

# Supporting Information

## Altering the Solubility of the Antibiotic Candidate Nisin – A Computational Study

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## Supplementary Tables

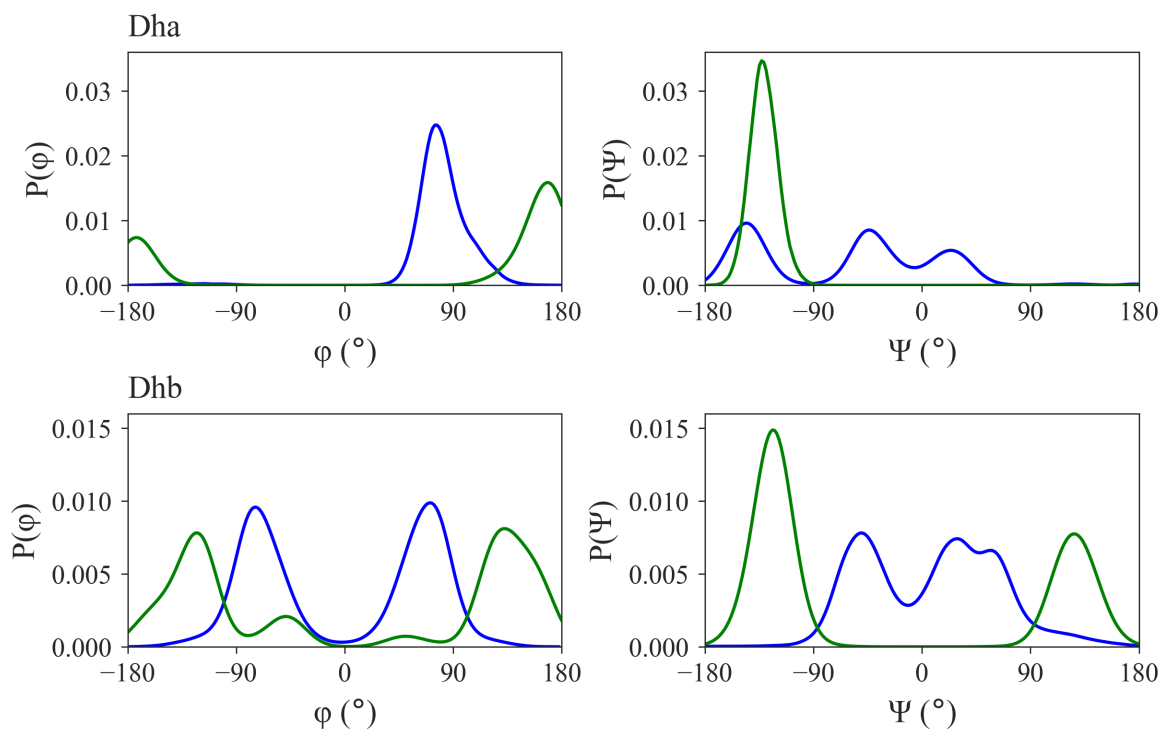
**Table S1.** Calculated p-values of statistical test (Welch's t-test) for solvation free energy (GBSA and PBSA) of nisin and its mutant forms as calculated from simulations using parameters of de Miguel *et al.*<sup>1</sup> and Turpin *et al.*<sup>2</sup>

