Biophysics and Physiological Modeling
Chapter 5: A new model of osmosis

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Introduction: A century-long controversy continues!

In this chapter we're going to develop a new **diffusive model of osmosis** based on the marble game we developed in **CHAPTER 1**. Osmosis is a process that's fundamental to the physiology of all living things on earth, but you might be surprised to discover that the approach taken in this chapter is currently controversial! Imagine…

> *You've been talking with Lyon (a friend who directs and produces YouTube science videos). Lyon says that you'll be working at the forefront of science, investigating one of the longest running controversies in all of science! – How does osmosis really work? Lyon says that despite its obvious importance in physiology and water purification technology, the marble game model of osmosis that you'll develop in this chapter contradicts papers published recently in the American Journal of Physics and Trends in Plant Science. Lyon says that if you want to learn how science is really done, then you should keep reading and do science!*

Lyon's challenge to do cutting edge science is a real one. Despite its simplicity, the approach outlined in this chapter is different from the standard quantitative model of osmosis. In addition, it directly contradicts a recent paper on the subject entitled "*Osmosis is not driven by water dilution*" [Kramer and Myers 2013]. According to Kramer and Myers:

> *"The kinetic explanation of osmosis familiar to most plant biologists appears in most introductory college-level textbooks on chemistry [Brown,* et al. *(2003)] [Moore,* et al. *(2010)], and is substantially incorrect. … [the incorrect] description equates osmosis with the familiar kinetic description of the diffusion of a compound down a concentration gradient"*

The marble game model of osmosis is based on the opposite assumption – that osmosis can be modeled, and hence explained, by a kinetic description of diffusion (**CHAPTER 1**). As we'll discover, the diffusive model is consistent with what is described in introductory chemistry and physiology textbooks (e.g. Silverthorn [2007]), but it goes significantly further and provides a quantitative model that is consistent with equilibrium thermodynamics and experiments investigating the kinetics of osmosis [Nelson 2017]. Lyon's challenge is for us to understand the diffusive model of osmosis, explore the network of predictions that it makes, and then compare those predictions with experiment and the traditional model of osmosis advocated by Kramer and Myers. Then *you* can decide whether the diffusive model of osmosis has scientific validity. A promo for the **SCIENCE CHANNEL** sums up this approach in two words – "question everything" [tomtom5418 2012].

A major goal of this book is to engage you in authentic research-level activities. This **CHAPTER 5** takes that approach to a whole new level. As this chapter was initially being written, this approach had not been published elsewhere. My hope is that you'll share in the excitement of doing real scientific research, and working on a model that no-one else has explored! You should note that as of 2019, the approach presented in this chapter is still controversial [Morris and Blyth 2019].

Humanity purifies over one trillion gallons of wastewater every day – in our kidneys using osmosis! – about 180 L per human per day! Osmosis is clearly an important phenomenon in human physiology, but it's even more central to the lives of plants and it is (in part) responsible for the turgor pressure that helps some plants, fungi and bacteria maintain their physical shape.

In **SECTION 5.1** we'll begin by applying the original marble game to the diffusion of water into and out of a red blood cell (RBC). As we'll discover, there is a technical problem with a straight-forward application of the marble game, but this can be overcome if we explicitly account for the semi-permeable nature of the RBC cell membrane. We'll take a short detour in **SECTION 5.2** to analyze how an energy difference between two boxes affects diffusion using the "gravity marble game." Then in **SECTION 5.3** we'll use what we learned from the gravity marble game to investigate how the energy stored in the pressurized fluid of a plant cell affects water diffusion, i.e. **osmosis**. We'll discover that the hydrostatic pressure difference slows down water diffusion from high to low effective water concentration. As a result, there can be an equilibrium between boxes with different effective water concentrations. This equilibrium pressure difference is known as the **osmotic pressure**. In **SECTION 5.4** we'll summarize the predictions of our diffusive model of osmosis and we'll discover that it predicts that the net rate of water diffusion, the **osmotic permeation rate**, can be thought of as having two thermodynamic "driving forces" – an effective water concentration difference and a hydrostatic pressure difference. These mathematical predictions are identical to the traditional model of osmosis. Hence, our diffusive model of osmosis is supported the same experimental evidence as the traditional model, allowing us to conclude that our diffusive model is indeed a valid scientific explanation of osmosis.

Any new scientific model should make predictions that distinguish it from previous models. Those new predictions should be testable by experiment (or molecular simulation). Our diffusive model does just that! It makes testable predictions that are different from the traditional model. We'll investigate those testable predictions in a later chapter. See also Nelson [2014][2017].

> **Q.5.1 DISCUSSION QUESTION** Using your knowledge from previous biology classes:
> **(a)** *briefly define* osmosis; and
> **(b)** *briefly discuss* the importance of osmosis using examples.

5.1 Red blood cells and osmosis

Human **red blood cells** (**RBC**s or **erythrocytes**) contain about 30% **hemoglobin** (**Hb**) by volume. Hemoglobin is a protein made up of four subunits that are each very similar to myoglobin (Mb).

A major role of Hb is to carry O_2. Hb also carries other gases such as CO_2 and NO. Like Mb, Hb is also red in color and it's responsible for the color of red blood cells (and hence the color of whole blood) (**CHAPTER 6**).

Fig.5.1 Cartoon representation of red blood cells (modified from an image by LadyofHats).

Red blood cells are shaped like deflated basketballs with dimples on both sides (Fig.5.1). An advantage of this shape is that it allows RBCs to change their volume without changing surface area – in an analogous way to how a deflated basketball can be partially inflated (or deflated even further) without stretching the cover. This arrangement is useful because it allows RBCs to change volume and shape in response to changing osmotic conditions in blood plasma (and to squeeze through narrow capillaries and past obstructions more easily). RBCs are *the* classic textbook example used to illustrate osmosis. We'll start our scientific journey by developing a simple two-box model of a RBC floating in blood plasma to introduce osmosis (see Fig.5.4 below).

Hypothesis: Osmosis is the diffusion of water across a semipermeable barrier

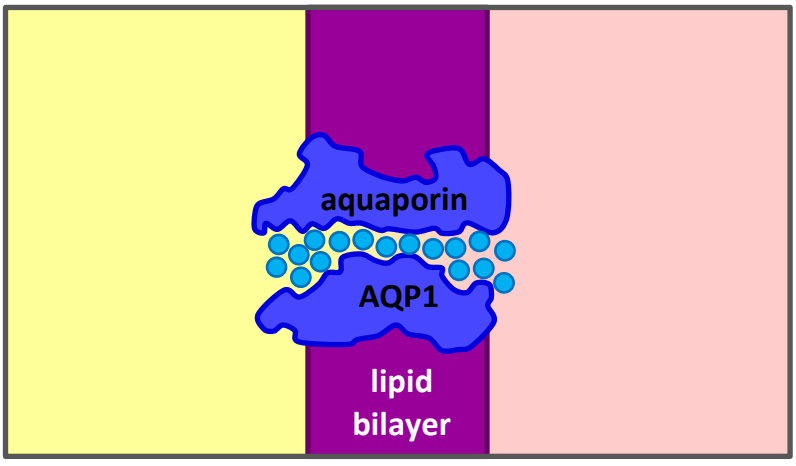

Fig.5.2 Schematic diagram of an AQP1 aquaporin protein (water channel) imbedded in a lipid bilayer membrane separating two solutions with differing effective water concentrations (after Murata *et al.* [2000]). The aquaporin provides a single-file pathway (shown in cross-section) for water molecules (circles) that makes the membrane semipermeable because only water molecules can pass through.

Osmosis occurs when two solutions are separated by a barrier that blocks solute molecules but allows water molecules to pass between the two solutions. In RBCs the barrier is the plasma

membrane – a lipid bilayer membrane that is impermeable to most hydrophilic solute molecules. It is only very slightly permeable to water molecules through a very slow dissolve-diffuse-dissolve mechanism making the bare lipid bilayer basically impermeable to water and many other solutes. Most biological membranes contain proteins known as **aquaporins**, or **water channels**, that span the lipid bilayer membrane and provide a path for water to diffuse across the membrane (Fig.5.2). This path is described as **single-file** because it is so narrow that water molecules must stay in line and cannot pass each other. This single-file property helps to make the path selective for water molecules over all other molecules. It can even exclude similarly sized hydronium H_3O^+ ions. This makes the membrane permeable to water but impermeable to polar and ionic solutes, i.e. the membrane is **semipermeable**. Rapid movement of water is thought to be advantageous when RBCs pass through the renal medulla (inner portion of the kidney) where the effective water concentration in blood plasma changes rapidly [Mathai *et al.* 1996].

Q.5.2 DISCUSSION QUESTION Diffusion in the bulk of a solution occurs by molecules being jostled by their neighbors resulting in a concerted random dance of all the nearby molecules. These concerted motions occasionally result in molecules swinging around each other and exchanging places or squeezing past each other. This type of diffusion can't occur in the single file of water shown at the middle of the APQ1 aquaporin (Fig.5.2) because the water molecules can't pass each other. *Briefly discuss* how you imagine diffusion might occur in this single-file situation.
Hint: It might help to think about a single file of cars in stop and go traffic on a narrow one-lane bridge.

About what you discovered: molecular dynamics simulation of single-file diffusion

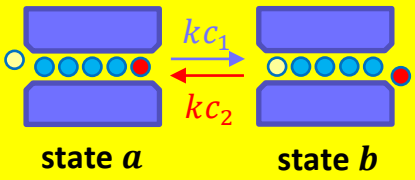

Fig.5.3 Schematic diagram of an AQP1 aquaporin selectivity filter showing the knock-on jump summary of single-file water permeation. The diagram shows two states (a and b) of the *same* AQP1 aquaporin. For the jump from state $a \rightarrow b$, the water molecule entering from box 1 is highlighted in yellow (lighter) and the water molecule knocked-on into box 2 is highlighted in red (darker). In the reverse $b \rightarrow a$ jump, the red water molecule enters from box 2 and the yellow water molecule is knocked-on into box 1.

After you've answered Q.5.2, watch the video [TCBG UIUC 2012]. Note that the red-and-white water molecules at the top and bottom of the video move according to regular diffusion. However, in the narrow channel formed by the aquaporin, they mostly move in a single-file manner. This single-file process can be summarized by **knock-on jump transitions** where a water molecule enters one side of the AQP1 aquaporin **selectivity filter** (**SF**) (the single-file portion of the AQP1 channel) and "knocks on" the furthest molecule in the file into the other side (Fig.5.3) [Hodgkin and Keynes 1955]. You should note that this knock-on jump mechanism doesn't need to be a

single elementary step. Just like the jumps in the original marble game (see Figure 1.4 or Fig.5.6 below), the transition shown in Fig.5.3 can be the result of many smaller jumps (**CHAPTER 10**).

Fig.5.3 conceptually summarizes these reversible **knock-on jump transitions**. It shows two states (a and b) of the *same* AQP1 selectivity filter. The transition from state $a \rightarrow b$ is caused by the diffusive single-file shuffling of all six water molecules along the pore as the yellow water molecule enters the selectivity filter from box 1 and the red water molecule leaves the selectivity filter into box 2. The net effect is a jump of a water molecule from box $1 \rightarrow 2$ with rate kc_1, where k is the **knock-on jump rate constant** and c_1 is the "**effective water concentration**" (or **activity** of water) in box 1. The reverse jump from box $2 \rightarrow 1$ occurs with rate kc_2 where c_2 is the effective concentration" (or activity) of water in box 2. ❑

FD model of a red blood cell in solution

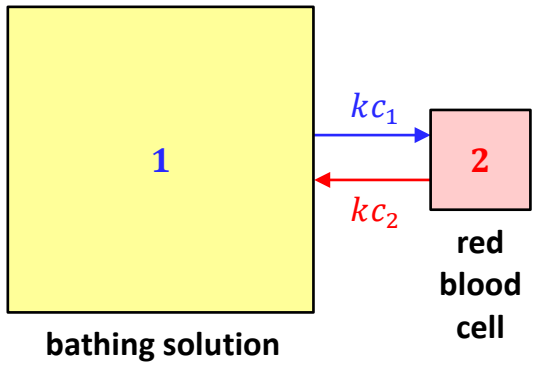

Fig.5.4 FD diagram of a red blood cell (box 2) floating in a large bathing solution (box 1). The water in the red blood cell has an effective concentration c_2 and the bathing solution has a constant effective water concentration c_1.

Fig.5.4 shows an FD diagram of a single red blood cell (box 2) floating in a large bathing solution (box 1) which we will call **the bath**. As shown in the FD diagram, we are assuming that water molecules can jump between the boxes according to the rules of the original marble game (with the same jump rate constant k in both directions). However, because we are assuming that the bath is much larger than the red blood cell, we can assume that the water concentration in the bath c_1 remains essentially constant during the time we're considering.

As a prelude to Peter Agre winning the Nobel Prize in 2003, Mathai *et al.* [1996] performed experiments with RBCs (see Q.5.12 below) and determined the value of the rate constant to be $k = 250\ \mathrm{s}^{-1}$ or $k = 0.25\ \mathrm{ms}^{-1}$. In this model we'll use the "**effective water concentration**" (or **activity** of water) in each box c_1 and c_2.

It might seem like a weird idea to you, but we can calculate the concentration of water in a solution (or even pure water) by simply dividing the amount of water in box 1 (n_1 [=] mol) by the volume of box 1 (V_1 [=] L) to get the concentration ($c_1 = n_1/V$) and similarly for box 2. For pure water at 22 °C, this concentration is $c_w^* = [\mathrm{H_2O}]_{\mathrm{pure}} = 55.386\ \mathrm{mol/L}$ see equation (5.1) for the

calculation. The notation c_w^*, is the technically correct way to indicate the concentration of pure water, but we'll drop the * and write c_w to make our notation easier to write, i.e.:

$$c_w = \frac{\rho(22\ ^\circ\text{C})}{M_r} = \frac{\left(0.99777\ \frac{\text{g}}{\cancel{\text{mL}}}\right)}{\left(18.015\ \frac{\text{g}}{\text{mol}}\right)}\left(\frac{1000\ \cancel{\text{mL}}}{1\ \text{L}}\right) = 55.386\ \frac{\text{mol}}{\text{L}} \tag{5.1}$$

If solutes are added to pure water, this "dilutes" the water, and the water concentration decreases. For a normal red blood cell, the **effective water concentration** is reduced by $0.290\ \text{mol/L}$ so that $c_2 = 55.096\ \text{mol/L}$.

