

Prologue

This is an exciting time for protein engineering and design: there have already been enough successes on many different fronts to convince even the skeptical; however, it is also clear that even greater developments lie ahead. It seems timely, then, to produce a book that surveys underlying fundamentals, describes a wide selection of present successes, and points to likely future developments on a variety of fronts.

As is clear from the present volume, the field of protein (or nucleic-acid) engineering and design is enormously diverse. What unites it, more than anything else, is an attitude or frame of mind – molecular designers all believe that our tools and our knowledge are mature enough to productively tinker with macromolecules, as well as just studying the ones provided by nature. Two different goals motivate this activist approach, mixed in different proportions for each researcher. One goal is to use protein engineering and design as a more effective way of learning about the molecules, particularly such difficult questions as protein folding and biological function. The second goal is to actually produce molecules with new and useful functions designed into them. Each goal can be served either by the modification of natural proteins or by inventing completely new ones out of “whole cloth”.

Already we can design some useful new functions by choosing the arena carefully, as is demonstrated by several authors in this volume. For example, catalytic antibodies (see Tarasow and Hilvert chapter) take advantage of a biological selection process to optimize the detailed design of a catalytic site, while subcellular localization can be changed in useful ways (see von Heijne chapter) by modifying or swapping signal regions whose properties are relatively simple functions of local sequence. If in parallel we also strike out boldly into the wilder sorts of design, we can hope to learn enough that some day fairly soon we might have engineering-level control of the entire process of protein production, folding, and functionality, at least for a useful set of specific cases. We are most definitely not there yet, nor are we within a few straightforward steps, but the benefits of such thorough understanding and control would be enormous, and we know many fruitful ways of proceeding toward that goal.

In approaching these long-range goals, the active meddling process of protein design and engineering is especially valuable because it can force us to stumble

over our incorrect assumptions. In other words, making good mistakes may be the most valuable part of the process.

For example, in the early days of protein engineering it was assumed that adding disulfide crosslinks between C_{α} atoms at the right distance would necessarily stabilize a protein. From several such attempts that actually decreased stability, we learned first that the details of geometry must be just right, and more recently that effective stabilization is easiest to achieve in the most mobile regions.

Another paradigm shift is taking place at present, driven by results from the sort of *de novo* design described in the Sander chapter and also done in our own laboratory. Ten years ago we all thought it would be extremely difficult (although worth trying) to come up with completely *de novo* protein designs that would fold up at all, but that once we got close it should be simple to make incremental improvements. However, it has turned out that it is actually fairly easy to achieve approximately-correct secondary and tertiary structure, that even after much effort no one has yet designed a unique, well-ordered structure that behaves like a natural protein. In other words, we were wrong about where the hard part was, and major research effort will now be concentrating on issues like internal packing, the determinants of uniqueness, and the attributes of the transition from a molten-globule-like state to a native state.

In summary, the field of protein engineering and design offers substantial rewards in both the intellectual and the practical spheres. I hope you enjoy reading about it.

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