

Tinkering a distorted α/β barrel

by Alexis Molfetas
Kokkinidis Lab

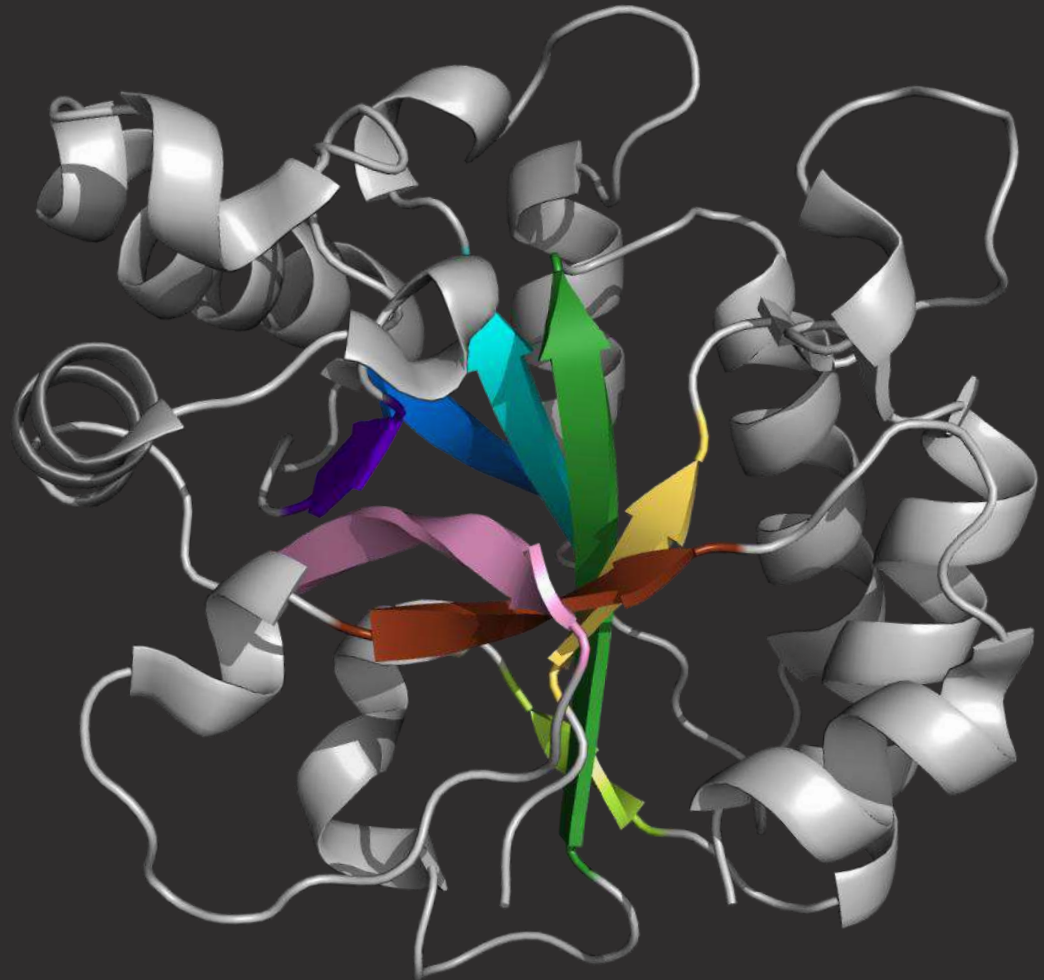


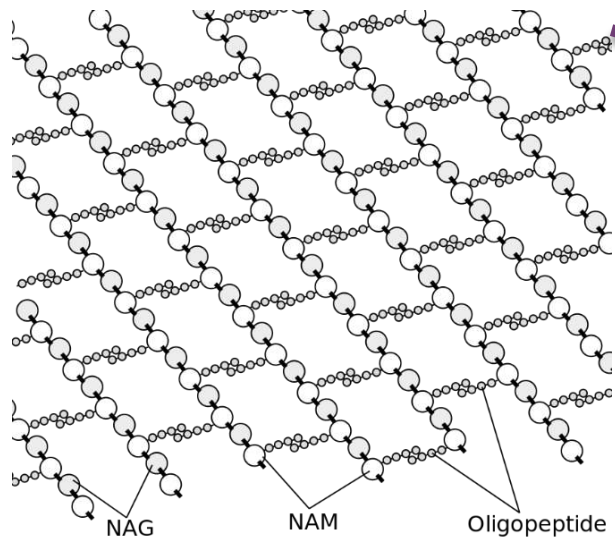
UNIVERSITY
OF CRETE



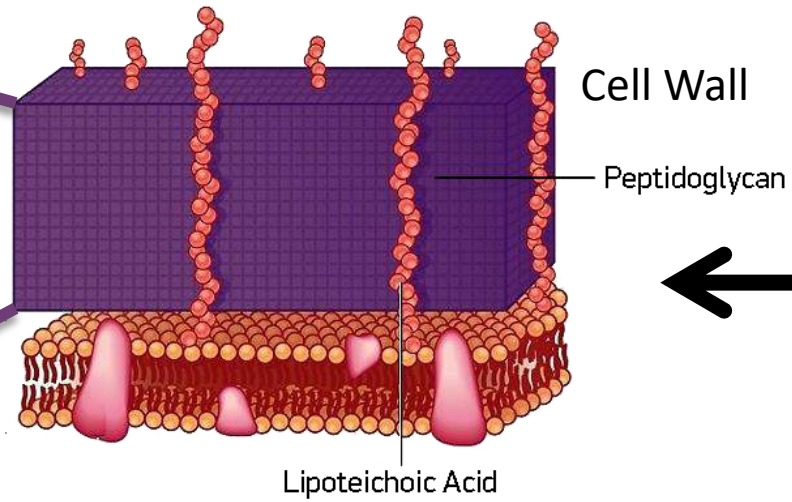
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

IΣN SNF

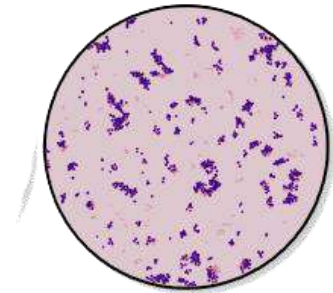




Edited from Quora



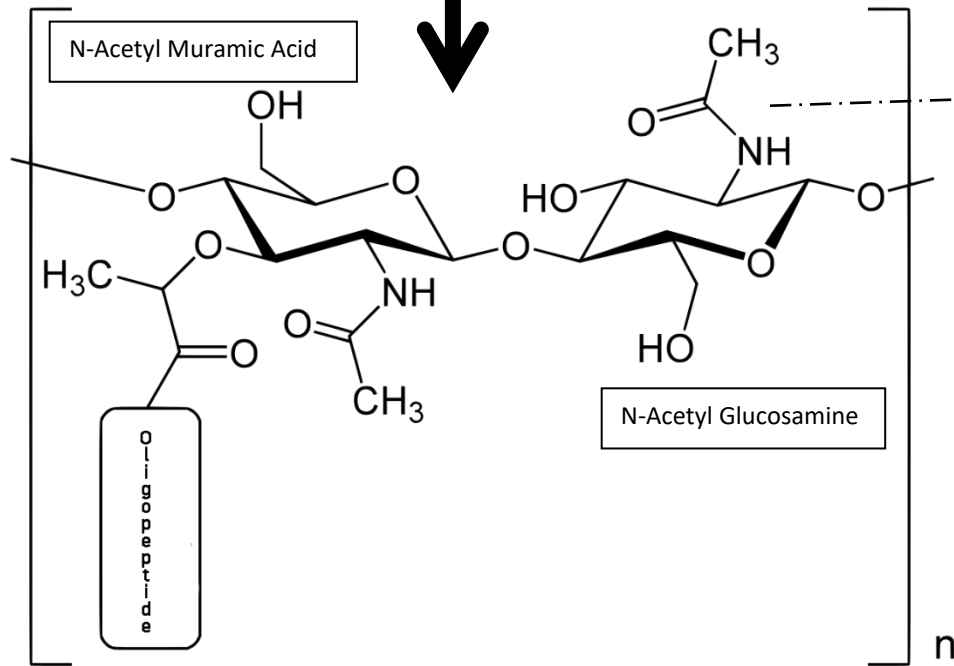
Edited from biologydictionary.net



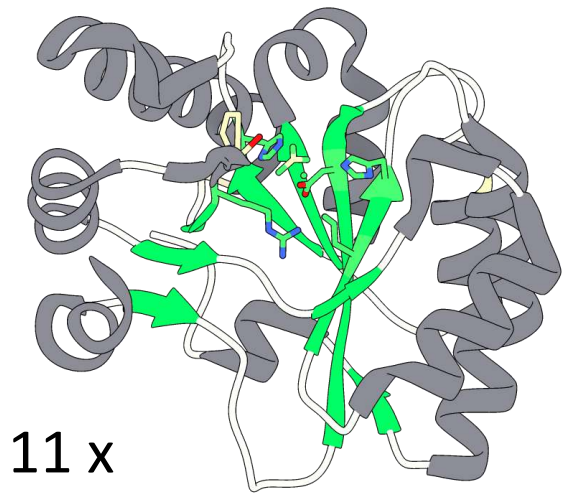
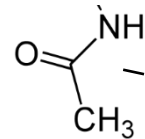
Gram +ve

Makes bacteria invisible to the immune system

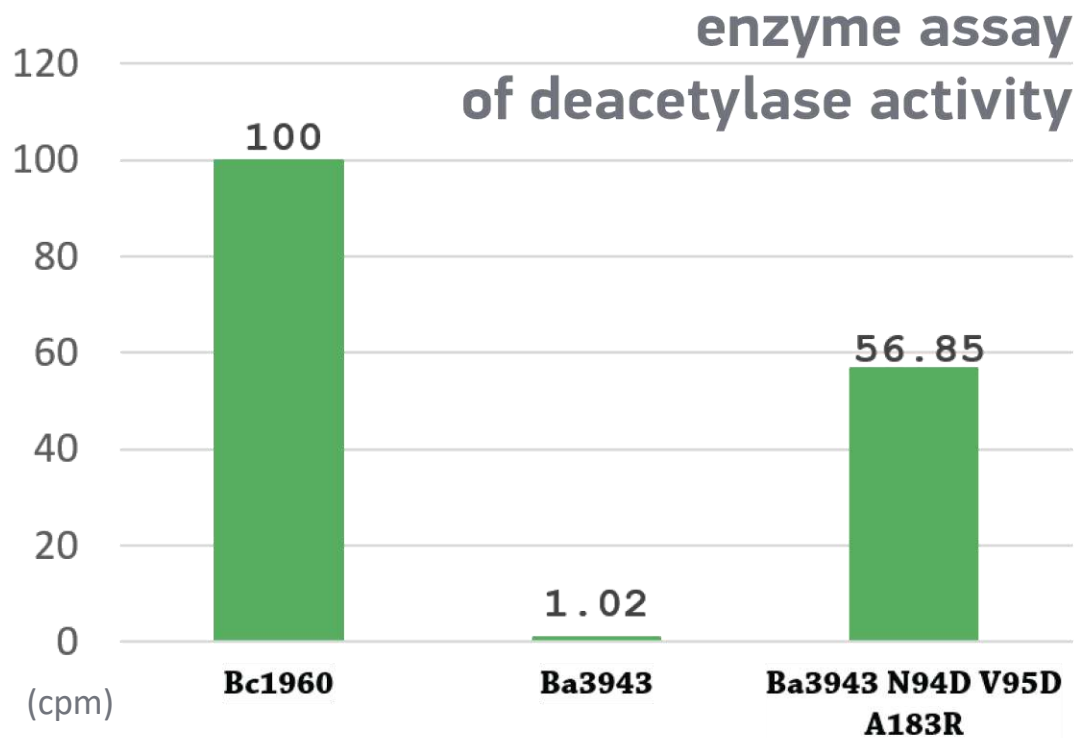
Edited from Wikipedia



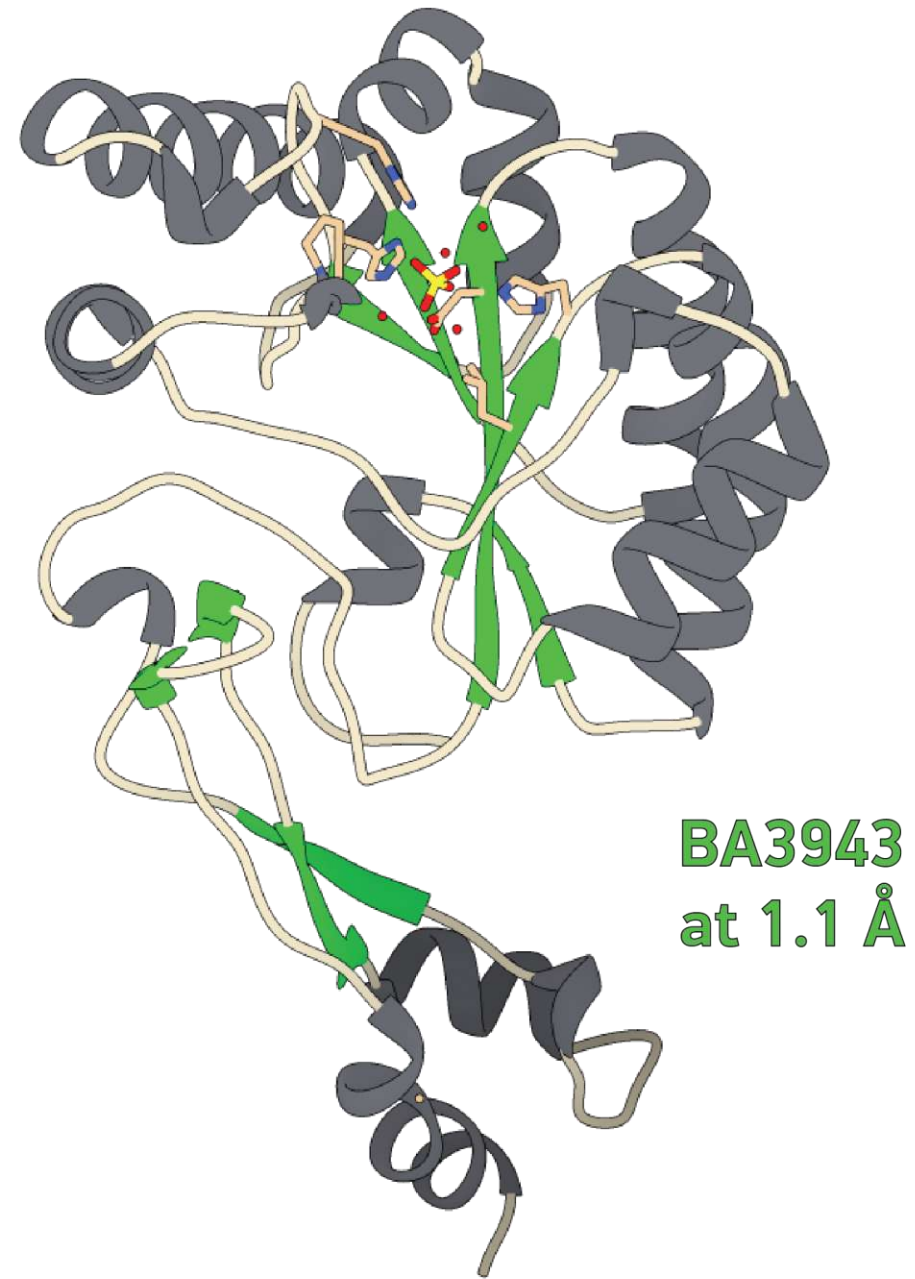
Polysaccharide Deacetylases catalyze the hydrolysis of N-linked acetyl group



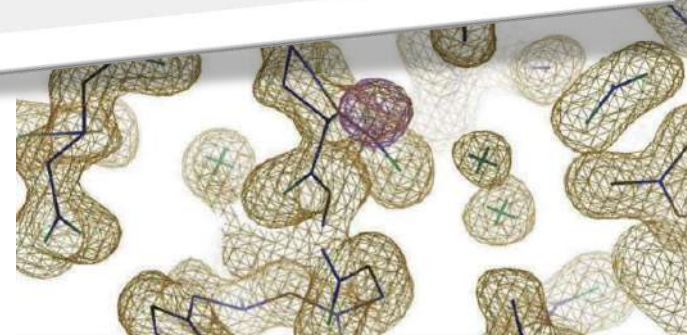
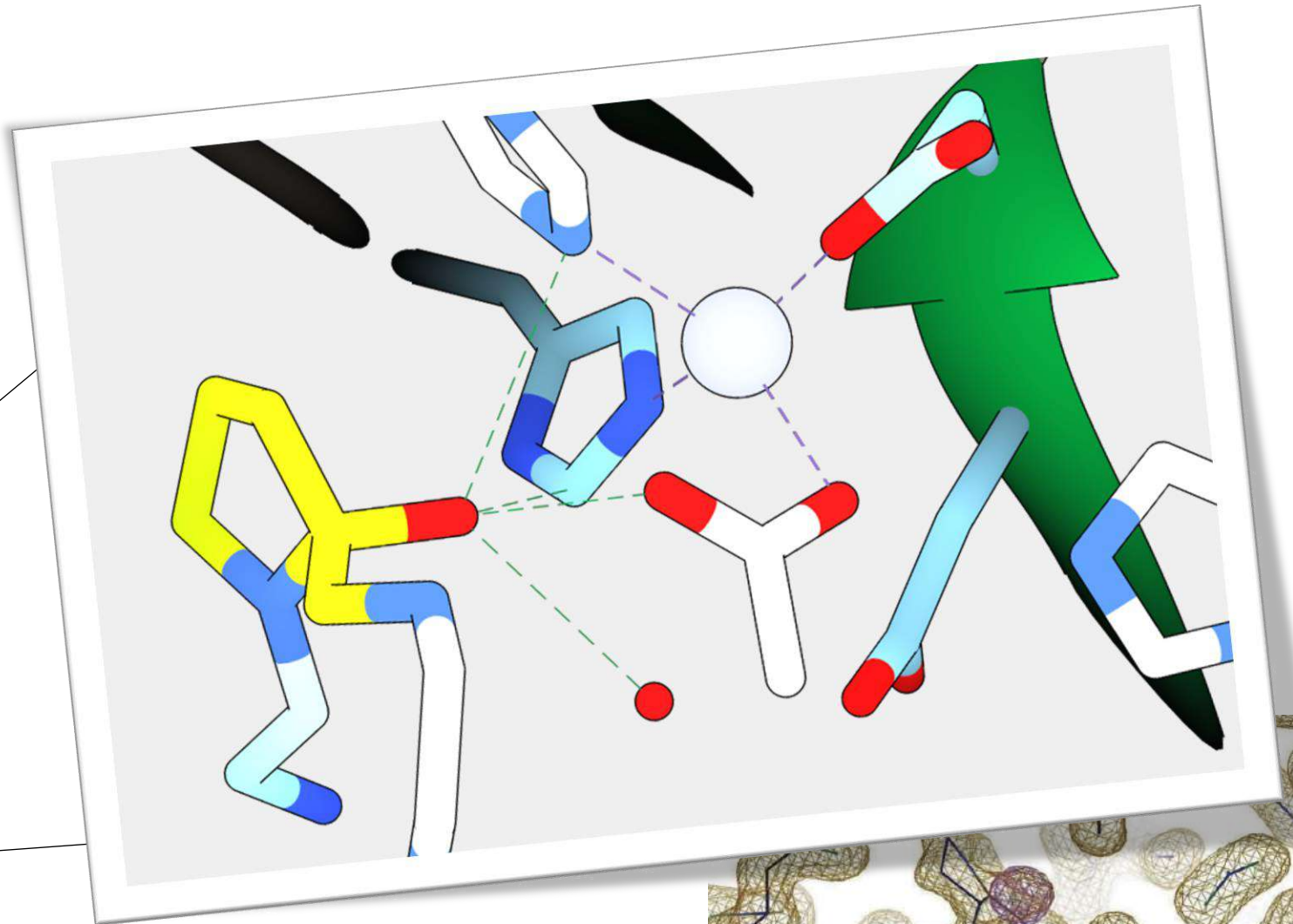
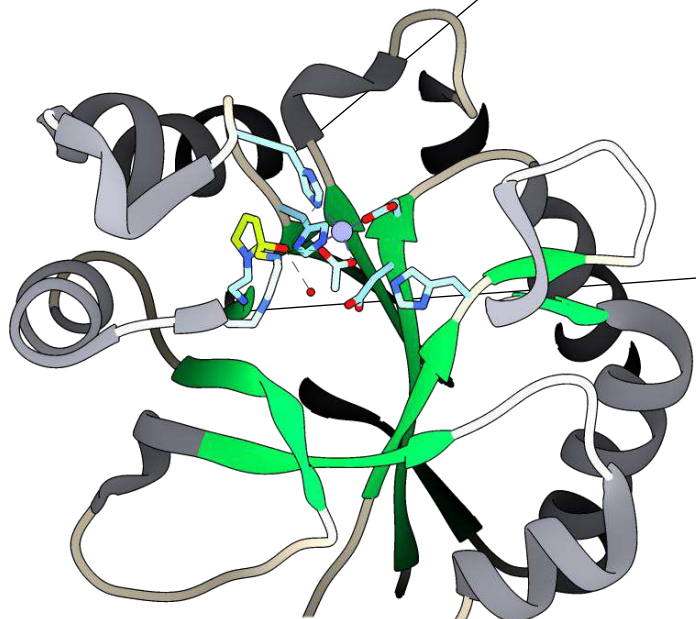
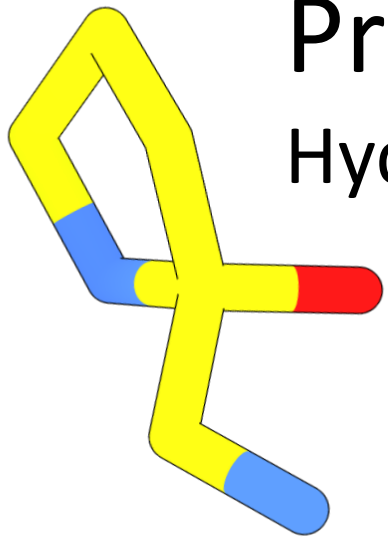
The Dead RELATIVE



