Supporting Information for

Fluorescence Lifetime Standards for Time and Frequency Domain Fluorescence Spectroscopy

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Table of Contents

1. Steady-State Spectroscopy

1.1. Steady-state spectra

The absorption measurements were performed on a Perkin Elmer Lambda 40 UV/vis spectrophotometer. Absorption spectra were measured using monochromator slits of 1 nm and a scanning speed of 120 nm/min. All fully corrected steady-state emission spectra were recorded on a SPEX Fluorolog-2. All spectra were collected at 20 °C for samples without deoxygenation.
The molar absorption coefficients ε were estimated by linear regression of the absorbances (> 0.1) as a function of concentration of 3–5 solutions of different concentrations. Each solution was made separately by weighing the appropriate amount of fluorescent dye (i.e., not by diluting the same stock solution).

1.2. Fluorescence quantum yield determination

The fluorescence quantum yield values ϕ_f were determined for three solutions of different concentrations. The absorbances of these solutions at the absorption maximum were adjusted to an absorbance lower than 0.1. The ϕ_f values were corrected for refractive index variation between the sample and reference solution. Values of the refractive indexes were taken from reference 1. All ϕ_f measurements were carried out on non-deaerated samples. The temperature of the samples was 20 °C, except for NATA and rubrene for which the temperature was set at 25 °C.

1.3. Quantum yields of used reference compounds

a: ϕ_f = 0.55 for quinine bisulfate/1 M H_2SO_4 for λ_{ex} = 355–366 nm.[2]
b: ϕ_f = 0.57 for acridine yellow/MeOH for λ_{ex} = 420 nm.[2]
c: ϕ_f = 0.72 for fluorescein/0.1 N NaOH for λ_{ex} = 486 nm.[3]
d: ϕ_f = 0.81 for rhodamine 6G/H_2O for λ_{ex} = 546 nm.[2]
e: ϕ_f = 0.55 for cresyl violet/MeOH for λ_{ex} = 546 nm.[2]
f: ϕ_f = 0.23 for naphthalene/cyclohexane for λ_{ex} = 275−292 nm.[4]
g: ϕ_f = 0.80 for 2,5-diphenyloxadiazole/cyclohexane for λ_{ex} = 280 nm.[5]

1.4. Literature data

Spectra, fluorescence quantum yields, and lifetimes of the following fluorescent dyes in cyclohexane are available from reference 4.
anthracene
9,10-diphenylanthracene (DPA)
N-methylcarbazole
1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP)
2,5-diphenyloxazole (PPO)
p-terphenyl

1.5. Websites with spectroscopic information (Lifetime data are missing)

The International Spectroscopic Data Bank (IS-DB) is a charitable society, which has developed an electronic database and archive for spectroscopic and associated data.

All scientists are encouraged to submit data to the IS-DB, which can then be shared with the rest of the scientific community who can access it free-of-charge. The IS-DB is collecting all types of spectroscopic data, from all spectroscopic techniques and from all disciplines, whether chemical, life or physical sciences.
http://www.is-db.org/

Alphabetical index of Photochem CAD spectra at the Oregon Medical Laser Center:
http://omlc.ogi.edu/spectra/PhotochemCAD/html/alpha.html

Table S1. Experimental conditions for the recording of the fluorescence emission spectra on the SPEX Fluorolog-2.

Compound [a]	Solvent	Excitation	Emission Monochromator Slits (mm)[b]	Scan Speed (nm/s)	Integration Time (s)
Anthracene	Methanol	1.25	1.25	2.0	0.5
	Cyclohexane	1.25	1.25	2.0	0.5
9-Cyanoanthracene	Methanol	1.25	1.25	2.0	0.5
	Cyclohexane	1.25	1.25	2.0	0.5
DPA	Methanol	1.25	1.25	2.0	0.5
	Cyclohexane	1.25	0.5	2.0	0.5
N-methylcarbazole	Cyclohexane	2.5	1.25	2.0	0.5
Coumarin 153	Methanol	1.25	1.25	1.0	1.0
Erythrosin B	Water	2.5	2.5	2.0	0.5
	Methanol	2.5	2.5	2.0	0.5
NATA	Water	5.0	5.0	1.0	1.0
POPOP	Cyclohexane	1.25	1.25	2.0	0.5
PPO	Methanol	1.25	1.25	1.0	1.0
	Cyclohexane	1.25	1.25	2.0	0.5
Rhodamine B	Water	1.25	1.25	2.0	0.5
	Methanol	1.25	1.25	2.0	0.5
Rubrene	Methanol	1.25	1.25	2.0	0.5
SPA	Water	1.25	1.25	2.0	0.5
p-Terphenyl	Methanol	2.5	2.5	2.0	0.5
	Cyclohexane	2.5	1.25	2.0	0.5

[a] Abbreviations used: DPA: 9,10-diphenylanthracene, NATA: *N*-acetyl-L-tryptophanamide, POPOP: 1,4-bis(5-phenyloxazol-2-yl)benzene, PPO: 2,5-diphenyloxazole, SPA: *N*-(3-sulfopropyl)acridinium.
[b] The bandpass can be calculated using the following formula:
Bandpass (nm) = Slit Width (mm) × Dispersion (nm/mm). The linear dispersion is 1.9 nm/mm for the SPEX Fluorolog-2 with double-grating monochromators and 1200 groove/mm gratings (the focal length of the monochromators of the Fluorolog-2 is 22.5 cm).

Table S2. Absorption (λ_{abs}) and emission (λ_{em}) spectral maxima, molar absorption coefficients at the maximum [$\varepsilon(\lambda_{max})$], and fluorescence quantum yields (ϕ_f) of the studied lifetime standards.

Compound	Solvent	λ_{abs} (max / nm)	$\varepsilon(\lambda_{max})$ (M^{-1} cm^{-1})[a]	λ_{em} (max / nm)	ϕ_f[b]	Ref. compound[e]
Anthracene	Methanol	356	9 500 ± 300	377	0.30 ± 0.09	a
	Cyclohexane	357	9 400 ± 200	377	0.43 ± 0.04	a
9-Cyanoanthracene	Methanol	364	5 100 ± 100	440	0.80 ± 0.04	a
	Cyclohexane	379	10 000 ± 200	402	0.92 ± 0.02	a
DPA	Methanol	371	14 100 ± 100	405	0.83 ± 0.04	a
	Cyclohexane	373	13 600 ± 100	405	1.00 ± 0.04	a
N-methylcarbazole	Cyclohexane	293	21 900 ± 600	344	0.78 ± 0.02[c]	f
Coumarin 153	Methanol	424	17900± 600	534	0.45 ± 0.02	b
Erythrosin B	Water	526	88 000 ± 2000	547	0.012 ± 0.002[c]	d
	Methanol	531	105 500 ± 800	551	0.09 ± 0.01	d
NATA	Water	279	5 800 ± 200	348	0.14 ± 0.01[d]	g
POPOP	Cyclohexane	358	42 000 ± 2000	387	0.97± 0.01	a
PPO	Methanol	303	32 000 ± 1000	360	0.86 ± 0.03	f
	Cyclohexane	303	39 000 ± 1000	357	0.94 ± 0.02[c]	f
Rhodamine B	Water	553	98 000 ± 3000	578	0.41 ± 0.01	e
	Methanol	544	101 000 ± 2000	569	0.66 ± 0.01[c]	e
Rubrene	Methanol	521	4 900 ± 100	553	0.27 ± 0.02[d]	c
SPA	Water	358	17 700 ± 900	490	0.57 ± 0.03[c]	a
p-Terphenyl	Methanol	277	30 000 ± 600	336	0.85 ± 0.03	f
	Cyclohexane	276	31 200 ± 200	340	0.89 ± 0.01[c]	f

[a] The molar absorption coefficients $\varepsilon(\lambda_{max})$ are expressed in the most commonly used units, namely M^{-1} cm^{-1}. In common usage for optical path length l /cm and concentration c /mol dm^{-3} (M), $\varepsilon(\lambda)$ results in dm^3 mol^{-1} cm^{-1} (M^{-1} cm^{-1}), which equals 0.1 m^2 mol^{-1} (coherent SI units). The quoted errors represent one standard error.

[b] All fluorescence quantum yields were determined at 20 °C by Dr. Wenwu Qin and Dr. Nikola Basarić (group of prof. N. Boens), except those of NATA and rubrene (see *d*). The quoted errors represent one sample standard deviation (standard uncertainty).[6]

[c] No refractive index correction necessary.

[d] Measured at 25 °C by Dr. Markus Grabolle and Dr. Ute Resch-Genger of the Bundesanstalt für Materialforschung und –Prüfung (or Federal Institute for Materials Research and Testing) (BAM), AG Optical Spectroscopy, Department I, Richard-Willstätter-Str. 11, D-12489 Berlin, Germany.

[e] Used reference compound for the determination of ϕ_f. The letters refer to section **1.3. Quantum yields of used reference compounds**.

Table S3. Fluorescence quantum yields (ϕ_f) of the lifetime standards in fluid solution at room temperature (20−25 °C) reported in the literature.

Compound	Solvent	ϕ_f[a]	Literature reference (ϕ_f value)
Anthracene	Cyclohexane	0.43	4 (0.36), 7 (0.30)
DPA	Cyclohexane	1.00	4, 8 (1.00), 5 (0.90, 0.97), 9 (0.93)
N-methylcarbazole	Cyclohexane	0.78	4 (0.51)
Coumarin 153	Methanol	0.45	10 (0.46)
Erythrosin B	Water	0.012	11 (0.02)
	Methanol	0.09	11 (0.08)
NATA	Water	0.14	12 (0.14)
POPOP	Cyclohexane	0.97	4 (0.93)
PPO	Methanol	0.86	13 (0.79),14 (0.50)
	Cyclohexane	0.94	4 ,13 (1.00), 5 (0.89, 0.94), 14 (0.85)
Rhodamine B	Water	0.41	15 (0.31)
p-Terphenyl	Cyclohexane	0.89	4 (0.93), 16 (0.77)

[a] Value measured in this study.

Figure S1. Normalized absorption (A/A_{max}) and fully corrected fluorescence (F/F_{max}) spectra of the fluorescence lifetime standards in the given solvents at 20 °C. Quantum yields ϕ_f were determined on nondegassed samples, while the lifetimes τ were measured for deoxygenated samples.

The molar absorption coefficients $\varepsilon(\lambda)$ are expressed in the most commonly used units, namely M^{-1} cm^{-1}. Conversion: 1 M^{-1} cm^{-1} (or 1 dm^3 mol^{-1} cm^{-1}) equals 0.1 m^2 mol^{-1} (SI units). See *Glossary of Terms Used in Photochemistry*, 3rd Ed., prepared for publication by S. E. Braslavsky for the International Union of Pure and Applied Chemistry, Organic and Biomolecular Chemistry Division, Sub-Committee on Photochemistry, 2007.

Figure S1-A. Anthracene in methanol.

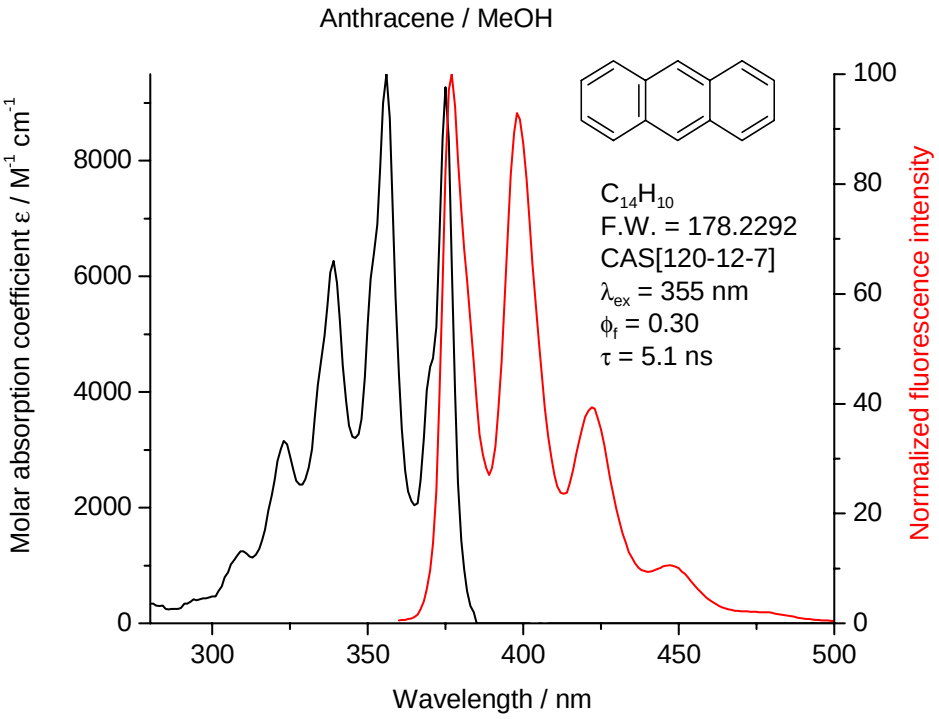


Figure S1-B. Anthracene in cyclohexane.

