# Physiological Effects of the Electrogenic Current Generated by the Na /K Pump in Mammalian Articular Chondrocytes

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**Research Article**

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# Abstract

Background: Although the chondrocyte is a non-excitable cell, there is strong interest in gaining detailed knowledge of its ion pumps, channels, exchangers and transporters. In combination, these transport mechanisms set the resting potential, regulate cell volume and strongly modulate responses of the chondrocyte to endocrine agents and physicochemical alterations in the surrounding extracellular micro-environment.

Materials and Methods: Mathematical modeling was used to assess the functional roles of energy-requiring active transport, the $Na^+/K^+$ pump, in chondrocytes.

Results: Our findings illustrate plausible physiological roles for the $Na^+/K^+$ pump in regulating the resting membrane potential and suggest ways in which specific molecular components of pump can respond to the unique electrochemical environment of the chondrocyte.

Conclusion: This analysis provides a basis for linking chondrocyte electrophysiology to metabolism and yields insights into novel ways of manipulating or regulating responsiveness to external stimuli both under baseline conditions and in chronic diseases such as osteoarthritis (OA).


# Introduction

The biomechanical and physiological functions of mammalian articular joints are essential for locomotion, postural stability, proprioception and motor learning [1–4]. At the tissue and cellular levels, this results in requirements for a wide range of dynamic motion coupled with remarkable stability. An enabling component of this system is articular joint lubrication, made possible by coordinated activity and secretion of biological lubricants from the two principal cell types in synovial joints: chondrocytes and synovial fibroblasts [5,6]. It is noteworthy that in the setting of chronic diseases of the articular joint e.g., rheumatoid arthritis (RA) or osteoarthritis (OA), significant changes in chondrocyte and synovial fibroblast function take place and these contribute to reduced secretion of joint lubricants [7], attenuated boundary lubrication and altered joint loading. Reduced tribology results in increased boundary friction, load-induced wear of articular cartilage, impaired joint function and eventual loss of proprioception [8].

The specific cell physiology-oriented focus of this study is the articular chondrocyte, which plays a key role in extracellular matrix (ECM) synthesis and degradation in all vertebrates [9]. The presence of mature healthy chondrocytes, and a full functional repertoire for these cells are essential for normal articular joint motion. Some of the important classes of transducer elements at the level of individual chondrocytes are ion channels, exchangers or pumps that are expressed in the surface membrane [10–16]. The significance of these integral membrane proteins, functioning individually or in concert, was first recognized with respect to the ability of chondrocytes to quickly and accurately volume regulate in response to even small changes in osmotic strength of the surrounding synovial fluid [17,18].

Within the last decade, a number of different ion selective channels and exchangers have been identified and shown to play essential roles in chondrocyte physiology [16,19–24]. Any such ion channel being functional in chondrocytes may seem somewhat surprising to researchers working on excitable cells, since the chondrocyte is considered to be a non-excitable cell. However, despite chondrocytes being non-excitable cells, their dynamic membrane potential serves many cellular roles [25], are metabolically active [16], especially during hypertrophy [26] and are capable of responses to commonly used drugs for treating hypertension [27]. Metabolic activity occurs at lower levels, especially in fully differentiated and senescent chondrocytes [28,29]. Indeed, chondrocyte {...}precursors (i.e. the chondroblasts), which proliferate during joint development, and even some developmentally mature chondrocytes, are more metabolically active, and are capable of robust ion channel mediated responses to biomechanical, pro-inflammatory [30–36] and immunometabolic factors [36,37]. Essential to this fundamental physiological regulation is the ability of the chondrocyte to set and maintain an appropriate, stable resting membrane potential as mediated by ubiquitous ion channels, pumps and exchangers in the plasma membranes of these cells [38].

Our main goal in this study was to obtain additional information concerning the role of the electrogenic $Na^+/K^+$ pump (also known as the $Na^+$, $K^+$-ATPase) in regulating the resting potential of the mammalian chondrocyte and thus ensure robust and stable physiological responses. A key starting point and motivation for our study was the previous demonstration of the presence of $Na^+/K^+$ pump proteins in the chondrocyte surface membrane using immunological and autoradiographic techniques, and the molecular characterization of its multiple a, b and g isoform subunits [39–41]. This dataset, based on demonstration of relatively high affinity, saturable binding sites for ouabain (a classical sodium potassium pump antagonist); combined with more recent molecular studies that have identified transcripts and proteins of each of the known subunits (a, b and g) of this integral membrane protein [42], strongly suggest that in the mammalian chondrocyte the electrochemical gradients for sodium and potassium are established and maintained by this active or ATP-requiring pump mechanism [43–45]. Very recent proteomic studies have taken an agnostic and unbiased molecular discovery approach to exploring the “surfaceome” of chondrocytes, confirming the presence of multiple $Na^+/K^+$ pump isoforms in these cells [24]. These findings considered in the context of the extensive literature on $Na^+/K^+$ pump cell physiology [46,47] make it very likely that in the chondrocyte the classical ‘coupled stoichiometry’ that is characteristic of this enzyme is 3 $Na^+$ pumped out of the cell, coupled with 2 $K^+$ pumped into the chondrocyte cytoplasm (Figure 1A). The resulting electrogenic current, oriented in the outward direction, would be expected to be one of the important factors in establishing the stable chondrocyte resting membrane potential. As shown in our previous paper [38], when this consideration is combined with the fact that the chondrocyte is a functional single cell with exceptionally high membrane resistance, it is likely that the electrogenic current due the $Na^+/K^+$ pump, (although it is very small) can provide a 10 – 20 mV contribution to the chondrocyte resting potential.