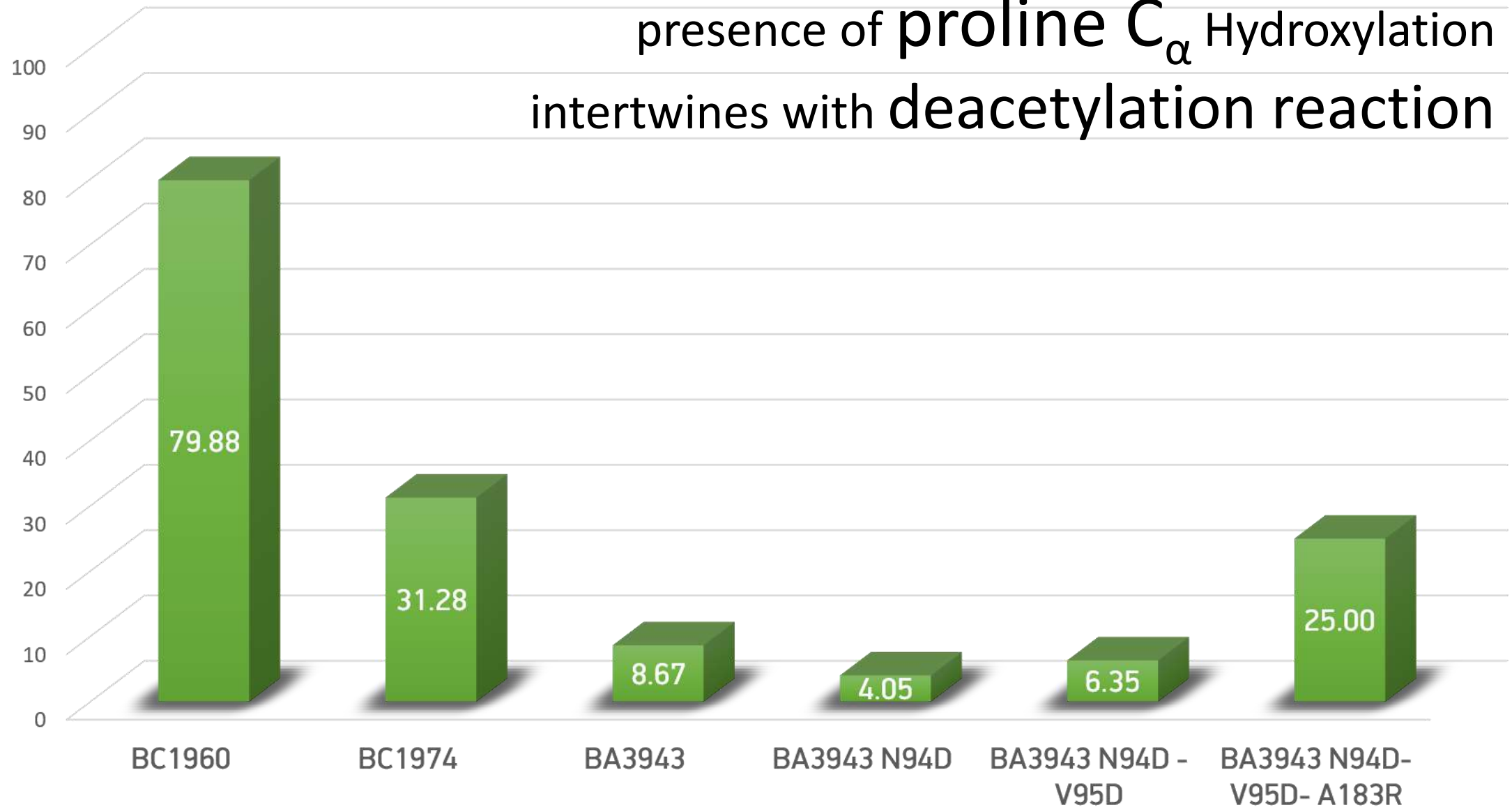
RESURRECTION



Proline C_α Hydroxylation



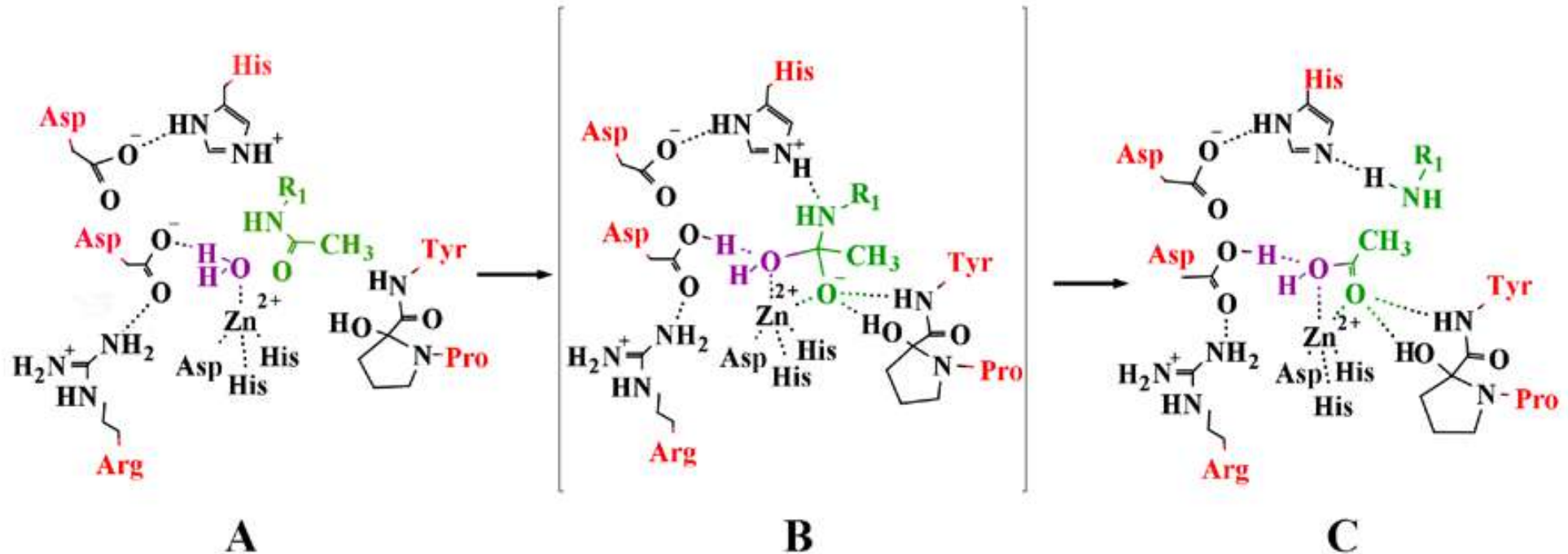
presence of proline C_{α} Hydroxylation
intertwines with deacetylation reaction



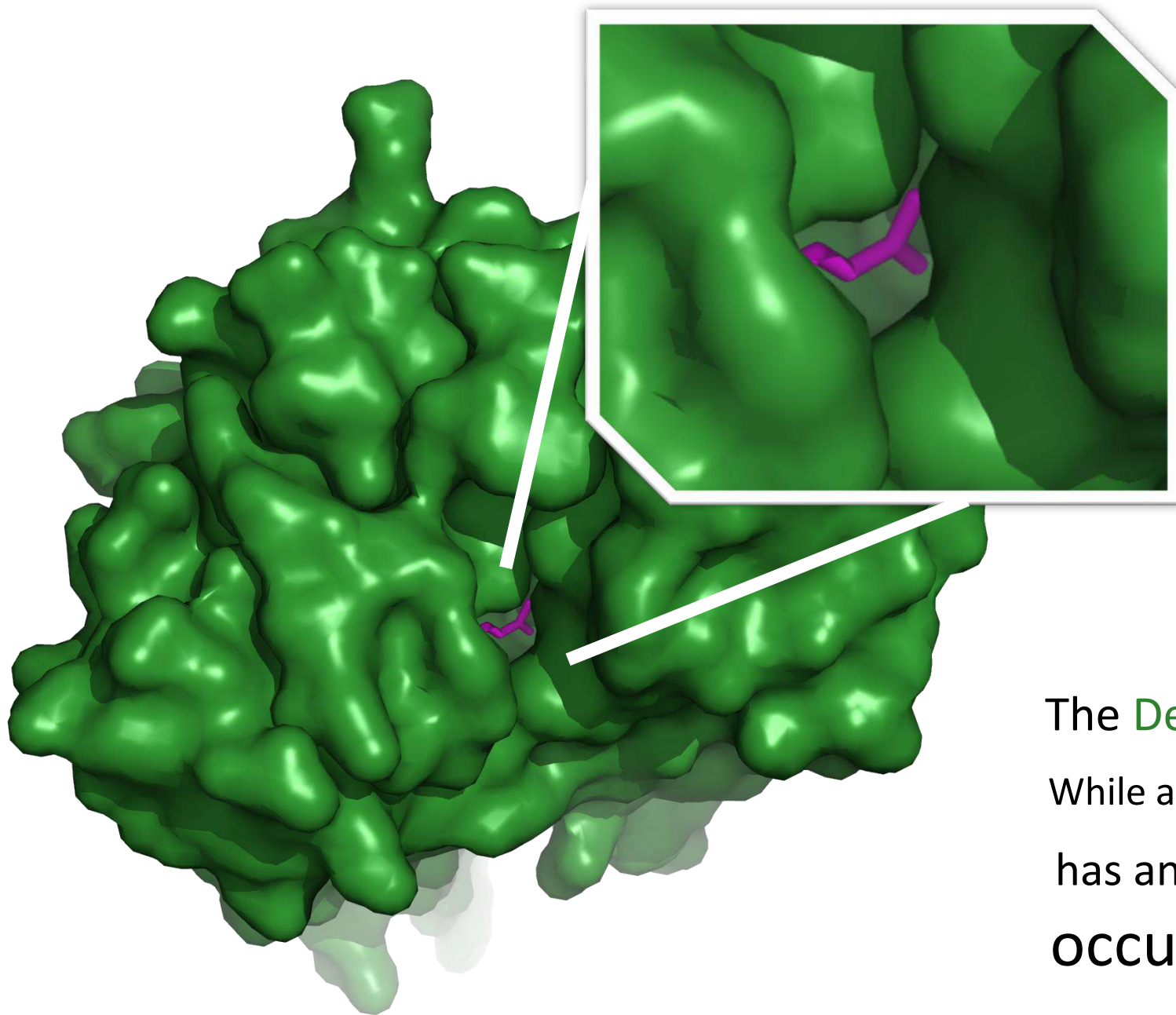
Masspec data by Prof. M. Aivaliotis

↓
RESURRECTION

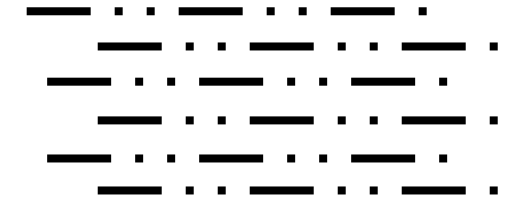
Transition state stabilization



Fadoulou et al. 2017. Unusual alpha-Carbon Hydroxylation of Proline Promotes Active-Site Maturation. J Am Chem Soc 139, 5330-5337.



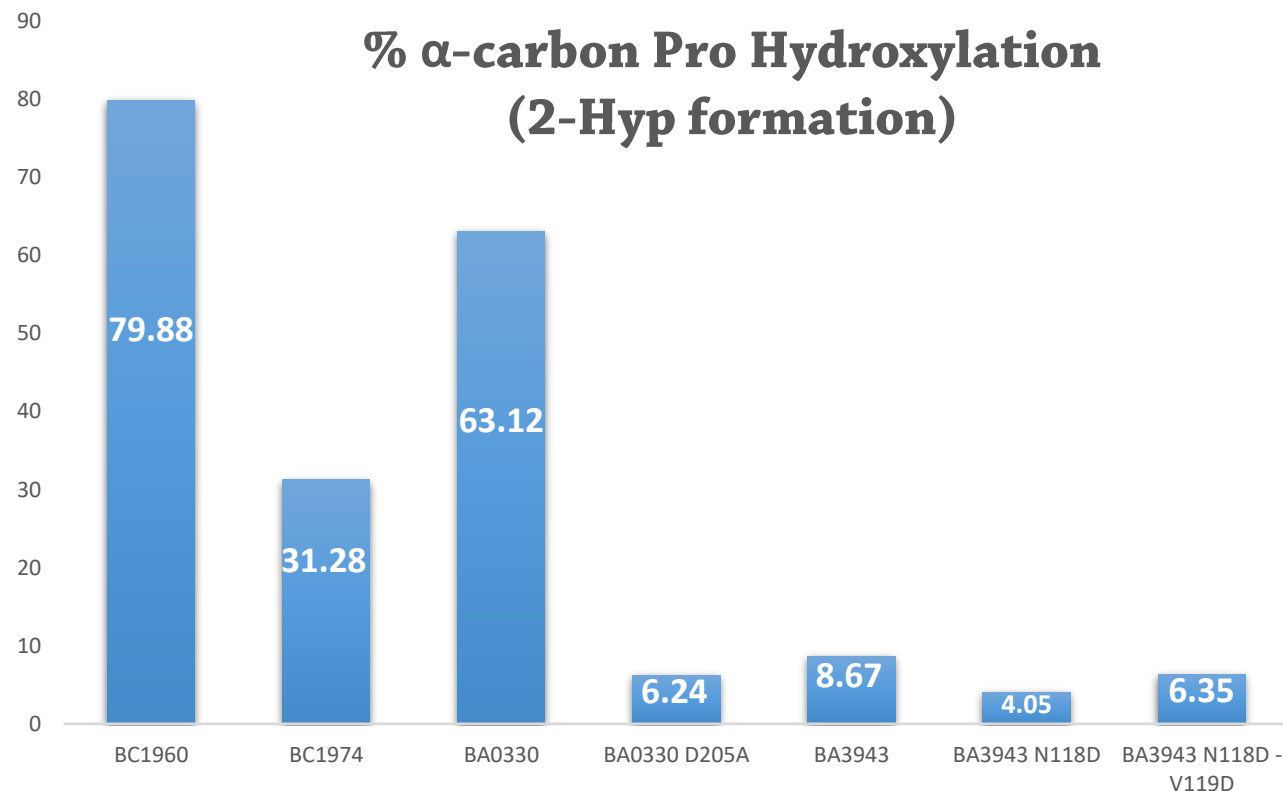
Surface Representation
and Superposition of
BA3943 Vs BC1960



The **Dead Relative** has a **cavity**
While an **active PDA** homologue
has an **Arginine side chain**
occupying the same site

Inactive Enzyme Homologues

**% α -carbon Pro Hydroxylation
(2-Hyp formation)**



BC1960 wt
 BC1974 wt
 BA0330 wt
 BA0330 D205A
 BA3943 wt
 BA3943 N94D
 BA3943 N94D V199D

77 LTFDDG
 73 LTFDDG
 202 VTFDDG
 202 VTFADG
 91 LTINVA
 91 LTI**D**V**A**
 91 LTI**D**D**A**

Motif 1

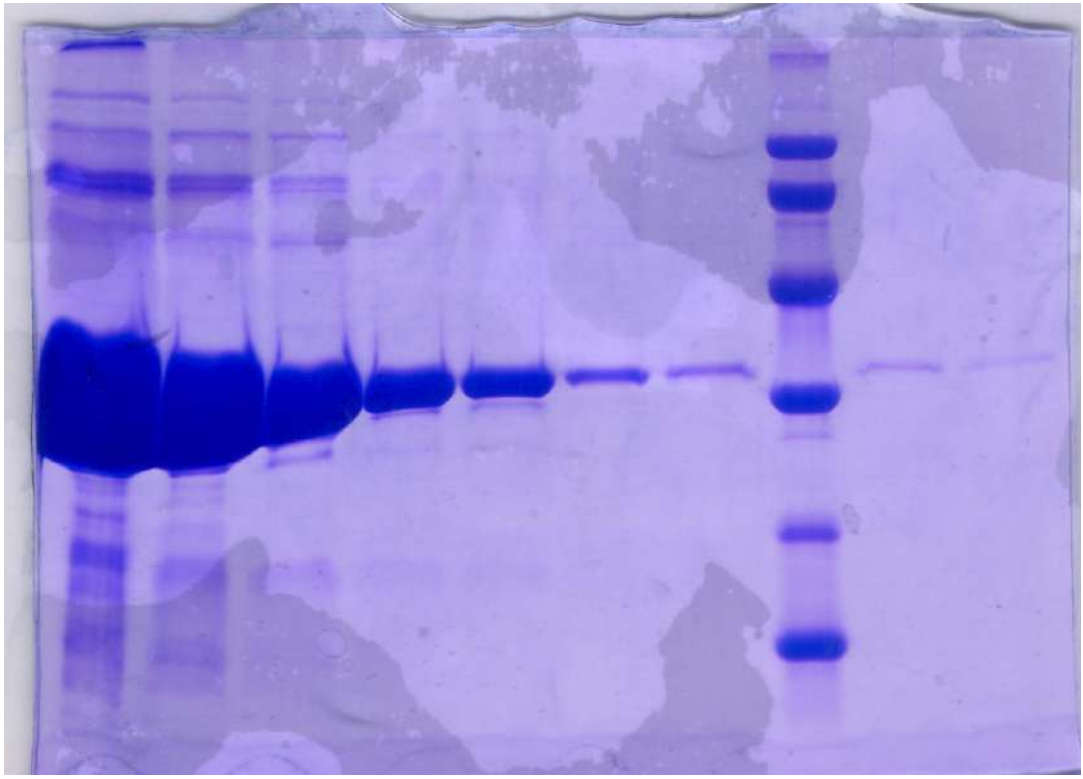
128 IGNHTY**S**H**P**
 123 VGMHSMTHN
 261 MQSHTATHA
 261 MQSHTATHA
 141 VGNHSYTHP
 141 VGNHSYTHP
 141 VGNHSYTHP

Motif 2

165 PKFIR**P**X**Y**G
 160 PKL**T**R**P**PY**G**
 296 VIAVAYXFG
 296 VIAVAYXFG
 178 VRWFAPPSG
 178 VRWFAPPSG
 178 VRWFAPPSG

