Crystal Structure of AGT: Insights for the Development of Therapeutic Agents

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"In human genetic disease, most point mutations in protein-encoding genes impair protein folding &/or oligomerization, producing aberrant conformations that result in protein aggregation, accelerated degradation and/or incorrect trafficking"

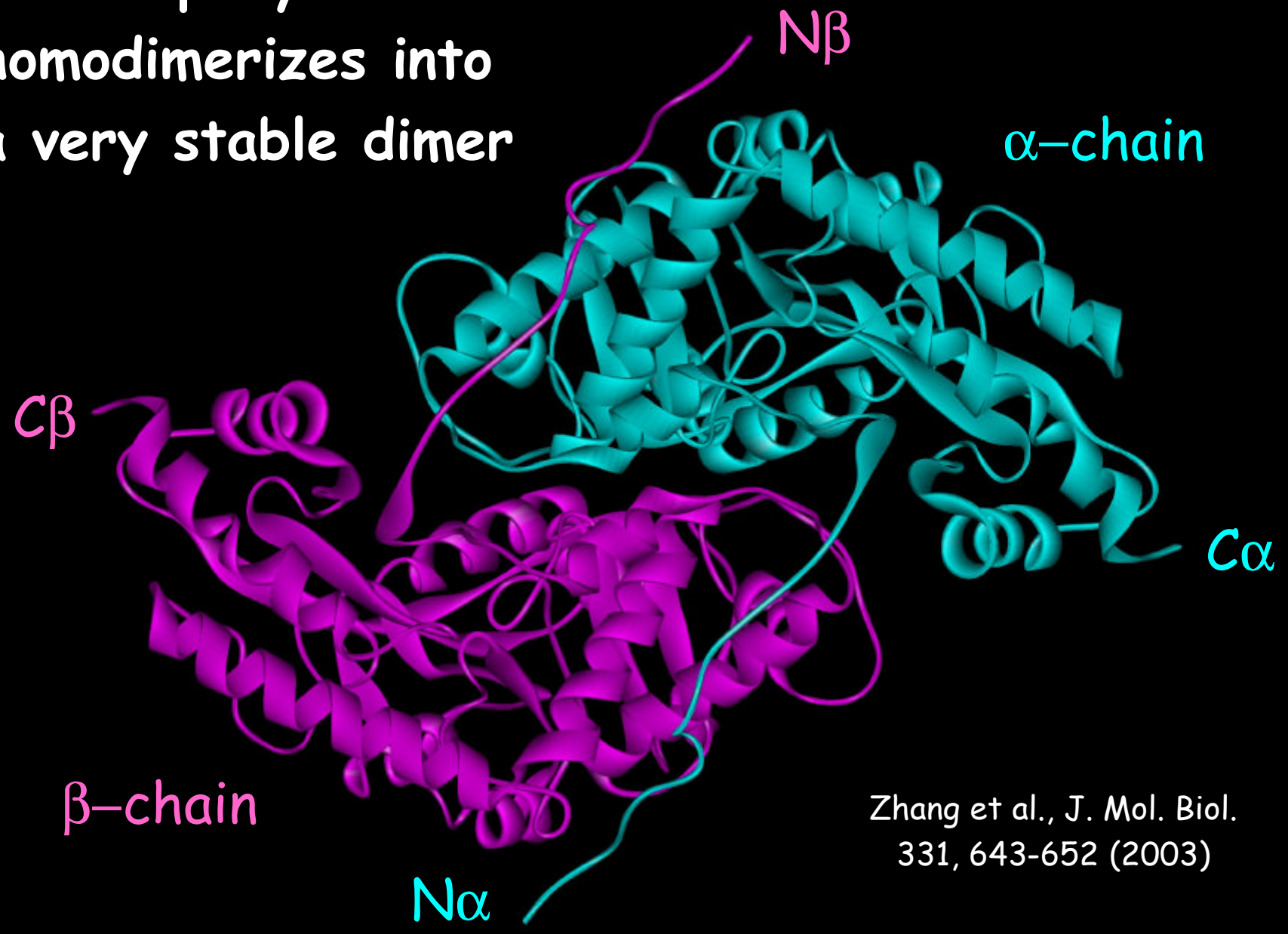
In PH1, there are mutations in AGT that result in:-

- aggregation
- accelerated degradation
- abnormal trafficking
- loss of catalytic activity

Challenge:-

to formulate (designer) therapeutic strategies that counter the effects of these mutations

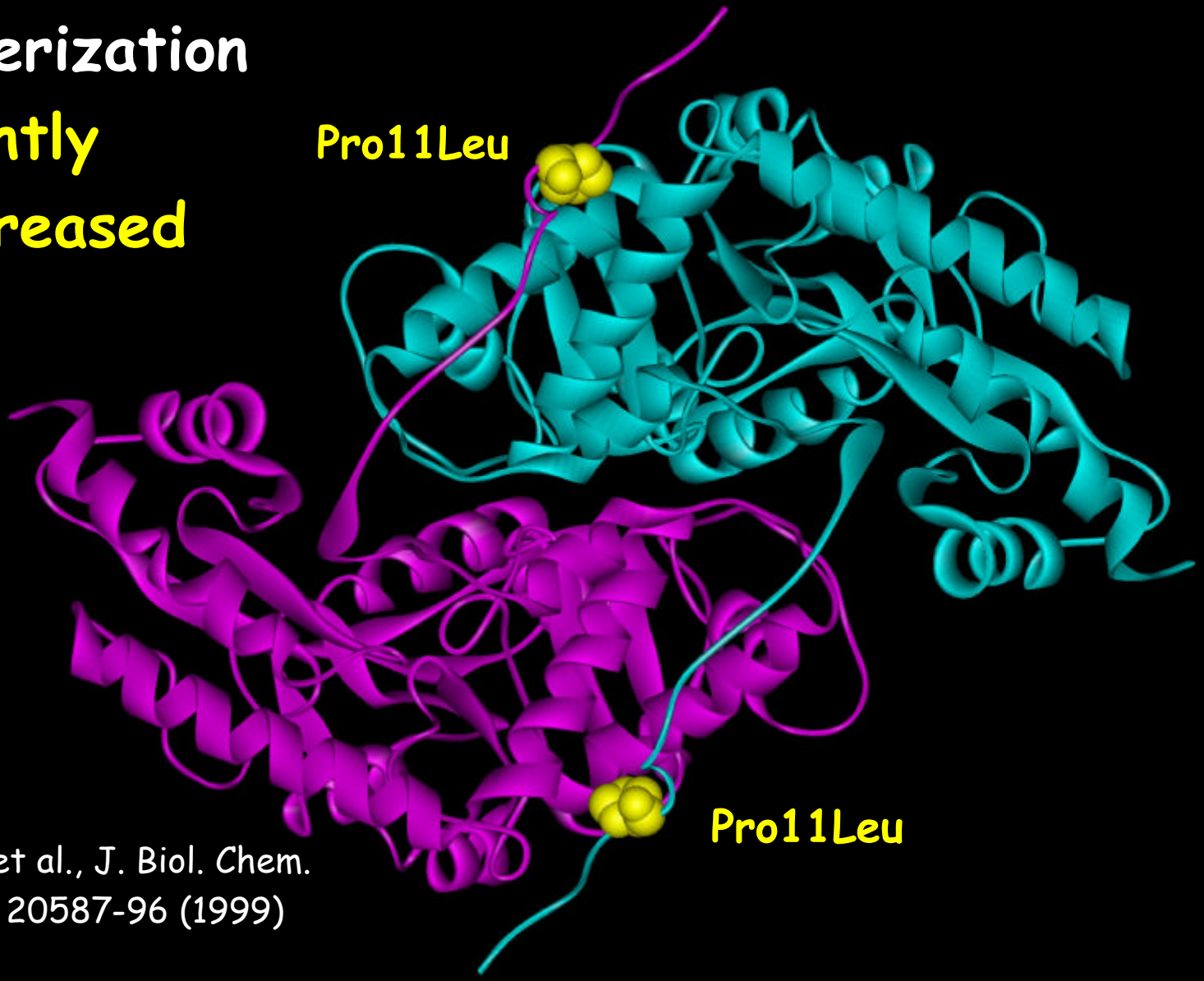
AGT rapidly homodimerizes into a very stable dimer



Zhang et al., J. Mol. Biol. 331, 643-652 (2003)

