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Supplementary information

Structural insights into vesicular monoamine storage and drug interactions

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Supplementary Discussion

Cytoplasmic-open transition is very slow in absence of a proton gradient

In the absence of a proton gradient, VMAT1 predominantly adopts a lumenal-open conformation that is maintained by extensive gating interactions (Fig. 2b). The cytoplasmic-open transition occurs very slowly, as inferred from the binding rate of reserpine, which recognizes VMATs in their cytoplasmic-open conformation[1,2] (Fig. 2). Without a proton gradient, reserpine binding takes over 24 h to reach equilibrium[3], indicating that although the unprotonated VMAT1 can still transition to the cytoplasmic-open state, this process occurs at a very slow rate. In contrast, proton gradient accelerates the reserpine binding, which reaches saturation in approximately 10 min[3,4]. Thus, protonation at vesicular acidic pH destabilizes the lumenal-open state and facilitates the cytoplasmic-open transition.

Cytoplasmic-open transition remains rate limiting for substrate transport with a proton gradient

In presence of a proton gradient, the cytoplasmic-open transition remains the rate-limiting step during the vesicular import mediated by VMATs[2]. Their K_M for substrate transport is 10-100 times lower (better) than substrate-binding K_D[5]. In other words, the uptake with a substrate-occupied binding site (K_M) is much faster than the reappearance of the unoccupied binding site (K_D), i.e., the protonation-driven cytoplasmic-open transition[2]. In addition, VMAT transports different substrates with similar V_{max}[6,7], suggesting that a conformational transition, instead of substrate binding or release, is the rate-limiting step for the transport process.

Reserpine treatment induces dimer formation

For cryo-EM studies, the cells were treated with reserpine for 10 min before membrane disruption. Because proton gradient is present at this stage, a fraction of VMAT1 can bind reserpine. The reserpine-bound fraction adopts a cytoplasmic-open conformation, while the unbound fraction is preferably lumenal open. Following membrane solubilization, a molecule at the cytoplasmic-open conformation is prone to dimerize, either with another reserpine-bound molecule or with an unbound, lumenal-open molecule. The membrane solubilization also eliminates the proton gradient, and under this condition, the lumenal-open monomer maintains a stable conformation (see above). The reserpine-bound monomer is also stable because this inhibitor is nearly irreversible[8]. Consequently, we observe reserpine/unbound or reserpine/reserpine dimers in alternate conformations.