The mathematical modeling that forms the basis of this paper represents a continuation of our studies of chondrocyte electrophysiology, with an emphasis on understanding the basis for the resting

potential and its physiological implications for cartilage function. Modeling and simulation here serve as knowledge integrators, unifying results from diverse tissues and experimental sources in order to offer new insight and to advance new hypotheses. Theoretical studies of this type are thus essential components of ongoing efforts to address important gaps in the background knowledge of the chondrocyte phenotype, mechanisms for cell volume regulation, transmitter and paracrine modulation, responses to anabolic and pro-inflammatory mediators in the context of ECM turnover, identification of early disease markers in chondrocytes, and perhaps most importantly, drug development for OA.

# Methods

Simulations essential for the illustrations in this paper were performed using our previously published model of the resting membrane potential of the chondrocyte and related intracellular calcium homeostasis [38]. In the present study, the physiological roles of the $Na^+/K^+$ pump are explored more fully by carrying out simulations at in consideration of the unique environment of the chondrocyte in the synovial joint in terms of the *temperature* and the *ionic composition* of the extracellular milieu.

*Temperature considerations.*

While temperature measurements of e.g. the human knee vary substantially dependent on the method, intra-surgical measurements suggest values between 31.5-33.5°C for a healthy knee joint [48–50]; as these values arise in the likely context of trauma and/or inflammation, a truly healthy knee joint at rest might have a temperature closer to 25±3°C, as suggested by external measurement methods. In contrast, joint temperatures are elevated in the context of RA (34-36°C) and OA (30-37°C), as probably attributable to chronic inflammation in RA and low-grade inflammation in OA. Thus, we have here considered the basal, "room" temperature of the previously published model, 23˚C, to reflect a near-physiological temperature for the healthy synovial joint and chondrocyte environment; simulations also consider, in contrast, an elevated joint capsule temperature of 37˚C to consider downstream effects on $Na^+/K^+$ pump function and the chondrocyte in the pathophysiological context.

*Extracellular ionic milieu.*

In addition, the parent model (Figure 1C) has been modified such that it can generate data sets that take into account the exceptional ionic milieu of the immediate microenvironment of the chondrocyte (see, e.g. Figure 2A inset). We use this expanded model to explore a putative role for the electrogenic $Na^+/K^+$ pump current in regulating the resting membrane in articular chondrocytes with particular focus on conditions likely to be found *in vivo*, i.e. given the unique ionic environment of the chondrocyte matrix.

*Differential subunit expression and pump function.*

In addition, knowledge that chondrocytes express a number of different isoforms of the subunits that make up functional sodium potassium pumps is taken into account. The contributions of subtype-specific combinations of functional sodium potassium pumps are illustrated by adjusting the affinity constants for $[Na^+]_i$ and $[K^+]_o$ binding, as explored further in Figure 4. We illustrate and discuss the resulting profiles and patterns of electrogenic pump currents and related changes in membrane potential.

Overall, our findings can be used to inform ongoing and future patch clamp electrophysiological studies. They also have the potential to guide investigators in the design and implementation of novel planar recording methods [51,52]. In the case of ion exchanger and pump activity generated by intracellular organelles, these approaches appear to be required for resolving and understanding these very small changes that can regulate fundamental properties of non-excitable cells such as the chondrocyte.

More specific information as to the current simulations performed using the model, including details regarding implementation in Matlab and parameter sets, may be found in the Supplementary Information. The model itself may be accessed at https://github.com/mmaleck/chondrocyte.

# Results

The sodium potassium ($Na^+/K^+$) pump makes an important contribution to setting up and maintaining the membrane potential [53]. The main goal of this study was to utilize mathematical modeling to explore the contribution of the electrogenic $Na^+/K^+$ pump to the resting membrane potential in chondrocytes isolated from healthy adult human articular joints. $Na^+/K^+$ pump expression has been demonstrated in primary bovine chondrocytes isolated from healthy joints [39], human chondrocytes in healthy and diseased joints *in situ* [41], and human chondrocyte-like cell lines [42]. The functional presence of the $Na^+/K^+$ pump in chondrocytes has been further documented by related analyses of the molecular properties (isoform composition and expression levels) [39], upregulation of the $Na^+/K^+$ pump in response to changes in extracellular $Na^+$ concentration [40,54] and activity as demonstrated by ouabain binding [40,43,44,54] and p-nitrophenylphosphatase activity *in situ* in both healthy and pathological samples [41]. Importantly also, the basis for the chondrocyte resting potential has previously been analyzed using mathematical modeling [38,55–57].

