Electronic Supplementary Information (ESI) for

DEERefiner-assisted Structural Refinement Using Pulsed Dipolar Spectroscopy: A Study on Multidrug Transporter LmrP

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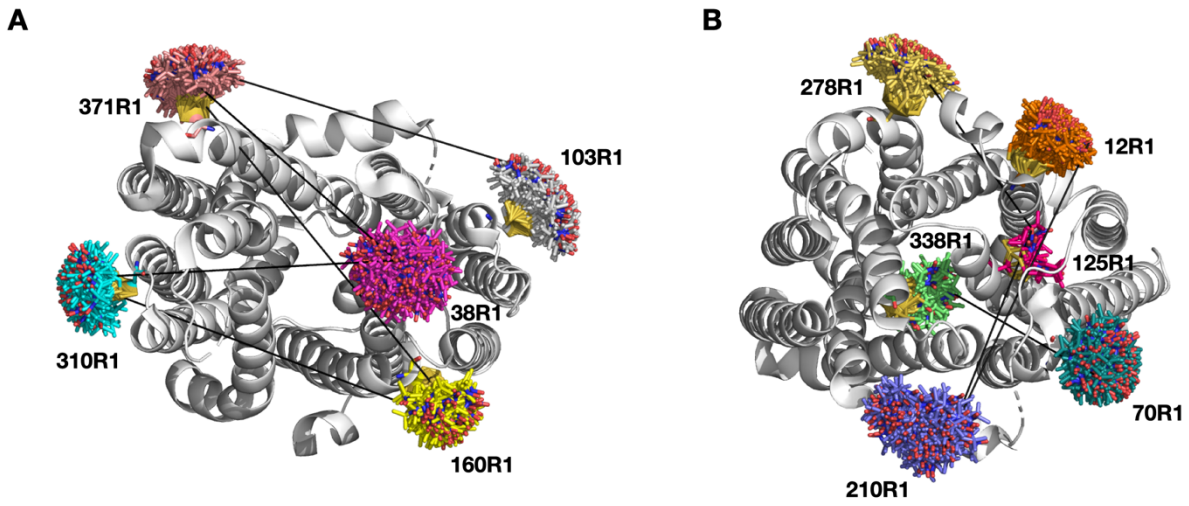

Figure S1. Cartoon models illustrating positions of spin-labeled residues and corresponding distance restraints. (**A**) 5 spin-labeled residues and 5 distance restraints (black solid line) at the extracellular side of LmrP. (**B**) 6 spin-labeled residues and 4 distance restraints (black solid line) at the intracellular side of LmrP.

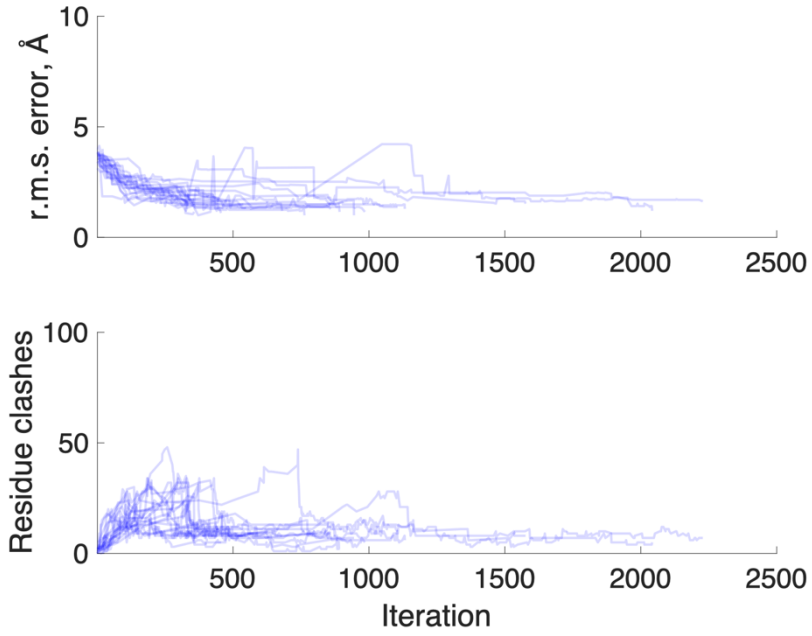

Figure S2. r.m.s. error and residue clashes evolutions of the 20 DEERefiner pH 8 models during computations. All computations reached convergence within 2500 iterations, with an average of 880 iterations.

