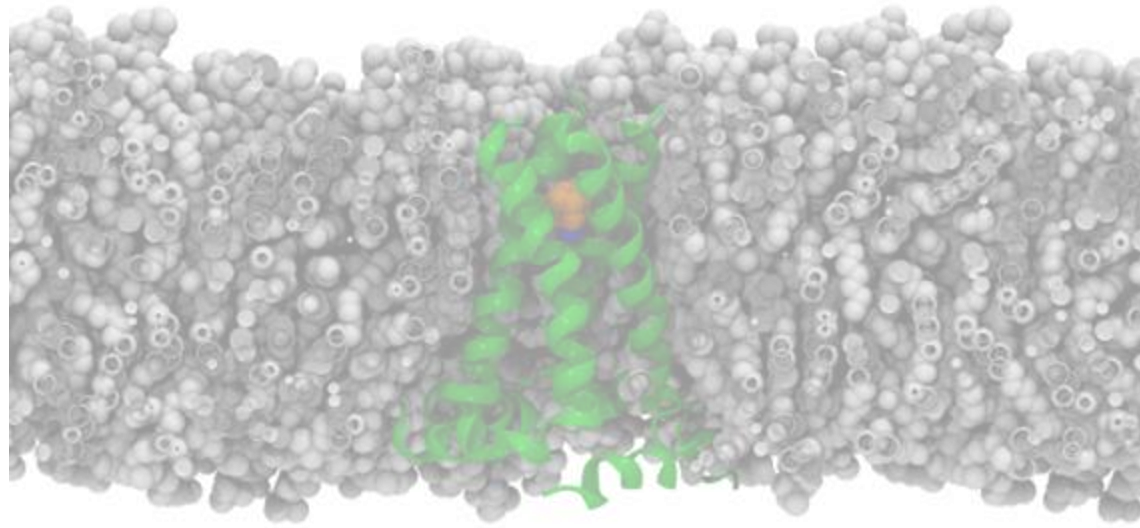


Modelling Drug Binding to the M2 Proton Channel Using Enhanced Sampling Methods



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Spain Conference

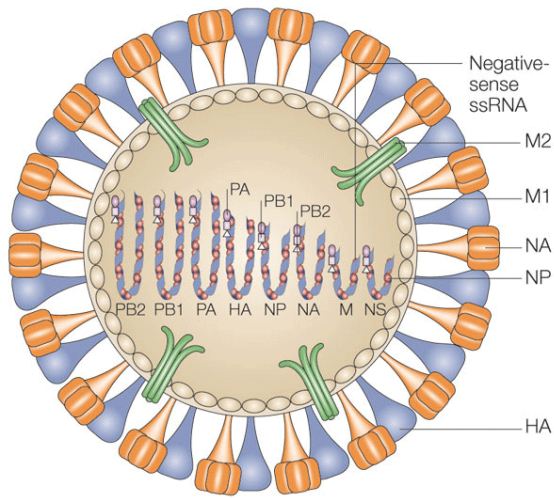


Universitat de Barcelona



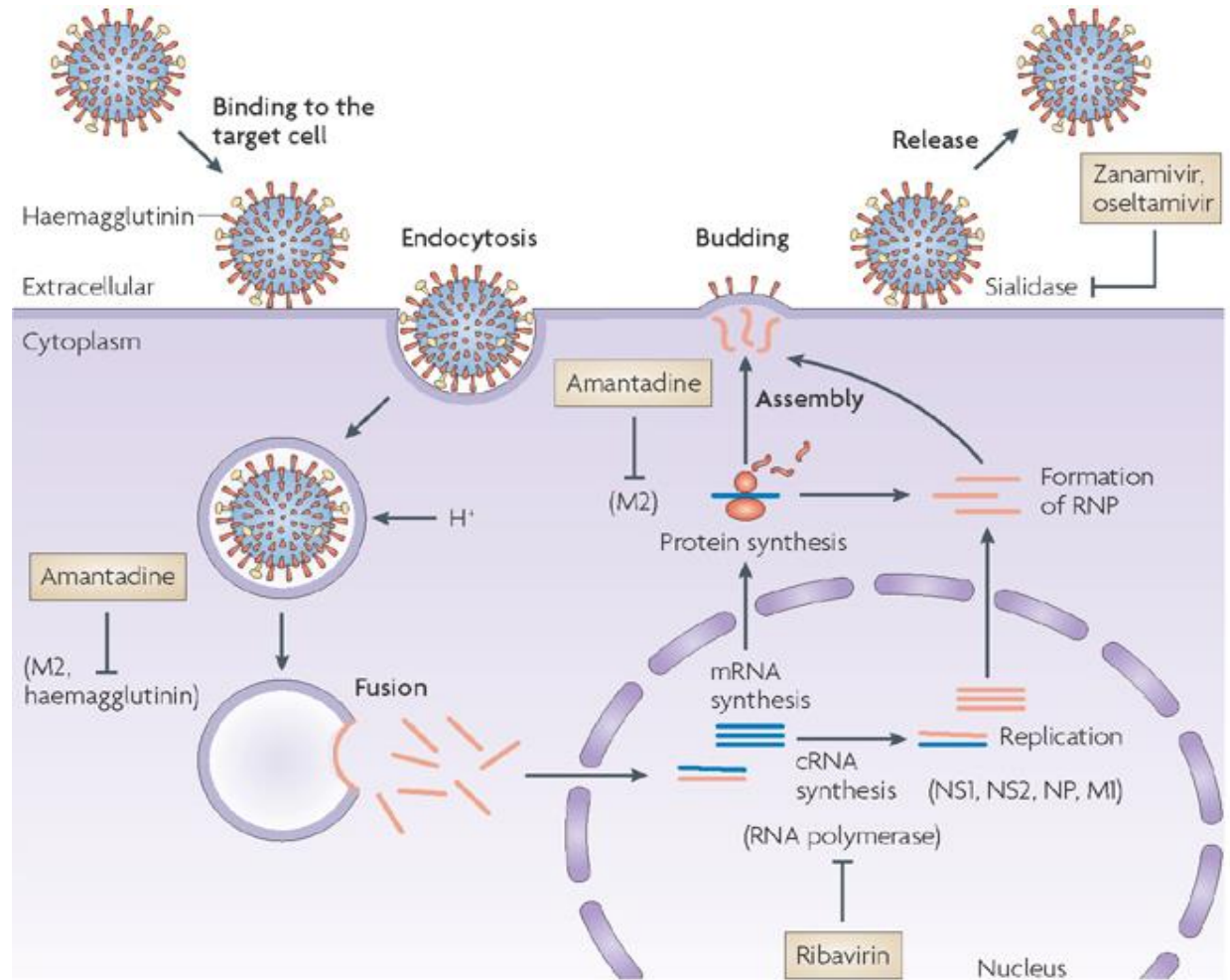
Campus
de l'Alimentació
Universitat de Barcelona

The Flu: Life cycle and drug target

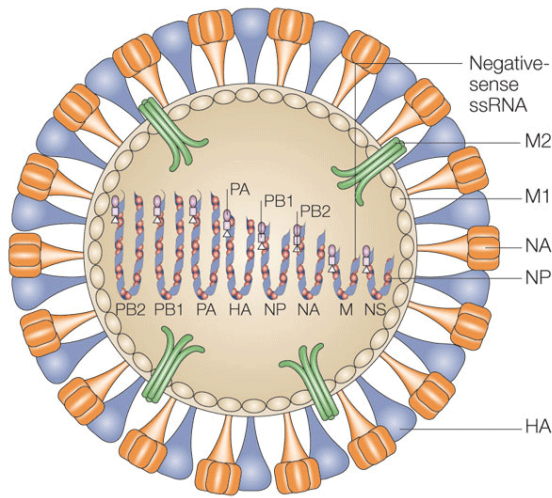


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Nature Reviews | Microbiology

