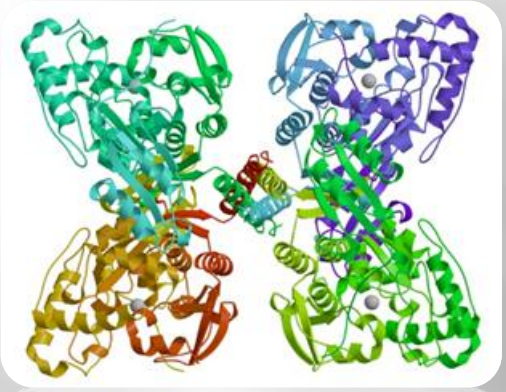
FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

FACULDADE DE FARMÁCIA
Universidade de Lisboa

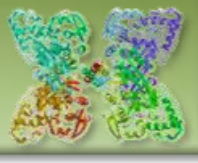


# PHENYLALANINE HYDROXYLASE:

## Towards 3D Structure Determination

Fábio Madeira
MSc Student

# Summary

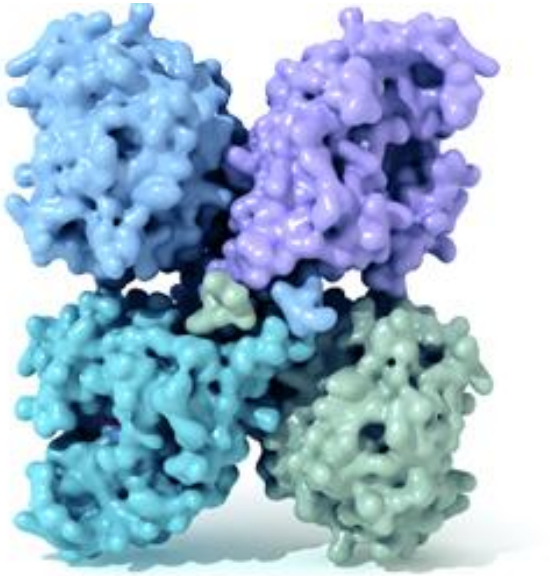


Structural Characteristics

Aims

Experimental Strategies

# Structural Characteristics



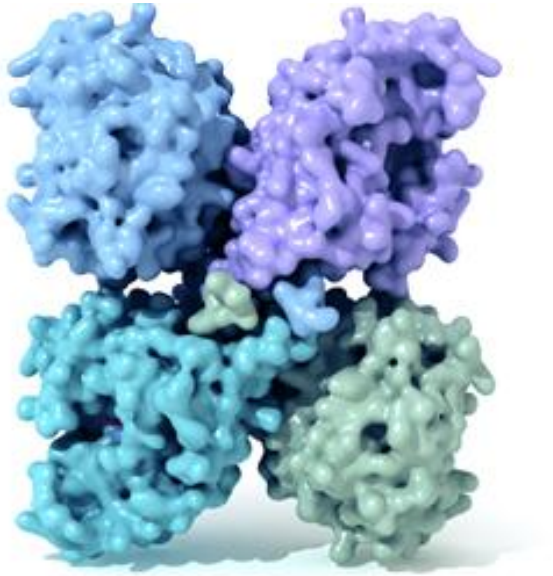
Phenylalanine hydroxylase protein consisting of 4 subunits

**Homotetramers** ⇄ **Homodimers**

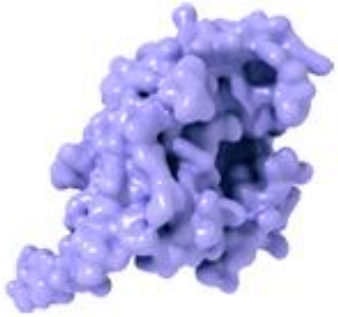
(Hufton, Jennings, & Cotton, 1995)

(Adapted from U.S. National Library of Medicine)

# Structural Characteristics



Phenylalanine hydroxylase protein consisting of 4 subunits



Single phenylalanine hydroxylase subunit

(Adapted from U.S. National Library of Medicine)

**Homotetramers** ⇄ Homodimers

(Hufton, Jennings, & Cotton, 1995)

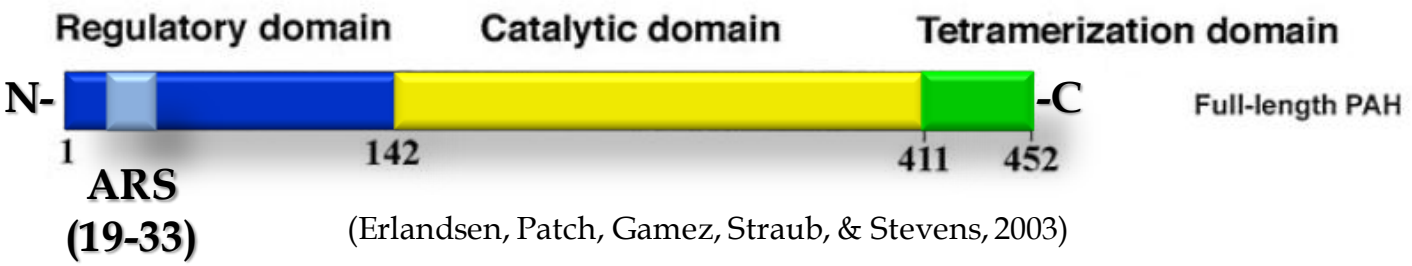
Requirement for ferrous iron, $BH_4$ and $O_2$ as cofactors

(Kappock & Caradonna, 1996)