However, none of these studies focused on defining the functional roles of the electrogenic current generated by the $Na^+/K^+$ pump under pathophysiological conditions, e.g., mimicking temperatures measured in OA (~37$^o$C), as opposed to the likely temperature found in healthy articular joints (~23$^o$C), also conditions that are typical of electrophysiological studies done using patch clamp methods under “room” temperature. Our first set of simulations involved making only one change to the parameters that govern the electrophysiological behaviour of our human chondrocyte model. In this formulation, the mathematical expression for the electrogenic $Na^+/K^+$ pump includes the ability to account for temperature differences by changing the $Q_{10}$ parameter that regulates the turnover rate of this enzyme. Based on the standard $Q_{10}$ (3.0) for a membrane-bound integral membrane enzyme [58] the change from

the baseline conditions (23$^{o}$C) in this model to pathophysiological conditions (37$^{o}$C) resulted in a substantial increase in the steady-state electrogenic current (Figure 1D). These two superimposed steady-state current-voltage (I-V) plots clearly demonstrate that over a broad range of membrane potentials, and in particular, in the range of membrane potentials (-70 to 0 mV) that is most relevant to chondrocyte physiology, the outward electrogenic current increases by a factor of approximately 3-4. The curvilinear characteristic of both steady-state I-V curves reflect the intrinsic voltage dependence of the enzymatic reactions that result in coupled net electrogenic (3 $Na^+$ in for 2 $K^+$ out) fluxes that produce the so-called 'pump current' [59]. These first findings strongly suggest that the $Na^+/K^+$ pump could modulate the resting membrane potential of the chondrocyte in health and disease.

The next set of mathematical simulations were performed in an attempt to illustrate the importance of the electrogenic current generated by the $Na^+/K^+$ pump for regulating or stabilizing the chondrocyte membrane potential. Panel A of Figure 2, based on our previously published mathematical model [38] illustrates our working hypothesis for the ionic basis for this resting membrane potential. In brief, a background $Na^+$ current (see also Supplementary Information, Figure S1) interacts with outward currents generated by 2-pore $K^+$ channels and the outward electrogenic $Na^+/K^+$ pump current. Current generated by the $Na^+/Ca^{2+}$ exchanger and a Cl- conductance are also present but these are not the focus of this study. Note that under room temperature conditions (23$^{o}$C) this complement of currents balances to generate a resting potential of approximately -40 mV, considering synovial ionic concentrations and generic affinities of the sodium-potassium pump for sodium and potassium ions, as published previously [38]. In the modified model used here (Supplementary Information), synovial concentrations generate a chondrocyte resting membrane potential of approximately -60 mV. The unique ionic milieu of the chondrocyte matrix already further hyperpolarizes this resting membrane potential significantly with respect to the predictions employing synovial ionic concentrations, even at room temperature/under healthy conditions (~-67 mV).

The analogous superimposed I-V plots that comprise Figure 2B were computed under conditions designed to mimic pathophysiological conditions (37$^{o}$C) the chondrocyte may experience, e.g. in OA. Specifically, the temperature dependence of the $Na^+/K^+$ pump was adjusted based on a $Q_{10}$ value of 3.0; whereas the temperature dependence of the ion channel-mediated fluxes was adjusted using a $Q_{10}$ value of 1.2 for each [60,61]{...}. The combination of the very small sizes of the baseline (resting conditions) channel-mediated current in this small and very high resistance cell and the strong temperature dependence of the electrogenic current of the $Na^+/K^+$ pump results in an increased outward current due mainly to the $Na^+/K^+$ pump. Although still relatively small, this current would be expected to result in a significant and maintained hyperpolarization of the resting potential (20-25mV) in the chondrocyte under pathophysiological temperature; indeed, the chondrocyte resting membrane potential apparently can be hyperpolarized to ~-92mV at 37˚C.

It is important to evaluate and understand overall homeostatic control of intracellular electrolytes and related changes in osmotic strength when studying active transport mechanisms in very small cells such as the chondrocyte [10,62]. Accordingly, we have tracked and illustrated time-dependent changes in intracellular $Na^+$ and $Ca^{2+}$ as well as extracellular $K^+$ in the *in silico* conditions in which our baseline mathematical model operates. Figure 3A confirms the steady-state changes in the $Na^+/K^+$ pump density due to a step change in temperature; four sets of time-dependent results are shown in Figure 3B. The upper left plot shows the time-dependent change in chondrocyte membrane potential that results from a step change in temperature (and hence pump turnover rate) from 23$^o$ to 37$^o$C. Corresponding changes in intracellular $Na^+$ and $K^+$ confirm that there are no significant changes from the starting conditions or baseline model values. Somewhat similarly, although intracellular $Ca^+$ increases approximately 2-fold soon after the temperature change, this stabilizes very near the 0.2 mM value still characteristic of most healthy mammalian cells. In summary, therefore, although the electrogenic current can contribute to a very substantial hyperpolarization of the resting membrane potential, its activity does not significantly alter intracellular electrolyte homeostasis in this model. This is important since the electrochemical gradient for $Na^+$ is a primary variable in regulating chondrocyte $Na^+/H^+$ ion exchange and hence intracellular pH; and in modulating $Na^+/Ca^{2+}$ exchange in chondrocytes [63] as it does in cardiomyocytes [64]. It is known that changes in intracellular $Ca^{2+}$ can alter $Na^+/K^+$ pump activity in most mammalian cells [65]. In addition, the ability of the $Na^+/K^+$ pump to stabilize intracellular $Na^+$ and thus contribute to medium and long term cellular volume regulation is well known [66].