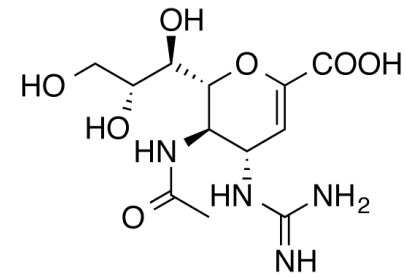
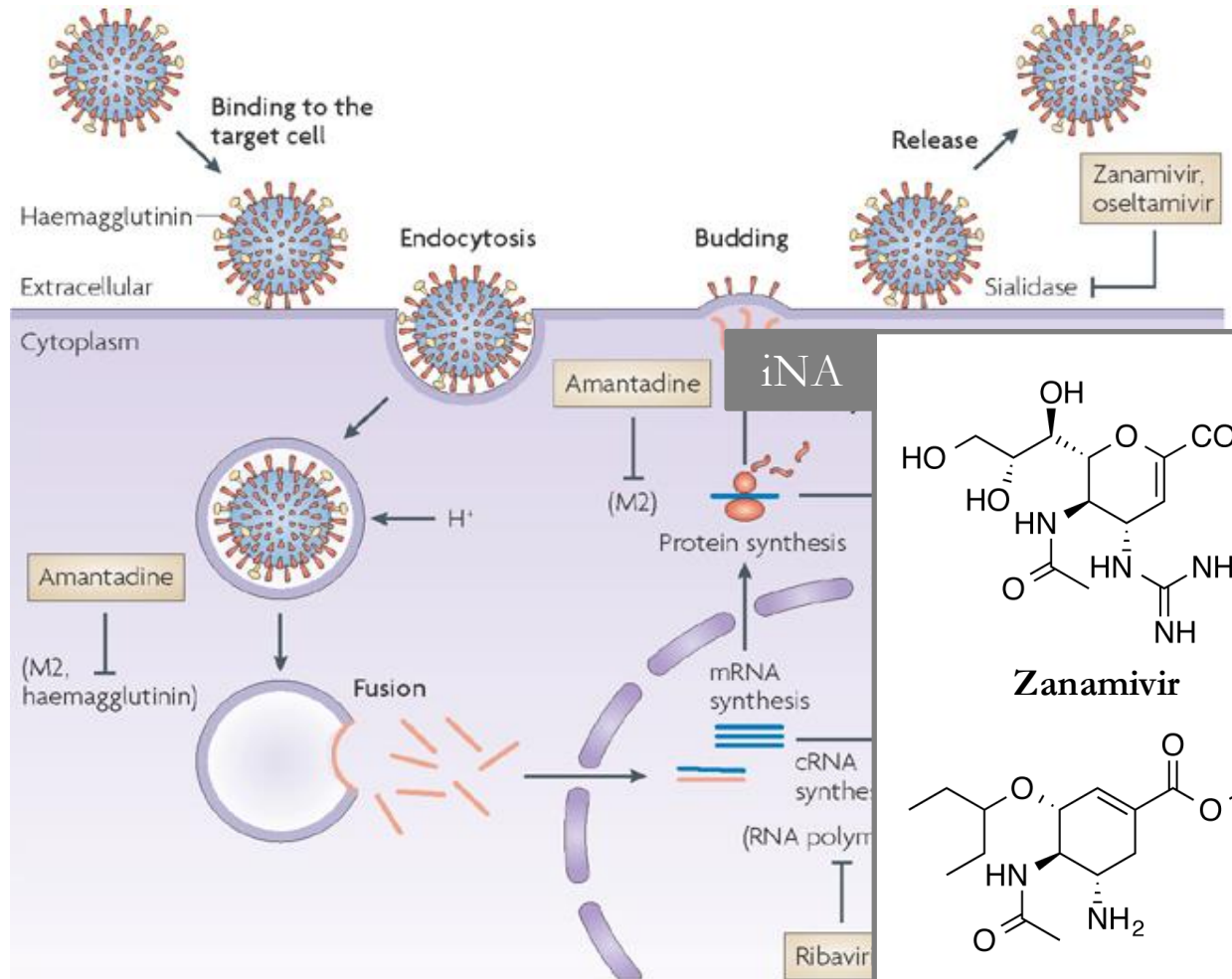
- Spanish Flu (1917-18)
20-50 Million deaths
- Asian Flu (1957-63)
2-4 Million deaths
- Hong Kong Flu (1968-69)
2 Million deaths
- “Swine” flu (2009)



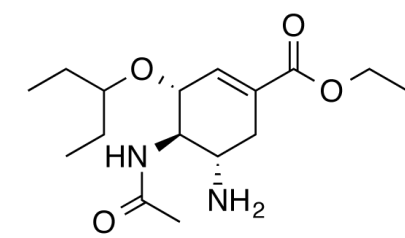
The Flu: Life cycle and drug target



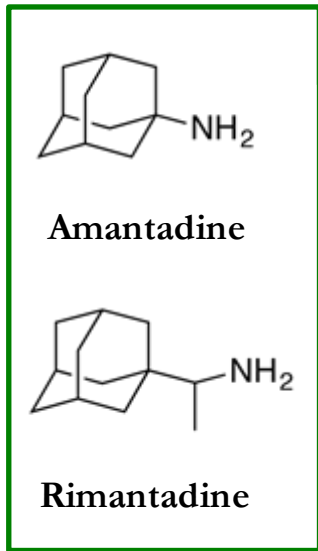
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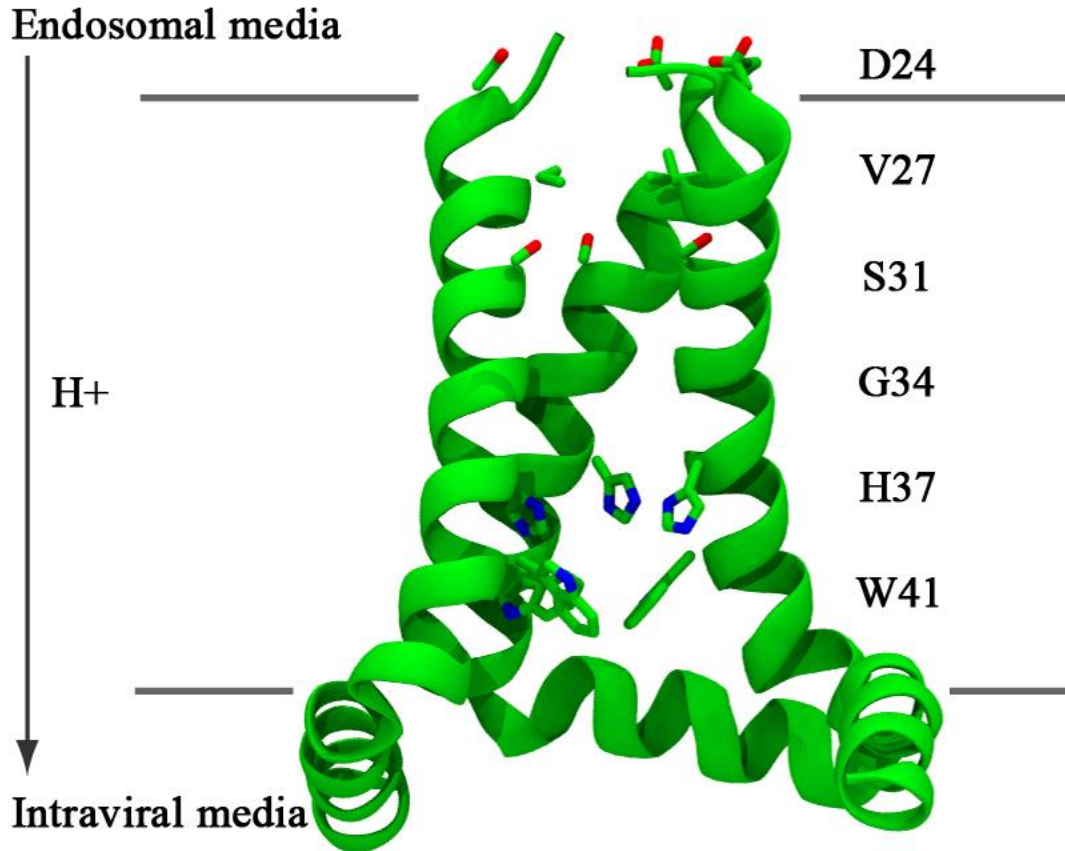
Zanamivir



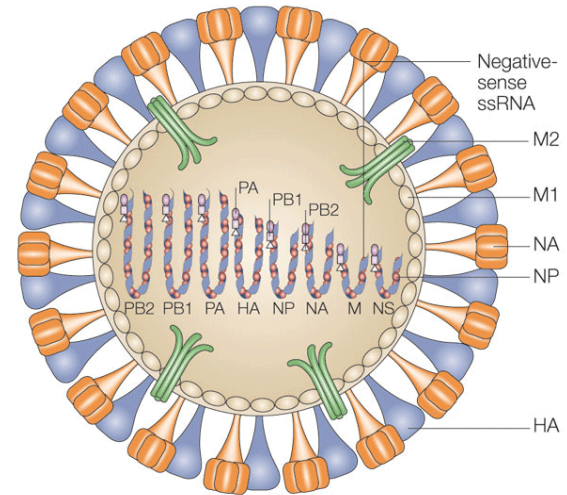
Oseltamivir



M2 proton channel

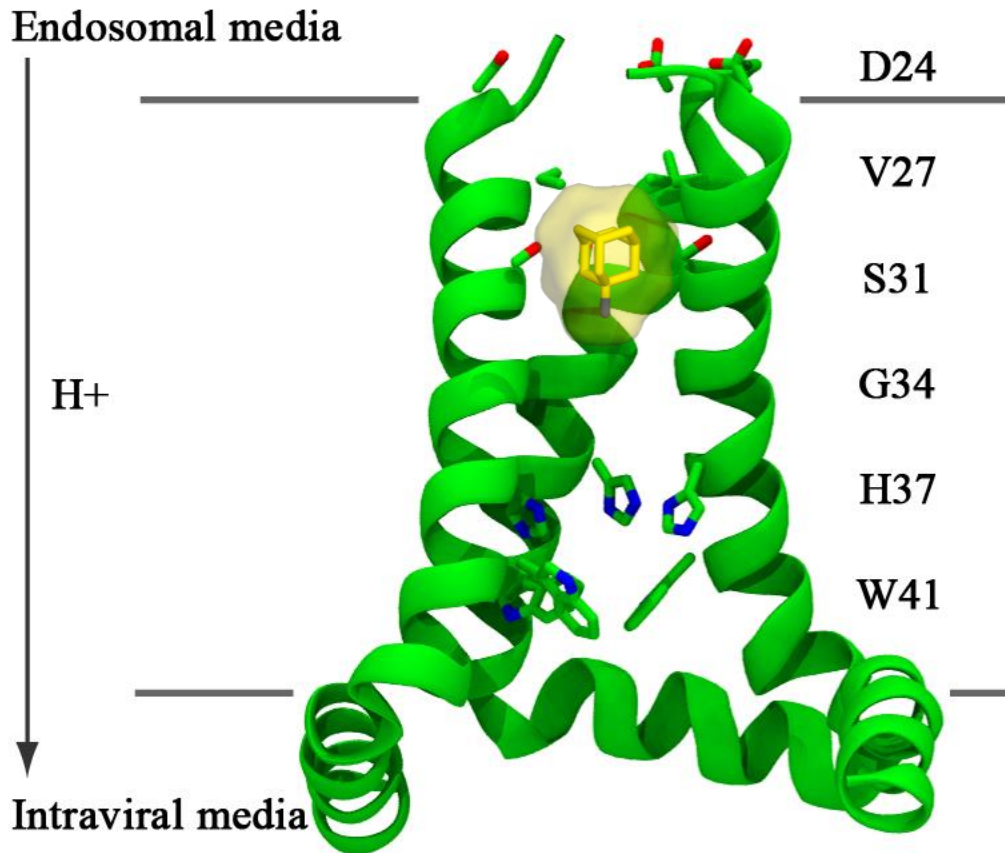


pKa: 8.2, 8.2, 6.3, <5.0 (*PNAS* 2006, 103, 6865)

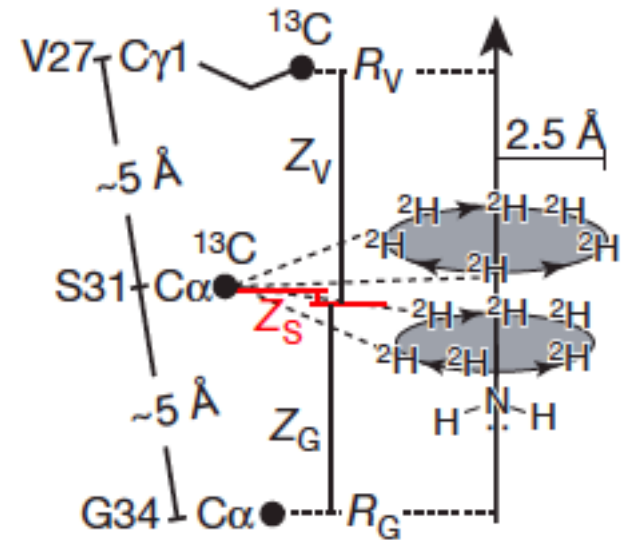


- Essential protein that transports H⁺ across the viral envelope.
- Acidification “activates” hemagglutinin, which triggers the fusion of endosomal and viral membranes
- Homotetramer.
- pH dependent conformation.
- Conserved motif: **H37xxxW41**.

