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Two different binding modes of α-Synuclein to lipid vesicles depending on its aggregation state

Supportive Information

Quantification of the bound fraction of αS - The fluorescence data were quantitatively evaluated using the *FIDA-Analyze* software (Evotec-Technologies, Hamburg, Germany). FIDA parameters (particles brightness Q and concentration C) and FCS parameters (diffusion time τ and particle number N) were calculated by fitting the measurement data (1-3). From SIFT measurements the red particle brightness of unbound monomeric αS (Q_1) was first determined from the 1D-FIDA analysis of a solution 10 nM of monomeric αS. Upon addition of vesicles the concentration of unbound αS (C_1), the concentration of vesicles with bound αS (C_2) and the red brightness of these vesicles (Q_2) were derived by fitting the parameters to the FIDA data (setting Q_1 to a fix value obtained from the brightness of αS in solution). Subsequently the fraction bound αS was derived from $C_2Q_2/(C_1Q_1+C_2Q_2)$ and the amount of αS bound per vesicle from Q_2/Q_1 (Table 1S, A). In aggregation experiments, a heterogeneous population of red particles was formed. Nevertheless the average concentration (C_2) and the brightness (Q_2) of the oligomers, and the concentration of the remaining monomers (C_1) were estimated by data fitting. The fraction of aggregated αS was derived from $C_2Q_2/(C_1Q_1+C_2Q_2)$.

From FCS measurements the diffusion time τ_{suv} of the vesicles and $\tau_{\alpha S}$ of unbound αS were first determined independently from the green and red fluorescence autocorrelation curves of stock solutions of 10 μM vesicles and 10 nM αS respectively. When the autocorrelation of αS was evaluated in the presence of vesicles the curve was in-between the curves of the vesicles and the free αS depending on the fraction bound αS. The fractions of unbound and bound αS were determined by fitting the autocorrelation curve of αS to τ_{suv} and $\tau_{\alpha S}$ (3)(Tabe 1S, B). The hydrodynamic radius (r_h) of the particles was derived combining Einstein's equation for 3-dimensional Brownian motion ($\Delta r^2 = 6Dt$)(4) and Stokes-Einstein equation ($r_h = k_BT/(6\pi\eta D)$), where *D* is the diffusion constant of the particles, η the viscosity of the medium. Δr is the radius of the focus of the laser (approximately 0.75 μm). FCS analysis was not useful for αS aggregates, since these structures diffuse very slowly, bleach, and are detected only inefficiently when no scanning device is used.

A.

FIDA	C_1 [αS_{free}]	Q_1 (fix) [αS_{free}]	C_2 [αS_{bound}]	Q_2 [αS_{bound}]	αS_{bound} [%]
α-Syn-647	3,06	27,00	1,82	125,51	74

B.

FCS	$\tau_{\alpha S}$ (unbound) [µsec]	τ_{SUV} [µsec]	αS_{free} [%]	αS_{bound} [%]
α-Syn-647 + DPPC-SUV	1000	6600	17	83

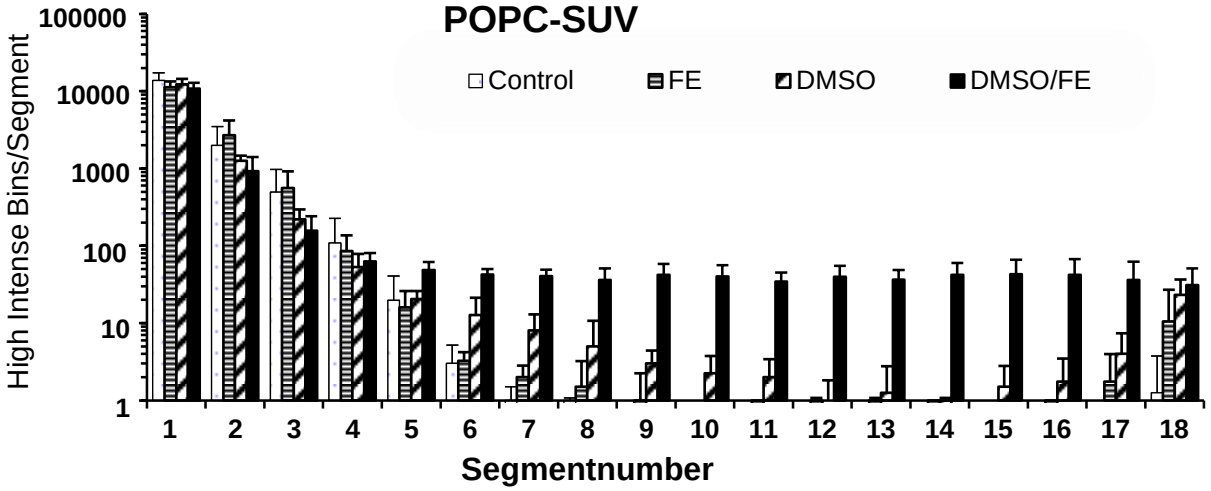
Table 1S. Quantitative evaluation of αS binding to DPPC-SUV

