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PHOTO-VOLTAGES OF BILAYER LIPID MEMBRANES IN THE PRESENCE OF CYANINE DYES

J. S. HUEBNER
Department of Natural Sciences, University of North Florida, Jacksonville, Fla. 32216 (U.S.A.)
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SUMMARY

The transmembrane photo-voltage waveforms induced by 10 different cyanine dyes absorbed to one side of bilayer lipid membranes are described. The membranes were prepared from lecithin, oxidized cholesterol, and mixed lecithin and oxidized cholesterol. An 8-μs flash illumination was used. Three dyes induced a photo-voltage which developed in a few milliseconds, then discharged in less than the membranes' resistance-capacitance time. Five dyes induced a photo-voltage which increased for much longer than the membranes' resistance-capacitance time. Two dyes did not induce any photo-electric effects. Models are presented which correlate the dye structure with the type of photo-voltage waveform induced.

INTRODUCTION

Photo-electric effects in bilayer and thin lipid membranes were initially investigated by Tien [1], and have subsequently been studied in many laboratories [2–10]. One impetus for this work is its possible relevance to photosynthesis and other photobiological processes [11, 12]. In addition, it seems clear that an improved understanding of membrane processes and structure [13–24] will result from these investigations. The type of photo-electric effects investigated here were first reported for cyanine dyes by Ullrich and Kuhn [25], and subsequently analyzed by them [5] and Trissel and Läuger [7].

METHODS

Bilayer lipid membranes were prepared at a 1.3-mm diameter punched hole in a teflon cup, using techniques and apparatus much as previously described [9, 18, 19, 26]. Unless otherwise noted, the membrane was shunted by a 10^9 Ω resistor. Flash illumination was from a xenon flash tube in a General Radio Corporation stroboscope (type 1538-A with a P4 capacitor). The peak light intensity was $1.2 \cdot 10^9$ lux at the membrane, as determined by comparing the resistance change in a silicon photoresistor with those produced by the flash tube under calibrated conditions [27], using grey filters to reduce the light intensity to the appropriate range. The 1/3 peak intensity points of the flash illumination were separated in time by about 8 μs.

The cyanine dyes and chromatographically pure egg L-α-lecithin were obtained from Koch-Light Laboratories, Ltd. The dye structure and nomenclature [28] are given in Fig. 1. These dye molecules probably remain planar [28], which allows the molecules' positive charge to be distributed along the molecule between the nitrogen atoms through a shifting of π electrons, providing resonance stabilization for the planar structure. Other chemicals used were either spectro or reagent grade. The water used was redistilled in an all-glass still. Three types of membrane-forming solutions were used: lecithin, oxidized cholesterol, and a mixture of equal parts by weight of lecithin and oxidized cholesterol. Decane was used as the solvent for the membrane-forming solutions. The oxidized cholesterol was prepared as described by Tien et al. [29].

Three different methods were used to create dye gradients across the bilayer lipid membranes. In the first two methods, the membranes were formed in symmetrical unbuffered solutions of 0.01 M NaCl, and were observed to thin to the black state. The resistance and capacitance were allowed to stabilize (15–30 min) before the dye was added to the inner solution. In method 1, 100 μl of dye solution of known concentration was added with a repeating dispenser. The amount of dye that could be added by this method was limited by the limited dye solubility in water. In method 2, dye crystals were dropped directly into the inner solution. The dye crystals often did not completely dissolve. Consequently, the solution concentration with respect to the dye was usually not known when method 2 was used. In a third method, method 3, the punched hole in the teflon (inside) cup was closed with a rubber plug. The outside cup was filled with a solution of 0.01 M NaCl, while the inside was filled with a solution of 0.01 M NaCl and a known concentration of dye. With the cell filled, in position with the electrodes in place, the rubber plug was punched out with a syringe

