

Final orthosteric site of hMT1

		10	TM1	20	30	ICL1	40	50	TM2					
6ME2-ram	23	P	SWLASALACVLIFTIVVDILGNLLVILSVYR	NKKLRN	AGNIFVVS	LAVADLVVA	77							
6ME3-2-fmel	23	P	SWLASALACVLIFTIVVDILGNLLVILSVYR	NKKLRN	AGNIFVVS	LAVADLVVA	77							
6ME4-2-lmel	23	P	SWLASALACVLIFTIVVDILGNLLVILSVYR	NKKLRN	AGNIFVVS	LAVADLVVA	77							
6ME5-agomel	23	P	SWLASALACVLIFTIVVDILGNLLVILSVYR	NKKLRN	AGNIFVVS	LAVADLVVA	77							
6PS8-2-fmel	23	P	SWLASALACVLIFTIVVDILGNLLVILSVYR	NKKLRN	AGNIFVVS	LAVADLVVA	77							
hMT1-site	23	P	SWLASALACVLIFTIVVDILGNLLVILSVYR	NKKLRN	AGNIFVVS	LAVADLVVA	77							
		60	70	ECL1	80	90	TM3	100						
6ME2-ram	78	I	YPYPLVLM	SIFN	NGWNL	GYLHCQVS	GFLMGLSV	IGSIFNITGIAINRYCYICH	S	132				
6ME3-2-fmel	78	I	YPYPLVLM	SIFN	NGWNL	GYLHCQVS	GFLMGLSV	IGSIFNITGIAINRYCYICH	S	132				
6ME4-2-lmel	78	I	YPYPLVLM	SIFN	NGWNL	GYLHCQVS	GFLMGLSV	IGSIFNITGIAINRYCYICH	S	132				
6ME5-agomel	78	I	YPYPLVLM	SIFN	NGWNL	GYLHCQVS	GFLMGLSV	IGSIFNITGIAINRYCYICH	S	132				
6PS8-2-fmel	78	I	YPYPLVLM	SIFN	NGWNL	GYLHCQVS	GFLMGLSV	IGSIFNITGIAINRYCYICH	S	132				
hMT1-site	78	I	YPYPLVLM	SIFN	NGWNL	GYLHCQVS	GFLMGLSV	IGSIFNITGIAINRYCYICH	S	132				
		120	ICL2	130	TM4	140	150	ECL2	160					
6ME2-ram	133	L	KYDKLYSSKN	SLCYVLL	IWLLTLA	AVLPN	LRAGT	LQYDPR	IYSC	TF	Q	SVSSAY	187	
6ME3-2-fmel	133	L	KYDKLYSSKN	SLCYVLL	IWLLTLA	AVLPN	LRAGT	LQYDPR	IYSC	TF	Q	SVSSAY	187	
6ME4-2-lmel	133	L	KYDKLYSSKN	SLCYVLL	IWLLTLA	AVLPN	LRAGT	LQYDPR	IYSC	TF	Q	SVSSAY	187	
6ME5-agomel	133	L	KYDKLYSSKN	SLCYVLL	IWLLTLA	AVLPN	LRAGT	LQYDPR	IYSC	TF	Q	SVSSAY	187	
6PS8-2-fmel	133	L	KYDKLYSSKN	SLCYVLL	IWLLTLA	AVLPN	LRAGT	LQYDPR	IYSC	TF	Q	SVSSAY	187	
