

Figure 23.2 D-1199 di-4-ANEPPS.

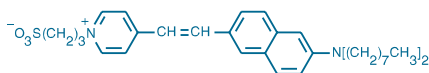


Figure 23.3 D-3167 di-8-ANEPPS.

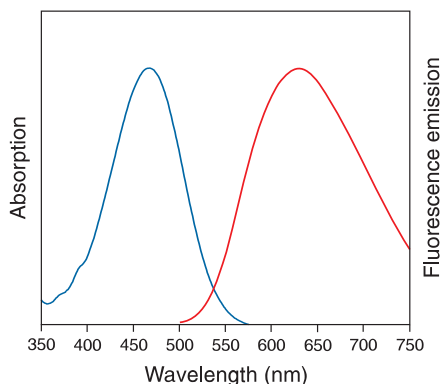


Figure 23.4 Absorption and fluorescence emission spectra of Di-8-ANEPPS (D-3167) bound to phospholipid bilayer membranes.

Numerous references are available for Di-4-ANEPPS and Di-8-ANEPPS at our Web site (www.probes.com/search).

23.2 Fast-Response Probes

Our fast-response potential-sensitive probes (Figure 23.1) are listed in Table 23.1, along with their charge, optical response and selected applications.

Styryl Dyes

Di-4-ANEPPS and Di-8-ANEPPS

The ANEP (AminoNaphthylEthenylPyridinium) dyes (Figure 23.2, Figure 23.3) developed by Leslie Loew and colleagues¹ exhibit the most consistently sensitive responses of the fast-response probes. Di-4-ANEPPS (D-1199) and di-8-ANEPPS (D-3167, Figure 23.4) exhibit fairly uniform 10% per 100 mV changes in fluorescence intensity in a variety of tissue, cell and model membrane systems.^{2,3} The millisecond-range temporal characteristics of the ANEP dyes compensate for this modest response amplitude (Figure 23.5). Di-4-ANEPPS is internalized in the cell rather rapidly, precluding its use in all but very short-term experiments, whereas di-8-ANEPPS (D-3167) is better retained in the outer leaflet of the plasma membrane. In addition, although both ANEP dyes exhibit good photostability and low toxicity, di-8-ANEPPS is reported to be slightly more photostable and significantly less phototoxic than di-4-ANEPPS.⁴⁻⁶

Like other styryl dyes, the ANEP dyes are essentially nonfluorescent in aqueous solutions and exhibit spectral properties that are strongly dependent on their environment.⁷ When bound to phospholipid vesicles, di-8-ANEPPS has absorption/emission maxima of ~467/631 nm (Figure 23.4), as compared to ~498/713 nm in methanol. The fluorescence excitation/emission maxima of di-4-ANEPPS bound to neuronal membranes are ~475/617 nm.⁸ Both di-4-ANEPPS and di-8-ANEPPS respond to increases in membrane potential (hyperpolarization) with a decrease in fluorescence excited at approximately 440 nm and an increase in fluorescence excited at 530 nm.^{7,9} These spectral shifts permit the use of ratiometric methods (see Loading and Calibration of Intracellular Ion Indicators in Section 20.1) to correlate the change in fluorescence signal with membrane potential.² Using di-8-ANEPPS, Loew and colleagues were able to follow changes in membrane potential along the surface of a single mouse neuroblastoma cell in their study of the mechanisms underlying cathode-directed neurite elongation¹⁰ and to define differences between transmembrane potentials of neurites and somata.¹¹ Potential-dependent fluorescence emission ratio measurements (ratio of emission intensities at 560 nm and 620 nm following excitation at 475 nm) have also been reported using both di-4-ANEPPS and di-8-ANEPPS¹²⁻¹⁵ (Figure 23.5). Some other applications are listed in Table 23.1.