Rate of AGT dimerization **slightly decreased**

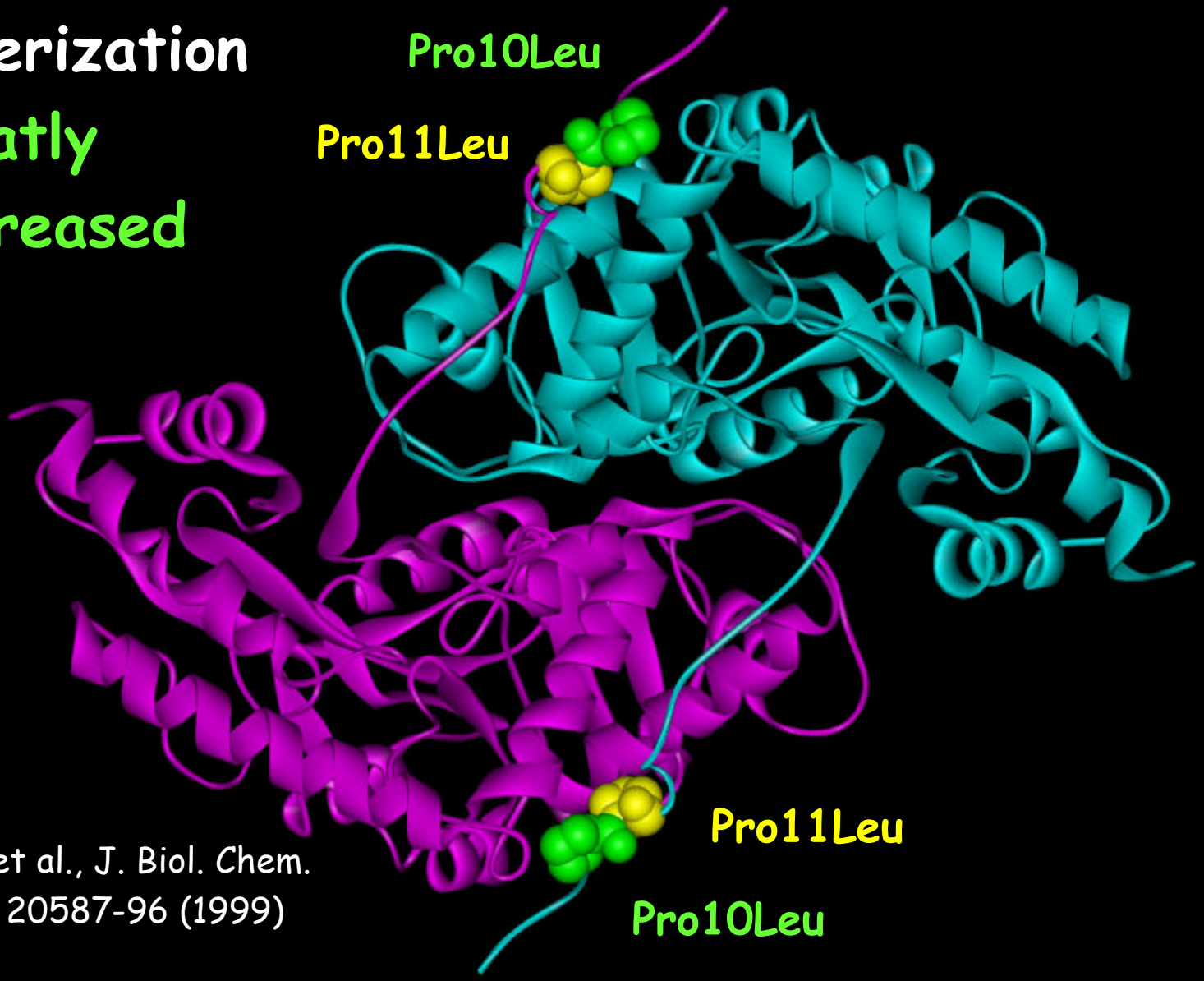
Zhang et al., J. Mol. Biol. 331, 643-652 (2003)



Lumb et al., J. Biol. Chem. 274, 20587-96 (1999)

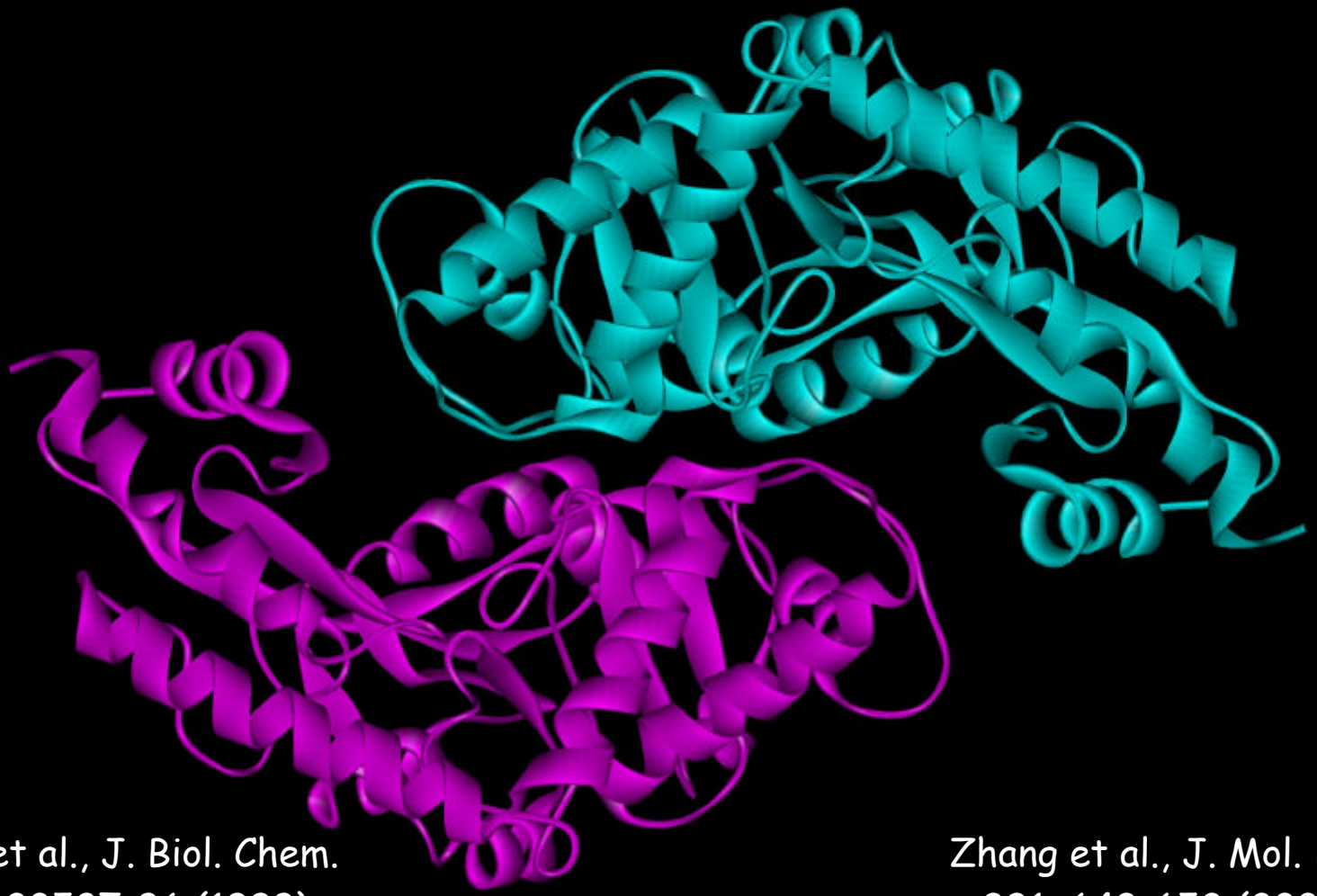
Rate of AGT dimerization greatly decreased

Zhang et al., J. Mol. Biol. 331, 643-652 (2003)



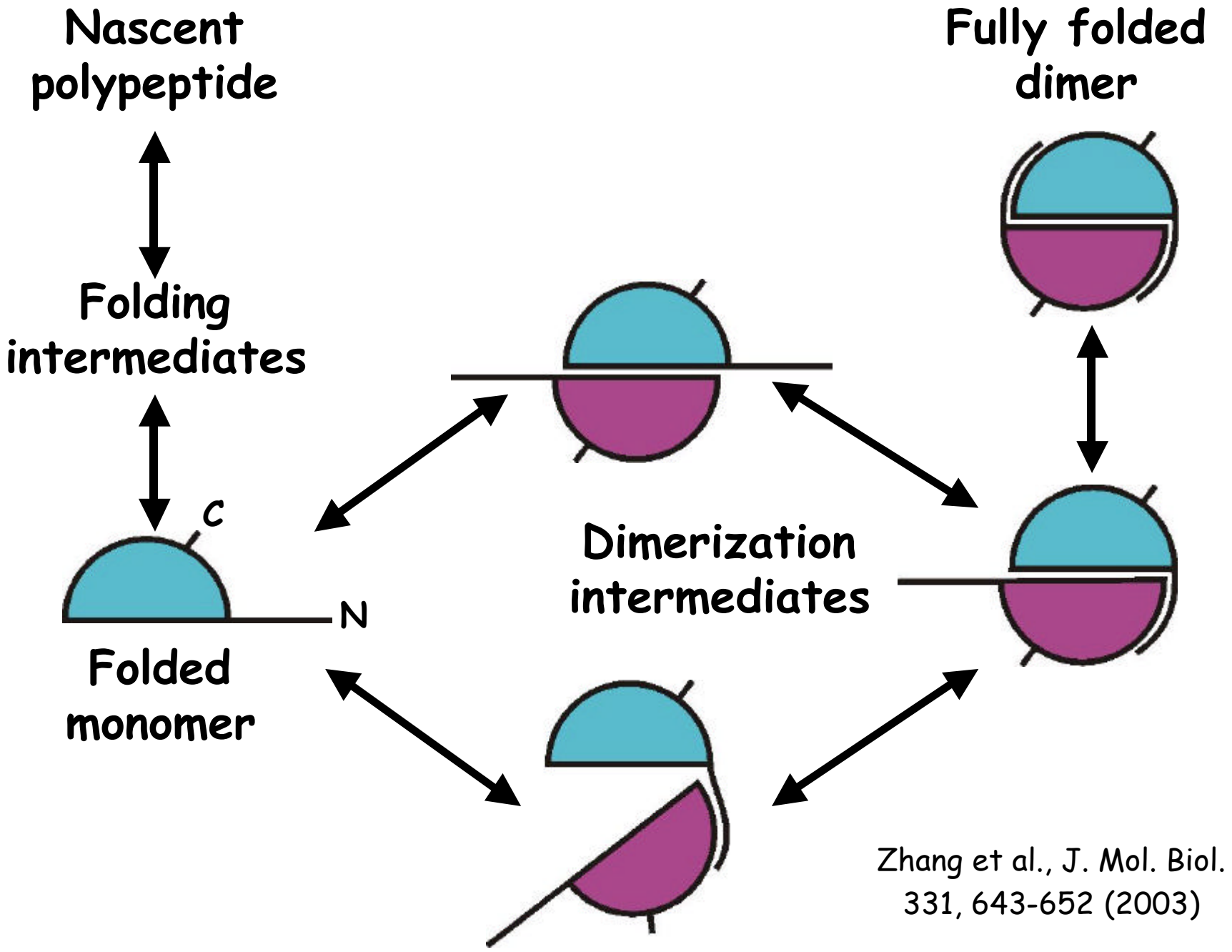
Lumb et al., J. Biol. Chem. 274, 20587-96 (1999)