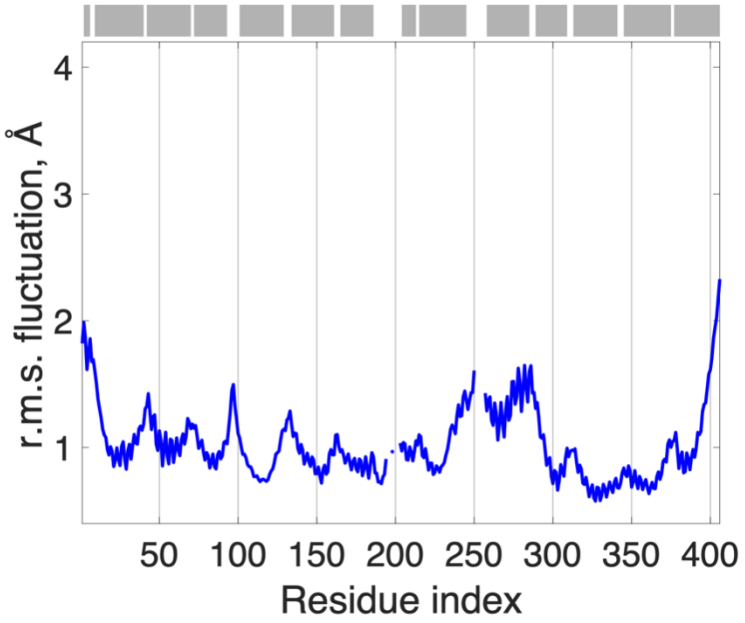

Figure S3. r.m.s. fluctuation of the protein C_α atom for the **20 DEERefiner pH 8 models.** The gray bars at the top show the regular α-helix structures.

