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Supplementary Materials
Novel Positive Allosteric Modulators of μ Opioid Receptor—Insight from In Silico and In Vivo Studies

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Figures S1–S3

Tables S1–S4

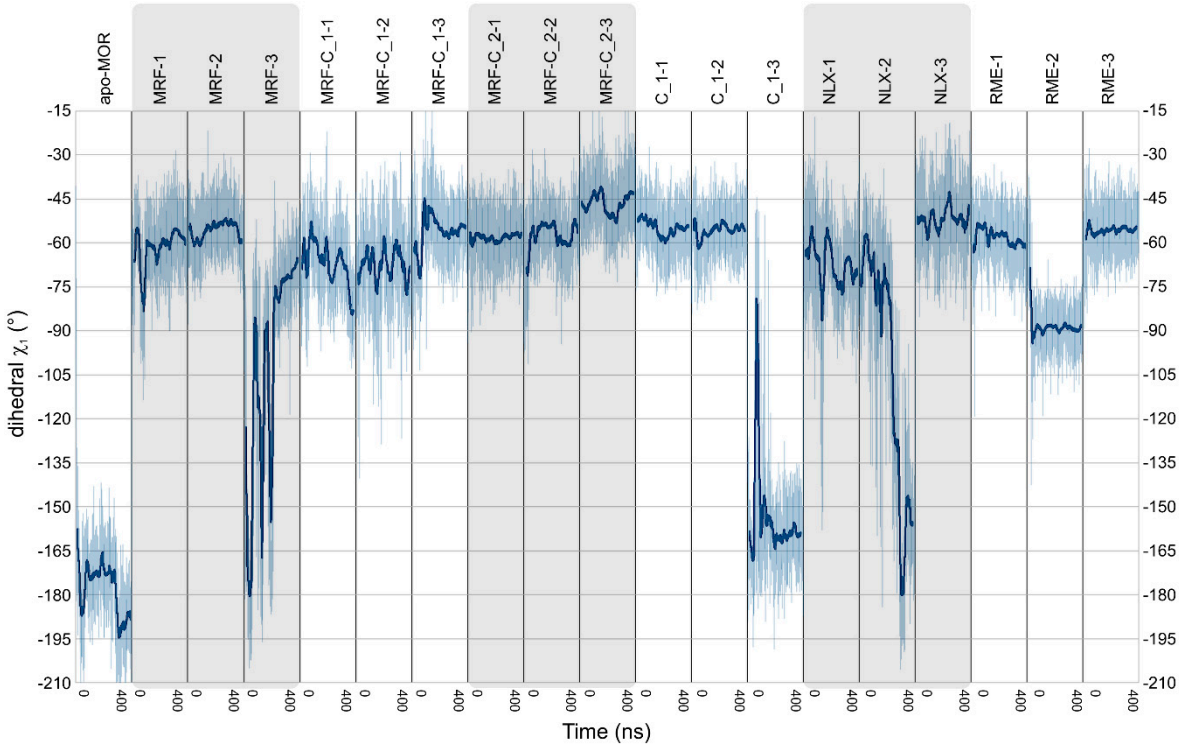


Figure S1. Values of χ_1 dihedral of Tyr 7.53 residue in all trajectories.

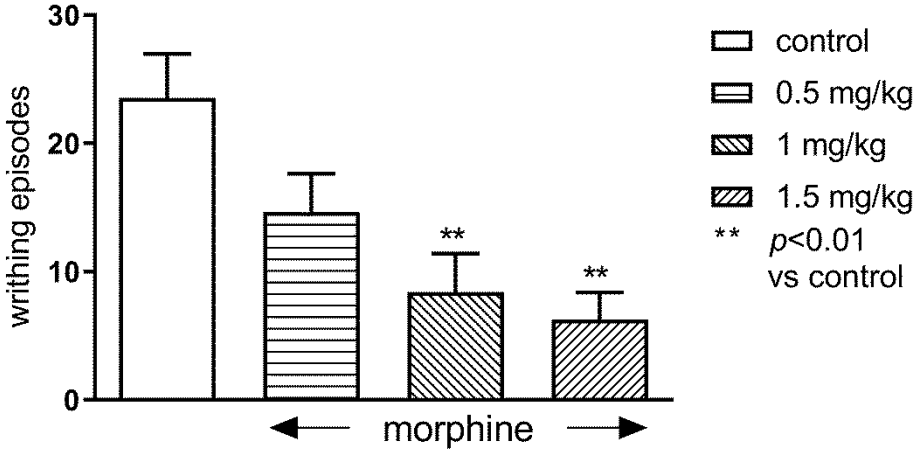


Figure S2. The effect of morphine (05, 1 and 2 mg/kg) on the nociceptive reactions in the 'writhing' test in mice. Morphine was administered s.c. 25 min before the test. The values represent means ± SEM. One-way ANOVA showed significant changes in the writhing episodes after administration of morphine in above doses $F(3,33) = 6.884$; p=0.001). Bonferroni's post hoc test confirmed a significant reduction in writhing episodes after the administration of morphine at the dose of 1 mg/kg (p< 0.001) and 1.5 mg/kg (p<0.01).

