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The pressure pixel—unit of life?

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Abstract

Life is based on the co-ordinated and efficient function of the molecular nanomachines that biochemists call enzymes. Popular models of these machines are miniature anthropomorphic devices, which function in empty space under conditions bearing little resemblance to the watery subcellular world. The concepts of force and work applicable in our macroscopic world are transposed down to the molecular level where the chaos of thermal energies dominate. Despite four decades of intense research effort, the thermodynamic explanation of water-protein interactions—the first level of living matter—is as remote as ever, because the disruptive thermal energies still remain dominant in these theories today. In this work, it is proposed that the important feature of the condensed medium is the formation of clusters, resulting from the bonded state of the molecules. This new view is the basis of the wave model of liquid structure. It is these water clusters, not single molecules, that are responsible for macroscopic pressure. Pressure is exerted on a size scale down to that of a single cluster, the hierarchical level defined by the 'pressure pixel'. Below this size, tension between molecules prevails. This tension explains the stability and co-ordinated movement of the subcellular world, where theories based on random collisions fail. It also explains the coherence displayed by the cell in its ability to act as a unit, rather than a collection of independent processes predicted by statistical theories. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Living processes imply movement. This in turn implies that they possess machines (enzymes) that are able to convert the chemical energy of metabolism into the physical energy of motion. This movement could be statistical and occur unpredictably, or it could be guided and con-

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trolled by the cell itself. The former view is the generally accepted one, i.e. that chemical reactions occur at random in the cell: a view that has a firm basis in chemical thermodynamics. The fundamental concepts of enzyme kinetics such as activation energy, rate constants, equilibrium constants, and so on, can be traced back to the statistical mechanics of chemical reactions in solution. Reactions proceed back and forth, but on average, they proceed in the direction of the downward Free Energy gradient. The overall picture is then, that (in the case of animal cells) a random mixture of glucose and oxygen of high Free Energy is converted into a random mixture of carbon dioxide and water of low Free Energy in a series of reversible steps. At these steps, increments of Free Energy can be captured from time to time by the energy transducing enzymes and converted into work. Hence, the overall macroscopic effect we observe at any given moment as the cell's activity, e.g. swimming, contraction, ion pumping etc, is the sum of a collection of random activities performed by its molecular machines. In other words, it expresses an uncoordinated average activity.

Cells are typically in the 1–10 μm range. Going down in size, we have around the 100 nm range integrated complexes (organelles) which, when isolated intact, can still function, i.e. they contain the order essential for living matter. However, at around 5 nm we pass from the world of ordered movement to that of random motion. This is the size range of single protein molecules (that of water being below 1 nm), and represents that mesoscopic intermediate world, where independent, coherent, protein mechanisms like enzyme action still operate. Alternatively, viewed in the opposite direction, protein is the first step on the upward path from thermal chaos to living matter.

Let us look in more detail at these machines of life. According to the principles of statistical thermodynamics, the probability that a protein molecule, a chain of some hundred amino acids, will fold into a unique 3D structure is next to zero. Yet as every biochemist knows, proteins—the building blocks of life—do not fold to give billions of states, they fold into a unique 3D structure (conformation) in adopting their functional state. Furthermore, thermodynamic studies tell us that this state is not stable. Such long chains, it is claimed, are constantly folding and unfolding in a chaotic manner due to the all-pervading random collisions of their thermal environment (Cooper, 1976). The subject has been under intense investigation for over 40 years using many techniques, including powerful computer simulations (see Rose and Wolfenden (1993) for a recent review and Richards (1991) for a readable non-technical overview). The fact that little progress has been made is demonstrated by comparing two reviews that follow one another in the same biophysical journal. Both refer to works of some 20 years standing which, it is claimed, still remain the correct view of protein structure. Yet that quoted by Frauenfelder et al. (1988) "…the molecules must be conceived as trying out every possible structure…" stands in direct contradiction to that by Goldenberg (1988) "…the time required for a peptide to randomly sample all possible conformations to find the structure of lowest energy would be ridiculously long". Flory (1969) proposed that there must be special molecular constraints in proteins which prevent them from populating the vast number of states of statistically favored conformations, but these constraints have not been identified to date. In fact, the detailed 3D structures of several hundred crystallized proteins have become available since Flory's analysis of macromolecular conformation, yet there is still no evidence of any unusual chemical bonding mechanism needed to hold the folded chain together in its unique fold. Clearly then, according to statistical thermodynamics, functional protein should not exist. Put another way: according to reductionist principles, living matter is not possible.