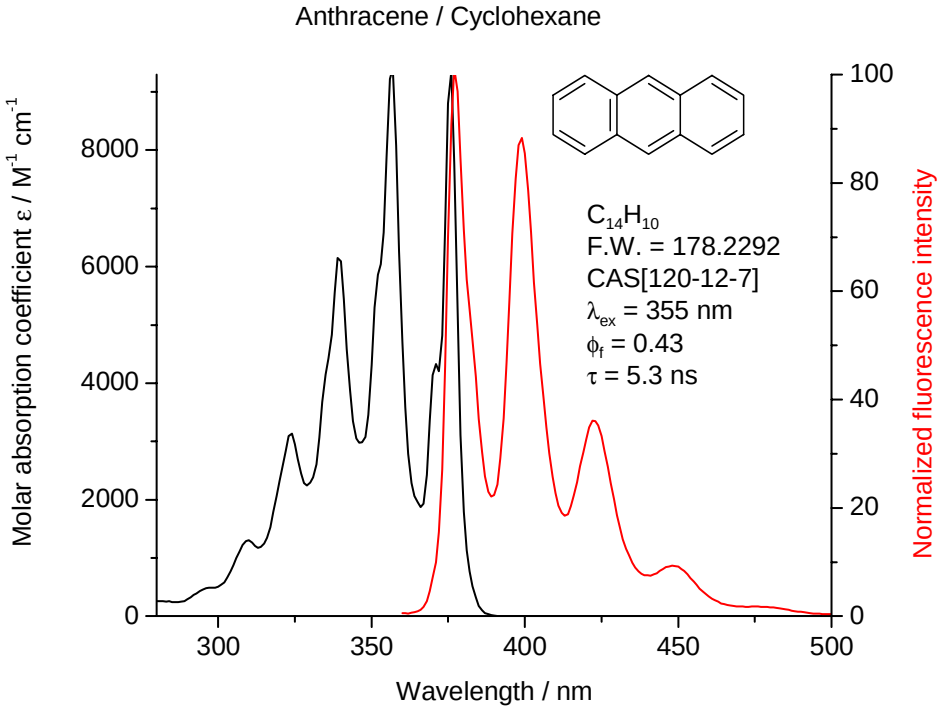


Figure S1-C. 9-Cyanoanthracene (anthracene-9-carbonitrile) in methanol.

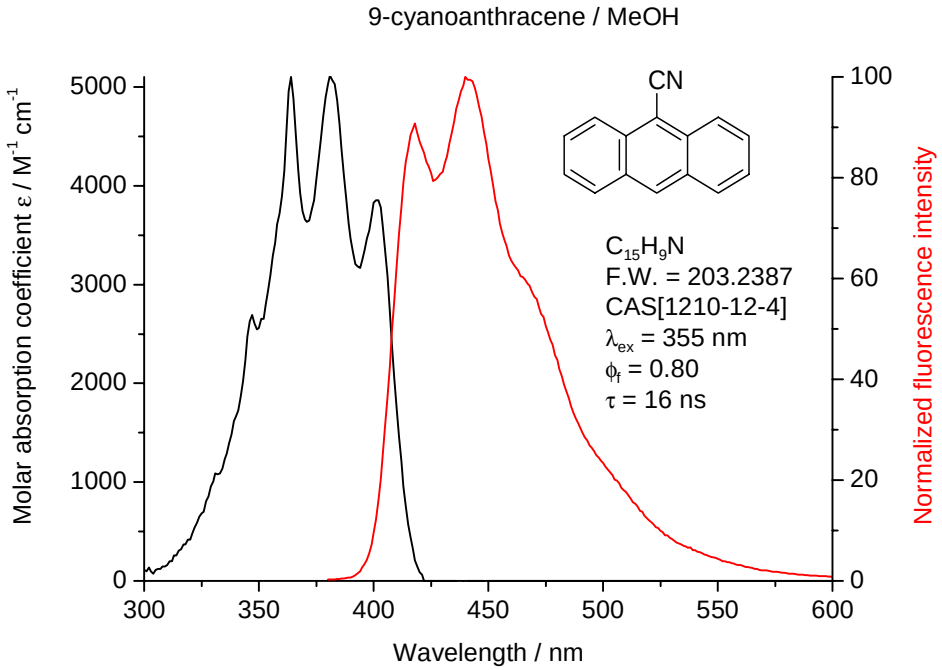


Figure S1-D. 9-Cyanoanthracene (anthracene-9-carbonitrile) in cyclohexane.

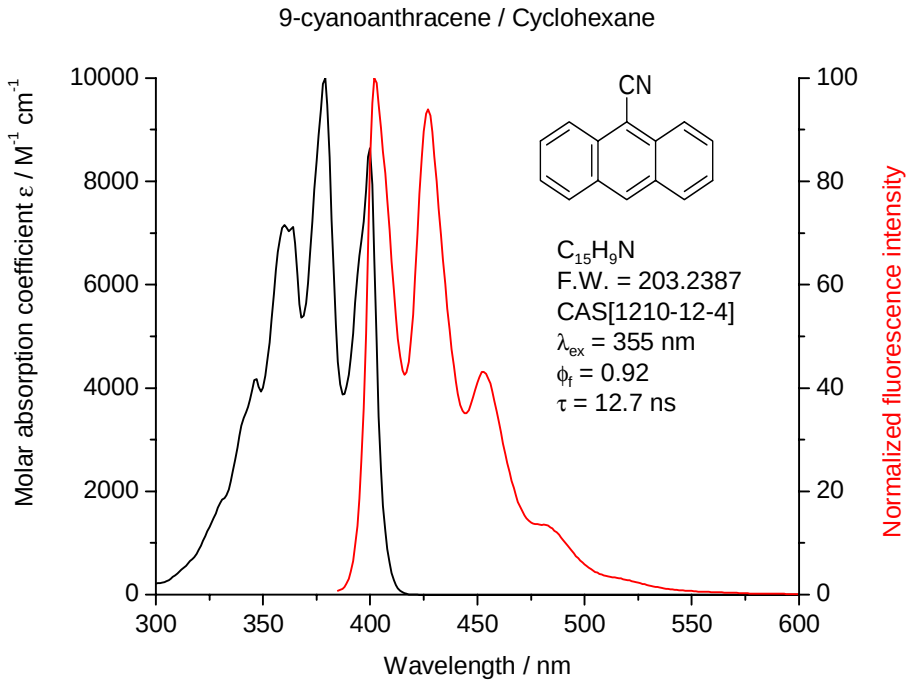


Figure S1-E. 9,10-Diphenylanthracene (DPA) in methanol.

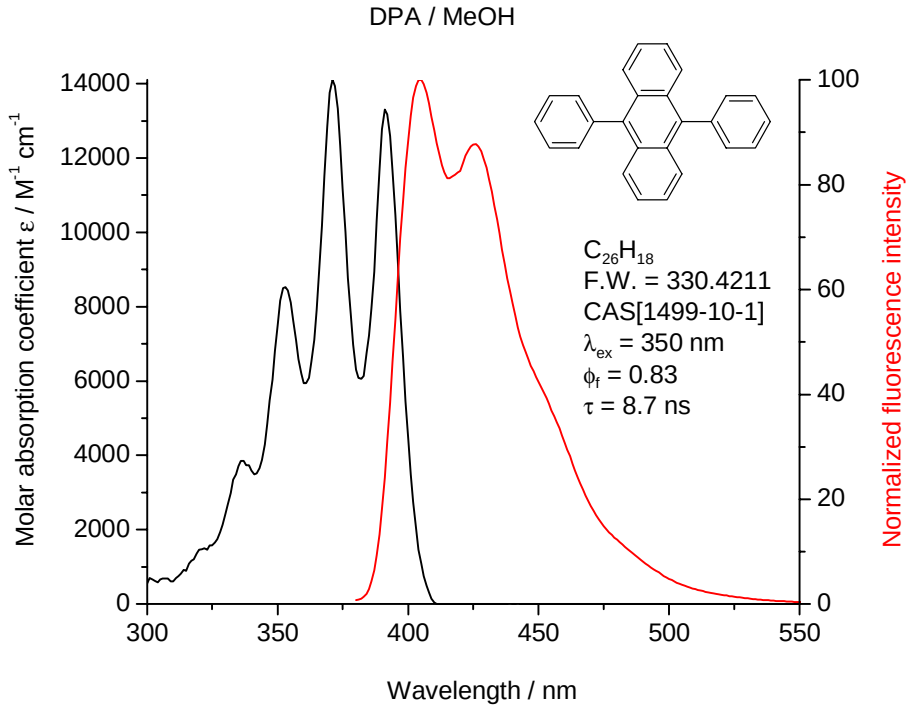


Figure S1-F. 9,10-Diphenylanthracene (DPA) in cyclohexane.

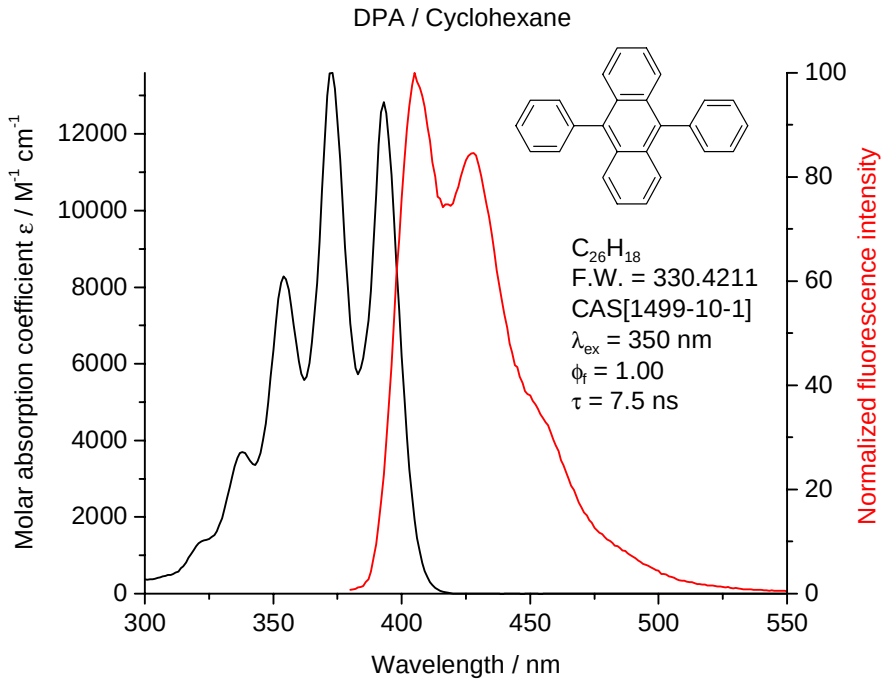


Figure S1-G. *N*-Methylcarbazole (9-methyl-9*H*-carbazole) in cyclohexane. The same fluorescence spectrum was obtained upon excitation at 328 and 343 nm.

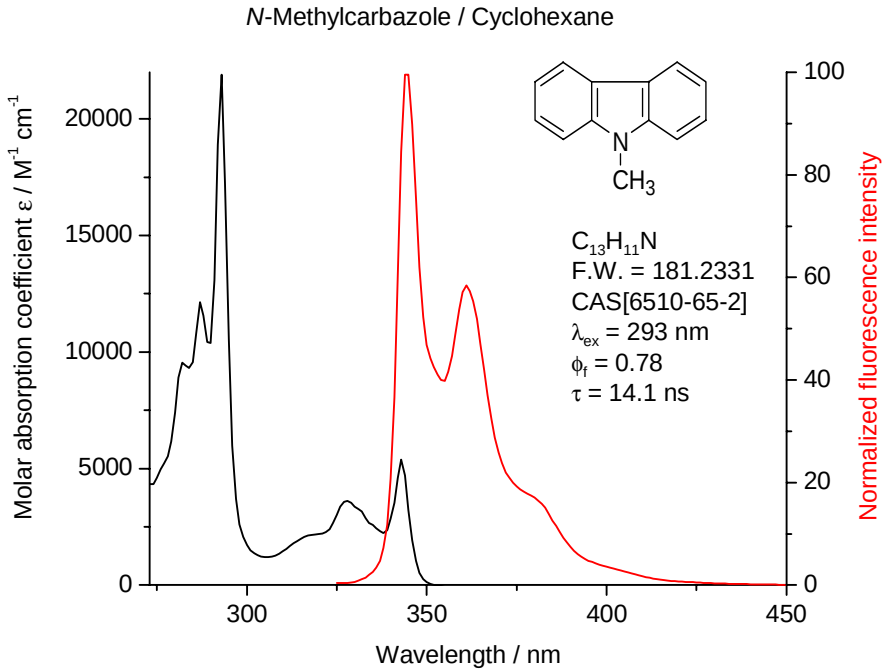


Figure S1-H. Coumarin 153 in methanol.

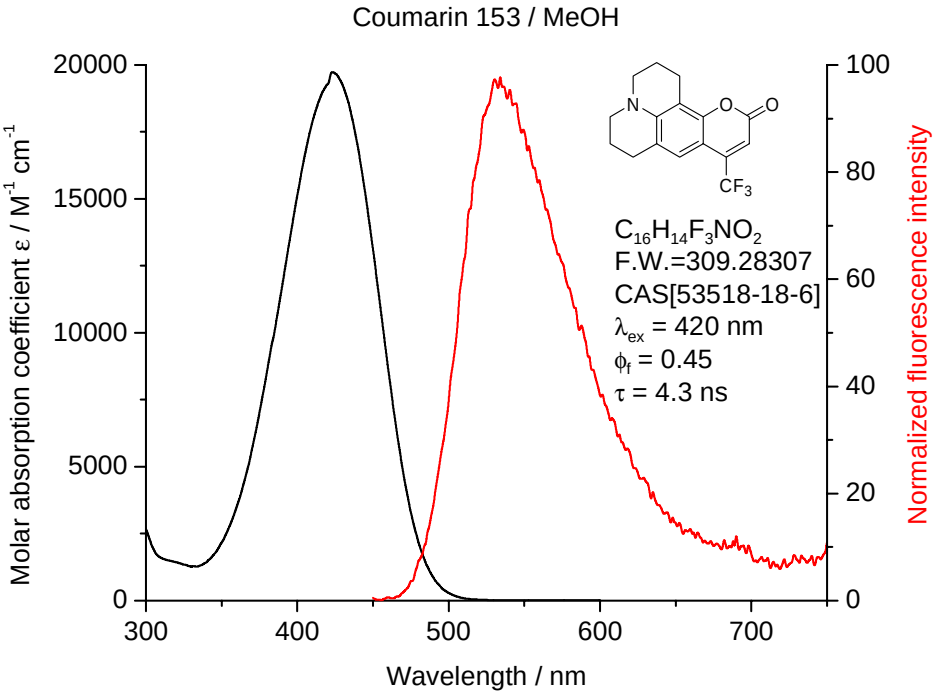


Figure S1-I. Erythrosin B in water.

