



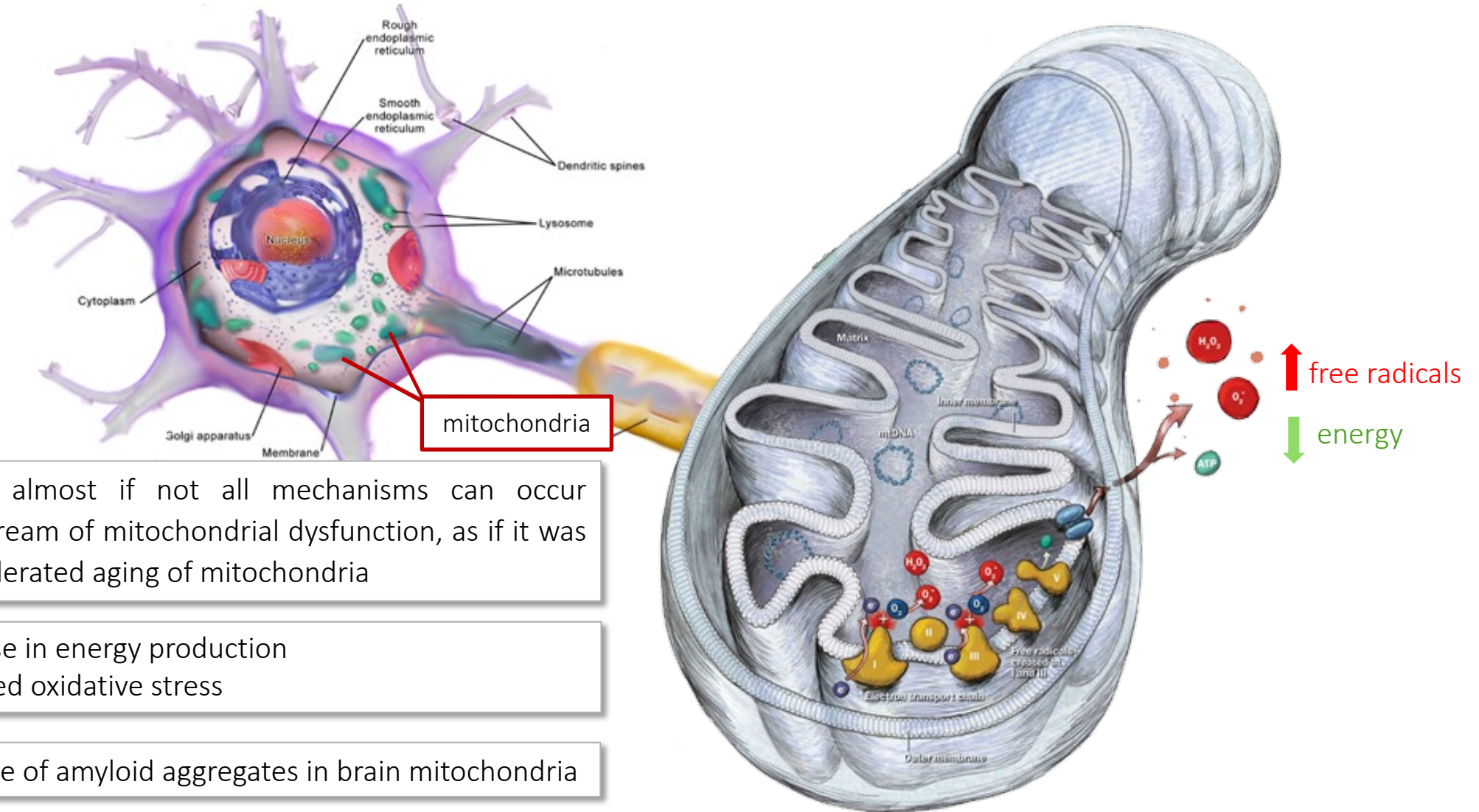
# Integrative Biology to tackle mitochondrial respiration in Alzheimer's pathogenesis