Are there forces operating at this level, which are not predicted by the statistical theories of the microscopic molecular level below? In Kauzmann (1959) it was proposed that the existence of the 'hydrophobic bond' is an explanation of protein shape and stability. This bond operated in the protein interior between non-polar amino acids, thereby keeping the chain fixed in a stable conformation. The idea was vigorously criticised by the thermodynamicists, as for example by Hildebrand

(1968), and during the 1970s the concept was discarded and replaced by the term 'hydrophobic effect' (Tanford, 1973). But to those of us interested in mechanism, this appeared more like a step backward than an advance. In discarding the old view, a physical model of a bond was replaced by thermodynamic jargon with no corresponding pictorial representation. Indeed, in the intervening time to the present day, the hydrophobic effect has slipped even further backwards to the extent that it is now considered by some physicists to be of only minor, if any, influence in protein folding (Finney, 1986), while other experts in the field agree that the term is so vague, that its use leads to misunderstanding among themselves (Privalov et al., 1990)!

Yet, the main point about proteins is not their puzzling stability, but that they function as molecular machines (enzymes). They are the nanomachines of living systems, manipulating energy and material within the cell in an apparently purposeful way. Enzymes gain Free Energy by catalyzing elementary chemical reactions in small molecules (metabolites). For example, a common type of enzyme activity associated with mechanical events is the hydrolysis of the triphosphate bond in ATP (adenosine triphosphate) to give ADP (adenosine diphosphate) and free phosphate. This reaction provides 20–30 kJ/mol, which is captured by the enzyme and then used to perform work. The problems arising from applying statistical principles to these nanomachines deepens when we take the cytoplasmic environment of the subcellular world into consideration. In the popular models describing how these machines work, the shape and stability of the protein is taken for granted as fixed properties. Biochemists are, in the main, unaware of the thermodynamic arguments against this assumption, and so see no difficulty in using it. In addition, the aqueous medium is too often ignored, so that the component parts of their models are depicted as moving in empty space. When we fill this space with water however, modelling difficulties arise which are the direct consequence of the facts, that the solvent has the same density as the moving parts and it also possesses the same thermal energy. In other words, enzymic processes should be depicted as taking place within an inert medium whose chaotic bombardment disperse their energies and hinder their progress. These processes therefore, should be shown to have control over their environment, because, in order that enzymes convert chemical energy into work with certainty, they must cycle through a series of precise physical steps, which cannot tolerate disruptive collisions from outside. But such models would have little chance of success, since the energy source, usually the phosphate bond of ATP mentioned above, is equivalent to 5, 10 or at most 20 H-bonds, and so could hardly be used to tame the violent surroundings of many hundreds of independently acting water molecules, let alone also then be used for the task at hand. This is simple arithmetic, it is not a sophisticated thermodynamic argument.

However, the nanomachines of life are conceptualized by biochemists in the absence of water. For example, the much discussed molecular motors of the myosin (Cooke, 1986), dynein and kinesin (Vale, 1992) systems, which achieve the transduction of energy gained from ATP hydrolysis into the mechanical work of vectorial motion, are depicted in terms of tight gripping actions and stiff lever-type movements of solid members about rigid hinges in free space, reminiscent of the inner workings of our car engines. But the network of strong covalent bonds that hold together the metals of our man-made machines have not been located in the cell. On the contrary, metabolic machinery is composed of the soft gel material of the cell interior, and so we need a fundamentally new concept to deal with the production of force in this condensed watery environment. I believe this new concept is one of participation and co-operation of the solvent in the functioning of molecular machines. To achieve this, the new concept must supplant the random collisions of traditional statistical theory with a coherent action that operates on the mesoscopic level. We need a new principle at this intermediate hierarchical level in order to explain how our nanomachines achieve the two-way interconversion of chemical energy and physical work, i.e. how they transduce energy between the metabolic molecular level below and the physical macroscopic level above.

The traditional view of liquid water as a structureless chaotic medium (Finney, 1979), does not allow the possibility of it being part of an energy transducing machine. Indeed, in such a view, its role in the production of directed action can only be a disruptive one. On the other hand, the cluster model presented in this work, provides us with an entity which has a size in the mesoscopic range along with proteins. This model is based on the idea that the making and breaking of H-bonds between individual water molecules is co-operative, so that the formation of bonded networks, their build-up and break-down, are on-going processes travelling through the liquid medium like successive waves of polymerization and depolymerization reactions (Watterson, 1981). In this wave-cluster model, clusters, although constantly changing, are at any instant held together internally by an unbroken linkage of intermolecular bonds. This means that the molecules exert tension on one another, although this tension obviously cannot extend beyond a break in their interconnections. Therefore, over spatial dimensions larger than the cluster size, pressure and not tension, operates throughout the liquid (Watterson, 1991). In this picture, these basic opposing forces do not cancel one another, but co-exist on different hierarchical levels: tension on the molecular scale below the size of the interconnected networks, i.e. inside the clusters, and pressure on the macroscopic scale in volumes larger than the cluster size (Watterson, 1995a). The possibility that intermolecular forces are tensile, removes the destructive randomizing action of collisions. On this basis, we can now explain how molecular machines are constructed out of their soft watery components, and how they can function in such an environment without the need of the steel and concrete of our man-made world.