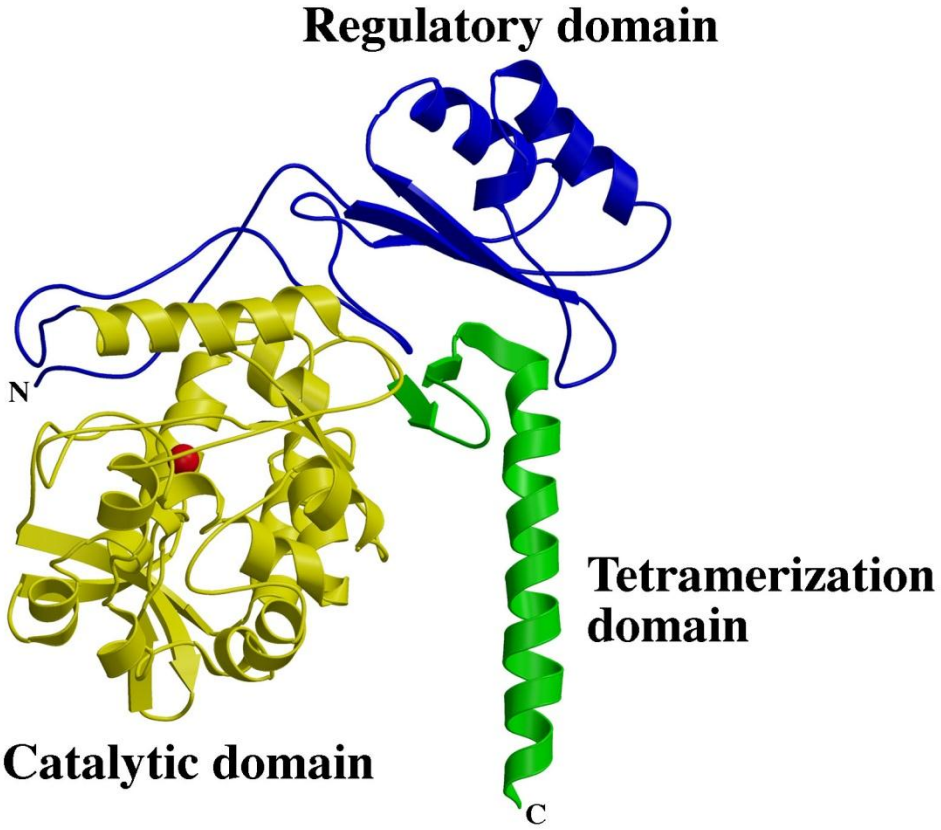
Each subunit ( ≈ 50 KDa)

(Flatmark & Stevens, 1999)

# Structural Characteristics

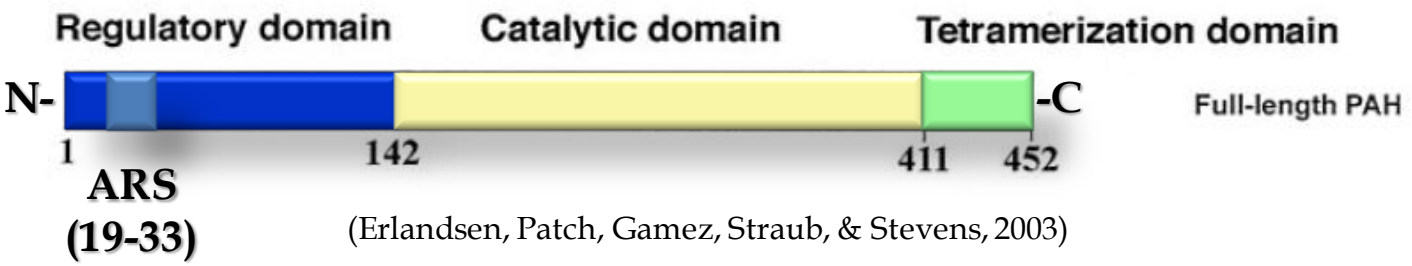



(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)

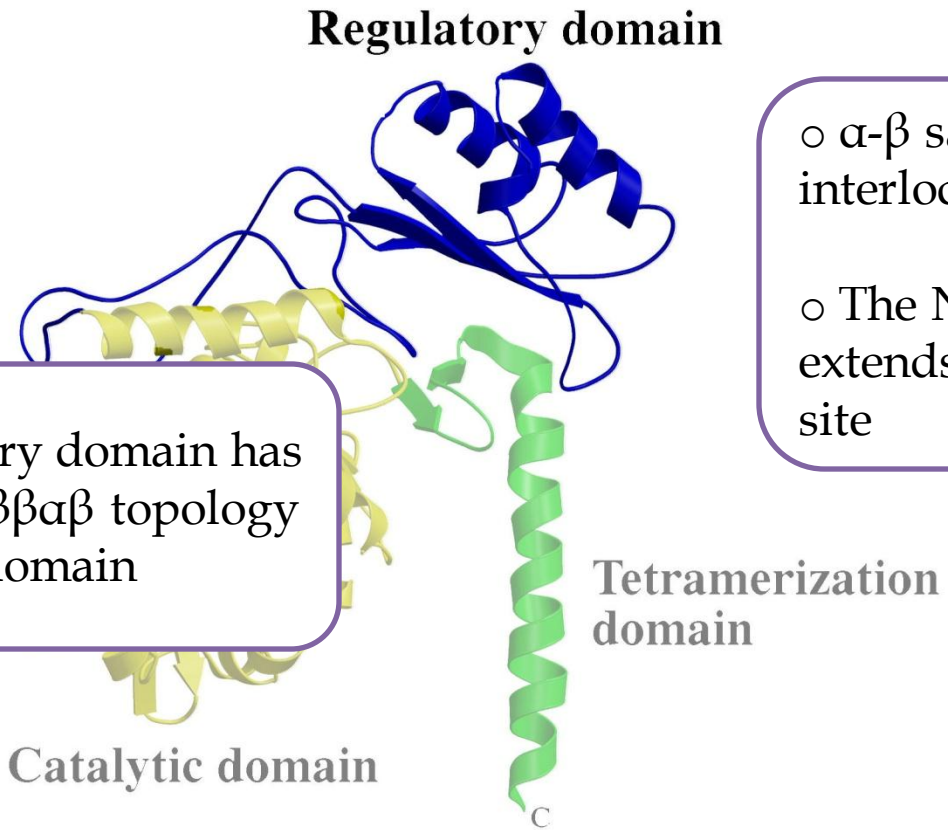



(Erlandsen & Stevens, 1999)

