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ARTICLE *in* ANALYTICAL CHEMISTRY · OCTOBER 1986

Impact Factor: 5.64 · DOI: 10.1021/ac00125a024

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Computerized Spectrofluorometric Titrator

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A simple, time saving computerized spectrofluorometric titrator is described. The system design accommodates titrations that require gas purging, have foaming problems such as surfactant solutions, and require high stability because of long titration times. An HP-85 computer, interfaced through a parallel card and an IEEE-488 card, controls the titration, data acquisition, and data reduction. A BASIC program controls a stepping motor driven microburet, a gas solenoid valve for gas purging and mixing, and an analog multiplexer for input data routing. Automatic source fluctuation compensation enhances accuracy and long-term stability. An improved cell for surfactant titrations minimizes sample foaming and decreases titration times. Applications to bimolecular luminescence quenching and to binding of luminescent metal complexes to micelles are presented.

We are currently studying the photophysics and photochemistry of luminescent species in homogeneous and organized media. Primary tools are luminescence titrations involving either the changes in luminescence intensity or lifetime vs. quencher or surfactant concentration. These measurements have traditionally been done manually and are labor intensive, especially the surfactant titrations. One manual titration with a large number of points may take 4–8 h because of the slow rate of deoxygenation (*1*, *2*); we had hoped to decrease the tedium of these measurements by automation.

For maximum utility, an automated system should provide easy data collection and reduction, convenient data storage and retrieval, and graphics display of results. While many of these features are available on automated potentiometric titrators (*3*–*7*), we found no luminescence system that addressed several problems inherent in our work.

We describe the design and performance of an automated spectrofluorometric titrator. This system is suitable for a variety of fluorometric titrations and brings, at modest cost, many of the advantages of the earlier potentiometric systems to luminescence titrations. Specific benefits include freeing the operator to perform other tasks during the titration as well as eliminating systematic errors from light source fluctuations and inadequate deoxygenation or mixing. Graphics display and complex data fitting enhance the system's utility.

EXPERIMENTAL SECTION

Materials. Triton X-100, a neutral surfactant octylphenoxyethoxyethanol $C_8H_{17}C_6H_{14}(OC_2H_4)_xOH$ (x = 8, 9), was used as received from Sigma Chemical Co. $[Ru(bpy)_3]Cl_2 \cdot 6H_2O$ (bpy, 2,2′-bipyridine) from G. F. S. Co. and $[Ru(5,6\text{-}Me_2phen)_3](ClO_4)_2$ (5,6-Me_2phen, 5,6-dimethyl-1,10-phenanthroline) were purified or synthesized as described earlier (*1*). All solutions were prepared with doubly distilled water and stored in lightproof containers.

Design Criteria. For our work a useful titrator must have the following attributes: (1) Ease of use and the ability to run unattended, (2) automatic sample addition, mixing, and deoxygenation, (3) data collection, display, and manipulation, (4) modest cost, (5) intrinsically high stability and freedom from systematic errors, and (6) the ability to analyze the data, automatically reject bad data, and take corrective action.

Difficulties common to all our titrations include excitation source fluctuations or drift during long titrations (up to 5–8 h), degassing and mixing, interference from gas bubbles in the deoxygenation stream or from bubbles clinging to the cuvette walls during emission measurements, and irreproducible sample addition. An additional problem was surfactant solutions that foam badly during purging, and require much longer times to achieve stable readings than nonfoaming solutions. Also, photostability was a problem during very lengthy titrations. Our system satisfactorily solves all these problems.

Instrument Overview. Figure 1 is a schematic diagram of the titrator. The system is based around a conventional homemade spectrofluorometer (*8*). A beam splitter/photocell combination monitors the excitation intensity to permit corrections for light source fluctuations. A stepper motor drives a microsyringe to deliver titrant to a thermostated (*9*) special spectrofluorometer cell (vide infra). The solution is mixed after each addition by bubbling with air or nitrogen. A solenoid valve controls gas flow so that bubbling is stopped while data are being read. An analog multiplexer selects data first from the signal photomultiplier and then from the beam monitor photodiode. The stepping motor, the multiplexer, and solenoid valve are all controlled by a microcomputer. The analog voltages are digitized with an interfaced digital multimeter (DMM).

