The potential benefits of using higher X-ray energies for macromolecular crystallography

Joshua L. Dickerson[a], Elspeth F. Garman[a]

[a]Department of Biochemistry, University of Oxford, South Parks Road, Oxford. OX1 3QU, UK

E-mail: jld62@cam.ac.uk

Hypothesis

Most macromolecular crystallography (MX) experiments are now performed at synchrotron sources with preferred X-ray wavelengths of around 1 Å (~12.4 keV). Increasing the X-ray energy may increase the amount of signal obtained per unit of dose.

Introduction

Structural biologists like to know the structure of proteins so they can better understand how they function and can then design drugs to bind to them and modulate their activity.
The most common way of determining the protein structure is to fire X-rays at them. However, the X-rays would destroy a single protein before we get enough signal so we grow our proteins into ordered arrays in crystals to amplify the signal and get a structure before the X-rays have destroyed the proteins.
However, there is a growing desire in the field to use smaller and smaller crystals so allow for serial crystallography, where we soak protein ligands (such as drugs) into the crystal and watch a movie of the protein changing structure in response, rather than just capturing the final state of the protein. Can higher X-ray energies be better for this?

1: Dose

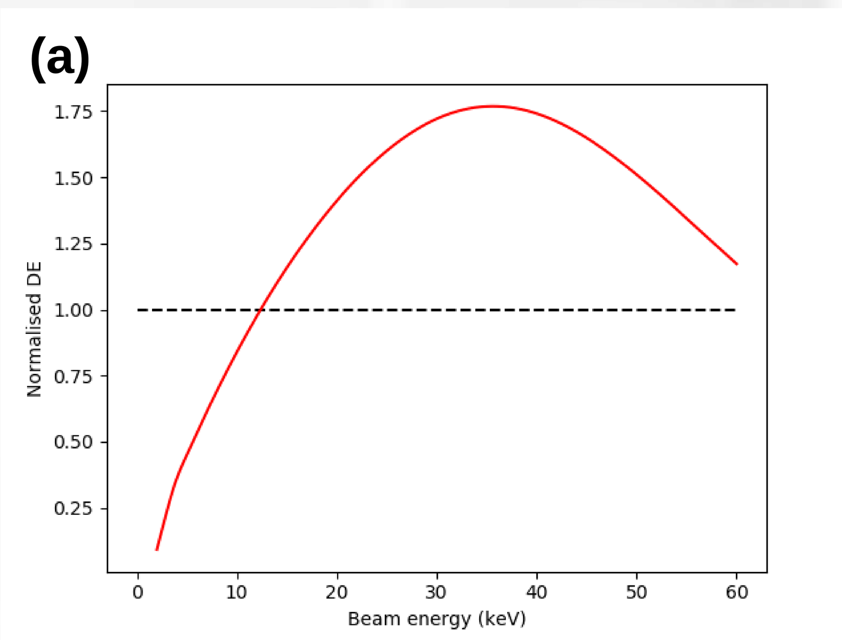
$$\text{Radiation damage progression} \propto \text{absorbed dose}$$

