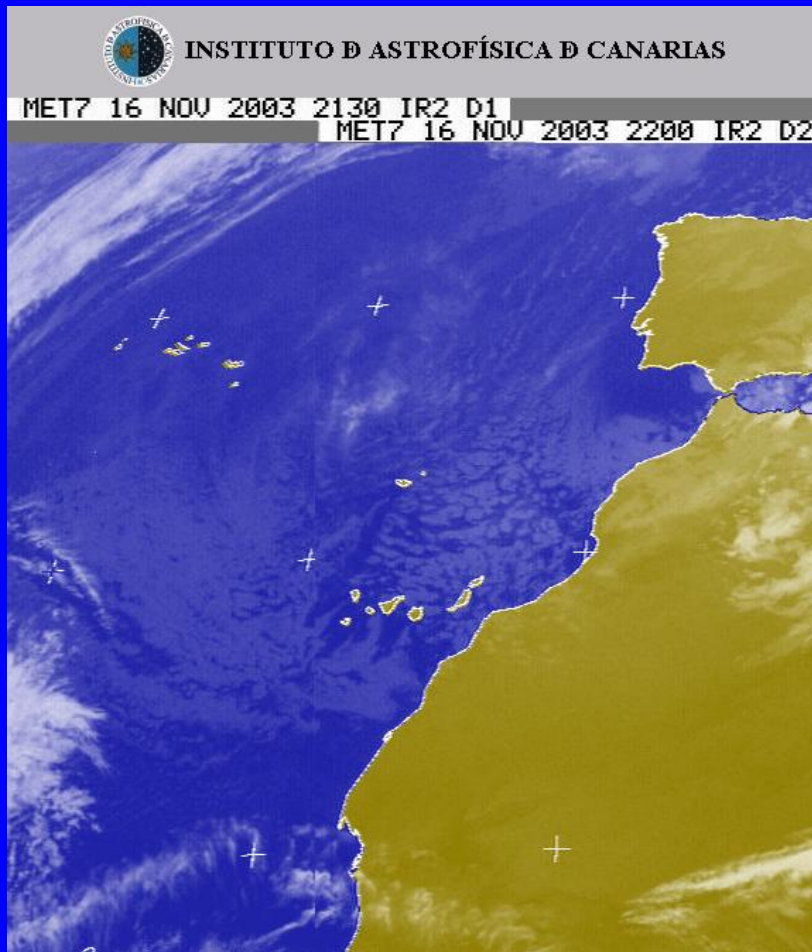


PH1: conformational disease due to P11L + I244T : is chemoprophylaxis possible?



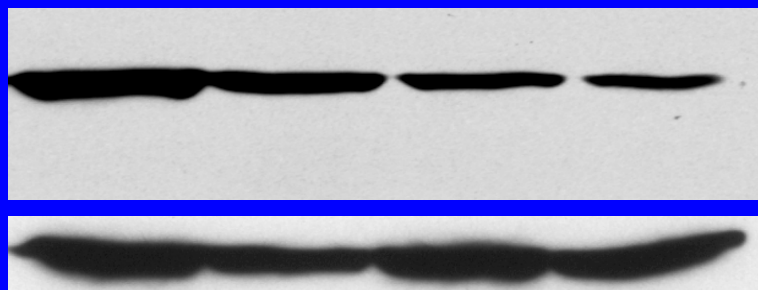
- Aut. Recessive: mutations @ *AGXT*
- 15 families (18 patients)
- at least 8 from Gomera (pop. 17,000)
- > 90% Ile244Thr (e7, T853C)
Pro11Leu, i1ins., C386T,
Ile340Met
- “founder effect”

Ile244Thr AGXT

- In vitro mutagenesis
- Expressed in Cos cells:
enzymatic activity similar to wt !!!
- Mutation + polymorphism (Pro11Leu):
loss of enzymatic activity
protein instability
degradation / aggregation of misfolded
protein?

