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Job Hunting with a Net

Richard Peters and Robert Sikorski

After many years of undergraduate, graduate, and postdoctoral training, scientists face the most important decisions of their professional lives: what jobs to apply for and, eventually, which job to take. Others may elect to switch careers for various reasons, but will also need to look for job opportunities.

Besides the usual classified advertisement sections of major print journals (such as *Science*) and local newspapers, another place to turn for help in your job search is the Internet; however, with literally thousands of Web sites claiming to have the best job database available, it is often hard to know where to start.

We attempt to provide the reader with a start-up kit of Web resources for job hunting. As a first step, you might turn to the Web site of the major scientific journals: most have implemented a searchable database of classified advertisements that appear in the print version of their journals. *Science* Professional Network (http://recruit.sciencemag.org/) has such a database of all the classified ads published in *Science*. International Job Opportunities (www.nature.com) lists the ads from the various *Nature* publications. Viewers can search the database by job, country, or employer. *Cell*'s recruitment database (http://server.cell.com/recruit/) includes the classified ads from the past two issues of the various *Cell* publications: *Cell*, *Immunity*, *Molecular Cell*, and *Neuron*.

The next step may be to turn to more general sites that have job listings for scientists. The Biotechnology Industry Organization (www.bio.org/job_bank/list1.dgw) has a database of open positions in various biotechnology companies, listed by company. Jobs that are available at the National Institutes of Health are also posted on the NIH's Web site (www.training.nih.gov): these jobs are categorized for students, postdoctoral candidates, and faculty.

The final step is to look to more general job bulletin boards on the Net. The Riley Guide is a directory of such sites on the Net. The most useful section of this directory is the "Education and Academe" section (www.dbm.com/jobguide/educate.html), which will point users to sites that list job openings for academicians. CareerPath.com (www.careerpath.com) has a database of more than 270,000 jobs in the United States. The site lists ads published in selected newspapers. Users can also submit resumes and ask to be notified when a new job matches their skills. The Monster Board (www.monsterboard.com) has a smaller database of more than 50,000 jobs, but it features a very friendly user interface and added bonuses such as relocation and apartment listings. The site also has an international section. The Job Openings Database of CareerMagazine (www.careermag.com/db/cmag_postsearch_form), a Web site that focuses on career issues, is very large. Every day the site operators download and index job postings from the major Internet newsgroups.

CareerWeb (www.careerweb.com) is another site that focuses on career issues; the best feature of its job database is the search capability by employer name. Finally, America's Job Bank (www.ajb.dni.us) features a database of more than 680,000 jobs nationwide. The site features fairly advanced search algorithms, so you can narrow your search

based on job skills and geographic location.

By routinely checking the sites listed above, the prospective job seeker is bound to find a job eventually that matches his or her specifications.

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Chemical Weddings

Richard Peters and Robert Sikorski

Scientists often rely on protein engineering techniques to introduce artificial molecules in protein sequences. Indeed, the introduction of these small molecules (artificial amino acids and peptides, fluorescent tags, resonance probes, or cross-linking agents) can be used to study their effects on the structure-function relationships of proteins and to gain biochemical insight into various biological systems. Of the many methods for introducing such molecules into proteins, most have practical, synthetic limitations. One general approach is to link a peptide thioester to a protein containing an NH_2-terminal cysteine: the chemical reaction between the two substrates leads to a product with a native peptide bond at the junction between the two moieties. The technical limitations of this approach, though, have commonly prevented the application of this method to proteins that are larger than 15 kD.

By combining molecular biology tools and chemical synthesis methods, the authors of a recent report in *Proceedings of the National Academy of Science* break down the size limitation barrier of the modified protein (*1*). They started with a commercially available bacterial expression vector, pCYB, which can be used to generate a recombinant protein that is fused via a thioester bound to an intein-chitin binding domain sequence. In standard laboratory procedures, treating the expressed fusion protein with a reducing agent such as dithiothreitol (DTT) or 2-mercaptoethanol releases the cloned protein. The transesterification reaction catalyzed by the reducing compound releases the protein of interest into the solution, while the fusion moiety remains attached to a chitin affinity column.

The authors reasoned that they could replace the reducing agent with a synthetic peptide carrying an NH_2-terminal cysteine that would react with the thioester bond; however, simply mixing the peptide with a thioester protein did not lead to any significant reaction, because the NH_2-terminal cysteine was not reactive enough. Next, the authors added a thiol cofactor (mercaptoacetic acid) to speed up the reaction, but this cofactor merely led to cleavage of the recombinant protein from the fusion protein, acting just as DTT would.

In the end, the trick was to add thiophenol in the reaction buffer: this led to greater than 90% ligation of the synthetic peptide to the recombinant protein, as determined by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The authors postulate that the thiophenol reacts first with the fusion protein via an initial transesterification reaction, followed immediately by chemical ligation with the synthetic peptide [figure 2 in (*1*)].

