cis-autoproteolysis of ThnT

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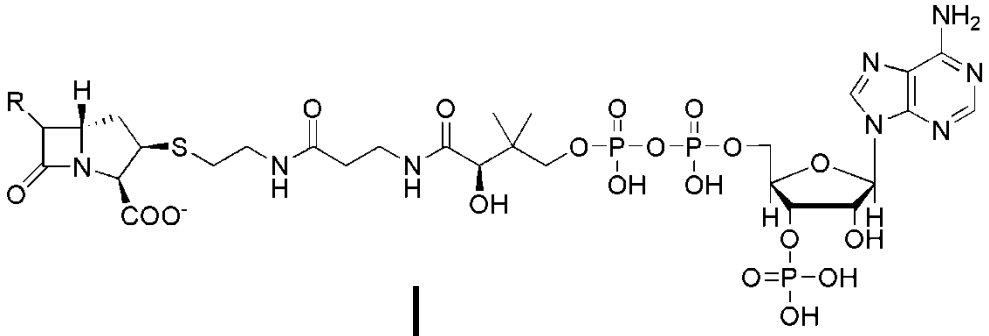
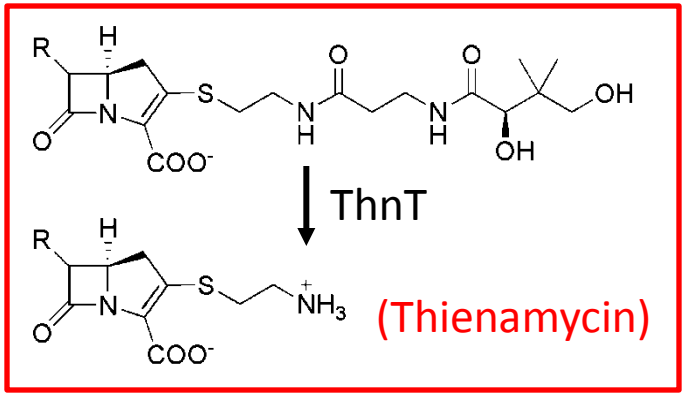
ThnT catalyzes the final step in the biosynthesis of Thienamycin

- Potent β-lactam antibiotic
  - Inhibitors of cell wall biosynthesis
- Developed and produced through total synthesis
- Biosynthetic pathway not yet fully elucidated
- Final step: hydrolysis of an amide bond

