



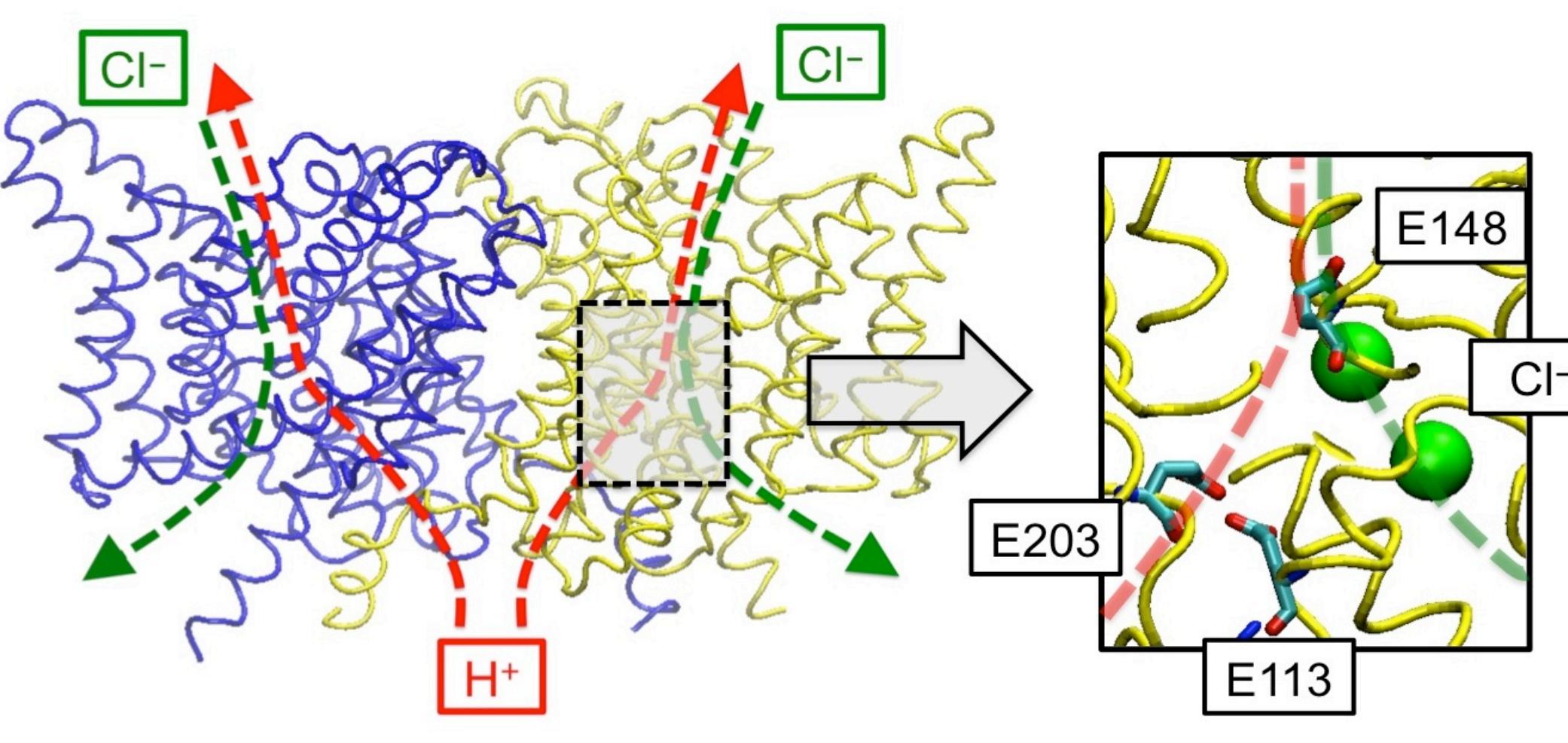
# Coupled charge transport in the Cl<sup>-</sup>/H<sup>+</sup> antiporter

Sangyun Lee, Andrew D. White, Jessica M. J. Swanson, and Gregory A. Voth\*

Department of Chemistry, James Franck Institute, Institute for Biophysical Dynamics, and Computation Institute, University of Chicago

## Introduction

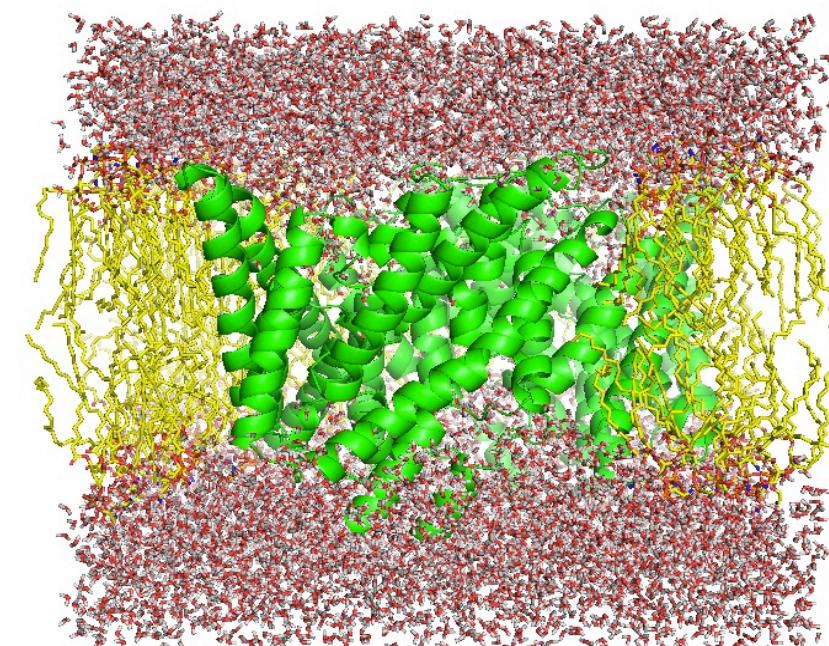
The chloride channels (CIC) are a family of proteins that transport Cl<sup>-</sup> across membranes either as selective ion channels or secondary active Cl<sup>-</sup>/H<sup>+</sup> antiporters. CIC-ec1, a prokaryotic homologue of CIC, uses Cl<sup>-</sup> gradient to pump H<sup>+</sup> thermodynamically uphill through the membrane, or vice versa. We have studied this **ion exchange process** by calculating free energy profile for migration of each ion through the protein channel. **Free energy calculation** was done with a suite of multi-scale methods, ranging from a classical MD simulation to use of semi-quantum mechanical reactive model and the hybrid QM/MM method. Here we report results on the proton transport process and coordinated movement of Cl<sup>-</sup> ions through the external and internal gate regions. A **Markov state model** was constructed to connect the intermediate states, identified in the free energy profile of Cl<sup>-</sup> transport. The model showed a good agreement with experimental results of the Cl<sup>-</sup> conduction rate and Cl<sup>-</sup>/H<sup>+</sup> exchange ratio. Our results suggest a plausible mechanism for coupled ion exchange.



