



# Mechanism of Proton Permeation through Influenza A M2 Channel

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## Abstract

The influenza A virus M2 channel (AM2) is crucial in the viral life cycle. In spite of many previous experimental and computational studies, the mechanism of the activating process in which proton permeation acidifies the virion to release the viral RNA and core proteins, is not well understood. Herein the AM2 proton permeation process has been systematically characterized using multiscale computer simulations, including quantum, classical, and reactive molecular dynamics methods. We report the first complete free energy profiles for proton transport through the entire AM2 transmembrane domain (AM2/TM) at various pH values, including explicit treatment of excess proton charge delocalization and shuttling through the His37 tetrad. The free energy profiles reveal that the excess proton must overcome a large free energy barrier to diffuse to the His37 tetrad, where it is stabilized in a deep minimum reflecting the delocalization of the excess charge among the histidines and the cost of shuttling the proton past them. At lower pH values the His37 tetrad has a larger total charge that increases the channel width, hydration, and solvent dynamics, in agreement with recent 2D-IR spectroscopic studies. The proton transport barrier becomes smaller, despite the increased charge repulsion, due to backbone expansion and the more dynamic pore water molecules. The calculated conductances are in quantitative agreement with recent experimental measurements. In addition, the free energy profiles and conductances for proton transport in several mutants provide insights for explaining our findings and those of previous experimental mutagenesis studies.

## Objectives

To characterize the multifaceted mechanism of proton permeation through AM2 channel by multiscale computer simulations.

## Results

### pH-Dependent Conformational Ensemble and Water Dynamics

- Lowering the pH value (i.e., increasing the number of positively charged His37 residues from +1, +2 states to the +3, +4 states) resulted in a more open packing of the  $\alpha$ -helices at C-terminus, allowing more water molecules to enter the channel (Figure 1).
- The low-pH simulations (+3, +4 states) features faster water reorientation than high-pH simulations (+1, +2 states) (Table 1).

