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Supporting information for article:

Structure of VDAC-tethered bilayer lipid membranes determined using neutron reflectivity

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S1. Neutron reflectivity (NR) curves and scattering length density profiles

Scattering length density (SLD) profiles are calculated from the composition space models described in the Methods section of the main text. The model NR curves are calculated from the SLD profile. Thus, the parameters of the composition space model determine the model NR curves, which are optimized to the experimental data. All error bars are 68% confidence intervals as determined by Poisson counting statistics. For clarity, error bars are not displayed for every data point.

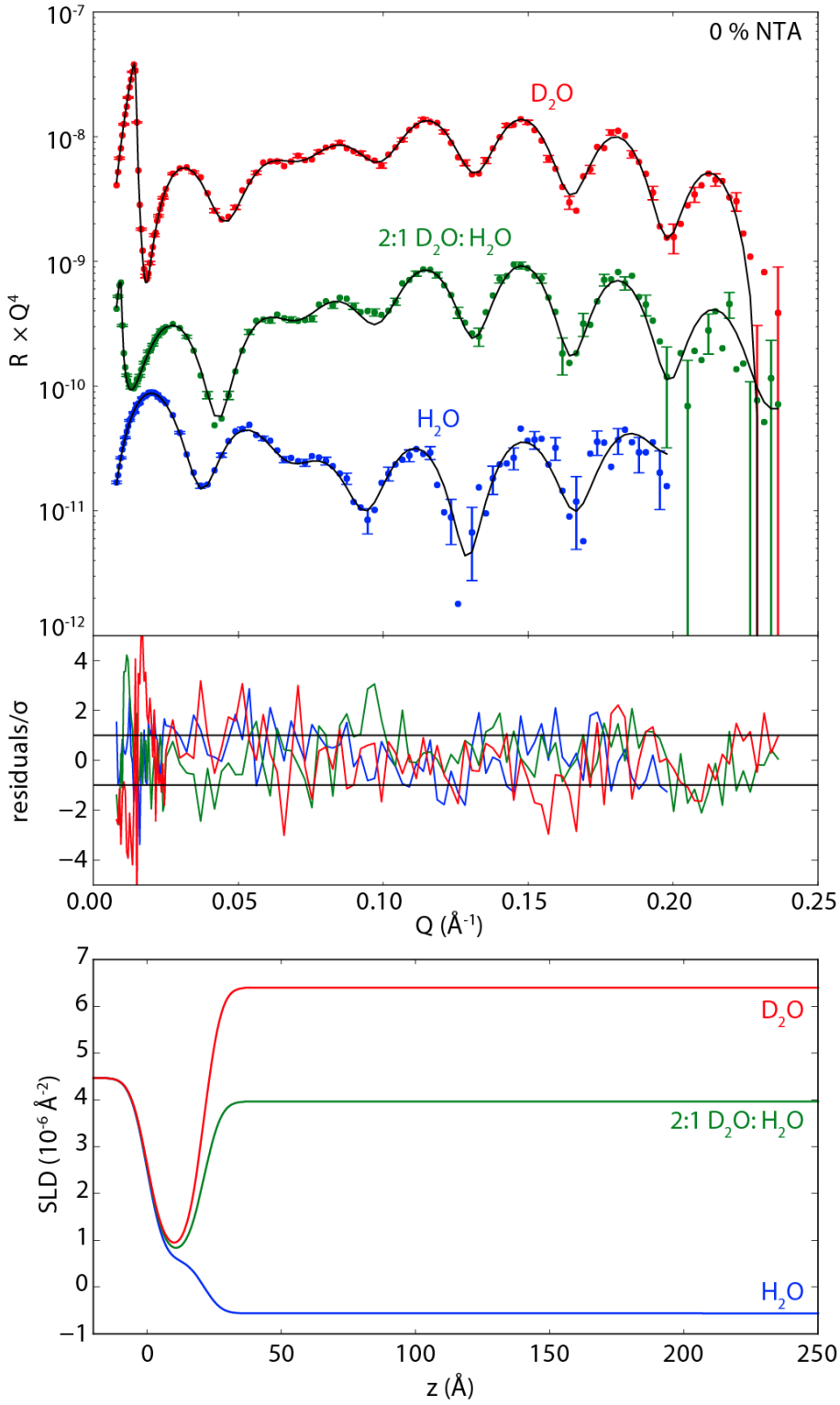

Figure S1 NR data, residuals, and SLD profiles for a 0% OEG-NTA (100% OEG) SAM.