2. The wave-cluster model of liquid structure

To begin the search for the role of liquid clusters in molecular forces, let us recall the explanation of pressure at the molecular level. Fig. 1 depicts the mechanism in classical terms. Two chambers of equal size containing different amounts of a gas are separated by a solid wall. The molecules each have mass, m, and component of velocity normal to the wall, v. From the Kinetic Theory of Gases we have the expression for pressure on the wall

$$P_0 = n_0 m v^2 \tag{1}$$

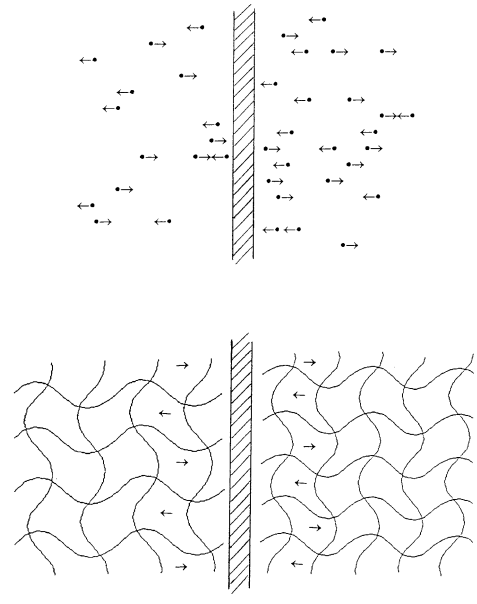

Fig. 1. Molecular mechanism of pressure. The upper panel shows the familiar collision mechanism of gas molecules moving in empty space. This is an illustrative scheme only, as the molecular movement in all directions, not just horizontally, must be considered. Nevertheless, rigorous derivation leads to the same expression for pressure as derived here in Eq. (1) using the much oversimplified scheme. The lower panel shows the structrue wave travelling through the liquid medium. In this case, the individual molecules are not colliding with one another and the wall, because they are bonded together in clusters by weak non-covalent bonds (H-bonds in water). It is the wave carrying momentum, not particles of given mass, that collides with and bounces back off the wall. Again this is a simplified scheme, since the directions of propagation are shown too ordered. In the bulk of a liquid away from the wall, there is no preferred direction of propagation, although there is some influence in the region next to the surface (Watterson, 1987b, 1988). In both cases the pressure on the right hand side of the wall is greater than on the left, due to the higher concentration (n) there. In addition, the pressures depicted in the liquid are similar to those in the gas, because the size of a cluster is given by the wavelength and so there is roughly the same number of clusters as gas molecules in this illustration.

in, say the left-hand chamber, where n_0 is their concentration (e.g. molecules/l). The frequency of collisions with the wall is $0.5n_0v$, whereby each molecule changes its momentum by 2 mv.

This equation represents the central result of the Kinetic Theory of Gases and in the last century physicists identified it as the molecular form of the Gas Law, $PV = RT$, since it can be rewritten

$$P_0u_0 = kT \quad (2)$$

where $u_o = 1/n_o$ is the volume occupied by one molecule, and kT (Boltzmann's Constant and temperature) is equated with the kinetic energy term, mv^2.

When a gas is compressed (at constant temperature), its volume decreases, thus increasing n. Consequently, its increase in pressure is a result of an increase in the frequency of collisions of the gas molecules with the wall. Put another way, the increase in frequency is a response (a reaction) of the gas to a compression imposed from outside (an action). However, liquids cannot be compressed into smaller volumes like gases, so how do they respond to imposed pressure? Here, the agents that cause pressure are not the individual molecules, but their aggregates. Clusters increase in concentration by decreasing in size, and because cluster formation is a wave motion, this means a decrease in the wavelength of the structure wave (Fig. 1). Viewing the liquid medium as a spring suggests a way to interpret pictorially this reaction to pressure. The turns of a familiar metal spring squeeze together as it is compressed, and so by analogy, the structure wave shortens its wavelength. In this picture, a liquid responds to pressure in the same way as a gas does, by increasing n and thus the collision frequency. To accommodate this increase, more turns in the spring appear, while its overall size remains constant. However, in contrast to the gas, the liquid molecules are not travelling through empty space, rather it is the structure wave which carries the momentum. The clusters are moving, while the molecules remain in the same locality. An aggregate of molecules forms a cluster, at that moment when they form a single structural unit as the crest of the wave passes.

The contents of the left and right chambers are not in equilibrium, in the case of neither the gas nor the liquid. If the walls became permeable, then material would be driven by pressure from right to left tending to distribute energy evenly over both sides. In the case of liquids however, we can have osmosis: a common phenomenon, well-known in the biological world. Then the situation remains static even while the pressure difference persists. How is this apparent non-equilibrium state maintained? Examining this question brings insight into the origin of molecular forces in liquids.

