

22.1 Fluorescent Na⁺ and K⁺ Indicators

SBFI and PBF1

Properties of SBFI and PBF1

SBFI¹ and PBF1^{1,2} are fluorescent indicators for sodium and potassium, respectively.³ Although the selectivity of SBFI and PBF1 for their target ions is less than that of calcium indicators such as fura-2, it is sufficient for the detection of physiological concentrations of Na⁺ and K⁺ in the presence of other monovalent cations.¹ Furthermore, the spectral responses of SBFI and PBF1 upon ion binding permit excitation ratio measurements (see Loading and Calibration of Intracellular Ion Indicators in Section 20.1), and these indicators can be used with the same optical filters and equipment used for fura-2^{4,5} (Table 24.8).

SBFI (Figure 22.1) and PBF1 (Figure 22.2) comprise benzofuranyl fluorophores linked to a crown ether chelator. The cavity size of the crown ether confers selectivity for Na⁺ versus K⁺ (or *vice versa* in the case of PBF1). When an ion binds to SBFI or PBF1, the indicator's fluorescence quantum yield increases, its excitation peak narrows and its excitation maximum shifts to shorter wavelengths (Figure 22.3), causing a significant change in the ratio of fluorescence intensities excited at 340/380 nm (Figure 22.4, Figure 22.5). This fluorescence signal is slightly sensitive to changes in pH between 6.5 and 7.5,^{6,7} but it is strongly affected by ionic strength⁸ and viscosity.⁹ Researchers have described the use of SBFI for emission ratio detection (410/590 nm, excited at 340 nm).¹⁰ More recently, the implementation of two-photon excitation of SBFI with infrared light has been reported for Na⁺ imaging in spines and fine dendrites of central neurons^{11,12} (Figure 22.6).

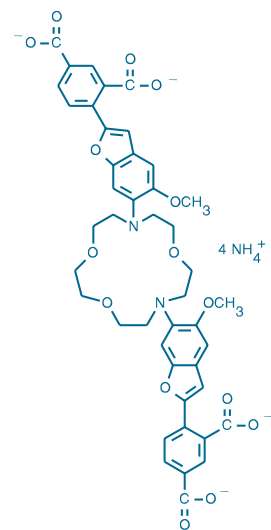


Figure 22.1 S-1262 SBFI, tetraammonium salt.

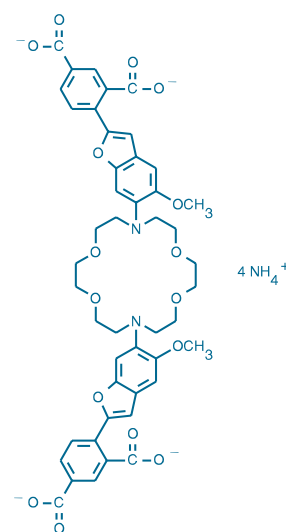


Figure 22.2 P-1265 PBF1, tetraammonium salt.

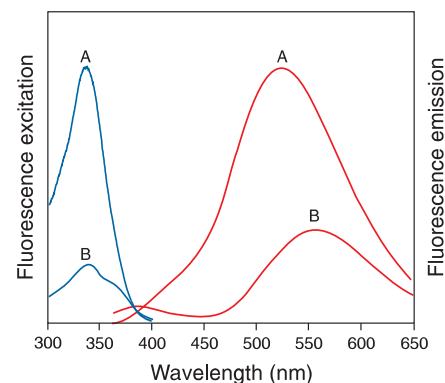
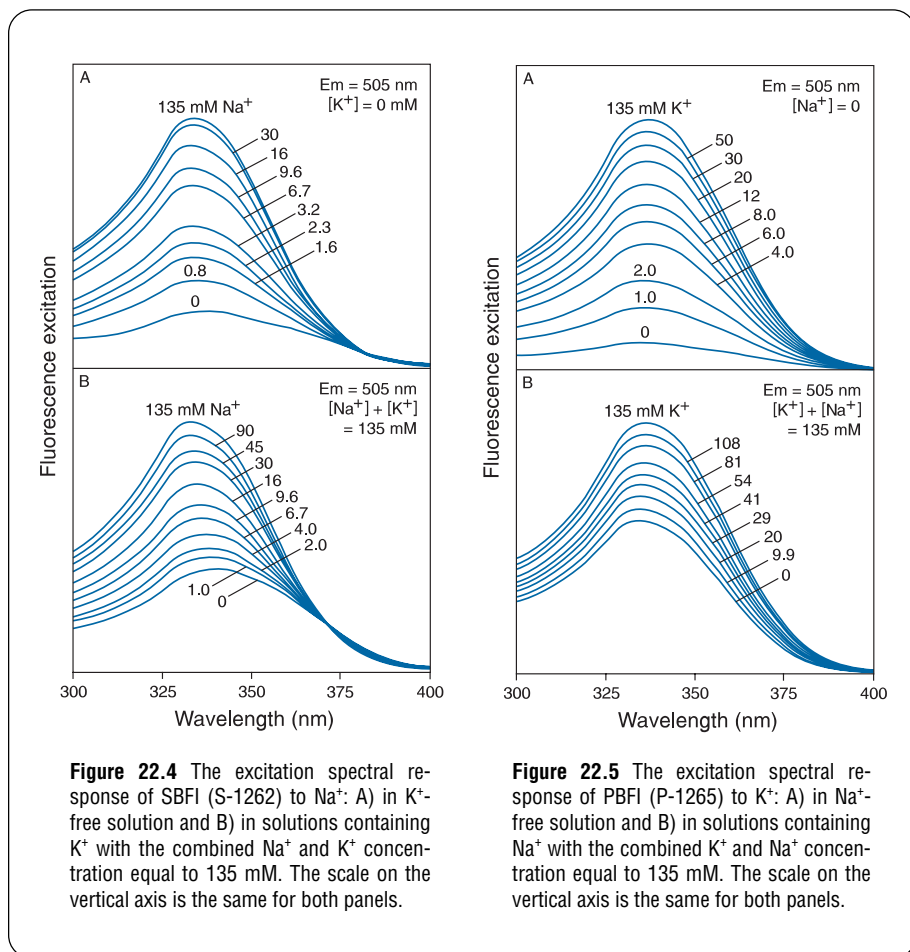


