Supporting Information

Bergdoll et al. 10.1073/pnas.1715464115

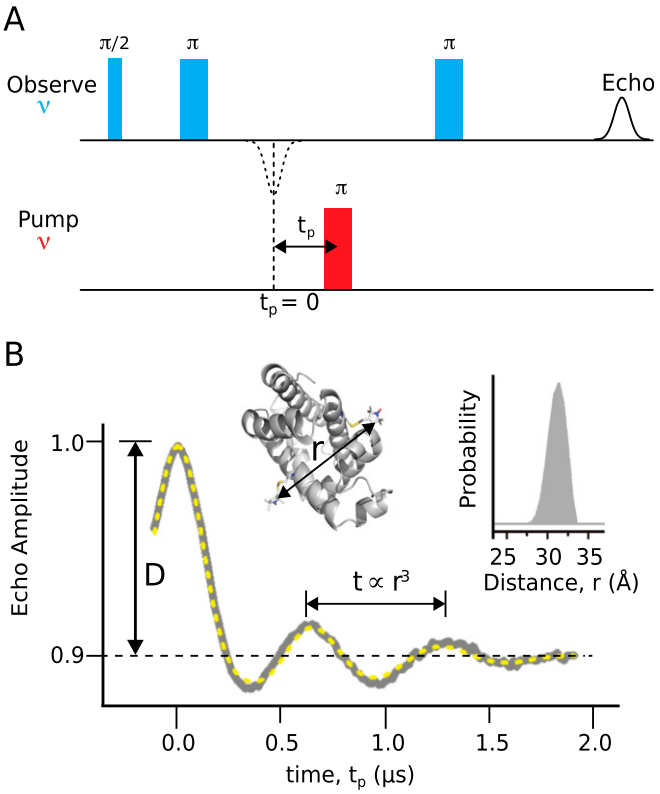

Fig. S1. The DEER experiment. (*A*) The four-pulse DEER sequence utilizes different frequencies (ν) for the observe (blue) and pump (red) pulses, corresponding to different positions in the spectral line shape and therefore separate spin populations. The observe pulses generate a refocused echo, and the echo amplitude is modulated as a function of the position of the pump pulse. The modulation depth (D), which is the amplitude of the DEER signal, reflects the number of pump spins interacting with each observe spin. In the case of protein dimerization, an increase in dimers increases the fraction of observe spins coupled to a pump spin, thus resulting in an increase in modulation depth. (*B*) The period of oscillation (t) in the time domain DEER data are a function of the cube of the interspin distance (r^3). The background-corrected dipolar evolution data (solid gray trace) may be fit (yellow trace) to determine the distance distribution for the coupled spins (*Inset*). For DEER experiments on proteins, typically site-directed spin labeling is used to attach side chains containing a stable unpaired electron in the form of a nitroxide.