Se. No.		$\Delta G_{\text{sol}}$ (GBSA)			$\Delta G_{\text{sol}}$ (PBSA) ( $\epsilon_{\text{in}} = 4$ )		
		de Miguel	Turpin	Significantly different? (p-value)	de Miguel	Turpin	Significantly different? (p-value)
1.	WT	-594.3 ± 31.5	-584.0 ± 26.8	Yes (<0.001)	-136.0 ± 7.4	-134.1 ± 6.1	Yes (<0.001)
2.	N20K	-732.2 ± 34.4	-743.6 ± 29.7	Yes (<0.001)	-170.0 ± 8.5	-171.8 ± 7.0	Yes (<0.001)
3.	M21K	-752.3 ± 36.8	-739.9 ± 34.6	Yes (<0.001)	-173.9 ± 8.7	-171.6 ± 8.0	Yes (<0.001)
4.	KK-NP	-592.9 ± 36.6	-610.0 ± 25.7	Yes (<0.001)	-136.0 ± 8.3	-139.4 ± 6.4	Yes (<0.001)
5.	KK-PP	-917.1 ± 29.3	-914.2 ± 31.9	No (0.101)	-214.5 ± 7.0	-212.9 ± 7.2	Yes (<0.001)
6.	N20Q	-595.6 ± 31.3	-603.0 ± 24.4	Yes (<0.001)	-136.5 ± 7.2	-137.9 ± 5.7	Yes (<0.001)
7.	N20R	-731.4 ± 36.3	-729.8 ± 31.1	No (0.412)	-168.4 ± 8.6	-168.7 ± 7.1	No (0.51)
8.	K22R	-581.6 ± 29.2	-589.9 ± 26.6	Yes (<0.001)	-131.6 ± 6.8	-134.0 ± 6.0	Yes (<0.001)
9.	RR	-731.3 ± 33.7	-710.1 ± 36.7	Yes (<0.001)	-168.0 ± 7.9	-164.2 ± 8.3	Yes (<0.001)
10.	N20H	-601.5 ± 25.0	-588.9 ± 25.1	Yes (<0.001)	-137.9 ± 5.9	-134.6 ± 5.6	Yes (<0.001)
11.	M21H	-611.8 ± 27.1	-602.9 ± 21.3	Yes (<0.001)	-140.1 ± 6.3	-138.1 ± 5.0	Yes (<0.001)
12.	K22H	-470.0 ± 31.7	-490.7 ± 28.3	Yes (<0.001)	-105.9 ± 7.2	-111.3 ± 6.5	Yes (<0.001)
13.	N20E	-549.7 ± 58.3	-576.5 ± 39.9	Yes (<0.001)	-124.0 ± 13.9	-131.6 ± 9.2	Yes (<0.001)
14.	M21E	-552.6 ± 47.2	-571.9 ± 37.6	Yes (<0.001)	-126.2 ± 10.9	-130.2 ± 8.8	Yes (<0.001)
15.	K22E	-486.9 ± 36.9	-495.7 ± 32.6	Yes (<0.001)	-108.8 ± 8.6	-111.4 ± 7.9	Yes (<0.001)
16.	N20V	-569.4 ± 42.2	-595.6 ± 24.3	Yes (<0.001)	-130.7 ± 9.5	-135.8 ± 5.6	Yes (<0.001)
17.	N20F	-600.3 ± 29.3	-596.8 ± 22.7	Yes (0.021)	-136.9 ± 6.8	-135.5 ± 5.2	Yes (<0.001)
18.	M21G	-593.1 ± 31.3	-601.1 ± 20.6	Yes (<0.001)	-135.8 ± 7.3	-137.5 ± 4.9	Yes (<0.001)
19.	K22G	-454.6 ± 34.4	-484.5 ± 23.5	Yes (<0.001)	-102.3 ± 7.8	-108.8 ± 5.5	Yes (<0.001)
20.	FLQ	-481.4 ± 23.4	-476.2 ± 22.4	Yes (<0.001)	-108.2 ± 5.4	-106.6 ± 5.1	Yes (<0.001)