Figure 22.3 Fluorescence excitation (detected at 505 nm) and emission (excited at 340 nm) spectra of SBFI (S-1262) in pH 7.0 buffer containing 135 mM (A) or zero (B) Na⁺.

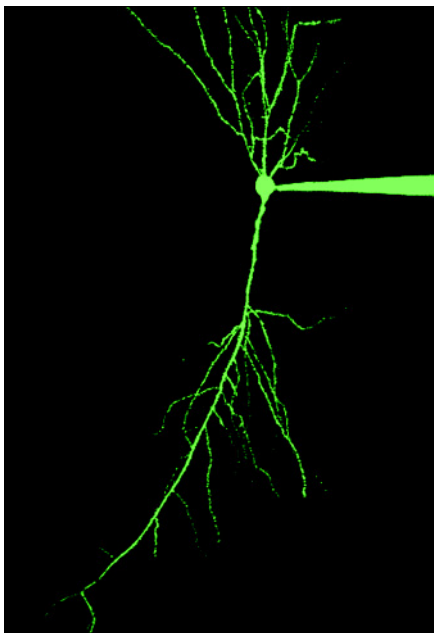


Figure 22.6 CA1 pyramidal neuron in a hippocampal slice filled with SBFI (S-1262) delivered from a patch pipette (visible on the right). The image was obtained using two-photon excitation of SBFI at 790 nm. Image contributed by Christine R. Rose, Physiological Institute, University of Munich.

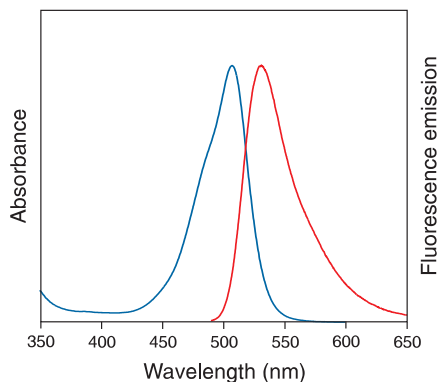


Figure 22.7 Absorption and fluorescence emission spectra of Sodium Green (S-6900) in pH 7.0 buffer containing 135 mM Na^+ .

There are only three fluorescent sodium indicators currently available: SBFI, which has dual excitation properties; Sodium Green, which has a large non-ratioable fluorescence enhancement upon binding Na^+ ; and the new CoroNa Red indicator, which localizes in the mitochondria and may be useful for measuring mitochondrial Na^+ flux.

Although SBFI is quite selective for the Na^+ ion, K^+ has some effect on the native affinity of SBFI for Na^+ (Figure 22.4). The dissociation constant (K_d) of SBFI for Na^+ is 3.8 mM in the absence of K^+ , and 11.3 mM in solutions with a combined Na^+ and K^+ concentration of 135 mM, which approximates physiological ionic strength. SBFI is ~18-fold more selective for Na^+ than for K^+ . Likewise, the K_d of PBFI for K^+ is strongly dependent on whether Na^+ is present (Figure 22.5), with a value of 5.1 mM in the absence of Na^+ and 44 mM in solutions with a combined Na^+ and K^+ concentration of 135 mM. In buffers in which the Na^+ is replaced by tetramethylammonium chloride, the K_d of PBFI for K^+ is 11 mM; choline chloride and *N*-methylglucamine are two other possible replacements for Na^+ in the medium. Although PBFI is only 1.5-fold more selective for K^+ than for Na^+ , this selectivity is often sufficient because intracellular K^+ concentrations are normally about 10 times higher than Na^+ concentrations.

