

DNA and RNA repair enzymes

One of the DNA and RNA repair enzymes in humans, ABH3 directly reverses methylated lesions into unmethylated bases. Its activity is important in keeping the integrity of the human genome; therefore, obtaining the crystal structure of this protein will give insights into bio-informatics studies for other DNA repair enzymes, and help to design future enzyme inhibitors.

Achieving protein stability and homogeneity is crucial for obtaining protein crystals. In this experiment, a DynaPro light scattering instrument was used to monitor the protein stability and homogeneity in different buffers.

Purified ABH3 was dialyzed into eight different buffers at various pH and at different salt and glycerol concentrations. The particle size and homogeneity of ABH3 in each buffer were measured by the DynaPro-M SXTC at 4°C and 25°C, respectively.

Based on the light scattering analysis, the buffer that best stabilizes ABH3 is 50 mM TAPS, pH 8.0, 300 mM NaCl, 10% glycerol. The light scattering of this sample shows that the protein has a molecular weight of approximately 36 kDa; the expected molecular weight of ABH3 is 35 kDa, and the polydispersity is 24% (figure 1).

ABH3 in this optimal buffer exists as monomer and the % polydispersity is under 25, which is the acceptable homogeneity for “good” protein preparations. The protein in other buffers is either non-monomeric, for example ABH3 in MOPS exists as dimer (the light scattering showed a molecular weight of 63 kDa, figure 2), or has a very high polydispersity.

Using the DynaPro-MSXTC light scattering experiments studying the stability and homogeneity of ABH3 in different buffers was undertaken. An optimal buffer for ABH3 was determined. In this buffer, ABH3 is in its monomeric state and has a relatively narrow polydispersity. By carrying out these experiments, our characterization enabled us to yield good crystals.

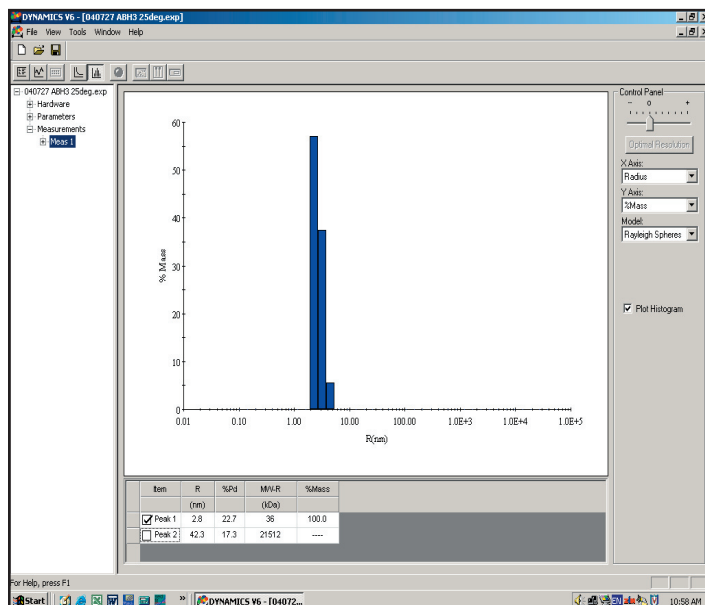


Figure 1. Histogram of ABH3 in optimized buffer (TAPS) showing a homogeneous sample of monomeric protein (36 kDa) with reasonable polydispersity (24%).

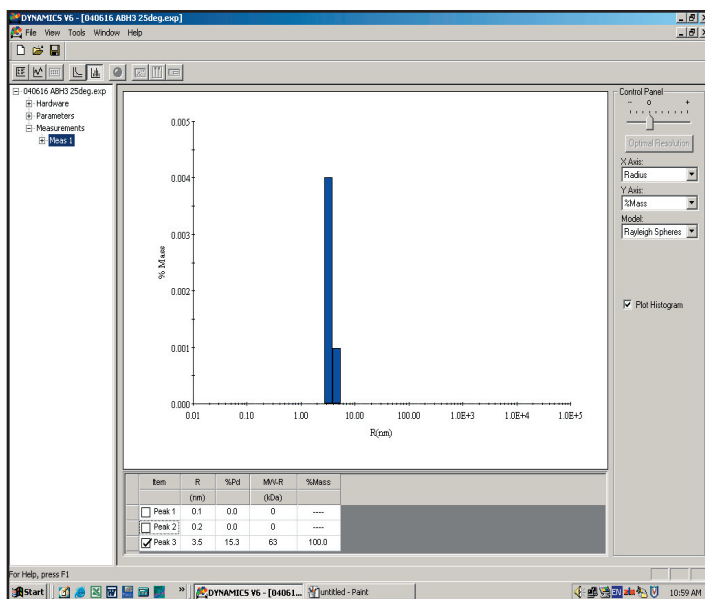


Figure 2. Histogram of ABH3 particles distribution in initial buffer (MOPS) showing the proteins dimerized (63 kDa), but still exhibiting reasonable polydispersity.