References

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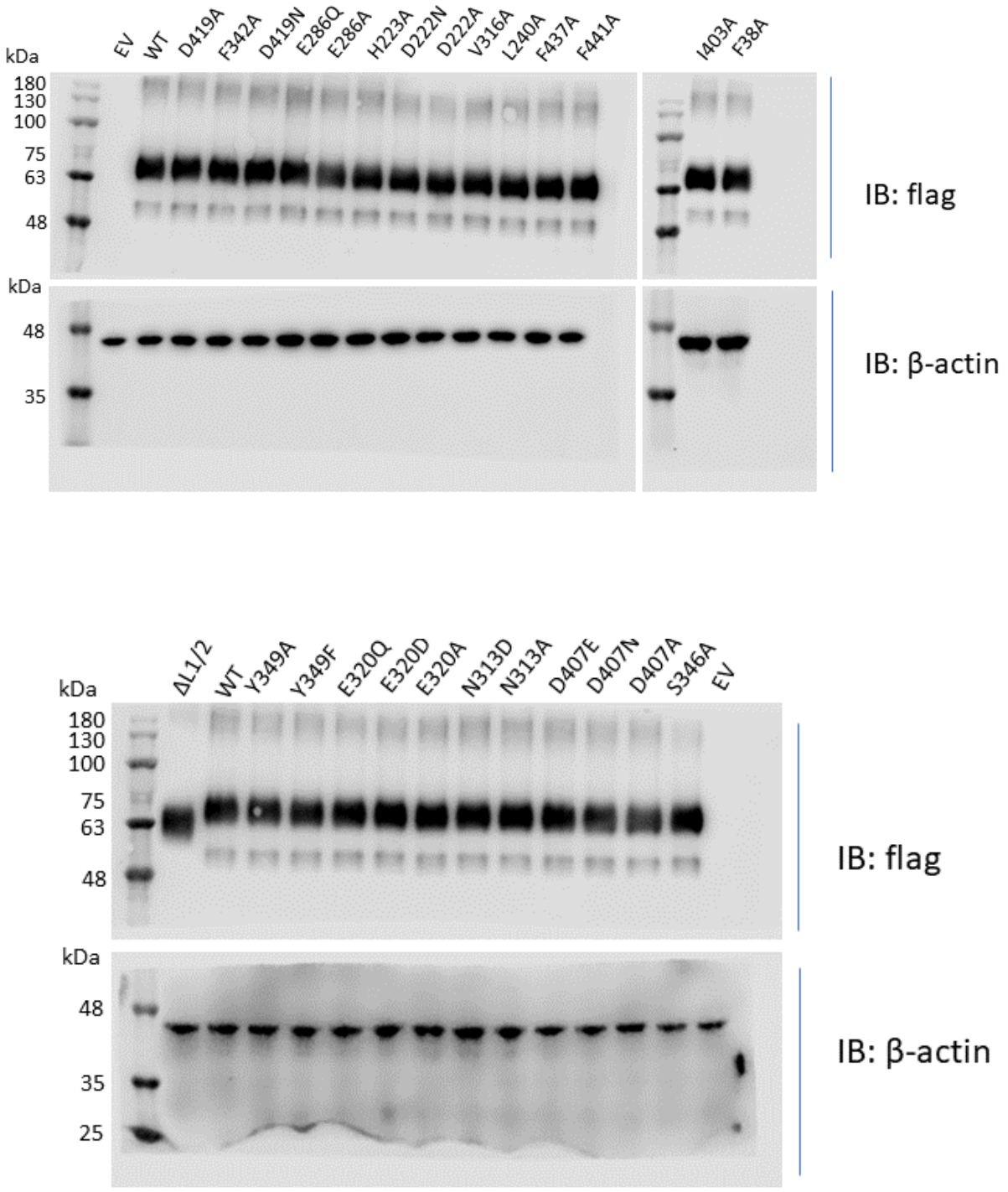
Supplementary Table 1. Alternate gating interactions formed at the cytoplasmic-open and vascular-open conformations. Same as above are indicated by "..".