hMT1-site	133	L	KYDKLYSSKN	SLCYVLL	IWLLTLA	AVLPN	LRAGT	LQYDPR	IYSC	TF	Q	SVSSAY	187	
		170	TM5	180	190	200	ICL3	210						
6ME2-ram	188	T	IAVVVFHFLVPMI	IVIFCYLR	IWILVVLQVR	QRVKPDR	KPKLK	PQDF	RNFV	TMFV			242	
6ME3-2-fmel	188	T	IAVVVFHFLVPMI	IVIFCYLR	IWILVVLQVR	QRVKPDR	KPKLK	PQDF	RNFV	TMFV			242	
6ME4-2-lmel	188	T	IAVVVFHFLVPMI	IVIFCYLR	IWILVVLQVR	QRVKPDR	KPKLK	PQDF	RNFV	TMFV			242	
6ME5-agomel	188	T	IAVVVFHFLVPMI	IVIFCYLR	IWILVVLQVR	QRVKPDR	KPKLK	PQDF	RNFV	TMFV			242	
6PS8-2-fmel	188	T	IAVVVFHFLVPMI	IVIFCYLR	IWILVVLQVR	QRVKPDR	KPKLK	PQDF	RNFV	TMFV			242	
hMT1-site	188	T	IAVVVFHFLVPMI	IVIFCYLR	IWILVVLQVR	QRVKPDR	KPKLK	PQDF	RNFV	TMFV			242	
		230	TM6	240	ECL3	250	TM7	260	270					
6ME2-ram	243	V	FVLFAICWAP	LNFI	GLAVAS	DPAS	MVPRIP	EWLF	FVAS	Y	YMA	Y	FNSCLNAIIYGL	297
6ME3-2-fmel	243	V	FVLFAICWAP	LNFI	GLAVAS	DPAS	MVPRIP	EWLF	FVAS	Y	YMA	Y	FNSCLNAIIYGL	297
6ME4-2-lmel	243	V	FVLFAICWAP	LNFI	GLAVAS	DPAS	MVPRIP	EWLF	FVAS	Y	YMA	Y	FNSCLNAIIYGL	297
6ME5-agomel	243	V	FVLFAICWAP	LNFI	GLAVAS	DPAS	MVPRIP	EWLF	FVAS	Y	YMA	Y	FNSCLNAIIYGL	297
6PS8-2-fmel	243	V	FVLFAICWAP	LNFI	GLAVAS	DPAS	MVPRIP	EWLF	FVAS	Y	YMA	Y	FNSCLNAIIYGL	297
hMT1-site	243	V	FVLFAICWAP	LNFI	GLAVAS	DPAS	MVPRIP	EWLF	FVAS	Y	YMA	Y	FNSCLNAIIYGL	297
		280	VIII-helix	290										
6ME2-ram	298	L	NQNF	RKEYRR	IIVSL	CTARV							318	
6ME3-2-fmel	298	L	NQNF	RKEYRR	IIVSL	CTARV							318	
6ME4-2-lmel	298	L	NQNF	RKEYRR	IIVSL	CTARV							318	
6ME5-agomel	298	L	NQNF	RKEYRR	IIVSL	CTARV							318	
6PS8-2-fmel	298	L	NQNF	RKEYRR	IIVSL	CTARV							318	
hMT1-site	298	L	NQNF	RKEYRR	IIVSL	CTARV							318	

