Electronic Supplementary Material (ESI) for Analytical Methods

A Facile Transport Assay for H^+ Coupled Membrane Transport Protein Using Fluorescence Probes

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Supplementary materilas:

Materials and methods:

Reagents. $MnCl_2$ (Manganese(Ⅱ) chloride tetrahydrate, NaCl (Sodium chloride) and KH_2PO_4 (Potassium dihydrogen phosphate) were purchased from Sinopharm Chemical Reagent Co., Ltd. Chloramphenicol (98-102%) was purchased from Bio Basic Inc. while IPTG was purchased (＞98%) from AMRESCO, Tryptone and Yeast extract were obtained from OXOID Ltd, Basingstoke, Hampshire, England. Fluorescence probe I 5(6)-FAM (5-(and 6)-carboxyfluorescein, MW 376.32g/mol) was purchased from Invitrogen. Fluorescence probe II was synthesized according to the literature procedure,[1] in which the carboxyl group was substituted by an piperazine. Elemental analysis of manganese ions concentration was determined with ICP-AES spectrometer (Varian 710ES).

Cell growth.

Cells were grown in YT media supplemented with chloramphenicol (25 μg/mL) and incubated for 3 hrs in the constant temperature air bath shake at 37°C. After growth for 2 hrs, cells were induced with 0.25 μM IPTG and were grown for an additional 2-3 hrs to reach OD_{600} 0.8 and cells were pelleted by centrifugation at 7000g for 5 min.[2] The resulting pellet was washed in 50 mM KH_2PO_4 buffer (pH 6.5). Cells obtained were stored at -20°C after harvesting.

Standard curves

To investigate and compare the sensitivity of the both FL probes, 50 mM KH_2PO_4 buffer at the range of pH 5.0-8.0 with the interval of 0.5 were prepared.[3] 5(6)-FAM (probe Ⅰ) and flurorescein derivative (probe Ⅱ) were dissolved in 50% DMSO and double distilled water to prepare a 1 mM solution. 3 μl, 1 mM probe solution was added into 3 mL buffer with a certain pH and the mixture was kept stirring for 1 min before FL measurement was performed. FL data were collected on F-4600 Spectroscopy (HITACHI) and the pH-dependent curve of probe was established as a standard pH-dependent curve for the following experiments.

Mn^{2+} uptake assay

The cells were resuspended in the same wash buffer KH_2PO_4 at a concentration of approximately OD_{600} 1.5 which was measured on Cary 50 Bio spectrophotometer (Varian, USA) and equilibrated at 37°C for 5-10 min before $MnCl_2$ was added. Mn^{2+} transport at different external manganese concentrations (0-125 μM final concentration) of the wild type and mutants were determined after 3 min reaction followed by placing the supernatant under fluorescence spectrometer by adding probe to detect the pH change caused by H^+ and Mn^{2+} co-transport. The kinetics data were obtained with the same procedure, except for the time. Each measurement was triplicates.

Table S1 H^+ and Mn^{2+} concentration changes in the supernatants with different initial Mn^{2+} concentrations under cell density of 1.5. $[Mn^{2+}]$ in supernatant was determined by elemental analysis.

Initial $[Mn^{2+}]$ (μM)	supernatant pH	$[H^+]$/μM	△$[H^+]$/μM	$[Mn^{2+}]$ /μM supernatant	△$[Mn^{2+}]$/μM supernatant
0	6.596	0.25	0	0	0
0.1	6.748	0.18	0.08	-	-
0.3	6.827	0.15	0.10	0.06	0.24
0.6	6.873	0.13	0.12	0.12	0.48

