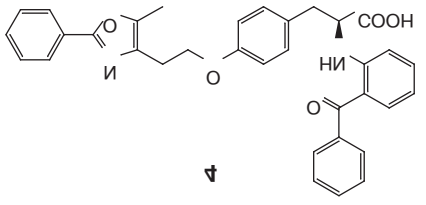
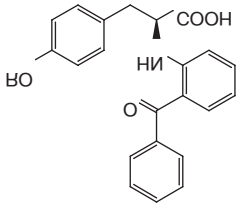
In the solid-phase SAR work, the caesium carboxylate (**5**, R = H) was attached to 2-chlorotrityl resin and Mitsunobu chemistry was employed to generate a range of aryl ethers. A total of 62 compounds were generated in parallel on solid-support, subsequently cleaved using 10% TFA in dichloromethane and were isolated with purities ranging from 16% to 98%. Of the products, six compounds demonstrated pK_i values greater than six, and these progressed to further biological assays.



4



5

Glycopeptide libraries

Many biological processes, including immune response, cellular adhesion, inflammation and cancer cell metastasis, are controlled by the recognition of glycoconjugates. In addition, many viral, bacterial and parasitic infections are also mediated by the interaction of glycoconjugates with protein receptors. As a result, compounds that mimic carbohydrates that can ameliorate these interactions have the potential for treating a range of diseases.

In the search for carbohydrate-based pharmaceuticals, a novel one bead–one compound library of glycopeptides has been prepared by using a novel encoding method [St Hilaire, P.M. *et al.* (1998) *J. Am. Chem. Soc.* 120, 13312–13320]. The method, named encoded ladder synthesis, is based on the ladder library method, in which in each round of synthesis a small proportion (10%) of the growing compound is capped with a carboxylic acid. This permits MALDI-TOF mass spectrometric identification of the product compounds by analysis of the unique mixture of terminated intermediates. This method has been extended by the use of a range of capping groups related to the monomers being added. Specifically, instead of adding an Fmoc-amino acid, the beads were also capped with a small proportion of the similarly reactive Boc-protected amino acid. Release of the glycopeptides from the resin support using a photolytic reaction generated 300,000 combinatorial library products tested as oligosaccharide mimics for the *Lathyrus odoratus* lectin.

Nick Terrett
Discovery Chemistry
Pfizer Central Research
Sandwich, UK
fax: +44 1304 655419
e-mail: nick_terrett@sandwich.pfizer.com

Bioinformatics: guide for evaluating bioinformatic software

There are hundreds of bioinformatic software programs available that serve a variety of applications. For someone new to bioinformatics there is a bewildering array of freeware, shareware, and commercial analytical programs, and it is difficult to make a decision between them. We are mainly influenced by what our peer group uses, but we still need a method to assess, or evaluate, the software to see if it fits our particular purpose. Established workers in bioinformatics also need some way to determine how best a particular software program integrates into the existing infrastructure. Software evaluation is an art, a science, and a business. It can be as casual as a 'let's have a play' approach or as formal as a rigorous, structured and systematic investigation. A large or networked organization would usually require detailed and specific criteria for evaluating software and also effective project management of the evaluation process. What follows, however, is a general guide for evaluating 'off-the-shelf' bioinformatics analysis software. Both an individual PC-user or a bioinformatics/IT manager, responsible for a suite of programs over a local or distributed network, may equally use these guidelines.

Why evaluate?

The first question that needs to be addressed is 'Does the software do the job it is supposed to do – is it fit for the purpose intended?' Evaluation also helps decisions to be made between competing products; to match software to the specific needs of the individual or organization; plan for integration within an existing infrastructure; ensure effectiveness, efficiency and quality; and finally to save time and money.

Who will evaluate?

The end-user is an obvious important evaluator. However, when organizational issues must be addressed then the information technology, bioinformatics and other appropriate departments need to be consulted for the evaluation process. A third party can also be consulted if necessary and this may be used to provide unbiased, independent advice and thus help to avoid internal politics or vested interests.