Removal of first 37 aa abolishes dimerization



Lumb et al., J. Biol. Chem.
274, 20587-96 (1999)

Zhang et al., J. Mol. Biol.
331, 643-652 (2003)



Zhang et al., J. Mol. Biol. 331, 643-652 (2003)

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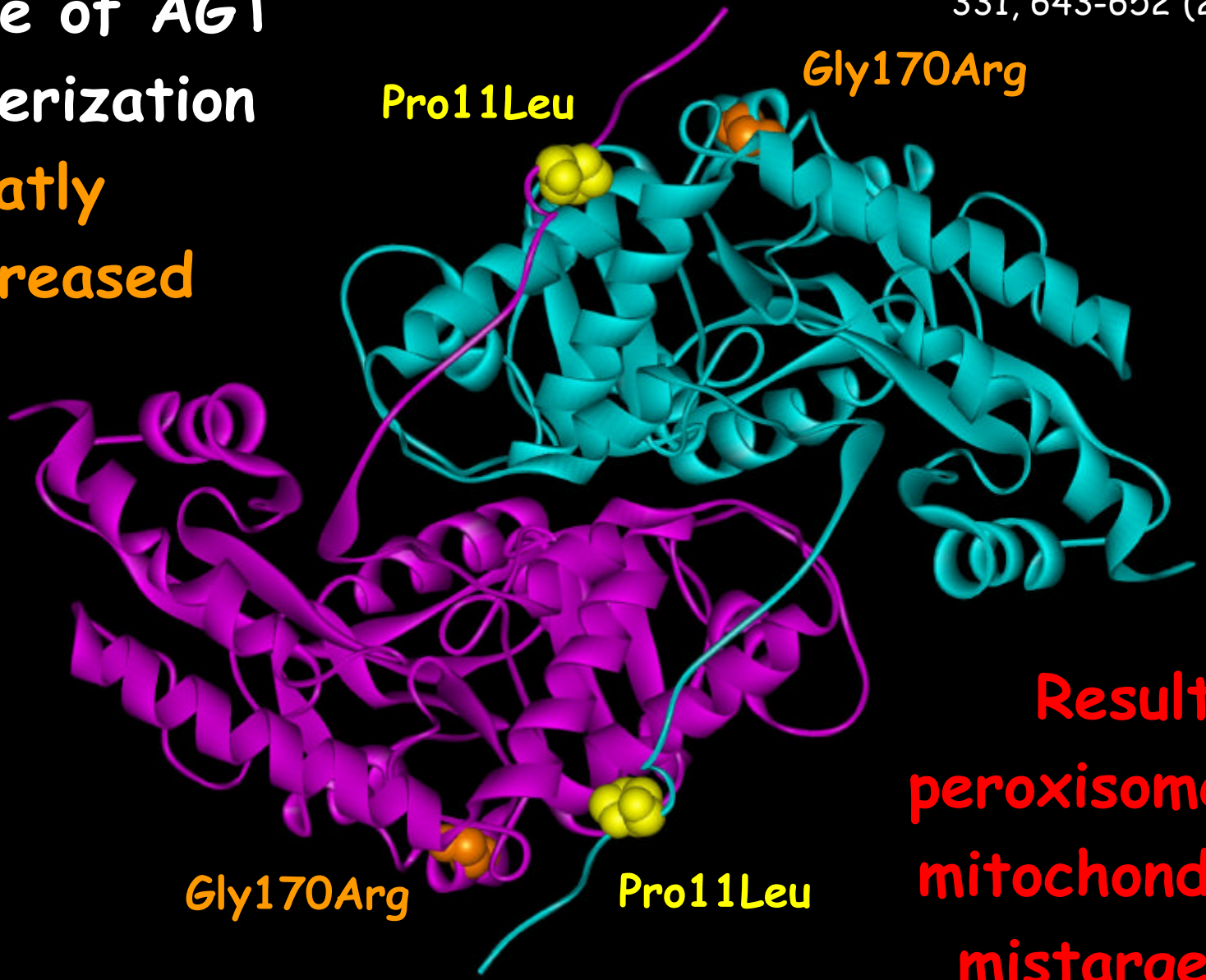
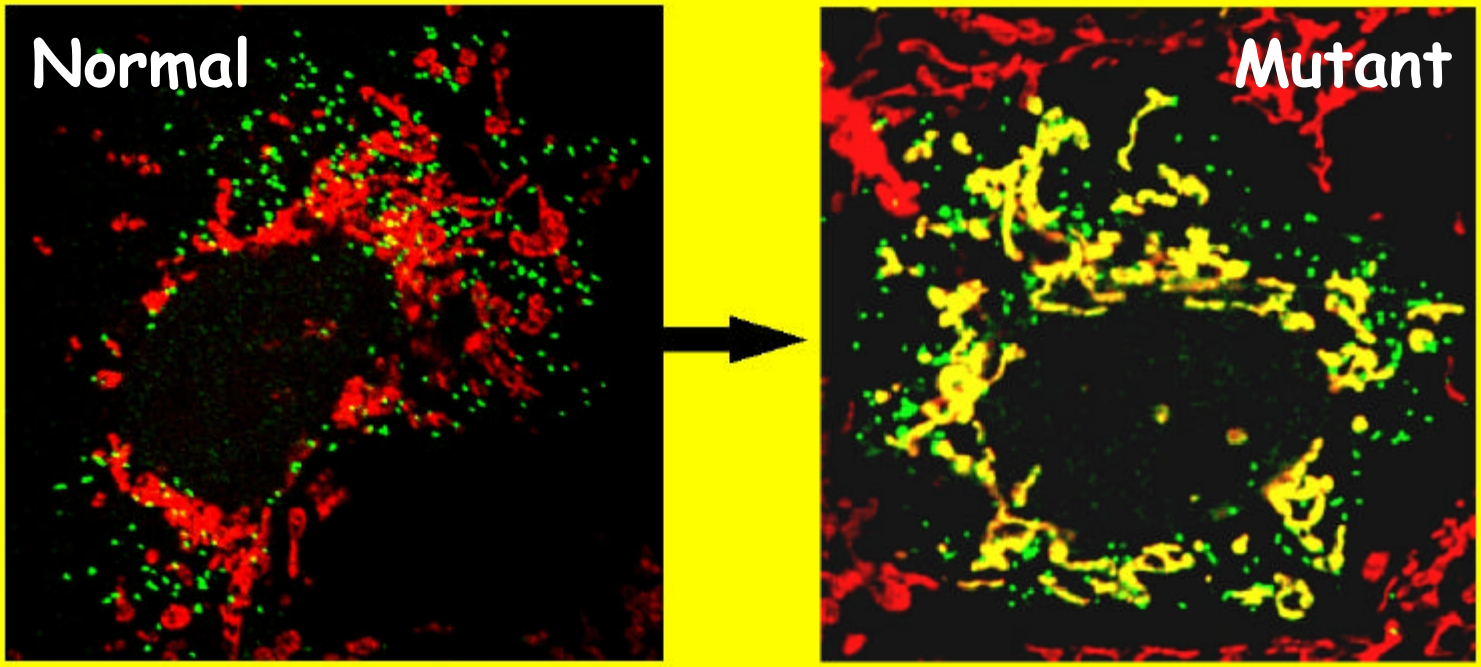


Results in peroxisome to mitochondrion mistargeting

Lieper et al., J. Cell Biol. 135, 939-951 (1996)

Normal & Mutant AGT cDNA Expressed in COS Cells
(Laser-Scanning Confocal Immunofluorescence Microscopy)