AGXT*LTM is expressed and not mistargeted

AGXT *L *T *LTM

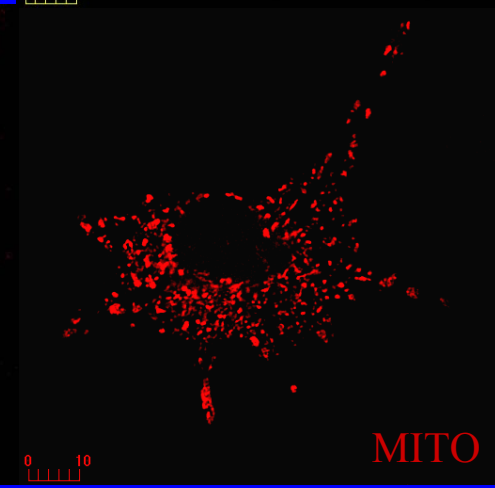
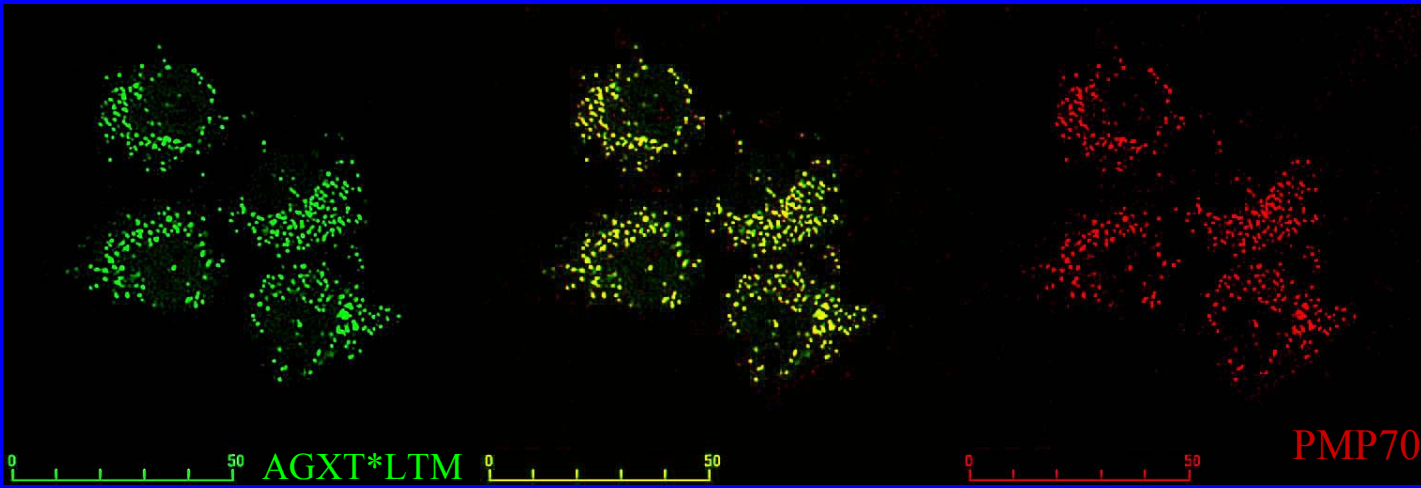
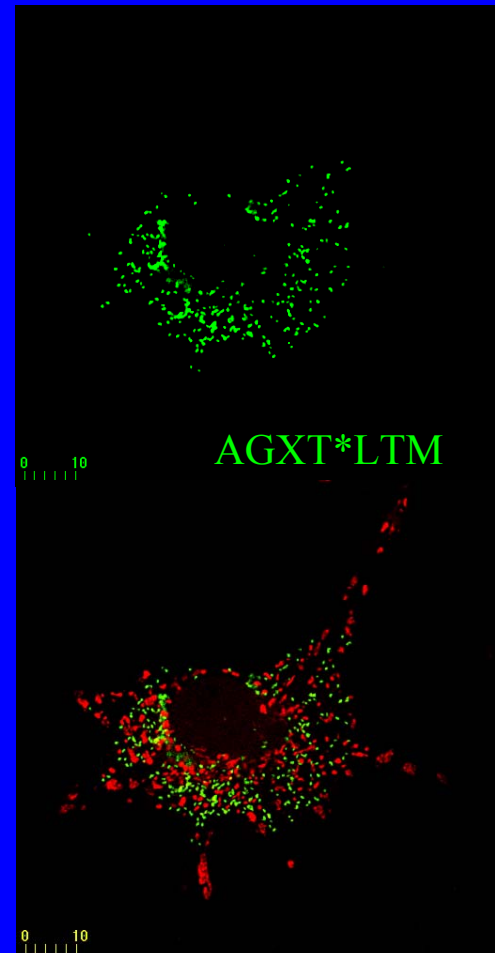


