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Structural analysis of fluid flow in complex biological systems

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Abstract

Biology is about structure. Structures within structures. Organs within animals, tissues within organs, cells within tissues, and molecules, often proteins within cells. The structures are so complex that they can only be described by numbers. No numbers are of more importance than those that describe proteins. The numbers that describe coordinates of its atoms, often determined by Patterson functions (which are inverse Fourier Transforms of intensities) of crystal diffraction. Without these numbers, structural biology would hardly exist. Without numbers, engineering would not exist. Numbers are surely needed by the engineers who produce the x-rays diffracting from atoms of protein crystals. Devices of engineering have function. They are built to implement equations. Engineering devices use structures to implement equations, when power is supplied at the right places, that produces appropriate flows. Flows are the essence of life. Equilibrium means death in most living systems. Flows in biological structures are hard to analyze because we do not know input output equations in advance. Sometimes we do not know the function of the structures. Flows, forces, and structures of life (like those of engineering) are related by field equations of conservation laws, partial differential equations, constrained by location and properties of structures. Constraints are boundary conditions located on the complicated domain of biological structure. The hierarchy of structures allows a handful of atoms (in proteins and nucleic acids) to control macroscopic function. Dealing with this complexity is simplified if one systematically analyzes structure using the most general field theory known, electricity described by the Maxwell equations, without significant known error. Currents are involved because flows of biology usually involve migration of charges, convection of water and solutes, diffusion of ions that form the plasma of life, and

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their interactions. Interactions can dominate function. Here I show how a few complex structures can be understood in engineering detail. This approach may be useful in dealing with biological and medical issues in many other cases. In one limited case—the clearance of a toxic waste (potassium ions) from the optic nerve—this approach seems to have succeeded.

Keywords: bidomain model, conservation laws, fluid flow, glymphatic system

1. Aim

My aim is to explain by example structural analysis of fluid flow in complex biological systems.

1.1. Fluid flow

A great deal of important biology involves fluid flow in large (cm) structures. The flows and biological function obviously depend on the structure, just as much as the work of an automobile engine depends on its structure. The flows of course depend on the atoms that flow and their properties. How could they not? But the forces that drive the flows and that allow biological control arise in proteins, as does so much of biological function. The central role of proteins is hardly surprising since the substrate of evolution, that creates biological systems, are genes, and genes make proteins! As a rule of thumb, it is much easier for evolution to mutate a protein, and select the mutation that produces more offspring, than to change a structure. And of course, as powerful as evolution is, it has a limited ability to change physical laws.

Proteins control flow in biology much as valves control flow in engineering. Channels and transporters are the transistors of life.[1-5] The transistors that make up our computers and smartphones are valves that control the flow of electrons and holes.[6-9] The transistors are most helpful when they are embedded in integrated circuits[10,11] that use the valves as switches (CMOS switches nowadays, for the most part). And the integrated circuits are embedded in groups that perform the main functions of the computers and smartphones they are in.

Life uses a hierarchy of systems much as engineers do.[12] Channels (and transporters) are embedded in membranes. Membranes form organelles (*e.g.*, mitochondria) and cells. Cells form tissues. And tissues form organs. Flows are important at all levels of the structural hierarchy that makes up biology. Function depends on the entire hierarchy of structures just as in a computer. The hierarchy allows a handful of atoms in a gene to control macroscopic function, including the reproductive behavior which is the substrate of evolution.

1.2. Multiscale flow

Understanding fluid flow in biological structures thus involves a large range of separate systems that involve many different forces and constraints, on a range of scales from atoms (literally, the ions of biological solutions), to membranes, organelles, cells, tissues, and organs. The complexity of these systems has been the joy of classical anatomists and zoologists for centuries, as they assemble the parts list of the system and describe how they are connected. It is not such a joy to the physical scientists who prefer analysis and equations to descriptions, and simplicity of unstructured fields of force to devices driven by power supplies.

The descriptive approach is as important in biology as it is in technology. No one could imagine understanding an automobile engine without a parts list, and drawings of its structure. A complete manual (usually oriented to what can go wrong, and so often called a repair manual) is almost a necessity nowadays.

1.3. Complexity in biology and engineering

Biological systems are often not much more complicated than an automobile engine, but we do not have a manual, and often we have only an incomplete set of drawings. We all seek a repair manual so we can take care of our loved ones in disease and decline as well as health and growth. Creating a repair manual is one of the main goals of modern medical and biological science.

The biological manual for organs and tissues that use fluid flow has to be built by scientists and clinicians from observations and experiments. We have to build the manual by reverse engineering. We look at outcomes and we try to infer mechanisms so we can predict other outcomes, and repair failures. These are the questions of inverse problems[13-17] and it will do a great deal of good if scientists confront that fact and the ambiguities and “ill posedness” (to use the jargon of the theory of inverse problems) inherent in reverse engineering and inverse problems. Mathematicians have shown in great detail that measurements of one type, even in a wide range of conditions, cannot determine underlying mechanisms in a robust way. No amount of measurement of the impedance of the black box can resolve that ambiguity. Think of the problem of measuring the values of resistors in a black box with a measured resistance of 10 ohms. That resistance could come from one resistor, or from two in parallel, or two in series, etc., etc.

1.4. Resolving ambiguity: inverse problems

Biologists know by training and intuition that the way to resolve ambiguity is often to make a different kind of measurement. To measure the total number of resistors, or better to determine the structure inside the box, with methods totally different from those used to measure the impedance of the box. Combining structural and functional measurements is usually needed to understand how biological systems work.

So, as we turn to our main issue of studying tissues and organs with fluid flow, we start with structure. We will do that in a few paragraphs, but we also must reiterate that we need to include the physical properties of fluids and the biophysical properties of proteins, chiefly membrane proteins that are channels and transporters, before we can make much sense of the structures.

1.5. Complex fluids

Biologists are fortunate that in the last decades physical scientists have learned to deal with flow in systems of this sort. The theory of complex fluids[18-26] has been developed to deal in a precise quantitative way with systems of fluids in which energy is stored, dissipated, and drives flows on many scales in many ways.

It is only quite recently that workers have realized that any ionic fluid (like the intracellular and extracellular fluids of life) is a complex fluid.[26-29] Traditionally, those electrolytes have been treated as simple fluids,[30-34] with some difficulties[35-40] as well known to leading experimental physical chemists.[41] Indeed, it still is not widely realized that quasi crystalline biological systems (think skeletal and even cardiac muscle) are quite similar to liquid crystals[19,42-46] that have been analyzed successfully with the theory of complex fluids for some decades now.

This review makes the case that a systematic approach to biological systems of complex structure is possible if it starts with structure, includes the structure (*i.e.*, location and density) of channels and transporters, and uses the theory of complex fluids to analyze the flows and forces.[47-57]

2. Methods

2.1. Biophysics of channels

The analysis depends on the still evolving representation and understanding of the biophysics of flow in channels and transporters. The hope is that details of that biophysics may not always dominate the biological function and that the details can be described at technological resolution, as the Hodgkin-Huxley model and equations describe channels and the current flows they produce.

It is reasonable to hope that physicists and mathematicians now entering the field will be able to use quantitative methods, inspired by the theory of complex fluids, to understand how these proteins work, at least well enough to understand their role in the transport of fluids. Structural and molecular biologists have made enormous progress in resolving the atomic details of channel and transporter function.

Sadly, models at atomic resolution, that use assumptions of periodicity and zero flow so atomic details can be computed in systems that function on biological time scales, cannot provide that understanding. Why? Because the biological systems are not periodic and depend on large flows for their function.

Computations that assume periodicity find it difficult to deal with irregular systems. Computations that deal with zero flows or nearly zero flows find it difficult to deal with systems that require large flows to function. It should be clearly understood that most of the devices of engineering depend on large flows. They need power supplies. Amplifiers without power supplies are no more worth studying than automobile engines with water in the gas tank. Most of biology requires flows.

Mathematics does not allow one to compute a finite value for something assumed to be zero. Ever. Approximations are possible of course, but they must be testable, either by mathematically defined error analysis, or by the scientific procedure of "guess and check". The approximation is the "guess", and experimental work is the "check" in most cases. When theories are made that are inconsistent with mathematics or ignore the need to check approximations, confusion and debate are almost certain to result.

Methods that assume periodicity and zero flow are unlikely to allow the consensus needed for scientific progress or clinical application in systems that are not periodic or systems that depend on flow. Different research groups deal with the inconsistency (between periodic assumptions and irregular reality for example) in different ways and so get different results. Discussion of inconsistencies is more likely to produce emotional heat than intellectual light, given the reality that science is done by human beings with practical needs that are in the foreground of their thoughts, understandably enough.

We begin with the structures and move from their relative certainty to the uncertainty of complex fluids later on.

2.2. Role of structure

The role of structure in the multiscale hierarchical analysis I will describe is best seen in the analysis of electrical properties started by Falk and Fatt[58] passed on to their graduate student[59] as they looked at the internal membranes of skeletal muscle. Falk and Fatt decided to measure the linear properties of cells by applying currents that crossed the membrane (with an intracellular microelectrode) instead of by applying currents that avoided the membrane and flowed around cells. In that way, they could use the highly developed methods of linear circuit theory or linear systems analysis[60-70] to work out how current and potential spread in the complex membrane systems of skeletal muscle. Instead of assuming that spread, as cardiac electrophysiologists did, Falk and Fatt measured it. That way skeletal muscle was spared the metastasis of artifactual conductances and ion channels[71,72] that produced a tangle of confusion in cardiology until single channel recording and the identification of channels as proteins cut through the tangle and brought the clarity of direct measurement to the field.[73,74]

The technical issues of measuring and analyzing linear circuits took some time to perfect but a variety of scientists[58,59,75-79] (reviewed by several others[80-99]) succeeded in making a powerful and general tool.

