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Data Article

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



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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 (His₆) to NTA(Ni) was utilized. Complementarily, this data article reports the average diffusion coefficient D of His₆-tagged enhanced green fluorescent protein (eGFP-His₆) 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-His₆ system are reported. Further, a conversion between K_d and K_P is provided.

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Specifications table

Subject area	Biophysics
More specific sub- ject 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-His₆/NTA(Ni) system with precise dissociation constants K_d for increasing percentages of DGS-NTA(Ni) in the membrane.
- The eGFP-His₆/NTA(Ni) dissociation constants could serve as reference for other His₆-tagged proteins reconstituted in GUVs.
- We provide a conversion between K_d and K_p for the His₆-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 (His₆) 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-(9Z-octadecenoyl)-sn-glycero-3-phosphocholin (DOPC) and 2, 3, 4 or 5 mol% 1,2-di-(9Z-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 His₆-tagged enhanced green fluorescent protein (eGFP-His₆) 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-His₆ and ATTO-647N-DOPE as well as the dissociation constant K_d of the NTA(Ni)/eGFP-His₆ 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 \pm combined s.e.m.).

DGS-NTA(Ni)	eGFP-His ₆ D in $\mu\text{m}^2/\text{s}$	ATTO-647N-DOPE D in $\mu\text{m}^2/\text{s}$
2%	4.36 \pm 1.12 ($n=548$)	10.03 \pm 0.68 ($n=549$)
3%	3.20 \pm 0.75 ($n=775$)	9.74 \pm 0.66 ($n=900$)
4%	3.14 \pm 0.94 ($n=740$)	9.67 \pm 0.76 ($n=969$)
5%	1.90 \pm 1.01 ($n=593$)	9.72 \pm 0.52 ($n=705$)

2. Experimental design, materials and methods

The materials, the preparation of eGFP-His₆ 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-His₆ attached to DGS-NTA(Ni) in the lipid bilayer and of ATTO-647N-DOPE (Table 1 and Fig. 1) by applying the following equation:

$$D = \frac{\omega_0^2}{4\tau_{2D}} \quad (1)$$

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-His₆ 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-His₆ average diffusion coefficients decreases from $D = 4.36 \pm 1.12 \mu\text{m}^2/\text{s}$ (mean \pm combined s.e.m., $n=548$) to $D = 1.90 \pm 1.01 \mu\text{m}^2/\text{s}$ (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 $D = 9.81 \pm 0.70 \mu\text{m}^2/\text{s}$ (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-His₆ 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

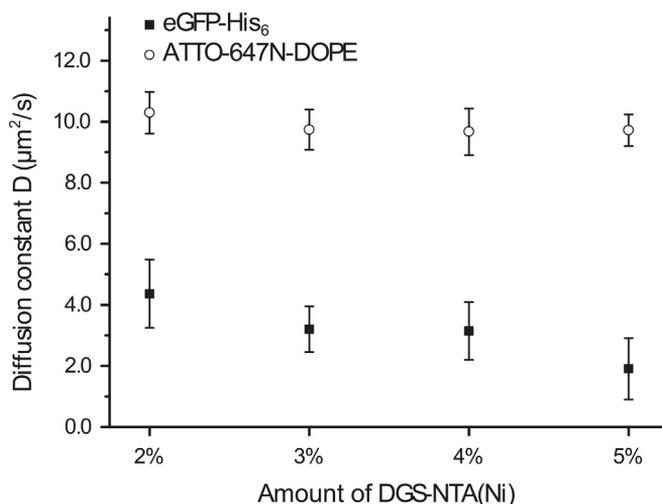


Fig. 1. Diffusion coefficients determined by GUV-FCS assay. D for eGFP-His₆ coordinated to NTA(Ni) (filled squares) and the membrane dye ATTO-647N-DOPE (circles) with increasing amounts of DGS-NTA(Ni). Error bars represent the combined standard error of mean. The D of ATTO-647N-DOPE shows no significant differences, whereas the D of eGFP-His₆ 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:

$$K_d = \frac{[P_f][L_f]}{[PL]} \quad (2)$$

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,

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

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

$$[L] = \frac{A}{A_L N_A V} \quad (4)$$

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:

$$[P_m] = [P_{2D}] \frac{A}{V} \quad (5)$$

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

A rearrangement of Eq. (3) gives:

$$[P_m] = \frac{[L]}{K_d} [P_f] \quad (6)$$

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

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

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 :

$$K_d = \frac{1}{a A_L N_A} \quad (8)$$

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

$$\frac{K_p}{W} = \frac{1}{K_d} \quad (9)$$

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

Assuming that the binding stoichiometry for the NTA(Ni)/eGFP-His₆ 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%	$1.15 \pm 0.27 \cdot 10^{-6}$

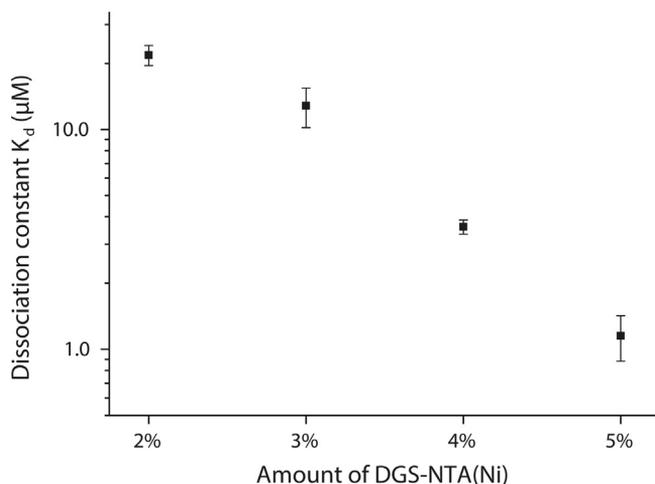


Fig. 2. Graphic presentation of dissociation constant K_d obtained by GUV-FCS assay. Error bars represent the combined standard error of mean.

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>.

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