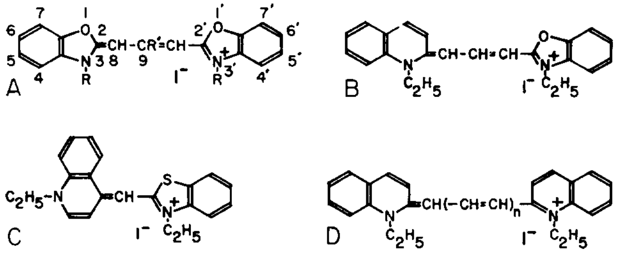

Fig. 1. The cyanine dye structure. (A) Shows dye 1 (3,3'-dimethyl-2,2'-oxacarbocyanine iodide) when R = CH_3 and R' = H; dye 4 (3.3'-diethyl-9-ethyl-2,2'-oxacarbocyanine iodide) when R and R' = C_2H_5; dye 5 (3,3'-dimethyl-2,2'-thiacarbocyanine iodide) when R = CH_3, R' = H, and the 1 and 1' oxygen atoms are replaced with sulfur; dye 6 (3,3'-diethyl-2,2'-thiacarbocyanine iodide) when R = C_2H_5, R' = H and the 1 and 1' oxygen atoms are replaced with sulfur; and dye 11 (3,3'-diethyl-2,2'-oxathiacarbocyanine iodide) when R = C_2H_5, R' = H, and the 1' oxygen atom is replaced with sulfur. (B) Shows dye 2 (1,3'-diethyl-2,2'-quinolyloxacarbocyanine iodide). (C) Shows dye 7 (1,3'-diethyl-4,2'-quinolylthiacyanine iodide). (D) Shows dye 8 (1,1'-diethyl-2,2'-cyanine iodide) when $n = 0$; dye 9 (1,1'-diethyl-2,2'-carbocyanine chloride) when $n = 1$ and chloride is substituted for iodide; and dye 10 (1,1'-diethyl-2,2'-tricarbocyanine iodide) when $n = 3$.

TABLE 1

MEAN VALUES FOR THE PROPERTIES OF BILAYER LIPID MEMBRANES IN THE PRESENCE OF CYANINE DYES

The dye addition methods are described in the text. L, L/C, and C, respectively, indicate solutions of lecithin, lecithin and oxidized cholesterol, and oxidized cholesterol were used to form the bilayer lipid membranes. F and S, respectively, refer to the fast and slow photo-effects described in the text and Figs 2 and 3. N indicates that no photo-effect was observed. The change in the membrane voltage resulting from flash illumination, with + indicating that the dye-containing (inside) solution became positive with respect to the outside solution.

Dye			Membrane		Photo-effect		Notes
No.	Concentration (μm/l)	Addition method	Lipid	Resistance (Ω)	Type	Amplitude (mV)	
1	60	1	L	$1 \cdot 10^9$	F	+4.6	Figs 2 b and c
	–	2	L/C	$3 \cdot 10^8$	F	+1.5	Fig. 2 a
	100	1	C	$1 \cdot 10^9$	F	+1.5	
2	50	1 and 2	L	–	–	–	unstable
	50	1 and 2	L/C	–	–	–	unstable
	400	3	C	$1 \cdot 10^7$	S	+0.6	Fig. 3 a
2*	100	1	L	$3 \cdot 10^9$	F and S	+1.0	
	60	1	L/C	$2 \cdot 10^8$	F and S	+0.4	
	60	1	C	$3 \cdot 10^7$	F and S	+0.3	
4	400	3	L	$2 \cdot 10^7$	N	0.0	
	400	3	L/C	$5 \cdot 10^6$	N	0.0	
	400	3	C	$2 \cdot 10^6$	N	0.0	
5	300	3	L	–	F	+0.3	unstable
	300	3	L/C	$1 \cdot 10^6$	F	+1.5	
	300	3	C	$1 \cdot 10^8$	F	+0.3	Fig. 2 d
6	100	3	L	$1 \cdot 10^7$	F	−2.0	Fig. 2 e, unstable
	100	3	L/C	–	F	+1.5	unstable
	100	3	C	$1 \cdot 10^7$	F	+1.0	Fig. 2 f
7	600	1 and 3	L	–	–	–	unstable
	600	3	L/C	–	–	–	unstable
	600	3	C	$5 \cdot 10^7$	S	+4.0	Fig. 3 b
8	300	1 and 3	L	–	–	–	unstable
	300	3	L/C	$3 \cdot 10^8$	S	−2.5	Fig. 3 d
	300	3	C	$3 \cdot 10^8$	S	+1.5	Fig. 3 c
9	300	3	L	–	–	–	unstable
	300	3	L/C	–	–	–	unstable
	300	3	C	$1 \cdot 10^7$	N	0.0	
	300	3	C	$1 \cdot 10^7$	S	−0.2	with KI added
10	300	3	L	$5 \cdot 10^8$	S	−0.1	
	300	3	L/C	$3 \cdot 10^8$	S	−0.1	
	300	3	C	$2 \cdot 10^8$	S	−1.0	Fig. 3 e
11	1 mM/l	3	L	$1 \cdot 10^8$	N	0.0	
	1 mM/l	3	L/C	$5 \cdot 10^7$	N	0.0	
	1 mM/l	3	C	$2 \cdot 10^7$	N	0.0	