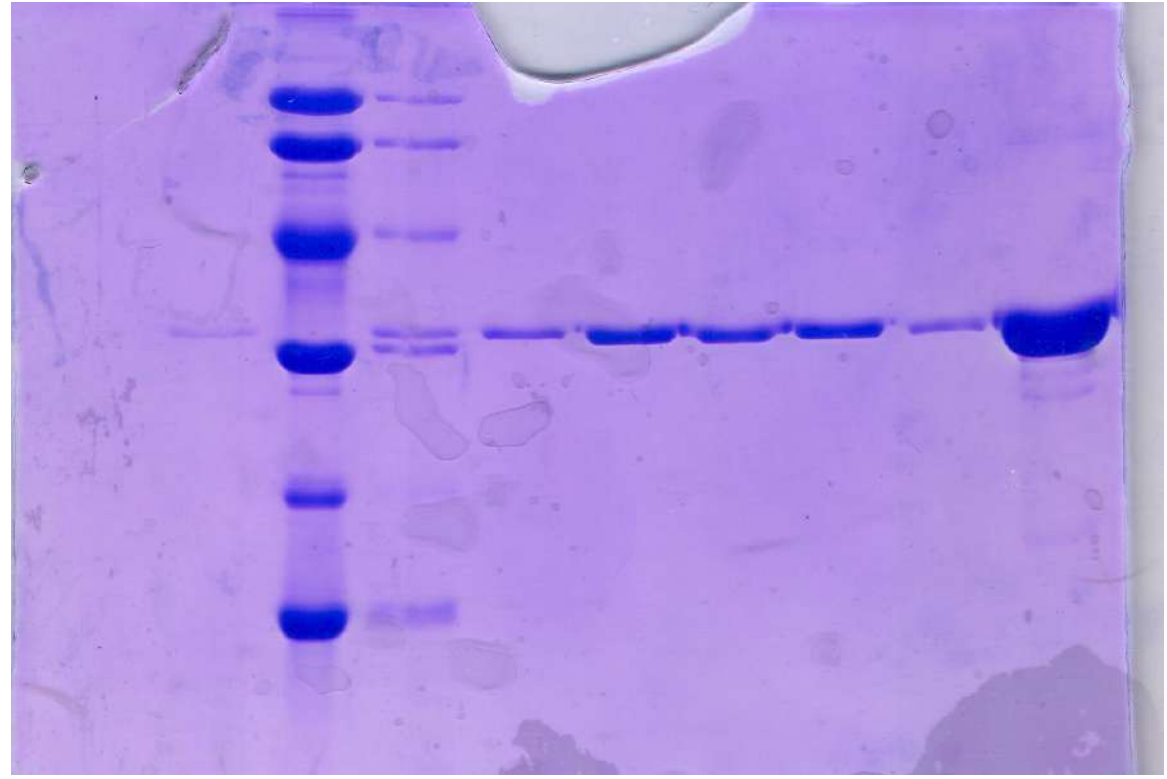
Motif 3

Normal High-yield



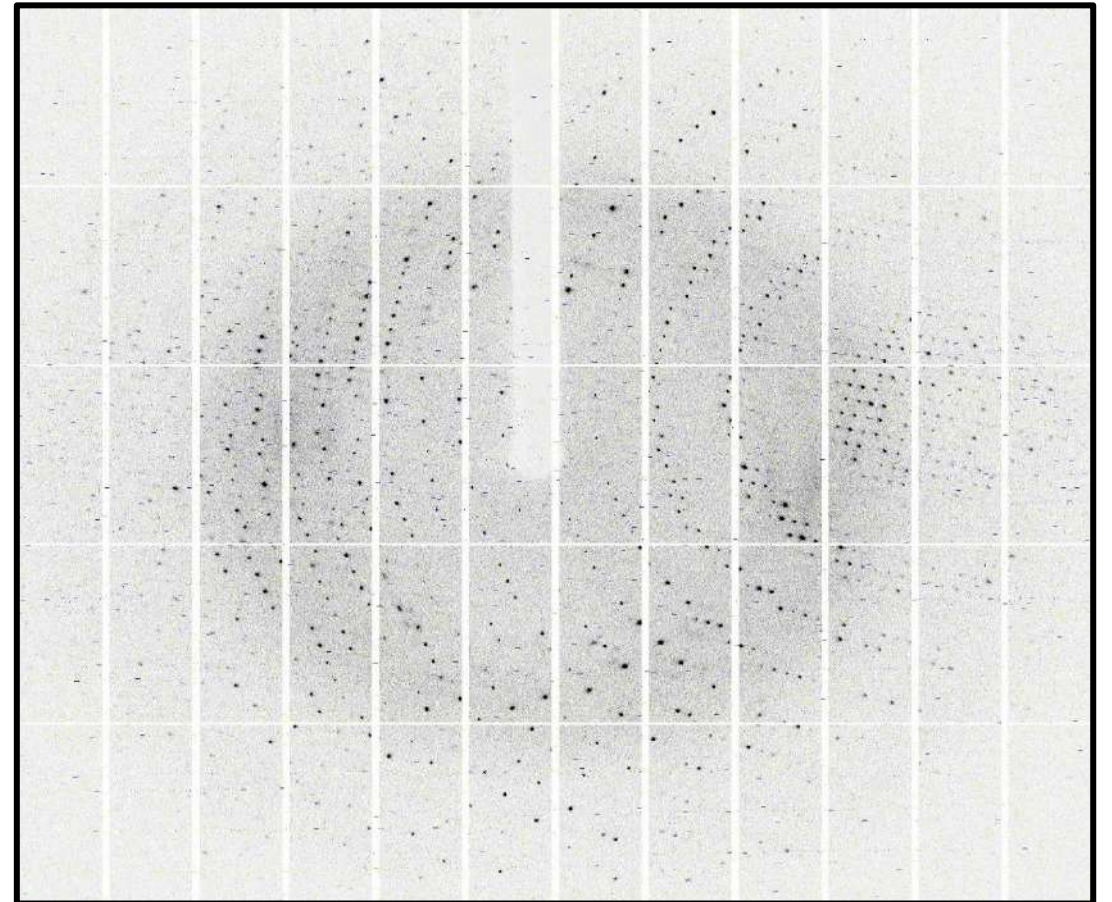
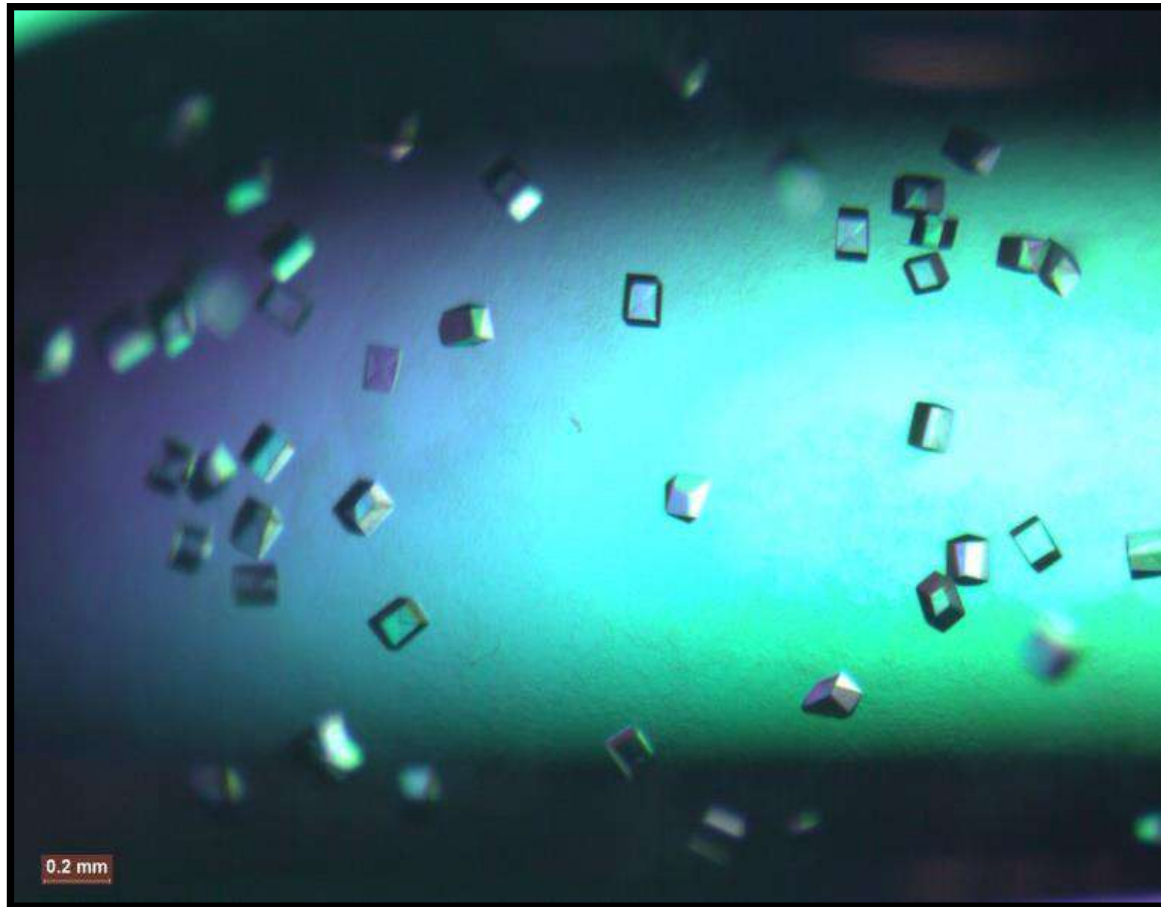
~120 mg of eluted protein

Low-yield



<5 mg of eluted protein

Structure determination of *Ba*3943 mutants



1-25 aa

Signal peptide

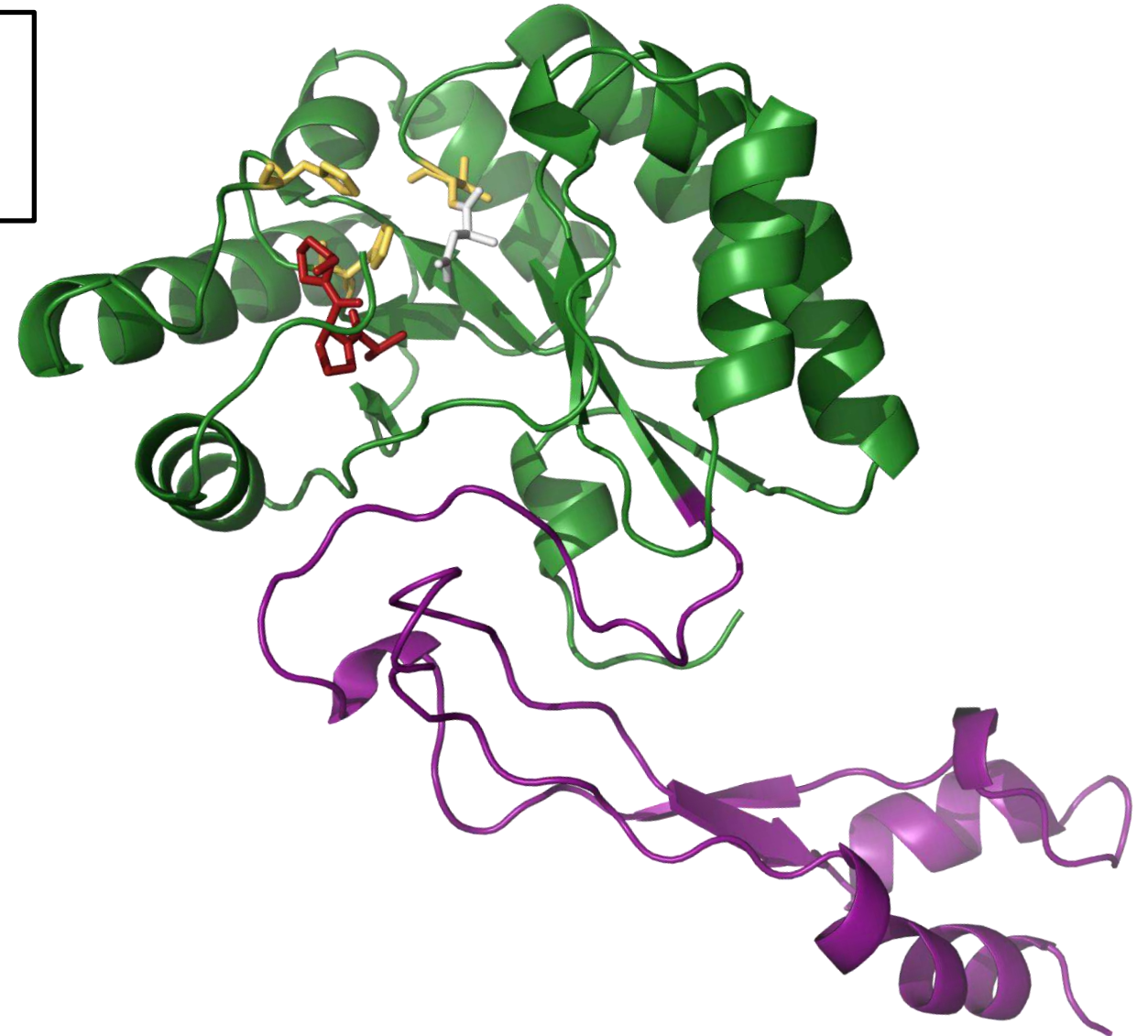
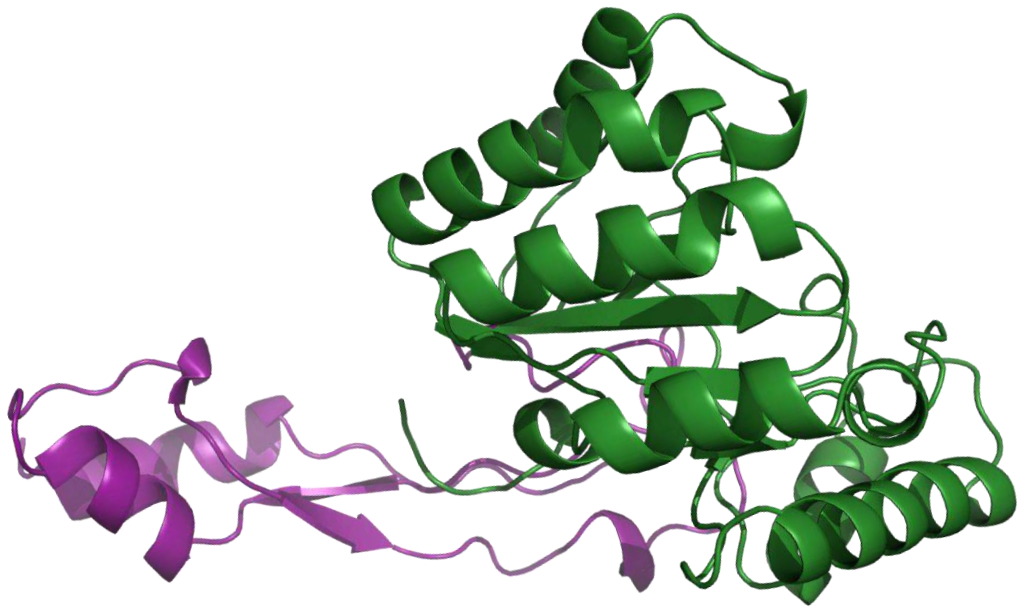
85 aa

N-terminus

188 aa

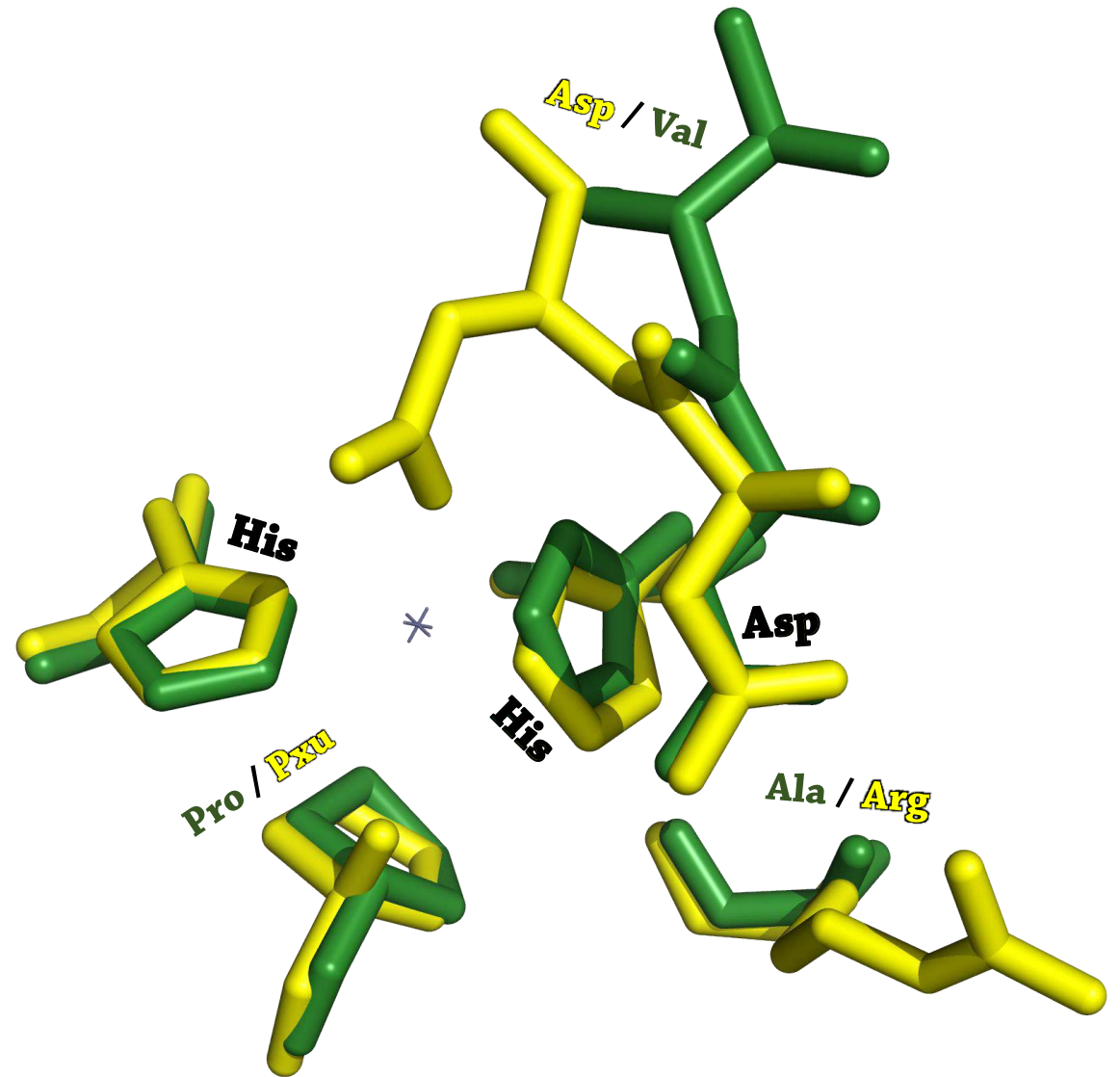
NodB

Structure determination at 1.5Å
via Molecular Replacement



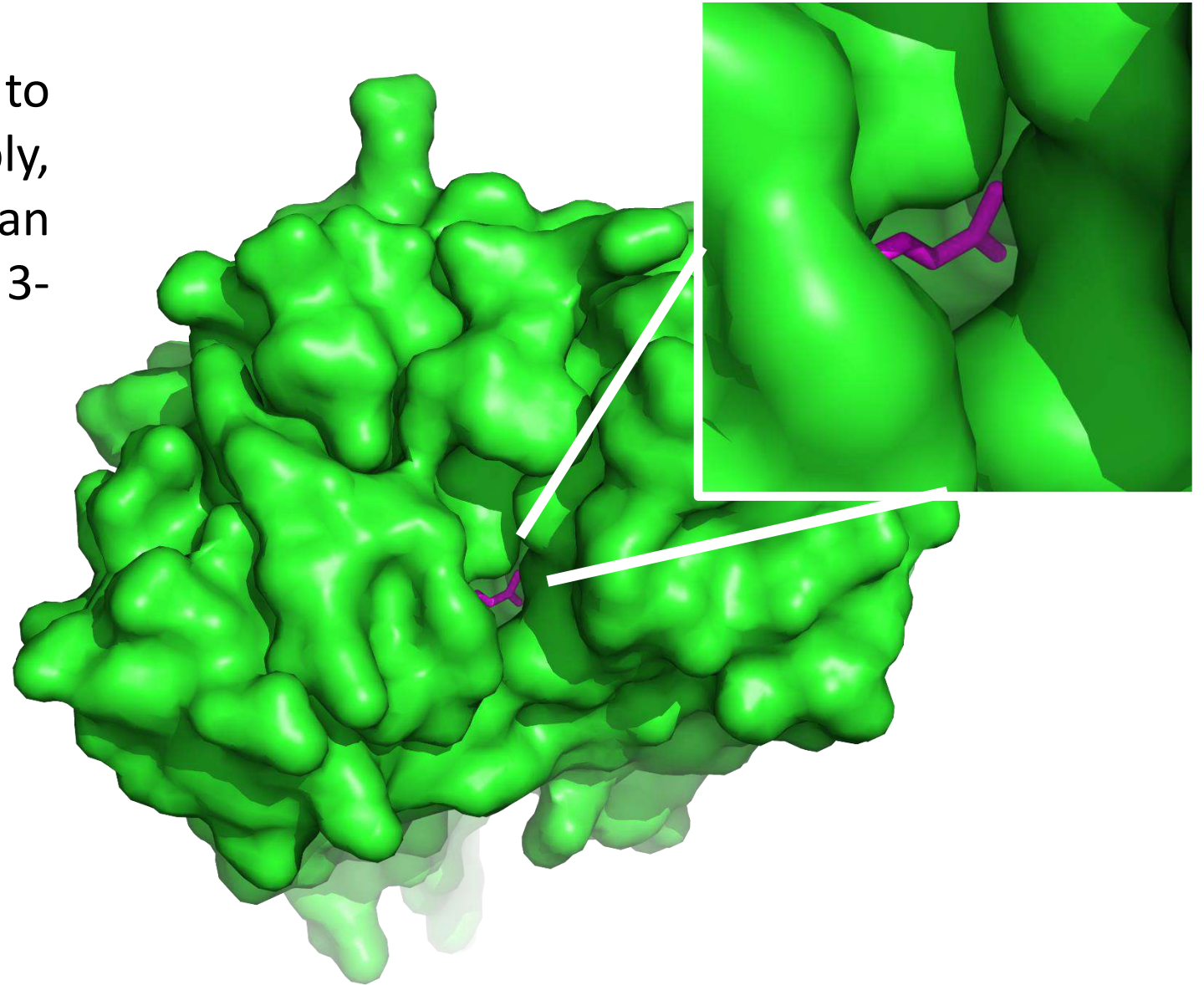
Structural Superposition of BA3943 N94D with BC1960⁴

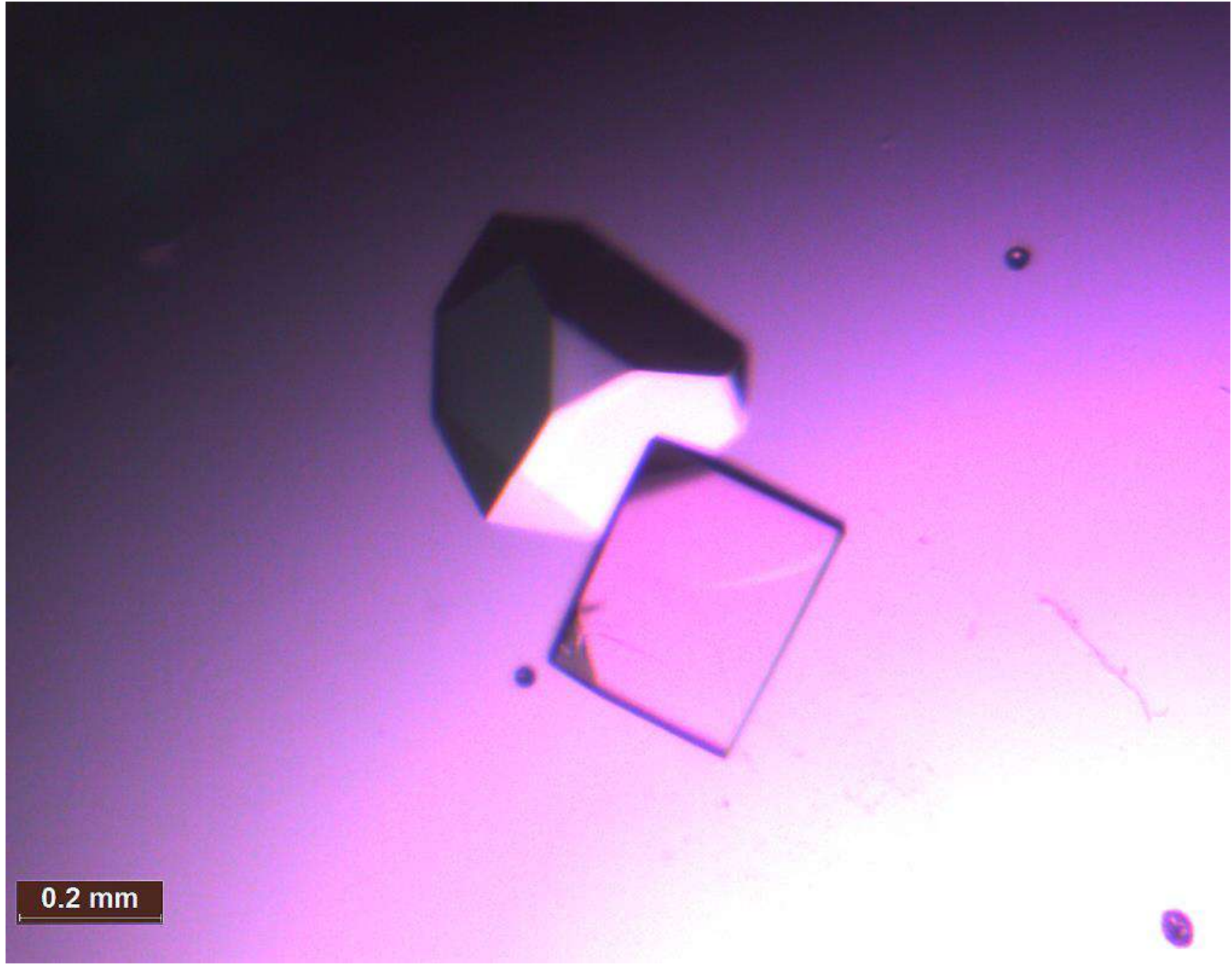
- 1) Pro residue lacks hydroxyl group.
- 2) The metal triad is disrupted.
- 3) The well conserved Arg in the vicinity of the catalytic Asp is replaced by an Ala, creating a cavity and potential de-stabilization of the active site.



