

Subject: Spc110 SAXS samples

From: Keenan Taylor <kctaylor@wisc.edu>

Date: 7/18/2012 7:59 AM

To: Seung Joong Kim <sjkim@salilab.org>, Ivan Rayment <ivan_rayment@biochem.wisc.edu>, Trisha Davis <tdavis@uw.edu>

Hi SJ,

The samples should be at your lab this morning. Here is the tracking number of the package: 8992 5572 8273. There are three proteins – the first I prepared for you and have divide into three samples at different glycerol concentrations as well as provided the buffers. The other two samples were prepared by our staff scientist Dima. I attached gel filtration traces for the three proteins.

1.

Gp7-Spc110_778-944_CaM is the sample I prepared for you. This is the ‘full’ c-terminus of spc110. The Cam is wild type *K. lactis* that has had the central helix mutated to serine. This sample has been split between 0%, 2.5%, and 5% glycerol containing buffers (150mM NaCl, 25mM HEPES pH 7.6) and is at 5.6 mg/mL. I have provided 100 mL of each buffer for you. There is ~ 150 – 200 uL of protein for each glycerol concentration frozen in 30uL aliquots.

2.

Spc110_891-944_CaM is the construct that was crystallized. The Cam is wild type *K.lactis* protein that has Cys in the central helix. To prevent cross-linking, it is essential to keep some reducing agent in the buffer. It is found in the vial labeled "Scp110-CaM, 15 c". In contains ~ 0.8 ml of ~ 12.5 mg/ml protein frozen in 30 ul aliquots. The buffer is HEPES 10 mM, NaCl 50 mM, imidazole 20 mM, TCEP 0.5 mM, CaCl2 2 mM, pH 7.5.

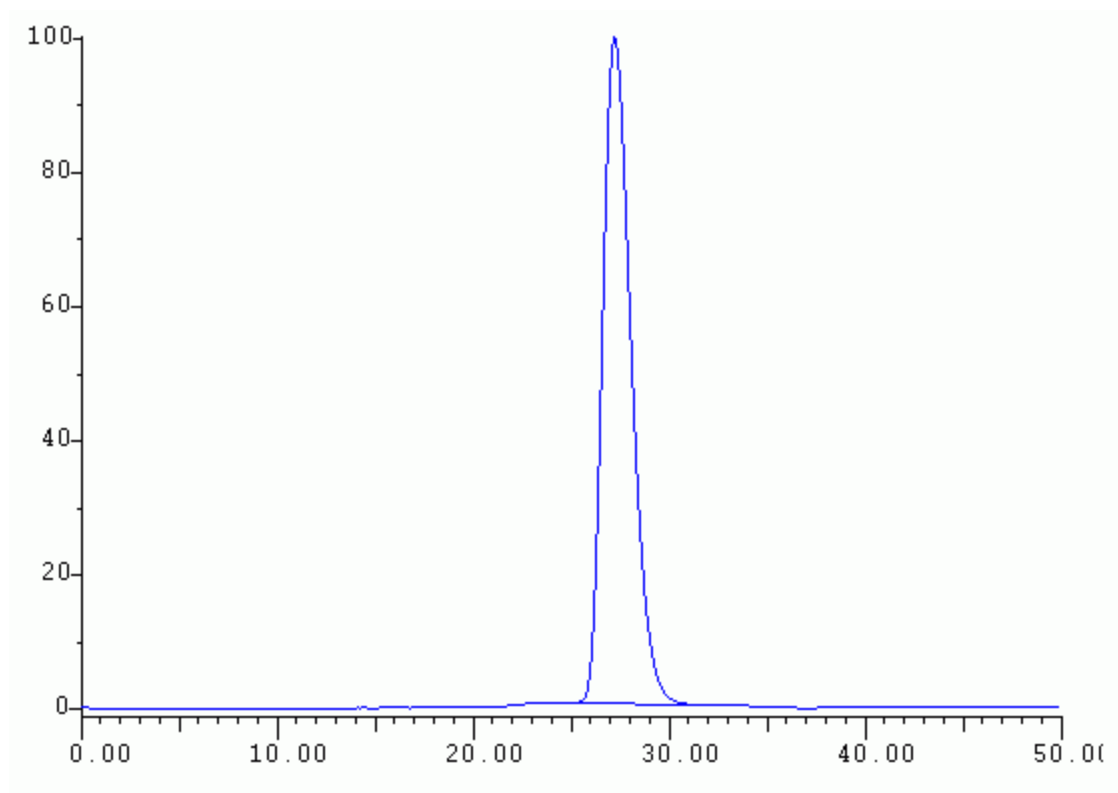
3.

Spc110_891-918_CaM is the C-terminal truncation with the entire CaM binding site intact. The CaM is C79S mutant, so the reducing agent can be safely omitted if needed. The vial labeled "Spc110-CaM delC2" has only a few frozen beads and their broken pieces left - I'd assume that the total is no more than 0.1 ml. Protein concentration is ~ 20 mg/ml in the same buffer as the wild type version of the complex.

