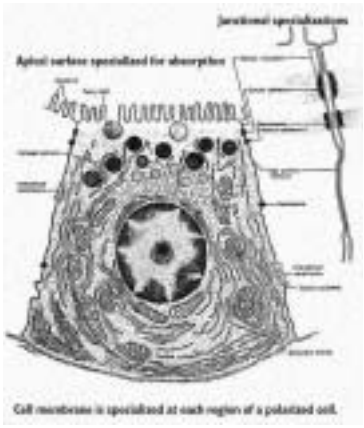
Amphi-philic Probes

Fluorescent dyes that are able to bind by polar/hydrophobic interactions allow labeling biological membranes for in vivo tracing of cells and liposomes in living organisms as well as for cell structure study and even cell function applications.

Technical tip - Membrane Constituants and Probes



Membranes en cells : localization and specializations.

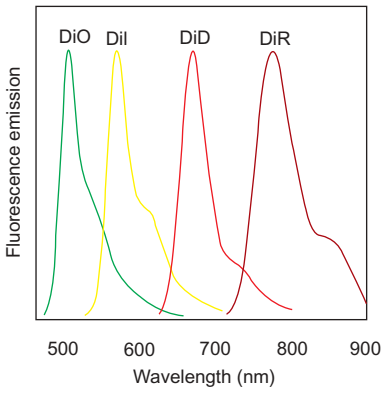
Biological membranes are described as a lipidic bilayer including proteins. Main lipids are phospholipids, sphingolipids (including ceramides), fatty acids, triglycerides and steroids. Main membrane proteins are cytochromes, channels. Biological membranes constitute the plasmatic membrane limiting the cell from outside environment, but also the membranes limiting several organelles inside cells, including the endoplasmic reticulum (and specialized forms of ER, as i.e. the sarcoplasm of muscular cells, the golgi apparatus, mitochondria, vacuoles, lysosomes, and nucleus).

Probes for membrane study include :

- Fluorescent derivatives of natural lipids ; i.e. main constituants for membrane visualization, and phosphoinosides for signal transduction ; see section "fluorescent lipids" page E75.
- Non lipidic fluorescent molecules with more or less lipophilic properties that anchor more or less deeply in membranes essentially through hydrophobic interactions (see page E70-E74).
- Some ion probes interact with membranes as Near membrane Ca2+ indicators (see Nomo i.e.).
- Fluorescein derivatives elicit hydrophobic (FAM) or ionic (Calceins) interactions than make them useful as membrane probes. See section "Cell Tracing" (page E97).
- CFSE and labeling agents (page E96, B41-B96) react chemically with membrane constituants to label cell membranes permanently.
- Molecular rotors are also used for cell membrane fluidity studies.
- Vital stains as Tryptan blue (T33190) are commonly used for dead cell staining (dye membrane exclusion test, or membrane integrity test). Please refer to page E148).
- SynapTracer™ dyes (page E138) are amphiphilic dyes used for living cell staining and vesicules study.
- Specific-ligand probes, including toxins that bind to membranes receptors (page E138) and membranes enzymes substrates (page E108).

The following sections E70-E85 include mainly fluorescent membrane probes used to study cell membranes, including structure, morphology and dynamics.

Reviews can also be found in the literature, as :
Maier O, et al ; Fluorescent lipid probes : some properties and applications (a review) ; Chem Phys Lipids 116, 3-18 (2002) PN47257.



Normalized fluorescence spectra in membranes

Dye	compatible filter set
DiIC(3) :	rhodamine (TRITC) filter
DiOC(3) :	fluorescein (FITC) filter

See also :
Membrane fluidity kit BP8331 1 kit
description page E200

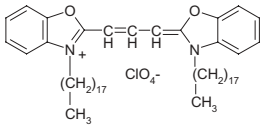
Dialkylcarbocyanine dyes

Carbocyanine dyes have hydrophilic/hydrophobic pattern (amphiphilicity), with strongest fluorescence when in membranes. This makes them suitable for many membrane studies (structure, morphology, dynamic, …), starting with the popular **DiI** (FP-46804) and **DiO** (FP-46805).
Several modifications are available, offering several color detections from green (DiO) to IR (DiR), and several lengths or saturation degree of hydrophobic tails.
Fluorescence, weak in water, is intense in lipidic environment thanks to high extinction coefficients, modest quantum yields, and short excited-state lifetimes (~1 nanosecond).
These dyes insert into the membrane, and diffuse rapidly staining the entire cell surface. They allow the synaptic terminals tracing in a single motor unit.