Our system is interfaced to, and controlled by, a Hewlett-Packard HP-85 computer via a general purpose parallel I/O interface (GPIO) and an IEEE-488 bus. The GPIO output lines control the stepping motor, the solenoid valve, and the analog multiplexer. The DMM is triggered and read by the HP-85A via the IEEE-488 bus.

Spectrofluorometer. The spectrofluorometer is described elsewhere (*8*). A quartz beam splitter reflects part of the excitation beam onto a United Detector Technology PIN 10DP silicon photodiode. The photocell current is amplified with an FET operational amplifier current-to-voltage converter. The Perkin-Elmer Model 99 emission monochromator has an internal chopper, which modulates the emission and provides a reference signal for the signal processing PAR Model 120 lock-in amplifier. Sample setup, such as excitation and emisson slit widths and wavelengths, amplifier gains, and PMT voltages are made manually. Neutral density filters in the excitation beam can be used to reduce the flux on photosensitive samples.

Sample Cell. With normal spectrofluorometer cells, loss of surfactant solutions due to foaming and spill-over during deoxygenation and mixing is a serious problem. To avoid these errors a low bubbling rate and longer deoxygenation and mixing times were required; this could unacceptably slow data acquisition. Recently, we described a cell that greatly reduced these problems (*10*), but complete deoxygenation was still not as rapid as desired, and solvent evaporation was a problem for very long titrations. We, thus, redesigned our cell to further reduce spill-over and evaporation problems. Our new cell permits even higher gas flows and shorter deoxygenation times.

Our new sample cell is shown in Figure 2. Gas and titrant are introduced through a rubber septum sealed Pasteur pipet. Titrant is delivered through a Teflon line to the narrow portion of the pipet. Gas, introduced at the top, forces the titrant out of the pipet and into the bottom of the cell. The gas bubbles also mix the solution after each addition. The titrant delivery tube is above

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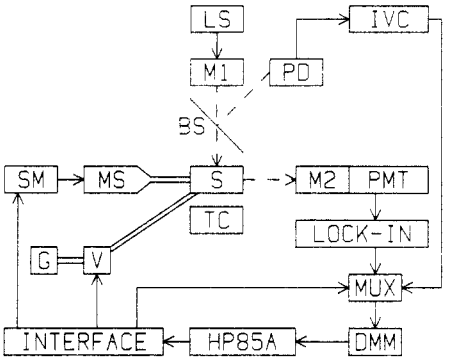

Figure 1. Schematic diagram of computerized spectrofluorometric titrator: SM, stepping motor controller and driver; MS, microsyringe; V, solenoid gas valve; G gas cylinder; S, sample; TC, temperature controller and magnetic stirrer; M1, M2, excitation and emission monochromators; LS, 1000-W xenon lamp; BS, beam splitter; PD, photodiode; IVC, current to voltage converter; PMT, signal photomultiplier; MUX, analog multiplexer; LOCK-IN, lock-in amplifier; DMM, digital multimeter; HP-85A, computer; INTERFACE, custom interface to stepping motor, gas valve, and multiplexer (see Figure 3 for details). Solid lines denote electrical paths, double lines are solution or gas paths, and dashed lines are optical paths.

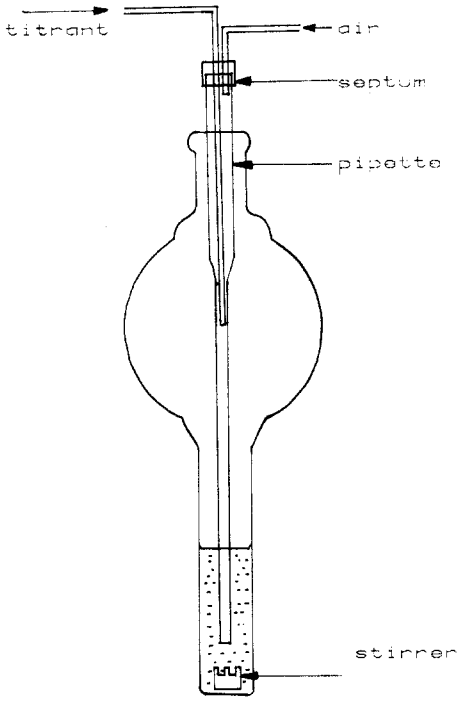

Figure 2. Diagram of deoxygenation cell. The stirrer can be used in addition to the bubbler to decrease deoxygenation time.

the sample solution, which prevents back diffusion of solution into the titrant when the gas flow is off.

