Transient Electrical Currents Mediated by the Na\(^+\)/K\(^+\)-ATPase: A Tour from Basic Biophysics to Human Diseases

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**ABSTRACT** The Na\(^+\)/K\(^+\)-ATPase is a chemical molecular machine responsible for the movement of Na\(^+\) and K\(^+\) ions across the cell membrane. These ions are moved against their electrochemical gradients, so the protein uses the free energy of ATP hydrolysis to transport them. In fact, the Na\(^+\)/K\(^+\)-ATPase is the single largest consumer of energy in most cells. In each pump cycle, the protein sequentially exports 3Na\(^+\) out of the cell, then imports 2K\(^+\) into the cell at an approximate rate of 200 cycles/s. In each half cycle of the transport process, there is a state in which ions are stably trapped within the permeation pathway of the protein by internal and external gates in their closed states. These gates are required to open alternately; otherwise, passive ion diffusion would be a wasteful end of the cell’s energy. Once one of these gates open, ions diffuse from their binding sites to the accessible milieu, which involves moving through part of the electrical field across the membrane. Consequently, ions generate transient electrical currents first discovered more than 30 years ago. They have been studied in a variety of preparations, including native and heterologous expression systems. Here, we review three decades’ worth of work using these transient electrical signals to understand the kinetic transitions of the movement of Na\(^+\) and K\(^+\) ions through the Na\(^+\)/K\(^+\)-ATPase and propose the significance that this work might have to the understanding of the dysfunction of human pump orthologs responsible for some newly discovered neurological pathologies.

The Na\(^+\)/K\(^+\)-ATPase is an integral membrane protein complex whose function relies on the assembly of an \(\alpha\)-subunit and a \(\beta\)-subunit. It exports three Na\(^+\) from the cell and imports two K\(^+\) into the cell. Because both ion species are moved against their electrochemical gradients, the Na\(^+\)/K\(^+\)-ATPase uses the energy derived from the hydrolysis of one molecule of ATP per transport cycle. Maintaining Na\(^+\) and K\(^+\) gradients is essential for cell survival and is part of numerous cell processes such as cell volume regulation, secondary active transport, and neuronal excitability. The Na\(^+\)/K\(^+\)-ATPase was discovered more than 60 years ago (1). Earlier biochemical work defined the transport kinetic scheme known as the Albers-Post model (2,3). Interestingly, the entire transport machinery of this model is confined within the \(\alpha\)-subunit (4,5). During the transport cycle, ions are transiently occluded or trapped within the permeation pathway. The existence of the occlusion states was proven experimentally using biochemical approaches (6–9) and confirmed structurally using crystallographic approaches for K\(^+\)-bound (4,5) and Na\(^+\)-bound states (10,11).

In principle, using electrophysiological approaches, ions could be monitored as they transit between their binding sites in the occluded state and the bulk solution. These electrical signals are expected to be transient because these ions are not moving across the cell membrane. In this perspective, we examine the transient electrical currents generated by Na\(^+\) and K\(^+\) as they move between the external solution and their binding sites, as depicted in Fig. 1 in green and orange, respectively. We describe their use to propose and define mechanistic models as well as their potential significance to understand dysfunctional Na\(^+\)/K\(^+\)-ATPases causing human disorders.

**The discovery of the Na\(^+\)-mediated transient electrical currents**

Transient displacement currents were first measured in cardiac cells by Nakao and Gadsby (12) using the whole-cell voltage-clamp technique in ventricular myocytes. They...
were recorded as strophanthidin-sensitive (pump) currents in K\(^+\)-free intracellular and extracellular solutions, as well as saturating intracellular Na\(^+\) and absence of ADP. Under these experimental conditions, the normal electrogenic 3Na\(^+\)/2K\(^+\) transport was blocked; therefore, these transient currents contained no steady-state component. Yet, they required intracellular ATP and Na\(^+\), as well as extracellular Na\(^+\). Further, they were abolished by oligomycin B and diminished by external K\(^+\). All these results strongly supported the idea that these transient currents originated from partial reactions by the Na\(^+\)/K\(^+\)-ATPase associated with the binding and release and occlusion and deocclusion of external Na\(^+\) (Fig. 1; encircled by green dashed lines). We will denote them as Na\(_o\)\(^+\)-mediated transient currents. The amount of charge moved by these transient currents showed clear signs of saturation at both negative and positive potentials (−120 to +80 mV), which could be well described by a two-state Boltzmann function with a steepness corresponding to almost one single elementary charge. With this information, the maximum amount of charge and the cell plasma membrane area experimentally estimated from the cell capacitance, these Na\(_o\)\(^+\)-mediated transient currents provided an electrophysiological approach to determine Na\(^+\)/K\(^+\)-ATPase site density, with ~1000 sites \(\mu\)m\(^{-2}\) for cardiac cells. The Na\(_o\)\(^+\)-mediated transient currents measured by Nakao and Gadsby decayed monoexponentially, with increasingly faster time constants at negative potentials but reaching an asymptotic minimum value at positive potentials.