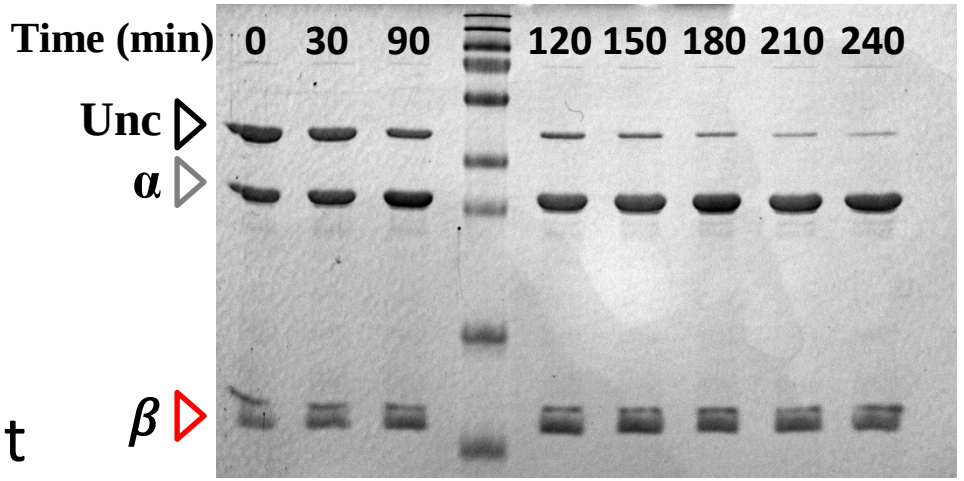


Thn R, K/L/P, H

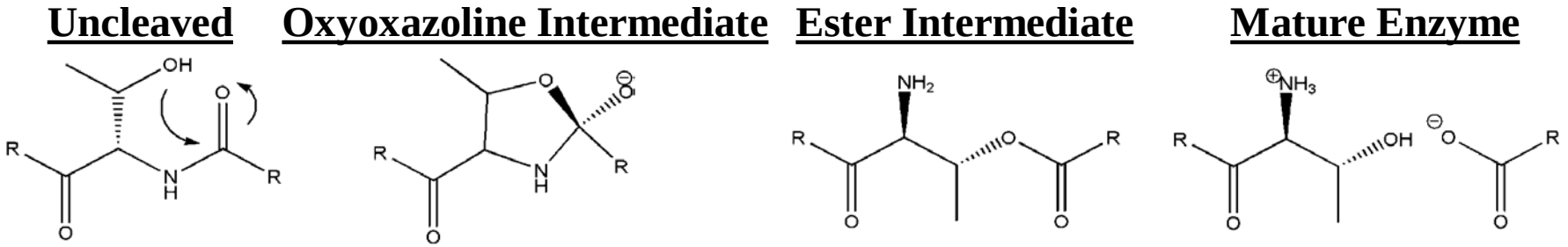


ThnT is an amidohydrolase activated through intramolecular proteolysis

- Maturation yields an N-terminal Nucleophile
  - "Threonine Proteases"
- *cis*-Autoproteolysis occurs from two different protein folds



Classical System: Ntn-Hydrolases

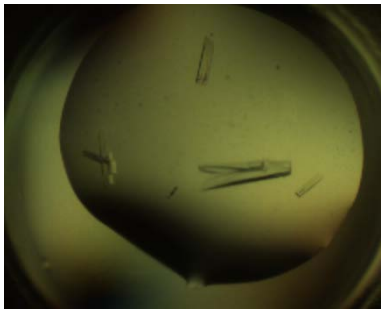


Our Questions

- What is the nature of the ‘single residue’ active site of ThnT?
- What factors promote self-cleavage?
  - Why is a Thr->Ser variant slower (~4.5 fold reduction in self-cleavage)?
- How does this bind Thienamycin?