AGT = Peroxisomal

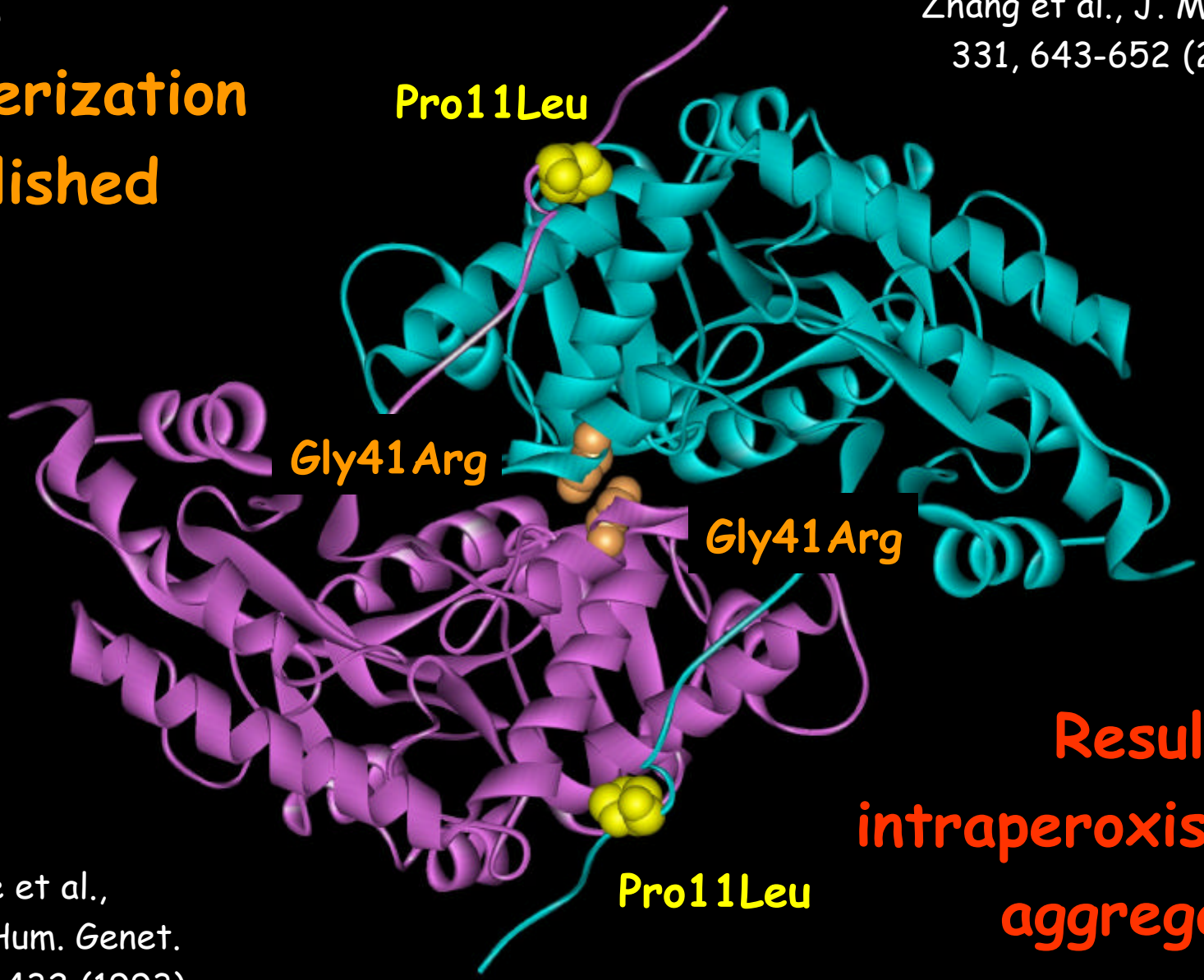
AGT = Mitochondrial
+ Peroxisomal

AGT = green
Mitochondria = red
Co-localization = yellow

Motley et al., J. Cell Biol. 131, 95-109 (1995)

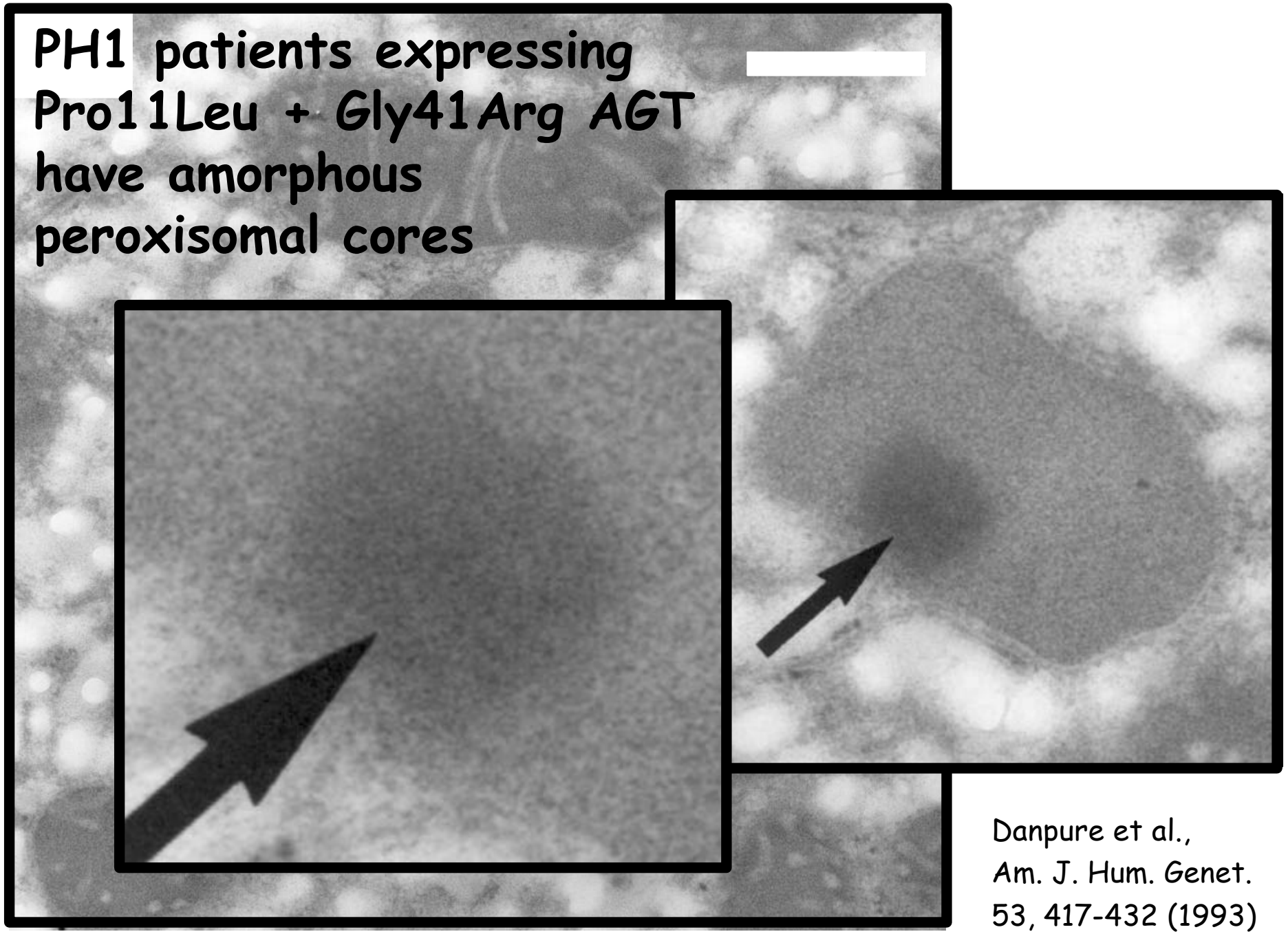
AGT dimerization abolished

Zhang et al., J. Mol. Biol. 331, 643-652 (2003)



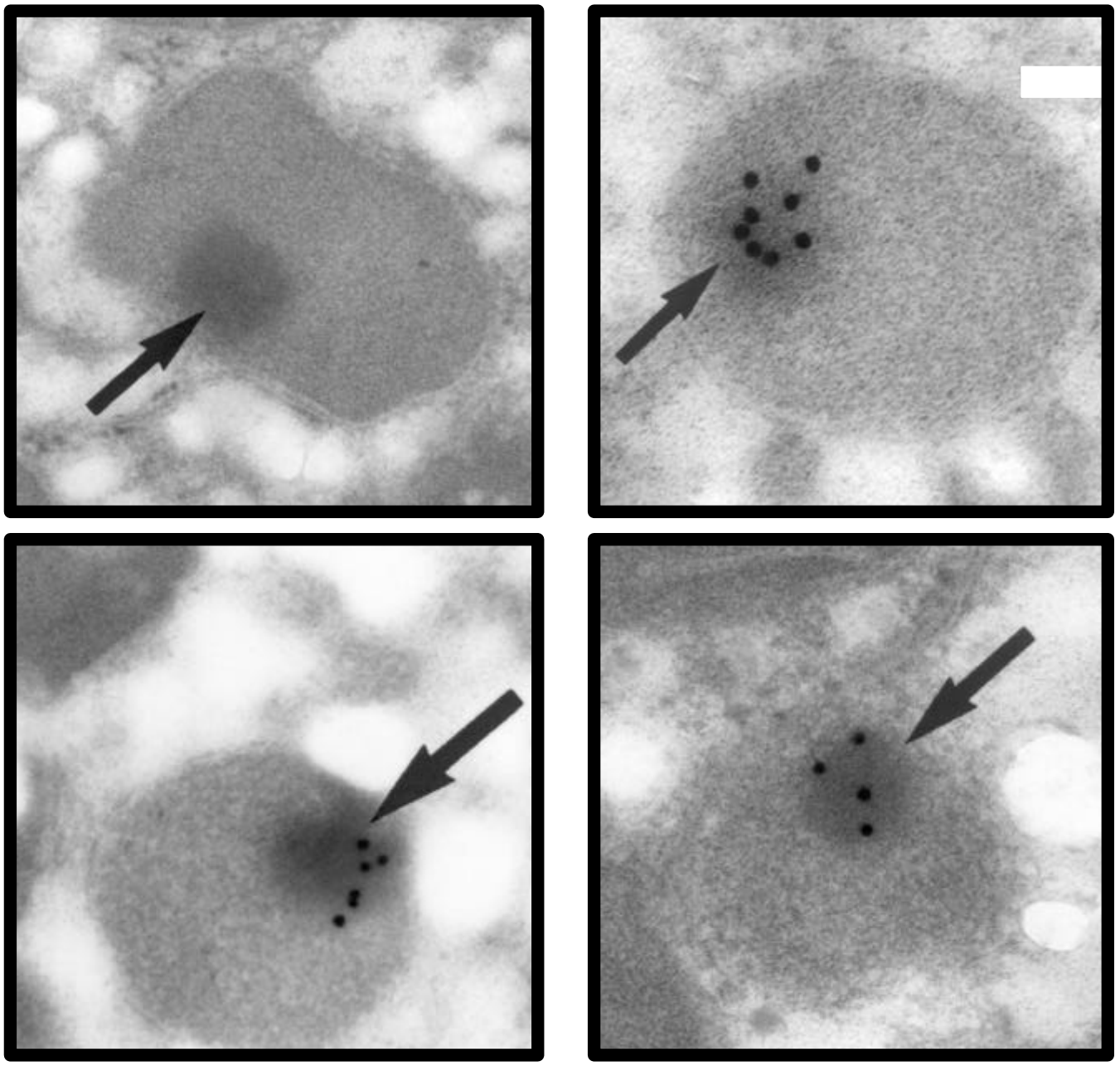
Results in intraperoxisomal aggregation

Danpure et al., Am. J. Hum. Genet. 53, 417-432 (1993)