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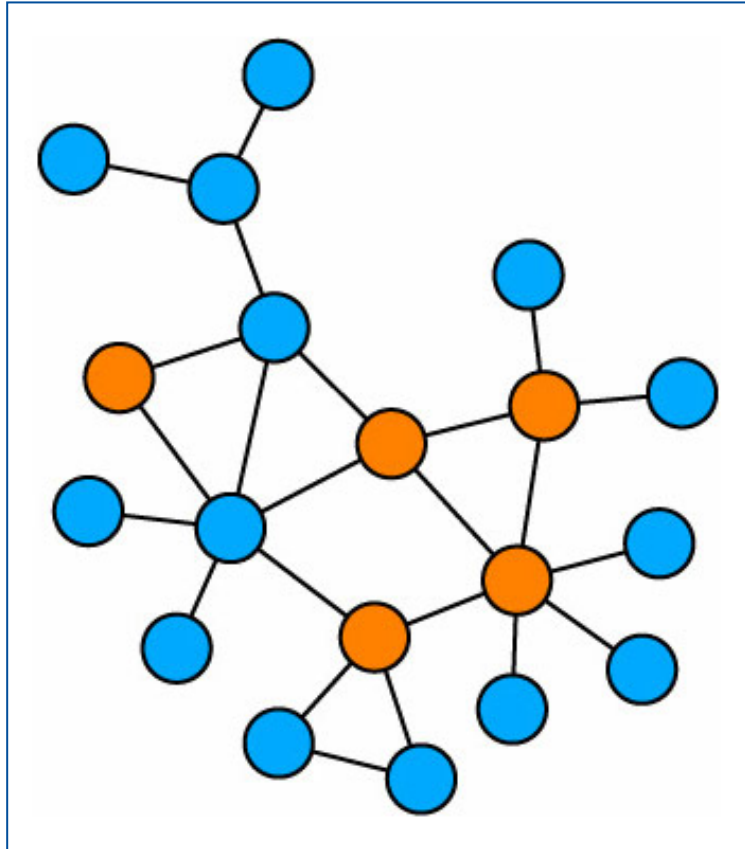


In AD, almost if not all mechanisms can occur downstream of mitochondrial dysfunction, as if it was an accelerated aging of mitochondria

Decrease in energy production  
Increased oxidative stress

Presence of amyloid aggregates in brain mitochondria

In fact, impaired energy metabolism is one of the earliest and most consistent features in AD



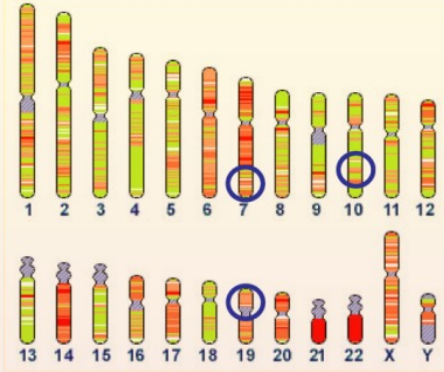
Disease-related proteins for many pathologies often work together within the same biological module (*Oti et al. 2007* & others)

AD well-established proteins  
(APP, PSEN1, PSEN2, APOE, ...)  
are highly interconnected

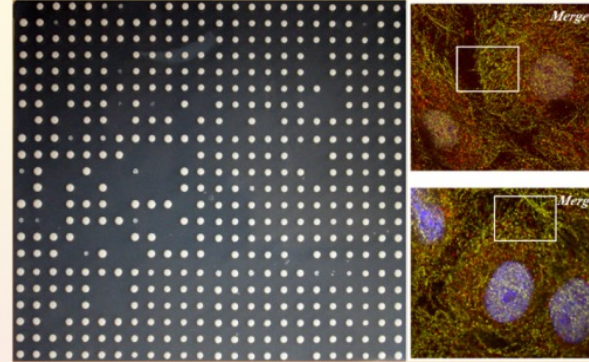
connectivity degree between two given nodes in the AD network is 3.2 compared to 4.7 in a random human interactome network ( $p < 10^{-17}$ )