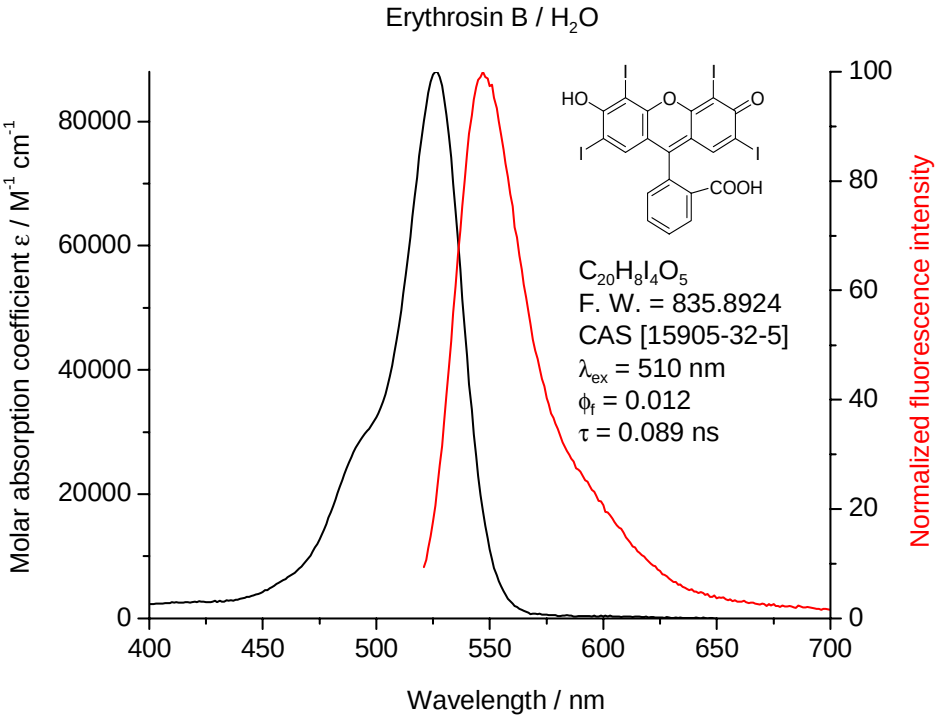


Figure S1-J. Erythrosin B in methanol.

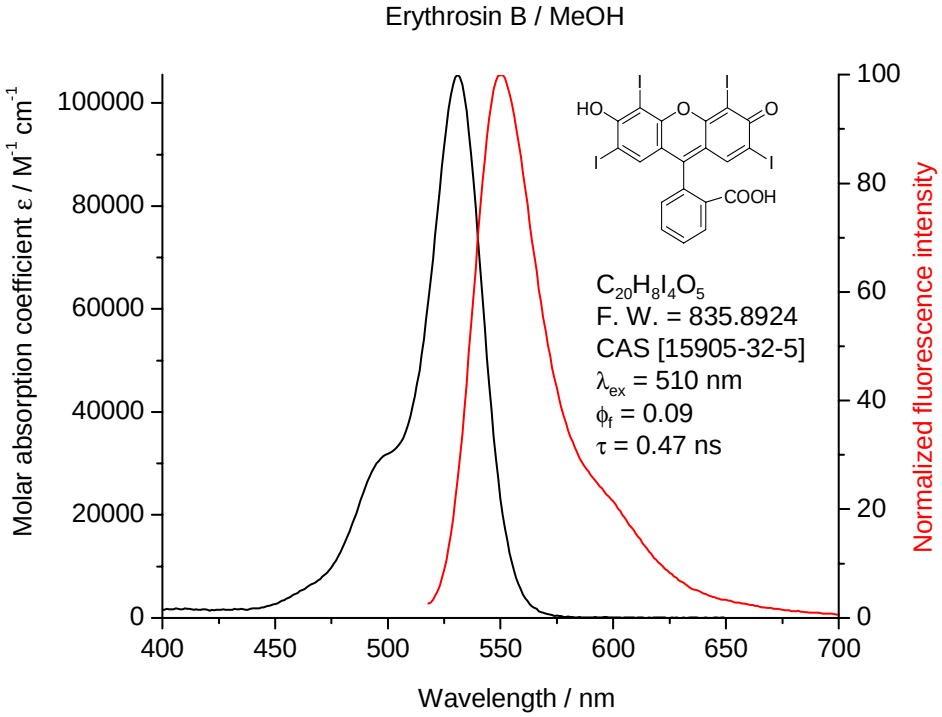


Figure S1-K. *N*-acetyl-L-tryptophanamide (NATA) in water.

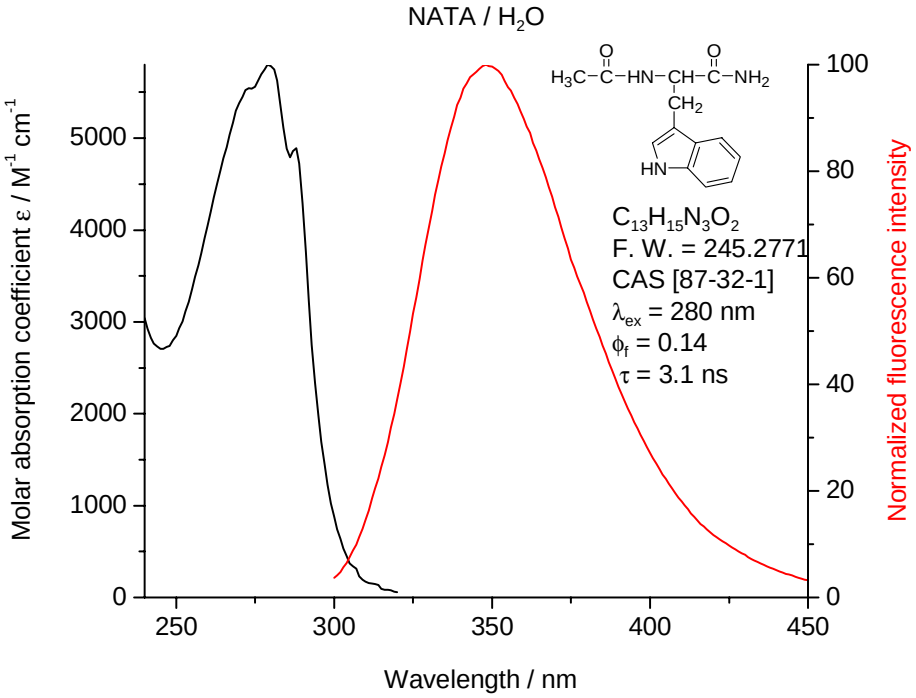


Figure S1-L. POPOP [1,4 bis(5-phenyloxazol-yl)benzene] in cyclohexane.

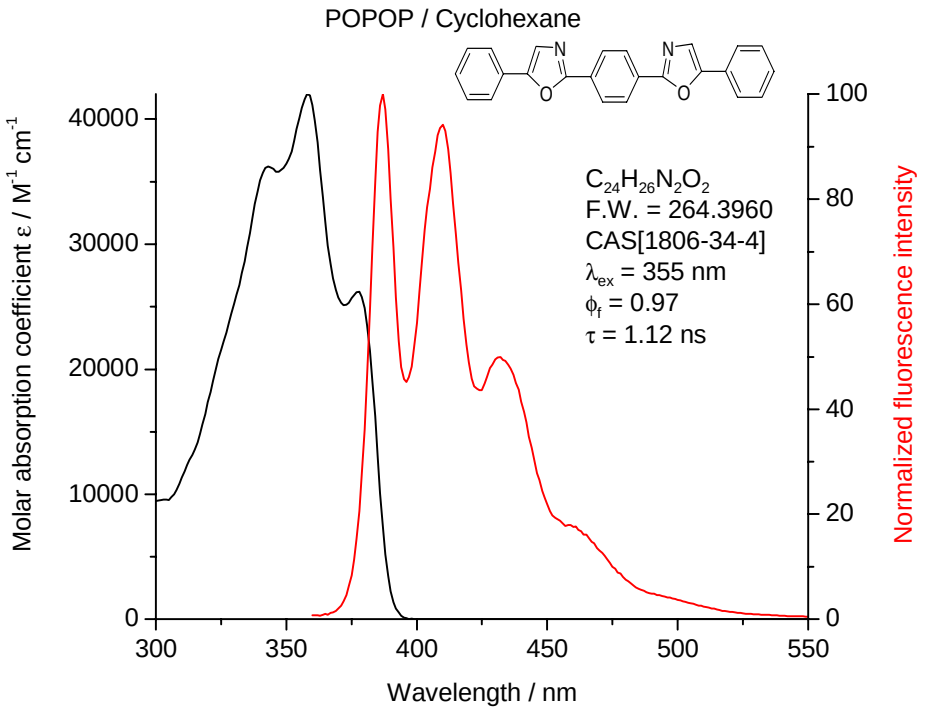


Figure S1-M. 2,5-Diphenyloxazole (PPO) in methanol.

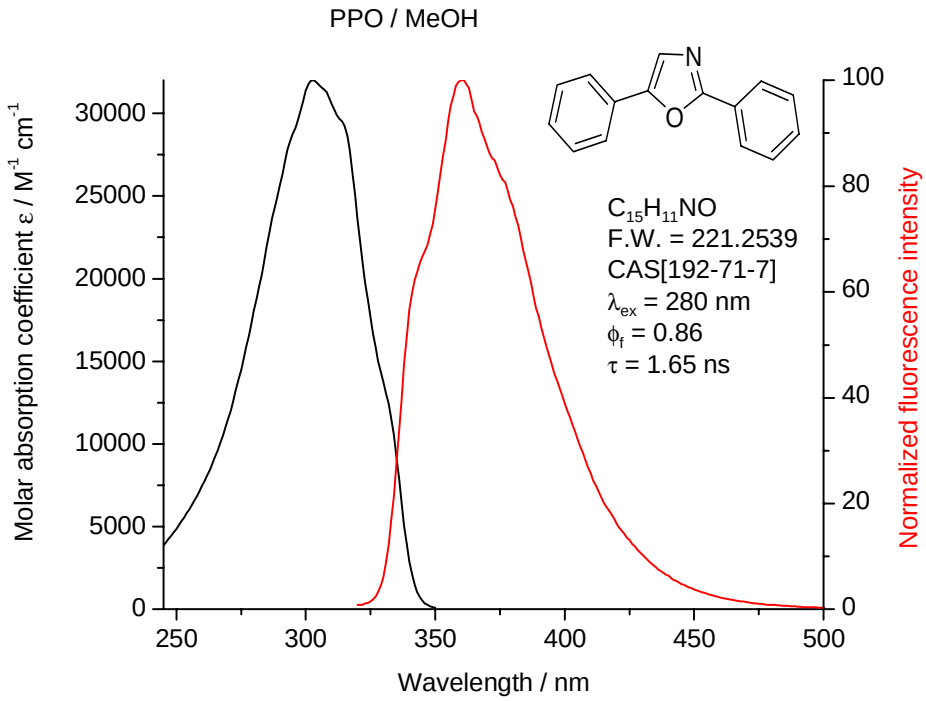


Figure S1-N. 2,5-Diphenyloxazole (PPO) in cyclohexane.

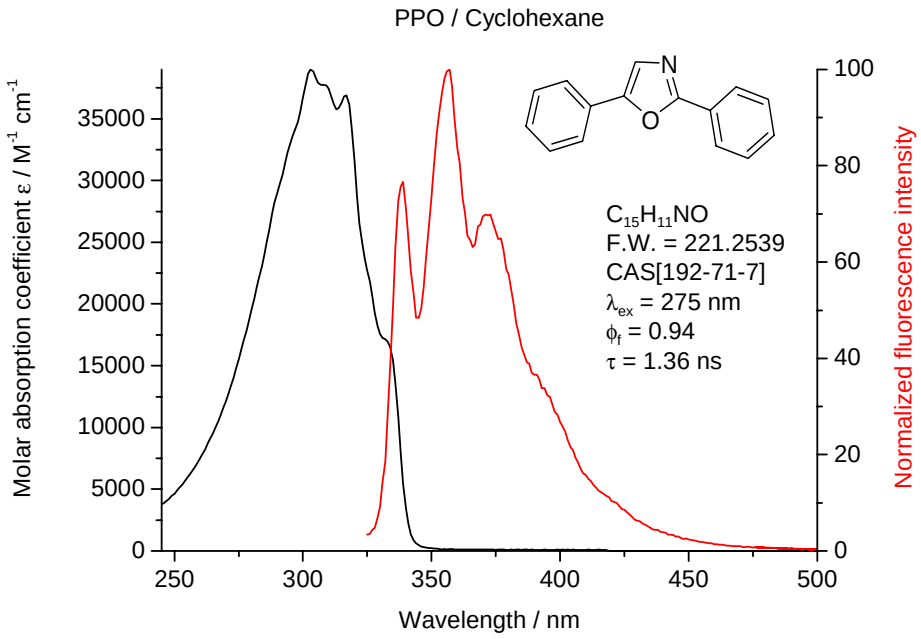


Figure S1-O. Rhodamine B in water.

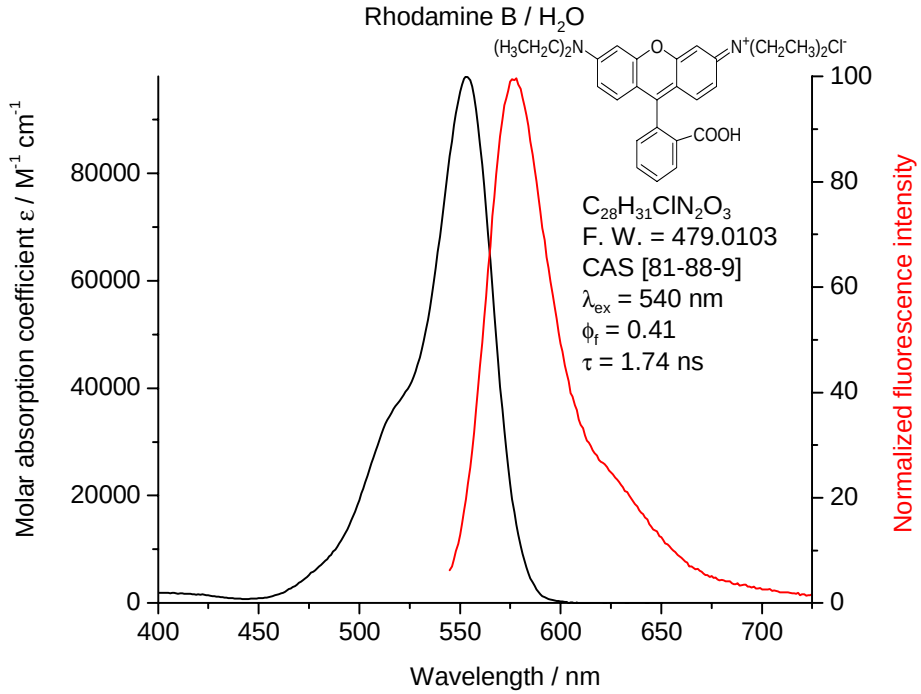


Figure S1-P. Rhodamine B in methanol.