About the same time as the Na\(_o\)\(^+\)-mediated transient currents were first recorded, Läuger and Apell (13,14) began adopting the concept of access channel (or ion well (15)) to the mechanistic interpretations of the voltage dependence of partial reactions of the Na\(^+\)/K\(^+\)-ATPase. A few years later, Gadsby et al. (16) determined that the \(^{22}\)Na efflux mediated by the electroneutral Na\(_o\)/Na\(_r\) exchange is sensitive to the transmembrane voltage of the axolemma from squid giant axons. Through an elegant kinetic treatment, it was concluded that the dependence on voltage arises entirely in the Na\(_o\)\(^+\) rebinding because the ions transit toward their binding sites through an access channel sensing ~70% of the transmembrane field in their path. With this new framework at hand, Na\(_o\)\(^+\)-mediated transient currents were used to confirm the channel-like structure at the external end of the permeation pathway of the Na\(^+\)/K\(^+\)-ATPase (17–20) or as tools to establish the origins of electrical transient currents elicited by ATP concentration jump (21,22).

**New and faster Na\(_o\)\(^+\)-mediated transient current components**

A major advance in our understanding of the events leading to Na\(_o\)\(^+\) release by the Na\(^+\)/K\(^+\)-ATPase arrived once faster voltage-clamp techniques were used. Hilgemann (17) hypothesized that if Na\(^+\) ions travel between their binding sites and the external milieu through narrow channels, a very fast charge movement, not detected before because of voltage-clamp speed limitations, would be expected. Using an integrating patch-clamp amplifier, Hilgemann showed that the charge generated by the Na\(_o\)\(^+\)-mediated transient currents contained two components: a fast event limited by the 4-\(\mu\)s temporal resolution of the voltage clamp carrying a weak voltage dependence and a slow transition with similar properties as those described earlier (12). Importantly, it was shown that the monoexponential rates of the slow component approached a maximum at negative potentials and a minimum at positive potentials, indicating that the protein dynamics involved in the occlusion and the deocclusion of the slow component are voltage insensitive. To account for these new observations, it was proposed that Na\(^+\) ions are released to the extracellular environment in two transitions: one Na\(^+\) is first released through a high-field access channel (slow component), and then two Na\(^+\) are released through a wider access channel with weak voltage dependence (fast component).

The squid axon offers the possibility of clamping the membrane potential in less than 10 \(\mu\)s while, at the same time, it has a high density of Na\(^+\)/K\(^+\) pumps. Thus, using high-speed voltage steps of the axolemma from squid giant axons, three components from the Na\(_o\)\(^+\)-mediated transient...
currents could be readily revealed (19,23): fast and slow ones, corresponding to those previously described (12,17), and a third one, the medium component, with time constants in between. This medium component was described in detail by analyzing the return of Na\textsuperscript{+}-mediated transient currents after test potentials of different durations, revealing the sequential release of the three Na\textsuperscript{+} ions. The magnitude and time courses of these three components at various extracellular Na\textsuperscript{+} concentrations are strongly correlated, consistent with a model in which each component of the Na\textsuperscript{+}-mediated transient currents corresponds to the protein dynamics of distinct Na\textsuperscript{+} as they transit through the permeation pathway from their binding sites to the bulk external solution.