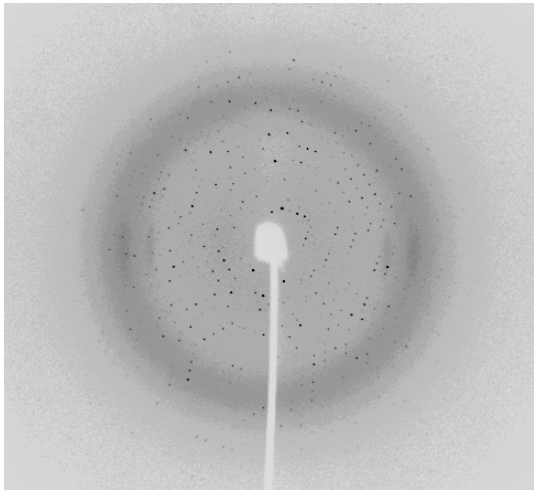
Our Strategy

- Crystallization of ThnT
  - T282C Inactive Precursor
  - wt-ThnT Mature enzyme



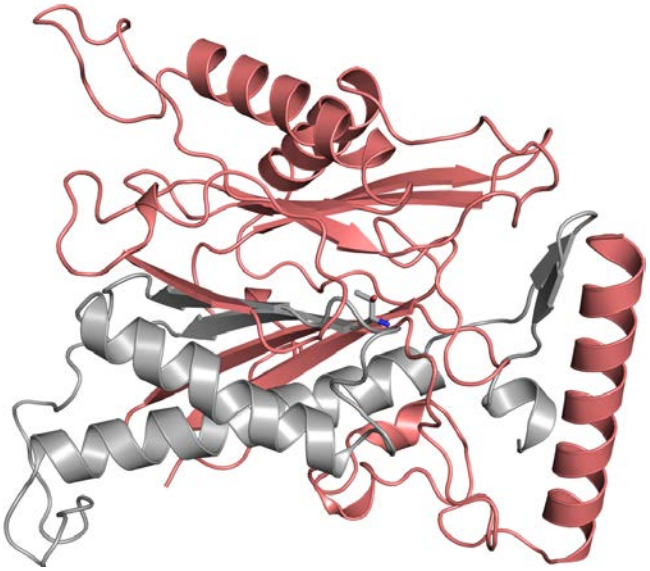
13% PEG3350, 0.5M NaAcetate

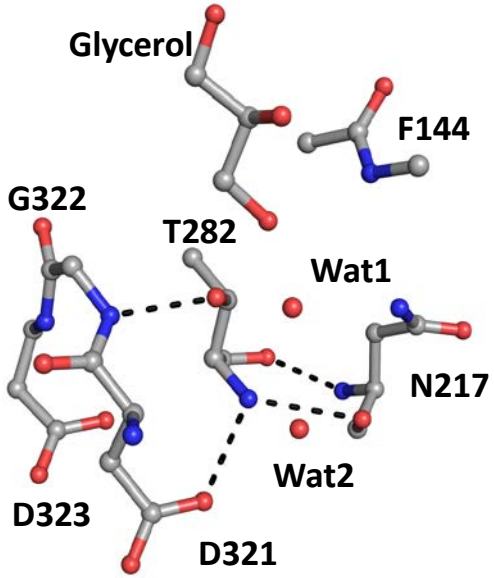
- Phasing…?
  - MR fails
  - No endogenous sulfur
  - EtHg derivitization of T282C and MAD phasing