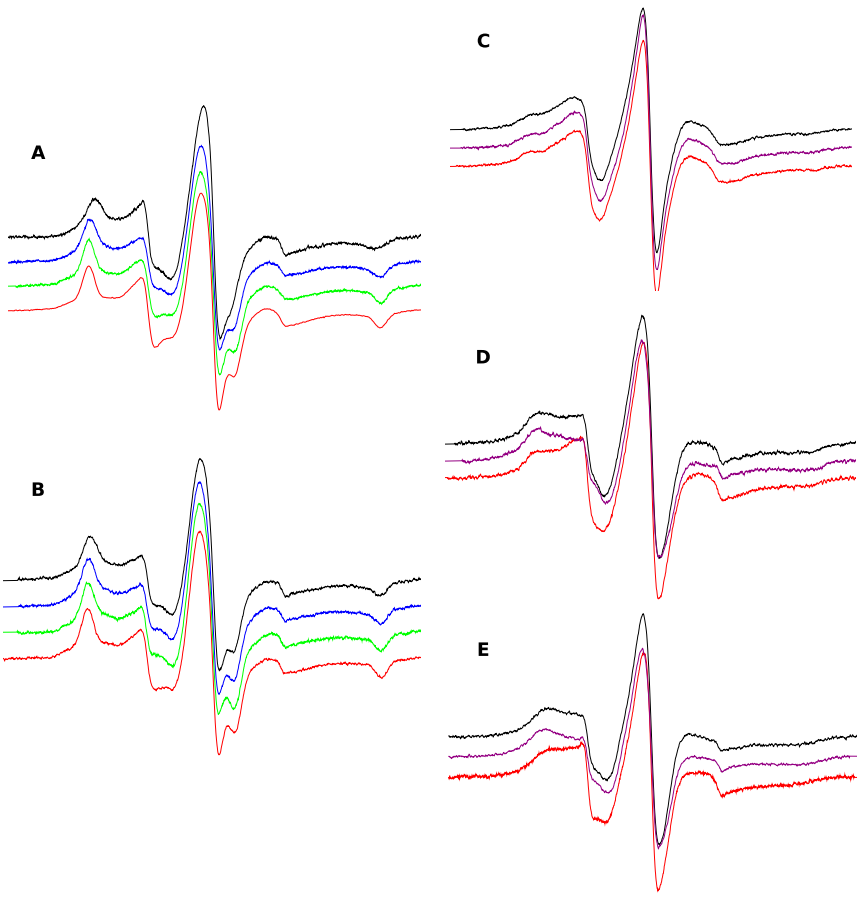

Fig. S2. Comparison of normalized room temperature CW EPR spectra at various pH. CW EPR spectra of D100R1 at pH 8 (black), pH 7 (blue), pH 6 (green), and pH 5 (red) in both (*A*) LDAO and (*B*) bicelles. CW EPR spectra for (*C*) D16R1 (*D*) K28R1, and (*E*) A141R1 in LDAO at pH 8 (black) and pH 5 (red) and in bicelles at pH 5 (purple). Data are vertically offset for clarity.