Software can also be used to evaluate software – although you'll then have the 'chicken and the egg' paradox of trying to evaluate the software which is evaluating the software!

How to evaluate?

Evaluation can be approached from several different perspectives encompassing scientific, business or procedural issues. Invariably, however, evaluation is based on a set of criteria

 1359-6446/99/$ – see front matter PII: S1359-6446(99)01352-5

Box 1. Databases and lists of bioinformatics software

- EBI BioCatalog (searchable database): http://www.ebi.ac.uk/biocat/biocat.html
- Steven Salzberg's links at Johns Hopkins: http://www.cs.jhu.edu/~salzberg/appendixa.html
- CAOS/CAMM: http://www.caos.kun.nl/
- Base4: http://telomere.base4.com/html/jacks_software.html
- Molecular Biology Notebook: http://www.res.bbsrc.ac.uk/molbio/soft.html
- Phylogenetic software:http://evolution.genetics.washington.edu/phylip/software.html
- Weizmann Institute of Science: http://bioinformatics.weizmann.ac.il/mb/software.html
- The Hospital for Sick Children: http://www.genet.sickkids.on.ca/bioinfo_resources/software.html
- Viviene's Bookmarks: http://ba-itumac1.lib.unimelb.edu.au/VivsBioinformatics.html
- Pedro's BioMolecular

Box 2. Commercial 'off-the-shelf' bioinformatic software packages

Company (Web address)	Software product[a]
ApoCom (http://www.apocom.com/)	Grail Toolkit, Gene Hound
Biosoft (http://www.biosoft.com/index.html)	GeneJockey II
Compugen (http://compugen.co.il)	GenCore
DNASTAR (http://www.dnastar.com/)	Lasergene
GeneCodes (http://www.genecodes.com/)	Sequencher
GeneData (http://www.genedata.com)	WorkBench
Hitachi Software (http://www.hitsoft.com/)	MacDNASIS, DNASIS
Incyte Pharmaceuticals (http://www.incyte.com/)	LifeTools 2.0, LifeSeq 3D, Life Array, GEM Tools
Informax (http://www.informaxinc.com/)	Vector NTI, SSBM
Molecular Biology Insights (http://www.mbinsights.com/)	Oligo 6
Molecular Applications Group (http://www.mag.com/)	Discovery Base, Gene Mine, Look, MacLook
Molecular Simulations Inc. (http://www.msi.com)	Gene Explorer
Oxford Molecular Group (http://www.oxmol.com/)	Omiga, MacVector, AssemblyLIGN, GCG Wisconsin Package, SeqWeb, SeqLab, SPS Cross Match, SPS Phrap, SPS Swat, AbM, Chameleon
Pangea Systems (http://www.pangeasystems.com)	GeneMill, GeneWorld
Premier Biosoft International (http://www.PremierBiosoft.com/)	Primer Premier 4, Plasmid Premier 2
SeqSearch (http://www.xs4all.nl/~marker/index.htm)	SeqSearch
Textco Inc. (http://www.textco.com/)	Gene Construction Kit, Gene Inspector

[a]The majority of these software products are stand-alone packages involved in sequence analysis.

and the software is assessed based on its ability to meet that criteria. The following criteria can be used for scientific evaluation and is subdivided into the four general areas of needs analysis, presentation, technical and performance sections.

Needs analysis

Probably the most important first step is to decide what you want to do and from this develop your criteria. What features do you want? What do you want to achieve? What benefits do you want from the software? What feel and aesthetics do you want? How does it fit within your existing infrastructure? How many users will there be and what is their competency? What do the users need to perform their duties? How do your requirements compare with the vendors' advertised solution?

The next step is to search for candidate software for your evaluation and to gather the necessary background information. Where do you find candidate programs to help you solve your particular needs? Well, you can check out software databases or lists (Box 1), vendors (Box 2), colleagues, journal

articles and advertisements, trade shows, conferences, industry associations, user groups, Internet newsgroups, and Web sites, such as BioMedNet (http://www.biomednet.com/). Some of the background information for the software will have to be provided by the vendor or manufacturer or programmer, provided that their inherent vested interest is recognized. Additional third party information can come from any literature reviews and testimonials from previous and existing users.

