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Multi-scale modelling and the IUPS physiome project

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Summary

We review the development of models of cellular and tissue function and in particular address issues of multi-scale modelling, including the transition from stochastic models to continuum models and the incorporation of cell and tissue structure. The heart is used as an example of linking models at the molecular level to cell, tissue and organ level function.

Introduction

Mathematical modelling provides a means of summarizing quantitative knowledge about complex systems. Models can be used both to interpret complex experimental data and to formulate new experiments (Noble 2002a), and to obtain information that cannot be directly observed. At the scale of tissues and organs the modelling approach has loosely been called 'computational physiology' (Noble 2002b, Hunter & Borg 2003). The methodology that has been the most successful in yielding physiological insights at the tissue and organ level has been continuum modelling. Here continuous spatially varying fields are defined to represent physical quantities such as voltage, stress, strain, ion concentrations and so on. These are typically represented by finite element meshes, where a quantity is represented over a computational element (a region of tissue) using basis functions (interpolating polynomials – see review by Smith *et al.* 2004c). The governing equations representing physical laws such as conservation of charge, mass and momentum are formulated in terms of the finite element fields and solved subject to appropriate boundary conditions. These equations and computational methods are well established in physical and engineering sciences (every new car and aircraft, for example, is designed using such methods).

As concerns continuum modelling, the unique aspect of biological tissues is not the governing physical laws, but rather the material properties of the tissues, and how these properties are affected by subcellular processes that reach down to proteins and gene regulation. The spatial scales relevant to physiological processes encompass nano-scale molecular events to metre-scale intact organ systems, a range of 10^9, and temporal scales from Brownian motion (microseconds) to a human lifetime (10^9s), a range of 10^{15}. Clearly this range of scales cannot be represented by one model but rather requires a hierarchy of models and modeling approaches such as stochastic models of ion channels and receptors for ligand binding calculations, ordinary differential equation lumped cell models, and partial differential equation continuum models at the tissue and organ levels. It also requires the model parameters at one scale to be linked to detailed models of structure and function at a smaller spatial scale – hence the need for "multi-scale modeling".

In this article we address the issue of multi-scale modelling in computational physiology, first discussing stochastic models of molecular events, spatial aspects of cell function, and model simplification based on analysis of spatial and temporal scales. We then illustrate multi-scale modelling by considering the development of a hierarchy of models addressing the electro-mechanical function of the heart, from stochastic molecular events through cell and tissue levels to whole organ function.

Modelling *in vivo* biochemical kinetics

At the molecular level, many if not all biological processes consist of sequences of discrete, random events. This includes processes such as interactions between protein molecules, protein-ligand reactions, ion channel gating, and so on. For many such processes the molecules are always present in large numbers and continuum limits can confidently be assumed. For example, biochemical reactions typically are described by continuum representations where each molecular species is described in terms of a concentration (or chemical activity), and reaction rate expressions are in

general predicated on this basis, using the law of mass action to relate reaction rates to concentrations. This empirical relation was postulated more than a century ago to describe observations about the rate of chemical reactions measured *in vitro*. It has in general been assumed that rate laws accurately describing *in vitro* dynamics will also be suitable and applicable to describe the biochemistry of *in vivo* reactions.

In many biological situations, however, only small quantities of molecules are involved. In cellular regulatory networks, for example, a single RNA polymerase binds to DNA to initiate gene transcription. mRNA is often produced in very low copy numbers, making a continuum description in terms of concentrations, and continuous time dynamics, an inappropriate framework for characterising such systems. Furthermore, this may be significant for the regulation of pathways downstream of this particular gene product (McAdams & Arkin 1997). In this case a stochastic modelling approach is necessary.