**Experimental challenges and the Humboldt squid**

**Thermodynamics of the slowest component of the Na\textsubscript{o}\textsuperscript{+}-mediated transient currents**

The Na\textsuperscript{+}/K\textsuperscript{+}-ATPase is a temperature-dependent enzyme, and as such, we expect that its activity be dramatically decreased for those animals living in the gelid waters of, for example, the Antarctic sea. Given the importance of this membrane protein in sustaining life, we expect to find robust adaptive changes of the ATPase activity in response to cold. Using the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase \(\alpha\)-subunits from an Antarctic and a temperate octopus as cases for study, Galarza-Muñoz et al. (24) expressed the enzymes in *Xenopus laevis* oocytes and determined the turnover rates and temperature sensitivities of these two pumps. The main finding of this study was that the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase adapts to cold environments by reducing thermal sensitivity. These experiments underscore the importance of temperature in defining the degree of functioning of this important membrane protein. However, given the complexity of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase transport cycle (Fig. 1), only a more formal thermodynamic analysis can allow us to determine to what degree the different transitions are affected by temperature. The thermodynamics of partial Na\textsuperscript{+} reactions can be studied by determining the temperature sensitivity of the different components of the transient pump currents described in the previous section of this review. However, these studies present us with challenges difficult to overcome without adequate biological preparations. Just consider that because the charge transported is not temperature dependent, lowering the temperature slows the kinetics of the transient currents originating from the binding and release of the external Na\textsuperscript{+}, making the currents too small to be detected accurately over the baseline noise. With its 1- to 2-mm-diameter axons, the squid *Dosidicus gigas* of the Humboldt current provided us with an axolemmal membrane area large enough to record the necessary number of pumps to make possible the characterization of the thermodynamics of the slowest current transition (25). This transition is associated with the extracellular release of the first Na\textsuperscript{+} from the deeply occluded state. First, the pump turnover rate as a function of temperature in this preparation was characterized by constraining to forward cycling of the pump. Under this condition and as previously reported (21,24,26), the turnover rate is 106 s\textsuperscript{-1} at 25°C. The temperature dependence of the turnover rate is well described using a transition enthalpy (\(\Delta H^*\)) of \(\sim 24\) kcal/mol corresponding to a \(Q_{10} = 4\) associated with a transition entropy (\(\Delta S^*\)) of 62 cal/molK. Thus, the free energy barrier (\(\Delta G^*\)) determining the rate-limiting step of forward pumping is 5.5 kcal/mol at 25°C. Second, to characterize the energy landscape associated with the extracellular release of the first Na\textsuperscript{+} from the occluded state, a three-state model considering that the Na\textsuperscript{+} binding reaction is in equilibrium with the occlusion-deocclusion (O-D) transition was used. This is a reasonable assumption because to reach the binding site, Na\textsuperscript{+} is traveling across a high-field access channel. It is noteworthy that the net \(\Delta H = 19.6\) kcal/mol equivalent to a \(Q_{10} = 3.3\) of the O-D transition is smaller than the enthalpy change obtained for the rate-limiting step of the turnover rate, indicating that the release of the first Na\textsuperscript{+} is not the transition that limits the pump cycle. In conclusion, although the net \(\Delta G\) of the slow reaction is quite small, the enthalpy and entropy changes defining the O-D and binding and unbinding reaction are large, indicating that these transitions involve relatively large protein-conformational changes. A small \(\Delta G\) should secure fast and reversible transport rates.

