer that dictates the tumbling of the OPV, whose wobbling can no longer be transmitted to the entire macromolecule. As a result, the reduction in \( r_0 \) is believed to reflect some residual loss in the rigidity of the foldamer with increasing chain length.

### 3.3.4 Anisotropy of rigid symmetric top macromolecules

The trend shown in Figure 3.5 between the rotational time (\( \phi \)) and the number of units (\( NU \)) of the OPV-Q\(_n\) constructs clearly indicates that the progressive addition of quinoline units onto OPV increases the rotational time in a stepwise manner, by relating the increase in \( \phi \) to a commensurate increase in volume of the oligoquinoline foldamer. Yet the simplicity of this result would appear somewhat fortuitous in view of the complex geometry of the OPV-Q\(_n\) foldamers. Indeed, helical foldamers are symmetric top macromolecules whose TRFA is best described by the triexponential function given in Equation 3.7:

\[
\begin{align*}
 r(t) &= 0.3 \sin^2(\beta_A) \sin^2(\beta_E) \cos(2\xi) \exp[-(4D_\| + 2D_\perp)t] \\
 &+ 0.3 \sin(2\beta_A) \sin(2\beta_E) \cos(\xi) \exp[-(D_\| + 5D_\perp)t] \\
 &+ 0.1 \times [3 \cos^2(\beta_A) - 1] \times [3 \cos^2(\beta_E) - 1] \times \exp[-6D_\perp t] \\
\end{align*}
\]

Equation 3.7

A representation of the different parameters used in Equation 3.7 is provided in Figure 3.6 for the three most common symmetric top macromolecules (oblate and prolate ellipsoids, and cylinders). The angles \( \beta_A \) and \( \beta_E \) in Figure 3.6 represent the angles between the absorption and emission dipole moments of the dye with the main axis of the helix, respectively, while the angle \( \xi \) corresponds to the angle between the projection of the absorption and dipole moments to the plane perpendicular to the main axis of the symmetric top macromolecule. The tumbling of the
dye solidly bound to the macromolecule in solution results in a triexponential TRFA, whose three rotational times are a function of the two diffusion coefficients $D_{//}$ and $D_{\perp}$. Rotation around the main axis is handled by $D_{//}$, whereas $D_{\perp}$ characterizes the tumbling of the symmetric top macromolecule around the secondary axis of the helix that is perpendicular to the main axis.

**Figure 3.6.** Geometries for A) an oblate ellipsoid, B) a prolate ellipsoid, and C) a cylinder. D) Structure of OPV-Q$_{24}$ determined by energy minimization with HyperChem.

Since Equation 3.7 predicts that three different rotational times, namely $\phi_1 = (4 \times D_{//} + 2 \times D_{\perp})^{-1}$, $\phi_2 = (D_{//} + 5 \times D_{\perp})^{-1}$, and $\phi_3 = (6 \times D_{\perp})^{-1}$, are required for the TRFA, of symmetric top molecules, the excellent fits obtained through global analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ decays with Equations 3.1 and 3.2 using a monoexponential TRFA would suggest a problem with the analysis. This apparent contradiction however can be reconciled, by representing the diffusion coefficients $D_{//}$ and $D_{\perp}$ as a function of $NU$ in Figure 3.7 for two types of ellipsoids (Ellipsoid-I and Ellipsoid-II), and a cylinder along with the corresponding decay times $\tau_1$, $\tau_2$, and $\tau_3$ that would be obtained in the expressions for $I_{VV}(t)$ and $I_{VH}(t)$ in Equations 3.1 and 3.2, if the TRFA took the form of Equation 3.7.
Figure 3.7. Plots of (■, □) $D_{\parallel}$ and (□, …) $D_{\perp}$ as a function of the number of units for A) Ellipsoid-I, B) Ellipsoid-II, and C) Cylinder. Plots of (□, □) $\tau_1$, (◊, …) $\tau_2$, and (○, …) $\tau_3$ as a function of the number of units for D) Ellipsoid-I, E) Ellipsoid-II, and F) Cylinder. The insets represent the ratio $\tau_3/\tau_1$ as a function of the number of units.

The calculation of the diffusion coefficients $D_{\parallel}$ and $D_{\perp}$ in Figure 3.7 requires the dimensions of the object along the vertical and horizontal axes, referred to as $L$ and $d$ for the length
and diameter, respectively. If the macromolecular object was well-described by a cylinder, as would be expected for the helical oligoquinoline foldamers, \( L \) would be represented by the product \( NU \times \Delta h \) where \( NU \) is the number of units constituting the foldamer, \( \Delta h \) is the helical rise per quinoline residue, equal to 0.136 nm, and \( d \) would be the helix diameter, estimated to equal 2.0 nm. The aspect ratio \( (p = L/d) \) is the most important parameter to calculate the diffusion coefficients describing the rotation of a symmetric top macromolecule around its one vertical (\( D_{//} \)) and two horizontal (\( D_{\perp} \)) axes of symmetry. As shown hereafter, the diffusion coefficients take different expressions depending on the type of symmetric top macromolecule considered in Figure 3.6.

In the case of an ellipsoid, the diffusion coefficients \( D_{//} \) and \( D_{\perp} \) are given by Equations 3.8 and 3.9, respectively.\(^{27}\)

\[
D_{//} = \frac{3p(p - S)}{2(p^2 - 1)} \times \frac{k_B T}{6\eta V_h} \tag{3.8}
\]

\[
D_{\perp} = \frac{3p(2p^2 - 1)S - p}{2(p^4 - 1)} \times \frac{k_B T}{6\eta V_h} \tag{3.9}
\]

In Equations 3.8 and 3.9, \( k_B \), \( T \), \( \eta \), and \( V_h \) are the Boltzmann constant \((1.38 \times 10^{-23} \text{ J.K}^{-1})\), the absolute temperature in Kelvin, the solvent viscosity \((0.536 \text{ mPa.s for chloroform at 25 }^\circ\text{C})\), and the hydrodynamic volume of a sphere with a volume equivalent to that of the ellipsoid, respectively. \( S \) is a function of \( p \), whose expression given in Equations 3.10 and 3.11, depends on whether the ellipsoid is an oblate \((p < 1)\) or a prolate \((p > 1)\).
For $p > 1$ (prolate)  
$$S = \frac{\text{Ln}\left(p + \sqrt{p^2 - 1}\right)}{\sqrt{p^2 - 1}}$$  \hspace{1cm} (3.10)

For $p < 1$ (oblate)  
$$S = \frac{\arctan\left(\frac{\sqrt{1 - p^2}}{p}\right)}{\sqrt{1 - p^2}}$$  \hspace{1cm} (3.11)

Two types of ellipsoids were considered for the calculation of $D_{\parallel}$ and $D_{\perp}$. Ellipsoid-I would have dimensions along the long and short axes given by $L$ and $d$, representing the helix length (= $NU \times \Delta h$) and diameter (= 2.0 nm), respectively. The volume of Ellipsoid-I with its round tips would thus be smaller than that of a cylinder with sharp tip edges of height $L$ and diameter $d$, as seen in Figures 3.6B and C. Yet Figure 3.5 suggests that the hydrodynamics of the OPV-Q$_n$ foldamers are well-represented by cylinders whose volume is larger than that of ellipsoids having the same $L$ and $d$ parameters. To account for this difference, Ellipsoid-II was considered, whose dimension along the secondary axis (perpendicular to the main axis) was given by $d$ equal to 2.0 nm, i.e. the diameter of a helical Q$_n$ foldamer, but whose length $L$ along the main axis was calculated so that its total volume, given by $(\pi/6)Ld^2$, would match that of a cylinder with an $NU$ value equal to $n$. The $D_{\parallel}$ and $D_{\perp}$ parameters are plotted as a function of the number of quinoline units in Figures 3.7A and B for Ellipsoid-I and Ellipsoid-II, respectively.

In the case of a cylinder, Tirado and Garcia de la Torre derived Equations 3.12 and 3.13 for $D_{\parallel}$ and $D_{\perp}$, respectively, for cylinder aspect ratios ($p = L/d$) between 2 and 30.33

$$D_{\parallel} = \frac{k_BT}{A_\alpha \pi \eta L^3} \left( \frac{4p^2}{1 + \delta_{\parallel}} \right)$$  \hspace{1cm} (3.12)

$$D_{\perp} = \frac{3k_BT}{\pi \eta L^3} (\text{Ln}(p) + \delta_{\perp})$$  \hspace{1cm} (3.13)
In Equations 3.12 and 3.13, $A_0$ equals 3.814, and the functions $\delta_// \text{ and } \delta_\bot$ accounting for end-effect corrections due to the cylindrical shape are given in Equations 3.14 and 3.15, respectively.\(^{34}\)

\[
\delta_// = 1.119 \times 10^{-4} + \frac{0.6884}{p} - \frac{0.2019}{p^2} \tag{3.14}
\]

\[
\delta_\bot = -0.662 + \frac{0.971}{p} - \frac{0.050}{p^2} \tag{3.15}
\]

Using $L = N_U \times \Delta h$ where $\Delta h$ equals 0.136 nm and $d = 2.0 \text{ nm}$ for the diameter of the cylinder, the $D_// \text{ and } D_\bot$ values obtained with Equations 3.12 – 3.15 were plotted as a function of $N_U$ in Figure 3.7C. We note that Equations 3.12 – 3.15 are valid as long as the aspect ratio $p$ takes a value between 2.0 and 30, which would correspond to $N_U$ values between, respectively, 28 and 441 for the oligoquinoline foldamers.

In order for TRF decay measurements to accurately retrieve decay times from TRF decay analysis, a commonly accepted practice is that every two decay times be separated by at least a factor of 2.\(^{27}\) The incorporation of Equation 3.7 for the TRFA into Equations 3.1 and 3.2 for the expression of the $I_{VV}(t)$ and $I_{VH}(t)$ decays would result in two tetraexponentials, with one exponential being the longest component equal to the lifetime of the dye ($\tau_0 = 1.6 \text{ ns}$), and the other three decay times $\tau_1$, $\tau_2$, and $\tau_3$, reporting on the rotational times $\phi_1$, $\phi_2$, and $\phi_3$, would be represented by Equations 3.16 – 3.18.

\[
\tau_1 = (\tau_0^{-1} + \phi_1^{-1})^{-1} = \left(\tau_0^{-1} + 4D_// + 2D_\bot\right)^{-1} \tag{3.16}
\]

\[
\tau_2 = (\tau_0^{-1} + \phi_2^{-1})^{-1} = \left(\tau_0^{-1} + D_// + 5D_\bot\right)^{-1} \tag{3.17}
\]

\[
\tau_3 = (\tau_0^{-1} + \phi_3^{-1})^{-1} = \left(\tau_0^{-1} + 6D_\bot\right)^{-1} \tag{3.18}
\]
The decay times $\tau_1$, $\tau_2$, and $\tau_3$ were then plotted as a function of the number of quinoline units in Figures 3.7D, E, and F for the Ellipsoid-I, Ellipsoid-II, and cylinder geometries, respectively. The ratio $\tau_3/\tau_1$ of the largest and the shortest decay times was calculated for each geometry and is plotted in the inset of Figures 3.7D, E, and F. To properly resolve the three decay times $\tau_1$, $\tau_2$, and $\tau_3$, the ratio $\tau_3/\tau_1$ would have to be larger than 4, to ensure that $\tau_2/\tau_1$ and $\tau_3/\tau_2$ be both greater than 2. As shown in the inset of Figures 3.7D – F, the ratio $\tau_3/\tau_1$ was never greater than 1.5 for all the geometries considered. This result explains why the $I_{VV}(t)$ and $I_{VH}(t)$ decays could be well-fitted by assuming a monoexponential TRFA instead of the triexponential function given in Equation 3.5, indicating that the recovery of the three decay times $\tau_1$, $\tau_2$, and $\tau_3$ would be challenging.

