Supporting information

Consensus 3D Model of μ-Opioid Receptor Ligand Efficacy based on a Quantitative Conformationally Sampled Pharmacophore

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Table 1. Experimental data used in model construction and evaluation

Training set		Test set	
compounds	% stimulation[a)]	Compounds	% stimulation
Morphine	93 ± 2.8	Methadone	116 ±20
Dihydromorphine	109 ± 5	Etonitazene	119 ±19
Normorphine	114 ±11	Fentanyl	100 ±12
Etorphine	117 ±24	(-)Ethylketozocine (EKC)	146 ±85
Buprenorphine	66 ±36	Funaltrexamine	19 ±0
Nalrexone	0	(-)Cyclazocine	33 ±18
Naloxone	0	(-)Pentazocine	35 ±4
Nalmefene	0	(-)Bremazocine	0
Nalorphine	0	DAMGO	100
Naltriben	0	PL017	109 ±22
Naltrindole	0	DSLET	134 ± 65
Diprenorphine	0	DADLE	89 ± 15
		Leu-Enkephalin	11 ± 4

* Lower limit (mean – standard deviation) of efficacies were used during model development.

a) Relative % stimulation with respect to that of DAMGO (100%) using a [^{35}S]GTPγS binding assay

Table 2. Overlap coefficient used in the ABNS and AB'NS models

ABNS

	AB	AN	AS	BN	BS	NS	ABN	ABS	ANB	ANS	ASB	ASN	BAN	BAS	BNS	BSN	NAS	NBS	efficacy
morphine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90
dihydromorphine	0.20	0.82	0.87	0.32	0.32	0.99	0.23	0.27	0.52	0.96	0.57	0.97	0.26	0.31	0.30	0.33	0.95	0.31	1.04
etorphine	0.50	0.52	0.55	0.24	0.24	0.98	0.53	0.46	0.24	0.71	0.23	0.72	0.24	0.24	0.33	0.41	0.63	0.25	0.93
normorphine	0.97	0.86	0.22	0.97	0.00	0.00	0.93	0.77	0.95	0.37	0.48	0.20	0.93	0.41	0.62	0.33	0.03	0.36	1.03
buprenorphine	0.50	0.41	0.03	0.16	0.00	0.00	0.49	0.85	0.20	0.32	0.01	0.10	0.19	0.24	0.17	0.27	0.30	0.16	0.30
naloxone	0.25	0.85	0.05	0.21	0.05	0.00	0.22	0.55	0.73	0.23	0.30	0.10	0.31	0.28	0.29	0.30	0.41	0.34	0.00
naltrexone	0.25	0.88	0.01	0.21	0.02	0.00	0.23	0.65	0.74	0.23	0.17	0.05	0.31	0.28	0.28	0.32	0.35	0.32	0.00
nalmefene	0.50	0.91	0.01	0.44	0.09	0.00	0.55	0.63	0.83	0.22	0.16	0.06	0.57	0.45	0.30	0.32	0.36	0.33	0.00
nalorphine	0.98	0.96	0.05	0.92	0.26	0.00	0.96	0.55	0.97	0.22	0.18	0.11	0.95	0.54	0.32	0.27	0.43	0.41	0.00
naltrindole	0.00	0.21	0.43	0.00	0.00	0.00	0.00	0.00	0.76	0.35	0.48	0.11	0.00	0.00	0.26	0.24	0.16	0.54	0.00
naltriben	0.00	0.30	0.36	0.00	0.00	0.00	0.00	0.00	0.32	0.32	0.84	0.11	0.00	0.00	0.33	0.30	0.19	0.59	0.00
diprenorphine	0.52	0.41	0.02	0.16	0.00	0.00	0.48	0.83	0.20	0.28	0.01	0.08	0.20	0.24	0.19	0.28	0.35	0.20	0.00
cyclazocine	0.75	0.84	0.03	0.75	0.21	0.00	0.77	0.43	0.66	0.27	0.22	0.09	0.73	0.48	0.31	0.26	0.33	0.35	0.15
pentazocine	0.75	0.84	0.03	0.75	0.17	0.00	0.77	0.46	0.66	0.34	0.14	0.10	0.73	0.48	0.28	0.32	0.22	0.29	0.31
EKC	0.01	0.78	0.11	0.22	0.00	0.00	0.02	0.40	0.04	0.26	0.71	0.06	0.08	0.25	0.66	0.40	0.29	0.52	0.61
bremazocine	0.00	0.79	0.03	0.01	0.35	0.00	0.05	0.75	0.00	0.48	0.26	0.12	0.00	0.07	0.32	0.59	0.14	0.03	0.00
etonitazene	0.00	0.20	0.26	0.00	0.02	0.01	0.12	0.17	0.09	0.53	0.04	0.52	0.36	0.34	0.41	0.44	0.16	0.10	1.00
methadone	0.01	0.50	0.57	0.01	0.06	0.00	0.02	0.07	0.54	0.33	0.56	0.39	0.08	0.16	0.18	0.23	0.03	0.25	0.96
fentanyl	0.46	0.03	0.00	0.00	0.00	0.00	0.22	0.41	0.04	0.04	0.00	0.01	0.01	0.03	0.21	0.11	0.30	0.44	0.88
funaltrexamine	0.00	0.50	0.32	0.00	0.00	0.00	0.00	0.00	0.24	0.30	0.12	0.09	0.16	0.12	0.45	0.39	0.24	0.63	0.19
DAMGO	0.00	0.17	0.32	0.00	0.00	0.00	0.00	0.00	0.08	0.27	0.06	0.25	0.18	0.27	0.20	0.12	0.25	0.04	1.00

DSLET	0.00	0.24	0.32	0.00	0.00	0.00	0.00	0.00	0.08	0.26	0.05	0.26	0.17	0.26	0.18	0.13	0.23	0.06	0.69
DADLE	0.00	0.23	0.32	0.00	0.00	0.00	0.00	0.00	0.11	0.29	0.07	0.28	0.16	0.26	0.20	0.12	0.25	0.04	0.74
PL107	0.00	0.42	0.38	0.00	0.01	0.00	0.00	0.00	0.04	0.27	0.02	0.29	0.26	0.31	0.21	0.16	0.12	0.06	0.87
Leu-Enkephalin	0.00	0.29	0.35	0.00	0.00	0.00	0.00	0.00	0.06	0.28	0.04	0.28	0.19	0.28	0.19	0.14	0.19	0.09	0.07

AB'NS

	AB	AN	AS	BN	BS	NS	ABN	ABS	ANB	ANS	ASB	ASN	BAN	BAS	BNS	BSN	NAS	NBS	efficacy
morphine	0.00	0.51	0.55	0.00	0.01	0.98	0.00	0.00	0.00	0.71	0.00	0.73	0.06	0.19	0.10	0.03	0.64	0.44	0.90
dihydromorphine	0.00	0.39	0.44	0.00	0.01	0.97	0.00	0.00	0.00	0.70	0.00	0.73	0.04	0.10	0.07	0.03	0.58	0.24	1.04
etorphine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93
normorphine	0.00	0.39	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.10	0.06	0.35	0.28	0.22	0.04	0.78	1.03
buprenorphine	0.29	0.78	0.00	0.43	0.35	0.00	0.03	0.01	0.04	0.37	0.02	0.14	0.05	0.10	0.59	0.78	0.24	0.05	0.30
naloxone	0.00	0.39	0.08	0.00	0.41	0.00	0.00	0.00	0.00	0.28	0.00	0.13	0.03	0.07	0.38	0.12	0.33	0.34	0.00
naltrexone	0.00	0.41	0.02	0.00	0.54	0.00	0.00	0.00	0.00	0.29	0.00	0.08	0.03	0.07	0.45	0.10	0.28	0.30	0.00
nalmefene	0.00	0.51	0.02	0.00	0.47	0.00	0.00	0.00	0.00	0.27	0.00	0.09	0.04	0.25	0.45	0.14	0.29	0.30	0.00
nalorphine	0.00	0.53	0.07	0.00	0.21	0.00	0.00	0.00	0.00	0.27	0.00	0.13	0.05	0.32	0.38	0.16	0.36	0.35	0.00
naltrindole	0.00	0.12	0.45	0.01	0.00	0.00	0.10	0.67	0.00	0.38	0.00	0.15	0.00	0.00	0.46	0.43	0.11	0.32	0.00
naltriben	0.00	0.21	0.39	0.01	0.01	0.00	0.10	0.62	0.00	0.33	0.00	0.14	0.00	0.01	0.48	0.44	0.15	0.39	0.00
diprenorphine	0.00	0.80	0.00	0.04	0.52	0.00	0.00	0.00	0.03	0.33	0.01	0.13	0.31	0.40	0.54	0.77	0.28	0.01	0.00
cyclazocine	0.00	0.40	0.03	0.00	0.07	0.00	0.00	0.00	0.00	0.34	0.00	0.13	0.06	0.41	0.34	0.19	0.26	0.34	0.15
pentazocine	0.00	0.39	0.03	0.00	0.16	0.00	0.00	0.00	0.00	0.37	0.00	0.14	0.06	0.39	0.33	0.19	0.18	0.27	0.31
EKC	0.00	0.38	0.12	0.00	0.47	0.00	0.00	0.00	0.00	0.33	0.00	0.09	0.02	0.03	0.11	0.02	0.22	0.30	0.61