# PREVIOUS WORK: NETWORK BIOLOGY TO IDENTIFY NEW PROTEINS INVOLVED IN AD



1. Identification of potential causative and susceptibility genes involved in AD

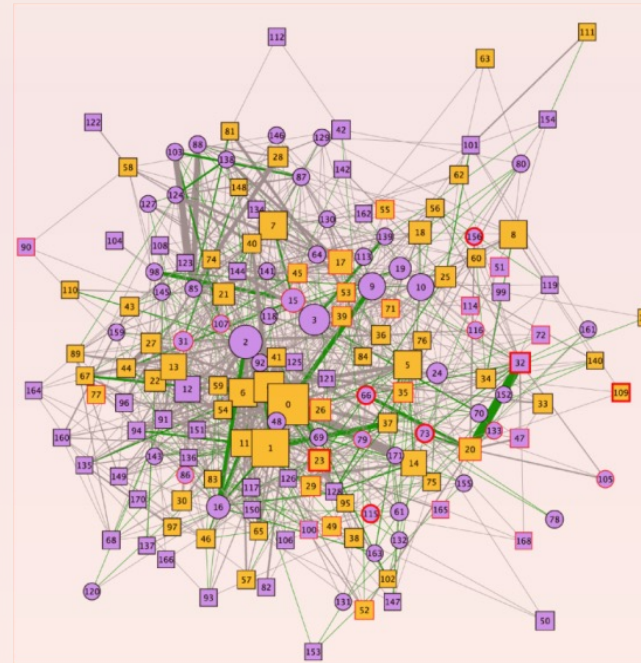


2. Experimental characterization of protein interactome network associated to AD



3. Computational analysis of the interactome. Integration with global-disease associated network to contextualize the interactome

5. Functional module analysis of AD-PIN. Elaboration of novel hypotheses to provide insights on the molecular mechanisms implicated in AD