## Method

### Components of System in Simulation

- A CIC-ec1 dimer (PDB code: 1OTS)
- 168 of POPE lipids
- ~11k of water molecules
- CHARMM22 force field



### H<sup>+</sup> Transport

- The potential of mean force (PMF) for H<sup>+</sup> transport from E113 to E203 and from E203 to E148 was calculated using a DFT-based QM/MM umbrella sampling simulation (BLYP(D3)/TZVP).
- The MS-RMD model for protonatable GLU residue was parameterized with adaptive force matching to reference *ab initio* data (QM/MM force). The PMF from E203 to E148 was then calculated doing umbrella sampling with the new MS-RMD model.

### Cl<sup>-</sup> Transport

- The two dimensional PMF of 2 Cl<sup>-</sup> ions with umbrella sampling and polarizable force field (Drude).

### Markov State Model

- The rates for change in protonation state were optimized, where the model gets the best fit to the experimental Cl<sup>-</sup> conduction rate and Cl<sup>-</sup>/H<sup>+</sup> exchange ratio.

## Resource

- The simulation was done in RCC midway, mostly at sandyb partition and the dedicated nodes to Voth group (gavoth).
- ~1.8 M SUs were used in the calculation of the 2D PMFs for H<sup>+</sup> transport (566 SUs/ns X 2 ns X 400 windows X 4 systems = 1.8 M), and ~0.6 M SUs for the 2D PMFs for Cl<sup>-</sup> transport (760 SUs/ns X 1 ns X 400 windows X 2 systems = 0.6 M),

## Results

### MS-RMD reactive Force-Field Modeling

- The model parameters were fit to QM/MM reference forces using FitEVB algorithm.
- MS-RMD models reproduced the free energy profile (potential of mean force, PMF) of PT through the central region as well as the local structure of the protein and the solvent.
- The MS-EVB will extend the limit of sampling time in QM/MM MD for typical biological systems from picoseconds to nanoseconds.