Foaming out of the cell is prevented by the enlarged bulb. As foam rises and enters the bulb, it is no longer able to support itself and collapses. The solution then runs back into the cell. The narrow top of the cell minimizes evaporation.

Computer and Interface. The computer system is an HP-85A equipped with IEEE-488 and GPIO interfaces. This relatively inexpensive system makes a powerful controller and data reduction instrument. The GPIO interface controls the analog multiplexer that selects the analog signal routed to the DMM, opens a solenoid valve for gas purging and sample mixing, and drives the microsyringe for titrant additions. The PMT and photodiode signals are digitized with an IEEE-488 controlled Keithley 177 DMM.

The GPIO digital interface to the multiplexer, titrator, and solenoid valve is shown in Figure 3. Four latched GPIO output logic data bits (D0, D1, D2, D3) and the control line strobe (CTL) are used; no inputs are needed. The output lines are set by software. Data bits D2 and D3 select one of four reed relays in an analog multiplexer through a 1-of-10 decoder. Only two relays

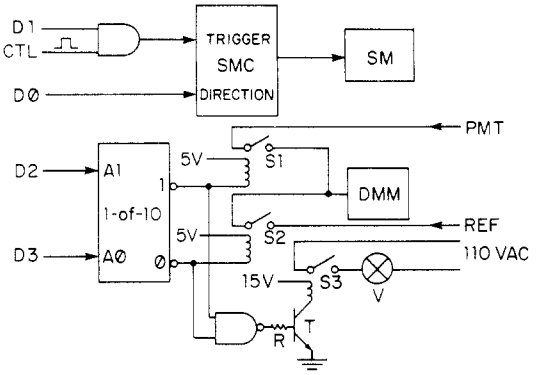

Figure 3. Schematic diagram of titrator interface: 1-of-10, 7442; S1, S2, S3, NO reed relays; R, 220-Ω resistor; T, 2N3904 transistor; SMC, SAA1027 stepping motor controller; SM, stepping motor; DMM, digital voltmeter; PMT, spectrofluorometer PMT signal source; REF, photodiode beam intensity monitor signal. The remaining IC's are standard 7400 series. Although not shown, the output lines from the computer are buffered with 74LS373 to protect the computer from failures in the interface.

(S1 and S2) are used in the current work.

D0 sets the titrator direction, and D1 is used to advance it a step. The stepping motor requires a pulse for each step; this is provided by ANDing D0 and CTL. If DO is a logic level high, then the stepping motor takes one step per output write, otherwise the motor does not move.

In order to prevent bubbles from disrupting the excitation and emission beams during the read cycle, the gas flow was turned off during the reading of data. Although we could have used another bit of the GPIO to control the gas flow, a simpler procedure was adopted. The two output lines of the 1-of-10 decoder were NANDed and used to drive the solenoid control valve. Whenever a data multiplexer relay (S1 or S2) was activated, one NAND input was low, and relay S3 was used to open the solenoid valve and interrupt the gas flow.

Once a signal source is selected by the multiplexer, the HP-85A triggers and reads the Keithley 177 DMM over the IEEE-488 bus. A reading takes 0.4 s; however, the DMM's RC time constant requires a 0.8-s wait before readings are stable.

Buret and Drive Mechanism. Precise titrant addition is made with a homemade semimicroburet. A stepper motor (North American Phillips) turns a Gilmont Corp. 2-mL micrometer syringe through an 8 to 1 reducing gear drive. One step delivers 0.2584 ± 0.0007 μL (based on seven replicates of 8000 steps). Flow rates of 100 pulses/s produced no missing steps. The stepper motor is interfaced via an SAA1027 stepper motor driver integrated circuit. The IC accepts the step and direction commands from D0 and D1 of the GPIO. The buret was filled under computer control from a reservoir.

