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**Supplemental Information**

**Mechanism of  $\mu$ -Opioid Receptor-Magnesium Interaction and Positive  
Allosteric Modulation**

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**Table S1.** Simulation lengths of all independent MD runs carried out on the MOP receptor.

<b>MD Simulation system</b>	<b>Length (ns)</b>
<b>Ligand-free inactive MOP receptor</b>	
Run 1	1450.1
Run 2	1500.7
Run 3	1658.2
Run 4	980.1
Run 5	798.7
Run 6	664.5
<b>Total</b>	<b>7052.3</b>
<b>Ligand-free active MOP receptor, charged D<sup>2.50</sup></b>	
Run 1	1453.8
Run 2	1518.0
Run 3	1603.4
Run 4	1470.4
<b>Total</b>	<b>6045.6</b>
<b>Ligand-free active MOP receptor, neutral D<sup>2.50</sup></b>	
Run 1	2243.9
Run 2	2397.3
Run 3	1591.1
Run 4	1531.7
<b>Total</b>	<b>7764.0</b>

**Table S2.** Average number of cation binding events observed during MD and GCMC simulations.

	Active MOP receptor with Charged D2.50	Active MOP receptor with Neutral D2.50	Inactive MOP receptor with Charged D2.50
<b>Mg<sup>2+</sup> binding (MD)</b>			
Orthosteric Site	586	-	-
ECL1/ECL2	923	159	110
ECL2		1028	637
ECL2/ECL3	614	1196	446
ECL1/ECL2/ECL3	-	70	-
ECL1/ECL3	-	43	-
<b>Mg<sup>2+</sup> binding (GCMC)</b>			
Allosteric Site	1970	1018	2913
Orthosteric Site	1442	714	808
ECL1/ECL2	168	173	187
ECL2	1049	943	614
ECL2/ECL3	263	428	479
ECL1/ECL2/ECL3	92	390	224
ECL1/ECL3	37	86	55
<b>Na<sup>+</sup> binding</b>			
Orthosteric Site	116	20	30
Allosteric Site	122	-	350
ECL2	344	484	345
ECL3	145	203	128
ECL1/ECL2	49	72	53

**Table S3. Thermodynamics of ECL2/3 gating.** Ratios of the open-bound and open-unbound states ( $J_O$ ), closed-bound and closed-unbound states ( $J_C$ ), and the equilibrium constant between the open and the closed states of the ECL2/3 region in the absence and in the presence of magnesium ( $H_0$  and  $H_M$ , respectively) for the three simulated MOP receptor systems. Confidence intervals are the obtained from 10 bootstrap samples.

	$J_O$	$J_C$	$H_0$	$H_M$
<b>Inactive MOP receptor</b>	2.54 (2.43, 2.63)	1.29 (1.29, 1.31)	0.087 (0.086,0.088)	0.169 (0.166,0.176)
<b>Active MOP receptor with charged <math>D^{2.50}</math></b>	0.57 (0.52, 0.60)	1.56 (1.56, 1.58)	0.053 (0.052,0.055)	0.019 (0.017,0.021)
<b>Active MOP receptor with neutral <math>D^{2.50}</math></b>	0.70 (0.65, 0.71)	1.64 (1.64, 1.66)	0.176 (0.174,0.181)	0.074 (0.070,0.077)

**Table S4. Allosteric coefficients.** Allosteric coefficients for the ECL2/3 gating model in the absence of  $\text{Mg}^{2+}$  ( $\rho_0$ ), for saturating  $\text{Mg}^{2+}$  concentration ( $\rho_\infty$ ), and their ratio. Confidence intervals are the obtained from 10 bootstrap samples.