bremazocine	0.00	0.35	0.05	0.00	0.01	0.00	0.00	0.00	0.02	0.59	0.00	0.19	0.09	0.64	0.53	0.09	0.11	0.02	0.00
etonitazene	0.00	0.16	0.20	0.43	0.59	0.01	0.04	0.33	0.02	0.48	0.03	0.43	0.08	0.24	0.21	0.22	0.18	0.10	1.00
methadone	0.00	0.35	0.34	0.62	0.64	0.00	0.02	0.07	0.00	0.35	0.01	0.38	0.04	0.11	0.07	0.07	0.04	0.21	0.96
fentanyl	0.08	0.02	0.32	0.37	0.11	0.00	0.07	0.03	0.04	0.08	0.00	0.06	0.03	0.00	0.12	0.09	0.01	0.55	0.88
funaltrexamine	0.03	0.40	0.33	0.10	0.03	0.00	0.51	0.28	0.01	0.34	0.00	0.12	0.02	0.02	0.49	0.36	0.19	0.48	0.19
DAMGO	0.11	0.16	0.25	0.03	0.07	0.00	0.19	0.07	0.28	0.23	0.23	0.21	0.23	0.30	0.29	0.26	0.28	0.04	1.00
DSLET	0.13	0.20	0.20	0.05	0.12	0.00	0.15	0.06	0.24	0.24	0.23	0.23	0.22	0.27	0.25	0.22	0.28	0.06	0.69
DADLE	0.11	0.22	0.25	0.03	0.07	0.00	0.20	0.05	0.22	0.26	0.23	0.24	0.18	0.24	0.30	0.27	0.28	0.04	0.74
PL107	0.15	0.28	0.21	0.08	0.14	0.00	0.28	0.10	0.39	0.27	0.25	0.28	0.28	0.39	0.25	0.23	0.19	0.06	0.87
Leu-Enkephalin	0.19	0.24	0.23	0.07	0.19	0.00	0.25	0.13	0.25	0.26	0.22	0.25	0.23	0.28	0.24	0.23	0.24	0.08	0.07

Table 3. Top 10 ABNS regression models for efficacy

no.	a	X_1	b	X_2	c	X_3	d	R^2	Q^2	P-value	Cor (X_1,X_2)	Cor (X_1,X_3)	Cor (X_2,X_3)
1	1.10	AS	2.09	BNS	-2.43	NBS	0.23	0.9602	0.95	0.00001	0.61	0.62	0.81
2	-1.73	BS	2.48	ANS	0.89	BAN	-0.80	0.9342	0.89	0.00004	0.78	0.57	0.18
3	0.82	BN	-1.54	BS	2.24	ANS	-0.68	0.929	0.90	0.00006	0.60	0.28	0.78
4	0.80	AB	-1.46	BS	2.30	ANS	-0.78	0.9286	0.94	0.00006	0.50	0.17	0.78
5	0.42	ABS	2.22	ASN	-1.70	NAS	0.18	0.928	0.95	0.00006	0.09	0.21	0.88
6	-1.46	BS	0.81	ABN	2.26	ANS	-0.77	0.9253	0.93	0.00007	0.53	0.78	0.20
7	0.46	AN	2.22	ASN	-1.79	NAS	0.14	0.9123	0.72	0.00014	0.27	0.42	0.88
8	0.73	BN	-1.49	BS	1.75	ASN	-0.21	0.9101	0.58	0.00015	0.60	0.32	0.80
9	0.70	AB	-1.41	BS	1.78	ASN	-0.29	0.9075	0.73	0.00017	0.50	0.21	0.80
10	0.31	AB	2.00	ASN	-1.44	NAS	0.22	0.9068	0.91	0.00018	0.21	0.19	0.88

Efficacy = $aX_1 + bX_2 + cX_3 + d$ where X_1, X_2 and X_3 represent the OC of combinations of pharmacophoric points used in the models.

Table 4. Top 10 AB'NS regression models for efficacy based on the use of the C19 substituent (B') as the B pharmacophoric point for the oripavines.

no	a	X_1	b	X_2	c	X_3	d	R^2	Q^2	P value	Cor (X_1,X_2)	Cor (X_1,X_3)	Cor (X_2,X_3)
1	1.86	BN	-2.51	BNS	0.62	NBS	0.97	0.8739	0.95	0.00059	0.81	0.51	0.32
2	3.34	AB	-3.39	BNS	-0.84	NAS	1.76	0.8728	0.59	0.00061	0.80	0.71	0.28
3	-1.97	BS	-1.37	ABS	3.38	ANB	0.89	0.8670	0.79	0.00073	0.27	0.72	0.71
4	-1.92	BS	3.93	ABN	-1.97	ABS	0.89	0.8634	0.90	0.00081	0.27	0.71	0.71
5	1.80	NS	1.01	BAS	-2.40	NAS	0.53	0.8617	0.85	0.00085	0.66	0.27	0.80
6	1.78	BN	0.72	BAS	-2.74	BNS	1.14	0.8604	0.92	0.00088	0.43	0.87	0.67
7	1.98	AB	-2.47	BNS	0.40	NBS	1.05	0.8505	0.77	0.00115	0.78	0.81	0.64
8	1.92	AB	0.53	BAS	-2.65	BNS	1.16	0.8445	0.84	0.00135	0.80	0.59	0.32
9	2.26	AB	-3.16	BNS	0.47	BSN	1.30	0.8434	0.74	0.00139	0.82	0.80	0.64
10	1.68	BN	0.83	BAN	-2.84	BNS	1.26	0.8330	0.87	0.00178	0.88	0.81	0.76

Efficacy = $aX_1 + bX_2 + cX_3 + d$ where X_1, X_2 and X_3 represent the OC of combinations of pharmacophoric points used in the models.

Table 5. RMSD of the training set with the ABNS and AB'NS models

	1	2	3	4	5	6	7	8	9	10	avg
ABNS	0.09	0.12	0.12	0.12	0.12	0.12	0.13	0.14	0.14	0.14	0.08
AB'NS	0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.18	0.14

Table 6. Predicted efficacies of fentanyl and methadone in test set 1 using ABNS and AB'NS models. Pharmacophoric point definitions are illustrated in Figure S3.