# Structural Characteristics




(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)

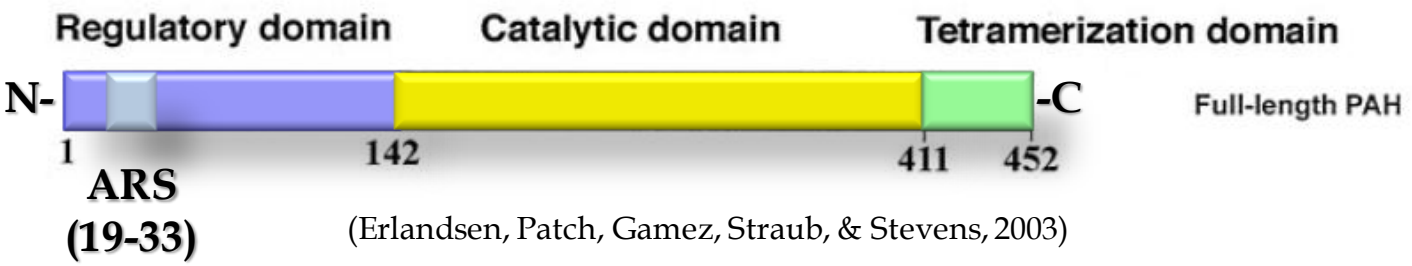



- α-β sandwich with interlocking βαβ motif
- The N-terminal ARS extends over the active site

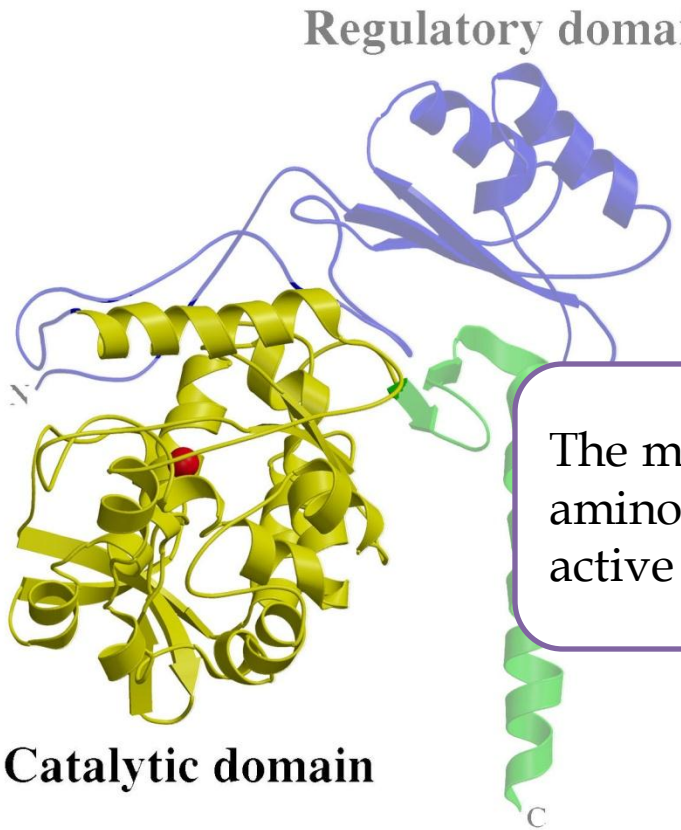
The regulatory domain has the same βαββαβ topology as the ACT domain

(Erlandsen & Stevens, 1999)

# Structural Characteristics




(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)



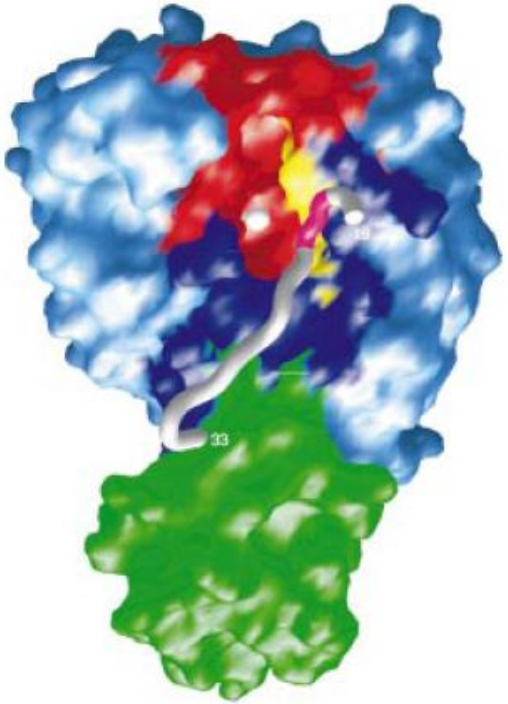

- Basket-like arrangment
- 13 α-helices and 8 β-strands

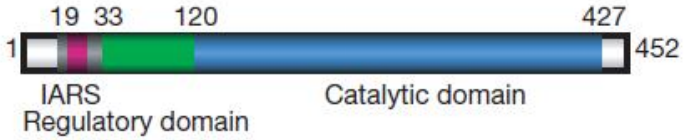
The majority of the 34 amino acids lining the active site are hydrophobic

(Erlandsen & Stevens, 1999)