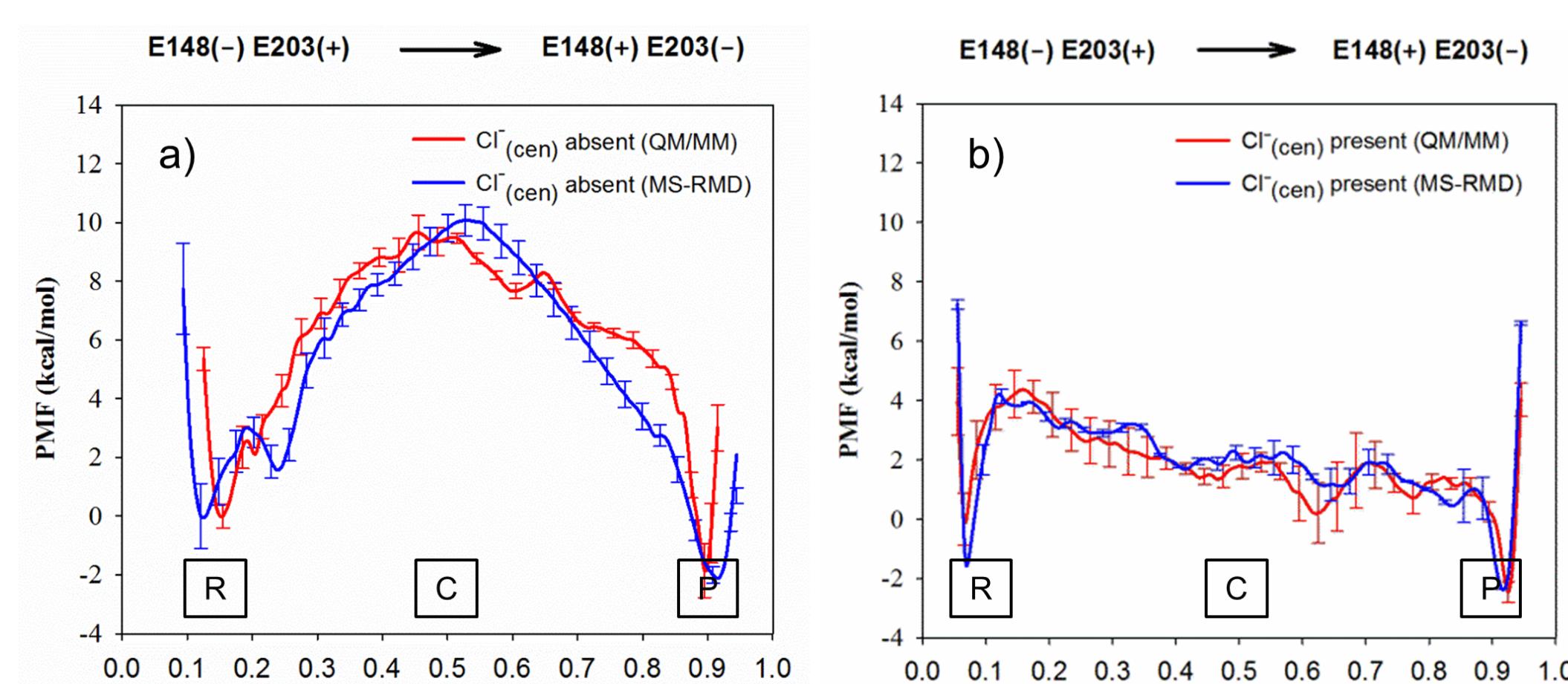


Figure 1. PMF of PT with Cl<sup>-</sup> (cen) absent (left) and present (right).

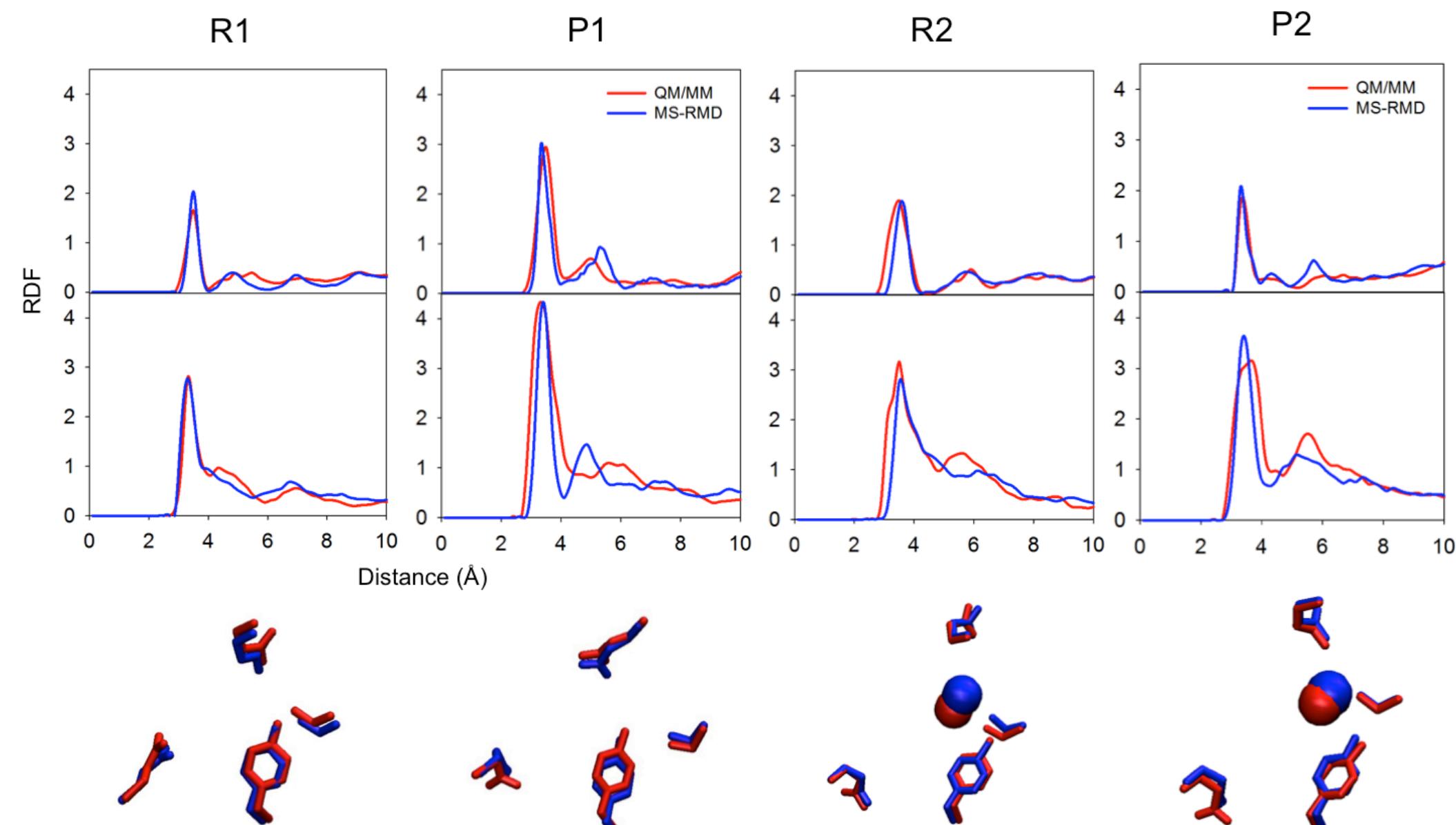


Figure 2. The RDF from carboxyl carbon of E148 (top) and E203 (middle) to water oxygen. The averaged configurations of the protein residues and Cl<sup>-</sup> (cen) in the central region (bottom). The plots and the figures are taken from R and P regions from each PMF in Figure 1. (R1 and P1 from the PMF with Cl<sup>-</sup> (cen) absent, and R2 and P2 from the PMF with Cl<sup>-</sup> (cen) present).

### H<sup>+</sup> transport

- Two acidic residues, E113 and E203, are close together.
- E113(-)E203(+) is more dominant protonation state than E113(+)E203(-) before PT from E203 to E148.