The K_d of all ion indicators depends on factors such as pH, temperature, ionic strength, concentrations of other ions and dye–protein interactions. Due to these environmental factors, the K_d determined *in situ* for intracellular SBFI is substantially higher than that determined in cell-free buffer solutions. K_d (Na^+) values of 29 mM, 26.6 mM and 18.0 mM have been determined for SBFI in lizard peripheral axons, porcine adrenal chromaffin cells and rat hippocampal neurons, respectively.^{6,7,13} Consequently, intracellular SBFI should be calibrated using the pore-forming antibiotic gramicidin⁴ (G-6888). Palytoxin, an ionophoric toxin isolated from marine coelenterates, is much more effective than gramicidin for equilibrating intracellular and extracellular Na^+ .¹³ Intracellular PBFI should be calibrated using the K^+ ionophore valinomycin¹⁴ (V-1644).

Cell Loading with SBFI and PBFI

SBFI and PBFI are available both as cell-impermeant acid salts (S-1262, P-1265) and as cell-permeant acetoxymethyl (AM) esters (S-1263, S-1264, P-1267). The anionic acid forms can be loaded into cells using our Influx pinocytotic cell-loading reagent (I-14402, Section 20.8), or by microinjection, patch-pipette infusion or electroporation. For AM ester loading (see Loading and Calibration of Intracellular Ion Indicators in Section 20.1), addition of the dispersing agent Pluronic F-127 and relatively long incubation times — up to four hours — are typically necessary.⁴ Pluronic F-127 is available as a 20% solution in dimethylsulfoxide (DMSO, P-3000), as a 0.2 μm -filtered 10% solution in water (P-6866) and as a solid (P-6867). ATP-induced permeabilization reportedly produces increased uptake of SBFI AM by bovine pulmonary arterial endothelial cells (BPAEC).¹⁵ Somewhat higher working concentrations of PBFI and SBFI than those used for fura-2 may be required because of the lower fluorescence quantum yields of these indicators. AM ester loading sometimes produces intracellular compartmentalization of SBFI.^{9,16} As with other AM esters, reducing the incubation temperature below 37°C may inhibit compartmentalization. Other practical aspects of loading and calibrating SBFI have been reviewed by Negulescu and Machen.⁴

Applications of SBFI

SBFI has been employed to estimate Na^+ gradients in isolated mitochondria,^{16–18} as well as to measure intracellular Na^+ levels or Na^+ efflux in cells from a variety of tissues:

- Blood — platelets,^{19,20} monocytes²¹ and lymphocytes²²
- Brain — astrocytes,^{23,24} neurons,^{6,7,25–29} and presynaptic terminals^{30,31}
- Muscle — perfused heart,^{32,33} cardiomyocytes^{34–37} and smooth muscle^{38,39}
- Secretory epithelia^{40–44}

SBFI has also been used in combination with other fluorescent indicators to correlate changes in intracellular Na^+ with Ca^{2+} and Mg^{2+} concentrations,^{30,45–48} intracellular pH^{49,50} and membrane potential.⁵¹

Applications of PBFI

PBFI² has fewer documented applications than SBFI. Renewed interest has been prompted by the observation that intracellular K^+ levels appear to be a controlling factor in apoptotic cell death pathways.⁵² Flow cytometric measurements using UV argon-ion laser excitation (351 nm and 364 nm) of PBFI indicate that K^+ efflux induces shrinkage of apoptotic cells and is a trigger for activation of caspases.^{53–56} Furthermore, PBFI pro-

vides a potential alternative to radiometric K^+ efflux assays using ^{86}Rb .¹⁴ Other applications of PBFI include:

- Investigating the relationship between cytoplasmic K^+ concentrations and NMDA excitotoxicity⁵⁷
- Evaluating the mediating effects of K^+ depletion on monocytic cell necrosis⁵⁸
- Detecting adrenoceptor-stimulated decreases of intracellular K^+ concentration in astrocytes and neurons⁵⁹
- Monitoring mitochondrial K_{ATP} channel activation^{60,61}
- Assessing the effects of mitochondrial K^+ on Ca^{2+} -dependent cytotoxicity in rat hepatocytes⁶²
- Measuring K^+ levels in plant cells and vacuoles^{63–65}
- Detecting elevated intracellular K^+ levels associated with HIV-induced cytopathology⁶⁶
- Following ionophore-induced K^+ transport and associated volume changes in PBFI-loaded liposomes^{67,68}
- Quantitating light-induced K^+ and H^+ fluxes in liposomes^{69,70}
- Using patch-clamp techniques to measure K^+ in isolated cochlear outer hair cells⁷¹ and in mammalian ventricles⁷²

Sodium Green Na^+ Indicator

Molecular Probes' patented Sodium Green indicator⁷³ can be excited at 488 nm (Figure 22.7), providing a valuable alternative to the UV light–excitable SBFI for use with confocal laser-scanning microscopes⁷⁴ and flow cytometers.⁷⁵ We offer the cell-impermeant tetra(tetramethylammonium) salt of the Sodium Green indicator (S-6900), as well as its cell-permeant tetraacetate (S-6901). Sodium Green comprises two 2',7'-dichlorofluorescein dyes linked to the nitrogen atoms of a crown ether (Figure 22.8) with a cavity size that confers selectivity for the Na^+ ion. Upon binding Na^+ , Sodium Green exhibits an increase in fluorescence emission intensity with little shift in wavelength (Figure 22.9). Although Sodium Green lacks the direct radiometric readout capability of SBFI, fluorescence intensity fluctuations due to cell size variability can be compensated to some extent by using forward light scatter as a reference signal in flow cytometry.⁷⁵ Ratiometric detection using a co-loaded Na^+ -insensitive reference should be feasible based on analogous Ca^{2+} detection techniques^{76–78} (Section 20.3) but apparently has yet to be demonstrated in practice.