Q.5.3 DISCUSSION QUESTION **(a)** By considering the FD diagram in Fig.5.4, *show that* the change in the water concentration in box 2 during a short time δt is given by

$$\delta c_2 = k(c_1 - c_2)\delta t \tag{5.2}$$

(b) Using equation (5.2) *write out* a complete FD algorithm, including unit checks, to calculate how the water concentration c_2 in the red blood cell changes with time using a jump rate constant of $k = 0.5\ \text{ms}^{-1}$ (0.5 per millisecond), a timestep of $\delta t = 1\ \text{ms}$ and a *constant* bath water concentration of $c_1 = 55.116\ \text{mol/L}$ and an intial value of the RBC water concentration of $c_{2_0} = 55.096\ \text{mol/L}$.
(c) Using your algorithm, calculate by hand what happens for steps 0, 1, and 2 and *write* your answer in the form of an output table.
Hint: As usual, you should do parts (b) and (c) of this question together. It's easier that way.

Open the preformatted spreadsheet BPM.Ch05_Marble_game.xlsx and implement your algorithm. Check that it generates exactly the same sequence that you calculated in Q.5.3(c). Then plot the RBC water concentration c_2 versus time and then (as usual) *adjust* the timestep δt until you're sure that your graph is accurate.

Q.5.4 **(a)** Change the bath water concentration to $c_1 = 54.806\ \text{mol/L}$ and *record* your graph.
(b) By inspecting your graph, *estimate* how long it takes the system to reach equilibrium.

Q.5.5 DISCUSSION QUESTION *Briefly discuss* what happens when you change the bath to
(a) $c_1 = 0\ \text{mol/L}$, and
(b) $c_1 = c_w = 55.386\ \text{mol/L}$.
(c) Before you read the following AWYD, *briefly discuss* whether the equilibrium values you obtained in parts (a) and (b) make sense for a real RBC.

About what you discovered: there's a problem with the marble game model!

Modelers often use extreme cases like $c_1 = 0$ (no water) or $c_1 = c_w$ (maximum water) to test the limits of a model and to see whether it makes sense in those cases. Your answer to Q.5.5(b) should have looked something like Fig.5.5. It shows that there's a problem with our marble game model of water diffusion when $c_1 = c_w$! Here's why – it predicts that the water concentration in a red blood cell placed in contact with pure water will increase until it reaches exactly the concentration of pure water (the dashed line). But that's impossible because an intact red blood cell still contains other molecules and ions like Hb, K^+ and Cl^- etc. … so the water concentration can never reach c_w (the concentration of pure water).

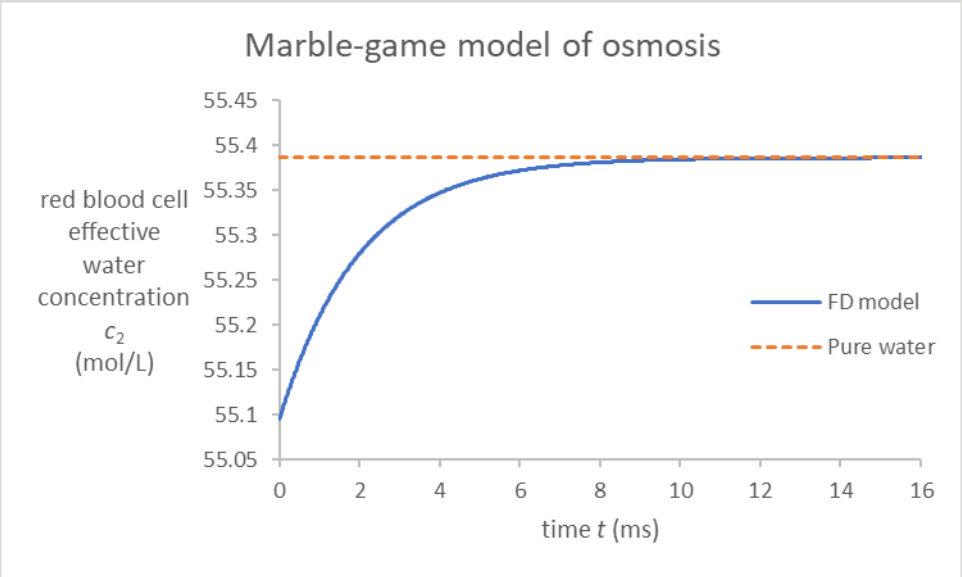

Fig.5.5 Excel 2016 chart of our initial two-box marble model of osmosis. There is a problem with this model when $c_1 = c_w$ because it *predicts* that the interior of a red blood cell can be diluted down to pure water.

The problem is even trickier than you might expect because it relates to assumptions implicit in the original marble game that you've probably never thought about before. We'll discuss these problems and how to resolve them next. ❑

Hidden marble game assumptions

As we discovered in Q.5.5 there is a problem with our first FD model of osmosis. The problem arises because we implicitly assumed that the boxes don't change when a marble jumps from one box to the other. This assumption is a good one for all of the situations we have considered so far, but it doesn't work for osmosis. That's because when a water molecule jumps from the bath into the RBC the volume of the RBC must increase by the volume v_w of one water molecule. In our derivation of FD equations such as equation (3.30) and (5.2), we implicitly assumed that the volume of the box remains constant. This is a good approximation if the concentration is low (as it was for the O_2 molecules in the blood plasma oxygenation example of **CHAPTERS 1** and **3**) or if solute and solvent molecules can exchange places during solute diffusion. This was an implicit

assumption in the discussion of the Brownian motion in Figure 1.4 (reproduced in Fig.5.6) where the tagged molecule diffuses by displacing solvent molecules.

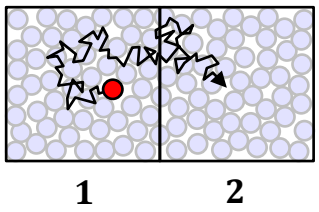

Fig.5.6 (same as Figure 1.4) Schematic representation of the physical basis for the original marble game. The black line is a "snail trail" of one possible trajectory that moves the tagged molecule from box 1 → 2. The net effect of this trajectory is that the tagged molecule jumps from box 1 → 2 during a short period of time δt. However, if the density of each box is to remain constant, then the jump of the tagged molecule must (on average) be accompanied by the net movement of one untagged molecule in the opposite direction, but that can't happen during osmosis.

However, osmosis is different from the situation considered in the original marble game, not only because the concentration of water is high (it's as large as it can possibly be for pure water c_w), but there is no mechanism for the "untagged" molecules to replace the tagged molecule. As a result, we need to change our model to account for the fact that the jump of a water molecule from box 1 → 2 results in an increase in the volume of the red blood cell and a jump from box 2 → 1 results in a decrease. This further assumes that the RBC is flexible enough that its volume can change without increasing (or decreasing) the hydrostatic pressure in the RBC. A two-box system like the one shown in Fig.5.4 in which both boxes are at constant temperature and pressure (constant T, P) is said to be in the **Gibbs ensemble** [Panagiotopoulos *et al.* 1988].

Thus, while FD equation (5.2) correctly accounts for the change in the number of water molecules entering or leaving the red blood cell, it fails to account the associated volume change at constant pressure within the Gibbs ensemble. Hence, our first task is to correctly calculate the number of moles of water molecules δn_2 that correspond to δc_2 in equation (5.2) and then we can calculate the associated change in volume δV_2 of the RBC. At the beginning of the experiment, when equation (5.2) is correct, the volume of the RBC is given by $V_2 = V_{2_0}$. Hence, we can write

$$\delta c_2 = \frac{\delta n_2}{V_{2_0}} \tag{5.3}$$

which can be combined with equation (5.2) to give

$$\delta n_2 = V_{2_0} k(c_1 - c_2)\delta t \tag{5.4}$$

Note: The net rate $\delta n_2/\delta t$ at which water molecules enter the RBC is always proportional to $(c_1 - c_2)$ so long as the surface area of the RBC doesn't change. Hence, equation (5.4) uses the parameter V_{2_0} (initial volume) and not the variable volume V_2.

Equation (5.4) predicts the increase δn_2 in the number of moles of water molecules n_2 in the RBC during a short time δt. These water molecules increase the volume of the RBC by an amount δV_2. Because the added molecules are pure water, the ratio of the small number to the corresponding volume is simply the concentration of pure water, i.e. $\delta n_2/\delta V_2 = c_w$. Hence, we can calculate the volume change of the RBC during a short time δt using

$$\delta V_2 = \frac{\delta n_2}{c_w} \tag{5.5}$$

We can then use equations (5.4) and (5.5) to calculate the *new* values of n_2 and V_2 using the FD update equation (3.31). The new values of n_2 and V_2 can then be used to calculate the *new* effective concentration of water in the RBC using the definition of concentration

$$c_2 = \frac{n_2}{V_2} \tag{5.6}$$

Q.5.6 **(a)** If we use **femtoliters** for volume and **femtomoles** for the number of water molecules in box 2 in equation (5.6), *show that* the concentration has units of mol/L. I.e. if V_2 [=] fL (1 fL $= 10^{-15}$ L) and n_2 [=] fmol, *show that* $c_2 = n_2/V_2$ [=] mol/L.
(b) Using equations (5.4)-(5.6) *write out* a complete FD algorithm, including unit checks, to calculate how the volume V_2 and water concentration c_2 change with time using a jump rate constant of $k = 0.5\ \text{ms}^{-1}$, a timestep of $\delta t = 300$ ms and a (constant) bath water concentration of $c_1 = 55.116$ mol/L and an intial value of the RBC volume of $V_{2_0} =$ 90 fL. The initial value of the RBC water concentration is $c_{2_0} = 55.096$ mol/L and the initial number of water molecules in box 2 can be calculated using $n_{2_0} = c_{2_0} * V_{2_0}$. You should use fL units for the volumes, n_2 [=] fmol and mol/L for the concentrations. As usual, don't forget to do unit checks to make sure that your algorithm calculations have the correct units.
(c) Using your algorithm, calculate by hand what happens for steps 0, 1, and 2 and *write* your answer in the form of an output table.
Hint: As usual, you should do parts (b) and (c) of this question together. It's easier that way.

Q.5.7 Implement your algorithm in the preformatted spreadsheet BPM.Ch05_RBC.xlsx and check that it generates exactly the same sequence that you calculated in Q.5.6(c). Then plot $V_2(t)$ and $c_2(t)$ in separate graphs and adjust the timestep δt and the duration of your sim until you're sure that your graphs are accurate and show the approach to equilibrium. Change the bath water concentration to $c_1 = 54.806$ mol/L and *record* your $V_2(t)$ and $c_2(t)$ graphs, i.e. *record* two separate graphs.
Hint: If you get crazy values, make sure that you adjusted δt. It shouldn't be 300 ms.

Q.5.8 DISCUSSION QUESTION **(a)** *Briefly discuss* how your graph of $c_2(t)$ (with $c_1 = 54.806$ mol/L) differs from your answer to Q.5.4.
(b) Why does it now take so much longer to reach equilibrium?
Hint: The rate at which the water molecules jump into and out of the RBC doesn't change, but the volume V_2 does. *Briefly explain* why that makes such a big difference.
(c) With $c_1 = c_w = 55.386$ mol/L, does c_2 ever reach c_w in your model? *Briefly explain*.
(d) With $c_1 = c_w = 55.386$ mol/L, carefully inspect your $V_2(t)$ graph and *briefly explain* why the volume graph is an unrealistic representation of what happens to a real red blood cell after about three to four seconds.

Osmolarity (osmotic concentration)

Biochemists and physiologists don't normally describe osmosis using effective water concentration. Instead they talk about **osmolarity** which is the effective concentration of osmotically active particles (solutes) in solution (BTW there is currently a move to replace this term with **osmotic concentration**). The idea behind osmolarity is that instead of specifying the effective water concentration in box 2 c_2, we specify the effective concentration $s_2 = n_{s_2}/V_2$ of the particles that are "diluting" the water in box 2, where n_{s_2} is the number of moles of solute particles in box 2. For simple molecular compounds (e.g. sucrose), the osmolarity s_2 is the solute concentration in box 2 in mol/L. However, many substances break up into more than one particle when they dissolve in solution. A familiar example is common table salt NaCl, which dissociates into sodium ions Na^+ and chloride ions Cl^-. From an osmotic point of view, each ion acts to dilute the water. Hence, the osmolarity of a dilute NaCl solution is twice the salt concentration [NaCl], i.e. $s_2 = [Na^+] + [Cl^-] = 2[NaCl]$. To avoid confusion with regular concentration, the osmotic concentration (osmolarity) is given in units of **osmoles** per liter (osmol/L or Osm/L). Hence, a solution with a salt concentration of $[NaCl] = 10$ mmol/L in a RBC has an osmolarity of $s_2 = 20$ mOsm/L (milliosmoles per liter). The total osmolarity is obtained by adding up the osmolarity of all solutes in the RBC. This results in an osmolarity of 290 mOsm/L for the normal RBCs in the study conducted by Mathai *et al.* [1996].
Please note: From a dimensional point of view Osm [=] mol, so that Osm and mol are able to cancel each other out in numerical calculations.

Osmolarity s_2 and the effective concentration c_2 are related by

$$c_2 = c_w - s_2 \tag{5.7}$$

and similarly for box 1

$$c_1 = c_w - s_1 \tag{5.8}$$

where c_w is the concentration of pure water. Equations (5.7) and (5.8) can be rearranged to give osmolarities s_1 and s_2 in terms of the effective water concentrations c_1 and c_2 that we used in our diffusive model of osmosis

$$s_1 = c_w - c_1 \tag{5.9}$$

$$s_2 = c_w - c_2 \tag{5.10}$$

About what you discovered: effective concentrations defined

Equations (5.7) and (5.8) *define* the effective concentration of water in dilute solutions. c_w is the real concentration of pure water and s_2 is the real osmotic concentration of solute particles in box 2. However, the effective concentration c_2 is only a real concentration (number divided by volume) if the solute particles have the same volume as water molecules. We'll talk more about effective concentrations and the origin of equation (5.7) in a later chapter [Nelson 2017]. ❑

Q.5.9 Using equations (5.4)-(5.10) *show that* equation (5.4) implies equation (5.11).

$$\delta V_2 = \frac{kV_{2_0}}{c_w}(s_2 - s_1)\delta t \tag{5.11}$$

In their experiment, Mathai *et al.* [1996] measured V_r the **relative volume** of the RBC as a function of time, which is defined as the current volume of the RBC V_2 divided by the initial volume of the RBC V_{2_0}

$$V_r \equiv \frac{V_2}{V_{2_0}} \tag{5.12}$$

Q.5.10 **(a)** Using the definition of solute concentration $s_2 = n_{s_2}/V_2$ and equation (5.12) *show that*

$$s_2 = \frac{s_{2_0}}{V_r} \tag{5.13}$$

Hint: $n_{s_2}/V_{2_0} = s_{2_0}$.