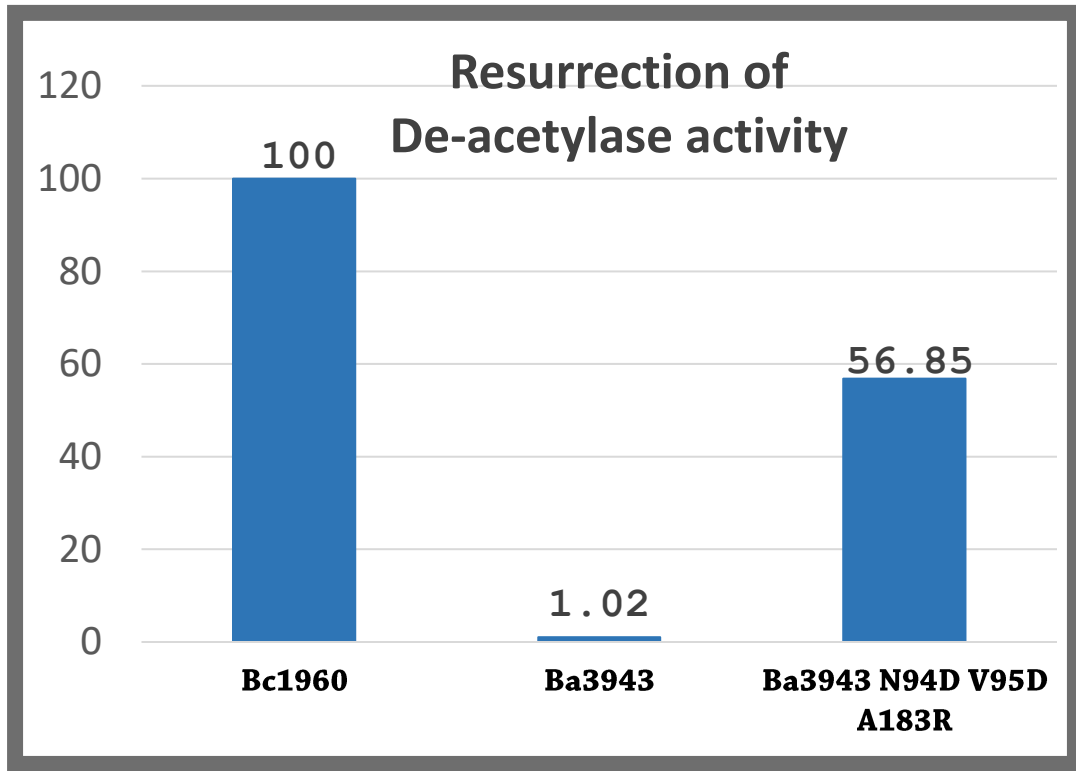
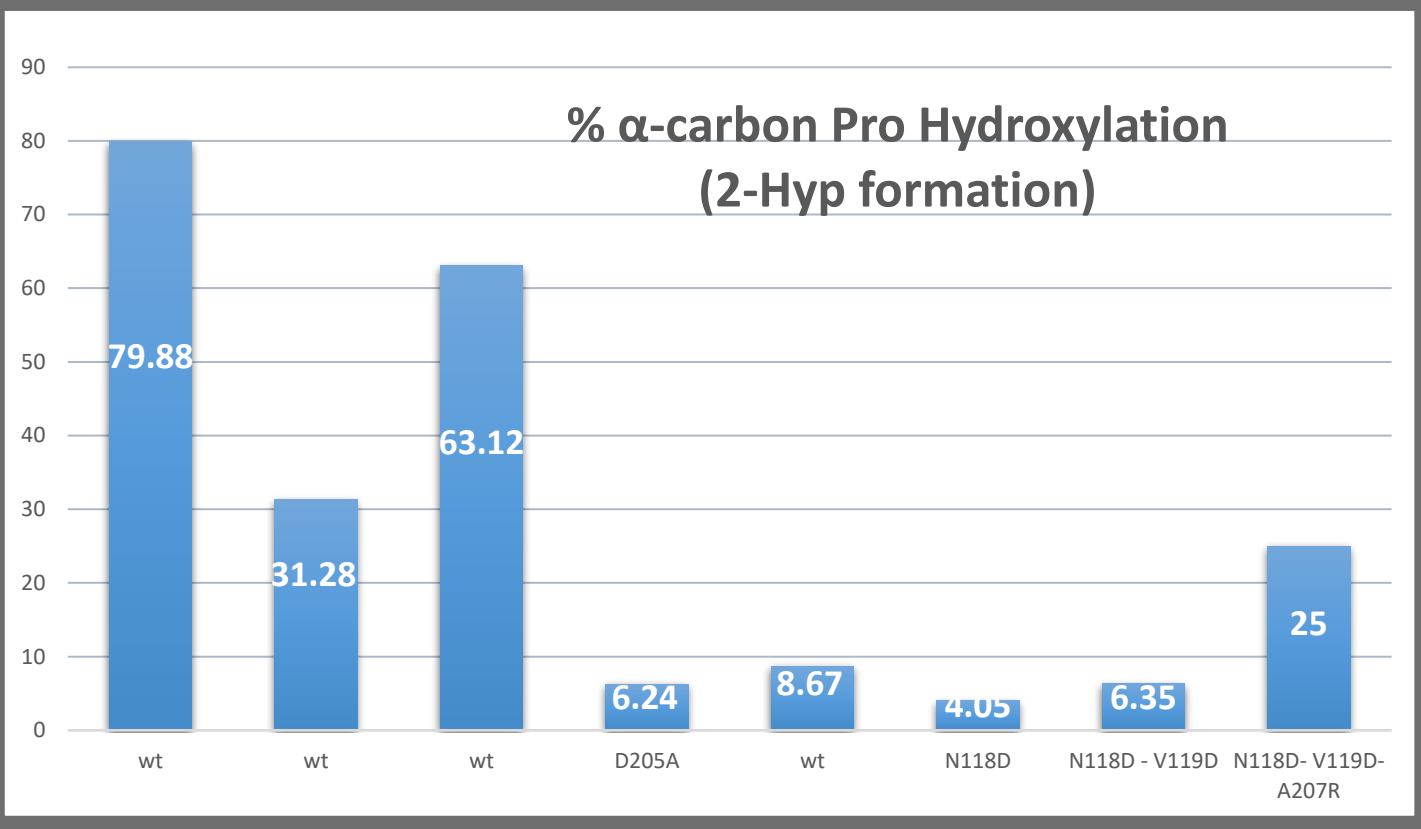
Structural Superposition of BA3943 N94D with BC1960⁴

The arginine not only acts to coordinate the base, but possibly, the long side chain occupies an important position in the 3-dimensional space.





0.2 mm



BC1960	wt	77	LT F DDG	128	IGNHTY S HP	165	PKFIR P XYG
BC1974	wt	73	LT F DDG	123	VGMHSMTHN	160	PKL T RPPYG
BA0330	wt	202	V T FDDG	261	MQSHTATHA	296	VIAVAYXFG
BA0330	D205A	202	V T FADG	261	MQSHTATHA	296	VIAVAYXFG
BA3943	wt	91	LTINVA	141	VGNHSYTHP	178	VRWFAPPSG
BA3943	N94D	91	LTID V A	141	VGNHSYTHP	178	VRWFAPPSG
BA3943	N94D V199D	91	LTID D DA	141	VGNHSYTHP	178	VRWFAPPSG
BA3943	N94D V199D A183R	91	LTID D DA	141	VGNHSYTHP	178	VRWF R PPSG

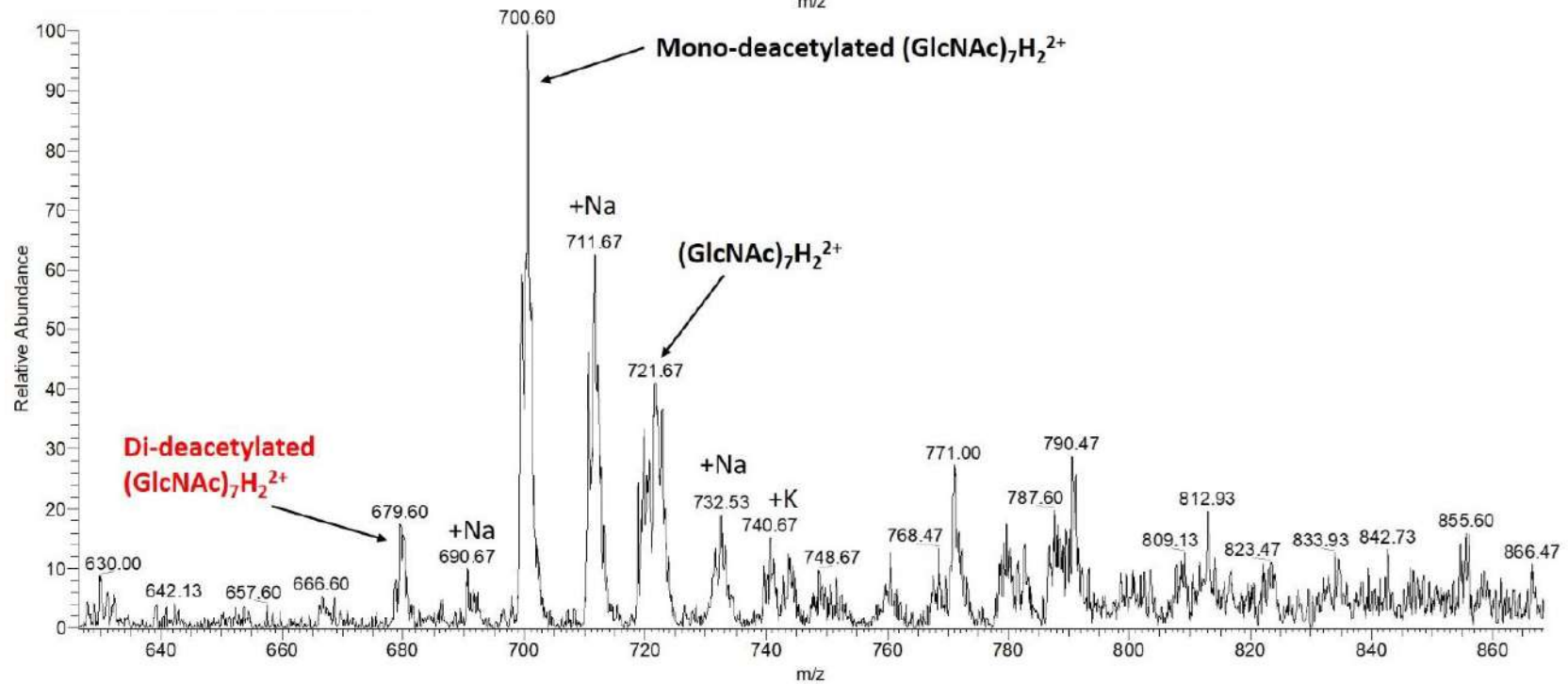
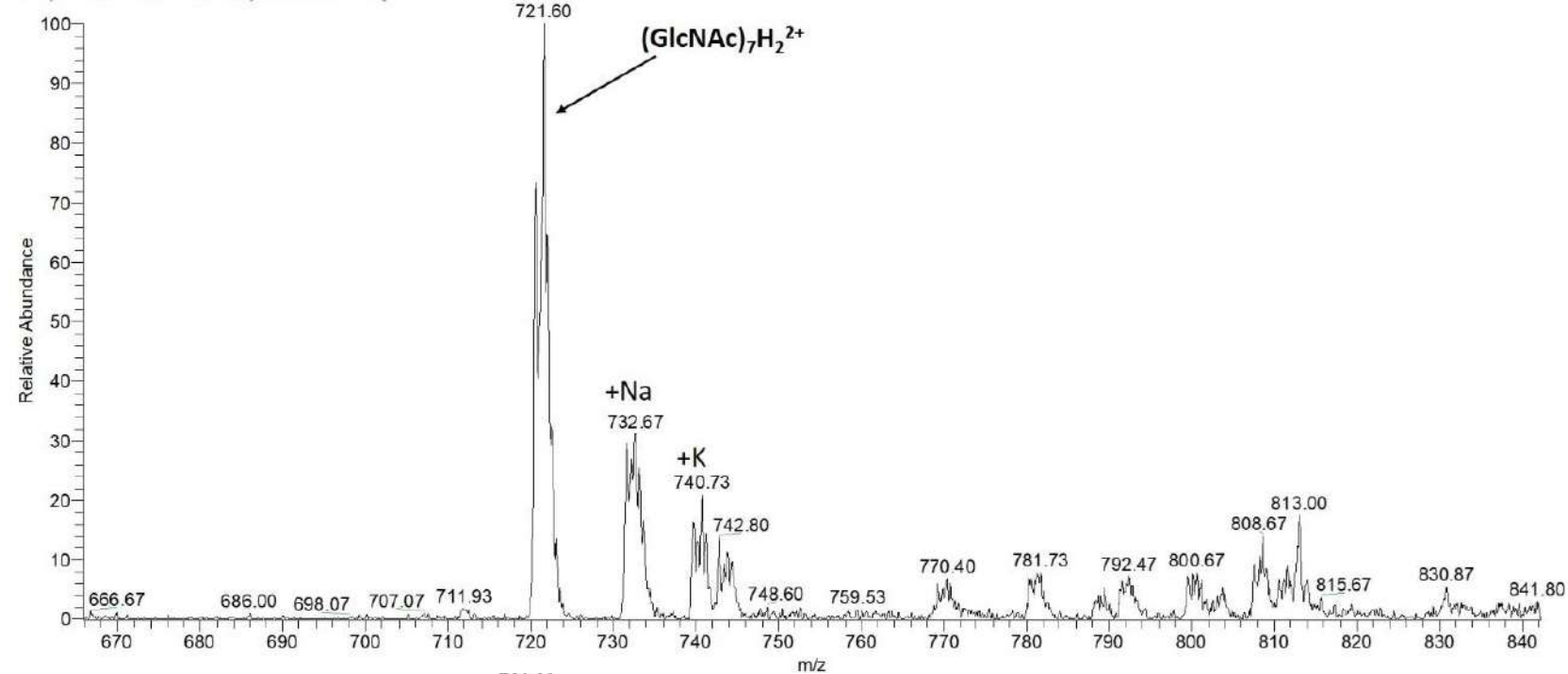
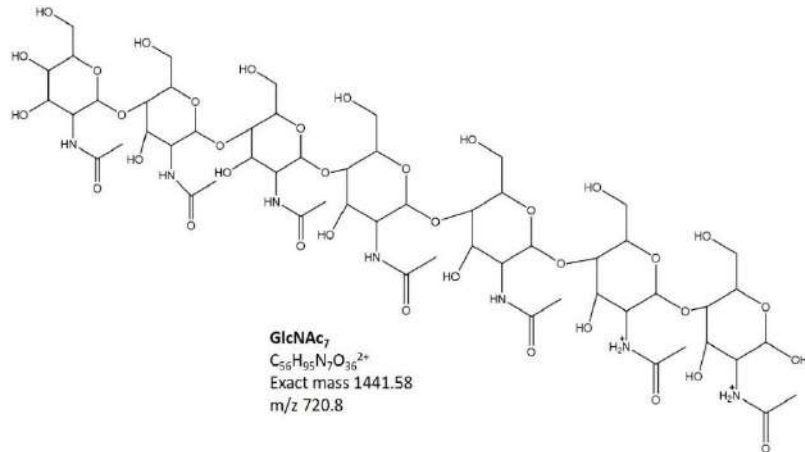
Motif 1
Motif 2
Motif 3

ESI-MS

Electrospray ionization –
Mass spectrometry

BA3943 N94D V95D A183R

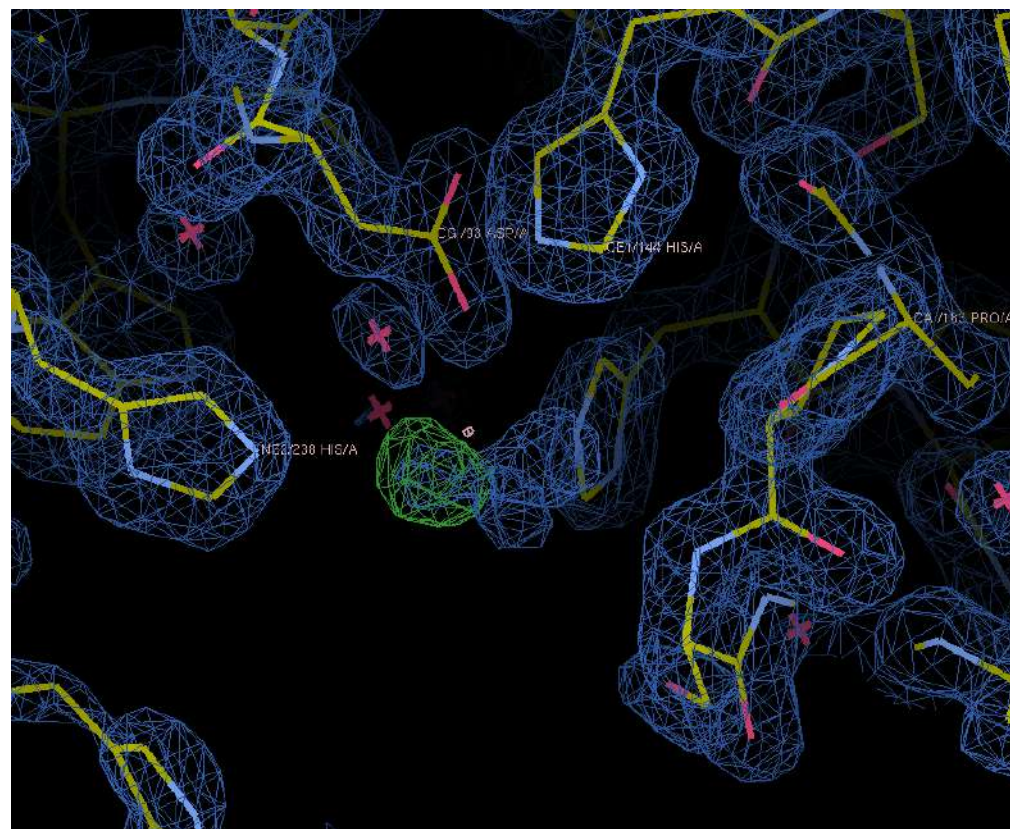
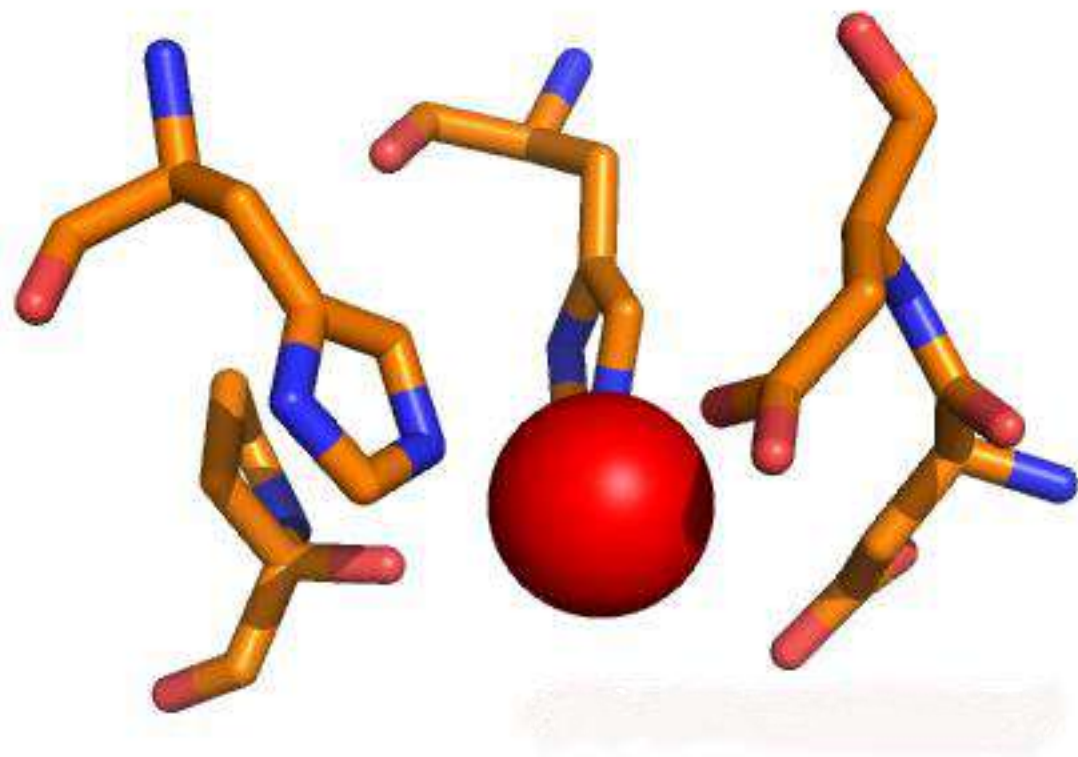
on



Identify divalent metal

2Fo-Fc $\sigma = 2.0$

Fo-Fc $\sigma = 4.5$



BA3943 N94D V95D A183R (0.2mgr/ml) was incubated with glycol chitin at different pH values, temperatures, hours of incubation and a variety of metals.

