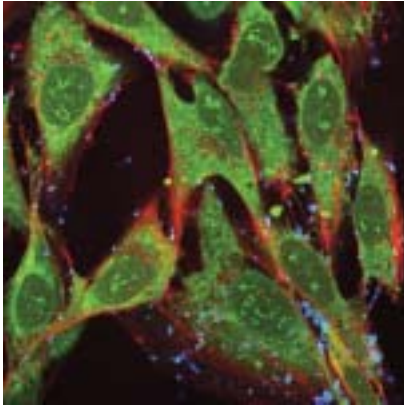


Cell Biology - Study/Probes

Membranes potential probes

The electric potential across biological membranes, from cells and organelles as mitochondria, drives many biological processes, including transports of biomolecules through membrane channels, ligands binding to membrane receptors and cell signaling vectors. Measurement of membrane potential is so required in various applications, and fluorescent probes have been shown to be superior tools for these studies, conjugated to imaging techniques, overcoming microelectrodes that have spatial and time response limitations. Besides our fluorescent probes for individual ions (K^+ , Na^+ and Cl^- concentration gradients contribute principally to transmembrane potential) that are described in sections E32-E65, several indicators change their fluorescence with electric potential :



Our two first groups of potential Indicators (with popular **ANEPPS** and **RH** dyes apply to important potential changes : hyperpolarization and depolarization occur in excitable cells like neurones, muscular cells, and during certain physiological responses. Styryl dyes are also interesting tools for endocytosis/exocytosis activity study.

The second group (page E94-E95, with leading dyes **DiOC6**, **TMRE** and **DiBAC**) elicits generally a much greater potential response, $\geq 10\%$ fluorescence change per 10mV electric variation, but with a slower response. They show distribution changes between compartments separated by the membrane.

See also JC-1 (FP-52314), a superior membrane potential probe for mitochondria.

The type of dye should be considered regarding response mechanism, toxicity, and method of use (ratiometric, potentiometric).

Potential probes for excitable cells and endo/exocytosis

Membranes hyperpolarization and depolarization occur in excitable cells like neurones (nerve impulse propagation), muscular cells (muscle contraction), and during certain physiological responses, induced by hormones or contact/adhesion (cell signaling, ion-channel gating).

Suitable fluorescent probes include styryl based dyes the most popular being ANEPPS. Styryl dyes are also interesting tools for endocytosis/exocytosis activity study.

Styryl indicators, (ANEP)

Styryl dyes are believed to partition between the aqueous phase, in a virtually non-fluorescent form, and the outer leaflet of synaptic membranes, in a highly fluorescent form. Resulting staining is dependent on endocytosis/exocytosis activity, fluorescence increasing during endocytosis ("on-rate") and declining during exocytosis ("off-rate"). ANEPS electronic structure depends on surrounding electric field, and consequently show an immediate fluorescence change, almost 2–10 % per 100 mV. This may also apply to mitochondria that show bigger potential (-150 mV) than plasmatic membrane (-70 mV).

ANEP indicators are essentially non-fluorescent in aqueous solutions and exhibit spectral properties that are strongly dependent on their environment. The main application is potential measurements, thanks to their fairly uniform 10 % change in fluorescence intensity per 100 mV potential variation, within milliseconds, that is documented in a variety of tissues, cells and membrane models systems. The emission maximum shifts, allowing to use a ratiometric method for accurate potential detection.

di-4-ANEPPS

$C_{28}H_{36}N_2O_3S$ MW : 480.67

Soluble in DMSO, EtOH

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$ (MetOH) : 496/705 nm ; EC : 39 000 $M^{-1}cm^{-1}$

A fast-responding membrane potential dye for short term experiments (as it is internalized in the cell rapidly for long term, so di-8-ANEPPS is preferred).

Description	Cat.#	Qty
di-4-ANEPPS	FP-56958A	5 mg

di-8-ANEPPS

$C_{36}H_{52}N_2O_3S$ MW : 592.89

Soluble in DMSO, EtOH

Store at 4°C

$\lambda_{exc.}/\lambda_{em.}$ (EtOH) : 498/713 nm ; EC : 35 000 $M^{-1}cm^{-1}$

A fast-responding membrane potential dye of choice: compared to di-4-ANEPPS it is better retained in the outer leaflet of the plasmatic membrane, and is reported to be slightly more photostable and significantly less phototoxic ⁽²⁾.