		ABNS	AB'NS
fentanyl	definition		
	1	-0.3	1.3
	2	-0.1	1.2
	3	0.1	1.4
methadone	1	0.5	1.1
	2	0.6	-0.1

Table 7. Comparison between experimental and average calculated values by the ABNS and AB'NS models of the test molecules (Figure 1). Numbers in parenthesis are differences from experimental values.

comp.	expt.	ABNS	AB'NS
cyclazocine	0.15	0.13 (-0.02)	0.50 (0.35)
pentazocine	0.31	0.30 (-0.01)	0.47 (0.16)
EKC	0.61	0.00 (-0.61)	0.58 (-0.04)
bremazocine	0.00	0.04 (0.04)	0.27 (0.27)
etonitazene	1.00	0.78 (-0.23)	0.41 (-0.59)
methadone	0.96	0.53 (-0.43)	1.07 (-0.11)
fentanyl	0.88	-0.31 (-1.19)	1.18 (0.30)
funaltrexamine	0.19	-0.02 (-0.21)	0.36 (0.17)
DAMGO	1	0.19 (-0.81)	0.85 (-0.15)
DSLET	0.69	0.19 (-0.50)	0.87 (0.18)
DADLE	0.74	0.24 (-0.50)	0.81 (0.07)
PL107	0.87	0.31 (-0.56)	1.05 (0.18)
Leu-Enkephalin	0.07	0.25 (0.18)	0.95 (0.88)

Root mean square deviation (RMSD) of top 10 models

	1	2	3	4	5	6	7	8	9	10	avg
ABNS	0.37	0.52	0.54	0.56	0.34	0.57	0.36	0.39	0.41	0.31	0.39
AB'NS	0.38	0.38	0.64	0.59	0.75	0.40	0.37	0.26	0.27	0.28	0.26

Table 8. Antinociceptive potencies (ED_{50} in mg/kg) in mouse

		tail-flicking assay	writhing assay	hot plate assay	relative % stimulation	AB'NS
training set	morphine	3.00[a] / 1.25[b] / 3.80[c] / 5.80[d]	0.60[e] / 0.28[f] / 0.43[a] / 0.23[d]	0.80[d]	0.93±0.03	1.0
	etorphine	0.0006[b]	0.0009[f]		1.17±0.24	0.9
	buprenorphine	2.40[c]	0.02[f]		0.66±0.36	0.3
test set 1	fentanyl	0.013[b]			1.00±0.12	1.2
	cyclazocine		0.10[f]		0.33±0.18	0.5
	pentazocine	inactive[c]	1.20[d] / 0.80[f]		0.35±0.04 (0.04[d])	0.5
test set 2	1	3.86[d]	0.39[d]	2.42[d]	0.52[d]	1.8
	2	0.6383[a]	0.1014[a]			1.9
	3	0.0001[d]	0.0002[d]	0.0001[d]	1.04[d]	0.4
	4	0.0032[d]	0.0062[d]	0.0023[d]		1.0
	5			1.18[i]	0.78±0.05[h]	0.8
	6	0.11[g]			0.79±0.01[h]	2.1
	7		0.8028[e]			0.4

* Experimental values from different publications are indicted by slash separator. Units in original papers were converted to mg/kg.

* Values in parenthesis are those found from other literatures. .

* Relative % stimulation is based on DAMGO's efficacy 1.00 (100%)

a. reference 1, b. reference 2, c. reference 3 d. reference 4, e. reference 5, f. reference 6, g. reference 7, h. reference 8, i. reference 9

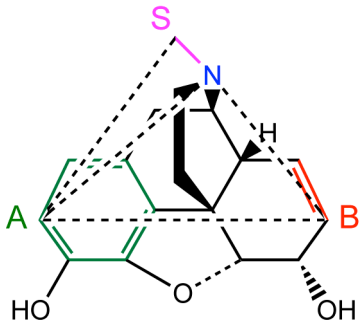


Figure 1. Pharmacophoric points used in CSP model development, from which six distances (AB, AN, AS, BN, BS, NS) and twelve angles (ABN, ABS, ANB, ANS, ASB, ASN, BAN, BAS, BNS, BSN, NAS, NBS) were derived and considered in model development. For the pharmacophoric points A and B, the definitions were based on the atoms yielding the maximum distance between A and B, pharmacophoric point N was simply the geometric position of the basic nitrogen, and definition of S was based on the geometric center of the atoms comprising that group.

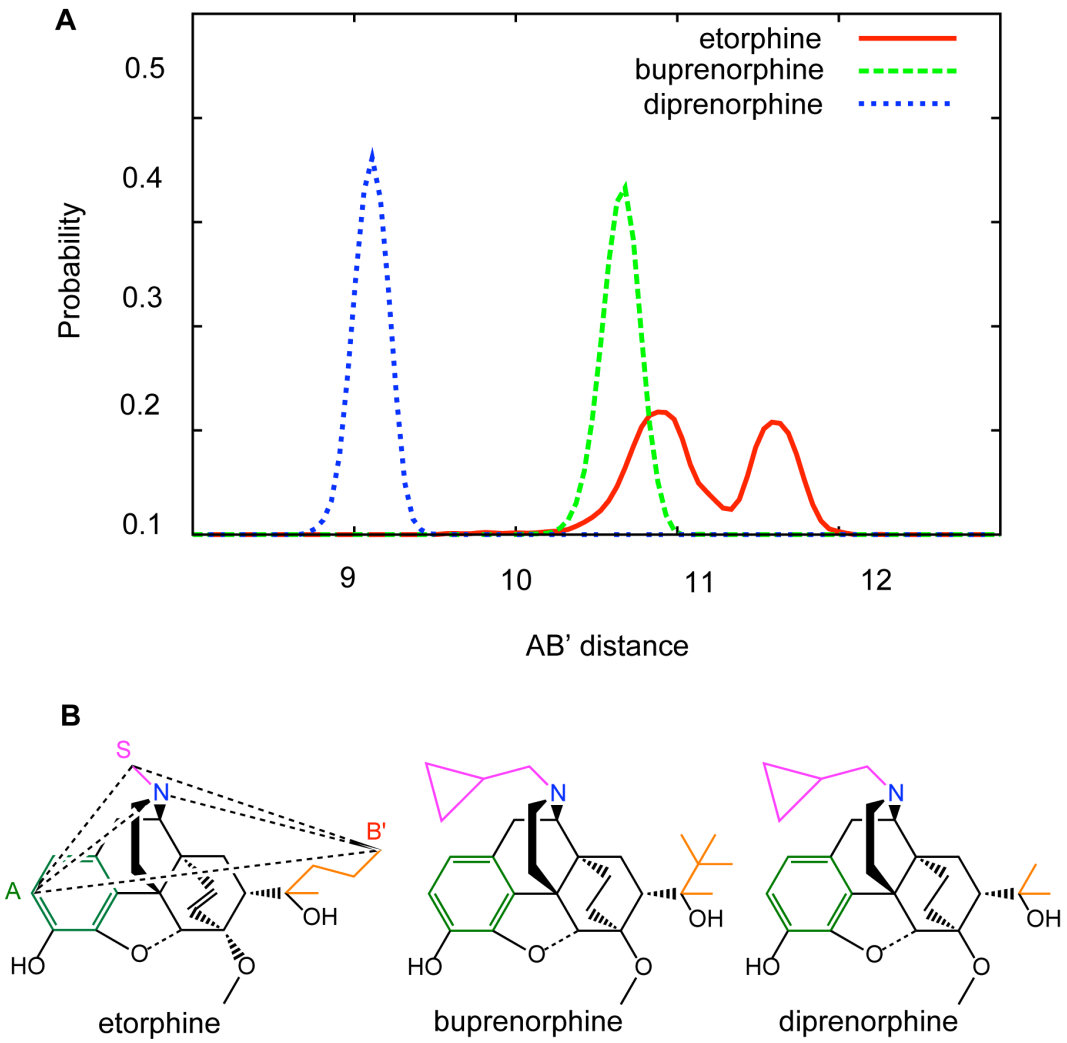


Figure 2. a) 1D probability distributions of AB' distance on etorphine, buprenorphine and diprenorphine using the alternate definition of the B group, designated B'. b) Pharmacophoric point definition for AB'NS models where the B' group is represented in orange.