4. Generation of the AD-protein interaction network (AD-PIN)



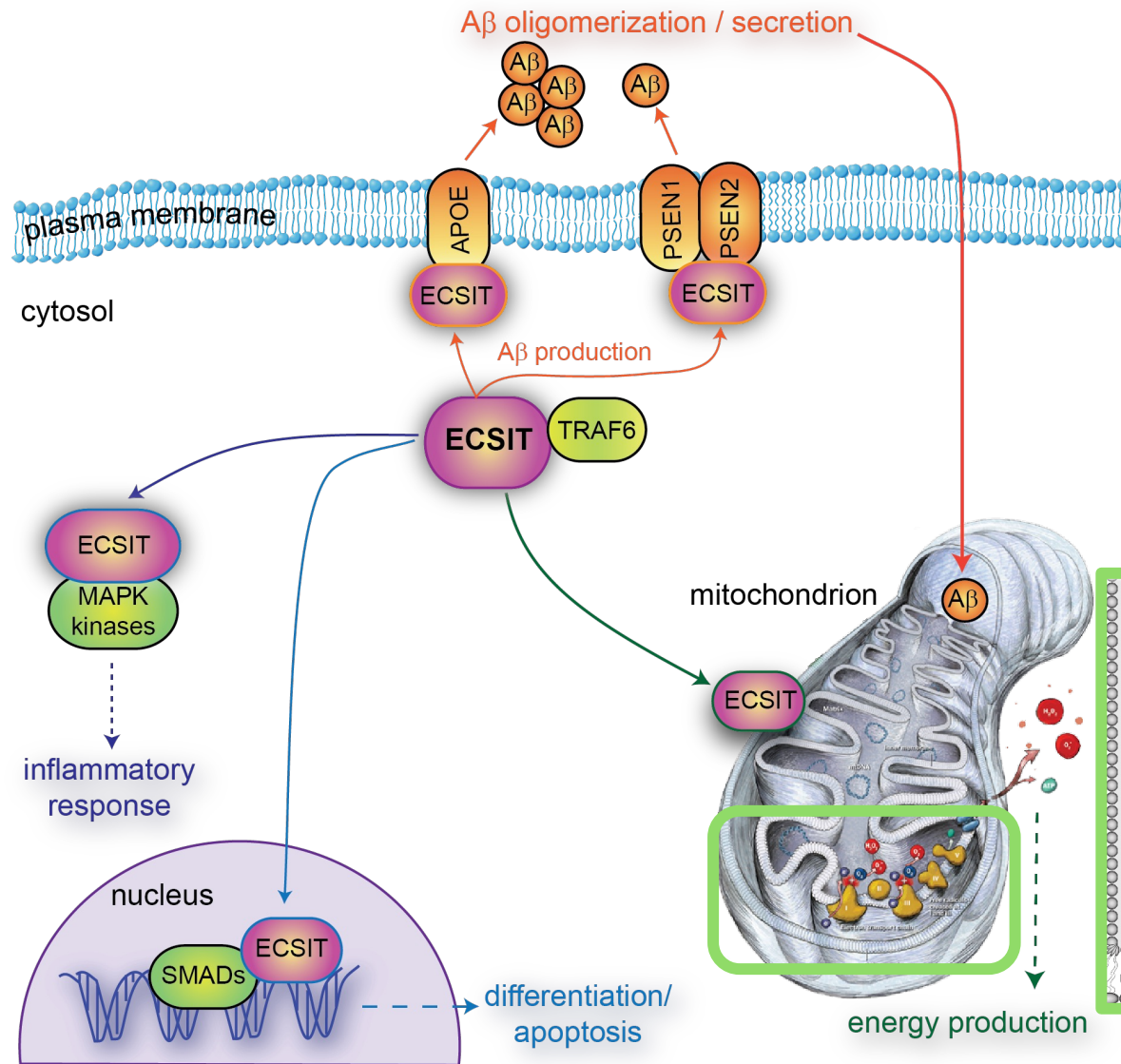
*Soler-López et al. Genome Res. 2011*



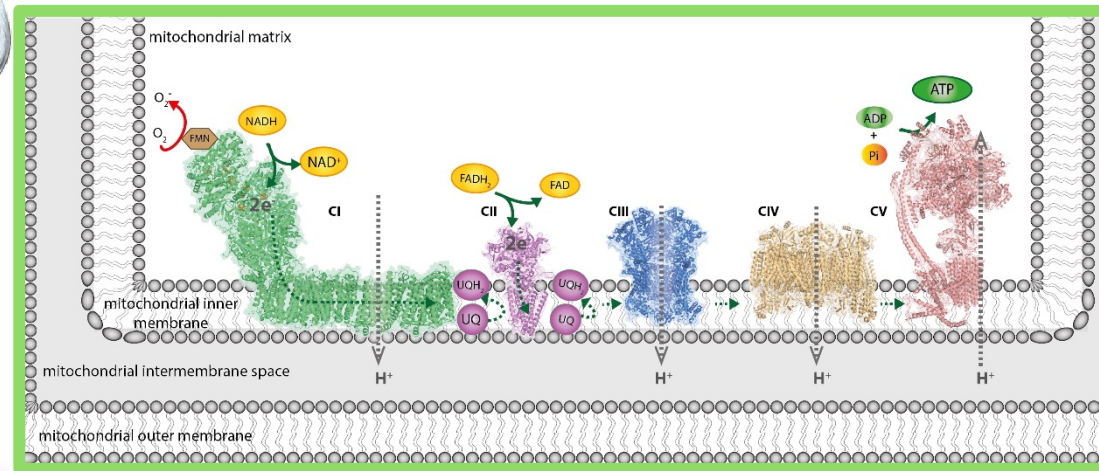
INSTITUTE FOR RESEARCH IN BIOMEDICINE

ECSIT IS A MULTITASK CHAPERONE...