2.3 Biophysical invariants: membrane capacitance and internal resistance

A crucial factor in the success of the circuit analysis of complex tissues was the fortunate fact that two properties of cells were simple. The interior of cells is a pure resistance. The membrane capacitance is essentially a constant independent of ionic conditions. Both statements are documented later in this paper. Thus, methods could be designed to exploit the constancy of these values.[76-79,84]

An essential step in analyzing the capacitance, and of course the circuit properties in general, was understanding what is intra and what is extracellular space. ***This seemingly trivial necessity needs to be stated explicitly when dealing with new systems that do not have a long history of biophysical analysis.*** Experiments are needed in those cases, not arguments. Localizing junction proteins is of course a help but nothing replaces direct measurements of accessible spaces with markers.

A variety of marker systems are available as a literature search will show (starting for example with these two papers[100,101]), but the horseradish peroxidase system seems to have been lost in that variety despite its significant advantages of black-and-white contrast yielding nearly 100% efficiency[102,103] in chemically treated, fixed, and sectioned material.

2.3. Morphometric measurement of area

Direct visualization of living material with modern microscopic techniques has the advantage of using native tissue without the artifacts of fixation. The advantage is profound when estimating volumes. ***But when determining electrical circuits, it is the area which is the main goal*** because the area is connected to the electrical parameter capacitance in a definite way, independent of ionic conditions. Measurements of area with light microscopy is dangerous because of its limited resolution, no matter what the wonders of modern techniques. The electron microscope will include fine structures (bends and wiggles) not apparent with the highest resolution light microscopy. These complexities can be significant, indeed very significant, both quantitatively and qualitatively.

It is important however that seeing not be believing. The visualization methods must be accompanied by objective validation before they can be trusted to give reliable results.

Making measurements of area is not easy. It is quite difficult to make such measurements with the obvious curve tracing methods, because of distortion and disruption in the sectioning methods used in most histology and because of the huge irreproducible systematic errors people make when asked to trace structures. There is also large systematic error in actually performing the tracing, even when the membrane is visible as morphometric calibrations against known systems will show. And then there are the frequent regions where structures are not clearly defined. What do you do when the membrane cannot be defined?

Another less known approach is the statistical sampling methods called "stereology".[81,104-107] A series of articles from Brenda Russell Eisenberg and her group[83,107-116] shows the power of these methods in a biological and functional context.

Stereological methods exploit the fact that it is not necessary or even wise to extract all the structural information in a single micrograph or section. Many sections and micrographs must be measured if the tissue or organ is to be adequately sampled. The variance of estimation produced by using only part of the data from each micrograph is often not significant in this process. The sampling process turns out to be much less tedious than one might expect, particularly when aided by modern computer technology and many clinically interesting systems might well benefit from stereological analysis. A search for stereological software will be helpful.

Once the extracellular and intracellular volumes, and membrane areas are determined, the next step is the construction of a model. An important part of constructing a model are the properties of the components of the model. The intracellular medium enters into essentially every biological model, and its representation was the source of controversy years ago.[117,118] The issue is now settled. In electrical models, the intracellular medium is a resistance.

The intracellular medium can safely be modeled as a resistance because current goes around obstacles, even very dense obstacles; see Sevostianov *et al.*[119] that builds on the still useful 1873 analysis of Maxwell.[120] Impedance measurements in which current flows around the structure are rarely useful.[121,122] The membrane resistance serves to isolate and define the inside of a cell. The membrane makes a cell an obstacle to electricity. The membrane is such a high resistance[123-129] that current flows in a parallel path even if that path is only a 10 nm width of salt solution. It is only when intracellular objects are within a Debye length or so (typically less than a nanometer in biological salt solutions) that current flow in the intracellular solution is significantly impeded, beyond the reduction of cross-sectional area.

This simple fact was not apparent to early workers in electrophysiology, some of whom insisted on attributing selectivity and other special properties to the cell cytoplasm[117,118] instead of the membrane where we now know they reside. We now know that biology uses proteins in membranes (as it uses proteins in so many other places) to provide selectivity and otherwise control many cellular processes. Few if any biological processes are controlled by the cytoplasm in general, although local concentrations of specific chemicals—organics and calcium ions—are often used for control.

The modern view is that complex electrical properties are not present even in the extraordinarily packed interior of skeletal muscle fibers.

2.4. Measurements of impedance

Measurements were made of the impedance of skeletal muscle[130,131] because they are the most extreme example of an organized dense cytoplasm. **If there is any system in which cytoplasm might produce significant complexities in current flow, it is skeletal muscle.**

Specifically, measurements were made of the longitudinal impedance down a very long length of a single muscle fiber. There is hardly any cell more solidly packed with proteins and structures than skeletal muscle, as a glance at its ultrastructure will show. If current is significantly impeded in its longitudinal flow in any tissue, it should be in skeletal muscle. The diameter of typical frog skeletal muscle fibers might be 50 μm and their inherent longitudinal periodicity is that of a sarcomere, say 2 or 3 μm (depending on stretch). Thus, measurements over a centimeter or so of fiber length give ample opportunity for interference in resistive current flow by the organelles and proteins within each sarcomere.

The dissection of centimeters of single fibers in these experiments was a significant personal achievement of graduate student Joe Leung, aided by his appropriate use of an air delivered pharmacological agent to reduce his already minimal hand tremor. The results were striking. The current and voltage down the length of a muscle fiber were related by a resistor with almost no phase change even though the muscle fiber appears entirely filled with contractile proteins and membranes when viewed in the electron microscope. The resistance representation was precise within 1% in the cases with the largest error, and was unmeasurable in the great majority of muscle fibers, whether skinned or intact.

The next choice of circuit component that must be made is the representation of the membrane itself. For strictly electrical measurements, as used in linear impedance analysis, the membrane was represented as a capacitor, with some success in a range of papers studying lipid bilayers starting with the life's work of Denis Haydon,[132-140] then moving to nerve axon,[141-145] skeletal muscle, cardiac muscle, and the lens of the eye.[84-99] Various epithelia[146-153] were analyzed this way in a literature starting with Clausen *et al.*[146] using the methods described by Clausen and Eisenberg.[76-78]

For analysis of more general properties beyond linear circuit properties, a realistic representation of transport properties of membrane proteins is required, and a linear representation is inadequate. The actual nonlinear properties of channels and transporters must be known, of course, but that is not enough. The spatial distribution of membrane proteins is needed. And it should go without saying that the properties of the channels and pumps must be measured in solutions that are a reasonable approximation to their natural environment. It is not satisfactory, for example, to study a channel or transporter in millimolar concentrations of calcium ion on both sides of the transporter molecules, when in life they face intracellular calcium concentrations of some 10^{-8} M that change the properties of the channel or transporter if the calcium concentration is changed.

3. Results

Decades of work has shown biologically what is obvious physically: the type, properties, and location of transporters (and their density) determine the flows and thus the function of many tissues and organs of great biological importance. Kidneys and transporting epithelia have been extensively studied and are a major success of physiology and biophysics.[154-158] The distribution of channels is also known from work on myelinated and unmyelinated nerve, and skeletal and cardiac muscle, to be dominant determinants of their function.

Unfortunately, many interesting tissues, of great clinical importance, have not been studied well enough experimentally to allow as specific prediction of flows as would be helpful.[159-164] The types of transporters and channels and their locations must be measured in those tissues and organs, and the input-output properties of those proteins must be known before any theory can be expected to provide robust realistic results. For example, one must expect the location of transporters to be as important for the glymphatic hypothesis of flow in the brain[47,50-53,55,56,165-167] as the location of channels and transporters is in the kidney.[168-171] Much can be done when that information is available as we shall see in studies of transport in the lens of the eye, and we will use that example of success in this paper.

3.1. The lens of the eye

The electrical properties of the lens of the eye were resolved by Rae and Eisenberg,[172] who explained the substantial differences between measurements made with single microelectrodes and two microelectrodes. The single microelectrode measurements obscured the interesting membrane properties because of three-dimensional effects[123] analyzed first here[173,174] and later by methods of singular perturbation theory.[124-129,175-177] The misleading three-dimensional effects can be easily removed once theory shows where they come from. Then single electrode and two electrode measurements are in agreement. Otherwise, they differ by orders of magnitude producing confusion and recrimination between laboratories using different methods.

The lens was described as a bidomain syncytium consisting of two domains, one intracellular and the other extracellular, connected by membranes very much in the spirit of the description of the transverse tubular system of skeletal muscle fibers, as reported (for example) here[58,59,75-79] and subsequently reviewed.[80,81]

The models of the lens exploited the important fact that membranes were capacitors, in parallel with resistors (in impedance measurements), while cytoplasm was a resistance. A rather complete analysis of the tissue was possible leading to many useful results.[84-99]

It is of great importance that the models were built from defined structures with components with definite known properties. Otherwise models degenerate into uncertainty, with approximations made in different laboratories in different

ways, that are hard to reconcile. Such approximations lead to more heat than light in the interactions of scientists, who are after all human beings, particularly when they are forced to compete for resources.