Reference: 1) Neurosci. 15, 1392(1995) 2) Neuron 9, 393(1992).

Description	Cat.#	Qty
di-8-ANEPPS	FP-17177A	5 mg

RH dyes/Dialkylaminophenyl polyenylpyridinium

Many derivatives of dialkylaminophenylpolyenylpyridinium exist, exhibiting various degrees of fluorescence excitation and emission spectral shifts in response to membrane potential changes, similarly to ANEP dyes. Their absorption and fluorescence spectra are also strongly dependent on the environment. They are principally used for functional imaging of neurons, but can likewise be used to detect activity-dependent synaptosomal recycling of live nerve terminals. RH421 yields the most sensitive response recorded for a fast potentiometric probe.

abs. em. RH 421

$C_{29}H_{42}N_2O_3S$ MW : 498.7

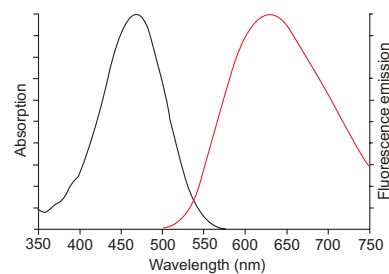
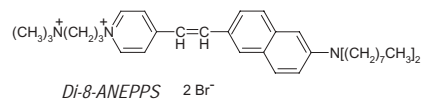
Soluble in DMSO, EtOH

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 515/704nm ; EC : 50 000 $M^{-1}cm^{-1}$

$\lambda_{exc.}/\lambda_{em.}$ (neuronal plasma mbr) : 493/638 nm

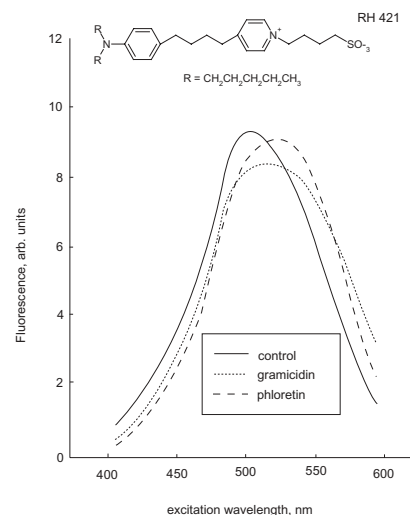
The most popular and useful dye of the RH dyes serie. Exhibits a >20% fluorescence change per 100 mV on neuroblastoma cells. Yields the most sensitive response recorded for a fast potentiometric probe. Also bind to RUBISCO enzyme (Frank 1997).

Description	Cat.#	Qty
RH 421	FP-46971A	25 mg

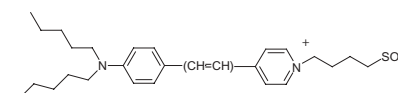


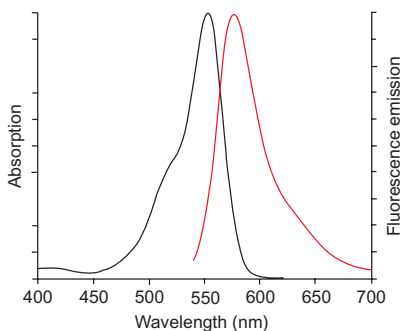
Absorption and fluorescence emission spectra of Di-8-ANEPPS bound to phospholipid bilayer membranes. Spectra of styryl dyes in membranes are blue shifted by ~ 20 nm for absorption and ~ 80 nm for emission.

Other useful styryl fluorescent probes are SynapTracer™ dyes, that are used mainly for nerve terminal synapses and neuro-muscular junctions studies.



Fluorescence excitation spectra of RH421 in the aqueous solution of 0.2 mg/ml DPPC vesicles (—) in presence of 5 μ M gramicidin A (.....) or 10 μ M phloretin (---). It shows that gramicidin A or phloretin incorporation in phospholipid monolayer decreases the surface potential.





Absorbance & emission spectra of TMR in pH 7 buffer.