Cos

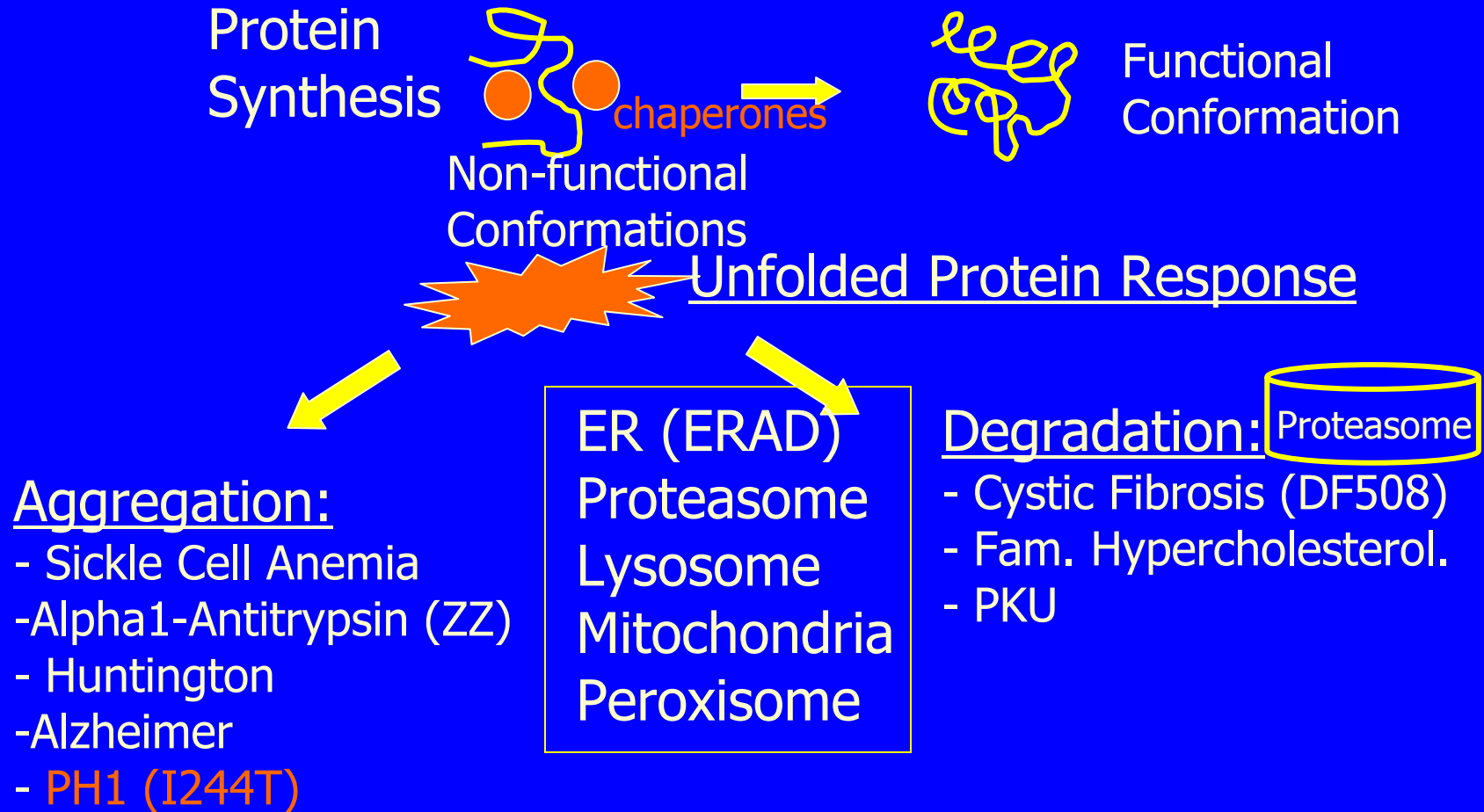
- FLAG

- actin

MDYKDDDDK



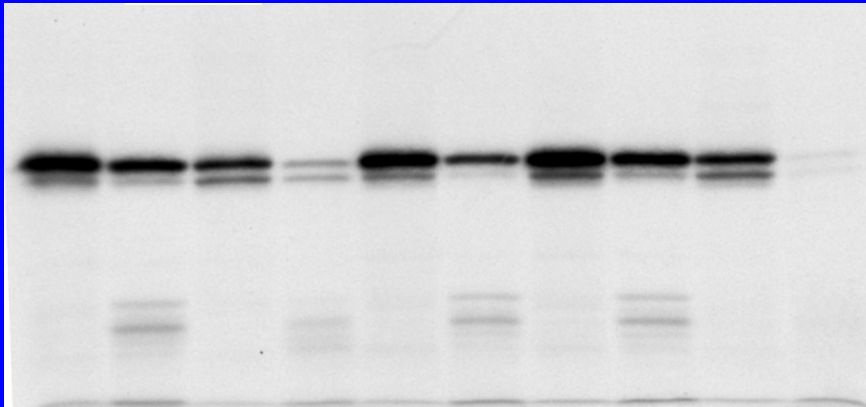
Conformational Diseases



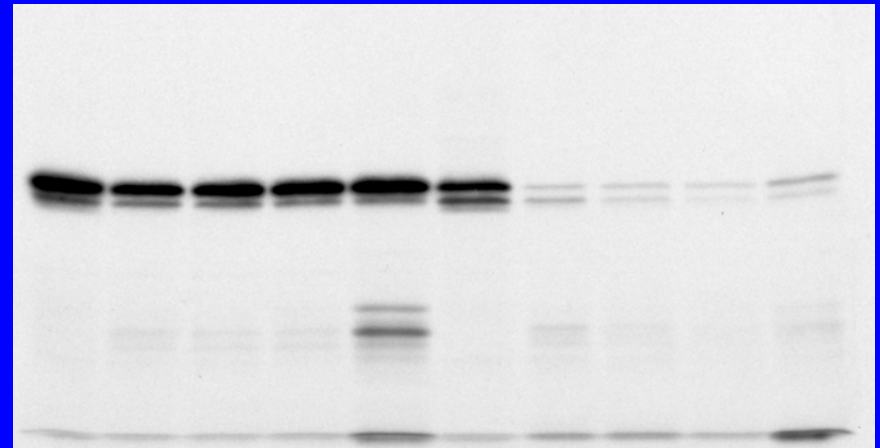
Limiting proteolysis:

in vitro TxTn proteins show differential accessibility

AGXT		*L		*T		*M		*LTM	
-	+	-	+	-	+	-	+	-	+



AGXT					*LTM				
0'	5'	10'	15'	20'	0'	5'	10'	15'	20'

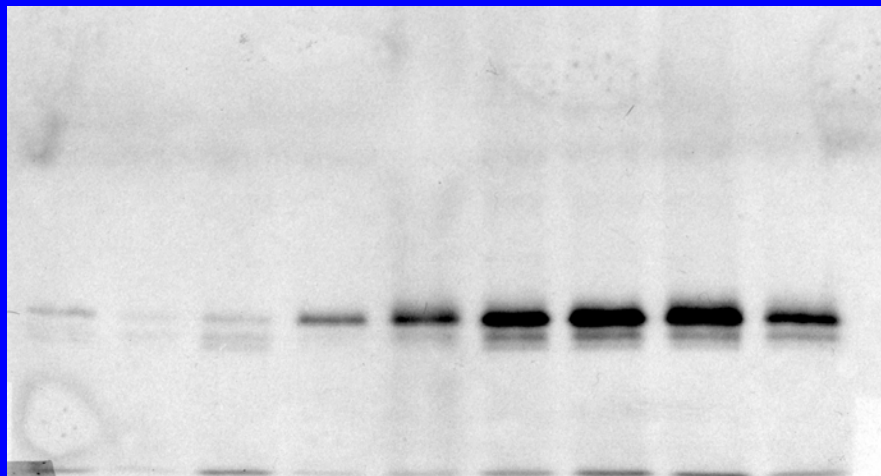


+ = trypsin 12 ug/ml 10 min

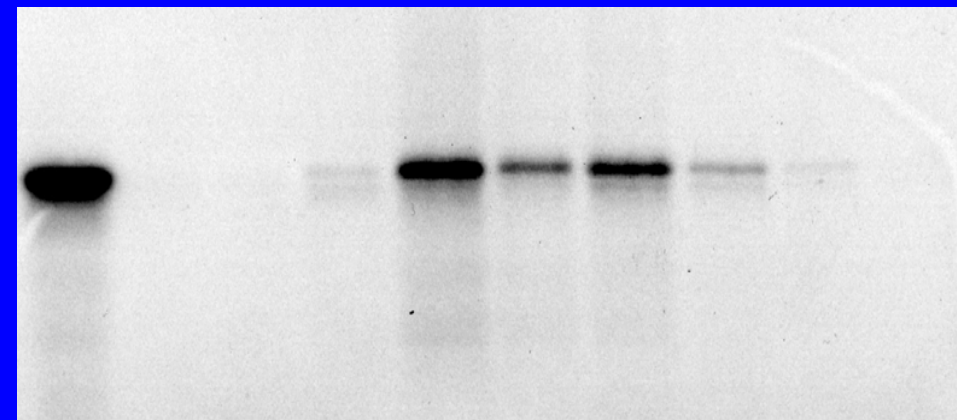
variations in limiting trypsinization most likely reflect conformation differences, # P11L has a significant conformation effect, which is magnified in *LTM