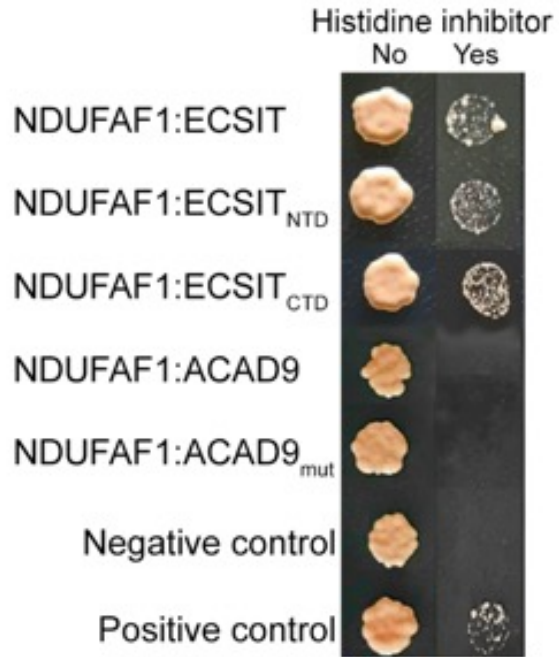
...AND A MOLECULAR LINK IN AD PATHOGENESIS?



Respiratory chain (OXPHOS)