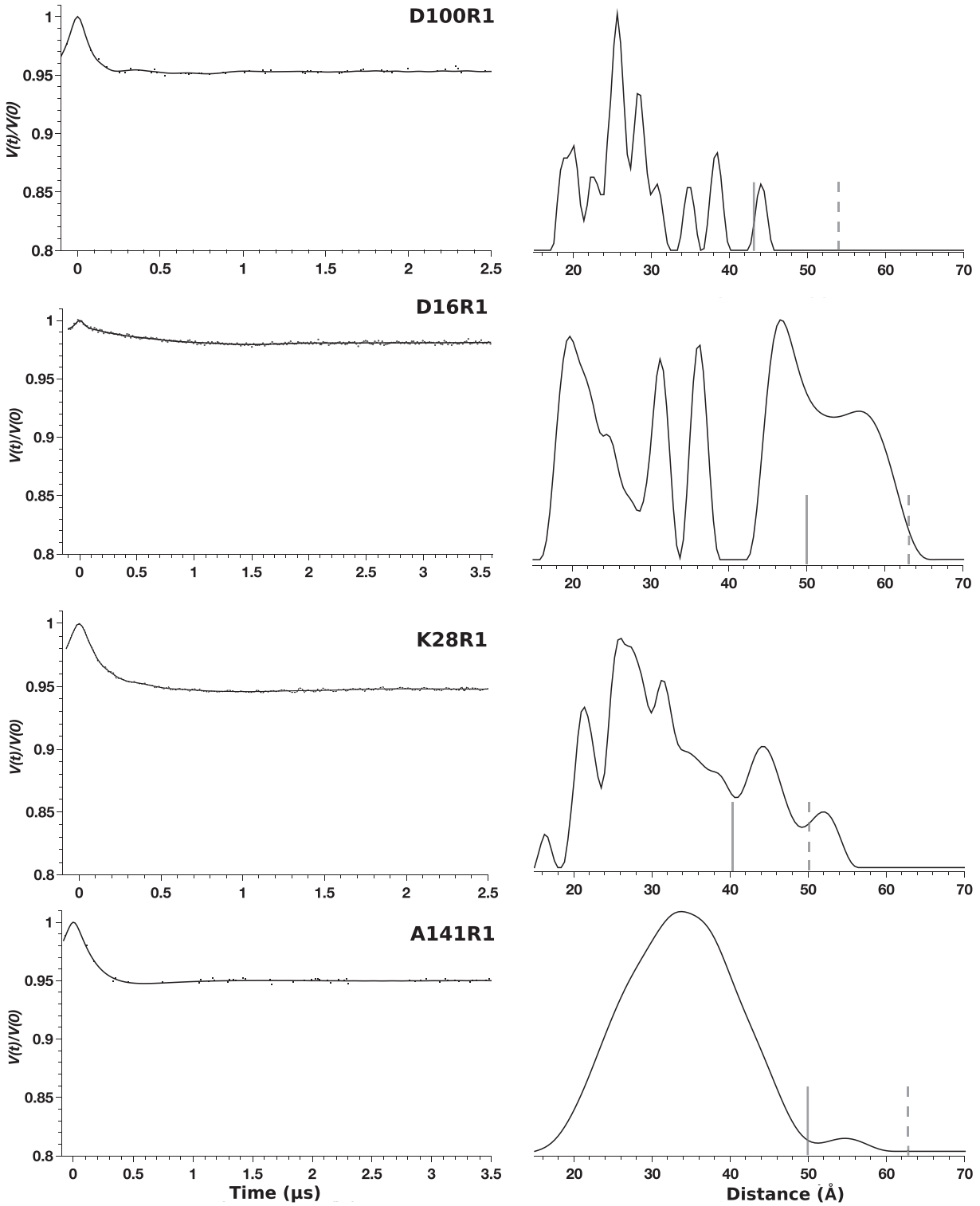

Fig. S3. pH 8 reveals multiple oligomeric forms of mVDAC1. (*Left*) Normalized, background-corrected dipolar evolutions. (*Right*) Normalized DEER distance distributions. The vertical bars indicate the upper limit of reliable width (solid line) and position (dotted line) of the distances in the distribution (*Materials and Methods*).

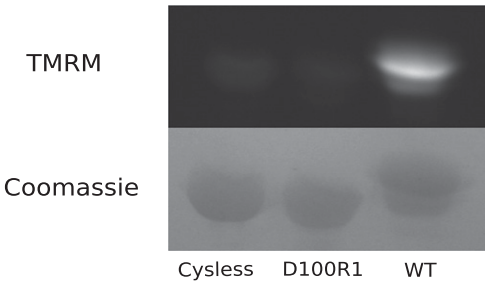

Fig. S4. TMRM assay shows full labeling for the D100R1 mutant. TMRM is a fluorescent label that binds to free Cysteine residues. Purified mVDAC1 (10 μg) was incubated with a 10-fold molar excess of TMRM for 15 min at room temperature. The reaction was stopped by the addition of SDS/PAGE loading buffer. The samples were run on a 12% SDS-polyacrylamide gel. Detection of TMRM was carried out using UV light. mVDAC1 cysless and labeled D100R1 samples displayed no fluorescence because no Cys residues were available to react with TMRM, revealing a full labeling of the spin-labeled D100R1.