(b) Using equation (5.11) and (5.13) and the equivalent equation for a small change in relative volume $\delta V_r = \delta V_2/V_{2_0}$, *show that*

$$\delta V_r = \frac{k}{c_w}\left(\frac{s_{2_0}}{V_r} - s_1\right)\delta t \tag{5.14}$$

(c) By comparing equation (5.14) with the fifth equation (unnumbered) in the left-hand column of page 1310 of Mathai *et al.* [1996] and by noting that $(\text{MVW}) = \bar{V}_w = 1/c_w$ is the **molar volume** of pure water, *show that* equation (5.14) is the same as the equation used by Mathai *et al.*, if our jump rate constant k is given by[1]

$$k = \mathcal{P}_f \times (\text{SAV}) = 0.5\left(22.8 \times 10^{-3}\,\frac{\text{cm}}{\text{s}}\right)\left(\frac{1}{4.57 \times 10^{-5}\,\text{cm}}\right) = 250\ \text{s}^{-1} \tag{5.15}$$

[1] The factor of 0.5 in equation (5.15) is needed because the initial volume V_{2_0} is twice the final volume.

In your answer, you should identify how each symbol in Mathai *et al.*'s equation matches up with those in equation (5.14). For example, $\mathrm{d}V(t) \longmapsto \delta V_r$ etc…
Hint: The full reference for Mathai *et al.* [1996] is listed in the **REFERENCES** section at the end of this chapter.
Note: The parameter SAV used by Mathai *et al.* is the surface area to volume ratio of the RBC. $(\mathrm{SAV}) = A_2/V_{2_0}$ [=] cm^{-1}, where A_2 is the surface area of the RBC and V_{2_0} is the initial volume of the RBC. $\mathcal{P}_f$ [=] cm/s is the **filtration permeability** or **osmotic permeability** of the RBC membrane. Permeabilities are discussed in more detail in a later chapter.

Q.5.11 **(a)** Using equation (5.14), *write out* a complete FD algorithm, including unit checks, to calculate how the relative volume V_r of a RBC changes with time in the Gibbs ensemble using a jump rate constant of $k = 250\ \mathrm{s}^{-1}$, a timestep of $\delta t = 0.2$ s and a *constant* bath osmolarity of $s_1 = 580$ mOsm/L an initial value of the RBC osmolarity of $s_{2_0} = 290$ mOsm/L.
Hint: By definition (5.12), $V_r = 1$ at $t = 0$. You will also need to match the units of c_w, s_{2_0} and s_1 (recall that from a dimensional point of view, mOsm/L [=] mmol/L [=] mM).
(b) Using your algorithm, calculate by hand what happens for steps 0, 1, and 2 and *write* your answer in the form of an output table.
Hint: As usual, you should do parts (a) and (b) of this question together. It's easier that way.

Q.5.12 Implement your FD algorithm in the FD table of the preformatted spreadsheet BPM.Ch05_Mathai_et.al.xlsx, which also includes experimental data from the control in Fig. 5*A* of Mathai *et al.* [1996]. Check that your FD table generates the exact same sequence that you calculated in Q.5.11(b). Your FD prediction for $V_r(t)$ should be automatically plotted as the **diffusive FD model** series in the premade graph. Check that, then reduce the timestep δt until you're sure that your FD graph is accurate.
(a) *Record* your graph.
(b) Your FD graph should look very similar to the experimental data for the first half second, but after that the experimental data becomes steadily higher than the prediction of equation (5.14). *Briefly discuss* why that might be?
Hint: Is water the only type of molecule that can cross a real RBC lipid bilayer?

About what you discovered: diffusive model predicts osmotic swelling/shrinking

The diffusive model predicts that osmotic swelling/shrinking is governed by equation (5.14). As you just discovered, this equation is identical to the traditional equation that Mathai *et al.* [1996] used to model their experimental data. Hence, our diffusive model makes exactly the same prediction as the traditional model of osmosis advocated by Kramer and Myers [2013]. Based on this mathematical equivalence we have no reason to prefer one model over the other. However,

from a conceptual perspective, our diffusive model is much simpler to understand. We'll return to this comparison at the end of **SECTION 5.3** after we've investigated how a pressure difference drives osmosis according to our diffusive model. ❑

About what you discovered: fitting models to validate them and find parameter values

Your answer to Q.5.12(a) should look something like Fig.5.7. Mathai *et al.* did the fit to the equation for us and found the values listed in equation (5.15) that we used to calculate our jump rate constant k. In **CHAPTER 4** we learned how to use regression in Excel to find fitted parameters. We'll wait until **CHAPTER 6** to learn how to do least-squares fits like those used by Mathai *et al.*, when we model ligand binding and enzyme kinetics.

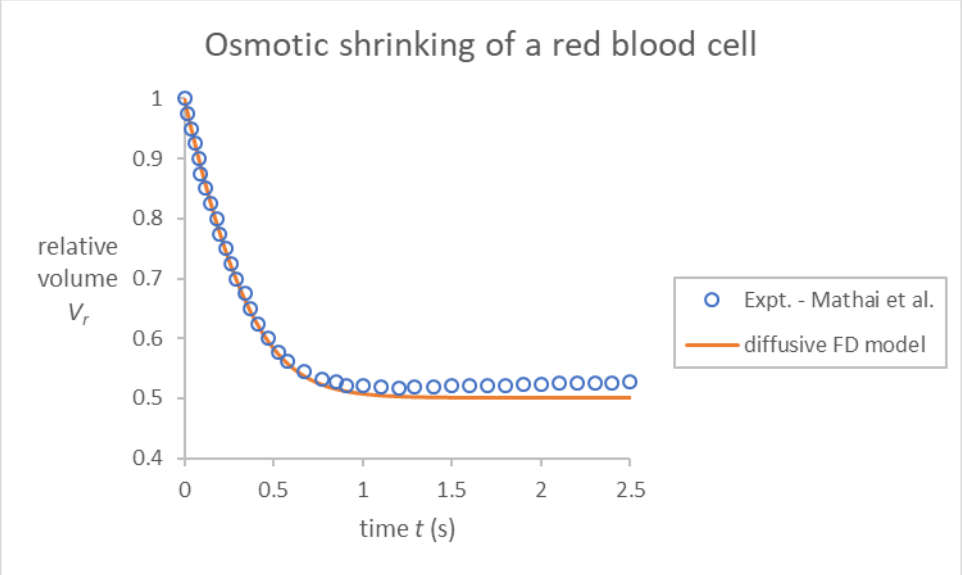

Fig.5.7 Excel 2016 chart showing the control (+/+) data from Fig. 5*A*. of Mathai *et al.* [1996] for the osmotic shrinking of a red blood cell, together with the diffusive model of osmosis calculated using FD equation (5.14). The experimental data were obtained by digitizing Fig. 5*A* and are reproduced here with permission from Mathai *et al.* [1996]).

❑

Q.5.13 **(a)** Delete the experimental data from the graph and adjust the initial value of the RBC osmolarity of s_{2_0} in your spreadsheet and *tabulate* how s_{2_0} affects the equilibrium relative volume V_r^{eq}.

(b) Plot the equilibrium relative volume versus s_{2_0}, add an appropriate trendline and *record* your graph.

(c) What mathematical relationship can you *hypothesize* from your graph?

(d) Using equation (5.14), *show that* the equilibrium relative volume is given by

$$V_r^{\text{eq}} = \frac{s_{2_0}}{s_1} \tag{5.16}$$

(e) If the relative volume of the RBC does not change, what does equation (5.16) predict for the equilibrium relationship between blood plasma s_1 and RBC osmolarity s_{2_0}. *Briefly discuss* why that makes sense.

About what you discovered: osmotic swelling/shrinking

Q.5.13 guided you to the discovery of equation (5.16). It provides a particularly simple prediction for what happens to a RBC when you drop it into a solution with an osmolarity that differs from blood plasma. This is a useful model to keep in mind while you're studying physiology. It tells you by how much a cell will swell or shrink if it is in contact with a fluid of differing osmolarity. It predicts that even a small change in osmolarity in the surrounding fluid can have a rather dramatic effect on the volume of a cell. As a result, the osmolarity of fluids in the body must be closely regulated to avoid swelling/shrinking. ❑

Q.5.14 DISCUSSION QUESTION Equation (5.16) implies that the RBC volume can change to any value. However, in practice RBC volume clearly must have limits.

(a) *Briefly discuss* what happens if the bathing solution has a very high or a very low osmolarity.

(b) *Briefly explain* what happens to a RBC that's dropped into (pure) water according to equation (5.16).

(c) *Briefly explain* what really happens to a red blood cell when it's dropped into distilled water.

(d) *Briefly explain* what happens to a limp piece of celery if you cut its base and place it in a glass of tap water.

(e) *Briefly explain* why the celery doesn't explode like an RBC when it's placed in distilled water.

(f) *Briefly explain* why you don't explode when you dive into a swimming pool or have a bath?

(g) *Briefly explain* why intravenous drugs are administered using "normal" saline solution rather than pure water.

About what you discovered: pressure and osmosis

The reason that you don't explode when you dive in a swimming pool (waterproof skin) is different from the reason why a celery stalk doesn't explode when you place it in a glass of water. The limp celery stalk becomes crisp again because of turgor pressure that is induced by the tap water. The rigid cell walls of the celery allow for the pressure inside the cell to increase and somehow that buildup of pressure slows down the diffusion of water into the plant cells. In order to understand how that happens, we'll need to take a detour from osmosis to discuss how differences in potential energy affect molecular diffusion. ❑

5.2 Marble game with gravity

In this section, we're going to investigate how potential energy differences affect molecular diffusion. This idea can be illustrated using a modified marble game where the marbles jump between two boxes that are at different heights (Fig.5.8). This might seem like a contrived example, but it will allow us to investigate one of the most important – and universal – principles in all of molecular physics and biochemistry. It also turns out that this gravity marble game is also a good model of N_2 and O_2 molecules in earth's atmosphere.

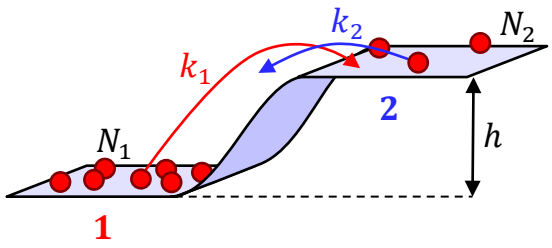

Fig.5.8 Schematic representation of the gravity marble game. The jump rate constant in the uphill direction (from box 1 → 2) is k_1. k_2 is the jump rate constant in the downhill direction (from box 2 → 1). The marbles each have mass m and the two boxes are separated by height h.

As shown in Fig.5.8, the gravity marble game has two boxes separated by a height h. Marbles jumping from box 1 → 2 have to jump uphill against gravity, whereas marbles jumping from box 2 → 1 can simply fall down the slope. Our plan is to make a mathematical model of this system that can be used to model molecules jumping between the two boxes by diffusion. In order to do that, we've allowed for the possibility that the jump rates might be affected by gravity. k_1 is the jump rate constant for uphill jumps (originating in box 1) and k_2 is the jump rate constant for downhill jumps (originating in box 2).

Q.5.15 DISCUSSION QUESTION *Briefly discuss* which rate constant you would expect to be higher and what factors are likely to affect the relative rates in each direction.

FD analysis of the gravity game

Q.5.16 *Draw* a properly labeled FD diagram of the marble game shown in Fig.5.8.

Constructing the FD diagram of the system shown in Fig.5.8 is very straight forward and would make for an easy test question, so before you look at the following AWYD, you should draw your own and focus on making sure that each of the arrows are labeled with a **rate** that has units of per second [=] s^{-1}.

About what you discovered: an easy test question!

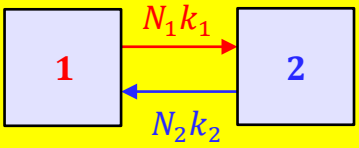

Fig.5.9 FD diagram of the gravity marble game.

Note: δt is *not* included in the rates! That is the most common error that I've seen from my students. ❑

Q.5.17 **DISCUSSION QUESTION** The FD diagram in your answer to Q.5.16 (Fig.5.9) should have been very similar to the plasma oxygenation model we investigated in **CHAPTER 3**. *Briefly describe* the analogy between the gravity marble game and the plasma oxygenation model from **CHAPTER 3**.
Hint: In your analogy, you should identify and explain the relationship between the rate constants and numbers in Figure 3.4 compared with those in Fig.5.9.

Q.5.18 By carefully considering the FD diagram 5.9, *show that* the (small) change in box 1 δN_1 during a short time δt is given by

$$\delta N_1 = (N_2k_2 - N_1k_1)\delta t \tag{5.17}$$

Q.5.19 Using equation (5.17), *show that* the ensemble-average *equilibrium ratio* of the numbers in the two boxes is given by

$$\frac{N_1}{N_2} = \frac{k_2}{k_1} \quad (only \text{ at equilibrium}) \tag{5.18}$$

Hint: Recall from **CHAPTER 3** that the condition for equilibrium is that N_1 does not change with time, i.e. $\delta N_1 = 0$.

About what you discovered: equilibrium ratios

Despite its simplicity, equation (5.18) is extremely important for any two-box marble game or any other system (e.g. chemical reactions) that can be represented by an FD diagram similar to Fig.5.9. It tells us that if we know one of the ratios at equilibrium (say N_1/N_2), then we also know what the other ratio (k_2/k_1) must be. In what follows, we'll use what we know about the ideal gas to determine how the density of the atmosphere changes with a small change in height δy. Once we have that information (about N_1/N_2) we can then use it to discover the mathematical form of the ratio k_2/k_1 (for small δy). ❑

Isothermal atmosphere – local change in conditions

Our task in this section is to determine the equilibrium ratio N_1/N_2 for a particularly simple molecular system – an ideal gas at constant temperature [Adkins 1987]. We can then use equation (5.18) to find the relationship between k_1 and k_2.

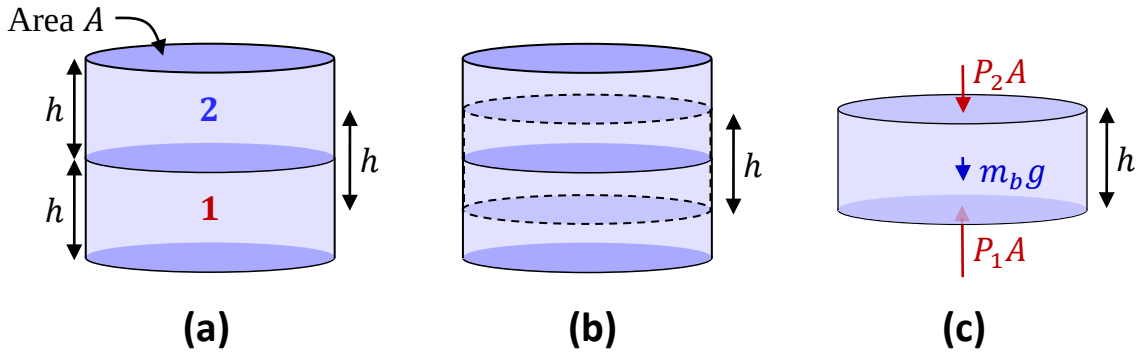

Fig.5.10 Imaginary boxes surrounding pillbox-shaped volumes of air. The cylindrical boxes have height h and circular end caps of area A. **(a)** Boxes 1 and 2 correspond to Figures 5.7 and 5.8. **(b)** Indicates the location of a central pillbox (dashed lines) with end caps located at the middle of boxes 1 and 2. **(c)** Isolated central pillbox with arrows indicating forces acting *on* it (after Adkins [1987]).