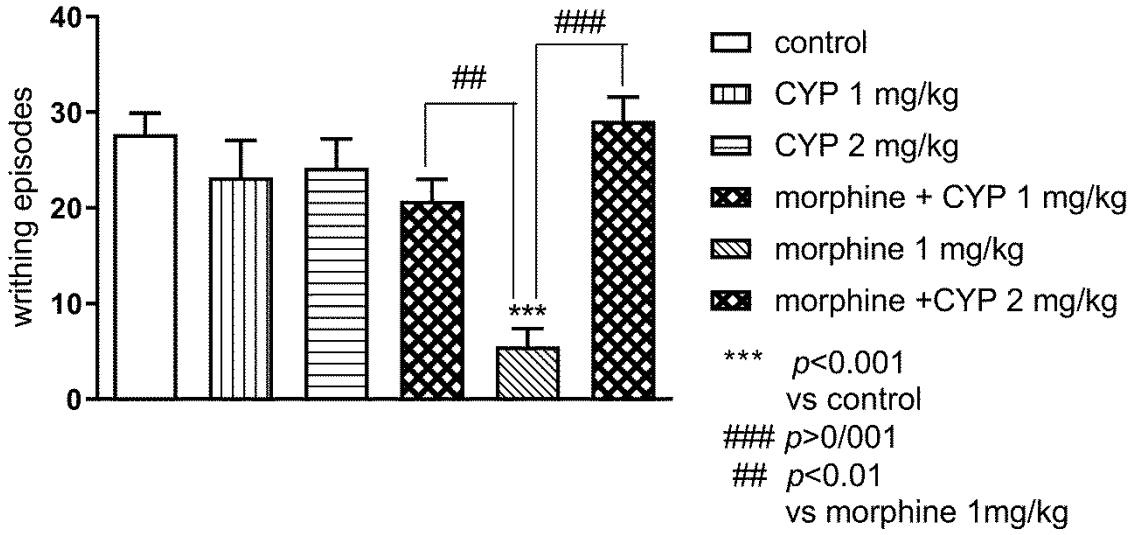


Figure S3. The influence of cyprodime (CYP, 1 and 2 mg/kg) on the antinociceptive activity of morphine in the 'writhing' test in mice. Morphine (1 mg/kg) and CYP were administered s.c. 25 min before the test. The values represent means ± SEM. One-way ANOVA showed significant changes in the writhing episodes after administration of these compounds ($F(5,51) = 10.05$; $p<0.0001$). Bonferroni's post hoc test confirmed a significant reduction in writhing episodes after the administration of morphine at the dose of 1 mg/kg ($p< 0.001$). CYP reversed antinociceptive effect of morphine at the dose 1 mg/kg ($p<0.01$) and at the dose 2 mg/kg ($p<0.001$).

Table S1. RMSF of the investigated compounds calculated in simulations in presence of morphine.

	Compound 1 (nm)	Compound 2 (nm)
Replica 1	0.2301	0.2283
Replica 2	0.2340	0.0961
Replica 3	0.1173	0.0969

Table S2. Ligand RMSD calculated for compound **1** and compound **2** in presence of morphine. Cα atoms of the main chain of the TM7 bundle were used for fitting. Plots show RMSD between a particular frame and a frame 500 ps before. Peaks represent changes of conformation compared to the frame 500 ps before.