Homesource diffraction to 2.2Å

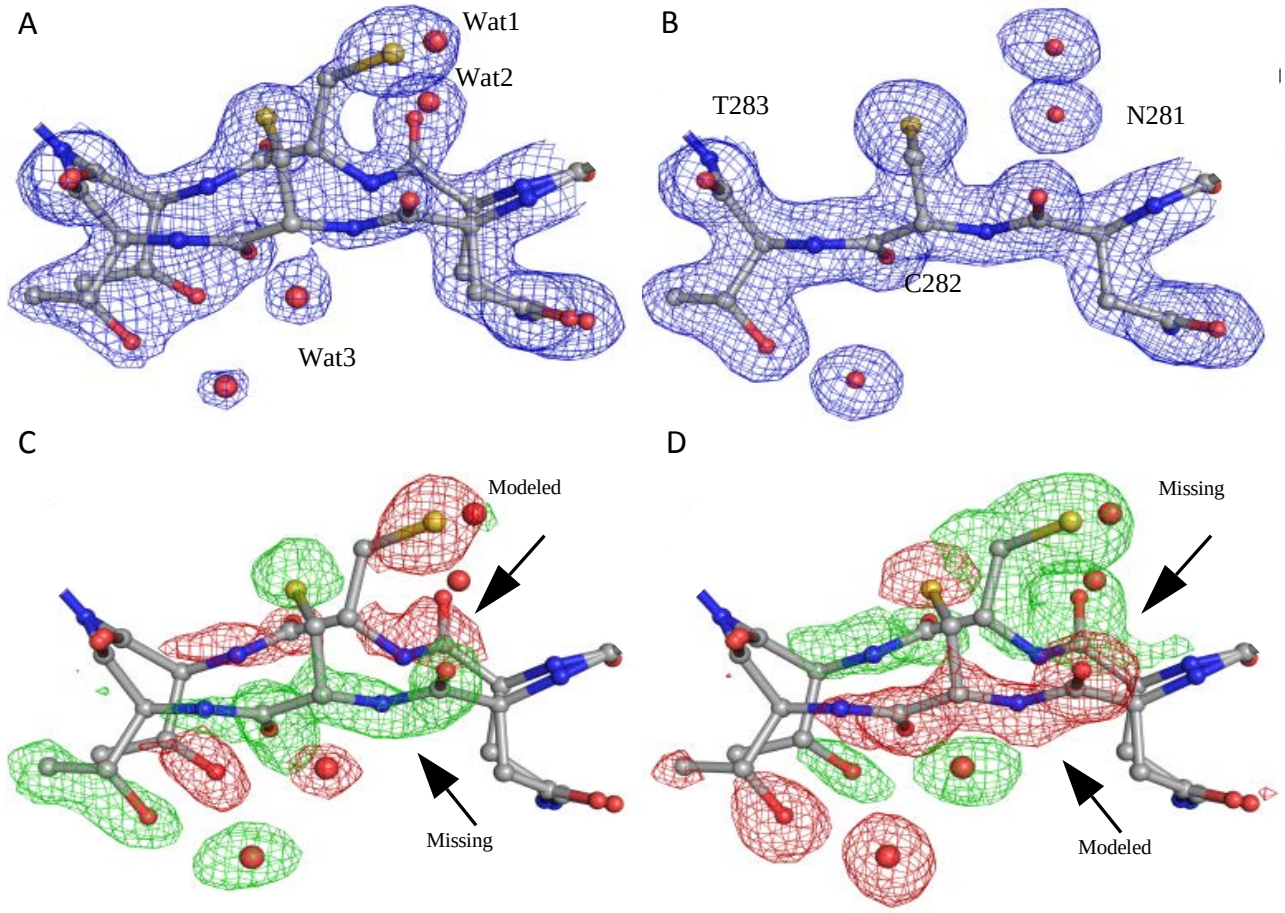
ThnT structure

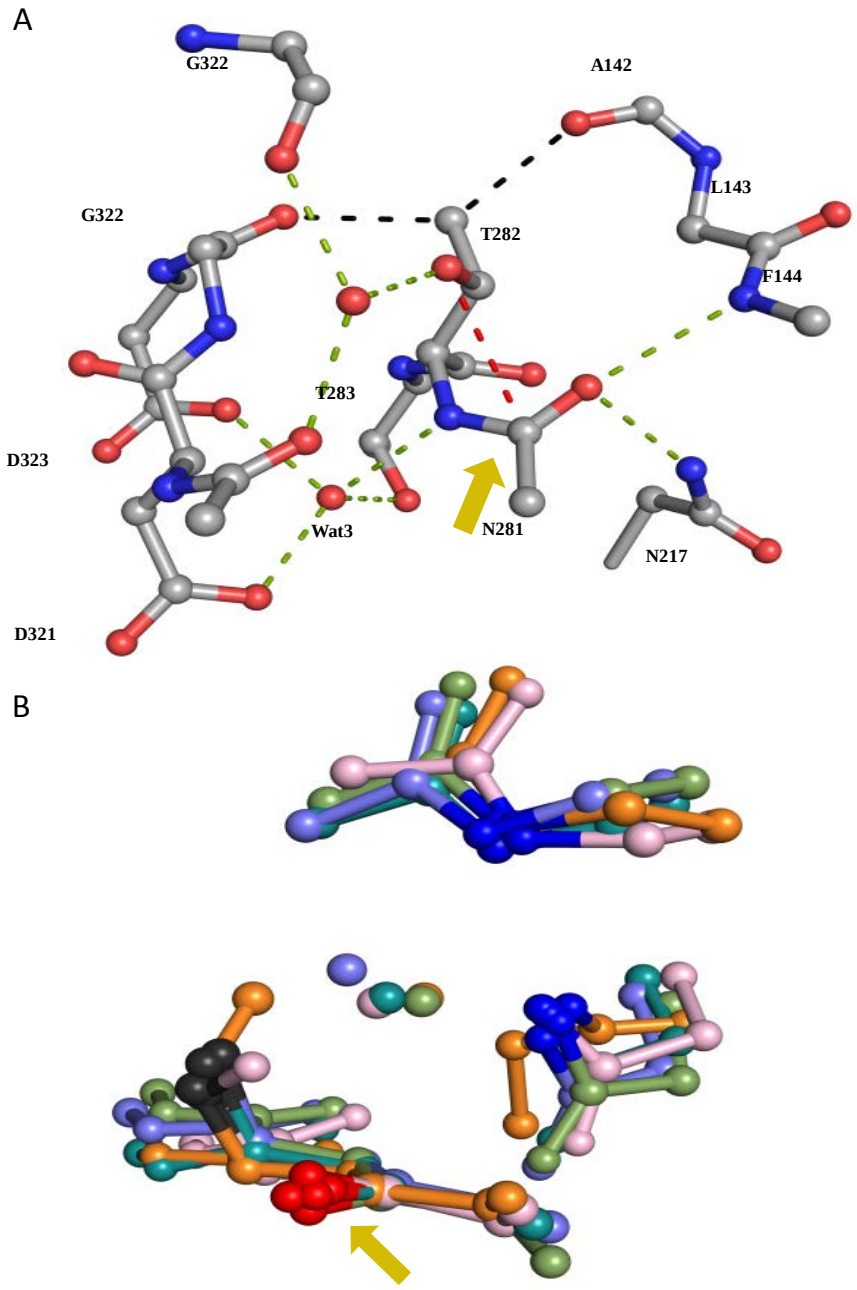




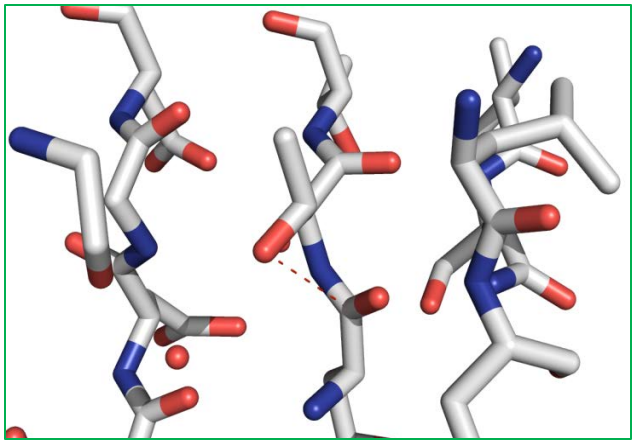
- Protein remains fully associated after proteolysis
- αββα fold- typical of the Ntn family of proteins

	ThnT T282C		ThnT T282C	ThnT
Modification		ethyl mercury phosphate		native	native
Wavelength (Å)	0.7336	1.0036	0.1010	0.8001	0.9788
Beamline		SSRL B12.2		SSRL B12.2	BNL X6A
Resolution (Å)	20 - 1.9	20 - 1.76	20 - 1.77	20 - 1.75	50 - 1.85
Last Bin	1.97-1.9	1.79-1.76	1.86-1.77	1.81 - 1.75	1.92 - 1.85
No. Observations	447,896	520,220	503,903	537,668	513,529