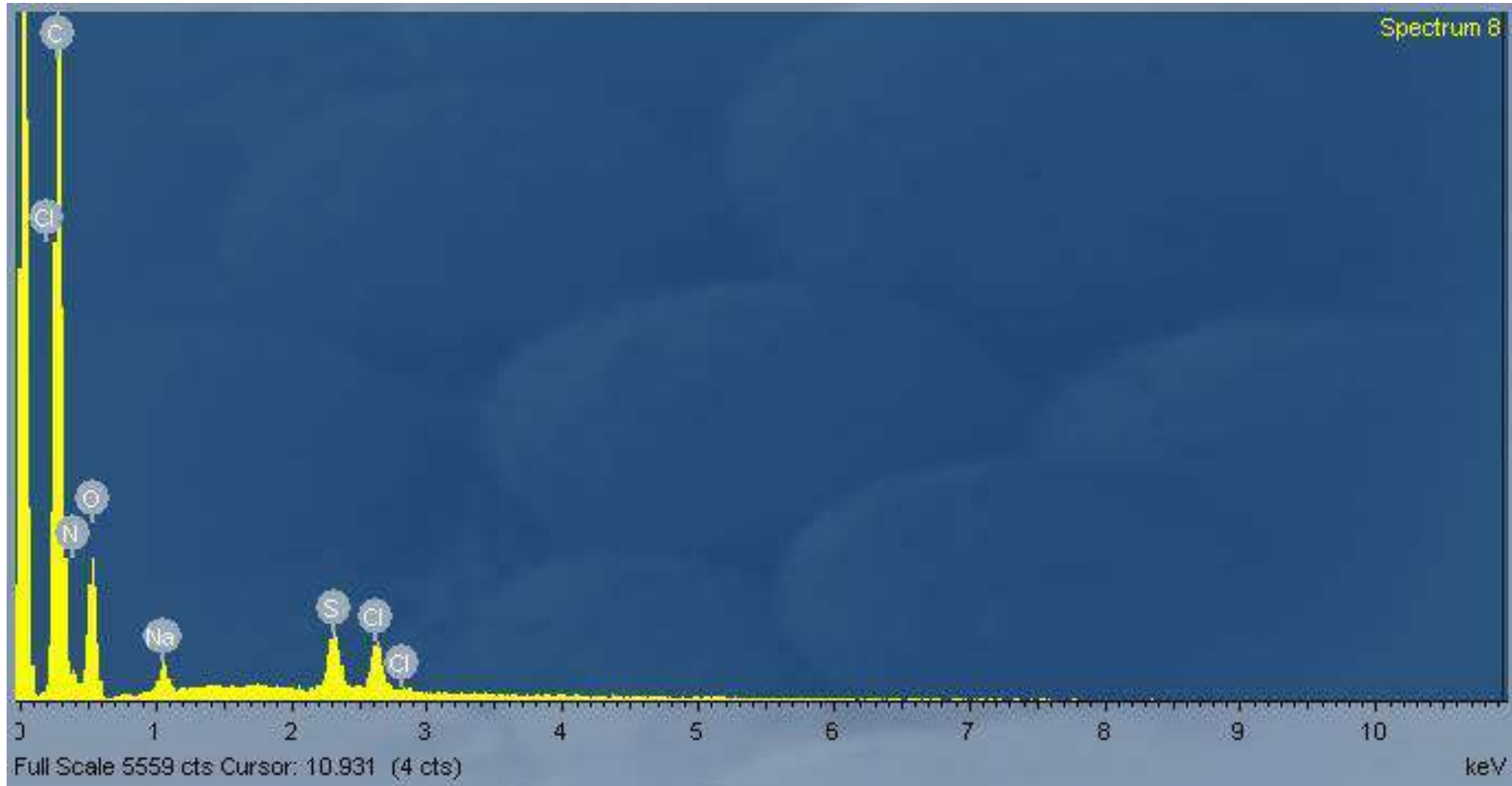
at pH=7.0 (25mM Tris-HCl), 37°C, overnight.

Metal	CPM
Control	54
Zn ⁺²	54
Ni ⁺²	320
Co⁺²	558
Mn ⁺²	360

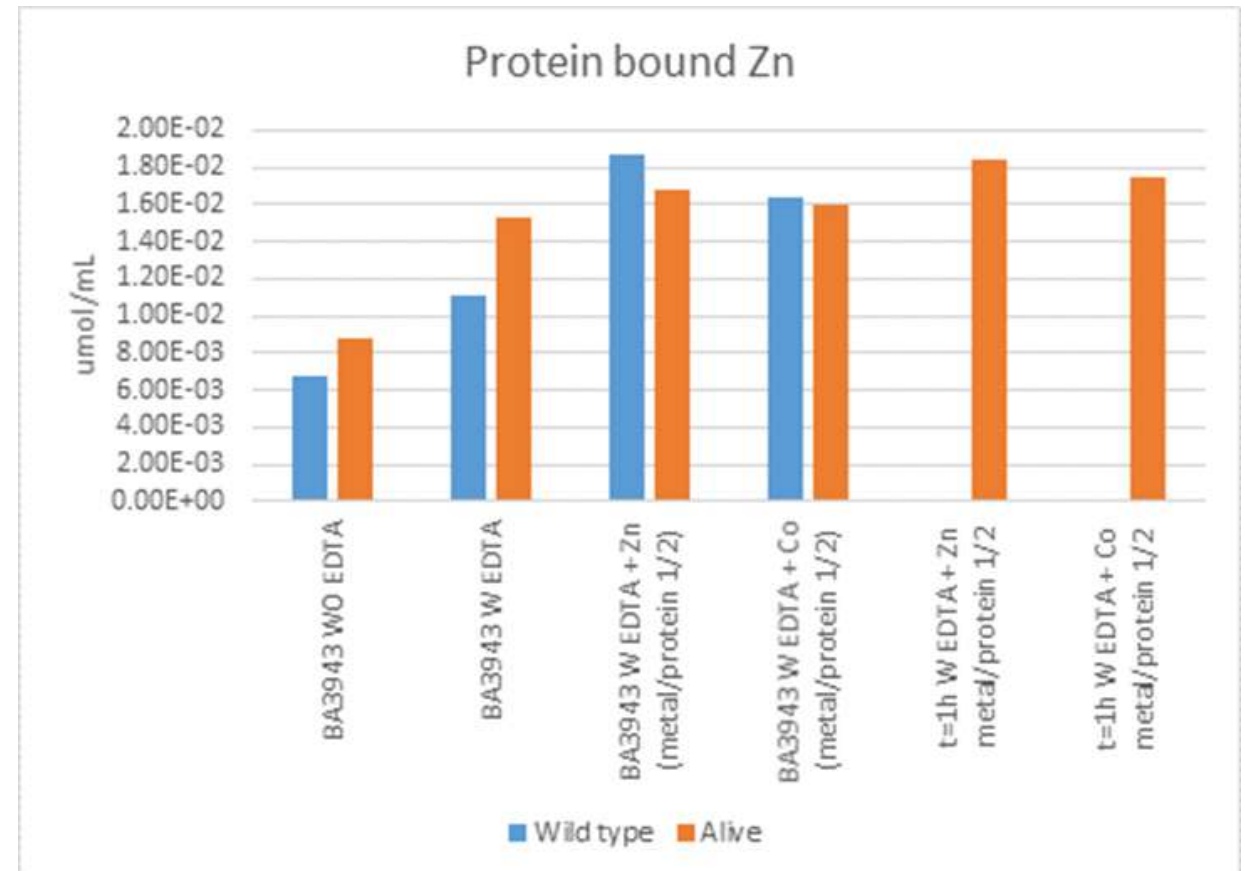
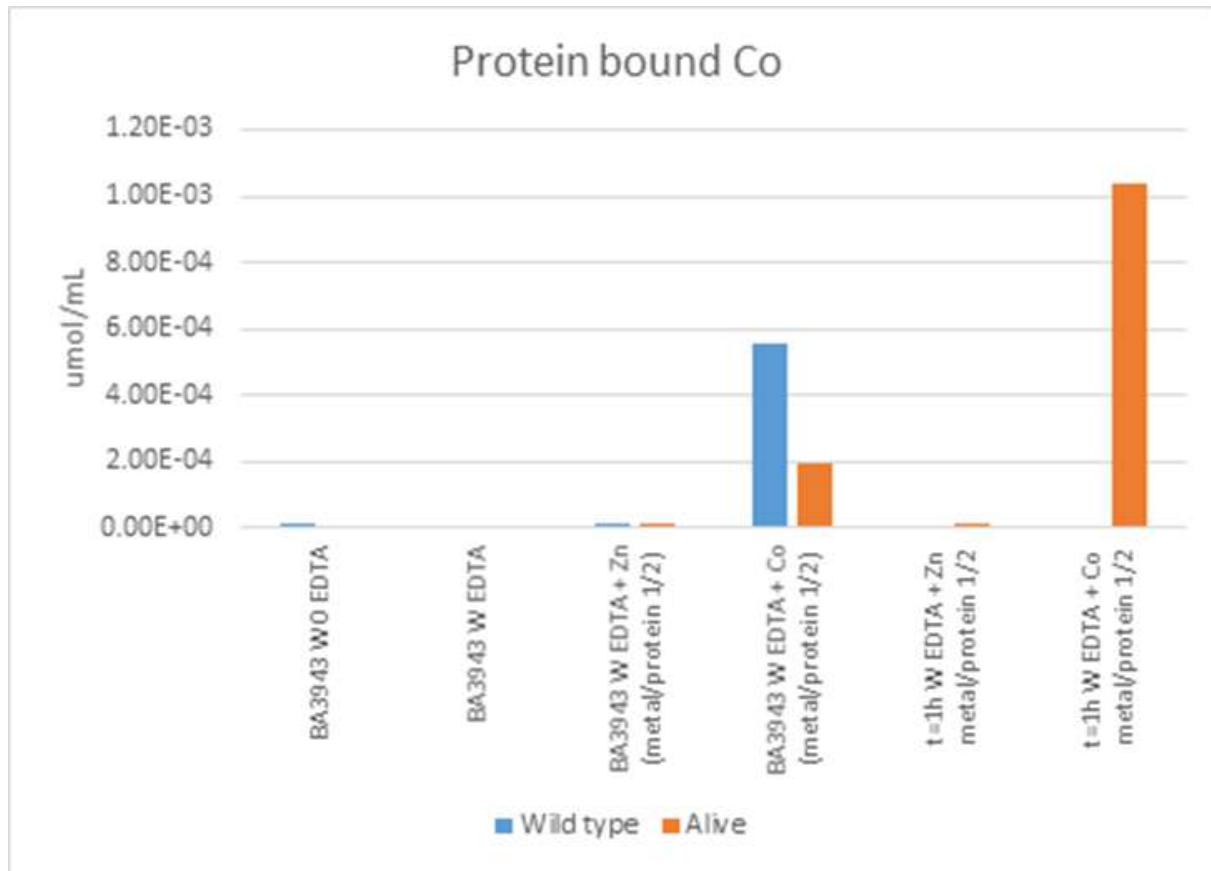
Table 4. Activity of BA3943 N94D V95D A183R in the presence of a variety of metals (1mM f.c.)

EDS energy dispersive spectroscopy

+ CoCl_2 during the growth of the cell culture.
+ ZnCl_2 during the growth of the cell culture.



ICP-MS for metal presence

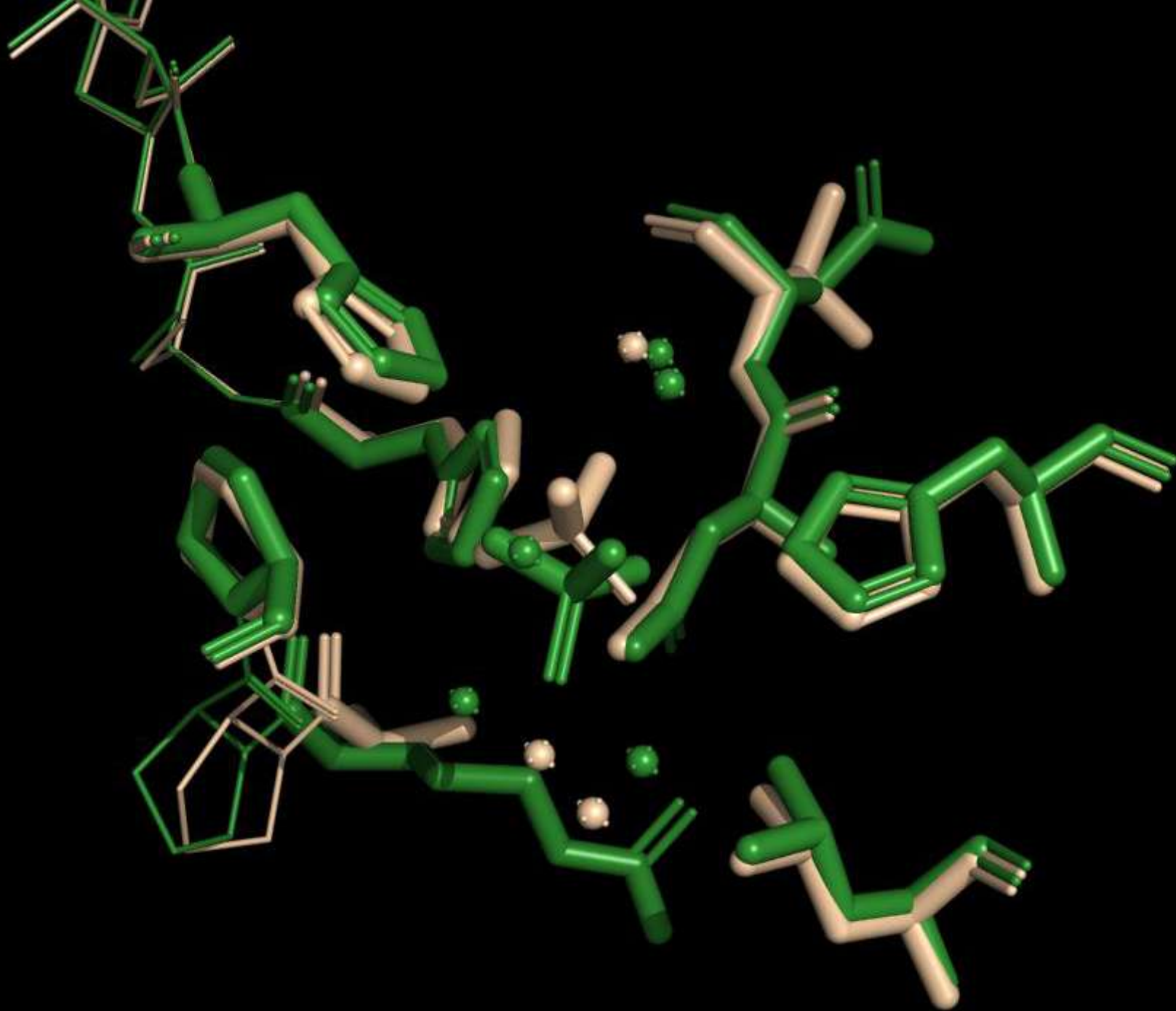


Is 2-hyp metal independent?

- α -carbon hydroxylation independent of metal presence
- BC1974 mutant (metal binding D to N) also hydroxylated

Protein	CPM	% 2-Hyp	Structural Data
Control	53	-	-
BA3943 wt	50	8,67 \pm 1,697	1.1 Å (submitted)
BA3943 N94D	55	4,05 \pm 1,923	1.5 Å (submitted)
BA3943 N94D V95D	50	6,39 \pm 2,960	-
BA3943 N94D V95D A183R	558	25 \pm 6,397	1.7 Å (submitted)
BA3943 N94D V95D A183K	48	ND	-
BA3943 N94D V95D A183R P185G	?	ND	1.2 Å
BA3943 N94D V95D A183R Hyp deletion	58	ND	0.9 Å
BA3943 N94D A183R	145	ND	-
BA3943 N94D V95N A183R	42	ND	Unreliable dataset
BA3943 N94D V95N A183R L235A	-	ND	-
BA3943 N94D V95N A183R L235D	-	ND	-