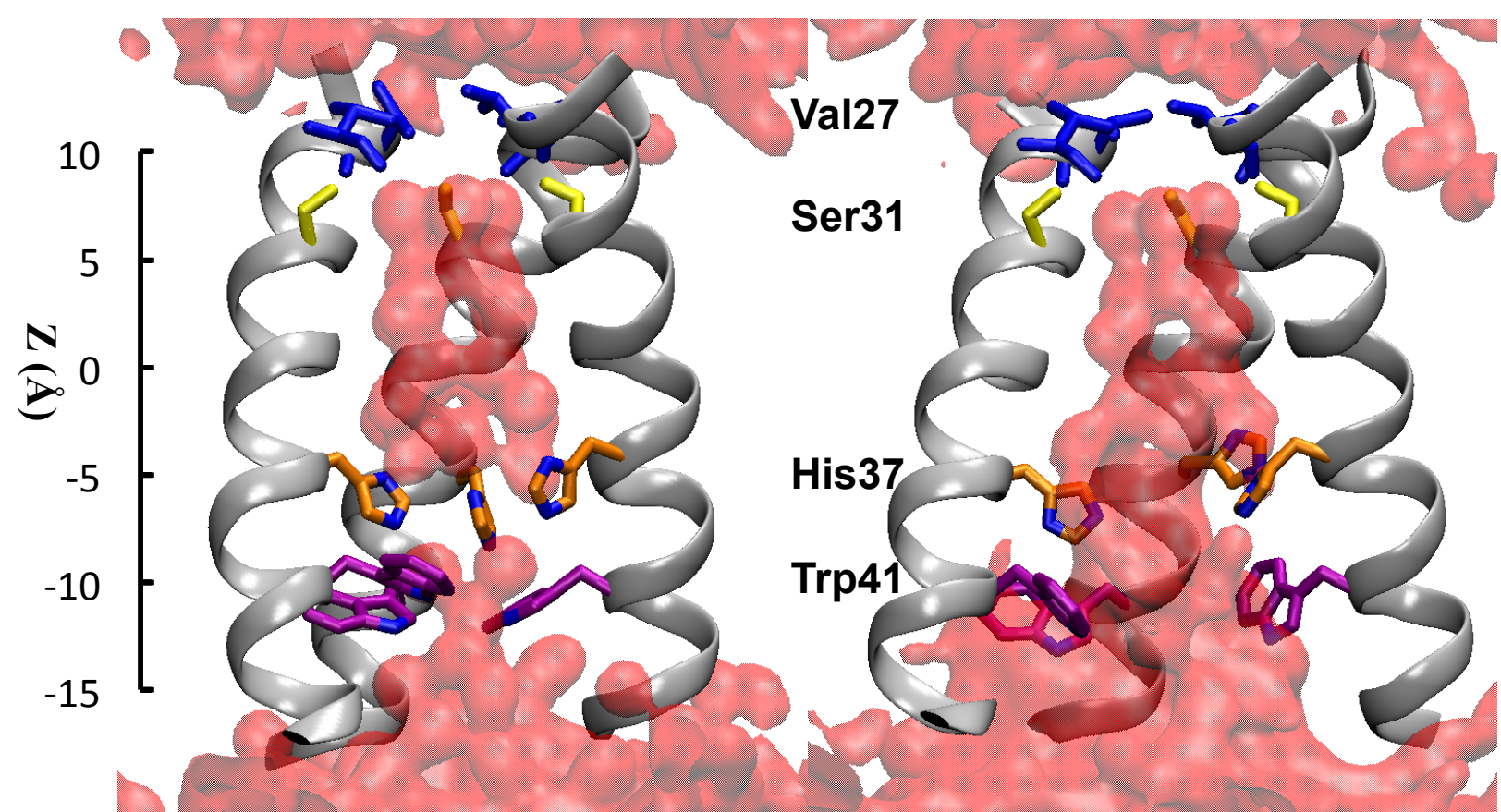


Fig. 1 Equilibrated structures of AM2/TM with His37 tetrad at +2 (left) and +3 (right) protonation states.