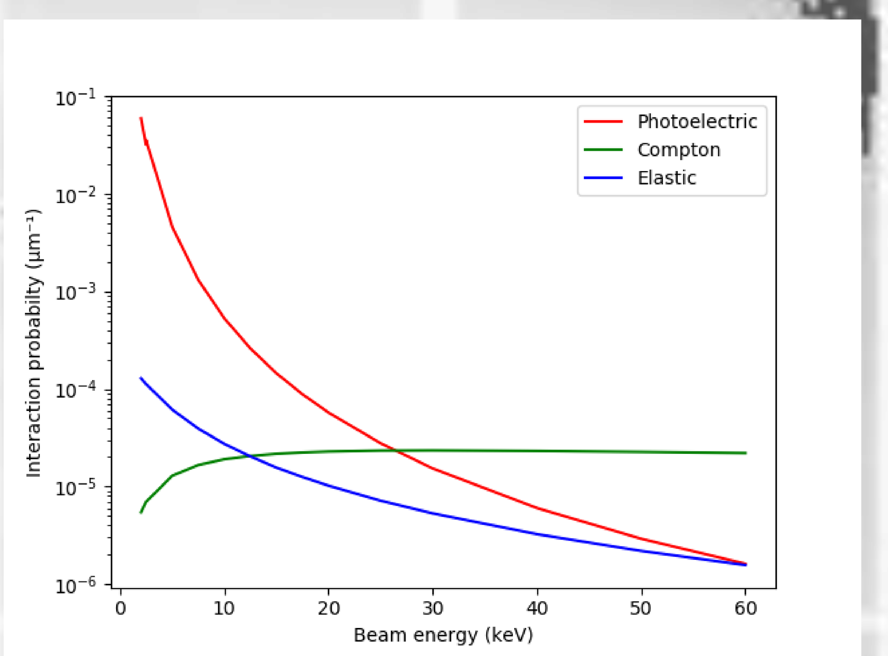
$$\text{dose} = \frac{\text{energy}}{\text{mass}} = \frac{\text{J}}{\text{kg}} = \text{Gy}$$

2: Diffraction efficiency

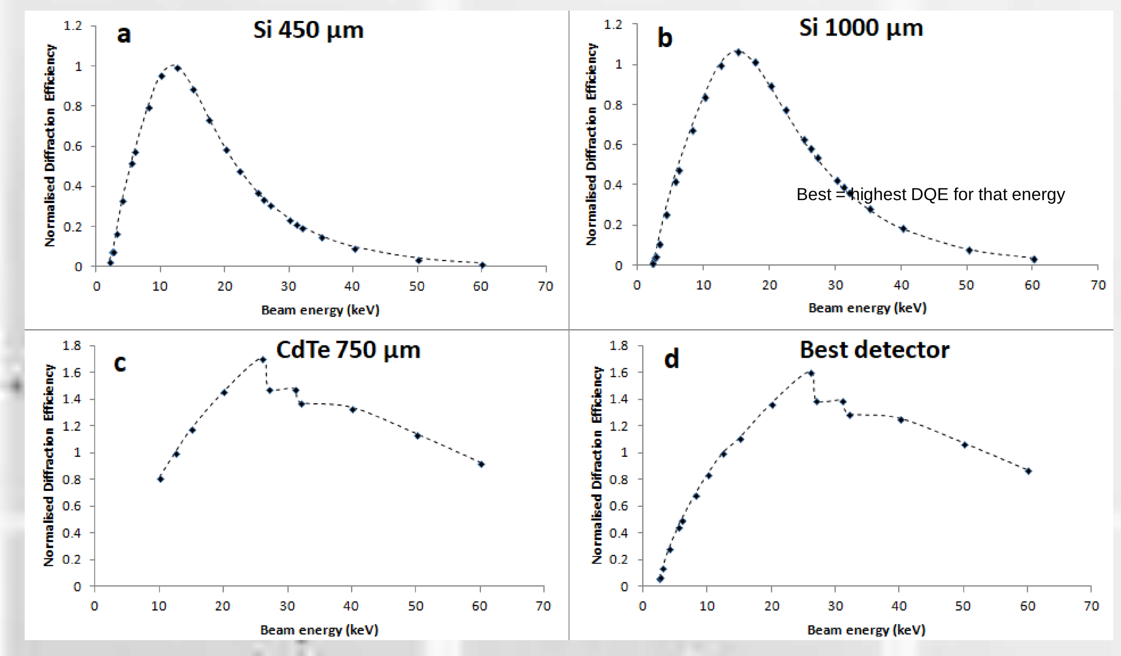
$$Diffraction\ efficiency\ (DE) = \frac{Number\ of\ elastically\ scattered\ photons}{dose}$$

- DE calculations in RADDOSE-3D [1], a program which estimates the absorbed dose during MX data collection at synchrotrons, indicate that there is an approximately 1.7-fold increase in DE at 35 keV incident X-ray energy compared to at 12.4 keV (a).
- The shape shown in (a) arises because the photoelectric absorption cross section falls at a faster rate than does the elastic scattering cross section as incident energy increases from 2 to 35 keV (b). However, the Compton scattering cross section increases with beam energy, accounting for the decrease in DE above ~35 keV.