Completeness (%)	100 (100)	99.9 (99.8)	99.3 (93.4)	99.9 (99.5)	98.3 (87.3)
Rmerge (%)	6.0 (51.1)	4.5 (35.0)	4.4 (45.9)	5.4 (48.1)	9.9 (43.0)
I/σI	36.4 (4.62)	55.2 (6.4)	50.0 (3.7)	41.4 (4.4)	23.6 (2.1)
Redundancy	7.5 (7.4)	7.3 (6.8)	7.2 (5.5)	7.4 (7.0)	8.5 (4.4)
			Refinement	Statistics	
FOM		0.871		0.881	0.854
R_{cryst} (R_{free})		0.168 (0.208)		0.164 (0.191)	0.175 (0.222)

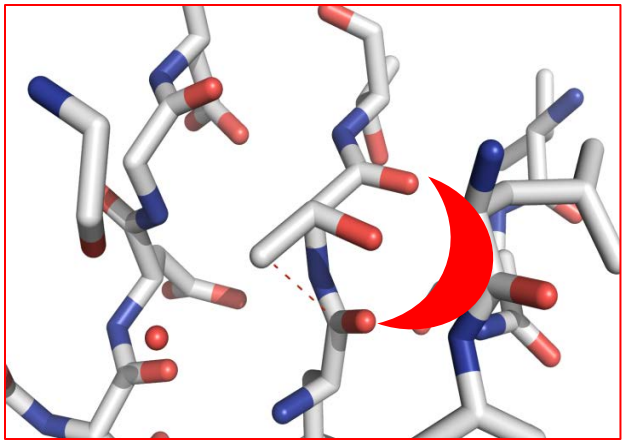

A
Wat1
Wat2
Wat3
B
T283
N281
C282
C
Modeled
Missing
D
Missing
Modeled


A
G322
A142
L143
G322
T282
F144
T283
D323
Wat3
N281
N217
D321
B

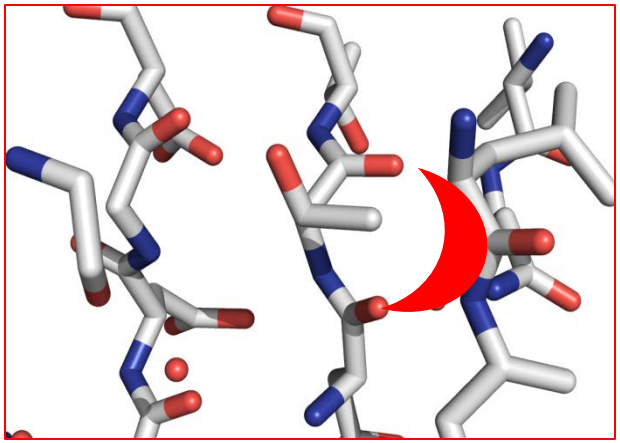
The γ-methyl raises the energy of an anti-conformation