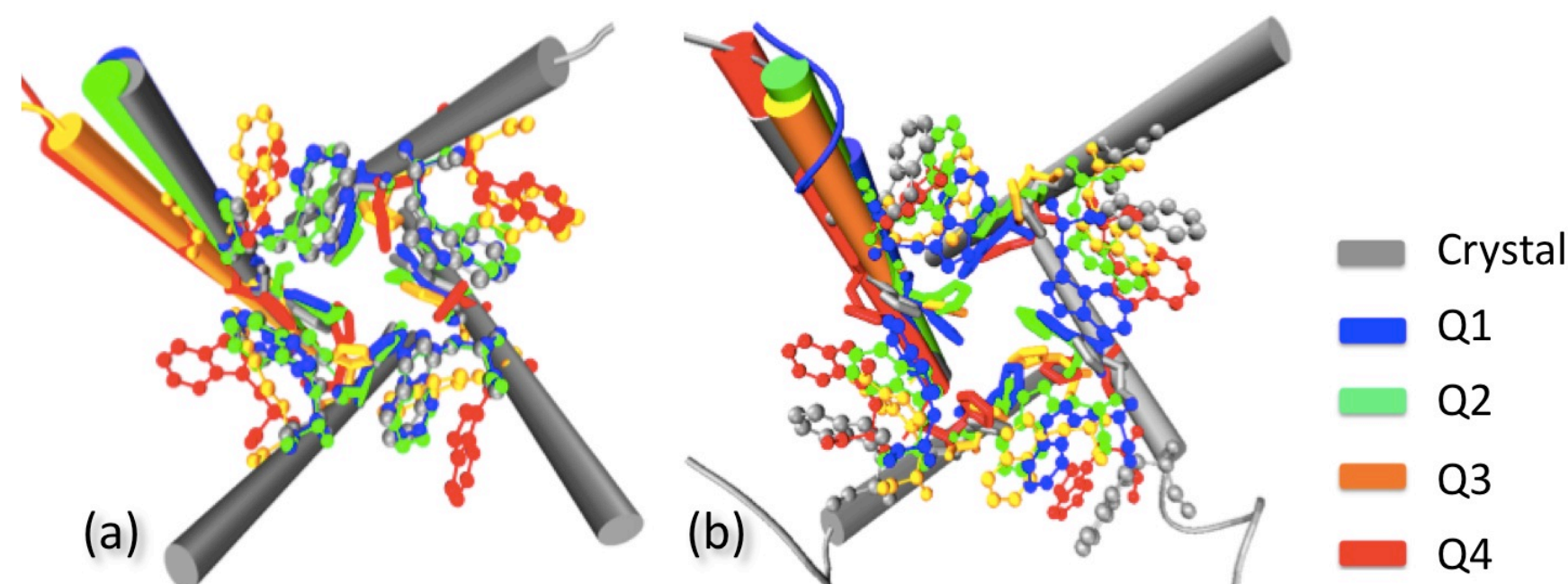


Fig. 2. Equilibrated AM2 channel transmembrane domain structures at various protonation states and aligned with either starting crystal structure 3LBW (a) or 3C9J (b). The notation Q1, Q2, Q3, and Q4 is used to denote the His37 tetrad in its +1, +2, +3 and +4 protonation states, respectively.

Table 1. Calculated dominant H-bond lifetime (ps), between pore-lining residue Gly34 amide-I groups and their nearest water molecules. Our simulations quantitatively capture the experimentally reported acceleration of channel water HB dynamics upon lowering the pH value (1).

Helix	+2	+3	+4
A	9.4	2.7	1.4
B	6.0	2.9	1.0
C	9.1	2.7	1.4
D	6.1	2.8	2.2
Experimental (1)	~10	~1.3	