Notes :
Short chain carbocyanines, i.e $DiOC_6$ (available with our FluoCD™ technology) are mainly dedicated to potential measurement. (see page E131).
Longer alkyl tails (>C12, i.e. $DiIC_{18}(3)$) are best for detection of particularly rigid gel phase. They are used for neuronal tracing long-term labeling (see page E92) of cells in culture and non-covalent labeling of lipoproteins.

abs em. DiOC18(3) [DiO]

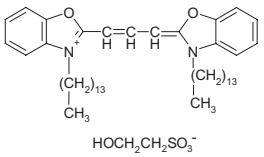
3,3´ -dioctadecyloxacarbocyanine, perchlorate
$C_{53}H_{85}ClN_2O_6$ MW : 881.73
Soluble in DMSO or DMF
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 484/501 nm ; EC : 152 000 $M^{-1}cm^{-1}$
One of the most classic dyes. Can be used with standard fluorescein and rhodamine optical filters.



Description	Cat.#	Qty
DiOC18(3) [DiO]	**FP-46805A**	50 mg

abs em. DiOC14(3)

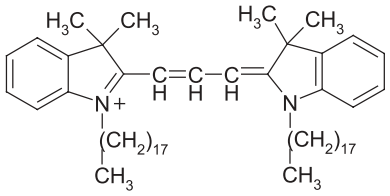
3,3´-ditetradecyloxacarbocyanine, hydroxyethanesulfonate
$C_{47}H_{74}N_2O_6S$ MW : 795.19
Soluble in methanol, ethanol or DMSO
Store at 4°C
DiO analog with shorter alkyl tails, but more soluble in aqueous media. Staining is accomplished by simple incubation of cells in the buffer containing the dye. Because the staining is usually nontoxic and very stable, the dye is useful for long term cells tracing.



Description	Cat.#	Qty
DiOC14(3)	**FP-AM329A**	50 mg

abs em. DiIC18(3) [DiI]

1,1' -dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate
$C_{59}H_{97}ClN_2O_4$ MW : 933.88
Do not freeze
Soluble in DMSO or ethanol
$\lambda_{exc.}/\lambda_{em.}$: 549/565 nm ; EC : 148 000 $M^{-1}cm^{-1}$
One of the most standard lipophilic dye for ER, Golgi studies. Can be used with standard fluorescein and rhodamine optical filters, and combined to DiO.
References : Afferent and efferent connections of the cerebellum of the chondrostean Acipenser baeri : a carbocyanine dye (DiI) tracing study. Huesa G, Anadon R, Yanez J. J Comp Neurol 460, 327-44 (2003). Honig, M.G. and Hume, R.I. Trends in Neurosci. 9, 333(1989) ; McConnell, S.K., et al. Science 245, 978(1989).



Description	Cat.#	Qty
DiIC18(3) [DiI]	**FP-46804A**	50 mg

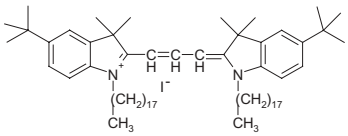
abs em. DiIC18(3) [DiI] solution, for microinjections

Characteristics of the dye : see DiI (FP-46804A)
A convenient formulation of DiI (FP46804A) in oil, with uniform dissolution.
Application : fate mapping and cell lineage studies(1) and for the determination of spatial organization and connectivity patterns of central nervous systems.
References : Ruiz, et al. in essential developmental biology : a practical approach Oxford : IRL Press at Oxford University Press, 81-95 (1993)2) International Journal for Parasitology 28, 363 (1998).

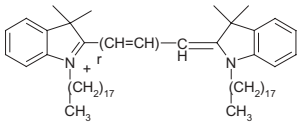
Description	Cat.#	Qty
DiIC18(3) [DiI] solution	**FP-AM328A**	0.5 ml

abs em. Neuro-DiI

$C_{67}H_{115}IN_2$ MW : 1075.58
Soluble in methanol, ethanol, DMF and DMSO.
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 550/565 nm ; EC : (MeOH) : 148 000 $M^{-1}cm^{-1}$
Diffuses faster than DiI in cells membranes and also may result in a more stable labeling.