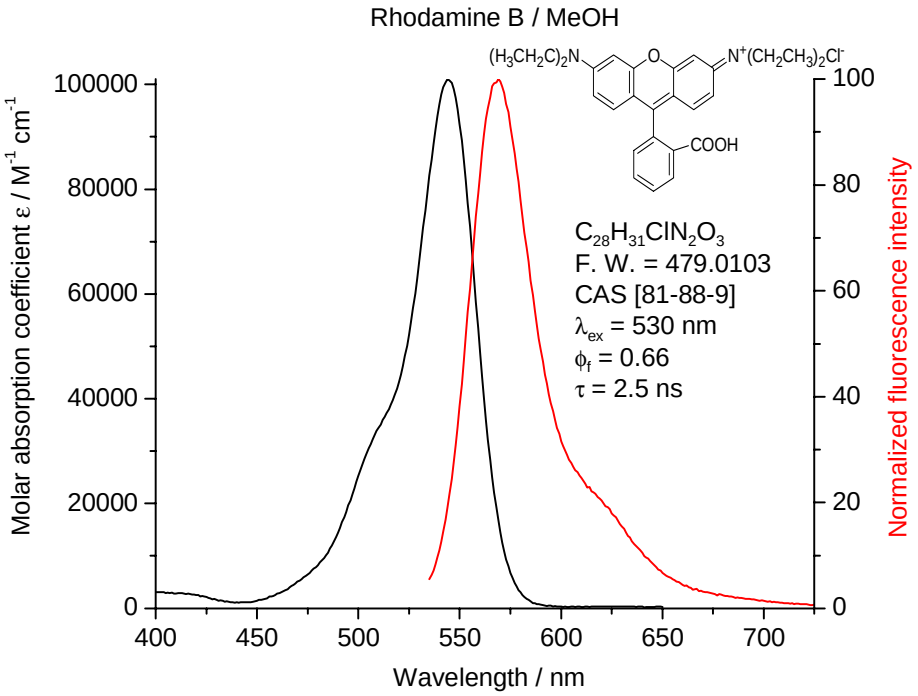


Figure S1-Q. Rubrene (5,6,11,12-tetraphenylnaphthacene) in methanol.

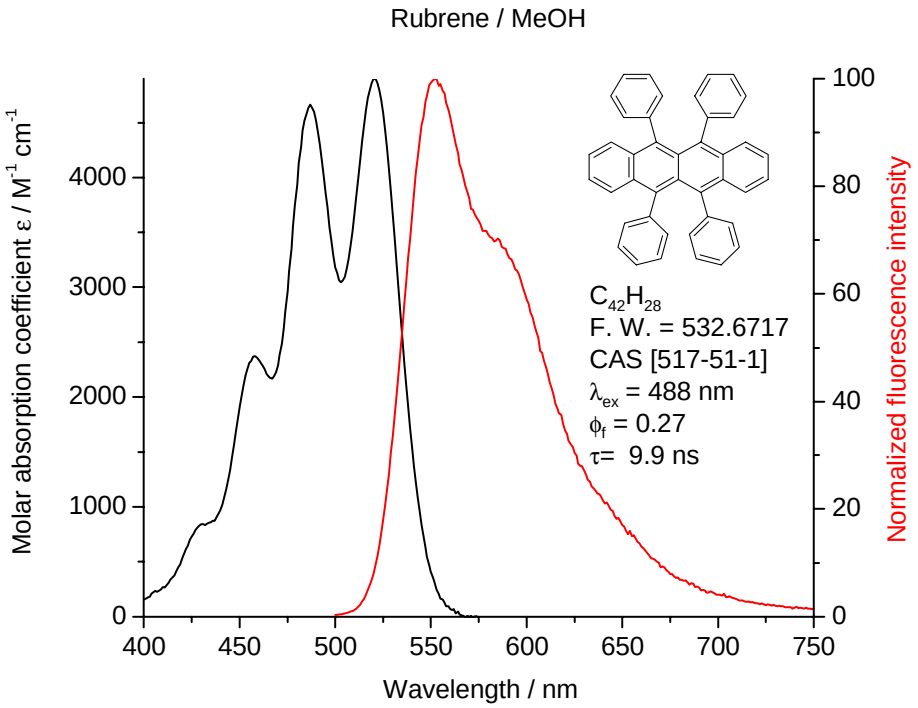


Figure S1-R. SPA [*N*-(3-Sulfopropyl)acridinum inner salt] in water. The same fluorescence spectrum was obtained upon excitation at 400 nm.

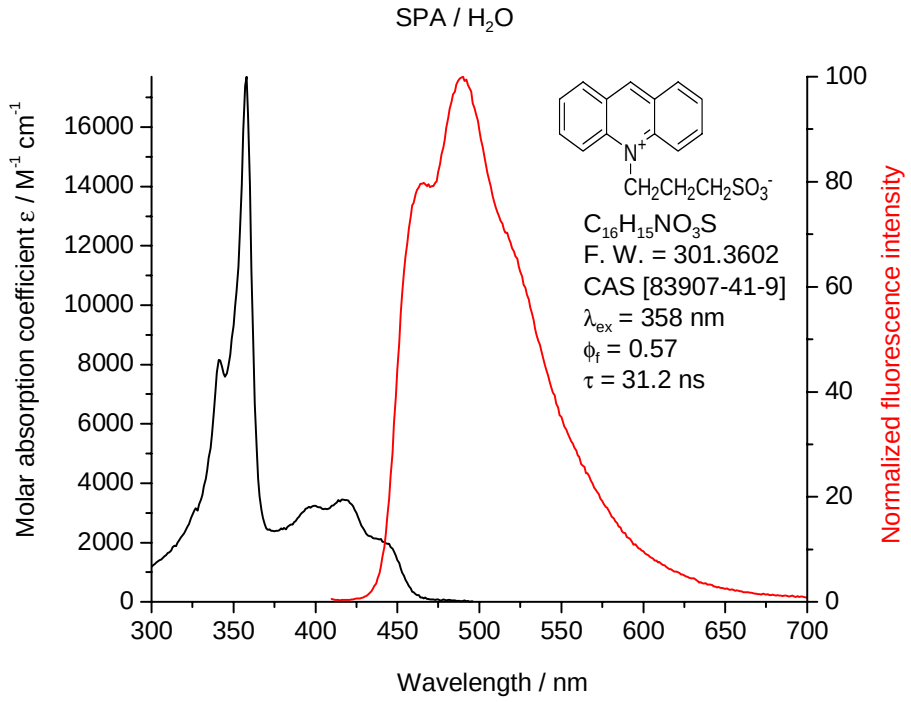


Figure S1-S. *p*-Terphenyl in methanol.

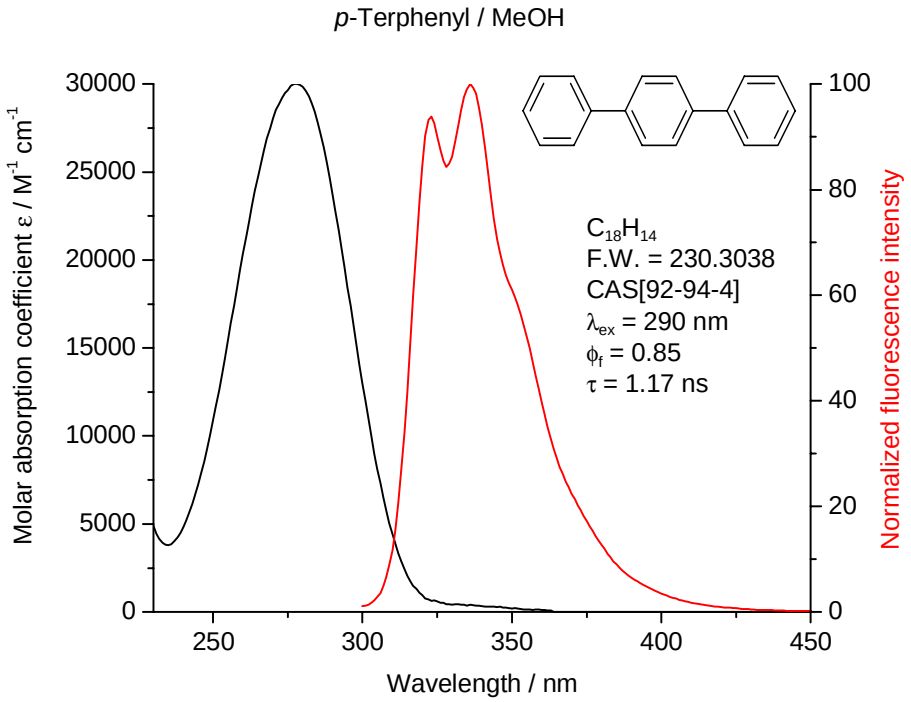
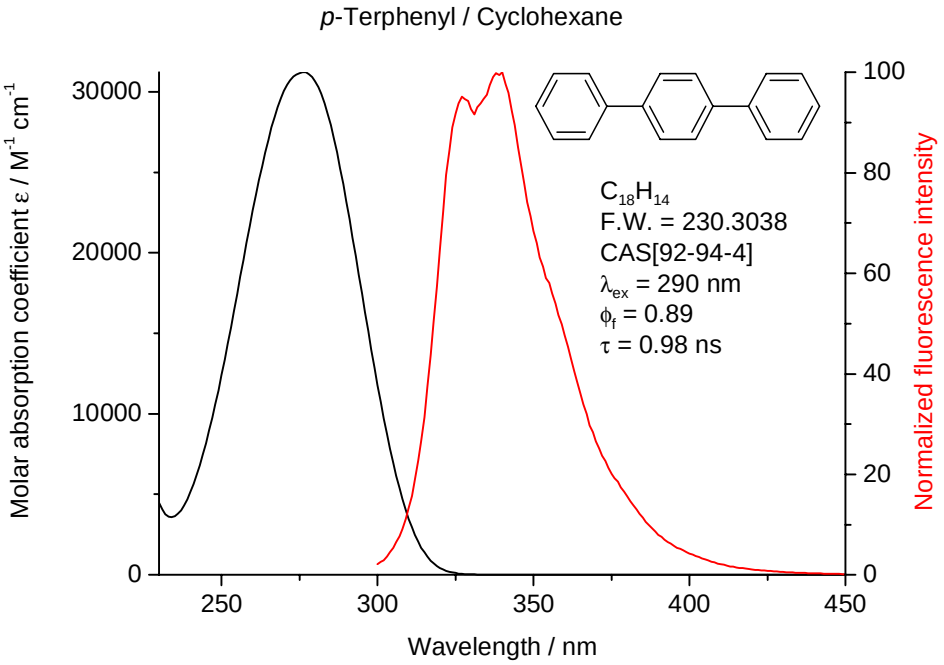


Figure S1-T. *p*-Terphenyl in cyclohexane.



2. Time-Resolved Fluorescence

2.1. Statistical tests for the quality of the fit in single-curve analysis

Graphical methods are popular techniques for examining residuals. Visual techniques are very informative and useful for observing patterns in TD as well as in FD data. Because time is the only observable, independent variable in TD measurements, it is useful to plot the observed sample fluorescence decay values y_i^o and their corresponding fitted values y_i^c against time t_i (or channel number i). Since the frequency $f = \omega/2\pi$ is the only observable, independent variable in FD measurements, one plots the observed phase and modulation data y_i^o and their associated fitted values y_i^c as a function of f_i. To highlight departures of the fitted values from their corresponding experimental values, one usually plots the differences $\left(y_i^o - y_i^c\right)$ or better the weighted residuals $R_i = \left(y_i^o - y_i^c\right)\sqrt{w_i} = \left(y_i^o - y_i^c\right)/\sigma_i$ against t_i (in TD experiments) or f_i (in FD fuorimetry). There should be no discernible trend in the plot of R_i vs. t_i (or channel number i in TD fluorometry) or vs. frequency f_i (for FD measurements); only a random scatter of points about the line $R_i = 0$ should be visible. The patterns of such a plot can often reveal bias (i.e., lack-of-fit of the proposed model) and outliers (i.e., extremely large deviations between experimental and fitted values in an otherwise satisfactory fit).

In detecting *heteroscedasticity* (error terms with unequal variances), it is helpful to plot the weighted residuals R_i against the fitted y_i^c. A graph of R_i vs. y_i^c should reflect a random scatter of points about a line with zero slope. Such a plot can detect model inadequacies, outliers, and unequal error variance. Systematic patterns would indicate an inadequate model for the decay law or the need to use weights other than those employed (changing variance).

Examination of the plot of the *autocorrelation function* C_j (eq S1) of the weighted residuals can reveal a tendency of the errors to be correlated:[17]

$$C_j = \frac{\frac{1}{m}\sum_{i=1}^{m} R_i R_{i+j}}{\frac{1}{n}\sum_{i=1}^{n} R_i^2} \qquad \text{(S1)}$$

In this expression m is the number of terms in the numerator. The index j can assume the values 0, 1, 2, ..., $(n - m)$. Usually one evaluates C_j for j values ranging from 0 ($C_0 = 1$) to $n/2$, so that m is always greater than $n/2$. In an acceptable fit, C_j for $j \neq 0$ is randomly scattered about zero, although, because of the finite value of m, some high-frequency low-amplitude fluctuations around zero are generally observed.