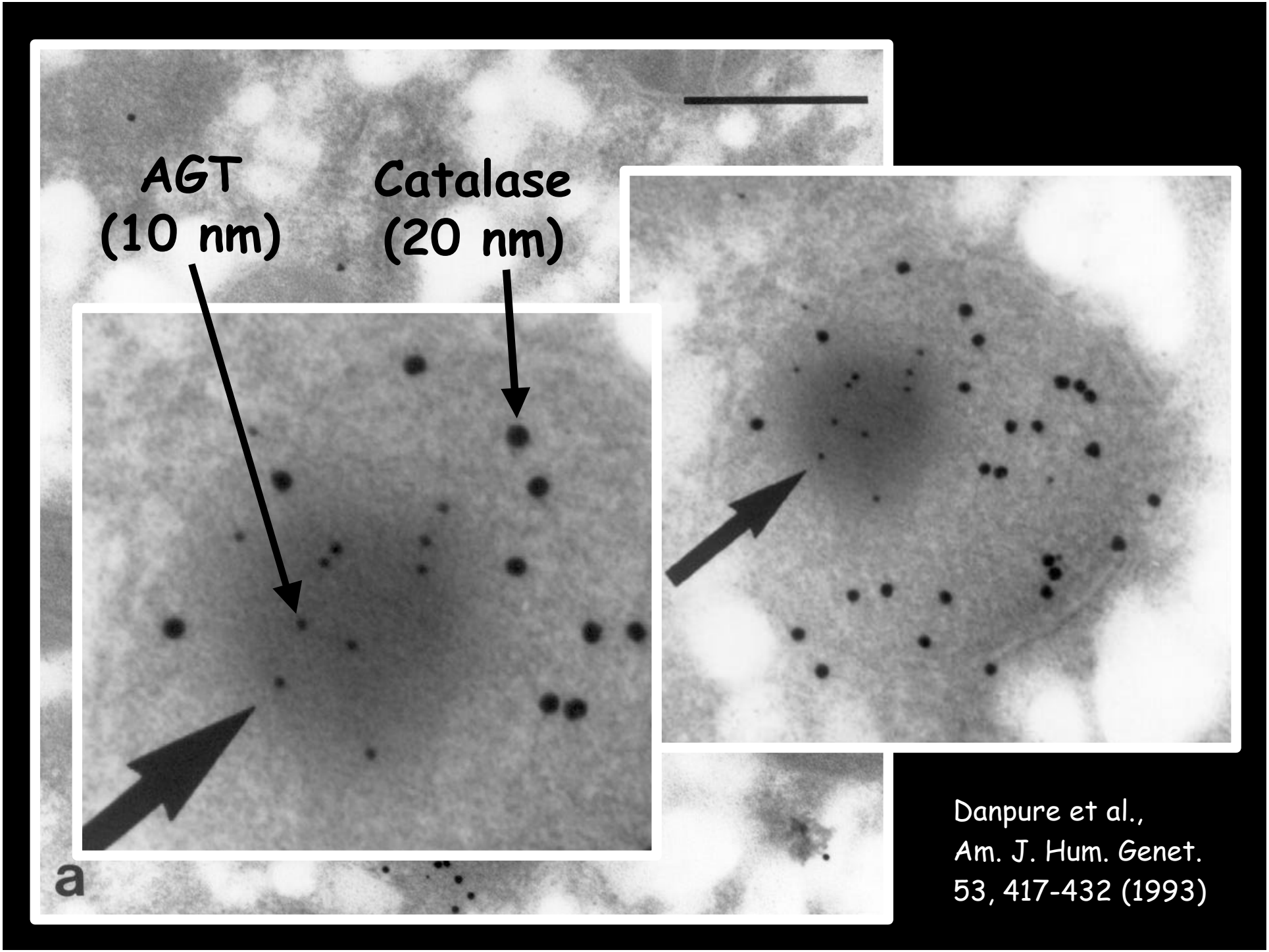


Danpure et al.,
Am. J. Hum. Genet.
53, 417-432 (1993)

Peroxisomal cores are made of AGT

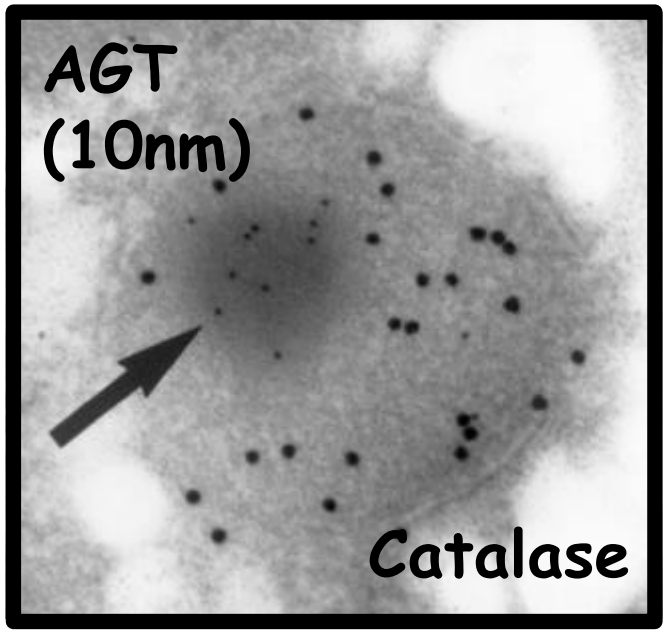


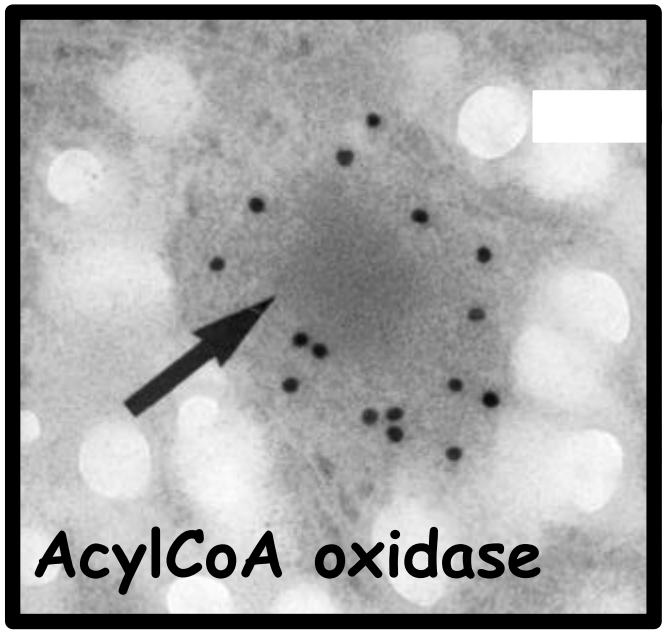
Danpure et al.,
Am. J. Hum. Genet.
53, 417-432 (1993)

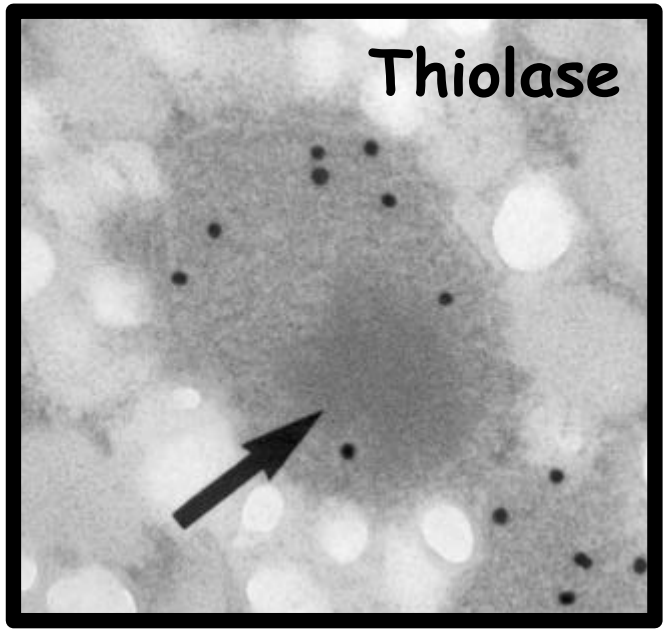


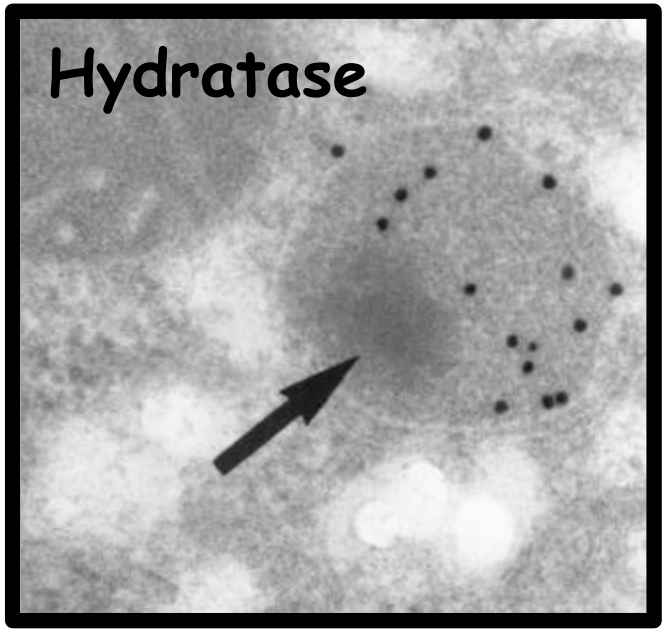
Danpure et al.,
Am. J. Hum. Genet.
53, 417-432 (1993)