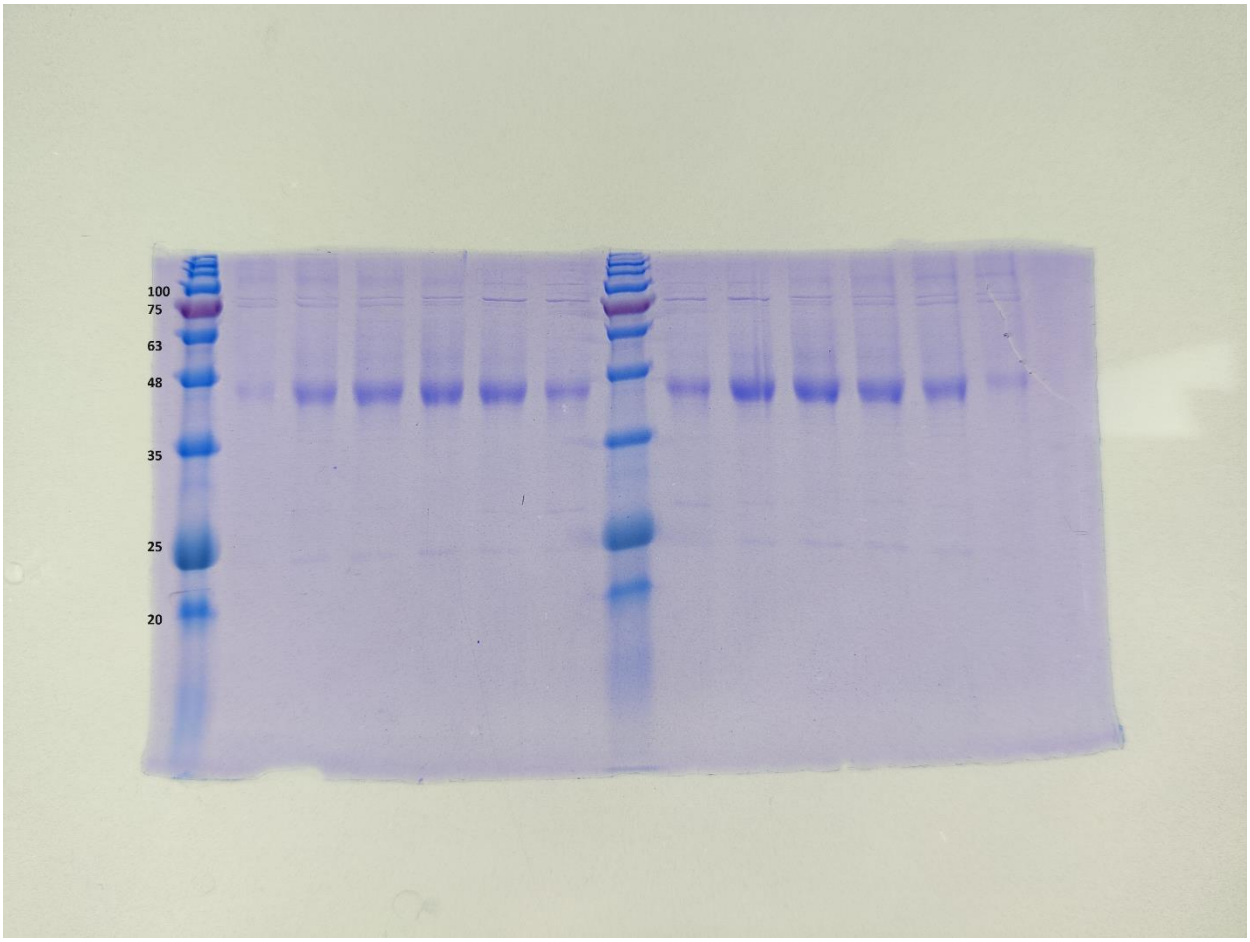
Cytoplasmic open (76 contacts)							Vascular open (145 contacts)						
NTD			CTD				NTD			CTD			
Region	**Residue**	**Atom**	**Region**	**Residue**	**Atom**	**Dist.**	**Region**	**Residue**	**Atom**	**Region**	**Residue**	**Atom**	**Dist.**
TM1	V42	CG1	TM7	P324	CG	3.98							
..	..	..	TM8	F342	CE2	3.69							
..	..	CG2	*TM7*	**E320**	CB	3.94							
..	..	..	..	..	CG	3.95							
..	..	..	..	..	OE1	3.96							
L1/2	T47	OG1	*TM8*	L338	CD1	3.94							
..	..	CG2	L7/8	K335	CD	3.55							
..	Q127	N	..	C332	SG	3.69							
..	..	CA	..	..	..	3.88							
..	..	CB	..	..	..	3.43							
..	..	CG	..	..	..	3.89							
..	L132	CB	..	Q329	OE1	3.93							
..	..	..	*TM7*	I325	CG2	3.68							
..	..	..	..	..	CD1	3.37							
..	..	CG	..	..	CD1	3.23							
..	..	CD1	..	..	CD1	3.44							
..	E133	O	L11/12	K453	NZ	3.41							
..	..	OE2	..	..	O	3.6							
..	E134	CA	..	..	NZ	3.72							
..	..	C	..	..	NZ	3.89							
..	..	O		..	NZ	3.35							
..	I136	CG2	TM11	G449	O	4							
..	T137	CG2	*L11/12*	*K453*	NZ	3.92							
TM2	G140	CA	*TM11*	P445	O	3.89							
..	..	O	..	..	CB	3.26							
..	..		..	..	C	3.86							
..	A144	CA	..	F441	O	3.93	TM2	A144	CB	TM11	S446	OG	3.03
..	..	CB	..	A442	O	3.72	..			..			
..	A147	CB	..	*F441*	C	3.69	..	A147	CB	..	F441	C	3.63
..	..	..	..	*A442*	N	3.36	..			..	A442	N	3.16
..	..	..	..	..	CA	3.51	..			..		CA	3.26
..	..	..	..	..	CB	3.29	..			..		CB	3.44
..	..	..	..	C438	O	3.28	..			..	C438	O	3.58
..	..	..	..	*F441*	CB	3.97	..			..			
..	Q150	CG	..	*C438*	SG	3.8	..	Q150	CG	..	*C438*	SG	3.41
..	..	CD	..	..	SG	3.5	..	..	CD	..	..	SG	3.27
..	..	OE1	..	..	SG	3.05	..	..	NE2	..	..	SG	2.53
								Q150	NE2	..	**D434**	CG	3.68
							..	..	..	..	..	OD1	3.01
							..	..	..	..	..	OD2	3.55
..	L151	CG	..	..	CB	3.83	..	L151	O	..	V435	CG2	3.84
..	..	CD1	..	M439	SD	3.78	..			..			
..	..	CD2	..	*C438*	CB	3.95	..			..			
								N154	CB	..	*V435*	CG2	3.61
							..	..	CG	..	*D434*	CG	3.96
..	N154	CG	..	D434	OD2	3.97	..	..	..	..	..	OD2	3.04
							..	..	OD1	..	..	OD2	2.97
							..	..	ND2	..	A431	O	3.84
							..	..	..	..	*V435*	CG2	3.55
							..	..	..	..	*D434*	CB	3.43
							..	..	..	..	..	CG	3.03
							..	..	..	..	..	OD1	3.92
..	N154	ND2	..	..	OD2	2.88	..	..	..	..	..	OD2	2.44
							..	..	..	..	..	C	3.63
							..	..	..	..	*V435*	N	3.44
							..	..	..	..	..	CA	3.85
							L2/3	N162	O	L6/7	P285	CA	3.59
							..	..		..	..	C	3.85
							..	..		..	**E286**	N	3.15
							..	..	CG	TM11	V425	CG1	3.44
							..	..	OD1	..	..	CG1	2.94
							..	..	ND2	..	A288	O	3.17
							..	..	..	..	*V425*	CG2	3.66
							..	..	..		G290	N	3.61
							..	..	..		*V425*	CB	4

