TECHVIEW
SOFTWARE

Looking Good from This Window

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The patch-clamp recording technique is widely used by electrophysiologists to measure currents produced by the flow of ions across biological membranes. Originally introduced by Nobel laureates Neher and Sakmann in 1976 (*1*), the patch clamp has revolutionized cell biology and physiology, and proven to be invaluable in the study of ion channel proteins.

During patch-clamp experimentation, the voltage of a patch of membrane (either attached to the cell or excised) is "clamped" (held constant) to a known value, while the current flowing through the membrane is measured. The technique requires a data acquisition and analysis system comprising a low-noise amplifier and voltage clamp, an analog-digital (A-D) computer interface, a computer, and software. The software must be capable of generating an output signal for control of the voltage stimulus, while concurrently conditioning and recording an input signal indicative of the membrane current.

Analysis of the acquired data is complex and requires a variety of features, including filters to reduce noise, baseline adjustment, event detection, specialized statistical analysis, and fitting functions specific for each recording configuration. The pCLAMP 7.0 suite is a 32-bit, Windows 95/98/NT–compatible version of the popular DOS-based pCLAMP data acquisition and analysis software package designed for these specialized electrophysiological applications.

pCLAMP programs were developed in the early 1970s at the California Institute of Technology. Since the purchase of developmental rights by Axon Instruments (Foster City, California), the pCLAMP suite of programs has evolved into one of the most reliable and powerful software tools for electrophysiology. In recent years, however, a variety of competing Windows-compatible data acquisition and analysis programs for electrophysiology have become available. With the advent of higher processor speeds and 32-bit operating systems, the DOS-based pCLAMP program needed a facelift to maintain its status among electrophysiologists and to take full advantage of advances in computer technology. The first generation of Axon's Windows-compatible software suite, pCLAMP 7.0, was released earlier this year. Clampex 7.0, the centerpiece of this release, is the data acquisition component of pCLAMP 7.0, incorporating both the Clampex and Fetchex data acquisition modules of earlier pCLAMP versions. The data analysis applications of the pCLAMP 7.0 software suite, Clampfit, Fetchan, and pStat, have not yet been upgraded to run under Windows.

Clampex 7.0 has a variety of features not found in earlier versions. The ability to create user-defined toolbars, along with the "dockable" component concept of the toolbar design allows the pCLAMP-Windows interface to be easily optimized for each user. The toolbar can be further individualized by the use of sequencing keys that can be configured to launch complex experimental protocols. As with most Windows applications, Clampex 7.0 supports multiple, simultaneously active Windows. This feature makes it possible to view an incoming data stream at different scales (current and time) and, with online charting of statistics, allows the researcher to more closely monitor the progress of an experiment.

Clampex 7.0
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Data acquisition with Clampex 7.0 is also more flexible and efficient than with earlier pCLAMP versions. The method for writing and editing data acquisition protocols is more intuitive and is guided by helpful prompts. Those familiar with pCLAMP 6.0 will find the layout of the tabbed menus to be similar between the two versions and thus relatively painless to master. Clampex 6.0 and Fetchex 6.0 acquisition protocols are compatible with Clampex 7.0, eliminating the need to rewrite protocols. A significant improvement in Clampex 7.0 is the ability to alter input-output parameters such as membrane holding potential, sampling rate, filtering, and scaling during execution of an experimental protocol. These allow the user to monitor voltage-sensitive processes, optimize signal amplification, and control file size of the acquired data.

Several new or greatly improved tools are available in Clampex 7.0. The Seal Test utility, originally introduced in pCLAMP 6.0, has been improved in this version in part due to the real-time control and enhanced resolution of the scope window graphics. Formation of a high resistance, electrically "tight" seal is critical during patch-clamp experiments. The seal resistance can be calculated from Ohm's law by using a small-voltage pulse and measuring the resultant changes in steady-state current level. The Clampex 7.0 Seal Test tool makes monitoring seal formation and subsequent capacitance compensation a much easier task.

Membrane Test, a new utility in Clampex 7.0, is an extension of the Seal Test function and allows the membrane capacitance and membrane resistance to be readily monitored and recorded throughout an experiment.

The Scale Factor Assistant aids in configuring the data acquisition system to reflect the correct scaling of experimental parameters. This feature will help students who are just beginning to understand the interrelationships between the many experimental parameters of an electrophysiological analysis and is a timesaving feature for the seasoned user.

Clampex 7.0 also has several useful tools for online record keeping. The Lab Book can be configured to record information about experimental protocols, results from the Seal and Membrane Tests, and to log time-tagged event input from the keyboard. In combination with the Lab Book, the Data File Index can be searched to identify experiments done on specific days, with particular reagents, or in unique sets of conditions.

Clampex 7.0 also provides some basic analysis functions. Multiple data files can be opened simultaneously and individual episodes can be selected and viewed independently. Data from current traces can be transferred to a results window, where basic statistics such as mean and standard deviations of current levels are calculated. These results can either be saved as an ASCII file or can be transferred to analysis programs directly via the clipboard. One drawback of the pCLAMP 7.0 release is that in-depth data analysis must be completed in the DOS-based companion modules, which lack the versatility of the updated Clampex 7.0. Axon promises Windows-based updates to the analysis components in the near future. Alternatively, other Windows-based software programs, compatible with the Clampex 7.0 file format, are listed at the Axon Web site and can be used for more extensive data analysis and presentation.