* Aged in an aqueous solution at room temperature in the dark for 3–5 days.

while the membrane-forming solution was ejected into the hole to form the membrane. Method 3 used solutions that were usually near saturation with respect to the dye. With exceptions noted below, the dye solutions used in methods 1 and 3 were prepared fresh each day.

RESULTS

All three membrane-forming solutions produced bilayer lipid membranes with resistance values greater than $3 \cdot 10^9\ \Omega$. The dye additions typically reduced the membrane resistance and induced photo-electric effects as listed in Table I. Two different types of photo-voltage waveforms were observed, identified as F or S in the table, depending upon the particular dye used. A reduced membrane resistance produces a predictable change in the photo-voltage waveform [9]. Apart from this change, and from variations in the amplitudes described in the table, the waveforms did not depend on the type of lipid used in the membrane-forming solution. The type of waveform induced by a particular dye (that is the waveforms' development in time following flash excitation) was reproducible to within the calibration accuracy of the equipment employed for the F waveforms on each of at least five membranes of each type for each dye listed in Table I. The amplitudes of the F waveforms varied by as much as 20 % from the values listed, probably because of the limited dye solubility in solution and inefficient stirring when unstable membranes were used. Variations in the S waveforms are described below. No photo-electric effects were observed using two of the dyes, dye 4 and dye 11.

Dyes 1, 5, 6 and aged solutions of dye 2 induced one type of photo-voltage waveform, illustrated in Fig. 2. The transmembrane voltage typically rose to a peak within a few milliseconds following the flash excitation, then fell back (discharged) to the baseline in a few tenths of a second, which was usually much less than the membranes' resistance-capacitance time. Consequently, this type of waveform is called the fast effect (F), to distinguish it from the other type of waveform observed, described below. Reducing the membrane's resistance-capacitance time to less than the discharge time by replacing the $10^9\ \Omega$ shunt resistor with a $10^7\ \Omega$ shunt resistor caused the discharge to undershoot the baseline, as illustrated in Fig. 2a. The polarity of the fast effect was such that the dye side of the membrane (i.e. the inside solution) always became initially positive in electrical potential. Observable amplitudes of the photo-voltage effect could be produced with dye 1 using all three methods of creating the dye asymmetry. Only method 3 produced easily observable photo-effects for dyes 5 and 6, while methods 1 and 3 produced observable effects with aged solutions of dye 2. Dye 2 was previously reported [5] to produce "very similar effects" to dye 1. Such effects could only be produced in this work using old solutions of dye 2, which had visibly discolored.

Dye 1 is the most extensively investigated of the dyes studied here. Increased concentratrations of the dye, or subsequent additions of more dye to the solution in contact with the membrane, increased the amplitude of the photo-effect from 25 to 40 % each time the dye concentration was doubled. The photo-voltage amplitude was not altered by repeated flash illumination, and it decreased linearly with reductions in the light intensity (Fig. 2c). No variations could be detected in the amplitude or waveform of the photo-voltages from lowering the oxygen partial pressure to near