References:

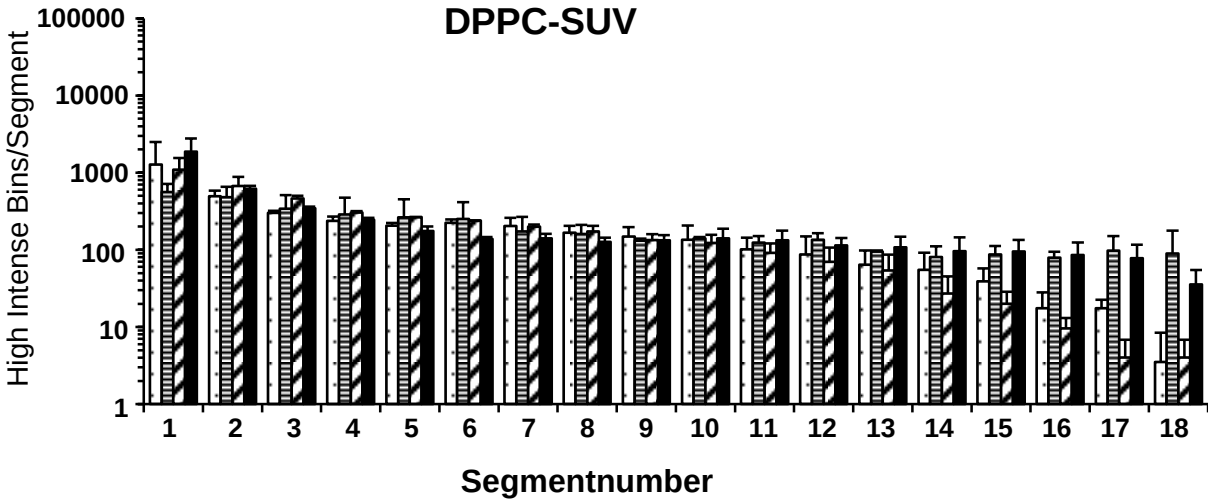
1. Bieschke, J., A. Giese, W. Schulz-Schaeffer, I. Zerr, S. Poser, M. Eigen, and H. Kretzschmar. 2000. Ultrasensitive detection of pathological prion protein aggregates by dual-color scanning for intensely fluorescent targets. Proc Natl Acad Sci U S A 97:5468-5473.
2. Kask, P., K. Palo, D. Ullmann, and K. Gall. 1999. Fluorescence-intensity distribution analysis and its application in biomolecular detection technology. Proc Natl Acad Sci U S A 96:13756-13761.
3. Rhoades, E., T. F. Ramlall, W. W. Webb, and D. Eliezer. 2006. Quantification of alpha-synuclein binding to lipid vesicles using fluorescence correlation spectroscopy. Biophys J 90:4692-4700.
4. Bloomfeld, V. A. 2000. Survey of Biomolecular Hydrodynamics. In On-line Biophysics Textbook. T. M. Schuster, editor. 1-16.

Supporting information.