**K\textsubscript{o}\textsuperscript{+}-mediated transient currents**

As is the case for Na\textsuperscript{+} binding, electrogenicity of extracellular K\textsuperscript{+} binding was established for some time (27,28), but the electrical transients associated with its movement in the access channel were difficult to measure. These transient currents were expected to be small and very fast. The first successful attempts at measuring K\textsuperscript{+} transients were made by Peluffo and Berlin (29) using Tl\textsuperscript{+} to replace K\textsuperscript{+}, which slows down the kinetics, thus making the measurements possible. Using the squid axon from *Dorithetus pealei*, attempts at measuring the K\textsuperscript{+} transients were frustrating because the signal/noise ratio was not high enough to get consistent recordings. The situation changed when we started using the Humboldt squid (D. gigas) because their larger diameter axons increased the available area while at the same time allowing a faster voltage clamp because the internal electrode resistance could be decreased (30). In this preparation, the K\textsuperscript{+} transient currents were reliably detected, and their properties were quite different from the Na\textsuperscript{+} transient ones. The experiments revealed a pump-mediated K\textsuperscript{+}-dependent pre-steady-state transient current that is the result of binding and unbinding and occlusion and deocclusion of K\textsuperscript{+} ions from the extracellular side of the pump. The currents were much faster than the transient Na\textsuperscript{+} currents, and contrary to the case of the Na\textsuperscript{+} currents, they
showed only one kinetic component. The data were consistent with a model in which the two K\(^+\) ions bind sequentially, but they are occluded simultaneously (one kinetic component). The occlusion rate was 13,000 s\(^{-1}\), whereas the deocclusion rate was 2000 s\(^{-1}\). By fitting the results to a simple model of two sequential binding and simultaneous occlusion, it was found that the binding of K\(^+\) showed K\(_d\)-values of 2.6 and 43 mM, which are orders of magnitude higher than the corresponding K\(_d\)-values of Na\(^+\) binding. This is to be expected because the forward pump direction is to take K\(^+\) ions from the outside with much higher affinity to compensate for its much lower concentration than Na\(^+\) in the extracellular medium. The model fit also showed that the fractions of the field traversed by the two K\(^+\) ions were 46 and 27%, smaller than the case of the Na\(^+\) ions and consistent with the fact that the K\(^+\) uptake is less voltage dependent than the Na\(^+\) release (31). Molecular dynamics simulations using the crystal structures of the Na\(^+\)/K\(^+\)-ATPase and homology model of the SERCA pump predicted binding sites that were at 49 and 27% of the transmembrane electric field, in good agreement with the electrophysiological data (30).

Structure and function of the Na\(_o^+\)-mediated transient currents

Na\(_o^+\)-mediated transient currents have been an important experimental tool to infer how function relates to protein structure. Some of these studies were performed even before crystal structures of the Na\(^+\)/K\(^+\)-ATPase were available, whereas others were guided by targeted mutagenesis or, more recently, by human disease-causing mutations. The latter will be reviewed separately in the next section. So far, K\(_o^+\)-mediated transient currents have not been used for structure and function studies, except for one molecular dynamics study that correlated the position of the binding sites determined by the transient currents with the pump’s crystal structures (30).

Most structure and function studies in which Na\(_o^+\)-mediated transient currents have been used as readouts consist of perturbing the Na\(^+\)/K\(^+\)-ATPase structure by mutagenesis and assessing the changes in the kinetics of the transient current to then infer a structure and function relationship. A few years before structures of the Na\(^+\)/K\(^+\)-ATPase were published (4,5), a comprehensive electrophysiological study was performed with mutations of four amino acids that were expected to be part of the cation binding pocket (32). These amino acids (N776, E779, D804, and D808) are in transmembrane segments TM5 and TM6. Based on biochemical (33,34) and electrophysiological (35) data, as well as homology modeling with crystal structures of SERCA (36), these amino acids were predicted to participate in ion binding. There were substantial qualitative and quantitative differences on the kinetics of the slow component from the Na\(_o^+\)-mediated transient currents (32), establishing and confirming the important role of these four amino acids in the transport mechanism.

In response to a voltage step, the Na\(_o^+\)-mediated transient currents represent the redistribution between the states (Na\(_3\)) E\(_1\)-P and P-E\(_2\) (Fig. 1). Therefore, changes in the voltage dependence of the charge carried by these currents can be interpreted as changes in apparent affinity for extracellular Na\(^+\). For example, C-terminal deletions substantially shifted the charge distribution of the slow component to more negative voltages, indicating a reduction of the apparent affinity for external Na\(^+\) (37). Similarly, Holm et al. (38) and Nielsen et al. (39) showed that changes in apparent affinity for external Na\(^+\) estimated by biochemical data are comparable with those obtained by electrophysiological methods. Further, the combination of both approaches provides strength to the specific structural inferences in these studies. Interestingly, shifts in the charge distribution by the Na\(_o^+\)-mediated transient currents had also been observed in the presence of extracellular inhibitory organic amines, providing insights into the occupancy of ion binding sites of the pump by these molecules (40).

