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Data Article

Diffusion coefficients and dissociation constants of enhanced green fluorescent protein binding to free standing membranes

Franziska A. Thomas [a], Ilaria Visco [a], Zdeněk Petrášek [a,b], Fabian Heinemann [a,c], Petra Schwille [a,*]

[a] Max Planck Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany
[b] Graz University of Technology, Institute of Biotechnology and Biochemical Engineering, Petersgasse 10-12/I, A-8010 Graz, Austria
[c] Roche Diagnostics, Nonnenwald 2, D-82377 Penzberg, Germany

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ABSTRACT

Recently, a new and versatile assay to determine the partitioning coefficient K_P as a measure for the affinity of peripheral membrane proteins for lipid bilayers was presented in the research article entitled, "Introducing a fluorescence-based standard to quantify protein partitioning into membranes" [1]. Here, the well-characterized binding of hexahistidine-tag (His_6) to NTA(Ni) was utilized. Complementarily, this data article reports the average diffusion coefficient D of His_6-tagged enhanced green fluorescent protein (eGFP-His_6) and the fluorescent lipid analog ATTO-647N-DOPE in giant unilamellar vesicles (GUVs) containing different amounts of NTA(Ni) lipids. In addition, dissociation constants K_d of the NTA(Ni)/eGFP-His_6 system are reported. Further, a conversion between K_d and K_P is provided.

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* Corresponding author. Tel.: +49 89 8578 2900; fax: +49 89 8578 2903.
E-mail address: schwille@biochem.mpg.de (P. Schwille).

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Specifications table

Subject area	*Biophysics*
More specific subject area	*Molecular Biophysics*
Type of data	*Table, figure*
How data was acquired	*Fluorescence Correlation Spectroscopy, Confocal Microscopy using a LSM 780 with a ConfoCor 3 unit (Zeiss, Jena, Germany)*
Data format	*Analyzed*
Experimental factors	*GUVs consisting of DOPC and 2, 3, 4 or 5 mol% DGS-NTA(Ni), labeled with 0.05 mol% ATTO-647N-DOPE*
Experimental features	*Titration of eGFP-His$_6$ to* the *GUVs*
Data source location	*Max Planck Institute of Biochemistry, Martinsried, Germany*
Data accessibility	The data are provided within this article

Value of the data

- **We provide the first valuable characterization of the eGFP-His$_6$/NTA(Ni) system with precise dissociation constants K_d for increasing percentages of DGS-NTA(Ni) in the membrane.**
- **The eGFP-His$_6$/NTA(Ni) dissociation constants could serve as reference for other His$_6$-tagged proteins reconstituted in GUVs.**
- **We provide a conversion between K_d and K_P for the His$_6$-NTA(Ni) system, which can be extended to any protein-lipid interaction with a known 1:1 stoichiometry.**
- **Protein diffusion coefficients could be used as an indicator of crowding effects.**
- **As for DOPC/DGS-NTA(Ni) the lipid dynamics is independent of increasing protein concentrations, the ATTO-647N-DOPE diffusion coefficient could serve as a standard.**

1. Data

Hexahistidine-tag (His$_6$) binding to Nickel (Ni) chelated with nitrilotriacetic acid (NTA) is a well-characterized process [2,3] and it is extensively used to reconstitute protein systems in giant unilamellar vesicles (GUVs) [4–6]. We made GUVs consisting of 1,2-di-(9*Z*-octadecenoyl)-*sn*-glycero-3-phosphocholin (DOPC) and 2, 3, 4 or 5 mol% 1,2-di-(9*Z*-octadecenoyl)-*sn*-glycero-3-[(*N*-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl] nickel salt (DGS-NTA(Ni)), labeled with 0.05 mol% ATTO-647N-DOPE. These GUVs were incubated with increasing amounts of His$_6$-tagged enhanced green fluorescent protein (eGFP-His$_6$) and point fluorescence correlation spectroscopy (FCS) was performed both at the top pole of the GUVs and in solution. From the obtained FCS auto-correlation functions the diffusion coefficient D of both eGFP-His$_6$ and ATTO-647N-DOPE as well as the dissociation constant K_d of the NTA(Ni)/eGFP-His$_6$ system were calculated.

Table 1
Diffusion coefficient D determined by GUV-FCS assay. Calculated diffusion coefficients by averaging all data points for increasing amounts of DGS-NTA(Ni) via the GUV method (mean ± combined s.e.m.).

DGS-NTA(Ni)	eGFP-His$_6$ D in μm^2/s	ATTO-647N-DOPE D in μm^2/s
2%	4.36 ± 1.12 (n=548)	10.03 ± 0.68 (n=549)
3%	3.20 ± 0.75 (n=775)	9.74 ± 0.66 (n=900)
4%	3.14 ± 0.94 (n=740)	9.67 ± 0.76 (n=969)
5%	1.90 ± 1.01 (n=593)	9.72 ± 0.52 (n=705)