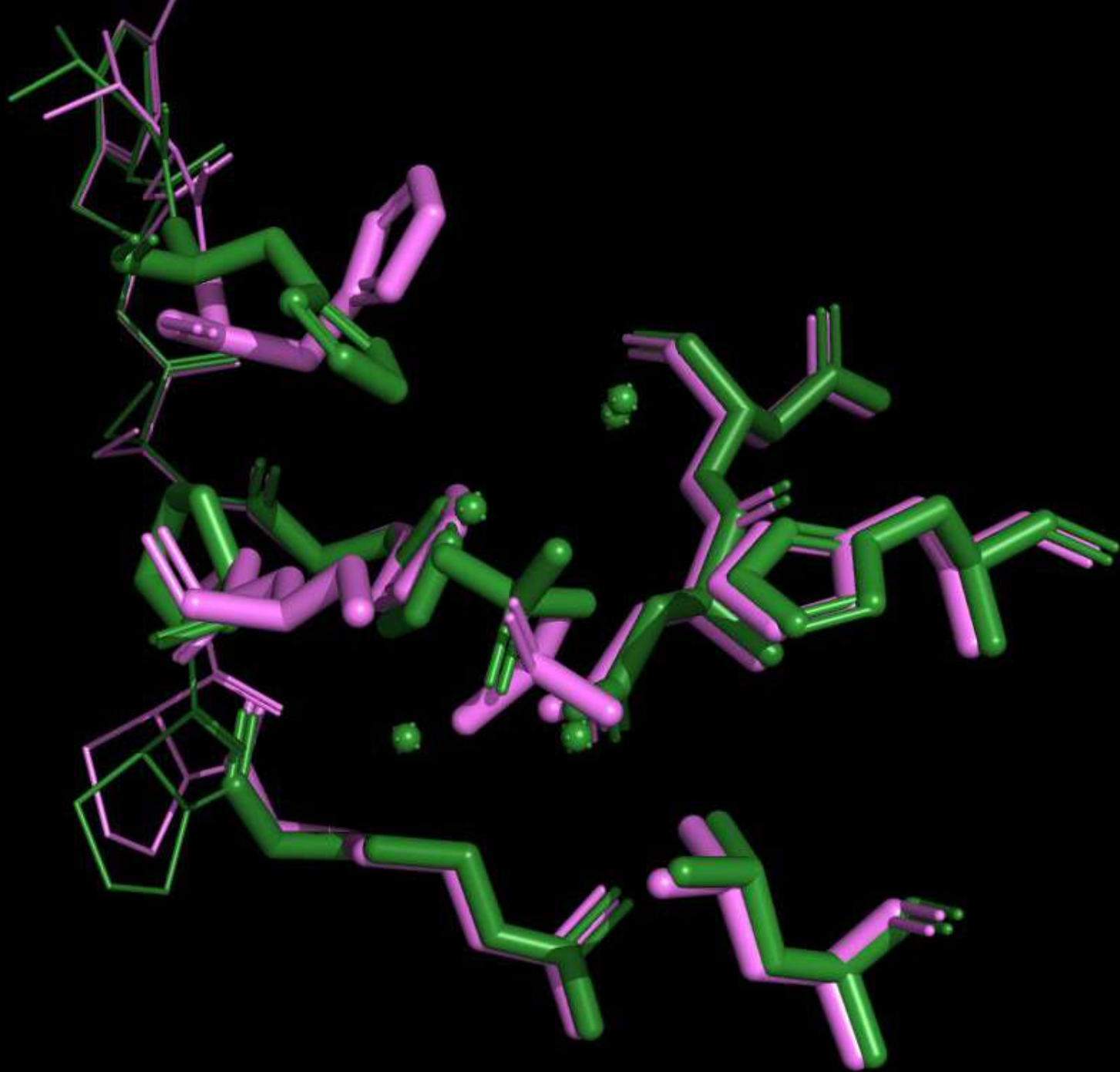
WT vs
alive



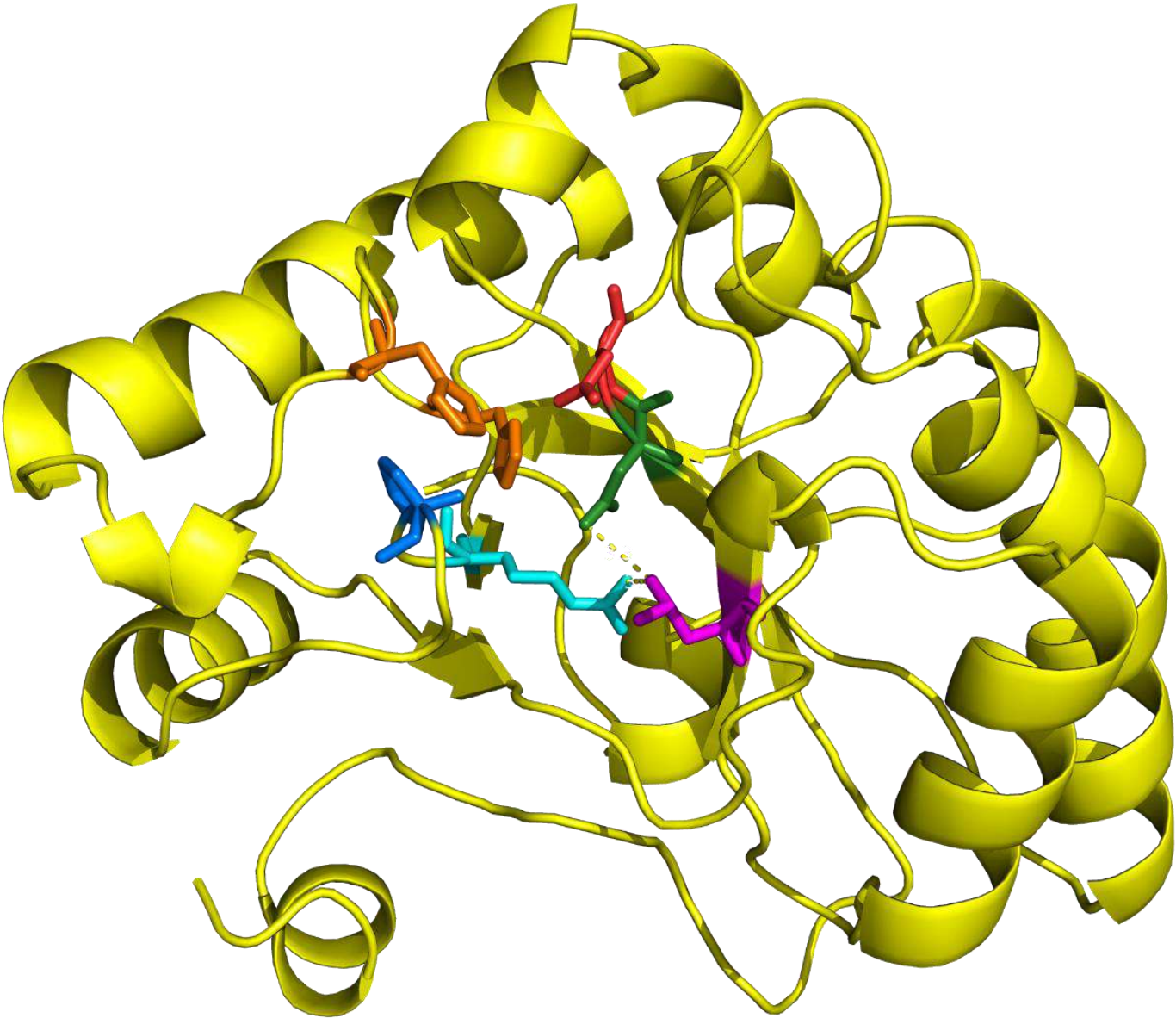
Alive

VS

P-G



Protein	CPM	% 2-Hyp	Structural Data
Control	53	-	-
BA3943 wt	50	8,67 \pm 1,697	1.1 Å (submitted)
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BA3943 N94D V95N A183R	42	ND	Unreliable dataset
BA3943 N94D V95N A183R L235A	-	ND	-
BA3943 N94D V95N A183R L235D	-	ND	-



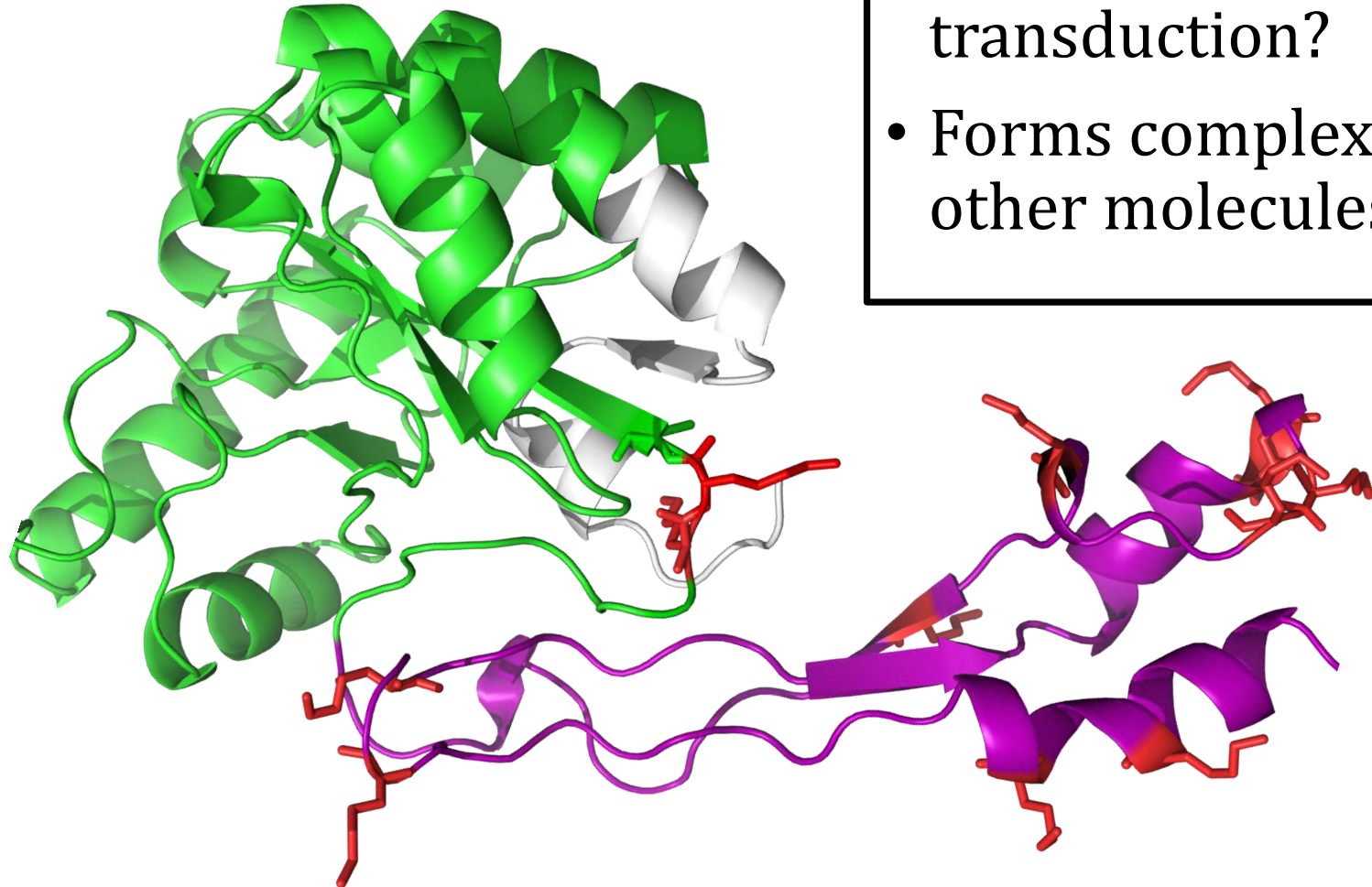
A mysterious N-terminal domain

N-terminus

NodB

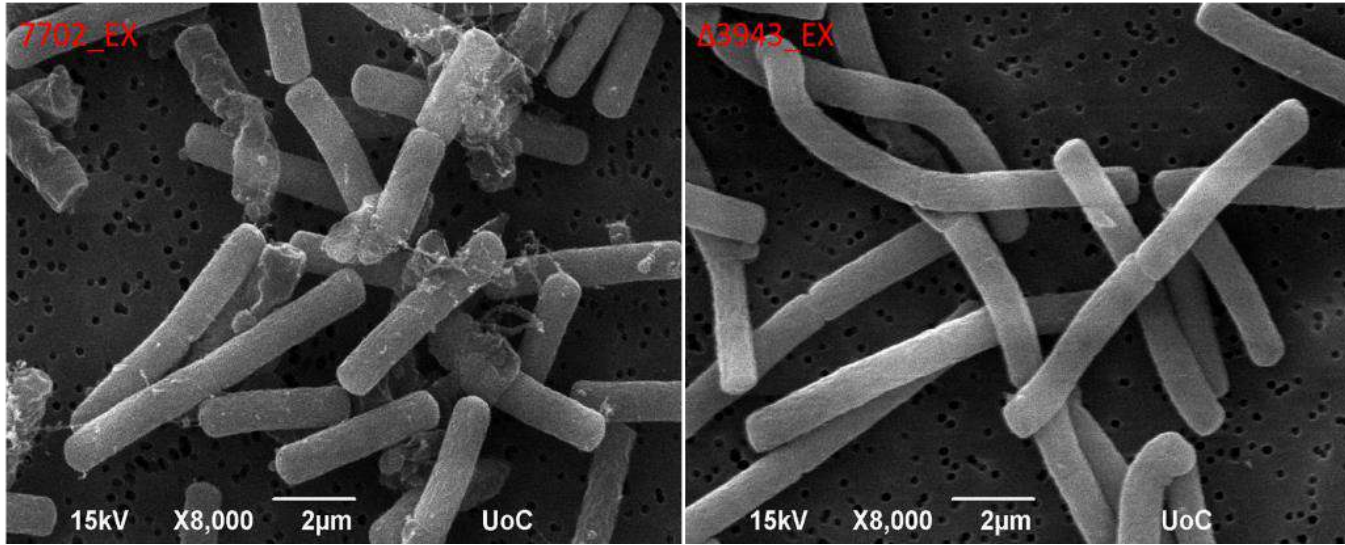
C-terminus

>Bacillus_cereus_26-111_99%
>Bacillus_sp.TD41_26-111_99%
>Streptococcus_pneumoniae_26-111_99%
>Bacillus_thuringiensis_26-111_99%
>Bacillus_wiedmannii_26-111_95%
>Bacillus_weihenstephanensis_26-111_92%
>Bacillus_mycoides_26-111_80%
>Bacillus_gaemokensis_26-111_78%
>Bacillus_bingmayongensis_27-111_80%
>Bacillus_cytotoxicus_27-111_73%
>Geobacillus_thermodenitrificans_37-121_66%
>Geobacillus_stearothermophilus_45-129_66%
>Geobacillus_icigianus_46-130_66%
>Parageobacillus_thermoglucoisidans_46-130_67%
>Geobacillus_thermoleovorans_45-129_65%
>Geobacillus_kaustophilus_45-129_65%

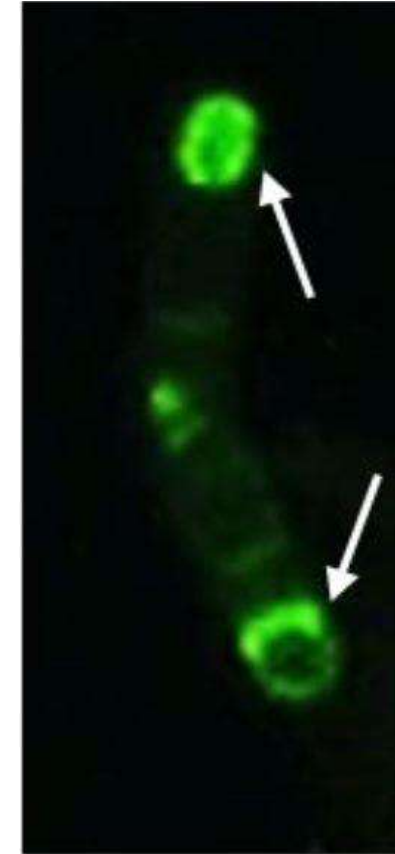


- 85 residues long
- 12 of them are Lysines.
- Involved in signal transduction?
- Forms complex with other molecules?

In vivo experiments performed by A. Tomatsidou



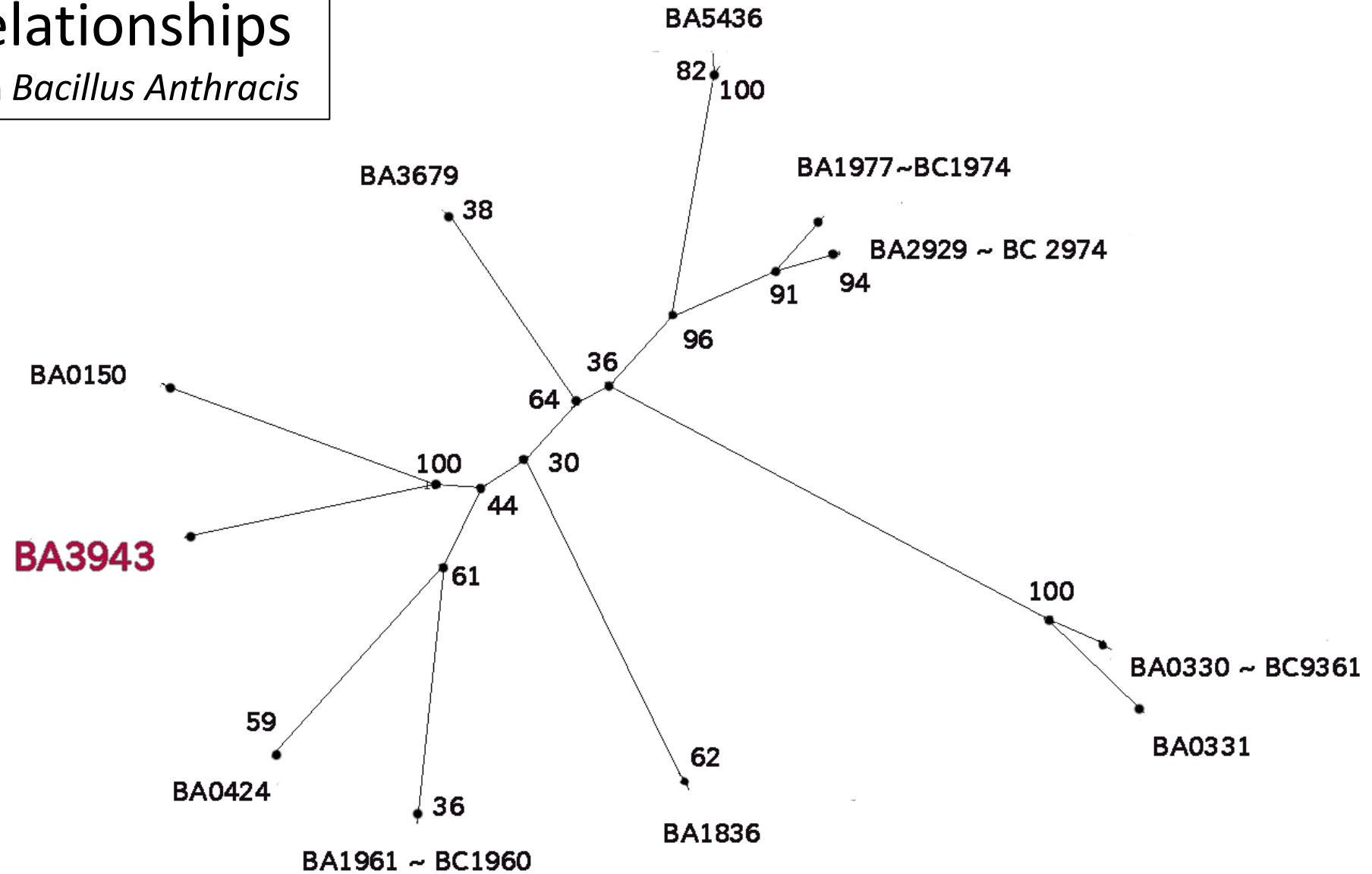
Scanning Electron Microscopy of 7702 and $\Delta ba3943$ mutant strain during exponential phase of growth.