Na\(_o^+\)-mediated transient currents have also been used as tool to understand biological questions. For example, how could an Na\(^+\)/K\(^+\)-ATPase function in marine osmoconformers, like cephalopods, whose cells are exposed to \(\sim\)0.5 M extracellular Na\(^+\)? If these marine Na\(^+\)/K\(^+\)-ATPases were to be like vertebrates’, transport would be drastically impaired. Colina et al. (41) showed that the midpoint of the voltage dependence of the charge moved by the slow component of the Na\(_o^+\)-mediated transient currents shifted toward positive potentials as the external mouth of the squid Na\(^+\)/K\(^+\)-ATPase was mutated to look like a vertebrate one. A change in the electrostatics of the external mouth provided the structural basis for adaptation to high salinity. In another study with biological context (42), it was shown that the conversion I877V at the external end of TM7 by RNA editing causes a redistribution of the amount of charge moved by the slow and fast components of the Na\(_o^+\)-mediated transient currents. This redistribution shifted the entire equilibrium toward the release of the last Na\(^+\) (fast component), thereby increasing the Na\(^+\)/K\(^+\)-ATPase’s velocity over the physiological range.

Site-directed fluorescence under voltage clamp (voltage-clamp fluorometry) is a technique that allows the measurement of conformational changes while the protein is functional (43,44). The group of Friedrich and Bamberg was the first to apply this technique to the Na\(^+\)/K\(^+\) pump (45,46). By inserting cysteines in the M5-M6 loop of the \(\alpha\)-subunit to conjugate tetramethylrhodamine-maleimide (TMRM), this group was able to measure fluorescence changes during pump operation that were abolished by ouabain. Fluorescence changes of \(\sim\)5% were observed when the external K\(^+\) was increased. Interestingly, voltage changes under conditions of the Na\(^+\)/Na\(^+\) exchange gave fluorescence changes that correlated with the transient...
currents under these conditions, indicating that during the binding and occlusion of the $\text{Na}^+$ ions, the protein changes conformation. The mechanism of the fluorescence change is not known in detail, but the most likely explanation is that the fluorophore contacts different residues that quench the fluorescence at different levels as the protein reacts to the voltage change. Therefore, these fluorescence changes indicate that during binding and occlusion, a conformational change occurred that moved the fluorophore between positions of different quenching efficacies. This group has also used this approach to show the close functional relation between the $\alpha$- and $\beta$-subunits as the complex transits between conformational states (47–49). These studies provided new insights on regions that move, but they do not pinpoint the exact movement because it is not known whether the residue where the fluorophore is attached or the region around the fluorophore have moved. To have a detailed map of the conformational changes during function, a series of site-directed fluorescence recordings, guided by the structure, are needed.

**Significance to human disorders**

In humans, four paralogous genes ($\text{ATP1A1}$ through $\text{ATP1A4}$) encode $\alpha$-subunit isoforms, which assemble into functional pumps with three $\beta$-subunit isoforms ($\text{ATP1B1}$ through $\text{ATP1B3}$). Heterozygous mutations in $\text{ATP1A1}$, $\text{ATP1A2}$, and $\text{ATP1A3}$ are known to cause disease.

Characterization of $\text{Na}_\text{o}^+$-mediated transient currents in pumps carrying human disease mutations has provided important clues to the pathogenesis of some $\alpha$-subunit-associated disorders.

Somatic, heterozygous $\text{ATP1A1}$ mutations occur in aldosterone-producing adenomas (APAs) in the adrenal cortex. In this disorder, impaired clearance of intracellular $\text{Na}^+$ by the pump leads to increased intracellular $\text{Ca}^{2+}$, which is normally cleared by the $\text{Na}^+/$$\text{Ca}^{2+}$ exchanger. Because $\text{Ca}^{2+}$ is the second messenger that activates aldosterone synthesis, these cells constitutively produce aldosterone, causing high blood pressure (50). An electrophysiological study by Azizan et al. (50) suggested that the effect of $\text{ATP1A1}$ mutations including L104R and V332G is to produce inward $\text{Na}^+$ “leak currents”: abnormal inward flow of $\text{Na}^+$ ions through the pump opposite to the direction of normal $\text{Na}^+$ transport proposed to cause a depolarizing shift in the resting membrane potential. However, subsequent studies by Meyer et al. showed that $\text{Na}^+$ leak currents are absent in some $\text{ATP1A1}$ mutants such as G99R and I327S, which still result in similar phenotypes, suggesting that $\text{Na}^+$ leak is not a necessary part of the disease mechanism (51,52).