Final orthosteric site of *hMT2*

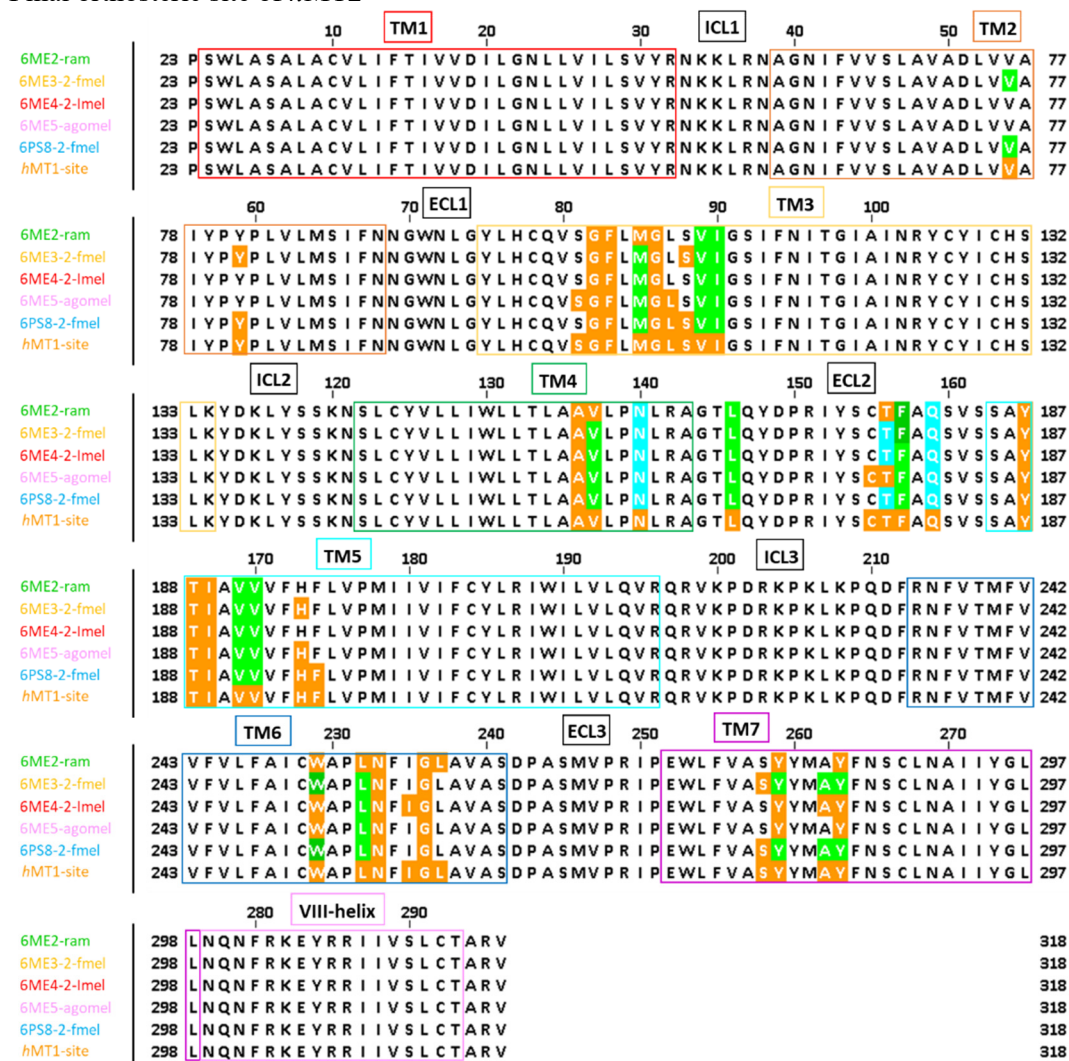


Figure S1. the amino acidic patterns of *hMTs* that participate to the intermolecular interactions.