							..	..	..		..	CG1	3.21
							..	R163	O		V283	CG1	3.57
							..	I164	C		S284	O	3.67
							..	..	O		..	O	3.64
							..	..	..		..	N	3.64
							..	..	CD1		*V283*	CG1	3.69
							..	..	..		..	CG2	3.81
							..	G165	N		*S284*	O	3.71
							..		CA			O	3.85
L2/3	Y166	OH	..	Y426	OH	3.87	..	Y166	CE1		**E286**	CD	3.77
							..					OE2	2.99
							..		CZ			OE2	2.98
							..		OH			OE1	3.92
							..					CD	3.47
							..					OE2	2.38
							..	**H167**	CB		P280	CB	3.97
							..	I168	CD1		K282	C	3.98
							..					O	3.18
TM4	S208	CB	..	Y430	OH	3.99	TM4	S208	OG		*D434*	CG	3.98
							..					OD1	3.97
							..	V209	CG2		A431	CA	3.71
							..	L212	CD2		M411	SD	3.97
TM4	A216	CB	..	..	CZ	3.81	..	A216	CB		Y426	CB	3.95
							..					CG	3.77
..	..	..	..	*Y426*	CD2	3.95						CD2	3.86
..	..	..	..	..	CE2	3.65						CE2	3.53
								D222	O		R365	NH2	3.53
									CG			NH2	2.96
									OD1			NH2	2.75
												CZ	3.83
									OD2			NH2	2.84
												NE	3.91
												CZ	3.82
								H223	CA		N361	ND2	3.16
									C			ND2	3.87
									O			ND2	3.68
									CB			ND2	3.17
									CG			CB	3.77
												CG	3.74
												ND2	3.19
									ND1			CB	3.45
												CG	3.87
												ND2	3.45
									CD2			CB	3.85
												CG	3.96
												ND2	3.82
									CE1			CB	3.35
									NE2			CB	3.59
L4/5	R225	NH1	..	*Y426*	CB	3.84		R225	NH1		*Y426*	CE2	3.75
												CZ	3.88
												OH	3.74
									NH2		G415	C	3.64
												O	3.73
											H416	N	3.8
											D419	OD2	3.59
								G226	CA		P412	CG	3.68
								M229	CE		Y430	CZ	3.43
												OH	2.99
											P412	CA	3.84
											Y430	CE1	3.22
											M411	O	3.29
												C	3.19
												CB	3.23
											P412	N	3.44
											M411	CA	3.73
								G230	CA		T353	O	3.87
									C			O	3.87
									O			O	3.46
												OG1	3.75
								L233	C			OG1	3.76
												CG2	3.81
									O			CG2	3.96
									CB			OG1	3.08
									CD1		**D407**	CB	3.64
								G234	N		T353	OG1	3.76