Fig.5.10(a) shows two volumes of air stacked one on top of the other. The two volumes correspond to our two-box gravity marble game, but the boundaries of the pillbox-shaped (cylindrical) boxes are completely imaginary. They have flat top and bottom end caps with area A and the cylinders have height h. There are no actual confining walls separating box 1 from box 2 (or either of them from the rest of the atmosphere). For the sake of simplicity, we'll consider the marbles to be nitrogen molecules at room temperature $T = 295$ K. From the ideal gas law, we know that for box 1, the pressure P_1 [=] Pa [=] N/m^2 and number of molecules N_1 [=] 1 are related by

$$P_1 V = N_1 k_B T \tag{5.19}$$

so that

$$N_1 = \frac{P_1 V}{k_B T} \tag{5.20}$$

Note that the volume V [=] m^3 and temperature T [=] K are the same for both boxes and $k_B = 1.38065 \times 10^{-23}$ J · K^{-1} is the **Boltzmann constant**. Similarly

$$N_2 = \frac{P_2 V}{k_B T} \tag{5.21}$$

Hence, by dividing equation (5.20) by (5.21), the ratio of the numbers in boxes 1 and 2 at equilibrium is given by

$$\frac{N_1}{N_2} = \frac{P_1}{P_2} \tag{5.22}$$

Equation (5.22) means that for an ideal gas at constant temperature we can use the pressure ratio to find the number ratio. To make that comparison, let's consider a third pillbox-shaped volume of air spanning the two original boxes (Fig.5.10(b)). As shown in Fig.5.10(c), the pressure on the top end cap of the central pillbox is P_2 and the pressure on the bottom end cap is P_1. Pressure is defined as force per unit area, so that $P_2 = F_2/A$, or $F_2 = P_2A$ and the downward force F_2 applied to the top surface of the pillbox by the surrounding atmosphere is P_2A (Fig.5.10(c)). Similarly, the upward force F_1 applied to the bottom surface of the central pillbox by the surrounding atmosphere is P_1A. If the gas in the central pillbox had no mass, then F_1 and F_2 would be the only external forces. However, because the gas does have a small mass, gravity exerts a body force on every molecule in the central pillbox which combines to give the downward gravitational force m_bg where m_b is the mass of all the nitrogen molecules in the central box

$$m_b = Nm \tag{5.23}$$

where N is the number of nitrogen molecules of mass m in the central pillbox.

There are also horizontal forces pushing in on the barrel-shaped curved surface of the cylinder, but all of the horizontal forces cancel out because there is an equal and opposite force applied on the far side of the cylinder. Hence, a free-body diagram of the central pillbox looks like Fig.5.11.

Reminder – free-body diagrams

As it might be a while since you used a **free-body diagram** (**FBD**), let's remind ourselves of what they are about and how they are used. The purpose of a free-body diagram is to find the net force $\vec{F}^{\text{net}}$ acting on an object free of any distractions caused by the surrounding objects. Once you have $\vec{F}^{\text{net}}$ you can determine the acceleration of the object using **Newton's second law** of motion $\vec{a} = \vec{F}^{\text{net}}/m$. The reason for taking the time to draw such a simple diagram is similar to the reason why we draw FD diagrams even for simple systems. If you don't do it – you might miss something important! The discipline of drawing the diagram makes you think about the problem in a very simple manner, with all forces illustrated as pulls even though they might really be pushes. The idea is to focus on the magnitude and direction of the force, not what is producing it.

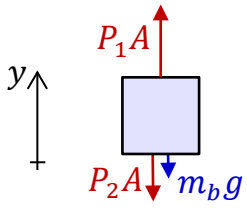

Fig.5.11 Free-body diagram (FBD) of the pillbox-shaped volume of air shown in Fig.5.10(c). **Note:** all of the forces are acting *on* the pillbox.

In a situation like Fig.5.10(c) there are always twice as many forces as shown in the free-body diagram. That's because a force is one half of the interaction between two objects. **Newton's third law** summarizes this observation by telling us that interactions always produce pairs of equal and

opposite forces that, as a force pair, describe either an attraction or a repulsion. The major purpose of a free-body diagram is to make us select the correct half of the interaction – the **external force** acting *on* the body (and not the forces produced *by* the object on other things). The word "external" in the previous sentence is extremely important, because internal forces also come in pairs, but by definition they both act on the same body and therefore can have no effect on the center-of-mass motion of the body because they cancel out when you consider the body as a whole.

The fluid in the pillbox of Fig.5.10(c) repels the surrounding fluid through a contact force per unit area, i.e. a pressure. The external fluid pushes in on the pillbox and the fluid in the pillbox pushes back on its surroundings with equal and opposite force at every point on the surface of the pillbox. However, it is only the inward forces that appear in the free-body diagram, because they act *on* the pillbox.

The other interaction shown in the free-body diagram is the gravitational attraction between the fluid in the pillbox and the earth. The earth pulls down on the air in the pillbox and the air pulls up on the earth. However, it is only the downward pull of gravity that appears in the free-body diagram because it's the only part of the attraction that acts *on* the pillbox. The arrow labeled $m_b g$ represents the sum of the forces acting on each molecule in the whole body of the fluid in the pillbox.

Wow – that was a much longer explanation than I expected! Perhaps that's why people find physics so difficult. Simple situations are often much more complicated that they first appear!

Now that we've reviewed the free-body diagram, let's use it for its intended purpose – to find the net force acting on the pillbox. By inspecting the free-body diagram we can write

$$F_y^{\text{net}} = P_1 A - P_2 A - m_b g \qquad \text{(FBD)} \tag{5.24}$$

and Newton II gives

$$F_y^{\text{net}} = m_b a_y \qquad \text{(NII)} \tag{5.25}$$

Q.5.20 By combining equations (5.24) and (5.25) *show that* that the acceleration of the pillbox is given by

$$a_y = \frac{(P_1 - P_2)A}{m_b} - g \tag{5.26}$$

Q.5.21 **DISCUSSION QUESTION** Using equation (5.26) *briefly explain* what conditions can make the parcel of air in the pillbox accelerate either upwards or downwards.
Hint: The values of P_1 and P_2 are determined by the rest of the atmosphere so that they don't change. What can be different is the temperature. Equation (5.20) shows how temperature can affect the number of molecules N and hence the mass $m_b = Nm$ of the parcel of air.

About what you discovered: thermal convection – hot air rises

When the air in the pillbox is hotter that the surrounding atmosphere, its density is less than the surrounding air. This makes m_b smaller than the equilibrium value. Equation (5.26) then predicts that the parcel of air will accelerate upwards with $a_y > 0$. This is often described as a **buoyant force** that makes hot air rise and cold air sink. ❑

Q.5.22 If the fluid in the pillbox is stationary, so that $a_y = 0$, *show that*

$$\Delta P = -\rho g h \tag{5.27}$$

where

$$\rho = \frac{m_b}{V} \tag{5.28}$$

is the **mass density** of the fluid in the pillbox and as usual $\Delta P = P_2 - P_1$.
Hint: The volume V of the pillbox is related to its height h and end cap area A by $V = Ah$.

About what you discovered: ocean deep

Equation (5.27) works for any fluid. An interesting and particularly simple example is the ocean. Because water is basically incompressible it has an almost constant density ρ – no matter what the pressure. The density of seawater is $\rho_{\text{sw}} \cong 1030\ \text{kg/m}^3$, so that equation (5.27) predicts that the pressure in the ocean (or a deep lake) increases by about one atmosphere for every $h = 10$ m of increased depth. ❑

Q.5.23 Unlike water, air *is* **compressible**, which means that its density is not constant. As a result, the number of molecules in each box is able to vary with height. This change in density is accompanied by a change in pressure. In order to use equation (5.27) we'll need to change the pressures and densities into numbers of molecules. Using equations (5.27), (5.28), the ideal gas law (5.20) and (5.21) and the fact that $m_b = Nm$ for the central pillbox, *show that* the number difference between boxes $\delta N = N_2 - N_1$ is given by

$$\delta N = -\frac{mgh}{k_{\text{B}}T} N \tag{5.29}$$

Equation (5.29) can be written as

$$\delta N = -\frac{\delta E}{k_{\text{B}}T} N \tag{5.30}$$

where we have defined $\delta E = mgh$ as the small gravitational potential energy difference between a molecule in box 2 and a molecule in box 1.

About what you discovered: the most important equation in molecular biophysics!

An amazing fact about equation (5.30) is that, as far as anyone knows, it's true for *any* molecular system at equilibrium! Even though we derived it for an ideal gas, the same equation is true for

any molecular system that has two states separated by a **small energy difference** δE. The energy need not be gravitational potential energy; it can be *any* kind of energy! All forms of energy are covered: including potential energies such as gravitational, electrical, chemical, magnetic and mechanical potential energy stored in a compressed spring or a fluid… The last example, energy stored in a compressed fluid, is the one that's important for osmosis. The mechanical PV work done moving water molecules from low to higher pressure is a key aspect of osmosis. As we'll see in later chapters, kinetic energy and heat are also covered by equation (5.30). ❑

Since equation (5.30) is so useful, and because we'll be using it so often, it's worthwhile defining a new **dimensionless variable** using the Greek letter psi (which rhymes with the name of the Korean singer – famous for dancing like a horse in a YouTube video [officialpsy 2012] that's been viewed more than 3.8 billion times!). See Greek letters go green! for more about the Greek letters and how they are used in Biophysics and Physiological Modeling [Nelson 2013]).

$$\delta\psi = \frac{\delta E}{k_B T} \tag{5.31}$$

This energy difference is dimensionless because the numerator δE [=] J and the denominator $k_B T$ [=] J are both energies so that the units cancel. As mentioned above, δE must be *small* – but what does that mean? Equation (5.30) and the definition of $\delta\psi$ in equation (5.31) answer that question. The **energy difference** δE must be small compared with the **thermal energy** $k_B T$ so that $\delta\psi$ is small compared with 1. The thermal energy is related to the energy that molecules have because they are hot (at some temperature greater than $T = 0$ K). The thermal energy is proportional to the **absolute temperature** T with the **Boltzmann constant** k_B telling us how big the thermal energy is per molecule for a given temperature. We'll return to this idea in **CHAPTER 9**.

Q.5.24 In order to get a feeling for how big the thermal energy is at room temperature $T = 295$ K,

(a) *calculate* the height difference h that makes $\delta E = mgh$ correspond to the thermal energy $k_B T$ for a nitrogen molecule in earth's gravitational field. BTW, this would give $\delta\psi = 1$, a value which is *definitely not* small;

Hint: If your answer were the height of a mountain, it would be as tall as Mt. Everest!

(b) *calculate* the height difference that corresponds to 1/100 of the thermal energy $k_B T$ for an *oxygen* molecule in earth's gravitational field (this corresponds to $\delta\psi = 0.01$, i.e. a small value of $\delta\psi$).

Now that we have some idea of what $\delta\psi$ is and what is does, we can rewrite equation (5.30) in a particularly simple form

$$\delta N = -N\delta\psi \tag{5.32}$$

Equation (5.32) highlights something very important – the *decrease* in the probability of being in box 2 is **directly proportional** to the dimensionless energy difference $\delta\psi$ and the probability of

being in box 1. In other words, the change δN in the ensemble average number as we change the energy ψ is proportional to how many N you already have.

About what you discovered: delta in space not time

Equation (5.32) describes how the number N decreases with potential energy as the height of the box is changed. This equation is of the same mathematical form as equations (3.12) and (4.2) that respectively described the time decay in the marble game order parameter (**CHAPTER 3**) and the time decay in the drug mass concentration during drug elimination (**CHAPTER 4**). We'll return to this important concept again in **CHAPTER 9**. ❑

Our final task for this section is to use equation (5.32) to determine the rate constants in the gravity marble game. In order to do that, we'll assume that the isothermal atmosphere can be modeled by the gravity marble game at equilibrium, so that equation (5.18) applies.

Gravity marble game rate constants

Q.5.25 Equation (5.18) tells us how the marble game rate constants are related to the equilibrium number ratio N_2/N_1, but equation (5.32) uses N and δN instead of N_1 and N_2. In order to relate them together, *show that* equation (5.32) can be converted into equation (5.33) by assuming that $N = N_1$ and $\delta N = N_2 - N_1$.

$$\frac{N_2}{N_1} = 1 - \delta\psi \tag{5.33}$$

Q.5.26 As shown in Fig.5.8, a marble that jumps far enough to the left simply falls down the slope. This implies that the downhill jump rate constant is approximately independent of h because once the marble goes far enough to the left, the size of the drop-off is irrelevant. As a result, we'll assume that for downhill jumps

$$k_2 = k \tag{5.34}$$

where k is a jump rate constant that does not dependent on $\delta\psi$. Using this assumption and equations (5.18) and (5.33), *show that* the uphill rate constant is given by

$$k_1 = (1 - \delta\psi)k \tag{5.35}$$

The energy factor: epsilon

The **dimensionless energy difference** $\delta\psi = \delta E/k_B T$ is the fraction of molecules attempting to jump up the energy step that *don't* have enough energy to make it to the top of the energy step. For example, if $\delta\psi = 0.01$ (Q.5.24), then $1/100$ of the uphill jumps are unsuccessful because they don't have enough energy to reach the top of the energy step. Another way to say the same thing is that the **fraction of successful uphill jumps** is $\varepsilon = 0.99$. Where ε is the **energy factor**

$$\varepsilon = 1 - \delta\psi = 1 - \frac{\delta E}{k_B T} \tag{5.36}$$

which tells us the factor by which the rate constant for uphill jumps is decreased compared with downhill jumps, so long as the dimensionless energy difference $\delta\psi$ is small (Fig.5.12). This means that the uphill jump rate constant is

$$k_1 = \varepsilon k \tag{5.37}$$

About what you discovered: connection with activation energies and introductory chemistry

The argument for why the upwards jump rate is reduced by a factor ε is similar to the one usually made in general chemistry to explain how chemical reaction rates depend on activation energies. However, we *derived* equation (5.37) from our gravity marble game. If you understand the derivation, then you should understand equation (5.37) and where it came from (Fig.5.12). In **CHAPTER 9** we'll return to this topic and discover that this energy factor must be changed to a "**Boltzmann factor**" if the dimensionless energy difference $\delta\psi$ is *not* small.