1



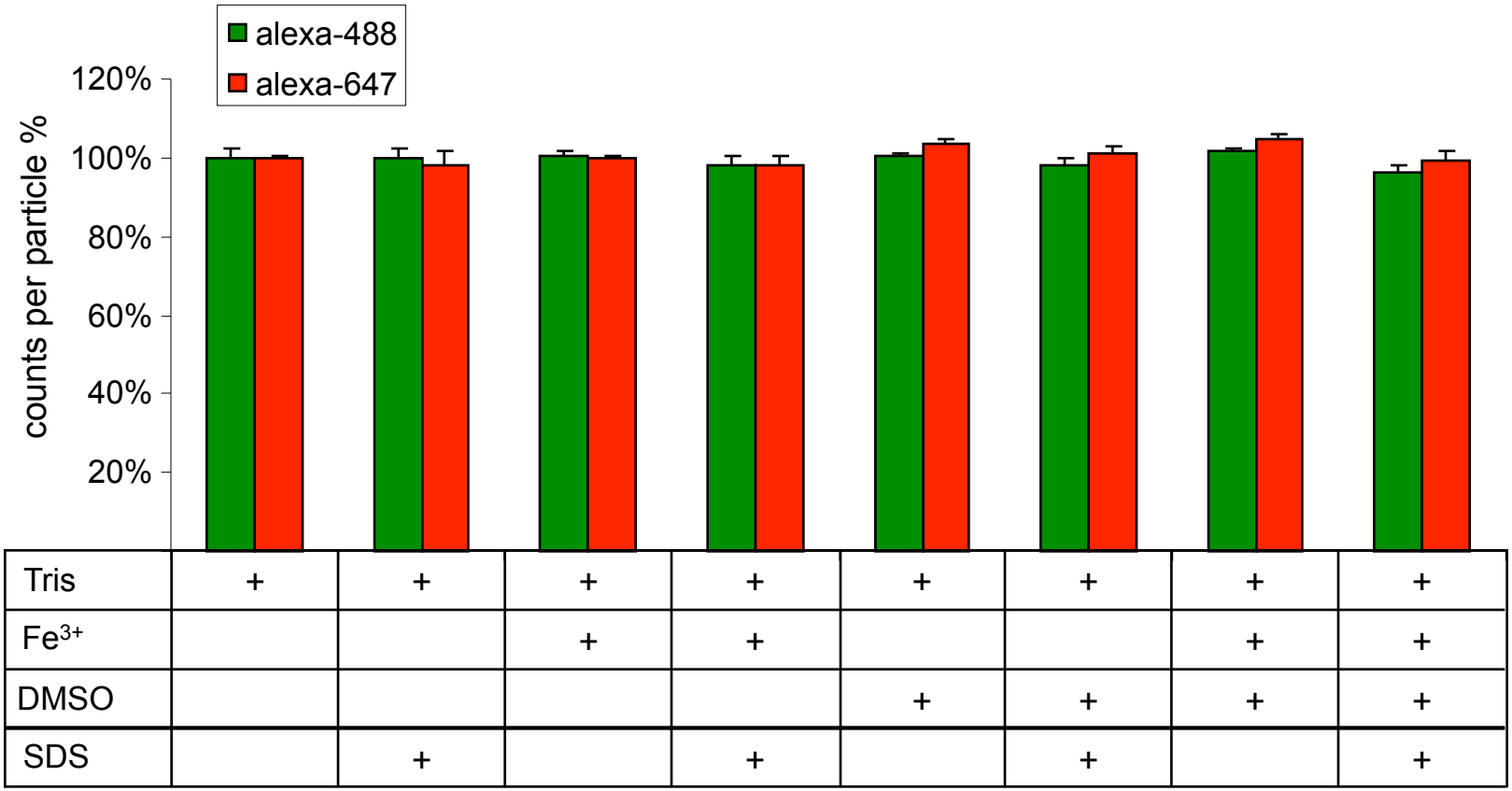
2



Figures S1 and S2.

Quantitative SIFT analysis for the experiments shown in Figure 2B and 2C.

For quantification of high-intensity bins, we used segments depicted in Figure 2A. S1 shows the mean ± S.E. of 20 measurements obtained in 4 experiments, S2 shows the mean ± S.E of 10 measurements obtained in 2 experiments. In presence of POPC-SUV, dual-colour high-intensity bins in segments 6-16 are found virtually only in presence of DMSO/Fe^{3+}. This indicates lipid binding of iron-induced αS-oligomers. In contrast, in the presence of DPPC-SUV the distribution of high-intensity bins among the different segments remains similar regardless of the addition of DMSO and/or iron. A further difference between S1 and S2 is that the number of high-intensity bins in segment 1, which indicates the presence of purely green particles (i.e. vesicles with no αS bound), is much higher for POPC-SUV in all conditions tested.



Supplementary Figure S3: Influence of buffer conditions on the fluorescence properties of fluorescent dyes Alexa488 and Alexa647 – Solutions containing Alexa-488 and Alexa-647 in 50 mM Tris buffer, pH 7.0, in a total assay volume of 20 µL were measured with a stationary focus of the FCS reader. In parallel, samples containing final concentrations of either 10 µM $FeCl_3$, DMSO (1% v/v), SDS (0,2% v/v) or all possible combinations, respectively, were measured. The measurement data were evaluated by autocorrelation analysis. Mean values and standard deviation of the individual particle brightness (counts per particle) [kHz] of five consecutive measurements were normalized to the mean counts per particle obtained for Alexa-488 or Alexa-647.