BOOKS: VIROLOGY

The New "Great Work"

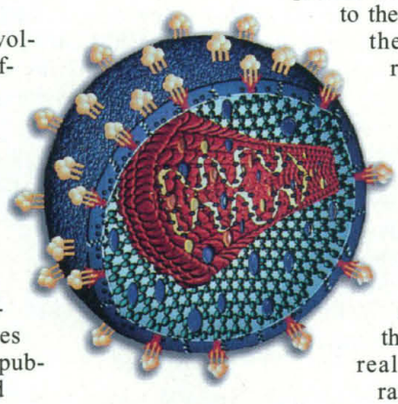
Simon Wain-Hobson

Many a scientific field has its standard reference work—a "great work" that some might go so far as to call a bible. For the study of viruses, Field's *Virology* (now in its third edition) is an obvious choice. Those whose interests are more focused on retroviruses have also had their own standard work for a number of years. Originally, it was the 1973 Cold Spring Harbor Laboratory publication *Molecular Biology of Tumor Viruses*, which spanned both RNA and DNA viruses. (For a long time the only interesting retroviruses were oncogenic. That some were pathogenic in natural outbred populations was beside the point; oncogenesis was foremost in the minds of retroviral researchers.) The "great work," encompassing all the advances resulting from cloning and sequencing, appeared in 1985 as the expanded paperback edition of *RNA Tumor Viruses*, also from Cold Spring Harbor. This text included the first cluster of sequence data from a novel retrovirus—HIV. It provided a compendium of the new discoveries that were reorienting molecular virology, and I remember taking the two volumes on holiday to Greece, thoroughly reading them from 5′ to 3′.

Retroviruses
by John M. Coffin, Stephen H. Hughes, and Harold E. Varmus, Eds.
Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998. 859 pp. $180. ISBN 0-87969-497-1.

Since the mid-1980s, retrovirology has grown explosively, producing vast amounts of data and spawning a new generation of researchers, many of whom have concentrated their efforts on HIV (and little else). For many years, the pace was such that edited volumes in the field were nearly out of date when published, and few risked writing books for fear these also would be rapidly superseded. Although some good volumes appeared, *RNA Tumor Viruses* seemed likely to remain the "great work." Yet during these past couple of years, the field has matured sufficiently to make one realize that something was lacking.

With the arrival of *Retroviruses*, a worthy successor to the 1985 classic has finally appeared. Despite its downsized title everything about this volume, edited by John Coffin, Stephen Hughes, and Harold Varmus, is top notch. Especially noteworthy are the many superb illustrations, which include color diagrams of protein structures, molecular mechanisms, and other aspects of retroviruses and their effects. (The publisher, once again Cold Spring Harbor Laboratory Press, is also offering a set of these illustrations as slides for use in teaching, research, or clinical presentations.)



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Retroviral virion. Surface and transmembrane proteins are inserted into the envelope of a cell-derived lipid bilayer. The interior contains the Gag structural proteins and the reverse transcriptase and integrase encoded by the *pol* region. (The cartoon drawing is from *The Art of Retroviruses: A Companion Slide Set from* Retroviruses.)

And the text? A series of chapters from 22 acknowledged researchers (all but two are from the United States) has been carefully assembled. Individual contributions were intended to be independent and accessible in any order, and indeed transitions among them are easily made. A historical introduction by Peter Vogt presents the principle retroviruses, their origins and classification, and a brief chronicle of the ideas and concepts that have shaped retrovirology. The following six chapters are resolutely molecular, describing key aspects of retrovirus structure, function, and replication. We are taken from the virion to membrane docking and penetration, proviral synthesis and integration, transcription, and virion assembly. One is struck by the breadth of topics and the care given to making comparisons interesting and informative. The attention to detail is remarkable. Quite a few times I was brought up short by a detail that I had missed. Any author who has ever written a chapter or review will wince in recognition of the effort the contributors must have exerted.