Stochastic models

A stochastic model of a biochemical pathway keeps track of the exact number of molecules of each type, known as the *state* of the system. The probability of a collision between molecules, and hence of a reaction occurring, is proportional to the number of molecules of each type present, i.e. on the current state of the system. In this way, at a given point in time the probability of a reaction occurring can be calculated for each potential interaction between molecules. The time evolution can, in principle, be described using the master equation approach, for which linear differential equations in the probabilities of different states of the system are solved for a chosen set of initial conditions. In practice, however, the number of different states is often prohibitively large, and a solution is found more efficiently using a probabilistic Monte Carlo simulation approach. Monte Carlo methods use random numbers to determine which, if any, of the possible reactions takes place during each time step, according to the reaction probabilities. If the time step is small enough then the assumption can be made that at most one reaction will occur per iteration. Following each reaction event in the simulation, the state of the system is updated and the reaction probabilities recalculated for the next iteration. Gillespie (1977) proposed a variation of this approach in which the time to the next reaction is calculated according to a Monte Carlo simulation on the probabilities. The algorithm proceeds by taking variable length time steps, and provides an exact equivalent simulation of the master equation. Repeated runs of the algorithm (using a different set of random numbers!) provide a number of different instances for the same biochemical pathway, which can be collated to calculate probability distributions and statistics on molecule numbers at a given time point. In the limit of large numbers of molecules, this approach converges exactly on to the law of mass action deterministic solution.

An alternative stochastic algorithm, StochSim, written by Carl Firth (Morton-Firth & Bray 1998) represents each molecule individually. For each iteration a molecule or a pair of molecules is selected at random, each molecule with equal probability. The probability of a reaction occurring is now dependent only on the type of molecules selected (as the probability of their selection is equivalent to the probability of a randomly occurring collision). This approach has been found to be well suited to systems with large numbers of molecular species (for example, proteins with several phosphorylation or methylation states) and with many possible reactions, and was developed specifically for modelling cell signalling networks (Shimizu & Bray 2001). However, because the iteration timescale must be shorter than the timescale of the fastest reaction overall, simulations can be very computationally intensive if the system contains a range of timescales. A different approach to modelling stochasticity is to include the randomness as a stochastic term in a differential equation, known as a Langevin equation. Recently Burrage *et al.* (2004) have shown how this approach can be used to deal efficiently with multi-scale problems, in which there is a range of different reaction timescales, where the direct Monte Carlo integration approach is not efficient.

Simulation of chemical reactions in inhomogeneous environments

The spatial organisation of the intracellular environment has only recently come to the fore in modelling *in vivo* reaction kinetics. The intracellular environment is far from the homogeneous, well mixed solution typically found in the *in vitro* experiments in which reaction rate laws are established and rate parameters are measured. An image of the internal environment of a cell (of the slime mold *Dictyostelium discoideum*) is reproduced in Figure 1, showing the high degree of macromolecular crowding within the cell (Medalia *et al.* 2002). It has been estimated that between 5% and 40% of total cell volume is occupied by macromolecular complexes (large molecules and molecular clusters) – much higher than in a typical biochemical experimental assay (Ellis & Minton 2003). This macromolecular crowding has been shown to affect the rates of reactions. Crowding has been mimicked *in vitro* (Rohwer *et al.* 1998, for example), and shown to affect enzymatic reactions by suppressing the dissociation of the enzyme-substrate complex (Laurent 1971, see also recent reviews by Hall & Minton 2003; Schnell & Turner 2004). Chemical reactions in crowded