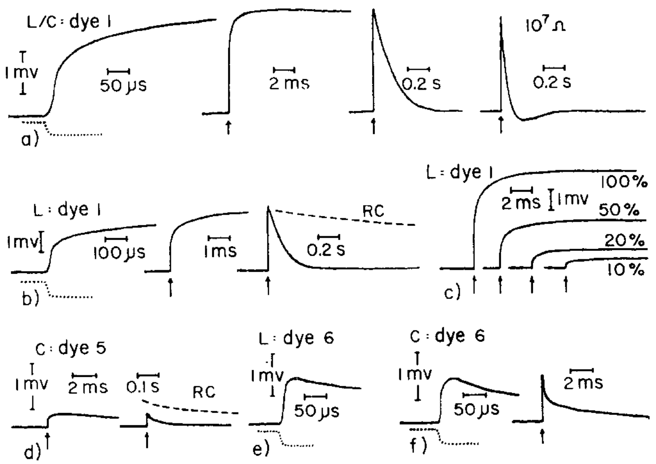

Fig. 2. The photo-voltage waveforms resulting from flash excitation, illustrating the fast effect. (a) Successive traces at progressively slower oscilloscope sweep speeds, using dye 1 and a lectithin-oxidized cholesterol bilayer lipid membrane. The resistance-capacitance discharge time with a $10^9 \Omega$ shunt resistor was 1 s. The trace on the right shows the response when a $10^7 \Omega$ shunt resistor was used. The dotted lines in a, b, e, and f show a solar cell output voltage, which is closely proportional to the negative time integral of the illumination intensity. Vertical arrows mark the time of the flash illumination on other traces. (b) Successive traces at progressively slower sweep speeds using dye 1 and a lecithin bilayer lipid membrane. The dashed lines in b and d show a portion of the resistance-capacitance discharge of the membrane when an external voltage source was removed. (c) The photo-voltage waveforms produced by another lecithin bilayer lipid membrane in the presence of dye 1, when the illumination intensity was unmodified (100 %), and reduced to 50, 20, and 10 % by neutral density grey filters. (d) The photo-response and resistance-capacitance discharge for an oxidized cholesterol bilayer lipid membrane in the presence of dye 5. (e and f). The photovoltage response of a lecithin, and an oxidized cholesterol bilayer lipid membrane, respectively, in the presence of dye 6.

zero. No variations in the photo-effects were detected with the addition of Fe^{2+} (from $FeCl_2$ crystals), Fe^{3+} (from $FeCl_3$ crystals), I^- (from KI crystals), or I^- and iodine (from solutions of KI of various ages) to the outside solution in quantities to produce millimolar concentrations. Similar concentrations of I^- added to the inner solution (the dye-containing solution) had no effect on the photo-effects, however, the addition of either Fe^{2+}, Fe^{3+}, or iodine and I^- to the inner solution in millimolar concentrations quenched (eliminated) the photo-effects.

The unmodified lecithin membranes were at times unstable after they had thinned to the black state, often rupturing during the period allowed for the resistance and capacitance to stabilize. Dye 1 had the effect of stabilizing these membranes against rupturing, and produced mean black membrane life-times of up to several hours, depending upon the particular lipid solution used. Fresh solutions of dye 2 made the lecithin bilayer membranes less stable, with no black membrane surviving for 10 min after stirring in the dye. Black lipid membranes prepared from oxidized cholesterol were more stable than those containing lecithin and were not made unstable by any of the dyes used.

Fig. 3 illustrates the photo-voltage waveform which resulted in membranes prepared from all three membrane-forming solutions, using dyes 2, 7, 8, and 10. The

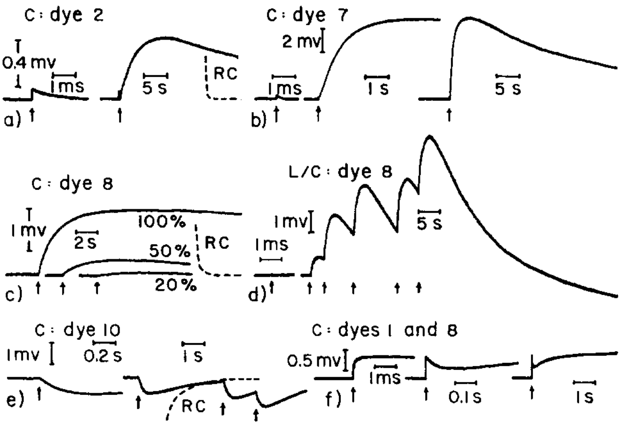

Fig. 3. The photo-voltage waveforms resulting from flash excitation, illustrating the slow effect. (a) The response of an oxidized cholesterol bilayer lipid membrane in the presence of a fresh solution of dye 2, at two oscilloscope sweep speeds. The vertical arrows mark the time of the flash illumination. The dashed lines in a, c, and e show the resistance-capacitance discharge of the membrane when an external voltage source was removed. (b) Successive traces at progressively slower sweep speeds using dye 7 and an oxidized cholesterol bilayer lipid membrane. (c) The photo-voltage waveforms produced by dye 8 and an oxidized cholesterol bilayer lipid membrane when the illumination intensity was unmodified (100 %), and reduced to 50, and 20 % by neutral density grey filters. (d) The photo-response produced by dye 8 and a lecithin-oxidized cholesterol bilayer lipid membrane to multiple flash illumination. (e) The photo-response produced by dye 10 and an oxidized cholesterol bilayer lipid membrane. (f) The photo-response produced by a mixture of dyes 1 and 8, and an oxidized cholesterol bilayer lipid membrane.