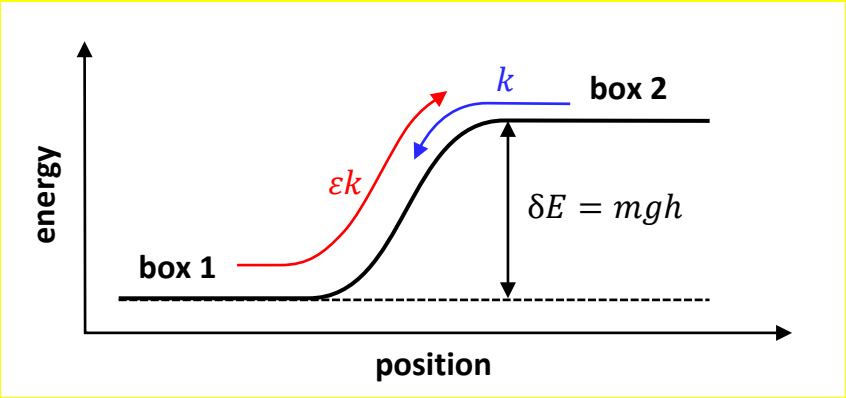

Fig.5.12 Simplified schematic energy diagram of the gravity marble game. The diagram shows a situation where box 2 is higher than box 1 by a height h. Each marble has mass m and the gravitational field g raises a marble's gravitational potential energy by $\delta E = mgh$ when it moves from box 1 → 2. The uphill jump rate is reduced by a factor $\varepsilon = 1 - \delta\psi$, where the small energy step is $\delta\psi = \delta E/(k_B T)$.

❑

5.3 How pressure affects osmosis

Now that we've figured out how a small step in energy affects the jump rates between boxes in the gravity marble game, we can use the same equations (5.34) and (5.37) to investigate how a pressure difference affects osmotic diffusion of water across a semipermeable membrane within the **Helmholtz ensemble** (constant T, V) [Nelson 1998][Nelson *et al.* 1999]. As you know from personal experience, it takes energy to blow air into a party balloon. In a similar way, it takes energy for a water molecule to move from low to high pressure. The energy that's required is a form of mechanical work (**CHAPTER 13**) that's stored in the pressure difference in an analogous manner to how the mechanical work is stored in the height difference in the gravity marble game. As you know from introductory physics, the energy δE stored in the gravitational field is related

to the **work done** δW *by* gravity *on* the molecule when it's height changes as it jumps from box $1 \rightarrow 2$ and moves upwards a distance δy, i.e.

$$\delta E = -\delta W = -F\delta y = -(-mg)\delta y = mg\delta y \tag{5.38}$$

Because the force of gravity is downward, $F = -mg$ (recall $g = 9.8\ \mathrm{m/s^2}$ is a positive quantity). Hence, $\delta E = -(-mg)\delta y = mg\delta y$ as shown in Fig.5.12 (where $h = \delta y$).

Using an argument similar to equation (5.38), we can show that the energy δE stored in the pressure difference is given by $\delta E = v_w \Delta P$ (equation (5.43)). The pressure difference between the two boxes is given by

$$\Delta P = P_2 - P_1 \tag{5.39}$$

Pressure is defined as force per unit area, so that $P_2 = F_2/A$, or $F_2 = P_2 A$ where A is the cross-sectional area of a water molecule in the single-file pore. Similarly, $F_1 = P_1 A$. Fig.5.13 shows the forces acting on the single-file water contained in the aquaporin selectivity filter. The pressure in box 1 pushes the water molecules towards box 2 and the pressure in box 2 pushes the water molecules towards box 1.

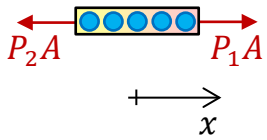

Fig.5.13 Free-body diagram (FBD) of the single-file water contained in the aquaporin selectivity filter. The net force in the x-direction is $F_x^{\text{net}} = P_1 A - P_2 A = -A\Delta P$, where A is the cross-sectional area of a water molecule.

As shown in Fig.5.13, the net force on the single-file water is

$$F_x^{\text{net}} = F_1 - F_2 = -(F_2 - F_1) = -(P_2 A - P_1 A) = -A(P_2 - P_1) = -A\Delta P \tag{5.40}$$

The *net* effect of a knock-on jump is the transfer of a single water molecule from box $1 \rightarrow 2$. The volume v_w of the water molecule is related to the concentration of pure water by

$$v_w = \frac{1}{N_{\mathrm{A}} c_w} \tag{5.41}$$

where N_{A} [=] mol^{-1} is the **Avogadro constant**, the number of molecules in one mole.
Note: v_w is lower case because it represents the volume of a single water molecule (in pure liquid water).

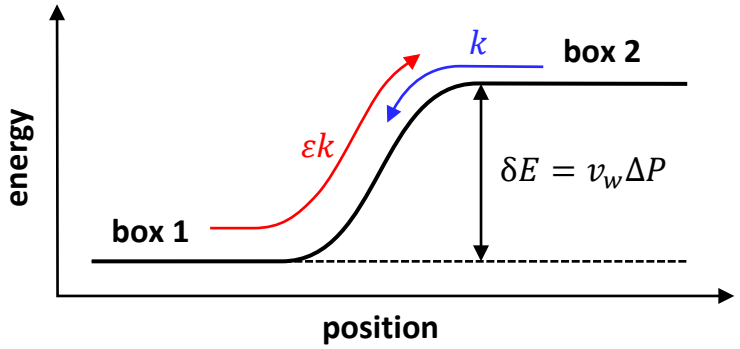

Fig.5.14 Simplified schematic energy diagram of osmosis with a (hydrostatic) pressure difference ΔP. The diagram shows a situation where box 2 has a higher pressure P_2 than box 1 with the difference given by $\Delta P = P_2 - P_1$. The water molecules each have volume v_w and the pressure difference ΔP raises the marble's potential energy by $\delta E = v_w \Delta P$ when it moves from box 1 → 2. The uphill jump rate is reduced by a factor $\varepsilon = 1 - \delta\psi$, where the small dimensionless energy step is $\delta\psi = \delta E/(k_B T)$.

If the water density in the selectivity filter is the same as pure water, then the file moves a distance δx during the knock-on jump that is defined by

$$v_w = A\delta x \tag{5.42}$$

Q.5.27 DISCUSSION QUESTION **(a)** Using equations (5.40) and (5.42) *show that* the potential energy stored by a knock-on jump from box 1 → 2, $\delta E = -\delta W = -F_x^{\text{net}}\delta x$, is related to the pressure difference ΔP by

$$\delta E = v_w \Delta P \tag{5.43}$$

as a water molecule jumps from box 1 → 2 (Fig.5.14).
Note: ΔP [=] N/m^2 [=] $\text{N} \cdot \text{m/m}^3$ [=] J/m^3, which is energy per volume, i.e. an energy density. Hence, if we multiply ΔP by the volume v_w of a single water molecule, as in equation (5.43), we get the corresponding energy δE.
(b) Hence, using equations (5.43) and (5.36), *show that* the associated energy factor is

$$\varepsilon = 1 - \frac{v_w \Delta P}{k_B T} \tag{5.44}$$

Fig.5.14 shows how the energy difference δE affects the rate constants using an energy factor ε that can be calculated from the small energy step δE given by equation (5.43) or the dimensionless energy (5.44) using equation (5.36). This allows us to construct the FD diagram for osmotic diffusion shown in Fig.5.15. The value of ε is determined by the pressure difference as shown in equation (5.44).

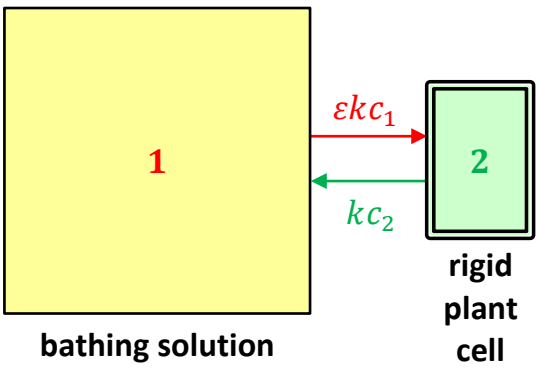

Fig.5.15 FD diagram of a rigid plant cell (box 2) in contact with a large bathing solution (box 1) within the **Helmholtz ensemble** (constant T, V). The water in the rigid plant cell has an effective water concentration c_2 and the bathing solution has a constant effective water concentration c_1. There is also a hydrostatic pressure difference $\Delta P = P_2 - P_1$ between the boxes that is maintained by the rigid cell wall of the plant cell. ΔP determines the value of the energy factor ε (5.44).

Q.5.28 By considering the FD diagram 5.15, *show that* the small change in water concentration δc_2 in box 2 during a short amount of time δt is given by

$$\delta c_2 = k(\varepsilon c_1 - c_2)\delta t \tag{5.45}$$

Q.5.29 If the bathing solution is pure water, then $c_1 = c_w$.

(a) Using equation (5.45) *show that* the effective water concentration in the rigid plant cell *at equilibrium* is given by

$$c_2 = \varepsilon c_w \quad \text{(pure water equilib.)} \tag{5.46}$$

Hint: Recall the condition for equilibrium from **CHAPTER 3**.

(b) Using equations (5.10), (5.46) and (5.36), *show that* the equilibrium osmolarity is given by

$$s_2 = c_w \delta\psi \quad \text{(pure water equilib.)} \tag{5.47}$$

(c) By substituting equations (5.43) and (5.41) into the definition of dimensionless energy (5.31), and then substituting that into equation (5.47) and solving for the pressure difference, *show that*

$$\Delta P = s_2 N_A k_B T \quad \text{(pure water equilib.)} \tag{5.48}$$

or

$$\Delta P = s_2 R T \quad \text{(pure water equilib.)} \tag{5.49}$$

Note: Equation (5.49) uses the **gas constant** $R = N_A k_B$ and not the Boltzmann constant k_B. If ΔP [=] kPa, it's convenient to use $R = 8.3145\ \text{kPa} \cdot \text{L} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$.

(d) Using the typical osmolarity of a red blood cell ($s_2 = 290$ mOsm/L), use equation (5.49) to *calculate* the pressure difference that would be required for a plant cell with the same osmolarity s_2 to be in equilibrium with pure water at room temperature ($T = 295$ K).

Q.5.30 DISCUSSION QUESTION **(a)** *Compare* your answer to Q.5.29(d) with 30 psi, which is the recommended pressure for the front tires of a small car.
Hint: As always, you should also use what you learned about making quantitative comparisons in the "talking numbers" AWYD in **CHAPTER 2**. You might find the preformatted spreadsheet BPM.Ch02_Talking_numbers.xlsx useful.
(b) *Compare* your answer to Q.5.29(d) with 100 psi, which is a typical recommended pressure for the road tires of a bicycle.
(c) *Briefly discuss* how the comparison you made in part (b) can explain the crisping of a limp celery stalk when it's placed in tap water.

Q.5.31 **(a)** By combining equations (5.31), (5.43) and (5.41), *show that*

$$\delta\psi = \frac{v_w \Delta P}{k_B T} = \frac{\Delta P}{c_w RT} \tag{5.50}$$

(b) Using the equilibrium pressure difference $\Delta P = 711$ kPa you found in Q.5.29(d) for a plant cell in equilibrium with pure water, *calculate* the corresponding dimensionless energy difference $\delta\psi$ and energy factor ε.
(c) *Briefly discuss* whether the dimensionless energy difference you calculated in part (b) is "small."

Osmotic pressure defined – the van't Hoff equation

Equation (5.49) tells us the pressure difference for a cell 2 that is in equilibrium *with pure water*. Even when box 2 is not in contact with pure water, the right-hand side of equation (5.49) can be used to *define* the **osmotic pressure** π_2 of box 2, i.e.

$$\pi_2 \equiv s_2 RT \tag{5.51}$$

Unlike the previous equations (5.46)-(5.49), equation (5.51) is always true even when box 2 is not at equilibrium with pure water. Equation (5.51) is called the **van't Hoff equation** for osmotic pressure and it tells us the pressure difference *that would be* required for a cell (box 2) to be in equilibrium with pure water. In physiology, this is called the **osmotic pressure** of the solution in box 2.

According to equation (5.51), the osmotic pressure π_2 is simply another way of specifying the osmotic concentration s_2 in cell 2. It is used in an entirely analogous manner to how **oxygen partial pressure** (**oxygen tension**) is used to indicate the oxygen concentration in blood plasma or other tissues (**CHAPTER 3**). Similar to the oxygen partial pressure, it does *not* usually indicate a real hydrostatic pressure, e.g. a car tire pressure. It is merely a convenient way to indicate the osmolarity of a solution in units of pressure. The only circumstance when it is a real pressure is if the cell is in equilibrium with pure water, and that doesn't ever happen in human physiology.

About what you discovered: osmotic pressure is a measure of chemical potential

The **osmotic pressure** defined by the van't Hoff equation (5.51) is another example of how a comparison with a *thermodynamically comparable* reference state can be used to predict how a system will behave when it's *not* at equilibrium. The osmotic pressure π_2 is used as a **measure** (an indication of the value of) the **chemical potential** of the *water* molecules in box 2. It tells us the pressure difference that *would be* required for it to be in osmotic equilibrium with pure water. Hence, we now have three ways to express the chemical potential of the water in a liquid – effective water concentration c_2, osmolarity or osmotic concentration s_2 and osmotic pressure π_2. All three provide the same information. **Please note:** A glass of freshly squeezed orange juice has an osmotic pressure of $\pi_2 \cong 7$ atm, but that pressure is not real. It would break the glass! π_2 is simply a **measure** of the osmotic concentration s_2 in the orange juice. ❑

How osmosis creates a hydrostatic pressure difference

So far, we have assumed that there is a pressure difference between the boxes, but we didn't talk about what is required to produce and maintain the pressure difference. Let's start with what is required to maintain a pressure difference. Our analogy of a RBC being similar to a basketball is useful once again. So long as the basketball has one or more dimples, the pressure inside and outside are approximately the same. If the basketball is inflated until the dimples are popped out, then the pressure will begin to increase as the cover becomes taut. This tension in the cover provides the mechanical force that is required to maintain the pressure difference between the inside and outside of the ball. If the pressure difference is too great, the basketball will burst. From a mechanical point of view, it is the ball cover that maintains the pressure difference. The cover of an animal cell, e.g. a red blood cell, is a lipid bilayer membrane. It is not strong, and hence can't maintain even a small pressure difference. However, plant cells have a fairly rigid cell wall outside of the membrane that is able to support a large pressure difference. These cells are like old-school basketballs (or soccer balls) that have a flexible inner rubber bladder and a strong leather cover. The flexible inner bladder is like the plasma membrane. The cell wall is like the leather cover. It provides the rigidity to maintain a non-zero pressure difference between the inside and the outside of the cell.