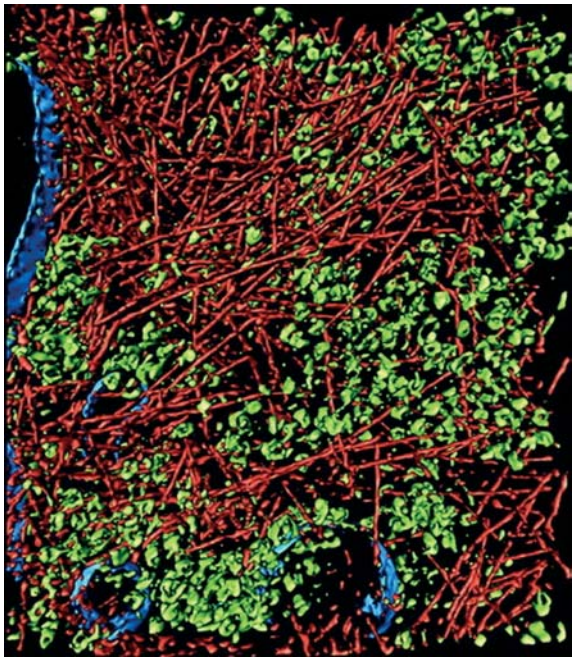

Figure 1. Internal structure of a *Dictyostelium discoideum* cell, showing the actin filament network (red), membranes (blue), and cytoplasmic macromolecular complexes (ribosomes and others, green) in a volume of approximately 815 nm by 870 nm by 100 nm. This image was produced by cryoelectron tomography, in which the cell is rapidly frozen and imaged over a range of tilt planes in the electron microscope. A three-dimensional image is then reconstructed from the two-dimensional slices to give an accurate representation of the structures within the cell. Reproduced from Medalia *et al.* (2002) Science 298, Figure 3A (with permission).

environments have been found to demonstrate fractal-like kinetic properties. Berry (2002) and Schnell and Turner (2004) have recently reported the results of simulations of the single enzyme-substrate reaction in crowded media, using a lattice gas automata model, finding that the enzyme reaction no longer follows the standard Michaelis–Menten relationship. The Monte Carlo approach used in these studies simulates the reaction kinetics on a spatial grid in which some of the lattice points are occupied by obstacles representing the macromolecular crowding of the intracellular environment. Molecules can move between vacant lattice points and react with other molecules encountered, with the reaction probabilities defined in the usual way. The net reaction rates, averaged over the spatial grid, have been found to decrease with time, following the empirical time-dependent relationship $k(t) = k_0(\tau + t)^{-h}$, where k_0 is the ideal (dilute solution) rate constant, and the positive parameters h and τ are found to depend on the number and arrangement of the obstacles (Schnell & Turner 2004).

There are, however, many other situations within the cell in which spatial organisation becomes important, and for which alternative simulation techniques can be applied. Because molecules are represented individually in the StochSim algorithm (Shimizu & Bray 2001, described above), each molecule can be ascribed chemical properties such as phosphorylation state (as we have described) and also physical properties including position. This can be used to simulate reactions within regions of cells in which the conventional 'dilute solution' approximation is not valid. For example, Shimizu *et al.* (2003) have simulated patterns of activity of clustered membrane receptors in a stochastic model of the chemotactic signalling pathway of *E. coli*.

Multi-scale modelling and model simplification

The physiological properties of any cell or organ system are determined and underpinned by a wide variety of sub-cellular processes, many of which may be inherently stochastic. When large numbers of molecules are present stochastic fluctuations are smoothed out and population-averaged properties can be accurately described using a continuum representation. Indeed, it is often the case that the finer detail is not necessary, nor useful even, for understanding function at a higher organizational scale. In the context of cellular electrophysiology, for example, the averaged channel conductance for a population of stochastically gated ion channels can be represented by deterministic ordinary differential equations, and is in general sufficient to understand cell membrane potential data.

In the same way, when a continuum modelling approach is appropriate the wide range of temporal and spatial scales present can often be exploited to produce a model which, although simplified, is quantitatively accurate when viewed at a higher level in the hierarchy of physiological organization. The microstructural detail of a specific biological tissue can be spatially averaged to represent effective tissue properties over macroscopic spatial scales, as for example in the bidomain model for the propagation of electrical excitation in cardiac tissue (Keener & Sneyd 1998). Using spatial averaging techniques ('homogenisation'), it is not necessary to represent cells as individual units in large scale simulations of tissue and organ function.