transmembrane voltage typically rose for several seconds following the flash excitation, and required 10–30 s to return to the baseline. This occurred in spite of the fact that the membrane's resistance-capacitance time as determined by applying and removing an external voltage source was often less than 0.1 s. Consequently, this type of waveform is called the slow effect (S). A higher concentration of dye was required in order that the slow effect could be easily distinguished from low frequency noise, necessitating that the dye asymmetry be created by method 3. No photo-voltage was observed using dye 9 unless iodide was also added. The slow effect photo-voltage amplitude was altered by repeated flash illumination, in some cases with the response amplitude becoming larger on subsequent flashes (Fig. 3d). The photo-voltage amplitude also decreased extra-linearly with reductions in the light intensity. The decrease was close to the square of the decrease in light intensity for dye 8 (Fig. 3c). The results were more complex for dye 7 in that the waveform shape changed with the light intensity, and repeated illumination. The addition of two types of dyes, dyes 1 and 8 for example, produced a linear addition of the slow and fast effect waveforms (see Fig. 3f).

DISCUSSION

The results reported here are that dyes 1, 5, 6, and aged solutions of dye 2 produce a fast photo-voltage waveform, that dyes 2, 7, 8, 10, and 9 (when iodide is added) produce a slow photo-voltage waveform and that dyes 4, and 11 do not produce any photo-electric effects. The fact that no photo-effects occur with dyes 4 and 11 indicates that neither type of photo-effect observed here is caused by iodide alone. Two different models are required to explain the appearance of the two types of waveforms. There is no conflict in having two different models since the two different types of effects require mutually exclusive dye structures. Indeed, Fig. 3f illustrates that both mechanisms can operate independently in the same membrane.

The slow effect is seen to require the presence of dye molecules with at least one quinoline ring, that the dye be present in solution in a relatively high concentration, and that iodide be present. The long duration of the slow effect compared to the membrane resistance-capacitance time in the presence of these dyes implicates some slowly proceeding photo-chemical reaction, a diffusion process, or a combination thereof. It is hypothesized that the slow effect results when the transmembrane diffusion equilibrium of the dye and iodide is temporarily upset by a photo-chemical reaction. Since the amplitude of the observed photo-voltage waveform varies with the square of the light intensity, the photo-chemical reaction apparently requires the interaction of two excited molecules. Since cyanine dye complexes are known [30], it may be useful to speculate here that such complexes transport ions across the membrane. If so, this would account for the resistance reductions observed in the presence of these dyes, and it would indicate that the photo-voltages observed here could be useful in kinetically analyzing various carrier-mediated transport models.

The fast effect waveform is seen to increase rapidly in the 20–80 μs following the flash illumination, reach a peak voltage, and then decrease, returning to the baseline, all in a time that can be much less than the membrane's resistance-capacitance time. The dyes which induce the fast effect all have mirror symmetry about the vinylene chain and have either two oxygen or two sulfur atoms (the 1 and 1′ atoms in Fig. 1A). From electrostatic considerations, isolated dye molecules would be more soluble in the membrane if their positive charge were distributed between the two nitrogen atoms. This requires that the molecule remains in the planar resonance-stabilized configuration [28].

