Biophysical Journal, Volume *118*

Supplemental Information

Structural Dynamics of the Paddle Motif Loop in the Activated Conformation of KvAP Voltage Sensor

Anindita Das, Satyaki Chatterjee, and H. Raghuraman

Membrane reconstitution of KvAP-VSD

Reconstitution in liposomes:

For reconstitution in liposomes, the wild-type and the labeled mutants of KvAP-VSD were reconstituted at a lipid-to-protein molar ratio of 100:1 in zwitterionic POPC membranes. Briefly, 640 nmoles of POPC in chloroform was mixed well and dried under a stream of nitrogen while being warmed gently (~35 °C). After the lipid was dried further under a high vacuum for at least 3 hr, the lipid film was hydrated (swelled) by adding 1 ml of 20 mM Tris, 100 mM KCl, pH 8.0 buffer and vortexed vigorously for 2 min to disperse the lipids and sonicated to clarity. Protein was then added to give a molar ratio of 100:1 lipid:VSD monomer. The sample was left at room temperature for 30 minutes on a rotator and 200 mg of pre-washed biobeads (SM-2, Bio-Rad, Hercules, CA) were then added and the mixture was incubated on a rotator overnight at 4 °C to remove the detergent. The biobeads were removed by filtering using a Bio-Rad 5 ml column filter before use.

Nanodisc incorporation of KvAP-VSD:

For reconstitution in Nanodiscs, the plasmid (pMSP1E3D1) encoding for a polyhistidine-tagged membrane scaffolding protein (MSP) was used for the Nanodisc assembly of 117-NBD mutant of KvAP-VSD, and was expressed and purified as described previously (S1). The MSP, sensor and lipids, solubilized with twice the concentration of sodium cholate with respect to lipid as recommended earlier (S2), were mixed with the molar ratios of 2:1:120 (MSP:VSD:POPC/POPG). The POPC and POPG lipids were used at 3:1 molar ratio. The mixture was incubated in ice for ~ 3 h, and the self-assembly of Nanodiscs was initiated by adding hydrated biobeads and incubated for ~ 3 h at room temperature with gentle rocking. The efficient Nanodiscs assembly was judged by gel filtration profiles.

Dynamic light scattering (DLS) measurements

The sizing of Nanodiscs in 20 mM Tris, 100 mM KCl, pH 8.0 buffer was measured in Zetasizer Nano S particle analyser from Malvern Instruments (Worcestershire, UK) by using He-Ne laser (633 nm) with 4 mW power as the light source. Since intensity-weighted particle size distributions overemphasize larger particles due to the fact that the scattering

intensity is proportional to the sixth power of the particle size (diameter), we derived volume-weighted size distributions in case of Nanodiscs.

TABLE S1: Fluorescence parameters of L115-NBD of KvAP-VSD reconstituted in liposomes composed of anionic and zwitterionic lipids

Liposomes	**Emission maximum (nm)**	**Polarization**	**REES (nm)**	τ_c **(ns)**	K_{SV} **(M^{-1})**	k_q **($M^{-1}s^{-1}$)**
POPC/POPG	530	0.29	1	7.18	3.2	0.75
POPC	530	0.28	1	6.99	3.9	0.72

τ_c, apparent rotational correlation times; K_{SV}, Stern-Volmer quenching constants; k_q, bimolecular quenching constants. See text for details.

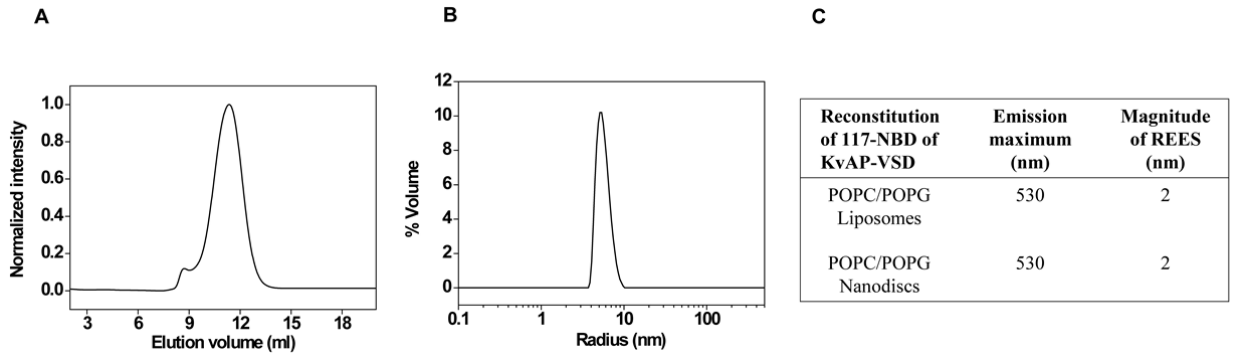

FIGURE S1 Fluorescence properties of 117-NBD mutant of KvAP-VSD in liposomes and Nanodiscs. (*A*) Gel filtration profile shows the efficient Nanodiscs assembly of the NBD-labeled KvAP mutant. (B) Volume-weighted particle size distribution of Nanodiscs obtained by DLS. The hydrodynamic diameter of Nanodiscs is found to be ~12 nm, which is in good agreement with previous studies (S2). (*C*) Comparison of fluorescence properties such as emission maximum and REES is shown for 117-NBD mutant of the sensor reconstituted in PC/PG liposomes and Nanodiscs. Interestingly, the identical values of fluorescence parameters in liposomes and Nanodiscs probably suggest negligible curvature effects on the structural dynamics of the sensor in membranes. See Materials and Methods for other details.

References

S1. Alvarez, F. J. D., C. Orelle, and Davidson, A. L. 2010. Functional reconstitution of an ABC transporter in nanodiscs for use in electron paramagnetic resonance spectroscopy. J. Am. Chem. Soc. 132:9513-9515.

S2. Ritchie, T. K., Y. V. Grinkova, T. H. Bayburt, I. G. Denisov, J. K. Zolnerciks, W. M. Atkins, and S. G. Sligar. 2009. Reconstitution of membrane proteins in phospholipid bilayer nanodiscs. Methods Enzymol. 464:211-23