Supporting Information

Quantifying the oligomeric state of hZIP4 on the surface of cells

Meghan E. Ahern[1], Elizabeth M. Bafaro[1], Ann Cowan[2], and Robert E. Dempski[1]*

(1) Department of Chemistry and Biochemistry, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609-2247, USA

(2) Department of Molecular Biology and Biophysics and Center for Cell Analysis and Modeling, University of Connecticut Health Center, Farmington, CT 06030

*To whom correspondence should be addressed:

Robert E. Dempski

Department of Chemistry and Biochemistry

Worcester Polytechnic Institute

100 Institute Road, Worcester, MA 01609-2247, USA

Tel. 1-508-831-4193. Email: rdempski@wpi.edu.

Methods and Materials

$^{65}Zn^{2+}$ Uptake Assay

cDNA for hZIP4 was cloned into pcDNA3.1 with a C-terminal monomeric enhanced GFP (eGFP). To quantify the function of this protein (hZIP4/eGFP), HEK293 cells were transiently transfected with the plasmid pcDNA3.1, as a negative control or pcDNA3.1 with hZIP4/eGFP. Functional expression was tested via a radioactive zinc ($^{65}Zn^{2+}$) uptake assay of transfected HEK293 cells 48 hours after transfection. Prior to the transport assay, cells were washed in Uptake Buffer (15 mM HEPES, 100 mM glucose, 150 mM KCl, pH 7.0) and added to prewarmed uptake buffer containing 5 uM $^{65}Zn^{2+}$ as previously described to initiate the reaction.[1] These cells were incubated in a 37°C water bath for 10 minutes. A second set of cells, treated the same way, was incubated at 4°C; these cells were used as a background reading and were subtracted from the final counts per minute (CPM) resulting from the cells at 37°C. Cells were filtered in 25 mm glass microfiber filters and the reactions were stopped with ice cold Uptake Buffer with 1 mM EDTA.[1] $^{65}Zn^{2+}$was measured using a scintillation counter and the number of cells per sample was determined prior to cell collection to normalize the data.

Analysis of fluorescence correlation spectroscopy data

A nonlinear least-squares fit using a 2D two-component model was used to fit the FCS data to autocorrelation curves. The two components reflect a fast component (τ_{D1}) generally ascribed to photophysical properties of the fluorophore, and a slow component (τ_{D2}) that reflects diffusion of the protein through the membrane.[2–4] The fast component diffusion time is on a scale of μs, while the slow component is on a scale of ms.

The average count rate k and the number of molecules N observed is used to calculate the molecular brightness, ϵ, using the following equation[4]:

$$1) \quad \epsilon = \frac{k}{N}$$

The molecular brightness corresponds to the number of fluorescent molecules in a protein complex, meaning that a monomer will have a brightness of 1x, a dimer of 2x, a tetramer of 4x, etc.[2–5] To establish the molecular brightness of monomer and dimer controls, the plasma membrane receptor CD86, a known monomer, was used with a single C-terminal eGFP as a monomeric control and a tandem C-terminal eGFP/eGFP to simulate a dimeric control.[2] For all experiments, reduced chi squared values similar to one another were used to determine the goodness of fit of the autocorrelation curve.

References

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