



# A Molecular Dynamics Study of Vasoactive Intestinal Peptide Receptor 1 and the Basis of its Therapeutic Antagonism

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## Supplementary materials

This file includes figure S1–S19 and table S1–S5 that corresponds to the main manuscript text.

### Tables

Table S1. Results of the SiteMap computations for both, ECD and TMD domains of VPAC1.

ECD domain							
	SiteScore	Dscore	Size (number of site points)	Enclosure Hydrophilic Hydrophobic			
Site 1 <sup>1</sup>	0.764	0.742	52	0.628	0.959	0.511	
Site 2 0.677 0.644 39 0.		0.591	0.913	0.179			
TMD domain							
Site 1 (allosteric)	1.049	1.087	92	0.751	0.814	1.354	
Site 2 (orthosteric)	1.024	1.044	188	0.729	1.024	0.502	
Site 3	0.809	0.839	25	0.714	0.183	3.704	
Site 4	0.722	0.724	28	0.637	0.478	2.190	
Site 5	0.671	0.622	31	0.647	0.887	0.819	

<sup>1</sup>Here, two binding sites of VPAC1, one in ECD and the other in TMD were bolded. Both of them were selected for the purpose of the study and both of them were assigned the highest SiteScore and Dscore by SiteMap.

		VPAC1 based on	VPAC1 based	VPAC1 based
	VPAC2 – 2X57	5NX2	on 5VAI	on 5YQZ
		(partially active)	(fully active)	(fully active)
	Comp	oound <b>31</b>		
Orientation 1	None found, the ligand on the surface (the lowest score: -8.3)	$-7.4^{1}$	-9.6	-8.6
Orientation 2	-7.2	-7.3	-9.3	-8.6
	Comp	ound 41		
Orientation 1	-6.8	-6.6	-9.0	-7.4
Orientation 2	-7.8	-7.0	-7.7	-8.1

**Table S2.** The Autodock VINA-approximated binding affinity for compounds **31** and **41** towards VPAC1 and VPAC2 ECD domains.

<sup>1</sup>Here, the Autodock VINA score for the first-ranked pose of a given orientation is provided.

**Table S3.** Results of MD simulations of VPAC1-antagonists complexes. Here, the ligand orientation obtained in molecular docking was shown in yellow lines.

Compound / pose no / major orientation	Autodock VINA score	MD-refined hypothetical binding mode	Ligand RMSD	Receptor ECD RMSD
Compound 31 – pose 1, orientation 1	-9.6		Compound 31 - pose 1 Ligand heavy atom RMSD vs. simulation time	Compound 31 - pose 1 Protein backbone RMSD vs. simulation time
Compound 31 – pose 2, orientation 2	-9.3		Compound 31 - pose 2 Ligand heavy atom RMSD vs. simulation time	Compound 31 - pose 2 Protein backbone RMSD vs. simulation time $f_{0}$ $f_{0}$ $f_{$





**Table S4.** Results of the extended to 50 ns (shown last 30 ns) MD simulations of compound **31** complexes (poses 1 and 5). Here, the last snapshot of the previous 20 ns MD simulation was shown in yellow.

Pose no.	MD-refined binding mode	Ligand RMSD	Receptor ECD RMSD	
Pose 1		Compound 31 - pose 1 - extended simulation Ligand heavy atom RMSD vs. simulation time $10^{-10^{-10^{-10^{-10^{-10^{-10^{-10^{-$	Compound 31 - pose 1 - extended simulation Protein backbone RMSD vs. simulation time $\left(\frac{1}{1000} + \frac{1}{1000} + \frac{1}{1000} + \frac{1}{1000} + \frac{1}{1000} + \frac{1}{1000} + \frac{1}{10000} + \frac{1}{10000000000000000000000000000000000$	
Pose 5		Compound 31 - pose 5 - extended simulation Ligand heavy atom RMSD vs. simulation time $7 = 6 = 6 = 10^{-10}$ $10 = 10^{-10}$	Compound 31 - pose 5 - extended simulation Protein backbone RMSD vs. simulation time $\int_{0}^{1} \int_{0}^{1} \int_{0}^{$	

Pose no.	Autodock VINA score	MD-refined binding mode	Ligand RMSD	Receptor TMD RMSD
Pose 1	-9.1		Compound 41 - pose 1 Ligand heavy atom RMSD vs. simulation time	Compound 41 - pose 1 Protein backbone RMSD vs. simulation time 1.0 0 5 10 15 20 25 30
Pose 5	-7.8		Compound 41 - pose 5 Ligand heavy atom RMSD vs. simulation time 7 6 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6 7 6	Compound 41 - pose 5 TMD region - protein backbone RMSD vs. simulation time 1.0 0.5 0 5 10 10 15 20 25 30

**Table S5.** Results of MD simulations of compound **41** complexes – the TMD region of VPAC1. Here,the ligand orientation obtained in molecular docking was shown in yellow lines.