# Structural Characteristics

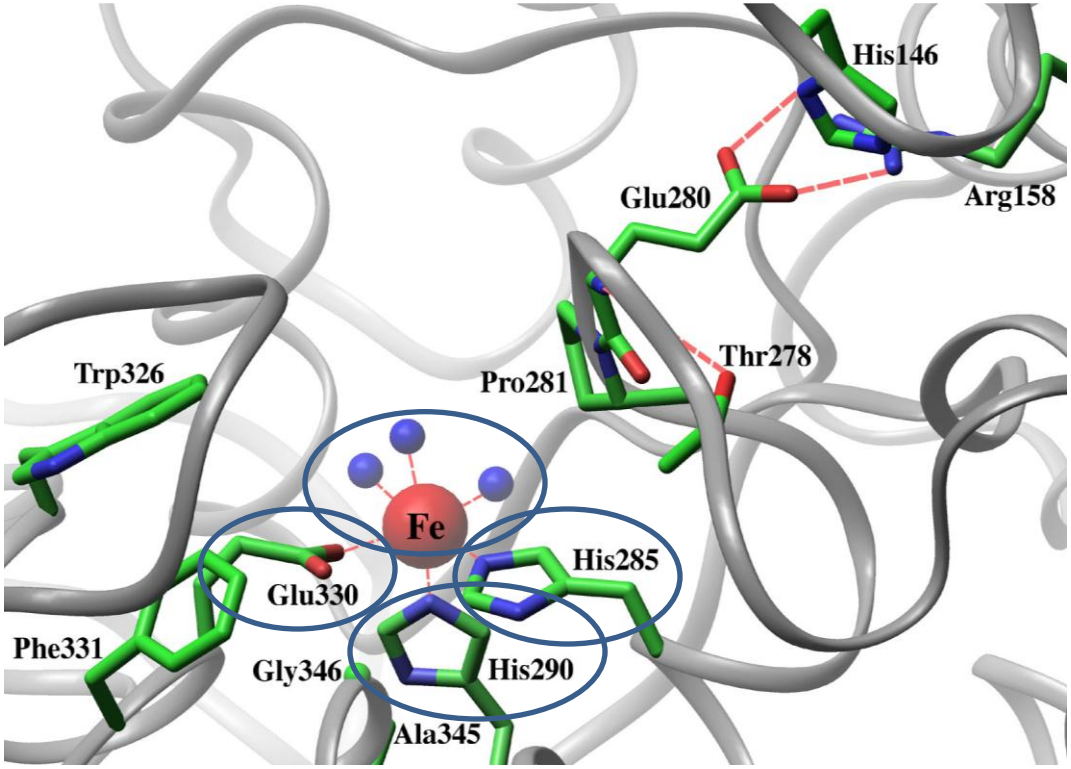
Active site pocket





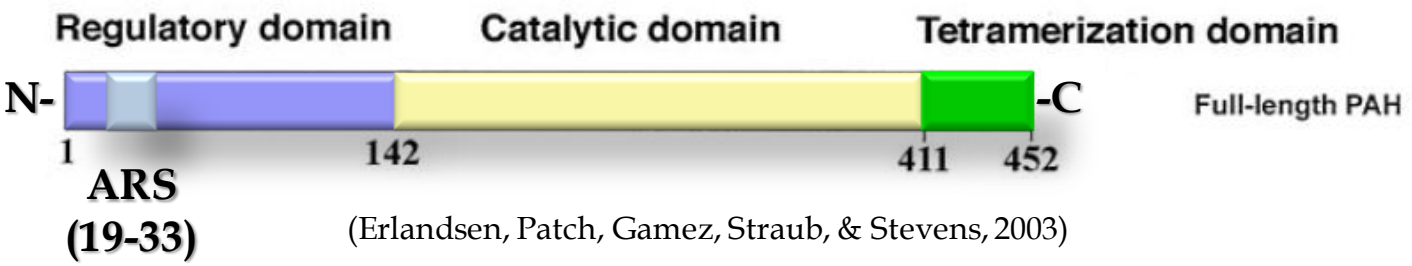

(Kobe & Kemp, 1999)

The iron ligands are arranged in an octahedral geometry, making the iron 6-coordinated

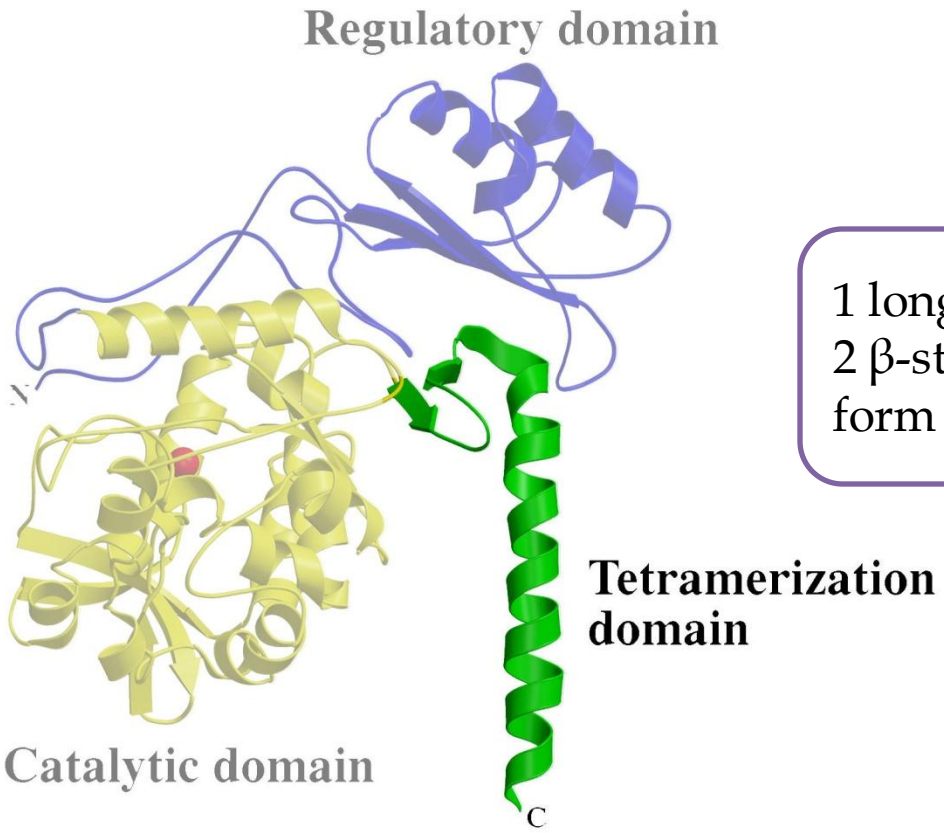



(Erlandsen & Stevens, 1999)