Despite these poor odds, an attempt was made to improve the resolving power of the fluorescence decay analysis program by adopting the following strategy. First, the $I_{VV}(t)$ and $I_{VH}(t)$ decays were acquired with 20,000 instead of 10,000 counts at the decay maximum, to improve the signal-to-noise ratio. Second, the program analyzed the $I_{VV}(t)$ and $I_{VH}(t)$ decays globally, which notably improves the resolving power of the fluorescence decay analysis program. Third, the program did not optimize the three rotational times $\phi_1$, $\phi_2$, and $\phi_3$ but rather optimized the diffusion coefficients $D_//$ and $D_\perp$ directly, thus reducing the number of floating parameters from three decay times to two diffusion coefficients. Fourth, the angles $\beta_\lambda$, $\beta_\varepsilon$, and $\xi$ describing the orientation of the absorption and emission dipole moments between themselves and with respect to the frame of the helical foldamer, were estimated through molecular mechanics optimization (MMO) with HyperChem and were used to calculate the pre-exponential factors in Equation 3.7, which were fixed in the analysis of the fluorescence decays. The angle $\xi$ was set to equal zero by noting that since $r_o$ for OPVA equals 0.4 (see Figure 3.5), the absorption and emission dipole moments of
OPV are parallel, thus implying that $\beta_A = \beta_E$ in Figure 3.6. To determine the angle $\beta_A = \beta_E$ between OPV and the main axis of the oligoquinoline helix, the published SCXRD structure of Q48 was imported into the modeling program HyperChem. Since the structure of Q48 is that of an anhydride between two Q24A moiety, one half of the dimer was deleted because the anhydride moiety induces a small bend in the helix. The OPVA was covalently attached to the N-terminal of Q24 in silico. Energy minimization of the OPV moiety while fixing the position of all the atoms of Q24 yielded the structure shown in Figure 3.6D, where $\beta_A = \beta_E$ was determined to equal 99.5 °. Having determined all the angles needed as inputs in Equation 3.5, Equation 3.19 was obtained for the anisotropy of the OPV-Qn foldamers.

$$
 r(t) = r_o \times \{ 0.71 \times \exp[-t \times (4D_\parallel + 2D_\perp)] 
 + 0.08 \times \exp[-t \times (D_\parallel + 5D_\perp)] 
 + 0.21 \times \exp[-t \times (6D_\perp)] \} \quad (3.19)
$$

The advantage of using Equation 3.19 as compared to Equation 3.7, with three floating rotational times and three floating pre-exponential factors, was to reduce the number of floating parameters from 6 to 3, namely $r_o$, $D_\parallel$, and $D_\perp$. All $I_{VV}(t)$ and $I_{VH}(t)$ decays acquired with 20,000 counts at the decay maximum were fitted by inputting Equation 3.19 into Equations 3.1 and 3.2. The recovered decay times $\tau_1$, $\tau_2$, and $\tau_3$, and diffusion coefficients $D_\parallel$ and $D_\perp$ were plotted as a function of the number of units in Figure 3.5. For all OPV-Qn constructs, the $\tau_1$, $\tau_2$, and $\tau_3$ decay times clustered together, as would be expected from these objects having a small aspect ratio ($p < 2.2$) and as found experimentally, since a single rotational time was sufficient in Equation 3.3 to fit the $I_{VV}(t)$ and $I_{VH}(t)$ decays. Only in the case of the longest OPV-Q33 sample did the $D_\parallel$ and $D_\perp$
diffusion coefficients and \( \tau_1 \), \( \tau_2 \), and \( \tau_3 \) decay times line up with the trends expected for an Ellipsoid-II or Cylinder geometry. The decay times retrieved for the smaller ellipsoids representing the shorter helical foldamers were longer than expected, thus indicating longer rotational times. These longer rotational times were probably due to the 18.7 Å long and 8.7 Å wide OPV moiety, as determined from MMO conducted with HyperChem. Considering its \( \phi \) value of 0.27 ns, OPVA would have a hydrodynamic volume of 2.1 nm\(^3\), equivalent to that of a pentaquinoline foldamer. OPVA will thus slow down the diffusion of the shorter foldamers, but its effect should decrease with increasing foldamer length, becoming negligible for the longer foldamers with 20 or more oligoquinolines, where the OPVA volume represents less than 20% of the overall volume of the macromolecule.

While it is clear that the dimension of the fluorescent OPV label affects the rotational time of the OPV-Q\(_n\) foldamers, particularly for low NU, this effect is much more difficult to handle quantitatively in the case of symmetric top geometries. As observed in Figure 3.5A, where the geometry of the OPV-Q\(_n\) foldamers was approximated to that of a sphere, the effect of the finite volume of the OPV-label manifested itself simply as a the non-zero intercept for the straight line. Every quinoline addition contributed a set volume to the hydrodynamic volume of the OPV-Q\(_n\) foldamer, resulting in the simple straight line found in Figure 3.5A. Although not mathematically correct, the representation of the foldamers as spherical objects appears to be fully justified for OPV-Q\(_n\) foldamers with 1 < \( n \) < 33 based on the trends generated in Figure 3.8, that demonstrate that the rotational times retrieved by assuming a symmetric top geometry for these objects would result in three similar rotational times, that are challenging to resolve experimentally.
3.3 Conclusions

It was demonstrated in this chapter that TRFA is ideally suited to probe the hydrodynamic behavior of rigid foldamers in solution. It was applied to a series of OPV-Qn foldamers, whose rotational time was found to increase linearly with increasing foldamer length. This result is taken as evidence that the addition of one quinoline residue increases the hydrodynamic volume of the foldamer by a set amount, as would be expected for rigid helical objects. Furthermore, the hydrodynamic volume of the OPV-Qn constructs matches perfectly that expected from the dimensions of oligoquinoline helices in the solid state retrieved from SCXRD. The excellent agreement between the structural information retrieved for the foldamers in the solid state by SCXRD and in solution by TRFA is evidence of the reliability of TRFA to probe the structure of rigid foldamers in solution. Consequently, this study opens the path to the use of TRFA in the characterization of foldamers in solution.
Chapter 4

Application of TRFA to probe foldamer self-assembly via metal coordination

4.1 Introduction

Unpublished work from the H-Lab in Bordeaux suggested that metal coordination of the terminal carboxylate anions of the foldamers yields larger foldamer complexes. Consequently, this procedure might offer an exciting non-synthetic pathway towards foldamer elongation. However, validation of this foldamer elongation procedure depends critically on one’s ability to probe the dimension of the putative foldamer complexes in solution. The sensitivity of TRFA to macromolecular size established in Chapter 3 led to the selection of this technique to probe the dimensions of the metal complexes generated by the OPV-Q\textsubscript{n}A foldamers. In this chapter, OPV-Q\textsubscript{4}A, OPV-Q\textsubscript{8}A, Q\textsubscript{8}A, Q\textsubscript{16}A, OPV-Q\textsubscript{17}A, and OPV-Q\textsubscript{33}A were employed to form the metal complexes referred to as (OPV-Q\textsubscript{4}A)\textsubscript{2}-Na, (OPV-Q\textsubscript{8}A)\textsubscript{2}-Na, OPV-Q\textsubscript{8}A-Na-Q\textsubscript{8}A, OPV-Q\textsubscript{8}A-Na-Q\textsubscript{16}A, OPV-Q\textsubscript{17}A-Na-Q\textsubscript{8}A, (OPV-Q\textsubscript{17}A)\textsubscript{2}-Na, OPV-Q\textsubscript{33}A-Na-Q\textsubscript{8}A, OPV-Q\textsubscript{33}A-Na-Q\textsubscript{16}A and (OPV-Q\textsubscript{33}A)\textsubscript{2}-Na where the overall number of units was expected to equal 8, 16, 16, 24, 25, 34, 41, 49, and 66, respectively. TRFA measurements of each metal complex samples were conducted in the same manner as described in Chapter 3. However, as the foldamer length increased, the geometry of the foldamer could no longer be approximated by that of an isotropic object and the $I_{VV}(t)$ and $I_{VH}(t)$ of the longer constructs could not be satisfyingly fitted by assuming a monoexponential TRFA as had been done in Chapter 3 for the shorter OPV-Q\textsubscript{n} foldamers with $n$
≤ 33. Analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ decays obtained for the longer constructs required that all the TRFA took into account the symmetric top geometry associated with a cylindrical shape for the foldamer complexes as well as wobbling of the OPV dye with respect to the frame of these constructs.

4.2 Experimental

4.2.1 Materials

Chloroform (HPLC grade) was used as received in all fluorescence experiments. Sodium hydroxide was bought from Sigma-Aldrich and was reagent grade. The doubly distilled Mili-Q water used to prepare the metal complexes was obtained from a Millipore Milli-RO 10 Plus or Milli-Q UFPlus, Bedford, MA system. The Q$_n$A, OPV-Q$_n$A and AQ$_2$PQ$_2$A samples used in this study were prepared by Dr. Victor Maurizot from the University of Bordeaux, France. Their chemical structure is presented in Figure 4.1 and their preparation has been reported earlier.\textsuperscript{10,26}

![Chemical structure of Q$_n$A, OPV-Q$_n$A and AQ$_2$PQ$_2$A.](image)

**Figure 4.1.** Chemical structure of Q$_n$A, OPV-Q$_n$A and AQ$_2$PQ$_2$A.
4.2.2 Preparation of the metal complex foldamer.

The (OPV-QnA)2-Na complexes were obtained by adding one NaOH pellet into 4 mL of a $1.4 \times 10^{-5}$ M OPV-QnA chloroform solution in the presence of 0.05 mL of water and stirring the mixture for 10 min. Similarly, the OPV-QnA-Na-QmA complexes were generated by adding a solid NaOH pellet and 0.05 mL of water to a 4 mL chloroform solution containing $2.8 \times 10^{-5}$ M OPV-QnA and $2.8 \times 10^{-4}$ M QmA and stirring the mixture vigorously for 10 min. The 10-fold excess of QmA used in these mixtures was meant to maximize the probability that all the fluorescently labeled OPV-QnA form a complex. After allowing the aqueous phase to separate from the organic phase, the foldamer solution in chloroform was withdrawn and placed in a fluorescence cell to conduct fluorescence measurements.

