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Dynamics and Kinetics in Structural Biology: Unravelling Function Through Time-Resolved Structural Analysis. By Keith Moffat and Eaton E. Lattman. Wiley, New York, 2023, pp. 288. ISBN 978-1-119-69628-5. Price USD 161 (hardback), USD 128 (Kindle)

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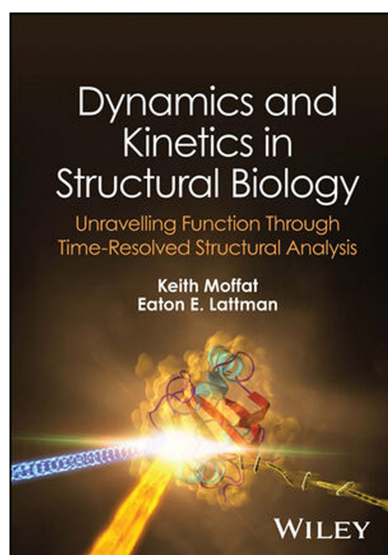

This book is authored by two senior figures active in the topics in the title. They describe in their author biographies that they have worked closely together when Eaton Lattman was lead PI of the USA BioXFEL project and Keith Moffat was Chair of the Advisory Committee. The scope of the book is broad, spanning macromolecular crystallography, solution scattering, cryoEM, and various other biophysical methods and their applications. These together reasonably justify the 'in structural biology' portion of the title. 'Dynamics and kinetics' is a very broad topic in physical chemistry as well as in computational simulations.

A critical, and correct, statement starts the book: *Whatever form the experimental sample takes when exploring structural dynamics, the molecules comprising the sample should be demonstrably active*. Furthermore *this requires the ability to generate and determine short-lived, intermediate structures whose populations vary with time as the biological reaction proceeds*. These statements, in effect, determine the content of the rest of the book.

This book extends two earlier books entitled *Time-resolved Macromolecular Crystallography* (Cruickshank *et al.*, 1992) and the more general *Time-Resolved Diffraction* (Helliwell & Rentzepis, 1997). The title of this new book is distinctly different though, spanning two distinct terms 'dynamics' and 'kinetics'. The experimental protocol of one *must study the biologically active state of a sample* opens the difficult, *i.e.* even broader, question *What is the structural chemistry of the living organism at its temperature and pressure?* (Helliwell, 2020). This new book addresses these topics in many ways and in considerable detail. Section 1.5 of the book identifies the focus in all but one of the book chapters as 'structural dynamics'. Chapter 2 entitled *Physical Chemistry of Reactions* describes the basics of thermodynamics and kinetics, and those well versed in physical chemistry are instructed to possibly skip this chapter. Section 2.1 declares that *these concepts are fundamental to structural dynamics* and yet Section 2.2, states that *thermodynamics does not deal directly with the 3D structures of molecules or the reactions they engage in*. In terms of the foundations of molecular biophysics and structures in motion, I was stimulated to look again at the book by Daune (1999) and I think I will still retain it on my bookshelf.

A good resume of studies for cryoMX and cryoEM is given in Section 2.6. A key paper on the limitations of cryostructures is Halle (2004), which is briefly cited but could have been expanded upon. Indeed, Section 2.6 signs off the whole chapter with a swingeing criticism of cryostructures being *potentially fatal* or *requiring cautious analysis* for structural dynamics. That conversation over with, Chapter 3 describes *The Experiment*. Section 3.2.1 is entitled *Signal, Accuracy and Systematic Errors*. Their definition of accuracy and precision is unconventional. Usually, accuracy is how well two or more different methods determine a quantity, each having their own systematic errors, which are attempted to be minimized. An important highlighted set of results is given in Box 3B, which exemplifies the long-standing experience of the authors gained with synchrotron radiation and then with the Stanford Linac Coherent Light Source (LCLS) whereby identical time points for the same biological system (photoactive yellow protein, PYP) could be compared in a control experiment. Difference electron density signals were

approximately doubled in the LCLS case. The advantages in the LCLS experiments over the synchrotron experiments were that much smaller crystals were used and their optical transparency to the laser pump was better. Section 3.3.2.3.2 *Practical considerations on photo initiation* sparkles and clarifies the balance between a smaller crystal for enhancing the light-stimulated reaction-intermediate fraction, and its impact on the change in X-ray diffraction intensity stimulated throughout the crystal, and the loss of X-ray diffraction intensity.