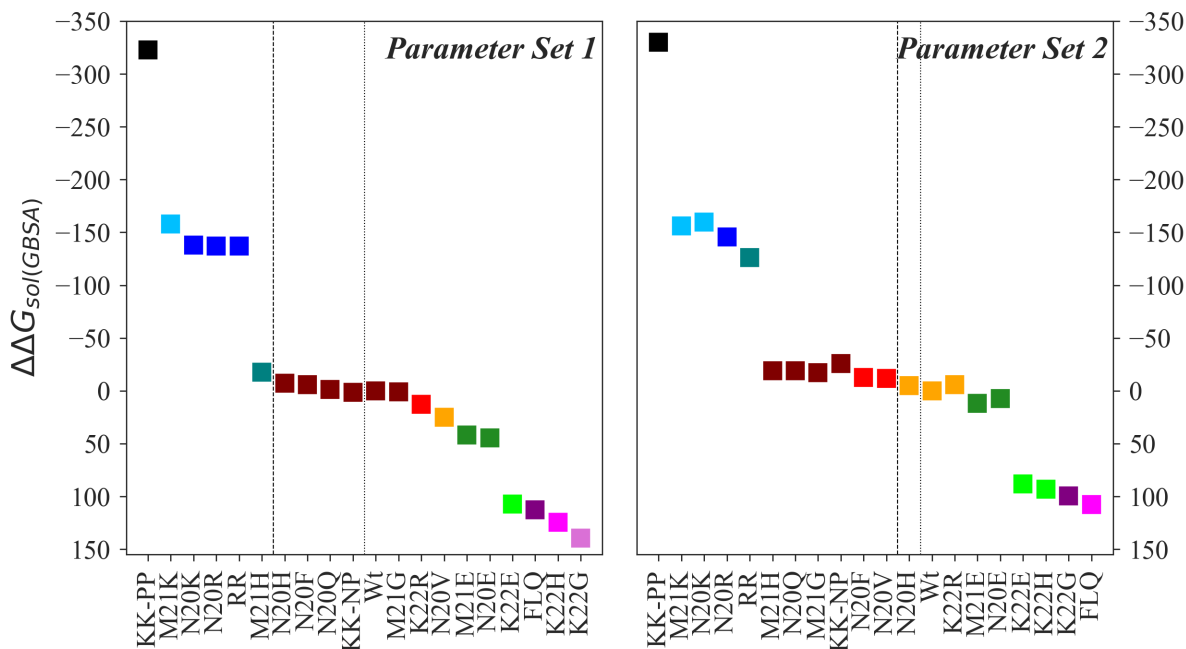
**Table S2.** Components of the solvation free energy ( $\Delta G_{\text{sol}}$ ) of nisin and mutants as measured in simulations using parameters of de Miguel *et al.*<sup>1</sup> and Turpin *et al.*<sup>2</sup> using PBSA.