In the mammalian articular joint, electrolyte levels and particularly those for *intracellular* $Na^+$ and $K^+$ may deviate significantly from those in standard plasma or intracellular levels found in other tissues, e.g. skin or muscle [10,67]. These differences and the resulting changes in $Na^+/K^+$ pump activity and baseline chondrocyte conductances were a focus of our previous paper [38] and have been studied previously in physiological and pathophysiological settings [10,14,16,43,44]. These considerations, when combined with the demonstration that the healthy bovine and human chondrocytes expresses a number of different isoforms of the a, b, and g subunits of the $Na^+/K^+$ pump protein complex [39,41], raise important questions concerning the relative sizes of the electrogenic current and related changes in membrane potential that would be expected due to predominant expression of specific combinations of the a, b, and g subunits [42,45]. We have approached this by simulating several of the combinations of expression of a and b subunits that are specified in a comprehensive review of the molecular physiology of the $Na^+/K^+$ pump [68] and more recent studies of the kinetic transitions of the movement of $Na^+$ and $K^+$ ions through the pump [69]. Results expressed in terms of temperature-dependent development of steady-state electrogenic currents for five subsets of data are shown in Figure 4. The most common physiological condition, assuming an exclusive a1, b1, subunit composition with the affinity constants as shown in the inset, is shown in black. Note that the maximal current is approximately 4.5 pA/pF and that the steady-state membrane potential is approximately -92 mV at 37$^o$C (membrane potential traces not shown). The red trace illustrates an identical calculation done under the assumption of a predominant a2, b1, subunit

expression. The corresponding higher affinity for the intracellular $Na^+$ site on the $Na^+/K^+$ pump predictably results in a somewhat larger steady state electrogenic current (nearly 5.0 pA/pF) and larger hyperpolarization of the resting membrane potential to approximately -95 mV. In contrast, the dark green trace simulates the effect of predominant expression of the a3, b1, subunits; based on the markedly decreased affinity for intracellular $Na^+$ of this combination, the maximal electrogenic current reaches approximately 2.5 pA/pF and the chondrocyte resting membrane potential stabilizes at -78 mV.

# Discussion

### a) Main Findings

This computational work, based on our published model of the healthy adult chondrocyte membrane potential [38], confirms that, the electrogenic current generated by the $Na^+/K^+$ pump is strongly temperature-dependent. This net outward current is relatively small. However, it can have substantial hyperpolarizing influences on the resting membrane potential of the chondrocyte due mainly to the very high input resistance, approximately 10 Giga-ohms [14,55,56] of this cell. In both physiological and pathophysiological settings it is likely that the $Na^+/K^+$ pump in the chondrocyte is strongly activated due to the relatively high intracellular $Na^+$ levels (~20 mM or more) in this cell type [10,43,70].

### b) Previous Findings

Hall and colleagues first reported evidence for energy-requiring active transport of $K^+$ across the surface membrane of healthy adult mammalian chondrocytes [71]. This initial observation was supported and put into a conventional cell physiology context by Mobasheri and colleagues [39] who adapted $^3H$-labeled ouabain binding methods to demonstrate substantial expression of the $Na^+/K^+$ pump alpha subunit in mammalian chondrocytes. Mobasheri et al. then confirmed and extended these findings based on experimental $^3H$-labeled ouabain binding and confocal immunofluorescence microscopy work [39,40]. They demonstrated regulation of surface expression by changes in levels of intra- and extracellular $Na^+$ in isolated cells [44,72] and in the extracellular matrix of articular cartilage from healthy bovine joints [43] and also reported a scheme for overall $Na^+$ regulation based on data that identified functional roles in the chondrocyte for $Na^+/Ca^{2+}$ exchange, $Na^+/H^+$ exchange, and antiporter exchange due to $Na^+/K^+/Cl^-$ expression [10]. These papers and subsequent work have specified the essential role of the $Na^+/K^+$ pump and overall regulation of intracellular $Na^+$ levels in volume regulation of the chondrocyte. It is also known that regulation of volume in the chondrocyte depends, in part, on the membrane potential of these cells [20,73].

### c) Physiological Effects of the Electrogenic $Na^+/K^+$ Pump in Chondrocytes

Insights gained from our mathematical modeling support the working hypothesis that the $Na^+/K^+$ pump can strongly regulate the resting membrane potential in chondrocytes from healthy and diseased

adult articular cartilage. Specifically, it is plausible that due to its expression levels, turnover rates [74] and intrinsic voltage dependence [75,76] this pump mechanism produces a hyperpolarizing influence that can be as large as 30 mV. This hyperpolarization would be expected to modulate volume regulation; in addition, however, it is also likely to markedly alter the overall electrophysiological function or electrophysiological operating point of the chondrocyte [77]. This is because a number of the other ion channels that are expressed in the chondrocyte, e.g., L-type $Ca^{2+}$ channels [15,27,78–80], delayed rectifier $K^+$ channels [55,57], and 2-pore $K^+$ channels [56] exhibit strong intrinsic voltage dependence in the range -40 to -80 mV. Accordingly, the hyperpolarizing influence of the $Na^+/K^+$ pump significantly regulates the activation and/or deactivation of these (and perhaps other) ion channels in the chondrocyte.

We note, however, that the singular focus on the physiological and pathophysiological effects of the electrogenic current produced by the $Na^+/K^+$ pump in this study could be somewhat misleading. When the chondrocyte is stimulated by stretch, or activated by ligands such as histamine or ATP, agonist-induced ion fluxes through either piezo [81], $Cl^-$ [77], or TRP channels [14,77,82,83] will reduce the input resistance of the cell. The resulting parallel conductance will partially 'shunt' the influence of the electrogenic current generated by the $Na^+/K^+$ pump. In addition, in both health and disease, the chondrocyte exists and functions in a relatively hypoxic environment [84–86]. In this setting, the supply of ATP as the principal energy source of energy for chondrocytes [87] may limit pump activity to a range that is less than the maximal currents shown in the Figures [88,89].