Chapter 4 is entitled *The Sample*. The chapter starts with the total energy in the reflected Laue reflection intensity of a reflection. I believe the correct expression, and the Laue diffraction Lorentz factor λ^4, not λ^2, is in Rabinovich & Lourie (1987). This chapter correctly stresses the importance of planning a full experimental runoff, with test experiments to evaluate the sample system at the preferred beamline of the users. This latter may simply be determined by location and thereby trying to contain the users' costs travelling to, and staying at, the relevant facility. These being complex experiments they do not lend themselves to remote access.

Section 4.3.1 is on radiation damage. It includes the statement that this depends strongly on X-ray energy, as well as temperature, crystal size, intensity and pulse length. There has been a debate about the X-ray energy dependence. Arndt (1984) and Helliwell & Fourme (1983) argued that there was an effect. The rest of this section of the book is a good description of radiation damage effects on the sample. There is an expert section on *Optogenetics and Photopharmacology* for starting reactions in a crystal through the light flash approach. The advantage, of course, is that the sample is pre-equilibrated with a caged molecule and so avoids time delays of diffusion of these molecules through the crystal.

Chapter 5 has three sections *Time-resolved crystallography*, *Time-resolved solution scattering* and *Molecular dynamics*. The authors very sensibly *concentrate here on the underlying experimental principles, likely to remain relevant*. A fulcrum point made is that for the authors *the structures and structure amplitudes differ from state to state but are themselves time-independent*. Section 5.1.2 describes *Time-resolved Laue diffraction*. A somewhat eccentric view is that diffraction at XFELS is Laue diffraction. Rather, given the basically monochromatic nature of an XFEL beam, it generates still diffraction patterns. A Laue diffraction experiment by definition requires a non-monochromatic beam. Section 5.2 is a clear explanation of solution X-ray scattering. Fig. 5.11, a Kratky plot, is very informative for the new user of the method as it clearly depicts the shape of the measured curve for different shapes and types of macromolecules. In the HIV protease case study, it is stated without comment that *as determined by SAXS, the radius of gyration of T80N was only 0.03 nm smaller than that of WT*. Is the reader to believe that a 0.3 Å change was significant? At least an estimated standard uncertainty should be given with the 0.03 nm value quoted. In fact, the original publication did provide it; Zhou *et al.* (2015) state that *the estimated standard deviation for the difference between these values for R_g was 0.36 Å, which is almost equal to the observed difference*.

There is an interesting and useful-to-the-beginner description of the computational method of molecular dynamics (MD). It would have been good to have included in the text a description of how these simulations seek to be validated. The method of starting a simulation at a different temperature and seeing if similar molecular dynamics evolves is a good check. This takes up c.p.u. time, rather than the much sought after longer time simulation to match biochemical time sequences, but surely is essential.

Chapter 6 is entitled *X-ray sources, detectors and beamlines*. Equation 6.1 defines brilliance, but this European preferred word was reviewed by the IUCr Nomenclature Committee (Mills *et al.*, 2005) whereby it was recommended that it be amended to 'spectral brightness' to comply with the earlier terminology of various scientific communities. Section 6.3 is on X-ray free-electron lasers, again a strong section of the book. Section 6.4 is on detectors. This is also a good description of detectors matched to time-resolved experiments. Section 6.5 is entitled *Beamlines and Experimental Stations*. It describes one particular beamline, at the LCLS. One important aspect is the timing tool, used to eliminate jitter as much as possible, which is described in a rather too condensed style.

Chapter 7 is on *Data Analysis and Interpretation*. Section 7.2 on *General Constraints on Analysis and Interpretation* is hard to follow and rather illustrates the maxim the authors state at the beginning of this chapter that *in time-resolved crystallography, innovations are being rapidly introduced into data analysis*. Such developments make this section of the book a challenge to write. The archiving of raw diffraction data is very important as these rapidly changing data analytics can then be applied to the actual experimental measurements and assess the reproducibility of earlier analyses and interpretations in publications. This is especially so where diffraction data changes are small, the occupancy of initiated intermediates are low and especially where there is an absence of error estimation of atomic coordinates or *B* factors (Helliwell, 2023). However, it is good that the role of raw diffraction data is acknowledged on page 163. This chapter is also rather theoretical and for a good practice example the authors ask us to read the supplementary material of Pandey *et al.* (2020), which is indeed excellent, and is acceptable if one is reading the book with internet availability. Section 7.8 is entitled *Making a Molecular Movie*. It is here that the whole topic of the book is challenged by the making of a movie using the concatenation of, and interpolation between, a sequence of static crystal structure models as snapshots. These are routinely available in molecular graphics packages. A direct comparison of the two types of methods (time-resolved and cartoon made up of static crystal structures) would be informative (see Blow *et al.*, 1992, for a laying down of this challenge).