3. The osmotic mechanism

Just over 100 years ago, van't Hoff proposed his famous equation for osmotic pressure

$$\Pi = zkT \quad (3)$$

where z is the solute concentration. Expressing z in terms of number of molecules and volume, $z = N/V$, this equation also takes the form of the Gas Law, $\Pi V = NkT = RT$. This identity led physicists earlier this century to speculate that the osmotic pressure must be produced by the solute molecules alone, as though they were colliding with the membrane in empty space (Fig. 2). This interpretation became known as the 'bombardment theory' and is the same as the gas case described above. It was further believed that, because the solvent permeates the membrane, it alone could not exert a net pressure from either side. This picture was given theoretical support by a thermodynamic argument called 'the gas analogy'. Instead of imagining the solvent as empty space, we can introduce an equal number of gas molecules into both solvent and solution sides, so that the pressures of this gas remain equal. This ensures the rigorous thermodynamic condition for equilibrium, that the Free Energy of this 'solvent gas' is the same in solvent and solution. As a consequence, the pressure on the solution side must be greater, because it has the solute molecules additionally mixed in with the solvent.

However, in the case of liquids, this mechanism is not correct. As we continue to introduce more

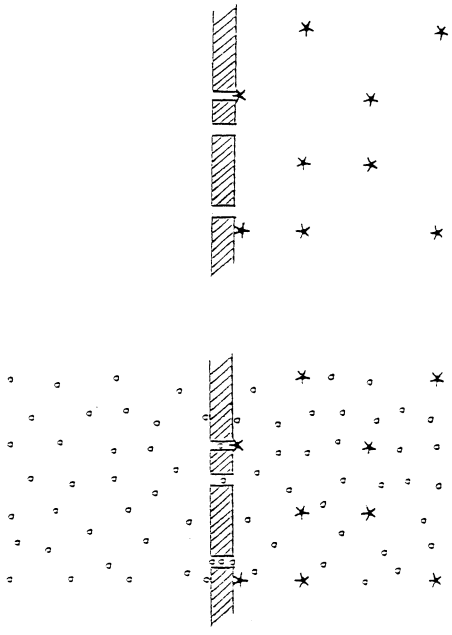

Fig. 2. Classical explanation of osmosis. The wall is now a semipermeable membrane separating a pure solvent on the left from a solution on the right. It is permeable to the solvent molecules (circles), but not to the solutes (stars). The upper panel illustrates the 'Bombardment Theory' in which the excess pressure is caused by the solutes colliding with the membrane on the right. The solvent passes through in both directions and so does not exert a net pressure. The lower panel illustrates the 'Gas Analogy' showing the solvent molecules, which are central to the thermodynamic argument stating that the Free Energy of the solvent must be equal on both sides. Since there is an equal number of them, their (partial) pressures must be equal and therefore this explanation reduces to the Bombardment Theory.

and more solvent molecules into both sides, they eventually come into contact, filling all the space, and then we have the liquid phase. But because of the presence of the solutes, we cannot introduce as many solvent molecules into the solution as into the pure solvent, and so the solvent now exerts a greater pressure on the pure solvent side than on the solution side, because of their greater number there. In fact, in the case of water, this situation is further exacerbated. It is well known that many solutes decrease its molar density in solutions, meaning that there are even fewer solvent molecules in a given volume of solution compared to pure solvent, than there would be if the solvent molecules were simply displaced by the solutes. Thus in the case of liquids, the concentration of solvent molecules is in general lower on the solution side, in contradiction to the gas analogy. We must conclude that liquid molecules exert pressure by a different mechanism than gas molecules do.

Many authorities, e.g. J. Chem. Soc., invoke 'the molecular theory of osmosis' to overcome this difficulty (Young, D., Ed in Chief, Faraday Trans, personal communication, 1987). According to this theory, the higher pressure in the solution is caused by solvent having faster molecular motion than in the pure solvent. This compensates for their fewer numbers by ensuring that an equal number pass through the pores of the membrane into the pure solvent side as in the opposite direction, so that equilibrium is maintained (Fig. 3). There is no experimental evidence for this. On the contrary, higher pressure usually causes an increase in viscosity of a liquid, and consequently a decrease in molecular motion.

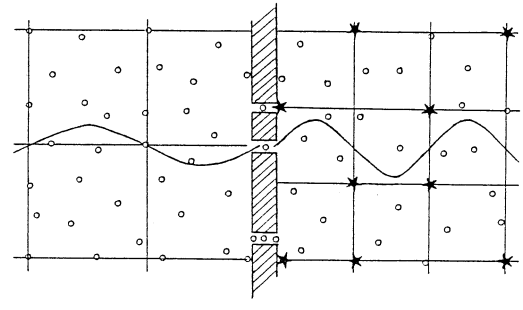

Fig. 3. Structural explanation of osmosis. Pictorial representation of the explanation of osmotic pressure based on the structrure wave. The grid on the left of the membrane is larger than on the right illustrating the larger size of the clusters in the pure solvent compared to the solution. The presence of the solutes (stars) results in an increase in the concentration of clusters in the solution. At equilibrium, these smaller clusters each have energy equal to that of the larger clusters in the pure solvent on the left. This condition causes the higher pressure observed on the solution side. To illustrate the liquid phase, the circles should be depicted everywhere in contact filling both sides of the diagram, but for reasons of clarity only a representative few have been drawn in. According to the 'Molecular Theory of Osmosis', these solvent molecules are diffusing faster on the right because of the higher pressure there (see text).