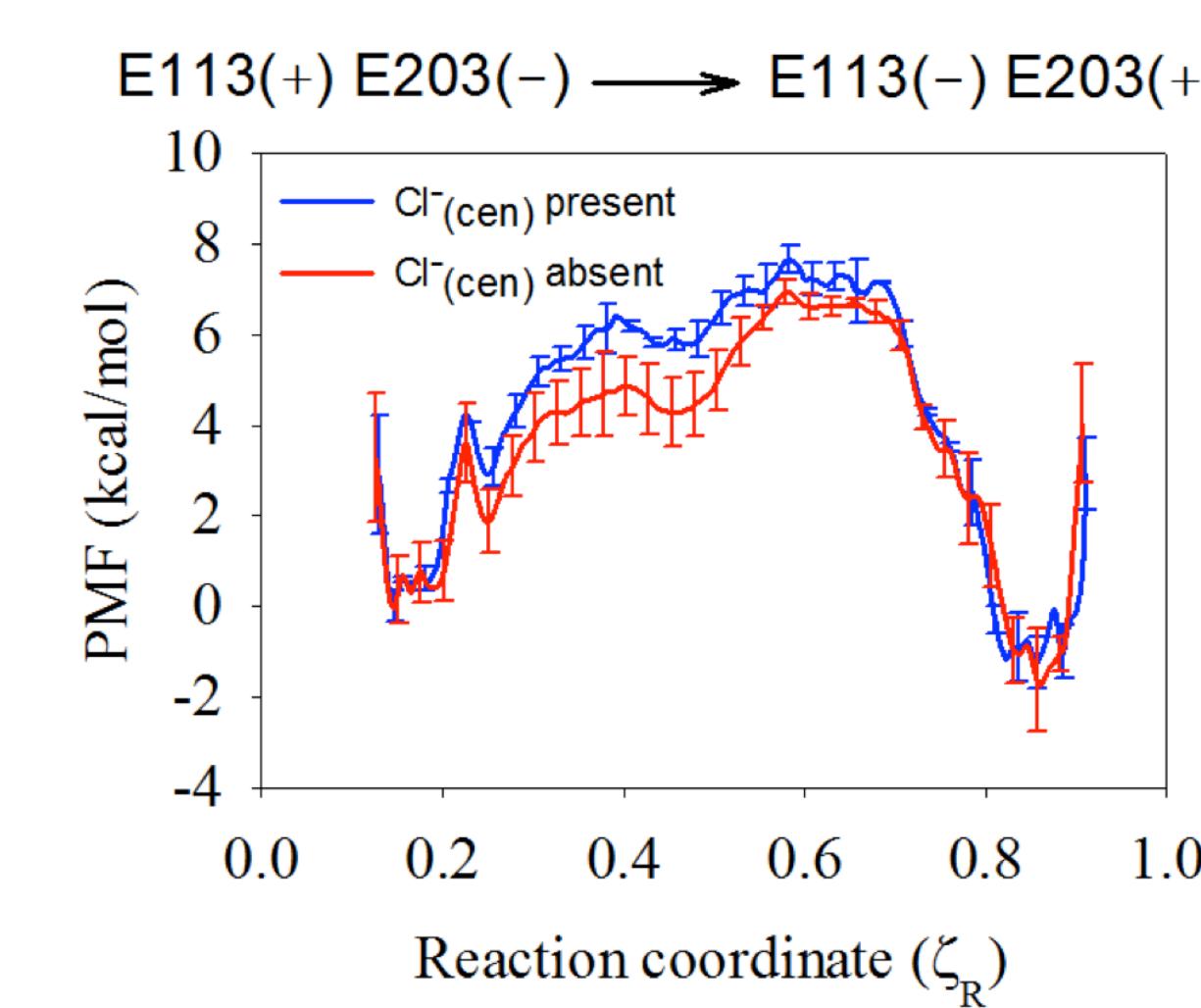


Figure 3. The PMF of PT from E113 to E203 (right), and E113(-) E203(-) to E113(-) E203(+) (left).

- The 2D PMFs as a function of two different sets of CVs show that,

- Cl<sup>-</sup> in the central site facilitates H<sup>+</sup> transport from E203 to E148.
- Hydration of the central region is coupled with the migration of the excess proton.
- The rotations of Glu residues are hindered by the presence of Cl<sup>-</sup> (cen).