Similarly, large scale simulations which include the wide range of timescales of different cellular processes, so-called stiff problems, are computationally expensive, prohibitively so for simulations of tissues or organs. Electrophysiological processes in the cell may take place on millisecond scales, or shorter, while metabolic changes take place over seconds or minutes. Therefore in many situations it is useful to derive time-averaged properties over molecular time scales to produce simplified macroscopic models which are quantitatively consistent with the underlying molecular events. Multi-scale approaches must take into account this range of timescales, typically by averaging over processes which are operating at a much faster rate than is of direct interest, and by assuming that much slower processes

remain essentially stationary. There is a standard set of mathematical tools available for these model reduction methods, recently illustrated for modelling ion transport processes in cell electrophysiology and metabolism models (Smith & Crampin 2004b). These techniques can be used to provide simplified representations of cellular processes to be included in higher-level models that couple multiple subcellular processes to form integrative models of cell function. In this fashion we can envisage constructing a hierarchy of models at different spatial and temporal scales, from subcellular processes up to whole organ function, where the parameters of higher level representations are consistent with, and informed by, more detailed models defined over shorter timescales and finer spatial scales. This hierarchical modelling process has been most fully developed for models of the electrical and mechanical properties of the heart, which we discuss below.

Multi-scale modelling of the heart

Over the past several decades, significant progress has been made towards modelling the structure and function of the heart, incorporating detailed electrophysiological, mechanical and structural information into a single computational framework (see Hunter *et al.* 2003, Smith *et al.* 2004c, for reviews). The mechanisms underlying whole organ function are characterised by a range of scales of spatial organization. The pumping capacity of the heart is largely determined by forces actively generated within cardiac myocytes by the cyclic interaction of the myofilament proteins actin and myosin, which in turn is regulated by intracellular ion and metabolite concentrations. Actin and myosin filaments interdigitate to form a regular lattice parallel to the longitudinal axis of the cell, comprising a repeating contractile unit, the sarcomere (which repeats about 50 times along the length of the cell). Projections from the myosin filaments ('myosin heads') attach to sites on the actin filaments to form tension-bearing cross-bridges. Cross-bridges form, undergo conformational change to generate tension, dissociate and reattach in a cycle to propel the thick filaments past the thin filaments, shortening the cell.

The molecular details of this cross-bridge cycle are becoming well understood. The cycle is initiated by a myosin head binding under strain to the actin filament. The probability of attachment is a function of the binding strain. During shortening of the muscle the strain on a cross-bridge is reduced, increasing the probability of its detaching from the actin filament. Once it does detach, it can then reattach at a new value of strain, completing the ratchet cycle, which is repeatedly performed by all cross-bridges in the sarcomere. The stochastic nature of the cross-bridge interactions has implications for the interpretation of data on the molecular details and energetic consequences of muscle contraction (Duke 1999, Smith *et al.* 2004a). In particular, stochastic models have been used to determine the effects of protein filament compliance (the degree to which the filaments yield elastically when force is applied, Forcinito *et al.* 1997, Daniel *et al.* 1998, Martyn *et al.* 2002). In the limit of non-compliant (rigid) filaments, each cross-bridge acts as a separate force generator, independent of the state of the other myosin heads in a sarcomere. In this case, stochastic models can be shown (Smith *et al.* 2004a, see Figure 2) to converge to the classical framework for the sliding filament theory, proposed by Huxley (1957).

Huxley's original model assumes that cross-bridges are either attached or unattached. For a population of independent cross-bridges, the change in the probability that a cross-bridge is attached at a given strain value can be described by a partial differential equation, using strain dependent transition rates for attachment and detachment. This continuum approach has subsequently been extended to incorporate multiple attached and detached states as increasing detail has become available on the energetics and molecular structure of the actin and myosin proteins (Eisenberg *et al.* 1980, Piazzesi & Lomberdi 1995, Smith 1998).