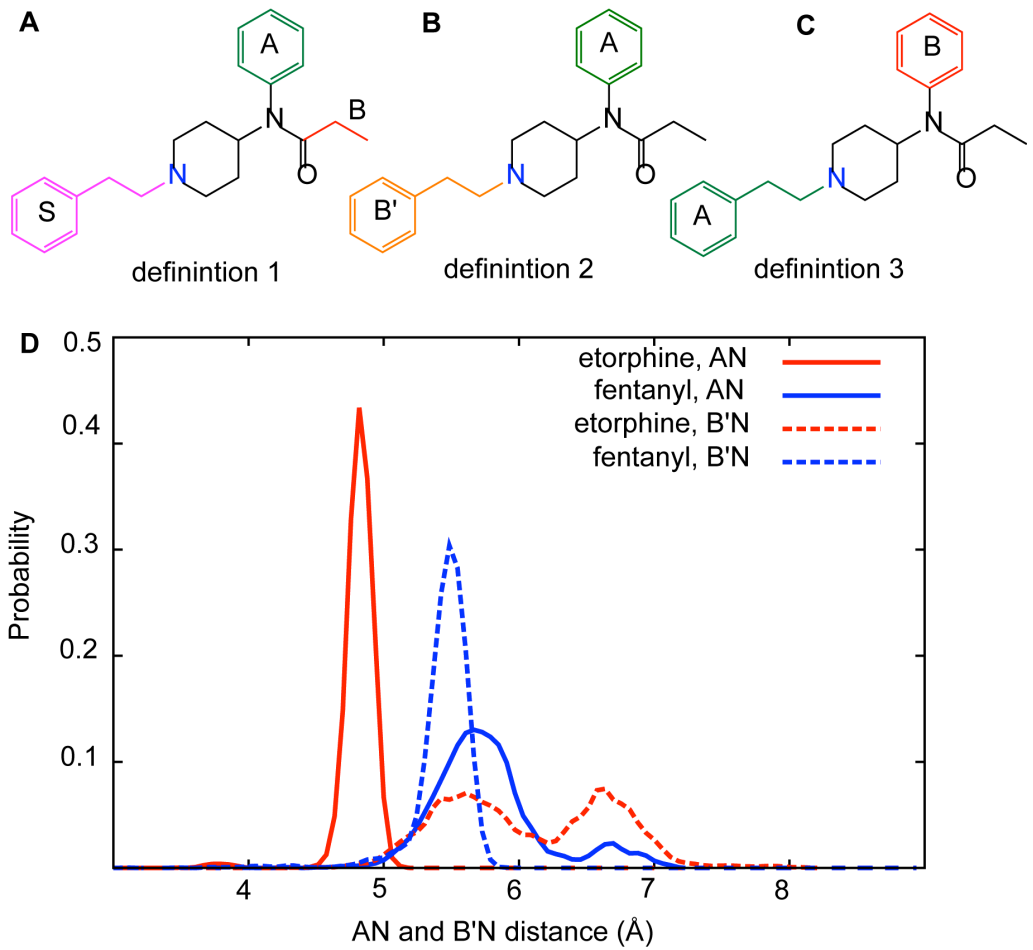


Figure 3. A) Conventional pharmacophore definitions used in ABNS models where phenethyl group is S point (N-substituent), the aromatic ring attached to the amide is A, and the propionyl group is B. B) Pharmacophore definitions used in AB'NS model where phenethyl group is alternative B' point. C) Third pharmacophore definition where A is the phenethyl group and B is the aromatic ring attached to the amide. In all cases N is the piperdine ring nitrogen and if not noted, S is the associated basic nitrogen. D) Probability distribution of the distance between the two aromatic rings and the N descriptor.

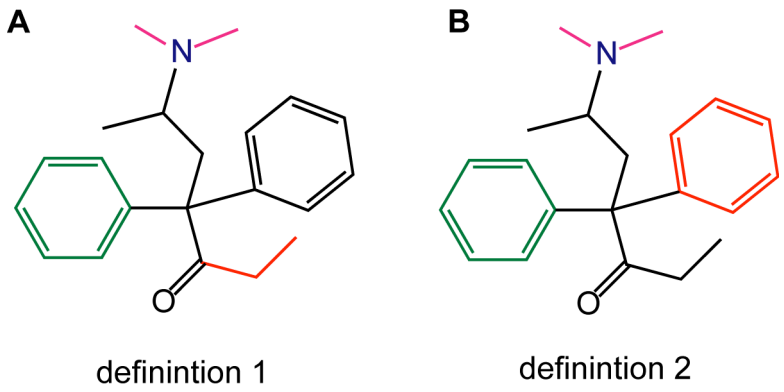


Figure 4. Pharmacophoric point definitions for methadone showing the A (green), B (red), N (blue), and S (pink) groups.

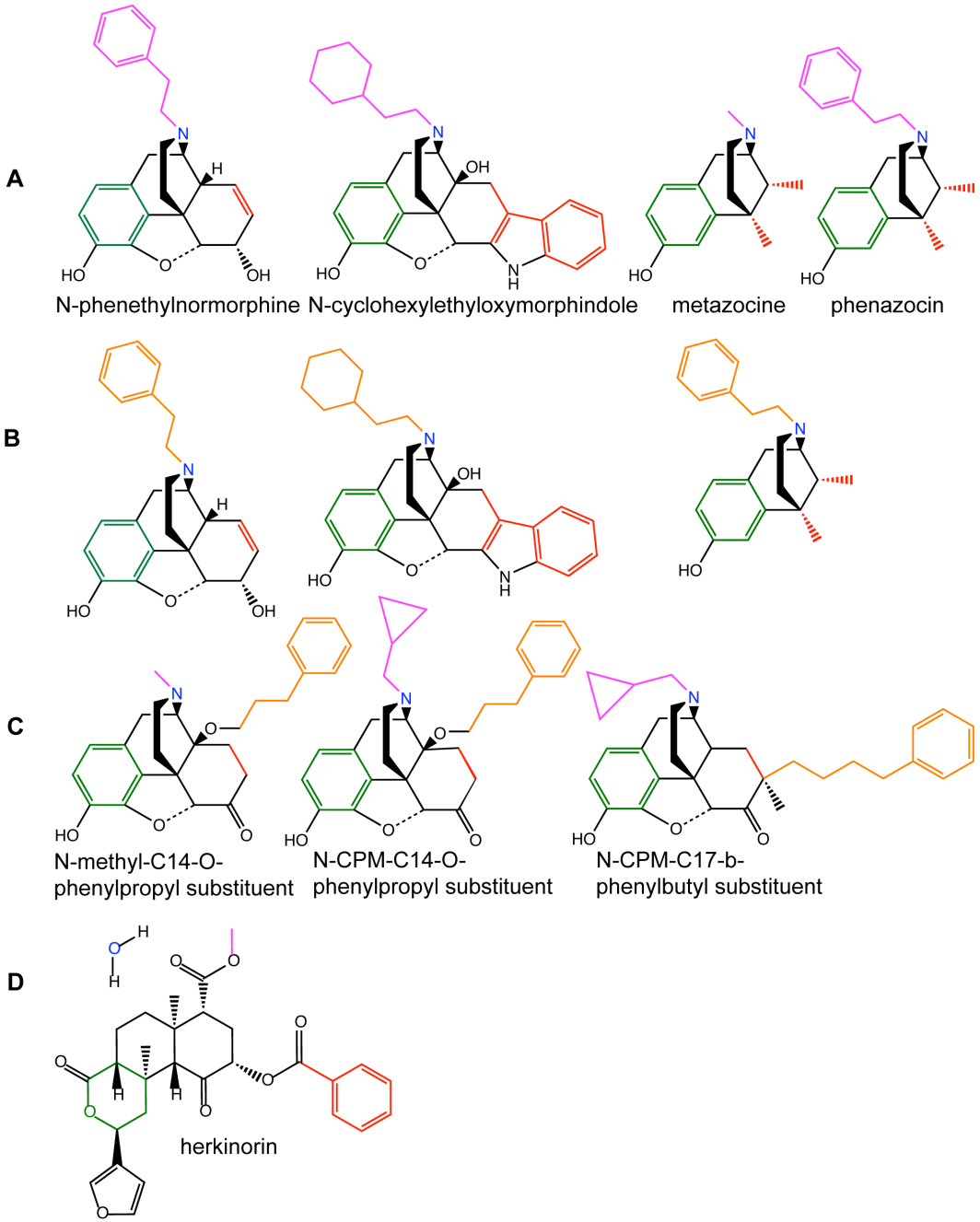

A
N-phenethylnormorphine
N-cyclohexylethyloxymorphindole
metazocine
phenazocin
B
C
N-methyl-C14-O-
phenylpropyl substituent
N-CPM-C14-O-
phenylpropyl substituent
N-CPM-C17-b-
phenylbutyl substituent
D
herkinorin