4.2.3 Dilution test on OPV-Q8A and Q16A mixtures.

Several solutions of 1:10 OPV-Q8A:Q16A mixture were prepared in chloroform where the concentration of OPV-Q8A was progressively diluted from 1.5 to 0.15 $\mu$M. One NaOH pellet and 0.05 mL of water were added to 4 mL of foldamer solution in chloroform. After vigorous stirring for 10 min and allowing the separation of the chloroform and aqueous phase, the foldamer solution was transferred to a fluorescence cell.

4.2.4 Polymerization of AQ2PQ2A with OPV-Q8A stoppers

AQ2PQ2A with isobutyl side chains was dissolved in chloroform at concentrations ranging from 17 to 136 $\mu$M, while chloroform solutions of AQ2PQ2A with hexyl side chains were prepared at concentrations ranging from 17 to 510 $\mu$M. A 4 mL solution of a given AQ2PQ2A concentration was prepared in chloroform with a fixed 16 $\mu$M OPV-Q8A concentration to yield solutions with different AQ2PQ2A:OPV-Q8A ratios. Then, one NaOH pellet and water (0.05 mL) were added into
the chloroform solution and the mixture was stirred for 10 min to induce the polymerization of AQ\textsubscript{2}PQ\textsubscript{2}A with OPV-Q\textsubscript{8}A stoppers through their complexation.

4.2.5 Absorption and fluorescence measurements and analysis of the fluorescence decays

The same instruments and protocols described in the Experimental section of Chapter 3 were employed in Chapter 4. The equations from Chapter 3 that are most relevant to the analysis of the fluorescence decays conducted in Chapter 4 are briefly reviewed hereafter. The fluorescence decays acquired with a vertically polarized excitation and a vertically ($I_{VV}(t)$) and horizontally ($I_{VH}(t)$) polarized emission were fitted globally according to Equations 4.1 and 4.2, respectively. To qualify as good, a fit was required to result in a $\chi^2$ value of less than 1.30, and to yield residuals and autocorrelation of residuals randomly distributed around zero.

\[
I_{VV}(t) = \frac{I_o}{3} e^{-t/\tau_r} \times (1 + 2r(t)) \tag{4.1}
\]

\[
I_{VH}(t) = \frac{I_o}{3G} e^{-t/\tau_r} \times (1 - r(t)) \tag{4.2}
\]

In Equations 4.1 and 4.2, the function $r(t)$ is the TRFA.\textsuperscript{27} The TRFA was well represented by the single exponential shown in Equation 4.3, where $\phi$ and $r_o$ are the rotational time and initial anisotropy, respectively, for OPV-Q\textsubscript{n}A complexes with overall dimensions smaller than an equivalent OPV-Q\textsubscript{n} foldamer with $n < 40$.

\[
r(t) = r_o e^{-t/\phi} \tag{4.3}
\]
For larger objects, poor fits were obtained when Equation 4.3 was used for the TRFA. These larger objects were assumed to adopt a cylindrical conformation resulting in Equation 4.4 for the TRFA.\(^{32}\)

\[
r(t) = \frac{r_o}{0.3 \sin^2(\beta_A) \sin^2(\beta_E) \cos(2\xi) + 0.3 \sin(2\beta_A) \sin(2\beta_E) \cos(\xi) + 0.1 [3 \cos^2(\beta_A) - 1][3 \cos^2(\beta_E) - 1]} \times \\
\{0.3 \sin^2(\beta_A) \sin^2(\beta_E) \cos(2\xi) \times \exp[-(4D_{//} + 2D_{\perp})t] \\
+ 0.3 \sin(2\beta_A) \sin(2\beta_E) \cos(\xi) \times \exp[-(D_{//} + 5D_{\perp})t] \\
+ 0.1 \times [3 \cos^2(\beta_A) - 1][3 \cos^2(\beta_E) - 1] \times \exp[-6D_{\perp}t]\} \\
(4.4)
\]

Consequently, the anisotropy in Equation 4.4 is a sum of three exponentials with the rotational times \(\phi_1, \phi_2, \text{ and } \phi_3\) whose expression is given in Equations 4.5 – 4.7.

\[
\phi_1 = \left(4D_{//} + 2D_{\perp}\right)^{-1} \\
(4.5)
\]

\[
\phi_2 = \left(D_{//} + 5D_{\perp}\right)^{-1} \\
(4.6)
\]

\[
\phi_3 = \left(6D_{\perp}\right)^{-1} \\
(4.7)
\]

In Equations 4.4 – 4.7, the diffusion coefficients \(D_{//}\) and \(D_{\perp}\) represent the rotation of the cylinder around its main and secondary axis, respectively; \(\beta_A\) and \(\beta_E\) are the angles between the major axis of the cylinder and the dipole moments of absorption (\(\mu_A\)) and emission (\(\mu_E\)) of the dye, respectively, and \(\xi\) is the angle between the projection of \(\mu_A\) and \(\mu_E\) in the plane perpendicular to the major axis of the cylinder (see Figure 3.6C).\(^{32}\) In the case of the OPV-Q\(_n\) foldamers, the angles \(\xi, \beta_A, \text{ and } \beta_E\) have been found to equal 0, 99.5, and 99.5°, respectively, so that \(r_o\) should equal 0.4.
An $r_o$ value lower than 0.4 implies that OPV undergoes isotropic wobbling that occurs on a timescale that is too short for detection with the time-resolved fluorometer.\textsuperscript{37}

The conclusion reached in an earlier study that the OPV-Q$_n$ foldamer could be viewed as cylindrical objects suggested that Equations 4.8 – 4.11, derived by Tirado and Garcia de la Torre, could be applied to estimate the diffusion coefficients $D_{\parallel}$ and $D_{\perp}$ of the foldamers.\textsuperscript{33}

\begin{equation}
D_{\parallel} = \frac{k_B T}{A_0 \pi \eta L^3} \left( \frac{4 p^2}{1 + \delta_{\parallel}} \right) \tag{4.8}
\end{equation}

\begin{equation}
D_{\perp} = \frac{3 k_B T}{\pi \eta L^3} \left( \ln(p) + \delta_{\perp} \right) \tag{4.9}
\end{equation}

Equations 4.8 and 4.9 are valid for aspect ratios $p = L/d$, where $L$ and $d$ are the length and the diameter of the helical foldamer, respectively, between 2 and 30. These equations use the parameter $A_0$ equal to 3.814 and the functions $\delta_{\parallel}$ and $\delta_{\perp}$ that account for end-effect corrections due to the cylindrical shape. The expressions of $\delta_{\parallel}$ and $\delta_{\perp}$ are given in Equations 4.10 and 4.11, respectively.

\begin{equation}
\delta_{\parallel} = 1.119 \times 10^{-4} + (0.6884/p) - (0.2019/p^2) \tag{4.10}
\end{equation}

\begin{equation}
\delta_{\perp} = -0.662 + (0.971/p) - (0.050/p^2) \tag{4.11}
\end{equation}

Considering that $L = NU \times \Delta h$ where $\Delta h$ equals 0.136 nm and $d = 2.0$ nm for the diameter of the cylinder, and that the aspect ratio $p$ can only take values between 2.0 and 30, it implies that the diffusion coefficients $D_{\parallel}$ and $D_{\perp}$ can only be determined for foldamers with $NU$ values between...
28 and 441, respectively. Equation 4.4 could be used to determine the number-average rotational time \(<\phi>\) of the object as shown in Equation 4.12, which was used to characterize its dimensions.

\[
<\phi> = \frac{0.3\sin^2(\beta_A)\sin^2(\beta_E)\cos(2\xi) + 0.3\sin(2\beta_A)\sin(2\beta_E)\cos(\xi) + 0.1[3\cos^2(\beta_A) - 1][3\cos^2(\beta_E) - 1]}{4D_{||} + 2D_{\perp} + 3D_{||} + 5D_{\perp}}
\]

\[
= \frac{0.3\sin^2(\beta_A)\sin^2(\beta_E)\cos(2\xi) + 0.3\sin(2\beta_A)\sin(2\beta_E)\cos(\xi) + 0.1[3\cos^2(\beta_A) - 1][3\cos^2(\beta_E) - 1]}{6D_{||}}
\]

(4.12)

4.3 Results and Discussion

4.3.1 Rotational time of metal complexes

All pairs of \(I_{VV}(t)\) and \(I_{VH}(t)\) decays, acquired for the complexes containing at least one OPV-Q_{nA} segment, were globally analyzed by first assuming a monoexponential TRFA as described in Equation 4.1 using the program aniso01d-4, which optimized \(r_0\), \(\phi\), the lifetime \((\tau_0)\) of the dye, and the G-factor. The \(\phi\) values of the foldamer complexes retrieved from this analysis are plotted in Figure 4.2 against their expected number of units. The solid straight line in Figure 4.2 represents the linear relationship obtained in Chapter 3 between the rotational time of the OPV-Q_{n} foldamers and their number of units, given as Equation 4.13. Within experimental error, the rotational times of the OPV-Q_{nA} foldamers terminated with a carboxylic acid at one end clustered around the straight line, indicating that the nature of the C-terminal of the Q_{n} foldamer, whether it was a carboxylic acid for OPV-Q_{nA} or a methyl ester for OPV-Q_{n}, had little effect on the tumbling of the macromolecules in solution.

\[
\phi = 0.057\times NU + 0.32
\]

(4.13)
The rotational time of the OPV-Q$_m$A-Na-Q$_n$A complexes was also plotted in Figure 4.2 as a function of the number of units expected for a foldamer made of $n + m$ quinolines. The rotational time increased linearly with increasing number of units and fell on the calibration curve for numbers of units lower than 41. For longer foldamers, $\phi$ reached a plateau at around 2.5 ns. When NU was lower than 41, the rotational time of the complexes corresponded to an equivalent foldamer having an NU value equal to the sum of the NUs of the constituting foldamers; in other words, the rotational time of the OPV-Q$_m$A-Na-Q$_n$A complex was equal to the rotational time of OPV-Q$_{m+n}$. This result strongly suggests that the complexation of two foldamers in solution generated a fully stacked foldamer. Unfortunately, this nice correlation breaks down for longer foldamers with NU greater than 41, whose rotational time appears to plateau when the TRFA was assumed to be monoexponential (Equation 4.3).