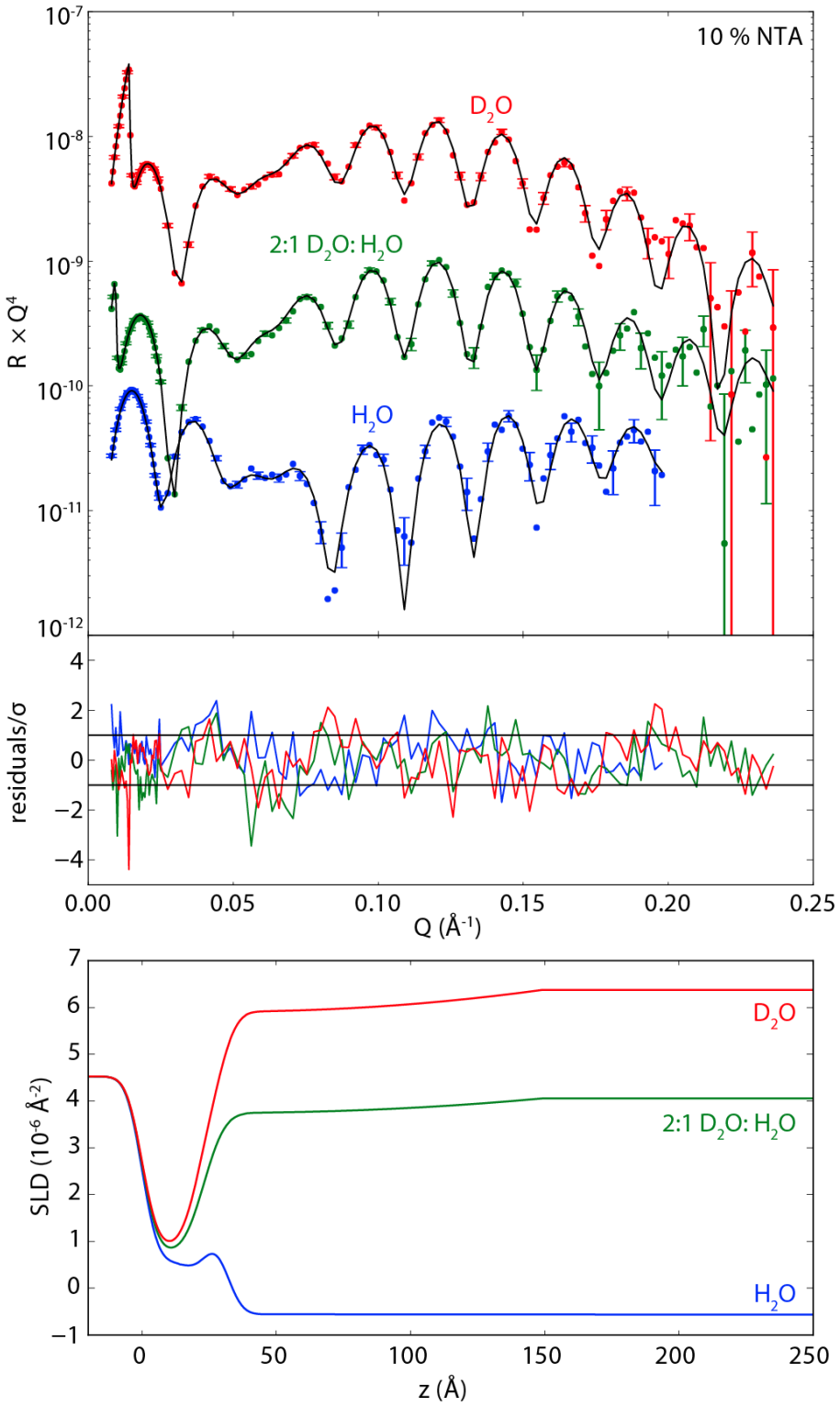

Figure S2 NR data, residuals, and SLD profiles for a 10% OEG-NTA (90% OEG) SAM.

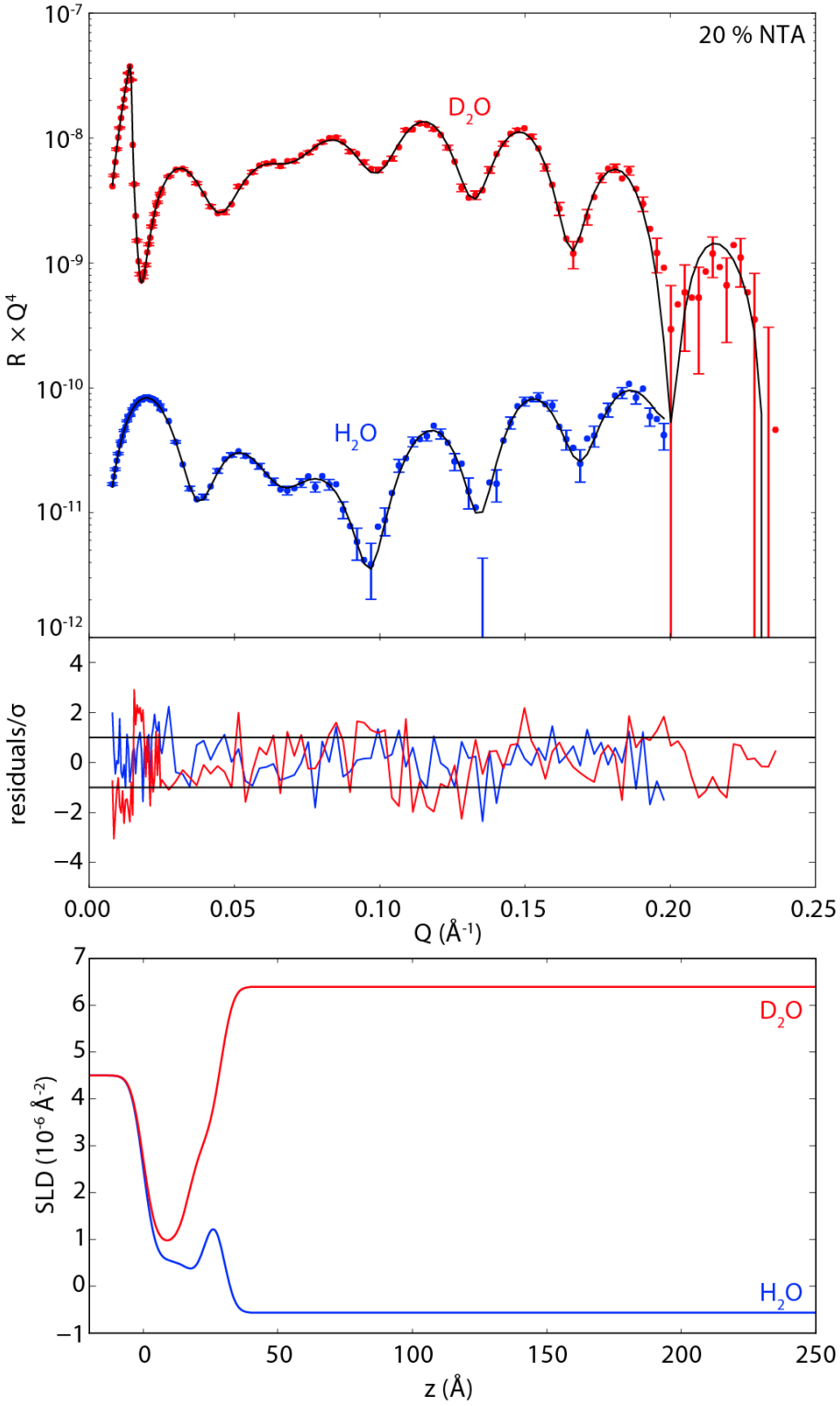

Figure S3 NR data, residuals, and SLD profiles for a 20% OEG-NTA (80% OEG) SAM.