Figure 5. Test set 2 compounds and their pharmacophoric point definitions for the A) ABNS and B) AB'NS models. Compounds in c) show both definitions B (red) and B' (orange) used for ABNS and AB'NS models respectively. D) Pharmacophoric point definition for herkinorin was used only in AB'NS models.

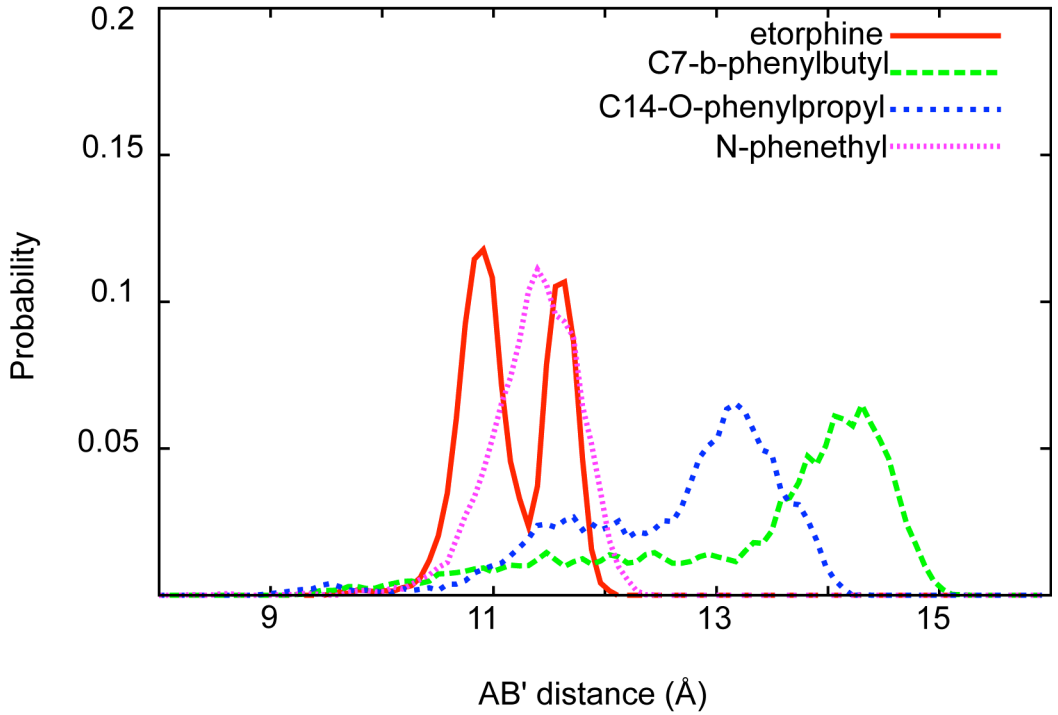


Figure 6. Probability distribution of AB' distance of etorphine, N-phenethylnormorphine, C7-β-phenylbutylmorphinans and C14-O-phenylpropylmorphinan.

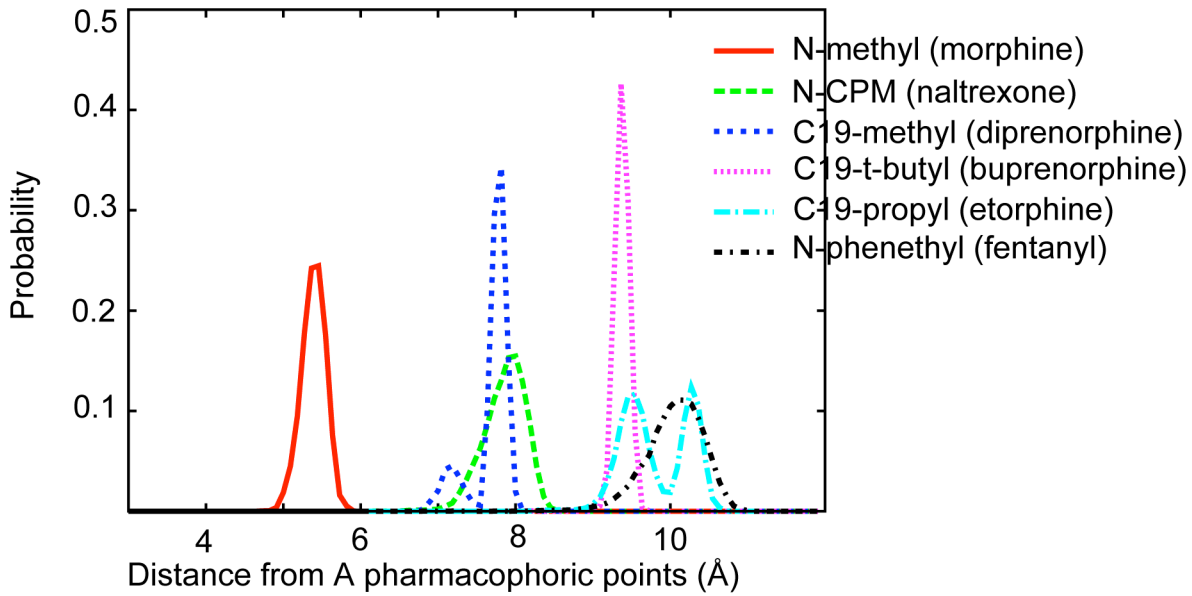


Figure 7. Distance distribution between the aromatic A ring and N-CPM of the antagonist naltrexone showing it to be similar to that between the A ring and C19-methyl substituent of the antagonist diprenorphin. In contrast the A to C19 substituent distance distributions in etorphine and buprenophine are significantly longer, allowing for interactions with the B site. A similar distribution occurs with fentanyl. Distances are from the centroid of aromatic ring to the indicated groups.

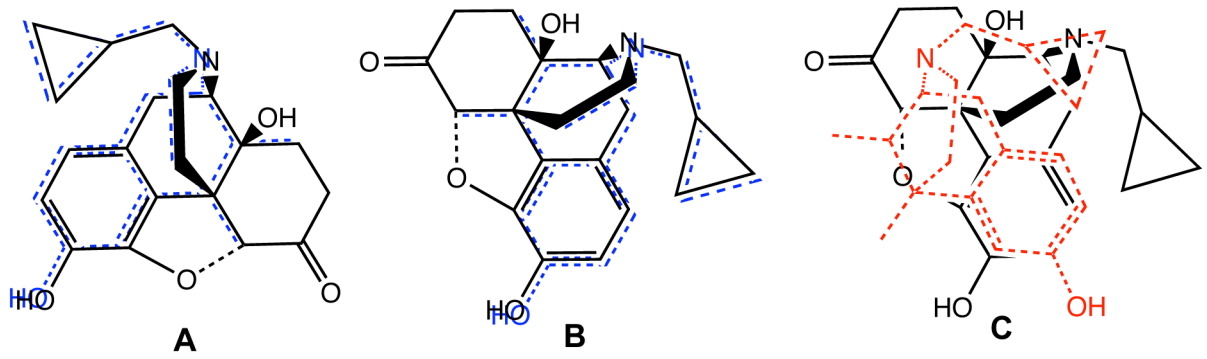


Figure 8. Reorientation of cyclazocine. Cyclazocine's structures are represented in dashed lines and overlaid on naltrexone. In b), structure a) is rotated around the axis connecting nitrogen and aromatic ring while c) shows cyclazocine in b) shifted towards the dihydrofuran ring of naltrexone illustrating the relative orientation in which cyclazocine assumes the binding orientation in Figure 5F.

