Supplementary Material (ESI) for New Journal of Chemistry

Electronic Supplementary Information

Experimental.

Calixarenes were synthesized following literature methods.(1) The compound used for the fluorescence experiments was 5, 11, 17 Tris[(carboxy)methyl] 25-mono heptyloxy -26,27,28 trishydroxycalix[4]arene (named C4:C7 later). NBD1 was purified as described in supplementary data. All chemicals used were commercially available and used without further purification. L-Tryptophan 99% was purchased from ACROS Organics. The buffer solution was prepared by mixing 2.5 mL of Hepes buffer 1M pH7.8, 5 mL of NaCl solution 5 M, 5 mL of Glycerol 10 %, 17.5 µL of ß-mercaptoethanol and completed to 50 mL with pure water.

Fluorescence spectra were measured using a Photon Technology International spectrofluorometer Quanta Master ^{TM} UV VIS (QM 4CW) using a conventional 10 x 10 x 45 mm quartz cell. The excitation wavelength was 295 nm and the emission spectrum was recorded from 310 nm to 370 nm. All measurements are expressed as Arbitrary Units, a.u. Fluorescence study was carried by measuring the fluorescence of the several ratios of calixarene:NBD1:salt in the buffer solution. Curve-fittings and determination of dissociation constants were performed with the Grafit program (Erithacus Software).[26]

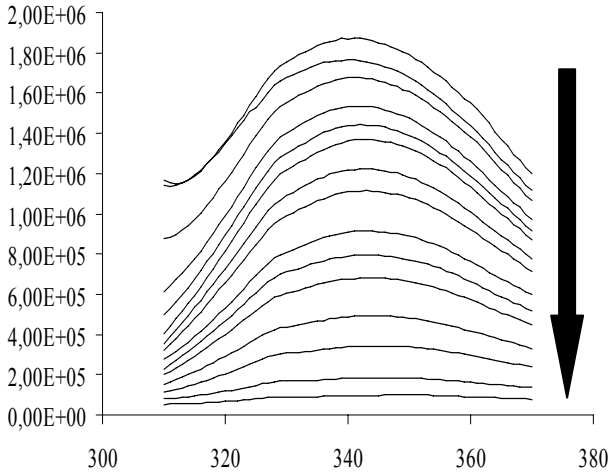

Figure ESI **Variation of the intensity of the fluorescence emission of NBD1 as a function of the concentration of tri-para-carboxylatmethylene-mono-heptoxy-calix[4]arene, the direction of the arrow shows the increasing concentration of the calix-arene.**

Expression of NBD1 (Asp^{628} to Asn^{881}) from MRP1 was performed as described (2) except for the purification steps which were modified. After centrifugation of bacterial cells lysate, the supernatant was first diluted two fold in a buffer containing 100 mMHepes pH 7.8, 20% glycerol, 1 MNaCl, 50 mM imidazole,10mM*β*-mercaptoethanol, and then applied to a Ni2+-nitrilotriacetic acid column equilibrated with 50 mMHepes Buffer pH 7.8, 0.5 MNaCl, 10% glycerol, 25 mM imidazole, 5 mMβ-mercaptoethanol. The column was first extensively washed with the same buffer, then with the same buffer containing 100 mM imidazole for contaminants elimination and finally with the same buffer containing 250 mM imidazole for NBD1 elution. The fractions were pooled and dialysed against anHepes Buffer pH 7.8 containing 10% glycerol, 5 mM*β*-mercaptoethanol, 500 mMNaCl. The dialysate was centrifuged to discard possible traces of precipitated material; the supernatant was aliquoted and kept frozen in liquid nitrogen. Protein fractions were analyzed on SDS-polyacrylamide gel electrophoresis (3) and protein concentration was routinely determined by the method ofBradford (4) with the CoomassiePlus Protein Assay Reagent Kit from Pierce (Brebières, France).

(1) K. Suwinska, O. Shkurenko, C. Mbemba, A. Leydier, S. Jebors, A. W. Coleman, R. Matarand P. Falson, *Tri-Anionic Calix[4]arene Monoalkyl Deriavtives: Synthesis, Solid-State Structures and Self-Assembly Properties, New J. Chem., (2008), 32, 1988-1998*

(2) D. Trompier, H. Baubichon-Cortay, X.-B. Chang, M. Maitrejean, D. Barron, J. R. Riordan and A. Di Pietro,*Multiple flavonoid-binding sites within multidrug resistance protein MRP1 Cell Mol Life Sci*, 2003, **60**, 2164–2177.

(3) U. K. Laemmli, *Cleavage of structural proteins during the assembly of the head of bacteriophage T4.Nature,*1970, **227,** 680–685.

(4) M. M. Bradford, *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding Anal Biochem* 1976, **72,** 248–254.