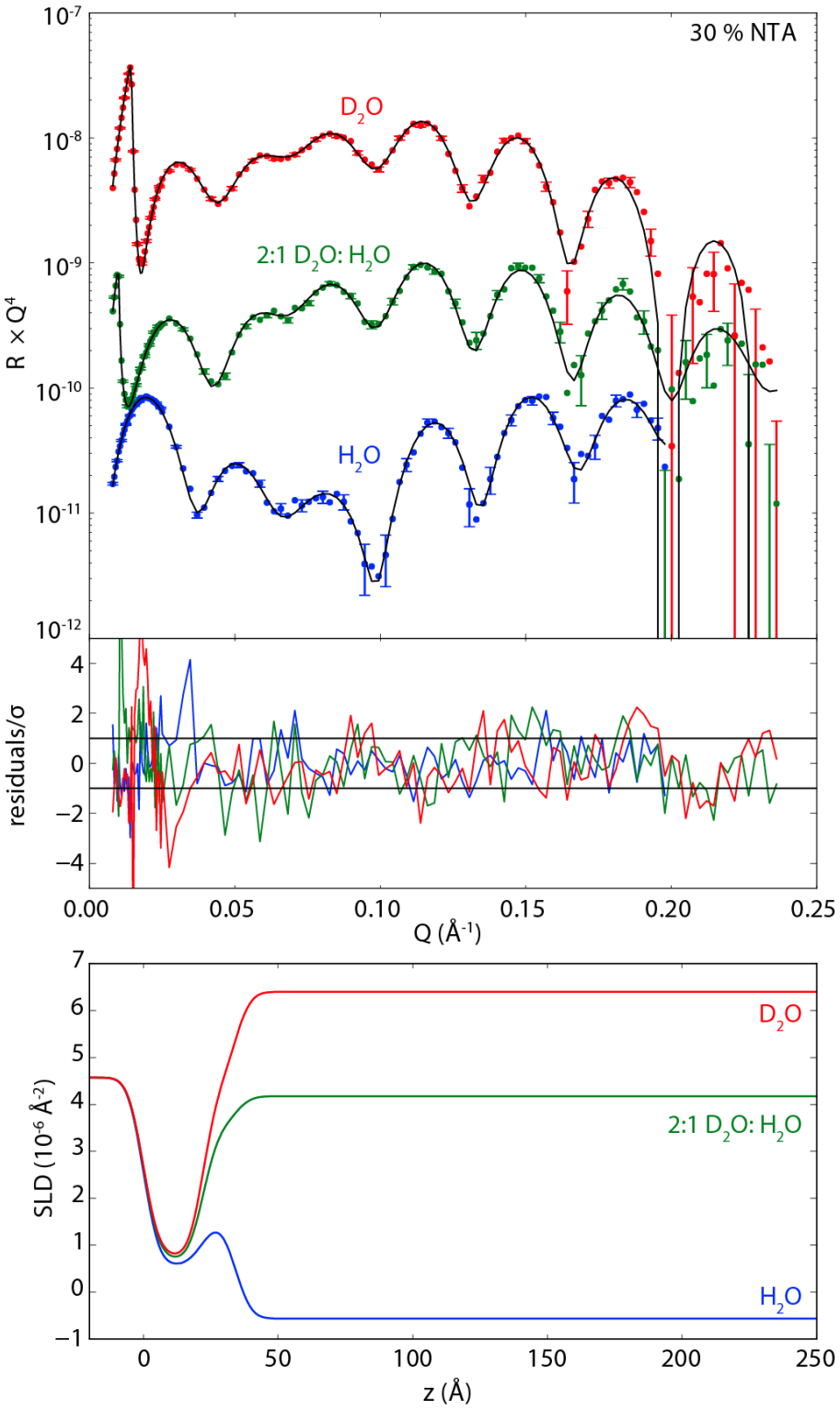

Figure S4 NR data, residuals, and SLD profiles for a 30% OEG-NTA (70% OEG) SAM.