Chapter 8 is entitled *Other Structural Biology Techniques* and has sections on cryoEM, energy landscape analysis, X-ray absorption and emission spectroscopies (XAS and XES), NMR (written by a guest coauthor Joseph Sachleben) and hydrogen deuterium exchange. This is clearly an important

chapter as it is the combination of methods, each of which may be precise, if taken together can determine the accuracy of research findings. For cyoEM, especially in the context of this book, the authors note that *structures with lifetimes shorter than tens of ms cannot be captured and imaged by cryo-EM at present*. This section is a lucid mini review of the cryoEM approaches. The spectroscopy section is skewed towards XFEL uses rather than the much more extensive synchrotron radiation range of XAS activities. Pages 199–202 describe the PS II studies by both XAS/XES and by XFEL crystallography at the stages of the Kok Cycle. This is a great example showing what this book is about. As it involves combined methods perhaps it should feature better in a finale chapter to the whole book? It is also a biological system that can show structural dynamics that is localized, and so does not disrupt the crystal lattice, is vital to biology and with potential for 'learning from nature' how to 'evolve oxygen'. It is also a system amenable to light flash reaction initiation with several clear structural and chemical states in the Kok reaction cycle.

The section on NMR immediately, helpfully, makes clear that *NMR is less powerful than crystallography in its ability to follow detailed 3D structural changes as a function of time. It is also unable to visualize motions correlated across distant locations of the molecule*. However, the author nicely and correctly explains that aromatic ring flips are discerned in the NMR spectra of a protein as I first learned from Bob Williams in Oxford during my doctorate in the 1970s. The author also nicely describes *one area where NMR stands out strongly from other methods is in the study of intrinsically disordered proteins, or of molecules having disordered regions or segments*. Figs. 8.12 and 8.13, in my ebook copy at least, have captions only *i.e.* no actual figure. There are equations as well in this section which are either garbled or not displayed at all. This section could have described NMR's important role in undertaking studies of a protein's histidine protonation state titration versus pH. The hydrogen deuterium exchange section is marred by two pages (214 and 215) having jumbled up symbols.

Chapter 9 is entitled *Looking Forward*. The authors openly state *this chapter briefly identifies some future directions that we see as promising. Without a doubt these are speculation; readers will choose their own directions*. The authors' often-used style of a stream of discussion works well in this chapter. On the topic of XFEL single particle diffraction, much highlighted as a prospect but not yet achieved, they state *issues of sample delivery, high repetition-rate data collection, and others remain. Yet we are optimistic about the ultimate power of the method, and of the desirability of studying particle dynamics in active samples*. They also ask *can room temperature EM of active, unstained, single particles at near-atomic resolution be developed?* I have attended lectures on this topic, and I conclude that it is hard to imagine reaching this stage. In a general observation, whilst assessing the future of AI/ML tools such as an extended-to-dynamics *AlphaFold2*, they re-emphasise that *mechanism and function in turn depend on both static structure and on the dynamic changes in structure essential to mechanism*.

There is an Appendix on the basics of crystallography which, given the advanced treatment of topics throughout the book, is extremely short and out of place. There is an extensive, and likely to be very useful, index. A strength of the book is the cited references at the end of each chapter. Obviously, there are some common references between chapters. Chapter 1 has 17, Chapter 2 has 28, Chapter 3 has 60, Chapter 4 has 85, Chapter 5 has 94, Chapter 6 has 39, Chapter 7 has 42. Chapter 8, spanning various biophysical methods naturally has a lot of references (86). Chapter 9 has 24 references.

This book should be bought, I think, as it expands the books devoted to the whole theme which I regard as important, as I imagine is obvious given that I have two of my own books on the time-resolved crystallography and diffraction topics. I would commend reading this new book when you have good wi-fi available as there will be many occasions where you will need to go to a cited reference to delve into the details required to fully understand the thrust of the book's text. As for the future, the final chapter is so wide-ranging that you will not be able to discern where these still very active researchers will themselves turn next. One of my major concerns, pressed by Durward Cruickshank, is the treatment of errors in protein crystal structures or rather the general lack of them (Helliwell, 2023). Chapter 9 reminded me of the paper by Wickstrand *et al.* (2020), which provides tools for looking for correlations between time-resolved difference electron-density maps. This will help address the challenges of not, so far, quantifying coordinate errors in atom movements. Likewise, another interesting approach is that of Ginn (2023) which by transforming protein atom coordinates to torsional space will effect a way forward to establish smaller atomic shifts in conformational dynamics, but will still need to estimate the errors on torsional angle shifts.

Having read the book, I turned again to David Blow's (2000) thoughtful essay *So do we understand how enzymes work?*. I think that this new book, great in scope and with marvellous researchers as authors, confirms that we are not there yet.

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