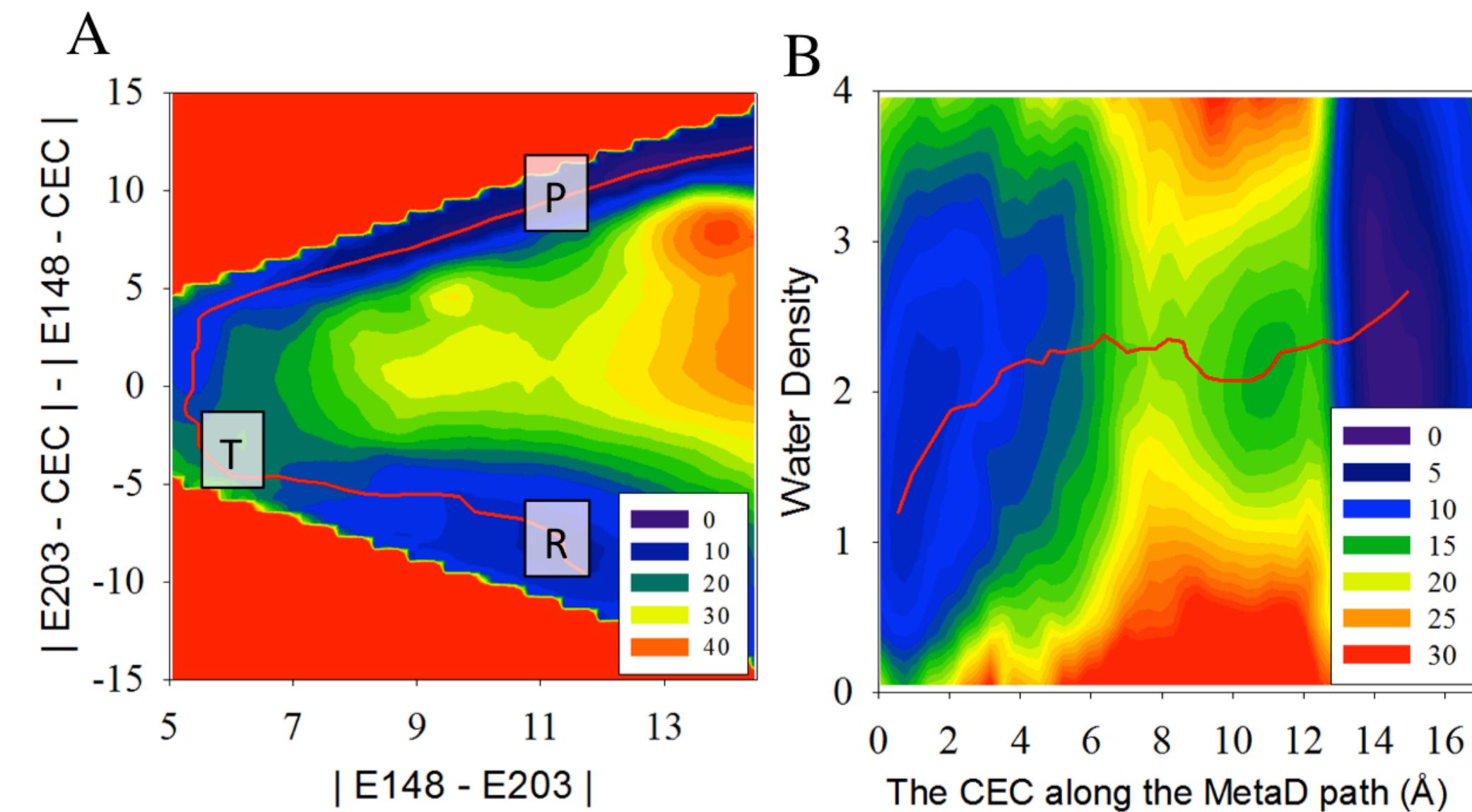


Figure 4. 2D PMF with Cl<sup>-</sup> (cen) absent. CV1 (the horizontal axis) is defined as the distance between E148 and E203, and CV2 (the vertical axis) is the difference in the two distances from the excess proton CEC to either E148 or E203. For panel B, CV1 is the distance of the CEC along the proton pathway sampled by the MetaD, and CV2 is the water density in the central region.