A curious little "intermezzo" from the editors after the seventh chapter is designed to remind the reader that there is more to retrovirology than molecular biology. Which it does—helping to place the findings on the viral life cycle discussed in the earlier chapters in the context of interactions between retroviruses and their hosts, which are the focus of the final five chapters. These deliver the goods: the endogenous retroviruses all around us, the pathogenesis of the exogenous ones, immune responses, control of infections, and the use of retroviruses as vectors. They are good, solid chapters with much to say and provide the bold new dimensions to the "great work." I found the one on endogenous retroviruses a particularly good read, if only because the topic is an area further from my territory, so there was much for me to learn. The two chapters on retroviral pathogenesis are nicely done; they get to grips with the real systems for reality, rather than the ex vivo substitutes.

Retroviruses conjures up that book shop feeling—the exhaustion and pleasure after having spent the best part of an afternoon squatting on the floor, absorbed in the book in front of you. The only problem I have come across so far is its size. (It doesn't quite lend itself to easy reading on the loo, a favorite place of mine.) The volume is a welcome reminder that, despite the advent of electronic browsing and retrieval, nothing can beat a good book.

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NEW MEDIA: SOFTWARE

Beyond Expert Advice

Larry S. Daley and Kevin Ahern

Nuclear magnetic resonance (NMR) spectroscopy is a powerful, noninvasive tool for prediction of molecular structures. In contrast to x-ray crystallography, which can only be used for crystallized molecules, NMR provides structural information about molecules in solution. The principle behind NMR is simple; certain biologically important nuclei—such as ^{1}H, ^{13}C, ^{15}N, ^{15}O, ^{19}F, and ^{31}P—behave as tiny magnets. When

HyperNMR 2.0
by Hypercube Inc.
Gainesville, FL. $495. [HyperChem Pro: $995]. Phone: 800-960-1871 www.hyper.com.

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placed in a magnetic field, these nuclei assume orientations that correspond to numerous quantized energy levels. Transitions between the orientations release energy, generating the NMR signal. In the magnetic field, each relevant nucleus in a molecule resonates with a characteristic frequency called a chemical shift. The chemical shift of nuclei can be affected by the electronic environment near each nucleus and the three-dimensional (3D) spatial orientation of each nucleus in the molecule. The components affecting chemical shift are thus a function of the molecular structure. By determining the unique contribution of each resonating nucleus to the observed chemical shifts in an NMR spectrum, one can solve a molecule's 3D structure.

One approach to interpreting chemical shifts in an NMR signal employs expert systems. In this scheme, the NMR spectra (chemical shifts) of the unknown sample are compared with spectra from molecules of similar chemical composition whose molecular structure and nuclear electronic environments are well understood. Nuclei in the unknown are assigned chemical and 3D orientations on the basis of their measured chemical shifts by simply matching them with chemical shifts of nuclei in a similar environment. Although this method works well for relatively small, simple molecules, it does not predict shifts well for larger molecules, such as proteins, whose 3D structure is not a simple function of the chemical groups in the molecule.

An alternative approach to predicting structure from chemical shift data is taken by HyperNMR, a Windows-based program that uses quantum mechanics to compute wave functions for a user-defined theoretical molecular structure. The one-dimensional NMR spectra that the program generates from these calculations are compared to the observed spectra for the unknown molecule. Successive refinements of the postulated structure for the molecule of interest are made (with subsequent updated chemical shift predictions by HyperNMR), to align the theoretical spectra with the actual spectra. The structure is assumed solved when these spectra match.

Understanding the 3D environment of each nucleus is central to being able to predict chemical shifts. Previous quantum mechanical approaches have not reliably predicted accurate chemical shifts. HyperNMR introduces two new semi-empirical methods, Typed Neglect of Differential Overlap versions 1 and 2 (TNDO/1 and TNDO/2) to improve chemical shift prediction. TNDO/1 and TNDO/2 define the chemical environment of each nucleus differently from earlier methods. For example, carbons can have orbital types of sp^3, sp^2, or sp, depending on their bonding in a molecule. Traditional quantum mechanical approaches have not taken these distinctions into account, but HyperNMR does.

