Supplementary Information

Reduction of radiation damage and other benefits of short-wavelength radiation for biocrystallography.

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Note S1. Derivation of equations (1), (2), (2') and (3).

The kinematical approximation for the integrated intensity of the Bragg reflection *hkl* is:

$$I_{hkl} = \overline{\Phi} r_e^2 \frac{V}{v^2} \frac{\lambda^3}{\omega} LPA \left| F_{hkl} \right|^2 \tag{S1}$$

with I_{hkl}: total number of photons in Bragg reflection *hkl*; $\overline{\Phi}$: mean flux of incident beam (number of photons.m^{-2}.s^{-1}); r_e: Thomson scattering length ($2.818 \cdot 10^{-15}$ m); *V*: illuminated volume of the crystal (m^3); *v*: volume of the crystal unit-cell (m^3); λ: X-ray wavelength (m); ω: angular velocity of crystal rotation (radian s^{-1}); *L*: Lorentz factor (dimensionless); *P*: polarization factor (dimensionless); *A*: attenuation coefficient for diffracted beam (dimensionless); F_{hkl}: structure factor of reflection *hkl* for the crystal unit-cell.

Derived from: i) Darwin, C.G. (1914). The theory of X-ray reflection. *Phil Mag* **27**, 315-333. ii) Woolfson, M.M (1970). *An Introduction to X-ray Crystallography*. Cambridge University Press. iii) Holton; J.M. & Frankel, K.A. (2010). The minimum crystal size needed for a complete diffraction data set. *Acta Cryst* D**66**, 393-408.

At first glance, one should think that the integrated intensity of a given reflection varies as λ^3. This is not true, because *L* varies almost as $1/\lambda$. More precisely:

$$L = \left| \frac{1}{\hat{\mathbf{u}}_\varphi \cdot (\hat{\mathbf{u}}_0 \times \hat{\mathbf{u}})} \right| = \left| \frac{1}{\sin 2\theta \cos \alpha} \right| = \frac{d_{hkl}}{\lambda \cos \theta |\cos \alpha|} \tag{S2}$$

where d_{hkl} is the interplanar spacing for reflexion hkl; $\hat{\mathbf{u}}_\varphi \cdot (\hat{\mathbf{u}}_0 \times \hat{\mathbf{u}})$ is the volume defined by unit vectors $\hat{\mathbf{u}}_0$ along the incident beam, $\hat{\mathbf{u}}$ along the scattered beam, and $\hat{\mathbf{u}}_\varphi$ along the rotation axis. α is the angle between the normal to the scattering plane and the rotation axis. Although the polarization factor and $1/\cos\theta$ term in the Lorentz factor depend further upon the diffraction geometry, they are much less sensitive to variations of λ.

The number of photons reaching during the time interval dt a small volume dV of mass ρdV located in the incident beam at a distance p from its entry point into the crystal is $\Phi(S) e^{-\mu_{ph} p} dS dt$, where μ_{ph} is the linear photon absorption coefficient. Energy is deposited to the sample only by the photoelectric and Compton absorption and thus one must use the linear energy-absorption coefficient μ_{en} for the deposited energy per photon: $dp\mu_{en}E$. The total deposited energy in the volume dV becomes $\Phi(S) e^{-\mu_{ph} p} \mu_{en} E dV dt$, which corresponds to a dose rate $\frac{dD}{dt} = \Phi(S) e^{-\mu_{ph} p} \frac{\mu_{en}}{\rho} E$.

Averaging over the radiated volume, the mean dose rate is:

$$\frac{dD}{dt} = \overline{\Phi} \mu_{en} E \frac{A'}{\rho} \tag{S3}$$

where A' is the attenuation coefficient for the transmitted beam.