The deviation of $\phi$ from the straight line in Figure 4.2 for larger foldamer complexes might simply reflect the inability of a monoexponential TRFA to describe a symmetric top macromolecule with a large aspect ratio such as that of an OPV-Q$_{66}$ foldamer, that would be equivalent to an (OPV-Q$_{33}$A)$_2$-Na dimer. Although global analysis with aniso01d-4 of the $I_{VV}(t)$ and $I_{VH}(t)$ fluorescence decays of (OPV-Q$_n$A)$_2$-Na assuming a monoexponential TRFA yielded a $\chi^2$ value of 1.27, smaller than the recommended value of 1.30, the residuals and autocorrelation of the residuals were not perfectly randomly distributed around zero in Figure 4.2A and B. The non-random distribution of the residuals and their autocorrelation function in Figure 4.2A suggests a poor fit, in agreement with the notion that a monoexponential TRFA was not suited to represent the larger foldamer complexes.
Figure 4.2. Plot of the rotational times of OPV-Q_nA (◇) and metal complexes determined by aniso01d-4 (□), aniso02o-3 (○), and aniso03c (△). Grey and empty symbols represent the (OPV-Q_nA)_{2}-Na dimers bearing two OPVs and the OPV-Q_{m}A-Na-Q_{n}A complexes with only one OPV, respectively. Rotational times according to (——) Equation 4.12 and (——) Equation 4.13.

One obvious reason for the worsening of the fits for larger OPV-Q_nA complexes could be their larger aspect ratio \( p = L/d \) that would equal 2.8, 3.3, and 4.5 for the OPV-Q_{33}A-Na-Q_{8}A, OPV-Q_{33}A-Na-Q_{16}A, and (OPV-Q_{33}A)_{2}-Na complexes, respectively. For such large aspect ratios, the difference in \( D_{//} \) and \( D_{\perp} \) might be too large and result in rotational times \( \phi_{1}, \phi_{2}, \) and \( \phi_{3} \) in Equations 4.5 – 4.7 too different to approximate the tri-exponential TRFA shown in Equation 4.4,
with the single exponential TRFA given in Equation 4.3. As shown in Figure 4.4A, the ratio $D_{\parallel}/D_{\perp}$ increases from 2.2 to 6.3 when the number of units of a helical foldamer increases from 30 to 70 and the rotational times $\phi_1$, $\phi_2$, and $\phi_3$ diverge dramatically in Figure 4.4B, thus rationalizing the failure of a monoexponential TRFA to fit the $I_{VV}(t)$ and $I_{VH}(t)$ decays of the longer foldamer complexes. Consequently, Equation 4.4 was employed to fit the $I_{VV}(t)$ and $I_{VH}(t)$ decays globally with Equations 4.1 and 4.2. However, before conducting the fits, the rotational times $\phi_1$, $\phi_2$, and $\phi_3$ given in Equations 4.5 – 4.7 were used to determine the decay times $\tau_1$, $\tau_2$, and $\tau_3$ given in Equations 4.14 – 4.16, that would need to be differentiated in the decay analysis.

$$\tau_1 = \left(\phi_1^{-1} + \tau_o^{-1}\right)^{-1}$$  \hspace{1cm} (4.14)

$$\tau_2 = \left(\phi_2^{-1} + \tau_o^{-1}\right)^{-1}$$  \hspace{1cm} (4.15)

$$\tau_3 = \left(\phi_3^{-1} + \tau_o^{-1}\right)^{-1}$$  \hspace{1cm} (4.16)

The trends obtained with the decay times plotted in Figure 4.4C indicate that $\tau_3$ is much too close to $\tau_o$ to be retrieved accurately. As a rule of thumb, two decay times can be retrieved with confidence from a multiexponential fit of fluorescence decays if one of the decay times is at least twice larger than the other decay time.\textsuperscript{27} As indicated in Figure 4.4C, this condition is clearly not obeyed for the three decay times $\tau_1$, $\tau_2$, and $\tau_3$, among themselves and as compared to $\tau_o$. Consequently, the following strategy was applied to fit the decays. Since the angles $\xi$, $\beta_A$, and $\beta_E$ were known to equal 0, 99.5, and 99.5$^\circ$, the pre-exponential factors in Equation 4.4 were fixed to their values during the decay analysis. Instead of optimizing the decay times $\tau_1$, $\tau_2$, and $\tau_3$, the analysis program optimized the diffusion coefficient $D_{\parallel}$, while $D_{\perp}$ was fixed to the value expected
from a foldamer of size equivalent to that of a given foldamer complex. In so doing, the program

\textit{aniso02o-3} optimized \(r_0, D_0, \tau_0\), and the \(G\)-factor as had been done earlier, reducing the number of floating parameters.\(^{37}\)

<table>
<thead>
<tr>
<th>(r(t)) : monoexponential (Equation 4.1)</th>
<th>(r(t)) : triexponential (Equation 4.4)</th>
<th>(r(t)) : triexponential with wobbling (Equation 4.17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(aniso01d-4; (\chi^2 = 1.27))</td>
<td>(aniso02o-3; (\chi^2 = 1.26))</td>
<td>(aniso03c; (\chi^2 = 1.17))</td>
</tr>
</tbody>
</table>

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{figure4.3.png}
\caption{Examples of fits from the global analysis of the A), C), and E) \(I_{VV}(t)\) and B), D), and F) \(I_{VH}(t)\) decays of (OPV-Q33A)\(_2\)-NA dimers in chloroform with the programs A) and B) \textit{aniso01d-4}, C) and D) \textit{aniso02o-3}, and E) and F) \textit{aniso03c}.}
\end{figure}

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With the diffusion coefficients $D_{\parallel}$ and $D_{\perp}$ being, respectively, optimized and fixed in the analysis, the averaged rotational time $<\phi>$ was calculated according to Equation 4.12 and plotted in Figure 4.2 as a function of the number of quinolines. Although $<\phi>$ approaches the trend shown as a dashed line in Figure 4.2, expected for cylinders, it still underestimates the expected value. However, using Equation 4.4 instead of Equation 4.3 only marginally improved the quality of the fit, with $\chi^2$ decreasing from 1.27 to 1.26 in Figure 4.3C and D. Furthermore, the fits of the $I_{VV}(t)$ and $I_{VH}(t)$ decays show clear distortions at early times. These distortions occurring on a fast time scale suggest wobbling of OPV with respect to the frame of the foldamer complex.

Figure 4.4. Plots of A) (△) $D_{\parallel}$ and (□) $D_{\perp}$, B) (□) $\phi_1$, (○) $\phi_2$, and (△) $\phi_3$, and C) (□) $\tau_1$, (○) $\tau_2$, and (△) $\tau_3$ obtained with aniso03c as a function of the number of units. The lines that pass closest to the symbols are the trends based on $D_{\parallel}$ and $D_{\perp}$ calculated from Equations 4.8 – 4.11. Solid horizontal line in C) represents $\tau_0 = 1.6$ ns.
OPV wobbling could be handled by using Equation 4.17, derived by Duhamel et al., by assuming that wobbling of the dye occurs between two reflecting barriers making an angle \(l\) between them, \(\omega(p) = p \pi l\), and \(D_w\) is the rotational diffusion coefficient for the wobbling of OPV around the helical axis. If anisotropic wobbling occurs on a fast time scale that cannot be observed with the fluorometer it would be handled by \(r_o\). The analysis program \texttt{aniso03c} used Equation 4.17 to fit globally the \(I_{VV}(t)\) and \(I_{VH}(t)\) fluorescence decays, where \(r_o, D//, D_w, \tau_o\), and the G-factor were optimized for a given \(l\) value which was fixed in the analysis. Varying \(l\) in 5° increments resulted in an optimal fit, as shown in Figure 4.3E and F, where the residuals and autocorrelation of the residuals were better distributed around zero and the \(\chi^2\) showed a substantial improvement by decreasing from 1.26 to 1.17. The average rotational time calculated from the \(D//\) and \(D_\perp\) values clustered around the dashed line predicted for a cylindrical geometry in Figure 4.2, suggesting that proper handling of the TRFA requires accounting for wobbling and the two diffusion coefficients \(D//\) and \(D_\perp\).

\[
\begin{align*}
\rho(t) &= \frac{r_o}{0.3 \sin^2(\beta_A) \sin^2(\beta_E) \cos(2\xi) + 0.3 \sin(2\beta_A) \sin(2\beta_E) \cos(\xi) + 0.1[3 \cos^2(\beta_A) - 1][3 \cos^2(\beta_E) - 1]} \\
&\times [0.3 \sin^2(\beta_A) \sin^2(\beta_E) \cos(2\xi) \times \exp[-(4D// + 2D_\perp)t]] \\
&\times \left[\frac{\sin^2 l}{l^2} + \frac{1}{l^2} \sum_{p=1}^{\infty} \frac{1 - (-1)^p \cos 2l}{1 - \omega^2(p) / 4} \exp[-\omega^2(p)D_w t] \right] \\
&+ 0.3 \sin(2\beta_A) \sin(2\beta_E) \cos(\xi) \times \exp[-(D// + 5D_\perp)t] \times
\end{align*}
\]
\[
\begin{align*}
\sin^2\left(\frac{l}{2}\right) + \frac{1}{(l/2)^2} \sum_{\ell=1}^{\infty} & \frac{1-(-1)^{\ell} \cos(\ell)}{1-\omega^2(\ell)} \exp\left(-\omega^2(\ell)D_{\ell}t\right) \\
+ 0.1 \times [3\cos^2(\beta_{A})-1] \times [3\cos^2(\beta_{E})-1] \times \exp[-6D_{\perp}t]
\end{align*}
\]

(4.17)

In summary, several analysis programs were implemented and their ability to fit globally the \(I_{VV}(t)\) and \(I_{VH}(t)\) decays obtained with the foldamer complexes was assessed. In the end, an analysis that included wobbling of the OPV and the two diffusion coefficients for symmetric top macromolecules could successfully probe the size of foldamer complexes equivalent to an OPV-Q\(_{66}\) oligoquinoline. Based on these results, metal coordination of oligoquinoline foldamer acids would appear to be a reliable method to elongate quinoline-based foldamers.

4.3.2 \(r_o\) values of metal complexes

The \(r_o\) values retrieved by the three different analysis programs, used to fit the \(I_{VV}(t)\) and \(I_{VH}(t)\) decays, were plotted as a function of the number of units of the foldamers in Figure 4.5. In general, the \(r_o\) values retrieved by assuming a monoexponential TRFA (Equation 4.3), a triexponential TRFA (Equation 4.4), and a triexponential TRFA with wobbling (Equation 4.17) were lowest, intermediate, and highest, respectively. The differences between the \(r_o\) values increased with increasing foldamer length, as longer foldamers required increasingly complex expressions for the TRFA to handle the cylindrical geometry of these symmetric top macromolecules and the wobbling of the OPV. When all the different decorrelation processes were accounted for with the program \textit{aniso03c}, \(r_o\) took its largest value equal to 0.35 ± 0.01. Since the two other programs did not formally account for the more rapid decorrelation processes, they yielded lower \(r_o\) values to reflect this rapid decorrelation.
Figure 4.5. Plot of $r_0$ of OPV-Q$_n$A (◇) and metal complexes determined by aniso01d-4 (○), aniso02o-3 (□), and aniso03c (△). Grey and empty symbols represent the (OPV-Q$_n$A)$_2$-Na dimers bearing two OPVs and the OPV-Q$_m$A-Na-Q$_n$A complexes with only one OPV, respectively. (——) $r_0$ values obtained for the OPV-Q$_n$ foldamers using Equation 4.3 for $r(t)$ up to $n = 33$ and (…) extrapolation for longer OPV-Q$_n$ foldamers.