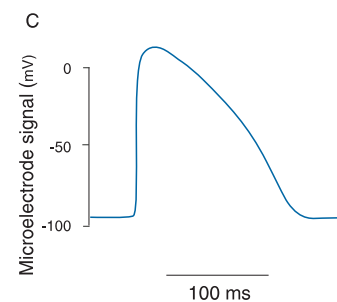
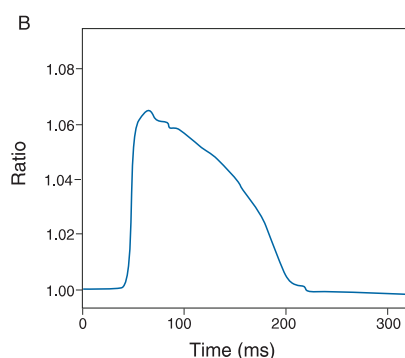
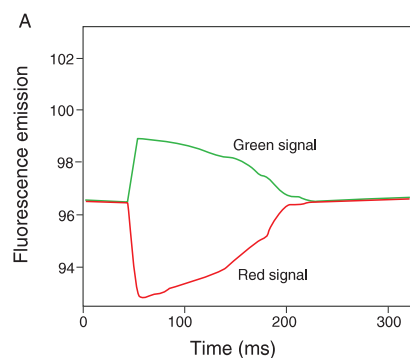


Figure 23.5 Detection of action potentials in intact rabbit hearts using the fast potentiometric probe di-4-ANEPPS (D-1199). Excised rabbit hearts were loaded with di-4-ANEPPS by perfusion with dye-containing medium. Fluorescence was excited at 488 nm by an argon-ion laser. Emission components at 540 ± 6 nm (green) and >610 nm (red) were detected simultaneously by two photomultipliers (panel A). The ratio of the green to red signals (panel B) dis-

played a larger fractional change during action potential cycles than either of the component signals, and also followed transmembrane voltage contours recorded simultaneously by an intracellular microelectrode (panel C). Fluorescence ratio measurements also reduce the motion artifacts that typically distort optical signals detected from contracting hearts. Figure reproduced with permission from *Am J Physiol* 279, H1421 (2000).

Table 23.1 Characteristics and selected applications of Molecular Probes' fast-response probes.

Dyes (Cat #)	Structure (Charge)	Optical Response	Selected Applications
Di-4-ANEPPS (D-1199) Di-8-ANEPPS (D-3167) Di-2-ANEPEQ (D-6923) Di-8-ANEPPQ (D-6925) Di-12-ANEPPQ (D-6927)	Styryl (cationic or zwitterionic)	FAST; fluorescence excitation ratio 440/505 nm decreases upon membrane hyperpolarization	<ul style="list-style-type: none"> • Mapping of membrane potentials along neurons¹⁻⁶ and muscle fibers⁷⁻¹⁰ • Combined potentiometric and Ca²⁺ measurements^{7,11,12} • Combined optical potentiometric and electrophysiological measurements¹³ • Detection of membrane potential changes in response to pharmacological stimuli^{14,15} • Imaging electrical activity from intact heart tissues¹⁶⁻¹⁸
RH 237 (S-1109) RH 414 (T-1111) RH 421 (S-1108) RH 795 (R-649)	Styryl (cationic or zwitterionic)	FAST; fluorescence decreases upon membrane depolarization	<ul style="list-style-type: none"> • Imaging of membrane potentials evoked by visual^{19,20} and olfactory²¹⁻²³ stimuli • Functional tracing of neurons²⁴⁻²⁷ • Detection of synaptic activity²⁸⁻³¹ and ion channel activity^{32,33}
RH 155 (R-1114)	Hybrid oxonol (anionic)	FAST; absorbance (at ~720 nm) changes	<ul style="list-style-type: none"> • Invertebrate neurons^{34,35} • Membrane potentials in muscle and nerve tissues³⁶⁻³⁸ • Voltage-sensitive calcium channels^{39,40}

1. J Neurosci 18, 9977 (1998); 2. J Neurosci 15, 1392 (1995); 3. Neuroimage 5, 154 (1997); 4. Neuron 13, 1187 (1994); 5. Neuron 9, 393 (1992); 6. Brain Res 700, 235 (1995); 7. Biophys J 78, 1777 (2000); 8. Biophys J 66, 719 (1994); 9. Nature 355, 349 (1992); 10. Biophys J 67, 1301 (1994); 11. J Physiol 508, 145 (1998); 12. Biophys J 71, 1057 (1996); 13. Biophys J 74, 48 (1998); 14. Am J Physiol 274, H60 (1998); 15. Pharmacol Res 34, 125 (1996); 16. Am J Physiol Heart Circ Physiol 280, H2053 (2001); 17. Biophys J 80, 516 (2001); 18. Am J Physiol Heart Circ Physiol 279, H1421 (2000); 19. J Neurosci 14, 2545 (1994); 20. Proc Natl Acad Sci U S A 94, 7621 (1997); 21. J Neurosci 20, 749 (2000); 22. Annu Rev Neurosci 15, 321 (1992); 23. J Neurosci 12, 976 (1992); 24. J Neurosci Methods 54, 151 (1994); 25. Proc Natl Acad Sci U S A 91, 4604 (1994); 26. Exp Brain Res 94, 371 (1993); 27. J Neurosci 12, 3054 (1992); 28. J Physiol 519 Pt 2, 427 (1999); 29. J Neurosci 14, 3319 (1994); 30. Nature 364, 635 (1993); 31. J Exp Biol 200, 161 (1997); 32. J Biol Chem 269, 21620 (1994); 33. Nature 367, 739 (1994); 34. J Neurosci 14, 1366 (1994); 35. Biophys J 56, 213 (1989); 36. Circulation 85, 1865 (1992); 37. Circ Res 69, 842 (1991); 38. J Neurosci 16, 313 (1996); 39. Proc Natl Acad Sci U S A 85, 4075 (1988); 40. J Neurosci 7, 3877 (1987).