In the published report, the authors applied their new method to Csk, a COOH-terminal Src kinase of 50 kD size. Csk lacks the regulatory COOH-terminal tyrosine tail that is found in the structurally related members of the Src family. The authors decided to investigate what the affect of adding such a tyrosine tail would be on

Csk's function or structure, or both. After cloning Csk in pCYB and co-transfecting bacterial cells with this vector and a GroEL-GroES expression plasmid (chaperones required for correct folding of Csk when expressed in bacteria), they isolated the fusion protein and reacted it for 24 hours at 25°C with an unphosphorylated or phosphorylated tyrosine tail peptide. Characterization of the semisynthetic Csks, using a number a biochemical and biophysical methods reported in the paper, seems to indicate that the Csk-phosphopeptide forms an intramolecular phosphotyrosine-SH2 interaction, like the interaction between the SH2 domain of Src and its endogenous COOH-terminal phosphotyrosine tail. To their surprise, the authors also noted a fivefold increase in catalytic activity of this Csk-phosphopeptide protein toward a specific target, the protein Lck. Although the biological significance of this finding is unknown, the results demonstrate that modifying proteins with this new method will lead to new hypotheses. This and a related approach have also been applied to several other proteins, hinting that the method will be broadly applicable as well (*2*, *3*).

By using basic chemical principles and commercially available molecular biology and biochemistry reagents, these authors have developed an elegant technique for ligating artificial molecules to proteins of virtually any size. We anticipate that this method will soon become part of the routine recipes used in protein engineering laboratories worldwide.

References

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The Heat Is On!

Richard Peters and Robert Sikorski

Manufacturers of proteins are constantly looking for ways to increase the stability and the shelf life of these molecules, whether they are to be used for industrial or medical purposes. Nowhere is this more true than in the field of biotechnology, where there is great interest in discovering methods that can increase the stability of recombinant proteins without affecting their function. A paper published last month in *Nature Structural Biology* seems to take us one step closer to this goal (*1*). Using a computer algorithm that they published in *Science* last year (*2*), the authors were able to design a hyperstable variant of streptococcal protein $G\beta_1$ domain. This domain binds with very high affinity to the Fc region of the immunoglobulin IgG and has already been characterized by x-ray crystallography, making it an ideal candidate for testing the computer optimization method.

Not surprisingly, in trying to design proteins that can withstand high temperatures, scientists have attempted to mimic the structure of proteins isolated from hyperthermophilic organisms; however, the thermal stability of such proteins does not result from unique structural characteristics, but instead from combinations of well-known interactions such as salt bridges, disulfide bonds, hydrogen bonds, or hydrophobic packing. For this reason, the authors' approach was to use their computer algorithm to optimize multiple interactions simultaneously. They succeeded in generating an amino acid sequence that conferred a significant increase in thermal stability. In the model they used, a combination of 1×10^6 amino acid sequences was possible. Because each amino acid can have a number of conformations (called rotamers), the total size of the search space ended up being 2.3×10^{31} rotamer sequences!

Pairwise interaction energies between amino acid side chains and between side chains and backbone residues were calculated and fed into an optimization algorithm. The computer selected the rotamers that yield the minimum energy solution for the system. For this experiment, the authors focused only on the optimization of a set of boundary residues (residues that lie at the surface of the buried core and solvent-exposed areas) in $G\beta_1$ that are located on a fold of the protein away from the IgG binding site so as not to disrupt IgG binding. These residues also had to be located on a contiguous stretch of the protein to facilitate the construction of the mutants for biophysical studies.

The sequence of the protein that was fed to the computer was not exactly the wild-type sequence, but rather a mutant sequence that already contained optimization of some core residues. In the computer simulation, almost all amino acids were considered at each boundary position except Pro, Met, Gly, and Cys to avoid disruption of the secondary structure and the addition of disulfide bonds. The computer generated an amino acid sequence with four boundary mutations: $Thr^{16} \rightarrow Ile^{16}$, $Thr^{18} \rightarrow Ile^{18}$, $Thr^{25} \rightarrow Glu^{25}$, and $Val^{29} \rightarrow Ile^{29}$.

Four different mutants with different combinations of the above mutations

were constructed, expressed, and purified for biophysical studies. Circular dichroism spectra (which report on the secondary structure of a protein) were identical among the four mutants and the wild-type protein, suggesting identical secondary folds. However, when thermal denaturation of the proteins was monitored by circular dichroism, the mutants failed to completely unfold or did not even reach unfolding transitions (melting points), even at 99°C. Denaturation of the mutants with guanidium chloride indicated that these proteins have increased thermodynamic stability, with the most stable mutant showing a change in ΔG of 4.3 kcal/mol.

The increase in stability of these mutants could result from the oligomerization of the proteins, but this was ruled out by analytical ultracentrifugation experiments. Binding to human IgG was monitored by surface plasmon resonance and showed intact affinity of the mutants. Finally, two-dimensional proton nuclear magnetic resonance studies were performed on one of the mutants and demonstrated that there was no significant change in the overall tertiary structure of the mutant compared with the wild-type protein.

There were several reasons for the increase in thermal stability of these mutants. An increase in the burial of hydrophobic surface areas that results from isoleucine mutations was the major contributor. This seemed to be achieved without significantly increasing the residual exposed area. Other factors that appeared to play a role are more favorable helix-dipole interactions and improvement of secondary structure propensity.

This study is an elegant blend of computer modeling and biophysical experimentation. The authors will now have to demonstrate that their approach can be applied to a number of other unrelated proteins. If they can pull this off, manufacturers of proteins will surely rejoice at the opportunity of having an objective method to increase protein stability without affecting function.

References

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