While the different trends obtained with $r_0$ could be rationalized based on the ability of the analysis programs to account for the various decorrelation processes, the low $r_0$ value obtained for the (OPV-Q$_4$A)$_2$-Na complex was somewhat surprising. This complex, which was expected to have a rotational time similar to that of OPV-Q$_8$, should have been short enough for a monoexponential TRFA. Although the fits appeared satisfactory, the retrieved $r_0$ value of $0.31 \pm 0.03$ was substantially lower than that of 0.37 expected for OPV-Q$_8$. Since $r_0$ equals
where $\alpha$ is the angle between the absorption and emission dipole moments, an $r_o$ value of 0.31 would be equivalent to an $\alpha$ value of 23 or 157 °, rather than 0 ° as found in this thesis and other studies for OPV. Since the (OPV-Q4A)$_2$-Na complex was relatively short and contained two OPV moieties, the possibility of having energy hopping between the two OPV moieties was considered. Rapid energy hopping between the two dyes would lead to the instantaneous delocalization of the absorption and emission dipole moments, that could result in the apparent decrease in $r_o$ observed for this complex.

The efficiency of fluorescence resonance energy transfer (FRET) between the two dyes can be characterized by determining the Förster radius ($R_o$), whose expression is given in Equation 4.18. In a typical FRET experiment, the integral in Equation 4.18 describes the overlap between the fluorescence spectrum of the donor and the absorption spectrum of the acceptor. In the present case where the donor and acceptor are two OPV labels on the same molecule, the integral represents the overlap between the absorption and fluorescence spectra of OPVA shown in Figure 3.2. In Equation 4.18, $\Phi_{OPV}$ is the quantum yield of OPVA, found to equal 0.62 ± 0.04, $N_A$ is the Avogadro number, $n$ is the refractive index of the solvent, $F_{OPV}(\lambda)$ is the normalized fluorescence intensity of OPVA, $\varepsilon_{OPV}(\lambda)$ is the extinction coefficient of OPVA, and $\kappa^2$ is the orientation factor describing the relative orientation in space of the transition dipoles of the donor and the acceptor, whose expression is given in Equation 4.19.

$$R_o = \frac{9000 L n(10) \kappa^2 \Phi_{OPV}}{128 \pi^5 N_A n^4} \int_0^\infty F_{OPV}(\lambda) \varepsilon_{OPV}(\lambda) \lambda^4 d\lambda$$  \hspace{1cm} (4.18)

$$\kappa^2 = \left(\cos \theta_T - 3 \cos \theta_{OPV \#1} \cos \theta_{OPV \#2}\right)^2$$  \hspace{1cm} (4.19)
The expression for $\kappa^2$ in a typical FRET experiment involves the orientation of the emission dipole moment of the donor ($\mu_D$) with respect to the absorption dipole moment of the acceptor ($\mu_A$) (see Figure 4.6). However, since the donor and acceptor are a same OPV molecule, whose absorption and emission dipole moments are parallel and oriented along the main axis of the OPV molecule, the expression for $R_0$, that would usually involve the energy donor ($D$) and acceptor ($A$), was modified to reflect this fact ($OPV = D = A$). In Equation 4.19, $\theta_T$ is the angle between the emission and absorption dipole moments, and $\theta_D$ and $\theta_A$ are the angles $\theta_{OPV#1}$ and $\theta_{OPV#2}$ between the emission or the absorption dipole moments of the OPV moieties located at each end of the complex and the vector joining them (Figure 4.6).\(^{27}\)

To obtain the structure of (OPV-Q4A)$_2$-Na, (OPV-Q8A)$_2$-Na, and (OPV-Q17A)$_2$-Na, the crystal structure of (Q8A)$_2$-Na provided by Dr. Maurizot from the University of Bordeaux was imported in HyperChem. Four quinoline residues were removed at the end of each octamer to yield the (Q4A)$_2$-Na complex, and nine quinoline residues were added at the end of each octamer to yield the (Q17A)$_2$-Na complex. The OPVA moiety was added via a peptide bond at the N-terminal of the two foldamers constituting the (OPV-QnA)$_2$-Na complex, and its energy was minimized while keeping the complex unchanged in the molecular mechanics optimizations to yield the structure of the complexes (OPV-Q4A)$_2$-Na, (OPV-Q8A)$_2$-Na and (OPV-Q17A)$_2$-Na shown in Figure 4.6.

The values of $\theta_T$, $\theta_D$, and $\theta_A$ for (OPV-Q4A)$_2$-Na were found by HyperChem to equal 29, 94.5 and 85.5 $^\circ$, respectively. Combining Equations 4.18 and 4.19 yielded a Förster Radius of 4.3
nm. However, since the distance between the centers of the two OPV moieties in (OPV-Q4A)₂-Na was only 1.4 nm, less than half the value of $R_o$, strong FRET was expected to take place between the two OPV units in this metal complex, thus resulting in the instantaneous delocalization of the dipole moments and a lower $r_o$ value as found in the TRFA measurements. In fact, the $\alpha$ value of 23 ° found from $r_o$ for the angle between $\vec{\mu}_A$ and $\vec{\mu}_D$ for the short complex agreed with the $\theta_T$ value of 29° predicted from the (OPV-Q₄A)₂-Na structure shown in Figure 4.6. This conclusion further strengthens the notion that the small $r_o$ value found for the short complex was a result of FRET occurring between the two OPV moieties. Applying the same protocol to (OPV-Q₈A)₂-Na, an $R_o$ value of 3.2 nm and a separation distance of 3.0 nm between the centers of the two OPV units were obtained. In a similar way, the $R_o$ value for (OPV-Q₁₇A)₂-Na was 1.15 nm and the distance separating donor from acceptor was 6.2 nm. Therefore, in these two latter cases, the two chromophores were separated by a long distance that was comparable to or much greater than $R_o$ which resulted in weaker FRET and a $r_o$ value that approached the $r_o$ value expected for whole foldamers having a same number of units.
### Figure 4.6.

Scheme representing the emission dipole moment of the donor ($\vec{\mu}_D$) and the absorption dipole moment of the acceptor ($\vec{\mu}_A$) and parameters retrieved from the optimized structures of (OPV-Q4A)$_2$-Na, (OPV-Q8A)$_2$-Na, and (OPV-Q17A)$_2$-Na.

<table>
<thead>
<tr>
<th></th>
<th>(OPV-Q4A)$_2$-Na</th>
<th>(OPV-Q8A)$_2$-Na</th>
<th>(OPV-Q17A)$_2$-Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_D$</td>
<td>95°</td>
<td>116°</td>
<td>106°</td>
</tr>
<tr>
<td>$\theta_A$</td>
<td>85°</td>
<td>64°</td>
<td>74°</td>
</tr>
<tr>
<td>$\theta_T$</td>
<td>29°</td>
<td>109°</td>
<td>81°</td>
</tr>
<tr>
<td>$R_o$ (nm)</td>
<td>4.3</td>
<td>3.3</td>
<td>1.1</td>
</tr>
<tr>
<td>$d_{D-A}$ (nm)</td>
<td>1.4</td>
<td>3.0</td>
<td>6.2</td>
</tr>
<tr>
<td>$r_o$</td>
<td>0.3</td>
<td>0.36</td>
<td>0.32</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>23°</td>
<td>15°</td>
<td>21°</td>
</tr>
</tbody>
</table>
4.3.3 Rotational time of OPV-Q₈A-Na-Q₁₆A as a function of OPV-Q₈A concentration

The solution of OPV-Q₈A-Na-Q₁₆A, obtained by mixing $1.4 \times 10^{-5}$ M OPV-Q₈A and $1.4 \times 10^{-4}$ M Q₁₆A, was diluted in an effort to probe the equilibrium leading to foldamer complexation. OPV-Q₈A was first neutralized by NaOH to generate the carboxylate anion, that would induce its self-assembly with the carboxylate anion of another strand in apolar chloroform. Therefore, diluting the solution was expected to induce the dissociation of the OPV-Q₈A-Na-Q₁₆A complex into its shorter constituting elements, namely the OPV-Q₈A and Q₁₆A foldamers, which would result in shorter rotational times. In turn, analysis of the trends obtained by plotting the rotational time as a function of foldamer concentration should yield the equilibrium constant.

In the OPV-Q₈A-Na-Q₁₆A dilution test, the OPV-Q₈A:Q₁₆A molar ratio was kept constant and equal to 1:10, to favor the formation of the OPV-Q₈A-Na-Q₁₆A complex over that of the (OPV-Q₈A)₂-Na symmetric dimer. The three equilibria given in Equations 4.20 – 4.22 accounted for the five species present in solution, namely OPV-Q₈A, OPV-Q₈A-Na-Q₁₆A, (OPV-Q₈A)₂-Na, Q₁₆A, and (Q₁₆A)₂-Na. The same equilibrium constant ($K$) was used for the three equilibria. Equations 4.23 and 4.24 reflected the conservation of the species OPV-Q₈A and Q₁₆A in the solution based on their overall concentrations $[\text{OPV-Q}_8\text{A}]_o$ and $[\text{Q}_{16}\text{A}]_o$, respectively. Among the five species present in solution, the OPV-labeled macromolecules were solely detected in the TRFA experiments. Therefore, the number-average rotational time $<\phi>$ obtained from the mixture was expected to take the expression given in Equation 4.25 for the weighted average of the OPV-labeled unimers ($r_{0u} = 0.37$ and $\phi_{0u} = 0.78$ ns for OPV-Q₈A), mixed foldamers ($r_{0M} = 0.35$ and $\phi_{M} = 1.69$ ns for OPV-Q₈A-Na-Q₁₆A), and dimers ($r_{0D} = 0.36$ and $\phi_{D} = 1.24$ ns for (OPV-Q₈A)₂-Na). Consequently, $<\phi>$ was expected to decrease from 1.7 ns for OPV-Q₈A-Na-Q₁₆A to 0.78 ns to OPV-Q₈A upon dilution of the solution.
2 OPV-Q\textsubscript{8}A $\xleftrightarrow{K}$ (OPV-Q\textsubscript{8}A\textsubscript{2}-Na) \hspace{1cm} (4.20A)

$[(\text{OPV-Q8A})\textsubscript{2}-\text{Na}]=K\times[\text{OPV-Q8A}]^2$ \hspace{1cm} (4.20B)

OPV-Q\textsubscript{8}A+Q\textsubscript{16}A $\xleftrightarrow{K}$ OPV-Q\textsubscript{8}A-Na-Q\textsubscript{16}A \hspace{1cm} (4.21A)

$[\text{OPV-Q8A-Na-Q16A}]=K\times[\text{OPV-Q8A}]^{\text{Q16A}}$ \hspace{1cm} (4.21B)

2 Q\textsubscript{16}A $\xleftrightarrow{K}$ (Q\textsubscript{16}A\textsubscript{2}-Na) \hspace{1cm} (4.22A)

$[(\text{OPV-Q16A})\textsubscript{2}-\text{Na}]=K\times[\text{OPV-Q16A}]^2$ \hspace{1cm} (4.22B)

