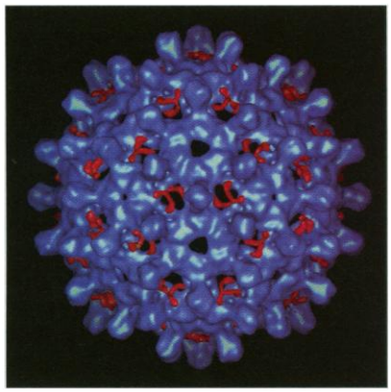
TECHSIGHTING
STRUCTURAL BIOLOGY

Difference Mapping Cryo-EM

Cryoelectron microscopy (cryo-EM) combined with three-dimensional image reconstruction techniques allows researchers to view macromolecular structures at resolutions below 10 Å. Cryo-EM has thus become an alternative method for solving the structure of biomolecules that remain beyond the reach of nuclear magnetic resonance (NMR) or x-ray crystallographic methods. Cryo-EM is an improvement over these methods in other ways as well. NMR is limited by the size of the molecule that can be studied (30 kD or less) and by the solubility of the molecule in question. X-ray crystallography is limited by the ability to make crystals that diffract appropriately. Cryo-EM does not suffer from these limitations and, in addition, offers a view of the hydrated conformation of biological molecules. The method has been extremely useful, for example, in the elucidation of the structure of whole viruses.



Study in blue and red. The outer surface of the Cpe capsid, determined by cryoelectron microscopy. The structure shown in red represents the amino terminus.

Typically, the cryo-EM approach first involves the preparation of thin, unstained specimens that can be examined by transmission electron microscopy. The samples are vitrified by rapid freezing and maintained at –160°C to –180°C in the microscope, while images are recorded under low-irradiation conditions to minimize electron beam damage to the specimen.

A number of variations of this basic approach have been developed to try to extract as much conformational information as possible. For example, one major goal of researchers is to delineate the overall paths of polypeptide chains through the density maps of biomolecules. The strategy consists of modifying the specimen in some way and visualizing the impact that the modification has on the structure. The modification can consist of labeling the biomolecule with heavy metals or antibodies that bind to epitopes on the molecule's surface.

Another strategy was recently described (*1*). The authors proposed inserting small peptides into biomolecules at defined sites and using those peptides as labels. They used this approach to localize the amino-terminus region of the hepatitis B virus capsid protein, also known as the hepatitis B core antigen. The hepatitis B virus is a small enveloped virus, with a diameter of about 300 Å, made of T-shaped dimers: The stems of the "T" project outward as spikes and the crosspieces form the contiguous shell.

The core antigen self-assembles in vivo into capsids and encloses the viral RNA pregenome and reverse transcriptase. In vitro studies indicate that additions to the amino-terminal end of the capsid protein are tolerated and do not prevent capsid assembly. The authors reasoned that they could add an octapeptide extension to the amino-terminus of the core antigen without otherwise changing its strucure. By creating difference maps between capsids generated from core antigen with and without the extension, they predicted that the location of the amino-terminus of the core antigen could be pinpointed in the capsid structure.

The capsid samples were frozen in thin films of vitrified ice. Micrographs were recorded at 38,000× on a CM200-FEG microscope operating at 120 kV and digitized to give a final sampling rate of 1.8 Å per pixel. Difference maps were constructed between capsids formed from core antigens with and without the octapeptide amino-terminal extension. Serial sections through the density maps demonstrated a significant density above background in the difference map starting at radii of 26Å, below the spike tips. This density represents extraneous octapeptides that emerge near the point where the stem of the "T" fuses with the crosspiece, and pinpoints the location of the amino-terminus in the model of the dimer.

This work demonstrates that the addition of small peptides (less than 1 kD in size) can be used to label macromolecular complexes. The location and conformation of these labels can be identified by difference mapping cryo-EM. Assuming that these labels do not alter the fold or oligomerization state of the targets, this approach can be used to gather additional information about macromolecular structures.

The choice of peptide label is, of course, very important. The smaller the peptide, the more precise the location will be, but the level of detection will also be lower. The primary sequence of the peptide can also be optimized to minimize the impact on the overall structure of the macromolecule. Finally, one needs to carefully decide where the peptide label will be added. The carboxyl termini and the hydrophilic regions of proteins are often solvent-exposed and are good insertion points. One can also be guided by the results of limited proteolysis or multiple sequence alignment before deciding where to insert the peptide label. In the end, a combination of trial and error and educated guesses will help decide the insertion point.

—RICHARD PETERS AND ROBERT SIKORSKI

CREDIT: J. CONWAY

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TECHSIGHTING
NET TIP

Digital Security, Part II

This month, we conclude our two-part series on digital security by describing how to use two security features: digital certificates and secure sockets layer (SSL), the most up-to-date security features available for Internet communications. Let's start where we left off last month, with digital certificates.

As we explained, a digital certificate is essentially a verified encryption key that serves two major purposes for the user: it authenticates the user on the World Wide Web, and it encrypts the user's communications. As an example, consider the case of a scientist who wants to connect to a secure server at a pharmaceutical company. The scientist could use a digital certificate to ensure security for the communications. When the scientist connects to the company's server, the browser initiates a behind-the-scenes message exchange that transmits the scientist's certificate to the server. The server decodes the certificate and decides whether the scientist is actually working at the company and what access permissions he or she should have. If the scientist leaves the company or the certificate is lost or stolen, the certificate can be revoked or deactivated. Indeed, a very important security aspect of digital certificates is that they can be deactivated at any time.