### Figures



**Figure S1.** The activation mechanism of class B GPCRs – superposition of active, partly active and inactive conformations of VPAC1. Here, we used VPAC1 models based on 5VAI (blue), 5NX2 (dark blue) and a set of inactive structural templates (grey). The active VPAC1 model based on 5VAI (GLP-1R) was constructed together with VIP (green) and G protein subunits (grey, magenta, yellow, pink). TMH6 is subject to the greatest conformational change – a sharp kink in the middle that enables interactions with the G alpha subunit (magenta). The ectodomain of VPAC1 deflects without any major conformational changes of secondary structure enabling interactions with a peptide hormone.



**Figure S2.** The comparison of conformational states of the activated class B receptors. The ECD domain of CGRP (CLR+RAMP) is the closest to its TMD domain because of additional interactions with the extracellular part of RAMP (not shown). Structures of GLP-1R (grey, PDB id: 5VAI), PTHR1 (red, PDB id: 6NBF), CGRP (green, PDB id: 6E3Y) were superimposed in their TMD regions to observe differences in the relative positions of ECD and TMD domains. TMD domains of shown here receptors undergo similar conformational changes upon the activation, e.g., the TMH6 deformation.



**Figure S3.** Comparison of the VPAC2 structure of ECD (grey, PDB id: 2X57) and models of the VPAC1 ECD domain constructed using: 5NX2 (yellow), 5VAI (orange) and 5YQZ (red) template structures.



**Figure S4.** The site-directed mutagenesis data for VPAC1 shown in its model of the extracellular domain. Orange residues are glycosylated (Asn58, Asn69, Asn100), residues shown in the spheres representation are crucial for the peptide binding (Asp107, Gly116, Cys122) which was confirmed by the site-directed mutagenesis. Other binding site residues are shown with the sticks representation (green – the two-tyrosines gate – see the main manuscript text). Here, we showed only three first residues out of five (Asp107, Gly116, Cys122 and Lys127 and Gln135) that were confirmed experimentally as important for the peptide binding. The remaining two residues are in the flexible region joining two domains that were not shown here.



Figure S5. A proposed evolutionary model of the PACAP-related genes



**Figure S6.** Negative allosteric modulators of class B GPCRs present in solved to date experimental structures. (a) PF-06372222 (GLP-1R – 5VEW), (b) NNC0640 (GCGR – 5XEZ), (c) MK-0893 (GCGR – 5EE7). All of them possess a distinct V-shape that blocks TMH6 from bending.



**Figure S7.** Potential ECD binding sites of VPAC1 detected by SiteMap. The binding site selected for the current study as more probable was bolded. The binding site representation is as provided by SiteMap (Schrodinger, LLC): grey spheres – site points, surfaces – interactions sites (yellow – hydrophobic, blue – hydrogen bonding donor, red – hydrogen bonding acceptor sites). The color scheme – red-to-blue (N-termini as red).



**Figure S8.** All potential TMD binding sites of VPAC1 detected by SiteMap (a). Two major binding sites (b) that were confirmed by solved-to-date structures of class B GPCRs: the orthosteric site for the peptide binding and the allosteric site for the allosteric modulators binding. The binding site representation was the same as in Figure S7.



**Figure S9.** Comparison of the binding site cavity located in ectodomains of VPAC2 (**a**) and VPAC1 (**b**, **c**, **d**). (b) – The VPAC1 model based on 5NX2, (c) – the VPAC1 model based on 5VAI and (**d**) – the VPAC1 model based on 5YQZ. The VPAC2 binding cavity is the shallowest and hydrophobic. The most spacious cavity-like binding site was observed for the VPAC1 model based on 5VAI.