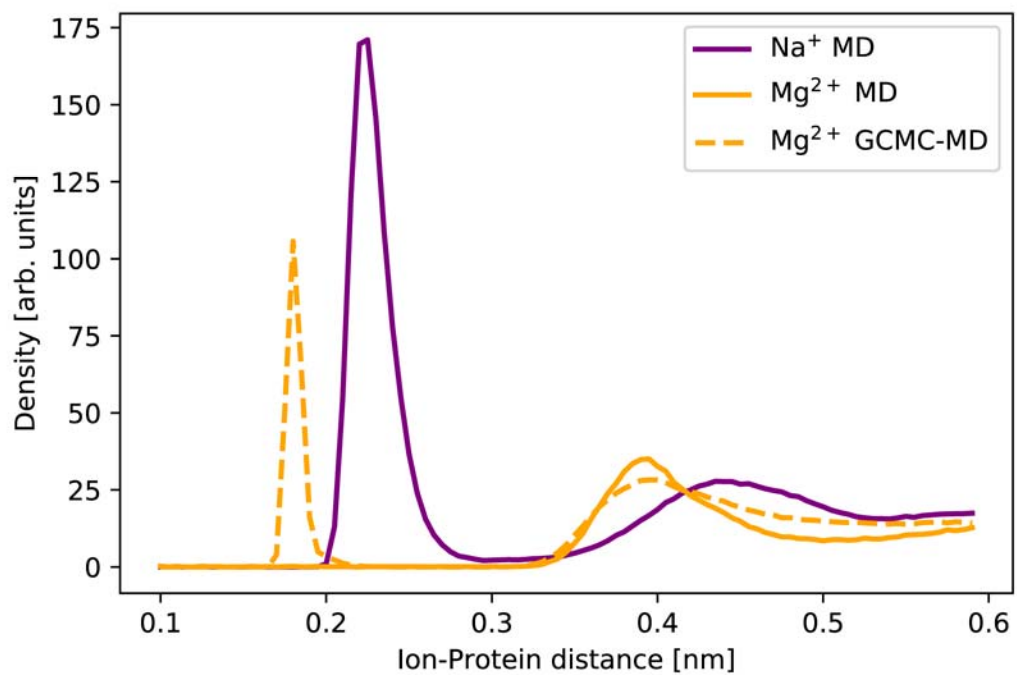
	$\rho_0$	$\rho_\infty$	$\rho_\infty/\rho_0$
<b>Inactive vs Active MOP receptor with charged D<sup>2.50</sup></b>	0.63 (0.61,0.66)	0.13 (0.12,0.14)	0.20 (0.18, 0.21)
<b>Inactive vs Active MOP receptor with neutral D<sup>2.50</sup></b>	1.85 (1.80, 1.88)	0.47 (0.45, 0.49)	0.25 (0.24, 0.27)

**Table S5.** Estimation of mouth and pocket areas for the selected receptor states with different loop conformations.

System	State	Loop conformation	Mouth Area ( $\text{\AA}^2$ )	Pocket Area ( $\text{\AA}^2$ )
Active MOP receptor with Neutral D <sup>2.50</sup>	ECL2/ECL3	Closed	20	719
	ECL2/ECL1	Open	100	850
	ECL2	Closed	29	673
Active MOP receptor with Charged D <sup>2.50</sup>	Orthosteric	Open	94	854
	ECL2/ECL1	Open	125	1,062
	ECL2/ECL3	Open	94	978
Inactive MOP receptor with Charged D <sup>2.50</sup>	ECL2/ECL1	Open	87	764
	ECL2	Open	116	827
	ECL2/ECL3	Closed	19	588

**Table S6.** Normalized co-information values between pairs of receptor residues and  $Mg^{2+}$  occupation of the cation binding macro-sites in the extracellular loop region of the receptor. Listed are the top 20 pairs ranked by the co-information absolute value.