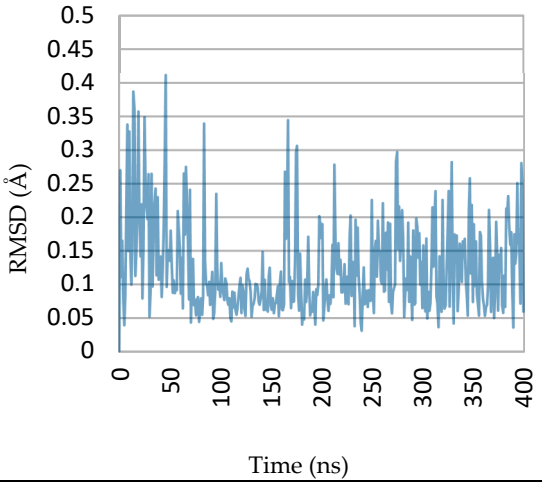
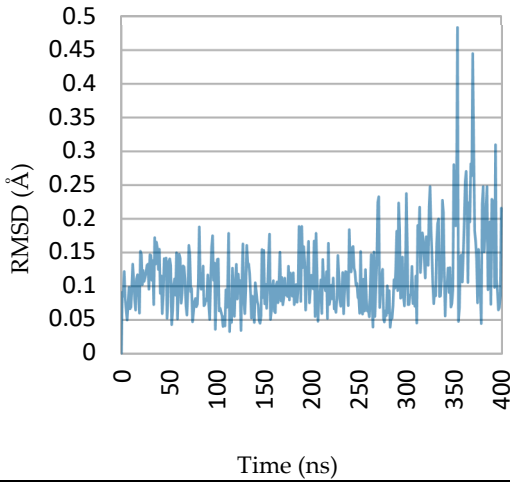
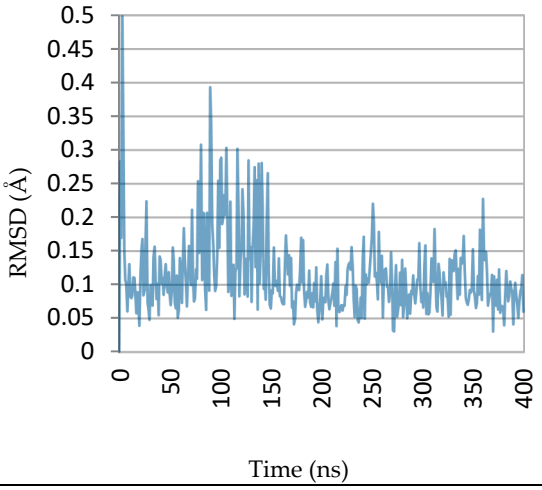
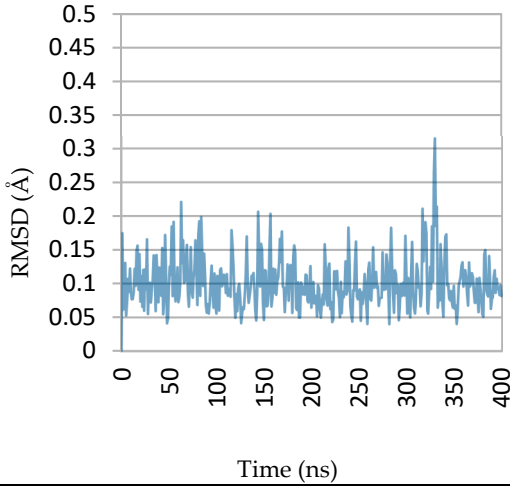
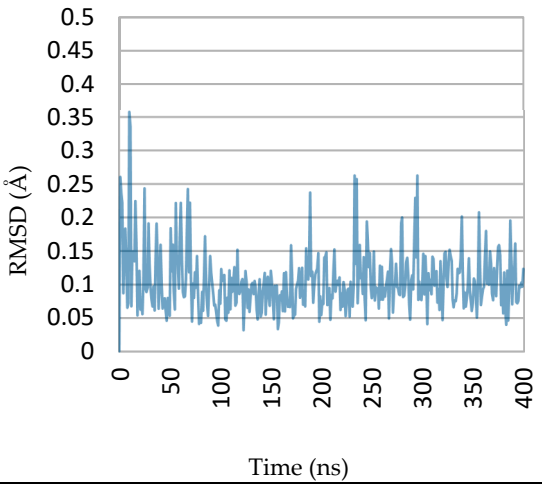
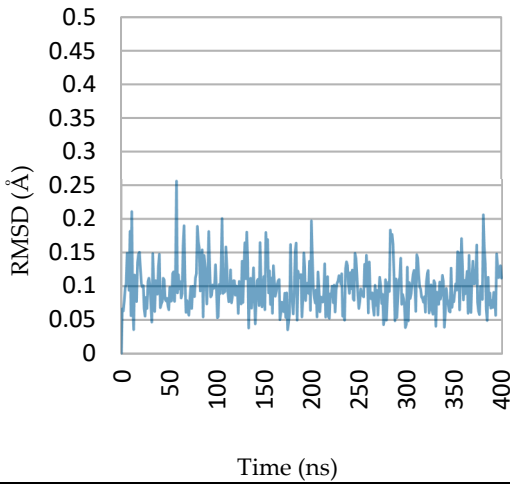
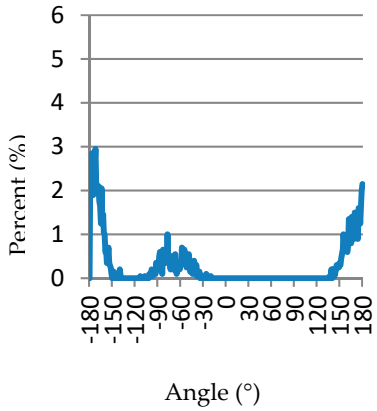