M2 proton channel

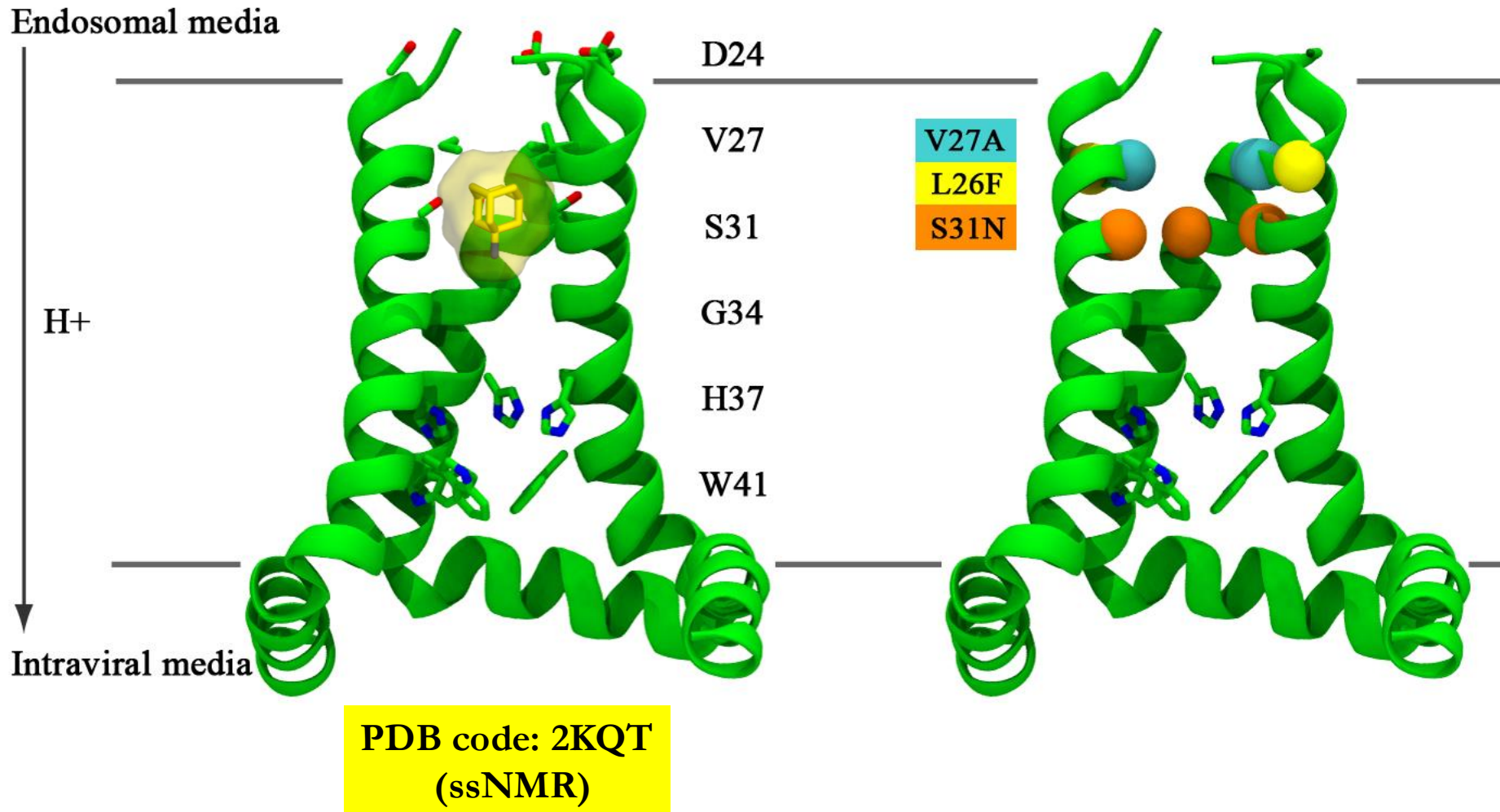


PDB code: 2KQT
(ssNMR)

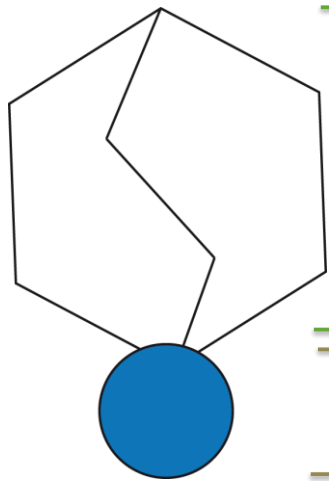


M2 inhibition occurs by physical occlusion, interrupting water wires involved in proton conduction

Amantadine-resistant variant

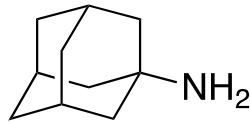


Representative inhibitors



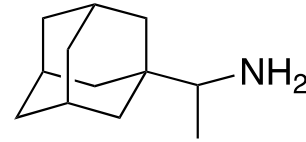
Hydrophobic
cage

Positive
charge

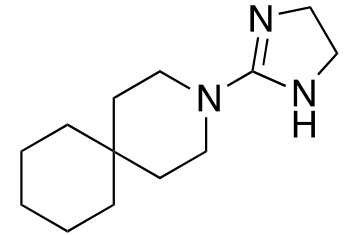


Amantadine

%: 91; IC₅₀: 16 μM

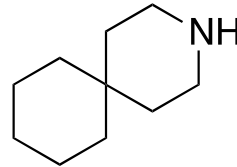


Rimantadine



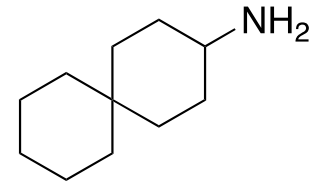
BL-1743

%: 75; IC₅₀: 41 μM



3-Azaspiro[5,5]undecane

%: 95; IC₅₀: 0.9 μM



Spiro[5,5]undecan-3-amine

%: 89; IC₅₀: 13 μM

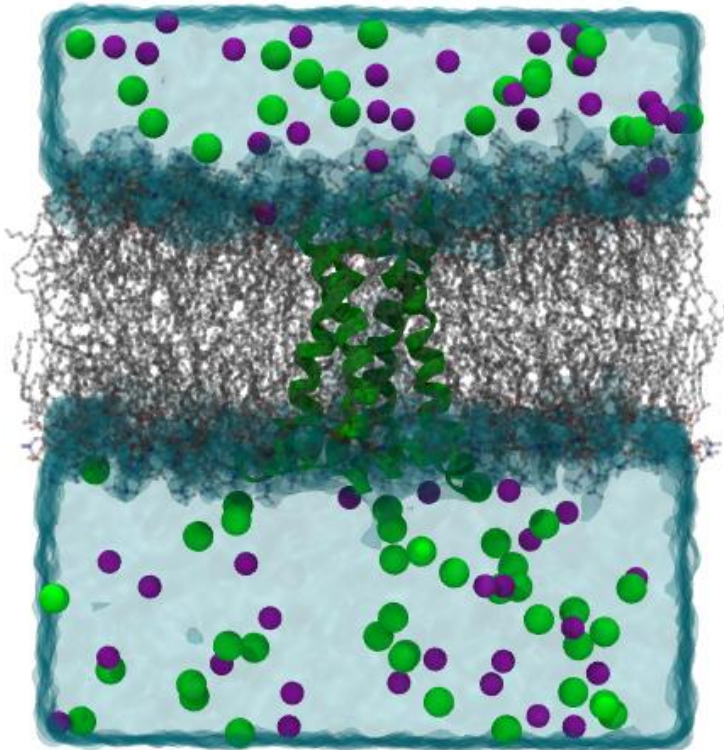
Aims

Development of inhibitors of M2 channel.

- Explore the interaction of Amt with the M2 channel.
- Rationalize the structure-activity relationships.
- Identify the binding mechanism.



Santiago Vázquez
Pharmaceutical Chemistry Unit
UB



~97000 atoms.

100x100 Å POPC membrane.

Protein: 2L0J (H37 protonation state +2).

150nM KCl.

GAFFlipid11 for POPC lipids.

GAFF & RESP charges for ligands.