$[\text{OPV-Q8A}]_0 = [\text{OPV-Q8A}]+[\text{OPV-Q8A-Na-Q16A}]+2\times[(\text{OPV-Q8A})\textsubscript{2}]$ \hspace{1cm} (4.23)

$[\text{Q16A}]_0 = [\text{Q16A}]+[\text{OPV-Q8A-Na-Q16A}]+2\times[(\text{Q16A})\textsubscript{2}-\text{Na}]$ \hspace{1cm} (4.24)

$$<\phi>=\frac{[\text{OPV-Q8A}]\times r_{oU} \phi_U + [\text{OPV-Q8A-Na-Q16A}]\times r_{oM} \phi_M + 2\times[(\text{OPV-Q8A})\textsubscript{2}-\text{Na}]\times r_{oD} \phi_D}{[\text{OPV-Q8A}]\times r_{oU} + [\text{OPV-Q8A-Na-Q16A}]\times r_{oM} + 2\times[(\text{OPV-Q8A})\textsubscript{2}-\text{Na}]\times r_{oD}}$$ \hspace{1cm} (4.25)

The $I_{VV}(t)$, $I_{VH}(t)$, and $I_{VM}(t)$ decays were acquired for the 1:10 OPV-Q\textsubscript{8}A:Q\textsubscript{16}A mixtures as a function of concentration with a slit width for the emission monochromator that was set to 1 nm (instead of 12 nm in the fluorescence experiments presented thus far), to lower the rate of fluorescence counts to within 2.0 % of the 20 MHz repetition rate of the time-resolved fluorometer. Unfortunately, the 1 nm slit width generated a 100 ps contribution in all the fluorescence decays due to scattering of photons being clipped off by the monochromator slits. This contribution could
be handled mathematically by the analysis program *aniso01d* and did not affect the rotational times retrieved from the TRFA measurements. All the TRFA measurements presented from this point on contained this 100 ps contribution. In the future, these decay acquisitions will be repeated with the wider 12 nm emission slits, used for the fluorescence measurements reported so far. The results of the dilution experiments are presented in Figure 4.7.

![Figure 4.7](image)

**Figure 4.7.** Rotation time of the 1:10 OPV-Q₈A:Q₁₆A mixture as a function of OPV-Q₈A concentration. Line is drawn to guide the eye.

The average rotational time plotted in Figure 4.7 increases with increasing foldamer concentration, from 0.75 ns at OPV-Q₈A concentrations below 0.1 μM to 1.7 ns at OPV-Q₈A concentrations greater than 6 μM. The rotational times of 0.75 and 1.7 ns were close to those of OPV-Q₈ and OPV-Q₂₄ foldamers, respectively. The S-shape profile shown for the average rotational time of the 1:10 OPV-Q₈A:Q₁₆A mixture in Figure 4.7 is what would be expected from the dissociation of the OPV-Q₈A-Na-Q₁₆A complex into its two constituting elements.
A mathematical protocol was implemented whereby the concentration of the three species, OPV-Q₈A, (OPV-Q₈A)₂-Na, and OPV-Q₈A-Na-Q₁₆A, were calculated for a given $K$ value and used to compare the $\langle \phi \rangle$ values calculated from Equation 4.25 based on these concentrations and the $\langle \phi \rangle$ values obtained experimentally in Figure 4.7. Unfortunately, poor agreement was obtained between the calculated and experimental $\langle \phi \rangle$ values. This disagreement could have two causes. First, the same equilibrium constant ($K$) was employed to account for the three equilibria depicted in Equations 4.20 – 4.22. This assumption might be erroneous, as $K$ might depend on foldamer length. This aspect of the dilution experiments will be investigated in the future. Second, the dilution experiments changed not only the foldamer concentration but also the ionic strength of the solution, since each foldamer was terminated by a carboxylate anion. Electrostatic repulsion at low foldamer concentration and screening effect at high foldamer concentration might complicate the analysis of the trend shown in Figure 4.7. Here again more experiments will be conducted in order to investigate the effect of ionic strength on these complexation experiments.

Despite the issues raised above, the most important result of the dilution study was the demonstration that the OPV-Q₈A-Na-Q₁₆A complex dissociates upon dilution. These experiments imply that complexation, and thus foldamer elongation, can only take place above a threshold concentration.

### 4.3.4 Oligomerization of AQ₂PQ₂A monomers in the presence of an OPV-Q₈A stopper

The examples presented thus far of the metal complexation of two oligoquinolines terminated at one end with a carboxylate anion into a homo- ((OPV-Q₈A)₂-Na) or hetero- (OPV-Q₈A-Na-Q₁₆A) dimer are examples of closed association mechanisms. The main advantage of a closed association
mechanism resides in the formation of well-defined products, which can be more easily characterized. Its disadvantage in the context of foldamer elongation is that the dimensions of the final product are constrained by the size of the reactants. By contrast, an open association mechanism results in poorly defined products but offers the advantage of generating products with a broad size distribution, including a small fraction of products that could be theoretically of infinite size. With this in mind, the H-Lab in Bordeaux envisioned that a foldamer functionalized with a carboxylic acid at both termini could polymerize by undergoing metal complexation. To this end, the building block referred to as AQ$_2$PQ$_2$A was a pyridine flanked by two quinoline dimers and terminated at both ends with a carboxylic acid (see Figure 4.1). The oligomerization of AQ$_2$PQ$_2$A had already been identified by the H-Lab by using NMR, but the characterization of the oligomer size had remained elusive up to this point. Consequently, the complexation of AQ$_2$PQ$_2$A with itself and OPV-Q$_8$A as a fluorescent stopper was investigated, as it was expected to result in the formation of AQ$_2$PQ$_2$A oligomers ((Q$_2$PQ$_2$)$_n$) terminated with 0, 1, or 2 OPV-Q$_8$A units. In turn, TRFA measurements conducted on the OPV moiety located at the end of (Q$_2$PQ$_2$)$_n$ should yield their number-average rotational time $<\phi>$, and thus their number average length.

Solutions containing a fixed 1.4×10$^{-5}$ M concentration of OPV-Q$_8$A and different molar ratios of AQ$_2$PQ$_2$A:OPV-Q$_8$A were prepared. Their $I_{VV}(t)$ and $I_{VH}(t)$ decays were acquired and fitted with the program aniso01d-4 by assuming a monoexponential TRFA (Equation 4.3). The rotational times retrieved from these experiments are shown in Figure 4.8.

Two types of AQ$_2$PQ$_2$A monomers were prepared in the H-Lab, depending on whether
isobutyl or hexyl side chains were employed to facilitate the solubilization of the resulting (Q2PQ2)n complexes. The hexyl side chain was found to provide better solubility than the isobutyl one. In both cases, $\phi$ increased with increasing AQ2PQ2A:OPV-Q8A molar ratio before reaching a plateau at higher molar ratios. Based on the calibration curve established in Figure 3.5A, the plateau value reached by $\phi$ corresponded to that of an OPV-Qn foldamer constituted of 24 or 30 units for the AQ2PQ2A monomer prepared with isobutyl or hexyl side chains, respectively. The traditional synthesis of Qn foldamers with $n$ equal to 24 or 30 would have been much more challenging to achieve by the traditional synthetic method, but further elongation of (Q2PQ2)n was prevented due to poor solubility.

Figure 4.8. Plot of rotational time as a function of the AQ2PQ2A:OPV-Q8A molar ratio obtained for mixtures of 1.4×10^{-5} M OPV-Q8A and different amounts of AQ2PQ2A with ( ), isobutyl and (□), hexyl side chains.
The progressive increase in $\phi$ observed with increasing molar ratio in Figure 4.8 is a clear indication that oligomerization of AQ$_2$PQ$_2$A took place in solution, and that it could be evidenced by conducting TRFA measurements. Furthermore, the calibration curve established in Chapter 3 for the OPV-Q$_n$ foldamers could be used to yield the size of the (Q$_2$PQ$_2$)$_n$ oligomers based on the size of an equivalent OPV-Q$_n$ foldamer. In conclusion, this study demonstrates the potential of this metal complexation procedure for foldamer elongation.

4.4 Conclusions

It was demonstrated in this chapter that TRFA is a convincing method to characterize the size of products obtained by the self-assembly of foldamer acids induced by metal coordination. The calibration curve determined in Chapter 3 was used to establish that the rotational time of a metal complex was the same as that of an OPV-Q$_n$ oligomer with a number of units $n$ equal to the sum of the number of units of the two foldamers constituting the complex. This result demonstrated that a foldamer complex remained a rigid object in solution. In turn, metal coordination opens a new venue to generate well-defined OPV-Q$_n$ foldamers with an $n$ value as large as 66. This conclusion was reached by taking advantage of the ability of our analysis programs to isolate the wobbling of the OPV dye from the tumbling of the foldamers with a number of units larger than 41.

The dilution experiments conducted with the OPV-Q$_8$A-Na-Q$_{16}$A complexes demonstrated that these complexes were the result of an equilibrium and that lowering the concentrations of the constituting foldamers resulted in the dissociation of the complexes. This equilibrium was taken advantage of to induce the oligomerization of two AQ$_2$PQ$_2$A building blocks whose average size
could be determined by TRFA measurements.

In conclusion, Chapter 4 demonstrated that the self-assembly of foldamer acids by metal coordination represents a viable procedure to elongate foldamers according to a closed or an open association mechanism. The size of the foldamer complexes generated in the process can be estimated by applying TRFA.
Chapter 5

Conclusions and future work

This study represented the first example in the scientific literature where TRFA was applied to characterize the size of whole or complexed foldamers in solution. Over the course of this project, a series of quinoline-based foldamers, terminated by either ester (Qₙ) or carboxylic acid (QₙA) groups, were successfully prepared and selectively labeled with OPV as a dye to form OPV-QₙA or OPV-Qₙ in the H-Lab or by myself.

Solutions of OPV-Qₙ in chloroform were excited with vertically polarized light, and the $I_{VV}(t)$ and $I_{VH}(t)$ fluorescence decays were acquired by monitoring the fluorescence intensity vertically or horizontally polarized, respectively. The decays were globally analyzed by assuming a monoexponential TRFA equation to yield the rotational time of the OPV-Qₙ foldamers. The rotational time was found to increase linearly with increasing foldamer length, which demonstrated that the hydrodynamic volume of the OPV-Qₙ foldamer was increased by a set amount upon addition of one quinoline unit to the foldamer. Furthermore, the hydrodynamic volume of the OPV-Qₙ foldamers in solution agreed perfectly with their dimensions expected from SCXRD analysis. This study established the reliability of TRFA to probe the structure of rigid helical foldamers in solution.