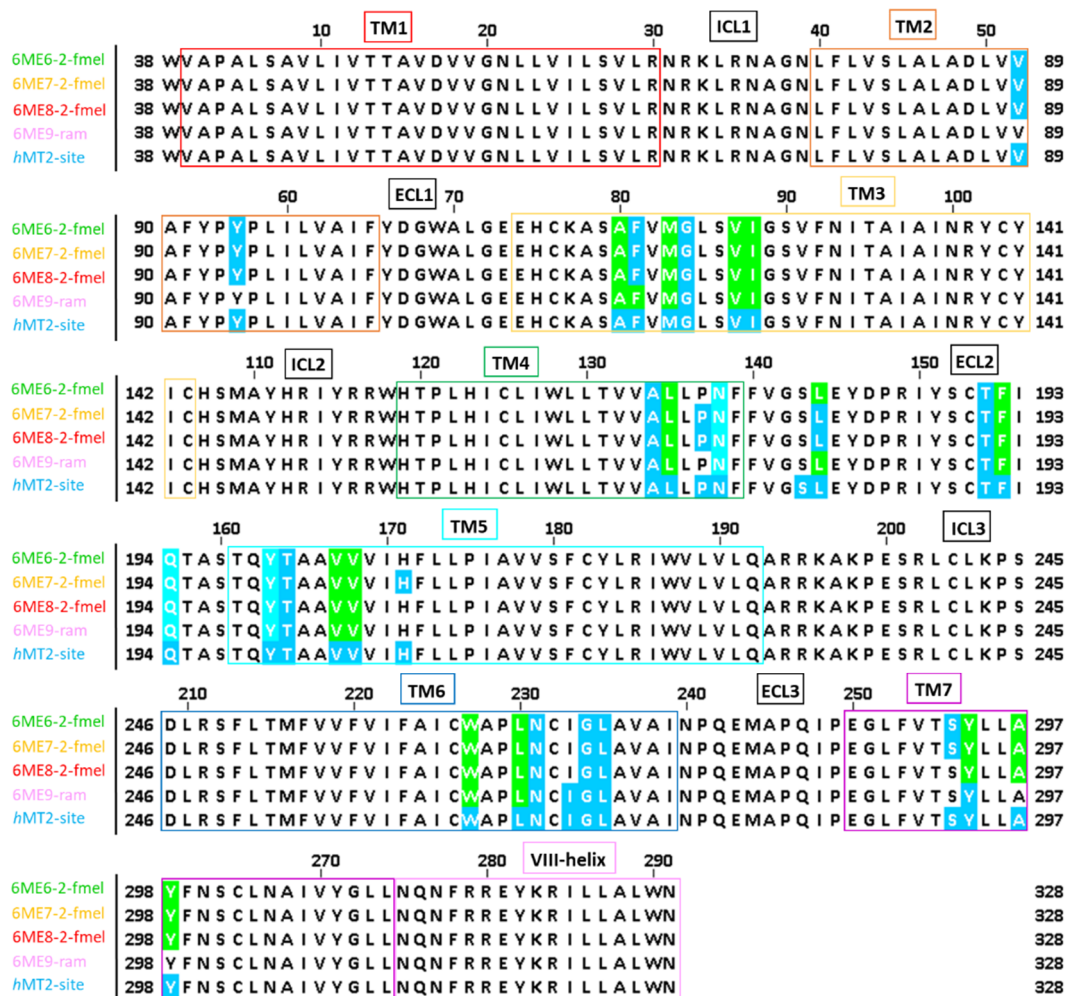
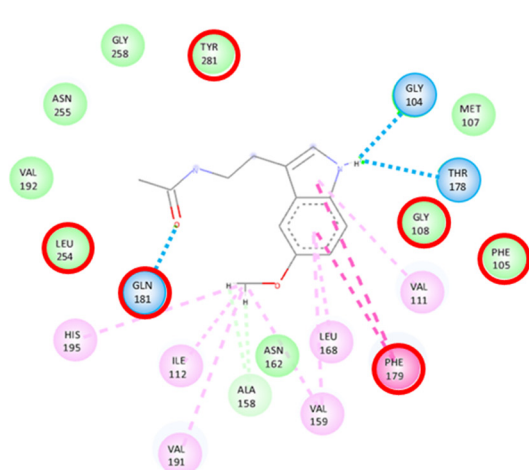


Figure S2. protein domains were squared to highlight where are localized the retrieved orthosteric site residues