Localization of BA3943 using GFP

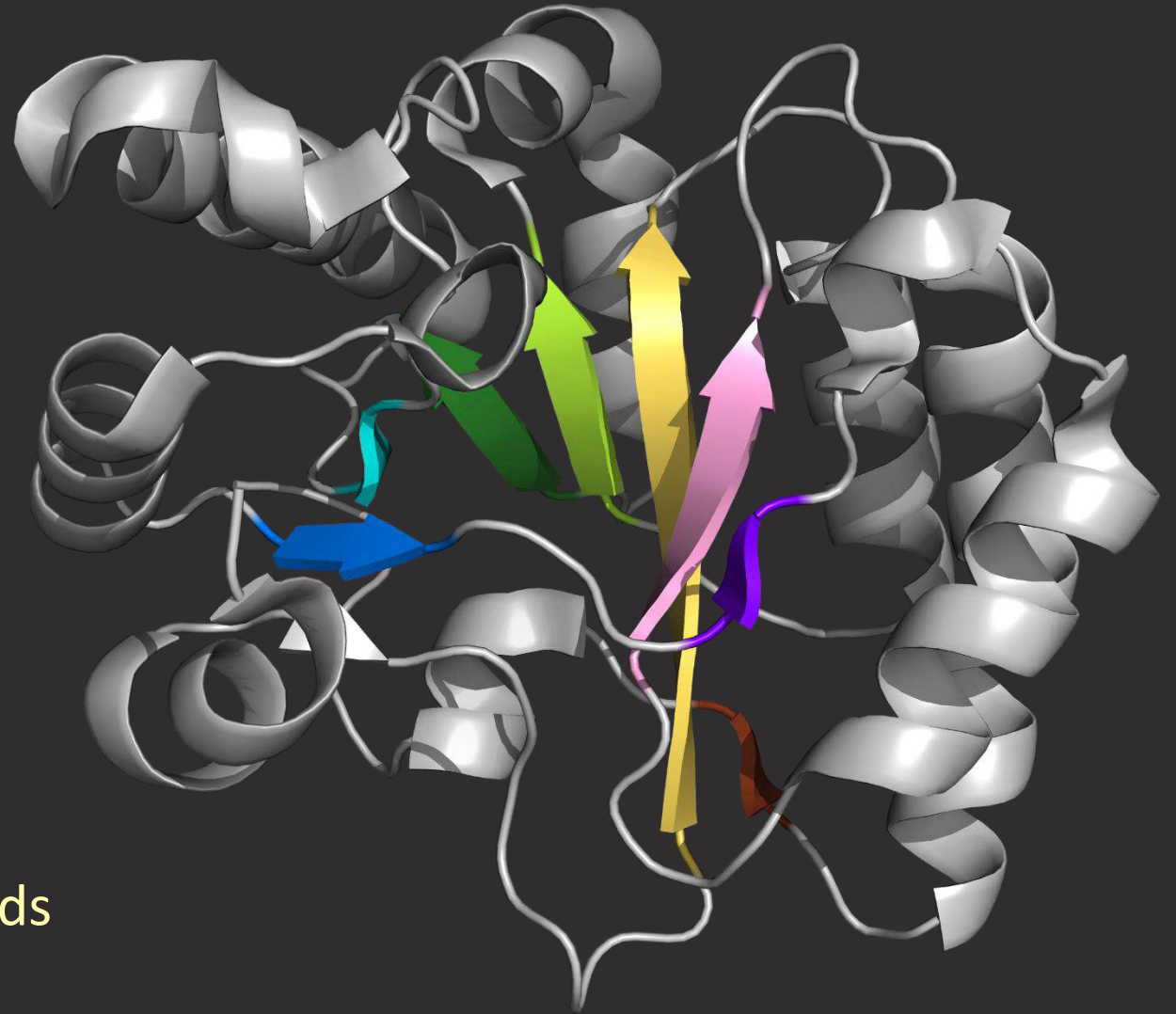
Evolutionary Relationships

between putative PDA's in *Bacillus Anthracis*

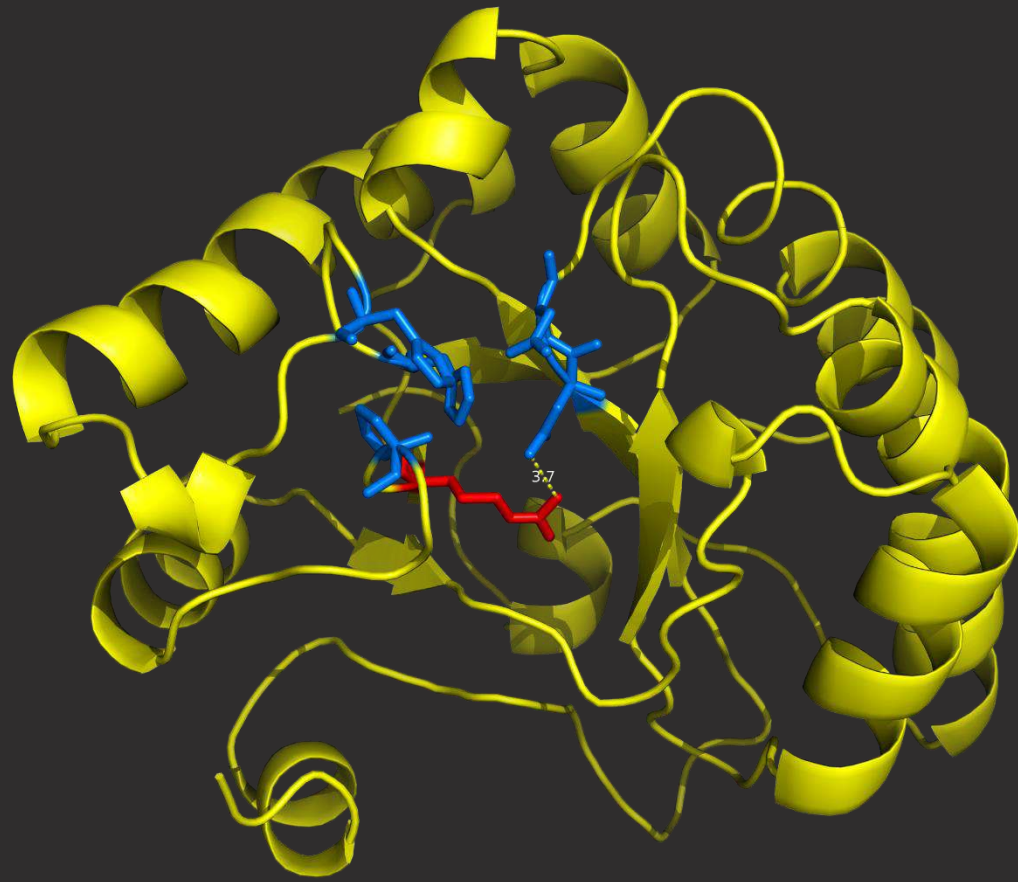


The NodB domain

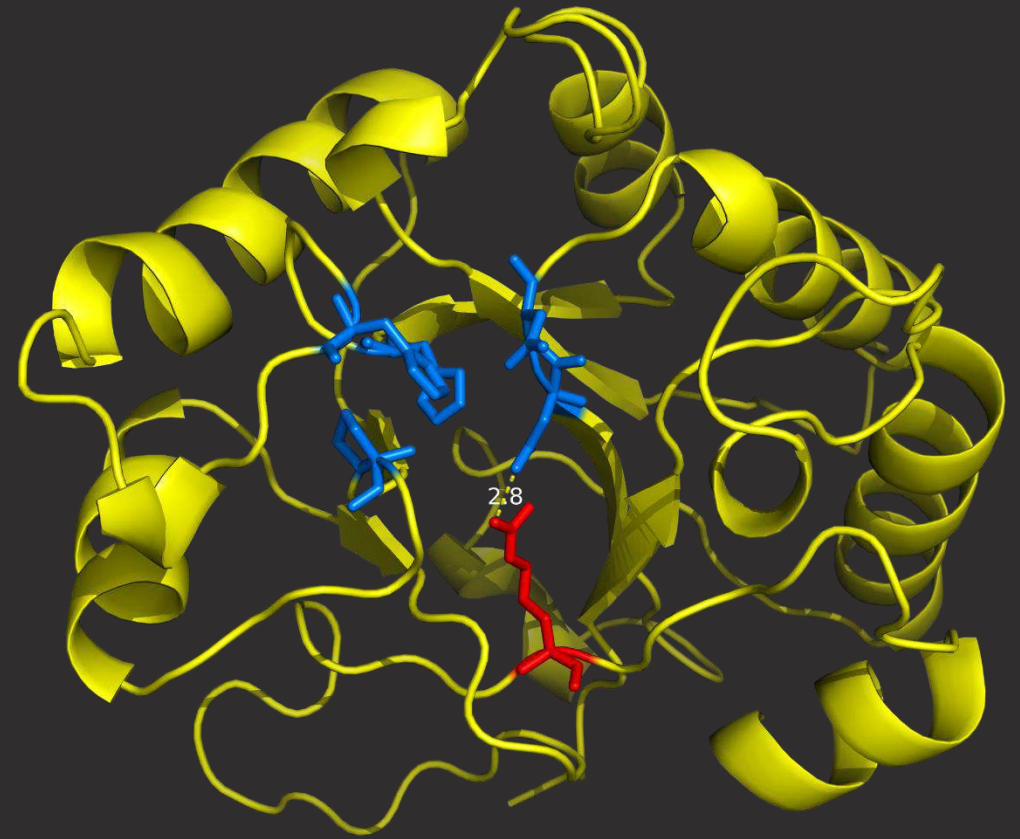
- Distorted α/β barrel
- 7 or 8 parallel β -strands form the hydrophobic core
- Equal no. of α -helices surround it
- Active site at the C-termini of β -strands



In Ba0330 the **arginine** occupies the same site, but originates from a different residue.



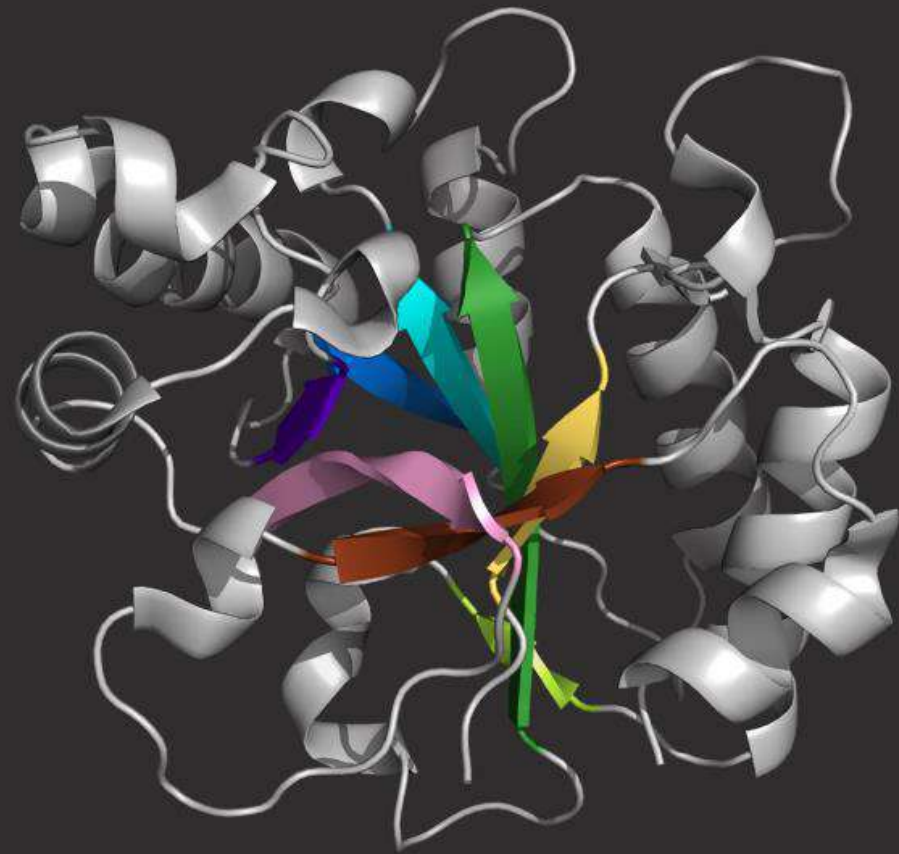
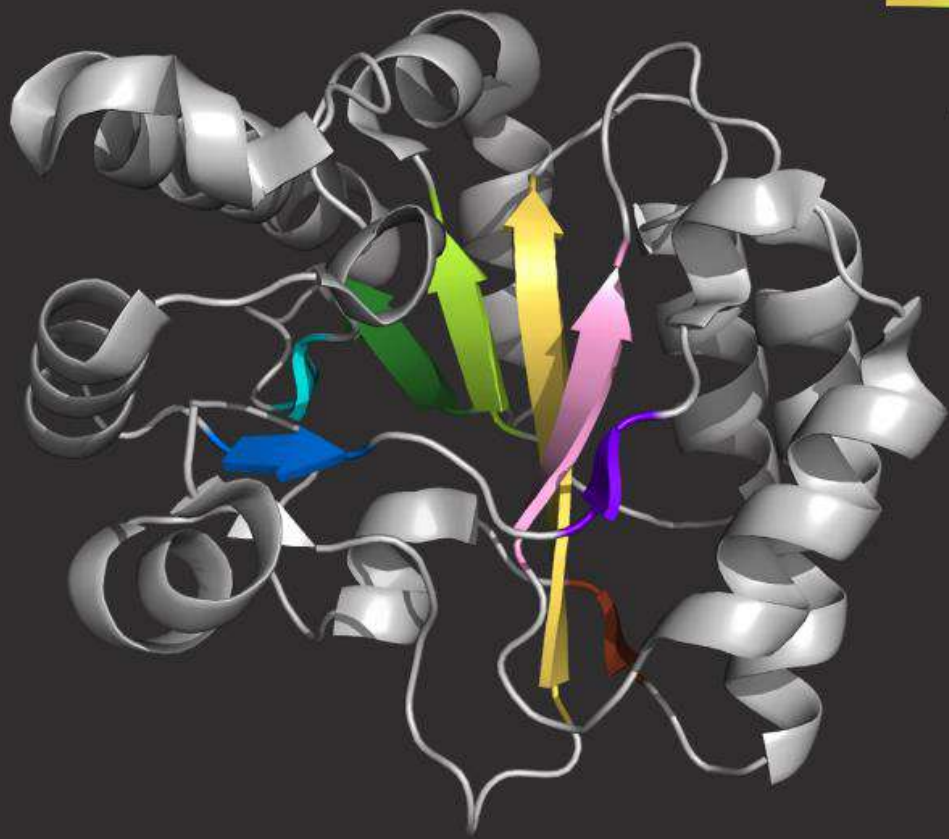
BC1960



BA0330

1 α/β
barrel

2 distinct
topologies

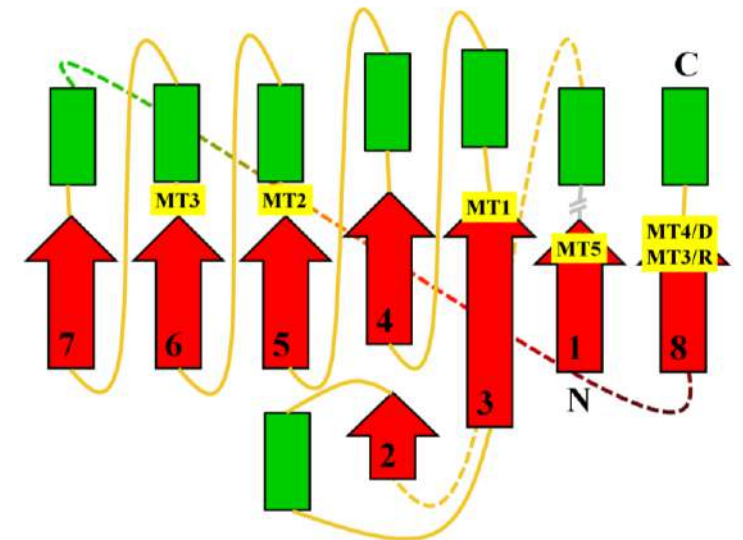
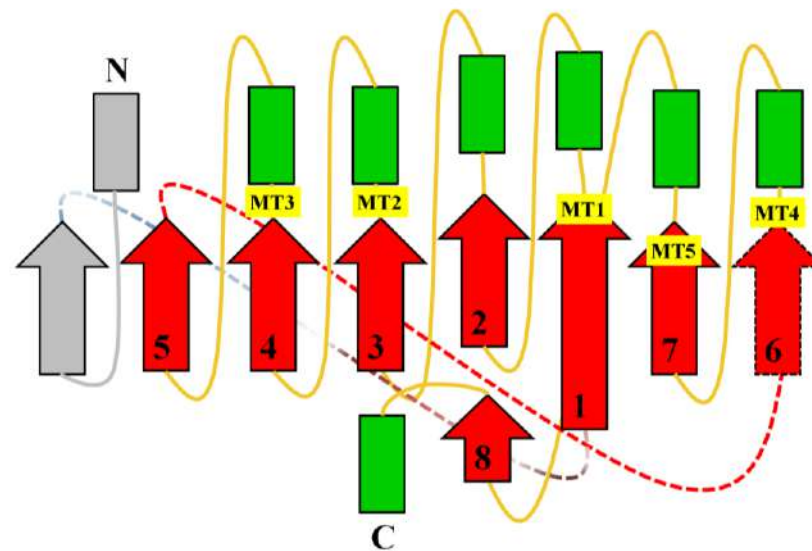
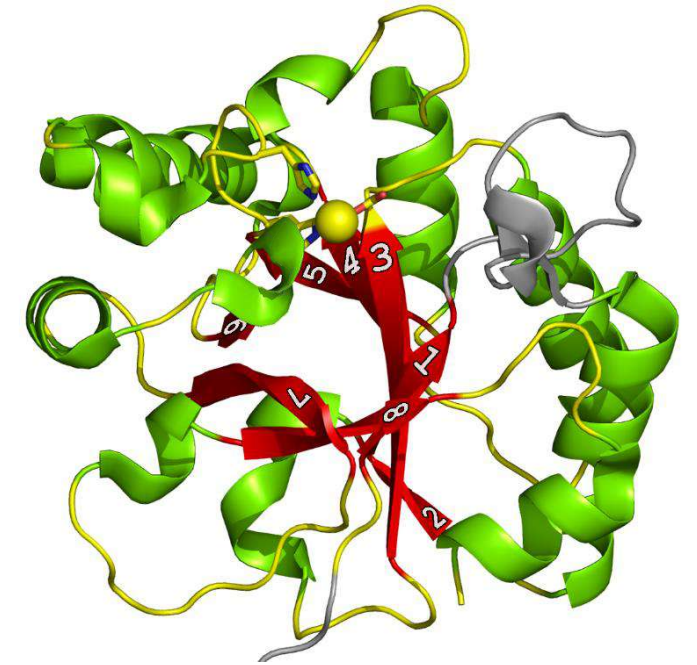
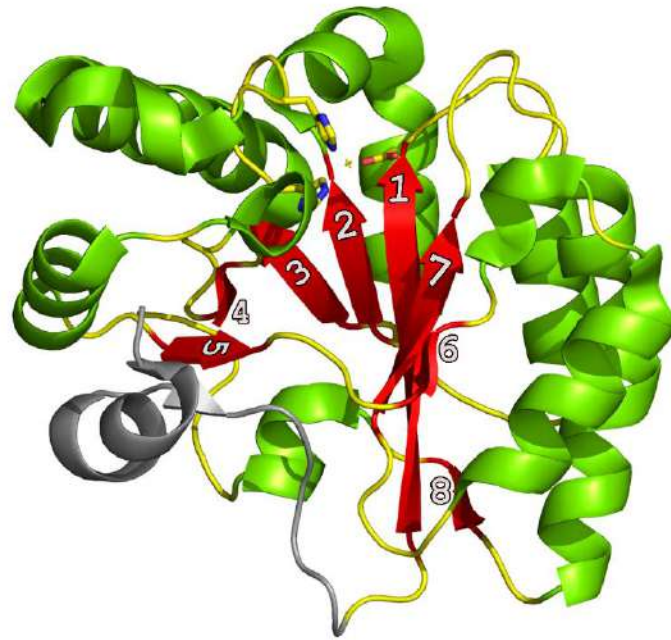