Finally, you need to have some way of scoring the evaluation. For example, a yes/no answer or a quantitative numbering scale for each of the selected criteria. This is to set a standard for evaluation, to enable fair comparison of proposed benefits and features of the program, and to help discriminate between competing products.

Presentation

In terms of presentation, is the software intuitive and user-friendly? Is the interface a command line, web-based, X-windows, or other graphical user interface (GUI)? How effectively does it use the monitor screen space – is it uncluttered, easily read, and does it make appropriate use of colour, graphics and sound?

Technical

Technical detail can be further subdivided into Method, Features and Programming.

Method

What algorithm is used and is it appropriate – is it based on old or new knowledge; what are the assumptions inherent in the algorithm, how good is it; is it fast enough; does it do what you expect? Are there any better algorithms? What sort of testing has been done and is this supported in the literature? Is it a 'black box' system using proprietary algorithms, or is it an auditable system open to inspection and assessment?

Program features or functions

The questions relating to a software's features and functions are numerous, but some general questions (in no particular order) include:

- What platform does it use: Unix, Windows NT, Windows, Mac, or other?
- What is the size of software and what is the minimum configuration required in terms of memory (hard disk and RAM) and central processing unit (CPU)? Is it CPU intensive and does it tend to 'hog' the CPU? Is a graphics card needed?
- Is the software networkable and what kind of interface does it have for distributed systems?
- Does it support multiuser, multitasking and background operations?
- What is the level of content and expertise within the program – is it for beginners, intermediate or advanced users?
- Is training or tutorials available for the software? If so, what form does it take – books, on-line or personal? How long does it take to learn and to become reasonably competent?
- Is it robust and reliable – does it have an ability to handle inappropriate calls or directions without crashing? Can the program be applied under varying conditions (adjusting parameters) and continue to give reliable and trustworthy results without crashing?
- Is it powerful – is it quick and can it do what you want it to do?
- Is it scaleable – does it have the ability to handle different sizes of data sets? What is the maximum data size it can handle?
- What file formats are used? Proprietary or non-standard file formats make it difficult to use elsewhere and limit flexibility.
- Does it have the ability to export and import different file formats? This affects its ability to share data and information with other programs.
- Does it have the ability to interface with the Internet and download data?
- How does it report results – in postscript files, text files or proprietary files? Can you edit the results?
- What is its printing ability – can it handle postscript, graphics and specialized text?
- What is the cost (initial and ongoing)? Does the cost cover a single user, multiple users, site licence, cost of upgrades, support and maintenance?
- How old is the technology it uses and has it been recently surpassed? Does it have a lineage and version history? Has it been recently upgraded?
- Have all the bugs been worked out and does it conflict with any other program?
- How much time and effort is required for installation and backup?
- Once installed, how long does it take to load and begin to work?
- Are there any benchmark performance data for the software – time required to open and read a particular file, draw a graphic, and export or import files? Is the benchmarking performance data independent and can it be validated by a third party?
- Is customization available and can the user set preferences?
- Are there any security measures available – password protection and prevention of online access with proprietary sequences?
- Is help available and what form does this help take – for example, documentation, on-line, context sensitive, menus and balloon help?
- How long has the vendor (if commercial), manufacturer or programmer been in existence? What experience and qualifications do they have? How likely is it that they will be operating in the future? What support systems are in place – such as a personal representative, e-mail, fax-back, toll-free number, support

for a fee, bulletin board, on-line chat and newsgroup?

Programming

Further questions relating to programming include:

- Is the source code provided and is it hackable? That is, is it possible to change and modify the program?
- Does it have the facility to accept plug ins/modules/sub-routines?
- Which programming language is used?
- What standards are incorporated into the software?
- For bundled programs (in which two or more programs are packaged together in one application) how good is the integration? Is it easy to move data from one program to the other, and what interim storage is necessary when moving between programs? How are the programs integrated – what language 'glues' the components together?

