

specificities among human CPT isoenzymes. This is obviously very valuable information for structure-based inhibitor design studies.

The three-dimensional structure reveals that membrane binding is mediated by a 30 amino acid segment, consisting of two short  $\alpha$  helices whose axes are predicted to be approximately parallel to and embedded in the membrane lipid bilayer. Comparison with the structure of the homologous carnitine acetyltransferase (Jogl and Tong, 2003) shows that this membrane binding segment corresponds to an insertion specifically present in CPT-2. It would be interesting to see whether the enzyme can be made water-soluble through the replacement of its membrane region with the corresponding segment of a soluble protein homolog. This approach has been successfully employed with mandelate dehydrogenase (Sukumar et al., 2001).

At present, there are five monotopic proteins of known three-dimensional structure ([http://blanco.biomol.uci.edu/Membrane\\_Proteins\\_xtal.html](http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html)): prostaglandin H2 synthase (Picot et al., 1994), cyclooxygenase-2 (Kurumbail et al., 1996), squalene hopene synthase (Wendt et al., 1999), monoamine oxidases (Binda et al., 2002), and fatty acid amide hydrolase (Bracey et al., 2002). These proteins are all dimeric with the molecular 2-fold axis perpendicular to the plane of the membrane; in this regard, the membrane bound monomer of ratCPT-2 appears to be unique. Another feature of interest is that in all these proteins the entrance to the substrate binding channel is located close to the membrane binding region as to allow the hydrophobic substrates to diffuse into the active site directly from the membrane. In ratCPT-2, it is likely that there is a similar mechanism because the CoA arm of the Y-shaped active site can be accessed through an opening that is close to the membrane binding insert. A fascinating hypothesis is that CPT-2 might form a complex with the acyl-carnitine transporter (Palmieri, 2004), which would allow direct channeling of the acyl-carnitine molecule from the transporter to the transferase enzyme.

The growing set of monotopic membrane proteins of known three-dimensional structure will assist the improvement of the algorithms that predict membrane binding regions, particularly for those cases, such as ratCPT-2, in which membrane binding is not mediated by a full trans-membrane segment.

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## Shedding UV Light on the Phase Problem

In this issue of *Structure*, Nanao and Ravelli (2006) describe the use of UV-induced radiation damage (UV-RIP) to solve the phase problem for proteins, employing single-wavelength X-ray radiation, without the need for derivatization. This should also permit data collection for many proteins on home sources, without travel to a synchrotron.

The Holy Grail for biological crystallographers remains a “mathematical solution” to the macromolecular phase problem, analogous to *Direct Methods* for small molecules, which resulted in Hauptman and Karle receiving the 1985 Nobel Prize in chemistry.

When Kendrew and Perutz commenced their pioneering work on solving the three-dimensional structures of myoglobin and hemoglobin, it was not at all clear how they would determine the phases. However, over the years, a series of elegant chemical and physical methods have yielded alternative ways of obtaining phases of macromolecules. The first of these was the ingenious approach of Bragg, Perutz, and their colleagues, resulting in the development of the multiple isomorphous replacement (MIR) method (Bragg and Perutz, 1954; Green et al., 1954), which has remained, until this day, the keystone for obtaining experimentally determined phases. The search for a suitable derivative, by the traditional method of soaking in heavy metals, is one of the most time-consuming steps in protein crystallography; often, after numerous attempts, the crystallographer just gives up in despair. The “quick cryo-soaking” procedure for derivatization with halides has

significantly speeded up the process (Dauter et al., 2000); unfortunately, however, it often affects the diffraction quality of fragile crystals.

The use of anomalous dispersion methods has proved invaluable, both in parallel, and as an adjunct to MIR, for experimentally obtaining phases (Blow and Rossmann, 1961). The pioneering development of multiwavelength anomalous dispersion (MAD) (Guss et al., 1988; Hendrickson et al., 1989) offered the possibility of obtaining phase information from a single crystal, as long as an anomalous scatterer was present. This approach has been extended to single anomalous dispersion (SAD) (Dauter et al., 2002). However, both SAD and MAD require synchrotron radiation, so as to permit tuning of the X-ray wavelength in order to optimize the weak anomalous signal generated by the anomalous scatterer. Moreover, the most common derivatization is replacement of Met by SeMet in the protein of interest. This substitution often requires significant molecular biology and biochemical manipulation; moreover, SeMet-containing proteins do not always crystallize as easily as the native ones, or even under the same conditions.

As the authors state (Nanao and Ravelli, 2006), “A general method that could overcome the need of heavy-atom derivatization would hold great promise for the growing number of structural biologists who are using macromolecular crystallography; such a method would save on labor, synchrotron time, toxic heavy atom solutions, and costs.”

In fact, such a method was indeed developed by Ravelli and colleagues. Rather than regarding radiation-damage as a problem to be avoided, they actually showed that specific radiation-damage can be used to phase macromolecular structures. The method they developed, radiation-damage induced phasing (RIP) (Ravelli et al., 2003), was a direct outcome of studies showing that exposure of crystals to third-generation synchrotron sources, even when cryo-cooled, causes not only general but also very specific damage to the structure of the irradiated protein (Burmeister, 2000; Ravelli and McSweeney, 2000; Weik et al., 2000). Such damage may include cleavage of specific disulphide bonds, loss of electron density for carboxyl groups, and harm to “strained” active sites (Weik et al., 2000). The RIP method represents a novel way to obtain phases from a single crystal, both in the absence of anomalous scatterers (Ravelli et al., 2003) and in combination with anomalous scattering (RIPAS) (Zwart et al., 2004). It has already been employed effectively by several other research groups (see references in Nanao and Ravelli [2006]).

Although RIP and RIPAS are very useful techniques, they require synchrotron radiation both to produce specific chemical damage and to take advantage of the anomalous scatter present in the protein. Nanao and Ravelli now describe a truly revolutionary and general method to overcome these limitations, making it possible, for the first time, to determine phases from a single crystal for many proteins, without the need for soaking in a heavy atom. The method is a variation on RIP; instead, however, of using synchrotron radiation, which results in both nonspecific and specific damage, an intense UV source is employed to produce the damage. As in RIP, the procedure involves three steps:

1. Initial X-ray data collection.
2. The “burn” stage, which involves exposure to an intense UV laser.
3. Postburn X-ray data collection.

Nanao and Ravelli describe application of this procedure to crystals of seven different proteins. The maps that they obtain are of extraordinary quality, showing peaks contoured in difference Fourier maps, [Final – Initial], at  $8\sigma$ . The damage caused by the UV burn, usually to sulfur atoms, appears to be much more specific than that achieved by synchrotron X-irradiation. What makes this technique so attractive is that it permits structure determination from native protein crystals, and it should be of utility for data collection on home sources, as well as at synchrotrons. Although the exact mechanism of the UV damage is not yet understood in detail, it is clear that the “proof of the eating is in the pudding,” and that the method works spectacularly.

Thus the UV-RIP method is a revolutionary new tool, which should permit structural biologists to solve many of their macromolecular structures without the need either for derivatization or for travel to a synchrotron. It can clearly be expected to have an enormous impact, inasmuch as it will make macromolecular crystallographic methods available to a much broader spectrum of scientists, and greatly simplify the solution of 3D structures in many cases.

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