### Proton Permeation in WT AM2

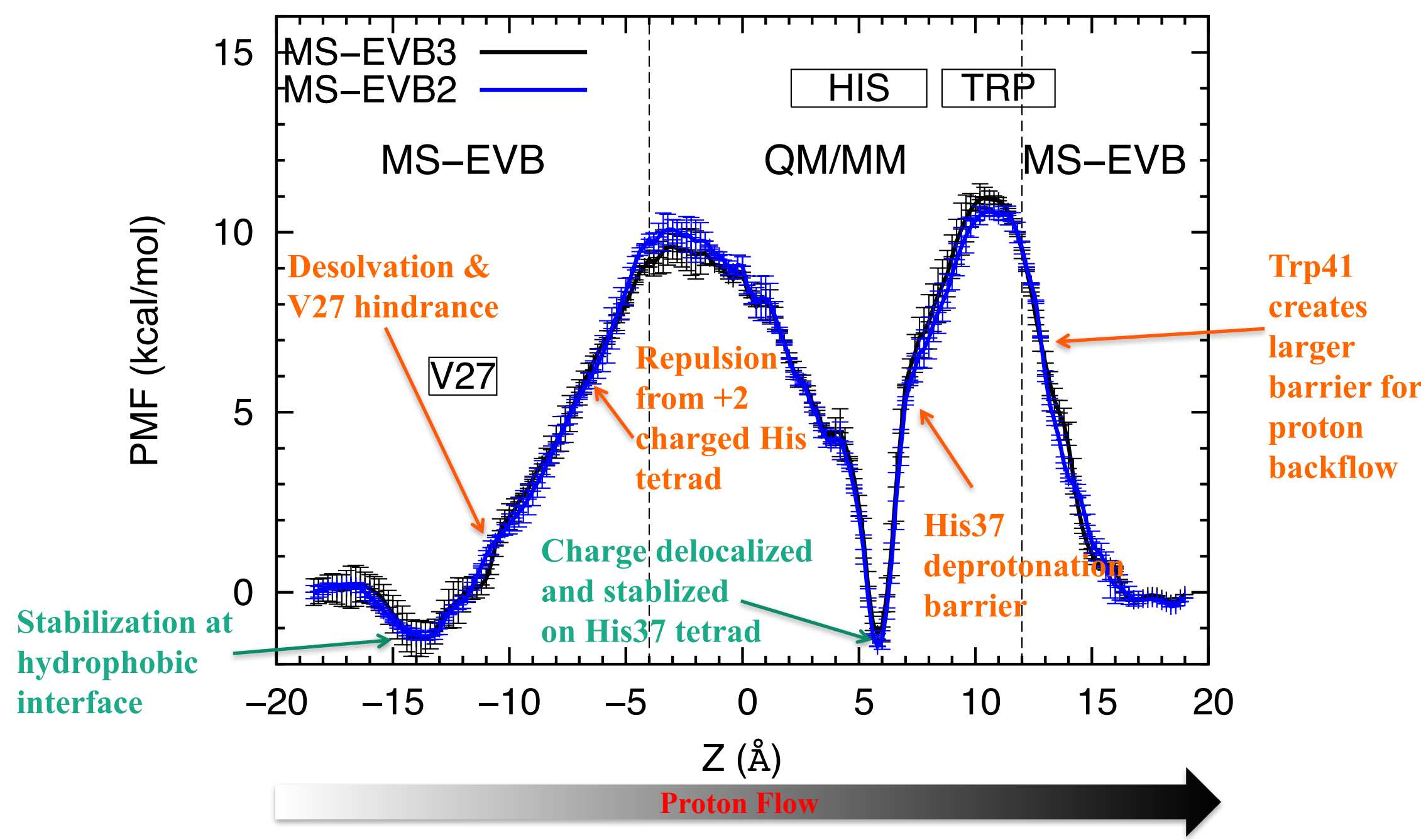


Fig. 3. Proton transport +2 free energy profiles (PMFs)

Table 2. Conductance comparison between simulation and experiments calculated from the +2 process. The quantitative agreement with experimental result (2) is encouraging.

Conductance (fs)	
Experimental (2)	1-4 fs
Simulation	1.2 fs

At lower pH (the +3 PMF compared to +2 PMF in Fig. 4), the barrier for proton diffusion to His37 is lower, resulting in higher conductance (14 fs), in reasonable agreement with the experimental result (3) and explains the unique acid-activation feature of this channel.