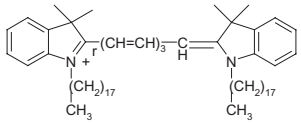
Description	Cat.#	Qty
Neuro-DiI	**FP-AM330A**	25 mg

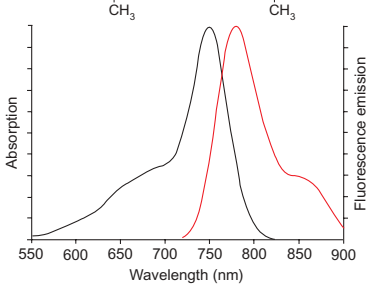


abs em. DiIC18(5) [DiD]

1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indodicarbocyanine, 4-chlorobenzenesulfonate salt
$C_{67}H_{103}ClN_2O_3S$ MW : 1052.1
Soluble in DMSO or ethanol
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 644/663 nm ; EC : 193 000 $M^{-1}cm^{-1}$
Similar to DiIC18(3), but excitable with longer wavelength than carbocyanines (He-Ne laser). It is useful when significant intrinsic fluorescence is observed with DiI or DiO.

Description	Cat.#	Qty
DiIC18(5) [DiD]	**FP-22574A**	50 mg



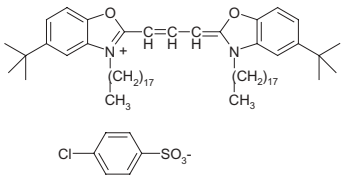


Absorption and fluorescence emission spectra of DiIC18(7) ("DiR") bound to phospholipid bilayer membranes.

abs em. DiIC18(7) [DiR]

1,1'-dioctadecyltetramethyl indotricarbocyanine Iodide
$C_{63}H_{101}IN_2$ MW : 1013.43
Soluble in DMSO or ethanol
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$ (MetOH) : 748/780 nm ; EC : 270 000 $M^{-1}cm^{-1}$
Lipophilic carbocyanine similar to DiI and DiO with near IR absorption and emission, allowing lowering the level of autofluorescence. Can be used in multicolor detection, combined to DiD (FP-22574A), DiI (FP-46804A) and Neuro-DiO (FP-AM330A).

Description	Cat.#	Qty
DiIC18(7) [DiR]	**FP-69084A**	25 mg



abs em. Neuro DiO

$C_{67}H_{105}ClN_2O_5S$ MW : 1086.11
Soluble in ethanol, hexane, DMSO and oil.
Store at -20°C and protect from light
$\lambda_{exc.}/\lambda_{em.}$: 484/501 nm
Similar features to DiO but with better solubility in membranes, less self quenching by aggregation, and higher diffusion rate in membranes.

Description	Cat.#	Qty
Neuro DiO	**FP-AM331A**	25 mg

abs em. NeuroDiO solution, for microinjections

Characteristics of the dye : see NeuroDiO (FP-AM331A)
Do not frezze.
A convenient formulation of NeuroDiO (FP-AM331A) in oil, with uniform dissolution.
Application : microinjection, combined to NeuroDiI solution (FP-AM330A).

Description	Cat.#	Qty
NeuroDiO solution	**FP-BA641A**	0.2 ml

SynapTracer™ dyes

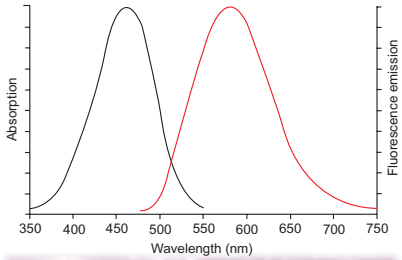
SynapTracer™ are cationic styryldyes, less lipophilic but still amphilic than DiO/D, I/DiR that became highly fluorescent once internalized in membranes. They are described in section "Neural Cell Study" as one important application is to follow synaptic activities. They are however very usefull as well for endocytosis of vesicules and vacuoles. The most popular SynapTracers™ are :

Description	Cat.#	Qty
Green SynpaTracer™ 1-4 (see page E139)	**FP-51254A**	2 mg
Red SynpaTracer™ 3-2 (see page E140)	**FP-41109A**	1 mg

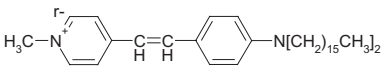
Tel 33 (0)4 70 03 88 55 ♦ Hot line 33 (0)4 70 03 73 06 ♦ Fax 33 (0)4 70 03 82 60

Dialkylaminostyryl

DialkylAminoStyryl dyes (Di-ASP) insert in membranes. They are slightly less hydrophobic than Synapt Tracer™ dyes.