A real cell wall has some flex, but it's easier for us to analyze the idealized situation where the cell wall is completely rigid. Mathematically, this means that the volume of the cell is constant no matter how high the pressure difference is. This system corresponds to the **Helmholtz ensemble** (constant T, V). Hence, the net result of moving a water molecule from box $1 \rightarrow 2$ is to increase the pressure P_2 in box 2 (it also increases the water concentration c_2). The pressure increase δP_2 occurs because if we squeeze one more water molecule into the same volume V_2, the water and everything else in box 2 is compressed. If we assume that box 2 is mostly water, then we can calculate the increase in pressure using the **compressibility** β_w of water at room temperature.

Compressibility β_w is a **material property** of water. It indicates how the volume V of a parcel of water changes in response to a small change in pressure δP, where the change in volume δV is given by

$$\frac{\delta V}{V} = -\beta_w \delta P \tag{5.52}$$

As a concrete example, imagine a limp sealed plastic bag full of water sinking into a deep lake. As we know from equation (5.27) the pressure increases by δP as the bag sinks a vertical distance h. In addition, the volume of water inside changes by an amount δV. The negative sign in equation (5.52) is needed because a positive δP indicates an increase in pressure that causes the volume to *decrease* resulting in a negative value for δV. The volume change is divided by the original volume V because the actual change in volume δV is proportional to the original volume. For example, if one liter shrinks by 1 mL then two liters should shrink by a combined 2 mL. I.e. each liter should compress by the same amount. Hence, the fractional volume change $\delta V/V$ should only depend on the pressure change δP and the compressibility β_w, as shown in equation (5.52).

The compressibility β_w is simply the proportionality constant between the fractional volume change and the pressure change. Because water is not very compressible, the value of its compressibility is very small $\beta_w = 4.6 \times 10^{-7}$ kPa^{-1}.

Q.5.32 In order to see how small the compressibility of water is:
(a) Use equation (5.52) to *calculate* the volume change δV when the pressure on 1 L (one liter) of water is increased by 1 MPa (one megapascal) which is about ten atmospheres. Express your answer as a percent decrease (**CHAPTER 2**); and
(b) Use your answer in part (a) and the definition of concentration to *calculate* the percent increase in the water concentration from the original 1 L.

We can now use the compressibility of water to investigate how pressure increases in a rigid plant cell as pure water diffuses in by osmosis. The effect of adding a small number δn_2 (in moles) of water molecules to cell 2 is to reduce the volume available to the rest of the molecules in box 2 by an amount

$$\delta V = -\bar{V}_w \delta n_2 = -\frac{\delta n_2}{c_w} \tag{5.53}$$

where the **molar volume** of pure water $\bar{V}_w = 1/c_w$ is the volume of one mole of water molecules. The negative sign is needed because adding water molecules to box 2 reduces the volume left for the original molecules. Combining equation (5.53) with equation (5.52) and solving for δP_2 gives

$$\delta P_2 = \frac{\delta n_2}{c_w \beta_w V_2} \tag{5.54}$$

for how the pressure changes in box 2 as water molecules are added by osmosis. You should note that this equation does not have a minus sign, consistent with the fact that increasing the number of water molecules in box 2 increases the pressure P_2. As $\delta c_2 = \delta n_2/V_2$, equation (5.54) can be rewritten as

$$\delta P_2 = \frac{\delta c_2}{c_w \beta_w} \tag{5.55}$$

Q.5.33 By substituting equation (5.41) into (5.44), *show that*

$$\varepsilon = 1 - \frac{\Delta P}{c_w RT} \tag{5.56}$$

Q.5.34 **DISCUSSION QUESTION** **(a)** Using equations (5.45), (5.55) and (5.56) *write out* a complete FD algorithm, including unit checks, to calculate how the pressure difference between the boxes $\Delta P = P_2 - P_1$ changes with time (using $\Delta P^{\text{new}} = \Delta P^{\text{old}} + \delta P_2^{\text{new}}$) for a rigid plant cell in contact with a bath solution of constant osmolarity $s_1 = 0$ (pure water) using a jump rate constant of $k = 0.50\ \text{ms}^{-1}$, a timestep of $\delta t = 0.1\ \text{ms}$ and an initial value of the plant cell osmolarity of $s_{2_0} = 0.290\ \text{Osm/L}$. The temperature is $T = 295\ \text{K}$, $\beta_w = 4.6 \times 10^{-7}\ \text{kPa}^{-1}$ and the initial value of ΔP is 0 kPa.
Hint: The units will work out easily if you use $R = 8.3144\ \text{kPa} \cdot \text{L} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$.
(b) Using your algorithm, calculate by hand what happens for steps 0, 1, and 2 and *write* your answer in the form of an output table.
(c) Implement your algorithm in the preformatted spreadsheet BPM.Ch05_Helmholtz.xlsx and check that it generates exactly the same sequence that you calculated in Q.5.34(b). Then plot ΔP versus time and adjust the timestep δt and the duration of your sim until you're sure that your graph is accurate and shows the approach to equilibrium. *Record* your graph.

Q.5.35 **DISCUSSION QUESTION** **(a)** *Briefly explain* why the time taken to reach equilibrium in your graph is so much shorter than your answer to Q.5.12(a).
(b) *Briefly describe* what your FD model predicts for the equilibrium pressure difference.
(c) *Compare* your FD prediction with the prediction of the van't Hoff equation (5.51).
Hint: Remember a "percent error" is an excellent way to answer this question, see the "talking numbers" AWYD in **CHAPTER 2**. You might find the preformatted spreadsheet BPM.Ch02_Talking_numbers.xlsx useful.

About what you discovered: a disagreeable result! What the heck is going on?

When I was first working on these materials, I performed the comparison asked for in question Q.5.35. I was expecting that the FD model would agree with the van't Hoff equation, but to my surprise, I discovered the same thing that you just did – the FD model does not agree with the van't Hoff equation. This frustrated me, because just like most students of science, I thought that a well-known equation *must* provide the correct answer – right?

Wrong! Just because an equation is in common use doesn't mean that it has to be correct – ever! I'm not ashamed to admit that it actually took me more than a month to figure out what was going

on. As a **CHALLENGE QUESTION**, you might like to see if you can solve this puzzle for yourself – right now before reading ahead. Q.5.36 (below) is designed to guide you to the answer that I ultimately discovered, using a series of simple questions. As it turns out, I had a misconception that led me to ignore the origin of the disagreement. Q.5.36 and Q.5.37 address that misconception. Correcting your own misconceptions is a slow and difficult process but it's essential to doing science. ❑

Q.5.36 DISCUSSION QUESTION **(a)** In many physics problems, such as calculating how the pressure increases with depth as you dive into the ocean or a deep lake, it's safe to assume that water is basically incompressible because its compressibility is so small $\beta_w = 4.6 \times 10^{-7}$ kPa^{-1}. Using equation (5.52) *calculate* the fractional change $\delta V/V$ in pure water exposed to a pressure difference of $\delta P = 669.5$ kPa.

(b) *Express* your answer to part (a) as the percent decrease from the starting volume to the compressed volume. You can use BPM.Ch02_Talking_numbers.xlsx.

(c) By comparing your answers in Q.5.36(b) and Q.5.35(c), *briefly discuss* whether you think that the finite (i.e. non-zero) value of water's compressibility is the cause of the disagreement between the van't Hoff equation and your FD model.

About what you discovered: misconceptions can be subtle

When I was first working on these materials, I already "knew" from my physics teaching that assuming water is basically incompressible is a good approximation when the pressure differences are "small". In this case 700 kPa is quite small – as you discovered in Q.5.36, but *that* was my mistake! Let's see what you can discover… ❑

Q.5.37 DISCUSSION QUESTION **(a)** *Calculate* the *initial* effective water concentration in the plant cell (box 2).

(b) If the water molecules in part (a) are compressed to 669.5 kPa, *calculate* the final water concentration.

Hint: You did a related calculation in Q.5.36(a).

(c) Using that final water concentration, *calculate* the final osmolarity in the compressed plant cell.

(d) *Calculate* the percent decrease from the initial to final osmolarity?

(e) *Briefly explain* how your answer to part (d) explains the disagreement between the FD model and the van't Hoff equation.

About what you discovered: water compression

As shown in Fig.5.16, there's a discrepancy between the prediction of the van't Hoff equation and the prediction of the FD model. This discrepancy arises because the van't Hoff equation is based on equation (5.10) which states that $c_2 = c_w - s_2$ and we have assumed that the effective water concentration c_2 and the osmolarity s_2 are constant (independent of pressure), whereas the FD model is based on calculating the effective water concentration using $c_2 = n_2/V_2$ where V_2 is

constant, but n_2 (the number of water molecules in box water) increases as box 2 is pressurized by the entering water. The FD model is consistent with $\Delta P = -\Delta cRT$ at equilibrium whereas equation (5.49) is $\Delta P = s_2 RT$. The concentration term $-\Delta c = c_1 - c_2$ decreases as water diffuses into box 2 whereas s_2 remains constant. Hence, the disagreement between the FD model and the van't Hoff equation is explained by the very small (but non-zero) compressibility of water.

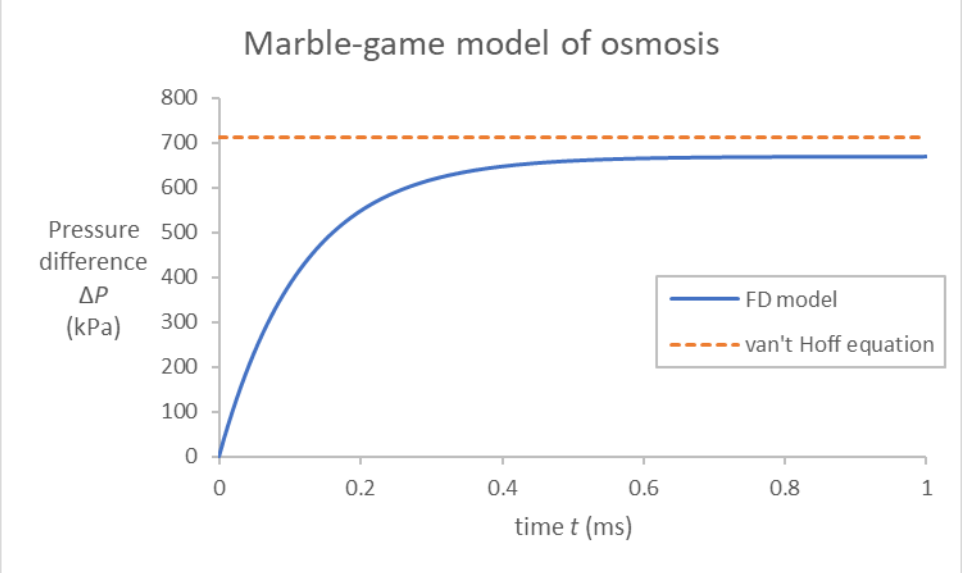

Fig.5.16 Excel 2016 chart of the FD model of pressure development due to osmosis, showing a comparison with the prediction of the van't Hoff equation for a starting osmolarity of $s_2 = 290$ mOsm/L.

Note: The traditional model of osmosis also assumes that water is incompressible, if we make that same assumption, then our diffusive model of osmosis is mathematically equivalent to the traditional model and the van't Hoff equation (5.51). ❑

Q.5.38 RESEARCH QUESTION Our FD model makes predictions that are slightly different from the van't Hoff equation. As discussed in the previous AWYD, these differences have to do with the compressibility of water. *Investigate* whether this difference has been discussed in the published literature. If not, what experiments could you design to verify what we just discovered?

About what you discovered: water compression and osmotic concentration

The research topic raised in Q.5.38 is a real one. As of this writing, I have no idea what the result would be. The topic is rather complex because in the traditional approach the van't Hoff equation (5.51) is used as a base empirical model and corrections are made to it to account for real behavior. The compressibility effect might be masked by these corrections. Consideration of this testable hypothesis of the marble game approach is beyond the scope of this book, but we'll return to the meaning of **osmotic concentration** in a later chapter, when we'll derive "Raoult's law" [Nelson 2017]. ❑

5.4 Osmotic permeation rate

In the following questions, we're going to rearrange equation (5.45) to show different ways of thinking about osmosis and what causes it to happen. Osmosis is one of the most misunderstood concepts in all of physiology, but if you carefully think about what the equations mean while you're working through the algebra, you should be able to use what you know about our marble game model to clearly understand the meaning and purpose of the equations. You should always keep in mind how the diffusive model resulted in equation (5.45). If the origin of equation (5.45) is still a mystery to you, now is a good time to review Section 5.3 and discuss it with your instructor/TA before continuing. Doing the algebra without understanding the equations is simply a waste of your precious time… so don't do it! The point is for you to understand the meaning of the equation, where it comes from, and how it explains osmosis.

Q.5.39 DISCUSSION QUESTION **(a)** Using equations (5.45) and (5.44), *show that* when there is no pressure difference ($\Delta P = 0$) between the bath and box 2 (Fig.5.15), the rate at which the concentration in box 2 changes (the **permeation rate**) is given by equation (5.57)

$$\frac{\delta c_2}{\delta t} = -k\Delta c \quad (\text{when } \Delta P = 0) \tag{5.57}$$

where

$$\Delta c = c_2 - c_1 \tag{5.58}$$

Note: $\delta c_2/\delta t$ is simply the small change in c_2 divided by the small amount of time δt taken for the change. This is another example of a **rate** (see **CHAPTERS 3** and **13**). Δc is the **effective water concentration difference** between boxes 1 and 2. This difference Δc need not be small, whereas δc_2 must be small for the equation to be true.

(b) *Translate* equation (5.57) into words.

