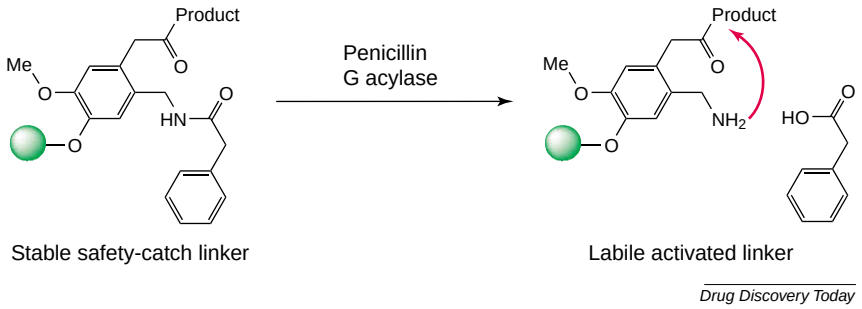

Figure 1. After enzymatic cleavage, the resulting labile activated linker is susceptible to intramolecular nucleophilic attack (red arrow) resulting in product release (not shown).

performed by using reactive chemicals, such as alkylating agents, but by instead resorting to mild and highly selective natural tools: enzymes. As was demonstrated by Grether and Waldmann recently, the penicillin-G-acylase-mediated cleavage of an amino group and subsequent lactam formation led to mild and selective cleavage of a safety-catch linker construct (Fig. 1) [5]. Using the combination of the safety-catch and enzyme-cleavable linker concepts, only one functional group of the anchor has to be transformed, which is much easier to achieve than enzymatic cleavage of the actual linkage.

Waldmann's group thus opened the way for the future synthesis of delicate, complex scaffolds bearing many different functional groups: natural products. This seems highly desirable, because natural-product-like molecules are progressively regarded as biologically validated drug leads.

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Don't underestimate the power of VS

Despite the rapid expansion of HTS throughout the pharmaceutical industry, virtual screening (VS) technology continues to play an important role in the lead discovery process. Indeed, as both technologies continue to mature, significant synergies are beginning to develop between them [1]. Schneider and Böhm provided a nice overview of VS techniques in a recent edition of *Drug Discovery Today* [2]. The review covered a wide range of topics from simple library filtering through to structure-based fast docking and *de novo* design methods.

An excellent example of the successful use of VS technology was conducted by Böhm *et al.* [3], which detailed the discovery of multiple inhibitor chemotypes for DNA gyrase using structure-based screening. These studies are particularly interesting because data is included for both lead discovery and lead optimization. It is also worth noting that a random screen undertaken by the same research facility produced no leads, thus highlighting the value-added potential that VS techniques can bring to a screening campaign.

One of the techniques used to good effect in this example was the application of pharmacophore constraints. These are included in the review but their potential use bears further emphasis. The authors touch on the problem of false positives in screening campaigns. The two major reasons for this in VS are insufficient configuration and conformation sampling and the use of simplistic scoring functions. Pharmacophore constraints provide a useful tool for mitigating both effects, by creating a significant drop in the number of binding modes a ligand can adopt, thus producing a subsequent reduction in the sampling undertaken.

Pharmacophores also force the presence of essential interactions (e.g. hydrogen bonds and salt bridges) in a given binding mode. This lessens the impact of the inability of many scoring functions to distinguish between hydrogen bond and salt-bridge strengths. There is also a more practical element to pharmacophore use. Baxter and colleagues presented a nice example of VS success for a search targeted at the estrogen receptor (http://www.lib.uchicago.edu/cinf/220nm/slides/220nm16/220nm16.pdf). In this presentation, the need for post-screen analysis and filtering criteria were emphasized. Pharmacophore constraints permit the user to build criteria for binding up-front, thus saving much time in post-screen hit evaluation.

The authors also touch on the recent progress in computing processing unit power available. With the advent of distributed computing, this is perhaps the single most important event to greet

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the VS world in the past decade. With distributed computing comes the potential to increase available processor power by several orders of magnitude. The simple manner in which VS databases can be divided up onto separate processors makes VS a prime candidate application for such technology. The resultant increase in power available is already being used to screen massive libraries (http://www.chem.ox.ac.uk/cancer/thinksoftware.html).

Using current technology, however, larger libraries bring with them more noise. Further post-screen analysis becomes a particularly daunting prospect given the huge increase in the hit numbers produced. Another, perhaps more promising, long-term alternative is to perform better, rather than more, calculations. By increasing sampling and using more accurate descriptors of the forces that govern binding, the number of false positives should be dramatically decreased, thus increasing the hit rates derived from VS calculations.

One final comment on a practical element of VS application that is generally neglected in technical reviews on the subject. A primary objective of such screens is to dramatically refine the list of compounds that is put forward for screening. Such focussing is often used to buy-in compounds that complement those screened in HTS. In addition, this number is often small enough that screening samples can be freshly made up from solid store. As a result, the issues of liquid sample stability and concentration inaccuracies are dramatically reduced, enabling a second 'bite' at compounds already screened but possibly missed in HTS campaigns.

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Conquering the proteome

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The fifth annual IBC *Proteomics and the Proteome* conference (18–20 February 2002, Geneva, Switzerland) began with a keynote presentation by Hanno Langen (F. Hoffmann-La Roche, Basel, Switzerland) who outlined state-of-the-art proteomics technologies. To maximize the identification of different proteins from complex biological material, such as biological fluids, he recommended that several fractionation steps (subcellular fractionation and classical chromatographic methods) should be performed before separation via two-dimensional (2D) gel electrophoresis. For example, albumin comprises 50% of the whole protein content of plasma and the other 40% includes immunoglobulins, transferrin and haptoglobulin. By removing these abundant proteins before separation, the sensitivity of subsequent 2D electrophoresis is increased 10-fold.

In his keynote address, Dennis Hochstrasse (Geneva University Hospital, Geneva, Switzerland) demonstrated interesting parallels between the way computers and cells generate and process information. Based on this, he shared the concept of connecting the virtual and the real worlds, that is, combining bioinformatics with experimental biology. He reiterated the problem of uneven distribution of protein concentrations inside the cell when resolving the identity of different proteins, and described a different approach to performing experiments or extracting proteins from large-scale batches of samples to isolate the least expressed proteins.

The use of free-flow electrophoresis (FFE) as a preparative method for fractionation and enrichment of proteins was described by Christoph Ekcerskorn (Tecan Group, Munich, Germany) as a way to reduce the complexity of samples. Enrichment facilitated the visualization of less abundant proteins for subsequent separation by 2D electrophoresis or LC–MS analysis. FFE has been used to process protein samples from intact cells, organelles, membranes and protein mixtures and is reported to be high throughput, with high quantitative recovery and high resolution.

Differential protein analysis without 2D electrophoresis was the topic of Pierre Thibault's presentation (Caprion Pharmaceuticals, Quebec, Canada). He

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