β -strand sequence of the central barrel



2 topological diagrams

Same basic fold



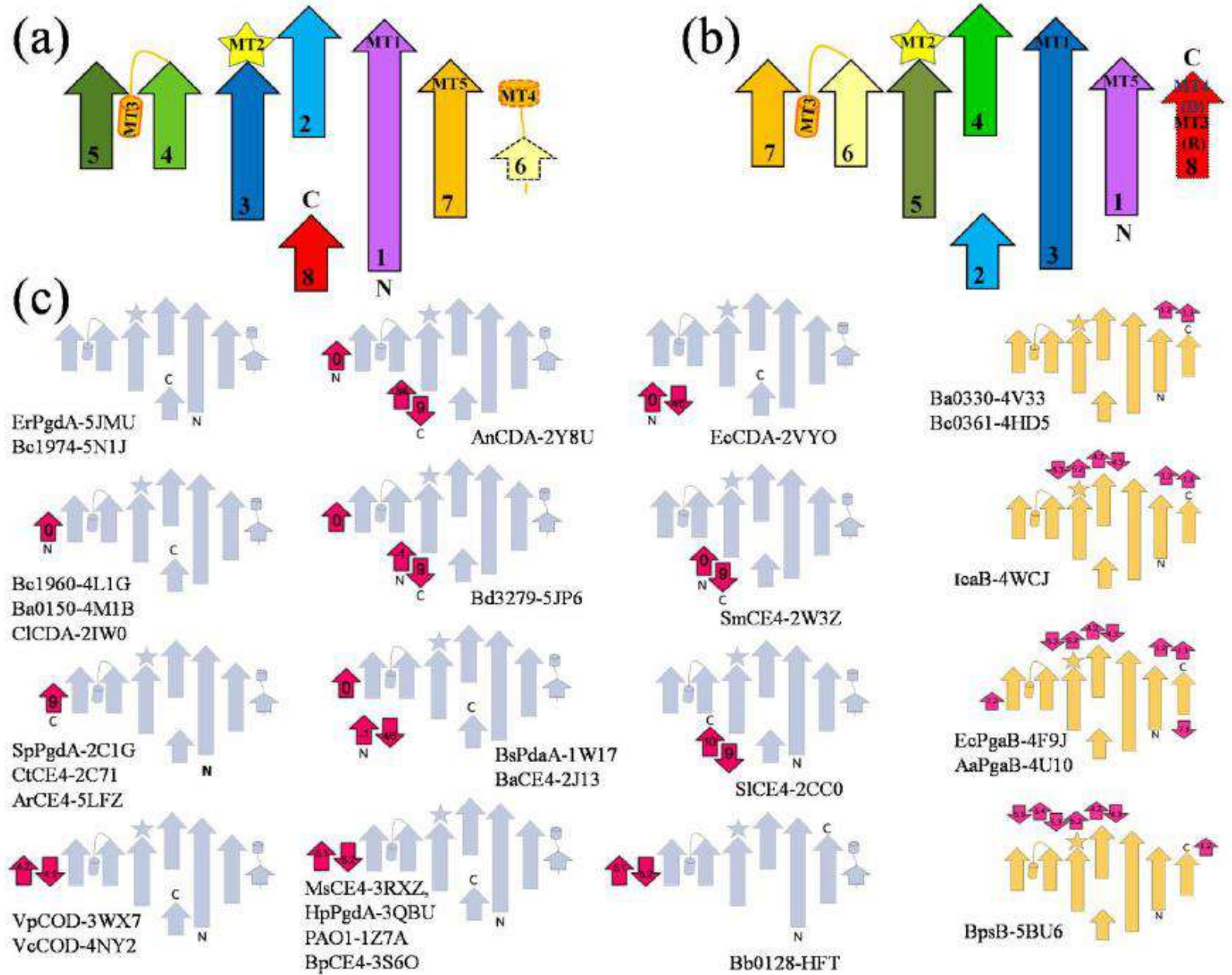
Collaboration with Prof. Fadouloglou

Categorisation of every known NodB structure in the CE4 family

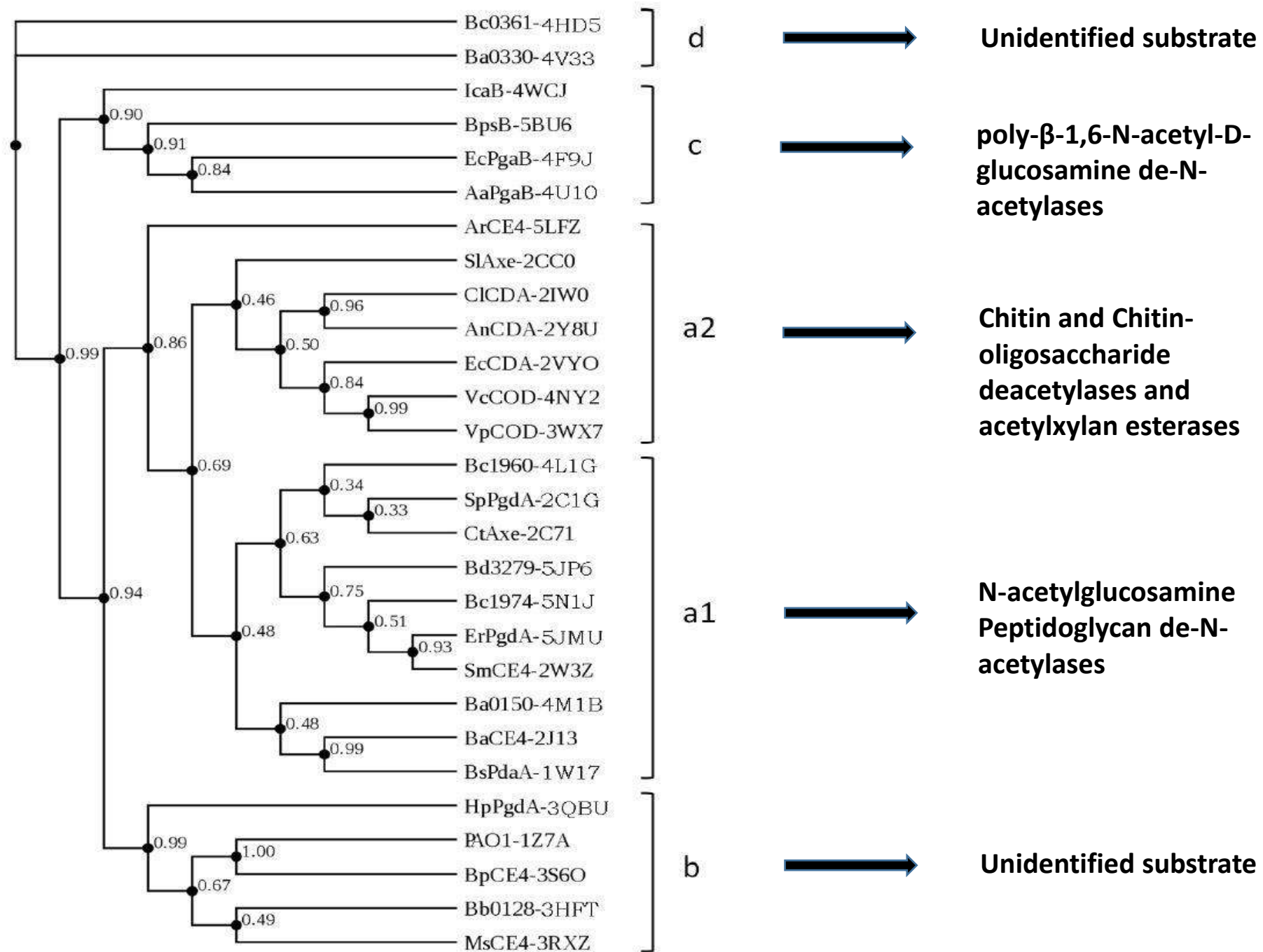
Topology a

Topology b

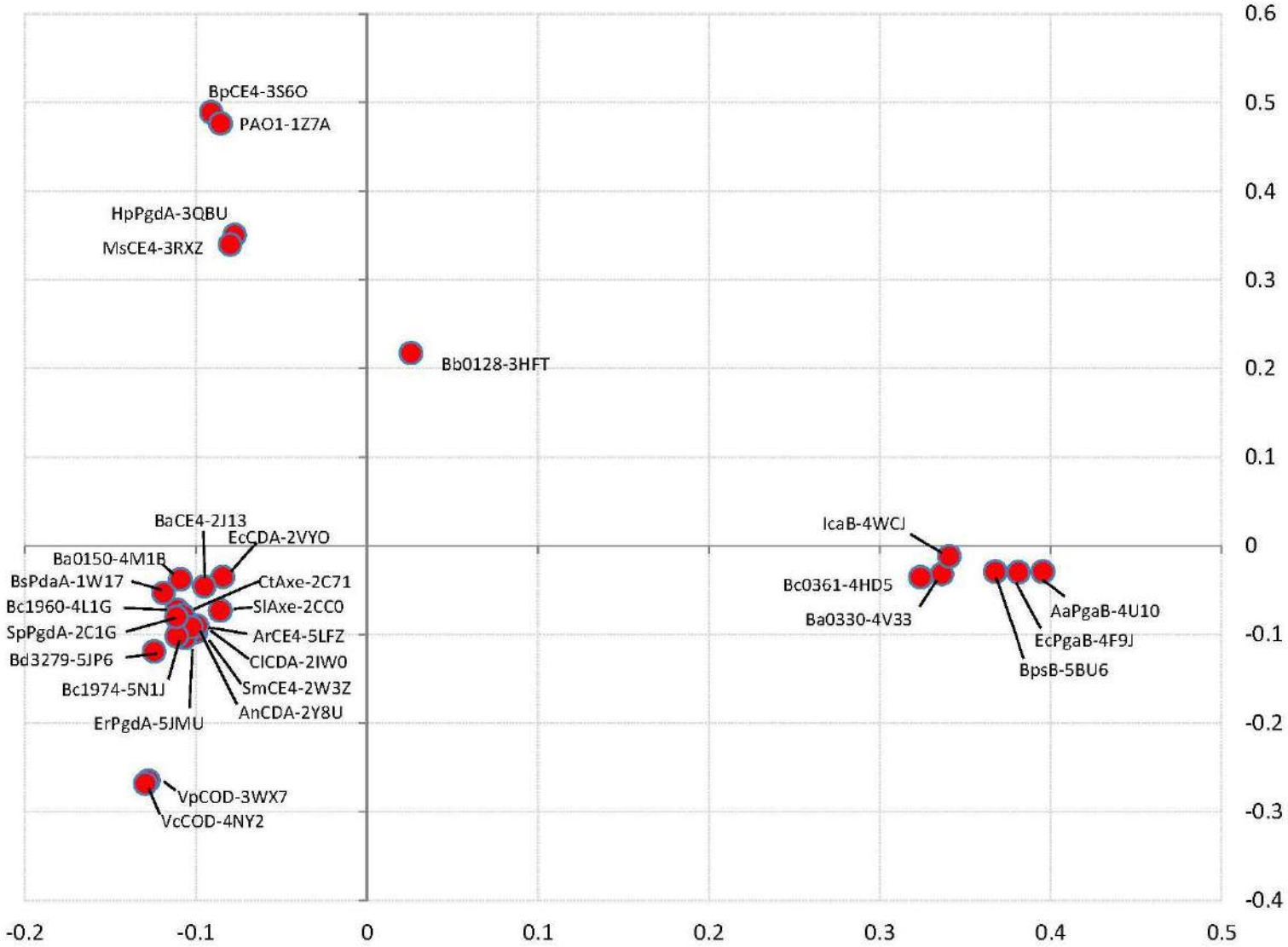
Extra α -helices



Collaboration with Prof. Fadouglou

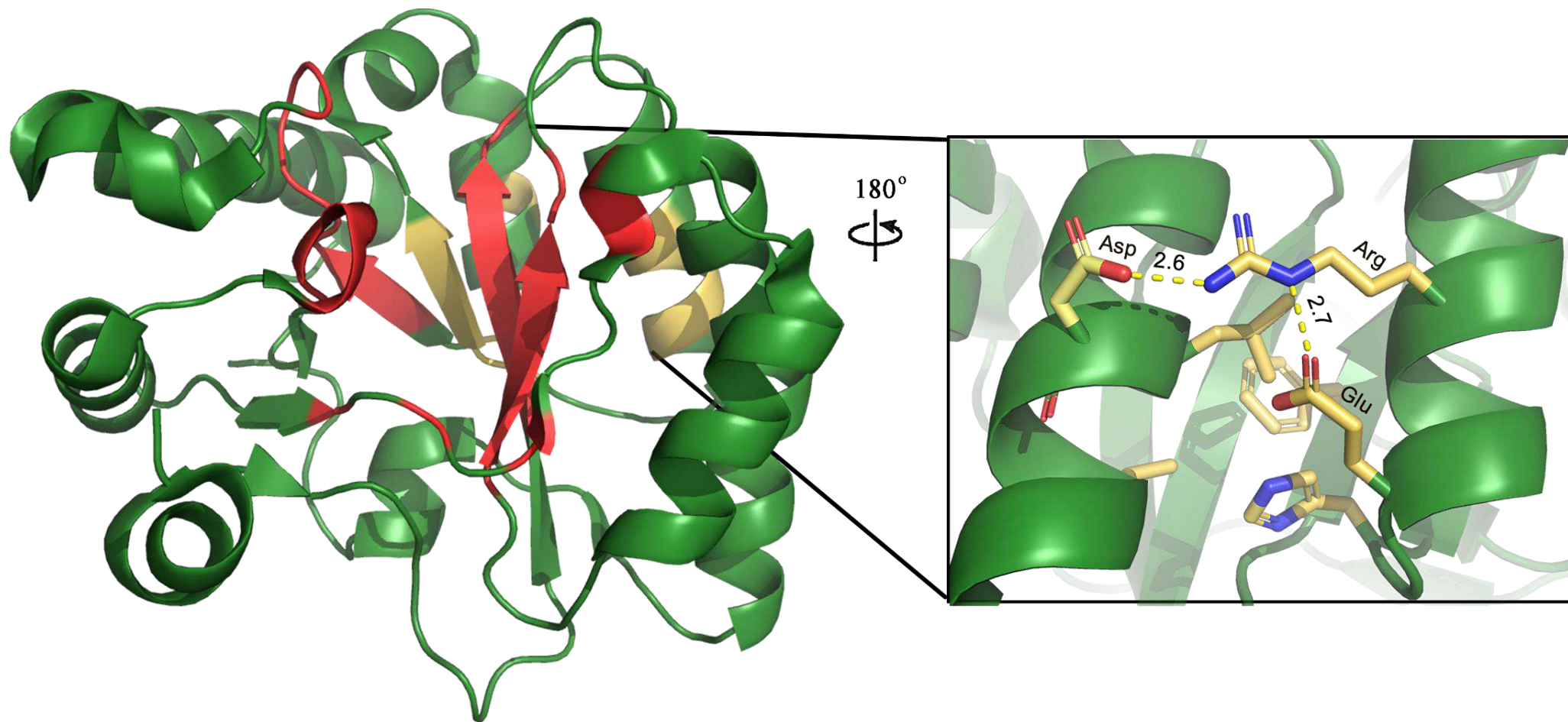


Correspondence Analysis



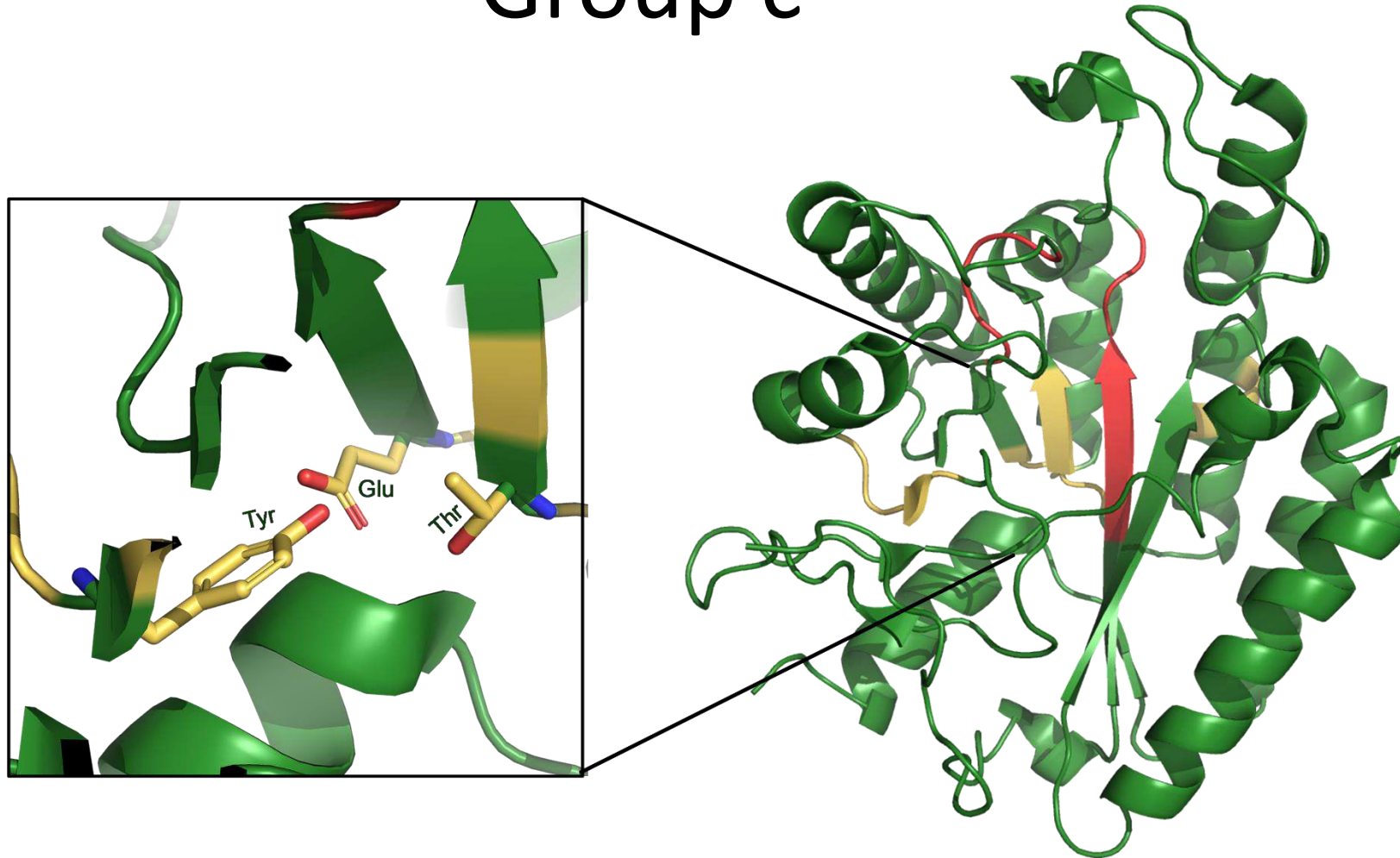
New emerging motifs

Group a

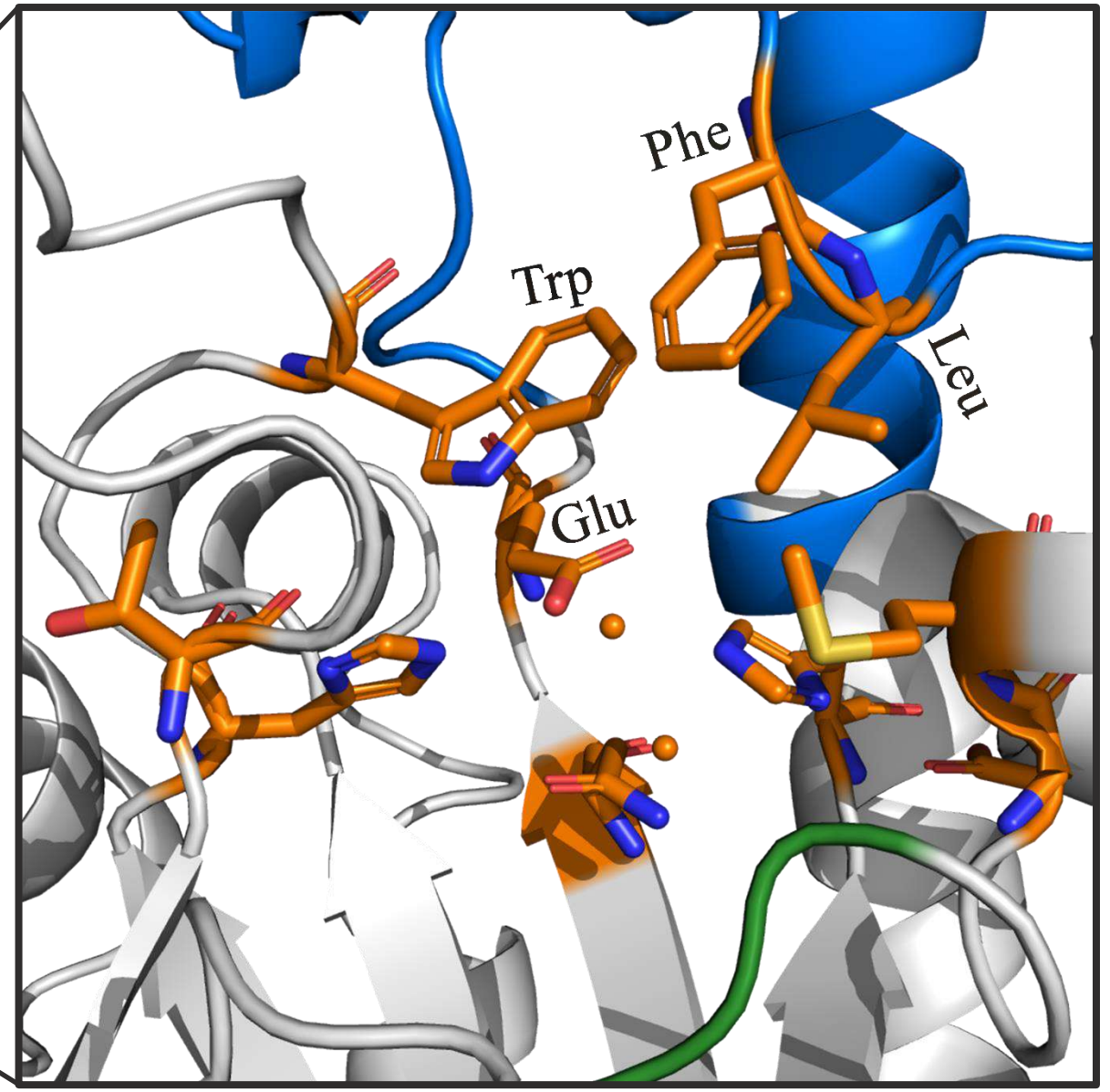
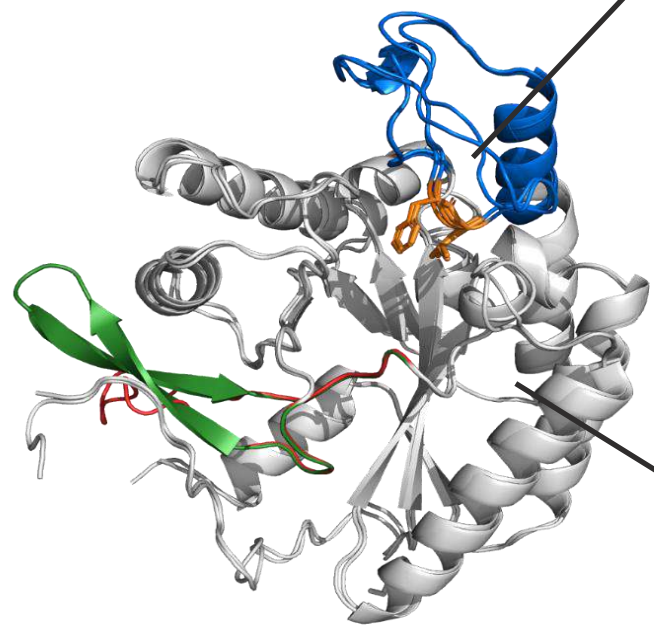


New emerging motifs

Group c

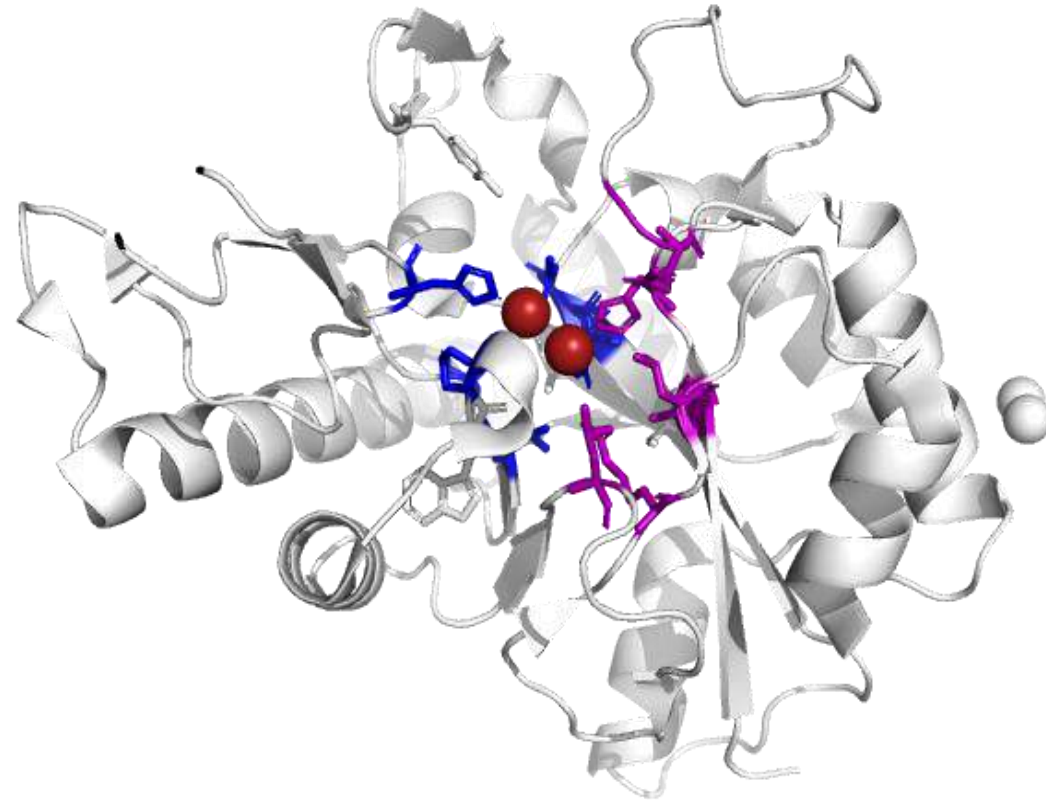


Group c



Group d

Second metal binding site



Conclusions

There is more than one way to make the same fold



New classification of the NodB domain on the basis of phylogeny relationships and topology or structural modifications. We show that this classification is correlated to the diverse functionalities of NodB.



Sequence and structural variations in the NodB core give rise to variations in substrate specificity.

Thank you for your time and patience!

SPECIAL THANKS TO

Prof. M. Kokkinidis

Prof. V. Fadouloglou

Prof. V. Bouriotis

Dr. Stratos Mylonas

Dr. Anastasia Tomasidou

Ntina Kotsifaki

+ a big list of students and lab colleagues



ΕΛΙΔΕΚ.
Ελληνικό Ίδρυμα
Έρευνας & Καινοτομίας



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