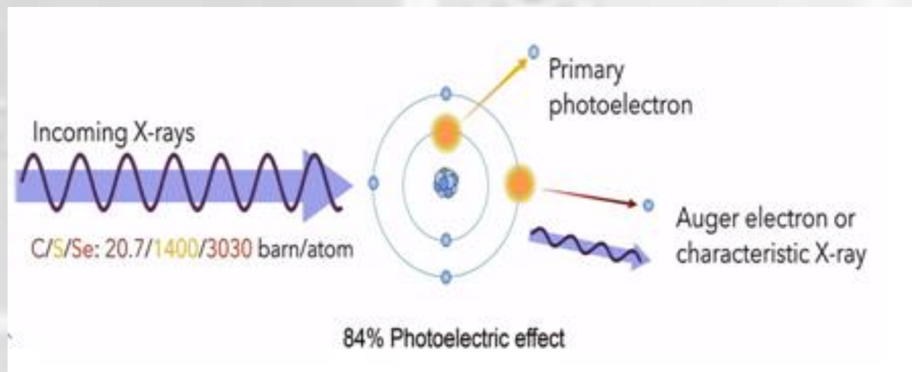
3: Detector efficiency



- A major issue with the use of higher X-ray energies for MX is the reduction in detector quantum efficiency at higher X-ray energies.
- A detector optimised for high energy X-rays, such as a CdTe based detector [2], must be used to realise the improvement in DE.

4: Microcrystals

- Data collection from microcrystals may benefit further from higher X-ray energies.
- When a photon is absorbed by an atom, a photoelectron is emitted with kinetic energy equal to the X-ray energy minus the binding energy of that shell.
- Photoelectrons are mobile at cryogenic temperatures and deposit energy in the sample by inelastic collisions before thermalising.
- These photoelectrons will have a finite probability of exiting the irradiated volume of the sample, reducing the absorbed dose.
- As X-ray energy is increased, the average energy given to photoelectrons will be higher so they will travel further and have a greater probability of escaping the irradiated volume [3, 4].



The Aim:

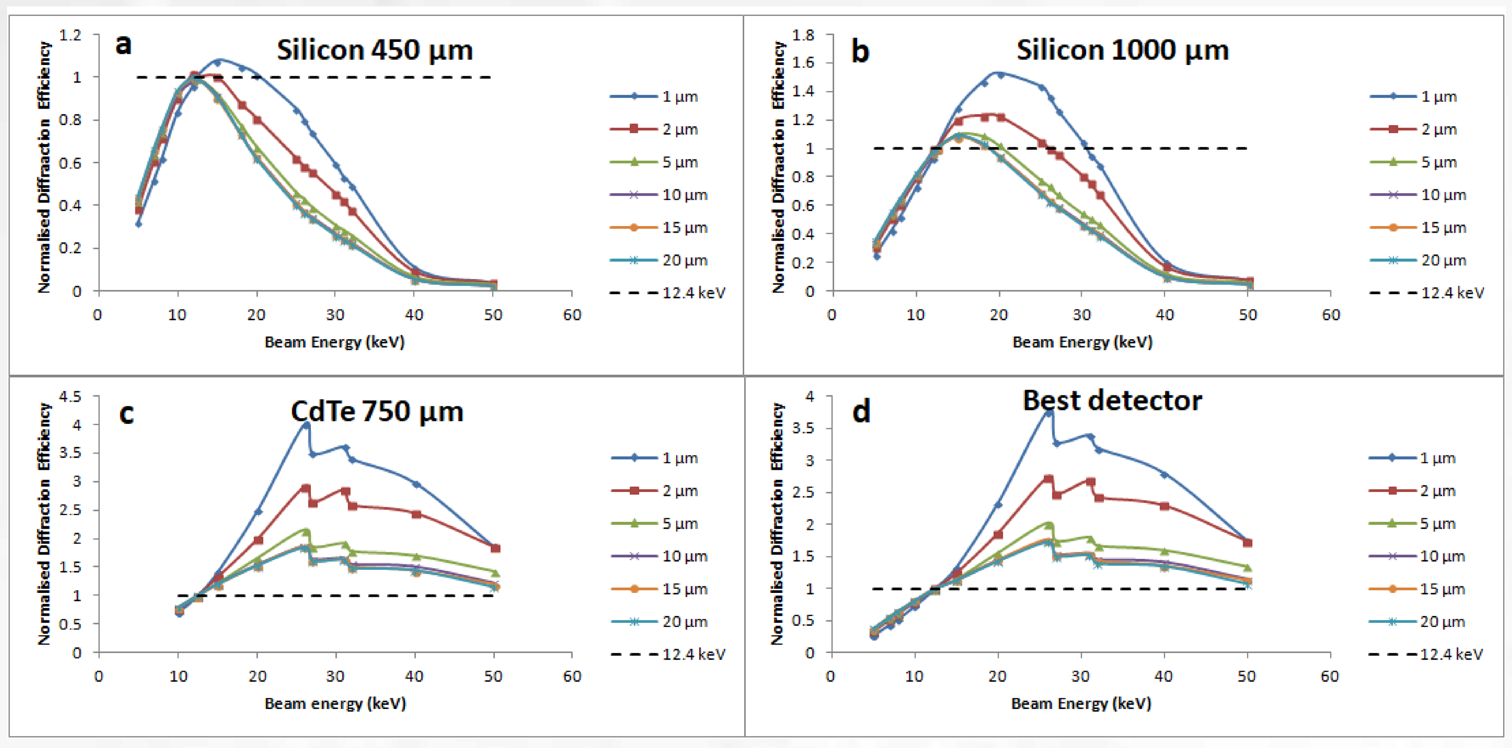
To write Monte Carlo simulations to investigate the effects of increasing incident X-ray energy on diffraction efficiency and experimentally verify the results.