Se. No.		de Miguel			Turpin		
		$\Delta G_{\text{sol,pol}}$	$\Delta G_{\text{sol,np}}$	$\Delta G_{\text{sol,pol}}$	$\Delta G_{\text{sol,np}}$	$\Delta G_{\text{sol,pol}}$	$\Delta G_{\text{sol,np}}$
1.	WT	-152.9 ± 7.5	16.8 ± 1.5	-136.0 ± 7.4	-150.6 ± 6.6	16.5 ± 1.1	-134.1 ± 6.1
2.	N20K	-186.0 ± 8.0	15.9 ± 1.5	-170.0 ± 8.5	-189.5 ± 7.1	17.7 ± 1.4	-171.8 ± 7.0
3.	M21K	-191.3 ± 8.9	17.4 ± 1.0	-173.9 ± 8.7	-189.1 ± 8.3	17.4 ± 1.5	-171.6 ± 8.0
4.	KK-NP	-152.8 ± 9.0	16.8 ± 1.4	-136.0 ± 8.3	-157.6 ± 6.0	18.2 ± 1.3	-139.4 ± 6.4
5.	KK-PP	-231.6 ± 6.9	17.1 ± 1.5	-214.5 ± 7.0	-230.9 ± 7.6	18.0 ± 1.0	-212.9 ± 7.2
6.	N20Q	-153.1 ± 7.4	16.6 ± 0.8	-136.5 ± 7.2	-154.6 ± 5.8	16.7 ± 0.7	-137.9 ± 5.7
7.	N20R	-185.7 ± 8.2	17.3 ± 1.1	-168.4 ± 8.6	-186.2 ± 7.5	17.5 ± 1.0	-168.7 ± 7.1
8.	K22R	-149.7 ± 7.2	18.1 ± 1.2	-131.6 ± 6.8	-151.5 ± 6.4	17.6 ± 1.1	-134.0 ± 6.0
9.	RR	-186.3 ± 7.9	18.2 ± 1.4	-168.0 ± 7.9	-181.0 ± 8.7	16.8 ± 1.2	-164.2 ± 8.3
10.	N20H	-154.8 ± 5.9	16.9 ± 0.8	-137.9 ± 5.9	-151.8 ± 6.1	17.2 ± 1.1	-134.6 ± 5.6
11.	M21H	-156.6 ± 6.6	16.5 ± 1.5	-140.1 ± 6.3	-155.1 ± 5.1	17.0 ± 0.7	-138.1 ± 5.0
12.	K22H	-122.8 ± 7.7	17.0 ± 1.2	-105.9 ± 7.2	-128.3 ± 6.9	17.0 ± 0.8	-111.3 ± 6.5
13.	N20E	-141.1 ± 15.1	17.1 ± 1.6	-124.0 ± 13.9	-148.3 ± 10.3	16.7 ± 1.5	-131.6 ± 9.2
14.	M21E	-142.5 ± 12.1	16.4 ± 1.7	-126.2 ± 10.9	-147.3 ± 9.2	17.0 ± 1.3	-130.2 ± 8.8
15.	K22E	-125.8 ± 9.5	17.0 ± 1.4	-108.8 ± 8.6	-128.0 ± 7.9	16.6 ± 1.0	-111.4 ± 7.9
16.	N20V	-146.5 ± 9.9	15.8 ± 1.1	-130.7 ± 9.5	-153.6 ± 6.1	17.8 ± 1.2	-135.8 ± 5.6
17.	N20F	-154.4 ± 7.1	17.5 ± 0.9	-136.9 ± 6.8	-154.2 ± 5.6	18.7 ± 1.3	-135.5 ± 5.2
18.	M21G	-152.4 ± 7.6	16.6 ± 1.1	-135.8 ± 7.3	-155.0 ± 5.0	17.4 ± 1.0	-137.5 ± 4.9
19.	K22G	-118.1 ± 9.1	15.8 ± 1.9	-102.3 ± 7.8	-126.3 ± 6.0	17.4 ± 1.4	-108.8 ± 5.5
20.	FLQ	-125.2 ± 5.9	17.0 ± 1.7	-108.2 ± 5.4	-124.1 ± 5.3	17.6 ± 0.9	-106.6 ± 5.1