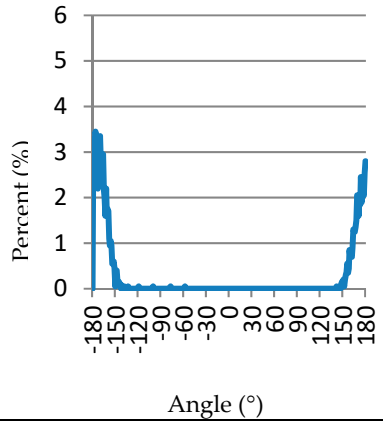
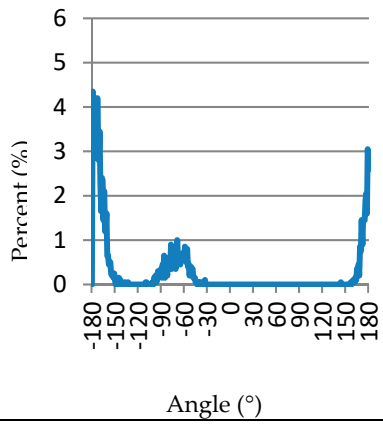
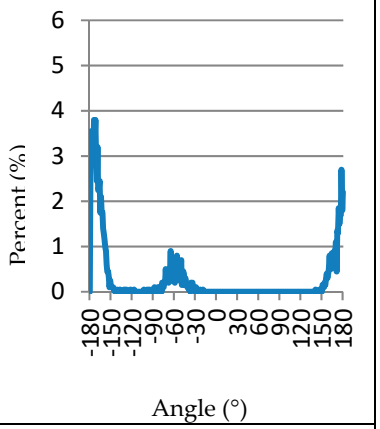
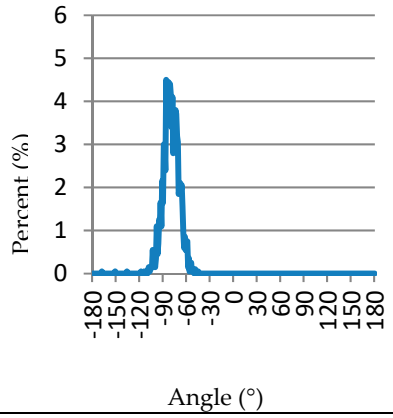
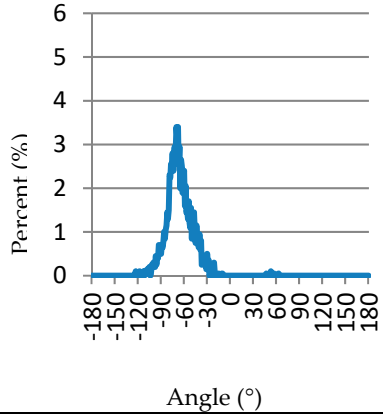
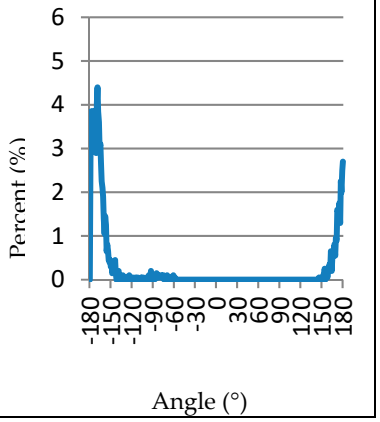
	Compound 1	Compound 2
Replica 1		
Replica 2		
Replica 3		