About what you discovered: thermodynamic driving forces

Equation (5.57) tells us that the permeation rate $\delta c_2/\delta t$ is proportional to the jump rate constant k and the effective concentration difference Δc. This tells us that the concentration difference Δc is the **thermodynamic driving force** for osmotic permeation (when there's no pressure difference). This is just like the original marble game, where the rate of diffusion is proportional to the marble number difference ΔN. The **thermodynamic driving force** Δc is *not* a Newtonian force [=] N (**CHAPTER 13**), but it is the thing that causes transport. In this context, the word "thermodynamic" has the meaning "generalized". The adjective "thermodynamic" is often dropped, but it's important that you realize that the **driving force** Δc is not the same kind of physics thing as the force F that accelerates a car. ❑

Q.5.40 DISCUSSION QUESTION **(a)** By substituting equations (5.7) and (5.8) into (5.58), and using

$$\Delta s = s_2 - s_1 \tag{5.59}$$

show that

$$\Delta c = -\Delta s \tag{5.60}$$

Note: Δs is the **osmolarity difference** (or **osmotic concentration difference**) between boxes 1 and 2.
Combining equations (5.57) and (5.60) gives

$$\frac{\delta c_2}{\delta t} = -k\Delta c = k\Delta s \quad (\text{when } \Delta P = 0) \tag{5.61}$$

(b) According to equation (5.61), *what's the driving force* for the osmotic diffusion of water into box 2 when there's no pressure difference?
(c) *What part* (letters) of the original FD equation (5.45) include the effect of Δs?

Q.5.41 DISCUSSION QUESTION By substituting equation (5.56) into equation (5.45) and using equation (5.60), *show that* when there is an osmolarity difference ($\Delta s \neq 0$) and a pressure difference ($\Delta P \neq 0$) between the bath and box 2, the permeation rate is given by

$$\frac{\delta c_2}{\delta t} = k\left(\Delta s - \frac{c_1}{c_w}\frac{\Delta P}{RT}\right) \tag{5.62}$$

When box 1 is dilute, $c_1 \cong c_w$ and equation (5.62) becomes

$$\frac{\delta c_2}{\delta t} = k\left(\Delta s - \frac{\Delta P}{RT}\right) \quad (\text{when } c_1 \cong c_w) \tag{5.63}$$

Q.5.42 DISCUSSION QUESTION When there is no osmolarity difference ($\Delta s = 0$) between the bath and box 2 and both solutions are dilute, then $c_1 = c_2 \cong c_w$ and the permeation rate is given by

$$\frac{\delta c_2}{\delta t} = -\frac{k}{RT}\Delta P \quad (\text{when } \Delta s = 0 \text{ and } c_1 \cong c_w) \tag{5.64}$$

(a) According to equation (5.64), *what's the driving force* for the osmotic permeation of water out of box 2 when $\Delta s = 0$?
(b) *What part* (letters) of the original FD equation (5.45) include the effect of ΔP?
(c) According to equation (5.63), *what are* the two driving forces for osmotic permeation and how are their directions related?

Q.5.43 DISCUSSION QUESTION **(a)** Using the van't Hoff equation (5.51) for the osmotic pressure in box 1 and box 2, *show that* equation (5.63) can be rewritten as

$$\frac{\delta c_2}{\delta t} = \frac{k}{RT}(\Delta\pi - \Delta P) \tag{5.65}$$

where

$$\Delta\pi = \pi_2 - \pi_1 \tag{5.66}$$

is the **osmotic pressure difference** between boxes 1 and 2.

(b) Equation (5.65) is a form of **Starlings law of filtration**. According to **Starling's law** (5.65), *what are* the driving forces for the osmotic permeation of water into box 2?

(c) *What part* (letters) of the original FD equation (5.45) include the effect of $\Delta\pi$?

Q.5.44 DISCUSSION QUESTION **(a)** Using equation (5.63) *derive* an equation for the pressure difference ΔP at equilibrium.

(b) Using equation (5.65) *derive* an equation for the pressure difference ΔP at equilibrium.

(c) *Briefly discuss* the relationship between your answers to parts (a) and (b) of this question and how an osmotic pressure difference $\Delta\pi$ is a useful concept for understanding the direction and magnitude of osmotic permeation in physiology.

About what you discovered: osmosis in a nutshell

As you discovered in Q.5.39, if a cell can't support a pressure difference (e.g. RBCs) then water will diffuse from low to high osmolarity (or from high to low effective water concentration). This process continues until the cell swells or shrinks until its osmolarity matches the bathing solution, or the cell **lyses** (bursts), or dehydrates completely. Equation (5.61) is another form of Fick's law of diffusion (**CHAPTERS 1** and **7**), consistent with the diffusive model of osmosis being based on the diffusion of water molecules through a single-file pore.

As you discovered in Q.5.42, if there is no osmolarity difference, but there is a real hydrostatic pressure difference ΔP between boxes 1 and 2 (e.g. a rigid plant cell in a bathing solution with $c_1 = c_2$) then water will *diffuse* from high to low pressure. This process continues until the pressure in the cell matches the bathing solution. Equation (5.64) is mathematically equivalent to **Darcy's law**, which states that the rate of fluid transfer is proportional to the pressure gradient. However, our diffusive model of permeation is not the same as the fluid **flow** of Darcy's law. It is a **diffusive flux** (**CHAPTERS 1** and **7**) rather than a **convective flow**. Water molecules jump in both directions, rather than being simply carried along by a bulk convective flow, such as water flowing in a pipe or the blood flowing in an artery or vein. The differences between convection and diffusion are discussed further in **CHAPTER 7**.

According to equations (5.62) and (5.63) there are two often competing "driving forces" for osmosis. The osmolarity difference Δs causes water molecules to diffuse from low to high osmolarity (or from high to low effective water concentration) and the pressure difference ΔP causes water molecules to *diffuse* from high to low pressure. An equilibrium can be reached when these two driving forces match each other and $\delta c_2/\delta t = 0$. From equation (5.63), this happens for dilute solutions when the term in parentheses is zero, i.e.

$$\Delta P = \Delta sRT \quad (only \text{ at equilibrium}) \tag{5.67}$$

Equation (5.67) allows us to *define* an **osmotic pressure difference** $\Delta\pi$ as

$$\Delta\pi \equiv \Delta sRT \tag{5.68}$$

which is another form of the **van't Hoff equation**. The osmotic pressure difference $\Delta\pi$ defined in equation (5.68) is simply another way of talking about the osmolarity difference Δs (or the effective water concentration difference Δc) between boxes 1 and 2. This equation can be rearranged to give

$$\Delta s = \frac{\Delta\pi}{RT} \tag{5.69}$$

If equation (5.69) is substituted back into equation (5.63), we get back to equation (5.65), which you derived in a different manner in Q.5.43. This confirms that equation (5.68) is consistent with our diffusive model of osmosis with $\Delta\pi = \pi_2 - \pi_1$ (equation (5.66)). We can therefore be confident that our diffusive model of osmosis provides a network of predictions that are self-consistent.

Equation (5.65) (**Starling's law**) is the pay-off equation for the concept of **osmotic pressure**. It says that there are two **driving forces** for osmosis. The osmotic pressure difference $\Delta\pi$ causes water molecules to *diffuse* from low to high osmotic pressure and the pressure difference ΔP causes water molecules to *diffuse* from high to low hydrostatic pressure. An equilibrium can be reached when these two driving forces cancel each other out and $\delta c_2/\delta t = 0$. In in Q.5.44, we saw from equation (5.65) that this happens (for dilute solutions) when the term in parentheses is zero, i.e.

$$\Delta P = \Delta\pi \qquad (only \text{ at equilibrium}) \tag{5.70}$$

Hence, using the concept of osmotic pressure, equilibrium occurs when the hydrostatic pressure difference equals the osmotic pressure difference. If $\Delta P < \Delta\pi$, then water molecules diffuse into box 2. This is **normal osmosis** because water diffuses from low to high osmolarity and the solution in box 2 is diluted. If $\Delta P > \Delta\pi$, then water molecules diffuse out of box 2. This is **reverse osmosis** because pure water diffuses from high to low osmolarity and the solution in box 2 is concentrated.

According to the United Nations Department of Economic and Social Affairs [UNDESA 2014], almost one-fifth of the world's population, live in areas with a physical scarcity of fresh drinking water. In principle, reverse osmosis could be used to purify *any* water source into pure drinking water. In Florida, this method is being used to purify seawater into freshwater. Box 1 contains freshwater and box 2 contains pressurized seawater. The main problem is that this technology is currently large and expensive.

Water purification also occurs inside your kidneys. The filtrate in kidneys can be thought of as a polluted water supply. Aquaporins in the kidneys filter out pure water that is transferred back into the blood supply. This amounts to about 200 L of filtered pure water per day per human. ❑

Q.5.45 DISCUSSION QUESTION **(a)** Read the case study "Osmosis Is Serious Business!" [Nash 2008] and *answer the questions* for "Part I—Too Much of a Good Thing" using what you've learned from our diffusive model of osmosis.
(b) Read the case study "Osmosis Is Serious Business!" [Nash 2008] and *answer the questions* for "Part II—Too Little, Too Late" using what you've learned from our diffusive model of osmosis.

About what you discovered: diffusive model correctly predicts osmotic permeation

Kramer and Myers' [2012] criticism of a diffusive explanation of osmosis was:

> "*Osmosis and simple diffusion are distinct phenomena. Not surprisingly, the analogy also fails to make quantitatively correct predictions.*"

However, as you just discovered, our diffusive model of osmosis does correctly predict that the osmotic permeation rate is given by equations (5.61)-(5.70) . These equations are identical to those of the traditional model of osmosis [Finkelstein 1987]. Hence, our diffusive model makes exactly the same quantitative predictions as the traditional model advocated by Kramer and Myers [2012], [2013]. Based on this mathematical equivalence, we have no experimental reason to prefer one model over the other. However, from a conceptual perspective, our diffusive model is *much* simpler to understand. We'll return to this comparison once again in a later chapter. ❑

Conclusion – about what you discovered

Congratulations! If you made it here, then you've successfully met Lyon's challenge to develop a marble game model of osmosis, explore the network of predictions that it makes, and then compare those predictions with experiment and the traditional model of osmosis. If you understood how you derived those equations – then you understand osmosis – one of the most confusing concepts in all of biology!

We began this chapter with a controversy, which can be summarized by our disagreement with "*Osmosis is not driven by water dilution*" [Kramer and Myers 2013]. The diffusive model of osmosis that we developed is based on the opposite premise, namely that osmosis can be explained by the jumps of water molecules though aquaporins via a diffusive process summarized by the knock-on jump mechanism.

The first situation we modeled was the textbook example of a red blood cell placed in solutions of differing osmolarity. We were able to show that the FD formulation of the original marble game (**CHAPTER 3**) successfully explains experimentally observed behavior. All we had to do was to

account for the volume change of the RBC and to express the effective water concentration using osmolarity. Once we did that, our diffusive model predicted the exact same equations that you'll find in Nobel Prize winning research [Mathai *et al.* 1996].

By analyzing the gravity marble game, we discovered how a small energy difference δE affects the jump rates between two boxes at different energies. By comparing the gravity marble game with earth's atmosphere, we discovered the concept of an energy factor ε that predicts the slight decrease in the uphill jump rate caused by the pull of gravity. This is an incredibly useful concept! In fact, a whole branch of physics, **statistical mechanics**, is based upon analyzing energy diagrams similar to that of the gravity marble game (Fig.5.12). We will return to this topic in **CHAPTER 9** where we'll use the gravity marble game to discover the Boltzmann factor – one of the most important concepts in all of statistical mechanics and biophysics.

Using the energy factor concept, we were able to generalize our osmosis model to account for pressure effects. The equations we derived from this model are identical to the standard ones advocated by Kramer and Myers [2013], showing that osmosis can indeed be explained by water diffusion. This puts us in the very interesting scientific position of having two competing models. As a scientist, you should be skeptical of the new model and look for evidence that distinguishes the new from the old – **question everything**! We will return to that challenging comparison in a later chapter, but before we finish up here, let's summarize what we've discovered from our diffusive model of osmosis.

Using our diffusive model of osmosis, we were able to derive (and hence understand) **Starling's law of filtration** as it applies to osmosis through an aquaporin

$$\frac{\delta c_2}{\delta t} = \frac{k}{RT}(\Delta\pi - \Delta P) \tag{5.65}$$

This equation shows that the **osmotic permeation rate** $\delta c_2/\delta t$ depends on two driving forces $\Delta\pi$ and ΔP. The first driving force is the osmotic pressure difference $\Delta\pi = \pi_2 - \pi_1$. This is the driving force that makes RBCs change volume when they are placed in solutions of different osmolarity. It is entirely diffusive within our model, as summarized by the van't Hoff equation

$$\Delta\pi = \Delta sRT = -\Delta cRT \tag{5.68}$$

which shows that the osmotic pressure difference $\Delta\pi$ is simply a measure of the osmolarity difference Δs, which in turn is a measure of the effective water concentration difference Δc. **Fick's law of diffusion** (**CHAPTER 1** and **7**) predicts that the concentration difference Δc, combined with random jumps between the boxes, will produce a flux of water molecules from high to low effective water concentration. Unlike the traditional model, our diffusive model doesn't require a hydrostatic pressure gradient within the pore during osmotic swelling or shrinking of a RBC [Nelson 2017].

The second driving force in Starling's law (5.65) is $\Delta P = P_2 - P_1$. This *is* a hydrostatic pressure difference that is measurable with a pressure gauge. As we discussed in the "osmosis in a nutshell" AWYD (after Q.5.44), if there is a hydrostatic pressure difference $\Delta P \neq 0$, but no osmolarity difference $\Delta\pi = 0$, then water will *diffuse* from high to low pressure. According to our model, the formal similarity of this situation with Darcy's Law is basically a coincidence because the flux is caused by random thermal motion biased by the energy factor, rather than by a pressure-driven convective flow, like water in a pipe.

Our diffusive model thus provides a diffusive explanation for osmosis through aquaporins. The marble game explanation of the first driving force is basically the same as what you'll find in chemistry and physiology textbooks [Brown, *et al.* 2003] [Moore, *et al.* 2010] [Silverthorn 2007]. However, the explanation of the second driving force is new and appears to address all of the criticisms raised by Kramer and Myers [2013], thus making it a valid competing model of osmosis.

This chapter has been our introduction to the gravity marble game and to osmosis. As we'll discover in **CHAPTER 9**, the gravity marble game illustrates basic principles that result in the Boltzmann factor, a central concept in statistical mechanics. In this chapter, we used the gravity marble game to develop a very simple conceptual picture of osmosis, based on a knock-on jump summary of water diffusion through the selectivity filter of aquaporins. While we haven't focused on the issue in this chapter, you should be aware that our conceptual picture is different from the traditional one. Briefly stated, the traditional model requires a real pressure gradient in the single-file pore whenever there is a non-zero permeation rate, whereas ours does not. In a later chapter, we'll take up the challenge of comparing our new model with the traditional one and discuss some of the experiments that might be done to investigate which one is better. Then *you* can decide if the diffusive model of osmosis is preferable to the traditional model.