According to classical concepts then, osmosis happens because random thermal collisions cause solvent molecules to diffuse from pure solvent into the solution where their collisions, by some unexplained mechanism, now exert greater pressure. Further examination also shows that the classical picture is based on a contradiction: on the one hand, it requires liquid to set up and hold pressure as though the liquid has the mechanical bulk properties of a solid, and on the other hand, it requires free diffusion, meaning that the liquid must flow with pressure and not against it. These problems arise because macroscopic concepts of force have been transposed down to the level where thermodynamics dictates the chaos of random motion must prevail. They are in fact the same problems we encountered in the Introduction, when discussing the popular models of molecular machines envisaged as microscopic replicas of man-made devices. I have shown formally elsewhere, that a fall in Free Energy, which is the rigorous thermodynamic criterion for a spontaneous process, cannot be identified as the potential that drives osmosis, since the Free Energy of a system increases as it develops osmotic equilibrium (Watterson, 1995b).

Another feature of the classical concepts is the assumption that the osmotic mechanism is independent of molecular interactions, since it is a colligative property, even though evidence from many fields of investigation ranging from the physical to the biological sciences have established the importance of these forces. The wave-cluster model presented here is based on the opposite view (Watterson, 1987a). When a solute molecule is introduced into a pure solvent medium, the ability of the individual molecules to rotate and facilitate smooth wave motion is disrupted. Those solvent molecules closest to the foreign solute can no longer co-operate with their neighbors in an unhindered way. As a result, the long-range extent of coherent interactions is reduced, slowing the wave down. In terms of structure dynamics, the solution is now a different material, in which the velocity of the structure wave, v_1, is lower compared to that in the pure solvent, v_0. Stated succinctly, osmosis happens precisely because the presence of solutes causes structural changes in the solvent.

From the physics of wave motion, we know that energy can pass smoothly back and forth across a boundary separating two materials. For the wave to maintain equilibrium, it must have the same frequency in both materials to ensure that the exit of a cluster from one side means the entry of just one cluster into the phase on the other side,

$$v_0 n_0 = v_1 n_1 \tag{4}$$

Now because $v_0 > v_1$, we have $n_1 > n_0$, i.e. we have a higher concentration of clusters in the solution. In addition, these clusters must transfer the same energy

$$m_0 v_0^2 = m_1 v_1^2 = \mathrm{kT} \tag{5}$$

so

$$P_1 - P_0 = n_1 m_1 v_1^2 - n_0 m_0 v_0^2 = (n_1 - n_0)\,\mathrm{kT} \tag{6}$$

Thus these two conditions, namely one-for-one exchange of clusters and each carrying the same energy, give the equilibrium pressure difference across the boundary (Fig. 3). Eq. (6) is a statement of osmotic pressure in molecular terms, just as Eq. (1) gave the pressure of a gas in molecular terms. For it to agree with the van't Hoff expression Eq. (3), the difference in cluster concentration, $(n_1 - n_0)$, must be identified with the solute concentration, z. I have explained elsewhere how each new solute forms a new cluster, because its introduction into the solvent medium causes a hindrance to local molecular motions thus producing a node in the structure wave (Watterson, 1987a). And since the number of nodes equals the number of clusters, the extra number of clusters in the solution phase are due to the solutes, z.

We see that the higher pressure is on the side where there are more clusters, just as in the case when pressure is imposed from outside on the pure solvent (Fig. 1). This means that these smaller clusters must have a higher internal tension than (but energy equal to) the larger ones on the left, because they are able to counteract the solvent flow from right to left caused by the pressure difference. The difference in tension is felt in the boundary region, where molecules periodically come under the influence of bonding forces operating in both sizes of clusters. In this

picture, it is easy to see how a molecule in this neighborhood can be pulled into a cluster and become part of its multimolecular structure. Because co-operative effects are long-range, clusters formed by strong bonding exert a pull on molecules in those where bonding is weaker, so that the smaller clusters can pull solvent across the membrane increasing the pressure on the solution side. At equilibrium, P_1 has become high enough to counteract their pull and equalize flow. We now have a mechanism for the action of a concerted and directed mechanical force operating at the molecular level within liquids.