### d) Concluding Remarks

Although we acknowledge the possibility that the $Na^+/K^+$ pump function in mammalian chondrocytes may be modulated by altered expression levels of selected isoforms of the a, b, and g subunits, our analysis is not sufficiently complete to fully examine the consequences as has been done in other tissues e.g., skeletal muscle [74]. It is known that the $Na^+/K^+$ pump can be strongly modulated by altered redox conditions [90] such as those that occur in sterile inflammation, or "low grade inflammation" in the context of chondrocyte biology and OA [91,92]. Our work provides a basis for this type of analysis but important pathophysiological effects such as this have not been studied. Finally, both classical findings and more recent detailed analyses have drawn attention to conditions under which changes in intracellular $Ca^{2+}$ can markedly alter the function of the $Na^+/K^+$ pump [93]. Our model, at its present state of development, cannot be used to simulate these $Ca^{2+}$-dependent effects due to the simplistic formulations now used for intracellular $Ca^{2+}$ buffering and $Na^+/Ca^{2+}$ exchange; in the absence of mathematical descriptors for $Ca^{2+}$ pumps and $Ca^{2+}$-sensitive channels localized to the endoplasmic reticulum [94]. These additions and other improvements will be needed before the mathematical modeling approach used in this study can be extended to analysis of ion homeostasis and the chondrocyte channelome [16,22] in a more physiological context, specifically in chondron units, which represent the chondrocyte and its immediate pericellular environment [95]. Further model development is also needed before our simulations can provide insights into the altered articular joint electrolyte homeostasis

resulting from disease-producing point mutations in one or more of the $Na^+/K^+$ pump subunits or accessory proteins [69].

From a tissue engineering perspective, there is ongoing interest in the $Na^+/K^+$ pump, ion transport and the modulation of intracellular $Na^+$ by pharmacological agents such as ouabain and bumetanide as *in vitro* treatments for altering intracellular ion concentrations as a viable method for manipulating ECM synthesis by chondrocytes and enhancing the mechanical properties of engineered articular cartilage [96].

Finally, in the context of drug development and screening for diseases such as OA, which is known to be characterized by cellular senescence, or "chondrosenescence" [29,97], the $Na^+/K^+$ pump has already been identified as a candidate target for modulation by cardiac glycosides, re-entering the limelight as classical cardiotonic drugs re-invented as senolytic compounds [98]. Thus, the modeling approaches described in this paper can support drug development for OA and related osteoarticular disorders as well as understanding of the basic cellular physiology and pathophysiology of joint tissues.

# Declarations


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## Author Disclosure Statement