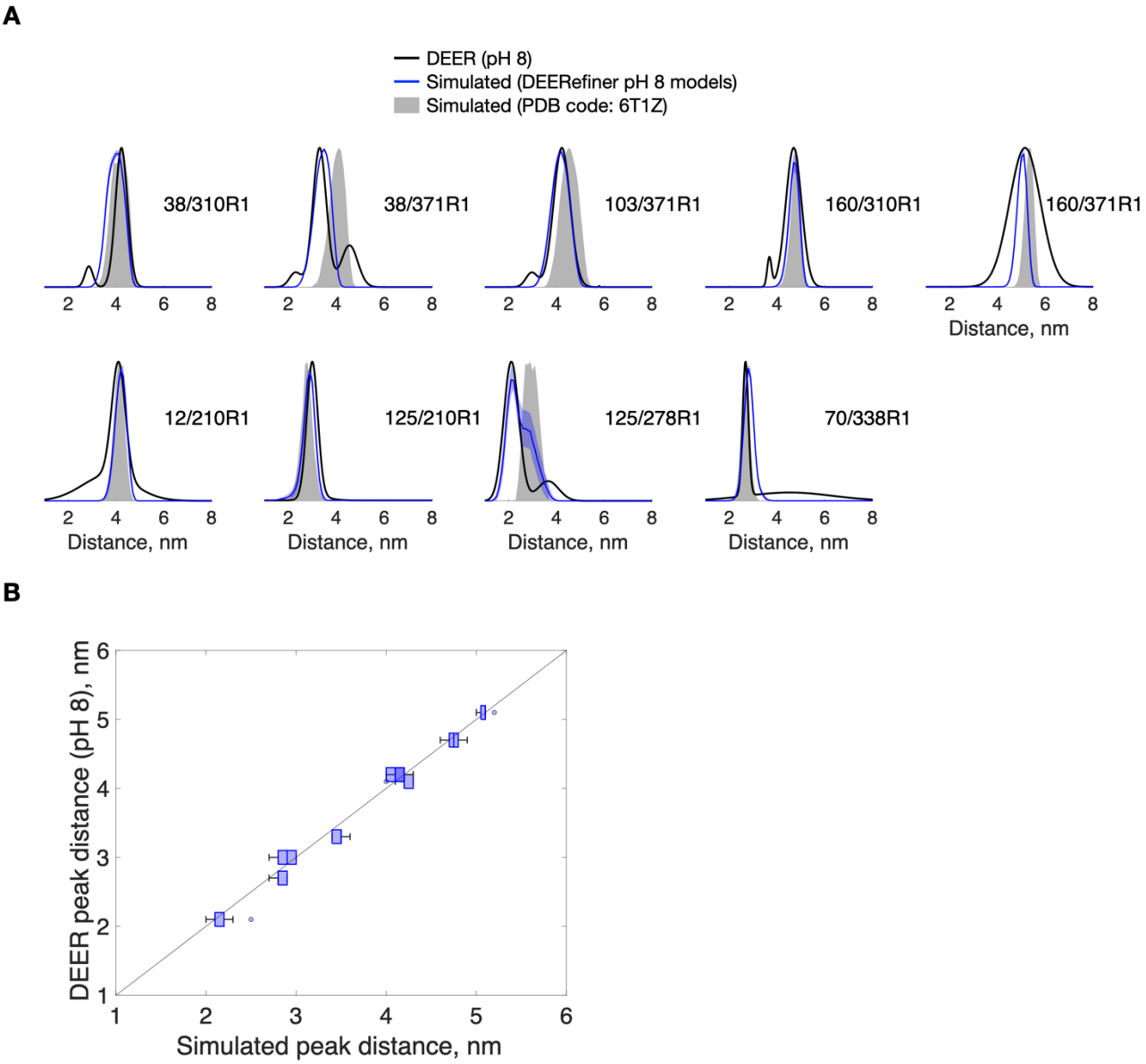

Figure S4. Consistency between experimental distance distributions and simulated distance distributions of the 20 DEERefiner pH 8 models. (**A**) Simulated distance distributions predicted from the crystal structure (gray shaded area), experimental DEER distance distributions (pH 8) taken from the previous study (black),[1] average simulated distance distributions predicted from the 20 DEERefiner pH 8 models (solid blue lines), and the resulting 95.4% confidence bands (2 standard error (SE) = $\sigma/\sqrt{n}$, blue shaded regions). (**B**) A correlation plot showing the relationship between experimental peak distances, as extracted from the DEER distance distributions collected at pH 8, and the simulated peak distances based on the 20 DEERefiner pH 8 models (as boxplot). The black line signifies x=y.

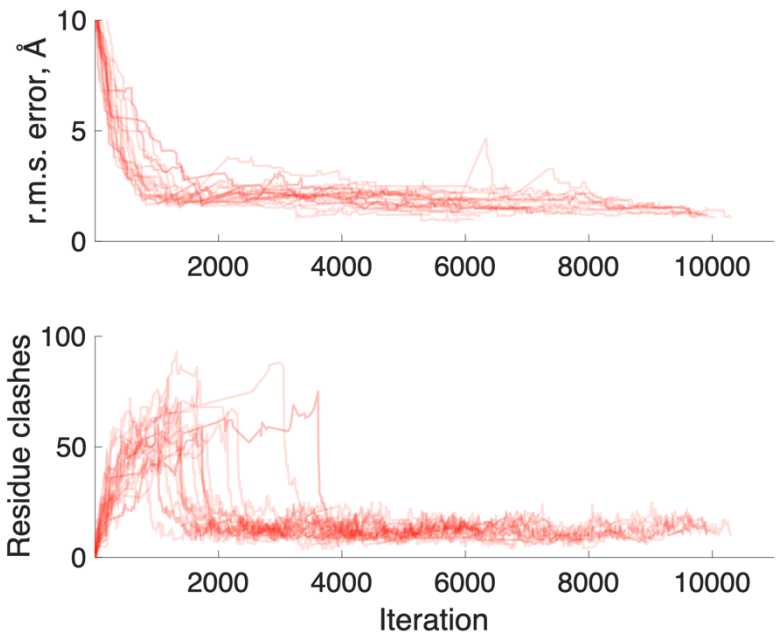

Figure S5. r.m.s. error and residue clashes evolutions of the 20 DEERefiner pH 5 models from crystal during computations. All computations reached convergence within 11000 iterations, with an average of 8082 iterations.

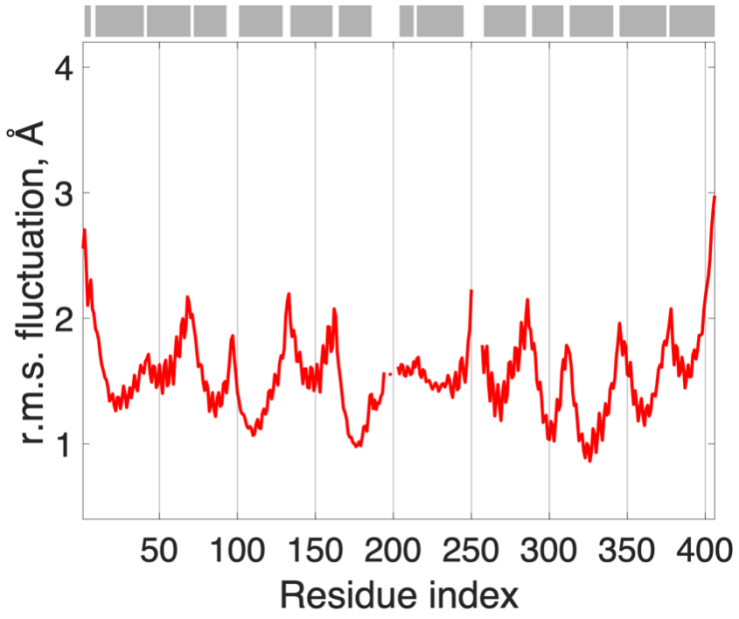

Figure S6. r.m.s. fluctuation of the protein C_α atom for the **20 DEERefiner pH 5 models from crystal.** The gray bars at the top show the regular α-helix structures.