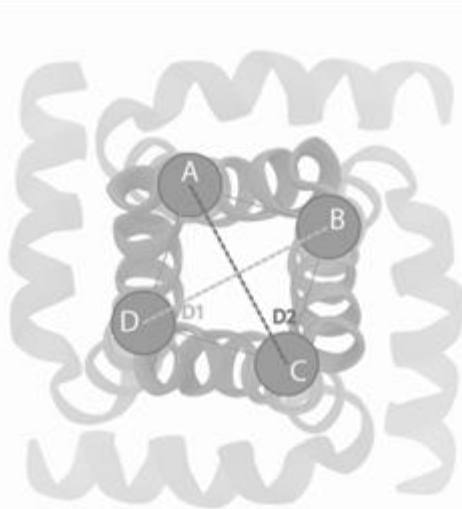
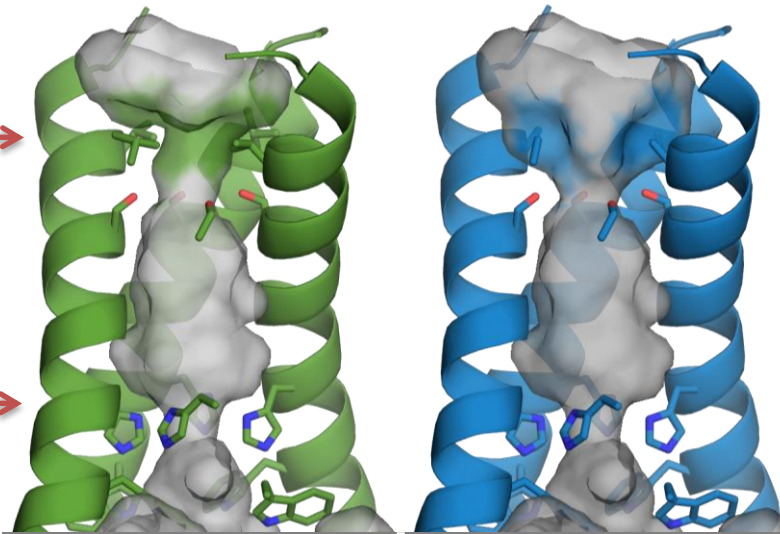
How does V27A mutation affect the channel pore?

wild type channel V27A mutant channel

Cavity expansion



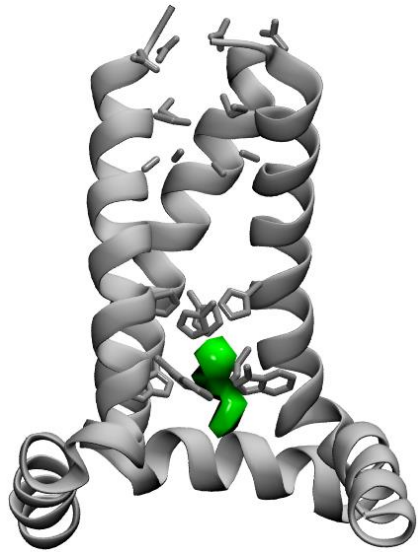
Mild cavity compression



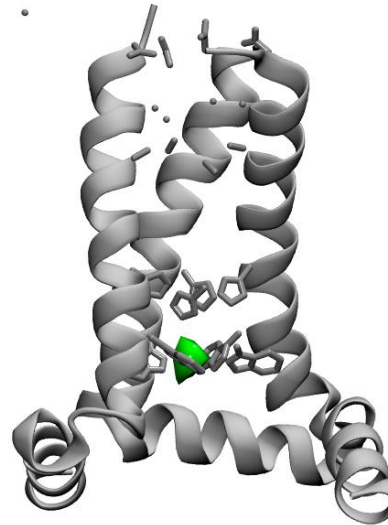
		D1	D2	D1	D2	Δ
V27 / A27	C α	10.3	10.6	11.6	11.5	+1.1
	C β	8.3	8.6	10.0	9.9	+1.5
S31	C α	11.0	10.7	11.4	11.3	+0.5
	C β	11.2	10.8	12.0	12.0	+1.0
G34	C α	10.1	9.3	9.6	9.4	-0.2
H37	C α	13.4	12.3	12.3	11.8	-0.8
	C β	10.6	9.9	9.6	9.2	-1.0

Chlorine distribution maps (apo channel)

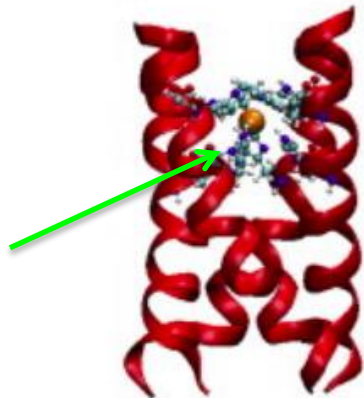
Wild Type



V27A

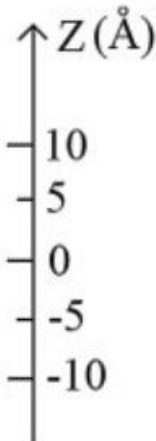


a

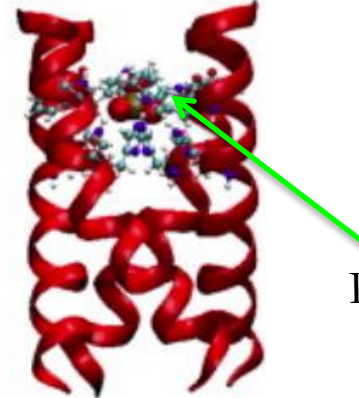


Chlorine

Trp41
His37



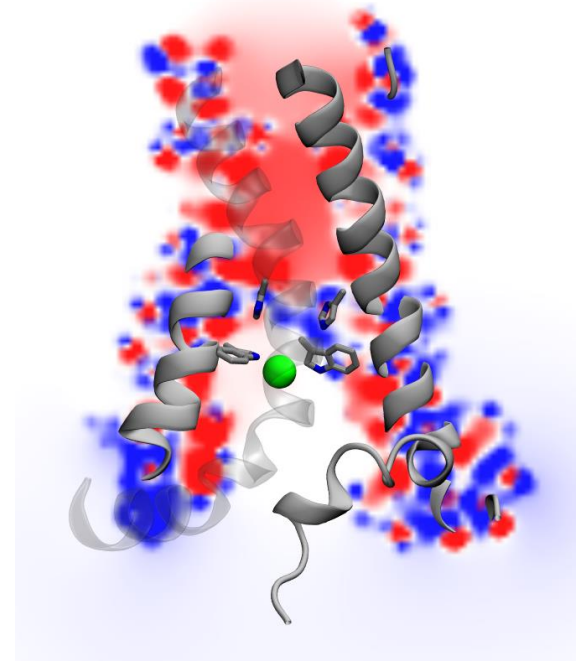
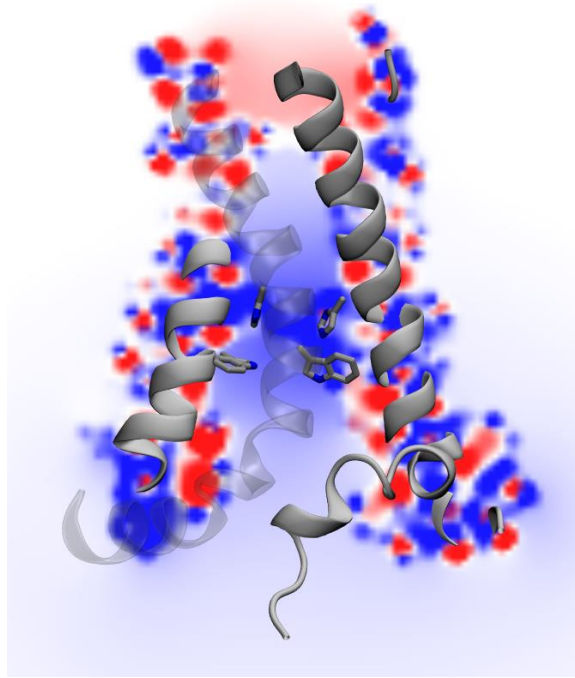
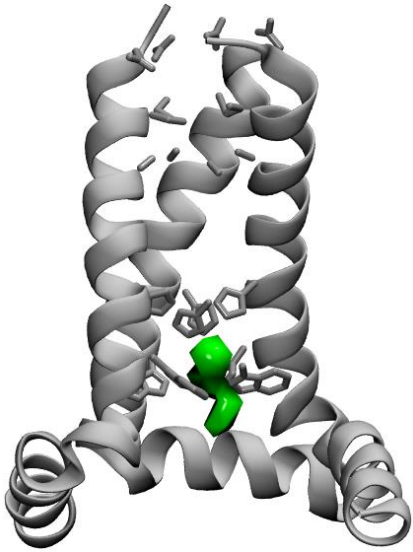
d



Wei, Pohorille
Biophys. J. 2013, 105,
2036
(CHARMM ff)
Phosphate

Electrostatic potential in the channel

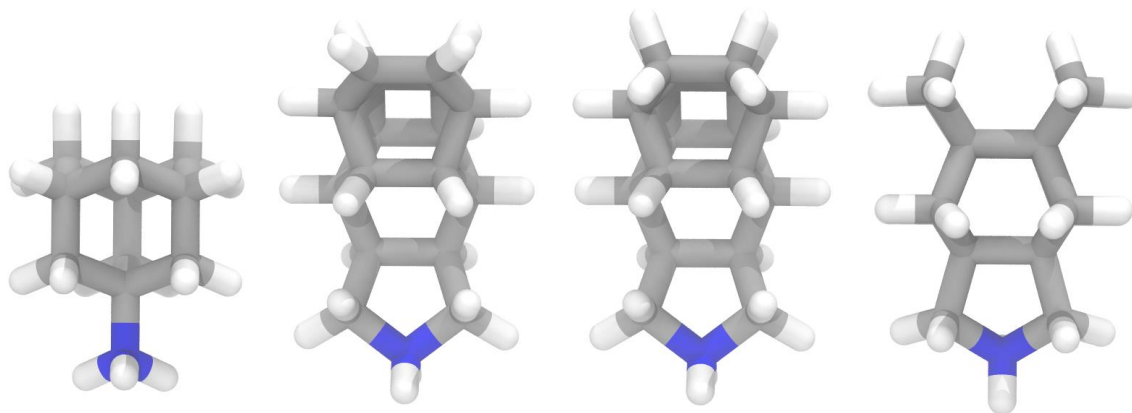
Wild Type



The presence of chlorine anion is required for assisting ligand binding.