The essential feature of the lens[86,172,178-184] that separates it from almost all other tissues is its transparency. That transparency means the lens cannot contain blood vessels, which would seriously degrade optical performance. The lens is a living tissue that is maintained in its function for some 60 or 70 years in most humans. It must clear metabolic wastes and supply nutrients throughout its large (centimeters) cytoplasm. Other cells in the body are only a few micrometers from blood vessels. Indeed, as students at University College (London) Physiology and Biophysics were taught in the 1960s: animals are made of cells decorating their circulatory system. That is how extensive a circulatory system is needed so that convection (*i.e.*, blood flow) can replace ineffective diffusion.

The **lens uses convection to supply and support its cytoplasm, but its convection is driven by an osmotic pump, not by the mechanical pump of the heart.** The electrical analysis of the lens is thus of limited interest and could only be a part of the understanding the lens itself when extended to include water flow by Richard (Rick) Mathias more than anyone else.

Mathias and his collaborators have extended the structural analysis of electrical properties of the lens to the analysis of the vital function of convection. It has been his life's work and is (in my view) a triumph of biophysics, theory and experiment.[95,179,180,182,183,185-202] Indeed, Mathias resolved (to my satisfaction) several damaging controversies in epithelial physiology[154,203-205] as he studied water flow in the lens and in a more general context.[206-208]

3.2. Two domain model

Mathias built his analysis on the two domain approach of the electrical models but included convection and water flow using an engineering circuit approach[95,185,206,207,209,210] written in the same spirit of approximation as the cable equation of Kelvin[211,212] that he used to design the Atlantic cable, "a thread across the ocean."[213] The Atlantic cable provided the first high speed (electrical) communication between Europe and the United States, cutting communication delays from weeks to seconds or minutes. The cable equation was used in biology, where it played a crucial role in our understanding of nerve conduction[214-216] and it was not thought necessary to derive it, because it seemed so obvious physically (with a few exceptions, important when cables had large diameters, or recording was done on or near point sources of current[123-129,173-177,217-220]).

As satisfying as the engineering treatment of the lens was to me personally, it was not found convincing I fear by biologists and clinicians unfamiliar with cable theory or even elementary circuit methods. And to be fair, water flow is not current flow, and the possibility existed that the engineering model might prove less solid a foundation for water flow than for the flow of electricity.

3.3. Fluid flow Starling equation

Huaxiong Huang (the leader of the project) at the Fields Institute (Toronto) and Shixin Xu (his immensely capable postdoctoral fellow and then faculty colleague) were interested in analyzing convection and flow in biological systems with significant structure and asked if I might help their graduate student Yi Zhu (at York University, Toronto) choose and formulate a problem. We chose the lens as our initial project because of the magnificent experimental work of the Mathias group, that went from molecules to pressure measurements, with electrical structure worked out along the way, not knowing of course whether we could do this problem at all, let alone how it would relate to the Mathias engineering approach.

Our essential challenge was that we approached the problem as mathematicians unwilling to start with anything except the structure of the tissue, conservation laws, and simple known representations of the properties of channels and membranes. No one had solved such a complex system of partial differential equations, as far as we knew, in this context, although Weinstein had done something in this spirit in the specialized (and very important) context of the kidney.[170,221-225]

We wished to avoid simplified models using ordinary differential equations for mathematical, scientific, and human reasons. Mathematically, the ordinary differential equations of (chiefly) models lumped in space could not be derived without dealing with the full field equations and conservation laws. Scientifically, it was entirely unclear to us how to make such lumped models (or other simplified models) that would be valid with one set of unchanging (more or less) parameters over the range of conditions of clinical and physiological interest. Different conditions and different questions might well need different approximate (or lumped) models. After all, the Taylor expansions of nonlinear differential equations are notoriously complex and different ranges of parameters yield very different approximations. (I have never forgotten the graph and apparently simple ordinary differential equation that Julian Cole used to start his course on asymptotics, shown in Fig. 2.3.2 in Kervorkian and Cole,[175] and the surrounding discussion.) Humanly, we had lived through controversies comparing simplified models in which investigators could not find a common ground for comparison, and found it easier to find fault than to solve problems.

We found to our surprise (and to my horror) that the fundament transport law called the Starling equation in the physiology literature for more than a century (since 1896[226]) did not have a mathematically satisfying derivation despite the best efforts of many.[227-233]

The central issue, from our point of view, was the classical treatment of ionic solutions as simple liquids (nearly ideal gases, so well presented in previous work[30-34]). It seemed clear to us that ionic solutions had too many too complex forms of energy (and flow and dissipation) to fit comfortably in a tradition that so

thoroughly depended on the theory of ideal (*i.e.*, perfect) gases (made of particles that did not interact at all, and had no internal structure) ***at equilibrium (with zero flows*** of all components) from which it grew.[234]

Consider for example the simple issue of incompressibility. While it is certainly true that ionic solutions as a whole are nearly incompressible, as is pure water, this fact is highly misleading. The individual components of ionic solutions change number density enormously (*i.e.*, concentration) as location and conditions change. Ionic solutions are mixtures of compressible fluids in which the 'entropic' energy stored by variable concentrations are major determinants of the behavior of these systems. These systems are nonideal: **the finite size of ions (and many other effects) mean that the free energy of any one species depends on every other.**

In other words, everything in these systems is coupled. Surprisingly, it is an historical fact that distinguished biochemists and biophysical chemists like John Edsall were unaware as late as the 1970s that activity coefficients were functions of the type and concentration of all ions. They taught students like the author about nonideal solutions, with activity coefficients less than one. The reality that these activity coefficients depend on everything, and thus that properties of components of solutions are coupled to concentrations of other components was not taught. Indeed, it is absent in the "independence principle" used indiscriminately by Hodgkin and Huxley for ions in solution, ions in selective channels, and ions in non-selective channels.

Even the compressibility of the total solution and water itself comes into play when nonideal solutions are considered. The small compressibility of water and ionic solutions turned out to be important later when Jinn Liang Liu and I tried to construct a theory of the free energy of ionic solutions in a series of some ten papers, reviewed in our paper.[38] If this compressibility were not included self-consistently, we could not compute properties of the so called simple primitive model in which water was spheres along with the spheres of ions. Perhaps others have had the same problem, although we are not sure. The practical consequences are important. Experimental properties of ionic solutions cannot be computed and, in that sense, cannot be understood, in my opinion, unless a self-consistent theory can be built. See p. 351 in Vera and Wilczek-Vera[41] for an experimental perspective and judgement as strong as my own.

3.4. Fields cannot be constant

Turning to more general issues that require a more complex theory, we look at the central fact that is present in a wide range of theories and simulations over an enormous range of scales.

Force fields like electrical potential can never be assumed to have a specific shape (like constant fields) independent of conditions.[2,12,15,235-246] As conditions change, even a little bit, the shape of the electrical potential (*i.e.*, the electrical force field) changes a lot. One suspects similar considerations apply to the force fields

used in molecular dynamics, for example. Can one really expect the force fields for sodium ions in bulk solution with low concentration (say 200 mM) and tiny electric fields to be the same as those for sodium ions in an ionic channel with molar (or higher) concentration and fields of 100 mV in say 2 nm?

These changes in electric field are dramatically illustrated by semiconductors. The changes in field determine the properties of bipolar transistors in which the charge carriers are nearly ions, namely holes and electrons.

The central property of bipolar transistors is rectification.[7,247-255] Rectification is produced by changes in the shape of the field. The field is never constant. The field must always be computed from the charges, even in Brownian motion theory[238,256] as Mott[257] quickly learned as he tried to understand real rectifiers. Mott quickly abandoned the idea of constant fields.[258,259] The constant field Goldman-Hodgkin-Katz (GHK) theory of selectivity (popularized by Hille[260]) leaned heavily on Mott's prewar work, as Hodgkin and Cole (Goldman's PhD supervisor) made clear to me in many conversations around 1962. But Mott abandoned that theory just as it was introduced to biophysics,[261,262] where it metastasized into nearly universal (mis) use.[260] This strong language is warranted because constant field GHK does not even include the main properties of a channel protein that surely control permeability. It does not include charge, diameter of ions, diameter of a pore, and so on.

Simple liquid theory, let alone the theory of ideal gases, cannot deal with the reality of ions in devices, channels, transporters, enzymes, and near nucleic acids.

3.5. Ions are often crowded where they are important

The ions in ionic solutions are usually crowded where they are most important:[263,264] inside ion channels[265-267] or near electrodes[38-40,268] (of batteries and electrochemical cells) or in active sites of enzymes[269] where number densities of more than 20 M are often found. (For comparison, solid NaCl is something like 40 M.) At these number densities, the ionic solutions cannot be described in any useful way as ideal gases, simple liquids, or fluids. **A great deal of energy is involved in their nonideal properties**. And interactions are profoundly important. The activity coefficient (or if you prefer the excess free energy) of each ion depends on the number densities of every other ion in a solution. Sadly this simple fact was not known to me as a student or for many years and I do not believe was understood by many biophysical chemists who taught me,[270,271] although I of course could be mistaken in this supposition.

The systems of biology usually depend on flow for their actual function. These systems are not close to equilibrium in the Green-Kubo sense.[272] Indeed, when used in devices, these systems always have power supplies needed for their function. The biological device of the nerve axon for example does not exist as a device if there are no power supplies (here gradients of concentration of ions).

Power supplies require spatially nonuniform boundary conditions that drive substantial flow. An equilibrium treatment of such devices is silly and is likely to be

fundamentally misleading as a study of amplifiers would be with zero volt power supplies, or the study of an automobile engine with water in the gas tank.