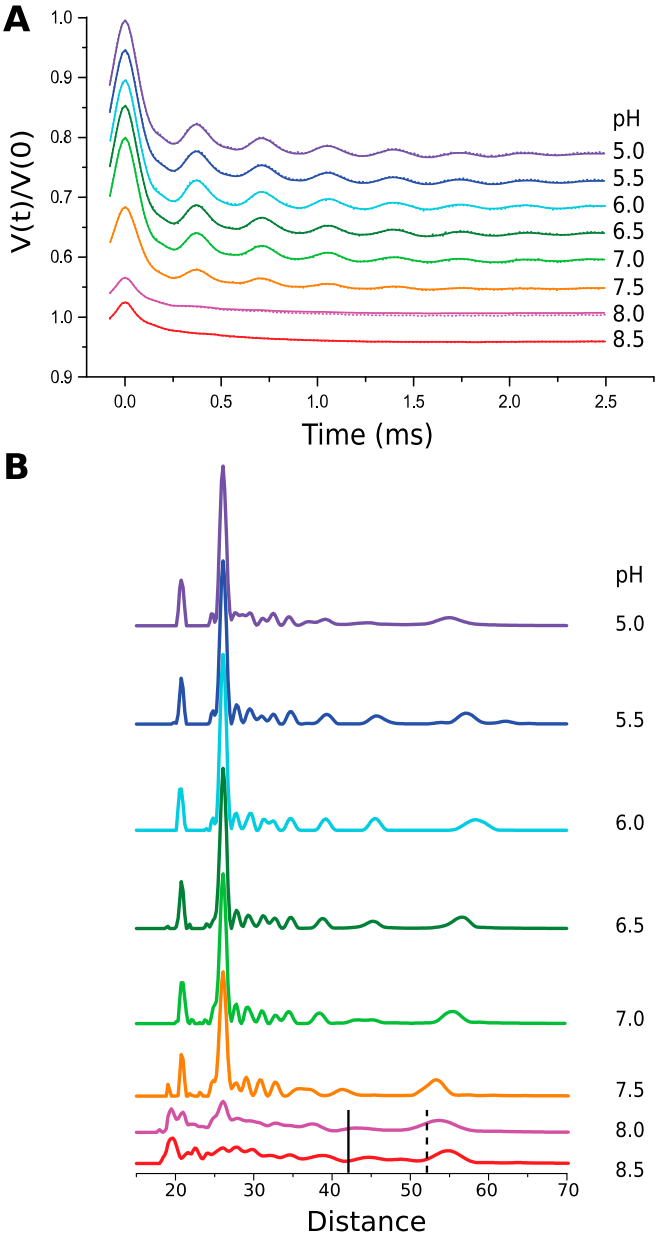

Fig. S5. mVDAC1 D100R1 titration reveals a dimerization induced by pH. (*A*) Normalized, background-corrected dipolar evolutions. These modulation depths were used for the titration curve displayed in Fig. 3. (*B*) Normalized DEER distance distribution. The titration was performed at eight different pH values each having a protein concentration of 100 μM. Data are vertically offset for clarity. The vertical bars indicate the upper limit of reliable width (solid line) and position (dotted line) of the distances in the distribution (*Materials and Methods*).