Chaperone interaction: Hsc70, Hsp90, immunoprecipitation

anti-Hsc70			anti-Hsp90			hrs.
AGXT	*LTM		*LTM			
2 4 6	2 4 6	2 4 6	2 4 6	2 4 6	2 4 6	



TnT	anti-Hsp90								
	AGXT			*LTM			*LTM/geld		
	2	4	6	2	4	6	2	4	6hrs



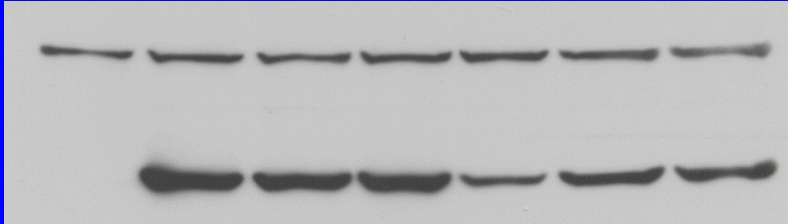
AGXT*LTM: solubility problem

Cos

AGXT

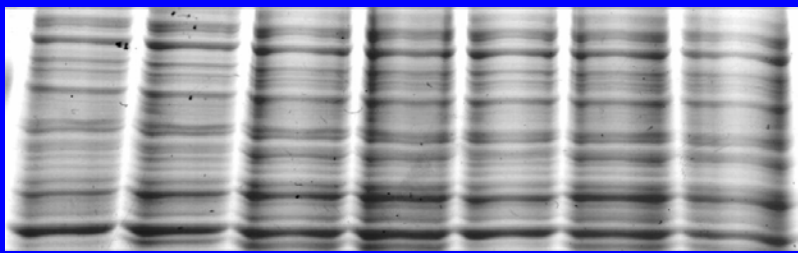
*LTM

Cos .05%T 1%S 6M U .05%T 1%S 6M U



Ku70

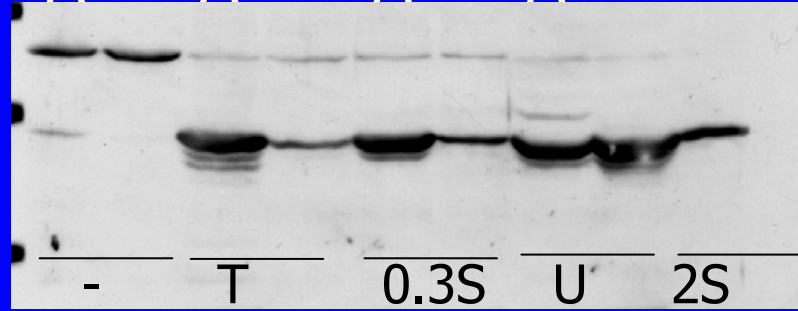
AGXT



Insect cells
baculovirus

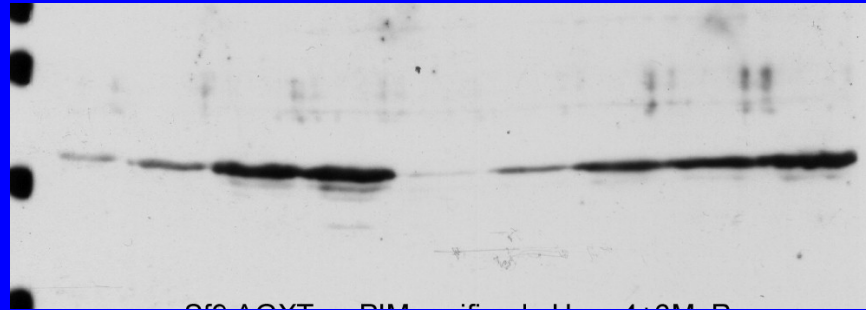
His-tagged protein
NTA purification
(Urea)