Significant computational gains are achieved by reducing a discrete stochastic model to a system of partial differential equations. However, the numerical solution of Huxley-type models is still computationally prohibitive for large-scale simulations of cardiac tissue. Essentially, this is because the strain characteristics for the population of cross-bridges are still represented, requiring the partial differential equation formulation. To simulate active force generation in a three-dimensional tissue model, one simplification approach is to

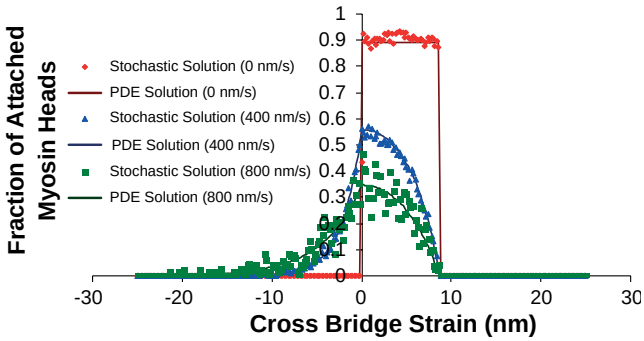

Figure 2. Fraction of attached cross-bridges as a function of cross-bridge strain at steady-state for different shortening velocities calculated using a stochastic model and compared to analytic solutions (solid lines) calculated from the Huxley (1957) sliding filament model of muscle contraction (results reproduced from Smith *et al.* 2004a).

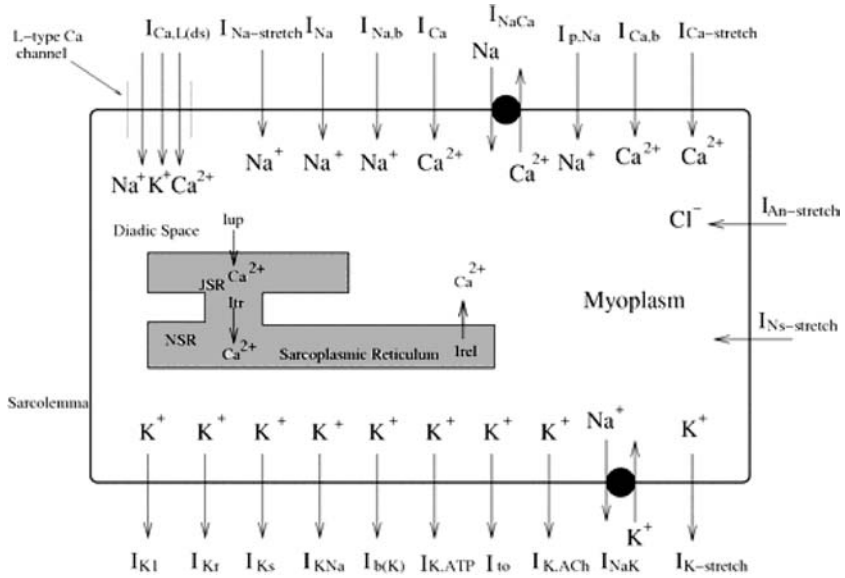

Figure 3. The Noble ventricular cell model, incorporating ion channels, pumps and transporters linked to intracellular calcium transport mechanisms (Noble *et al.* 1998), available from the CellML website (www.cellml.org).

approximate the probability distributions of the sliding filament model with Gaussian functions. Ordinary differential equations can then be derived for the moments of each distribution, corresponding to the population-averaged stiffness, tension and stored elastic energy for the sarcomere (Zahalak 1981, Guccione *et al.* 1998). Alternatively, empirically based models have been developed from experimental measurements of the properties of intact muscle fibres. Kawai and Brandt (1980) measured muscle stiffness as a function of the frequency of an applied sinusoidal length perturbation. In these data, state transitions corresponding to molecular events that occur at rates much faster than the frequency of perturbation will be in phase with the length perturbation. Conversely, processes that are much slower will appear to be stationary in the response of the system, and those occurring at intermediate rates will show a change in magnitude and phase lag with change in frequency. The frequency-stiffness experimental data can be fitted using a transfer function to describe the mechanical response of the tissue (Kawai *et al.* 1993a,b). The model of Hunter *et al.* (1998)[1] combines this characterisation of the mechanical properties of cardiac muscle with the kinetics of calcium-dependent regulation of the proportion of available actin binding sites (and hence the maximum force) to describe active tension generation in the myocyte in response to an intracellular calcium transient, which is the trigger for contraction during each heart beat. Smith (2003) has recently investigated the connection between these approaches, and has developed an efficient computational method for linking the parameters of the biophysically based Huxley-type of model to the more computationally efficient data-driven model of Hunter *et al.* (1998). Such a coupling serves two purposes; firstly, it provides a method of explicitly determining microscopic properties from macroscopic measurement and secondly, it is a way of introducing model detail (and associated computational expense) only when it is required.