**Figure S10.** The allosteric binding site of VPAC1 located in its transmembrane domain. Here, we presented the VPAC1 model in its inactive conformation, constructed using a set of inactive template structures. The binding site is mostly occupied by polar residues with a single aromatic residue Tyr7.57 in the middle.



Figure S11. The MD-refined pose 5 of compound 31 (the 20 ns simulation).



**Figure S12.** Results of the extended (from 20 ns to 50 ns) simulation of the compound **31** and VPAC1 complex (pose 1). Here, the last frame of the simulation (50 ns) is shown in brown and the last frame with extended N-terminal helical fragment of ECD (~45 ns) is shown in grey. A further extension of this MD simulation should be carried out in the presence of the TMD domain because of too significant mobility of N and C-terminal fragment that might influence the binding site and crucial tyrosine residues (Tyr118 and Tyr39) positions.



Figure S13. A multiple sequence alignment that was used to build an inactive VPAC1 model.



**Figure S14.** A multiple sequence alignment that was used to build an active VPAC1 model based on the GLP-1R structure (5NX2) including the ECD domain.

VPAC1 VPAC1_IMD 2RRI 2X57 5YQZ_R	1 ARIQEECDYVOM  REHIB QVMDRIFEK	IEVQHKQCLEEA IQEHETKCAELL WKLYCDQCHHNL	QLENETI-GC RSQTEKHKAC SLLPP-PTELVC	SKMWDNLTCWPA SGVWDNITCWPP NRTFDKYSCWPD	TPRGQVVVLACP ANVG <mark>H</mark> TVTVPCP TPANTTANISCP
VPAC1 VPAC1_IMD 2RRI 2X57 5YQZ_R	61 LIFKLESSIQG-  MYLPWHHKVQE-	RN VESNEYSKA-GN RE	VSRSCIDEG-NI ISKNCISEG-NS VEKRCGPEGOWV	TPPPVP RGP-RGQPWRDA	IACGLDDKAASL 
12 VPAC1 VPAC1_TMD 2RRI 2X57 5YQZ_R	21 Deqqime ygsvk Tme ygsvk 	TGYTIGYGLSLA TGYTIGYGLSLA V <mark>MYT</mark> VGYSLSLG	TLLVATAILSLF TLLVATAILSLF ALLLALAILGGL	RKIHCTRNYIHM RKIHCTRNYIHM SKIHCTANAIHA	IL ISPILBAAA HLFISFILBAAA NLFASFVLKASS
18 VPAC1_ VPAC1_TMD 2RRI 2X57 5YQZ_R	31 VFIKDLALFDSG VFIKDLALFDSG ULVIDGLLRTRY	SOKICDELSVST	NCSEGSVGCKA	amv <b>ffqyq</b> vman amv <mark>ffqyq</mark> vman amv <mark>ffqyq</mark> givan	FWLLVEGLYLY FFWLLVEGLYLY YCWLLVEGLYL
24 VPAC1 VPAC1_TMD 2RRI 2X57 5YQZ_R	41 TLLAVSFFSERK TLLAVSFFSERK NLLGLA-TFRS	YFWGYILIGWGV YFWGYILIGWGV FFSLYLGIGWGA	PSTFTMVWTIAR PSTFTMVWTIAR PMLFVVPWAVVK	IHFEDYGCWDTI IHFEDYGCWDTI CIFENVQCWTSN	NS-SLWWIIKGP NS-SLWWIIKGP DNMGEWWILREP
30 VPAC1 VPAC1_TMD 2RRI 2X57 5YQZ_R	D1 ILTSILVNFILF ILTSILVNFILF  VFLAILINFFIF	ICIIRILLOKIR ICIIRILLOKIR VRIVQLLVAKIR	PPDIRKSDSSPY PPDIRKSDSSPY ARQMHHTDYK	SRLARSTLLLIP SRLARSTLLLIP FRLA <mark>KST</mark> LLLIP	LEGVHYIMPAFF LEGVHYIMPAFF  LIGV <mark>HE</mark> VVFAFV
30 VPAC1 VPAC1_TMD 2RRI 2X57 5YQZ_R	61 PDNFKPEV PDNFKPEV 	KMVFELVVGSFQ KMVFELVVGSFQ KIFFDIFLSSFQ	GFVVAILYCFLN GFVVAILYCFLN GLLVAVLYCFLN	G <mark>e</mark> vQa <mark>e</mark> l <mark>RRKWR</mark> GevQael <mark>RRKWR</mark> KevQselrRRWH	RWHLQGVLGWNP RWHLQGVLGWNP RWRLGKVLWE
42 VPAC1 VPAC1_TMD 2RRI 2X57 5YQZ_R	21 <b>KY/HSDAVFIDN</b> <b>KY</b>	YTRLRKQMAVKK YTRLRKQMAVKK SKYL <mark>D</mark> SRRAQ <mark>D</mark> F	YLNSILNG YLNSILNG VKWLLNT-		