How is a digital certificate actually used? After it is obtained from a certificate authority [see Part I (*1*)] it is installed on a computer and loaded into the browser. Browsers from both Netscape (Navigator and Communicator) and Microsoft (Internet Explorer) come with a pre-installed collection of certificates from various or-

ganizations. The certificates bound to Internet Explorer can be reviewed by going to the menu View:Options:Content. In Communicator, go to the menu Communicator:Security Info:Certificates.

Although certificates provide good digital security, they have some shortcomings. Setting up and managing a network of digital certificates can be difficult, as can educating the user community about how to use them.

Other security methods can be used to authenticate a user and encrypt communications. One of the most popular technologies that provides the foundation for an alternative to digital certificates is SSL.

SSL is a technology originally developed by Netscape, but it is now widely deployed on all major servers and browsers. SSL works this way: When a browser connects to a server that hosts SSL, the browser and server exchange a series of messages. These messages create a transient encryption "tunnel" that effectively scrambles all communications between the server and browser. In the SSL scheme, both the server and browser can share an identical, symmetric encryption key. The browser can use this key to encrypt a message, and the server can use the same key to decrypt the message.

If you have ever securely purchased an item online, chances are that you have already used SSL. Unlike digital certificates, SSL does not require any configuration on the browser end. All a user needs to do is type in an SSL URL that begins with "https" instead of "http." Although the simplicity of SSL solves the encryption problem of Internet transmissions, it does not address the authentication problem. How does the SSL server know who you are? The answer: passwords.

You can log into sites that have an "http" URL simply by typing the address into a text box and hitting submit. The individuals that run the server usually are the ones that give out the passwords. By looking at the passwords, a server can know who you are. The problem is that the passwords are not sent over the Internet in an encrypted form. A hacker can use tools to intercept passwords as they are in transit. Armed with your password, the hacker can log onto a computer and cause harm. This can be prevented by encrypting the passwords before they are sent to the server. A solution that is quite common today is to send the passwords over the internet via an SSL connection. Using this setup, the server knows who you are, and all further transmissions are safely encrypted.

Why is digital security important for scientists? Consider a real-life situation in which experimental MRI (magnetic resonance imaging) data is obtained by scanning the brains of volunteers. The data, shared among researchers at three universities, are sent over the Internet. But the scans also contain sensitive information, such as the patients' names, that should not be made public. Tools like SSL and digital certificates could be used to link the researchers in a secure communications network. Other scenarios might include a group of researchers at a pharmaceutical company who wish to use the Internet for exchanging patient data, a biotech supply company selling cloning kits online, or a university giving remote access to its databases. Armed with the fundamentals of digital security, users and administrators can apply today's security to a wide variety of sensitive communications in the fields of science and medicine.

—ROBERT SIKORSKI AND RICHARD PETERS

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TECHSIGHTING

SOFTWARE

Graphically Speaking

At a time when scientific software support on the Macintosh has been reported to be slowing (*1*), along comes release of a new version of one of the premier plotting packages designed for the Mac, DeltaGraph. One of the major players in the category of all-purpose charting and graphing, DeltaGraph excels in creating visually appealing, customized charts for publication and, notably, for presentations, too. Like earlier versions of the program, DeltaGraph 4.5 draws on the Mac's powerful graphic capabilities, enabling users to extensively edit and annotate graphs for striking visual presentations.

DeltaGraph 4.5

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The program is organized around four different views: Data, Chart, Outline, and Sorter. The user interface for data entry in the Data view resembles a spreadsheet. Data can be entered manually or imported from another program. Charts and graphs are created in the Chart view, following the selection of the desired data range. The Outline view contains a summary of all the charts and graphs created with a set of data and allows for the addition of speaking notes or comments, creation of a bulleted list, or the placement of text within a chart. The Sorter view allows a user to see the charts as a list or as thumbnail presentations of each chart. An interesting function of the Sorter is its ability to display charts as a slideshow, eliminating the need to export them separately to Microsoft PowerPoint for presentations.

Providing functions to assist users in choosing plots for their data has been a trend noted in recent reviews of data analysis packages, such as InStat (*2*), Data Desk (*3*), and the Data Analysis Toolbox (*4*). With 80 chart types to choose from, DeltaGraph 4.5 provides a Chart Advisor that can help users select appropriate chart types for their data. The program's suggestions should be sufficient for most situations. If a different type of chart is desired for illustrating a particular set of data, charts can also be selected manually in the chart gallery, where examples of each chart type can be viewed as a small graphic illustration.

DeltaGraph 4.5 is the first major upgrade to this software line since it was acquired by SPSS, Inc., in 1997. Significant additions in the new version include 11 new chart types, resulting in the availability of over 80 charts and 200 chart styles in the updated program. The upgrade also adds additional graphic file support with the inclusion of new file formats for both import and export. New data import options include support for Excel 98, Excel 3.0 to 5.0, and Trapeze formats. Other welcome new features include AppleScript support (a powerful macro-like environment for automating the program), the ability to create press-ready color, enhanced automation features, and compatibility with Macintosh OS 8.5.

DeltaGraph 4.5 requires Macintosh system 7.5.5 or later and a computer with at least a 68030 processor. Minimum memory requirements are 8 MB of RAM and 20 MB of hard disk space. Documentation includes a comprehensive manual and online help within the program. Although the 4.5 upgrade to DeltaGraph is only for the Macintosh platform, DeltaGraph is also available for Windows 3.1 and Windows 95.

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