# Structural Characteristics



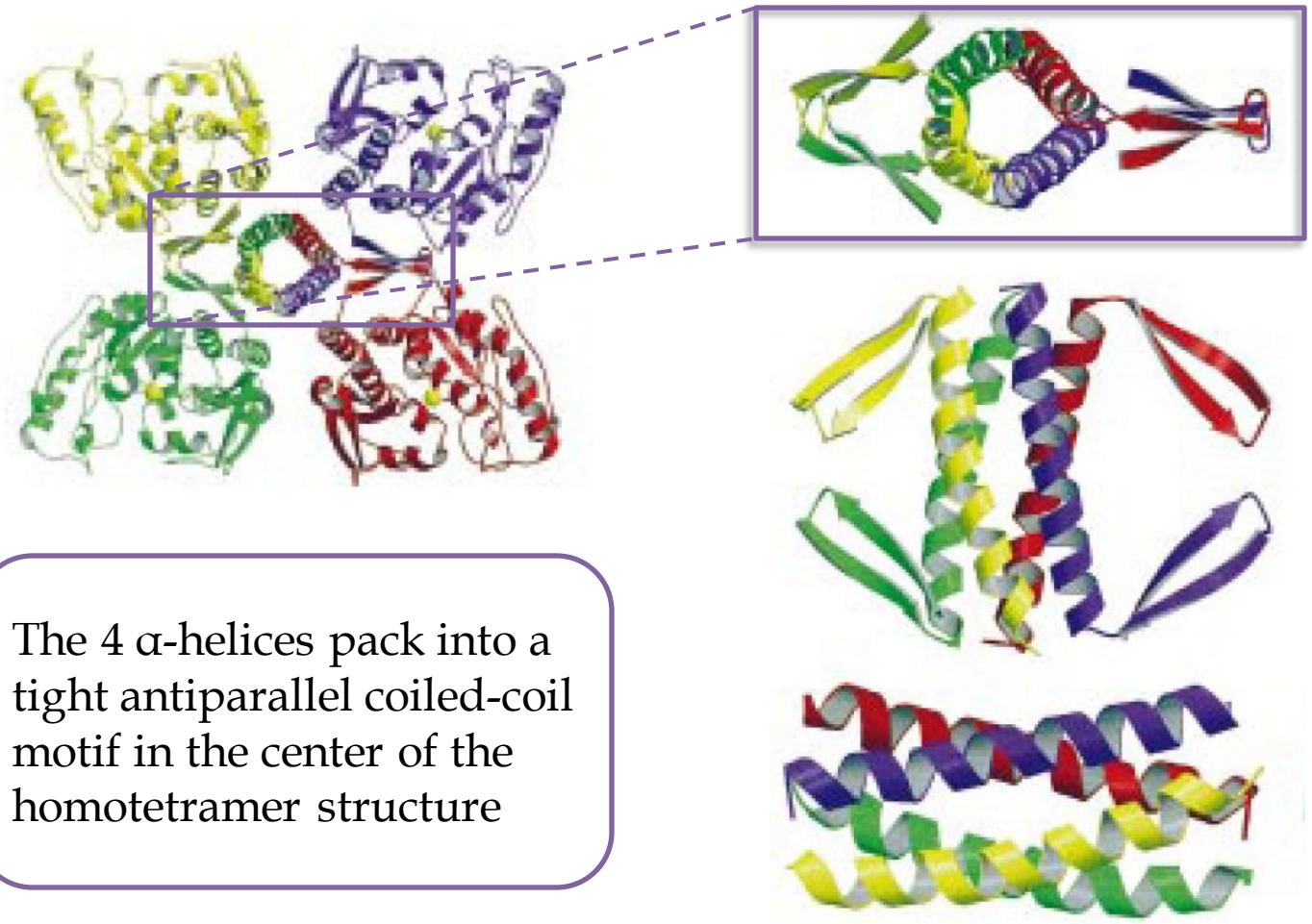

(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)




1 long α-helix and 2 β-strands which form a β-ribbon

(Erlandsen & Stevens, 1999)

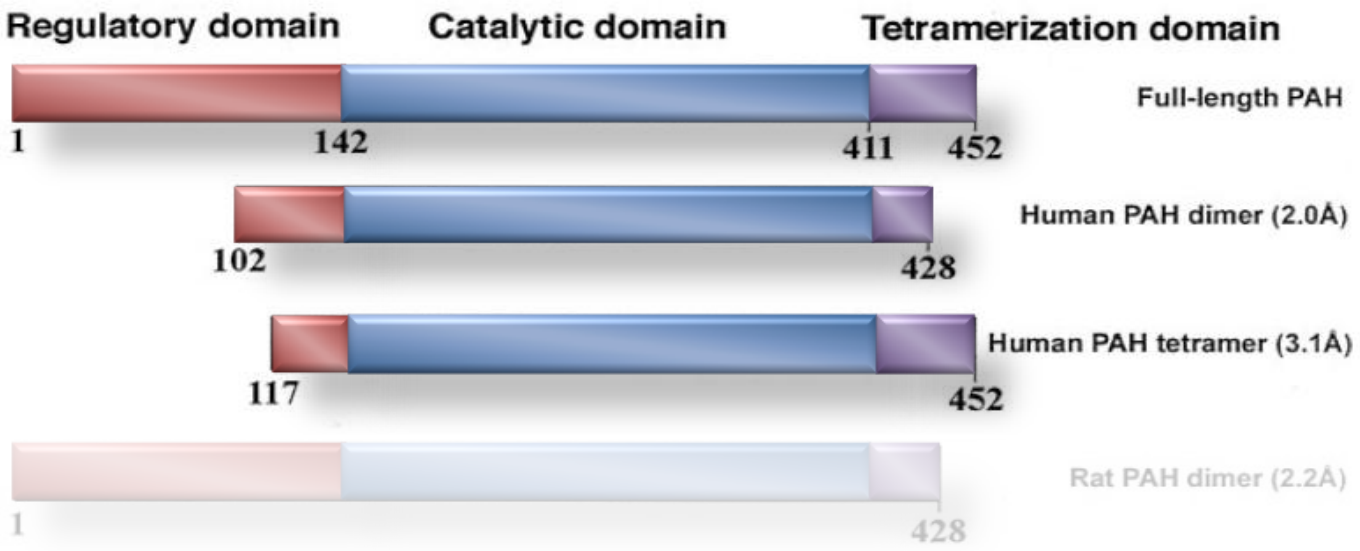
# Structural Characteristics



The 4 α-helices pack into a tight antiparallel coiled-coil motif in the center of the homotetramer structure