Peroxisomal cores contain AGT but not four other peroxisomal proteins









Danpure et al.,
Am. J. Hum. Genet.
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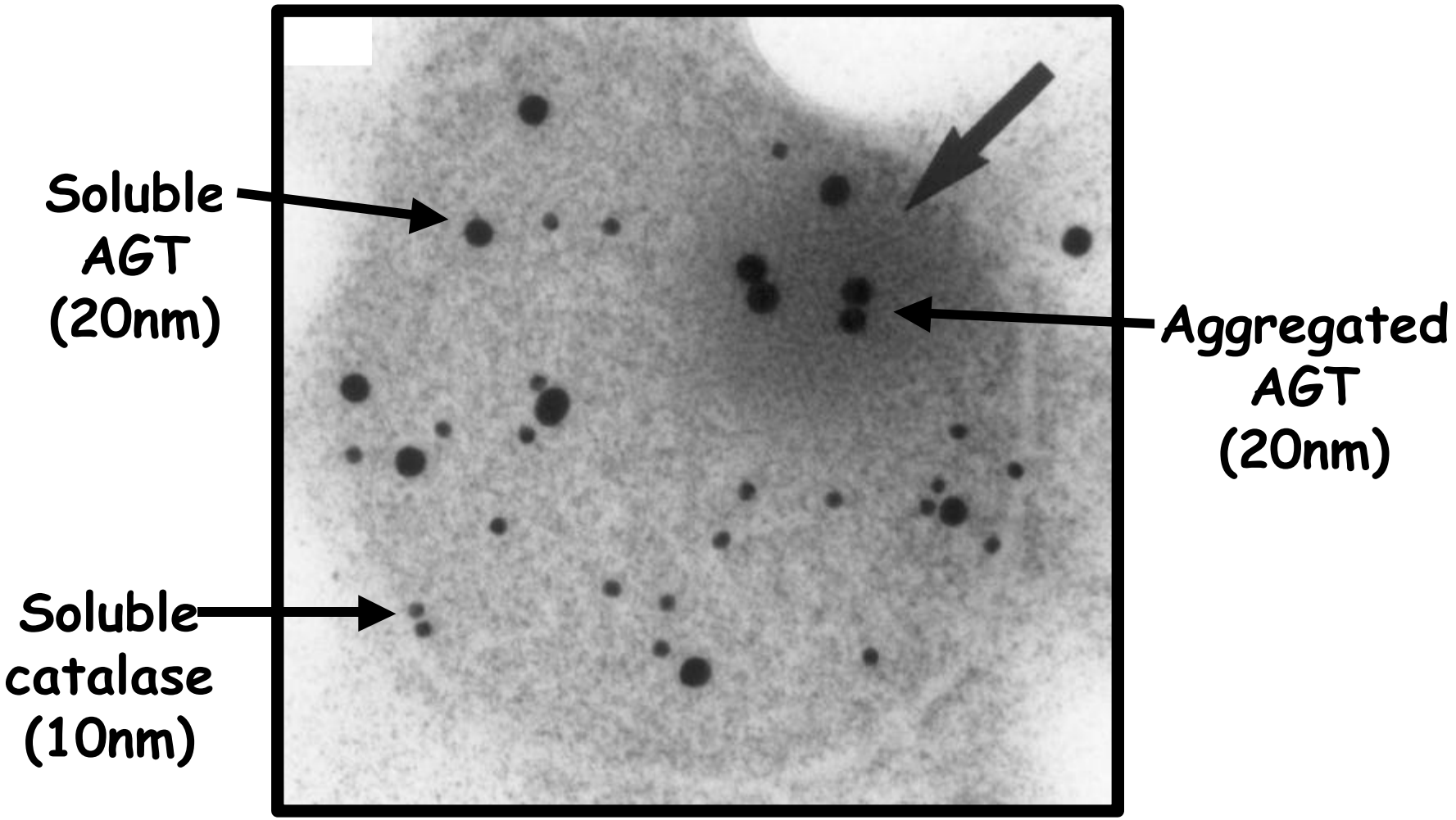
Normal Pro11Leu+Gly41Arg heterozygotes have both aggregated and soluble AGT



Danpure et al., Am. J. Hum. Genet. 53, 417-432 (1993)

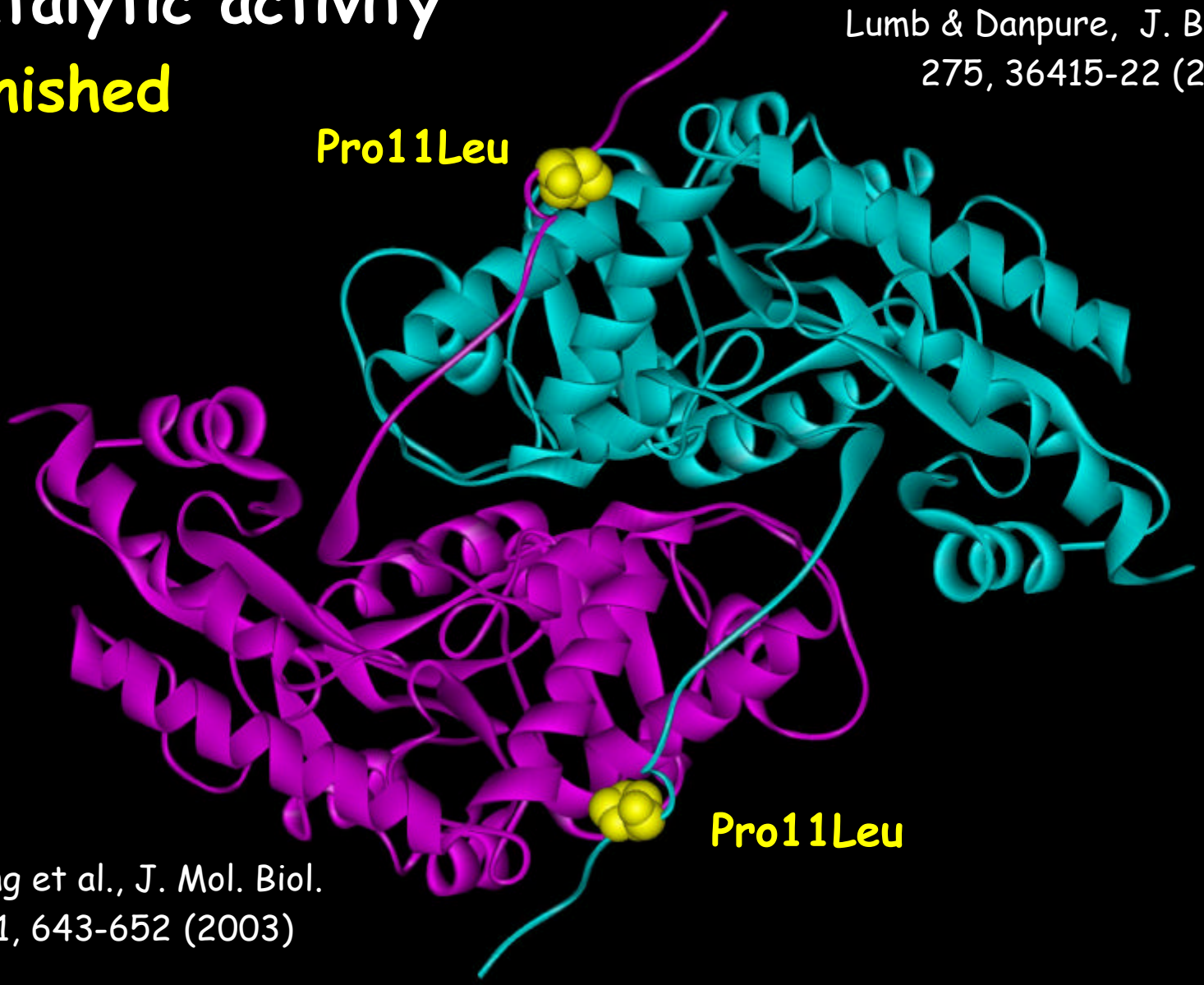
Mutations that interfere with AGT folding &/or dimerization result in:-

1) Peroxisome-to-mitochondrion mistargeting (Pro11Leu + Gly170Arg)
   - DIMERIZATION DELAYED

2) Intraperoxisomal aggregation (Pro11Leu + Gly41Arg)
   - DIMERIZATION ABOLISHED