The *normal probability plot of the weighted residuals*[18, 19] is one of the most popular graphical techniques for detecting departures from normality and outliers. In a normal probability plot, the n ordered residuals R_i ($R_1 < R_2 < ... < R_n$) are plotted vs. the inverse cumulative standard normal distribution function $F^{-1}[i/(n+1)]$.[18, 19] If the errors are normally distributed, the points should lie roughly on a straight line. Some wobbling will occur in this type of plot, especially when the sample size is small. It has been recommended[20] that at least 20 and preferably more than 50 observations be used to effectively evaluate the plot. Minor variations in the extremes are common in

normal probability plots even when the data are truly normally distributed. Gross systematic departure from linearity, however, is evidence of violations of the assumption of normality of the errors.

Although visual techniques are very helpful in detecting points that do not fit the model under consideration, their interpretation is somewhat subjective. Therefore, several additional numerical statistical tests are proposed to make the residual analysis more reliable.

The quantity χ^2_ν (eq 9) has a precise interpretation beyond that of a number that – when minimized – gives the *best* fit. Tables of the percentage points of the χ^2-distribution in terms of the number of degrees of freedom ν compile the probability α that χ^2_ν exceeds $\chi^2_{\nu,\alpha}$ (α is the probability in one tail of the distribution). For $\nu > 100$, it is convenient to calculate the standard normal deviate $Z\chi^2_\nu$ of χ^2_ν, given by

$$Z\chi^2_\nu = \sqrt{\nu/2}\left(\chi^2_\nu - 1\right) \tag{S2}$$

The theoretical probability that $Z\chi^2_\nu$ exceeds a certain value can be obtained from tables of the standard normal distribution. If there is lack of fit, the model used for the analysis must be rejected. Although the χ^2_ν (or $Z\chi^2_\nu$) test is a powerful test for lack of fit, it does not take any account of the signs of the residuals, of their distribution and of their correlation.

To test for serial correlation, one can calculate the *Durbin-Watson test statistic d* defined by[21]

$$d = \frac{\sum_{i=2}^{n}\left(R_i - R_{i-1}\right)^2}{\sum_{i=1}^{n} R_i^2} \tag{S3}$$

Single-exponential fits yielding values of *d* greater than 1.65 are generally acceptable.

One method to detect if it is reasonable to assume that the errors associated with the experimental time-resolved fluorescence data are randomly distributed is to investigate the arrangement of the signs (+ or −) of the corresponding residuals. In the *ordinary runs test*,[19, 22] the number of sequences of residuals vs. channel number (TD) or frequency $f = \omega/2\pi$ (FD) with the same sign (runs), *r*, is counted and compared with the number expected, μ, for a set of random numbers. When the number of residuals with plus (n_+) and minus (n_-) signs both exceed 20, the normal approximation can be made and the hypothesis of randomness rejected at the α significance level if

$$Z = \frac{r - \mu + \frac{1}{2}}{\sigma} < -Z_{1-\alpha/2} \text{ or } Z = \frac{r - \mu - \frac{1}{2}}{\sigma} > Z_{1-\alpha/2} \tag{S4a}$$

where

$$\mu = \frac{2n_+n_-}{n_+ + n_-} + 1 \tag{S4b}$$

and

$$\sigma^2 = \frac{2n_+n_-(2n_+n_- - n_+ - n_-)}{(n_+ + n_-)^2(n_+ + n_- - 1)} \quad \text{(S4c)}$$

Here, σ is the standard deviation of the runs distribution. The variable Z in eq S4a is distributed approximately as $N(0,1)$ and the required probability can be determined from a table of standard normal probabilities. In TD experiments, because of the high number of data points in a fluorescence decay trace, the normal approximation can usually be made (i.e., $n_+ > 20$ and $n_- > 20$). In FD measurements where the number of used modulation frequencies is generally low(er), tables of upper and lower critical values of r in the runs test at the 0.05 significance level (two-tailed significance level of 0.10) are available when $n_+ \leq 20$ and $n_- \leq 20$.[22]

Additionally, the mean, the standard deviation (standard uncertainty)[6], and the percentage of weighted residuals in the [−2, 2] interval can be calculated and compared to the predicted values of 0.0, 1.0 and 95.44%, respectively.

2.2. Outlier detection

An easy test for detecting an outlier in a small sample from a normal distribution is Grubbs's test,[23] which is useful when a single outlier is suspected. First, one calculates the mean lifetime $\bar{\tau}$ and corresponding standard deviation s (standard uncertainty u)[6] using all lifetime data of the sample. The test statistic for testing if the largest or smallest lifetime value $\tau_{(n)}$ is an outlier is

$$T_n = \frac{|r_{(n)}|}{s} = \frac{|\tau_{(n)} - \bar{\tau}|}{s} \quad \text{(S5)}$$

where $r_{(n)}$ is the residual corresponding to the largest or smallest observation. Several percentage points of the distribution of T_n are given in Table S5.

If the calculated statistic is larger than the tabled value, the largest or smallest lifetime value $\tau_{(n)}$ is an outlier and should be removed from the data set for the calculation of the statistical tests [mean lifetime, standard deviation (standard uncertainty)[6], variance, *F*-test, *t*-test, etc.]. The largest τ-value for DPA in cyclohexane (data point from LEU, 9.74 ns) yields a T_8 value of 2.20, which is larger than the tabled T_8 value (2.03) at $\alpha = 0.05$, indicating that this observation is an extreme value at this significance level. The value of 2.20 is very similar to the tabled T_8 value (2.22) at $\alpha = 0.01$. For NATA in water, the largest lifetime value (observation from BAL, 3.28 ns) gives a T_7 value of 1.95, which is marginally larger than the tabled T_7 value (1.94) at $\alpha = 0.05$. This observation is thus a borderline outlier at this significance level. The T_7 value of 1.95 is smaller than the tabled T_7 value (2.10) at $\alpha = 0.01$, showing that this observation cannot be considered extreme at this significance level. The T_6 test statistic ($T_6 = 2.04$) for the data point for rhodamine B in water obtained by IRV (2.8 ns) confirms that this value is clearly an outlying observation.

Another simple test suitable for detecting multiple outliers in small samples from a normal distribution is that of Dixon.[24] First, the lifetime data are ordered from smallest to largest, i.e., $\tau_{(1)} < \tau_{(2)} < \ldots < \tau_{(n-1)} < \tau_{(n)}$, where $\tau_{(i)}$ is the ith smallest ordered lifetime value in a random sample of size n from a normal distribution. To test whether the smallest observation $\tau_{(1)}$ is an outlier, we calculate the test statistic

$$r_{10} = \frac{\tau_{(2)} - \tau_{(1)}}{\tau_{(n)} - \tau_{(1)}} \quad \text{(S6)}$$

To test whether $\tau_{(n)}$ is an outlier, the statistic r_{10} is used:

$$r_{10} = \frac{\tau_{(n)} - \tau_{(n-1)}}{\tau_{(n)} - \tau_{(1)}} \quad \text{(S7)}$$

The calculated r_{10} is compared with the entries in Table S6, and the observation is considered an outlier at a significance level α if the calculated r_{10} exceeds the tabled value. If more than one data point within the same data set is a potential outlier, the above described procedure has to be repeated for each data point *separately*, after deleting the previous outlier from the sample.

The largest τ-value for DPA in cyclohexane ($\tau_{(8)}$ = 9.74 ns) yields an r_{10} value of 0.567, larger than the corresponding tabled r_{10} value (0.468) at α = 0.05. Hence, this observation can be marked as an outlier at this significance level. The largest τ-value for NATA in water ($\tau_{(7)}$ = 3.28 ns) gives an r_{10} value of 0.477, which is smaller than the tabled r_{10} value (0.507 at α = 0.05) for 7 observations. Hence, the Dixon outlier test reveals that this observation is not an extreme point at this significance level. Grubbs's test already indicated that the largest τ-value for NATA in water can hardly be considered an extreme observation. Finally, comparison of Dixon's r_{10} test statistic calculated for $\tau_{(6)}$ (2.8 ns) for rhodamine B in water (r_{10} = 0.964) with the tabled r_{10} value (0.560 at α = 0.05) for n = 6 confirms that this point is indeed an outlying observation.

Other tests for detecting outlying observations are less transparent and always involve more calculations than the Dixon and Grubbs test. For additional discussion on other outlier measures, we refer to the literature.[25]

Table S4. Lifetime (ns) data in deoxygenated fluid solution at 20 °C measured in the present study. All estimated τ values are obtained from acceptable fits as judged by the quality-of-fit criteria.

Compound[a]	Solvent	LEU/HAS[b] g-TD	PAR[b] FD	IRV[b] FD	LEU[b] g-FD	LON[b] g-TD	WAG[b] TD	BAL[b] FD	WLU[b] TD	NIS[b] TD
Anthracene	Methanol	5.45	4.95	5.46	4.59		5.210		5.19	4.948
	Cyclohexane	5.38	5.16	5.45	5.46		5.153	5.20	(4.94[c])	5.421
9-Cyanoanthracene	Methanol	17.43	16.70	15.8	13.37	17.00	16.25		14.39	
	Cyclohexane	13.47	12.79	12.6				11.77	(12.63[c])	
DPA	Methanol	8.73	8.18	8.4[d]	9.55	8.56	8.87[d]		8.34	9.34
	Cyclohexane	7.67	7.53	7.35[d]	9.74	7.60	7.79[d]	6.62	(7.80[c])	7.97
N-methylcarbazole	Cyclohexane	14.03	14.32	14.3[d]	13.56	15.50	12.907		(14.04[c])	
Coumarin 153	Methanol	4.43	4.23		4.12[d]		4.06[d]			4.50
Erythrosin B	Water	0.090	0.092	0.087			0.085		0.087	0.093
	Methanol	0.476	0.457	0.451			0.477		0.502[d]	0.458
NATA	Water	2.990		3.03	3.113		3.06	3.28[d]	3.04	2.93[d]
POPOP	Cyclohexane	1.101	1.07	1.18	1.163	1.11[e]	1.163	1.08	(1.12[c])	1.121
PPO	Methanol	1.62	1.588	1.69	1.608	1.693[e]	1.66		1.62	1.707
	Cyclohexane	1.354	1.371	1.38	1.293	1.382[e]	1.38	1.34	(1.36[c])	1.414
Rhodamine B	Water	1.753	1.727	2.8[d]			1.766		1.74[d]	1.729[d]
	Methanol	2.494	2.469	2.47	2.378[d]		2.281	2.62[d]	2.47	2.512
Rubrene	Methanol	10.37	9.95	9.63			9.58[d]		9.96	
SPA	Water	31.8	31.09[d]	30.7			31.38[d]		30.94	
p-Terphenyl	Methanol	1.31		1.10	1.091	1.20[e]	1.19		1.12	1.163
	Cyclohexane	0.991		0.99	0.948	1.00[e]	1.01	0.93	(1.01[c])	1.016

[a] Abbreviations used: DPA: 9,10-diphenylanthracene, NATA: *N*-acetyl-L-tryptophanamide, POPOP: 1,4-bis(5-phenyloxazol-2-yl)benzene, PPO: 2,5-diphenyloxazole, SPA: *N*-(3-sulfopropyl)acridinium. All solutions are deoxygenated by repetitive freeze-pump-thaw cycles or by bubbling N_2 or Ar through the solutions. The number of significant digits of the tabled values is determined by the corresponding standard deviation (standard uncertainty)[6].

[b] The sequence of the laboratories follows that of the title page. The labels of the laboratories are based on a three-letter code for the University or the associated University City: LEU = Leuven, HAS = Hasselt, PAR = Paris, IRV = Irvine, LON = London, WAG = Wageningen, BAL = Baltimore, WLU = Wilfrid Laurier University, NIS = Nishinomiya. g-TD and g-FD indicate that global analysis in time domain and frequency domain was used to estimate the fluorescence lifetimes.

[c] Measured in methylcyclohexane as solvent.

[d] Estimated τ value of main decay component of biexponential fit.

[e] Estimated τ value (eq 15) of biexponential fit due to the fact that no magic angle detected was used.

Table S5. Percentage points of T_n.

Number of observations *n*	1−α .95	.99
3	1.15	1.15
4	1.46	1.49
5	1.67	1.75
6	1.82	1.94
7	1.94	2.10
8	2.03	2.22
9	2.11	2.32
10	2.18	2.41

Values are taken from reference 23.

Table S6. Percentage points of the distribution of r_{10}.

Number of observations *n*	1−α .95	.99
3	.941	.988
4	.765	.889
5	.642	.780
6	.560	.698
7	.507	.637
8	.468	.590
9	.437	.555
10	.412	.527

Values are taken from reference 24.

Table S7. Student's *t*-distribution.

Degrees of freedom *n*	α	
	.05	.01
1	12.706	63.657
2	4.303	9.925
3	3.182	5.841
4	2.776	4.604
5	2.571	4.032
6	2.447	3.707
7	2.365	3.499
8	2.306	3.355
16	2.120	2.921

The table is two-sided, meaning that the probability for the variable to lie outside the interval [$-t$, t] equals α.

Table S8. Lifetime data in fluid solution at room temperature (20−25 °C) reported in the literature.