The electrophysiological mechanisms underlying the action potential and calcium transient in heart cells have been studied in great detail. Detailed models of myocyte electrophysiology and calcium handling have been developed by a number of authors including Noble and coworkers (DiFrancesco & Noble 1985[2], Noble *et al.* 1998[3]), the Luo and Rudy models (1991[4], 1994[5]) and the Winslow group (Jafri *et al.* 1998[6]). Following Hodgkin and Huxley's (1952) characterization of electrical excitation in the squid axon, these cell models consist of a system of coupled ordinary differential equations describing the dependence of the transmembrane voltage on the various ion channel, pump and exchanger currents, along with their gating variables, and the flux of intracellular calcium from intracellular stores (see Figure 3). Nickerson *et al.* (2001) have combined the models of Noble *et al.* (1998) and Hunter *et al.* (1998) to characterise excita-

[1]http://www.cellml.org/examples/repository/HMT_model_1998_doc.html

[2]http://www.cellml.org/examples/repository/DFN_model_1985_doc.html

[3]http://www.cellml.org/examples/repository/N_model_1998_doc.html

[4]http://www.cellml.org/examples/repository/LR_I_model_1991_doc.html

[5]http://www.cellml.org/examples/repository/LR_II_model_1994_doc.html

[6]http://www.cellml.org/examples/repository/JRW_model_1998_doc.html

tion-contraction coupling in the myocytes, to include stretch-induced changes in membrane conductance and cell extension-dependent binding of calcium to troponin C to regulate the availability of actin sites.

In order to simulate tissue and whole organ function, anatomically based tissue models are developed based on experimental measurements of passive tissue properties, stiffness and conductivity (see Smith *et al.* 2004c, for a review). These properties are strongly influenced by the tissue microstructure, which in the heart is determined by the arrangement the connective tissue that binds individual myocytes into layers of interconnected sheets separated by cleavage planes (Le Grice *et al.* 1995). To simulate the spread of electrical excitation and the generation of tension, the cellular level model is embedded as a spatially distributed grid in an anatomically based tissue framework. Advection-diffusion partial differential equations for the cellular variables (membrane potential, developed tension) are integrated to determine the spread of excitation in the tissue and finite deformation equations are solved to for the change in geometry due contraction (Smith *et al.* 2004c). Figure 4 demonstrates the influence of the heterogeneous microstructure on the spread of excitation though a small block of ventricular tissue and shows how the conduction anisotropy can be approximated as a continuous field at higher spatial scales.

At the whole organ scale, anatomically based models of coronary blood flow embedded in the contracting myocardium provide a means of determining regional oxygen delivery, such that the balance between ATP synthesis and its utilisation (shown in Figure 5(a), as calculated in a model of the heart) within the tissue can be predicted in both health and disease. A mathematical model of coronary geometry is shown in Figure 5(b). The deformation of this geometry, Figure 5(c), as a result of the active tension, is calculated using the cellular mechanics model of Hunter *et al.* (1998) combined with passive constitutive laws and the equations of finite deformation. The coronary blood flow velocity in these vessels induced by contraction of the tissue is shown in the figure.