The intermolecular forces hold a cluster together like a giant 3D molecule. Its size, u, is defined by the Gas Law (Eq. (2)), which gives the minimum volume of aqueous medium which experiences the action of pressure. The term 'pixel' borrowed from the information sciences, describes this minimum concept very aptly. A pixel is that region of space (a dot on your computer screen or on a printed page) containing one bit of information. Further, subdivision does not bring further information, in fact just the opposite, resolution begins to deteriorate. By analogy then, the size of a cluster defines the pressure pixel. Although liquids are macroscopically under pressure, we cannot speak meaningfully of pressure in volumes smaller than this basic unit, (for a fuller explanation see Watterson, 1995a). Since Eq. (2) applies equally to gases and liquids, the pressure pixel is the same size in both physical phases. Under conditions prevailing in the biosphere, i.e. 1 atmosphere pressure and 300 K, it has a volume of about 40 cubic nm, represented by a cube with an edge 3.5 nm long. This unit of space contains some 1400 water molecules and has a molecular weight of around 25 000 Da.

4. Pixel size and dynamics

We began in the Introduction with the fundamental question of how does a chain of amino acids fold with certainty into a domain with its own unique shape. Yet this is not the only puzzle presented by proteins. Another question often asked by biochemists runs: Why are enzymes so big? Indeed, we can go on and ask a number of related questions such as, why is the shape compact rather than open or extended, why do single-domain proteins have sizes in the 20–25 000 Da range independent of function, and why is this size apparently the maximum, larger proteins being subdivided into domains of this basic size. In other words: why are proteins quantized in size?

The pressure pixel answers this question. Like water clusters, protein domains fit the size of the pressure pixel. Therefore, within the domain, tension and not pressure, prevails. It is the size of the pressure pixel that explains protein stability. As I have pointed out before, domain size is better indicated by the volume occupied in the crystal than by the amino acid chain length (Watterson, 1991). The pixel defines a region of 3D space, and can be occupied by proteins ranging in length from around 150–250 amino acids, which are known to occupy roughly the same volume in the crystal. Because the amino acids are linked together by covalent bonds, proteins behave like permanent clusters. Water clusters and protein domains fit together, they are compatible in size and internal dynamics. In this picture, the subcellular world consists of assemblies of water clusters and protein domains stacked together in regular array. It is not a protein solution (Watterson, 1987b, 1988).

These supramolecular aggregates constitute the protein gel, which in the case of cytoplasm, can be up to 80% water. Although the gel is composed of many separate pixels, it can act as one. An array of pressure pixels can fuse into one large pixel, in which the tensile force then extends throughout its entirety. Because harmonic transitions are inherent to wave motion, the transition between the multicomponent and single unit states is a reversible, dynamic one. This type of transition is illustrated in Fig. 4. The row of squares represents an assembly of cubic cluster-domains. In this state they are independent entities separated at their interfaces by nodes in the structure wave. Seen as a whole, the assembly is initially under pressure, but after the transition the wavelength has become large enough to encompass the whole assembly, holding it together as a single entity. These structures can become indefinitely large

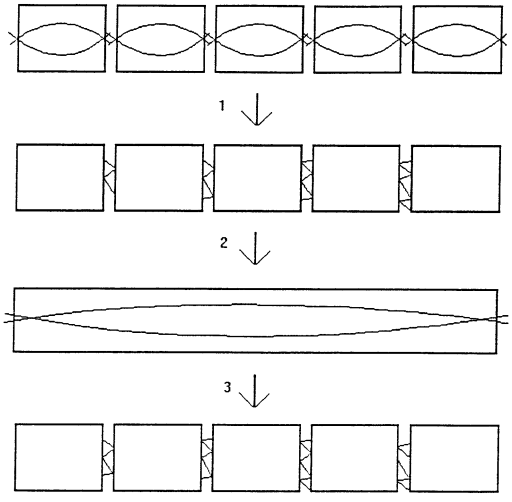

Fig. 4. Co-operative enzyme function. The row of squares represents a group of equally sized water clusters and protein domains in contact. Although only schematic, the high spatial ordering depicted reflects the order found in cytoplasmic complexes and protein crystals. The wavelength of the structure wave passing through the complex corresponds to the basic pixel size, so nodes occur at the junctions between the cluster-domains. The strong spatial correlation induces harmonic transitions in the wave motion. Transitions are facilitated by insertion of small molecules (zig-zag lines) between the cluster-domains, which take up specific linking positions (binding sites) such that the H-bond network now extends throughout the whole complex (Step 1). The transition occurs without the need for any special energetic mechanism, because tension is now being exerted across the entire region (Step 2). The resulting structural oscillations cause chemical changes in the bound molecules (substrate into product), disrupting the interconnections, so that the wave returns to its initial short wavelength state (Step 3). The cycle is completed by co-ordinated movement of the new forms of the linking molecules one position to the right. This step (not shown) involves the concerted action of mechanical forces of pixel size. Although details would be necessarily too speculative at this stage, this step should not be seen as an ad hoc introduction of some entirely new energetic element into the scheme. The force causing these displacements is just the same tensile force operating in steps 1–3. It can move solvent as packages in predetermined directions, and so could also readily displace solutes in discrete steps, as in DNA processing.

reaching macroscopic proportions. In a protein crystal, for example, there are alternating regions of protein and solvent, yet even though there is no attraction and no direct bonding between the protein solutes, the crystal remains a solid gel. The multi-cluster-domain units are condensed into a single coherent entity.