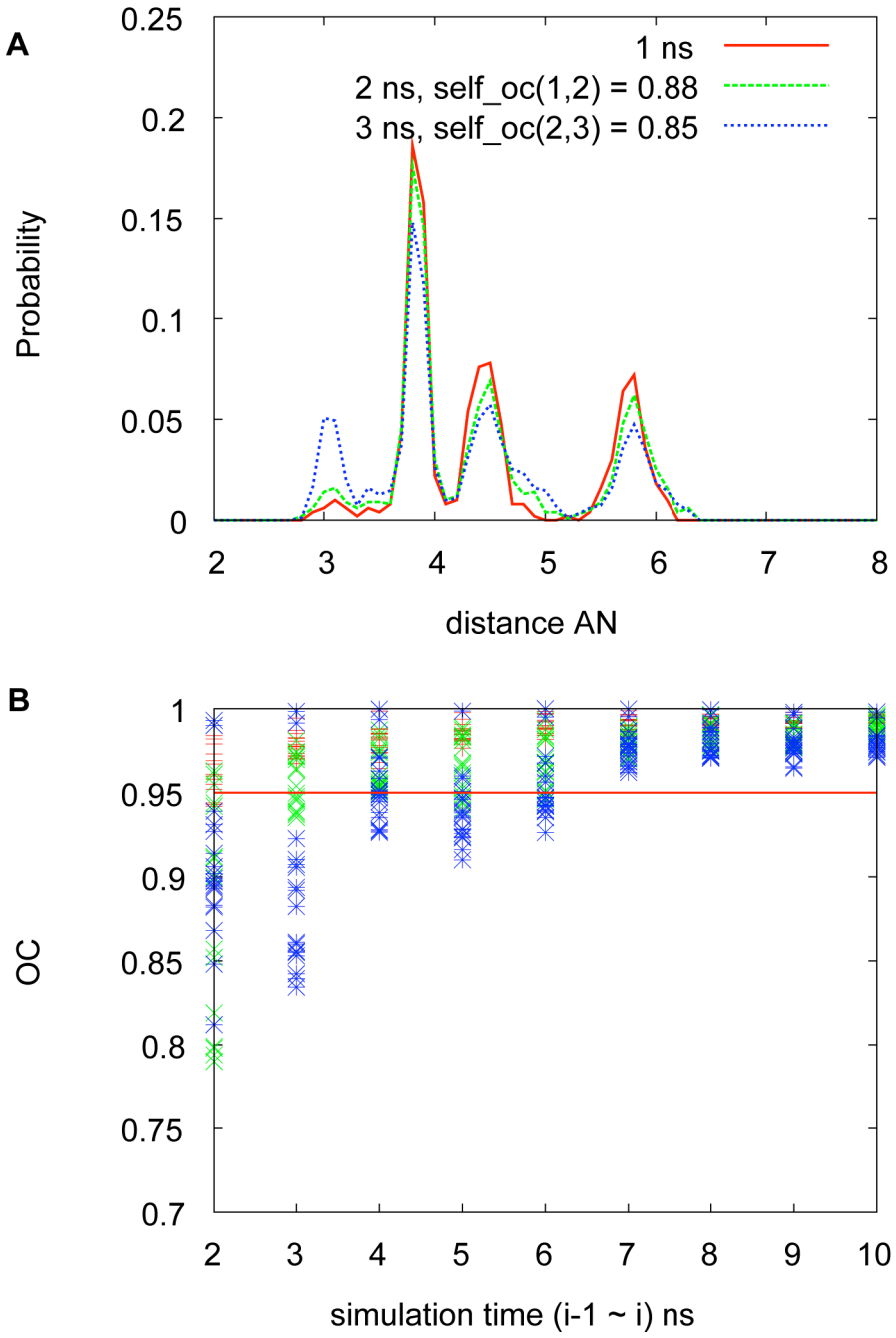


Figure 9. Convergence evaluation based on self OC values. a) Self OC values of the DAMGO AN probability distributions calculated between distributions at 1 and 2 ns (green line) and between 2 and 3 ns (blue line). In b) each point represents the self OC of a distance or angle parameter as a function of simulation time. Morphine's self OC values are shown in red, etonitazene in green, and

DAMGO in blue. Self OC values were calculated using equation S1 as a function of simulation time,

$$self\ OC\ (i, i+1) = \sum_j\ \min\ (P^A_{i,j}, P^A_{i+1,j})$$

$$self\ OC = \sum \min(P^A_i, P^{A+\Delta t}_i) \qquad \text{Eq. S1}$$

where P^A_i is the probability distribution of distance or angle between pharmacophoric points, at bin *i* out to *A* ns. The eighteen distances and angles described above (Figure S8) were subjected to this analysis and convergence was defined based on the magnitude of the self OC values as a function of simulation time. As an example, Figure S9a shows probability distributions for the distance between the aromatic ring (A) and protonated nitrogen (N) of DAMGO at 1, 2 and 3 ns. Figure S9b shows the change in all self OC values as a function of time for three compounds of varying flexibility. Self OCs of the flexible compounds etonitazene and DAMGO grow slowly (Figure S9b), whereas rigid morphine reaches 95 % similarities at 3ns. In all cases the convergence of conformational space was greater than 95 % following 10 ns of simulation time. This level of convergence was deemed satisfactory for CSP model development.

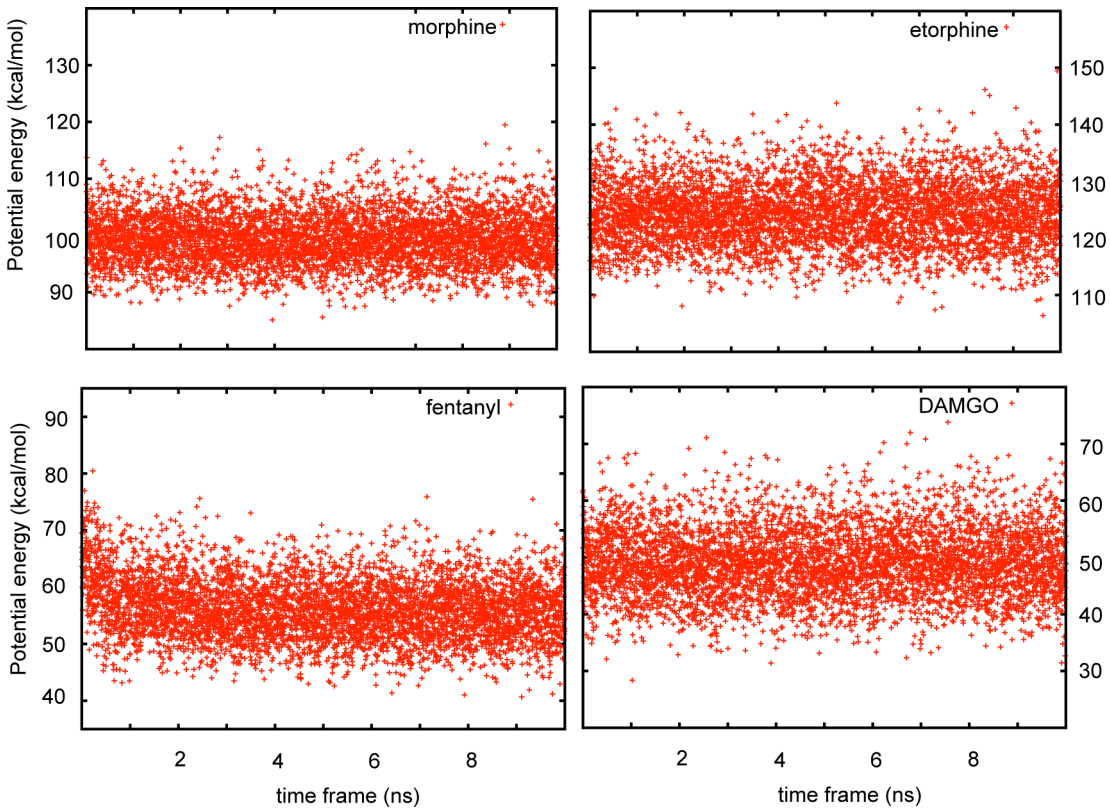


Figure 10. Potential energy as a function of time in the ground state replica. Results for four compounds, morphine, etorphine, fentanyl and DAMGO are shown. Notably, the use of temperature replica exchange MD in this study allowed for energy barriers of 30~40 kcal/mol to be overcome.

