Data in Brief 5 (2015) 537–541

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

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

ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form
29 September 2015
Accepted 1 October 2015

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.

DOI of original article: http://dx.doi.org/10.1016/j.bbamem.2015.09.001
* Corresponding author. Tel.: +49 89 8578 2900; fax: +49 89 8578 2903.
E-mail address: schwille@biochem.mpg.de (P. Schwille).

http://dx.doi.org/10.1016/j.dib.2015.10.002
2352-3409/

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 $\mu m^2/s$	ATTO-647N-DOPE D in $\mu m^2/s$
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)

2. Experimental design, materials and methods

The materials, the preparation of eGFP-His_6 and GUVs, the optical setup used and the FCS data acquisition/analysis were described elsewhere [1].

2.1. Determination of average diffusion coefficients

We determined the average diffusion coefficients D of eGFP-His_6 attached to DGS-NTA(Ni) in the lipid bilayer and of ATTO-647N-DOPE (Table 1 and Fig. 1) by applying the following equation:

$$D = \frac{{\omega_0}^2}{4\tau_{2D}} \tag{1}$$

The average focal waist w_0 obtained from a calibration with Alexa488 and with ATTO-655, were $w_0 = 218.0 \pm 6.0$ nm (mean $\pm$ s.e.m, $n=19$) and $w_0 = 246.2 \pm 4.6$ nm (mean $\pm$ s.e.m, $n=19$), respectively. The diffusion times τ_{2D} were determined fitting the auto-correlation curves with a weighted $2D-3D+T$ model function. The D values were averaged and the significance of their deviation was tested using a one-way analysis of variance (ANOVA) in SigmaPlot 12.3 (Systat Software, Inc., San Jose, CA). This statistical analysis indicated a significance of deviation for the average diffusion coefficients of eGFP-His_6 in presence of different DGS-NTA(Ni) concentrations ($F(3,78)=19.48$, $p<0.001$). With increasing amount of DGS-NTA(Ni), the eGFP-His_6 average diffusion coefficients decreases from $D = 4.36 \pm 1.12\ \mu m^2/s$ (mean $\pm$ combined s.e.m., $n=548$) to $D = 1.90 \pm 1.01\ \mu m^2/s$ (mean $\pm$ combined s.e.m., $n=593$). In contrast, the average diffusion coefficient of ATTO-647N DOPE for all concentrations DGS-NTA(Ni) was $D = 9.81 \pm 0.70\ \mu m^2/s$ (mean $\pm$ combined s.e.m., $n=3123$) and did not show any statistical significant difference ($F(3,86)=3.24$, $p=0.026$).

2.2. K_d for eGFP-His_6 DGS-NTA(Ni) system

Only in cases where the protein-lipid binding is purely stoichiometric and if the stoichiometry is known, the protein affinity for the lipid membrane can be expressed by the dissociation constant K_d. In equilibrium, an identical number of molecules P will dissociate from and associate to the lipid

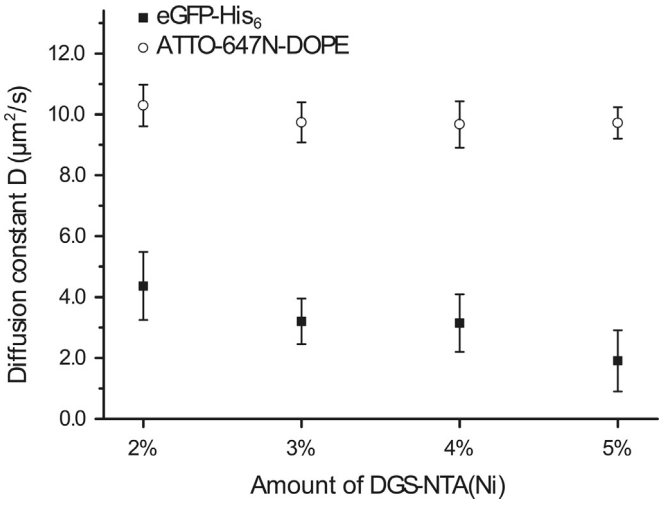

Fig. 1. Diffusion coefficients determined by GUV-FCS assay. D for eGFP-His_6 coordinated to NTA(Ni) (filled squares) and the membrane dye ATTO-647N-DOPE (circles) with increasing amounts of DGS-NTA(Ni). Error bars represent the combined standard error of mean. The D of ATTO-647N-DOPE shows no significant differences, whereas the D of eGFP-His_6 decreases with increasing amounts of DGS-NTA(Ni).

phase L per area and time $P+nL \rightarrow nPL$. For 1:1 binding stoichiometry ($n=1$), K_d is defined as:

$$K_d = \frac{[P_f][L_f]}{[PL]} \tag{2}$$

where $[P_f]$ is the freely diffusing species in solution, $[PL]=[P_m]$ the membrane associated fraction and $[L_f]=[L]-[L_m]$ with the total accessible lipid concentration $[L] \gg [L_m]$. Thus,

$$K_d = \frac{[P_f][L]}{[P_m]} = \frac{k_{off}}{k_{on}} \tag{3}$$

$[L]$ is constant in a given sample and can be expressed by:

$$[L] = \frac{A}{A_L N_A V} \tag{4}$$

Here, A is the total accessible lipid area, A_L the area per lipid, N_A the Avogadro's constant and V the volume of the sample chamber. $[P_f]$ and $[P_m]$ can be determined by FCS [1]. In particular, $[P_m]$ is obtained by:

$$[P_m] = [P_{2D}]\frac{A}{V} \tag{5}$$

where $[P_{2D}]$ is the surface concentration on the top pole of a GUV.