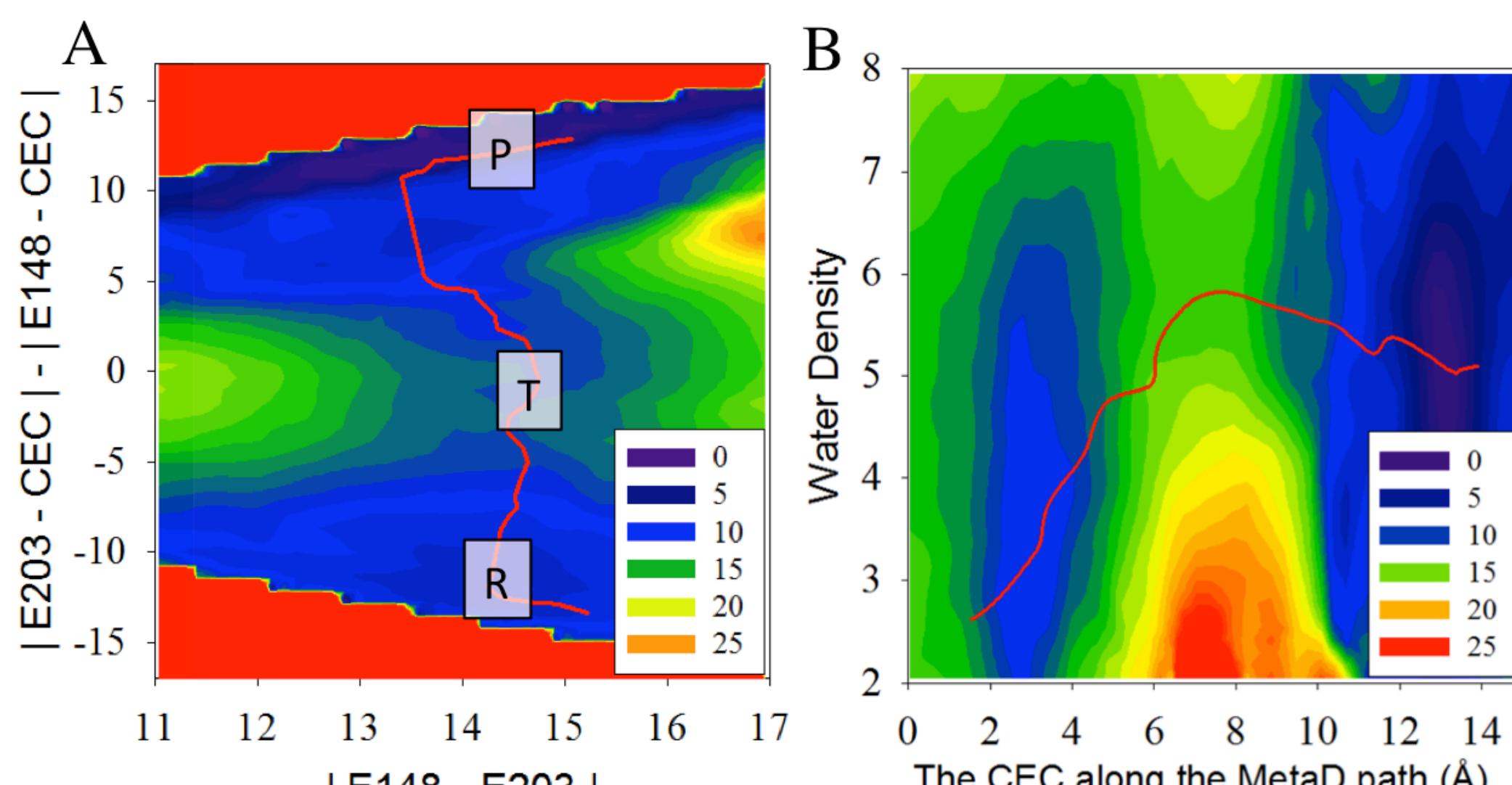


Figure 5. 2D PMF with Cl<sup>-</sup> (cen) present.

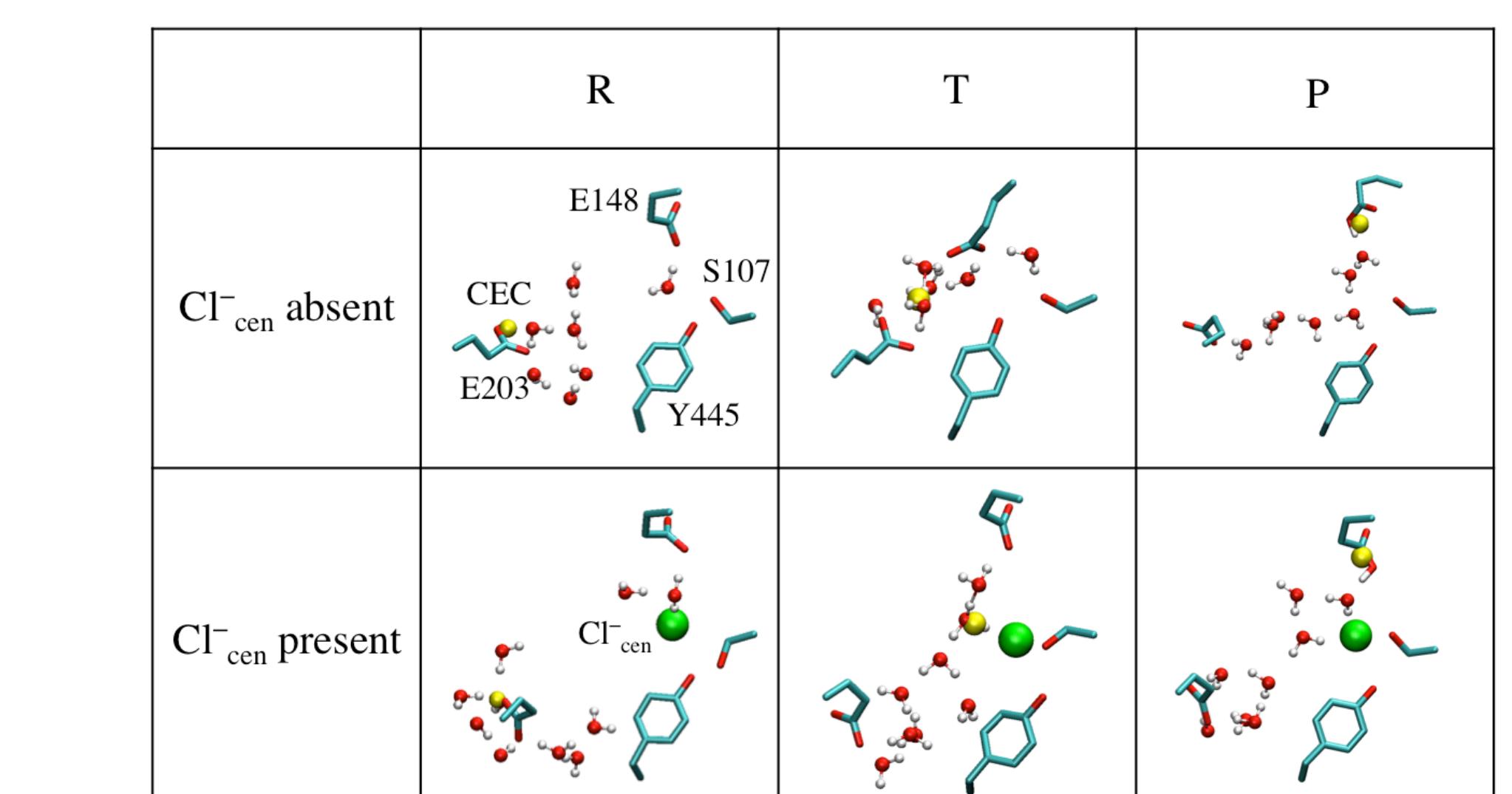


Figure 6. Representative configurations of the central region for each of the boxes R, T, and P in the 2D PMFs.

- The calculated rate constants are much greater than the experimentally measured values for the proton pumping. (~1 ms<sup>-1</sup> for pH values between 4 and 7)
- PT through the central region is not rate limiting.