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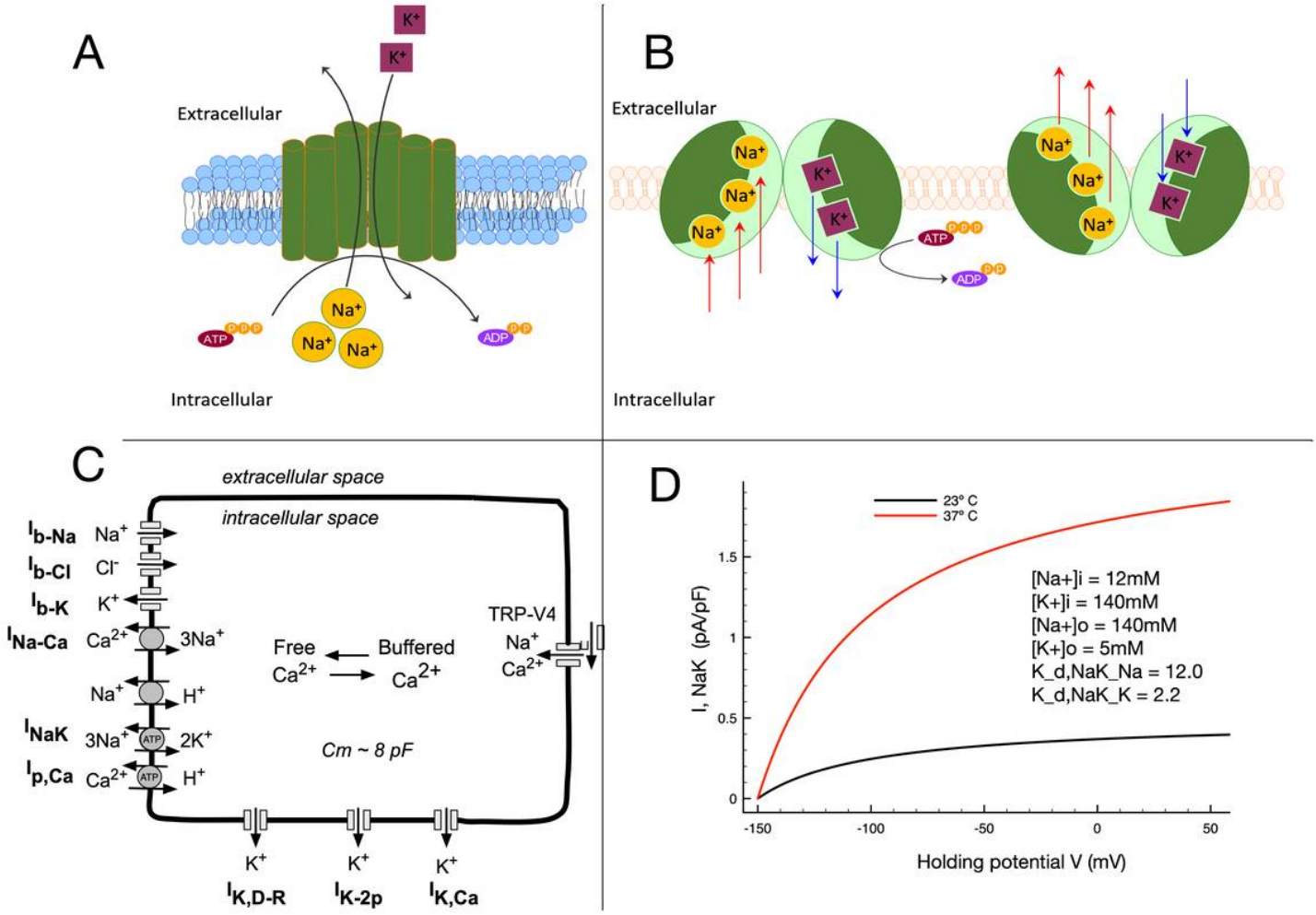
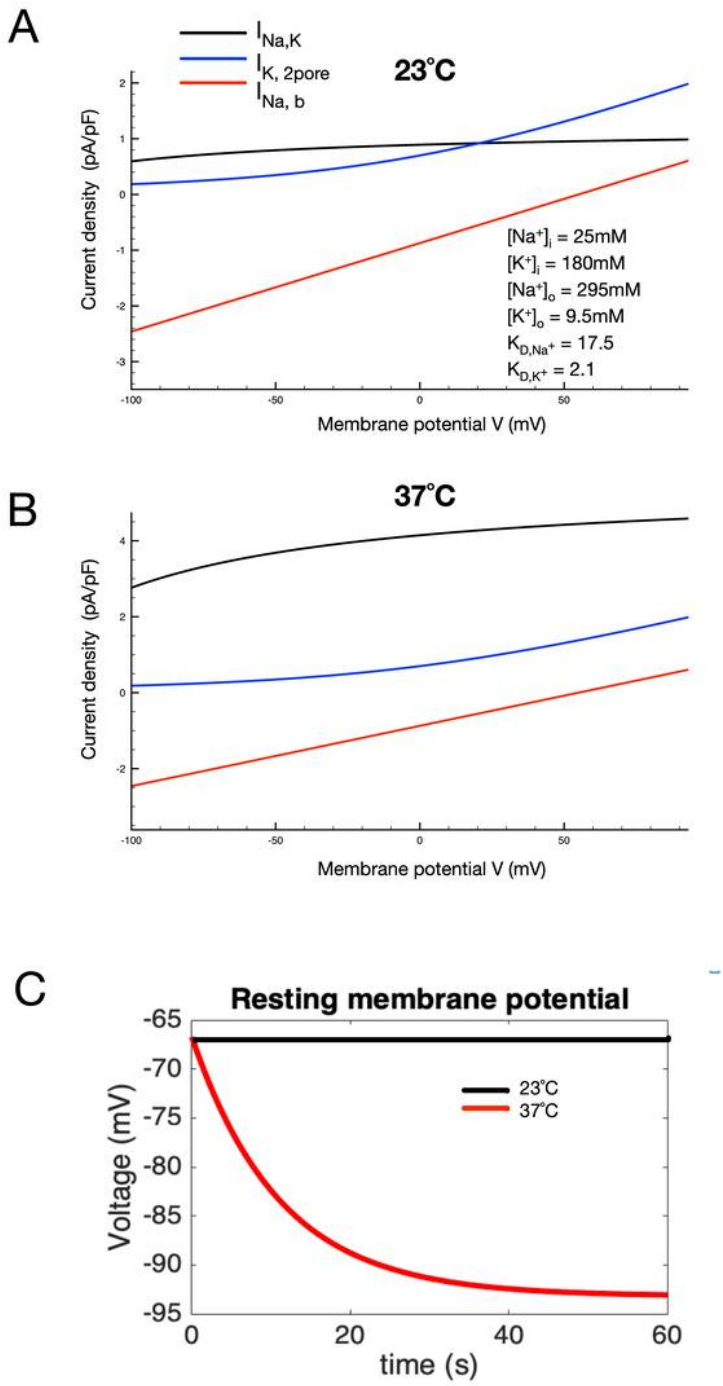
# Figures