Flow produces friction[273] as is obvious when one remembers that ionic solutions are condensed phases with little empty space. Ideal gases have no velocity or flow or friction for that matter. Real atoms and molecules are not at all ideal. They collide (*i.e.*, change direction) at enormous rates (think 10^{15} times per second). Even four collisions of deterministic trajectories produce nearly random (Gaussian) noise. All motion in ionic solutions is dissipative, and theories[260,274] that do not include friction or other form of dissipation are as pointless as theories of reproduction of mammals that do not deal with two classes of structure (and function), male and female. These issues were discussed some time ago[3,275-284] and became clear to me as I learned of the thesis work of Kim Cooper,[285] which was later extended in our work together.[286-288]

For all these reasons, the classical approach based on the theory of ideal gases and simple liquids that described ion membranes without mention of the charge or structure of membranes or proteins[261,262] (popularized by Hille[260]) needed to be replaced by something that was not so obviously false.

Fortunately, in the last decades, a theory of complex fluids has been developed that allows treatment of remarkably complex problems,[289] in which energy is stored ***and dissipated*** as it flows far from equilibrium. The theory of complex fluids deals with many types of fields and flows consistently (*i.e.*, the diffusion, migration, and bulk flow of the ions and water in electrolyte solutions), and is mathematically well defined, as a variational principle developed by Chun Liu, more than anyone else. The EnVarA approach (for Energy Variational Approach) was named[26] with the help of Fred Cohen and is described in the tutorial lectures[18] that yield well defined field theories—usually partial differential equations—in structures defined by boundary conditions.[19] The partial differential equations are fully coupled field theories including dissipation in which "everything interacts with everything else" as it obviously must in the concentrated salt solutions so important to life, and our electrochemical technology.[20-25] Ionic solutions need to be dealt with by mathematics appropriate for these physical realities.[29,38,239,263,264,290-295]

3.6. Plasmas of life are complex fluids

The first task of our group (led by Huaxiong Huang, Shixin Xu, and Zilong Song) was then to apply EnVarA to derive a transport equation like Starling had written, but one that included all interactions consistently. To do this required a reworking of the techniques of EnVarA. The usual methods had to be modified to accommodate membranes within the domain as found in the lens and most other syncytial tissues and not just in boundary conditions on the membranes defining the outer borders of the system. That took some time, but the result was pleasing: we rederived the Starling equation, rather to our surprise.

It should be clearly understood, however, that we were not able to deal with

nonideal ionic components. The full treatment of flow for ions with substantial excess (electro)chemical potentials (*i.e.*, with activity coefficients much less than one) remains for the future. The essential difficulty is dealing with the dependence of the excess electrochemical potential (*i.e.*, activity coefficient) on every ionic species in real solutions. The classical assumption that activity coefficients of an ion (say Na^+) depend only on the Na^+ concentration must be replaced by the reality that the activity coefficient depends on all ion concentrations, *e.g.*, the activity coefficient of Na^+ depends on the concentration of K^+. This cross-dependence can have profound practical effects throughout chemical science as Wolfgang Nonner[266] discovered when we evaluated the activity coefficients of Ca^{2+} from tables of EGTA solutions used throughout chemistry and biochemistry,[296-300] particularly the biochemistry of contractile proteins. The activity coefficient of Ca^{2+} was assumed to be the same in equimolar Na^+ and K^+ solutions. Our results showed it was not. And of course it could not be, given the different excess chemical potentials and activity coefficients of these ions.

3.7. Structural model of the lens

Using variational treatment of bulk solutions, the next task was to construct a structural model of the lens. This was an easy task, following in the footsteps of the Mathias group (*op. cit.*) whose structural model we essentially copied using their representation of the anatomy and histology of the lens, and the distribution of connexins that connect lens fibers. We used a much more primitive spatial distribution of channels and that needs to be improved in future work. The results have been recently published in a bidomain model of lens circulation[301] with even more details in the thesis of Yi Zhu.[302]

3.8. More detail: derivation of the field equations

It is tempting to just write down the physically intuitive field equations for the bidomain model[129,177]– correct, or rather not incorrect, as that approach might be–using the general approach of the structural analysis of electrical properties[79,84,123-125,303-305] conjoined to a representation of fluid flow. An abundance of caution and mis-steps using this approach in our several careers, motivated a complete derivation from conservation laws themselves, and indeed a derivation of the underlying fluid flow laws like Starling's equation.[306] Such an approach also makes it more difficult to quibble about the best of multiple representations, since conservation laws are conservation laws, with representations as integral or differential equations that are not in dispute. The following derivation follows that of Zhu *et al.* closely, lest simplification lead to inadvertent ambiguities.

The derivation follows the approach used for the analysis of electrical structure[85,88,176] joined with the laws of fluid flow[306] using the variational approach to the theory of complex fluids[26] of Liu and collaborators.[18-25] The conservation law for each species of ions is:

$$\begin{cases} \int_{S_e} J^i_{ex} \cdot n_v dS + \int_{S_i} J^i_{in} \cdot n_v dS = 0, \\ \int_{S_e} J^i_{ex} \cdot n_v dS - \int_{S_m} J^i_m \cdot n_m dS = 0, \end{cases} \tag{1}$$

where we define symbols below. A physical discussion of the symbols and their meaning is found in the original paper.[302]

$$\begin{aligned} J^i_l &= u_l C^i_l - C^i_l M^i \nabla \mu^i_l \qquad l \in \{ex, in\}, \\ \mu^i_l &= k_B T ln(C^i_l) + z^i e \varphi_l, \\ J^i_m &= \alpha \left(j^i + a^i \right) n_m, \\ j^i &= g^i \frac{1}{e z_i} \left[(\varphi_{in} - \varphi_{ex}) + \frac{k_B T}{e z_i} ln\left(\frac{C^i_{ex}}{C^i_{in}} \right) \right]. \end{aligned} \tag{2}$$

The j^i are passive fluxes driven only gradients of electrical and chemical potential of the ions. ATP hydroysis is not involved. Following classical derviations of conservation partial differential equations, and vector differential operators, we approximate surface integral on S_e and S_i:

$$\int_{S_e} J^i_{ex} \cdot n_v dS = \int_{S^x_e} J^i_{ex} \cdot \overrightarrow{dS} + \int_{S^y_e} J^i_{ex} \cdot \overrightarrow{dS} + \int_{S^z_e} J^i_{ex} \cdot \overrightarrow{dS}. \tag{3}$$

$$\begin{aligned} \int_{S^x_e} J^i_{ex} \cdot \overrightarrow{dS} &\approx \Delta V \left[\frac{\mathcal{M}^x_{ex}\ (x + \frac{\Delta x}{2}) J^i_{ex} \cdot e_x (x + \frac{\Delta x}{2}, y, z) - \mathcal{M}^x_{ex} (x - \frac{\Delta x}{2}) J^i_{ex} \cdot e_x (x - \frac{\Delta x}{2}, y, z)}{\Delta x} \right] \\ &\approx \Delta V \frac{\partial \left(\mathcal{M}^x_{ex}\ J^i_{ex} \cdot e_x \right)}{\partial x}. \end{aligned} \tag{4}$$

By Equation (2), we have the approximation:

$$\int_{S_m} J^i_m \cdot n_s dS \approx \Delta V \mathcal{M}_v \alpha \left(j^i + a^i \right). \tag{5}$$

By Equations (3), (4), and (5), we have:

$$\begin{cases} \nabla\cdot\left(\mathcal{M}_{ex}J^i_{ex}\right)+\nabla\cdot\left(\mathcal{M}_{in}J^i_{in}\right)=0, \\ \nabla\cdot\left(\mathcal{M}_{ex}J^i_{ex}\right)-\alpha\mathcal{M}_v\left(j^i+a^i\right)=0, \end{cases} \tag{6}$$

where the $\mathcal{M}_{ex}$ and $\mathcal{M}_{in}$ are both diagonal matrices with:

$$\mathcal{M}_{\ell}=\begin{bmatrix} \mathcal{M}^x_{\ell}(x), & 0 & , & 0 \\ 0 & , & \mathcal{M}^y_{\ell}(y), & 0 \\ 0 & , & 0 & , & \mathcal{M}^z_{\ell}(z) \end{bmatrix}, \qquad \ell \text{ is either } ex \text{ or } in\}.$$

Mass conservation law gives:

$$\begin{cases} \int_{S_e}u_{ex}\cdot n_v dS+\int_{S_i}u_{in}\cdot n_v dS=0, \\ \int_{S_e}u_{ex}\cdot n_v dS-\int_{S_m}u_{ex}\cdot n_m dS=0. \end{cases} \tag{7}$$

Trans-membrane velocity u_m is given by:

$$\begin{aligned} u_m\cdot n_m &= -L_p(P_{ex}-P_{in})+L_p\sigma RT(O_{ex}-O_{in}). \\ O_l &= \sum_i C^i_l, \qquad l\in\{ex,in\}. \end{aligned} \tag{8}$$

Similarly, Equation (7) is approximated by:

$$\begin{cases} \nabla\cdot\left(\mathcal{M}_{ex}u_{ex}\right)+\nabla\cdot\left(\mathcal{M}_{in}u_{in}\right)=0, \\ \nabla\cdot\left(\mathcal{M}_{ex}u_{ex}\right)-\mathcal{M}_v\left[-L_p(P_{ex}-P_{in})+L_p\sigma RT(O_{ex}-O_{in})\right]=0. \end{cases} \tag{9}$$

Darcy's law[307] is used to eliminate u_{ex} and u_{in} in Equations (6) and (9).

$$\begin{cases} u_{ex}=-\dfrac{\kappa_{ex}}{\mu}\left[\nabla P_{ex}+e\nabla\varphi_{ex}\left(\sum_i z^i C^i_{ex}\right)\right], \\ u_{in}=-\dfrac{\kappa_{in}}{\mu}\left[\nabla P_{in}+e\nabla\varphi_{in}\left(\sum_i z^i C^i_{in}\right)\right]. \end{cases} \tag{10}$$