A * A * A * A * *

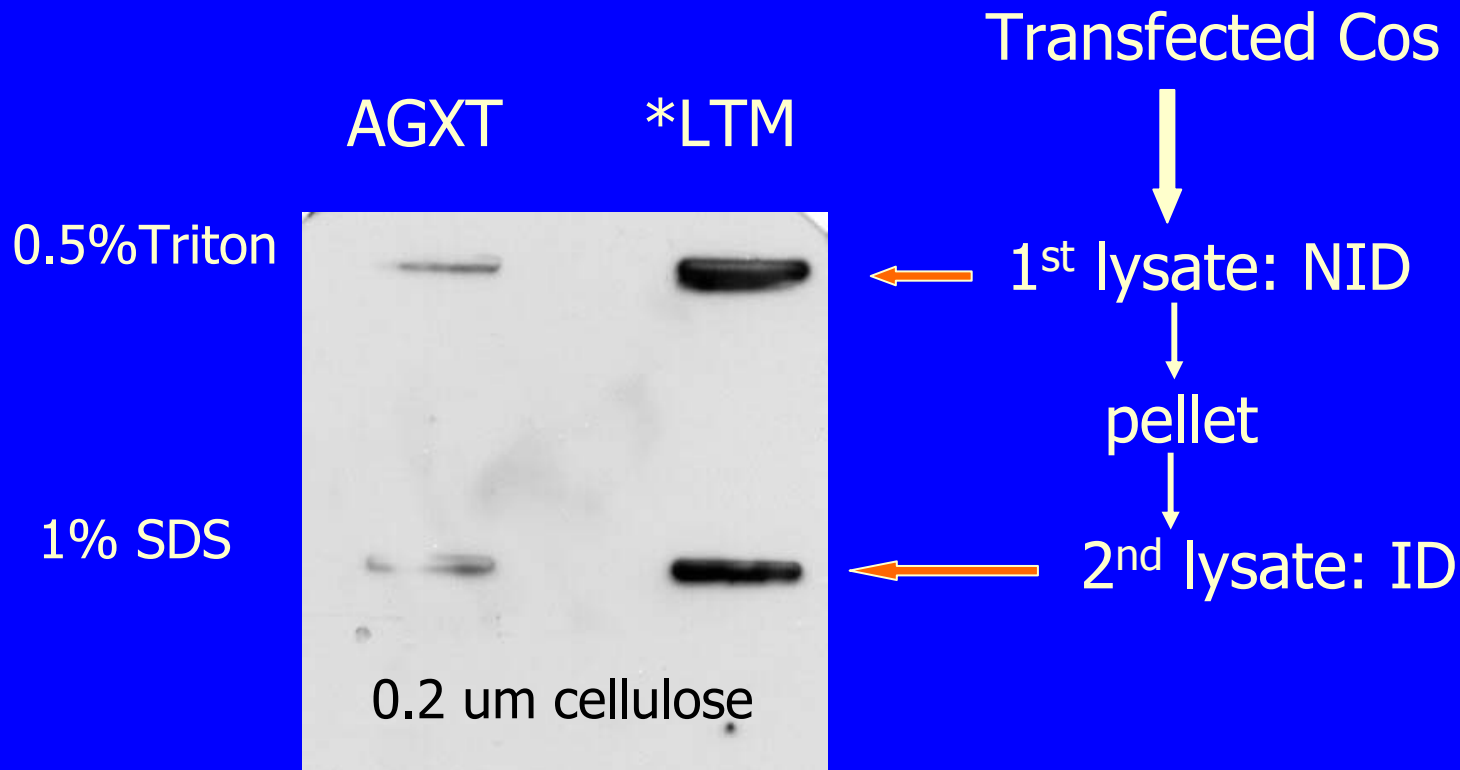


AGXT

*LTM

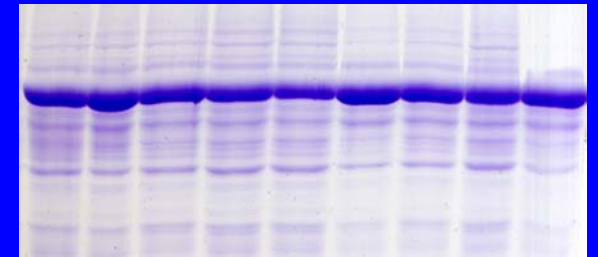
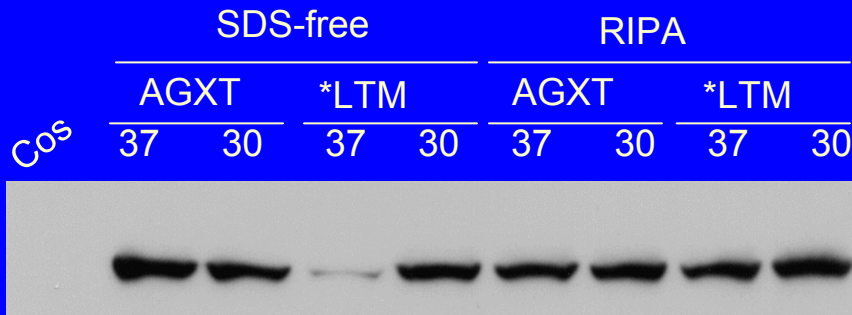
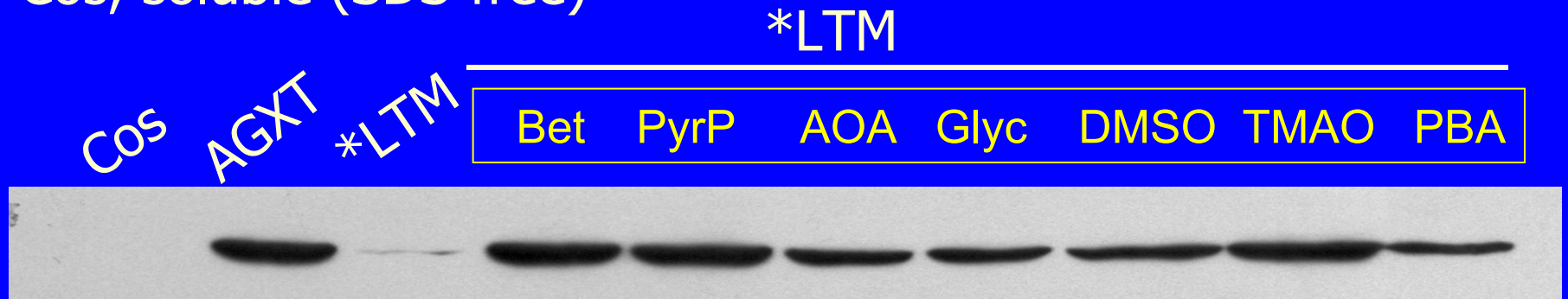


