Supplementary Figure 1

Protein microcrystals used for SFX measurements.

(a) lysozyme, (b) glucose isomerase, (c) thaumatin and (d) FABP3 crystals. Scale bars represent 20 μm.
Supplementary Figure 2

Room-temperature structure of glucose isomerase.

(a) A typical diffraction pattern from an individual microcrystal. Resolution at the edges corresponds to ~1.6 Å. (b) A close-up view of glucose isomerase structure with \((2Fo - Fc)\) electron-density map (contoured at 1.0σ). This figure was drawn with the program PyMol (http://www.pymol.org).
**Supplementary Table 1.** Summary of crystal size, crystal number density and storage solutions for four proteins.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lysozyme</th>
<th>Glucose isomerase</th>
<th>Thaumatin</th>
<th>FABP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal size (μm)</td>
<td>7–10</td>
<td>10–30</td>
<td>10–30</td>
<td>10–20</td>
</tr>
<tr>
<td>Crystal number density (Number of crystals/ml)</td>
<td>6.0×10⁷</td>
<td>2.0×10⁷</td>
<td>1.0×10⁷</td>
<td>0.9×10⁷†</td>
</tr>
<tr>
<td>Storage solution</td>
<td>10%(w/v) sodium chloride</td>
<td>30%(w/v) PEG4000</td>
<td>0.1 M ADA*</td>
<td>43%(v/v) PEG400</td>
</tr>
<tr>
<td></td>
<td>1.0 M sodium acetate pH 3.0</td>
<td>0.2 M lithium sulfate</td>
<td>0.75 M K/Na tartrate pH 7.0</td>
<td>73 mM Tris–HCl pH 8.0</td>
</tr>
<tr>
<td></td>
<td>0.1 M Tris–HCl pH 8.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†About 30% of this includes crystals formed in cluster.

*ADA: N-(2-acetamido) iminodiacetic acid
**Supplementary Table 2.** Crystallographic statistics. Values in parentheses are for the outermost shell.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Needle ID (μm)</th>
<th>Flow rate (μl min⁻¹)</th>
<th>Space group</th>
<th>Unit-cell parameter</th>
<th>Number of collected images</th>
<th>Number of indexed patterns</th>
<th>Indexing rate (%)§</th>
<th>Consumed protein per 1,000 indexed patterns (μg)</th>
<th>Number of total reflections</th>
<th>Number of unique reflections</th>
<th>Resolution range (Å)</th>
<th>Completeness (%)</th>
<th>R_split (%)†</th>
<th>CC₁/₂ (%)</th>
<th>&lt;I/σ(I)&gt;</th>
<th>R/R_free (%)</th>
<th>Refinement</th>
<th>Bond lengths (Å)</th>
<th>Bond angles (°)</th>
<th>PDB code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>110</td>
<td>0.48</td>
<td>P4₁₂₂</td>
<td>a (Å) 79</td>
<td>101,330</td>
<td>34,438</td>
<td>34.0</td>
<td>31</td>
<td>4,566,054</td>
<td>8,548</td>
<td>30–2.0 (2.04–2.00)</td>
<td>99.9 (100)</td>
<td>8.7 (8.3)</td>
<td>97.8 (98.7)</td>
<td>12.4 (10.8)</td>
<td>20.7/23.0</td>
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<td></td>
</tr>
<tr>
<td>Lysozyme</td>
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<td>0.24</td>
<td>P4₁₂₂</td>
<td>b (Å) 79</td>
<td>48,743</td>
<td>14,595</td>
<td>29.9</td>
<td>18</td>
<td>1,950,298</td>
<td>8,549</td>
<td>30–2.0 (2.04–2.00)</td>
<td>99.9 (100)</td>
<td>12.7 (15.3)</td>
<td>96.3 (96.5)</td>
<td>7.9 (5.9)</td>
<td>19.8/23.0</td>
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<tr>
<td>Lysozyme</td>
<td>110</td>
<td>0.12</td>
<td>P4₁₂₂</td>
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<td>29,323</td>
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<td>8</td>
<td>3,932,325</td>
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<td>7.7 (9.9)</td>
<td>98.7 (98.1)</td>
<td>13.3 (8.8)</td>
<td>18.5/21.1</td>
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<td>31</td>
<td>3,508,692</td>
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<td>99.9 (100)</td>
<td>9.5 (8.6)</td>
<td>97.1 (97.9)</td>
<td>11.8 (11.4)</td>
<td>21.8/22.6</td>
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</tbody>
</table>

§Percentage of images that were indexed.