This does not imply co-transport with proton!

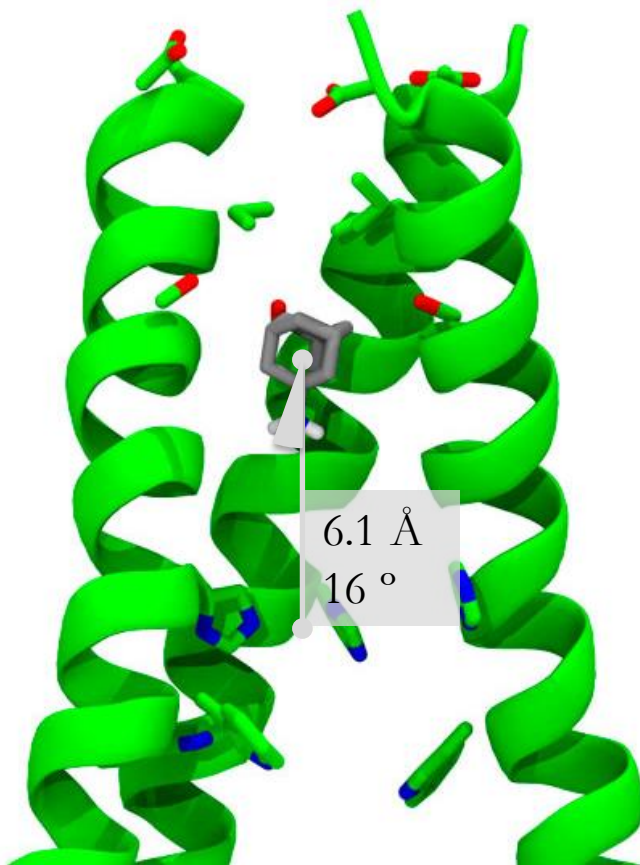
New inhibitor size-expanded scaffolds



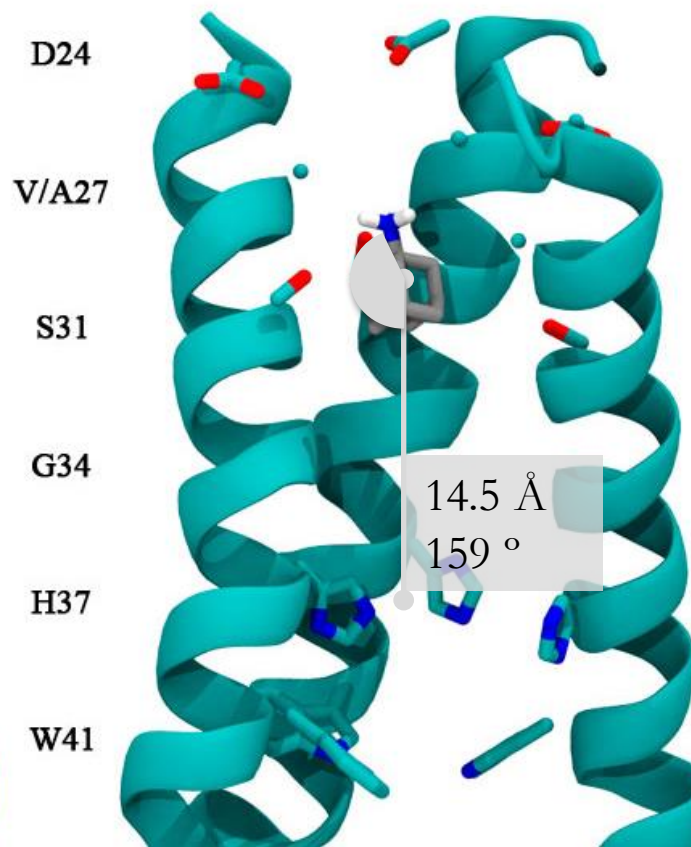
		Amantadine	ETCA40	ETCA41	MFV3
Structural trends	Length (Å)	4.06	6.6	6.6	5.6
	Area (Å ²)	214.2	254.9	259.7	308.5
	Volume (Å ³)	213.1	292.6	304.1	321.3
Wild type	Inhibition (%)	91.0	96.4	98.1	90.2
	IC ₅₀ (μM)	16.0	2.6	2.1	18
V27A mutant	Inhibition (%)	10.8	49.0	83.9	96.4
	IC ₅₀ (μM)	ND	184.6	17.2	0.7