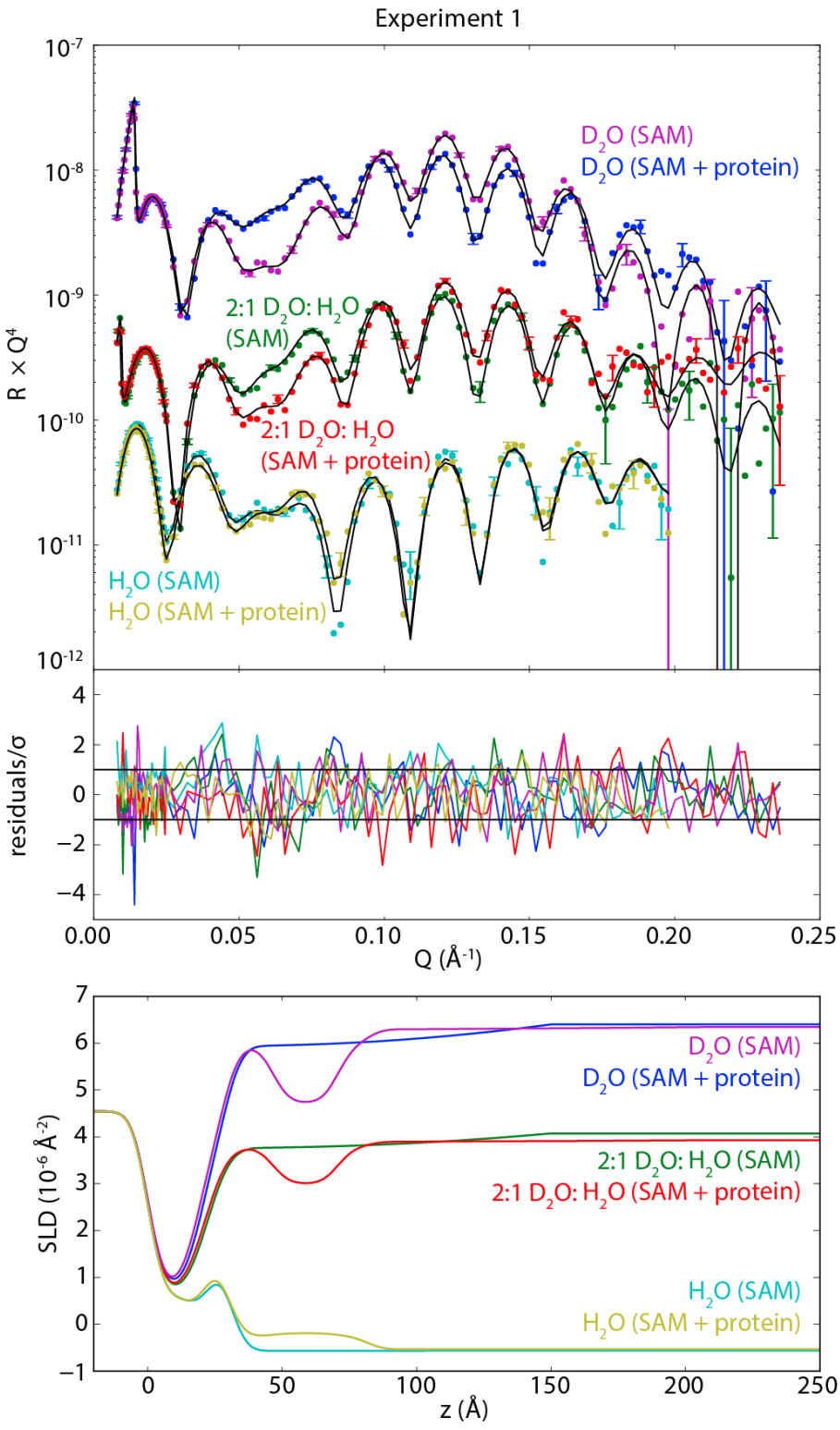

Figure S5 NR data, residuals, and SLD profiles for VDAC capture experiment 1.

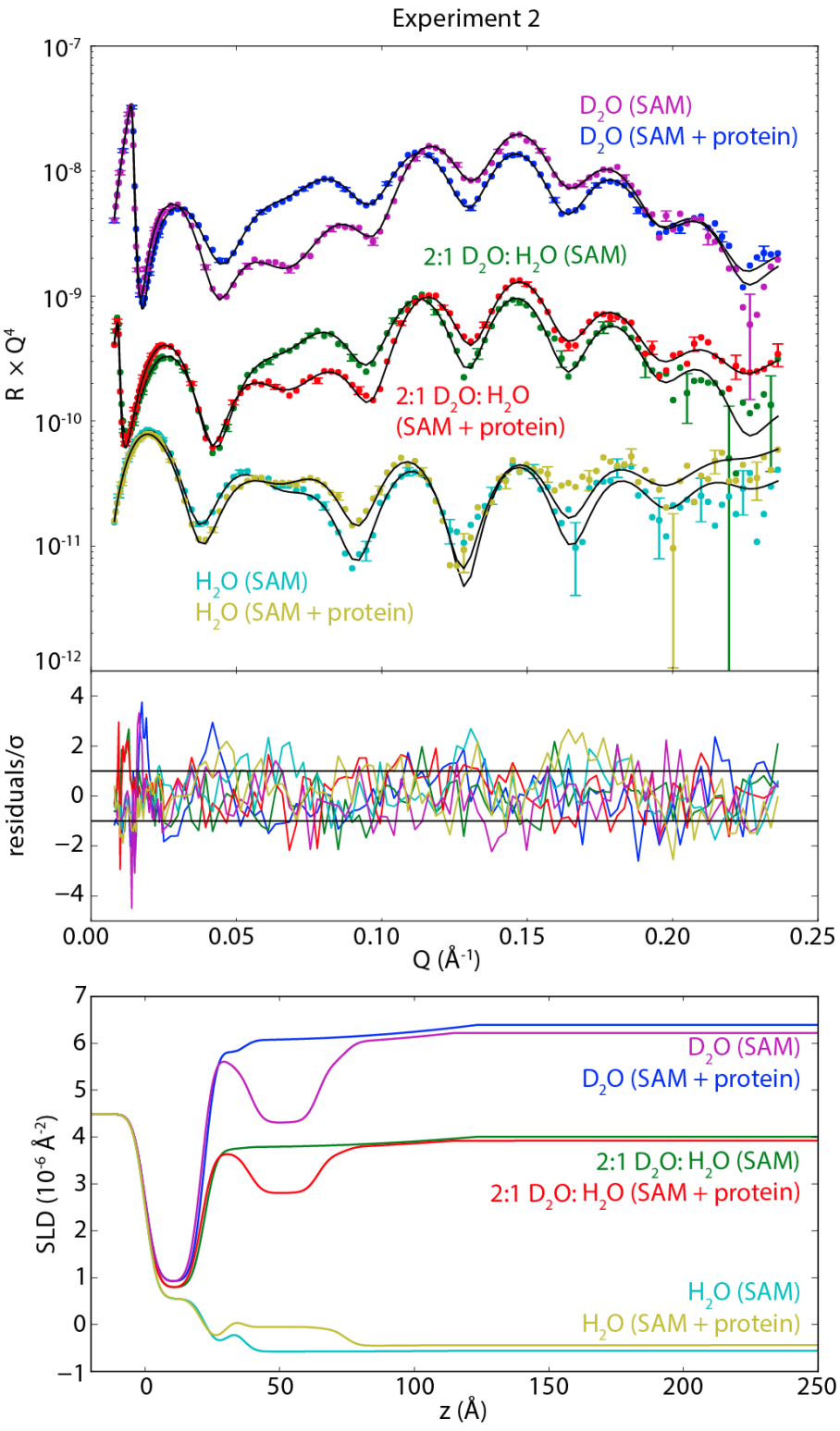

Figure S6 NR data, residuals, and SLD profiles for VDAC capture experiment 2.