A rearrangement of Eq. (3) gives:

$$[P_m] = \frac{[L]}{K_d}[P_f] \tag{6}$$

Combining Eq. (6) with Eqs. (4) and (5) gives the following main equation (A and V cancel out):

$$[P_{2D}] = \frac{1}{K_d A_L N_A}[P_f] \tag{7}$$

When a set of $[P_f]$ and $[P_{2D}]$ is plotted and fitted with a linear equation passing through the origin of the axis, K_d can be calculated from the slope a:

$$K_d = \frac{1}{a A_L N_A} \tag{8}$$

Comparing Eq. (8) with Eq. (7) in Thomas *et al.* [1] leads to the following conversion between K_d and partition coefficient K_P:

$$\frac{K_P}{W} = \frac{1}{K_d} \tag{9}$$

with the water concentration $[W]$ being constant with $[W]=W=55.5$ M.

Assuming that the binding stoichiometry for the NTA(Ni)/eGFP-His_6 system is 1:1 [2,7], we could calculate the dissociation constant K_d from the reported partitioning coefficient K_P [1] with Eq. (9) or directly from the slope a with Eq. (8). In Table 2 and Fig. 2 the values of the dissociation constant K_d are given for the different content of DGS-NTA(Ni). They correspond to the upper range of values reported in the literature, which vary from 10 nM to 10 μM [7–9].

Table 2
K_d determined by GUV-FCS assay. Calculated dissociation constants by fitting all data points for increasing amounts of DGS-NTA(Ni) via the GUV-FCS method (mean ± combined s.e.m.).

DGS-NTA(Ni)	K_d in M
2%	$2.18 \pm 0.23 \cdot 10^{-5}$
3%	$1.28 \pm 0.26 \cdot 10^{-5}$
4%	$3.60 \pm 0.27 \cdot 10^{-6}$
5%	$1.15 \pm 0.27 \cdot 10^{-6}$

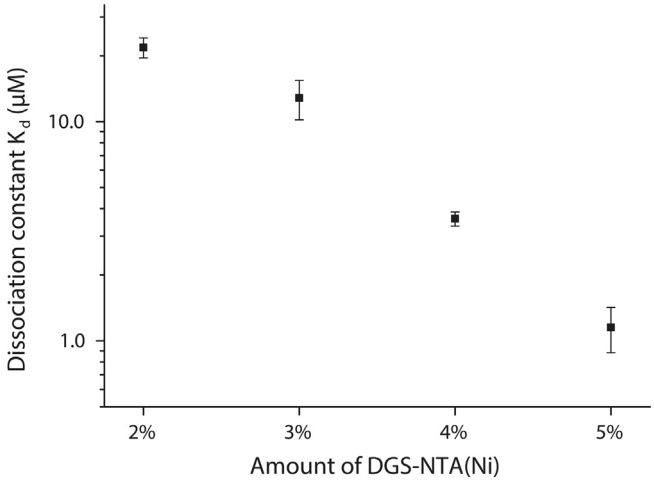

Fig. 2. Graphic presentation of dissociation constant K_d obtained by GUV-FCS assay. Error bars represent the combined standard error of mean.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.10.002.

References

[1] F.A. Thomas, I. Visco, Z. Petrášek, F. Heinemann, P. Schwille, Introducing a fluorescence-based standard to quantify protein partitioning into membranes, *BBA–Biomembranes*, **1848** (2015), 2932-2941.
[2] S. Knecht, D. Ricklin, A.N. Eberle, B. Ernst, Oligohis-tags: mechanisms of binding to Ni^{2+}-NTA surfaces, J. Mol. Recognit. 22 (2009) 270–279.
[3] F. Khan, M. He, M.J. Taussig, Double-hexahistidine tag with high-affinity binding for protein immobilization, purification, and detection on Ni-nitrilotriacetic acid surfaces, Anal. Chem. 78 (2006) 3072–3079.
[4] I. López-Montero, P. López-Navajas, J. Mingorance, M. Vélez, M. Vicente, F. Monroy, Membrane reconstitution of FtsZ–ZipA complex inside giant spherical vesicles made of *E. coli* lipids: large membrane dilation and analysis of membrane plasticity, BBA–Biomembranes 1828 (2013) 687–698.
[5] W. Römer, L.L. Pontani, B. Sorre, C. Rentero, L. Berland, V. Chambon, C. Lamaze, P. Bassereau, C. Sykes, K. Gaus, L. Johannes, Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis, Cell 140 (2010) 540–553.
[6] A.J. García-Sáez, J. Ries, M. Orzáez, E. Pérez-Payà, P. Schwille, Membrane promotes tBID interaction with BCL(XL), Nat. Struct. Mol. Biol. 16 (2009) 1178–1185.
[7] E.G. Guignet, R. Hovius, H. Vogel, Reversible site-selective labeling of membrane proteins in live cells, Nat. Biotechnol. 22 (2004) 440–444.
[8] M. Hintersteiner, T. Weidemann, T. Kimmerlin, N. Filiz, C. Buehler, M. Auer, Covalent fluorescence labeling of His-tagged proteins on the surface of living cells, ChemBioChem 9 (2008) 1391–1395.
[9] S.A. Lauer, J.P. Nolan, Development and characterization of Ni-NTA-bearing microspheres, Cytometry 48 (2002) 136–145.