Ligand-bound complexes: Amantadine binding modes

wild type channel
“down” binding mode

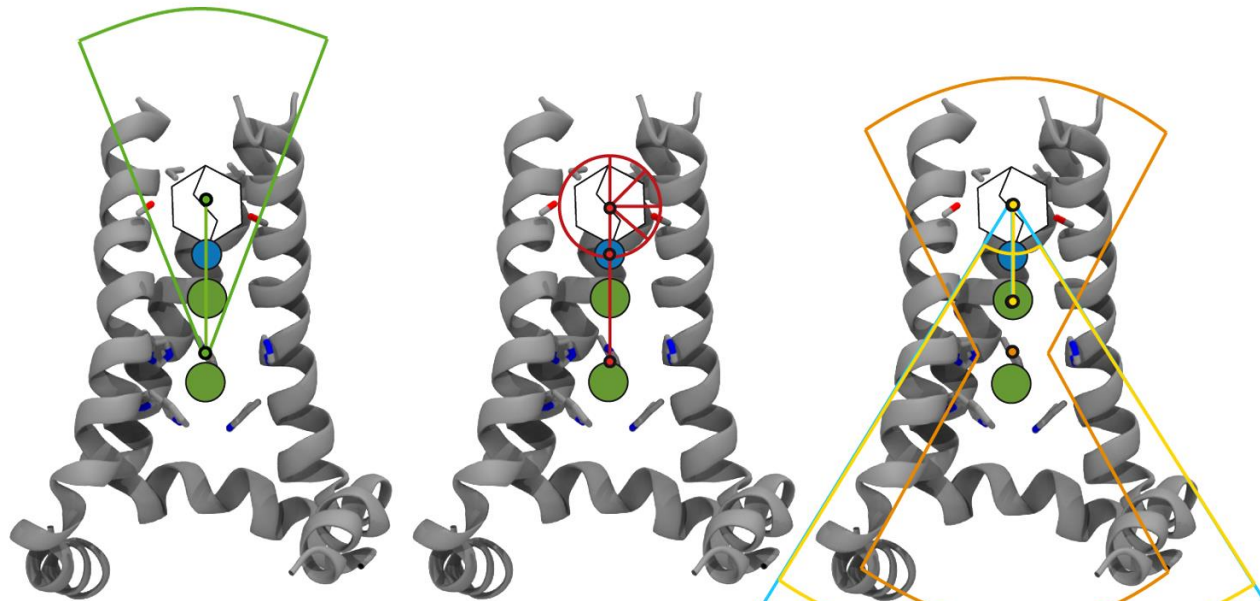


V27A mutant channel
“up” binding mode



Multiple-walker metadynamics

Chosen Collective Variables



CV1

Amantadine binding

CV2

Amantadine tilting

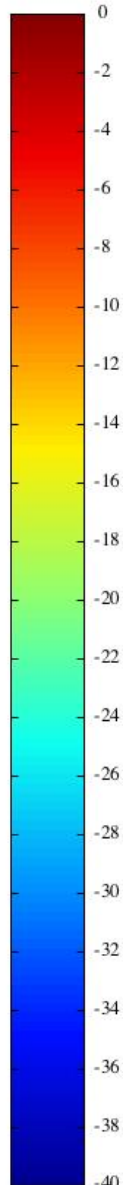
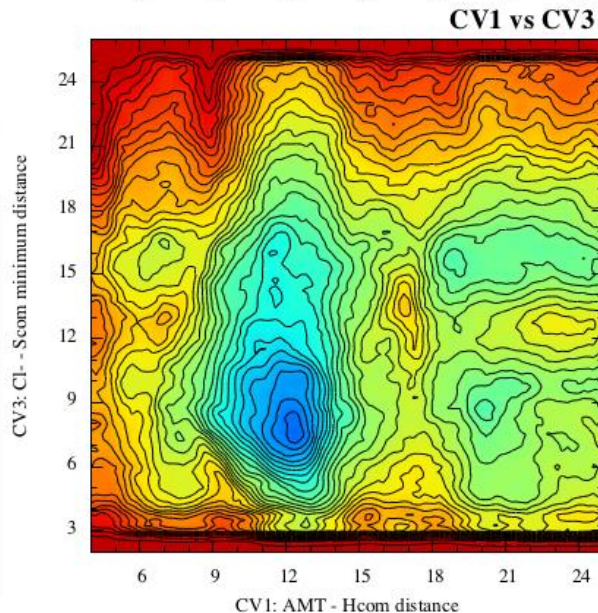
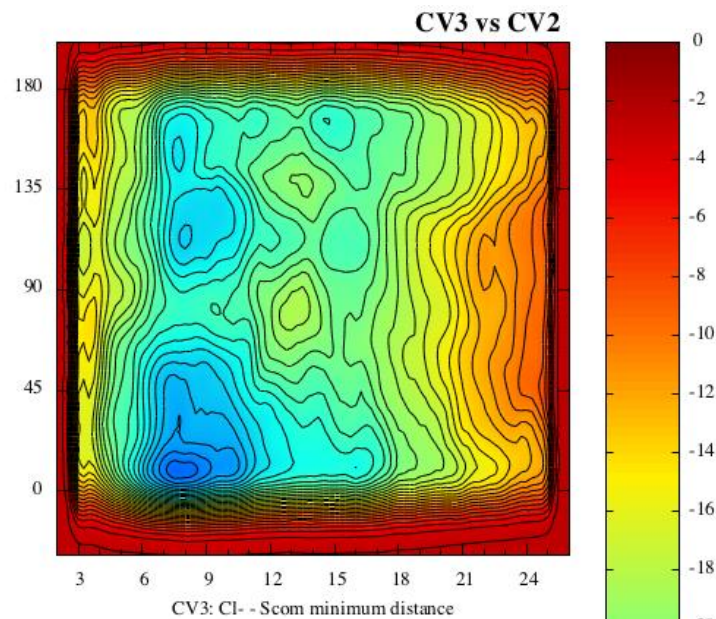
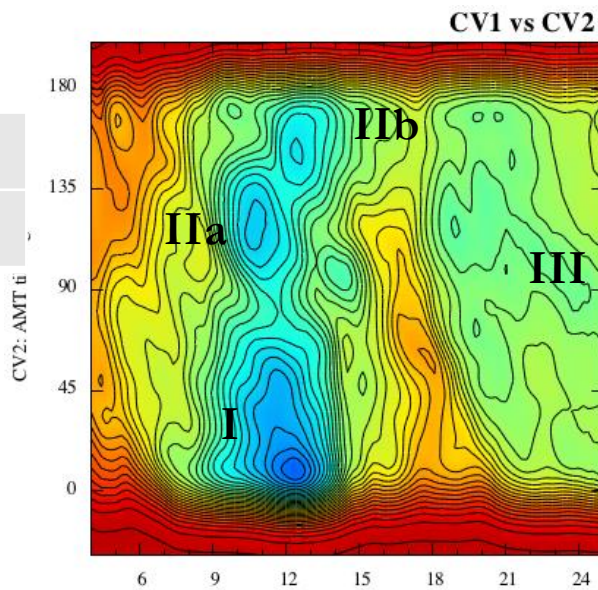
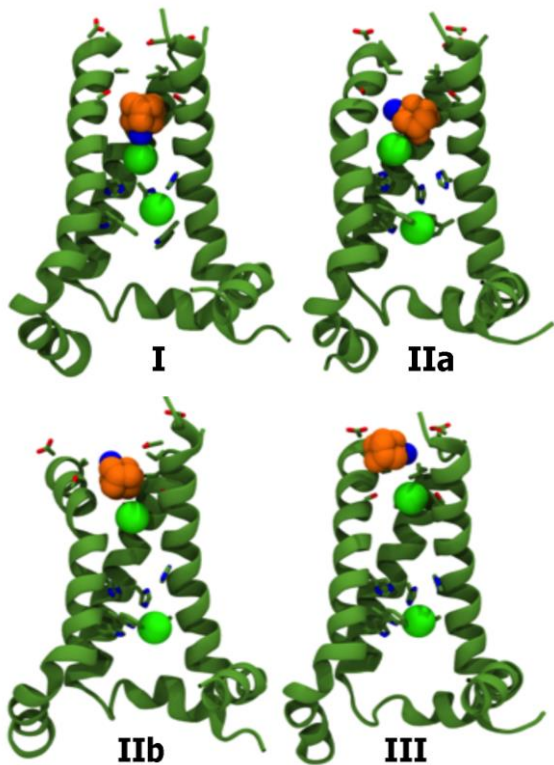
CV3

Chlorine anions
inside the channel

Amt-Wild Type

Inhibition (%)	91.0
----------------	------

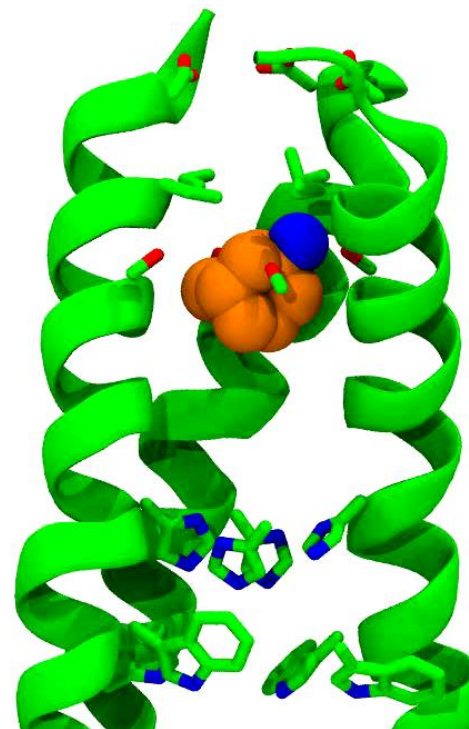
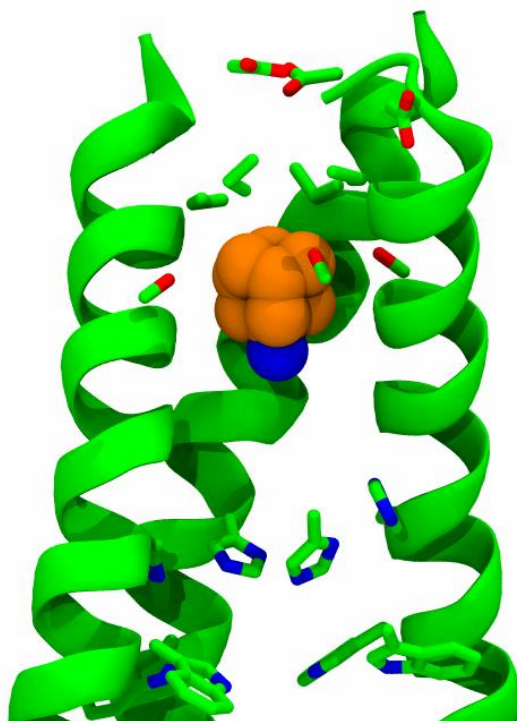
IC ₅₀ (μM)	16.0
-----------------------	------



Unbiased MD simulations

10 μ s
unbiased MD
Simulations

	Initial Binding Mode	Final Binding Mode		
		<i>down</i>	<i>up</i>	<i>unbound</i>
Wild type	<i>down</i>	50/50	0/50	0/50
	<i>up</i>	3/50	47/50	0/50



Electrophysiological proton conductance assays

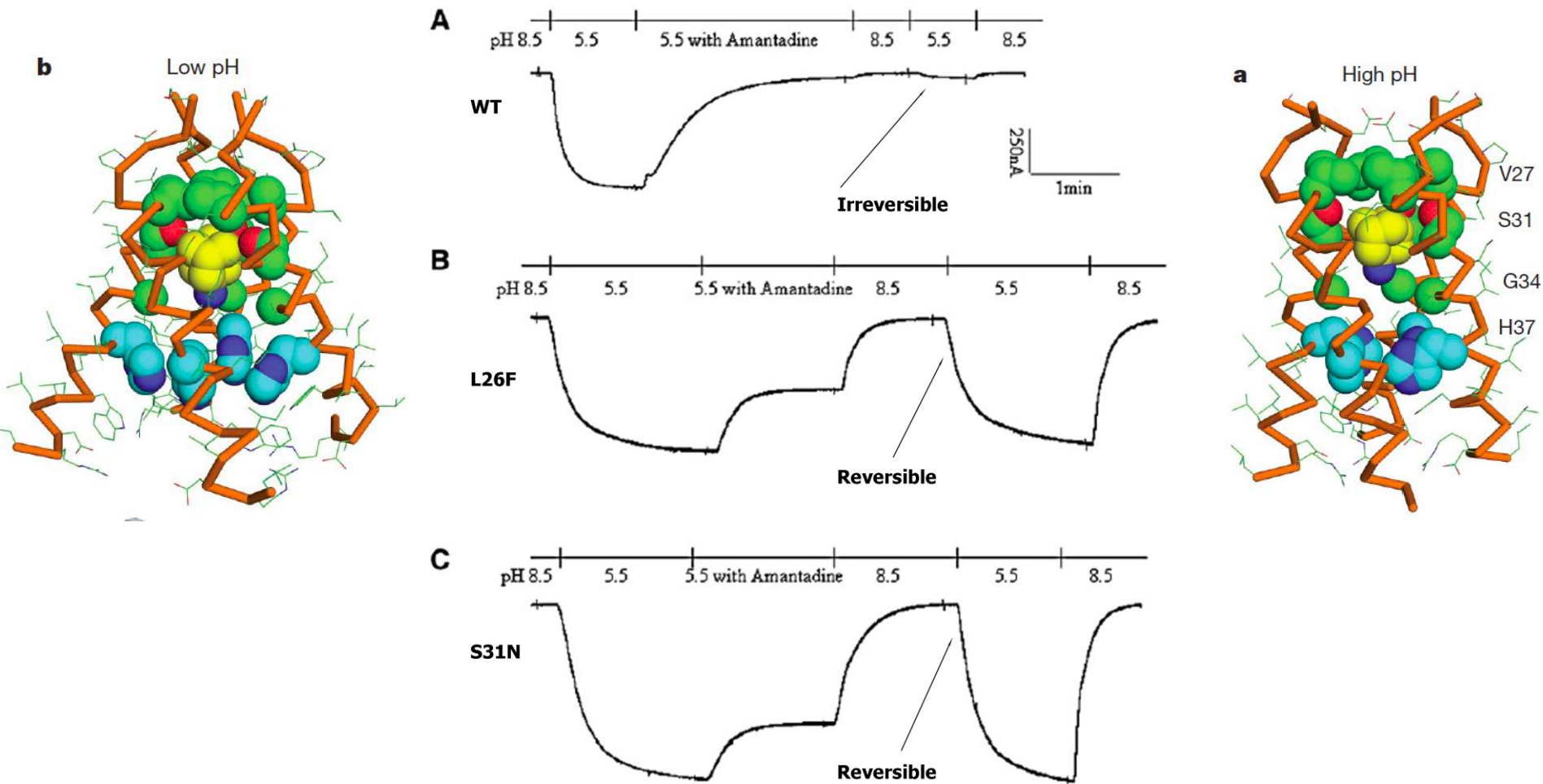
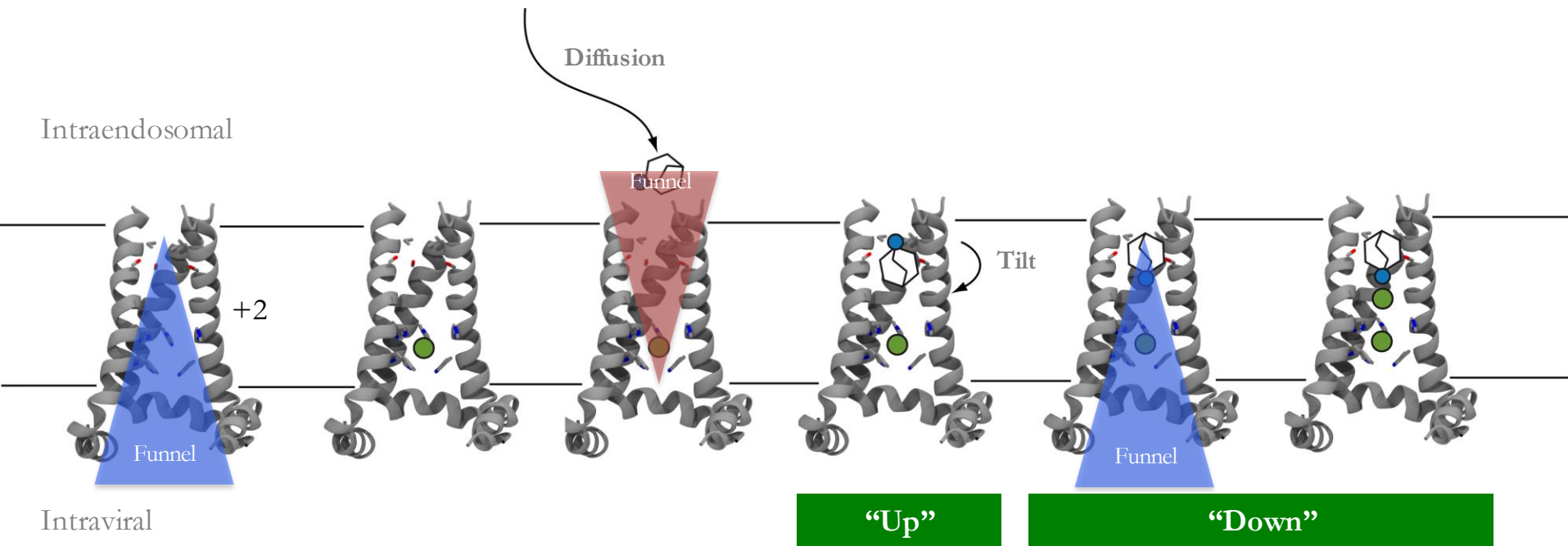