The potentials φ_{ex} and φ_{in} are determined from our previous work:[88,176]

$$\begin{cases} \nabla\cdot(g_{ex}\nabla\varphi_{ex})+\nabla\cdot(g_{in}\nabla\varphi_{in})=0, \\ \nabla\cdot(g_{in}\nabla\varphi_{in})-\mathcal{M}_v Y_m(\varphi_{in}-\varphi_{ex})=0, \end{cases} \tag{11}$$

where:

$$g_{ex}=\begin{bmatrix} \tau_{ex}^x\sigma_{ex}^x\mathcal{M}_{ex}^x, & 0, & 0 \\ 0, & \tau_{ex}^y\sigma_{ex}^y\mathcal{M}_{ex}^y, & 0 \\ 0, & 0, & \tau_{ex}^z\sigma_{ex}^z\mathcal{M}_{ex}^z \end{bmatrix}, \tag{12}$$

$$g_{in}=\begin{bmatrix} g_c^x+\tau_{in}^x\sigma_{in}^x\mathcal{M}_{in}^x, & 0, & 0 \\ 0, & g_c^y+\tau_{in}^y\sigma_{in}^y\mathcal{M}_{in}^y, & 0 \\ 0, & 0, & g_c^z+\tau_{in}^z\sigma_{in}^z\mathcal{M}_{in}^z \end{bmatrix}.$$

We combine Equations (6), (8), (9), and (11), to produce:

Bidomain Model

$$\begin{cases} \nabla\cdot\left(\mathcal{M}_{ex}J_{ex}^i\right)+\nabla\cdot\left(\mathcal{M}_{in}J_{in}^i\right)=0, \\ \nabla\cdot\left(\mathcal{M}_{ex}J_{ex}^i\right)-\alpha\mathcal{M}_v\left(j^i+a^i\right)=0, \\ \nabla\cdot\left[\frac{\kappa_{ex}}{\mu}\mathcal{M}_{ex}\left(\nabla P_{ex}+e\nabla\varphi_{ex}\left(\sum_i z^i C_{ex}^i\right)\right)\right]+\nabla\cdot\left[\frac{\kappa_{in}}{\mu}\mathcal{M}_{in}\left(\nabla P_{in}+e\nabla\varphi_{in}\left(\sum_i z^i C_{in}^i\right)\right)\right]=0, \\ \nabla\cdot\left[\frac{\kappa_{ex}}{\mu}\mathcal{M}_{ex}\left(\nabla P_{ex}+e\nabla\varphi_{ex}\left(\sum_i z^i C_{ex}^i\right)\right)\right]+\mathcal{M}_v\left[-L_p(P_{ex}-P_{in})+L_p\sigma RT(O_{ex}-O_{in})\right]=0, \\ \nabla\cdot(g_{ex}\nabla\varphi_{ex})+\nabla\cdot(g_{in}\nabla\varphi_{in})=0, \\ \nabla\cdot(g_{in}\nabla\varphi_{in})-\mathcal{M}_v Y_m(\varphi_{in}-\varphi_{ex})=0. \end{cases} \tag{13}$$

where:

$$J_l^i=-C_l^i\frac{\kappa_l}{\mu}\left[\nabla P_l+e\nabla\varphi_l\left(\sum_i z^i C_l^i\right)\right]-C_l^i M^i\nabla\mu_l^i, \qquad l\in\{ex,in\}. \tag{14}$$

The bidomain model is complex because the underlying tissue is complex. Biology imposes a structure. Physics and biology impose boundary conditions on the structure, by the properties of the channels and lipids of the structure. Biophysics imposes the specific values of these properties. The specific values matter. Just as engineering devices behave properly and perform their function only in a specific environment of power supply voltages and so on, biological devices only function under specific conditions. They, and engineering devices, "die", *i.e.*, cease to function in other conditions. We are interested in functioning bidomain models, so we present the parameters under which the model has been shown to function properly (Table 1).

Table 1. Parameter values

Parameter	Value	Ref	Parameter	Value	Ref
M_v	$6 \times 10^1 m$	[3]	e	$1.6 \times 10^{-19}\ C$	
R_{ex}	$4.85 \times 10^6\ ohm \cdot m$	[3]	k_B	$1.38 \times 10^{-23}\ J \cdot K^{-1}$	
R_{in}	$6.25 \times 10^4\ ohm \cdot m$	[3]	R	$8.314\ J \cdot mol^{-1} \cdot K$	
μ	$7 \times 10^{-4}\ Pa \cdot s$	[9]	T	$310\ K$	
$\boldsymbol{\tau_{ex}}$	0.16	[8]	M^{Na}	$0.3249\ m^2 \cdot s^{-1} \cdot volt^{-1} \cdot C^{-1}$	[6]
$\boldsymbol{\tau_{in}}$	1	[8]	M^K	$0.4769\ m^2 \cdot s^{-1} \cdot volt^{-1} \cdot C^{-1}$	[6]
$\boldsymbol{\sigma_{ex}}$	$1/(4.85 \times 10^6 \times 0.16 \times S_{ex}/S_T)$	[1]	M^{Cl}	$0.4956\ m^2 \cdot s^{-1} \cdot volt^{-1} \cdot C^{-1}$	[6]
$\boldsymbol{\sigma_{in}}$	$1/(6.25 \times 4 \times 1 \times S_{in}/S_T)$	[1]	g^{Na}	$2.2 \times 10^{-3}\ S \cdot m^{-2}$	[8]
σ	1	[9]	g^{Cl}	$2.2 \times 10^{-3}\ S \cdot m^{-2}$	[8]
L_p	$1.34 \times 10^{-13}\ m^3 \cdot N^{-1} \cdot s^{-1}$	[7]	κ_{in}	$5.159 \times 10^{-13}\ m^2$	[9]
C_m	$7.9 \times 10^{-3}\ F \cdot m^{-2}$	[1]	κ_{ex}	$1.33 \times 10^{-16}\ m^2$	[9]
G_m	$4.38 \times 10^{-3}\ ohm^{-1} \cdot m^{-2}$	[1]			

3.9. More detail

The full model could be computed with little difficulty using a combination of home-grown programs and library programs in **MATLAB**. Parameters could be robustly determined by the extensive experimentation available. Fits to experimental data required no further adjustment of parameters and were very good, almost embarrassingly so as far as at least one of the authors was concerned (however, good structural models are often found to fit good data well, as exemplified elsewhere[59,76,78,88]).

The perturbation properties of the full model could be studied. That is to say, classical Taylor expansions (with occasional consideration of singular situations as done in previous work[124,172-175] a long time ago) allow construction of a simplified model. The errors in the simplified model were determined by direct comparison with the full computed solution, over a range of parameters and conditions although all pertinent parameters and conditions could not be tested of course. Thus, this computing of errors remains an unbounded iterative process. We are always

confronted with new conditions, new experiments, new testing of the reduced models. I point out this process because the reduced perturbation models give important physical and biological insight that is easily lost amongst the complete calculation of the model, which correctly determine everything (within the accuracy of the model specification itself, of course). But everything is such a large set that nothing may be understood as a result (of our human limitations).

The perturbation model allowed derivation of the Mathias engineering model. Our simplified model (determined, I repeat, by actual perturbation and Taylor expansion not by guessing, however inspired) was a slight but significant generalization of the Mathias engineering model.

We could study the effects of the effective resistance of connexins, showing an interesting qualitative bifurcation in quantitative properties predicted (and measured). The intracellular pressure changed dramatically as the effective connexin conductance was changed but many variables were essentially unchanged. In our model, all variables are related to the potential of the extracellular space within the lens, except the hydrostatic pressure. The potential of the extracellular phase within the lens hardly changes with effective resistance of the connexins, and so only the hydrostatic pressure changes significantly as the effective resistance of the connexins is changed.

The perturbation structure of the problem shows the mathematical reason the potential of the extracellular phase within the lens does not vary with connexin density. Physically and physiologically it seems obvious that changes in the intracellular domain would have only second order effects on the potential of the extracellular phase within the lens because they are separated by a high resistance membrane that buffers the extracellular from the intracellular region (see similar effects in other systems, such as a qualitative general discussion[123] and other quantitative special cases that were computed[125-128]).

The insensitivity of measurements to changes in connexin effective resistance has been a cause of concern in the lens experimental literature as I read it.[183,198] It seems likely that much of the experimental literature of the lens (sampled by several works[184,200,201,308-314]) will benefit from an understanding of the role of the potential of the extracellular phase within the lens and its insensitivity to many parameters.

4. Optic nerve of the amphibian salamander

We turn now to the generalization of the bidomain model of flow in the lens to the tridomain model of the optic nerve of the salamander *Necturus*.

The approach to tissue complexity described and advocated here depends on careful connection of structural and experimental work, emphasizing known biophysical properties of membranes, channels, and transporters. It grew out of the tradition of Cambridge (England) Physiology, driven by Alan Hodgkin more than anyone else, of choosing preparations given us by evolution in which complexity is not overwhelming, and physiological function is evident.