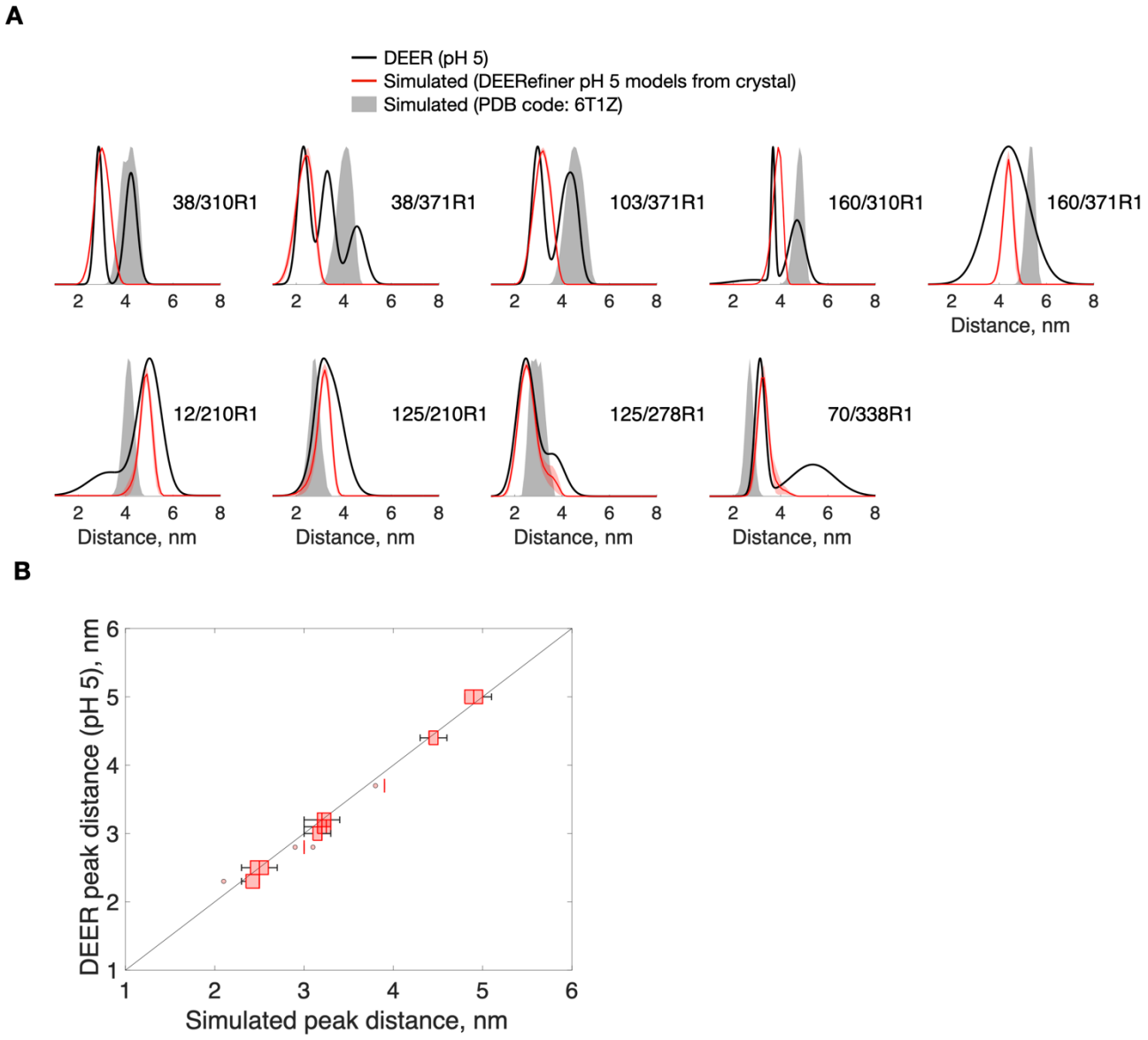

Figure S7. Consistency between experimental distance distributions and simulated distance distributions of the 20 DEERefiner pH 5 models from crystal. (**A**) Simulated distance distributions predicted from the crystal structure (gray shaded area), experimental DEER distance distributions (pH 5) taken from the previous study (black),[1] average simulated distance distributions predicted from the 20 DEERefiner pH 5 models from crystal (solid red lines), and the resulting 95.4% confidence bands (2 SE = $\sigma/\sqrt{n}$, red shaded regions). (**B**) A correlation plot showing the relationship between experimental peak distances, as extracted from the DEER distance distributions collected at pH 5, and the simulated peak distances based on the 20 DEERefiner pH 5 models from crystal (as boxplot). The black line signifies x=y.