Thr – Favored



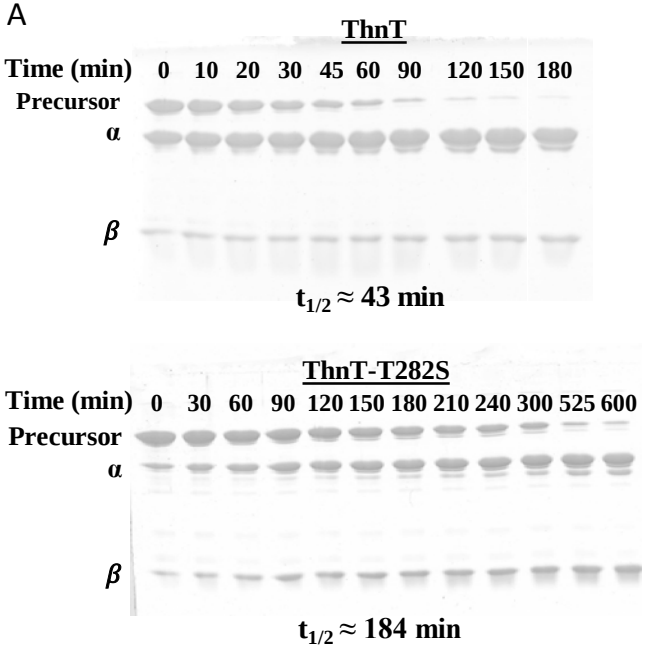
Thr – Unfavored



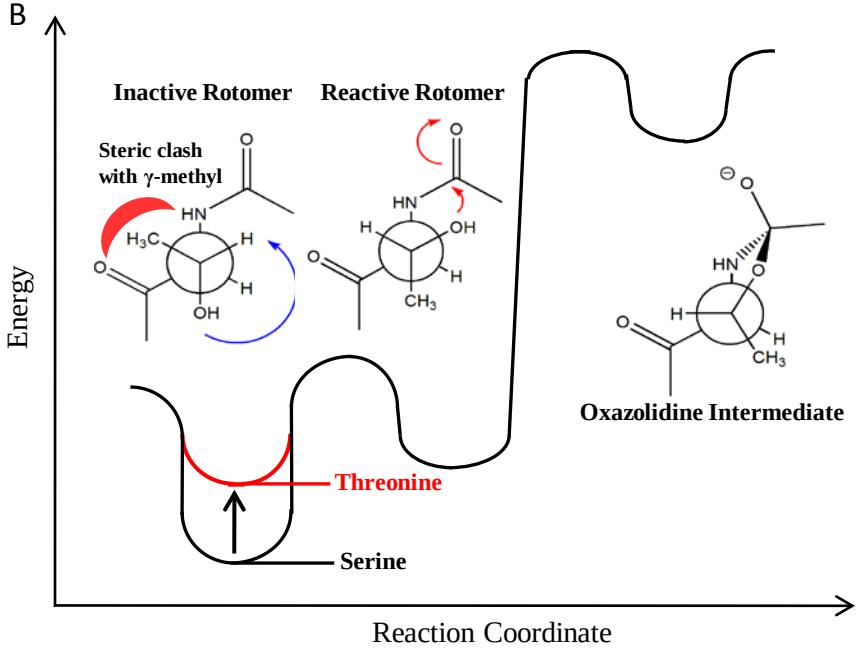
Thr – Unfavored

Reactive rotomer

A

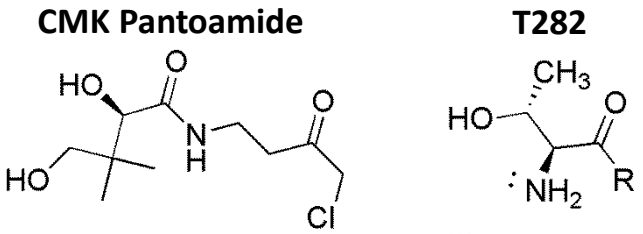

ThnT
Time (min) 0 10 20 30 45 60 90 120 150 180
Precursor
α
β
t1/2 ≈ 43 min
ThnT-T282S
Time (min) 0 30 60 90 120 150 180 210 240 300 525 600
Precursor
α
β
t1/2 ≈ 184 min