The history is evident in the study of the nerve impulse that carries information throughout the nervous system. The squid axon[315-317] was chosen as a model preparation[318-321] after Hodgkin, as a student, showed that the nerve impulse was conducted by electric current[322,323] as opposed to the chemical hypothesis of the then leader of English Biophysics, the Nobel Laureate AV Hill.[324] The squid axon was large enough to allow control of potential using the voltage clamp method of KS Cole,[318-321,325] but results were difficult to interpret because of the fear that concentrations of ions would change (in a process quaintly called "concentration polarization" then and still today[326-328]). Hodgkin, Huxley, and Katz seemed to have always left out the word "concentration", thus creating singular confusion between their "concentration polarization" (which was a concentration change driven by current flow) and the dielectric (and vacuum) polarization of Maxwell, Heaviside, and followers (*i.e.*, all physicists), for whom polarization did not include the translation of ions (rather for them polarization was the rapid small reversible change in distribution of charge in solids, molecules, and atoms and the mysterious displacement current found everywhere, including a vacuum). Wang[329] has recently shown how the energetic variational approach EnVarA[26] of Liu and collaborators[18-25] can be used to reunite both meanings of polarization, in a way that would have brought a smile to Hodgkin's face, more likely than not.

Once a dual electrode system was placed inside the squid axon, making what is usually called a four electrode recording system, the artifactual concentration polarization around the current electrode was of no importance, but Hodgkin, Huxley, and Katz were surprised to find a slow (say 4 msec) change in current (in their voltage clamped axon) that might represent the change of concentration of ions near the squid membrane. It did not for the most part, but Hodgkin and Huxley were careful to leave open the possibility later demonstrated by Frankenhaeuser and Hodgkin[330] that some of the slower changes were the result of accumulation of potassium in the narrow space outside of the nerve membrane, a result made even more plausible by the theory of Taylor and Bezanilla.[331] The arguments for potassium accumulation were however somewhat indirect, and required a greater knowledge of channel biophysics than was widespread in the 1950s, so it was desirable to find a way to measure the potassium concentration in the narrow extracellular space

outside the nerve directly, or to find an optical way to measure the membrane potential that depended on potassium flow in a different way.[332]

Following the tradition of choosing the right preparation, Kuffler investigated the accumulation of potassium[333,334] in the optic nerve of an amphibian salamander, the mudpuppy *Necturus* found in the wetlands of Eastern North Carolina. Kuffler had studied the optic nerve[335-337] because it, as a part of the central nervous system, is made of three domains: nerve cell, extracellular space, and glia. Kuffler had shown that the glia are an electrical syncytium, so all three domains are regions of salt solutions of relatively small longitudinal and three-dimensional effective resistivity. The glia have a membrane potential (*i.e.*, measured at very low frequencies) that responds to potassium. So Kuffler could use the membrane potential recorded inside the glia to measure the potassium concentration immediately outside the nerve membrane.

I present here the precise specification of the electrical part of the tridomain model[338] lest an important detail be inadvertently omitted. Presenting the precise specification of the flow part of the tridomain would be too much for this paper. It is also not necessary: the material is presented in complete detail in the overlapping publications.[167,302,338,339] The many pages devoted to validation and calibration are of particular importance, although not presented here because of space limitations.

4.1. Ion transport

We define the regions:

1. Ω_{OP} consist of axon compartment
2. Ω_{ax} axonal interior
3. Ω_{gl} glial compartment
4. Ω_{ex}^{OP} extracellular space.

In addition there are regions. For example, the subarachnoid space Ω_{SAS} that has only extracellular space.

The conservation of chemical species implies the following system of partial differential equations to describe the dynamics of ions in each of the three regions, for $i = \mathrm{Na}^+, \mathrm{K}^+, \mathrm{Cl}^-$

$$\frac{\partial(\eta_{gl} c_{gl}^i)}{\partial t} + \mathcal{M}_{gl} J_{gl}^{m,i} + \nabla \cdot (\eta_{gl} \mathbf{j}_{gl}^i) = 0, \text{in } \Omega_{OP}, \tag{15}$$

$$\frac{\partial(\eta_{ax} c_{ax}^i)}{\partial t} + \mathcal{M}_{ax} J_{ax}^{m,i} + \frac{\partial}{\partial z}(\eta_{ax} j_{ax,z}^i) = 0, \text{in } \Omega_{OP}, \tag{16}$$

$$\frac{\partial(\eta_{ex} c_{ex}^i)}{\partial t} - \mathcal{M}_{ax} J_{ax}^{m,i} - \mathcal{M}_{gl} J_{gl}^{m,i} + \nabla \cdot (\eta_{ex} \mathbf{j}_{ex}^i) = 0, \text{in } \Omega_{OP}, \tag{17}$$

where the last equation reduces to the following in the subarachnoid Ω_{SAS} region:

$$\frac{\partial c_{ex}^{i,SAS}}{\partial t} + \nabla \cdot \mathbf{j}_{ex}^{i,SAS} = 0. \tag{18}$$

The transmembrane ion flux $J_k^{m,i}(k = gl, ax)$ consists of active ion pump source $J_{p,k}^i$ and passive ion channel source $J_{c,k}^i$ on the k membrane:

$$J_k^{m,i} = J_{p,k}^i + J_{c,k}^i, k = gl, ax, i = \mathrm{Na}^+, \mathrm{K}^+, \mathrm{Cl}^-.$$

On the glial cell membranes, $J_{c,gl}^i$ is defined as:

$$J_{c,gl}^i = \frac{g_{gl}^i}{z^i e}(\varphi_{gl} - \varphi_{ex} - E_{gl}^i), i = \mathrm{Na}^+, \mathrm{K}^+, \mathrm{Cl}^-, \tag{19}$$

where the Nernst potential is used to describe the gradient of chemical potential

$$E_{gl}^i = \frac{k_B T}{e z^i} \log\left(\frac{c_{ex}^i}{c_{gl}^i}\right)$$

for ith ion species in the glial membrane and the conductance g_{gl}^i is a fixed constant independent of voltage and time. On the axon's membrane, $J_{c,ax}^i$ is defined as:

$$J_{c,ax}^i = \frac{g_{ax}^i}{z^i e}(\varphi_{ax} - \varphi_{ex} - E_{ax}^i), i = \mathrm{Na}^+, \mathrm{K}^+, \mathrm{Cl}^-,$$

where:

$$g_{ax}^{Na} = \overline{g}^{Na} m^3 h + g_{leak}^{Na}, g_{ax}^K = \overline{g}^K n^4 + g_{leak}^K, g_{ax}^{Cl} = g_{leak}^{Cl}.$$

The time dependent dynamic of open probability, often loosely called 'gating' is governed by the Hodgkin-Huxley model:[319,321,325,340,341]

$$\begin{aligned}
\frac{dn}{dt} &= \alpha_n(1-n) - \beta_n n, \\
\frac{dm}{dt} &= \alpha_m(1-m) - \beta_m m, \\
\frac{dh}{dt} &= \alpha_h(1-h) - \beta_h h,
\end{aligned}$$

Here,n is the open probability of the K^{+}channel. m is the open probability of the Na^{+}activation gate, and is the open probability of the h inactivation gate.

We assume that the only pump is the Na/K active transporter. We are more than aware that other active transport systems can and likely do move ions and thus water in this system. They will be included as experimental information becomes available.

In the case of the Na/K pump $J_{p,k}^{i}(k=ax,gl),$ the strenght of the pump I_k depends on the concentration in the intracellular and extracellular space[342], *i.e.*:

$$J_{p,k}^{Na}=\frac{3I_k}{e},J_{p,k}^{K}=-\frac{2I_k}{e},J_{p,k}^{Cl}=0,k=gl,ax, \tag{21}$$

where:

$$\begin{aligned} I_k &= I_{k,1}\left(\frac{c_k^{Na}}{c_k^{Na}+K_{Na1}}\right)^3\left(\frac{c_{ex}^{K}}{c_{ex}^{K}+K_{K1}}\right)^2 \\ &+I_{k,2}\left(\frac{c_k^{Na}}{c_k^{Na}+K_{Na2}}\right)^3\left(\frac{c_{ex}^{K}}{c_{ex}^{K}+K_{K2}}\right)^2,k=ax,gl. \end{aligned} \tag{22}$$

$I_{k,1}$ and $I_{k,2}$ are related to the maximum current of α_1- and α_2- isoform of Na/K pump on the glial membrane$(k=gl)$ or axon membrane $(k=ax)$.

The definitions of ion flux in each domain are as follows, for $i=\mathrm{Na}^{+},\mathrm{K}^{+},\mathrm{Cl}^{-}$:

$$\begin{aligned} &\mathbf{j}_l^i=c_l^i\mathbf{u}_l-D_l^i\tau_l\left(\nabla c_l^i+\frac{z^ie}{k_BT}c_l^i\nabla\varphi_l\right),l=gl,ex, \\ &j_{ax,z}^i=c_{ax}^iu_{ax}^z-D_{ax}^i\left(\frac{\partial c_{ax}^i}{\partial z}+\frac{z^ie}{k_BT}c_{ax}^i\frac{\partial\varphi_{ax}}{\partial z}\right). \end{aligned}$$

For the axon compartment and glial compartment boundary condition, we have:

$$c_{ax}^i=c_{ax}^{i,re},\text{on}\,\Gamma_2\quad\Gamma_6, \tag{23}$$

and:

$$\begin{cases} \mathbf{j}_{gl}^i\cdot\widehat{\mathbf{r}}=0, & \text{on}\,\Gamma_1, \\ c_{gl}^i=c_{gl}^{i,re}, & \text{on}\,\Gamma_2\quad\Gamma_6, \\ \mathbf{j}_{gl}^i\cdot\widehat{\mathbf{r}}=0, & \text{on}\,\Gamma_7, \end{cases} \tag{24}$$