Compound[a]	Solvent[a]	Conc. (M)	Lifetime (ns)	Lit. ref.
Anthracene	Methanol (d)	[b]	5.12–5.5	26
	Cyclohexane (d)	9.52×10^{-6}	5.42	27
	Cyclohexane (d)	1×10^{-6}	5.15	27
	Cyclohexane (d)	4×10^{-5}	5.28	28
	Cyclohexane (d)	2.1×10^{-5}	5.03	29
	Cyclohexane (d)	2.7×10^{-5}	5.22	7
	Cyclohexane (d)	$< 10^{-5}$	5.23	30
	Cyclohexane (u)	$< 10^{-5}$	4.10	30
	Cyclohexane (u)	5×10^{-6}	3.97	31
	Cyclohexane (u)	2.1×10^{-5}	3.99	29
9-Cyanoanthracene	Methanol (d)	[b]	16.9	26
	Cyclohexane (d)	[b]	12.8	29
	Cyclohexane (u)	[b]	10.5	29
	Cyclohexane (d)	[b]	12.8	32
Coumarin 153	Methanol (u)	5×10^{-6}	4.2	10
DPA	Methanol (d)	[b]	8.3	26
Erythrosin B	Water (u)	[b]	0.096	33
	Water[c]	1×10^{-4}	0.115	11
	Water (ns)	$< 10^{-5}$	0.066	34
	Water (ns)	[b]	0.110	35
	Water (ns)	[b]	0.090	36
	Water (ns)	[b]	0.075	37
	Water (ns)	[b]	0.057	38
	Water (ns)	10^{-5}	0.060	39
	Methanol (u)	[b]	0.459	33
	Methanol[c]	1×10^{-4}	0.500	11
NATA	Water (u)	$< 5 \times 10^{-6}$	3.153	40
	Water (u)	7.24×10^{-5}	2.75–2.84	41
	Water (d)	[d]	2.98	42
	Water (ns)	$< 10^{-6}$	2.95	43
POPOP	Cyclohexane (d)	5×10^{-6}	1.10	29
	Cyclohexane (u)	[f]	1.12–1.15	28
	Cyclohexane (u)	2×10^{-6}	1.13	44
PPO	Cyclohexane (d)	10^{-5}	1.36	45
	Cyclohexane (d)	$< 10^{-5}$	1.42	30
	Cyclohexane (u)	$< 10^{-5}$	1.28	30

	Cyclohexane (d)	[b]	1.38	46
	Cyclohexane (ns)	5×10^{-5}	1.7	13
	Methanol (ns)	5×10^{-5}	2.0	13
Rhodamine B	Methanol (ns)	$(1-2) \times 10^{-6}$	2.28	47
	Methanol (ns)	4×10^{-5}	2.3	48
	Methanol (ns)	1×10^{-5}	2.85	49
	Water (ns)	1×10^{-5}	1.50	49
	Water (ns)	$(1-2) \times 10^{-6}$	1.50–1.775	47
	Water (ns)	1×10^{-6}	1.65	50
	Water (ns)	[b]	1.52	15
SPA	Water (u)	[d]	31.56/32.78[e]	51
p-Terphenyl	Cyclohexane (d)	10^{-5}	0.96	45
	Cyclohexane (ns)	10^{-3}	0.945	52

[a] Abbreviations used: d: deoxygenated, u: undegassed, ns: not specified whether solvent is degassed. For the other abbreviations see footnote *a* of Table S4.
[b] The absorbance at the excitation wavelength was < 0.1.
[c] No difference in lifetime was detected in N_2-saturated compared to air-saturated solutions.
[d] No information available.
[e] The first value is measured vs. a glycogen scatterer, the second vs. monoexponential compound.
[f] A saturated solution of POPOP in cyclohexane was filtered and diluted by 100.

Table S9. Mean lifetime data $\bar{\tau}$ of some fluorescent reference dyes from Table S8 in fluid solution at room temperature measured in the literature.

Compound[a]	Solvent	Lifetime $\bar{\tau}$ ± s (ns)[b]	100 $s/\bar{\tau}$	n[b]	95% confidence interval of τ
Anthracene	Cyclohexane[c]	5.2 ± 0.1	2.5	6	5.2 ± 0.3
	Cyclohexane[d]	4.02 ± 0.07	1.7	3	4.0 ± 0.3
Erythrosin B	Water[e]	0.08 ± 0.02	28.6	8	0.08 ± 0.05
NATA	Water[e]	3.0 ± 0.1	4.9	4	3.0 ± 0.5
POPOP	Cyclohexane[e]	1.12 ± 0.02	1.7	3	1.12 ± 0.08
PPO	Cyclohexane[c]	1.39 ± 0.03	2.2	3	1.4 ± 0.1
Rhodamine B	Water[f]	1.58 ± 0.08	4.9	4	1.6 ± 0.2
	Methanol[f]	2.5 ± 0.3	13.1	3	2 ± 1

[a] Abbreviations used: see footnote *a* of Table S4.

[b] Average lifetime $\bar{\tau}$. The quoted errors are sample standard deviations $s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(\tau_i - \bar{\tau})^2}$.

n is the number of measurements used in the calculation of $\bar{\tau}$ and *s*. **Standard deviation** *s* is also termed **standard uncertainty** *u*.[6]

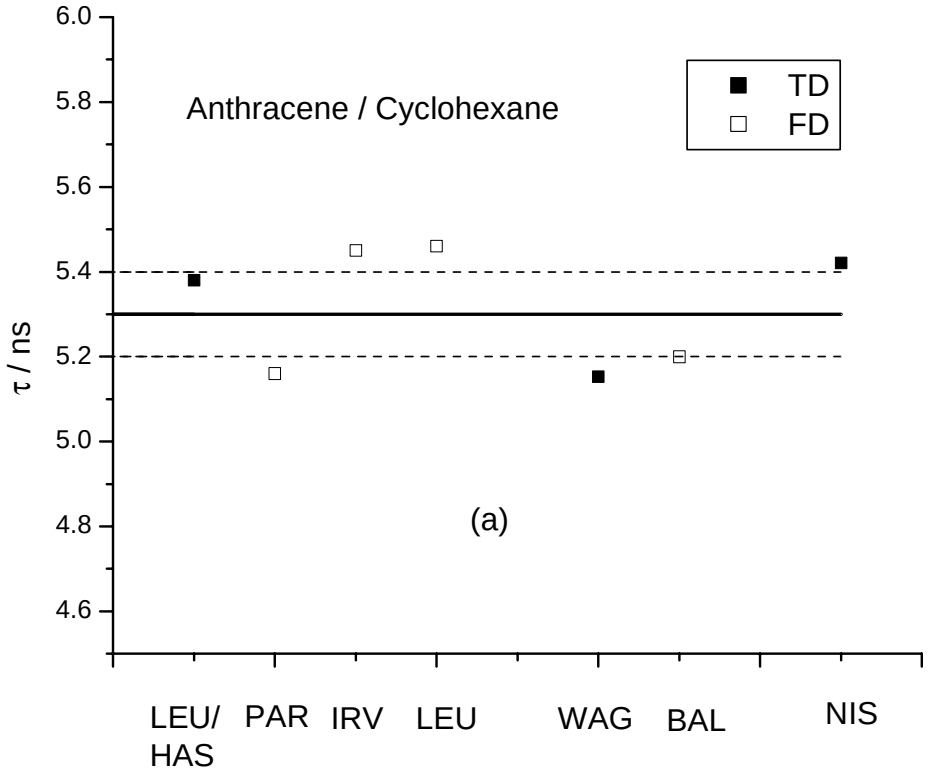
[c] Deoxygenated solutions.

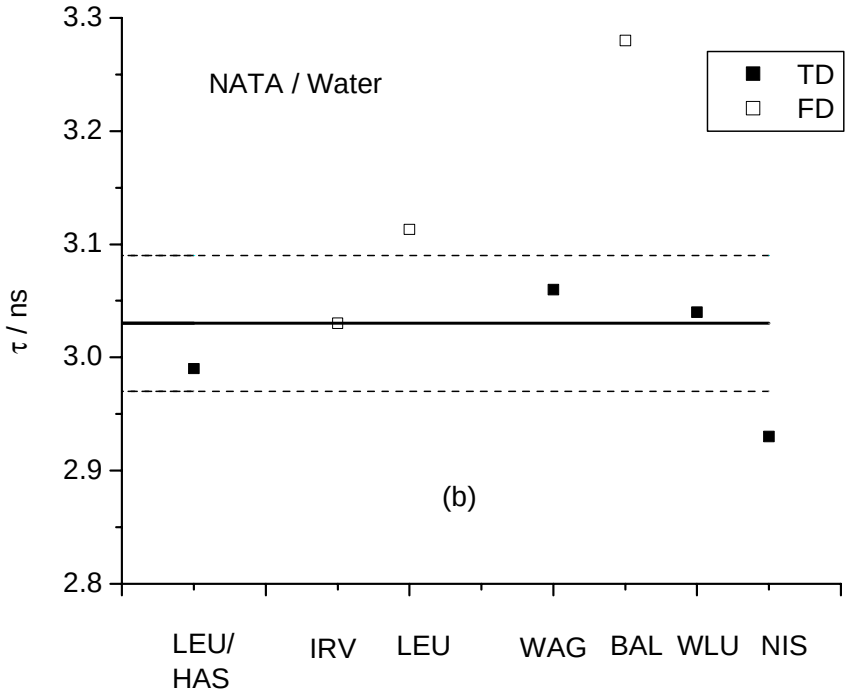
[d] Undegassed solutions.

[e] Undegassed and deoxygenated solutions.

[f] Not specified whether solutions are deoxygenated.

Figure S2. (a) Lifetimes τ of anthracene in cyclohexane at 20 °C estimated by the various laboratories. The bold solid line indicates the average value $\bar{\tau}$, the dashed lines represent $\bar{\tau}$ ± one sample standard deviation *s* (standard uncertainty *u*)[6]. (b) Corresponding figure for NATA in water. (c) Analogous figure for rhodamine B in water. The point determined by IRV is an outlier and was not taken into account in the calculation of the average lifetime $\bar{\tau}$ and its standard deviation *s* (standard uncertainty *u*)[6]. All samples were deoxygenated and measured under magic angle.




NATA / Water
TD
FD
τ / ns
3.3
3.2
3.1
3.0
2.9
2.8
(b)
LEU/
HAS
IRV
LEU
WAG
BAL
WLU
NIS

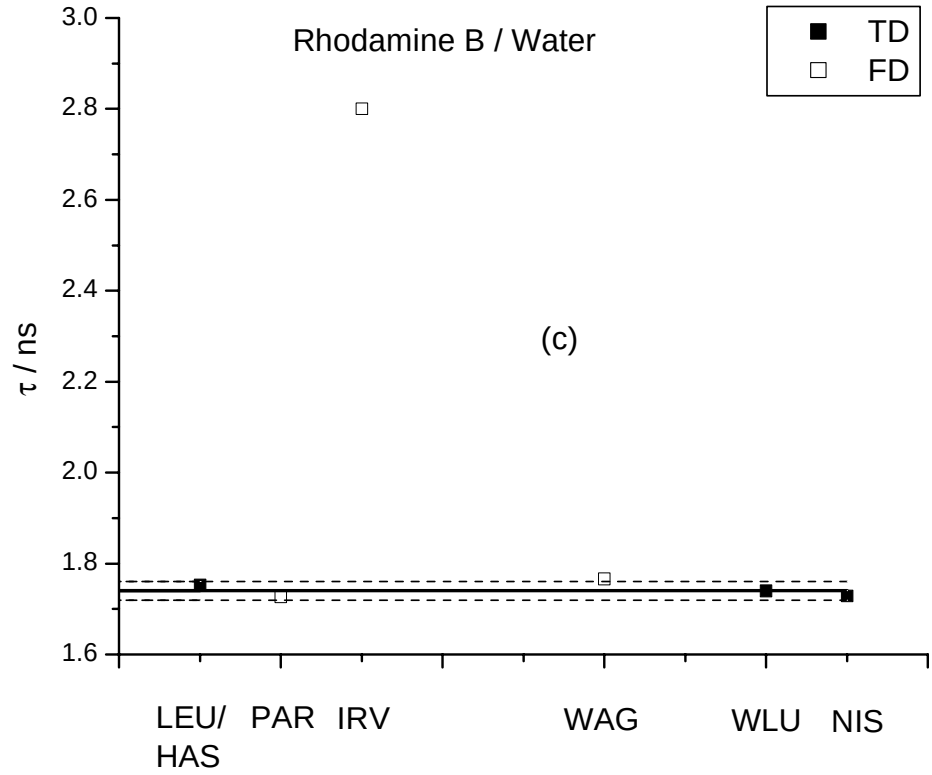

Rhodamine B / Water
TD
FD
τ / ns
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
(c)
LEU/
HAS
PAR
IRV
WAG
WLU
NIS

Figure S3. Experimental fluorescence decay trace (red) of fluorescence lifetime standards measured under magic angle in degassed solution at 20 °C. The instrument response function $u(t)$ measured with a Ludox scattering solution (in water) is displayed in black. The green line shows the best mono-exponential fit to the experimental decay data. The plot of the weighted residuals R_i vs. time is also given. The quoted error represents one standard deviation (standard uncertainty)[6]. Data from LEU/HAS.

Figure S3-A. Anthracene in methanol (excitation wavelength λ_{ex} = 360 nm, observation wavelength λ_{em} = 375 nm, number of channels used in the fitting = 3500, channel width = 6.43 ps). Results: τ = (5.44 ± 0.01) ns, χ^2_v = 1.135.

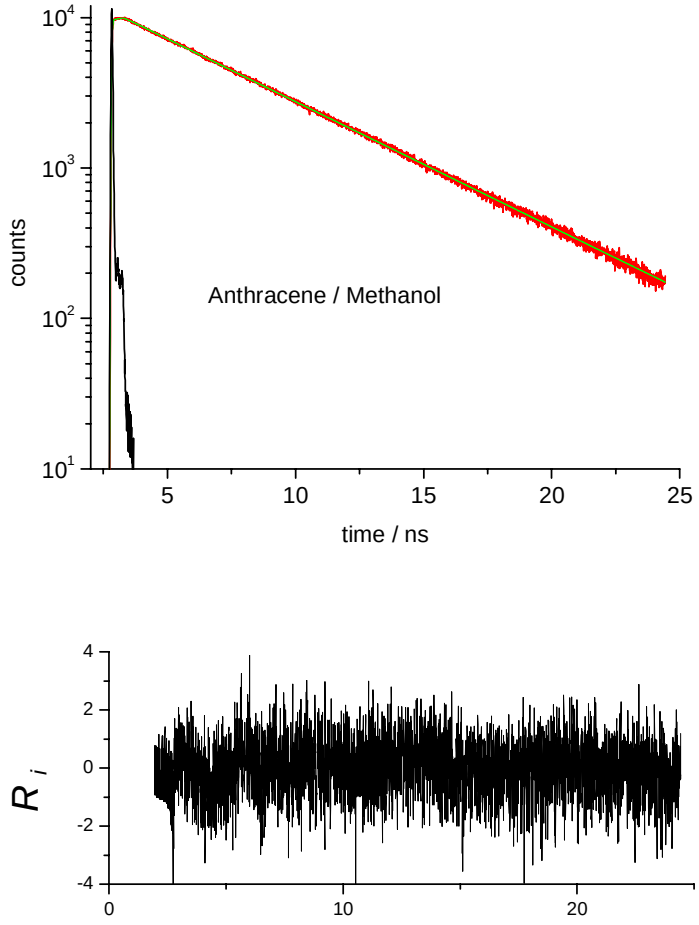


Figure S3-B. 9-Cyanoanthracene in cyclohexane (λ_{ex} = 360 nm, λ_{em} = 410 nm, number of channels used in the fitting = 3500, channel width = 20.8 ps). Results: τ = (13.46 ± 0.03) ns, χ^2_v = 1.115.