**Figure S15.** A multiple sequence alignment that was used to build an active VPAC1 conformation model based on the GCGR structure (5YQZ) including the ECD domain and VIP.

	1				
VPAC1 VPAC1_TMD 2RRI	HSDAVF IDNYIR HSDAVF IDNYIR	LRECMAVERYLN	SILNG/ARLQ SILNG		HKQCLEEAQLEN
2×57 5VAI_R	HAEGIE ISDVSS	YL <mark>E</mark> GQAA <mark>KEF</mark> TA	NLVKGRG/TV	SLSETV <u>CKWR</u> EY	RECONFLITERS
VPAC1 VPAC1_TMD 2BB1		NI TOMPATPRO	QVVVLA <mark>C</mark> PLI <mark>PK</mark>	I <mark>- SS</mark> TQG	
2X57 5VAT_R	EKIKACSGVW PLATCI <mark>CNRTP</mark>	NITCHRPANVG MYACWPDCAPC	ETVTVP <mark>C</mark> P S <mark>F</mark> VNVSCPWYTP	M <mark>asnvi Q</mark>	TYSKA-GNISKN GHVYR
1 VPAC1 VPAC1_TMD 2001	21 CIDEG-MINIEP	GP <mark>Y</mark> PIA <mark>C</mark> G	L <mark>DDK</mark> AASL <mark>DF</mark> QQ	T <mark>MF</mark> YGSVKTGYT T <mark>MF</mark> YGSVKTCYT	IGYGLSLATLEV IGYGLSLATLEV
2X57 5VAI_R	CTSDG-WSEIFP CTALCIWLPKDN	DEV <mark>D</mark> ACG SSIP <mark>WRD</mark> LS <mark>ECE</mark>	YS <mark>D</mark> P ESSP <mark>DE</mark>	RLLSLYIIYT	VG <mark>Y</mark> AL <mark>SF</mark> SALVI
1 VPAC1 VPAC1_TMD	81 ATAILSIFRKIH ATAILSIFRKIH	CIRNYIHMHIFI CIRNYIIMHIFI	SFILRAAAVFIK SFILRAAAV <mark>F</mark> IK	DIALFDSGE DIALFDSGE	S <mark>D</mark> QCS S <mark>D</mark> QCS
2X57 5VAI_R	ASAILLG <mark>FRHI</mark> H	CIRNYIHINI <mark>B</mark> A	SFILRALSVFIK	AAL <mark>KMMYST</mark> AA	QQ <mark>H</mark> -Q <mark>WD</mark> GLLSY
2 VPAC1 VPAC1_TMD	41 EGSVGCKAAMVE EGSVGCKAAMVE	F <u>QYCVMANFFW</u> L F <u>QYCVMAN</u> FFWL	LV <mark>E</mark> GLYLYTLLA LV <mark>E</mark> GLYLYTLLA	VSFES <mark>ERKY</mark> FWG VSFE <mark>SERK</mark> YFWG	YILIGMGVP <mark>ST</mark> YILIGMGVP <mark>ST</mark> F
2X57 5VAI_R	SLG <mark>OR</mark> LV <mark>E</mark> LL	<mark>MQYC</mark> VAANYY <mark>M</mark> L	LV <mark>E</mark> GA <u>YLYT</u> LLA	AV <mark>ES CR</mark> IFKL	YLSIG <mark>W</mark> GVPLL
3 VPAC1 VPAC1_IMD	01 TMAWTIARIHEE TMAWTIARIHEE	DYG <mark>CWDTINS-S</mark> DYG <mark>CWDTINS-</mark> S	L <mark>MN</mark> II <mark>K</mark> GPIL <mark>TS</mark> LMNIIK <mark>GPILTS</mark>	ILV <mark>NF</mark> ILFI <mark>C</mark> II ILVNFILFI <mark>C</mark> II	RILIOKI RPP <mark>D</mark> I RILIOKI RPP <mark>D</mark> I
2X57 5VAI_R	VIP <mark>W</mark> GIV <mark>KYLY</mark> E	<mark>O™</mark> C <mark>CWTRNSN</mark> ⊻N	YMLII <mark>R</mark> lpil <mark>b</mark> a	IGV <mark>NF</mark> LI <mark>F</mark> IRVI	<mark>CIVVSK</mark> IKANIM
3 VPAC1 VPAC1_TMD	61 RKS <mark>D</mark> SSPYS <mark>R</mark> LA RKS <mark>D</mark> SSPYS <mark>R</mark> LA	<mark>RST</mark> LLLIPL <mark>F</mark> GV RSTLLLIPLFGV	<mark>HYIMI AFFPO</mark> NF HYIMFAFFPONF	KP <mark>BVKMVP</mark> KP <mark>B</mark> VKMVP	ELVVG <mark>SFOGF</mark> VV ELVVG <mark>SFOGF</mark> VV
2X57 5VAI_R	<mark>okte</mark> ikoria	KSTLTLIPLLGT	nevi parvmeen	A <mark>r</mark> gtl <b>rfvklf</b> t	ELSETSFOGLMV
4 VPAC1 VPAC1_IMD	21 AILYCFLNGEVO AILYCFLNGEVO	A <mark>J</mark> I RRKWRRWHI A <mark>JI RRKWRRW</mark> II	QGVLG <mark>MNPKY</mark> /I QGVLG <mark>MNPKY</mark>	ED <mark>orneek</mark> aore	ANKKI <mark>E</mark> KQLQKD