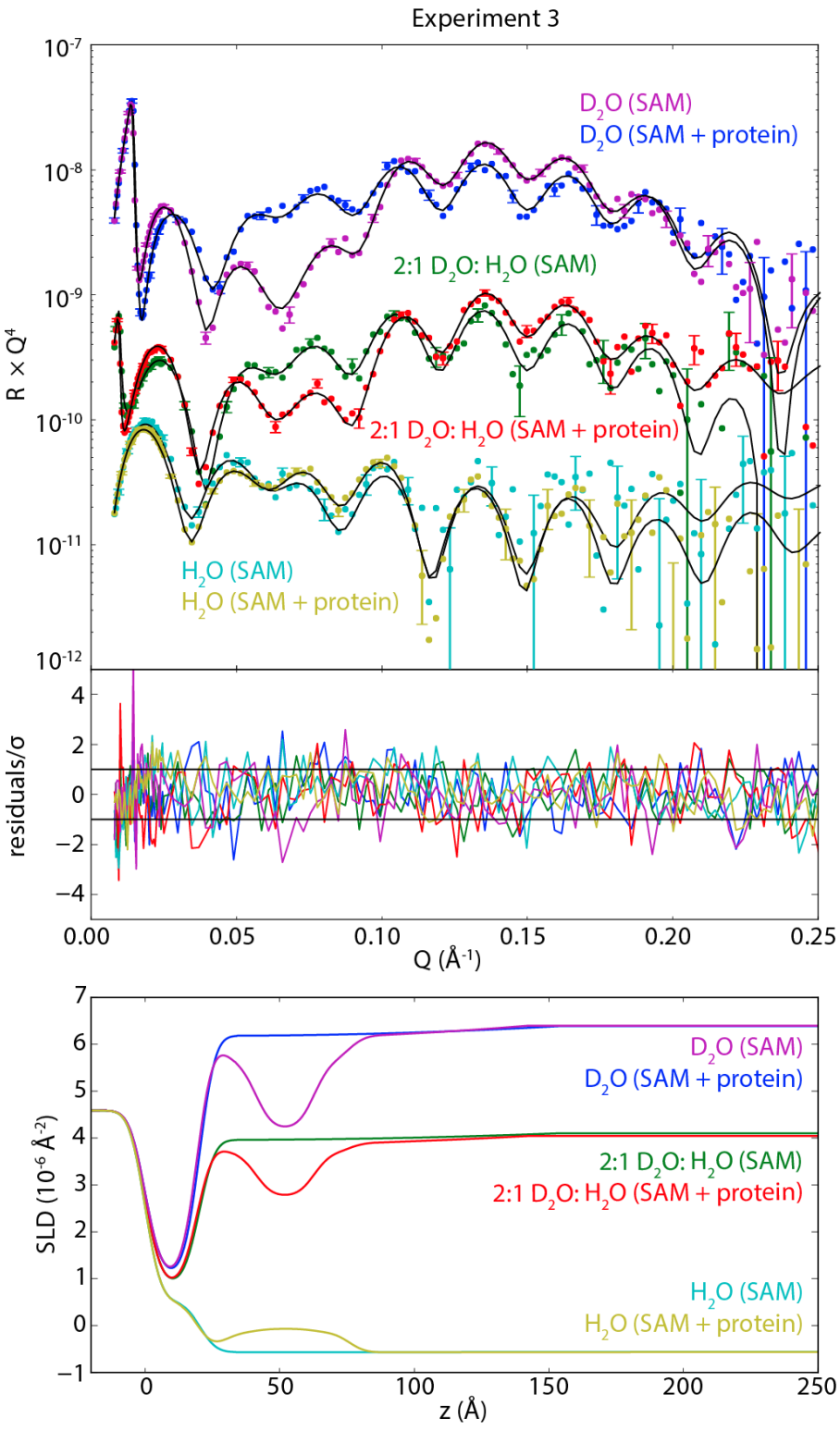

Figure S7 NR data, residuals, and SLD profiles for VDAC capture experiment 3.