Following these preliminary TRFA experiments, the linear relationship found between rotational time and oligomer chain length was used as a calibration curve to determine the size of the self-assembled foldamers prepared by metal complexation. Comparison of the rotational times obtained for the complexes with this calibration curve indicated that the complexation of foldamers QₙA and QₘA with one sodium ion resulted in the formation of an extended foldamer with a NU value equal to the sum of the NU values of its constituting parts (i.e. n + m). This result suggested
that the foldamer complex remained rigid after self-assembly of the constituting foldamers induced by metal coordination. Consequently, this study demonstrated that the complexation of foldamers by metal coordination provides a novel experimental means to elongate foldamer strands into rigid folded complexes that is much easier and faster than traditional elongation methods based on synthesis.

The dilution test conducted for the OPV-Q8A-Na-Q16A indicated that complex formation between two foldamers is based on an equilibrium and is driven by foldamer concentration. Addition of increasing amounts of AQ2PQ2A to an OPV-Q8A solution induced the formation of oligomeric compounds whose average chain length was determined by TRFA measurements. Unfortunately, extension of the oligomers was limited by their poor solubility. The solubility issue will need to be resolved in the future to prepare longer foldamers.

In summary, the TRFA offers an experimental means to characterize the size and dynamics of oligoquinoline-based foldamers labeled with OPV in solution.

In terms of future work, the experiments described in this thesis have open a new means to probe foldamers in solution. A lot still remains to be done. First, the dilution test conducted with the OPV-Q8A-Na-Q16A complexes needs to be repeated using 12 instead of 1 nm slit width for the emission monochromator to avoid the 100 ps artefact in the $I_{VV}(t)$ and $I_{VH}(t)$ decays. The binding constant for the equilibrium describing the formation of the foldamer complexes needs to be determined. Current derivations fail to properly describe the trends obtained when plotting $<\phi>$ as a function of foldamer concentration. A study of electrostatic effects, that might affect the equilibrium between these charged foldamers, must be conducted. Also, the foldamer size might affect the association mechanism. The possible dependency of the binding constant on foldamer length needs to be resolved to better understand the oligomerization of the AQ2PQ2A monomers.
More work needs to be carried for the oligomerization experiments that would cover a wider range of monomer concentration as compared to what has been accomplished so far in Figure 4.8.
References


Appendices

A] \(^{1}\)H-NMR (300MHz, CHCl\(_3\))

\(\text{OPV-Q}^2\text{E}\)

\(^{1}\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) (ppm): 11.59 (s, 1H), 11.47 (s, 1H), 11.40 (s, 1H), 11.21 (s, 1H), 11.05 (s, 1H), 11.01 (s, 1H), 10.99 (s, 1H), 8.27 (d, \(J = 7.5\) Hz, 1H), 8.21-8.14 (m, 3H), 8.10 (d, \(J = 7.5\) Hz, 1H), 7.99-7.88(m, 4H), 7.86 (d, \(J = 7.8\) Hz, 1H), 7.84-7.76 (m, 3H), 7.54 (s, 2H), 7.52-7.30 (m), 7.23-7.14 (m, 3H), 7.11-7.01 (m, 2H), 6.93 (s, 1H), 6.88 (s, 1H), 6.87 (s, 1H), 6.81 (s, 1H), 6.77 (s, 1H), 6.71 (s, 1H), 6.65 (s, 1H), 6.60 (s, 1H), 6.44 (s, 1H), 5.92 (s, 1H), 4.26-4.15 (m, 3H), 4.11-4.01 (m, 2H) 4.01-3.87 (m, 9H), 3.84 (s, 2H), 3.81 (s, 2H), 3.80-3.70 (m, 5H), 3.68-3.57 (m, 2H), 3.08 (s, 3H), 2.64-2.12 (m, 14H), 2.32 (s, 3H), 1.95 (sept, 1H), 1.69 (s, 3H), 1.44-1.35 (m, 13H), 1.34-1.10 (m, 55H), 1.02 (d, \(J = 6.6\) Hz, 3H), 0.88 (d, \(J = 6.6\) Hz, 3H), 0.84-0.74 (m, 6H). 0.02 (d, \(J = 6.6\) Hz, 3H), -0.05 (d, \(J = 6.6\) Hz, 3H); HRMS (ES\(^+\)): m/z calculated for C\(_{147}\)H\(_{168}\)O\(_{22}\)N\(_{14}\) \([\text{M}+2\text{H}]^{2+}\): 1242.1318; Found: 1242.13057

\(\text{Q}^4\)

\(^{1}\)H NMR(CDCl\(_3\), 300 MHz): \(\delta\) 11.30 (s, 1H), 11.18 (s, 1H), 11.08 (s, 1H), 11.01 (s, 1H), 10.98 (s, 1H), 10.96 (s, 1H), 10.83 (s, 1H), 8.32 (dd, \(J = 8.3, 1.5\) Hz, 1H), 8.23 (dd, \(J = 7.7, 1.2\) Hz, 2H), 8.18 (dd, \(J = 4.2, 1.3\) Hz, 1H), 8.16 (dd, \(J = 3.6, 1.3\) Hz, 1H), 8.12 (dd, \(J = 8.3, 1.3\) Hz, 1H), 8.05 (dd, \(J = 7.7, 1.2\) Hz, 1H), 7.91 (dd, \(J = 3.0, 1.3\) Hz, 1H), 7.90 – 7.85 (m,2H), 7.84 (s, 1H), 7.81 (s, 1H), 7.66 (dd, \(J = 7.7, 1.3\) Hz, 1H), 7.54 (dd, \(J = 7.6, 1.2\) Hz, 1H),7.44 (td, \(J = 8.0, 4.2\) Hz, 2H), 7.35 –7.21 (m, 6H), 7.19 – 7.12 (m, 1H), 7.06 (s, 1H), 7.02(s,1H), 6.98 (d, \(J = 8.0\) Hz, 1H), 6.80 (s, 1H), 6.69 (s, 1H), 6.52 (s, 1H), 6.48 (s, 1H), 6.45 (s, 1H),6.15 (s, 1H), 4.18 – 4.04 (m, 3H), 4.04 – 3.81 (m, 10H), 3.74 (d, \(J = 6.4\) Hz, 2H), 3.66 (dd, \(J = 9.1, 7.4\) Hz, 1H), 2.51 (m, 2H), 2.44 – 2.25 (m,5H), 2.25 – 2.18 (m, 1H), 1.38 – 1.33 (m, 8H),1.27 – 1.10 (m, 40H). MS (ES\(^+\)): m/z calculated for C\(_{112}\)H\(_{112}\)N\(_{16}\)O\(_{19}\) \([\text{M}+\text{H}]^{+}\) 1986.8 found 1986.8.

80
$Q_{16A}$

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 10.93 (s, 1H), 10.73 (s, 2H), 10.56 (s, 1H), 10.54 (s, 1H), 10.38 (s, 1H), 10.36 (s, 1H), 10.24 (s, 1H), 10.22 (s, 1H), 10.17 – 9.94 (m, 6H), 8.21 (dd, $J = 8.1$, 1.5 Hz, 1H), 8.08 – 7.98 (m, 2H), 7.91 (dd, $J = 7.3$, 0.9 Hz, 1H), 7.88 – 7.57 (m, 15H), 7.28 – 6.74 (m, 31H), 6.59 (s, 1H), 6.49 (s, 1H), 6.36 (s, 1H), 6.16 (s, 1H), 6.13 (s, 1H), 5.97 – 5.88 (m, 3H), 5.85 – 5.76 (m, 5H), 5.75 (s, 1H), 4.01 – 3.48 (m, 32H), 2.45 – 2.10 (m, 16H), 1.42 – 0.95 (m, 96H).

MS (ES$^+$): m/z calculated for C$_{224}$H$_{224}$N$_{32}$O$_{35}$ [M]$^+$ 3924.68 found 3924.69.

$OPV-Q_{17E}$

$^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ ppm = 11.10 (1H, s), 11.05 (1H, s), 10.81 (1H, s), 10.52 (1H, s), 10.47 (1H, s), 10.37 (1H, s), 10.27 (1H, s), 10.20 (1H, s), 10.15 (1H, s), 10.11 (1H, s), 10.01 (1H, s), 9.98 (1H, s), 9.96 (1H, s), 9.92 (1H, s), 9.87 (3H, s), 7.93 (1H, d, $J = 7.40$ Hz), 7.86 (1H, d, $J = 7.35$ Hz), 7.77 (2H, d, $J = 7.91$ Hz), 7.72 (3H, d, $J = 7.99$ Hz), 7.49-7.69 (14H, m), 7.43 (3H, s), 7.06-7.23 (7H, m), 6.69-7.06 (28H, m), 6.65 (1H, s), 6.55 (2H, d, $J = 9.88$ Hz), 6.38 (2H, d, $J = 9.28$ Hz), 6.20 (1H, s), 6.13 (2H, d, $J = 5.68$ Hz), 5.90 (2H, d, $J = 4.87$ Hz), 5.61-5.78 (9H, m), 3.80-3.96 (5H, m), 3.43-3.79 (41H, m), 2.82 (3H, s), 1.93-2.38 (26H, m), 0.27-1.26 (120H, m), 0.85 (3H, d, $J = 6.67$ Hz), 0.71(3H, d, $J = 6.67$ Hz), 0.64 (3H, d, $J = 6.67$ Hz), 0.60 (3H, d, $J = 6.67$ Hz), -0.25 (3H, d, $J = 6.48$ Hz), -0.31 (3H, d, $J = 6.70$ Hz).

HRMS (ESI$^+$): m/z calculated for C$_{287}$H$_{310}$N$_{34}$O$_{42}$ [M+2H]$^{2+}$: 2453.6634; found 2453.6588.