Discussion

The examples in this paper outline one of the most significant challenges for integrative modelling of physiological processes. One is the challenge of linking models at different spatial scales – for example, interpreting the

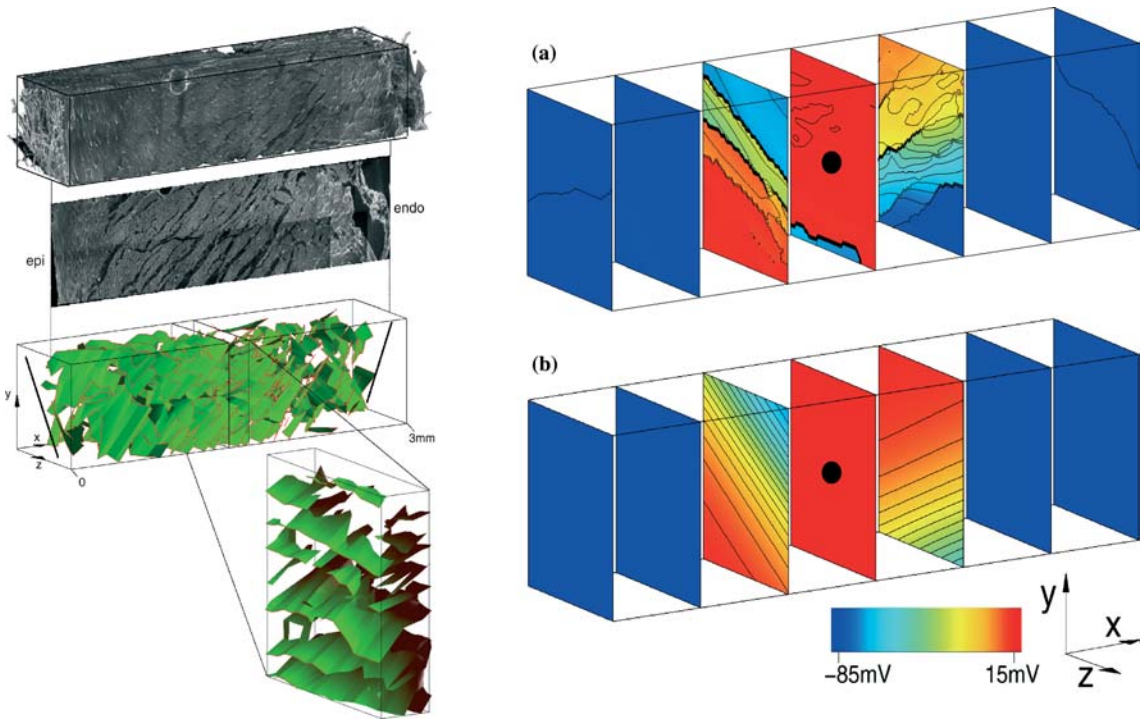

Figure 4. Left upper: reconstructed volume of rat left ventricular free-wall myocardium. *Left middle:* transmural slice from the reconstructed volume showing a complex network of cleavage planes which course between myocyte laminae. *Left lower:* the bilinear finite element geometrical description of the cleavage planes through the entire rat tissue block, and a smaller midwall subsection. Myofiber orientation is shown on the epi- and endo-cardial surfaces. *Right*. Discontinuous model (a) and continuous model (b) potential maps. Transmembrane potentials are mapped on 7 equi-spaced surfaces through the reconstructed rat tissue volume, at 8ms following midwall stimulation. Isopotential lines at 5 mV intervals are shown in black. Site of stimulation is shown with black dot at centre of volume. The cleavage plane obstacles in (a) lead to a highly discontinuous form of propagation, which is however well approximated by the continuous model. From Hooks *et al.* (2002) with permission.