General Procedure. Excitation and emission wavelengths were chosen to obtain the maximum emission intensities. To avoid decomposition of photosensitive samples, the excitation intensity could be reduced with neutral density filters while still providing good signal-to-noise ratio. Care was taken in filling the microburet to avoid introduction of any bubbles. All samples were initially thermally equilibrated for 5 min.

Immediately after each titrant addition, the sample was mixed by bubbling for 3 min. Alternating source intensities, I(source), and sample intensities, I(sample), were then read every 3 s. Starting and ending with a source reading, 11 source and 10 sample intensities were read. For each I(sample), a corrected sample emission intensity, I(corr), was calculated. I(corr) was the ratio of I(sample) to the average I(source) for the points immediately before and after each I(sample).

Before further additions were made, data quality was judged by two criteria. First, the standard deviation of the ten values of I(corr) was calculated. If the relative deviation (standard deviation of the data divided by its average value) exceeded 1%, the data set was discarded and a new data set was recorded. In a second test for adequate sample mixing, the least-squares slope of I(corr) vs. time was calculated. If the slope exceeded 0.1%/min the data set was discarded and the sample was bubbled for an

additional minute. Both tests had to be satisfied before making the next addition. Typically, for a 3-mL sample the first mixing period was adequate for complete mixing. Larger sample volumes tended to fail the slope vs. time test more often, resulting in a second mixing being performed.

After accepting the data, the values of I(corr) were plotted on the computer's display. Concentration of titrant and the average value of I(corr) were printed and recorded on digital tape. The titrate–mix–read cycle was continued until the titration was complete or the operator intervened. Data could then be fit by such methods as linear and nonlinear least squares to appropriate models.

Applications. Two types of luminescence titrations were performed. We measured luminescence quenching of [Ru(bpy)$_3$]Cl$_2$ (bpy, 2,2′-bipyridine) by Cu^{2+} and Ni^{2+} and binding of [Ru(5,6-Me$_2$phen)$_3$](ClO$_4$)$_2$ to the nonionic surfactant Triton X-100. These are systems that we have previously studied using much more tedious manual titration methods.

The quenching studies involved titrating solutions containing 2–10 μM [Ru(bpy)$_3$]Cl$_2$ with a solution containing the same concentration of [Ru(bpy)$_3$]Cl$_2$ and 50–100 mM of the metal ion quencher. The surfactant studies involved titrating an aliquot of 5 μM [Ru(5,6-Me$_2$phen)$_3$](ClO$_4$)$_2$ with a 0.100–0.200 M Triton X-100 solution containing the same concentration of the Ru(II) complex. An equal concentration of Ru(II) complex in both the sample and the titrant prevented changes in emission intensity due to dilution.

RESULTS AND DISCUSSION

The beam monitor and ratio correction turned out to be essential for satisfactory system performance. Ratio correction successfully reduced short- and long-term source fluctuations as well as the larger fluctuations occasionally observed on our system. In one extreme example, where the lamp was particularly noisy (perhaps due to arc wander), the uncorrected sample intensities fluctuated by up to 50%, but the corrected intensities only varied by 10%. Typically, our long- and short-term fluctuations were much smaller (±5%), and the ratio mode reduced fluctuations in I(corr) by a factor of at least 3–5 over I(sample).

Bimolecular excited-state quenching is governed by Stern–Volmer quenching kinetics

$$I_0/I = 1 + K_{sv}[Q] \quad (1)$$

where I_0 is the unquenched luminescence intensity and I is the quenched intensity at a quencher concentration [Q]. Plots of I_0/I vs. [Q] should be linear with slopes equal to the Stern–Volmer quenching constant K_{sv}. Quenching of Ru(bpy)$_3^{2+}$ by numerous transition-metal ions has been reported (*11*).

For quenching of Ru(bpy)$_3^{2+}$ by Cu^{2+} and Ni^{2+}, we obtained linear Stern–Volmer plots. The K_{sv} for $CuSO_4$ of 20 ± 2 M^{-1} compares well with the literature value of 22 ± 4 M^{-1} (*11*). Likewise, the value of K_{sv} for $NiSO_4$ of 0.9 ± 0.2 M^{-1} agrees satisfactorily with the literature value of 1.9 ± 0.7 M^{-1} (*11*).