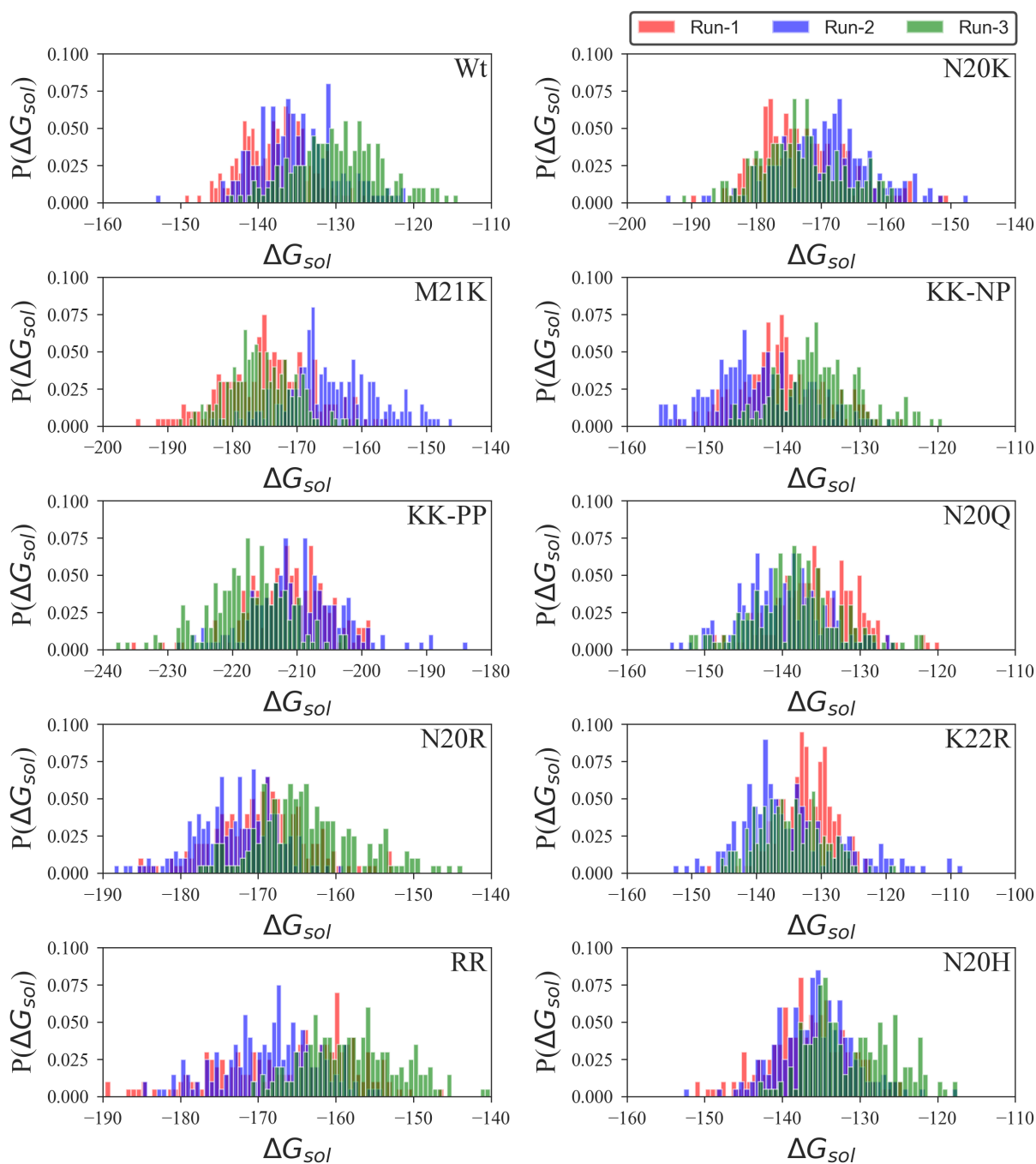
## Supplementary Figures



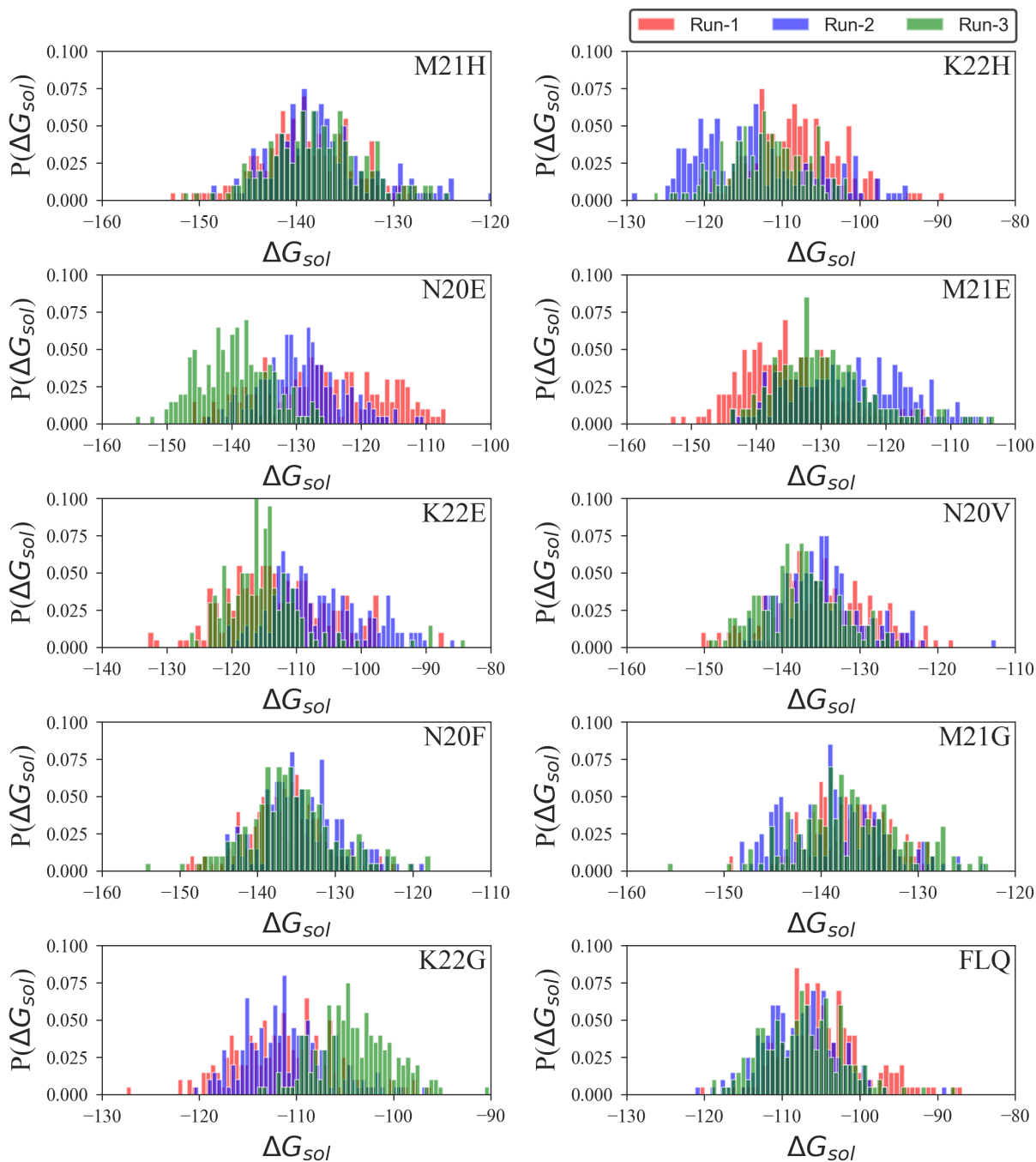
**Figure S1.** Phi-psi ( $\phi/\psi$ ) backbone dihedral angle distribution of Dehydroalanine and Dehydrobutyrine as measured in simulations relying either on the parameter set of de Miguel *et al.*<sup>1</sup> (blue) or the one by Turpin *et al.*<sup>2</sup> (green).



**Figure S2.** Effect of mutation on the solubility of nisin estimated using GBSA. The figure shows the difference between the solvation free energies of mutant and wild-type nisin ( $\Delta\Delta G_{sol} = \Delta G_{mutant} - \Delta G_{wt}$ ). Nisin and mutants are ranked in decreasing order of solubility. A different color is used for each rank, and a matching color is assigned to the mutants with the same rank. Statistical significance is determined by using multiple comparison Tukey's test at  $\alpha=0.05$ . Data in the left figure are from simulations relying on the parameters of de Miguel *et al.*<sup>1</sup> (*Parameter Set 1*) and the ones in the right figure on the parameter set of Turpin *et al.*<sup>2</sup> (*Parameter Set 2*)



**Figure S3 (a).** Distribution of solvation free energy of wild-type nisin and mutants estimated using PBSA using parameters of Turpin *et al.*<sup>2</sup>



**Figure S3 (b).** Distribution of solvation free energy of wild-type nisin and mutants estimated using PBSA using parameters of Turpin *et al.*<sup>2</sup>

## References:

- (1) de Miguel, A.; Tapia-Rojo, R.; Utesch, T.; Mroginski, M. A. Structure, Dynamics and Kinetics of Two-Component Lantibiotic Lichenicidin. *PLoS One* **2017**, *12* (6), e0179962.
- (2) Turpin, E. R.; Mulholland, S.; Teale, A. M.; Bonev, B. B.; Hirst, J. D. New CHARMM Force Field Parameters for Dehydrated Amino Acid Residues, the Key to Lantibiotic Molecular Dynamics Simulations. *RSC Adv.* **2014**, *4* (89), 48621–48631.