Table S3. Distributions of Tyr 2.64 χ_1 dihedral in all simulations.

	Replica 1	Replica 2	Replica 3
Apo			
MRF			
MRF-C_1			

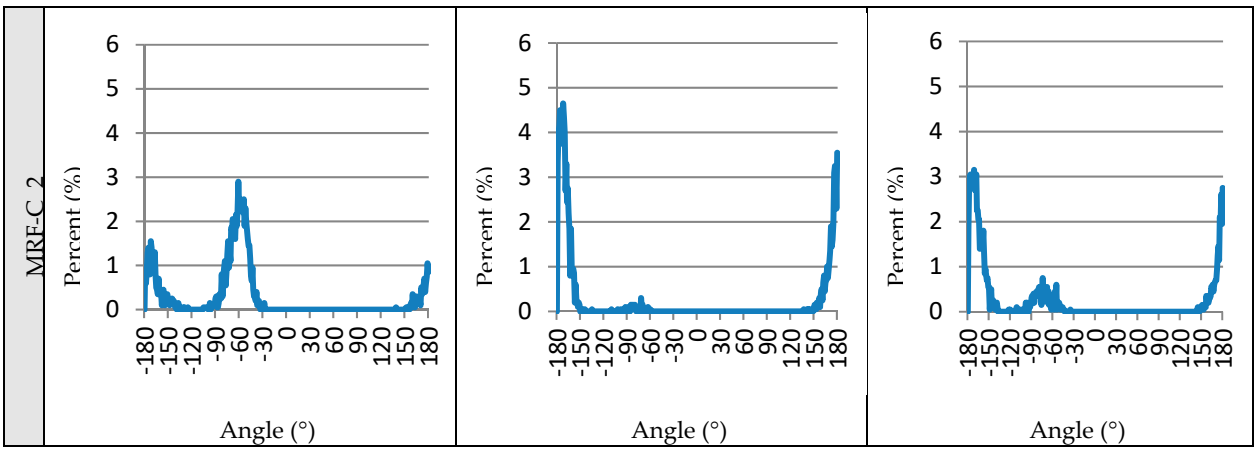
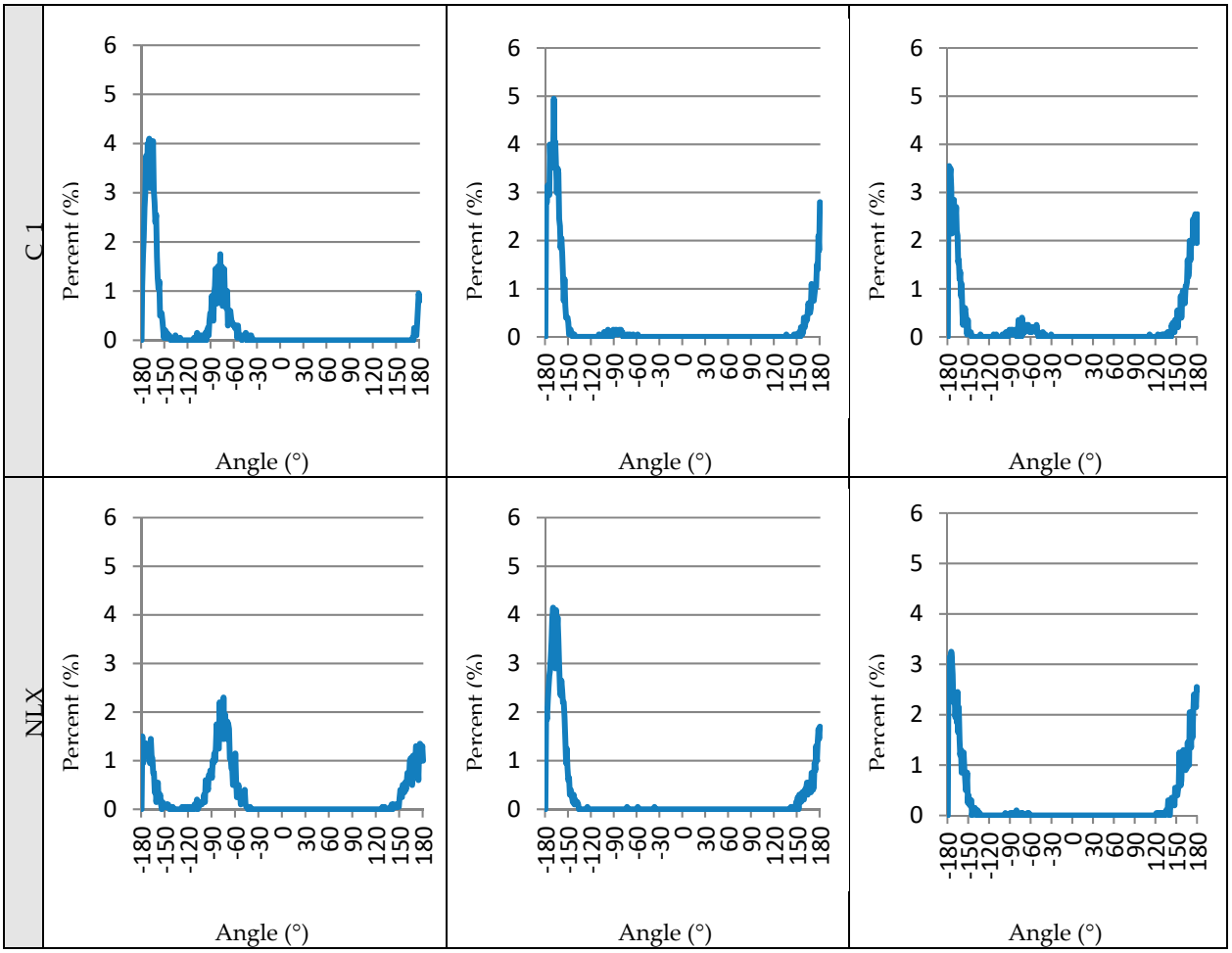