CGenFF parameters for nonpeptidic opioids: As internal parameters for many of the non-peptidic opioids were not explicitly treated in the CHARMM General Force Field (CGenFF) it was necessary to extend the force field to assure accurate modeling of these compounds. This was performed by analogy with the resulting parameters then validated based on calculations on morphine, cyclazocine, and etorphine. The resulting bond and angle parameters reproduced target geometries quite well, having root mean square differences (RMSD) within 0.02 Å and 2.3˚ with respect to QM data and 0.03 Å and 3.1° with respect to high resolution crystal structures for the bonds and angles, respectively (Table P1). In addition, CGenFF vibrational frequencies were compared with QM results for aminomethylcyclopropane and benzomorphan (see Tables P2 of the Supporting information). The overall agreement was considered acceptable and further optimization of the internal parameters was not undertaken. Further validation of the parameters was based on the crystal MD simulations. Results from the simulations showed that interactions in the condensed-phase crystal environments were well balanced, with similar changes occurring for the A, B and C lattice parameters. However, the force field appears to overpredict the unitcell volumes. A similar phenomenon has been observed with carbohydrate parameters[10] and has been suggested to be associated with limitations in the additive potential energy function. Accordingly, additional optimization of the force field parameters was not undertaken.

Vibrational frequencies, including potential energy distributions, were obtained at the MP2/6-31G* level and examined using the CHARMM VIBRAN and MOLVIB modules[11]. QM vibrational frequencies were scaled by 0.971 to account for limitations in

the level of theory[12]. Final validation of the force field was performed with crystal simulations using the CRYSTAL module in the CHARMM program. Atomic coordinates of morphine, etorphine, and naltrexone (CSD ID: MORPHC, SUNVOM, PABCEA, respectively) were relaxed by minimization with the lattice parameters fixed. Nonbond interactions were truncated at 26 Å with smoothing from 24 Å of the Lennard-Jones interactions by a switching function while long-range electrostatic interactions were included using the particle mesh Ewald method[13]. The Velocity-Verlet 2 algorithm[14] was used to propagate the simulations with an integration timestep of 1fs in conjunction with Nose-Hoover thermostat and Anderson-Hoover barostat. The crystal simulations were run for 7 ns with the last 5 ns used for analysis.

Table P1. Comparison of empirical and target bond and angles from the CGenFF minimized structures and from the crystal simulations.

	Geometry (RMSD)				Crystal simulation (%error)			
	QM		Xtal		Unitcell parameters			
	Bond	Angle	Bond	Angle	A	B	C	Volume
Cyclazocine	0.02	2.01						
Morphine	0.02	2.33	0.02	1.83	0.6	0.6	0.6	1.7
Etorphine			0.03	2.62	5.5	5.5	5.5	17.5
Naltrexone			0.02	2.38	2.4	2.4	2.4	7.3

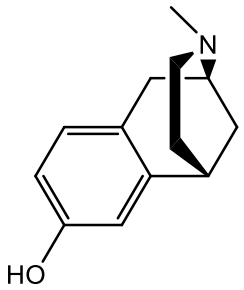
* units: Å and degrees for bonds and angles, respectively, RMSD= $\sqrt{\frac{\sum(x_{mm}-x_{qm\ or\ xtal})^2}{N}}$,

% error = $\frac{x_{mm}-x_{exp}}{x_{exp}} * 100$

Table P2. Vibrational spectra of Aminomethyl cyclopropane and 1,2,3,4,5,6-hexahydro-3-methyl-2,6-Methano-3-benzazocin-8-ol. Frequencies in cm^{-1}.

MP2/6-31G* scaled by a factor 0.971							CGenFF						
Freq.	Assignment	%	Assignment	%	Assignment	%	Freq.	Assignment	%	Assignment	%	Assignment	%
123.4	torC1-C9	100					92.6	torC1-C9	99				
212.1	torC9-N1	58	rC1C9a	26			178.6	torC9-N1	94				
231.4	torC9-N1	32	rC1C9a	32	cC9X2	25	242.2	rC1C9a	59	cC9X2	34		
367.5	rC1C9	73					382.1	cC9X2	28	rC1C9a	24	rC1C9	15
453.9	cC9X2	40	rC1C9a	31			444.7	rC1C9	62	cC9X2	17		
785.6	STRETCH	29	tCrH2	23	sC1-C9	16	690.5	tCrH2	54	sC1-C9	24		
811.4	rCrH2	37	tCrH2	20	rC9H2	20	757.8	rC9H2	30	wCrH2	29	tCrH2	22
861.6	rCrH2	32	rC1Ha	22	SLIDE	16	785.1	tCrH2	45	rC1Ha	43		
865.4	rC9H2	23	rCrH2	22	sC9-N12	17	802.0	wCrH2	65	rC9H2	18		
910.0	sC9-N12	55	STRETCH	15			838.9	tCrH2	46	wCrH2	34		
941.9	SLIDE	62					864.6	rN12H3a	19	sC1-C9	17	tCrH2	16
994.3	STRETCH	31	sC1-C9	20	tCrH2	16	900.1	wCrH2	47	rC1Ha	38		
1034.3	rN12H3a	37					963.4	rN12H3	41	sC9-N12	32		
1096.4	wCrH2	95					984.6	rN12H3	31	rN12H3a	19	sC9-N12	17
1121.9	wCrH2	98					1006.0	rN12H3a	35	sC9-N12	31		
1124.8	rN12H3	24					1086.2	rCrH2	38	STRETCH	18		
1158.6	rC1Ha	59	tCrH2	18			1106.7	STRETCH	60	rCrH2	18		
1221.5	tCrH2	48	rCrH2	44			1111.7	SLIDE	54				
1247.0	tCrH2	25	rC9H2	15			1205.5	rCrH2	99				
1256.5	PULSE	44	rC1H	26			1253.3	PULSE	56				
1351.6	tC9H2	61					1291.5	tC9H2	72				
1392.0	wC9H2	62	rC1H	15			1348.0	wC9H2	56	rC1H	23		
1458.2	rC1H	35	wC9H2	23			1457.5	dsN12H3	80				

1503.3	cCrH2	98			1463.4	cCrH2	96				
1527.5	cC9H2	69	dsN12H3	26	1471.2	cCrH2	55	rC1H	27		
1533.6	dsN12H3	70	cC9H2	28	1486.6	cC9H2	56	dsN12H3	16		
1547.8	cCrH2	76			1513.9	rC1H	37	PULSE	21	cCrH2	19
1686.9	daN12H3	89			1620.3	daN12H3	74	daN12H3a	21		
1697.7	daN12H3a	89			1624.1	daN12H3a	74	daN12H3	21		
3104.8	ssC9H2	100			2773.4	ssC9H2	99				
3153.5	ssCrH2	95			2805.0	saC9H2	100				
3168.7	sC1-H1	48	saC9H2	47	3029.4	ssCrH2	96				
3175.5	ssCrH2	94			3039.4	ssCrH2	99				
3181.7	saC9H2	50	sC1-H1	49	3054.6	sC1-H1	94				
3253.3	saCrH2	95			3087.9	saCrH2	100				
3271.3	saCrH2	98			3102.0	saCrH2	98				
3360.8	ssN12H3	100			3153.5	ssN12H3	100				
3472.1	saN12H3	93			3256.6	saN12H3a	51	saN12H3	49		
3476.7	saN12H3a	93			3257.2	saN12H3	51	saN12H3a	49		