Figure 4. pH Activation and Amantadine Sensitivity of L26F, S31N, and WT AM2 Channels

Amantadine Binding Mechanism Hypothesis



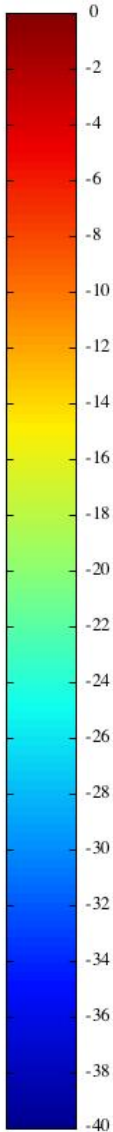
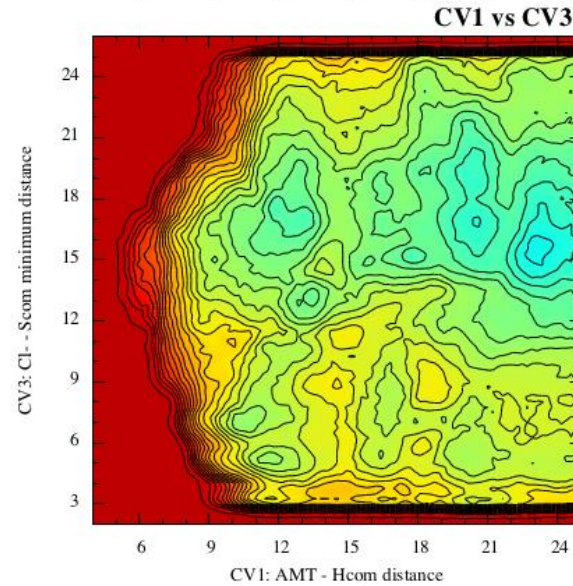
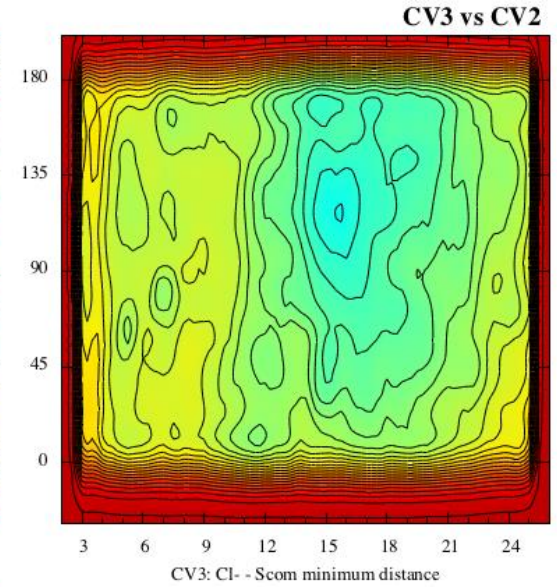
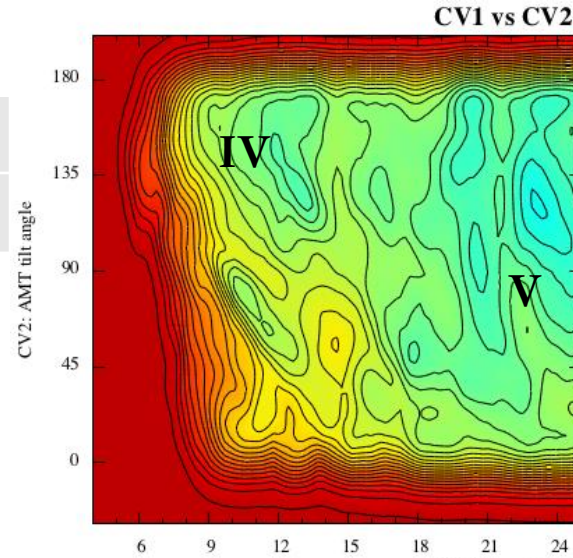
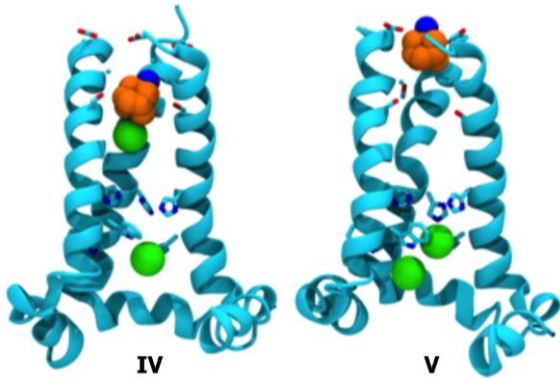
Amt-V27A

Inhibition (%)

10.8

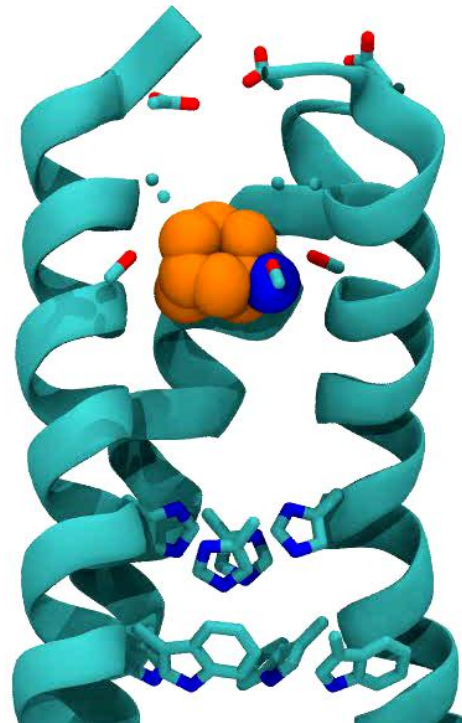
IC₅₀ (μM)

ND



Amt-Wild Type

		Initial Binding Mode	Final Binding Mode			
			<i>down</i>	<i>up</i>	<i>unbound</i>	
10 μ s	Unbiased MD Simulations	V27A mutant	<i>down</i>	0/50	44/50	6/50
				<i>up</i>	0/50	45/50



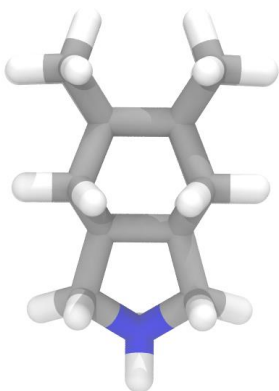
MFV3-V27A

Inhibition (%)

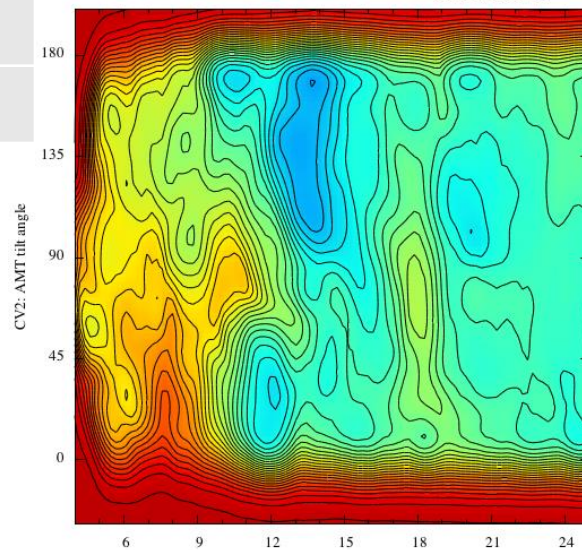
96.4

IC₅₀ (μM)