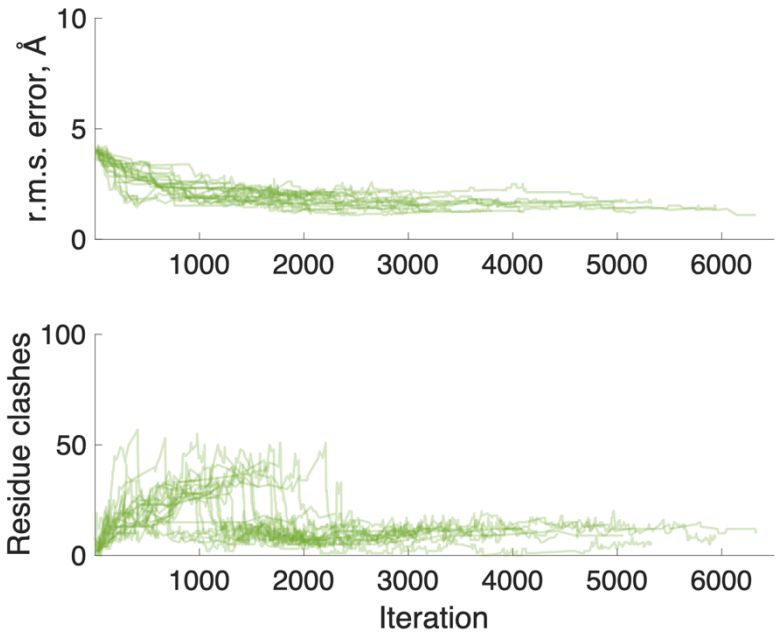

Figure S8. r.m.s. error and residue clashes evolutions of the 20 DEERefiner pH 5 models from AF during computations. All computations reached convergence within 7000 iterations, with an average of 3485 iterations.

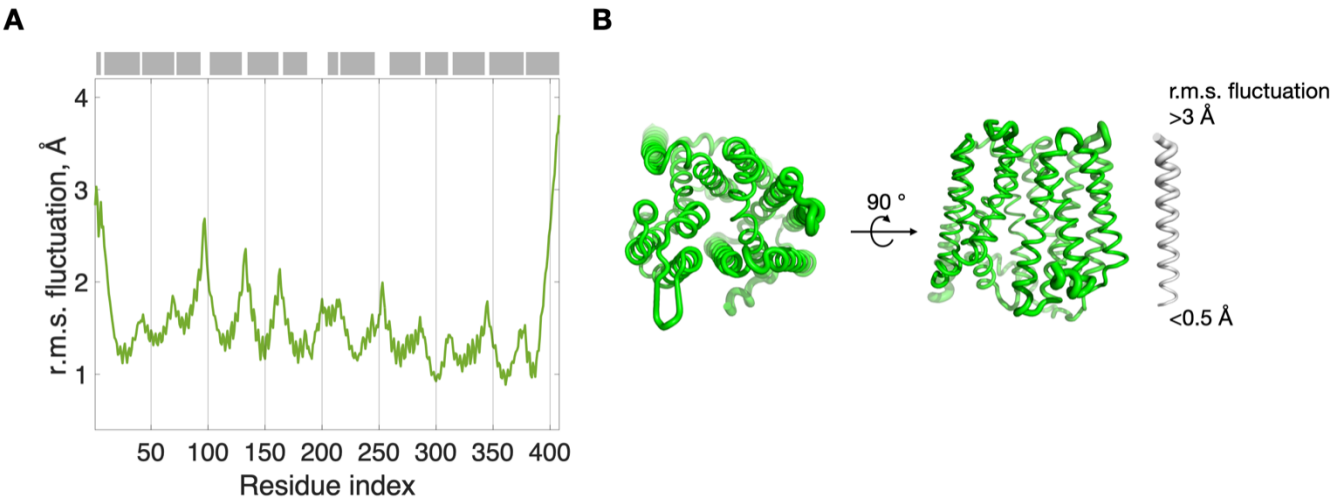

Figure S9. Precision analysis of the DEERefiner pH 5 models from AF. (**A**) r.m.s. fluctuation of the protein C_α atom for the 20 DEERefiner pH 5 models from AF. The gray bars at the top show the regular α-helix structures. (**B**) The DEERefiner pH 5 medoid model from AF with chain thickness representing the C_α r.m.s. fluctuation across 20 DEERefiner pH 5 models from AF.

