FOCUS ON STRUCTURAL BIOLOGY

A Guide to Protein Isolation

2nd edition

by
Clive Dennison

Kluwer Academic Publishers

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I am grateful to the reviewers of the 1st edition, and the reviewers of the draft of this edition, for pointing out shortcomings and areas where the book could be improved. In answer to their critiques, I have added more study questions and have provided answers. I have also added a section on HPLC and a chapter on practical methods, in the hope of making the book more useful in the lab. I wish to acknowledge the contribution of Ron Berry, Senior Customer Assurance Specialist, Waters Australia, Melborne. The section on HPLC was distilled, with permission, from a teaching manual which he prepared when he was a staff member in the same Department as myself.

My fundamental philosophy is that whenever one is doing something practical, *you should understand what you are doing!* In the hope of increasing students' understanding, I have added a prefatory chapter, dealing with the physics on which protein isolation methods are based. In writing this chapter, I was obliged to clarify my own thinking on some matters and I wish to thank my colleagues in Physics for answering my sometimes naive questions, for correcting the formatting of equations etc. and for pointing out some conventions in physics, of which I was not aware.

Preface

It is a truism of science that the more fundamental the subject, the more universally applicable it is. Nevertheless, it is important to strike a level of "fundamentalness" appropriate to the task in hand. For example, an in-depth study of the mechanics of motor cars would tell one nothing about the dynamics of traffic. Traffic exists on a different "level" - it is dependent upon the existence of motor vehicles but the physics and mathematics of traffic can be adequately addressed by considering motor vehicles as mobile "blobs", with no consideration of how they become mobile. To start a discourse on traffic with a consideration of the mechanics of motor vehicles would thus be inappropropriate.

In writing this volume, I have wrestled with the question of the appropriate level at which to address the physics underlying many of the techniques used in protein isolation. I have tried to strike a level as would be used by a mechanic (with perhaps a slight leaning towards an engineer) - i.e. a practical level, offering appropriate insight but with minimal mathematics. Some people involved in biochemical research have a minimal grounding in chemistry and physics and so I have tried to keep it as simple as possible.

Besides trying to find the right level, I have tried to show that the physical principles which can be employed in protein isolation are, in fact, ubiquitously applicable principles with which students may be well familiar, though perhaps in different contexts. These "ubiquitously applicable principles" - once identified as such - turn out to be old and familiar friends, with whom one can have a great deal of fun when applied to the challenges of protein isolation.

In an uncertain world one never knows what the future will bring - who knows whether the economy, the state of world politics, or the weather, will be better or worse this time next year than it is now? - but one of the enduring attractions of science is that, because of the labours of scientists throughout the world, it is almost certain that, "this time next year we'll have greater understanding and insight". This book is offered in the spirit of sharing some of the insights that I have gained in my career in Biochemistry. In some instances, I might have got hold of the wrong end of the stick. Where this is the case, I would welcome comment so that we might all learn - as we always do - from the errors.

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Preface to the 2nd edition

In the 1st edition of this book, I made the assumption that the reader would have some background in physics and would at least understand the elementary concepts of Newtonian physics. This edition is aimed at undergraduate students, and my experience has been that students may indeed have attended and passed courses in physics and may indeed have acquired some grasp of the concepts in the context in which they were given. However, many students have difficulty in transferring their knowledge to a new situation. They have a tendency to package their knowledge in mental boxes - one being labelled "physics" - but seldom reopen those boxes to use the material again in a subject which is not called "physics".

One of the attractions of physics is that it greatly simplifies the natural world. Only a few physical principles are necessary to explain almost everything - at least at the scale at which we live our daily lives and at which biochemists operate. Many students try to "learn" the material presented in a course - filling their minds with a large number of unconnected facts which soon become overwhelming and the material is "lost" as soon as the examinations are over and, more importantly, no tools are acquired with which to tackle new, presently-unimaginable problems. In fact, the tools are much more important than the "facts". Who can say what problems current students will face in, say, 20 years time? All we can say with certainty is that technology will change - probably becoming more complex - but the principles of physics will apply and if the erstwhile student has a good grasp of these, they will be able to deal creatively with any new challenges.

One of the challenges facing teachers of science, in my opinion, is to show how the real world is not divided into "boxes". Biochemistry may be taught separately from physics but, in the real world, there are no divisions. Similarly, in the real world, there is no division between the biochemical separations one may do in the lab and one's everyday life - the same physical principles apply. In this book, a recurrent theme, therefore, is to make connections between seemingly-unrelated phenomena, in order to show the connections between every-day events and the things one may do in the lab., i.e. *how the same principles apply!* And the more one sees the bigger picture, the simpler everything becomes.

As most of the separation methods used in protein isolation have a basis in physics, it is necessary to have a good understanding of the physical principles, so that one can properly understand how the separation methods work. For this reason, I have added a prefatory Chapter on some of the relevant principles of physics - as a revision or limbering-up exercise before these principles are applied in the protein isolation methods to follow.

A note to students.

The purpose of learning should be to increase one's insight and understanding. However, there is much debate about what is meant by the word "understanding". In some respects it has to do with familiarity. This is because of the way our brains are structured. Because a particular neural network is reinforced by repetitive usage, we become better and better at the things that we do often - "practice makes perfect", as the adage goes. However, we can become very good at doing something, without really understanding what we are doing. We can learn to catch a ball or ride a bicycle, for instance, without knowing Newton's laws of motion. Doing these things requires the development of very quick, but subconscious skills.