Integrating this dose rate over a time Δt and taking into account equation (S2) and that $\omega \Delta t = \Delta \varphi$, the number of diffracted photons obtained for a given dose is

$$\frac{I_{hkl}}{D} = r_e^2 \frac{V}{v^2} \frac{d_{hkl} \rho}{\Delta \varphi \cos \theta |\cos \alpha|} P \frac{A}{A'} |F_{hkl}|^2 \frac{\lambda^2}{E \mu_{en}}. \tag{S4}$$

In practical cases, the evaluation of the attenuation coefficients for diffracted and transmitted beams, A and A', respectively, are rather complicated with:

$$\overline{\Phi} V A = \iint \Phi(S) \left[\int e^{-\mu_{ph}(p+q)} dp \right] dS \tag{S5}$$

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$$\overline{\Phi}VA' = \iint \Phi(S)\left[\int e^{-\mu_{\mathrm{ph}}p}\mathrm{d}p\right]\mathrm{d}S \tag{S6}$$

with $\Phi(S)$: flux of the incident beam entering into the crystal at position S of its surface; q: distance along the scattered beam from scattering point to exit point from the crystal. Generally, and especially in the case of low absorption, $A/A' \approx 1$.

It should be noted that whereas the integrated intensity of the Bragg reflection is almost proportional to the volume of the crystal, the dose rate is almost independent of the volume, which means that the number of photons diffracted for a given dose at a given energy is almost proportional to the volume of the crystal.

For high energy photons, the dose is uniformly distributed and diffracted rays are close to forward direction,

$$\frac{I_{hkl}}{D} \propto \frac{V}{E^3 \mu_{\mathrm{en}}}. \tag{S7}$$

The accuracy of the structural parameter definition depends on the total diffraction information and therefore, the intensities of all the reflections observable at a given resolution should be summed to estimate the realistic intensity-dose ratio. When the Lorentz factor in the equation (S1) is replaced by the δ-function describing the diffraction conditions, $L = (2\pi/\lambda)^3 \iint \delta\big(2\pi(\hat{\mathbf{u}}-\hat{\mathbf{u}}_0)/\lambda - \mathbf{H}_{hkl}\big)\mathrm{d}\hat{\mathbf{u}}\mathrm{d}\varphi$, and the sum over all hkl is replaced by an integration over the reciprocal space, $\Sigma_{hkl} \rightarrow 1/v^*\int\mathrm{d}\mathbf{H}_{hkl}$, the total elastic scattering can be then approximated as

$$I = \sum_{hkl} I_{hkl} \approx \overline{\Phi}r_e^2 \frac{V}{v} \iint PA|F_{hkl}|^2 \mathrm{d}\hat{\mathbf{u}}\mathrm{d}t. \tag{S8}$$

Furthermore, the structure factor can be written as

$$|F_{hkl}|^2 = \sum_i |f_i|^2 + \sum_{i \neq j} f_i f_j^* e^{i\frac{2\pi}{\lambda}(\hat{\mathbf{u}}-\hat{\mathbf{u}}_0)\cdot(\mathbf{R}_i - \mathbf{R}_j)}, \tag{S9}$$

where the second term is oscillating around zero. Now we obtain

$$I \approx \overline{\Phi}r_e^2 \frac{V}{v} \iint PA \sum_i |f_i|^2 \mathrm{d}\Omega\mathrm{d}t. \tag{S10}$$

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where $d\Omega$ is the solid angle element.

As can be expected, the total elastic scattering is proportional to the sum of the elastic scattering by individual atoms in the sample. The I/D ratio can now be written as

$$\frac{I}{D} \approx r_e^2 \frac{\rho V}{v} \frac{\int PA \sum_i |f_i|^2 d\Omega}{A'E\mu_{en}}. \tag{S11}$$

Note S2. Relation between absorbed dose and radiation damage at the various photon energies.

