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**Mother Goose's Insights into Cryo-Cooling.** Jeffrey J. Lovelace<sup>1</sup>, Cameron R. Murphy<sup>1</sup>, Reinhard Pahl<sup>2</sup>, Keith Brister<sup>2</sup>, Gloria E. O. Borgstahl<sup>1</sup>, <sup>1</sup>Eppley Institute for Cancer Research and Allied Diseases, 987696 Nebraska Medical Center, Omaha, NE 68198, <sup>2</sup>BioCARS – Univ. of Chicago, Argonne, IL 60439.

In an attempt to better understand how cryo-cooling affects the mosaic domain structure of protein crystals a complex experiment was undertaken. The experiment involved the use of both super fine  $\phi$  slicing and digital topography to aid in the analysis of these effects. For the experiment, a single lysozyme crystal was immobilized in a capillary with epoxy to eliminate slippage. The orientation was adjusted until a group of reflections were positioned to minimize Lorentz effects. The reflection group also had an arrangement that allowed them to be collected on a digital topography system in one pass. For the first part of the experiment a super fine  $\phi$  slicing run was collected followed by a digital topography run at room temperature. Next the crystal was cryo-cooled in the capillary maintaining the same orientation. After cooling, a run of digital topography followed by a super fine  $\phi$  slicing run was carried out on the same reflections. After processing, the sequences were analyzed to determine how the cryo-cooling had affected the mosaic domains. Room temperature data showed that the crystal was initially composed of 4 to 5 major domains relative to the angle at which the maximum intensity occurred (on a per pixel basis). The profiles showed a FWHM of around  $0.100^\circ$ . After cooling, the profiles swelled to  $0.460^\circ$ . The crisp mosaic domains visible in the room temperature data were shattered during cooling, the borders became highly irregular and some regions failed to diffract at all. Although exaggerated, the angular relationships between the major domains appeared to be conserved. Therefore, the crystal after cryo-cooling was like Humpty Dumpty's great fall. All the kings' horses and all the kings' men couldn't put Humpty Dumpty back together again.