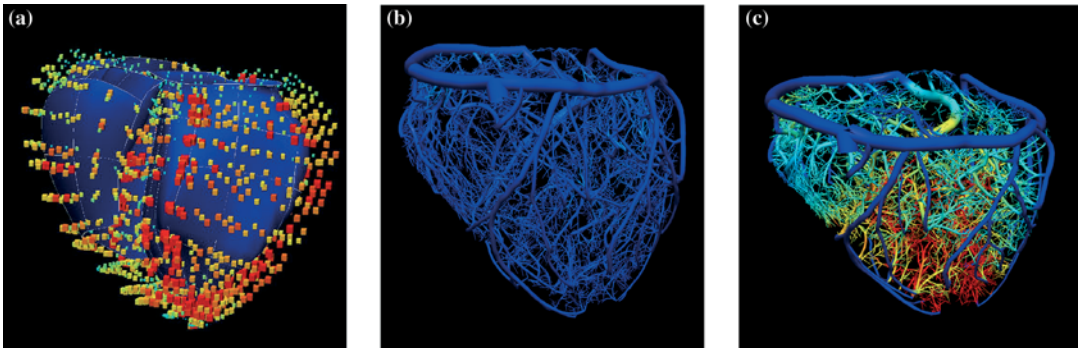

Figure 5. Using cellular models embedded in an anatomically based model of the heart, (a) the regional ATP consumption resulting from the contraction cycle, (b) undeformed coronary vessel geometry (c) deformed coronary vessel geometry and blood induced resulting from contraction.

parameters of a constitutive law describing macroscopic tissue properties in terms of a more detailed model of the tissue microstructure, or using a model of molecular events to predict the average rate constants for a biochemical process. Another multi-scale issue is bridging from stochastic models dealing with individual molecules to continuum models, in particular as the significance of stochastic processes in cells becomes more apparent. A second challenge is to incorporate three-dimensional cell and tissue structure into models of cell and tissue function. For example, at the tissue level the spatial organization of the components of the extra-cellular matrix (ECM) is essential to the mechanical function of the tissue (LeGrice *et al*. 1995), and detailed microstructural information must be incorporated into simulations of the electrical and mechanical properties of tissues as we have discussed. However, the importance of the three-dimensional structure of cells and the organization within the cell is much less well represented in models. Current models of calcium handling in cells distinguish compartments such as T-tubules, the dyadic space, sarcoplasmic reticulum and mitochondria as being distinct from the cytosol, but detailed information on calcium sparks and waves has only recently started to be incorporated into models. Information on the roles of the many different proteins of the cytoskeleton, for example, which maintain the alignment of the cell and transmit molecularly developed stress to adjacent cells, could be obtained from spatially extended cell models. These data on intracellular stresses cannot readily be measured *in situ*, and there is little qualitative understanding of the role of each component, or of how deletion or modification of these proteins, known to lead to various forms of heart failure, may lead to disruption of cell and tissue function.

Five years ago the International Union of Physiological Sciences (IUPS) created a new commission called the 'Physiome Commission' chaired by one of us (PJH)[7]. The IUPS Physiome Project was then begun as an internationally collaborative open-source project to provide a public domain framework for computational physiology, including the development of modeling standards, computational tools and web-accessible databases of models of structure and function at all spatial scales (Noble 2002a,b, Kitano 2002a,b, Hunter *et al*. 2002, Hunter & Borg 2003). The goal of the Physiome Project is to establish a publicly accessible framework for handling the hierarchy of computational models, associated experimental data and publications, that will help integrate knowledge, from the genomic and proteomic levels to whole organ and body scale, into an understanding of physiological function for intact organisms.

[7] The inaugural meeting for the Physiome Project, entitled "On Designing the Physiome Project", was held on July 5–8, 1997, in Petrodvoret, St. Petersburg, Russia, immediately after the 33rd World Congress of IUPS, held in St Petersburg. The IUPS Physiome Commission was established in 1998. This was later merged with the Bioengineering commission into a new 'Physiome and Bioengineering' Committee, chaired by PJH and Prof Aleksander Popel. For further details see www.bioeng.auckland.ac.nz/physiome/physiome_project.php.

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