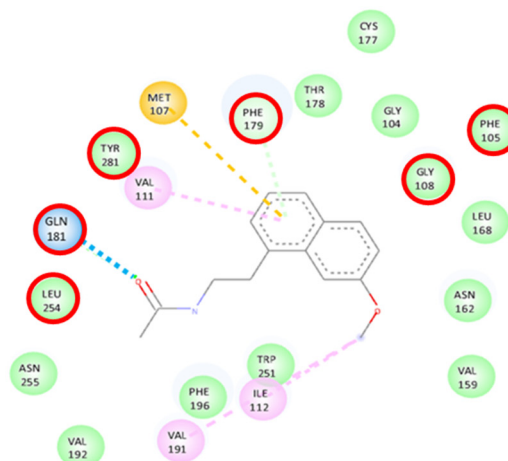
<i>hMT1</i>	<i>hMT2</i>
TM2: V76 ^{2.53} Y81 ^{2.58}	TM2: V89 ^{2.53} Y94 ^{2.58}
TM3: S103 ^{3.28} G104 ^{3.29} F105 ^{3.30} M107 ^{3.32} G108 ^{3.33} L109 ^{3.34} S110 ^{3.35} V111 ^{3.36} I112 ^{3.37}	TM3: A117 ^{3.29} F118 ^{3.30} M120 ^{3.32} G121 ^{3.33} V124 ^{3.36} I125 ^{3.37}
TM4: A158 ^{4.56} V159 ^{4.57} N162 ^{4.60}	TM4: A171 ^{4.56} L172 ^{4.57} N175 ^{4.60}
ECL2: L168 ^{ECL2} C177 ^{ECL2} T178 ^{ECL2} F179 ^{ECL2} Q181 ^{ECL2}	ECL2: L181 ^{ECL2} T191 ^{ECL2} F192 ^{ECL2} Q194 ^{ECL2}
TM5: Y187 ^{5.38} T188 ^{5.39} I189 ^{5.40} V191 ^{5.42} V192 ^{5.43} H195 ^{5.46} F196 ^{5.47}	TM5: Y200 ^{5.38} T201 ^{5.39} V204 ^{5.42} V205 ^{5.43}
TM6: W251 ^{6.48} L254 ^{6.51} N255 ^{6.52} I257 ^{6.54} G258 ^{6.55} L259 ^{6.56}	TM6: W264 ^{6.48} L267 ^{6.51} N268 ^{6.52} I270 ^{6.54} G271 ^{6.55} L272 ^{6.56}
TM7: S280 ^{7.38} Y281 ^{7.39} A284 ^{7.42} Y285 ^{7.43}	TM7: S293 ^{7.38} Y294 ^{7.39} A297 ^{7.42} Y298 ^{7.43}
Res. 50: N45 ^{1.50} D73 ^{2.50} R125 ^{3.50} W152 ^{4.50} P199 ^{5.50} P253 ^{6.50} A292 ^{7.50}	Res. 50: N58 ^{1.50} D86 ^{2.50} R138 ^{3.50} W165 ^{4.50} P212 ^{5.50} P266 ^{6.50} A305 ^{7.50}

Table S1. The table below collects all amino acids involved in stabilizing interactions; the Ballesteros–Weinstein numbering scheme for *hMTs* is also reported

hMT2

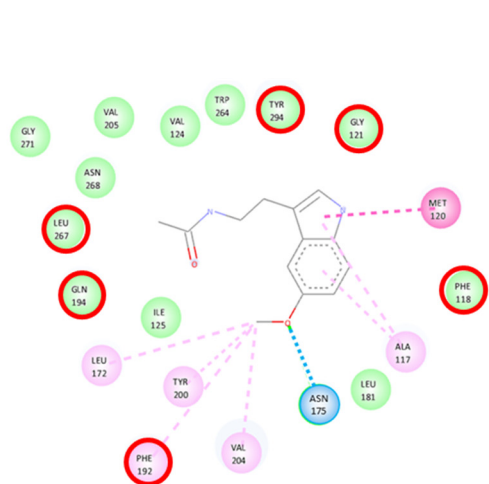


melatonin

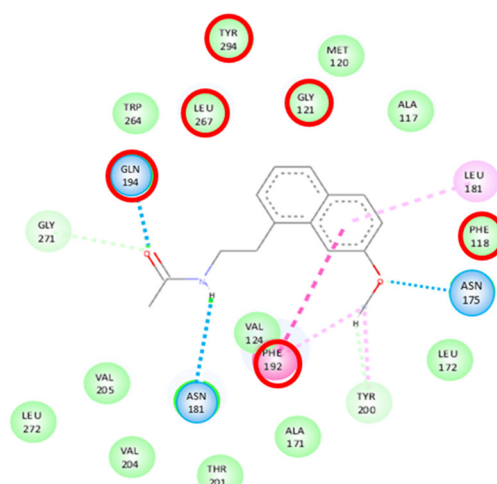


agomelatine

hMT2



melatonin



agomelatine

Legend: Van der waals π -alkyl π - π interaction Hydrogen bond π -sulfide

Figure S3. 2D Representation of the final poses of melatonin and agomelatine at the orthosteric site of hMT1 and hMT2, according to molecular dynamics simulations