Methods

- A program to run Monte Carlo simulations was written and used to calculate the diffraction efficiency of synchrotron experiments at different X-ray energies.
- For the experiment –
  - Lysozyme microcrystals were used with a microfocus beam to match the size of the crystal. A 1M EIGER2 X CdTe detector (kindly on loan from DECTRIS) was used on the microfocus FMX beamline at NSLSII.
  - On this beamline the beam energy could be varied between 12.4 and 26 keV.
  - A polymer chip specially designed for serial crystallography was trialled to aid these technically challenging experiments

Results

- For silicon based detectors, the simulations predict there is little advantage in increasing incident X-ray energy, irrespective of the crystal size.
- When using the ideal sensor material for a given energy (d, bottom right), simulations predict there is a greater than two-fold (ranging from two- to four-fold) improvement in DE on increasing X-ray energy from 12.4 keV to 26 keV for all crystals of size 5 μm and below [5].
- The results of the experiment at NSLSII are currently being analysed. We successfully collected good data from ~20 crystals at two beam energies. Early data analysis is looking promising!



Conclusions:

The results reveal a `sweet spot' at an incident X-ray energy of 26 keV when a CdTe based detector is used, and show a greater than factor of two improvement in diffraction efficiency at this energy when using microbeams and microcrystals of 5 μm or less.

References

1. Zeldin, O. B., Gerstel, M. & Garman, E. F. (2013). J. Appl. Cryst. 46, 1225–1230.
2. Zambon, P., Radicci, V., Trueb, P., Disch, C., Rissi, M., Sakhelashvili, T., Schneebeli, M. & Broennimann, C. (2018). Nucl. Instrum. Methods Phys. Res. A, 892, 106–113.
3. Cowan, J. A. & Nave, C. (2008). *J. Synchrotron Rad.* **15**, 458–462.
4. Marman, H., Darmanin, C. & Abbey, B. (2018). *Crystals*, **8**, 267.
5. Dickerson, J. L. & Garman, E. F. (2019). *J. Synchrotron Rad.* **26**, 922–930.

Acknowledgements

We thank Andreas Förster (DECTRIS) for providing detector quantum efficiency data on their detectors.
I also thank the Laidlaw scholarship for their funding, allowing me to carry out the work.