Table S3. *(continued)*



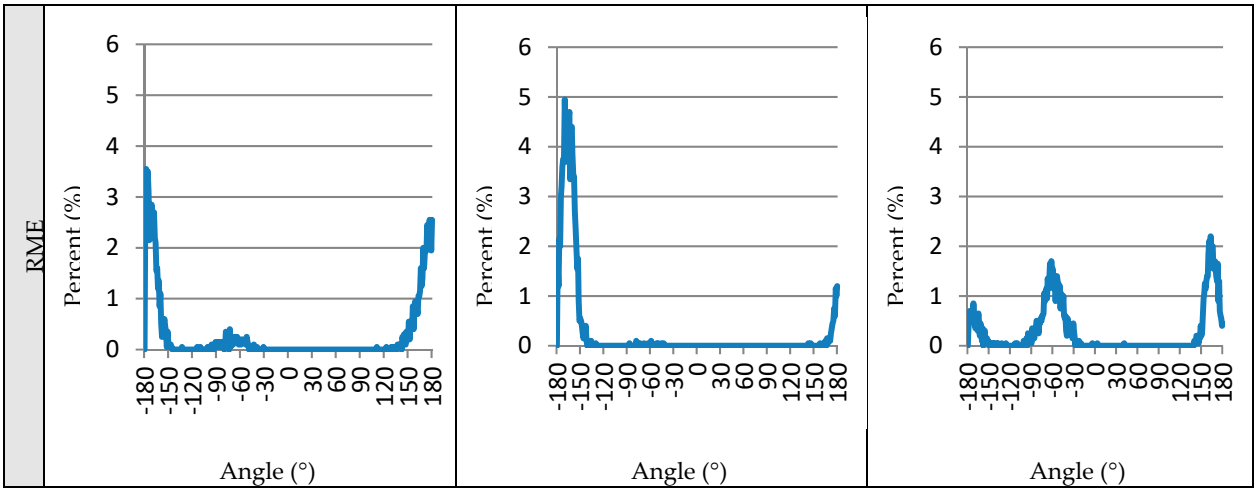
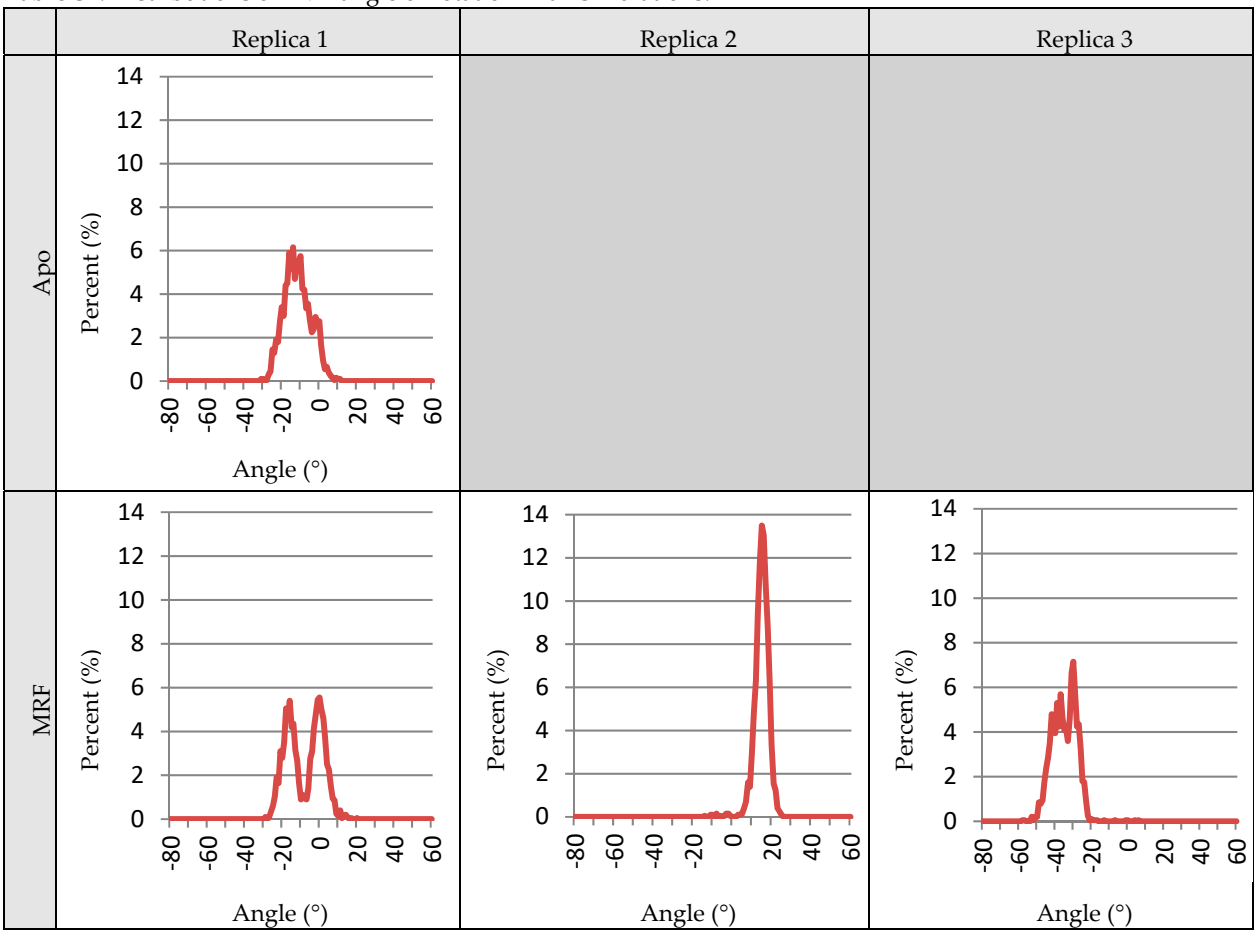


Table S4. Distributions of TM7 angle of rotation in all simulations.