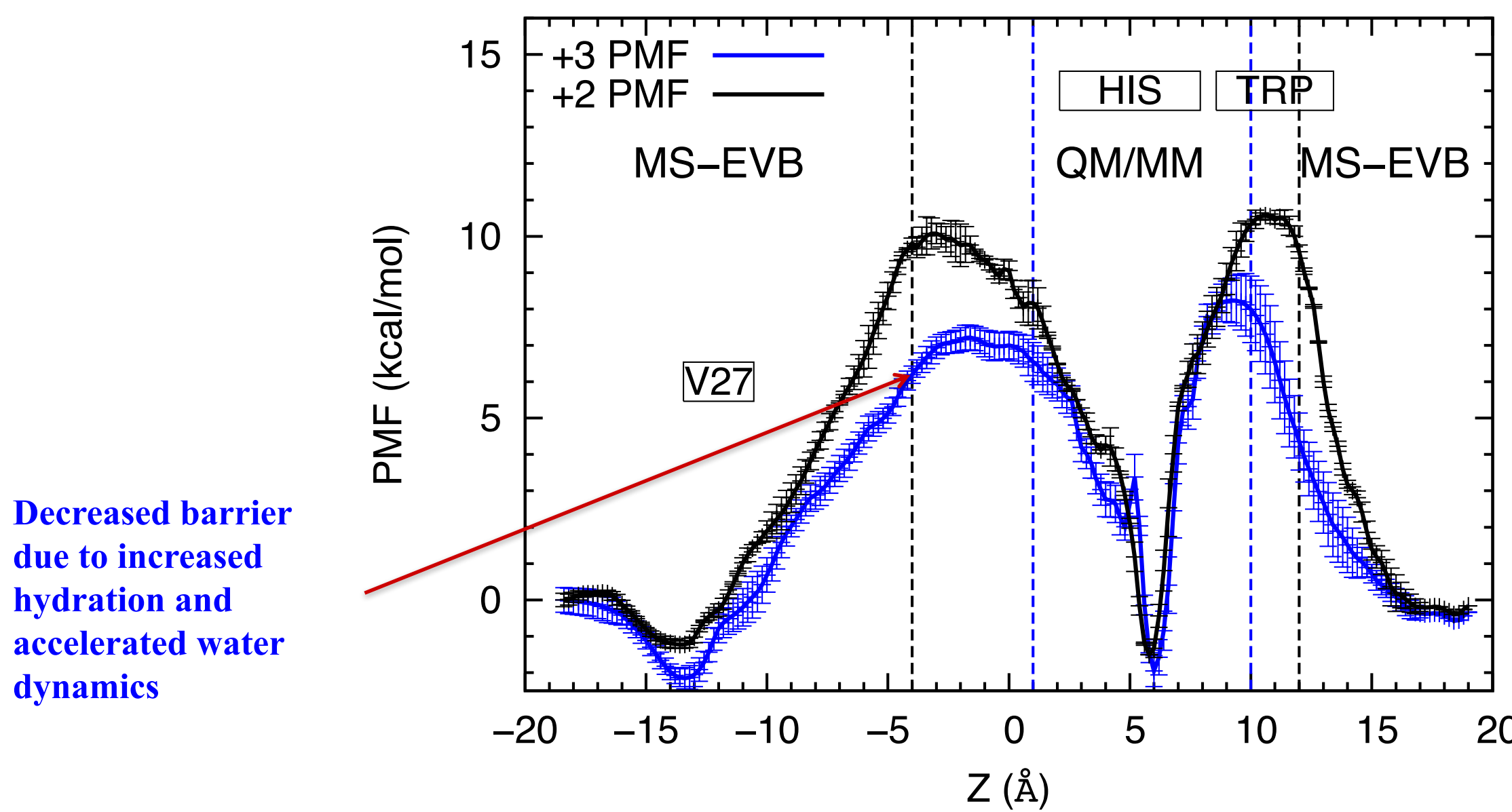


Fig. 4. Proton transport +3 free energy profiles (PMFs) (blue) compared with +2 (black).