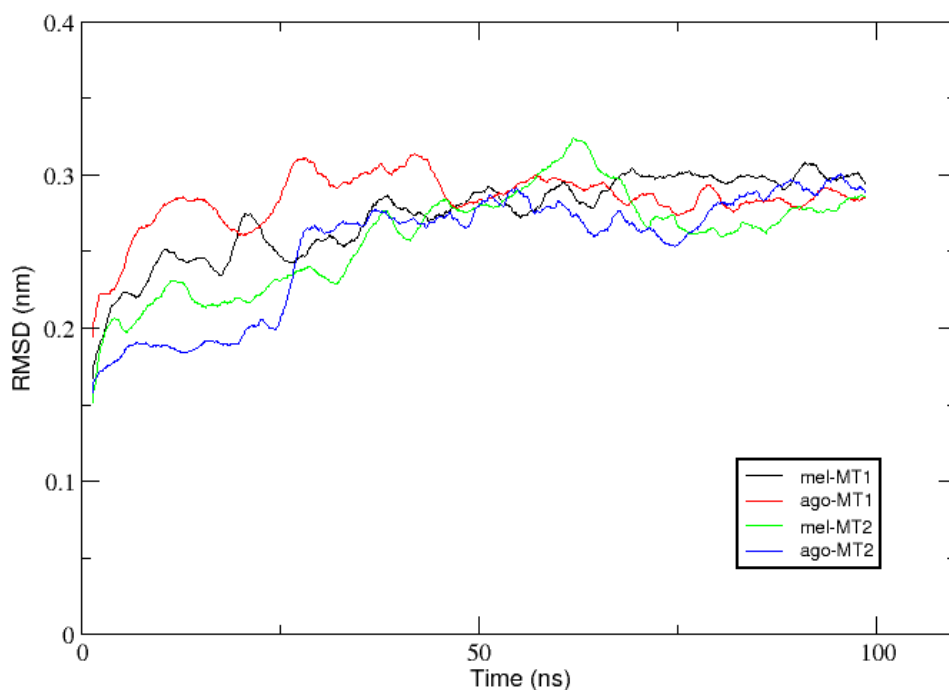


Figure S4. RMSD plot as function of time for the protein not considering hydrogen atoms. Each colour represents a different protein-ligand complex.

Receptor	Ligand	RMSD
<i>hMT1</i>	Melatonin	0.0901 nm
	Agomelatine	0.1039 nm
<i>hMT2</i>	Melatonin	0.1054 nm
	Agomelatine	0.1187 nm

Table S2. Calculation of RMSD from starting and final point of molecular dynamics simulations, using UCSF Chimera⁶⁵.

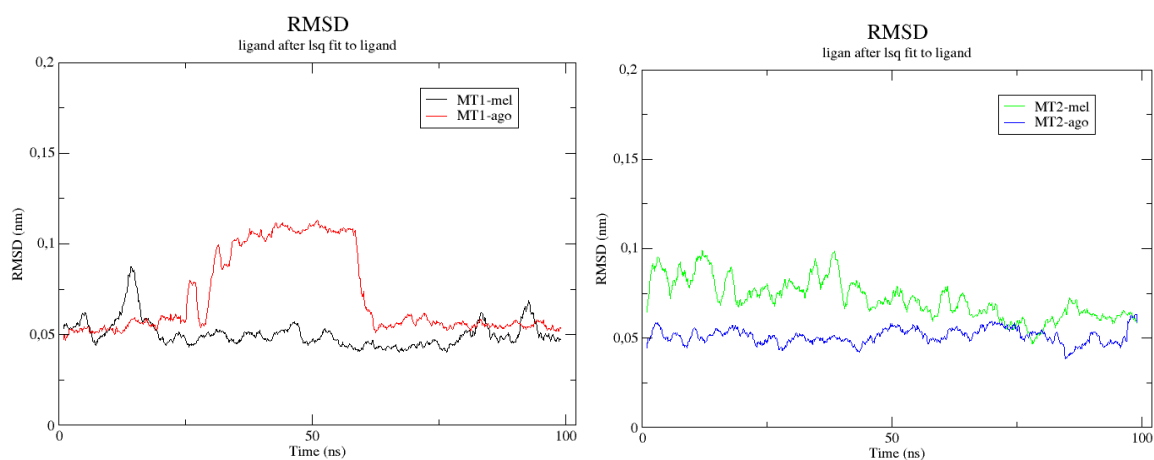


Figure S5. Time-RMSD plots for ligands in *hMT1* and *hMT2*.