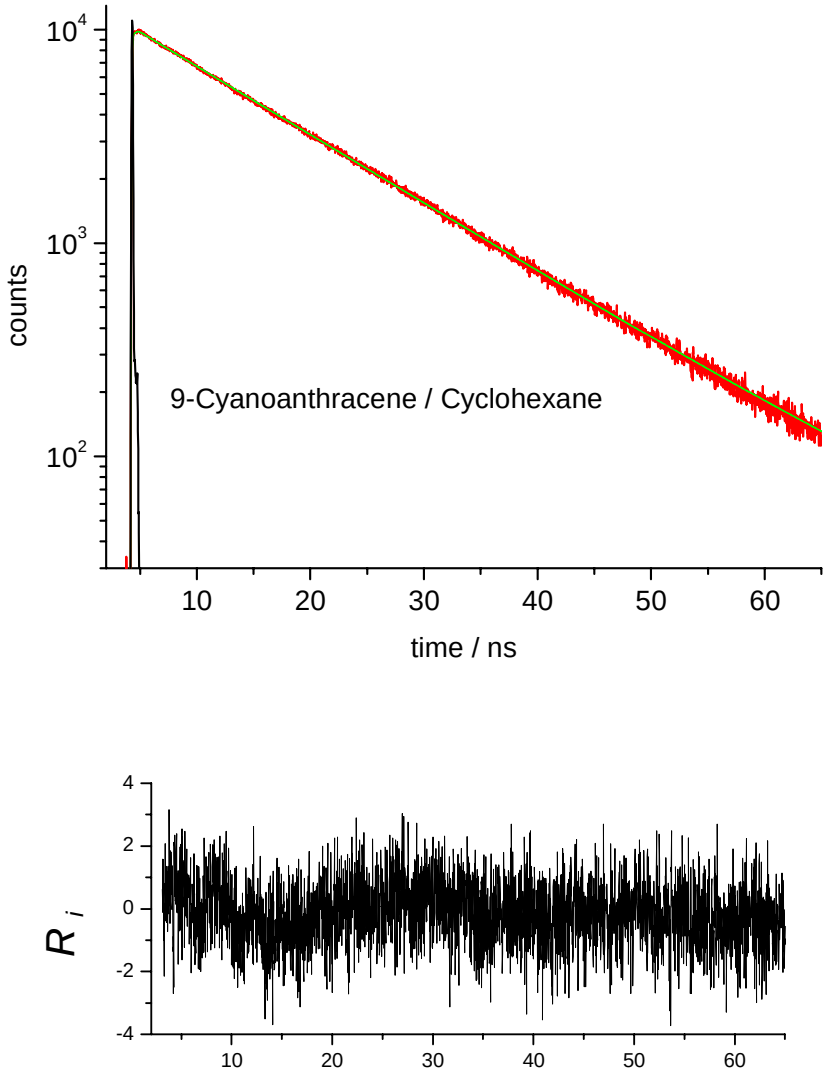


Figure S3-C. Erythrosin B in methanol (λ_{ex} = 543 nm, λ_{em} = 555 nm, number of channels used in the fitting = 1600, channel width = 1.63 ps). Results: τ = (0.475 ± 0.002) ns, χ^2_v = 1.036.

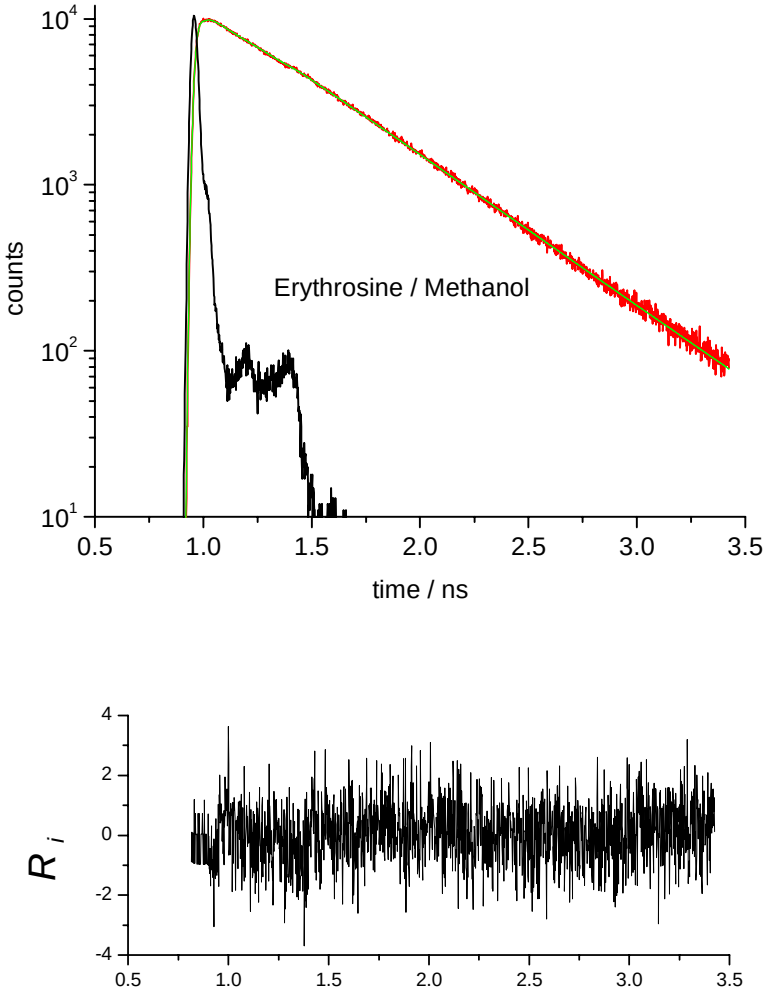


Figure S3-D. POPOP in cyclohexane (λ_{ex} = 360 nm, λ_{em} = 410 nm, number of channels used in the fitting = 3500, channel width = 1.62 ps). Results: τ = (1.102 ± 0.003) ns, χ^2_v = 1.167.

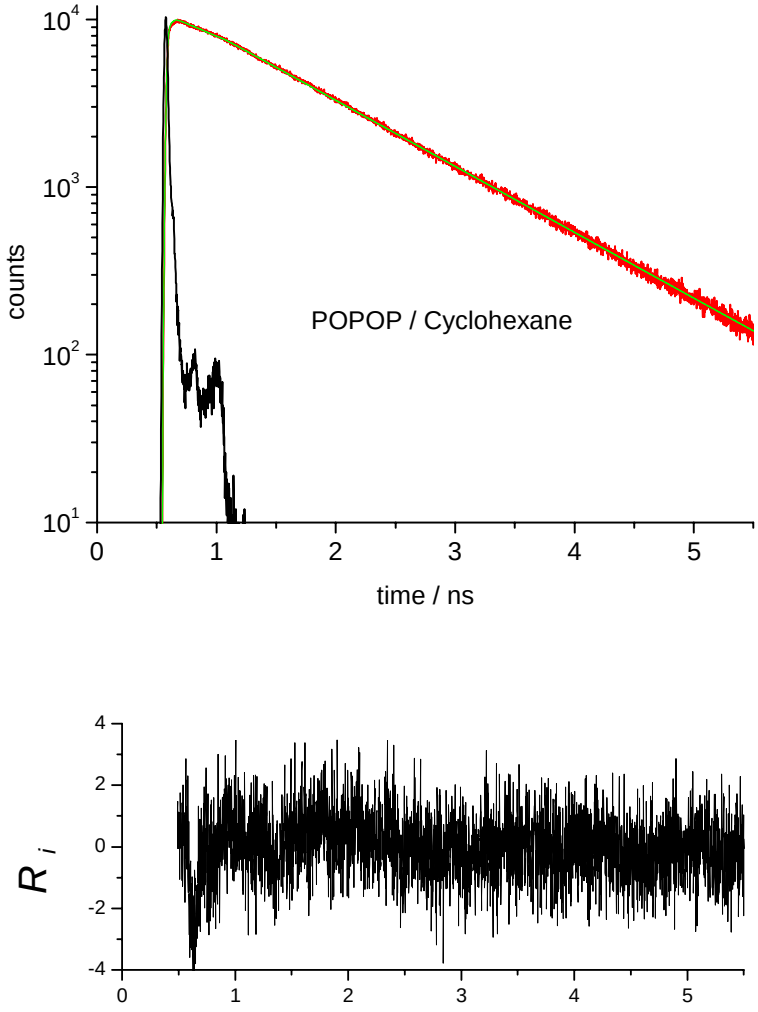


Figure S3-E. Rhodamine B in methanol (λ_{ex} = 543 nm, λ_{em} = 600 nm, number of channels used in the fitting = 3350, channel width = 4.23 ps). Results: τ = (2.501 ± 0.003) ns, χ^2_v = 1.045.

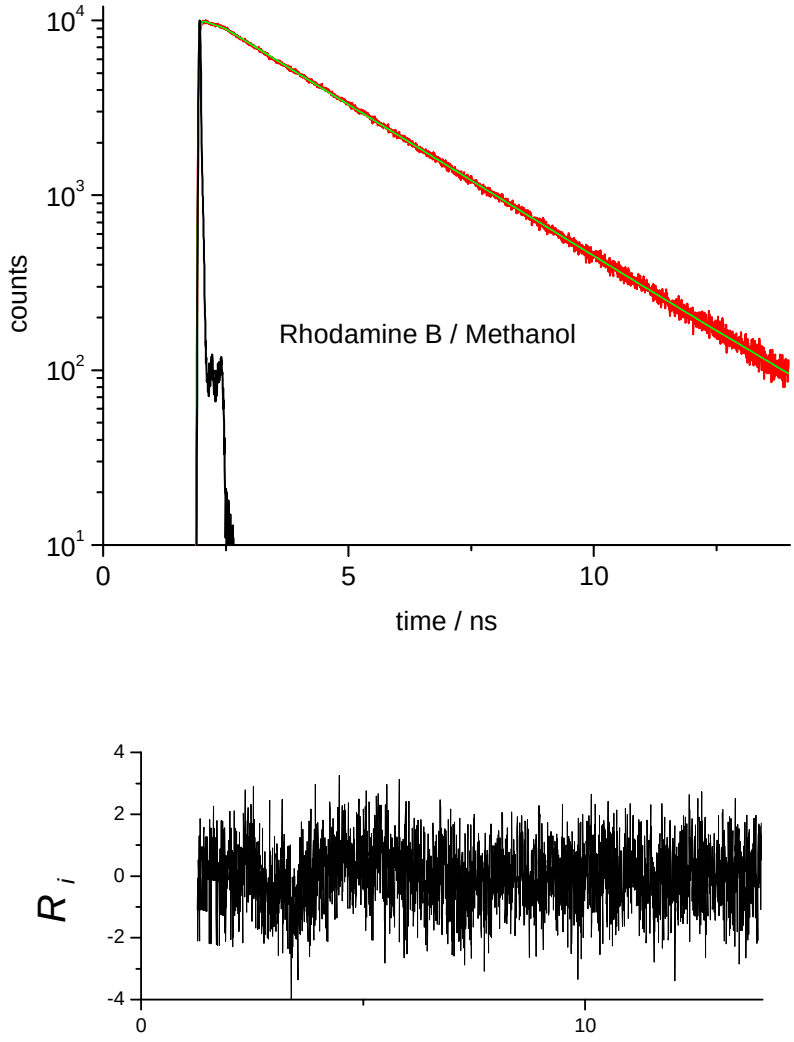


Figure S4. Experimental phase shift (black dots) and modulation data (red dots) of fluorescence lifetime standards measured under magic angle in degassed solution at 20 °C vs. a Ludox scattering solution (in water). Number of phases/modulations determined per frequency = 50. The black and red lines show the best mono-exponential fit to the experimental data. Plots of the weighted residuals for phase shift and modulation data are also given. Data from BAL.

Figure S4-A. Anthracene in cyclohexane (excitation wavelength λ_{ex} = 295 nm, observation wavelength λ_{em} > 345 nm, number of frequencies = 21, from 7.8 to 140 MHz). Results: τ = 5.20 ns, χ^2_v = 1.2.

