

# Binding of hemopressin peptide with cannabinoid CB<sub>1</sub> receptor: structural insights

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# 1 Supplementary results

## 1.1 Proton Chemical Shifts of Hemopressin in DPC/SDS micelles 90:10 M/M.

**Table 1S.** The  $^1\text{H}$  chemical shifts of Hemopressin in water DPC/SDS 90:10 M/M and HFA/H<sub>2</sub>O at 300K and 600 MHz

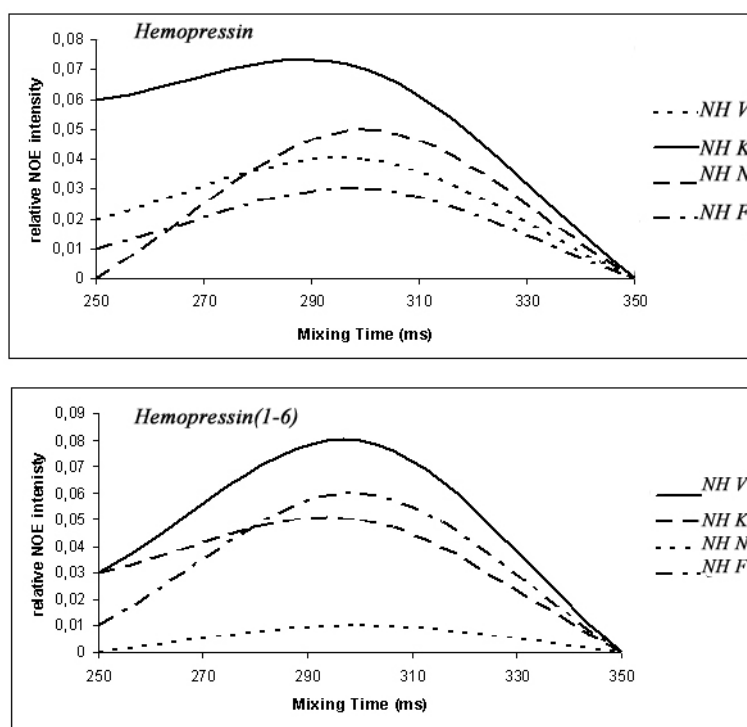
Hemopressin in DPC/SDS							
Residue	HN	C <sup><math>\alpha</math></sup> H	C <sup><math>\beta</math></sup> H	C <sup><math>\gamma</math></sup> H	C <sup><math>\delta</math></sup> H	C <sup><math>\epsilon</math></sup> H	Others
Pro1		4.368	2.397	1.891	3.290		
Val2	8.788	3.918	1.856	0.796/0.640			
Asn3	8.323	4.618	2.724/2.620		7.594/6.780		
Phe4	8.143	4.477	2.936/2.718		7.125	7.201	HZ7.040
Lys5	8.397	4.078	1.728	1.283	1.562	2.883	QZ7.791
Phe6	7.785	4.401	3.101/3.032		7.209	7.047	HZ7.140
Leu7	7.456	4.219	1.539/1.474	1.1755	0.791/0.755		QQD0.780
Ser8	7.814	4.307	3.749				
His9	7.990	4.382	3.166/3.040		7.990	7.173	QB3.166

## 1.2 Proton Chemical Shifts of Hemopressin(1-6) in DPC/SDS micelles 90:10 M/M.

**Table 2S.** The  $^1\text{H}$  chemical shifts of Hemopressin(1-6) in water DPC/SDS 90:10 M/M and HFA/H<sub>2</sub>O at 300K and 600 MHz

Emopressina 6aa in DPC/SDS							
Residue	HN	C <sup><math>\alpha</math></sup> H	C <sup><math>\beta</math></sup> H	C <sup><math>\gamma</math></sup> H	C <sup><math>\delta</math></sup> H	C <sup><math>\epsilon</math></sup> H	Others
Pro1		4.400	2.425	1.965	3.345		
Val2		4.036	1.958	0.867/0.796			
Asn3	8.472	4.650	2.725/2.638		6.807/7.550		
Phe4	8.055	4.494	2.945/2.871		7.105	7.176	
Lys5	7.910	4.245	1.696	1.256	1.577	2.912	
Phe6	7.691	4.349	3.120/2.902		7.234		

**1.3** *Plots of relative NOE cross peak intensities, versus the mixing time  $\tau$ . NOE cross peak intensities involving NH protons of Val, Lys, Asn, Phe, were respectively used to produce the buildup curve.*