2RRI 2X57 5VAI_R	AIL <mark>YCFVNNE</mark> VQ	MEFRKSWERMR-	/I	ED <mark>ORNEEK</mark> AORE	ANKKI <mark>EK</mark> QL <u>OK</u> D
4 VPAC1 VPAC1_IMD	81 KQVYRA_HRLL_	LGAG <mark>E</mark> SG <mark>KST</mark> EV	KQSGI <mark>FE</mark> TKEQV	DKVNEHMED VGG	<u>ORDERRKW</u> IQCF
2KRI 2X57 5VAI_R	KOVYRATHRLLT	LGAG <mark>.</mark> SG <mark>KST</mark> IV	KQSGI <mark>FE</mark> TKEQV	<mark>dk</mark> v <mark>ne h</mark> me <b>d</b> vgg	OR <mark>DERRKW</mark> IQCF
VPAC1	541 NUVTAIIEVVAS	SSYNMINELO	INIFESTENNES	I <mark>RTIS</mark> VII <mark>FINK</mark>	ODLLA <mark>RK</mark> VLAG <mark>K</mark>
VPAC1_TMD 2RRI 2X57					
VPAC1	601	VTTOPRATORO			
VPAC1_TMD 2RRI 2V57					
5VAI_R	SKIEDYFPEFAF	YTTP <mark>ED</mark> ATP <mark>E</mark> PC	CEPRVIRAKYFI	RDEF <mark>LRISTAS</mark> S	CENYCYPHFIC
VPAC1 VPAC1_TMD 2RRI	AVOTENIRRVEN		QYELL/QSELEC	I ROLATQI KNQI	R <mark>d</mark> ark <mark>acad</mark> atti
2X57 5VAI_R	AV <b>TENIRR</b> VEN		QYELL/QSELEC		R <mark>d</mark> arkaca <mark>d</mark> atl
VPAC1 VPAC1_IMD 2RRI	SQITNNI PVG	IQMRIRRIL	LA <mark>KIYAMHN</mark> GT <mark>D</mark>	S <mark>r</mark> llvSASO <mark>L</mark> GK	LII <mark>MD</mark> SYITN <mark>K</mark> V
2X57 5VAI R	SQITINI PVOR	IOMRURRUIRO	LA <mark>KIMAMIN</mark> GT	SPLLVSASO <mark>D</mark> OK	LII <mark>WD</mark> SYTIN <mark>K</mark> V
VPAC1 VPAC1_TMD 2RRI	HAIPIRSS VMI	CAYAPSGNYVA <mark>C</mark>	GGL <mark>ONICSIYN</mark> I	KER GNV VSR	LAG <mark>HT</mark> GYL <mark>SCC</mark> R
2x57 5VAI_R	HAIPLRSS VMI	CAYAPSGNYVA <mark>C</mark>	GGL <mark>I</mark> NICSIMI	KTREGNVRVSRE	LAG <mark>HT</mark> GYLS <mark>CCR</mark>
VPAC1 VPAC1_IMD 2RRI	FIDDNQIVTSSG	TTCALWD INTO	<u>QOTIT<b>F</b>TGHTG</u>	V <mark>MSLSLAP<mark>DTR</mark>L</mark>	FV <mark>S</mark> GAC <mark>T</mark> ASA <mark>K</mark> L
2X57 5VAI R	FIDENCIVISSO	DTTCALNOI TO	COTTT <mark>F</mark> TCHICL	V <mark>MSLSLAP<mark>U</mark>TR</mark> L	VSGAC ASAKL
VPAC1 VPAC1_TMD 2RRI	NUVRECKOROTE	TG <mark>HE</mark> S <mark>I IN</mark> AIC	FPNGNA <mark>F</mark> ATGS <mark>D</mark>	DATCELFOLEAD	Q <mark>e</mark> lmtysh <mark>o</mark> nii
2x57 5VAL_R	WIVE GMCROIF	TG <mark>HE</mark> S <mark>D INAIC</mark>	FPNGNAFATGS	DATCRIFTIRAD	Q <mark>e</mark> lmiys <mark>ho</mark> nii
VPAC1 VPAC1_IMD 2BBI	961 CGITSVS <mark>F</mark> SKSG	ELLA( <mark>YDDE</mark> N)	NV <mark>NT</mark> AL <mark>KADR</mark> AG	VLAG <mark>H<mark>O</mark>NR<mark>VSC</mark>L</mark>	gv <mark>iidd</mark> gmava <mark>ii</mark> g
2X57 5VAI_R	<mark>ocitsvs<mark>t</mark>sks</mark> c	ELLACY <mark>DDEN</mark> C	NV <mark>MD</mark> AL <mark>K</mark> A <mark>DR</mark> AC	VLAC <mark>H<mark>D</mark>NR<mark>VSC</mark>L</mark>	CV <mark>TDD</mark> CMAVA <b>I</b> C
1 VPAC1 VPAC1_TMD	021 SMLSFIKIWN/N	TASIAQA <mark>RK</mark> LV	QL <mark>KME</mark> ANI <mark>DR</mark> IK	V <mark>SK</mark> AAA <mark>D</mark> IMAYC	AHAKED PLLTP
2X57 5VAL_R	SWUSFLKINN/N	TASIAQA <mark>RK</mark> LV	QL <mark>KMB</mark> ANI <mark>DR</mark> IK	V <mark>SK</mark> AAA <mark>U</mark> LMA <mark>Y</mark> C	<b>TAHAKED</b> PLLTP