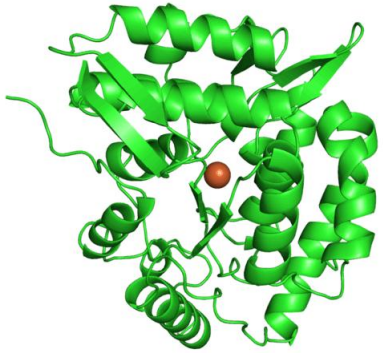
(Flatmark & Stevens, 1999)

# Structural Characteristics



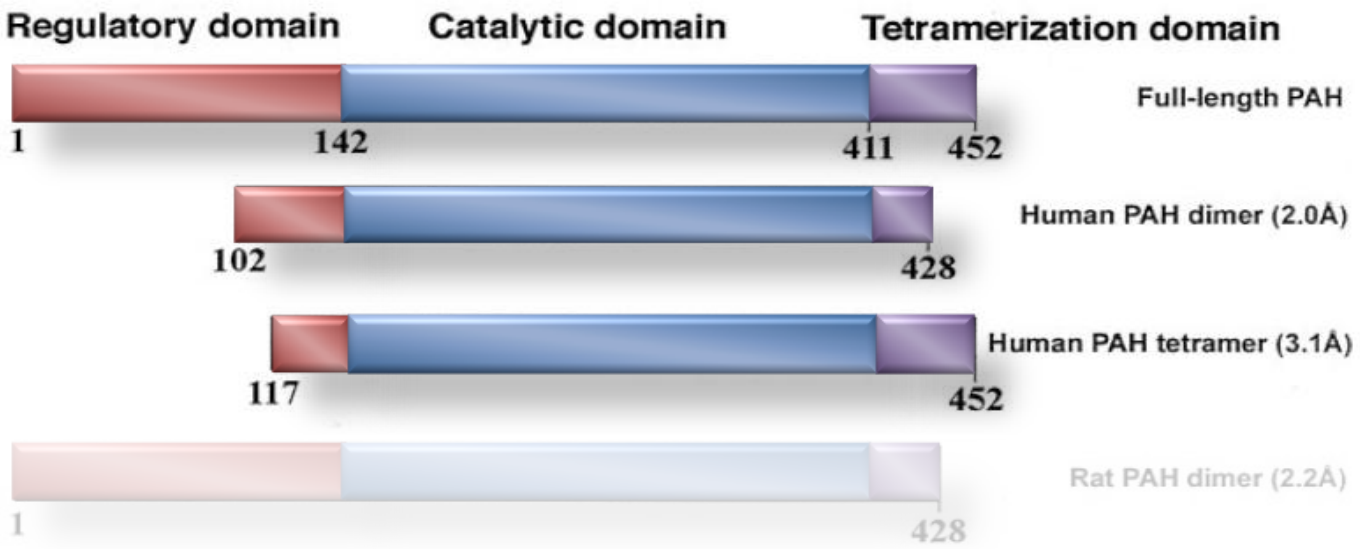

(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)

A high-resolution dimeric double-truncated form



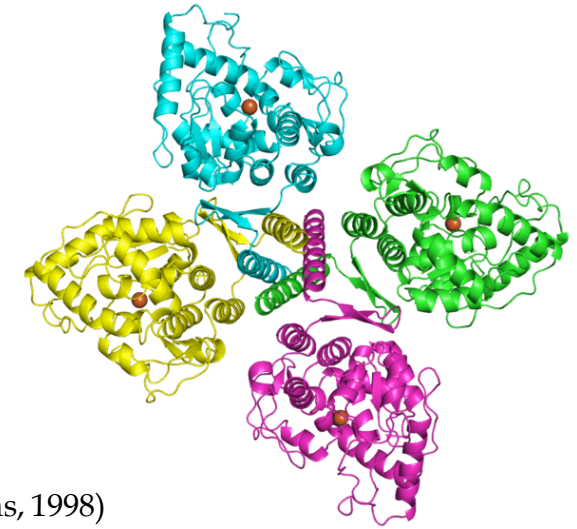
(Erlandsen et al., 1997)