The *Clampex 7.0 for Windows User's Guide* is provided in the form of an online help interface. The chapters designated as "must read" are particularly helpful for the experienced or the novice user. Step-by-step instructions, including example dialog boxes, are presented for implementation of all the program features. The tutorial is beneficial for the novice, and those upgrading from previous versions will bene-

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fit from both the tutorial and the "assistant" in the online help.

To upgrade or not to upgrade? Although new purchasers of Axon amplifiers or the Digidata 1200B A-D board will certainly opt for the pCLAMP 7.0 program suite, users of previous pCLAMP versions will wonder whether to upgrade now. A key consideration is whether the existing data acquisition computer hardware and operating system are compatible with pCLAMP 7.0. Hardware recommendations are as follows, with minimum requirements in brackets: IBM AT-compatible 133 MHz+ Pentium [80486], Windows 95/98/NT 4.0 [Windows 95], 32 to 128 MB RAM [16 MB RAM], a 1 GB [200 MB] hard drive, a CD-ROM drive [floppy drive], one full-length Industry Standard Architecture (ISA) slot, a parallel port, one free Interrupt Request (IRQ), and two [one] free Direct Memory Access (DMA) channels. In addition, pCLAMP 7.0 is not compatible with the oldest Digidata board and requires a Digidata 1200 A, AE, or B.

The availability of full-length ISA slots, required not only by the Digidata board, but also by modems, Ethernet, and sound cards, can be a limiting factor in standard computer configurations. Windows resource allocation for other devices may also conflict with the IRQ and DMA settings required for the Digidata acquisition board. To eliminate possible conflicts with the Windows Plug-and-Play utility, the DMA and IRQ settings used by the Digidata board should be reserved as described by the Axon technical support Web page. This site also gives other helpful information for troubleshooting during installation and provides access for downloading the latest debugged versions of pCLAMP 7.0. Technical support is readily available by e-mail or telephone.

One deciding factor in upgrading to pCLAMP 7.0 may be whether an experienced electrophysiologist or a novice will be the primary user of the application. The user-friendly, tutorial-based system of pCLAMP 7.0 makes it an effective teaching tool for electrophysiology.

A possible deterrent to upgrading to pCLAMP 7.0 is that in its present incarnation, pCLAMP 7.0 falls short of being a Windows program suite, because it still includes DOS-based data analysis applications. Therefore, it may be worthwhile to wait for improvements.

Overall, the transition to the Windows environment has been done well. The basic design of the data acquisition environment is familiar, and many features were added to make Clampex 7.0 a substantially more versatile application. One hopes that Axon is as successful with upgrading the data analysis components of the pCLAMP suite—Clampfit, Fetchan, and pStat—to the Windows environment.

References

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TECHVIEW
SOFTWARE

Protein Structure at Your Desk

Beth Basham

Software that can identify protein function on the basis of amino acid sequence is needed by the biotechnology industry, which is awash in protein sequences of unknown function. The BioTools package, PepTool, integrates many existing, validated algorithms and databases to help identify protein structure and function.

Identification of function from amino acid sequence usually starts with homology searching—identifying sequence similarities between the unknown protein and others of known function. PepTool performs homology searches against the PepTool database, which is an amalgam of public sequence databases. Homologous sequences can be gathered and transferred into PepTool's alignment editor. This program performs multiple sequence alignments and, unlike similar products, allows easy editing of the result. The alignment editor also permits one to color code matches based on chemical similarity, and to create publication-quality output. Sequences may be transferred from one window to another to allow seamless alternation between sequence analysis and sequence alignment.

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PepTool recognizes structural domains or amino acid sequence patterns that lead to known secondary structures and uses these to predict biological function in unknown sequences. Many of the programs in PepTool are derived from algorithms that have previously been qualified, documented, and published in the literature by David Wishart and Brian Sykes (*1*). Multiple sequence alignments are done with XALIGN, a modification of the pairwise Needleman-Wunsch algorithm (*2*). Homology searches are done with the SEQSEE algorithm (*3*), called FAST ALIGN in PepTool. PepTool provides flexibility in running these programs, with various options to score similarity and gaps in the alignment. The protein secondary structure prediction algorithms are also standard, such as Chou-Fasman (*4*) and GOR (*5*). PepTool displays the results of analyses graphically and as dot plots and helical wheels.

A strong point of PepTool is the way the databases are implemented. Sequence and annotation information in the public protein sequence databases (SWISS-PROT and PIR) have been compressed by BioTools, making it possible to house the current databases on a desktop computer or CD-ROM. BioTools provides subscription-based updates of the databases on a daily, weekly, or monthly basis. PepTool also includes a protein structure database (formerly called SEQBANK), which contains the secondary structures of the sequences for which three-dimensional configurations are known. This database is a reduced form of the protein structure database (Protein Data Bank) and contains only sequences that are not more than 50% identical to one another. These databases are searchable by sequence homology, keyword, or patterns. PepTool also permits users to import their own databases.

The documentation and online help are thoughtfully designed. All technical terms are described in detail, and some are hyperlinked to relevant references or, in some cases, to the abstract of the original paper. The computational methods are clearly described and include a summary of the method, its strengths, when it is most accurate, and its general predictive ability. With this information, the scientist can evaluate the results of PepTool's calculations to determine whether the outcome is biologically meaningful.

PepTool is available for Windows 95/98/NT, Power Macintosh, Sun, Solaris, and SGI. For some of these operating systems, a network version is available. A network parallelism option may be purchased for UNIX platforms, which allows for computationally intensive calculations to be distributed over a computer network.

References

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