Our research is also heavily involved in studying the interactions of luminescent species with micelles. The model is

$$D + h\nu \rightarrow {}^*D \quad (2)$$

$$DM + h\nu \rightarrow {}^*DM \quad (3)$$

$${}^*D + M \rightleftarrows {}^*DM \quad K_{DM} \quad (4)$$

$${}^*D \rightarrow D + h\nu \quad (5)$$

$${}^*DM \rightarrow D + h\nu' \quad (6)$$

where D is the sensitizer, M is the micelle, and DM is the sensitizer associated with the micelle (*1*, *12*). Micelles only form above the critical micelle concentration (cmc). K_{DM} is the binding constant for binding of the sensitizer to the micelle. *D and *DM have different luminescence efficiencies, lifetimes, and emission spectra. We use luminescence intensity titration curves to determine the important K_{DM}'s and cmc's.

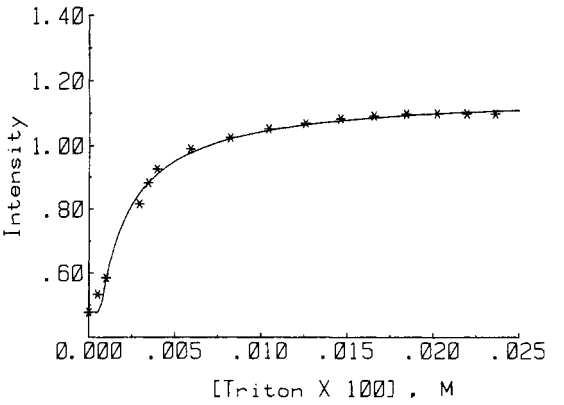

Figure 4. Intensity titration curve for Ru(5,6-Me$_2$phen)$_3^{2+}$ by Triton X-100: experimental points (asterisk); best fit (solid line).

This model leads to the following equations:

$$I = (I_0 + I_f K_{DM}[M])/(1 + K_{DM}[M]) \quad (7)$$

$$[M] = ([S] - ICMC)/N \quad (8)$$

where [S] is the formal added surfactant concentration, N is the aggregation number, [M] is the micelle concentration, I_0 and I are the luminescence intensities without and with added surfactant, and I_f is the emission intensity at infinite surfactant concentration (i.e., when all the sensitizer is micelle bound). ICMC is the induced critical micelle concentration, which may differ from the normal one because of induced aggregation around the sensitizer.

For Triton X-100, N is 140. For the sensitizer Ru(5,6-Me$_2$phen)$_3^{2+}$ interacting with TX-100, the bound form, DM, emits 3 times more intensely than the unbound D form in aerated solutions. The bound form's emission maximum is shifted to 620 nm compared to 605 nm for the unbound form.

The curves were fit to eq 7 and 8 using a simplex minimization method while varying K_{DM}, ICMC, and I_f. With the titrator we measured K_{DM} = 50000 ± 5000 M^{-1} for binding of Ru(5,6-Me$_2$phen)$_3$(ClO$_4$)$_2$ to Triton X-100. The indicated uncertainty is the average deviation for three determinations. This value is in good agreement with the 53000 ± 5000 M^{-1} reported earlier using the much more tedious manual method (*12*). A representative titration curve and best fit is shown in Figure 4.

The system's enhanced performance is especially evident in the TX-100 titrations. A manual titration with degassing takes 4–8 h and requires essentially constant operator supervision. With the titrator, the operator only spends 15 min initially setting up the titration, which then runs unattended. With the automatic titrator and the new purging cell, a complete deaerated titration takes only 2–4 h, and the data are more reliable than previously.

In summary, we present an automated titrator that is optimized for luminescence titrations. It satisfies all of our design criteria including low cost and ease of use. It is particularly well suited for systems that require long titration times, sample gas purging, and complex data reduction. It handles foaming systems well and corrects automatically for a variety of potential error sources.

ACKNOWLEDGMENT

E. R. Carraway, R. Hammond, and J. Love were responsible for the construction of a prototype automatic titrator. P. T. Rieger synthesized the Ru(5,6-Me$_2$phen)$_3$(ClO$_4$)$_2$ and performed the manual titrations in Triton X-100. We thank J. R. Bacon, James Beach, and Marvin Grubb for electronics assistance and William Shoup for constructing the sample cell.