# Structural Characteristics



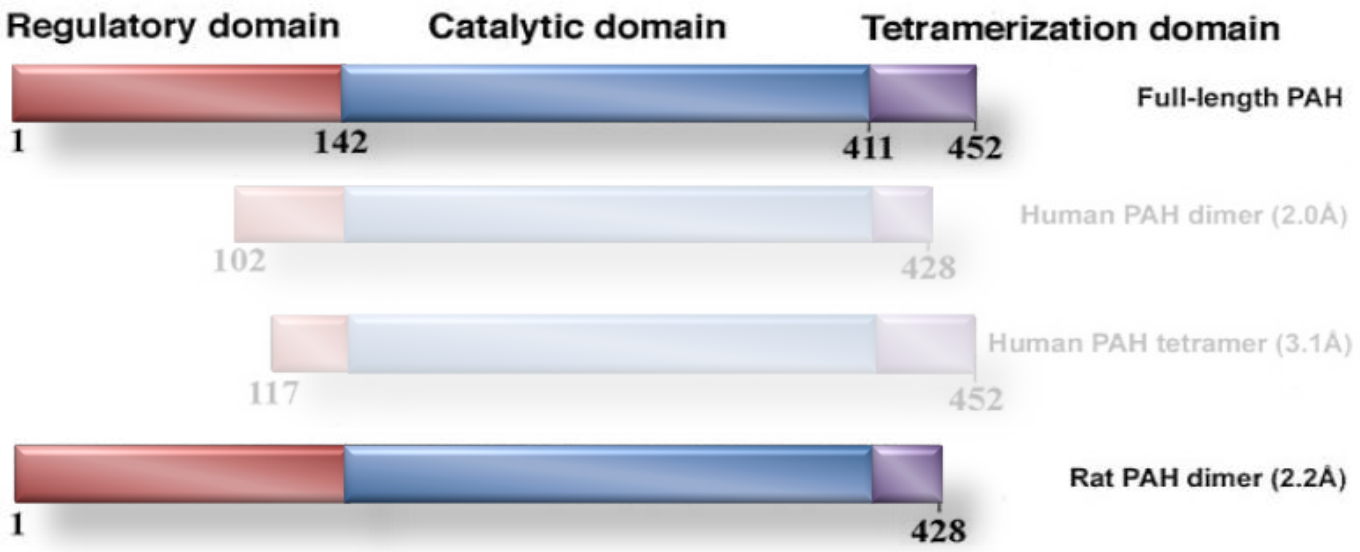

(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)

A tetrameric form containing the catalytic and the tetramerization domains



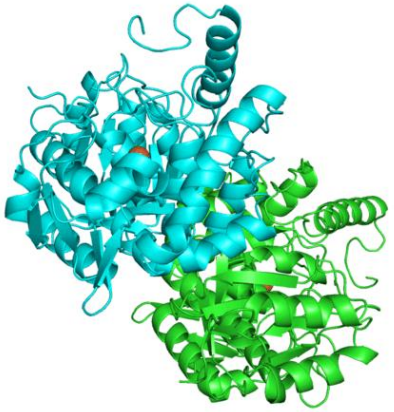
(Fusetti, Erlandsen, Flatmark, & Stevens, 1998)

# Structural Characteristics



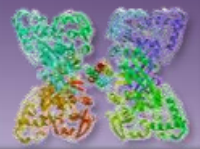

(Erlandsen, Patch, Gamez, Straub, & Stevens, 2003)

A dimeric form containing the regulatory and catalytic domains from rat

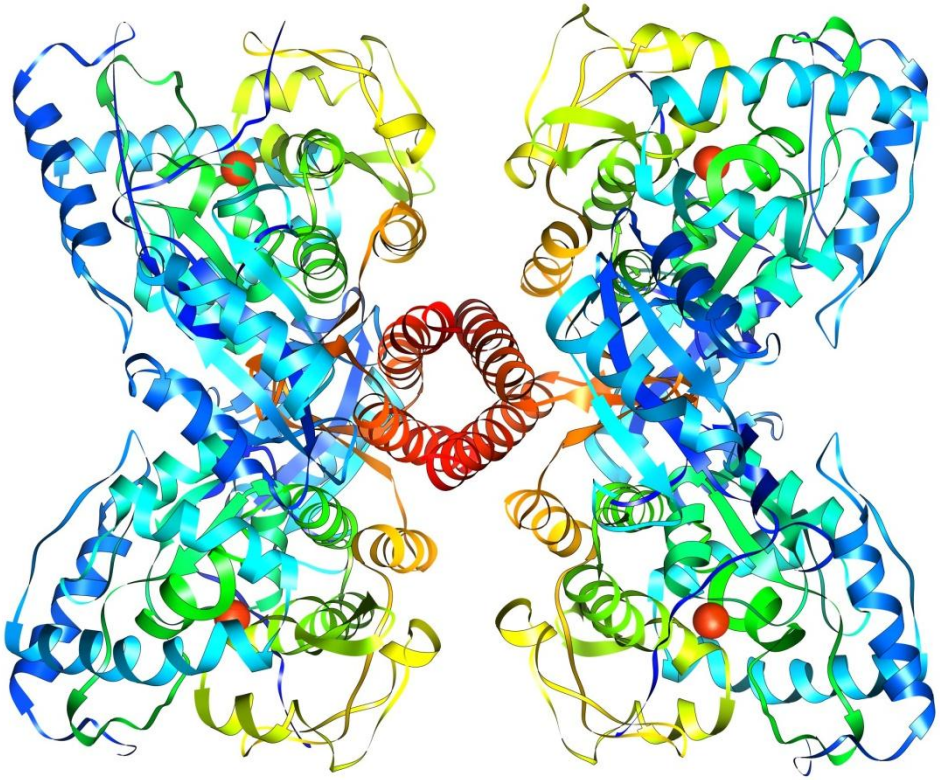


(Kobe et al. 1999)

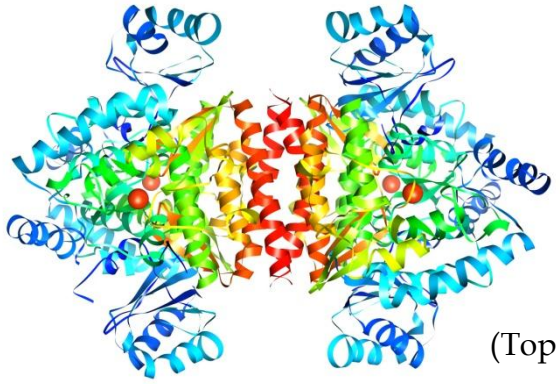
# Structural Characteristics



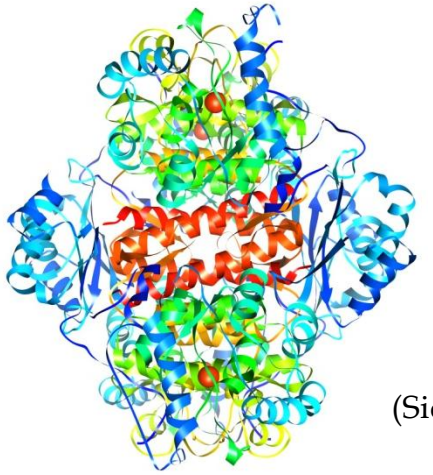
A composite full-length structure model was constructed by superimposing the respective catalytic regions domain



(Erlandsen & Stevens, 1999)

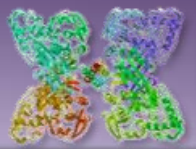


(Top view)



(Side view)