Absorption and Emission spectra of DiA (FP-66096) in membranes.



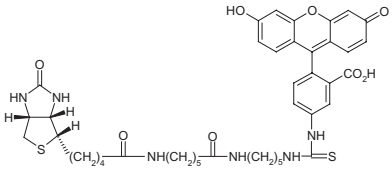
abs em. **4-Di-16-ASP [DiA]**
4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide
$C_{46}H_{79}IN_2$ MW : 787.06
Soluble in DMSO or ethanol
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 491 / 613 nm ; EC : 52 000 $M^{-1}cm^{-1}$
Commonly used for neuronal membrane tracing : diffuses faster than DiO.
Has a very broad emission spectrum (can be detected with green, orange or even red filters), combined notably DiIC18(3) for 2 colors staining.

Description	Cat.#	Qty
4-Di-16-ASP [DiA]	**FP-66096A**	25 mg

Other +/- polar dyes

Fluorescent biotins are polar probes that can also be used for probing membranes, and also to localize biotin-binding proteins.
Aminated (NH2) biotins can be fixed in cells with aldehyde, facilitating detection.

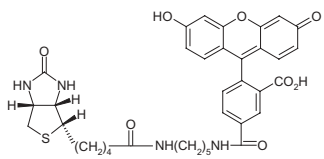
abs em. **Fluorescein-Biotin**
$C_{42}H_{50}N_6O_8S_2$ MW : 831.03
Soluble in DMF, pH > 6
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 494/518 nm ; EC : 75 000 $M^{-1}cm^{-1}$ (pH9)
(greatly reduced < pH7)
Used to detect and quantify biotin binding sites and the biotinylation degree of proteins, and also as a non-fixable polar probe in cell biology.



References : Biochim Biophys Acta 1381, 203 (1998) ;
Gruber HJ, et al ; Accurate titration of avidin and streptavidin with biotin-fluorophore conjugates in complex, colored biofluids ; Biochim Biophys Acta 1381, 203-212 (1998).
Buranda T et al ; Peptides, antibodies, and FRECT on beads in flow cytometry : A model system using fluoresceinated and biotinylated beta-endorphin ; Cytometry 37, 21-31 (1999).

Description	Cat.#	Qty
Fluorescein-Biotin	**FP-959145**	5 mg

abs em. **Biotin-4-fluorescein**
$C_{33}H_{32}N_4O_8S$ MW : 644.71
Soluble in DMF, DMSO, pH >7
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 494/523 nm
A single isomer of fluorescein biotin (FP-959145). Improves results for quantitating biotin binding sites (binds quicker to avidin).

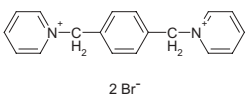


References :
Biochim Biophys Acta 1427, 44 (1999).
Kada G et al; Rapid estimation of avidin and streptavidin by fluorescence quenching or fluorescence polarization ; Biochim Biophys Acta 1427, 44-48 (1999).

Description	Cat.#	Qty
Biotin-4-fluorescein	**FP-M1769A**	10 mg

Other

Many other amphiphilic and +/- polar dyes can be useful, as described in sections 'Fluorescent lipids (E75), cell and organelles tracing (E86), mitochondria study (E129), and nerve study (E136).



abs ■ **DPX**

Pyridinium, 1,1'-(1,4-phenylenebis(methylene)bis-, dibromide
$C_{18}H_{18}N_2Br_2$ MW : 422.18
Soluble in Water
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 259/none ; EC : 8 800 $M^{-1}cm^{-1}$
A positively charged quencher that is often used as polar tracer with ANTS to study membrane fusion or permeability.

Description	Cat.#	Qty
DPX	**FP-47017A**	2 x 500 mg

abs em. **ANTS**

$C_{10}H_7NNa_2O_9S_3$ MW : 427.34
Store at 4°C
$\lambda_{exc.}/\lambda_{em.}$: 353/520 ; EC : 7 200 $M^{-1}cm^{-1}$
A yellow fluorescent and anionic dye. It is used as a polar tracer in conjunction with the quencher DPX to study membrane fusion or permeability Ti^+ ans Cs^+ also quench it.

Description	Cat.#	Qty
ANTS	**FP-46574A**	500 mg