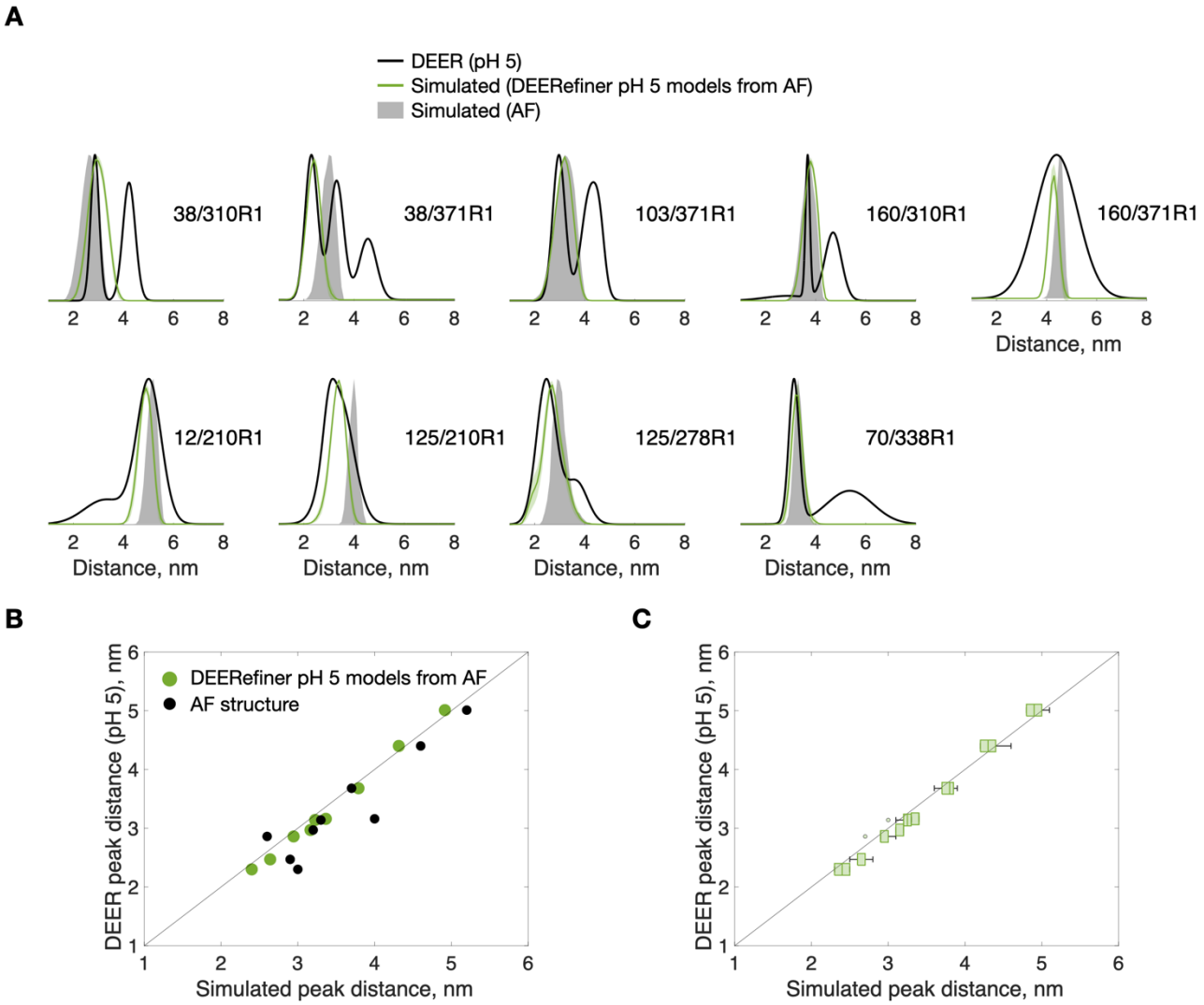

Figure S10. Consistency between experimental distance distributions and simulated distance distributions of the 20 DEERefiner pH 5 models from AF. (**A**) Simulated distance distributions predicted from the AF structure (gray shaded area), experimental DEER distance distributions (pH 5) taken from the previous study (black),[1] average simulated distance distributions predicted from the 20 DEERefiner pH 5 models from AF (solid green lines), and the resulting 95.4% confidence bands (2 SE = $\sigma/\sqrt{n}$, green shaded regions). (**B**) A correlation plot showing the relationship between experimental peak distances, as extracted from the DEER distance distribution collected at pH 5, and the average of simulated peak distances based on the 20 DEERefiner pH 5 models from AF (green filled circle) and the AF structure (filled black circle). The black line signifies x=y. Error bars (1 SE = $\sigma/\sqrt{n}$) of simulated peak distances based on the pH 5 ensemble from AF are within the symbol size. (**C**) A correlation plot showing the relationship between experimental peak

distances, as extracted from the DEER distance distributions collected at pH 5, and the simulated peak distances based on the 20 DEERefiner pH 5 models from AF (as boxplot). The black line signifies x=y.

Table S1. Simulated peak distance of 20 DEERefiner pH 8 models as well as their statistical analysis. Standard errors are calculated as, $\sigma/\sqrt{n}$, where σ is the standard deviation and n is the number of models.