Cl <sup>-</sup> (cen)	PMF figure	k (μs <sup>-1</sup> )	ΔF <sup>‡</sup> (kcal/mol)
absent	A	0.71 ± 0.25	10.2
	B	0.24 ± 0.09	10.9
present	A	(3.8 ± 1.8) × 10 <sup>2</sup>	5.1
	B	(6.4 ± 1.5) × 10 <sup>2</sup>	5.9

## Cl<sup>-</sup> transport

- 2D PMFs were calculated for the upper and the lower Cl<sup>-</sup> ions.

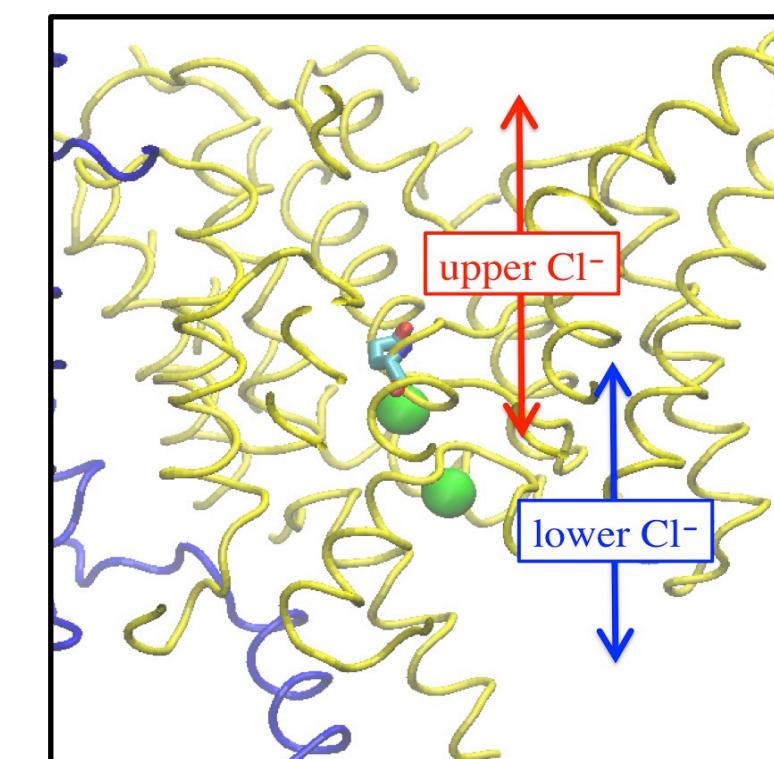


Figure 7. The CV range for each Cl<sup>-</sup> in the 2D PMFs in Figure 8.

- The protonation state of E148 acts as a switch for Cl<sup>-</sup> permeation from the external to the central binding site (transition along the horizontal axis in the 2D PMFs).

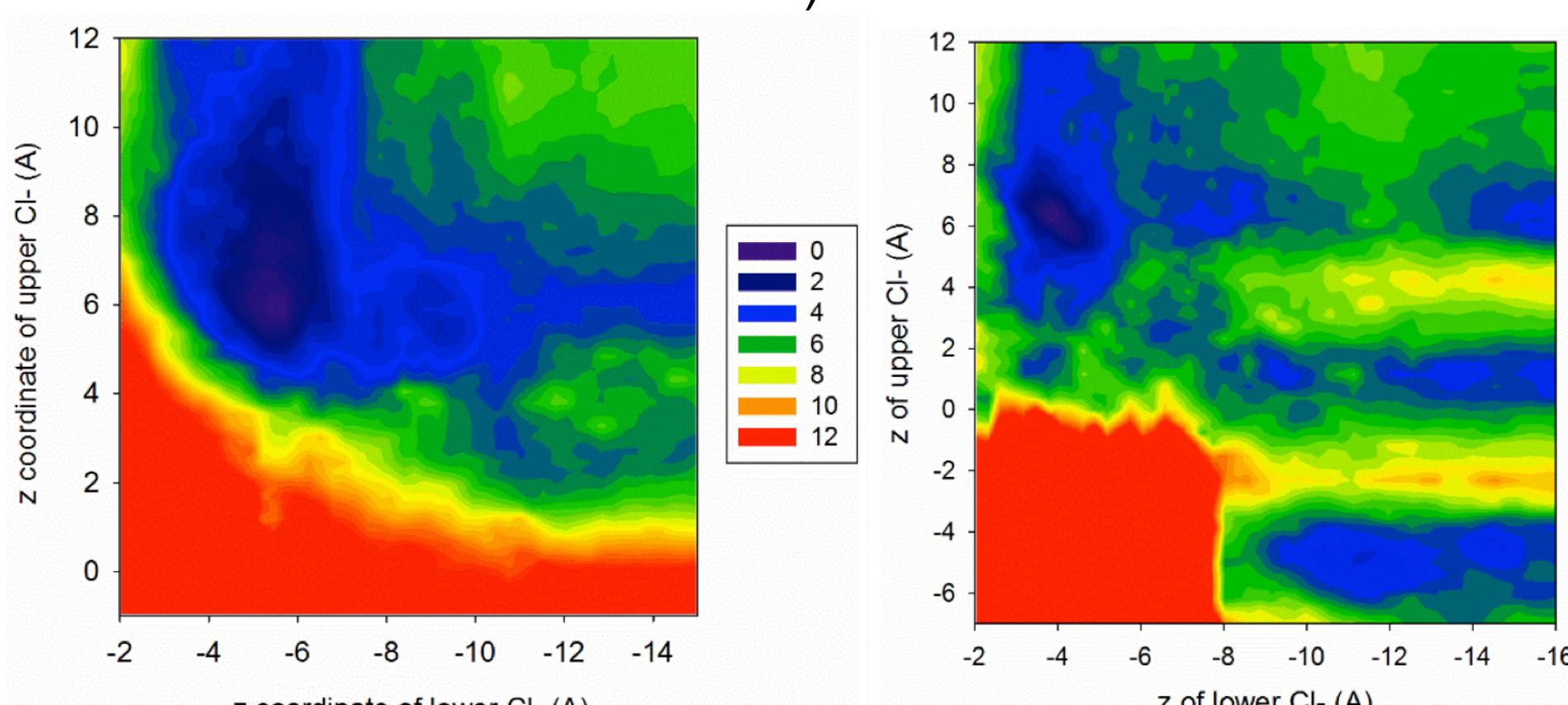


Figure 8. The 2D PMF for transport of two Cl<sup>-</sup>s with deprotonated E148 (left) and protonated E148 (right).

