Data Article

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

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\textbf{A R T I C L E  I N F O}

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\textbf{A B S T R A C T}

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\textsubscript{6}) to NTA(Ni) was utilized. Complementarily, this data article reports the average diffusion coefficient \( D \) of His\textsubscript{6}-tagged enhanced green fluorescent protein (eGFP-His\textsubscript{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\textsubscript{6} 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 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-His₆ 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 was determined.

#### 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-His₆ $D$ 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-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 ± s.e.m, $n = 19$) and $w_0 = 246.2 \pm 4.6$ nm (mean ± s.e.m, $n = 19$), respectively. The diffusion times $\tau_{2D}$ 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 $D = 4.36 \pm 1.12 \mu m^2/s$ (mean ± combined s.e.m., $n = 548$) to $D = 1.90 \pm 1.01 \mu m^2/s$ (mean ± 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 ± 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).](image-url)
phase $L$ per area and time $P + nL \rightarrow nPL$. For 1:1 binding stoichiometry ($n = 1$), $K_d$ is defined as:

$$K_d = \frac{[P_f]}{[P_l]}$$

(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 $[L] > [L_m]$. Thus,

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

(3)

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

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

(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:

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

(5)

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

A rearrangement of Eq. (3) gives:

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

(6)

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

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

(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 $a$:

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

(8)

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

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

(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].

| 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%         | $1.15 \pm 0.27 \cdot 10^{-6}$ |
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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