Here, the Dirichlet boundary conditions are used at locations for axons and glial cell, and a nonflux boundary condition is used for glial cells, ions, flux, and pia matter. For the extracellular space boundary condition, similar boundary conditions are imposed except on the pia matter The flux across the pia matter is assumed

continuous and Ohm's law is used.[338] Additionally, a nonpermeable boundary condition is used at location, and a homogeneous Neumann boundary condition is applied at the location of the dura matter .

$$\begin{cases} \mathbf{j}_{ex}^{i} \cdot \widehat{\mathbf{r}} = 0, & \text{on } \Gamma_1, \\ c_{ex}^{i} = c_{csf}^{i}, & \text{on } \Gamma_2 \quad \Gamma_3, \\ \nabla c_{ex}^{i} \cdot \widehat{\mathbf{r}} = 0, & \text{on } \Gamma_4, \\ \mathbf{j}_{ex}^{i} \cdot \widehat{\mathbf{z}} = 0, & \text{on } \Gamma_5, \\ c_{ex}^{i} = c_{eye}^{i}, & \text{on } \Gamma_6, \\ \mathbf{j}_{ex}^{i,OP} \cdot \widehat{\mathbf{r}} = \mathbf{j}_{ex}^{i,SAS} \cdot \widehat{\mathbf{r}} = \dfrac{G_{pia}^{i}}{z^{i} e}(\varphi_{ex}^{OP} - \varphi_{ex}^{SAS} - E_{pia}^{i}), & \text{on } \Gamma_7. \end{cases} \tag{26}$$

We define:

$$\delta_{7,l}^{i} = \frac{\Delta c_{l}^{i,*}}{c_{l}^{i,*}}, i = \mathrm{Na}^{+}, \mathrm{K}^{+}, \mathrm{Cl}^{+}, l = ax, gl, ex.$$

so the ion fluxes can be written as:

$$\widetilde{\mathbf{j}}_{l}^{i} = Pe_{l}^{i} \delta_{7,l}^{i} \widetilde{c}_{l}^{i} \widetilde{\mathbf{u}}_{l} - (\delta_{7,l}^{i} \widetilde{\nabla} \widetilde{c}_{l}^{i} + z^{i} \widetilde{c}_{l}^{i} \widetilde{\nabla} \widetilde{\varphi}_{l}), l = gl, ex,$$

$$\widetilde{j}_{ax,z}^{i} = Pe_{ax}^{i} \delta_{7,l}^{i} \widetilde{c}_{l}^{i} \widetilde{u}_{ax}^{z} - \left(\delta_{7,l}^{i} \frac{\partial \widetilde{c}_{l}^{i}}{\partial \widetilde{z}} + z^{i} \widetilde{c}_{l}^{i} \frac{\partial \widetilde{\varphi}_{l}}{\partial \widetilde{z}} \right),$$

with Peclet numbers:

$$Pe_{ax}^{i} = \frac{u_{ax}^{*} z^{*} c_{ax}^{i,*}}{D_{ax}^{i} \Delta c_{ax}^{i,*}}, Pe_{l}^{i} = \frac{u_{l}^{*} r^{*} c_{l}^{i,*}}{D_{l}^{i} \tau_{l} \Delta c_{l}^{i,*}}, l = gl, ex. \tag{27}$$

If we let $g_{l}^{*}, l = ax, gl$ be the characteristic membrane conductance $k_B T/e$ be the characteristic electric potential, the dimensionless form of transmembrance flux is: $\widetilde{J}_{l}^{m,i} = \widetilde{J}_{c,l}^{i} + \widetilde{J}_{p,l}^{i}$,

where for $i = \mathrm{Na}^{+}, \mathrm{K}^{+}, \mathrm{Cl}^{-}, l = gl, ax,$

$$\widetilde{J}_{c,l}^{i} = \frac{\widetilde{g}_{l}^{i}}{z^{i}}(\widetilde{\varphi}_{k} - \widetilde{\varphi}_{ex} - \widetilde{E}_{gl}^{i}), \widetilde{J}_{p,l}^{i} = \frac{J_{p,l}^{i} e^{2}}{k_B T g_{l}^{*}}.$$

The governing equations for ions become:

$$\frac{\partial(\eta_{gl}\widetilde{c}^{i}_{gl})}{\partial\widetilde{t}}+\delta^{i}_{8}\widetilde{J}^{m,i}_{gl}+\delta^{i}_{9}\widetilde{\nabla}\cdot\left(\eta_{gl}\widetilde{\mathbf{j}}^{i}_{gl}\right)=0, \tag{28}$$

$$\frac{\partial(\eta_{ax}\widetilde{c}^{i}_{ax})}{\partial\widetilde{t}}+\delta^{i}_{10}\widetilde{J}^{m,i}_{ax}+\delta^{i}_{11}\frac{\partial}{\partial\widetilde{z}}\left(\eta_{ax}\widetilde{j}^{i}_{ax,z}\right)=0, \tag{29}$$

$$\frac{\partial(\eta_{ex}\widetilde{c}^{i}_{ex})}{\partial\widetilde{t}}-\delta^{i}_{12}\delta^{i}_{10}\widetilde{J}^{m,i}_{ax}-\delta^{i}_{13}\delta^{i}_{8}\widetilde{J}^{m,i}_{gl}+\delta^{i}_{14}\widetilde{\nabla}\cdot(\eta_{ex}\widetilde{\mathbf{j}}^{i}_{ex})=0, \tag{30}$$

where:

$$\widetilde{\nabla}\cdot(\eta_{l}\widetilde{\mathbf{j}}^{i}_{l})=\frac{1}{\widetilde{r}}\frac{\partial(\widetilde{r}\eta_{l}\widetilde{j}^{r,i}_{l})}{\partial\widetilde{r}}+(\delta_{0})^{2}\frac{\partial(\eta_{l}\widetilde{j}^{z,i}_{l})}{\partial\widetilde{z}},l=gl,ex,$$

$$\delta^{i}_{8}=\frac{t^{*}\mathcal{M}_{gl}g^{*}_{gl}k_{B}T}{c^{i,*}_{gl}e^{2}},\delta^{i}_{9}=\frac{D^{i}_{gl}\tau_{gl}t^{*}}{(r^{*})^{2}},$$

$$\delta^{i}_{10}=\frac{t^{*}\mathcal{M}_{ax}g^{*}_{ax}k_{B}T}{c^{i,*}_{ax}e^{2}},\delta^{i}_{11}=\frac{D^{i}_{ax}t^{*}}{(z^{*})^{2}},$$

$$\delta^{i}_{12}=\frac{c^{i,*}_{ax}}{c^{i,*}_{ex}},\delta^{i}_{13}=\frac{c^{i,*}_{gl}}{c^{i,*}_{ex}},\delta^{i}_{14}=\frac{D^{i}_{ex}\tau_{ex}t^{*}}{(r^{*})^{2}}.$$

The symbol Δf is used to denote the variation of the variable f from its resting state. Multiplying the Equations (23-25) with $z_i e$ respectively, summing up, and using the charge neutrality condition, we have the following system for the electric fields in ax,gl,ex ,

$$\sum_{i}z^{i}e\mathcal{M}_{gl}J^{m,i}_{gl}+\sum_{i}\nabla\cdot(z^{i}e\eta_{g}\mathbf{j}^{i}_{gl})=0, \tag{31}$$

$$\sum_{i}z^{i}e\mathcal{M}_{ax}J^{m,i}_{ax}+\sum_{i}\frac{\partial}{\partial z}(z^{i}e\eta_{ax}j^{i}_{ax,z})=0, \tag{32}$$

$$\sum_{i}z^{i}e\nabla\cdot(\eta_{gl}\mathbf{j}^{i}_{gl})+\sum_{i}\frac{\partial}{\partial z}(z^{i}e\eta_{ax}j^{i}_{ax,z})+\sum_{i}\nabla\cdot(z^{i}e\eta_{ex}\mathbf{j}^{i}_{ex})=0, \tag{33}$$

In the subarachnoid space Ω_{SAS}, the extracellular equations reduce to:

$$\sum_i \nabla \cdot \left(z^i e \sum_i \mathbf{j}_{ex}^{i,SAS} \right) = 0. \tag{34}$$

The boundary conditions for electric fields $\varphi_{ax}, \varphi_{gl}$ and φ_{ex} are given below.
In the axon compartment:

$$\begin{cases} \nabla \varphi_{ax} \cdot \widehat{\mathbf{z}} = 0, \ \text{on } \Gamma_2, \\ \nabla \varphi_{ax} \cdot \widehat{\mathbf{z}} = 0, \ \text{on } \Gamma_6, \end{cases} \tag{35}$$

In the glial compartment:

$$\begin{cases} \nabla \varphi_{gl} \cdot \widehat{\mathbf{r}} = 0, \ \text{on I} \\ \nabla \varphi_{gl} \cdot \widehat{\mathbf{z}} = 0, \ \text{on I} \\ \nabla \varphi_{gl} \cdot \widehat{\mathbf{z}} = 0, \ \text{on I} \\ \nabla \varphi_{gl} \cdot \widehat{\mathbf{r}} = 0, \ \text{on I} \end{cases} \tag{36}$$

and in the extracellular space:

$$\begin{cases} \nabla \varphi_{ex} \cdot \widehat{\mathbf{r}} = 0, & \text{on } \Gamma_1, \\ \nabla \varphi_{ex} \cdot \widehat{\mathbf{z}} = 0, & \text{on } \Gamma_2 \quad \Gamma_3, \\ \nabla \varphi_{ex} \cdot \widehat{\mathbf{r}} = 0, & \text{on } \Gamma_4, \\ \nabla \varphi_{ex} \cdot \widehat{\mathbf{z}} = 0, & \text{on } \Gamma_5, \\ \nabla \varphi_{ex} \cdot \widehat{\mathbf{z}} = 0, & \text{on } \Gamma_6, \\ \sum_i z^i e \mathbf{j}_{ex}^{i,OP} \cdot \widehat{\mathbf{r}} = \sum_i ? z^i e \mathbf{j}_{ex}^{i,SAS} \cdot \widehat{\mathbf{r}} & \\ = \sum_i G_{pia}^i (\varphi_{ex}^{OP} - \varphi_{ex}^{SAS} - E_{pia}^i), & \text{on } \Gamma_7. \end{cases} \tag{37}$$

This concludes the formal presentation of this component of the tridomain model. The rest is found in the original overlapping papers;[167,302,338,339] the entire model, and its components, are validated and calibrated at length in those publications. To repeat: the validation and calibration are at least as important as the derivation.