As compared to SBFI, Sodium Green shows greater selectivity for Na^+ than K^+ (~41-fold versus ~18-fold) and displays a much higher fluorescence quantum yield (0.2 versus 0.08) in Na^+ -containing solutions. The longer-wavelength absorption of the Sodium Green indicator results in reduction of the potential for photodamage to the cell because the energy of the excitation light is lower than that of the UV light required for excitation of SBFI. The K_d of the Sodium Green indicator for Na^+ is about 6 mM in K^+ -free solution and about 21 mM in solutions with combined Na^+ and K^+ concentration of 135 mM, approximating physiological ionic strength. Because its K_d may be shifted as a result of intracellular interactions, Sodium Green should be calibrated *in situ* using the pore-forming antibiotic gramicidin⁷⁵ (G-6888). In some cases, dye–protein interactions may cause severe dampening or even complete elimination of the Na^+ -dependent fluorescence response of intracellular Sodium Green. Nevertheless, flow cytometric measurements in Chinese hamster ovary (CHO) are well correlated with spectrofluorometric measurements using SBFI.⁷⁵ Other applications include:

- Assessing the regulation of Na^+ , K^+ -ATPase by persistent Na^+ accumulation in rat thalamic neurons⁷⁹
- Detecting anoxia-induced Na^+ influx in neurons⁸⁰
- Fluorescence lifetime imaging of intracellular Na^+ ^{81–83}
- Investigating the effects of amiloride on transport of Na^+ in submandibular salivary ducts without interference from UV light–excited amiloride fluorescence⁸⁴
- Measuring the effect of hyperthermia on Na^+ concentration in Chinese hamster ovary and HeLa cells^{85,86}
- Confocal imaging of Na^+ transport in rat colonic mucosa⁸⁷ and cochlear hair cells by flow cytometry⁸⁸
- Measuring Na^+ flux in colonic crypts⁸⁷

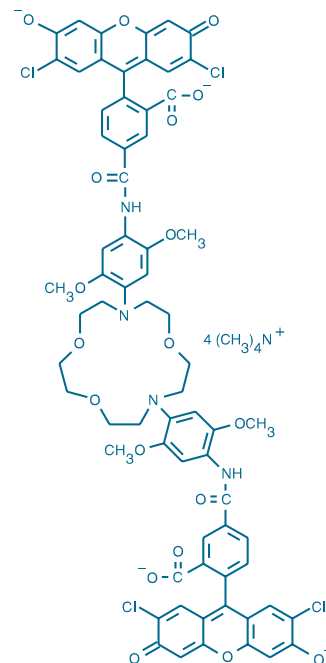


Figure 22.8 S-6900 Sodium Green, tetra(tetramethylammonium) salt.

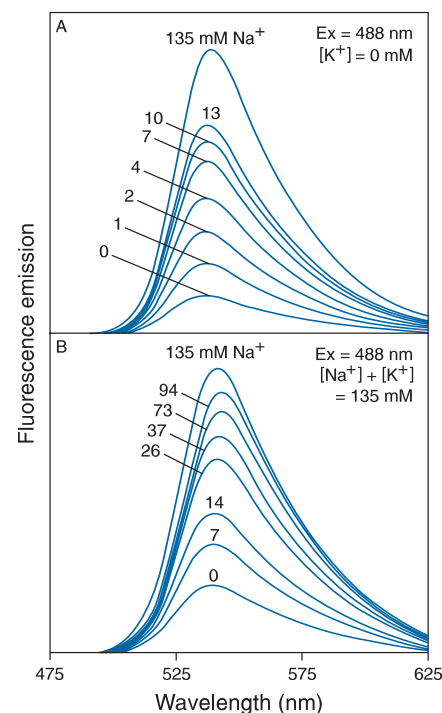


Figure 22.9 Emission spectral response of the Sodium Green indicator (S-6900) to Na^+ : A) in K^+ -free solution and B) in solutions containing K^+ with the combined Na^+ and K^+ concentration equal to 135 mM. The scale on the vertical axis is the same for both panels.

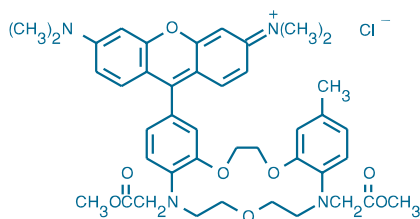


Figure 22.10 C-24430 CoroNa Red chloride.

