It's time to be rational: enzymes under power

Voltage-sensitivity and fluorescence of Arch3 variants

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In Short

- Arch-3 is a voltage-sensitive fluorescence reporter serving as template in optogenetics and neuroscience research.
- Arch-3 based mutants show improved voltage-sensitivity and fluorescence.
- Computational electrophysiology based on molecular dynamics simulations provides key information to understand the voltage-sensitivity.
- Rational design of novel Arch-3 variants based on a computational approach.

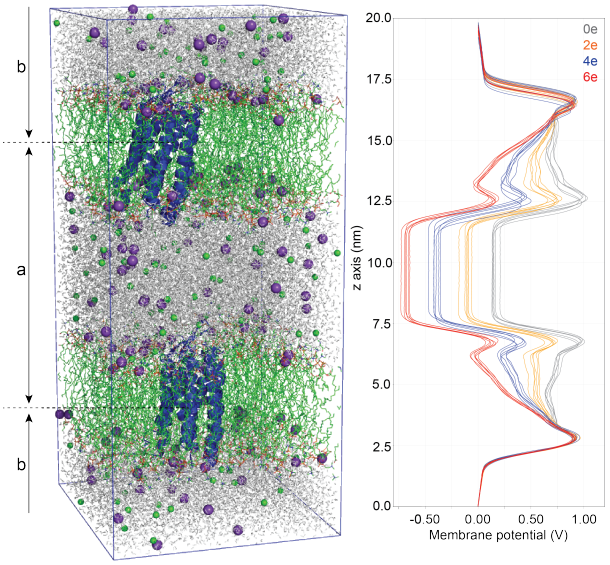

***Figure 1:** Computational Electrophysiology: Scheme of the computational electrophysiology approach (left). The simulation cell consists of two membranes (green), each including one protein (blue), surrounded by water (grey sticks), K^+ ions (purple sphers) and Cl^- ions (green sphers). By using periodic boundary conditions two compartments, a and b, with distinct ion concentrations are created. Thus, a transmembrane voltage gradient is generated across each membrane. The course of an example transmembrane potential dependent on different ion imbalances is illustrated (right).*

Enzymatic reactions at the plasma membrane play a central role in many biological processes. One way to trigger the enzymatic activity of membrane proteins is to change the transmembrane potential. This activation mechanism is, *inter alia*, used in neuronal cells during the propagation of action potentials. Unfortunately, the tempospatial detection and imaging of these signals is still a demanding and difficult process.

The proton pump Archaerhodopsin-3 (Arch-3) is a transmembrane protein and acts as fast and sensitive voltage-dependent fluorescent sensor [1], which makes it to a promising tool as a voltage indicator. For applications as an optogenetic reporter, however, the voltage-sensitivity and fluorescence intensity are not sufficient. To overcome these limitations, a number of variants with higher efficiency (e.g. QuasArs [2] and Archons [3]) have been generated by high-throughput methods without understanding the underlying molecular mechanism. This lack of knowledge on the atomic level, however, precludes the rational design of even better variants with higher fluorescent quantum yields and a higher voltage sensitivity compared to Arch-3.

Since the structural dynamics of the voltage-activation are very difficult to investigate experimentally, computational approaches often become the only method of choice. Here, we use a atomic molecular dynamics (MD) based approach called "computational electrophysiology" [4], where the transmembrane potential is generated by an explicit ion gradient across the membrane in a double membrane system (Figure 1). With this setting, we are able to systematically study the functional dynamics of the proton pump Arch-3 and its optimized variants in response to different transmembrane voltage.

Using this approach, we identified key residues for voltage-sensing in Arch-3 and in the non-pumping variant Archon1. These residues are spread over the entire proposed proton transfer pathway which also includes the retinal binding site. Surprisingly, the voltage dependent structural rearrangements were not identical in both variants. This behaviour was induced by mutations at critical sites (Figure 2), which not only changed the local environment, such as hydrogen bonding and salt bridges, but presumably also the proton transfer within the variant. Furthermore, these rearrangements within the variants altered the structural flexibility/rigidity which could drastically affect the fluorescence. Although Arch-3 and Archon1 showed different voltage-sensing mechanisms, both enzyme cores around the chromophore became more rigid upon action potentials. In summary, proton transfer and fluorescence, which are highly relevant for optogenetic application, are controlled by the applied voltage.