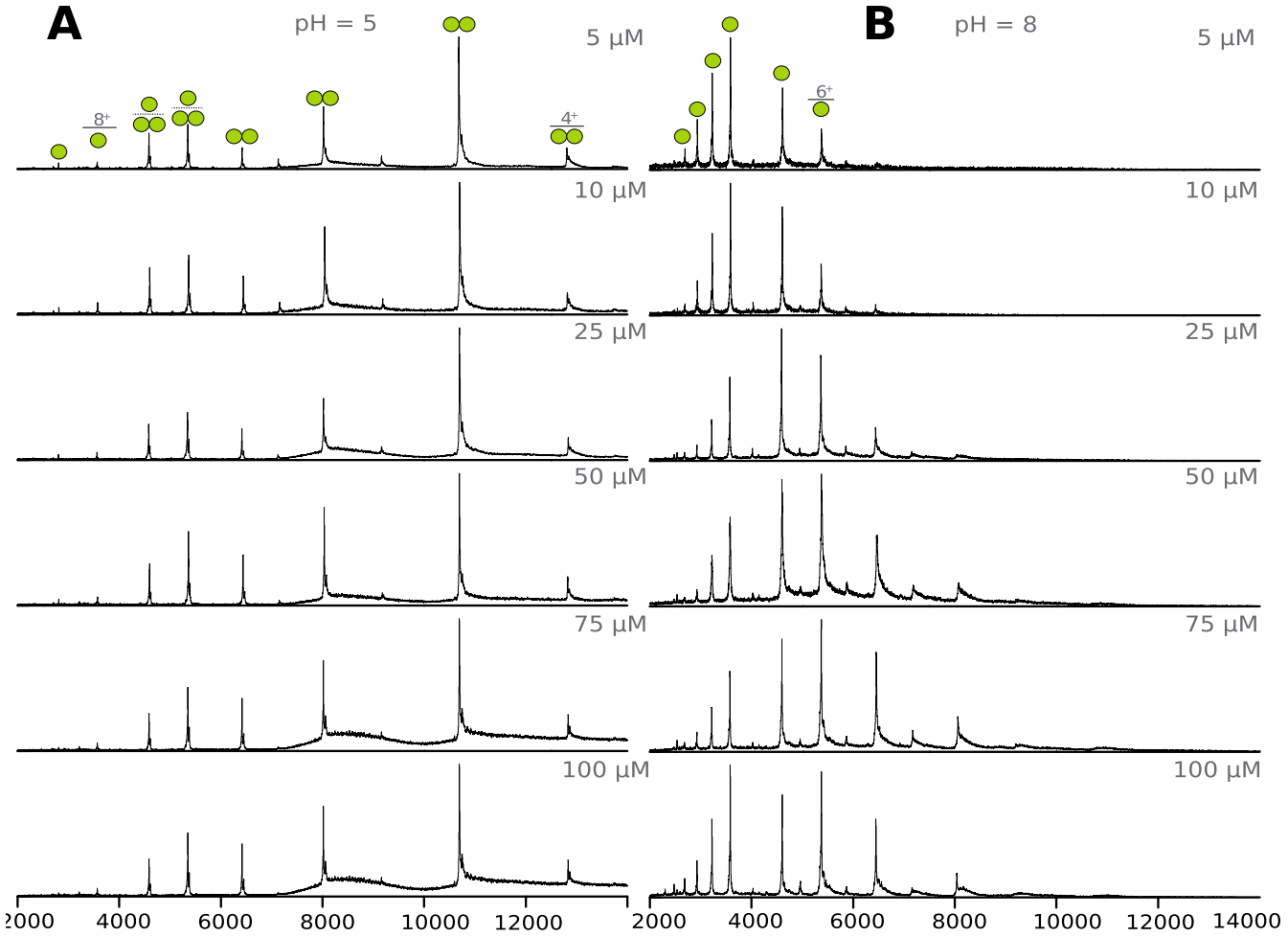

Fig. S6. Native mass spectra for VDAC1 D100R1 acquired at different protein concentrations. (*A*) Native mass spectra recorded at pH 5.0. (*B*) Native mass spectra recorded at pH 8.0. Labeled are the ions corresponding to either monomer (one sphere) or dimer (dual spheres) and total protein concentration.