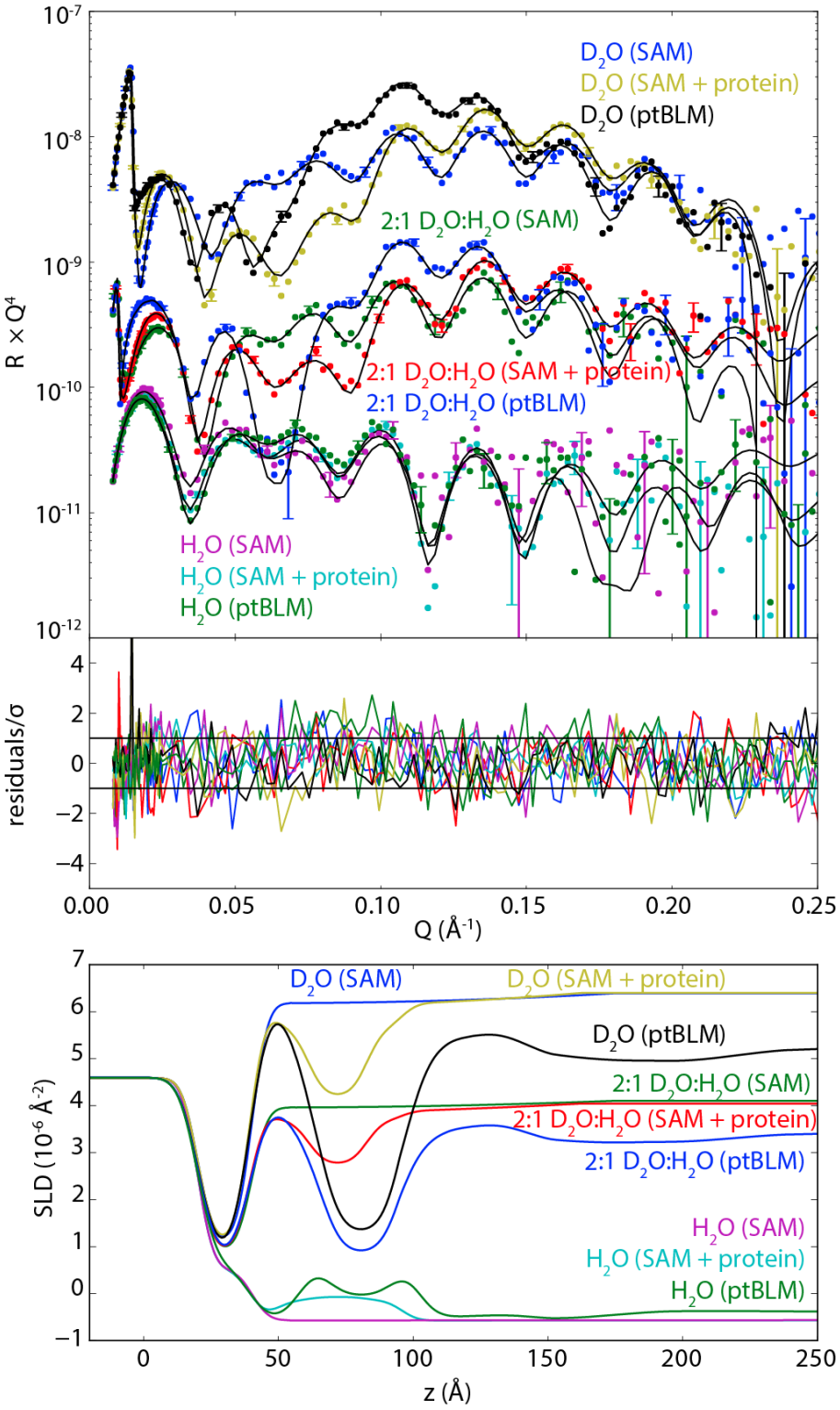

Figure S8 NR data, residuals, and SLD profiles for a complete VDAC reconstitution experiment.

