

**Supplementary Table.**

Comparison of rt porcine and cryo human insulin structures.

Protein <sup>a</sup>	Change in <sup>b</sup> backbone (Å)	$\Delta R_g$ <sup>c</sup> (%)	Rotation <sup>d</sup> (deg)	Intramolecular <sup>e</sup> contacts		Intermolecular <sup>e</sup> contacts	
				rt	cryo	rt	cryo
Insulin		-1.33					
Chain A.1	0.54		1.48	none	none	42	59
Chain B.1	0.53		1.28	8	8	76	89
Chain A.2	0.60		0.54	none	6	41	49
Chain B.2	0.54		1.83	8	8	87	90
Total				16	22	246	287

<sup>a</sup> The molecular mass of insulin is 11.5 kDa, the A chains are 2.4 kDa and the B chains are 3.4 kDa.

<sup>b</sup> Calculated from root-mean-square deviation of main-chain atoms after superposition.

<sup>c</sup>  $\Delta R_g$  is the fractional change in the radius of gyration with cooling.

<sup>d</sup> Rotation is the rotation in degrees for superposition.

<sup>e</sup> All solvent and hydrogens were removed from the coordinate files (4INS and 1MSO) and then contacts were calculated with the EDPDB program (Zhang & Matthews, 1995). Intramolecular contacts are those between different residues in the same insulin subunit. Intermolecular contacts are those between insulin subunits (e.g. across subunit interfaces and crystal lattice). Two atoms are considered to be in contact when their center-to-center distance is less than the sum of their van der Waals radii plus 0.25 Å.

**Supplementary Figure** Selection of reflection profiles from the  $\mu\text{g}$  insulin crystal ( $\mu\text{g6}$ ) that had data sequentially collected at rt, and then cryo (See Table 6). (A) rt [11,8,9] reflection, 2.85 Å,  $\phi_R = 0.005^\circ$ ,  $\eta = 0.004^\circ$ ,  $\eta_{\text{Gauss1}} = 0.004^\circ$ ,  $\eta_{\text{Gauss2}} = 0.003^\circ$ ,  $\Delta\phi_{\text{Gauss}} = 0.005^\circ$ ; (B) rt [-22,7,7] reflection, 2.93 Å,  $\phi_R = 0.005^\circ$ ,  $\eta = 0.003^\circ$ ,  $\eta_{\text{Gauss1}} = 0.004^\circ$ ,  $\eta_{\text{Gauss2}} = 0.002^\circ$ ,  $\Delta\phi_{\text{Gauss}} = 0.003^\circ$ ; (C) cryo [-9,8,-11] reflection, 2.84 Å,  $\phi_R = 0.193^\circ$ ,  $\eta = 0.192^\circ$ ,  $\eta_{\text{Gauss1}} = 0.206^\circ$ ,  $\eta_{\text{Gauss2}} = 0.055^\circ$ ,  $\Delta\phi_{\text{Gauss}} = 0.041^\circ$ ; (D) cryo [24,-20,-4] reflection, 2.93 Å,  $\phi_R = 0.238^\circ$ ,  $\eta = 0.236^\circ$ ,  $\eta_{\text{Gauss1}} = 0.196^\circ$ ,  $\eta_{\text{Gauss2}} = 0.359^\circ$ ,  $\Delta\phi_{\text{Gauss}} = 0.054^\circ$ . The fit Gaussian curves are in solid lines with their deconvoluted widths in dashed lines (Gauss 1 is in blue and Gauss2 in green). The sum of the two Gaussians is shown in a thick black line. Pink X represent measured intensity values, red circles the averaged intensity values from the traveling averaging window. Reflections were selected from the vertical sectors where the instrument correction was minimal.