Author's afterword

As mentioned in the introduction to this chapter, the new **diffusive model of osmosis** we developed in this chapter contradicts the standard **hydrodynamic flow model of osmosis** that you'll find in current biophysics and physics textbooks [Nelson 2017] and in the current botany literature [Morris and Blyth 2019]. The difference is best illustrated by the model of RBC osmotic swelling/shrinking that we developed in **SECTION 5.1**. The equation (5.14) that we derived is *mathematically identical* to the traditional equation that Mathai *et al.* [1996] used to model their Nobel-prize winning experimental data. Osmotic swelling of an RBC dropped into pure water is caused by the diffusion of water from high to low effective water concentrations. Because the marble game model is diffusive, there is no pressure drop required in the pore. However, the hydrodynamic flow model requires a hydrostatic pressure drop along the pore that produces a pressure driven flow within the pore that's similar to blood flow in capillaries – see Figs. 17(b) and 17(d) of Nelson [2014]. I initially developed the concept of an **effective water concentration** so that osmosis could be explained using the conceptual framework provided by the marble game. The initial idea was that the effective water concentration could be justified based on the traditional thermodynamic arguments that lead to the concept of **activity** in physical chemistry and chemical physics. However, because of the controversial nature of the new diffusive model,

I sought to find a more intuitive kinetic explanation for the effective water concentration. The resulting **solute blocking model** was first presented in an *arXiv* manuscript [Nelson 2014] and has subsequently been published in the *European Biophysics Journal* [Nelson 2017]. This model not only provides a novel kinetic explanation of the **effective water concentration** concept, but it also provides a simple kinetic explanation for the thermodynamics and colligative properties of ideal solutions. We'll return to the solute blocking model in a later chapter.

Summary: A new model of osmosis

Gravity marble game

- The jump rates are different because the boxes are at different energies (Fig.5.17).

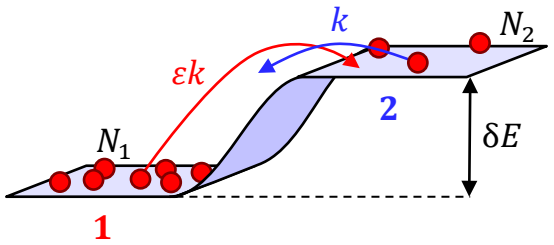

Fig.5.17 Schematic representation of the gravity marble game. The jump rate constant in the uphill direction is εk and k is the jump rate constant in the downhill direction. The gravitational potential energy difference is $\delta E = mg\delta y$, where the marbles each have mass m and the two boxes are separated by height δy in a gravitational field of magnitude g (based on Fig.5.8).

The energy factor

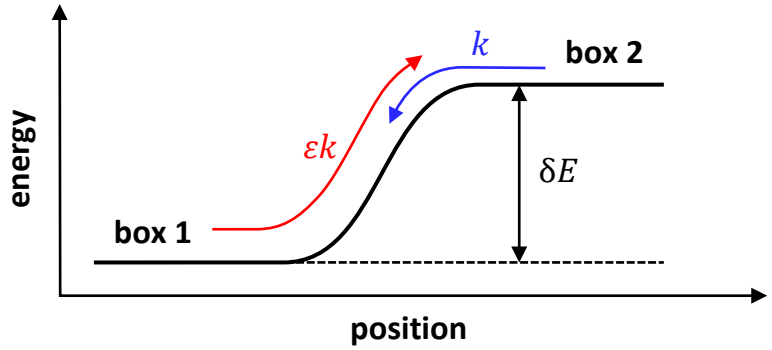

Fig.5.18 Simplified schematic energy diagram of the gravity marble game. The diagram shows a situation where marbles in box 2 have an energy δE higher than box 1 (based on Figures 5.12 and 5.14).

- The energy diagram shown in Fig.5.18 is common to any situation for which there is a small energy difference δE [=] J.
  - $\delta E = mg\delta y$ for the gravity marble game (and molecules in the atmosphere).
  - $\delta E = v_w \Delta P$ for osmosis (see below).

- The **energy factor** ε is the fraction of molecules that have enough energy to make it up the jump of energy δE

$$\varepsilon = 1 - \delta\psi \tag{5.36}$$

- $\delta\psi$ is the **dimensionless energy difference** for the jump. It's the ratio of the energy difference δE to the **thermal energy** $k_B T$

$$\delta\psi = \frac{\delta E}{k_B T} \tag{5.31}$$

where k_B is the **Boltzmann constant** and T is the **absolute temperature** in kelvins.

- $\delta\psi$ must be small compared with 1 for equation (5.36) to be valid. As we'll see in **CHAPTER 9**, the energy factor becomes a Boltzmann factor if $\delta\psi$ is not small.
- The equilibrium ratio of concentrations in box 1 and 2 is

$$\frac{c_2}{c_1} = \varepsilon \tag{5.71}$$

Key osmosis concepts

- **Osmosis** is the selective diffusion of water through a semipermeable membrane.
- In physiology, water permeates via an **aquaporin** protein **selectivity filter**.
- Permeation through the **selectivity filter** can be summarized by a diffusive **knock-on jump mechanism** (Fig.5.19).

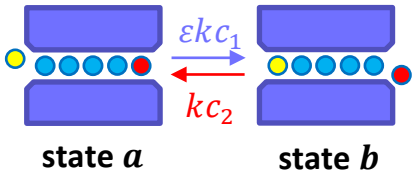

Fig.5.19 Schematic diagram of an AQP1 aquaporin selectivity filter showing the knock-on jump summary of single-file water permeation. The diagram shows two states (a and b) of the same AQP1 aquaporin. For the jump from state $a \rightarrow b$, the water molecule entering from box 1 is highlighted in yellow (lighter) and the water molecule knocked-on into box 2 is highlighted in red (darker). In the reverse $b \rightarrow a$ jump, the red water molecule enters from box 2 and the yellow water molecule is knocked-on into box 1 (based on Figures 5.3 and 5.12).

- k is the **knock-on jump rate constant**.
- c_1 and c_2 are the **effective water concentrations** in box 1 and box 2 respectively. The **effective water concentration** in box 2 is defined by

$$c_2 = c_w - s_2 \tag{5.7}$$

where $c_w = 55.386$ mol/L is the concentration of pure water and s_2 is the osmolarity in box 2 (see below).

- Uphill jumps require PV work (energy)

$$\delta E = v_w \Delta P \tag{5.43}$$

where v_w is the volume of a single water molecule and $\Delta P = P_2 - P_1$ is the pressure difference between boxes 1 and 2.

- Equations (5.36), (5.31) and (5.43) can be combined with Fig.5.20 to provide a diffusive model of osmosis to predict the **osmotic permeation rate** $\delta c_2/\delta t$

$$\frac{\delta c_2}{\delta t} = k(\varepsilon c_1 - c_2) \tag{5.45}$$

where the **energy factor** for osmosis is given by

$$\varepsilon = 1 - \frac{\Delta P}{c_w RT} \tag{5.56}$$

- **Osmolarity** (or **osmotic concentration**) s_2 [=] Osm/L is the standard physiological **measure** of effective water concentration. It's the total concentration (activity) of solute particles in box 2. Ions count as separate particles so that one mole of NaCl (salt) has an osmolarity of 2 Osm/L. Equation (5.7) can be rearranged to give

$$s_2 = c_w - c_2 \tag{5.10}$$

Osmotic pressure

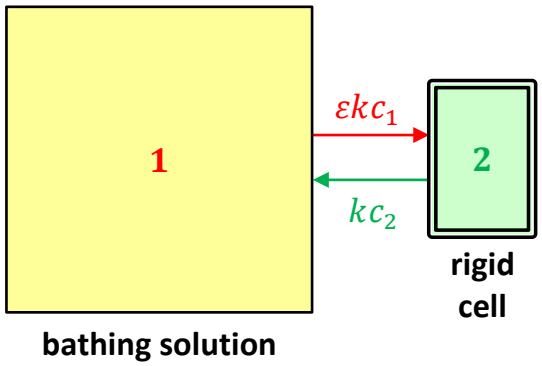

Fig.5.20 FD diagram of a rigid cell (box 2) in contact with a large bathing solution (box 1) within the **Helmholtz ensemble** (constant T, V). The water in the rigid cell has an effective water concentration c_2 and the bathing solution has a constant effective water concentration c_1. There is also a hydrostatic pressure difference $\Delta P = P_2 - P_1$ between the boxes that is maintained by the rigid cell wall. ΔP determines the value of the energy factor ε (based on Fig.5.15).

- If the bathing solution in Fig.5.20 is pure water, then $c_1 = c_w$ and equilibrium occurs when $\varepsilon c_w = c_2$. Combining this with equations (5.36), (5.31), (5.43) and (5.10) results in

$$\Delta P = s_2 RT \quad (only \text{ at equilibrium}) \tag{5.49}$$

- Equation (5.49) is used to *define* the **osmotic pressure** π_2 of the solution in box 2 (even when it is not in equilibrium with pure water)

$$\pi_2 \equiv s_2 RT \tag{5.51}$$

- The osmotic pressure defined by the **van't Hoff equation** (5.51) is the pressure difference that a solution would need to be under to be in equilibrium with pure water. The osmotic pressure π_2 is a standard physiological **measure** of the osmolarity s_2 and hence the effective water concentration c_2. This osmotic pressure π_2 indicates the **chemical potential** of water in an analogous manner to how oxygen tension (partial pressure) P_{O_2} indicates the **chemical potential** of oxygen dissolved in plasma (**CHAPTER 3**). It's *not* a real (hydrostatic) pressure.

Osmotic swelling/shrinking

- A red blood cell has a flexible membrane that cannot withstand any significant pressure difference, hence $\Delta P = 0$ and $\varepsilon = 1$.
- Using the FD model, it is possible to show that the relative equilibrium volume V_r^{eq} of a cell placed in solution with osmotic concentration s_1 is given by

$$V_r^{eq} = \frac{s_{2_0}}{s_1} \tag{5.16}$$

This equation is only valid for values of V_r^{eq} that are physically possible. E.g. if V_r^{eq} is too large, the cell will burst.

Osmotic permeation rate

- Using the FD model based on Fig.5.20, the osmotic permeation rate is predicted to be

$$\frac{\delta c_2}{\delta t} = k\left(\Delta s - \frac{\Delta P}{RT}\right) \quad (\text{when } c_1 \cong c_w) \tag{5.63}$$

or

$$\frac{\delta c_2}{\delta t} = \frac{k}{RT}(\Delta\pi - \Delta P) \tag{5.65}$$

where

$$\Delta\pi = \pi_2 - \pi_1 \tag{5.66}$$

- Equation (5.65) is a form of **Starling's law of filtration**, which shows that both hydrostatic and osmotic pressure differences are the **thermodynamic driving forces** for osmotic diffusion into/out of box 2 with the same rate constant k.

- Using equation (5.65), it's easy to see that osmotic equilibrium occurs when the physical (hydrostatic) pressure difference matches the osmotic pressure difference

$$\Delta P = \Delta\pi \tag{5.70}$$

- The osmotic pressure difference $\Delta\pi$ is not a real pressure difference, it's simply a way to account for the osmolarity difference Δs. The relationship between them is summarized by an alternate form of the **van't Hoff equation**

$$\Delta\pi = \Delta sRT = -\Delta cRT \tag{5.68}$$

which shows that an osmotic pressure difference $\Delta\pi$ is equivalent to an osmolarity difference Δs or an effective water concentration difference $-\Delta c$.

References

Adkins, C. J. (1987). *An introduction to thermal physics.* Cambridge University Press, Cambridge.

Brown, T.E. *et al*. (2003). *Chemistry: The Central Science.* (9th Ed.) Prentice Hall.

Finkelstein, A. (1987). *Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes: Theory and reality*. John Wiley & Sons, New York.

Hodgkin, A.L., and R.D. Keynes. (1955). The potassium permeability of a giant nerve fibre. *J Physiol*. 128:61-88.

Kramer, E.M., and D.R. Myers. (2012). Five popular misconceptions about osmosis. *Am. J. Phys.* **80**:694-699.

Kramer, E.M., and D.R. Myers. (2013). Osmosis is not driven by water dilution. *Trends Plant Sci*. **18**:195-197.

Mathai, J.C., S. Mori, B.L. Smith, G.M. Preston, N. Mohandas, M. Collins, P.C. van Zijl, M.L. Zeidel, and P. Agre. (1996). Functional analysis of aquaporin-1 deficient red cells. The Colton-null phenotype. *J. Biol. Chem*. **271**:1309-1313.

Moore, J.W. *et al*. (2010). *Principles of Chemistry: The Molecular Science.* Brooks Cole.

Morris, R.J., and M. Blyth, (2019). How water flow, geometry, and material properties drive plant movements. *Journal of experimental botany*. **70:**3549-3560.

Murata, K., K. Mitsuoka, T. Hirai, T. Walz, P. Agre, J.B. Heymann, A. Engel, and Y. Fujiyoshi. (2000). Structural determinants of water permeation through aquaporin-1. *Nature*. **407**:599-605.

Nash, T.R. (2008). *Osmosis is serious business!* National Center for Case Study Teaching in Science. http://sciencecases.lib.buffalo.edu/cs/files/osmosis.pdf

Nelson P.H. (1998) *Simulation of self-assembled polymer and surfactant systems.* PhD Thesis, Massachusetts Institute of Technology, Cambridge, MA.

Nelson P.H., Hatton T.A., Rutledge G.C. (1999) Asymmetric growth in micelles containing oil. *J Chem Phys* **110**:9673–9680

Nelson, P. H. (2013) *Greek letters go green!* http://circle4.com/biophysics/videos/

Nelson, P.H. (2014) Osmosis, colligative properties, entropy, free energy and the chemical potential. *arXiv:*1409.3985.

Nelson, P.H. (2017). Osmosis and thermodynamics explained by solute blocking. *Eur. Biophys. J.* 46:59-64. https://doi.org/10.1007/s00249-016-1137-y, http://rdcu.be/nify (free access).

officialpsy (2012) *PSY - GANGNAM STYLE (강남스타일) M/V* http://www.youtube.com/watch?v=9bZkp7q19f0&feature=youtu.be

Panagiotopoulos, A.Z., N. Quirke, M. Stapleton and D.J. Tildesley (1988) Phase equilibria by simulation in the Gibbs ensemble, *Molecular Physics*, **63**:527-545

Silverthorn, Dee U. (2007). *Human physiology: an integrated approach* 4th Edition. Pearson Education Inc., San Francisco.

TCBG UIUC (2012) *Water Channels in Cell Membranes* http://www.youtube.com/watch?v=GSi5-y6NHjY&feature=youtu.be

tomtom5418 (2012) *Question Everything" Promo 2012 (Science Channel)* http://www.youtube.com/watch?v=IH5SQEKIGhA

UNDESA (2014). *International Decade for Action 'WATER FOR LIFE' 2005-2015* retreived April 12, 2014. http://www.un.org/waterforlifedecade/scarcity.shtml

This material is based upon work supported by the National Science Foundation under Grant No. 0836833. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.