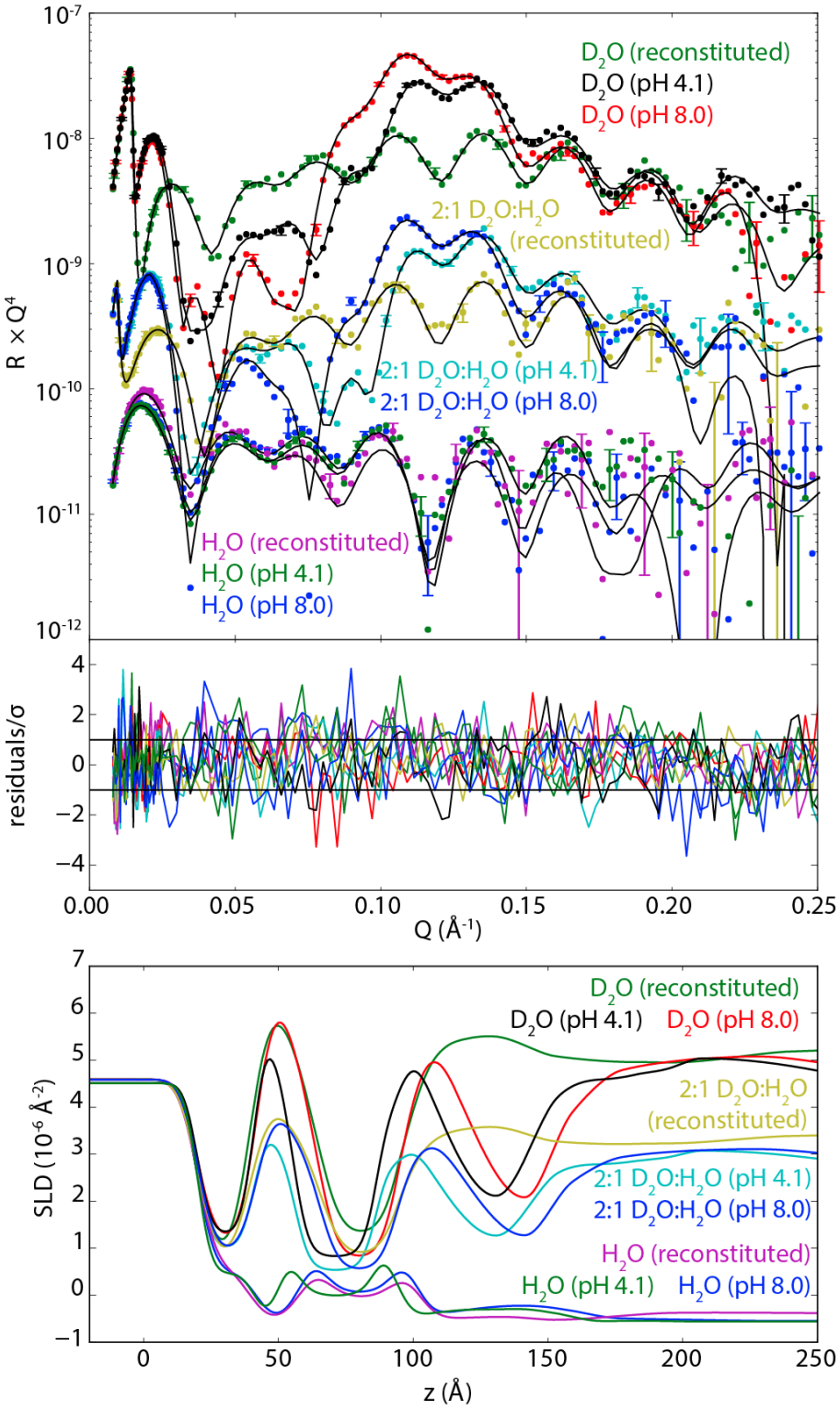

Figure S9 NR data, residuals, and SLD profiles for the reconstituted bilayer, showing differences at pH 4.1 and after return to pH 8.0.

S2. Substrate parameters for VDAC capture experiments

Table S1 Measured substrate parameters.

Si SLD was fixed at 2.071×10^{-6} Å^{-2}. Ranges are 95 % confidence intervals as calculated from the DREAM optimization algorithm.

Substrate parameters	**Experiment 1**	**Experiment 2**	**Experiment 3**
SiO_2 thickness (Å)	$20.8^{+3.4}_{-6.6}$	$12.5^{+8.9}_{-4.1}$	17^{+22}_{-9}
SiO_2 SLD ($\times10^{-6}$ Å^{-2})	$3.35^{+0.24}_{-0.05}$	$3.45^{+0.33}_{-0.25}$	$3.42^{+0.35}_{-0.12}$
SiO_2/Cr roughness (Å)	$2.4^{+1.6}_{-0.4}$	$3.9^{+1.2}_{-1.8}$	$2.4^{+1.6}_{-0.4}$
Cr thickness (Å)	$27.0^{+5.7}_{-4.1}$	$30.8^{+3.2}_{-7.7}$	28^{+10}_{-21}
Cr SLD ($\times10^{-6}$ Å^{-2})	$2.92^{+0.07}_{-0.12}$	$2.980^{+0.030}_{-0.063}$	$3.14^{+0.56}_{-0.79}$
Cr/Au roughness (Å)	$8.6^{+1.1}_{-0.9}$	$6.73^{+0.80}_{-0.58}$	$12.7^{+4.3}_{-3.0}$
Au thickness (Å)	$217.7^{+1.1}_{-0.7}$	$128.03^{+0.52}_{-0.35}$	$144.6^{+8.2}_{-6.9}$
Au SLD ($\times10^{-6}$ Å^{-2})	$4.541^{+0.014}_{-0.022}$	$4.495^{+0.012}_{-0.016}$	$4.578^{+0.015}_{-0.025}$
Au surface roughness (Å)	$4.88^{+0.35}_{-0.32}$	$3.54^{+0.39}_{-0.40}$	$4.67^{+0.42}_{-0.43}$

S3. Atomistic Molecular Dynamics (MD)

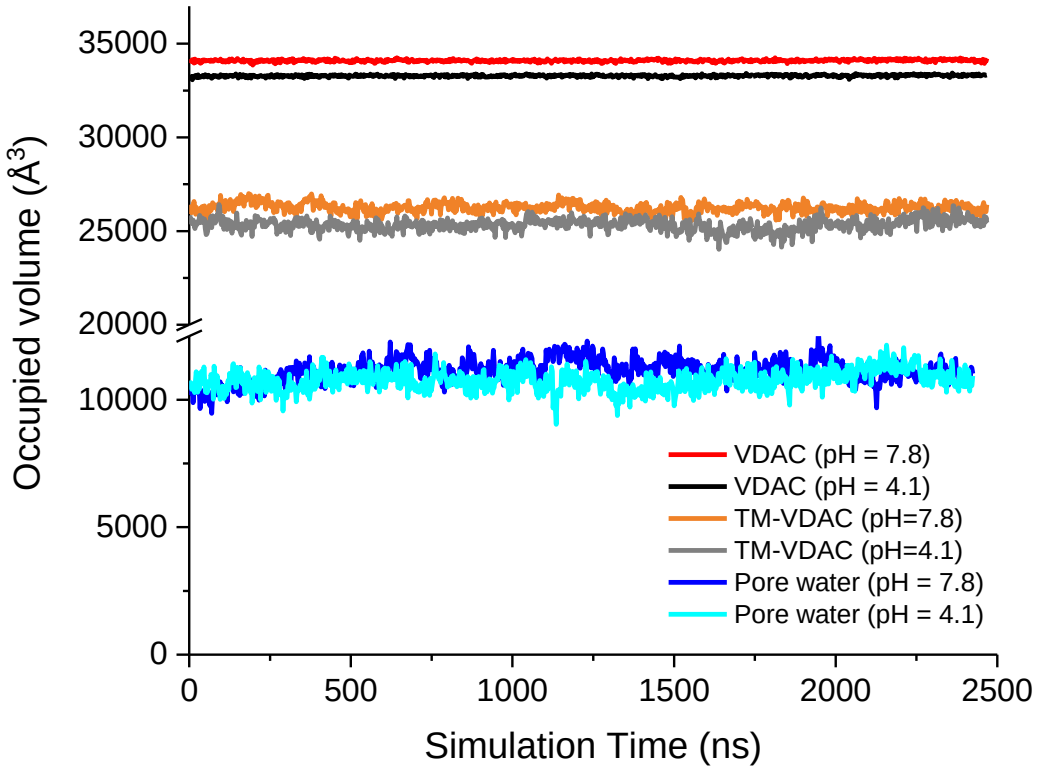

Figure S10 Molecular volumes determined by MD simulation. Total molecular volume of VDAC at pH 7.8 and pH 4.0 are shown as red and black curves, respectively. The total volume occupied by the trans-membrane section of VDAC (TM-VDAC) is shown for pH 7.8 (orange) and pH 4.1 (gray), respectively. The TM region was defined for consistency with NR models as ± 13.5 Å from the bilayer center. The total volume of water molecules confined in the TM-VDAC pore lumen are also shown for pH 7.8 (blue) and pH 4.0 (aqua-blue), respectively. The time-traces shown were calculated from 1000 frames evenly spaced in the 2.5 micro-seconds long equilibrium MD simulations. Each frame has been re-centered and re-oriented relative to the X-ray structure of VDAC protein. The occupied volumes were calculated using vdW radius (CHARMM-36) for protein or water atoms. The 3D grid search was used with grid covering entire simulation system. The grid resolution was set to 0.1 Å.