Instead, measurements of $\text{Na}_\text{o}^+$-mediated transient currents in G99R pumps showed an 8- to 16-fold decrease in apparent extracellular $\text{Na}^+$ affinity compared with the wild-type, which occurs with either of the $\beta$-isoforms present in the adrenal cortex, $\beta$1 or $\beta$3 (51,52). On the other hand, I327S pumps have $\text{Na}_\text{o}^+$-mediated transient currents similar to wild-type but greatly decreased intracellular $\text{Na}^+$-stimulated pump activity (51,52). Thus, the combination of $\text{Na}_\text{o}^+$-mediated transient currents and pump current measurements allowed more precise distinction between the effects of $\text{ATP1A1}$ mutations in APAs and established that the common disease mechanism is a decreased $\text{Na}^+$ export by the pump.

Leak currents have also been described with the S779N $\text{ATP1A2}$ mutation found in a patient with seizures and hypokalemic periodic paralysis. $\text{Na}_\text{o}^+$-mediated transient current measurements showed a reduced extracellular $\text{Na}^+$ affinity, altered binding rate constants, and persistent inward leak currents (53). Most $\text{ATP1A2}$ mutations instead decrease pump turnover rates and cause familial hemiplegic migraine (FHM). Even though many of these mutations are far away from the ion binding sites, they changed the kinetics and voltage dependence of the $\text{Na}_\text{o}^+$-mediated transient currents (54,55), suggesting changes in the E1P-E2P conformational equilibrium or long-range structural changes to the ion coordination site.

Heterozygous $\text{ATP1A3}$ mutations cause a broad spectrum of disease, including alternating hemiplegia of childhood (AHC) (56,57), rapid-onset dystonia-parkinsonism (RDP) (58), and CAPOS, a complex syndrome characterized by cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (59). Notably, some phenotypic differences may be explained by the location of the mutation in the protein structure; for example, CAPOS and RDP syndromes result mostly from cytoplasmic mutations, whereas mutations close to the $\text{Na}^+$ and/or $\text{K}^+$ binding sites often cause more severe phenotypes, such as AHC (60). Numerous functional investigations have been made to identify the molecular defect in $\text{ATP1A3}$-related diseases. Although expression studies pointed to a reduced expression level as a common disease mechanism for many RDP mutations (57), biochemical and two-electrode electrophysiological studies in multiple AHC cases are consistent with a disruption of function. Interestingly, many AHC mutations are at or near the ion binding sites of the $\text{Na}^+/\text{K}^+$-ATPase. It is conceivable that a systematic study of these mutations using $\text{Na}_\text{o}^+$-mediated currents recorded with enough resolution to discern the fast, medium, and slow components would provide insights into the contribution of each ion binding site to the kinetics of ion binding and occlusion.

Finally, $\text{Na}^+$ transient currents have helped characterize a physiological proton leak through the pump (I11) and its alteration in disease. Proton import was shown to occur through $\text{Na}^+$-selective site III by comparing mutants of acidic residues in each site. Because some mutations abrogated canonical pump current, transient current measurements of total charge allowed comparison of ion flux per pump between mutants, as well as estimation of proton import rate at 1/30 transport cycles under physiological conditions (61). In fact, the $\text{ATP1A3}$ D923N disease mutation...
located in site III was studied in ATP1A1 pumps and resulted in loss of both I$_{1}$ and the slow component of sodium off transients (55). Although the significance of I$_{1}$ in disease is unknown, both decreases and increases that might lead to disturbed cellular pH regulation have been found with disease mutations in ATP1A1 (50), ATP1A2 (55), and ATP1A3 (62,63).

In summary, the study of transient currents in the Na$^{+}$/K$^{+}$-ATPase over the past three decades has yielded important insights into the structure and dynamics of Na$^{+}$ and K$^{+}$ transport. Transient currents have shed light on questions ranging from evolutionary adaptations of the pump in cold and high-salinity environments to specific biophysical mechanisms of disease-causing mutations in humans. Combining transient current measurement with new approaches, such as using fluorescence resonance energy transfer (FRET) to probe conformational changes or studying mutations informed by human phenotypes, may lead to further advances.

**AUTHOR CONTRIBUTIONS**

M.H., R.L., and F.B. outlined the manuscript. All authors contributed to the writing and discussion of the manuscript.

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