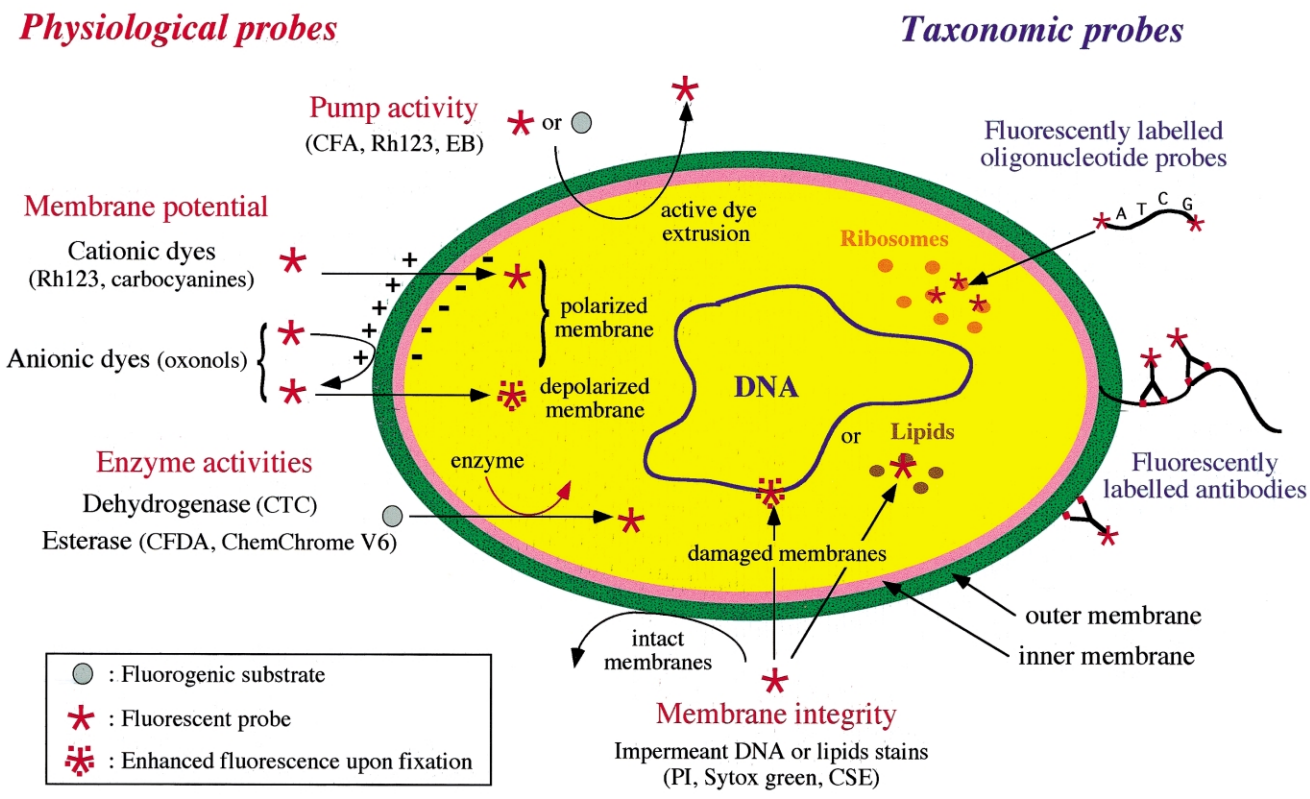

Figure 1. Different cellular target sites for physiological and taxonomic fluorescent dyes.

4. Physiological target sites

This section presents the different probes used to assess different physiological functions and cellular structures. The interpretation of these dyes in terms of cellular activity or viability is discussed in more details in sections 5 and 6. *Figure 1* summarises the different physiological target sites of these probes.

4.1. Membrane potential

The electrochemical potential occurring through the plasma membrane of metabolising bacteria is generated by respiration or by ATP hydrolysis. It results from the selective permeability of biological membranes to a variety of cations and anions leading to a difference of electric potential across the membrane. Inside, the cell is negatively charged compared with outside the cell, and membrane potential (MP) plays a central role in different cell-life processes (ATP synthesis, active transport, mobility, regulation of intracellular pH, etc.). Voltage-sensitive dyes have been developed to measure MP in bacteria. Depending on the charge of the dye, they are accumulated in polarised (cationic dyes) or depolarised (anionic dyes) cells. Reliability of staining is confirmed by observing if dye uptake is sensitive to uncouplers (e.g., carbonyl cyanide *m*-chlorophenyl hydrazone; CCCP) or ionophores (e.g., nigericin, valinomycin). In appropriate conditions, the amount of dye taken up can be directly related to the level of energy metabolism in the cell.

Rhodamine 123 (Rh123) is a lipophilic, cationic dye commonly used to detect MP [16]. However, staining with Rh123 often requires a pretreatment step of the cells, generally performed by adding EDTA, to permeabilise the outer membrane of Gram-negative bacteria [17]. When antibiotic or disinfectant treatments are studied, the permeabilisation step can introduce some bias by enhancing the toxic effects of these compounds (i.e. they penetrate more easily inside the cells). Furthermore, the pretreatment conditions may vary depending on the environment (e.g., salinity) and between species. This is why MP dyes have received little applications at the community level. An additional problem is that Rh123 staining requires several cell-washing steps, which are time consuming and may result in cell losses. The cationic carbocyanine dyes (e.g., 3,3'-dihexyloxacarbocyanine; $DiOC_6(3)$) have also been used to estimate MP of bacteria. Advantages of these dyes are the absence of permeabilization and washing steps. However, nonspecific binding of carbocyanine dyes to hydrophobic regions of the cell and quenching of the fluorescence of intracellular dye have been reported [17]. Thus, careful calibration of the staining procedure is required to avoid false positive signals.

MP can also be determined by the anionic lipophilic oxonols. Accumulation inside bacterial cells is favoured by a reduction in the magnitude of the MP, allowing dye molecules to concentrate within the cell, and bind to lipid-rich components. Bis-(1,3-dibutylbarbituric acid) trimethine oxonol [$DiBAC_4(3)$] (BOX) has been reported to