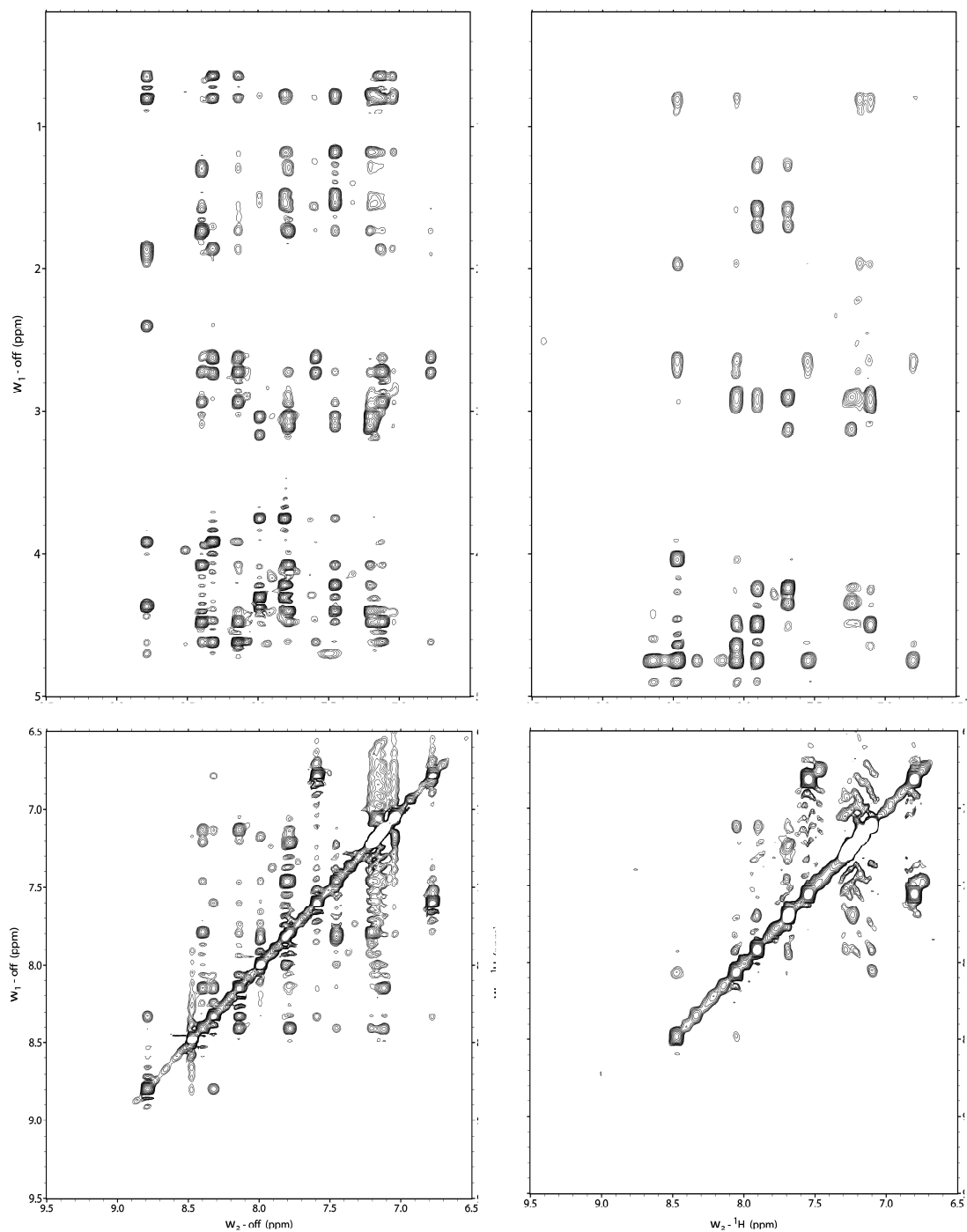


**1.4 Structural statistics for the final 20 structures of Hemopressin and Hemopressin(1-6) in DPC/SDS micelles**

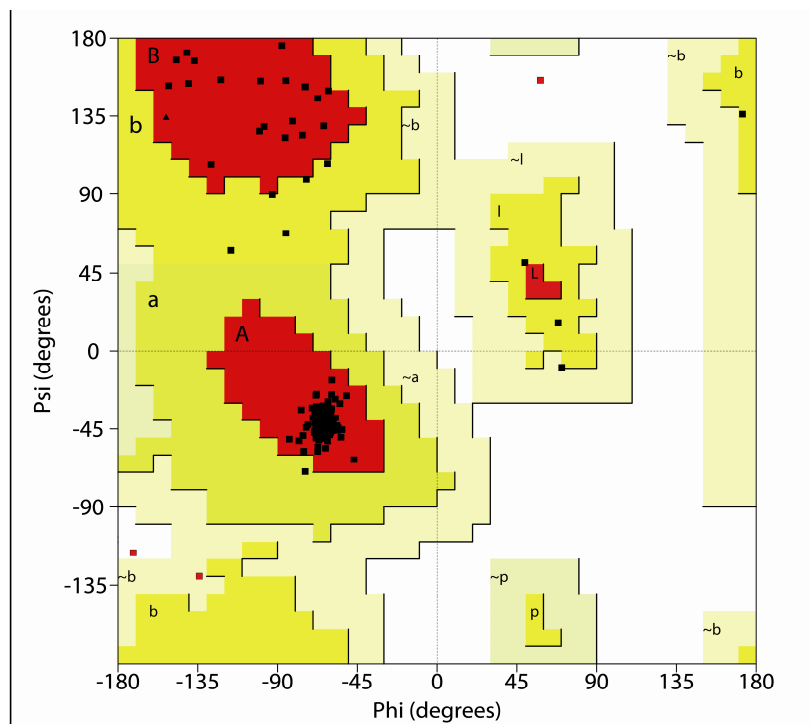
**Table 3S.** Structural statistics for the final 20 structures of Hemopressin and Hemopressin(1-6) in DPC/SDS micelles