	Simulated peak distance, nm								
	38/310R1	38/371R1	103/371R1	160/310R1	160/371R1	12/210R1	125/210R1	125/278R1	70/338R1
Model 1	4.00	3.40	4.30	4.80	5.10	4.20	2.90	2.10	2.90
Model 2	4.00	3.50	4.00	4.90	5.10	4.30	3.00	2.30	2.80
Model 3	4.10	3.50	4.30	4.80	5.10	4.10	2.80	2.10	2.90
Model 4	4.20	3.40	4.20	4.60	5.20	4.30	2.90	2.30	2.80
Model 5	4.00	3.50	4.20	4.80	5.10	4.30	2.80	2.10	2.80
Model 6	4.00	3.50	4.10	4.70	5.10	4.30	2.80	2.20	2.90
Model 7	4.00	3.60	4.00	4.90	5.10	4.30	2.90	2.00	2.90
Model 8	4.10	3.50	4.10	4.70	5.10	4.20	2.90	2.10	2.80
Model 9	4.30	3.50	4.20	4.70	5.10	4.20	3.00	2.10	2.80
Model 10	4.00	3.50	4.20	4.70	5.10	4.20	2.80	2.10	2.90
Model 11	4.10	3.50	4.20	4.70	5.10	4.30	3.00	2.20	2.70
Model 12	4.10	3.50	4.10	4.70	5.00	4.00	3.00	2.20	2.80
Model 13	4.20	3.50	4.20	4.90	5.20	4.30	2.90	2.10	2.90
Model 14	4.00	3.50	4.20	4.60	5.00	4.20	2.80	2.10	2.90
Model 15	4.10	3.40	4.10	4.80	5.10	4.10	2.70	2.20	2.80
Model 16	4.20	3.40	4.00	4.90	5.10	4.30	2.80	2.30	2.80
Model 17	4.10	3.40	4.20	4.80	5.20	4.30	2.90	2.20	2.90
Model 18	4.20	3.40	4.10	4.80	5.00	4.20	3.00	2.50	2.80
Model 19	4.20	3.40	4.20	4.70	5.00	4.10	3.00	2.10	2.70
Model 20	4.10	3.50	4.20	4.70	5.00	4.20	2.80	2.10	2.80
Average	4.10	3.47	4.16	4.76	5.09	4.22	2.89	2.17	2.83
Standard error	0.0201	0.0134	0.0201	0.0201	0.0134	0.0201	0.0201	0.0246	0.0157

Table S2. Simulated peak distance of 20 DEERefiner pH 5 models from crystal as well as their statistical analysis. Standard errors are calculated as, $\sigma/\sqrt{n}$, where σ is the standard deviation and n is the number of models.

	Simulated peak distance, nm								
	38/310R1	38/371R1	103/371R1	160/310R1	160/371R1	12/210R1	125/210R1	125/278R1	70/338R1
Model 1	3.00	2.10	3.20	3.90	4.40	5.10	3.40	2.50	3.30
Model 2	3.00	2.50	3.10	3.90	4.40	4.90	3.10	2.50	3.00
Model 3	3.00	2.50	3.10	3.90	4.30	5.00	3.30	2.50	3.20
Model 4	3.00	2.40	3.20	3.80	4.30	4.80	3.20	2.70	3.30
Model 5	3.00	2.30	3.20	3.90	4.40	4.80	3.20	2.70	3.30
Model 6	3.00	2.50	3.10	3.90	4.60	4.80	3.30	2.60	3.30
Model 7	3.00	2.50	3.10	3.90	4.50	4.80	3.30	2.40	3.30
Model 8	3.00	2.40	3.10	3.90	4.30	4.80	3.10	2.60	3.20
Model 9	3.00	2.10	3.20	3.90	4.40	4.90	3.20	2.50	3.30
Model 10	3.00	2.50	3.20	3.90	4.60	4.80	3.20	2.60	3.30
Model 11	3.00	2.30	3.20	3.90	4.40	4.80	3.10	2.50	3.30
Model 12	3.00	2.50	3.20	3.90	4.40	4.90	3.30	2.50	3.10
Model 13	2.90	2.50	3.00	3.90	4.40	5.00	3.10	2.50	3.20
Model 14	3.00	2.50	3.10	3.90	4.60	5.10	3.30	2.40	3.20
Model 15	3.00	2.50	3.10	3.90	4.40	4.80	3.20	2.40	3.10
Model 16	2.90	2.50	3.20	3.90	4.40	5.10	3.20	2.30	3.20
Model 17	3.10	2.50	3.20	3.90	4.40	4.80	3.40	2.50	3.30
Model 18	2.90	2.40	3.30	3.90	4.40	4.90	3.40	2.30	3.30
Model 19	2.90	2.30	3.20	3.90	4.60	4.90	3.20	2.60	3.00
Model 20	3.00	2.40	3.10	3.90	4.50	5.10	3.00	2.40	3.10
Average	2.99	2.41	3.16	3.90	4.44	4.91	3.23	2.50	3.22
Standard error	0.0112	0.0291	0.0157	0.0045	0.0224	0.0268	0.0246	0.0246	0.0224

Table S3. Simulated peak distance of 20 DEERefiner pH 5 models from AF as well as their statistical analysis. Standard errors are calculated as, $\sigma/\sqrt{n}$, where σ is the standard deviation and n is the number of models.

