General Principles for Solution X-Ray Diffraction.

Coordinate-based simulations of solution x-ray scattering (or diffraction) patterns from model structures were performed using computational approach described previously(1-5). Briefly, molecular scattering is calculated as the Fourier transform of the position of N atoms of the molecular assembly:

$$I(\mathbf{q}) = \sum_{j}^{N}\sum_{k}^{N} A_j A_k e^{i\mathbf{q}\cdot\mathbf{r}_{j,k}} \quad , \qquad \text{S1a}$$

where A_j is the atomic scattering amplitude for the jth atom, and $r_{j,k}$ is the distance between the jth and kth atoms. In solution the molecular scattering is orientationally averaged, yielding the following form

$$I(q) = \left\langle I(\mathbf{q}) \right\rangle_{\Omega} = \sum_{j}^{N}\sum_{k}^{N} A_j A_k \frac{\sin qr_{j,k}}{qr_{j,k}}. \qquad \text{S1b}$$

The atomic scattering amplitudes are of the form:

$$A_j = f_j(q) - \rho_0 g_j(q) \quad , \qquad \text{S1c}$$

where $f_j(q)$ is the atomic x-ray scattering form factor of atom j, ρ_0 is solvent electron density, and g_j is the form factor for the dummy atom, or group of atoms, with volume V_j:

$$g_j(q) = G(q)V_j e^{-q^2 V_j^{2/3}/4\pi} \quad , \qquad \text{S1d}$$

and $G(q)$ is a volume expansion factor written as

$$G(q) = \frac{V_o}{V_m} e^{-q^2 (V_o^{2/3} - V_m^{2/3})/4\pi} \quad , \qquad \text{S1e}$$

where V_o is the expanded atomic volume, and V_m is the average atomic volume for the group. The expansion factor account for solvent displacement by the solute and is adjusted by changing the ratio of the dummy atom radius to average atomic van der Waals radius, $R_{om} = r_o/r_m$. The ratio R_{om} has a significant effect on the scattering intensity at low angles but a progressively minor effect at high angles, as shown in Fig. 5. In all calculations of SXD patterns, R_{om} was set to 0.96, which was chosen to bring the calculated and experimental low angle scattering amplitudes into better agreement. Decrease in R_{om} has the effect of raising the scattering contrast. It is likely that the R_{om} adjustment is partially accounting for the lack of counter ions in the calculated scattering patterns. A counter ion "cloud" will similarly contribute significantly to small angle

scattering, but because of the absence of short-range order will show progressively diminished effects at high angle. It should be noted that these adjustments do not alter the SXD peak pattern, but alter the scattering background.

Experimental Data Treatment

Solution-state x-ray diffraction patterns were obtained subtracting solvent background scattering patterns from DNA solution scatterings patterns (Fig. 6*A*). A scaling factor was applied to the solvent background to account for the volume fraction occupied by the DNA molecules in the solution. In principle the scaling factor can be determined from the DNA solution concentration and measured x-ray transmissions for the solution and solvent samples. In practice, it was found necessary to make slight adjustments (±0.1%) to the scaling factor to prevent oversubtraction, as indicated by anomalously steep or negative scattering intensities at the high q limits, or undersubtraction, as indicated by the presence of a discernable solvent peak in the difference scattering patterns. DNA model-base scattering calculations were used to provide a target for diffuse scattering in the region $q > 2.2$ Å^{-1}. The normalization constant for background subtraction was finely adjusted to achieve a slope in the high q region that approximated slopes in model calculations. The broadness of the solvent diffraction peak compared with those of DNA (Fig. 6*B*) were found to make measurement of DNA solution diffraction peak positions and linewidths relatively insensitive to uncertainties in the accuracy of solvent background subtraction.

Measured scattering patterns were adjusted to account for the angle-dependent variation in the scattering solid angle subtended by each pixel in the linear detector. Calculations were made for corrections that account for cylindrical sample cell x-ray absorption, Compton scattering, fluorescence, x-ray beam polarization, but each of these was found to be negligible in the experimental q-range, in part due to the high energy of the x-ray (20 keV) beam and the absence of high Z elements in the aqueous DNA samples.

The DNA solution scattering patterns were used as “fingerprints” of DNA conformation and conformational dispersions in solution. Experiments and simulations were quantitatively compared by measurements of scattering pattern peak positions. Peak positions determined from zero crossing points in first derivative plots. Experimental peak positions were determined from

10 point smoothed derivative plots of the SXD data and peak uncertainties determined from experimental half-width noise about the smoothed curves.

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