## Figure 1

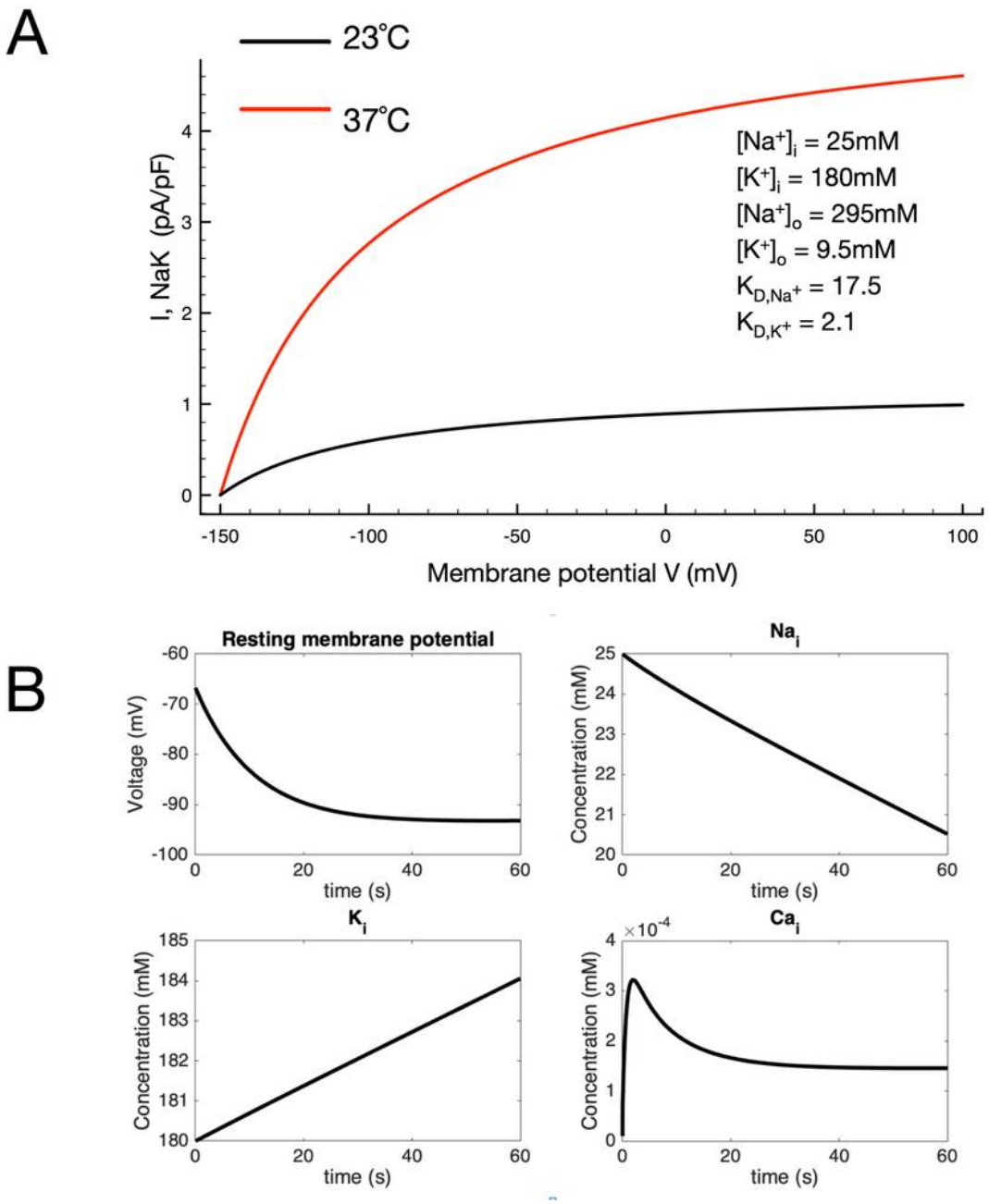
Modeling of the Chondrocyte Resting Membrane Potential and Demonstration of the Effects of Temperature on the Electrogenic Current Generated by the Na+/K+ Pump in a Human Chondrocyte Preparation Panel A and B present an illustration of the structure and function of the electrogenic sodium-potassium ATPase whose role in chondrocyte electrophysiology is investigated here; each pump turnover results in 3 Na+ ions' expulsion from and 2 K+ ions' inclusion into the cell, generating a net outward current. Panel C shows an illustrated schematic of the mathematical model of the chondrocyte resting membrane potential used here, as previously published 38. Panel D explores the steady-state voltage dependence of the electrogenic pump Na+/K+ pump current density, given via our previously published model 38. Strong temperature dependence is revealed, and the steady-state current at healthy joint temperature (23˚C, black trace) as well as at pathophysiological temperature (37˚C, red trace) are illustrated. Both I-V curves also show the curvilinear waveform that is due to the intrinsic voltage dependence of the Na+/K+ pump in mammalian cells. Intracellular and extracellular ion concentrations as initial modeling conditions are assumed (as previously) to be those measured in synovial fluid, (as shown in inset).