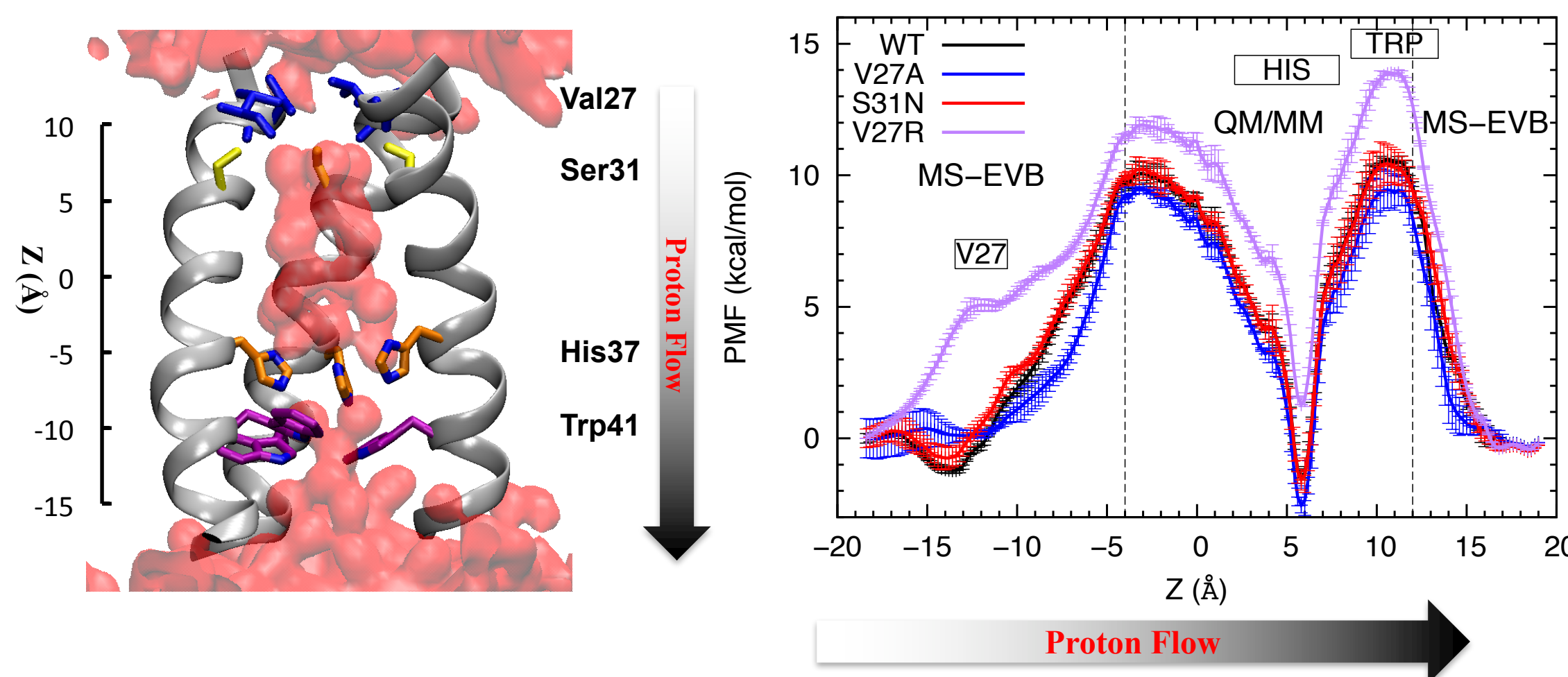


Fig. 5. Proton transport free energy profiles (PMFs) for mutants compared with the WT AM2 channel.

### Proton Permeation in AM2 mutants

The free energy profiles for the AM2 mutants are plotted in Fig. 5:

- S31N: The overall free energy profile and conductance are similar to WT
- V27A: The proton entrance barrier height is reduced, resulting in increased conductance.
- V27R: The Arg27 electrostatically repels the proton at channel entrance, lowering the conductance.

It is encouraging that our simulation results reproduce the experimental conductance trends (Table 3) (4) (in nearly quantitative agreement).

Table 3. Relative experimental (4) and calculated (present work) conductances for AM2 mutants normalized to the conductance of wild type (WT)

	Experiment	Simulation
WT	1.0	1.0
S31N	1.3	1.4
V27A	1.5	2.2
V27R	0.2	0.3

## Conclusions

- Classical MD simulations revealed that as the pH is lowered, the channel is expanded to accommodate more water molecules with increased mobility, in agreement with experimental 2D-IR results.
- Free energy profiles (PMFs) provide explicit confirmation of current proposals for the AM2 conduction mechanism, such as the importance of V27 for proton channel entry, the role of the His37 tetrad in excess proton charge delocalization (i.e., the 'shuttle' mechanism), and the barrier for His37 deprotonation.
- The complete free energy profiles also provide new insights, such as presence of a large barrier (~ 10 kcal/mol) for an excess proton to shuttle to the His37. Interestingly, this barrier decreases at lower pH value despite the increased charge of the His37 tetrad. The decreased barrier is induced by channel broadening, increased solvation, and more mobile hydrogen bond dynamics.
- The mutant studies, which are in good agreement with experimental conductance results, not only help to validate our computational methodology for studying the AM2 channel, but point to various physical explanations for the altered mutant conductance rates.

## Methodology

### Modeling of the pH-dependent conformational ensemble and water dynamics

Two high-resolution structures for the G34A mutant (3BLW and 3C9J), were used as the initial structures for MD simulations. To provide a clear picture of the channel conformation change upon acidification, the His37 tetrad was modeled at each possible protonation state.

### Modeling of proton permeation

To characterize free energy profiles of PT in the AM2/TM, umbrella sampling was used. The simulations of the windows centered outside the His37 tetrad were performed with the multi-state empirical valence bond version 3 (MS-EVB3) model. For the simulations of windows centered near and within the His37 tetrad, QM/MM MD approach was used to describe the excess charge delocalized around the ionizable His37 tetrad and nearby water molecules.

## References

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