$OPV-Q_{24E}$

$^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ (ppm): 11.102 (s, 1H), 11.057 (s, 1H), 10.531 (s, 1H), 10.471 (s, 1H), 10.364 (s, 1H), 10.252 (s, 1H), 10.172 (s, 1H), 10.132 (s, 1H), 10.084 (s, 1H), 9.970 (s, 1H), 9.926 (s, 1H), 9.890 (s, 1H), 9.839 (s, 1H), 9.780 (s, 1H), 9.738 (s, 1H), 9.728 (s, 1H), 9.710-9.578 (m, 8H), 7.946 (d, $J = 7.5$ Hz, 1H), 7.883 (d, $J = 7.5$ Hz, 1H), 7.803-7.416 (m, 28H), 7.299-7.022
(m, 10H), 7.022-6.715 (m, 28H), 6.715-6.559 (m, 12H), 6.542 (s, 1H), 6.408 (s, 1H), 6.382 (s, 1H),
6.220 (s, 1H), 6.146 (s, 1H), 6.116 (s, 1H), 5.896 (s, 1H), 5.887 (s, 1H), 5.738 (s, 1H), 5.726 (s,
1H), 5.707(s, 1H), 5.687 (s, 1H), 5.674-5.629 (m, 2H) 5.629-5.576 (m, 4H) 5.576-5.509 (m, 5H)
3.985-3.826 (m, 5H), 3.826-3.492 (m, 39H), 3.492-3.316 (m, 13H), 2.8372 (s 3H), 2.405-1.926 (m,
31H), 1.865-1.750 (m,3H), 1.288-0.934 (m, 158H), 0.934-0.868 (m, 5H), 0.726 (d, J =6.7 Hz, 3H),
0.6653(d, J =6.7 Hz, 3H), 0.6314(d, J =6.7 Hz, 3H), -0.2387(d, J =6.7 Hz, 3H), -0.2979(d, J =6.7
Hz, 3H). HRMS (ES\textsuperscript{+}): m/z calculated for C\textsubscript{38}H\textsubscript{40}O\textsubscript{5}N\textsubscript{48} \([M+2H]\textsuperscript{2+: 2201.3583; Found: 2201.37726}
**B| Time-Resolved Fluorescence Decay Analysis:**

As discussed in the Experimental section of Chapter 3, the standard procedure applied to determine the time-resolved fluorescence anisotropy (TRFA = $r(t)$) is by acquiring the $I_{VV}(t)$ and $I_{VH}(t)$ fluorescence decays simultaneously by continuously alternating the orientation of the emission polarizer and acquiring the fluorescence signal for short durations at the time. This procedure accounts for fluctuations in the intensity of the excitation source which would affect the overall fluorescence intensity of the $I_{VV}(t)$ and $I_{VH}(t)$ decays over the decay acquisition time. The decays are then combined into Equation B1 to yield the TRFA. Since fluctuations in the intensity of the excitation source are accounted for, the $G$-factor handles the difference in the detection efficiency of the instrument between the two orientations of the emission polarizer and needs to be determined independently.

$$r(t) = \frac{I_{VV}(t) - G \times I_{VH}(t)}{I_{VV}(t) + 2G \times I_{VH}(t)}$$  \hspace{1cm} (B1)

Yet the expression of $r(t)$ in Equation B1 is derived from Equations 3.1 and 3.2 in the main text where $G$ is a simple scaling factor which can be optimized through global analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ fluorescence decays. This procedure was successfully implemented in 2004\textsuperscript{1} for the global analysis of the monomer and excimer fluorescence decays of pyrene-labeled macromolecules in solution and its efficacy in fluorescence decay analysis has led to the publication of a number of reviews.\textsuperscript{2,3} The same logic was applied to the global analysis with Equations 3.1 and 3.2 of the $I_{VV}(t)$ and $I_{VH}(t)$ fluorescence decays acquired with the OPV-Q\textsubscript{n} constructs studied in this report.
Acknowledging that the procedure applied to globally analyze the polarized fluorescence decays according to Equations 3.1 and 3.2 might appear somewhat unorthodox, the parameters retrieved from the analysis of the fluorescence decays acquired with the 9 foldamers and listed in Tables D1 and D2 in Appendix D were utilized to generate 20 $I_{VV}(t)$ and 20 $I_{VH}(t)$ fluorescence decays for each foldamer using Equations 3.1 and 3.2, respectively. The decays were convoluted to the experimental instrument response function (IRF) and different patterns of Poisson noise were added, thus generating a grand total of 360 decays. The analysis of the decays was then conducted globally with the program aniso01c and the $r_0$ and $\phi$ parameters obtained from the analysis of the experimental and simulated fluorescence decays were compared in Figure B1.

![Figure B1](image-url)

**Figure B1.** Comparison of the parameters A) $r_0$ and B) $\phi$ retrieved from the global analysis of the experimental and simulated fluorescence decays.

The $r_0$ and $\phi$ parameters were found to cluster perfectly along the diagonals in Figure B1 indicating that the program retrieved accurately these parameters. Furthermore, the error on the parameters retrieved from the analysis of the simulated fluorescence decays was minuscule compared to that
obtained from the analysis of the experimental fluorescence decays. This observation confirmed that experimental errors, most probably due to the instrument and sample solubility, dwarfed those generated by the analysis program. The excellent agreement found in Figure B1 between the analysis of the simulated and experimental decays validated the procedure applied in this study to analyze the polarized fluorescence decays of the OPV-Q₉ constructs. A follow up study will provide further evidence of the robustness of this new experimental procedure and will be submitted shortly.
C] Dilution study of OPV-Q$_{33}$.

The rotational time of OPV-Q$_{33}$ was monitored as a function of foldamer concentration. As can be seen in Figure C1, $\phi$ remained constant with foldamer concentration indicating that these molecules were not aggregated in chloroform.

![Figure C1. Plot of $\phi$ as a function of OPV-Q$_{33}$ concentration in chloroform.](image)
D] Parameters obtained from the fluorescence decay analysis.

Table D1. Parameters retrieved from the analysis of the \( I_{VV}(t) \) and \( I_{VH}(t) \) decays acquired with 10,000 counts at the decay maximum fitted globally when the anisotropy is a monoexponential (aniso01c) and with the triexponential given in Equation 3.19 (aniso02n-3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>( r_0 ) (ns)</th>
<th>( \phi ) (ns)</th>
<th>( \tau_0 ) (ns)</th>
<th>( \chi^2 )</th>
<th>( D_{//} ) (ns(^{-1}))</th>
<th>( D_{\perp} ) (ns(^{-1}))</th>
<th>( r_0 )</th>
<th>( \langle \phi \rangle ) (ns)</th>
<th>( \tau_0 ) (ns)</th>
<th>( \chi^2 )</th>
</tr>
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<td>OPV-Q4</td>
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<td>0.578</td>
<td>1.67</td>
<td>1.28</td>
<td>0.285</td>
<td>0.285</td>
<td>0.387</td>
<td>0.586</td>
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<td>1.27</td>
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<td>0.283</td>
<td>0.362</td>
<td>0.586</td>
<td>1.69</td>
<td>1.20</td>
</tr>
<tr>
<td>OPV-Q7</td>
<td>0.356</td>
<td>0.727</td>
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<td>1.09</td>
<td>0.223</td>
<td>0.240</td>
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<td>0.230</td>
<td>0.387</td>
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<td>1.22</td>
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<td>0.132</td>
<td>0.289</td>
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<td>1.59</td>
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<td>0.108</td>
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Table D2. Parameters retrieved from the analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ decays of foldamer ester acquired with 20,000 counts at the decay maximum fitted globally when the anisotropy is a monoexponential (aniso01c) and with the triexponential given in Equation 3.19 (aniso02n-3).

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<td>$r_0$</td>
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<td>0.376</td>
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<td>OPV-Q7</td>
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Table D3. Parameters of OPV-QnA retrieved from the analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ decays acquired with 20,000 counts at the decay maximum fitted globally when the anisotropy is a monoexponential (aniso01c) and with the triexponential given in Equation 3.19 (aniso02n-3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$r_0$</th>
<th>$\phi$ (ns)</th>
<th>$\tau_0$ (ns)</th>
<th>$\chi^2$</th>
<th>$D_\parallel$ (ns$^{-1}$)</th>
<th>$D_\perp$ (ns$^{-1}$)</th>
<th>$r_0$</th>
<th>$\tau_0$ (ns)</th>
<th>$\chi^2$</th>
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<td>0.268</td>
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<td>1.279</td>
<td>0.316</td>
<td>0.217</td>
<td>0.310</td>
<td>1.701</td>
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<td>OPV-Q8A</td>
<td>0.341</td>
<td>0.809</td>
<td>1.608</td>
<td>1.238</td>
<td>0.206</td>
<td>0.206</td>
<td>0.341</td>
<td>1.609</td>
<td>1.219</td>
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<td>0.323</td>
<td>0.781</td>
<td>1.617</td>
<td>1.256</td>
<td>0.343</td>
<td>0.100</td>
<td>0.333</td>
<td>1.610</td>
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<td>0.807</td>
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<td>1.603</td>
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<td>OPV-Q17A</td>
<td>0.346</td>
<td>1.289</td>
<td>1.580</td>
<td>1.274</td>
<td>0.159</td>
<td>0.101</td>
<td>0.347</td>
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<td>1.262</td>
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<td>0.341</td>
<td>1.356</td>
<td>1.583</td>
<td>1.193</td>
<td>0.189</td>
<td>0.063</td>
<td>0.348</td>
<td>1.582</td>
<td>1.207</td>
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Table D4. Parameters retrieved from the analysis of the $I_{V\nu}(t)$ and $I_{V\mu}(t)$ decays of metal complex acquired with 20,000 counts at the decay maximum fitted globally when the anisotropy is a monoexponential (aniso01c) and with the triexponential given in Equation 3.19 (aniso02o-3).

<table>
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<tr>
<th>Sample</th>
<th>aniso01d-4</th>
<th>aniso02o-3</th>
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<tbody>
<tr>
<td></td>
<td>$r_0$</td>
<td>$\phi$</td>
</tr>
<tr>
<td></td>
<td>(ns)</td>
<td>(ns)</td>
</tr>
<tr>
<td>OPVQ4-Na-Q4-OPV</td>
<td>0.27</td>
<td>0.83</td>
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<tr>
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<td>0.31</td>
<td>0.72</td>
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<tr>
<td>OPVQ8-Na-Q8-OPV</td>
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<td>0.63</td>
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<tr>
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<td>0.34</td>
<td>1.25</td>
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<tr>
<td>OPVQ8-Na-Q8</td>
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<td>1.26</td>
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<td>OPVQ17-Na-Q17-OPV</td>
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<td>0.24</td>
<td>2.68</td>
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<td>2.75</td>
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<td>-------------------------------------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>OPVQ&lt;sub&gt;33&lt;/sub&gt;-Na-Q&lt;sub&gt;33&lt;/sub&gt;-OPV</td>
<td>0.23</td>
<td>2.63</td>
</tr>
</tbody>
</table>
Table D5. Parameters of longer metal complexes retrieved from the analysis of the $I_{VV}(t)$ and $I_{VH}(t)$ decays acquired with 20,000 counts at the decay maximum fitted globally when the anisotropy is the function given in Equation 4.17 (aniso03c).

| Sample          | $D_{||}$ (ns$^{-1}$) | $D_{\perp}$ (ns$^{-1}$) | $r_0$ (ns) | $<\phi>$ (ns) | $\tau_o$ (ns) | angle | $\chi^2$ |
|-----------------|----------------------|--------------------------|------------|---------------|---------------|-------|---------|
| OPVQ33-Na-Q8    | 0.08                 | 0.03                     | 0.35       | 0.34          | 1.59          | 40    | 1.15    |
|                 |                      |                          |            |               |               |       |         |
| OPVQ33-Na-Q16   | 0.08                 | 0.03                     | 0.34       | 3.39          | 1.59          | 40    | 1.16    |
|                 | 0.07                 | 0.02                     | 0.34       | 4.11          | 1.62          | 45    | 1.15    |
| OPVQ33-Na-Q33-OPV | 0.07               | 0.01                     | 0.38       | 6.26          | 1.61          | 60    | 1.15    |
|                 |                      |                          |            |               |               |       |         |
|                 | 0.06                 | 0.01                     | 0.35       | 6.74          | 1.60          | 45    | 1.18    |
|                 | 0.06                 | 0.11                     | 0.35       | 6.77          | 0.35          | 50    | 1.17    |

E] REFERENCES