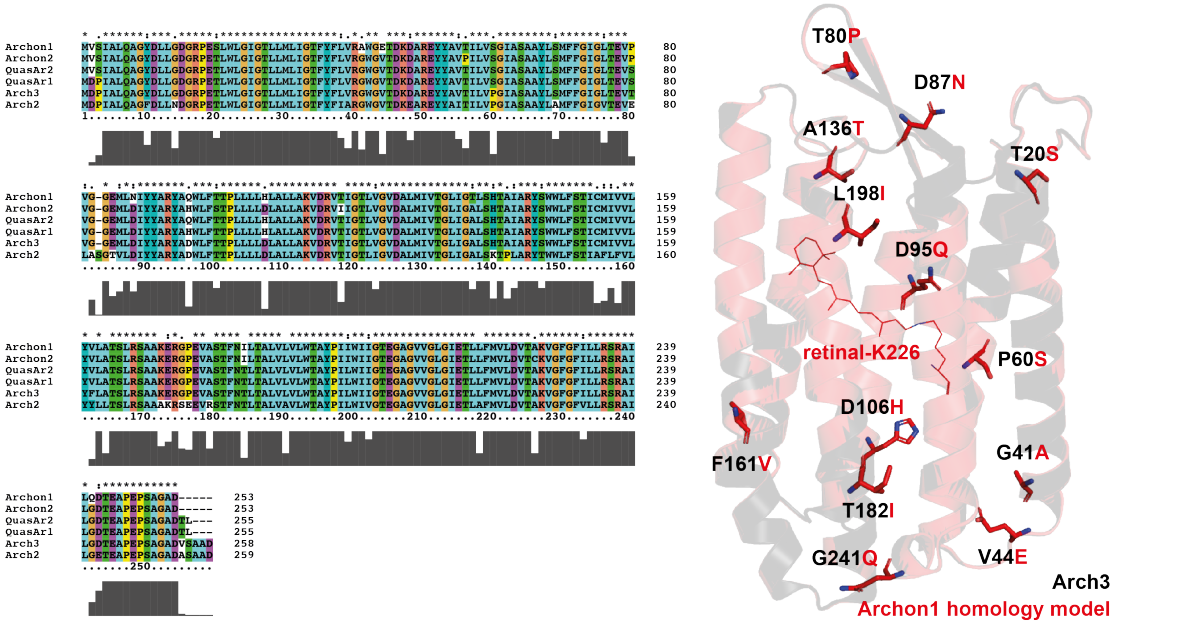

Figure 2: *Sequence alignment of Arch3 and its variants (left side). Structural comparison of Arch3 (black) and Archon1 (red) highlighting the mutated residues (right side).*

Beside these observations, molecular dynamics simulations of the relatively voltage-insensitive Arch-2 showed nearly no voltage-dependent reorientation within the protein. This behaviour was a result of overall decreased structural flexibility and, thus, ion binding properties remote from the retinal binding pocket. Here, the identification of key residues reducing voltage-sensitivity in Arch-2 is a main aim since it would contribute to the understanding of the underlying voltage-sensing mechanism. Targeted mutation of identified key residues in Arch-2 could offer another voltage-sensitive template for generating new variants.

Our findings, hypotheses and structural models are carefully compared to experimental data obtained by our cooperation partners (AG Peter Hegemann, HU Berlin). In this way the simulations and the suggested mechanisms are validated and improved. Extending the calculations to other known voltage-sensitive variants will further increase the mechanistic understanding and allow us then to rationally design novel proteins.

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http://www.leibniz-fmp.de/de/lange.html

More Information

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Project Partners

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