Inactive MOP receptor		Active MOP receptor with charged $D^{2.50}$		Active MOP receptor with neutral $D^{2.50}$	
Residue pairs	CI	Residue pairs	CI	Residue pairs	CI
N150 <sup>3.35</sup> -R211 <sup>ECL2</sup>	-0.13	T153 <sup>3.38</sup> -R211 <sup>ECL2</sup>	-0.22	P181 <sup>4.39</sup> -R211 <sup>ECL2</sup>	-0.31
T153 <sup>3.38</sup> -R211 <sup>ECL2</sup>	-0.13	N150 <sup>3.35</sup> -R211 <sup>ECL2</sup>	-0.20	M99 <sup>ICL1</sup> -R211 <sup>ECL2</sup>	-0.29
N150 <sup>3.35</sup> -Y210 <sup>ECL2</sup>	-0.12	S154 <sup>3.39</sup> -R211 <sup>ECL2</sup>	-0.19	Q212 <sup>ECL2</sup> -P309 <sup>ECL3</sup>	-0.28
R211 <sup>ECL2</sup> -L331 <sup>7.48</sup>	-0.11	R211 <sup>ECL2</sup> -L331 <sup>7.48</sup>	-0.18	V94 <sup>1.58</sup> -R211 <sup>ECL2</sup>	-0.27
R211 <sup>ECL2</sup> -V288 <sup>6.43</sup>	-0.11	N109 <sup>2.45</sup> -R211 <sup>ECL2</sup>	-0.17	K209 <sup>ECL2</sup> -P309 <sup>ECL3</sup>	-0.26
A102 <sup>2.38</sup> -S222 <sup>ECL2</sup>	-0.11	R211 <sup>ECL2</sup> -T327 <sup>7.44</sup>	-0.17	I93 <sup>1.57</sup> -R211 <sup>ECL2</sup>	-0.24
S222 <sup>ECL2</sup> -L324 <sup>7.41</sup>	-0.11	T157 <sup>3.42</sup> -R211 <sup>ECL2</sup>	-0.17	S64 <sup>Nterm</sup> -R211 <sup>ECL2</sup>	-0.24
I215 <sup>ECL2</sup> -E341 <sup>ICL4</sup>	-0.11	S154 <sup>3.39</sup> -Y210 <sup>ECL2</sup>	-0.16	S222 <sup>ECL2</sup> -P309 <sup>ECL3</sup>	-0.24
N109 <sup>2.45</sup> -R211 <sup>ECL2</sup>	-0.10	Y210 <sup>ECL2</sup> -C292 <sup>6.47</sup>	-0.16	M99 <sup>ICL1</sup> -P309 <sup>ECL3</sup>	-0.24
A113 <sup>2.49</sup> -R211 <sup>ECL2</sup>	-0.10	Y210 <sup>ECL2</sup> -T327 <sup>7.44</sup>	-0.16	R211 <sup>ECL2</sup> -K260 <sup>5.66</sup>	-0.23
Y210 <sup>ECL2</sup> -N342 <sup>H8</sup>	-0.10	R211 <sup>ECL2</sup> -L324 <sup>7.41</sup>	-0.15	R211 <sup>ECL2</sup> -S222 <sup>ECL2</sup>	-0.23
R211 <sup>ECL2</sup> -C292 <sup>6.47</sup>	-0.10	R211 <sup>ECL2</sup> -C292 <sup>6.47</sup>	-0.15	R211 <sup>ECL2</sup> -P309 <sup>ECL3</sup>	-0.22
Y149 <sup>3.34</sup> -R211 <sup>ECL2</sup>	-0.10	M151 <sup>3.36</sup> -Y210 <sup>ECL2</sup>	-0.15	V94 <sup>1.58</sup> -P309 <sup>ECL3</sup>	-0.22
Y210 <sup>ECL2</sup> -C235 <sup>5.41</sup>	-0.10	F156 <sup>3.41</sup> -R211 <sup>ECL2</sup>	-0.15	R211 <sup>ECL2</sup> -D340 <sup>ICL4</sup>	-0.22
Y210 <sup>ECL2</sup> -C330 <sup>7.47</sup>	-0.10	I155 <sup>3.40</sup> -Y210 <sup>ECL2</sup>	-0.15	V173 <sup>ICL2</sup> -R211 <sup>ECL2</sup>	-0.21
Y210 <sup>ECL2</sup> -L339 <sup>7.56</sup>	-0.10	R211 <sup>ECL2</sup> -N328 <sup>7.45</sup>	-0.15	D272 <sup>6.27</sup> -P309 <sup>ECL3</sup>	-0.21
M151 <sup>3.36</sup> -Y210 <sup>ECL2</sup>	-0.10	Y210 <sup>ECL2</sup> -Y326 <sup>7.43</sup>	-0.14	V94 <sup>1.58</sup> -S222 <sup>ECL2</sup>	-0.21
D147 <sup>3.32</sup> -P309 <sup>ECL3</sup>	-0.10	R211 <sup>ECL2</sup> -L335 <sup>7.52</sup>	-0.14	V173 <sup>ICL2</sup> -P309 <sup>ECL3</sup>	-0.21
N150 <sup>3.35</sup> -L176 <sup>ICL2</sup>	-0.10	F152 <sup>3.37</sup> -R211 <sup>ECL2</sup>	-0.14	R211 <sup>ECL2</sup> -R273 <sup>6.28</sup>	-0.21
Y210 <sup>ECL2</sup> -E341 <sup>ICL4</sup>	-0.10	F108 <sup>2.44</sup> -Y210 <sup>ECL2</sup>	-0.14	C170 <sup>3.55</sup> -R211 <sup>ECL2</sup>	-0.20



**Figure S1.** Radial distribution function (RDF) of distances between heavy atoms of protein residues and either the  $\text{Na}^+$  (purple line) or  $\text{Mg}^{2+}$  (orange lines) cations.