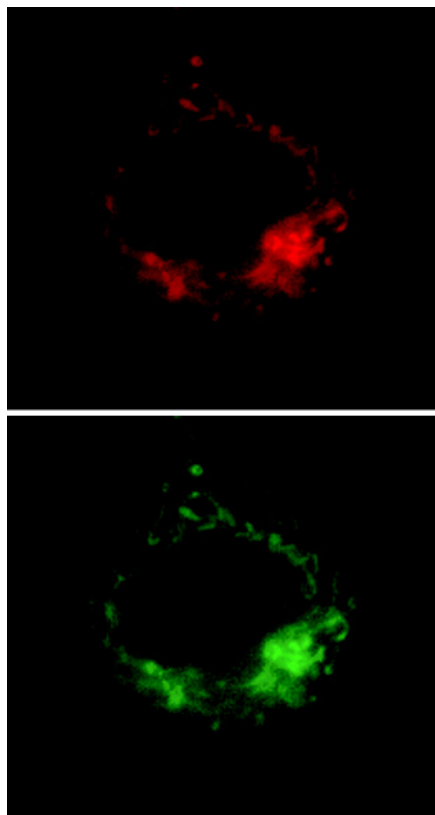


Figure 22.11 Images of an NIH 3T3 cell showing colocalization of the CoroNa Red sodium indicator (top panel; C-24430, C-24431) with the MitoTracker Green FM mitochondrial marker (bottom panel, M-7514). A cell loaded with both dyes was imaged consecutively using Omega Optical bandpass filter set XF41 for CoroNa Red sodium indicator and set XF23 for MitoTracker Green FM.

The Corona Red sodium indicator has an exceptionally large fluorescence response to Na⁺, particularly at high levels of Na⁺.

CoroNa Red Na⁺ Indicator

Our newest Na⁺ indicator is CoroNa Red chloride^{89,90} (C-24430, C-24431), which is based on a crown ether that has structural similarity to the Ca²⁺ chelator BAPTA (Figure 22.10). Unlike SBF1 and Sodium Green, the net positive charge of CoroNa Red targets the indicator to mitochondria (Figure 22.11) and loading of cells does not require use of a permeant ester derivative of the dye. Cells are typically loaded by adding 0.5–1.0 μM CoroNa Red from a 1 mM stock solution in DMSO, incubating for 10–30 minutes at 37°C and finally washing with dye-free medium before commencing fluorescence analysis. CoroNa Red is only weakly fluorescent in the absence of Na⁺ and its fluorescence increases ~15-fold upon binding Na⁺ (Figure 22.12). Despite its relatively high K_d for Na⁺ of ~200 mM, CoroNa Red indicator exhibits sensitive responses to cellular Na⁺ influxes through voltage-gated channels and ATP-gated cation pores. Verkman and co-workers have immobilized CoroNa Red indicator on polystyrene microspheres and used the complex to measure Na⁺ concentrations around 100 mM in the tracheal airway–surface liquid (ASL) of cultured epithelial cells and human lung tissues.^{89,90} The CoroNa Red indicator is available as a single 1 mg vial (C-24430) or as a set of 20 vials, each containing 50 μg of the indicator (C-24431).

Alternative Fluorescence Techniques for Measuring Na⁺ and K⁺

Researchers have discovered a variety of other fluorescence techniques for measuring Na⁺ and K⁺:

- Ion channel-mediated K⁺ fluxes are frequently detected via the accompanying membrane potential changes registered by voltage-sensitive dyes such as DiBAC₄(3)^{91–93} (B-438; FluoroPure Grade, B-24570; Section 23.3).
- Quenching of polyanionic fluorophores such as ANTS (A-350, Section 14.3) by the K⁺ analogs thallium (Tl⁺) or cesium (Cs⁺) is often exploited for monitoring K⁺ fluxes in reconstituted-vesicle preparations.^{94–96}
- The fluorescent pH indicator BCECF AM (B-1150, B-1170, B-3051; Section 21.2) is useful for flow cytometric measurements of intracellular K⁺ by exploiting the K⁺/H⁺ clamping action of the ionophore nigericin⁹⁷ (N-1495, Section 22.2).
- Various amphiphilic fluorophores have been used in combination with ion-selective carriers to construct membrane- or polymer-based sensors for Na⁺ or K⁺.^{98–102}
- A unique fiber-optical sensor for K⁺ that is sensitive over the range of 1 μM to 1 mM employs our red-fluorescent FluoSpheres microspheres (Section 6.5) in combination with a pH-sensitive dye.¹⁰³

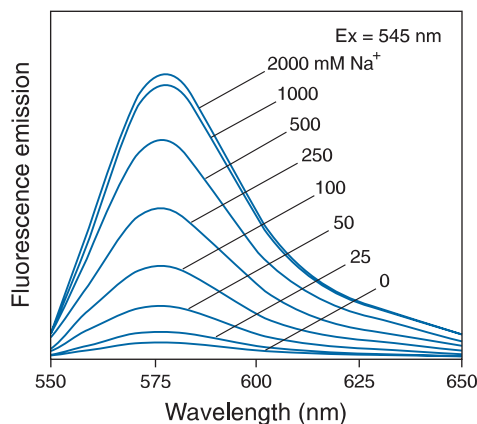


Figure 22.12 Fluorescence emission spectra of CoroNa Red (C-24430, C-24431) in 50 mM MOPS (pH 7.0, adjusted with tetramethylammonium hydroxide) containing 100 mM K⁺ and variable concentrations of Na⁺, as indicated.