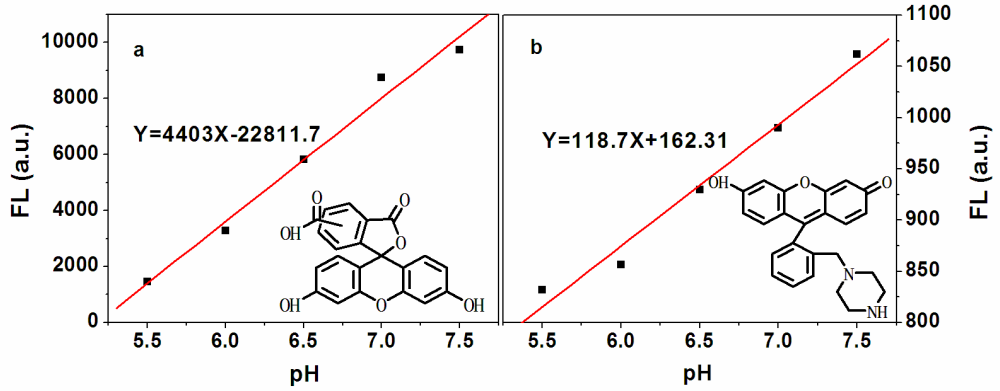

Figure S1. Calibration curve of the pH-dependent fluoresce probes, probe I (a) and probe II (b). The structures of the two probes and the slopes of the two curves are indicated in the figure.

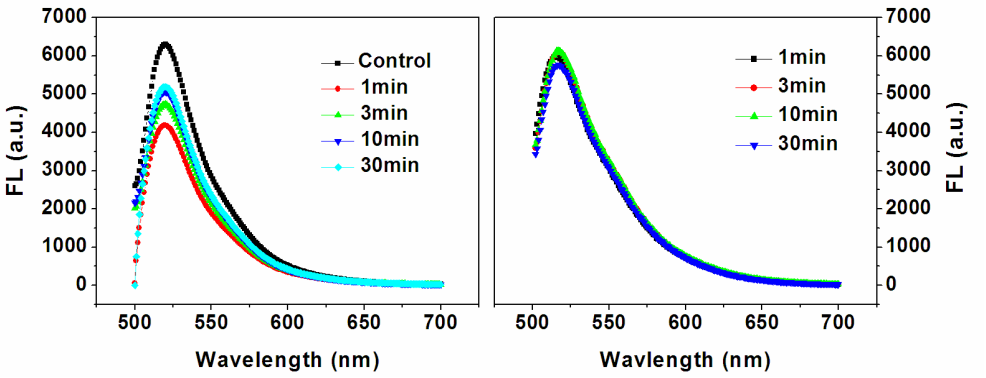

Figure S2. a) Influence of the cell on the FL intensity of the probe I. The cells (OD_{600}=1.5) were equilibrated for 1 min (red), 3 min (green), 10 min (blue), and 30 min (verdant) respectively, the FL intensity of the each sample was measured. b) Same as a), except that the cells of each sample were removed from the system, the fluorescence intensity of the supernatant was measured.

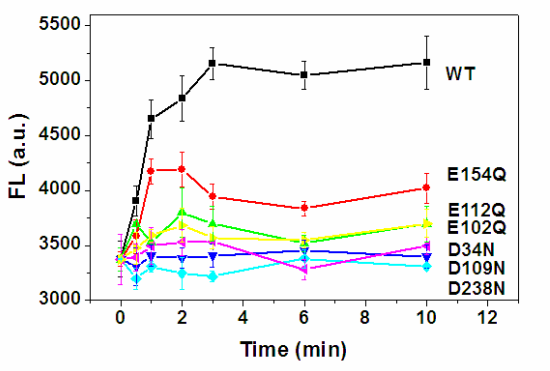

Figure S3. Mn^{2+} uptake of the wild type and mutant strains as function of time. Mn^{2+} was 0.3 μM, OD_{600} was 1.5.

References:

(1) P. Pfeiffer, R. W. *Liebigs Ann. Chem* **1928**, *461*.

(2) Haemig, H. A. H.; Moen, P. J.; Brooker, R. J. *Biochemistry* **2010**, *49*, 4662.

(3) Knöpfel, M.; Schulthess, G.; Funk, F.; Hauser, H. *Biophysical Journal* **2000**, *79*, 874.