Filter retardation assay



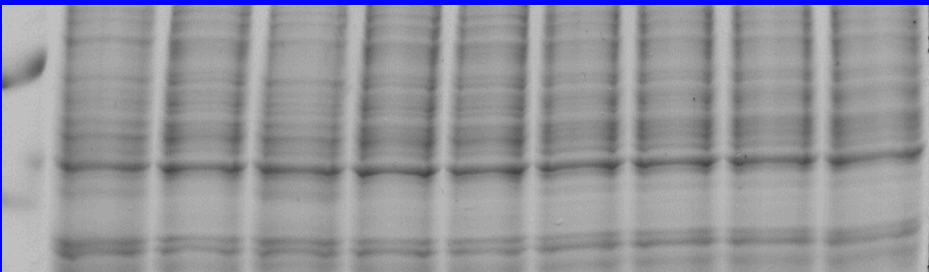
Temperature, chemical chaperones, and solubility

Cos, soluble (SDS-free)

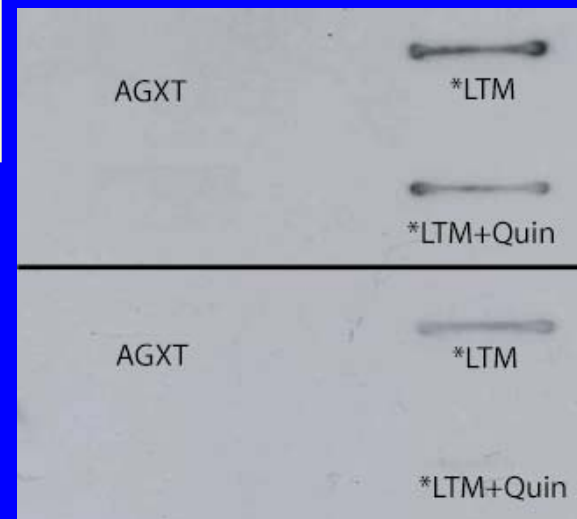
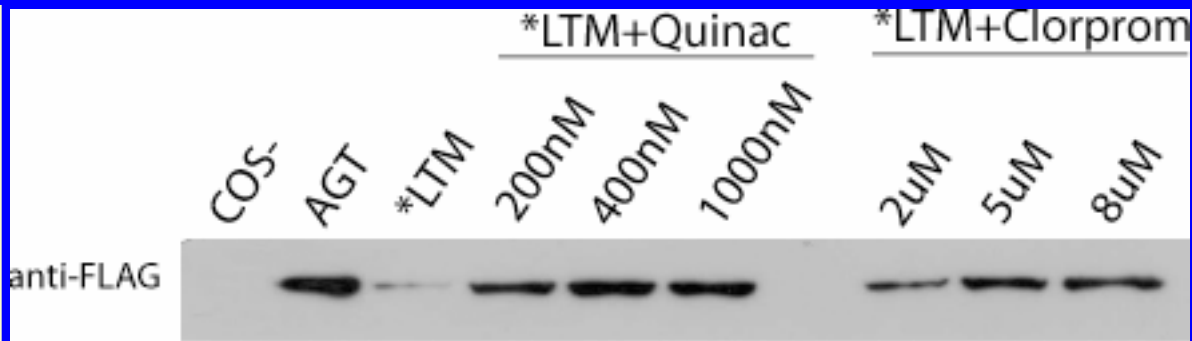
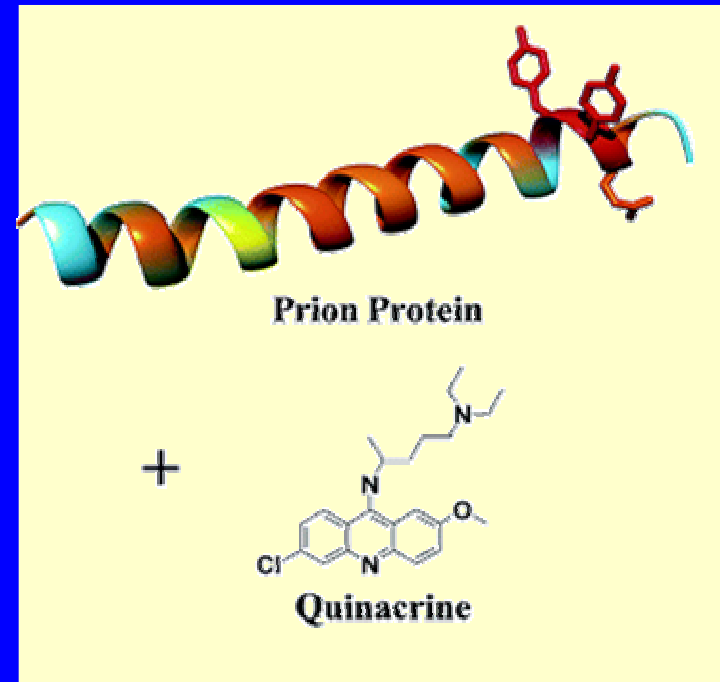