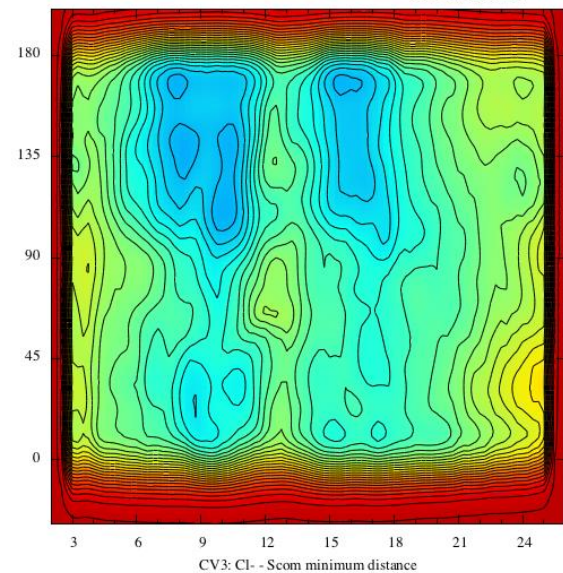
0.7



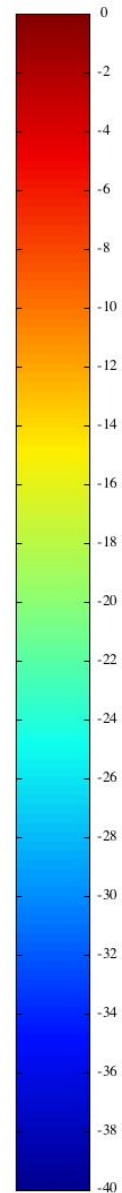
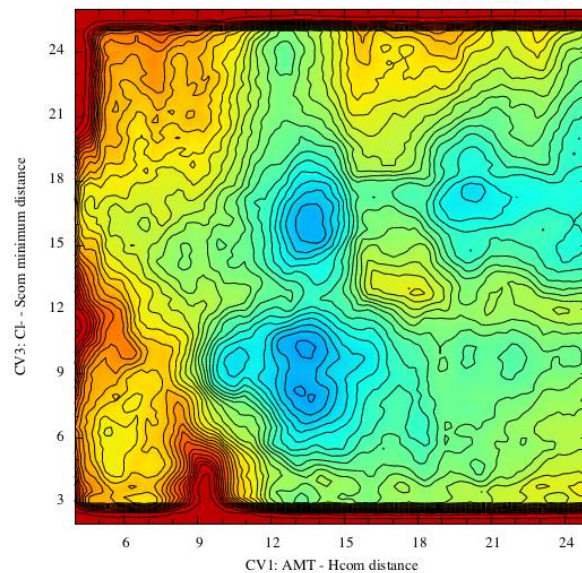
CV1 vs CV2



CV3 vs CV2



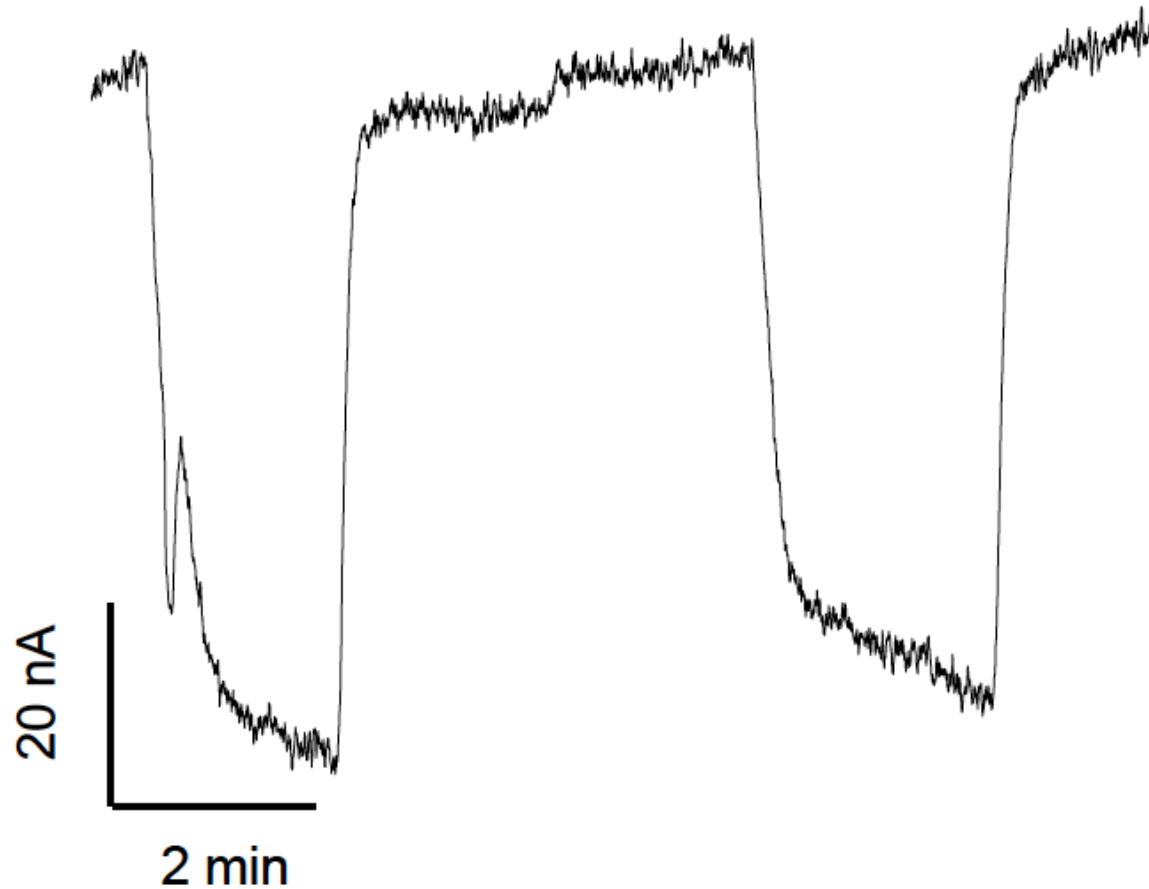
CV1 vs CV3



Proton conductance assays

V27A

8.5	5.5	+ MFV-3	8.5	5.5	8.5
-----	-----	---------	-----	-----	-----



Conclusion

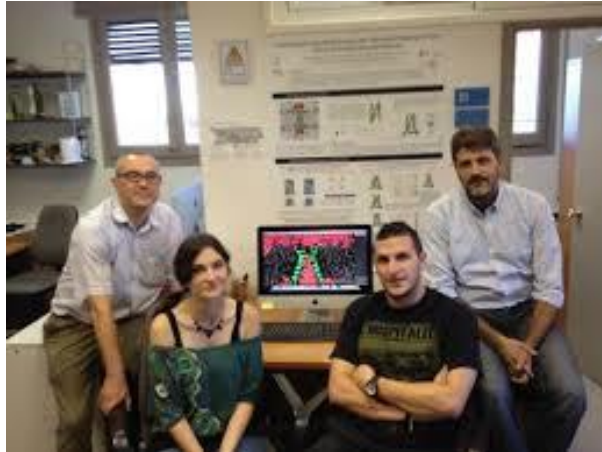
The results suggests a binding mechanism that involves two binding sites in the interior of the channel.

Only drugs able to tilt in the interior of the channel and fill the **inner cavity** (*down* binding mode) appear to be good candidates as inhibitors of wild type channel, showing pseudo-irreversible binding.

For V27A, though structural changes are apparently minor, they lead to a significant change in the free energy surface.

Drugs with larger cages appear to be required to fill the larger cavity in the **outer part** of the pore, but they are reversible.

Hybrid compound: Size-expansion/reduction in outer/inner sites?



Santiago Vázquez
Salomé Llabrés Jordi Juárez



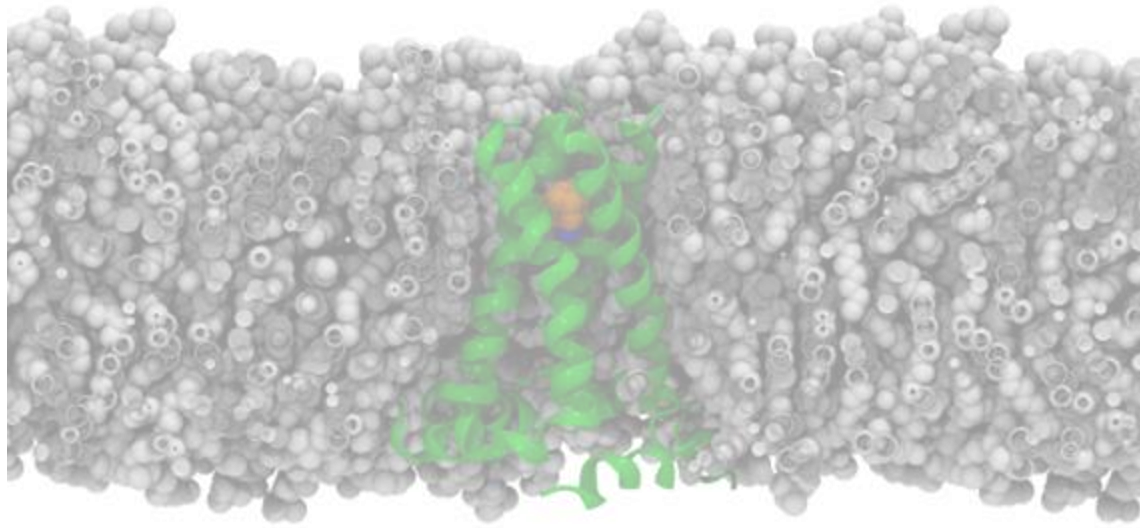
Matteo Masetti



Andrea Cavalli



Thank you!



9th RES & 4th Annual HPC Advisory Council
Users' Conference Spain Conference