SUPPLEMENTARY INFORMATION

High-yield production in *E. coli* and characterization of full-length functional p13^{II} protein from human T-cell leukemia virus type 1

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Supplementary Figure S1. Size exclusion chromatograms of Ni-affinity purified WT and mutant p13^{II}-Ub.



Supplementary Figure S2. (A and B) A log-linear background (gray) was subtracted from the raw DEER data plotted on semi-logarithmic scale (black) for $p13^{II}$ -Ub spin-labeled at position S47C in LPPG. Protein concertation was 50 μ M, LPPG concentration was 6 mM in A and 18 mM in B. (C) The background-subtracted and normalized DEER data are compared for the same protein in 6 mM and 18 mM LPPG. The differences in the DEER signals for LPPG ratios of 1:120 (black) and 1:360 (red) are small and within the uncertainty typical for background subtraction. (D) The reconstructed inter-spin distances for the data in C. Only minor variations in the distributions for 1:120 (black) and 1:360 (red) ratios are within the variations originating from the change of SVD cutoff.



Supplementary Figure S3. (A) The DEER data on a semi-logarithm scale for the sample made of 50 μ M MTSL and 6 mM LPPG. Nearly linear signal trace was recorded suggesting random distribution of spins in the sample. To prepare the sample, the MTSL/LPPG mixture was incubated ca. 10 min at RT and then 25 % (w/v) glycerol was added right before freezing it in a capillary tube. (B) The RT cw EPR spectrum of the same MTSL/LPPG sample without glycerol.



Supplementary Figure S4. The DEER data for p13^{II}-Ub, spin-labeled at residue H63C, are plotted for LPPC and LPPG containing samples. The DEER signal intensity at zero evolution time, which is equal to "the DEER modulation depth", is significantly greater for the protein in LPPG compared to LPPC emphasizing the effect of lipid charge on p13^{II}-lipid interactions and oligomerization.



Supplementary Figure S5. cw EPR spectra of spin-labeled at residue H63C p13^{II}-Ub in buffer solution, LPPG, and DOPC/POPG liposomes. The spectra were normalized to maximum intensity.



Supplementary Figure S6. p13^{II} fluorescence-based Tl⁺ uptake assay in liposomes.

(A) Plotted is the drop, Δ FI, of the fluorescence intensity recorded at 18 min waiting time after transferring the liposomes to the bath buffer for the cases of protein-loaded and free liposomes. The data taken with and without pH gradient are plotted in red and blue, respectively. The bars corresponding to the presence of protein are rendered with pattern. (B) The difference between Δ FI measured with and without p13^{II} (in A) is plotted for samples with and without pH gradient. These data are from the second independent liposome uptake assay supportive of the increase of Tl⁺ uptake caused by p13^{II} in agreement with Fig. 8 in the main text. The data shown in panel A as well as in Fig. 8 panel B were normalized in the same manner using the maximum value of fluorescence intensity drop. The pH gradient dependent difference in the background fluorescent intensity for empty liposomes observed in this experiment is the result of less favorable experimental arrangement rather than caused by a change of liposome permeability. This deviation from Fig 8 required from us to inspect the pH gradient dependence of the background, which was performed as shown in Suppl. Fig S7 below.



Supplementary Figure S7. The difference, Δ FI, between the fluorescence intensities recorded at 18 min waiting time and at zero time that is right after the transfer of liposomes to the quencher bath buffer for protein-free liposomes. These data were taken from independent control experiment in which three samples with pH gradient and tree samples without pH gradient were measured within 2 h after their preparation to ensure that liposome leakage was not sensitive to pH gradient. The fluorescence intensities were normalized by dividing to the larger of average value obtained for each set of data (with and without pH gradient). The effect on Δ FI caused by the gradient is clearly quite small and is well within the experimental error.