# Structural Characteristics



**Difficulty of crystallizing hPAH**

Quaternary structure heterogeneity (dimers and tetramers)

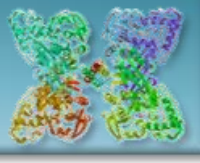
Micro-heterogeneity (deamidation of labile asparagines)

Inter and intra-domain hinge bending regions involved in cooperativity (slow conformational transition)

(Solstad, Carvalho, Andersen, Waidelich, & Flatmark, 2003)

**No full-length tetrameric structure exist for PAH, yet!**

# Aims



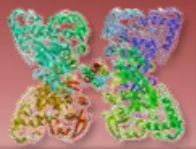
**Full-length tetrameric hPAH 3D structure determination**

Information on the active site structure and binding of substrates and inhibitors

Amino acid residues involved in enzyme catalysis and regulation

Molecular basis of PAH disease-causing mutations

# Experimental Strategies



**1- Enhance stability of the wild-type PAH**

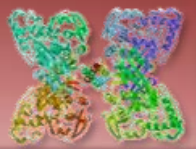
Stabilizing additives

+

Cryo-protectant agents

- Mannitol
- Glycerol
- Threalose
- …

# Experimental Strategies



**2- Produce chimerical proteins with no micro-heterogeneity or with higher stability**

**Suppress PAH micro-heterogeneity**

- Asn → Asp (Residues 32 and/or 376)

**Suppress PAH motions**

- Residues in the oligomerization domain
- Residues in the regulatory domain
- Residues in the flexible intra-domain regions

# Experimental Strategies

Protein production and purification

*IMAC*

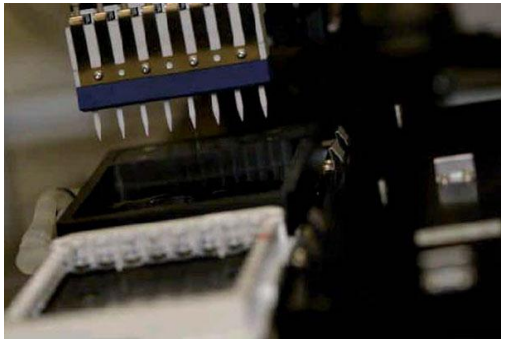
*SEC*

(Leandro, Rivera, Lechner, Almeida, & Konecki, 2000)

# Experimental Strategies

Crystal structure determination

**Crystal growth** → **Data collection** → **Model build and refinement**



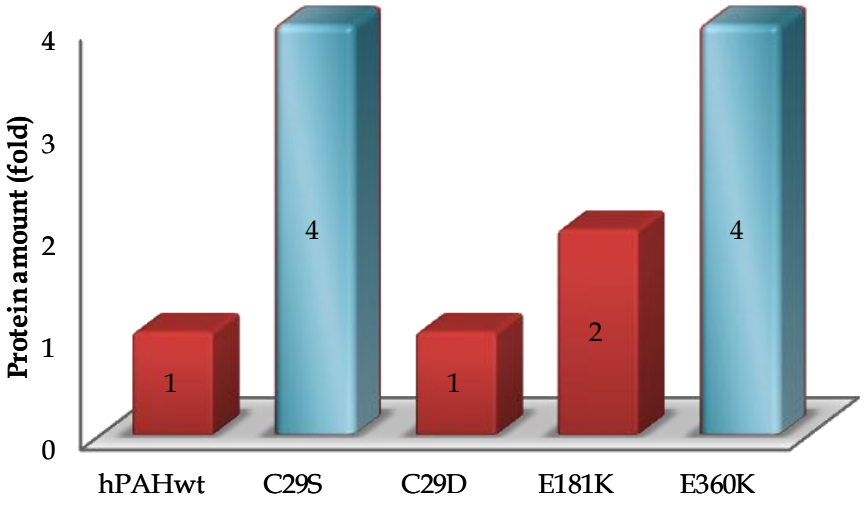
High throughput Crystallization robot, EMBL, Grenoble

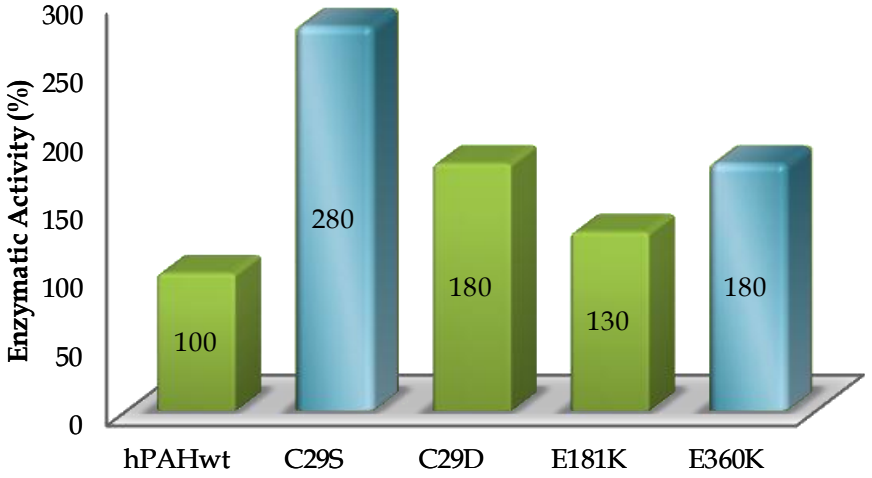
X-ray generator, REQUIMTE, FCT

Synchrotron, ESRF, Grenoble

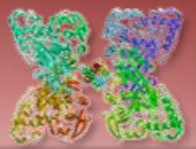
# Experimental Strategies

Chimerical human PAH with enhanced stability and enzymatic activity



# Experimental Strategies



Crystal structure determination

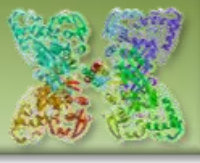
Crystal growth → Data collection → Model build and refinement

Preliminary results:

Using PEG 8000 as precipitant and concentrated protein

← C29S

# Acknowledgements

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