SPECTRAL VALUES

Carbon number	Experimental (ppm)	HyperNMR model (ppm)	Expert system (ppm)
C1	173.0	173.76	175.4
C2	92.9	92.93	102.3
C3	68.6	62.14	69.3

Spectral values for the carbons in phosphohydroxypyruvate. HyperNMR–generated values are compared to a previous estimate from an expert system (*2*) and to real data. The data were standardized with tetramethylsilane (TMS) from handbook tables (*3*). Under the HyperNMR conditions calculated, TMS had a single major envelope with a maximum of 11.57 ppm. The experimental data had an assigned value for TMS of 10, so the calculated data were generated by dividing the values of each peak by 1.157.

To test the enhanced prediction abilities in HyperNMR, we used the program to predict the chemical shifts for a relatively simple molecule. We chose to study phosphohydroxypyruvate because the structure provides a cross comparison to spectral modeling by expert systems, which work well on small, easily described molecules. Also, phosphohydroxypyruvate has been experimentally characterized (*1, 2*).

The first step in operation of HyperNMR requires user entry of a 3D model of the test compound into the program. This model is only an approximation of the structure and provides a blueprint for HyperNMR to perform quantum mechanical calculations necessary to predict the environment and resulting chemical shifts of the nuclei. Model structures may be either user-generated or imported from a structural database. HyperNMR accepts direct input in the common HIN or Z Matrix structure formats; both are in the public domain. Molecular structures created in other programs can also be read by HyperNMR if converted with the public domain software package called BABEL (www.eyesopen.com/babel.html).

HyperChem, a companion product, is also made by Hypercube, but is not required for use with HyperNMR. It is, however, very convenient, because it helps users to draw 3D models of molecules. Structures drawn in HyperChem Pro are easily transferred to HyperNMR.

We drew a theoretical structure for phosphohydroxypyruvate in HyperChem and optimized it for conformation in an aqueous solvent by using general guidelines for bond lengths, angles, etc. Similar structural predictions could be made by modeling the molecule in other solvents, such as acetone. HyperNMR's ability to predict shifts in a wide range of solvents is a significant advantage over expert systems, which can only accurately predict spectra for molecules dissolved in the same solvent as the model molecules they base their predictions upon. After the structure was transferred to HyperNMR the conditions of the experimental simulation of spectra were optimized and calculations were performed. The program's predicted spectra with chemical shifts, coupling constants, and shielding tensors were output in numerical form and in a graphical view, where the NMR spectra were depicted as a collection of vertical lines having line heights proportional to the intensity of the signal. The HyperNMR-generated spectral values for the carbons in phosphohydroxypyruvate were compared to a previous estimate from an expert system (*1*) and to experimental data.

Relative to the predictions of the expert system, HyperNMR's prediction for carbons 1 and 2 were closer to the experimental data, but they were somewhat less accurate for carbon 3 (see the table). Differences between the actual spectra of a compound and those predicted by HyperNMR arise from discrepancy between the modeled molecular structure and the actual structure in solution or HyperNMR's implementation of quantum mechanical theory to predict spectra. It is possible, with HyperChem and HyperNMR, to iteratively remodel the structure of a compound to match its theoretical spectra with experimental values, a process one would normally follow for structure determination with the program. After theoretical and experimental spectra have been matched, one has in hand the structure or structures of the compound in solution, assuming, of course, that all theoretical assumptions in the program are correct.

HyperNMR works best with abundant computer memory. The manufacturer claims that spectra for 12 nonequivalent NMR atoms are practical in 8 to 16 Mb of RAM, if virtual memory is used. HyperNMR requires considerably more computer processor time than do expert systems, but the computationally intensive approach employed by the program has several advantages. For example HyperNMR

is not constrained to predicting chemical shifts for molecules similar to common, well-characterized molecular structures. HyperNMR is particularly useful for predicting the spectra of compounds, such as large biomolecules, for which few or no related structures have been determined. In this regard, predictions from expert systems, which depend heavily on libraries of NMR spectra of existing compounds, can be very disappointing. HyperNMR is also not limited to a single molecular species. It can evaluate several molecules and their interactions simultaneously, especially with regard to the solvent matrix in which the molecule or molecules are found. Other possible uses of HyperNMR include time-course spectral predictions for both chemical and enzymatic reactions, spectral modeling for unstable compounds, reaction intermediates, or transition states, and comparison of the spectrum of a crystallized molecule (PDB format) to the actual spectrum of the same molecule in solution. Information from the last type of study is sought by biophysicists attempting to understand the physiological conformation of biopolymers.