The relation between dose and damage at the various photon energies depends on the pattern of energy deposit in the tracks of high-energy electrons and their relationships with chemical yields. Following Magee and Chatterjee [31], consider an electron with initial energy E which is completely absorbed in the system ; the radical yield per 100 eV of absorbed energy of some particular product x is designated by $G_x(E)$. The total number of molecules of this product formed as the electron is stopped is $N(E) = 10EG_x(E)$, where E is measured in keV and $G_x(E)$ is an integral yield averaged over the track. Calculation and experiments show that in the range 5-100 keV the variations of $G_x(E)$ with E for various products are small and either increase or decrease as a function of E. As an example, variations of $G_x(E)$ for primary decomposition products of water (OH, $-H_2O$, H, H_2O_2 and H_2) are given in Figure S2. For OH, H_2O and H, there is a slight minimum around 0.1-2 keV, whilst for H_2O_2 and H_2, there is a slight maximum at the same energy range. On this basis, considering all possible radical products, one can consider that the damage at the various energies is proportional to the absorbed dose with only small deviations, as explicitly or implicitly granted in most articles on radiation damage. Then, our simulations on I/D can be extended to variations of I/damage.

Supplementary Figure legends

Figure S1. Variation of mass photon-absorption coefficient and mass energy-absorption coefficient versus photon energy for a protein. Coefficients for liquid water are very similar. (http://physics.nist.gov/PhysRefData/XrayMassCoef/).