												CG2	3.32
									CA		N354	OD1	3.35
											T353	CG2	3.05
TM5	A237	CB	*TM8*	L350	CD1	3.63		A237	CB		L350	CA	3.88
											Y349	CD1	3.15
												CE1	3.32
								L238	N		L350	CD1	3.84
									CA			CD1	3.73
									CB			CD1	3.75
								L241	CD1		L343	CD1	3.51
									CD2		V347	CG2	3.85
..	P245	CA	..	G339	C	3.93							
..	..	CG	..	L343	CG	3.99							
..	S248	O	*L7/8*	W336	CA	3.56							
..	..	..	..	..	CB	3.56							
..	..	CB	..	..	O	3.86							
..	..	..	*TM8*	*G339*	N	3.36							
..	..	..	..	..	CA	3.66							
..	..	OG	*L7/8*	Q337	N	3.66							
..	..	..	*TM8*	L338	N	3.68							
..	..	..	*L7/8*	K335	C	3.86							
..	..	..	..	..	O	3.05							
..	..	..	..	*W336*	N	3.88							
..	..	..	..	..	CA	2.98							
..	..	..	..	..	C	2.89							
..	..	..	..	..	O	2.85							
..	..	..	*TM8*	G339	N	3.37							
L5/6	E252	CD	*L7/8*	*W336*	CD1	3.51							
..	..	OE1	..	*K335*	N	3.61							
..	..	..	..	..	CA	3.71							
..	..	..	..	..	CB	3.49							
..	..	..	..	*W336*	CG	3.51							
..	..	..	..	..	CD1	3.05							
..	..	..	..	*K335*	C	3.54							
..	..	..	..	*W336*	N	3							
..	..	..	..	..	CA	3.67							
..	..	..	..	..	CB	3.27							
..	..	OE2	..	..	CD1	3.58							
								Q274	O		P280	CD	3.88
								L275	CA			CD	3.98
									CD2			CD	3.87
								L278	C			N	3.61
												CD	3.87
									O			N	3.74
								Q279	N			N	3.02
												CD	3.07
									CA			N	2.46
												CA	3.85
												CD	2.94
									C		S281	N	3.42
											P280	N	1.33
												CA	2.46
												CG	3.63
												CD	2.5
												C	3.31
												CB	3.58
									O		*S281*	N	3.16
											P280	N	2.24
												CA	2.76
												CD	3.62
												C	3.43
									CB			N	3.74



Supplementary Figure 1. Western blot of human VMAT1 constructs showing their similar expression levels. The anti-flag antibody (Cell Signaling Technology) and anti-actin antibody (Cell Signaling Technology) were used. These constructs are used for the monoamine uptake and binding assays in Figs. 1b, 1c, 3g, 3h, 5f, 5g, and Extended Data Figs.1 and 7f.



Supplementary Figure 2. Uncropped gel for Extended Data Figure 2c.