The transition needs suitable conditions at the interfaces between the clusters and domains. If the arrangement of atoms is such as to prevent bond formation, then tension cannot be transmitted across this interface and the structure wave would therefore maintain a node at this location. Bonding conditions can however be changed by insertion of a small molecule metabolite, as happens in enzymes where the active site is known to lie in a cleft between domains. We can extend this concept and propose that the row of domains represents the supramolecular complex composed of enzymes involved in a metabolic pathway. Then only when the metabolites are located at their respective catalytic sites between the clusters and domains, can the transition take place. Since it encompasses the whole complex, each metabolite is converted into its product, which can then all be moved along one position in the sequence of active sites. This co-operative mechanism ensures that each molecule entering the complex as the initial metabolite is fully converted into the final product. The cell must operate in some way such as this, in order to avoid the cytoplasmic space from becoming filled with metabolic debris. There is no experimental evidence that the subcellular space is a concentrated solution of metabolites, each waiting for its chance encounter with the correct enzyme to continue along the pathway. This problem cannot be solved in the classical picture in which we have soluble metabolites colliding with soluble enzymes, because the diffusion mechanism is random and can therefore only disrupt the order needed for the operation and maintenance of sequential processes. If enzyme catalysis were diffusion controlled, the cell would become clogged with metabolites of all types. Furthermore, once freely dissolved in the cytoplasm, a portion of them would never re-enter the metabolic flow because of the chance nature of diffusion. To illustrate this point, the classical picture of cellular activity can be likened to traffic flow in a busy city where there are no rules of the road.

Many enzyme complexes are associated with membranes, which often are stacked in layers. This additional lipid component induces spatial

order on both the protein domains and the intervening water layers. As discussed elsewhere, the 3–4 nm thickness of lipid membranes means that they fit neatly with water clusters (Watterson, 1988), and this architectural compatibility would facilitate coherent cluster dynamics. A simpler but analogous system is offered by swelling clay, which provides us with a dramatic, clear-cut example of cluster dynamics resulting from transitions in the structure wave. Clay particles are extremely asymmetric in shape, having length and breadth thousands of times greater than their thickness (1–2 nm). Familiar clay material is composed of stacks of these microscopically thin sheets, which swells when it comes in contact with water (a problem well known to construction engineers). Water flows spontaneously in between the sheets forcing them apart, even against pressures of hundreds of atmospheres imposed on the stack (Fig. 5). As with other examples of such everyday osmotic phenomena, there is still today no satisfactory description in terms of statistical concepts (Watterson, 1989). However, the force

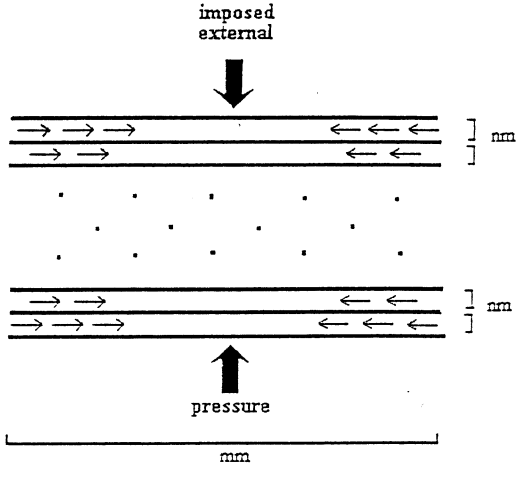

Fig. 5. Mechanism of clay swelling. Clay particles are thin sheets with thickness in the nm size range but length and breadth in the μm–mm range. As indicated here, they pack by aligning in parallel array to form stacks which can become mm high. The thin arrows show the flow of water into the stack from the surroundings at 1 atmosphere pressure, which forces the sheets apart, even though there may be a pressure of thousands of atmospheres exerted normal to the direction of flow on the stack.

opposing the applied pressure has been recognized for several decades, and has been directly measured in many systems. Because it is always manifested in the interaction between solutes and water, it has become known as the 'hydration force'. Classical theories fail in their explanations, and here again the reason is because single water molecules in random motion are seen as the agents that cause pressure. With this view, we are always faced with the immediate problem of how can molecules under one atmosphere external pressure in the surroundings, flow by diffusion into a region where the pressure is 1000 times greater?

Again, the answer lies in the size of the pressure pixel. The introduction of the solid 2D surface into the liquid medium causes an ordering of the clusters. This flat surface induces clusters to take up position forming a layer of clusters packed side-by-side, or in other words, this immobile 2D boundary forces a nodal plane in the structure wave. With the clusters aligned in a regular array, they can induce harmonic transitions producing larger and larger clusters in the layer between the sheets. This fusion produces a cluster as wide and as broad as the clay particles themselves, resulting in a pressure pixel of macroscopic proportions. Pressure no longer operates within the unified layer, since tension has spread laterally throughout the entire region.