Rate of AGT dimerization **slightly decreased**
& catalytic activity
diminished

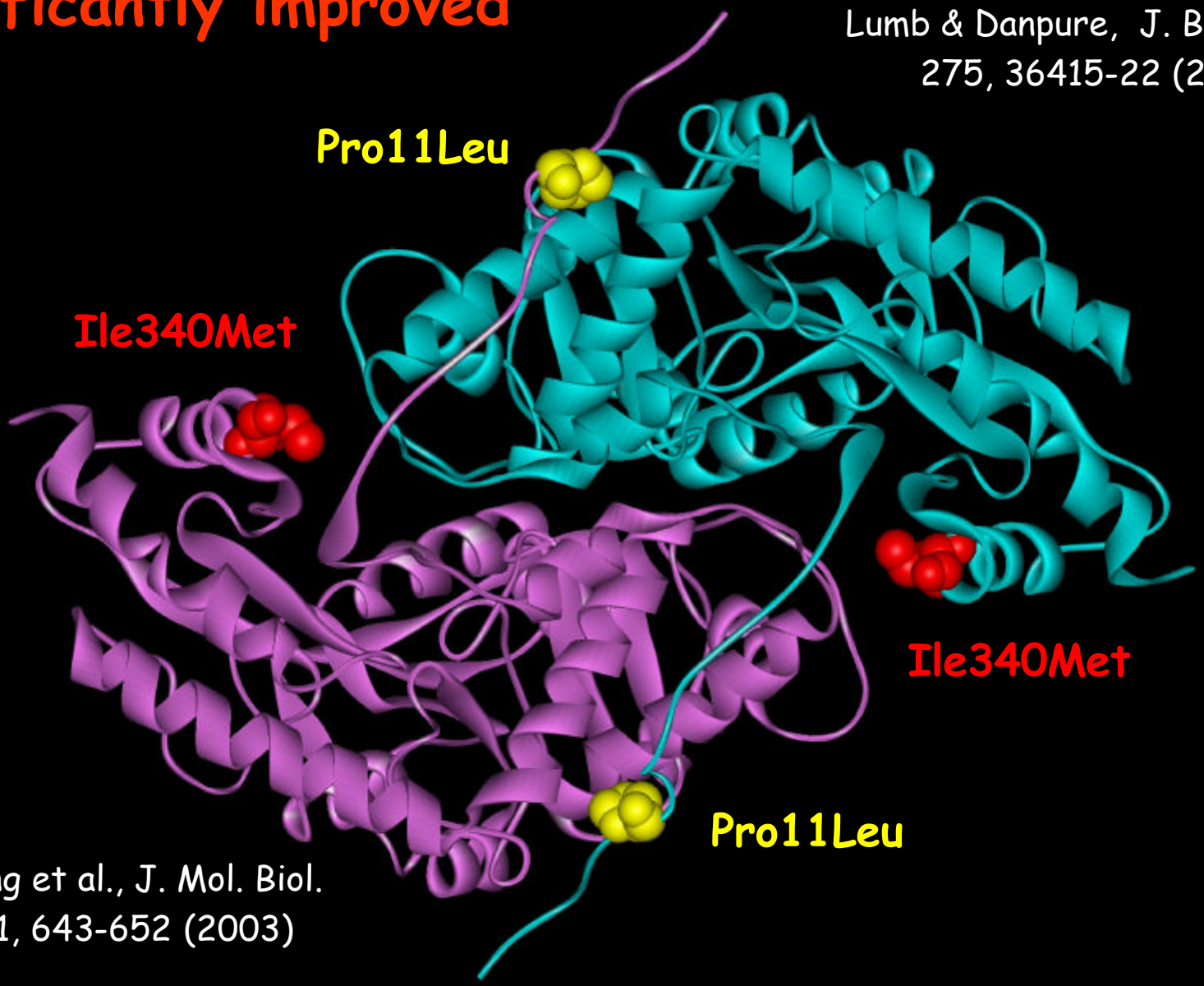
Lumb & Danpure, J. Biol. Chem.
275, 36415-22 (2000)



Zhang et al., J. Mol. Biol.
331, 643-652 (2003)

Rate of AGT dimerization & catalytic activity significantly improved

Lumb & Danpure, J. Biol. Chem. 275, 36415-22 (2000)



Zhang et al., J. Mol. Biol. 331, 643-652 (2003)

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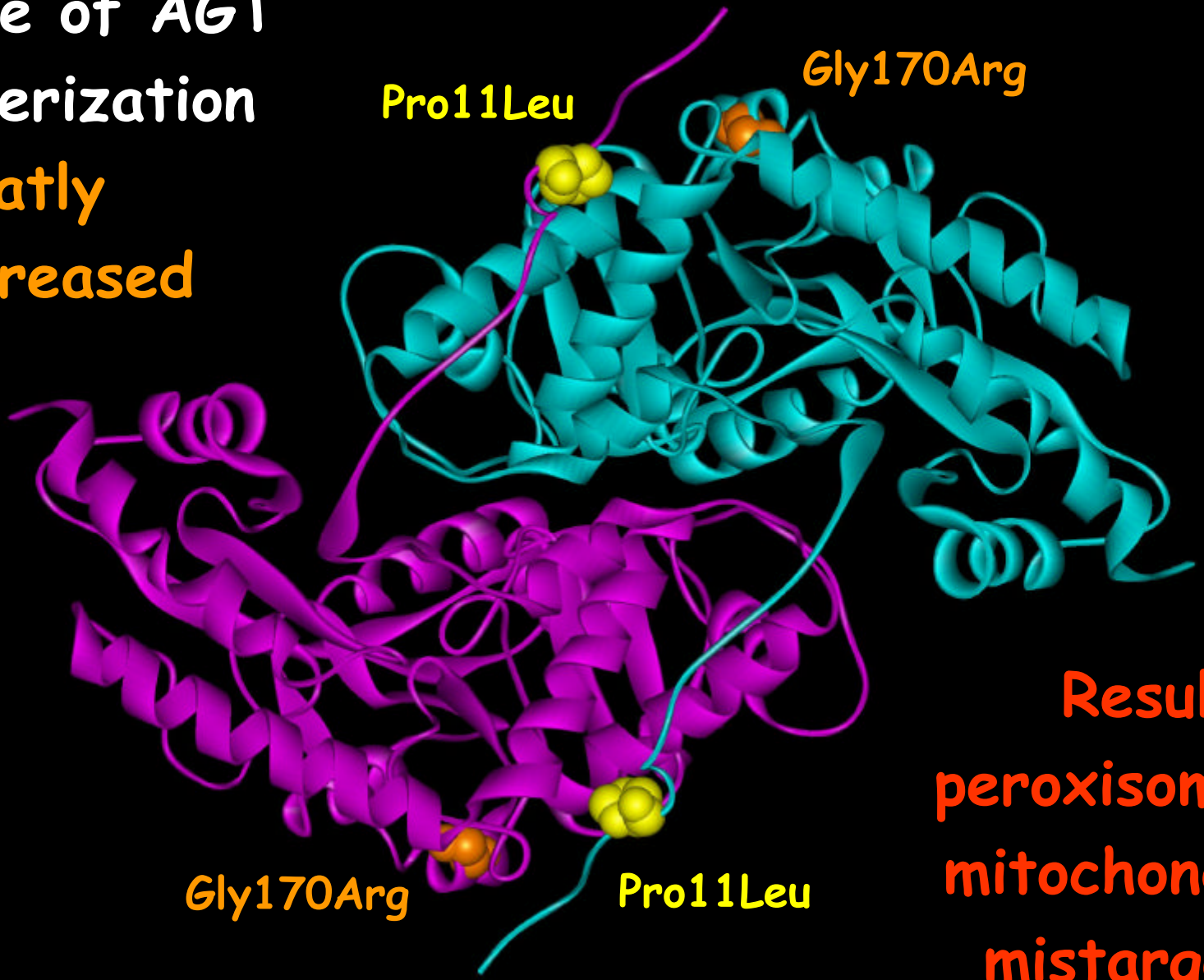
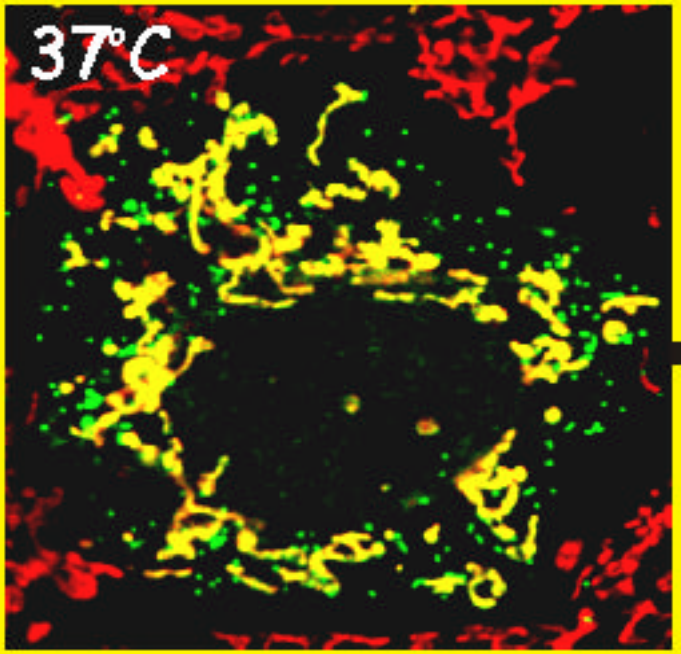
Rate of AGT dimerization greatly decreased



Results in peroxisome to mitochondrion mistargeting

Mutant AGT

(Pro11Leu + Gly170Arg)



AGT = Mitochondrial + Peroxisomal

Mutant AGT

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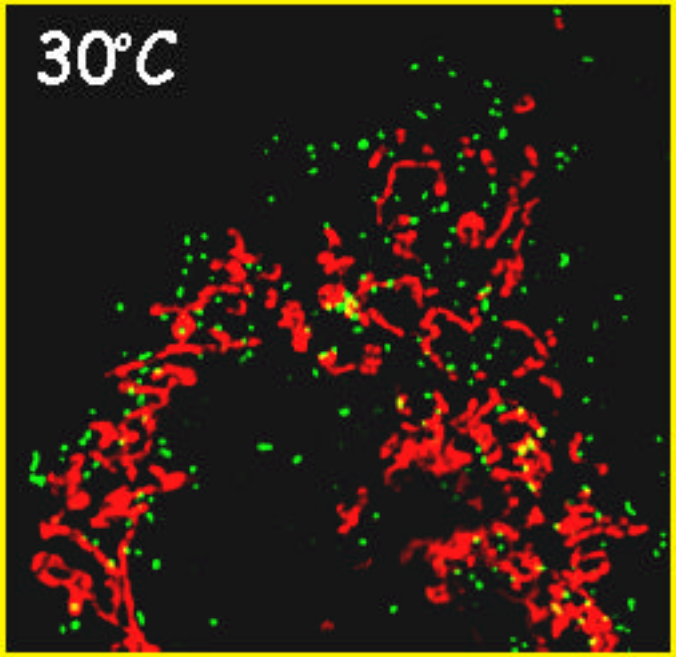
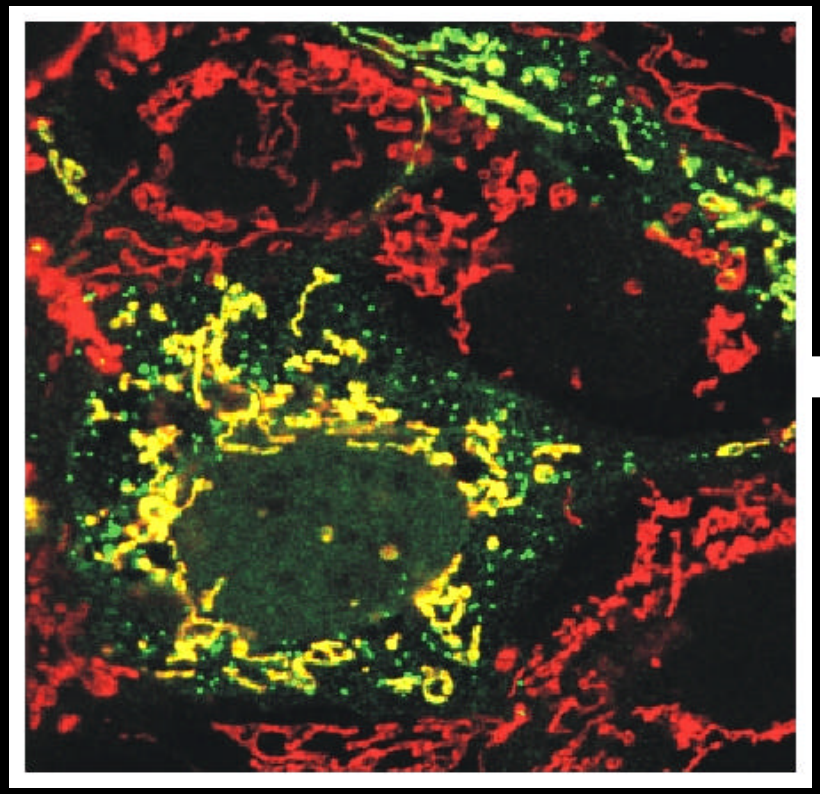


