How to Solve Protein Structures with an X-ray Laser

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For over a decade, biologists have asked whether x-ray lasers can be used to determine the structures of biomolecules such as proteins. Such methods have the potential to allow structure determination from micro- or even nanoscale crystals, but radiation damage can be extensive and data interpretation is fraught with difficulty. On page 227 of this issue, Redecke et al. (1) overcome these problems to determine the room-temperature structure of a protein of importance to drug discovery.

The authors studied Trypanosoma brucei cathepsin B (TbCatB), a protein that is important in structure-based drug discovery aimed at eradicating sleeping sickness. They first grew protein microcrystals with a typical volume of just ~4 µm³ [see the figure (A)]; the flow was asynchronous, reducing the efficiency of an x-ray flash hitting a crystal. Nearly 300,000 microcrystals with a combined crystal volume of 100 by 100 by 100 µm³ were used (a volume that is quite typical for single protein crystals used in structure determination). The total x-ray exposure time, with allowance for misses, was only 126 nanoseconds. The authors used a known structure of a similar protein to solve the new structure (a method called molecular replacement).

Synchrotrons have long been used to interrogate micrometer-scale protein crystals. Early tests showed that protein crystals with diameters of 20 µm would yield sufficiently strong diffraction data to allow structure determination (2), which led to the establishment of microbeam instruments at many synchrotrons (3). Use of such synchrotron x-ray beams with diameters of about 10 µm has also become very popular in chemical crystallography (4). The current European Synchrotron Radiation Facility upgrade program (www.esrf.fr) is making x-ray beams with even smaller, submicrometer, diameters more readily accessible.

X-ray laser sources deliver the same intensity within tens of femtoseconds that a typical synchrotron beamline delivers in a millisecond. The short, intense pulse of the x-ray laser allows radiation damage mechanisms that occur on time scales longer than tens of femtoseconds to be avoided. Furthermore, the exceptional brightness of the laser reduces the required sample volume by several orders of magnitude. However, there have been many questions regarding data interpretation. For example, would useful intensity be measurable from the diffraction pattern over all the usual scattering angles? Would there, at much higher scattering angles, be details of femtosecond time–dependent changes in the x-ray scattering factor? What about the ejection of core shell electrons (5), which could potentially ruin anomalous scattering phasing? Would the sample withstand the x-ray beam both thermally and in terms of overall and specific radiation damage?

Redecke et al. now show that useful electron density details can be derived from an x-ray beam diffracted by tiny single crystals. Time-dependent changes to the x-ray scattering factor may still be hidden and may emerge when a sample is used that diffractions to higher scattering angles.

The authors used a focused x-ray beam with a diameter of 4 µm, reasonably well matched to the crystal lengths of up to 10 µm but quite a lot bigger than the cross section (~1 by 1 µm³). A smaller focus would be needed for crystals in the submicrometer size range. Also, the x-ray wavelength was 1.32 Å, but could, in principle, be shifted to a longer wavelength, which would give a gain in sample scattering efficiency (6). The combination of these useful gain factors means that protein structure determination from protein nanocrystals, which have a smaller x-ray scattering strength than do microcrystals, is within reach of protein x-ray crystallography.

To get a full view of the protein structure, single crystals are conventionally rotated in the x-ray beam. Redecke et al. instead measure many tiny crystals, each of which is in a different orientation. This serial method leads to a myriad of still diffraction patterns. Each x-ray flash destroys the tiny crystal that is hit owing to the intense x-ray power. In addition, each diffraction pattern can be of limited statistical quality—that is, it has a low number of counts per diffraction spot. Therefore, a
large number of crystals are needed, although it may be possible to use fewer than the current study did.

To determine the crystal structure, the authors used the conventional crystal structure of solubilized and recrystallized TbCatB (Protein DataBank code 3MOR), determined at 100 K (7). The new structure provided additional structural details at room temperature and provided higher resolution (2.1 Å, compared with 2.5 Å for 3MOR). This extra detail is potentially important at the physiological temperatures at which a drug must act (8). Detailed comparison of the TbCatB crystal structure (1) with the equivalent enzyme from humans (9) revealed structural differences that may be important for drug design, because any drug targeting the TbCatB protein should not affect the human enzyme.

Another distinctive feature of the study is the use of femtosecond x-ray pulses. To date, studies of time-resolved evolution of small changes in a protein crystal structure extend into the picoseconds regime (10). The use of x-ray lasers should now allow such studies to be taken into the femtosecond regime.

There will be very wide interest in this result from the structural biology and structural chemistry research communities as well as pharmaceutical companies, which are frequent users of synchrotron sources. As demonstrated with this successful application reported by Redecke et al., x-ray lasers are set to enable protein structure determination of proteins that are difficult to crystallize. Getting next into the nanometer crystal-size range looks doable and exciting. The fact that the structure reported by the authors may aid the discovery of drugs against sleeping sickness is an important added bonus.

References

BIOMEDICINE

Improving Metabolism by Throwing Out All the JNK

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Obesity activates a complex immune response that includes the production of proinflammatory molecules and the recruitment of immune cells to key metabolic organs including adipose tissue (1, 2), liver (3), pancreas (4), and hypothalamus (5). This response has been implicated in derangements of local tissue metabolism and the subsequent development of obesity-associated disorders—especially type 2 diabetes, nonalcoholic fatty liver disease, and dyslipidemia. Defining regulators of obesity-induced activation of the proinflammatory response remains a challenge that offers the potential to identify therapeutic strategies to treat several metabolic diseases. On page 218 of this issue, Han et al. (6) have established that signaling by the enzymes c-Jun NH2-terminal kinases (JNK1 and JNK2) in myeloid cells is required for obesity-induced immune cell recruitment, inflammation in adipose tissue, and the development of insulin resistance and impaired glucose homeostasis.

Almost a decade ago, Hirosumi et al. found that obesity increases JNK activity in adipose tissue and liver of mice (7) and hypothesized that JNK1 and JNK2 are part of a cell signaling cascade that senses metabolic stress and activates obesity-induced inflammatory responses. Although deletion of JNK2 in mice had no discernible metabolic effect, mice genetically deficient in JNK1 were protected from weight gain in both dietary and genetic mouse models of obesity (7). However, it was neither clear whether deficiency of JNK1 had beneficial metabolic effects independent of its effects on weight, nor in which cells’ populations JNK activity contributed to adverse metabolic signaling.

Studies to determine whether JNK1 deficiency reduced obesity-induced inflamma-

JNK activity in adipose macrophages promotes inflammation and insulin resistance in mouse models of obesity.