MP2/6-31G*							CGenFF						
Freq.	Assignment	%	Assignment	%	Assignment	%	Freq.	Assignment	%	Assignment	%	Assignment	%
52.5	torR2	55	bfR1R2	20			52.2	torR2	51	bfR1R2	21		
100.6	torR2'	51	torR1	43			105.5	torR2'	59	torR1	23		
123.6	torR3a	50	bfR1R2	39			125.7	torR3a	47	bfR1R2	42		
208.6	torCH3	39					205.2	wR1CO	22				
228.8	torCH3	43					242.0	rR1CO	18				
258.3	torR3a'	38					295.0	torCH3	21	puckR2	17		
289.6	torR3a'	45	dR3XCZ	16			298.3	torR3a'	27	dR2a	20		
322.6	puckR2	26	puckR1	20	puckR3	20	324.0	dR3YCZ	47	dR3XCZ	15		
334.4	torR1'	30	torR2'	19			328.0	torR1'	26				
349.4	dR3YCZ	45					340.2	torCO	77				
361.8	torCO	48					357.4	torCH3	54				
363.0	torCO	57					369.0	torR3a'	52	dR3a'	17		
390.4	puckR2	22					406.9	puckR2	18				
426.4	rR1CO	29	torR1'	18			413.7	dR3XCZ	20				
444.6	torR1'	24					448.5	rR1CO	20				
470.2							453.9	torR1'	30	torR1	15		
490.6	dR2a'	19	dR3a	18			471.4	dR1a	18				
511.7	dR3a	16					512.4	torR1	25				
528.7	wR1CO	73	puckR1	26			534.3	dR3a	22	dR2	19		
558.1	dR2a'	17					550.1	puckR3	21	dR2a'	20	sR3CN	15
600.9	dR2a	25	puckR2	16			616.8	puckR1	15				

673.8	dR3a'	16					649.4	puckR1	61							
708.3	dR1	24	sR2CC	17	dR1a'	15	670.6	puckR1	21							
725.0	sR3CN	52					700.7	sR3CN	28							
755.4	sR1CC	31	sR2CC	24			736.3	dR1a'	34	sR1CC	23					
765.4	wR1CH	90					781.4	rR3CH2'	38							
813.3	wR1CH	104					826.6	wR1CH	38	rR2CH2	34					
821.8	rR3CH2'	24					843.3	sR3CC	27	twR2CH2'	18					
852.4	wR1CH	107					849.2	wR1CH	60							
863.0	sR3CC	39	sR2CC	20			882.5	sR1CC	25	twR2CH2'	23					
894.6	sR2CC	48					899.5	sR2CC	39							
919.2	rR2CH2	40					916.7	wR1CH	102							
937.7	twR2CH2'	20	dR2	20			919.2	twR2CH2'	22	rR3CH2	18	sR1CC	17			
964.0	sR2CC	29	sR1CC	17			944.7	sR2CC	18	sR3CN	18					
975.8	sR1CC	17					980.0	wR1CH	118							
1016.7	rR3CH2	23	sR3CN	20	sR2CC	19	994.3	rR3CH2	28	sR3CC	25					
1025.9	sR3CN	21	sR3CC	20			1030.2	sR3CN	31							
1053.6	dR1	18					1050.3	sR3CN	30	sR2CC	21					
1074.9	sR3CC	24	sR2CC	16			1074.1	sR3CN	24	sR3CC	16					
1085.8	sR3CN	27					1086.6	sR1CC	16							
1120.2	sR3CC	19	sR2CC	18			1112.1	sR1CC	52	rR1CH	16					
1142.0							1112.2	sR2CC	19	sR3CN	15					
1156.5	rCH3	34					1126.1	sR1CC	33	sR2CC	17					
1171.9	rR1CH	27					1147.1	sR2CC	25							
1187.7	rR1CH	30					1173.4	twR3CH2'	41							
1197.7	rR1CH	23	rR2CH2'	17			1184.7	rCH3	38	rCH3'	20					
1216.5	dCOH	54	sR1CC	19			1200.2	rR2CH2'	36							
1221.8							1227.5	sR1CC	22							
1231.2	sR2CC	19					1238.4	twR2CH2	22							
1268.4	rR1CH	27					1250.9	rR1CH	31	sR1CC	29					
1278.0							1282.9	rR3CH	28							
1292.3	twR2CH2	34					1291.6	sR1CC	27	rR1CH	19					
1320.3	sR1OH	24					1294.4	twR2CH2	31							
1321.8	rR2CHb'	26					1303.7	sR3CC	18							

1355.1							1326.9	wR3CH2'	47							
1370.8	rR2CH'	20	wR3CH2'	18			1337.6	twR3CH2	55							
1378.6	wR2CH2'	17	twR3CH2	15			1360.1	wR2CH2'	62	sR2CC	15					
1387.3	rR2CH	19	wR2CH2	17			1374.2	sR2CC	20							
1403.3	wR3CH2	20	wR2CH2	18	wR2CH2'	16	1393.8	rR1CH	26							
1405.6	rR2CH'	31					1406.8	wR3CH2	45							
1414.9	wR3CH2'	22					1415.5	scR2CXY	30	rR1CH	19					
1443.7	rR3CH	51	wR3CH2	19			1424.7	dCH3	52							
1457.1	rR3CH'	40					1429.1	sR1CC	42							
1470.0	sR1CC	72					1444.5	scR3CXY'	58							
1482.6	dCH3	74					1445.8	dCH3	17							
1497.9	sR1CC	45	rR1CH	19			1447.6	scR3CXY'	27	rR1CH	15					
1509.6	scR2CXY	51	scR2CXY'	21			1458.9	scR2CXY'	79							
1515.3	scR3CXY'	58					1470.8	rR1CH	28	scR2CXY	26	sR1CC	18			
1524.4	dCH3a'	50	scR3CXY	26			1479.3	rR2CH'	23	dCH3a	22	rR3CH'	17			
1528.0	scR3CXY	41	scR2CXY'	21	dCH3a'	19	1492.1	dCH3a'	50							
1529.8	scR2CXY'	41	scR3CXY'	21	dCH3a'	15	1493.0	sR1CC	17	scR3CXY	16					
1542.4	dCH3a	48	scR3CXY	19			1503.8	scR3CXY	52							
1545.0	sR1CC	37	rR1CH	34			1517.9	sR1CC	22	dCH3a	19					
1633.6	sR1CC	72					1531.1	dCH3a	23							
1672.1	sR1CC	65	rR1CH	15			1552.7	sR1CC	31	rR1CH	26					
3042.7	sR2CH2'	67	sR3CH2'	19			2819.7	sR3CH2	99							
3044.6	sR2CH2	90					2848.0	sR3CH2a	100							
3046.1	sR3CH2'	67	sR2CH2'	18			2855.9	sR2CH2	98							
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3118.3	sR3CH2a'	86					2935.4	sR2CH2a'	89							
3162.9	sR1CH	100					2970.0	sR2CH	100							
3164.8	sR3CH2a	96					2987.3	sCH3a	87							

3180.4	sR1CH	61	sR1CO	38	2990.0	sCH3a'	87		
3180.7	sR1CO	61	sR1CH	38	3054.8	sR1CH	79	sR1CO	21
3203.1	sCH3a	82	sCH3a'	18	3055.7	sR1CH	88		
3223.5	sCH3a'	81	sCH3a	18	3058.9	sR1CO	67	sR1CH	33
3365.2	sR3NH	100			3317.3	sR3NH	100		
3691.5	sR1CH	100			3683.3	sR1CH	100		

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