AGT = Peroxisomal

Lumb, Birdsey & Danpure.
Biochem. J. 374, 79-87 (2003).

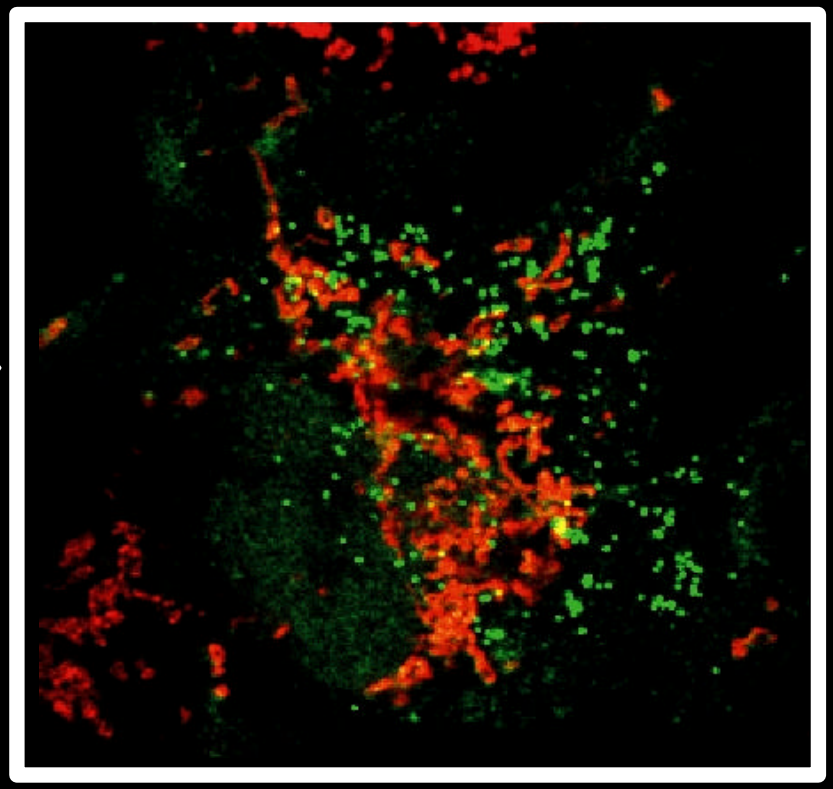
AGT = green
Mitochondria = red
Co-localization = yellow

Pro11Leu + Gly170Arg



PEROX + MITO

Pro11Leu + Gly170Arg
+ GLYCEROL



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Lumb, Birdsey & Danpure. Biochem. J. 374, 79-87 (2003).

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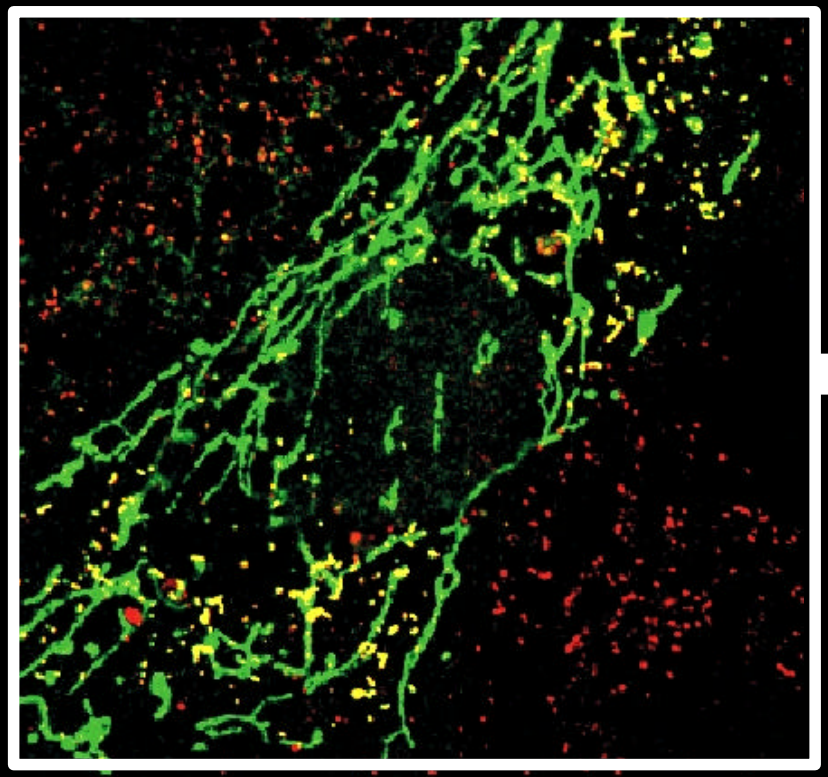

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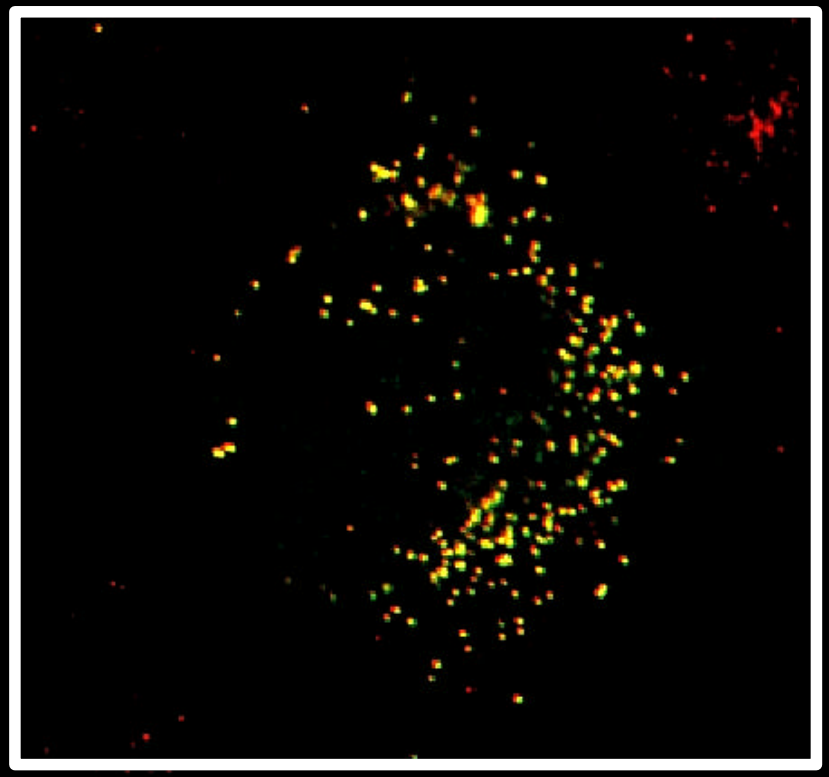

PEROX

Lumb, Birdsey & Danpure. Biochem. J. 374, 79-87 (2003).

CONCLUSIONS

1) Several mutations in AGT interfere with its dimerization, leading to its aggregation, accelerated degradation &/or peroxisome-to-mitochondrion mistargeting.

2) Knowledge of AGT crystal structure provides insights into the mechanism of AGT dimerization & provides an explanation for how it is perturbed by several mutations.

CONCLUSIONS

3) Treatments that stabilise the AGT dimer or increase rate of AGT dimerization counteract the effects of at least some mutations.

4) Knowledge of AGT crystal structure & the identification of binding sites for chemical chaperones, such as glycerol, should enable design of pharmacological agents of high affinity and specificity that can stabilise the AGT dimer.

AGT mistargeting

J. Cell Biol. 108 (1989)

J. Cell. Biol. 111 (1990)

AGT aggregation

Am. J. Hum. Genet. 53 (1993)

AGT targeting/dimerization

J. Cell Biol. 131 (1995)

J. Cell Biol. 135 (1996)

J. Biol. Chem. 274 (1999)

Polymorphism-mutation synergism

J. Biol. Chem. 275 (2000)

Normalisation of AGT targeting

Biochem. J. 374 (2003).

AGT crystal structure

Acta Cryst. D57 (2001)

J. Mol. Biol. 331 (2003).

THE END