## Markov State Model

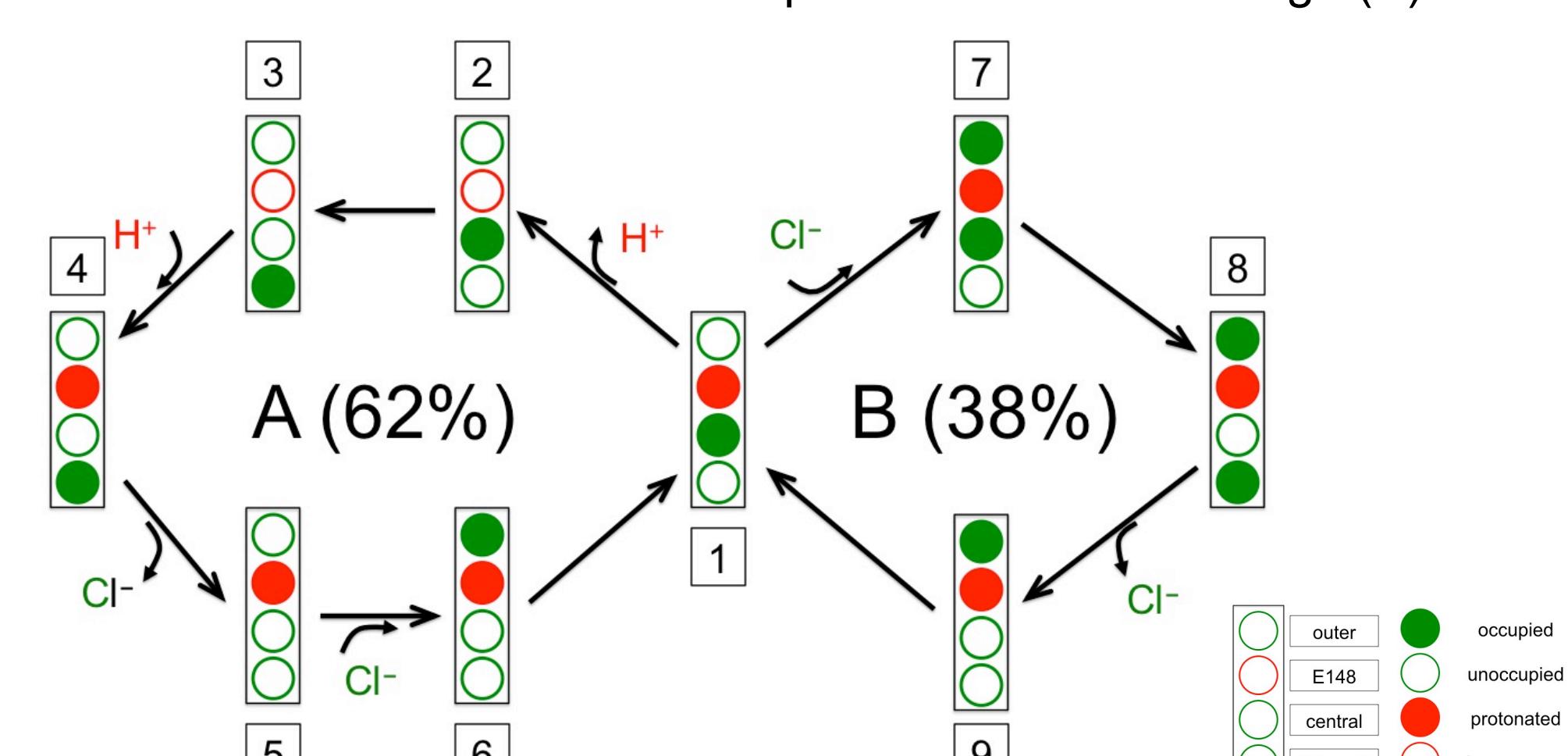
- A Markov state model is constructed with 16 discrete states represented by transition between 0 and 1 at 4 sites.

state	protonation	outer	central	internal
1	0	0	0	0
2	0	1	0	0
3	0	0	1	0
4	0	0	0	1
15	1	0	1	1
16	1	1	1	1

- The net flux at each state is defined by master equation.

$$\frac{dp_i(t)}{dt} = \sum_{j=1}^M k_{ij} p_j(t) - \sum_{i=1}^N k_{ji} p_i(t) \quad p_i(t): \text{the probability of the state } i \\ k_{ij}: \text{the rate constant of the in-flux from } j \text{ to } i \\ k_{ji}: \text{the rate constant of the out-flux from } i \text{ to } j$$

- The model predicts branched two sub-cycles as the most probable pathway in the entire cycle: one for 1:1 Cl<sup>-</sup>/H<sup>+</sup> exchange (A), and the other for additional Cl<sup>-</sup> transport without H<sup>+</sup> exchange (B).



## Acknowledgement

This investigation was supported by the University of Chicago Research Computing Center (RCC) and the National Institutes of Health (NIH grant R01 GM053148).