Of course, clay is dead material. Its lack of complexity means it does not possess the internal flexibility needed by the living system. Returning to biological examples, the force at work in a contracting cell is a living analogy of the tension observed in swelling clay. The linear protein filaments which direct the activity of the molecular motors (see Section 1), are one dimensional cables, not two dimensional sheets, however they are invariably arranged in parallel array within the cytoplasm and do not show random orientation. Ordered alignment of solutes, whether cables or sheets, produces ordered alignment of clusters and ensuing macroscopic tension within cytoplasmic water. Nanomachines associated with these cables do not each independently produce an increment of force, because the cytoplasm itself is active, as recently demonstrated by stiffness mea-

surements of cytoplasmic water in contracting cells using acoustic microscopy (Luers et al., 1992). Rather, their role is to control and co-ordinate energy transduction, so that it flows in the bottom-up direction from the lower chemical level of the phosphate bond of ATP up to the higher physiological level of the contracting fiber, and is not lost to thermal chaos through independent action. In other words, they are involved in maintaining coherence in the release of their energy increments to ensure that these 'many energies' are transformed into a 'single energy' and thus a large-scale single force.

The stacked lipid bilayers of the Golgi, mitochondria and chloroplasts are more closely analogous to a stack of parallel clay particles. These organelles perform a lot of mechanical work, and although mechanical force has not yet been directly measured using intact systems, there is no doubt concerning the existence of the hydration force which operates within the intervening water layers in multilayer preparations of isolated lipids (Watterson, 1989). Pressure-tension effects have, however, been directly measured on the single bilayer outer cell membrane using the patch-clamp technique on intact living systems. A large body of cell physiological data shows that stretching the membrane switches the open/closed state of several types of ion gates, the so-called 'mechanosensitive' channels (Morris, 1990; Kung et al., 1990). Stretch activation of ion-pumping and contraction are important observations, since they demonstrate a co-ordinated transfer of information from the large down to the small scale. A coherent top-down flow of this type is not possible with classical mechanisms, because energy in large macroscopic packages necessarily becomes randomized and lost as thermal motion when it is subdivided into packages of molecular size.

These working proteins embedded within membranes possess sizes and shapes similar to the metabolic nanomachines (enzymes) described above in Fig. 4. For example, the water channel protein recently characterized by van Hoek et al. (1995) folds in the environment of the lipid bilayer into the same dimensions as protein domains do in water. Two-dimensional electron crystallography reveals that, as well as bridging the 3 nm span through the membrane, this protein presents a pore of around 3 nm across to the aqueous phase at the membrane interface (Mitra et al., 1995). There is, therefore, ever-mounting evidence, that the physical nature of proteins designed to function in membranes is quantized, like that of their cytoplasmic counterparts.

5. Conclusion

Enzyme action, whether it be addition of phosphate to glucose or effecting vectorial motion along a microtubule filament, involves mechanical force. In order for it to proceed, small molecule metabolites as well as whole protein domains undergo movement in a definite controlled way. It is of course possible to construct theories that describe the various molecular mechanisms independently of one another with the use of diverse principles applicable to particular isolated cases only. This has indeed been the main thrust of work until now. But as argued in the Introduction, the statistical approach will not lead to a co-ordinated view of cell function. On the contrary, cell activity will always be equated with an average activity: the result of disparate forces which happen by chance to be operating at the same time.

In the wave-cluster-domain model, the force underlying the operation of molecular nanomachines is the same force that holds proteins in their stable form and is also the same force that moves water across membranes against pressure. It derives from weak but dynamic interactions, the H-bonds, which can extend their bonding action over long ranges set by the size of the pressure pixel. Below this spatial limit a unifying tension operates. It is an inward-directed force: a force that supports the build-up of multimolecular structures and opposes the randomization caused by thermal motion.

The statistical collision mechanism of pressure and diffusion is, on the other hand, an outward-directed force. Therefore, machines based on this principle need to be strong structures with bulk properties which are not perturbed by the collisions of solution chemistry. Furthermore, energy

transfer, such as effected by a power stroke, must be produced by equally strong pistons and levers, whose movements cannot be thermalized and lost into the molecular motion of the environment. But such subcellular apparatus has not been located. The molecular motors referred to in the Introduction are associated with the cytoskeleton, which is an array of protein filaments, and therefore has physical attributes closely related to those of its aqueous environment with which it is in intimate contact. Protein cannot be seen as physically stronger, separate and energetically independent from the water of the cytoplasm. Production of macroscopic work is better understood in terms of pixel dynamics, in which co-operation between individual molecular structures on the mesoscopic level transforms them into a single force-producing entity.

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