†\( R_{\text{split}} = \frac{1}{\sqrt{2}} \frac{\sum_{hkl} |I_{\text{even}} - I_{\text{odd}}|}{\frac{1}{2} \sum_{hkl} |I_{\text{even}} + I_{\text{odd}}|} \)
<table>
<thead>
<tr>
<th>Protein</th>
<th>Glucose isomerase</th>
<th>Thaumatin</th>
<th>FABP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle ID (μm)</td>
<td>110</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Flow rate (μl min⁻¹)</td>
<td>0.48</td>
<td>0.46</td>
<td>0.48</td>
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</table>

**Data collection**

<table>
<thead>
<tr>
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<th>Glucose isomerase</th>
<th>Thaumatin</th>
<th>FABP3</th>
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</thead>
<tbody>
<tr>
<td>Space group</td>
<td>I222</td>
<td>P4;212</td>
<td>P2;212</td>
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<tr>
<td>Unit-cell parameter</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a (Å)</td>
<td>94</td>
<td>58</td>
<td>34</td>
</tr>
<tr>
<td>b (Å)</td>
<td>100</td>
<td>58</td>
<td>55</td>
</tr>
<tr>
<td>c (Å)</td>
<td>103</td>
<td>150</td>
<td>71</td>
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<tr>
<td>Number of collected images</td>
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<td>84,078</td>
<td>54,256</td>
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<td>Number of indexed patterns</td>
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<td>13,231</td>
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<tr>
<td>Indexing rate (%)</td>
<td>15.2</td>
<td>10.5</td>
<td>24.4</td>
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<tr>
<td>Consumed protein per 1,000 indexed patterns (μg)</td>
<td>73</td>
<td>32</td>
<td>11</td>
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<tr>
<td>Number of total reflections</td>
<td>2,817,002</td>
<td>2,415,636</td>
<td>2,147,575</td>
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<tr>
<td>Number of unique reflections</td>
<td>33,122</td>
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<td>18,204</td>
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<tr>
<td>Resolution range (Å)</td>
<td>30–2.0 (2.04–2.00)</td>
<td>30–2.0 (2.04–2.00)</td>
<td>30–1.60 (1.63–1.60)</td>
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<tr>
<td>Completeness (%)</td>
<td>99.9 (100)</td>
<td>99.9 (99.9)</td>
<td>100 (100)</td>
</tr>
<tr>
<td>R_splis (%)</td>
<td>28.9 (33.9)</td>
<td>16.4 (15.9)</td>
<td>20.0 (38.2)</td>
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<tr>
<td>CC₁/₂ (%)</td>
<td>85.2 (75.5)</td>
<td>91.8 (94.9)</td>
<td>91.5 (79.9)</td>
</tr>
<tr>
<td>&lt;I/σ(I)&gt;</td>
<td>3.60 (3.0)</td>
<td>6.4 (5.6)</td>
<td>4.9 (2.5)</td>
</tr>
</tbody>
</table>

**Refinement**

<table>
<thead>
<tr>
<th></th>
<th>Glucose isomerase</th>
<th>Thaumatin</th>
<th>FABP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/R_free</td>
<td>15.9/19.6</td>
<td>15.7/20.0</td>
<td>17.8/22.2</td>
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<td>Bond lengths (Å)</td>
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<td>0.017</td>
</tr>
<tr>
<td>Bond angles (°)</td>
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<tr>
<td>PDB code</td>
<td>4w4q</td>
<td>3wxs</td>
<td>3wxq</td>
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</tbody>
</table>

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