Other ANEP Dyes

In collaboration with Leslie Loew and Joe Wuskell of the University of Connecticut, Molecular Probes offers a series of three potential-sensitive ANEP dyes.^{16,17} The very water-soluble di-2-ANEPEQ¹⁸ (JPW 1114, D-6923; Figure 23.6) can be either microinjected into cells, a mode of delivery that intensifies the staining of remote neuronal processes, or applied topically to deeply stain brain tissue.¹⁹ Microinjection of di-2-ANEPEQ into neurons in ganglia of the snail *Helix aspersa* produced an approximately 50-fold improvement in voltage-sensitive signals from distal processes over that obtained with conventional absorption and fluorescence staining methods.²⁰ Di-8-ANEPPQ (D-6925) and di-12-ANEPPQ (D-6927) are useful for potential-sensitive retrograde labeling of neurons^{16,21} using techniques similar to those employed for lipophilic carbocyanine and aminostyryl tracers (Section 14.4).

RH Dyes

Originally synthesized by Rina Hildesheim, the RH dyes include an extensive series of dialkylaminophenylpolyenylpyridinium dyes that are principally used for functional imaging of neurons (Table 23.1). The existence of numerous RH dye analogs reflects the observation that no single dye provides the optimal response under all experimental conditions.^{5,22,23} Currently, the most widely used RH dyes are RH 414 (T-1111, Figure 23.7), RH 795 (R-649, Figure 23.8) and RH 237 (S-1109). The RH 421 probe (S-1108) has yielded the most sensitive response recorded for a fast potentiometric probe, exhibiting a >20% fluorescence change per 100 mV on neuroblastoma cells.²⁴ Physiological effects of staining with different analogs are not equivalent. For example, staining of the cortex with RH 414 causes arterial constriction, whereas staining with RH 795 does not.²⁵ RH 795 produced negligible side effects when tested *in vitro* using hippocampal slices and *in vivo* using single-unit recordings in cat and monkey visual cortices.²⁶ Electrophysiological measurements indicate a broadening of action potentials that is attributable to the staining of cultured neurons with RH 237.²⁷

Like the ANEP dyes, the RH dyes exhibit varying degrees of fluorescence excitation and emission spectral shifts in response to membrane potential changes.⁸ Their absorption and fluorescence spectra are also strongly dependent on the environment.²⁸ Spectra of RH 414 bound to phospholipid vesicles are similar to those obtained on neuronal plasma membranes.⁸ The RH dyes belong to the same structural class as our FM dyes

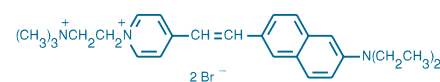


Figure 23.6 D-6923 di-2-ANEPEQ.

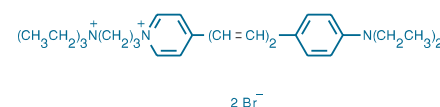


Figure 23.7 T-1111 RH 414.

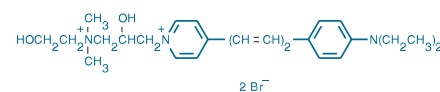


Figure 23.8 R-649 RH 795.

All of the fast-response dyes in this section are essentially nonfluorescent unless bound to membranes and, with the exception of the nonfluorescent RH155 dye, become fluorescent with large Stokes shifts on binding to membranes.