PARAMETER	Hemopressin DPS/SDS	Hemopressin(1-6) DPC/SDS
<i>Backbone RMSD from mean structure (Å)</i>	<b>Peptide region</b>	
	<b>2-8</b>	<b>2-6</b>
	0.164	0.1991
<b>NOE distance restraints</b>		
<i>total</i>	221	96
<i>Intra</i>	102	57
<i>Short</i>	66	25
<i>Medium</i>	53	14
<i>Long</i>	0	0
<b>Ramachandran Plot</b>		
<i>Residues in most favoured regions</i>	55.8%	20.3%
<i>Residues in additional allowed regions</i>	7.5%	76.6%
<i>Residues in generously allowed regions</i>	36.7%	1.6%

**1.5 Amide and fingerprint regions of NOESY spectra for Hemopressin and Hemopressin(1-6) in DPC/SDS 90:10 M/M**



**1.6 Ramachandran Plots for the CB1 final structure from MODELLER calculation.**



### 1.7 Molecular interactions of Rimonabant, Hemopressin and Hemopressin(1-6) into CB1 receptor and calculated values of inhibition constant.

**Table 4S.** Molecular interactions of Rimonabant, Hemopressin and Hemopressin(1-6) into CB1 receptor and calculated values of inhibition constant

vdW interaction residues (scaling factor = 1.50 Å)			
RIMONABANT			
Ligand	Protein		
<i>phenyl portions</i>	<i>Phe200, Tyr275, Trp279, Trp356</i>		
<i>Pyrazole group</i>	<i>Val196, Met363, Leu387, Tyr275</i>		
<i>Amidic portion</i>	<i>Lys192,Asp366,Ser383,Tyr275</i>		
<i>piperidinyl moiety</i>	<i>Phe379, Phe177, Ile375, Leu387</i>		
HEMOPRESSIN			
<i>Pro1</i>	<i>,Asp176, Phe180, Ile375, Arg182, His181</i>		
<i>Val2</i>	<i>Ser185,Arg182, His181</i>		
<i>Asn3</i>	<i>Phe189, ,Lys192,Ser185, Arg182</i>		
<i>Phe4</i>	<i>Phe189 , Ser185, Leu190, Arg182, Trp255</i>		
<i>Lys5</i>	<i>Asp272, Phe189, Met363, Tyr275, Asp366, Lys192, Leu193</i>		
<i>Phe6</i>	<i>Asp272, Val363, Phe368, Lys370, Gly369, Asp366</i>		
<i>Leu7</i>			
<i>Ser8</i>	<i>Trp255</i>		
<i>His9</i>	<i>Asp272, Glu273, Thr274, Leu193, Phe189, Asn256, Tyr275, Trp255</i>		
HEMOPRESSIN(1-6)			
<i>Pro1</i>	<i>Phe180, Ile375, Phe177, His181,Asp176</i>		
<i>Val2</i>	<i>Ile375,Asp176, His181, Arg182, Phe180</i>		
<i>Asn3</i>	<i>Asp176,Lys192, Ile375, Phe177, Phe379</i>		
<i>Phe4</i>	<i>Phe189 , Ser185, Lys192, Leu193,Asp176, Arg182</i>		
<i>Lys5</i>	<i>Met363, Leu193, Tyr275, Phe189, Asp366, Lys192,Phe379</i>		
<i>Phe6</i>	<i>Gly369, Asp272, Val367, Phe368, Lys370,</i>		
Values of inhibition constant <sup>a</sup> and energies <sup>b</sup>			
	LIGAND		
	Rimonabant	Hemopressin	Hemopressin(1-6)
Inhibition Constant, Ki	20.84pM	1.64mM	1.44uM
Free Energy of Binding	-14.57	-3.80	-7.97
Torsional Free Energy	1.19	10.44	6.86
<sup>a</sup> the inhibition constant is measured at 298.15 K.			
<sup>b</sup> energies are expressed in Kcal/mol.			