New users may discover that HyperNMR is not intuitively easy to use the first time. Fortunately, the learning curve is not substantial. The HyperNMR manual comes with three tutorials, which can be mastered in about 30 minutes each, if followed in the prescribed step-by-step fashion. The lessons are clear and, for the most part, unambiguous. Chapters following the tutorials provide a better understanding of the program and the scientific principles behind it. For further information on the accuracy and theory of predictions, Hypercube maintains an excellent e-mail support and FAQ section (located at www.hyper.com/support/default.htm). Manufacturer listed minimal system requirements for HyperNMR include an Intel 386-, 486- (with math coprocessor), or Pentium-compatible CPU, 4 Mb of RAM, 8 Mb of free hard disk space, and Windows 3.1, Windows 95, or Windows NT. For working with complicated structures, Hypercube recommends a fast Pentium-based system with at least 32 Mb of RAM. We did not experience unusual or excessive computer instability while using the program on a 150 MHz Pentium-based system with 80 Mb of RAM under Windows 95.

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PERSPECTIVES: NEUROSCIENCE

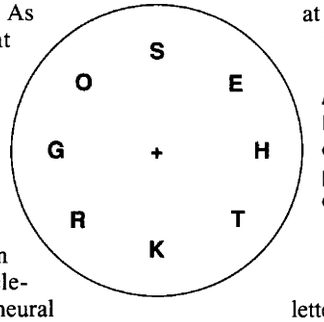
Separating the Wheat from the Chaff

Nancy Kanwisher and Paul Downing

Seeing the world around you is like drinking from a firehose. The flood of information that enters the eyes could easily overwhelm the capacity of the visual system. To solve this problem, a mechanism—attention—allows selective processing of the information relevant to current goals. As the eminent physiological psychologist Helmholtz noted over a hundred years ago, even without moving our eyes we can focus our attention on different objects at will, resulting in very different perceptual experiences of the same visual input (*1*). Visual attention has been the focus of several decades of elegant behavioral research, but the neural basis of this process has come under intensive investigation only recently. A report by Kastner *et al.* on page 108 (*2*), in which the authors used functional magnetic resonance imaging (MRI) of the brain in awake human subjects performing visual tasks, provides new clues about how our brains deal with the onslaught of sensory input.

Enhanced online at www.sciencemag.org/content/full/282/5386/57

Try a version of Helmholtz's experiment for yourself. Fix your eyes on the cross at the center of the figure (below), and without moving your eyes read the letters around the circle one letter at a time, starting at the top. Attention enhances your awareness of the selected letter, relegating the rest to the margins of consciousness.



Attention is distinct from gaze. Maintain fixation on the central cross, and read one letter at a time, progressing around the circle without moving your eyes.

Why might we have such a system in the first place? Why not just move our eyes to place objects of interest on the fovea, the high-resolution central region of the retina? Several reasons have been suggested. First, as social primates, we are acutely aware of where others are looking (*3*). The ability to move attention while holding our eyes fixed allows us to keep our interests and intentions private (*4*). Second, having an attentional system that is independent of eye movements allows us to attend to objects whose images would not fit neatly within the fovea, as well as to track several independently moving objects at once (*5*, *6*). Basketball players, for example, can mentally track several other players on the court—not just the one they could follow if they had to rely on eye movements alone.

How does selective attention work? According to one recent hypothesis (*7*, *8*), the neural representations of different objects in the image suppress each other, and attention acts by biasing this competition: The visual attributes of the relevant object are strengthened while those of irrelevant objects are weakened. In the new study, Kastner and colleagues tested this idea in humans by using functional MRI to measure the summed neural responses from each of four areas of the brain that participate in processing visual signals (V1, V2, V4, and TEO), while the subjects' attention was engaged with a difficult task at the center of gaze.

In their first experiment, the overall neural response from each of these brain areas was lower when four objects were presented simultaneously above and to the right of the central display in the peripheral parts of the subjects visual field than when the same four objects were presented sequentially in the same locations, even though the total amount of retinal stimulation (integrated over time)

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