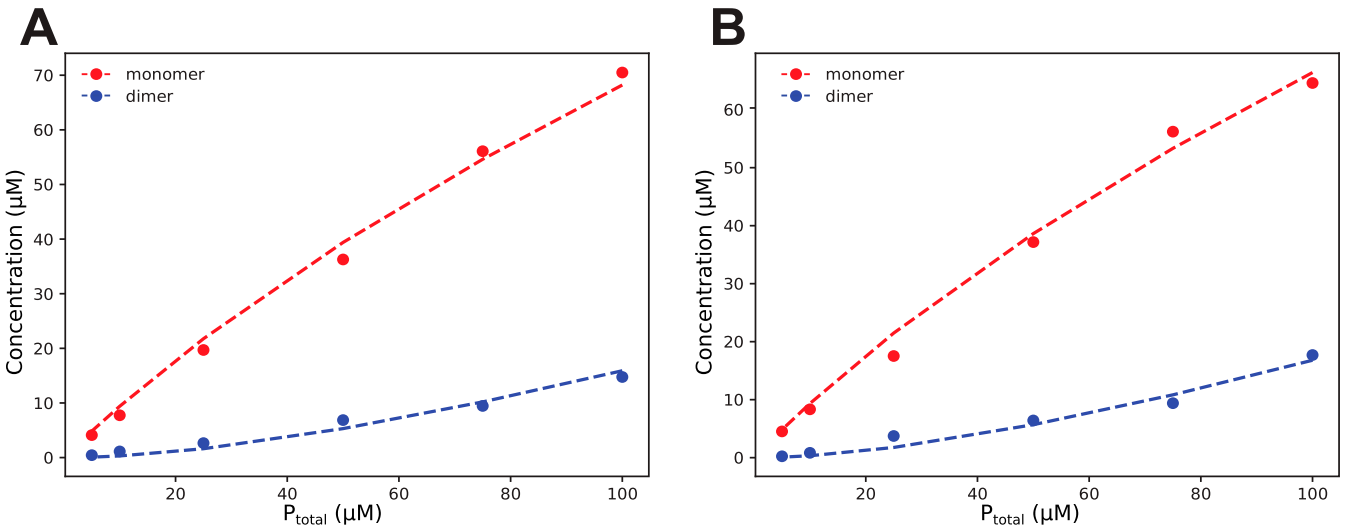

Fig. S7. Plot of monomer and dimer concentration of VDAC1 D100R1 as function of total protein concentration (P_{total}) at (*A*) pH 6 and (*B*) pH 7. Shown are the experimental concentrations (dots) and resulting fit from a monomer–dimer binding model (dashed lines). Fitting results are reported in Table S1.

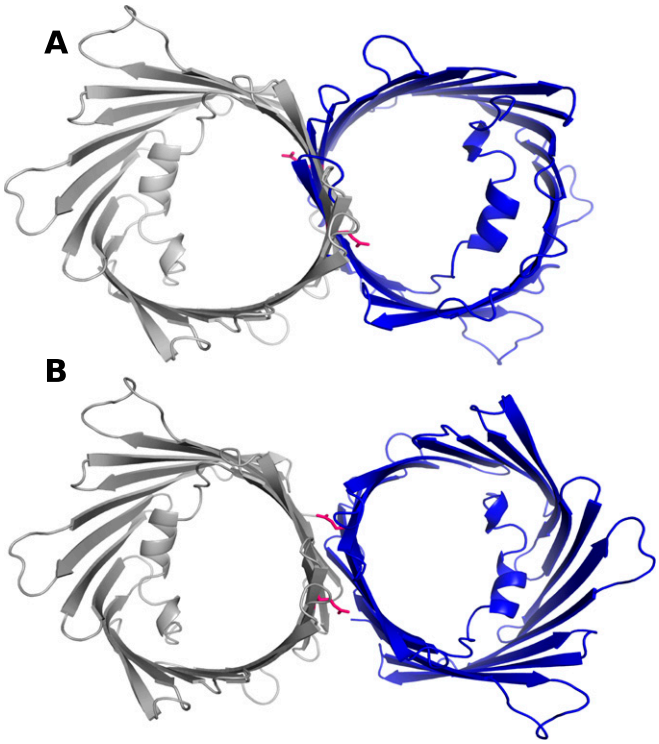

Fig. S8. Comparison of antiparallel vs. parallel model built with DEER data. Both models were generated without taking into account steric overlap through surface complementarity. (*A*) The antiparallel dimer reveals unreasonable overlap between the two channels and still does not satisfy the DEER distance values. (*B*) The parallel model built solely with the DEER data is nearly identical to the model displayed in Fig. 5.