References

1. *J Biol Chem* 264, 19449 (1989); 2. *Biophys J* 68, 2469 (1995); 3. SBFI and PBFI are licensed to Molecular Probes under US 5,134,232; 4. *Methods Enzymol* 192, 38 (1990); 5. *Proc Natl Acad Sci U S A* 94, 7053 (1997); 6. *Am J Physiol Cell Physiol* 280, C1623 (2001); 7. *J Physiol* 498, 295 (1997); 8. *Cell Regul* 1, 259 (1990); 9. *J Biol Chem* 264, 19458 (1989); 10. *J Mol Cell Cardiol* 29, 3375 (1997); 11. *J Neurosci* 21, 4207 (2001); 12. *Pflügers Arch* 439, 201 (1999); 13. *J Neurosci Methods* 75, 21 (1997); 14. *J Biol Chem* 265, 10522 (1990); 15. *J Appl Physiol* 81, 509 (1996); 16. *J Physiol* 448, 493 (1992); 17. *J Biol Chem* 270, 672 (1995); 18. *Am J Physiol* 262, C1047 (1992); 19. *Biochim Biophys Acta* 1220, 248 (1994); 20. *J Endocrinol* 138, 565 (1993); 21. *J Biol Chem* 272, 4753 (1997); 22. *Biochim Biophys Acta* 1137, 143 (1992); 23. *J Neurochem* 68, 1451 (1997); 24. *J Neurosci* 14, 2464 (1994); 25. *Eur J Neurosci* 12, 2199 (2000); 26. *J Physiol* 517, 135 (1999); 27. *Eur J Neurosci* 10, 3572 (1998); 28. *J Neurochem* 71, 112 (1998); 29. *J Neurophysiol* 78, 1188 (1997); 30. *Biophys J* 73, 2476 (1997); 31. *J Neurochem* 70, 1513 (1998); 32. *Am J Physiol Heart Circ Physiol* 280, H280 (2001); 33. *Am J Physiol* 273, H1246 (1997); 34. *Am J Physiol Heart Circ Physiol* 279, H1661 (2000); 35. *Am J Physiol Heart Circ Physiol* 279, H2143 (2000); 36. *J Mol Cell Cardiol* 29, 2653 (1997); 37. *Am J Physiol* 270, H2149 (1996); 38. *Biophys J* 73, 3371 (1997); 39. *Proc Natl Acad Sci U S A* 90, 8058 (1993); 40. *Am J Physiol Cell Physiol* 279, C1648 (2000); 41. *Am J Physiol Gastrointest Liver Physiol* 278, G400 (2000); 42. *J Biol Chem* 273, 32602 (1998); 43. *J Biol Chem* 272, 287 (1997); 44. *Am J Physiol* 267, G601 (1994); 45. *J Biol Chem* 276, 13657 (2001); 46. *J Physiol* 509, 103 (1998); 47. *Neuron* 17, 969 (1996); 48. *Am J Physiol* 266, H568 (1994); 49. *J Biol Chem* 272, 20179 (1997); 50. *Am J Physiol* 262, C348 (1992); 51. *Neuroscience* 68, 1051 (1995); 52. *Curr Opin Cell Biol* 13, 405 (2001); 53. *J Biol Chem* 275, 19609 (2000); 54. *J Biol Chem* 274, 21953 (1999); 55. *J Biol Chem* 272, 30567 (1997); 57. *Mol Pharmacol* 56, 619 (1999); 58. *Am J Physiol* 276, C717 (1999); 59. *Neurosci Lett* 238, 33 (1997); 60. *J Biol Chem* 273, 13578 (1998); 61. *J Biol Chem* 271, 8796 (1996); 62. *Biochem J* 299, 539 (1994); 63. *J Exp Botany* 48, 1609 (1997); 64. *J Biol Chem* 270, 4368 (1995); 65. *Planta* 195, 525 (1995); 66. *J Virol* 70, 5447 (1996); 67. *Biochim Biophys Acta* 1146, 87 (1993); 68. *Biophys J* 71, 3242 (1996); 69. *J Biol Chem* 271, 6199 (1996); 70. *J Biol Chem* 271, 2615 (1996); 71. *Brain Res* 636, 153 (1994); 72. *Circ Res* 74, 829 (1994); 73. *US* 5,405,975 and *US* 5,516,864; 74. *Methods Enzymol* 307, 119 (1999); 75. *Cytometry* 21, 248 (1995); 76. *Cytometry* 11, 923 (1990); 77. *Cell Calcium* 24, 71 (1998); 78. *J Gen Physiol* 105, 95 (1995); 79. *J Physiol* 525, 343 (2000); 80. *Brain Res* 663, 329 (1994); 81. *Anal Biochem* 281, 159 (2000); 82. *Anal Biochem* 250, 131 (1997); 83. *Methods Enzymol* 240, 723 (1994); 84. *J Biol Chem* 270, 19606 (1995); 85. *Radiat Res* 146, 283 (1996); 86. *J Membr Biol* 152, 217 (1996); 87. *J Physiol* 514, 211 (1999); 88. *Hear Res* 119, 1 (1998); 89. *Proc Natl Acad Sci U S A* 98, 8119 (2001); 90. *J Clin Invest* 107, 317 (2001); 91. *J Exp Med* 191, 1167 (2000); 92. *Br J Pharmacol* 129, 1323 (2000); 93. *J Physiol* 517, 781 (1999); 94. *Proc Natl Acad Sci U S A* 77, 4509 (1980); 95. *Methods Enzymol* 207, 501 (1992); 96. *J Biol Chem* 273, 27620 (1998); 97. *Cytometry* 28, 42 (1997); 98. *Anal Chim Acta* 334, 125 (1996); 99. *Biophys Chem* 33, 295 (1989); 100. *Analyst* 113, 693 (1988); 101. *Anal Chim Acta* 251, 184 (1986); 102. *Analyst* 115, 353 (1990); 103. *Anal Chem* 65, 123 (1993).