The model offered here to explain the fast effect makes three presumptions. (i) That some of the dye molecules are absorbed into the membrane-aqueous interface with the vinylene bridge parallel to the surface and the 1 and 1′ atoms in contact with water, while the remaining hydrocarbon portions of these molecules remain buried in the oil phase. Fluorescence spectroscopy indicates that 3,3′-dioctadecyl-2,2′-oxacarbocyanine iodide orients in this manner in oxidized cholesterol bilayers [21]. (ii) That illumination raises some of these dye molecules to an electronically asymmetric excited state which concentrates the positive charges near one end of the molecules. Electrostatic forces then reorient the molecules that are in the membrane-aqueous interface, transporting their positive charges toward the aqueous phase. This yields a net positive electric displacement toward the dye solution and produces the observed rising portion of the fast effect. (iii) That upon de-excitation the molecules return to their initial positions, transporting the electric charges back, discharging the photo-voltage. Dye molecules inside the membrane or in solution, being randomly oriented and relatively far from the interface (the dielectric discontinuity) would not be expected to contribute to the generation of a transmembrane voltage. Conformational changes of the excited dye molecules may also be involved. The excited dye molecules which are able to extend their charged ends into the aqueous phase (the higher dielectric constant medium) will probably be more stable in that configuration than excited dye molecules completely inside the oil phase of the membrane or entirely in the aqueous solution. Thus, the excited state life-time in either solvent cannot be used directly to predict the time lapse between the voltage generation (the rising part of the waveform) and discharge (the falling part of the voltage waveform).

The model just presented predicts: (i) the polarity of the fast effect, (ii) that the risetime of the fast effect should be in the order of molecular rotation times, and should be longer for dyes with longer hydrocarbon tails, (iii) that the photovoltage should discharge in the dye relaxation time, which is often much less than the membrane's resistance-capacitance time, (iv) that dyes with two oxygen or sulfur atoms in positions to hydrogen bond at the interface (dyes 1, 5, and 6) will produce the fast effect, while dyes without these atoms (dyes 2, 7, 8, 9, and 10) will not. Note that dye 4 has mirror symmetry and oxygen atoms in the 1 and 1′ positions, yet does not induce the fast effect. This is explained by examining a space-filling model of the dye molecule (Fig. 4), which shows that the ethyl group attached to the number 9 carbon atom effectively covers the oxygen atoms, and thus prevents their bonding to water. This prevents dye 4 molecules from lying parallel to and in the plane of the membrane interface. The failure of dye 11 to produce a fast photo-voltage effect is due to the oxygen-sulfur asymmetry in the 1 and 1′ positions. The increased ability of oxygen to bond to water apparently prevents the molecule from lying parallel to the plane of the membrane while at the interface.

This model is able to make quantitative predictions. For example, the shape of the rising part of the waveforms should be predictable from calculations involving the kinetics of the excited dye molecule rotation under the dielectric forces. Work toward this end and additional experiments are in progress. Preliminary results with 3,3′-dipropyl-2,2′-oxacarbocyanine iodide and 3,3′-dibutyl-2,2′-oxacarbocyanine io-

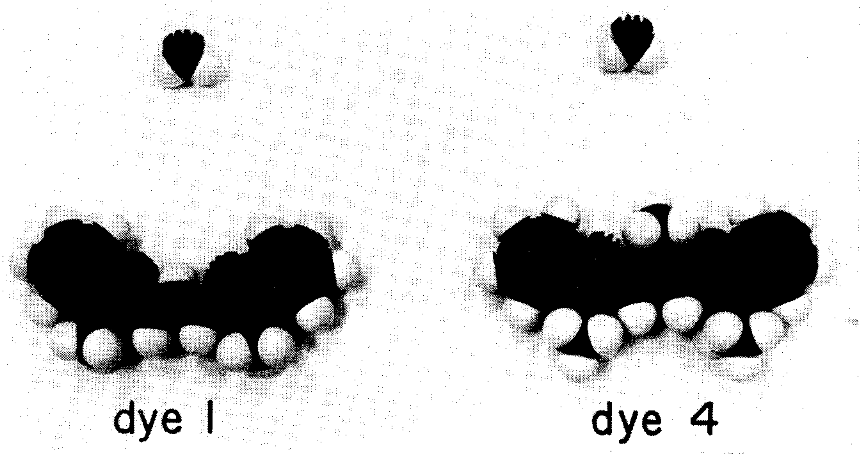

Fig. 4. Space-filling models of dye 1 (3,3′-dimethyl-2,2′-oxacarbocyanine iodide) and dye 4 (3,3′-diethyl-9-ethyl-2,2′-oxacarbocyanine iodide) in a planar configuration. Note that the 9-ethyl group overfills the gap between the two oxygen atoms in dye 4, slightly distorting some of the bond angles. Two water molecules are shown for comparison.

dide, which will be described in more detail in a subsequent publication, indicate that progressively longer risetimes are obtained with longer hydrocarbon tails, providing additional confirmation for this model.

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