Table S2 Volume of molecular components in Figure S10.

TM-VDAC and pore water volumes are calculated between ± 13.5 Å from the bilayer center (so that the average area over the bilayer can be determined by dividing the volume by 27 Å), while the VDAC volume is the total protein volume.

Quantity	**pH 7.8**	**pH 4.1**
VDAC vdW volume (Å^3)	34098 ± 57	33288 ± 52
TM-VDAC vdW volume (Å^3)	26251 ± 259	25339 ± 323
Pore water vdW volume (Å^3)	11141 ± 401	10772 ± 401

S4. Electrochemical Impedance Spectroscopy

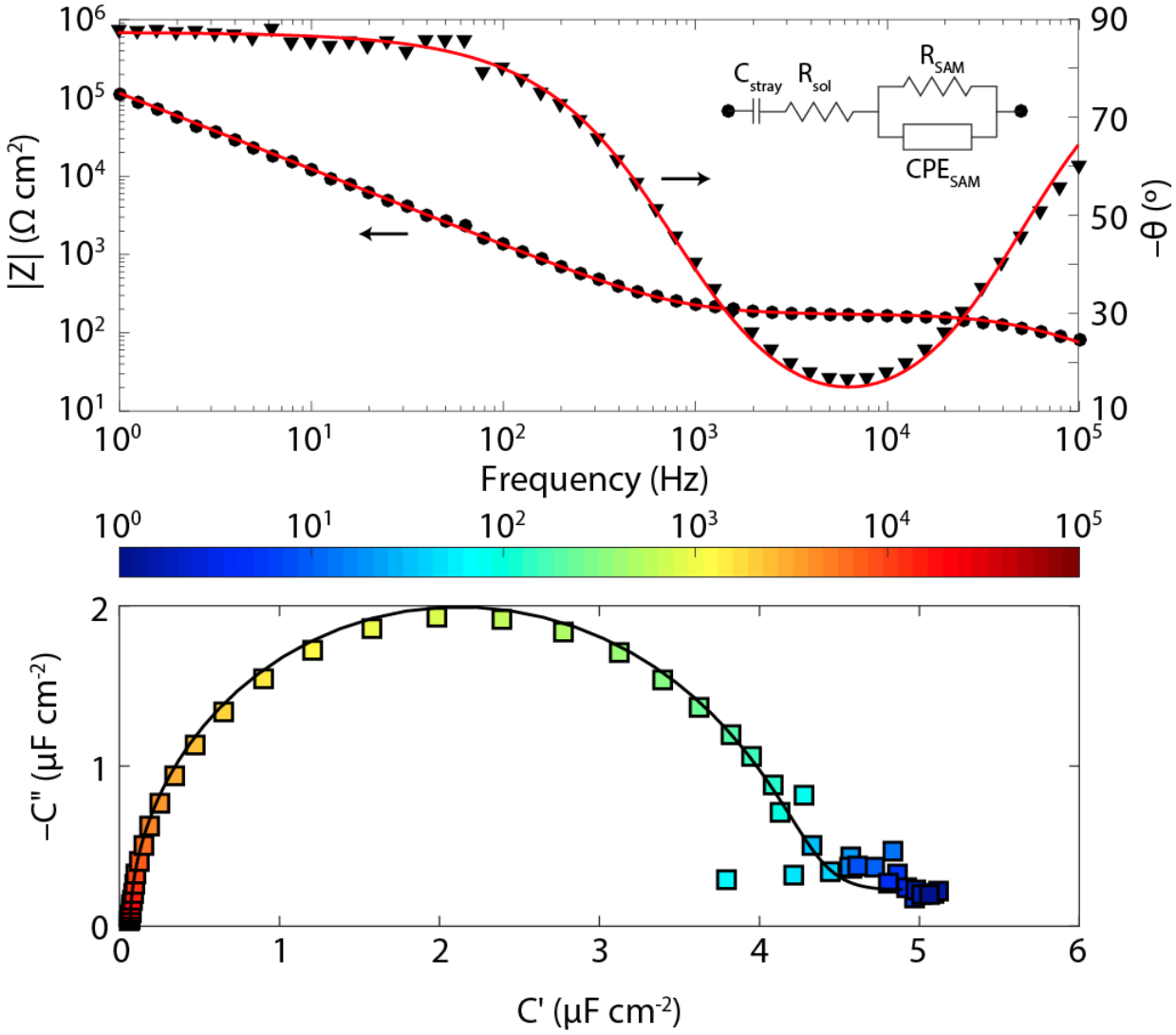

Figure S11 EIS spectra of a 30% OEG-NTA SAM. (Top) Impedance (left axis, circles) and phase angle (right axis, inverted triangles). At low frequencies the SAM is purely capacitive (phase angle near 90°) with a very large impedance (> 10^5 Ω cm^2). (Top inset) Equivalent circuit used to model the data (solid lines). "CPE" refers to a "constant phase element" that accounts for nonlinear diffusive processes, but for present purposes can be considered a simple capacitor. (Bottom) Cole-Cole representation, showing the capacitance of the SAM to be about 4 μF cm^{-2}.