Confirmation of even loading by duplicate Coomassie gels

- # 75 mM betaine
- # 80 uM Pyridoxal PO4
- # 150 mM TMAO



other compounds tested in protein aggregation diseases



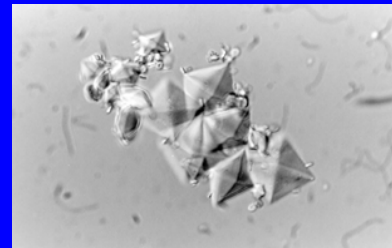
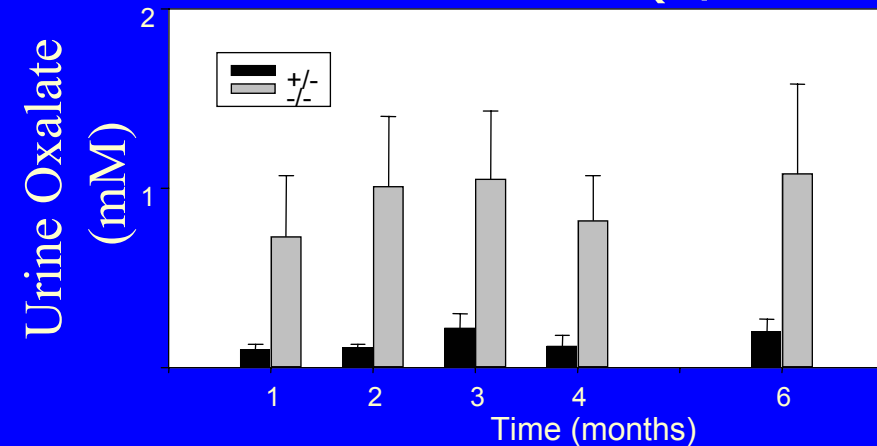
Filter retardation

non-ionic detergent

ionic detergent (SDS)

Agxt deficient mice: hyperoxaluria

- HIGH URINE OXALATE LEVELS
- CRYSTALLURIA
- URINARY STONES (2/3 of males by 6mo.)



hAGXT transgenic mice:

hAGXT promoter-Bglobintron-AGXT (wt., LTM, LRM)

**hAGXT haplotypes expressed in Agxt deficient mice
is chemoprophylaxis possible?**

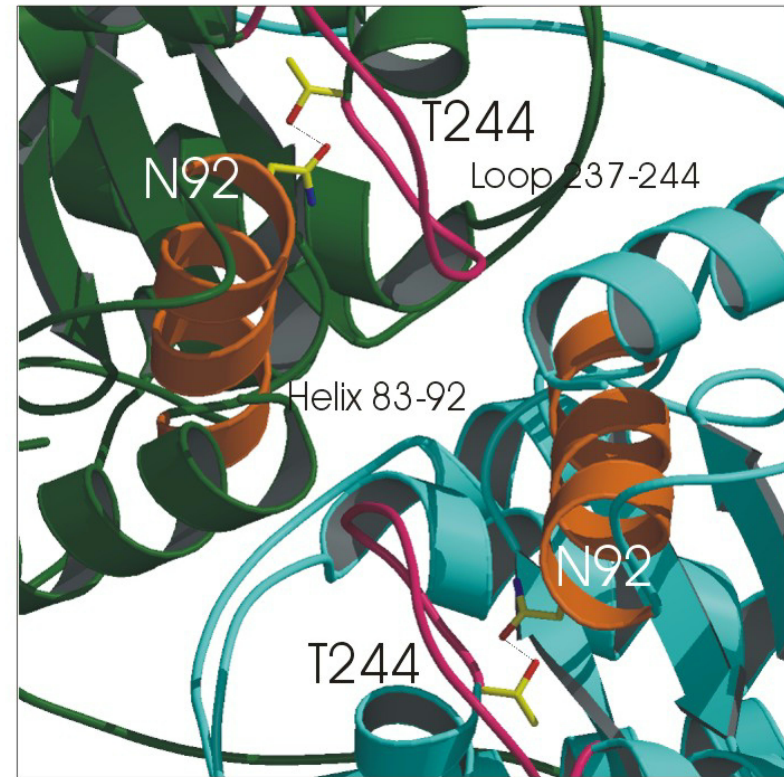
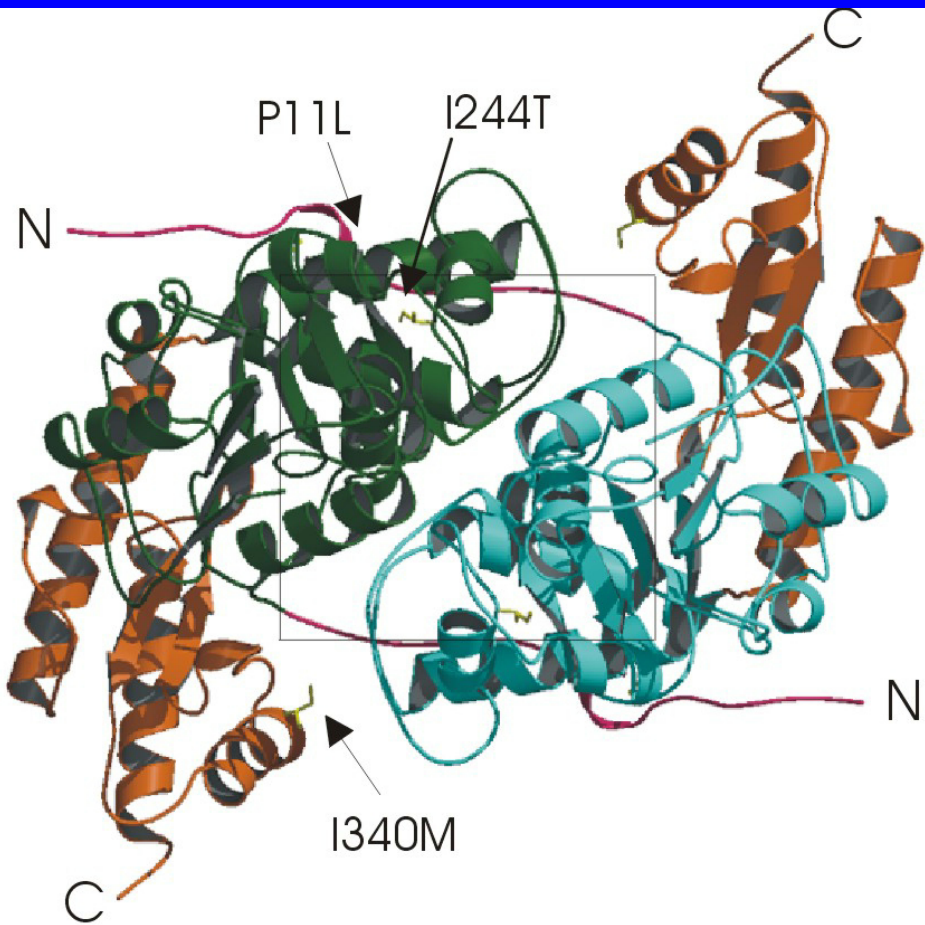
Secondary structure prediction AGXT vs. LTM