B

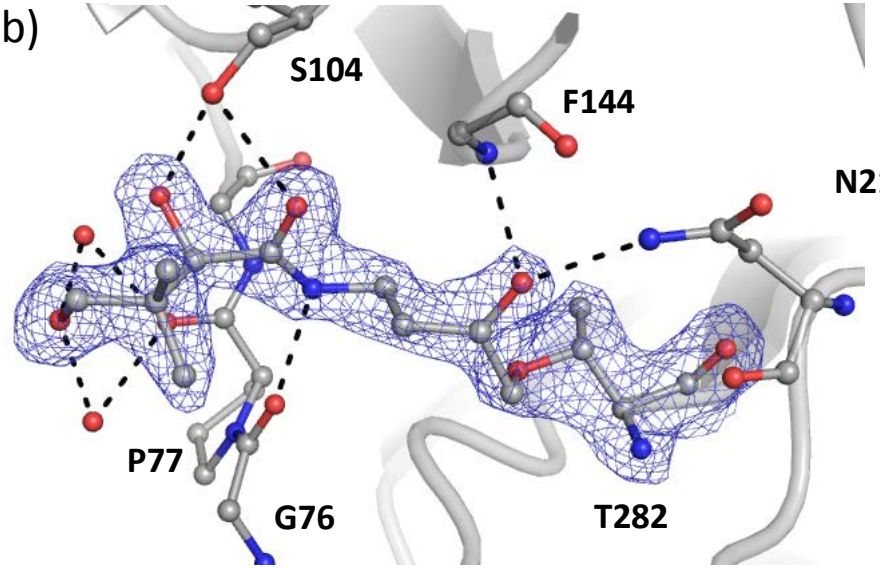

Inactive Rotomer
Reactive Rotomer
Steric clash with γ-methyl
Oxazolidine Intermediate
Threonine
Serine
Energy
Reaction Coordinate

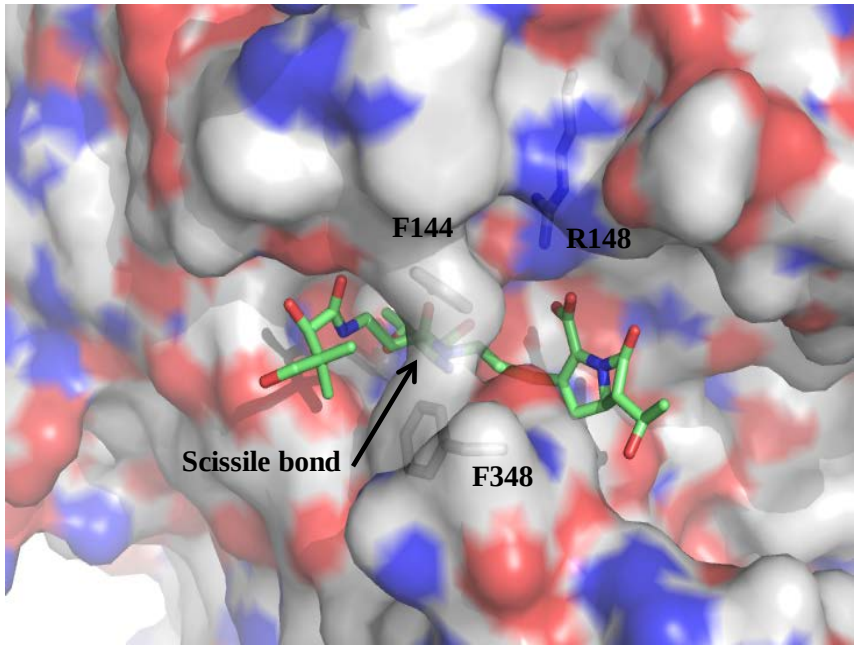
Inhibitor and carbapenem substrate studies of ThnT

(a)



(b)