Figure S2. $G(E)$ **curves for the primary decomposition products of water as a function of photon energy.**
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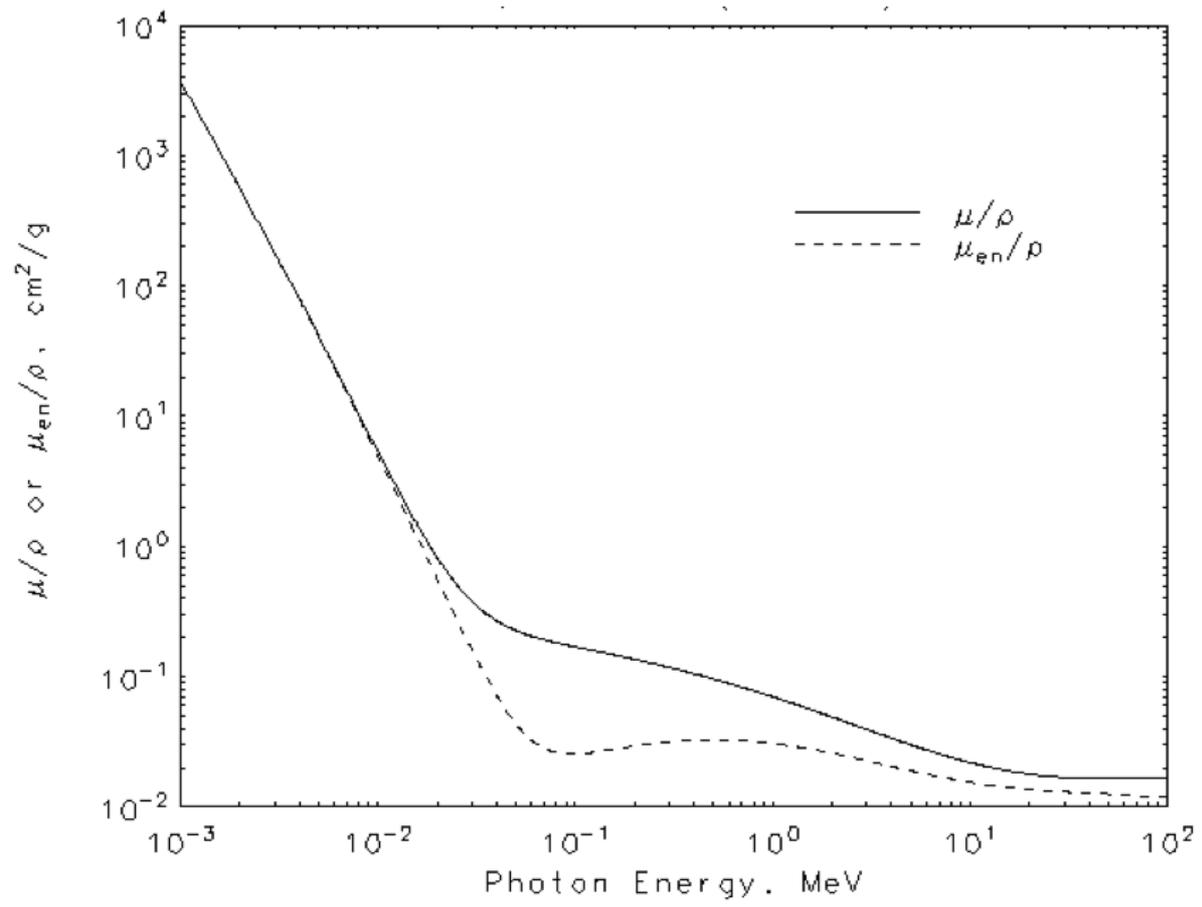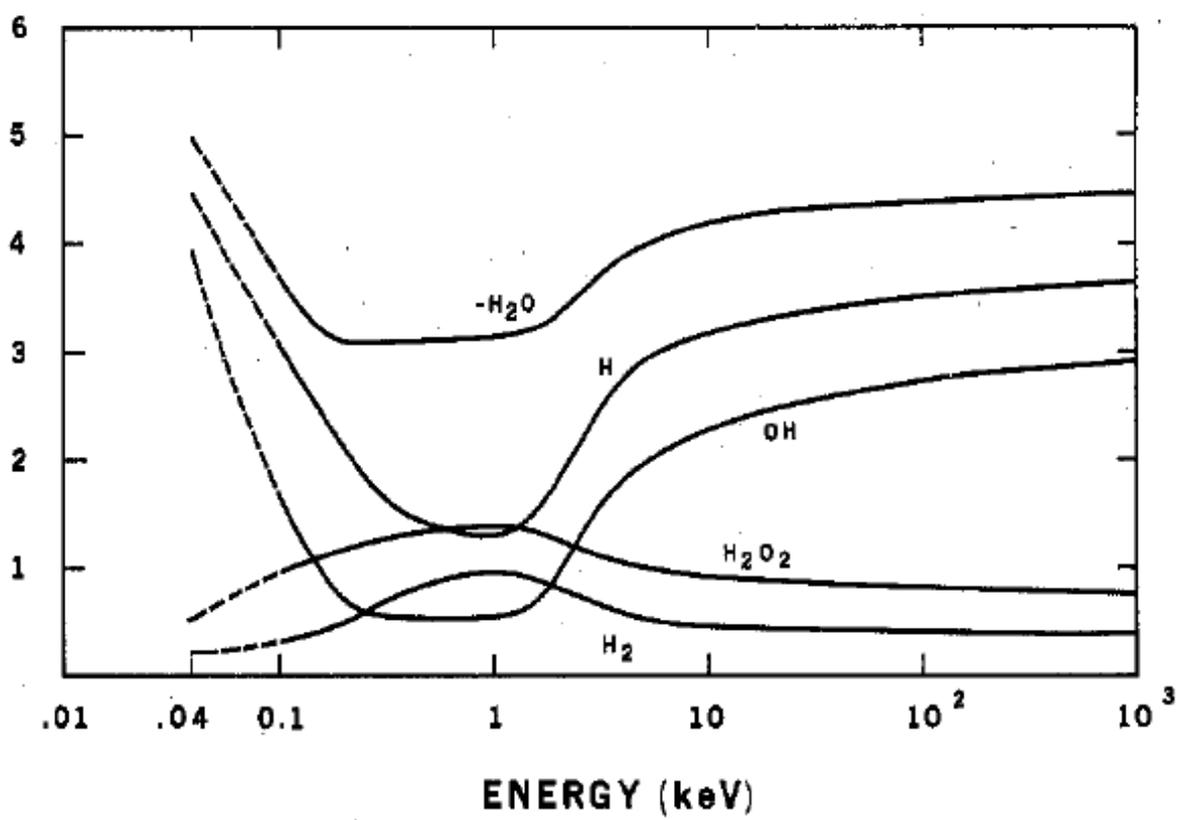
Figure S1

Figure S2.