(H = helix, E = strand, - = no prediction)

```
-----H-----E-----EEHH--EEE-----HHHHHHHH  
HH-HEEEE-----EEEE-----HHHHHHHHH-----EEE-----H-----HHHHH-  
-----HHHHHHHHH--HEEEE-----E-----HHHHHHH-EE  
EHH-H-----EEEEH-----EEEEHHHHH--H-----E  
EENHHHHH-----EEEE-----HHHHHHHHHHH-HHHHHHHHHHHHHHHHHHH  
HHHHHHHHE-----EEE-----EEEEEEH-----HEEE-----EEE  
EENH-----HHHHHHHHHH-----
```

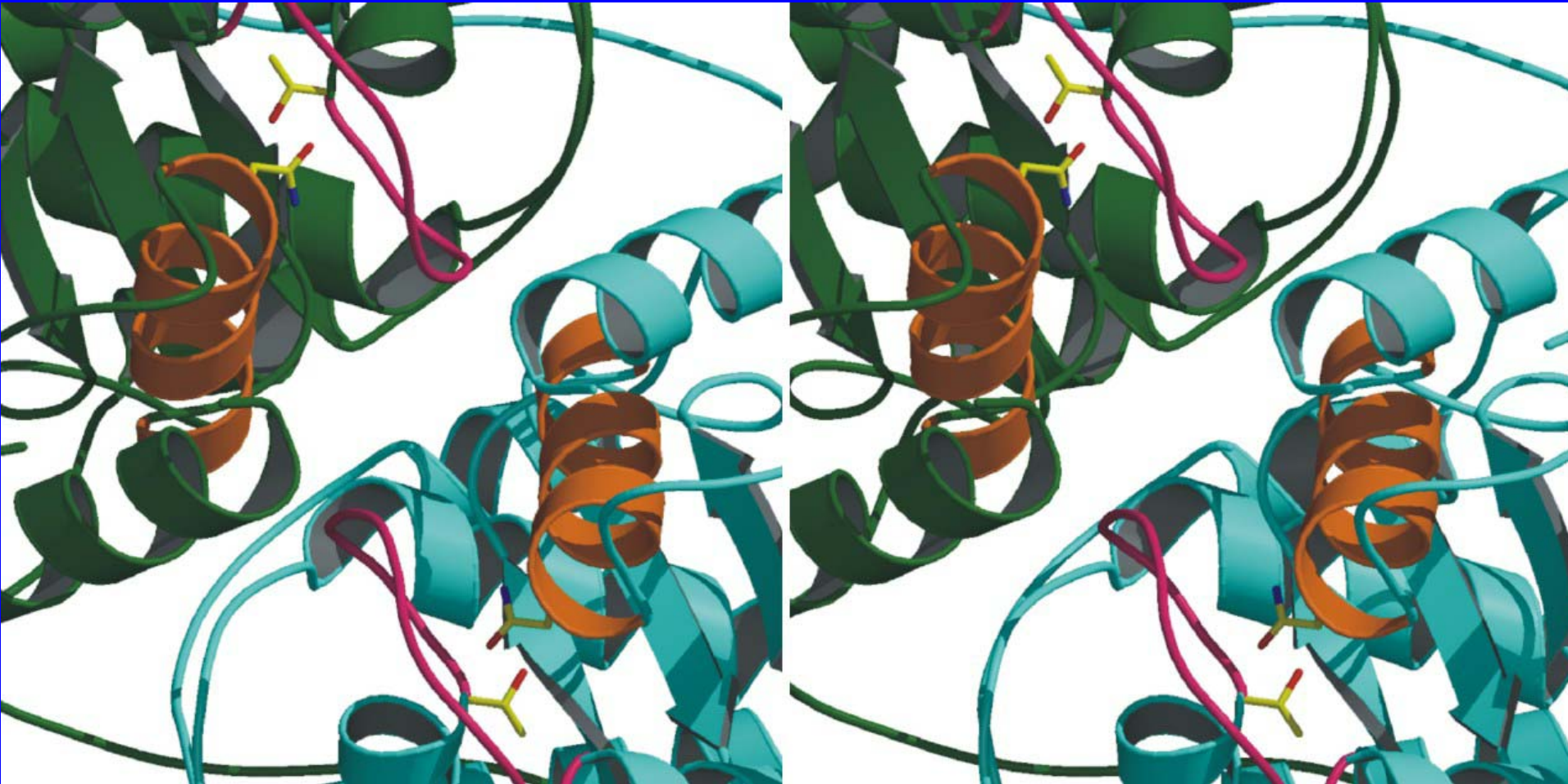
```
----[HHHHHHH]-----E-----EEHH--EEE-----HHHHHHHH  
HH-HEEEE-----EEEE-----HHHHHHHHH-----EEE-----H-----HHHHH-  
-----HHHHHHHHH--HEEEE-----E-----HHHHHHH-EE  
EHH-H-----EEEEH-----EEEEHHHHH--H-----E  
EE--[HHHH]-----EEEE-----HHHHHHHHHHH-HHHHHHHHHHHHHHHHHHH  
HHHHHHHHE-----EEE-----[EHHEE]-----HEEE-----EEE  
EENH-----HHHHHHHHHH-----
```

Ile244Thr possible structural implications



Ile244Thr

possible structural implications



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