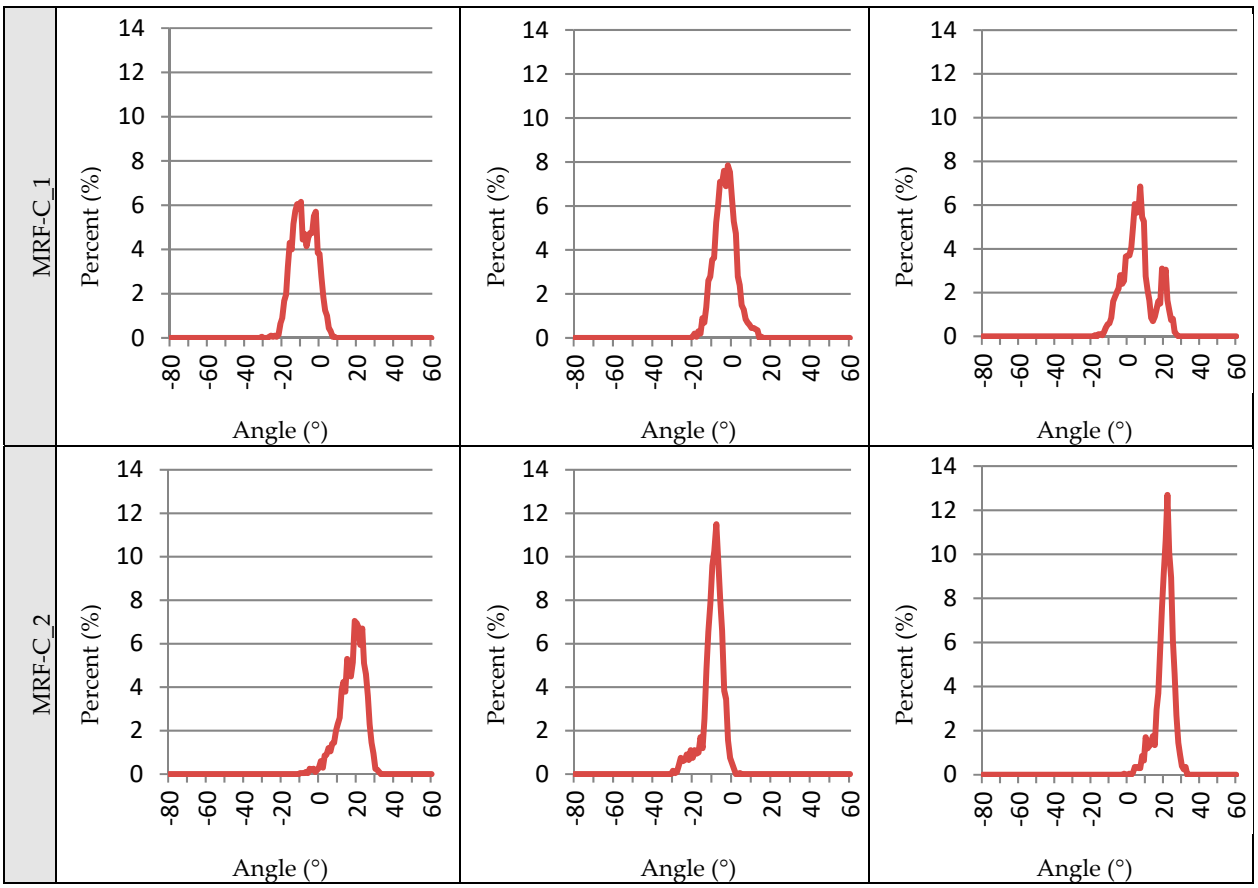
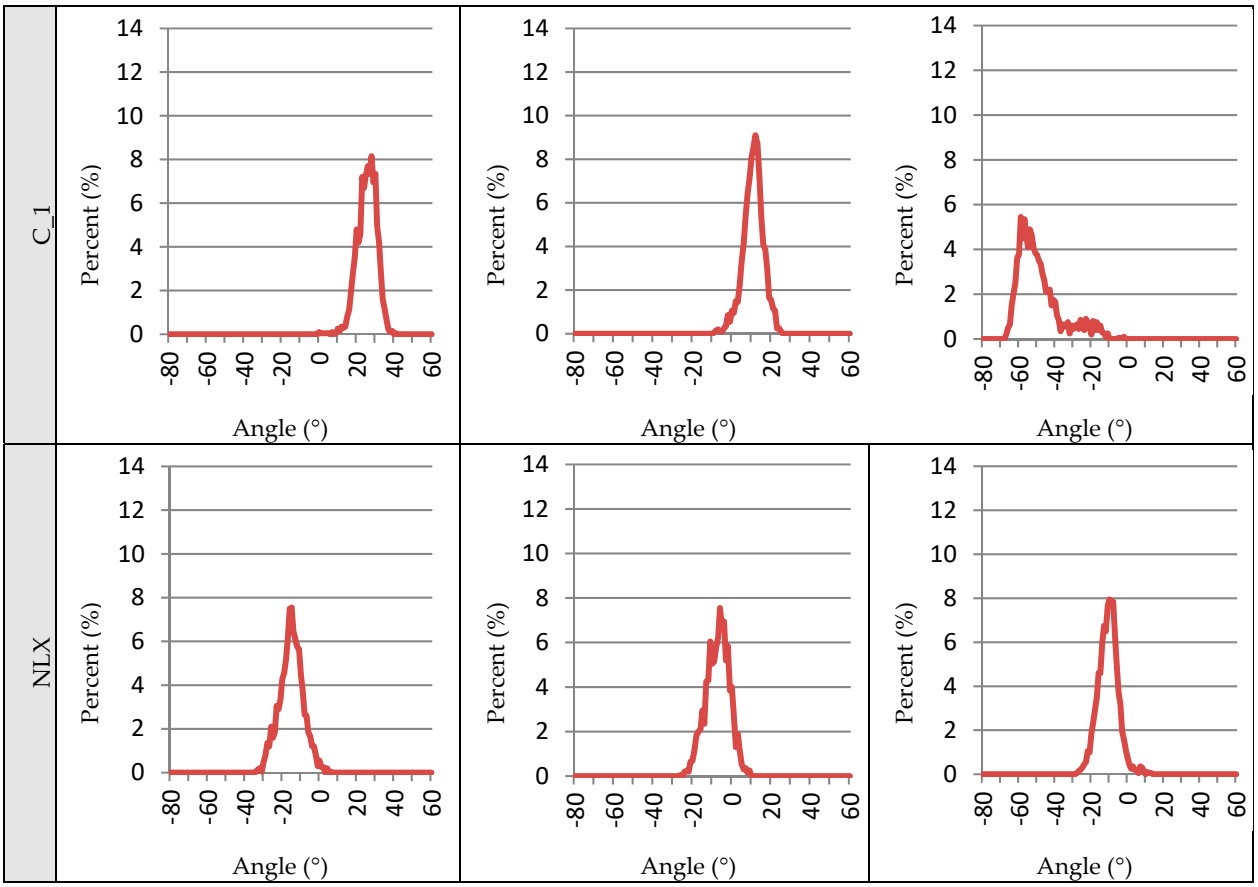
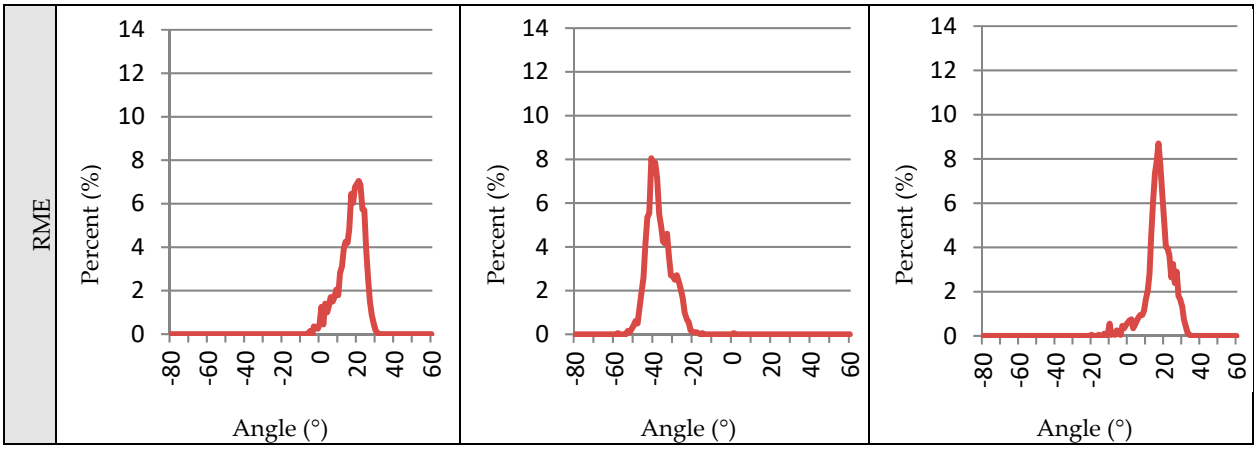


Table S4. *(continued)*




RME
Percent (%)
Angle (°)
14
12
10
8
6
4
2
0
-80
-60
-40
-20
0
20
40
60