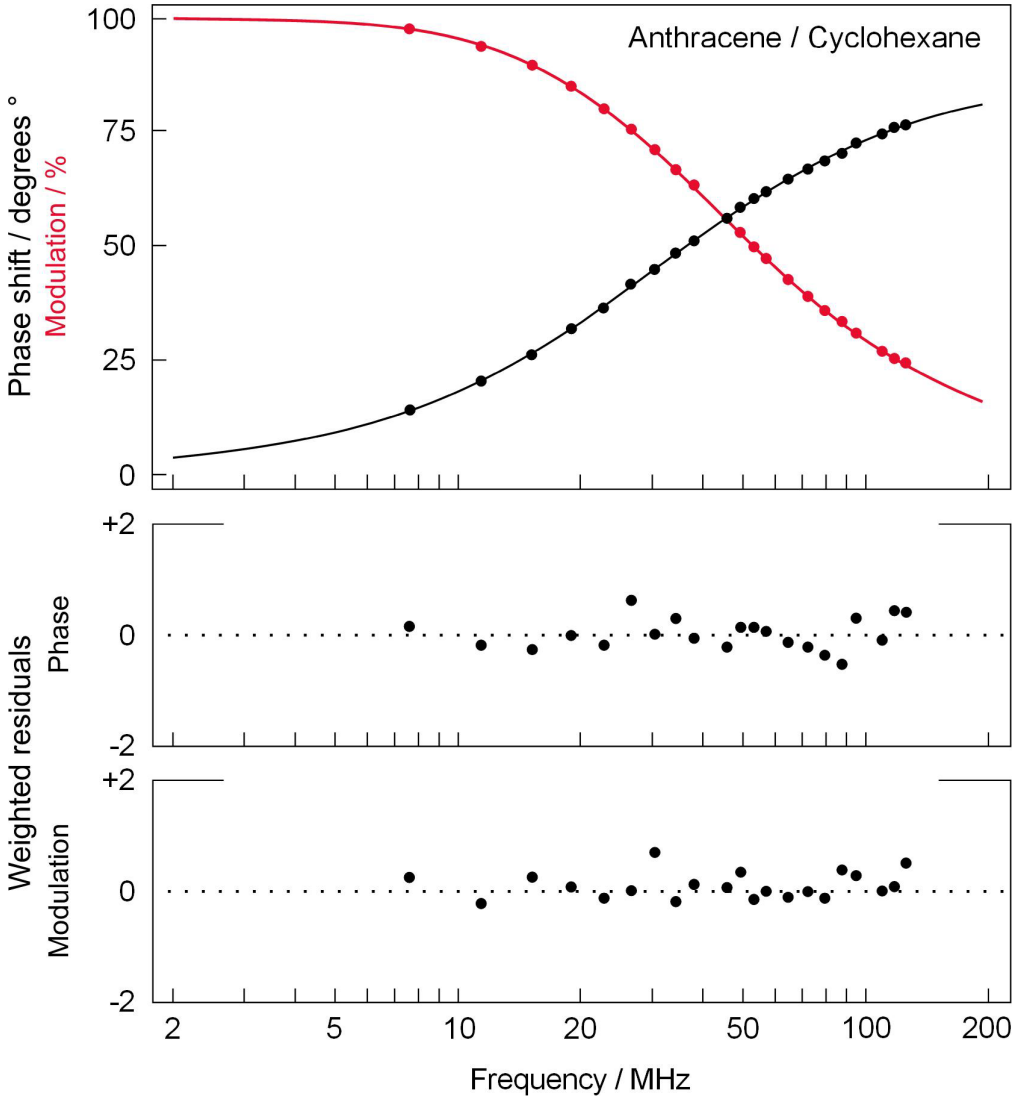


Figure S4-B. 9-Cyanoanthracene in cyclohexane (λ_{ex} = 295 nm, λ_{em} > 345 nm, number of frequencies = 21, from 7.8 to 130 MHz). Results: τ = 11.77 ns, χ^2_v = 1.5.

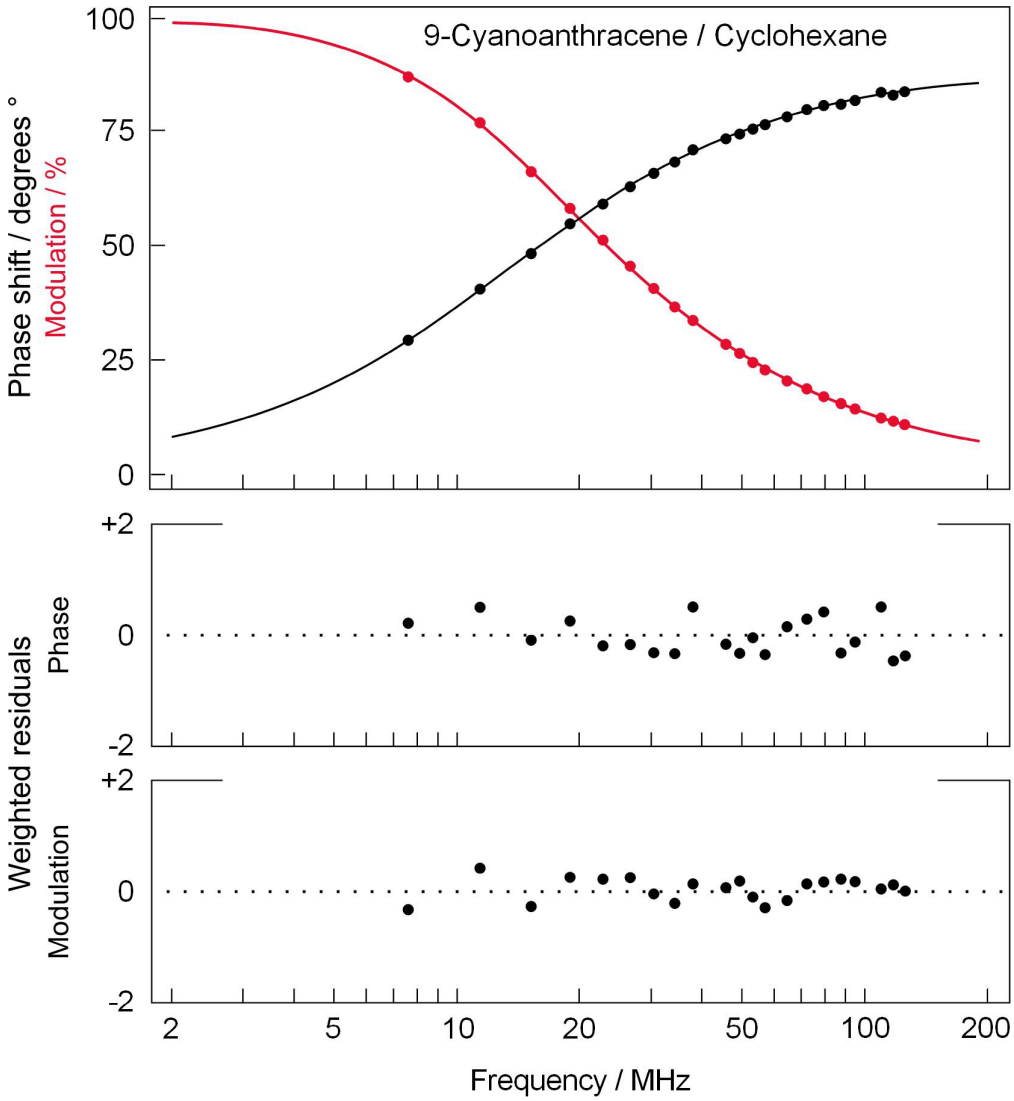


Figure S4-C. 9,10-Diphenylanthracene (DPA) in cyclohexane (λ_{ex} = 295 nm, λ_{em} > 345 nm, number of frequencies = 25, from 11 to 280 MHz). Results: τ = 6.62 ns, χ^2_v = 1.1.

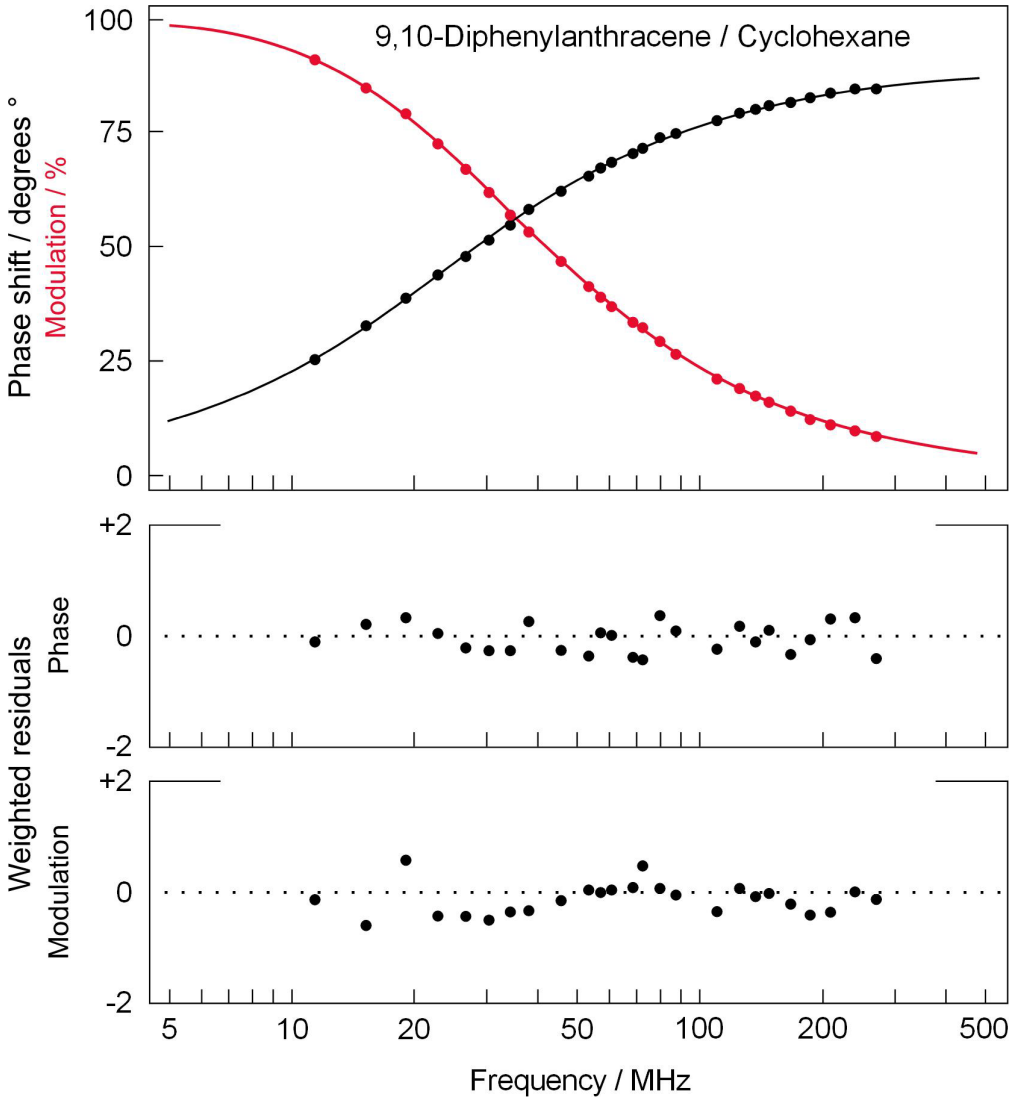
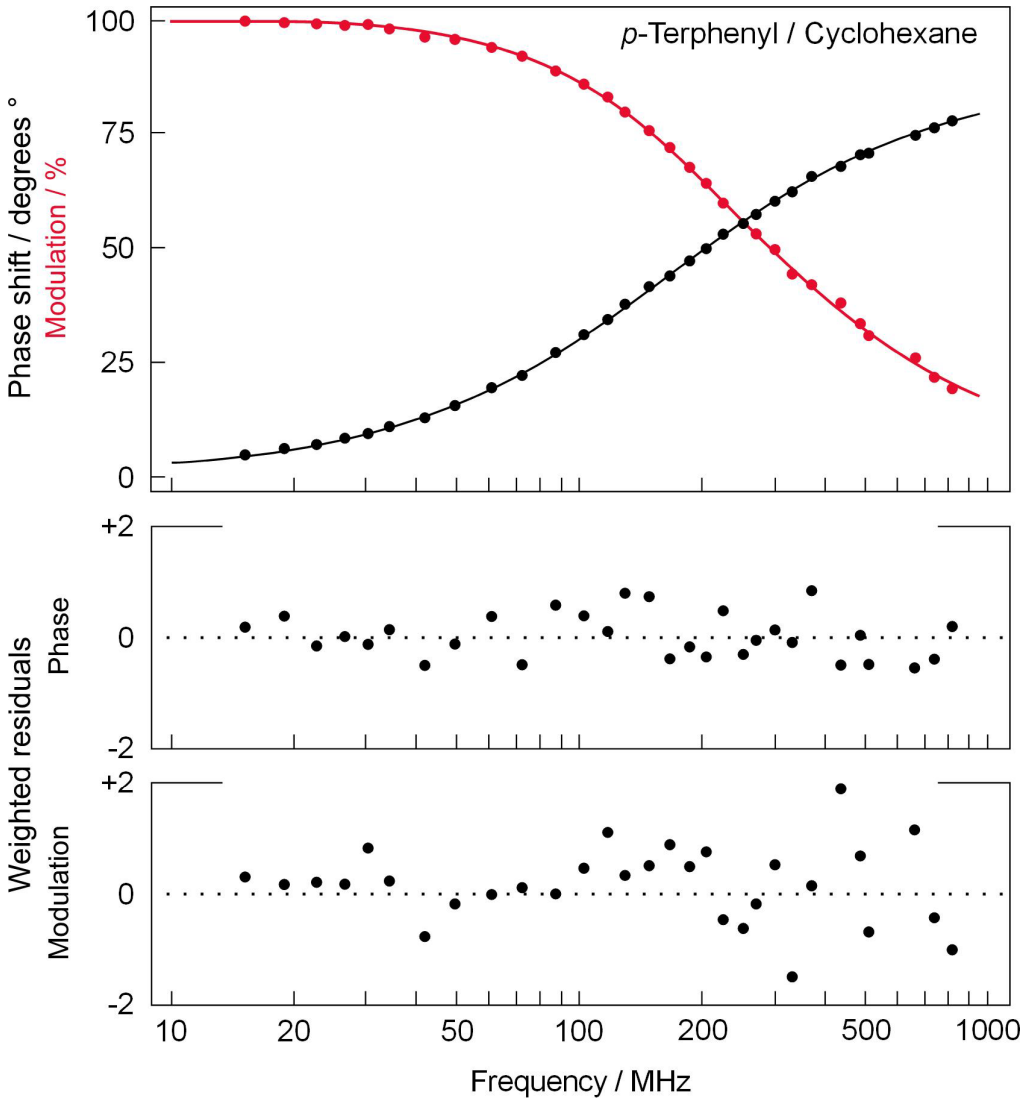


Figure S4-D. *p*-Terphenyl in cyclohexane (λ_{ex} = 300 nm, λ_{em} = 340 nm, number of frequencies = 30, from 15 to 800 MHz). Results: τ = 0.93 ns, χ^2_v = 1.6.



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