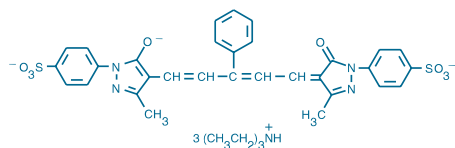


Figure 23.9 R-1114 RH 155.

The full citations and, in most cases, links to PubMed for all references in this Handbook are available at our Web site (www.probes.com/search).

(Section 16.1), and can likewise be used to detect activity-dependent synaptosomal recycling at live nerve terminals.^{29,30}

Impermeant Oxonol: RH 155

RH 155 (R-1114, Figure 23.9) is an oxonol dye with phenylsulfonate substituents that tend to make it impermeant to cell membranes. Consequently, its potentiometric response is faster but of smaller magnitude than those of other oxonol dyes³¹ (Section 23.3). Because it is anionic, adsorption of this dye to the membrane increases upon depolarization. RH 155 is nonfluorescent; its potentiometric response is detected using absorption changes at approximately 720 nm.^{32,33}

References

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4. *Biophys J* 76, 2272 (1999);
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6. *Pflugers Arch* 426, 548 (1994);
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19. *Proc Natl Acad Sci U S A* 94, 7621 (1997);
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21. *J Neurosci Methods* 70, 111 (1996);
22. *Physiol Rev* 68, 1285 (1988);
23. *Annu Rev Neurosci* 8, 263 (1985);
24. *Biophys J* 42, 195 (1983);
25. *Nature* 324, 361 (1986);
26. *J Neurosci* 14, 2545 (1994);
27. *Biophys J* 69, 299 (1995);
28. *Biophys J* 68, 1406 (1995);
29. *J Exp Biol* 200, 161 (1997);
30. *Science* 255, 200 (1992);
31. *Chem Phys Lett* 129, 225 (1986);
32. *Annu Rev Physiol* 51, 507 (1989);
33. *J Neurosci* 7, 649 (1987).

Data Table — 23.2 Fast-Response Probes

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
D-1199	480.66	D,L	DMSO, EtOH	496	39,000	705	MeOH	1
D-3167	592.88	D,L	DMSO, EtOH	498	35,000	713	MeOH	1
D-6923	549.39	D,L	DMSO, EtOH	517	36,000	721	EtOH	
D-6925	731.74	D,L	DMSO, EtOH	516	36,000	721	EtOH	1
D-6927	843.95	D,L	DMSO, EtOH	519	36,000	719	EtOH	1
R-649	585.42	D,L	DMSO, EtOH	530	33,000	712	MeOH	1
R-1114	950.26	D,L	DMSO, EtOH	650	120,000	none	MeOH	
S-1108	498.72	D,L	DMSO, EtOH	515	50,000	704	MeOH	1, 2
S-1109	496.71	D,L	DMSO, EtOH	528	53,000	782	MeOH	1, 2
T-1111	581.48	D,L	DMSO, EtOH	532	55,000	716	MeOH	1

For definitions of the contents of this data table, see “How to Use This Book” on page viii.

Notes

1. Abs and Em of styryl dyes are at shorter wavelengths in membrane environments than in reference solvents such as methanol. The difference is typically 20 nm for absorption and 80 nm for emission, but varies considerably from one dye to another.
2. Abs/Em for these dyes adsorbed on neuronal plasma membranes are 493/638 nm (S-1108) and 506/687 nm (S-1109) (*Biochim Biophys Acta* 1150, 111 (1993)).

Product List — 23.2 Fast-Response Probes

Cat #	Product Name	Unit Size
D-6923	di-2-ANEPEQ (JPW 1114)	5 mg
D-6925	di-8-ANEPPQ	5 mg
D-6927	di-12-ANEPPQ	5 mg
D-1199	di-4-ANEPPS	5 mg
D-3167	di-8-ANEPPS	5 mg
R-1114	RH 155	25 mg
R-649	RH 795	1 mg
S-1109	<i>N</i> -(4-sulfobutyl)-4-(6-(4-(dibutylamino)phenyl)hexatrienyl)pyridinium, inner salt (RH 237)	5 mg
S-1108	<i>N</i> -(4-sulfobutyl)-4-(4-(4-(dipentylamino)phenyl)butadienyl)pyridinium, inner salt (RH 421)	25 mg
T-1111	<i>N</i> -(3-triethylammoniumpropyl)-4-(4-(4-(diethylamino)phenyl)butadienyl)pyridinium dibromide (RH 414)	5 mg