4.2. Implications: potassium ions are a toxic waste

I turn now to the implications of this analysis. It should be clearly understood that accumulated potassium is a toxic waste, a hazard to both the signaling function of neurons and their survival as cells. Signaling is blocked by high potassium (because of inactivation of sodium channels more than anything else). Cell volume changes when potassium is elevated and with enough external potassium, cells will burst and die.

Potassium accumulation is important in many biological and clinical applications. Potassium accumulation and flow in the extracellular space have been shown to have important roles in aging, Alzheimer's disease, anesthesia, dementia, diabetes, epilepsy, migraine, sleep, stroke, and traumatic brain injury, as well as an important role in the biology of the central nervous system.[47,49,50,52,53,55,56,100,101,343-362] Indeed, Filipidis *et al.*[363] have identified many such systems in biology containing three intercalated sets of tissues, including pleura, peritoneum, pericardium, fetal membranes, and leptomeninges citing the significant references.[364-393] Each of these tissues likely include intercalated syncytia and thus require multidomain models of the type we have studied. Hopefully, a structural analysis using conservation laws and stereological estimates of structure, along with the biophysics of the type and distribution of channels and transporters will help understand each of these tissues in health and disease.

While I was aware of the classical physiological literature on potassium accumulation (as the many words of this review illustrate), I was insensitive, and mostly unaware of the important literature on other related systems. I apologize for my inadvertent neglect of appropriate references.

The reader will benefit from reading different approaches from ours in the work of several laboratories, including those of Mori,[55,354,394-404] Ellingsrud,[405-410] Sacco,[411] and the many groups interested in glymphatics, sampled elsewhere.[47-57] While few if any of these papers deal with potassium accumulation in tridomain systems, no doubt their methods could be usefully applied to those issues. Sadly, I am so distracted by other work—on the Maxwell equations, models of polarization as a stress strain relation of charge density, the voltage sensor of sodium and potassium nerve channels, and most recently (and speculatively) the stochastic analysis of the mechanics of hydrogen bonds—that I have been unable to focus on those other methods.

Huaxiong Huang led our efforts (with his graduate student Yi Zhu and postdoctoral fellow then faculty colleague Shixin Xu) to create a tridomain model able to deal with the Orkand data on potassium accumulation in optic nerve[333,334] as he had led the team analyzing the lens. See Acknowledgement.

4.3. Clearance of potassium ions from the extracellular space

Turning to the results of our analysis and simulations,[338,339,412] I discuss the clearance of potassium from the extracellular space. Only in the idealized world of the classical squid axon experiments is a single action potential enough to study. In the real world, trains of action potentials are frequent. Potassium accumulated after these many action potentials acts as a toxic waste. As it accumulates, potassium will depolarize the nerve membrane (*i.e.*, make the resting potential more positive) and that in turn will inactivate sodium channels and so block the action potential. Extracellular potassium poisons nerve signals this way. Extracellular potassium can also kill the nerve fiber itself (as well as its signal). When potassium accumulates sufficiently it will diffuse into the nerve fiber, along with water, and that diffusion can overwhelm the pumping mechanisms that maintain homeostasis of cell volume. In plain English, the potassium swells the nerve until it bursts, killing the nerve. The mechanism of potassium clearance thus is of particular biological interest since it is needed to maintain nerve life, and thus animal life, as well as the nerve signal.

To determine that mechanism, we built a model[338,339,412] of all three of the domains of the optic nerve including the coupled migration (in the electric field), diffusion (in the concentration field), and convection (in the diffusion field of water) of sodium, potassium, and chloride ions, and water itself. We could not predict ahead of time which of the flows in which of the domains would be used by evolution for potassium clearance. And we hasten to say that we do not know which of the flows (and the channels and transporters that control the flow) will be most important for other functions of tridomain systems in the brain, and elsewhere.

We could not build a simplified transport model, or a lumped model in which compartments had a single concentration of ions and water independent of location, before we analyzed the entire system. And we were convinced from study of similar systems, that if we did guess a compartment model, it would not be the same as that of other investigators, with just as good intuition and probably greater knowledge than ours.

Confronted with different models of the same results, it seemed unlikely that progress would be rapid. The unavoidable competition of different research groups for resources (and recognition) and the absence of an agreed upon universal model has slowed research in other epithelia for decades,[413-415] and it seemed it would do so here as well. So ***we constructed what seemed to us to be a complete model of 21 partial differential equations and 3 ordinary differential equations.*** We used this model to describe the structures actually used by the optic nerve to clear potassium from the extracellular space during a train of action potentials.

This much structural detail is not arbitrary. It is needed because that is how evolution has built its system of potassium clearance. There is some arbitrariness, particularly in the choice of channels open at long times and the choice and distribution of transporters, but our results were robust enough to allow some conclusions. Experimental data will resolve these issues and be easy to incorporate

into our model and its implementations. It is important to note that other functions may depend critically on the distribution and properties of channels and pumps and so the experimental measurement of those properties and distributions will be critical, in my opinion, in the application of these methods to crucial biological and clinical problems, from waste clearance in sleep to glaucoma.

When the differential equations were solved (including the full computation of the Hodgkin-Huxley nerve model on the time scale of the action potential), clearance was found to be greatly enhanced by the syncytial nature of the glial domain. The syncytium allowed the glia to function as a pipe to clear waste, with the flow in the pipe driven by the diffusion of water, more than anything else. The diffusion of water was in turn the result of the difference in sodium and potassium movements across the glial membrane.

We had not foreseen this mechanism, and hasten to add that we cannot conclude that the same mechanism occurs in other related systems, as clear as the mechanism of waste clearance was in this optic nerve. For example, if the glia are connected to blood vessels through aquaporins, it is possible (although unlikely in my opinion) that qualitative properties will change significantly.

A calculation of this sort must be extensively checked to see how sensitive its results are to its complexity. We were surprised to find how sensitive flows were to the spatial distribution of transporters and channels. When these were varied stochastically (in space) we found substantial effects.

Thus, we are led to the unavoidable reality. While we can understand quite well the mechanisms of waste clearance in the optic nerve of *Necturus*, we cannot be sure the same mechanisms dominate in the brain in general.

We can however be reasonably certain that the model we have used can be modified to accommodate flow into blood vessels. We can be sure it can be modified to include other transporters and channels, in other distributions, because we have actually shown that by building such models. We can be reasonably certain that a full structural model is feasible and almost certainly needed to understand the structural and molecular basis of flow in cells and tissues in general, including the brain, including in sleep.

5. Conclusions and future perspectives

It is tempting to reach sweeping conclusions from the powerful tridomain theory and the successful analysis of the optic nerve of a salamander. But it is after all only a salamander that we have studied, and its optic nerve does not contain blood vessels and so is not a wise preparation in which to study flow into blood vessels, as occurs in the mammalian brain.

What does seem clear is that the approach and model we have constructed can be generally applied, because it is based on observed biological structure, measured

biophysical properties of those structures, and of the protein transporters and channels embedded in those structures, using conservation laws likely to be as true in biology as in the rest of the world.

Our approach is as faithful as it can be to the details of that structure because we know from long experience that details matter in physiological systems as they do in engineering systems. What seem small inconsequential details often wind up as controls for the entire system. After all, the action potential is a big system involving meters of nerve fibers, but it is controlled by a few protein channels in the cells that initiate the action potential. Thus, I do not speculate. I advocate real work.

It seems clear that the path to understanding (and clinical control) of fluid flow in brain function requires:

1. Construction of a structural model based on conservation laws and the structure(s) of clinical and biological interest. For example, studying the glymphatic hypothesis (as sampled in the literature[47-57]) is likely to require construction of a four domain model involving blood vessels (of variable size and shape and properties) along with glia, extracellular space, and axon. We see no sign that there will be serious theoretical, mathematical, or numerical obstacles to building such a model, although the work involved should not be underestimated, nor the skill level needed to construct its equations, and compute them, with appropriate perturbation expansions for understanding, as Shixin Xu, Yi Zhu, and Zilong Song have done, with the help and leadership of Huaxiong Huang.
2. The identification of the types and locations (and densities) of the various pumps and channels used by the genome to control function. Experiments to acquire this data can be done but they are as tedious as they are necessary. Frankly, it is not clear they will be done until the community of biologists and clinicians are convinced that they are needed. **One of the main roles of a complete structural model is to convince biologists to make the measurements needed to understand function.**
3. The calculation of the biological function. Is the waste actually cleared? Can the spatial distribution of transporters (for example the sodium potassium pump) or its upregulation in sleep actually explain waste clearance in the brain, thus supporting the glymphatic hypothesis in a more or less quantitative way?

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