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RECEIVED for review December 2, 1985. Resubmitted May 28, 1986. Accepted May 28, 1986. We gratefully acknowledge support by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Science Foundation (CHE 82-06279).

Multidimensional Data Formats for Phase-Resolved Fluorometric Multicomponent Determinations Using Synchronous Excitation and Emission Spectra

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The use of multidimensional data formats combining wavelength and fluorescence lifetime as selectivity parameters is described for determinations of two-, three- and four-component systems of polycyclic aromatic hydrocarbons (9-phenylanthracene, 9,10-diphenylanthracene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene). In the data formats, phase-resolved fluorescence intensity is measured as a function of either emission wavelength or synchronously scanned excitation and emission wavelengths, as well as detector phase angle and excitation modulation frequency. Results were compared with results for determinations using wavelength selectivity alone. Best results for the two- and three-component systems were obtained with the steady-state measurements, but the PRFS-synchronous data format was much better for the four-component system (−1.2% average relative error) and gave the best, most consistently good overall results for the multicomponent systems.

The use of phase-resolved fluorescence spectroscopy (PRFS) to incorporate fluorescence lifetime selectivity into multicomponent determinations has been explored in recent years (*1*). Previous studies describe PRFS determinations of two-, three-, and four-component systems (*2–6*). All of these studies used experimental conditions chosen for the quantitation of the known components in the mixtures, although none of the conditions were rigorously optimized for those particular components. In one of these studies (*3*), it was demonstrated that the phase-resolved approach gave better accuracy and smaller average error magnitudes than the equivalent steady-state (non-phase-resolved) determinations based on wavelength selectivity alone. Determinations described in two of the other studies (*2*, *4*) could not be accomplished with wavelength selectivity due to extensive spectral overlap of the components.

One of our goals for multicomponent PRFS determinations is the development of a generalized data format for multicomponent determinations for identification and subsequent quantitation of the components. One possible data format consists of a three-dimensional representation of phase-resolved fluorescence intensity (PRFI) as a function of synchronously scanned wavelength on one axis and detector phase angle setting (providing the fluorescence lifetime information) on the other, independent axis (*1*). The use of synchronous excitation spectroscopy for the determination of polynuclear aromatic hydrocarbons has been previously described (*7–9*).

The work described here compares results obtained by using the PRFS-synchronous excitation data format with results obtained by using a similar data format in which emission spectra are used rather than synchronous spectra. The results for both PRFS formats are compared with results for steady-state determinations using only synchronous or emission spectra and no fluorescence lifetime dimension. Four polycyclic aromatic compounds were used in these studies, including 9-phenylanthracene (9PA), 9,10-diphenylanthracene (9,10DPA), benzo[*a*]pyrene (BaP), and benzo[*k*]fluoranthene (BkF). These compounds were chosen because of the high degree of overlap between both their emission and their synchronous spectra. Their fluorescence lifetimes are within 10 ns of each other, with less than 1.5 ns difference between 9,10DPA and BkF. Four different systems were studied, including two two-component systems (9PA/9,10DPA and 9,10DPA/BkF), a three-component system (9PA/9,10DPA/BkF), and a four-component system containing all four of the compounds.

EXPERIMENTAL SECTION

Materials. The BaP (98%), 9PA (98%), and 9,10DPA (99%) were purchased from Aldrich and recrystallized from absolute ethanol (U.S. Industrial Chemical Co.). The BkF (99%) was purchased from Foxboro and used without further purification. Dimethylbis(5-phenyl-2-oxazolyl)benzene (Me_2POPOP) used as a reference for fluorescence lifetime determinations was purchased from Aldrich (scintillation grade) and used without further purification.

Stock solutions of each PAH were prepared in absolute ethanol. Mixtures were prepared from the stock solutions. The compositions of the solutions used for each of the two-, three-, and four-component systems (five solutions per system) are shown in Table I.

Apparatus. An SLM 4800S phase-modulation spectrofluorometer (SLM Instruments, Inc., Urbana, IL) was used for all steady-state and phase-resolved fluorescence measurements.

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