Learning by repetition can be corrupted into rote learning, where the object is to retain some knowledge simply by repetition, but without proper, conscious, understanding and without integrating it with one's existing knowledge. To a limited extent this can work - I can still recite parts of some foreign language poems which I was obliged to learn by rote over 40 years ago - but the problem with rote learning is that it relies excessively on memory and, in the absence of real understanding or integration, one is not in a position to be creative.

A much better approach is to build your understanding together with your knowledge, by continual reflection and integration of any new knowledge with one's existing knowledge. Continual reflection and

introspection is needed to get proper integration: in those cases where one fails to achieve proper integration, it means that either one's previous conceptions or one's new conceptions are faulty and both must be revisited until they are reconciled. Failure to do this means that one's knowledge will be of a glib and superficial nature - somewhat like that of a confidence trickster.

To be more than a journeyman of science, one must be intellectually creative. This requires developing habits of thought that involve careful observation and questioning. If you ride a bicycle, for instance, you should observe that in order to turn right, you have to turn the handlebars to the left and you should ponder why this is. If you use a "cell 'phone" (called a "mobile 'phone" in some countries) you should question why it is so-called - what is the "cell" that is being referred to?

Properly developed, this way of thinking becomes habitual and it enables one to continually build and integrate one's knowledge - mostly going forward, incrementally, but sometimes going back to dismantle some misconception which may be familiar but which doesn't fit with some new insight. This way of thinking and learning requires introspection, retrospection, cogitation (thinking) and metacogitation (thinking about how you think) - and it is by developing these processes that one can learn to be creative.

Intellectual creativity essentially involves the ability to manipulate concepts in the abstract - to move them around and try new combinations. "What if" questions are a part of this process and so is having, or making, sufficient leisure time for abstract reflection. It is no accident that Archimedes had his "eureka" moment while taking a bath or that Newton achieved his great insights during a period of enforced inactivity, the universities in England having been closed due to the plague. In this way one can become a "life-long learner", to use a currently fashionable phrase. Rote learning is problematic in that it hinders development of the processes required for one to become a creative thinker. The challenge for teachers is to encourage creative thinking in a system governed by grades. Real education is much more than grades, though (Einstein, for example, achieved only mediocre grades), and is largely a process which you have to do for yourself. Although it is a painful process, which involves much work, I wish you success because there are few things more rewarding than a rich intellectual life.

Clive Dennison

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Chapter 1

Basic physical concepts applicable to the isolation of proteins

This chapter is provided as a reference source to some of the basic physical concepts applicable to the isolation of proteins. Don't be put off by the mathematics in this chapter, or the fact that it seems somewhat removed from actual protein isolation. If you wish, you could skip this chapter and start at Chapter 2, coming back here when you need a definition or to gain greater clarity on an underlying physical concept.

Linear motion: Motion is important in protein isolation because, in order to separate molecules, at least some of them must be moved. Linear motion is a vector quantity, having magnitude and direction. The magnitude is the displacement: in the S.I. system the unit is the meter.

Linear velocity: Velocity v is the displacement per unit time. In the S.I. system the unit is meters per second ($m.s^{-1}$).

$$v = \frac{dx}{dt}$$

Since displacement is a vector, velocity is also a vector.

Acceleration: An acceleration is a change in velocity in time. The change may be in either magnitude or direction. The unit of acceleration in the S.I. system is $m.s^{-1}.s^{-1}$, or $m.s^{-2}$. This precise definition of the word "acceleration" is not to be confused with the every-day meaning which is, roughly, "to go faster".

Force: A force is a 'push' or a 'pull' exerted on a body. It is a vector quantity, so it has magnitude and direction. In the S.I. system the unit of magnitude of a force is the newton (N).

Newton's laws of motion.

1. A body will maintain its state of rest or of uniform motion (i.e. at constant speed along a straight line) unless acted on by an unbalanced force. This is also called the law of inertia.
2. An unbalanced force F acting on a body produces in it an acceleration a in the direction of the force. The acceleration is directly proportional to the force and inversely proportional to the mass m of the body.

   i.e., $$a = k\frac{F}{m} \qquad (1.1)$$

   where, k is a proportionality constant.

   Eqn 1.1. defines the unit of **mass**, which refers to the *inertia* of a body, or its tendency to resist acceleration. In the S.I. system, the unit of mass is the kilogram which is that mass, when acted upon by a force of 1 newton, will have an acceleration of 1 $m.s^{-2}$. Hence, in the S.I. system of units, $k = 1$ and, so:

   $$F = ma \qquad (1.2)$$

An important caveat applies to the 2nd law. To apply this law, an observer must be in an inertial frame, i.e. a non-accelerating reference frame in which the law of inertia (Newton's 1st law) applies.

3. If one object exerts a force on a second object, then the second object exerts an equal and opposite force on the first.

Weight: The *force* resulting from the effect of gravity acting upon a body (i.e. on a mass). Since weight is a force, in the S.I. system its units of magnitude are newtons.

As $F = ma$,

so. weight (newtons) = mass (kg) x g ($m.s^{-2}$).

Where g is the acceleration due to gravity (on Earth, g averages 9.8 $m.s^{-2}$).

Uniform circular motion: Uniform circular motion is relevant to centrifugation. one of the standard tools in biochemistry. When a particle undergoes uniform circular motion, its speed in the direction of the tangent is the same at all points and its acceleration is also constant