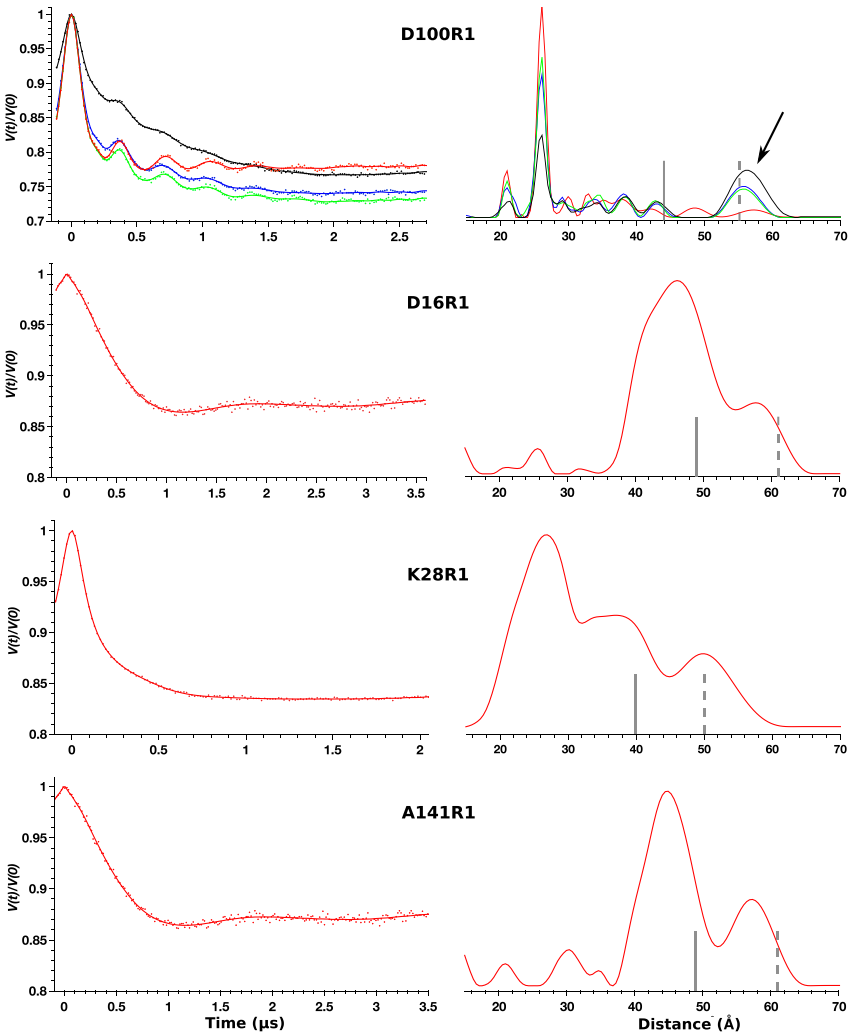

Fig. S9. Acidification induces oligomerization of mVDAC1 in a lipidic environment. (*Left*) Normalized, background-corrected dipolar evolutions. (*Right*) Normalized DEER distance distribution. mVDAC1 D100R1 in DMPC:CHAPSO bicelles, at pH 8 (black), pH 7 (blue), pH 6 (green), and pH 5 (red). The arrow represents the dimer described for zfVDAC2 (11). mVDAC1 D16R1, K28R1, and A141R1 spin-labeled mutants in bicelles at pH 5. The vertical bars indicate the upper limit of reliable width (solid line) and position (dotted line) of the distances in the distribution (*Materials and Methods*).

Table S1. Equilibrium dissociation constants (K_D) and fitting results for VDAC D100R1 at different pH values derived from native mass spectrometry

pH	K_D, µM	χ^2	R^2
5.0	32	1.52×10^{-10}	0.94
6.0	293	3.09×10^{-11}	0.99
7.0	263	3.80×10^{-11}	0.99
8.0	247	9.75×10^{-12}	1.00

Table S2. Comparison between experimental values and generated dimer models of mVDAC1

Mutant	Measured distance, Å	Parallel model, Å	Antiparallel model, Å
D16	46.2	46.2	50.2
K28	26.0	26.0	27.0
D100	26.1	27.0	34.3
A141	47.9	47.6	50.6

Measured distances are the most probable distance obtained by DEER data at pH 5 (Fig. 2) and used to assess dimer models. The best parallel model [green (cross)] and antiparallel model [purple (circle)] are presented in Fig. 5*A*.