

Ligation with varying thioester concentrations (100-800 μg / 40 μL rxn.)

TEV cleavage:

378 μg GST-ENLYFQC-Pax(38-557)-FLAG in 600 μL

70 μL of 500 mM Tris-HCl, pH 8.0

16 μL mTEVp

- the above reagents were combined in a 1.5 mL eppendorf tube, flicked to mix, then incubated at 37°C for 4 1/2 hours. The protein solution was then concentrated to 50 μL .

Ligation to AchHHHHHDDLADLESTTSHISKRPVFLSEETPYSYPTG-cosBn:

- 4 separate aliquots of a thioester stock soln. were lyophilized in .65 mL eppendorf tubes. They contained 100, 200, 400, and 800 μg of thioester

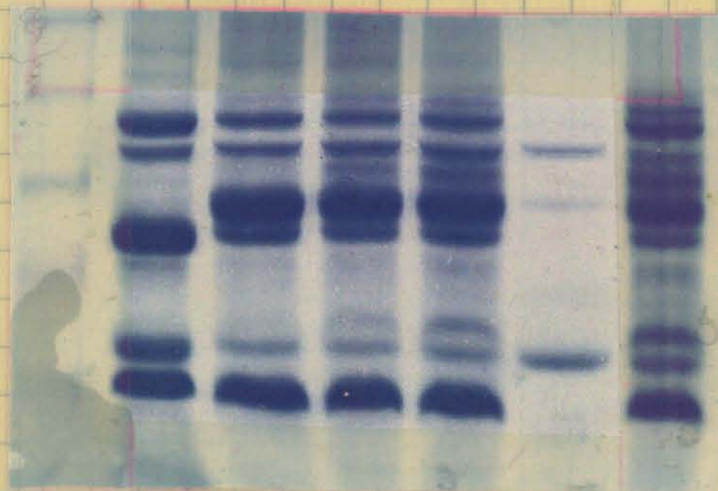
- 10 μL of the 50 μL protein solution (5 $\mu\text{g}/\mu\text{L}$) were removed for gel analysis (this was combined w/ 10 μL water and 20 μL 2x SDS reducing gel loading buffer.) The following 4 reactions were set up and then incubated for 17 hours at 27°C: (No precip. formed in Ba-Bc)

Ba : 10 μL protein (50 μg), 10 μL (100 mM Tris, pH 8.0, 600 mM NaCl) soln., pH 8
100 mM MESNA, 100 μg thioester (5 mg/mL , 1 mM)

Bb : 10 μL protein (50 μg), 10 μL Tris soln.
100 mM MESNA, 200 μg thioester (10 mg/mL , 2 mM)

Bc : 10 μL protein soln. (50 μg), 10 μL Tris soln.
100 mM MESNA, 400 μg thioester (20 mg/mL , 4 mM)

Bd : 10 μL protein soln., 10 μL Tris
100 mM MESNA, 800 μg thioester. The thioester in rxn. Bd never fully dissolved. Precipitation was visible also after 17 hours.



↑ uncleaved full length

← ligation product

← Cys-Pax(38-557)-FL

ladder

Cys-Pax-FL

Ba

Bb

Bc

Bd in
solution

Bd
pelleted

19 μL (from 40 total) loaded per lane