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).

Data Table — 22.1 Fluorescent Na⁺ and K⁺ Indicators

| Cat # | MW | Storage | Soluble | Low Ion* | | | | High Ion* | | | | Product | K _d | Notes |
|---------|---------|---------|---------|----------|---------|-----------|------------------|-----------|---------|-----|----------------------------------|---------|----------------|------------|
| | | | | Abs | EC | Em | Solvent | Abs | EC | Em | Solvent | | | |
| C-24430 | 773.32 | L | DMSO | 547 | 92,000 | 570 | H ₂ O | 551 | 92,000 | 576 | H ₂ O/Na ⁺ | | 200 mM | 1, 2, 3, 4 |
| C-24431 | 773.32 | L | DMSO | 547 | 92,000 | 570 | H ₂ O | 551 | 92,000 | 576 | H ₂ O/Na ⁺ | | 200 mM | 1, 2, 3, 4 |
| G-6888 | ~1880 | D | MeOH | <300 | | none | | | | | | | | |
| P-1265 | 950.99 | L | MeOH | 336 | 33,000 | 557 | H ₂ O | 338 | 41,000 | 507 | H ₂ O/K ⁺ | | 5.1 mM | 1, 5, 6 |
| P-1267 | 1171.13 | F,D,L | DMSO | 369 | 37,000 | see Notes | MeOH | | | | | P-1265 | | 7 |
| S-1262 | 906.94 | L | pH >8 | 339 | 45,000 | 565 | H ₂ O | 333 | 52,000 | 539 | H ₂ O/Na ⁺ | | 3.8 mM | 1, 5, 8 |
| S-1263 | 1127.07 | F,D,L | DMSO | 379 | 32,000 | see Notes | MeOH | | | | | S-1262 | | 7 |
| S-1264 | 1127.07 | F,D,L | DMSO | 379 | 32,000 | see Notes | MeOH | | | | | S-1262 | | 7 |
| S-6900 | 1667.57 | L | pH >6 | 506 | 117,000 | 532 | H ₂ O | 507 | 133,000 | 532 | H ₂ O/Na ⁺ | | 6.0 mM | 1, 2, 5, 8 |
| S-6901 | 1543.17 | F,DD,L | DMSO | 302 | 21,000 | none | MeOH | | | | | S-6900 | | |
| V-1644 | 1111.33 | F,L | EtOH | <300 | | none | MeCN | | | | | | | |

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

* For "Low Ion" spectra, the concentration of Na⁺ or K⁺ is zero. For "High Ion" spectra, the concentration of Na⁺ or K⁺ is in excess of that required to saturate the response of the indicator.

Notes

- Dissociation constant values vary considerably depending on presence of other ions, temperature, pH, ionic strength, viscosity, protein binding and other factors. It is essential that the spectral response of the probe be calibrated in your system.
- This indicator exhibits fluorescence enhancement in response to ion binding, with essentially no change in absorption or emission wavelengths.
- K_d determined at Molecular Probes in 50 mM MOPS, pH 7.0 (adjusted with tetramethylammonium hydroxide) containing 40% DMSO at 22°C.
- Spectra measured in aqueous buffers containing 40% DMSO and zero (H₂O) or 1 M Na⁺ (H₂O/Na⁺).
- Spectra measured in aqueous buffers containing zero (H₂O) or a >10-fold excess of free cation X (H₂O/X) relative to the dissociation constant (K_d) for cation X listed.
- K_d(K⁺) has been determined at Molecular Probes in 10 mM MOPS, pH 7.0 (adjusted with tetramethylammonium hydroxide) at 22°C. K_d(K⁺) is strongly dependent on the concentration of Na⁺. In solutions with [Na⁺] + [K⁺] = 135 mM, K_d(K⁺) = 44 mM.
- Fluorescence of SBFI, AM and PBFI, AM is very weak.
- K_d(Na⁺) has been determined at Molecular Probes in 10 mM MOPS, pH 7.0 (adjusted with tetramethylammonium hydroxide) at 22°C. Na⁺ dissociation constants for these indicators are dependent on K⁺ concentration. In solutions with total [Na⁺] + [K⁺] = 135 mM, K_d(Na⁺) = 11.3 mM (S-1262) and 21 mM (S-6900).