## Figure 2

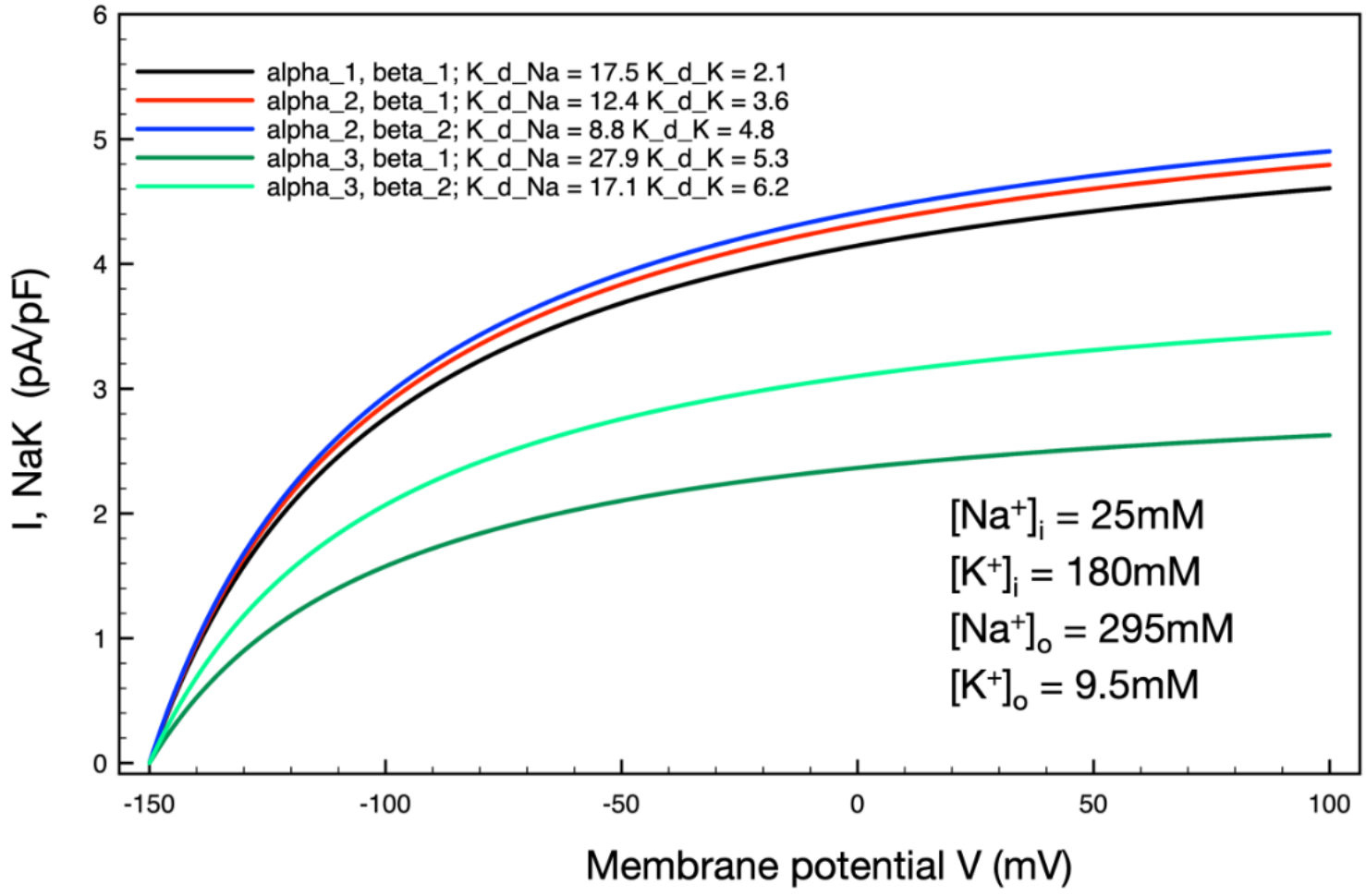
Temperature-dependent Contribution of the Na+/K+ Pump Electrogenic Current to the Chondrocyte Resting Membrane Potential. The superimposed I-V curves in Panel A were generated using our published model of the chondrocyte resting potential as shown, assuming a temperature of 23oC and measured and estimated ionic concentrations within the chondrocyte matrix. The analogous superimposed I-V curves shown in Panel B were generated using the same model after the Na+/K+ pump current was

adjusted to pathophysiological temperature (37oC) based on a Q10 of 3.0 and the channel-mediated background currents were adjusted using a Q10 of 1.2. The two records of membrane potential in Panel C illustrate the significant but relatively small effect of the electrogenic current due to the Na+/K+ pump at 23oC contrasted with the much larger hyperpolarization generated by this pump current at 37oC. As shown, the additional Na+/K+ pump current density simulated at pathophysiological temperature in the chondrocyte matrix hyperpolarizes the chondrocyte resting membrane potential by ~25mV (~-67mV at 23˚C and ~-92mV at 37˚C).



## Figure 3

Intracellular Electrolyte Homeostasis in the Setting of Altered Na+/K+ Pump Activity/Turnover Rate Due to a Step Change in Temperature (23o to 37oC). As noted, the electrogenic Na+/K+ pump current density is changed significantly when intracellular and extracellular ion concentrations are altered to reflect the ionic milieu of the chondrocyte matrix found in vivo (shown in inset, Panel A); compare to simulations performed assuming a baseline synovial fluid environment (Fig 1D). In Panel A, we illustrate steady-state voltage dependence, and the temperature dependence of the Na+/K+ pump current. The steady-state current at 23˚C (black trace) as well as at 37˚C (red trace) are shown. The additional Na+/K+ pump current density simulated at a pathophysiological temperature hyperpolarizes the chondrocyte resting membrane potential by ~25mV at 37˚C in the chondrocyte milieu. Panel B also shows the time-dependent hyperpolarization of chondrocyte membrane potential resulting from a step change in temperature from 23o to 37oC, as well as the corresponding alterations in intracellular Na+, K+, and Ca2+. In the cases of Na+ and K+ these changes are small: the shift in e.g. [Na+]i is about a 20% change over the time course measured, and likely to be of small physiological relevance overall. The intracellular Ca2+ level changes transiently (perhaps due to the intrinsic voltage dependence of the Na+/Ca2+ exchanger or specific model-dependent features of intracellular Ca2+ buffering) but quickly stabilizes near 0.2 □M, an accepted level for a resting mammalian cell.




## Figure 4

Simulations of the Effects on Steady-state Electrogenic Na+/K+ Pump Currents Due to Assumed Changes in the alpha and beta Subunit Composition of this Pump Complex in an Adult Mammalian Chondrocyte. This Figure illustrates five different combinations of alpha and beta subunits and the corresponding changes in maximum steady state electrogenic currents and alterations in chondrocyte resting membrane potential. The steady-state voltage dependence and overall Na+/K+ pump expression held constant, the altered expression of alpha and betasubunits (as specified in the inset, upper left), were modeled by changing the Na+/K+ pump affinities for [Na+ ]i and [K+]o known to be associated with these isozymes 68. All ionic concentrations reflect the chondrocyte milieu and all simulations were performed at 37˚C to reflect a putative pathophysiological state e.g. OA.

## Supplementary Files

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