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Lecture Title:

Probing coordinated gating of inner and outer gates in a CLC antiporter through mutational and structural analyses

CLC family proteins regulate chloride (Cl^-) homeostasis across cellular membranes and are classified into two functional classes:

Cl^- channels and Cl^-/H^+ antiporters. While channels mediate passive Cl^- transport, antiporters stoichiometrically exchange two Cl^- for one H^+ . In both cases, ion transport is regulated by inner and outer gates, which respond to stimuli such as voltage, pH, and permeant ions. Structural and functional studies have identified a key glutamate residue (GluGate or GluEx) acting as the outer gate, which undergoes multiple conformational changes during pH- and voltage-dependent gating in both CLC channels and antiporters.

In contrast, the inner gate, formed by two hydroxyl-bearing residues, conserved serine and tyrosine at central positions (Sercen and TyrCen), has been considered rather a static kinetic barrier. However, this kinetic barrier model appears inefficient to achieve 2:1 Cl^-/H^+ coupling, compared to the classical alternating access mechanism of other coupled transporters.

To test whether the inner gate actively gates and coordinates with the outer gate, we introduced mutations at pore-lining residues and examined their effects on ion transport rates, chloride-binding affinity, and structure. In this conference, I will present the functional and structural impact of two key mutations: one stabilizes the outer gate while destabilizing the inner gate, and the other selectively stabilizes the inner gate. These findings support a model in which the CLC antiporter exchanges Cl^- and H^+ through a semi-alternating access mechanism involving coordinated gating of the inner and outer gates.