Probes for high potential changes

TMRE, TMRM/Rhodamine derivatives

TMRE and TMRM, the methyl and ethyl esters of tetramethylrhodamine, are preferred as rapid membrane potential sensors for quantitative measurements using Nernst equation. They pass through the plasmatic membrane better than related rhodamine 123, and accumulate in mitochondria where fluorescence self quenches. The transmembrane distribution is directly related to the membrane potential, because they do not form aggregates in cell membranes or interact with membrane proteins. They are used to obtain unbiased images of potential-dependent dye distribution.

Highly selective, potential-dependent staining of mitochondria is obtained by setting the extracellular K^+ concentration close to intracellular values (~137 mM), thereby depolarizing the plasmatic membrane.

They are also used for mitochondria, and also cytotoxic assays.

abs em. TMRE

(Tetramethylrhodamine Ethyl Ester, perchlorate)

$C_{26}H_{27}ClN_2O_7$ MW : 514.96

Soluble in DMSO, EtOH

Store at $-20^\circ C$ and protect from light

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 549/573 nm ; EC : 109 000 $M^{-1}cm^{-1}$

Description	Cat.#	Qty
TMRE	FP-41391A	25 mg

abs em. TMRM

(Tetramethylrhodamine Methyl Ester, perchlorate)

$C_{25}H_{25}ClN_2O_7$ MW : 500.93

Soluble in DMSO, MeOH

Store at $-20^\circ C$ and protect from light

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 549/573 nm ; EC : 115 000 $M^{-1}cm^{-1}$

Description	Cat.#	Qty
TMRM	FP-21089A	25 mg

DiBAC/oxonol

DiBAC4(3) is an anionic oxonol (often cited as "bis-oxonol.") with relatively low potential-dependent fluorescence changes (i.e. ~1% per mV). It enters depolarized cells and binds to intracellular proteins or membranes and then exhibits enhanced fluorescence and red spectral shift. Hyperpolarization is then detected by a decrease in fluorescence. Its overall negative charge exclude it from mitochondria, making it superior to carbocyanines for measuring plasmatic membrane potentials by flow cytometry. DiBAC4(3) has been the membrane potential dye of choice for HTS both because it can be optimally excited by the Ar laser (488 nm) equipped in the FLIPR system, besides application in flow cytometry, and confocal microscopy.

abs em. DiBAC4(3)

(bis-(1,3-dibarbituric acid)-trimethine oxanol)

$C_{27}H_{40}N_4O_6$ MW : 516.64

Soluble in DMSO/EtOH

$\lambda_{exc.}/\lambda_{em.}$: 493/516 nm ; EC : 140 000 $M^{-1}cm^{-1}$

Fluorescence of DiBAC4(3) increases about 3-fold relative to H_2O on binding to proteins.

Description	Cat.#	Qty
DiBAC4(3)	FP-46600A	25 mg

abs em. DiSBAC2(3)

$C_{19}H_{24}N_4O_4S_2$ MW : 436.56

Soluble in DMSO/ECtOH

$\lambda_{exc.}/\lambda_{em.}$: 535/560 nm ; EC : 170 000 $M^{-1}cm^{-1}$

Description	Cat.#	Qty
DiSBAC2(3)	FP-46603A	100 mg

DiOC dyes/Carbocyanines

Carbocyanine derivatives with short alkyl tails (<7 carbon atoms) accumulate on hyperpolarized membranes, by translocation into the lipid bilayer and aggregation that decrease their fluorescence.

They were widely used for cell membrane potential measurements, including by flow cytometry, but some limitations make DiBAC often preferable, excepted for specific cell types (i.e. bacteria) or applications.

- ◆ Carbocyanine dyes with C1–C6 alkyl chains stain mitochondria of live cells when used at low concentrations (~0.5 μ M or ~0.1 μ g/mL).
- ◆ C5 and C6 carbocyanines also stain the endoplasmic reticulum when used at higher concentrations (~5–50 μ M or ~1–10 μ g/mL).

DiOC, Dil and DiS carbocyanines are however available on request.

abs em. DiOC₆(3)

C₂₉H₃₇IN₂O₂ MW : 572.53

See complete description page E131. The most widely used carbocyanine dye for membrane potential measurements, including plasmatic membranes, mitochondria, ECR.

Description	Cat.#	Qty
DiOC ₆ (3)	FP-46764A	100 mg

See also JC-1 (FP-52314), a superior membrane potential probe for mitochondria.