Product List — 22.1 Fluorescent Na⁺ and K⁺ Indicators

| Cat # | Product Name | Unit Size |
|---------|--|------------|
| C-24430 | CoroNa™ Red chloride | 1 mg |
| C-24431 | CoroNa™ Red chloride *special packaging* | 20 x 50 µg |
| G-6888 | gramicidin | 100 mg |
| P-1267 | PBFI, AM *cell permeant* *special packaging* | 20 x 50 µg |
| P-1265 | PBFI, tetraammonium salt *cell impermeant* | 1 mg |
| P-6867 | Pluronic® F-127 *low UV absorbance* | 2 g |
| P-3000 | Pluronic® F-127 *20% solution in DMSO* | 1 mL |
| P-6866 | Pluronic® F-127 *10% solution in water* *0.2 µm filtered* | 30 mL |
| S-1263 | SBFI, AM *cell permeant* | 1 mg |
| S-1264 | SBFI, AM *cell permeant* *special packaging* | 20 x 50 µg |
| S-1262 | SBFI, tetraammonium salt *cell impermeant* | 1 mg |
| S-6901 | Sodium Green™ tetraacetate *cell permeant* *special packaging* | 20 x 50 µg |
| S-6900 | Sodium Green™, tetra(tetramethylammonium) salt *cell impermeant* | 1 mg |
| V-1644 | valinomycin | 25 mg |

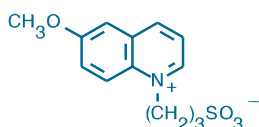


Figure 22.13 M-440 6-methoxy-*N*-(3-sulfopropyl)quinolinium, inner salt (SPQ).

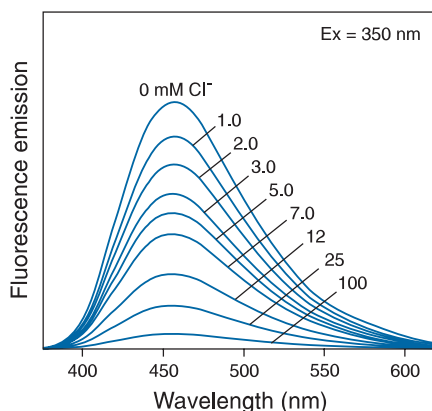


Figure 22.14 Fluorescence emission spectra of MQAE (E-3101) in increasing concentrations of Cl⁻.

Chloride indicators have all depended on fluorescence quenching by diffusion of the Cl⁻ to the indicator during its excited-state lifetime. Consequently, Cl⁻ measurements are susceptible to viscosity effects that are difficult to control. MQAE (E-3101) seems to be the best Cl⁻ indicator in common use.

22.2 Detection of Chloride, Phosphate, Nitrite and Other Anions

This section describes fluorescent indicators for intracellular and extracellular chloride together with an assortment of analytical methods for direct or indirect quantitation of other inorganic anions, including bromide, iodide, hypochlorite, cyanide, sulfide, sulfite, nitrite, nitrate, phosphate, pyrophosphate and selenide.¹

Fluorescent Cl⁻ Indicators

Most of Molecular Probes' fluorescent chloride indicators are 6-methoxyquinolinium derivatives, the prototype of which is 6-methoxy-*N*-(3-sulfopropyl)quinolinium^{2,3} (SPQ, Figure 22.13). Cl⁻ detection sensitivity has been improved by modifications of the quinolinium *N* substituent.^{4,5} Our current range of Cl⁻ indicators consists of:

- 6-Methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ, M-440)
- *N*-(Ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE, E-3101)
- 6-Methoxy-*N*-ethylquinolinium iodide (MEQ, M-6886)
- Lucigenin (L-6868)

All of these indicators detect Cl⁻ via diffusion-limited collisional quenching.⁶ This detection mechanism is different than that of fluorescent indicators for Ca²⁺, Mg²⁺, Zn²⁺, Na⁺ and K⁺. It involves a transient interaction between the excited state of the fluorophore and a halide ion — no ground-state complex is formed. Quenching is not accompanied by spectral shifts (Figure 22.14) and, consequently, ratio measurements are not directly feasible. Quenching by other halides, such as Br⁻ and I⁻, and other anions, such as thiocyanate, is more efficient than Cl⁻ quenching.⁶ Fortunately, physiological concentrations of non-Cl⁻ ions do not significantly affect the fluorescence of SPQ and other methoxyquinolinium-based Cl⁻ indicators. With some exceptions,⁷ fluorescence of these indicators is not pH sensitive in the physiological range.⁴ Because Cl⁻-dependent fluorescence quenching is a diffusional process, it is quite sensitive to solution viscosity and volume. Exploiting this property, SPQ has been used to measure intracellular volume changes.⁸

The efficiency of collisional quenching is characterized by the Stern–Volmer constant (K_{SV}) — the reciprocal of the ion concentration that produces 50% of maximum quenching. For SPQ, K_{SV} is reported to be 118 M⁻¹ in aqueous solution and 12 M⁻¹ inside cells.⁹ For MQAE, *in situ* K_{SV} values of 25–28 M⁻¹ have been determined in various cell types,^{10,11} compared to the solution value of 200 M⁻¹. Intracellular Cl⁻ indicators are