	Simulated peak distances, nm								
	38/310R1	38/371R1	103/371R1	160/310R1	160/371R1	12/210R1	125/210R1	125/278R1	70/338R1
Model 1	2.90	2.50	3.20	3.90	4.20	4.90	3.30	2.60	3.30
Model 2	3.00	2.40	3.10	3.80	4.40	4.90	3.40	2.60	3.30
Model 3	2.70	2.30	3.20	3.70	4.20	4.80	3.30	2.50	3.30
Model 4	2.90	2.40	3.10	3.80	4.30	4.80	3.40	2.70	3.20
Model 5	2.90	2.40	3.10	3.90	4.60	5.00	3.40	2.60	3.20
Model 6	2.90	2.40	3.20	3.80	4.20	4.80	3.30	2.70	3.20
Model 7	3.10	2.30	3.20	3.80	4.30	4.80	3.40	2.60	3.20
Model 8	3.10	2.50	3.20	3.70	4.40	4.90	3.30	2.70	3.30
Model 9	2.90	2.50	3.10	3.80	4.30	5.00	3.40	2.70	3.30
Model 10	2.90	2.50	3.20	3.80	4.20	5.00	3.40	2.60	3.30
Model 11	2.90	2.50	3.20	3.90	4.40	5.00	3.30	2.80	3.30
Model 12	2.90	2.40	3.20	3.80	4.30	4.80	3.40	2.60	3.10
Model 13	2.90	2.30	3.20	3.90	4.30	5.00	3.30	2.60	3.10
Model 14	3.00	2.40	3.20	3.80	4.40	5.10	3.40	2.70	3.20
Model 15	3.00	2.40	3.10	3.80	4.30	4.90	3.40	2.50	3.30
Model 16	3.00	2.40	3.10	3.60	4.30	4.90	3.30	2.60	3.00
Model 17	3.00	2.30	3.20	3.70	4.30	4.90	3.40	2.70	3.20
Model 18	3.00	2.50	3.20	3.70	4.50	5.00	3.40	2.60	3.10
Model 19	2.90	2.30	3.20	3.70	4.20	4.80	3.40	2.70	3.30
Model 20	3.00	2.30	3.10	3.90	4.20	5.00	3.40	2.70	3.30
Average	2.95	2.40	3.17	3.79	4.32	4.92	3.37	2.64	3.23
Standard error	0.0201	0.0179	0.0112	0.0201	0.0246	0.0201	0.0112	0.0179	0.0201

Table S4. α and β angles of DEERefiner models as well as their statistical analysis. Standard errors are calculated as, σ/√n, where σ is the standard deviation and n is the number of models.

	DEERefiner pH 8 models		DEERefiner pH 5 models from crystal		DEERefiner pH 5 models from AF	
	α, °	β, °	α, °	β, °	α, °	β, °
Model 1	32.2	24.5	26.2	26.7	24.1	28.3
Model 2	32.2	24.9	25.9	26.8	24.9	27.5
Model 3	32.0	25.9	25.6	27.4	24.3	30.3
Model 4	31.8	23.2	25.6	27.4	24.9	27.2
Model 5	31.8	24.8	25.3	26.2	24.3	28.1
Model 6	31.4	23.2	24.6	26.6	24.5	28.8
Model 7	30.9	24.8	24.3	26.9	24.0	27.8
Model 8	31.0	25.0	25.6	25.9	25.4	27.9
Model 9	31.8	23.9	25.9	28.2	25.7	27.9
Model 10	31.2	23.3	25.1	25.6	24.6	29.1
Model 11	30.9	24.1	26.6	26.1	25.4	29.7
Model 12	30.8	23.2	25.4	26.0	25.3	27.3
Model 13	31.9	24.7	24.2	26.1	25.4	27.9
Model 14	31.0	23.3	25.8	27.5	25.6	27.7
Model 15	31.3	23.8	24.0	27.5	25.3	28.4
Model 16	31.2	24.1	24.2	26.9	24.7	27.1
Model 17	31.2	23.6	25.8	27.5	24.8	27.3
Model 18	31.2	24.2	25.5	28.1	25.5	27.8
Model 19	30.5	23.4	25.0	26.3	25.2	29.5
Model 20	31.6	24.4	24.3	26.7	24.7	29.1
Average	31.4	24.1	25.2	26.8	24.9	28.2
Standard error	0.02	0.04	0.04	0.04	0.03	0.05

Video S1. Representative modeling of pH 5 model from crystal during computation. This video represents the pH 5 modeling from crystal in Fig. 3. Modeling details were described in Materials and Methods.

REFERENCES

(1) del Alamo, D.; Govaerts, C.; Mchaourab, H. S. AlphaFold2 predicts the inward-facing conformation of the multidrug transporter LmrP. *Proteins: Struct., Funct., Bioinf.* **2021**, *89*, 1226–1228.