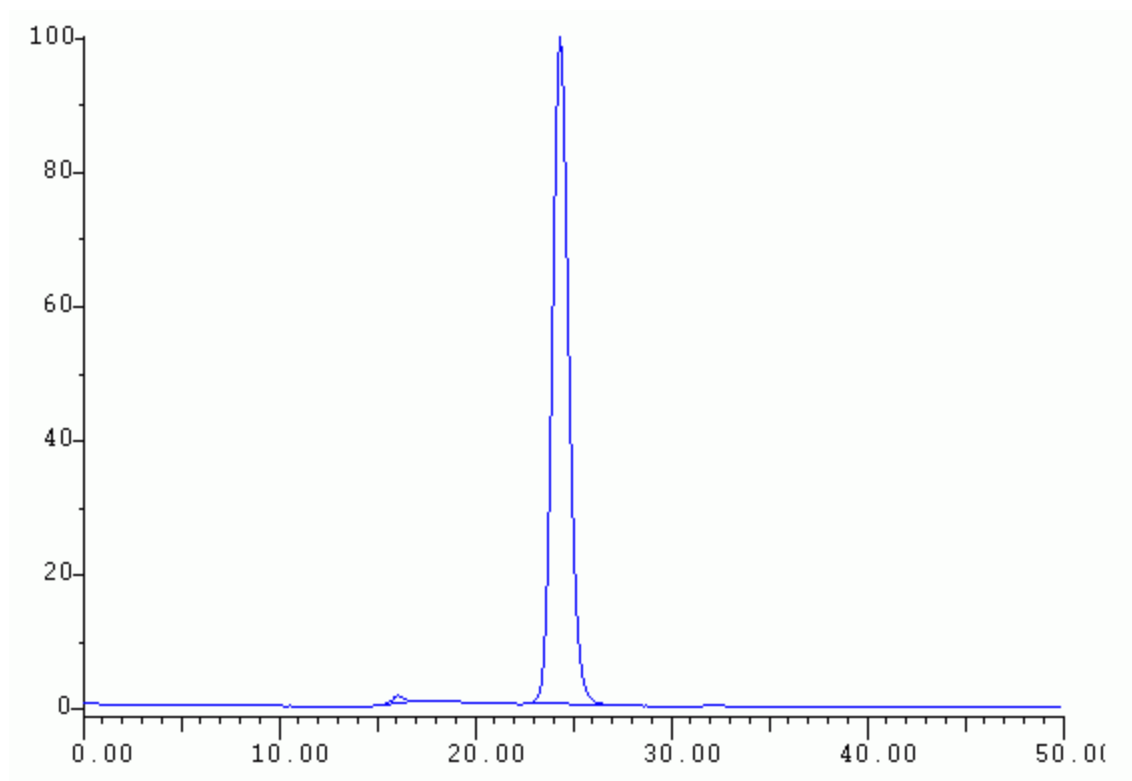
I hope your data collection goes well!

Thanks,
Keenan

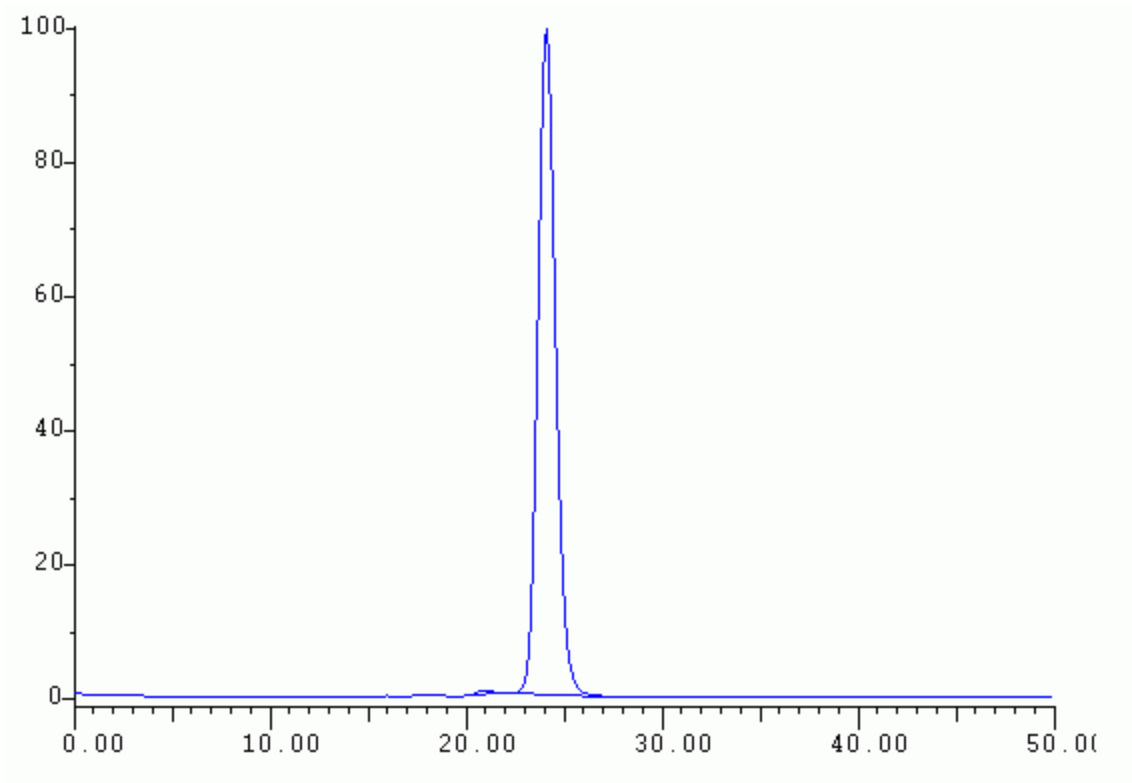
—Gp7_Spc110_778-944_CaM_Superdex200.gif—



—Spc110_891-918_CaM_Supredex75.gif



—Spc110_891-944_CaM_Superdex75.gif



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Gp7_Spc110_778-944_CaM_Superdex200.gif	3.5 KB
Spc110_891-918_CaM_Superdex75.gif	3.6 KB
Spc110_891-944_CaM_Superdex75.gif	3.5 KB