**Figure S16.** A multiple sequence alignment that was used to build an active VPAC1 conformation model based on the GLP-1R structure (5VAI) including the ECD domain, VIP and G protein subunits.



**Figure S17.** A validation of VPAC1 models presented in this study by all-atom 100 ns MD simulations. Here, protein backbone RMSD of the transmembrane helices was presented, similarly to data presented in Table A5. (a) The VPAC1 model based on 5VAI. Here, VIP and G protein subunits were included in the simulation system. (b) The VPAC1 models based on 5YQZ. Here, the VIP was included in the simulation system. (c) a VPAC1 model based on 5NX2. Here, only the receptor was included in the simulation system. The least change in the receptor structure was observed for (b), most probably due to a well-defined secondary structure of the N-terminal helical fragment of TMD joining with ECD. The largest changes in the receptor structure were observed for (a), due to the presence of the whole G protein subunits complex in the simulation system.



**Figure S18.** Results of 100 ns MD simulations of the VIP – VPAC1 complex. Here, two models of the VIP-VPAC1 complex were presented: (a) the model based on the 5VAI template structure, (b) the model based on the 5YQZ. Heavy atom RMSD values were computed for the interface residues of VIP that interact with ECD (see Figure 2b in the main manuscript text, the upper part, residues shown in sticks). In both cases, this peptide interface remained stable.



**Figure S19.** Results of the extended simulation (to 80 ns) of the compound **41** in complex with TMD of VPAC1 (pose 1 - see Table S5). Here, ligand and protein RMSD was computed, similarly to data presented in Table S5.