Performance

The crucial test of any bioinformatic 'off-the-shelf' software is if it makes biological sense. For example, a program may be designed to find patterns, but do the patterns have any biological significance? Does the software do the job you thought it could do? Is it fit for the purpose you intended? Does it give you error-free, accurate and precise results? Can you trust the results – especially if it is a 'black box' system? What sort of quality control has been performed and does it adhere to your own company's quality control standards? Finally, is a test data set available to determine a software's performance and is it validated by a third party or from the literature?

Steve Bottomley
School of Biomedical Sciences
Curtin University of Technology
Perth, Australia
e-mail: ibttoml@info.curtin.edu.au

Nitric oxide disbalance and structural alterations of the cardiovascular system

Nearly two decades have passed since Furchgott and Zawadzki revealed the obligatory role of endothelium for arterial relaxation to acetylcholine [Furchgott, R.F. and Zawadzki, J.V. (1980) *Nature* 288, 373–376]. More than ten years have passed since endothelium-derived relaxing factor was identified as a nitric oxide (NO). At present, it is generally accepted that NO, one of the simplest molecules in nature, is actively engaged in many biological processes in the organism. The findings that NO is a potent vasodilatory molecule, continuously produced by endothelial cells, gave rise to the question of whether its decreased production might be one of the main factors contributing to the development of some types of hypertension. An experimental model of NO deficiency has been developed. The pathogenic background of NO deficiency consists of inhibition of NO synthase, an enzyme of crucial importance in the virtually ubiquitous arginine–citrulline pathway with concomitant coproduction of NO. Blockade of NO synthase disturbs the balance between vasodilatory and vasoconstrictory agents, resulting in a pronounced increase in blood pressure.

Long-term NO synthase inhibition in rats resulted not only in increased blood pressure but also in cardiac hypertrophy and an increase in arterial-wall thickness. We found that in conduit arteries – thoracic aorta, carotid artery and coronary artery – the arterial-wall thickness (tunica intima and tunica media) was increased by more than 70% [Kristek, F. and Gerová, M. (1996) *Physiol. Res.* 45, 361–367].

The question remains open as to whether these pathological changes are primary consequences of NO deficiency or whether NO deficiency only triggers the underlying processes that induce the changes. Moreover, pressure elevation itself probably also affects metabolic and proliferation processes in the vessel wall. Because NO may have an antiproliferative effect, NO synthase blockade would be expected to act in the opposite way. In NO-deficient hypertension, both stimuli – a decrease of NO level and an increase in blood pressure – seem to operate synergistically, and thus we suppose that the two factors could enhance the contribution of smooth muscle to the increase in arterial-wall mass.

From both the pathophysiological and mechanistical point of view, an important issue is whether the cellular- or the non-cellular component of the arterial wall is responsible for the described increase. Morphometric analysis of the arterial wall (tunica intima and tunica media) revealed that all of these components of the arterial wall (endothelial cells, smooth muscle cells, and extracellular matrix) increase their volume. Nevertheless, our findings indicated that the extracellular matrix significantly increased its volume and thus became the main contributor to the wall thickness [Kristek, F. *et al.* (1996) *Physiol. Res.* 45, 329–333]. Long-term NO deficiency was found to result in hypertrophic remodelling of the arterial wall. Moreover, the increased smooth-muscle contractility induced by inhibition of NO production appears to cause damage to the areas supplied by the arteries.

NO deficiency

Does supplementation of NO in the form of exogenous NO donors correct NO deficiency after NO-synthase blockade? It is well documented that exogenous donors of NO initiate a chain of events leading to vascular relaxation. However, NO donors may also be a source of superoxide radicals, and this may affect their therapeutic profile. Molsidomine, an NO donor, is frequently used to provide prophylaxis

 1359-6446/99/$ – see front matter PII: S1359-6446(99)01353-7