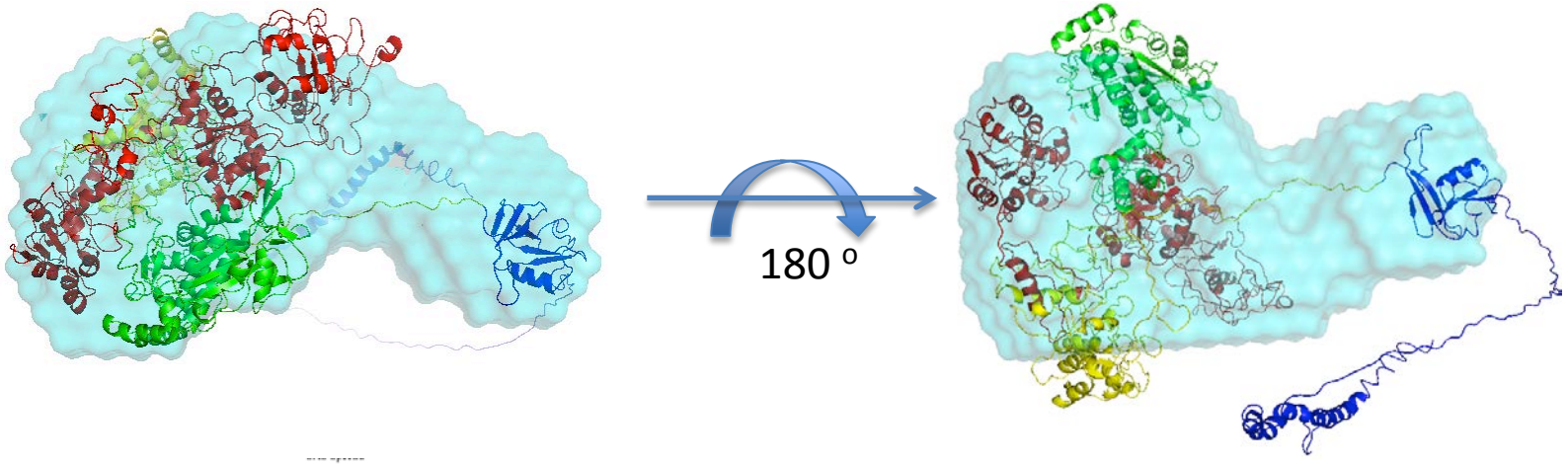


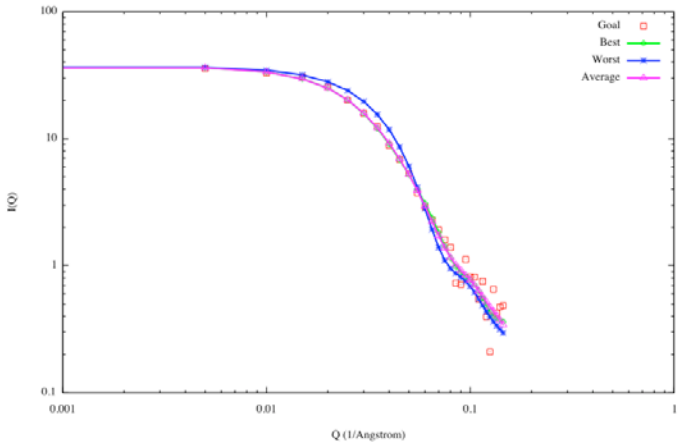
What have we learned?

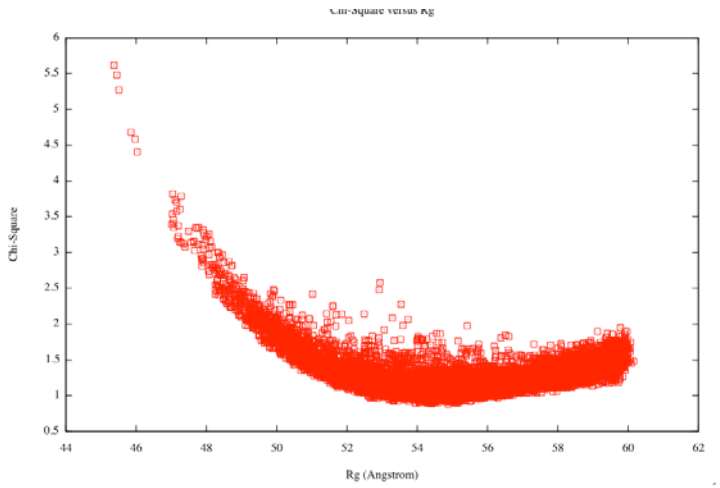
1) Used X-ray crystallography elucidate a mechanism of protein autoprocessing

2) Co-crystallization with an inhibitor a) defined substrate-binding interactions and b) identified the catalytic components of thienamycin synthesis.

In silico construction of TraI using SAXS data for model verification







Acknowledgements

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