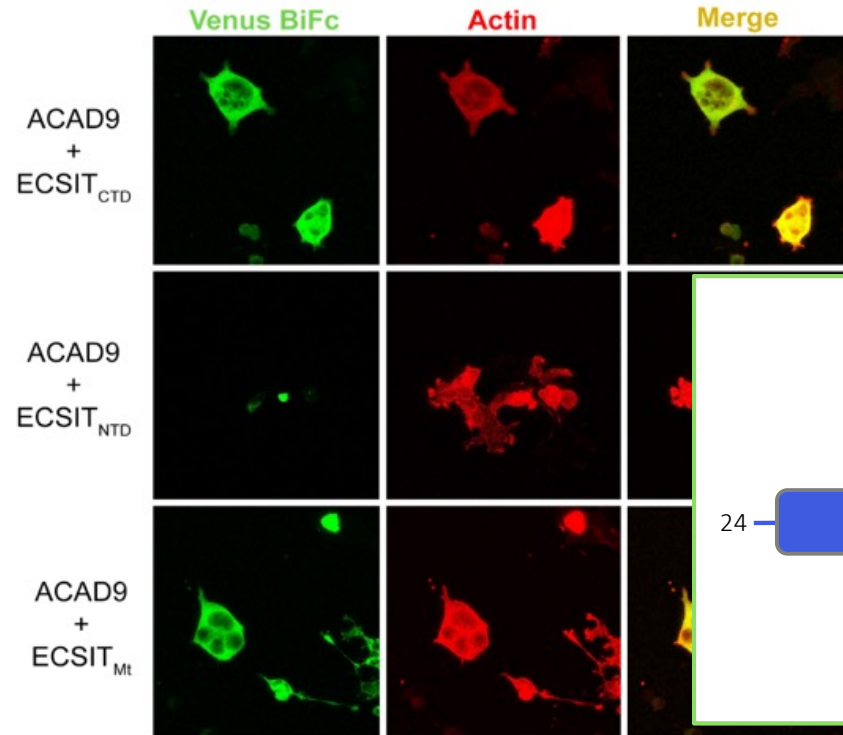


## Two-hybrid interaction assays in yeast

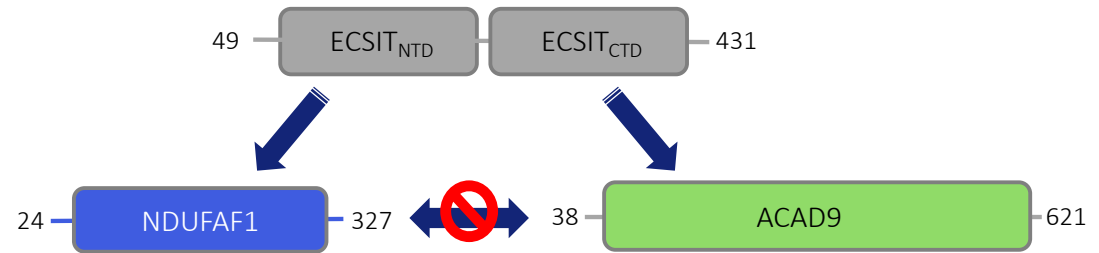


NDUFAF1 interacts with ECSIT but not with ACAD9

## BiFC interaction assays in human cells



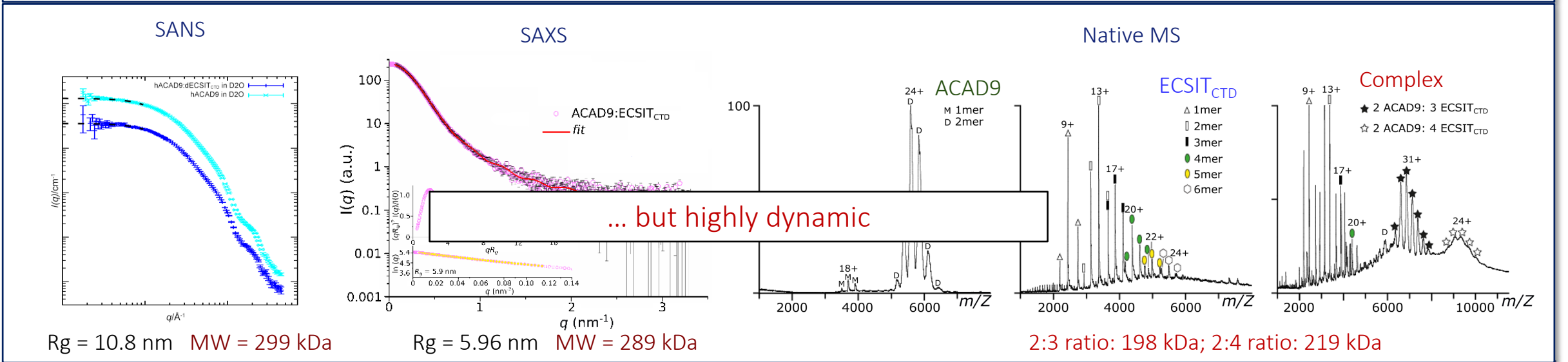
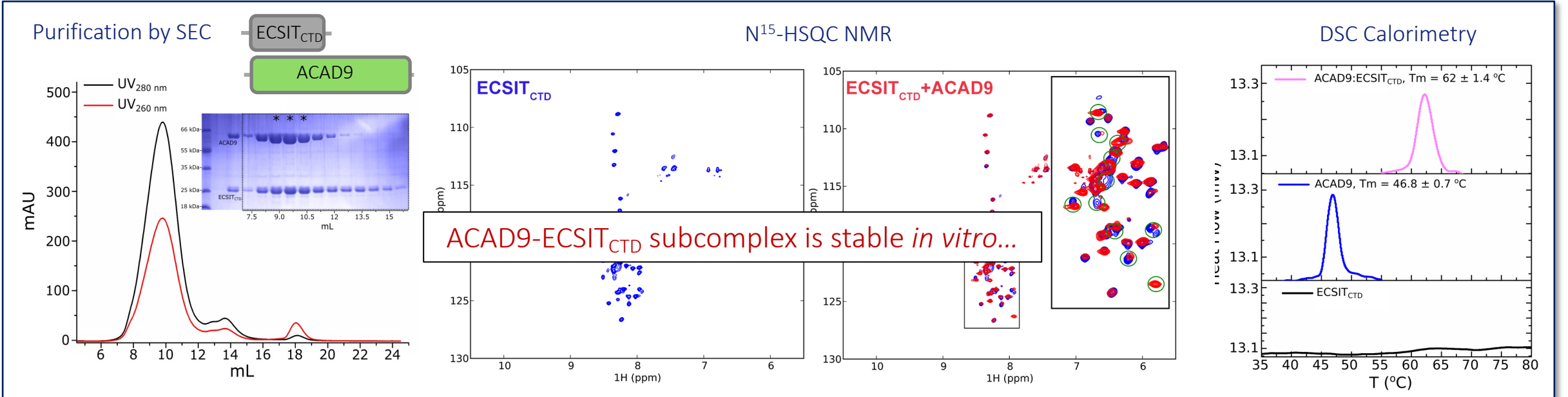
ECSIT<sub>NTD</sub> does not interact with ACAD9



ECSIT is a framework protein for MCIA complex formation



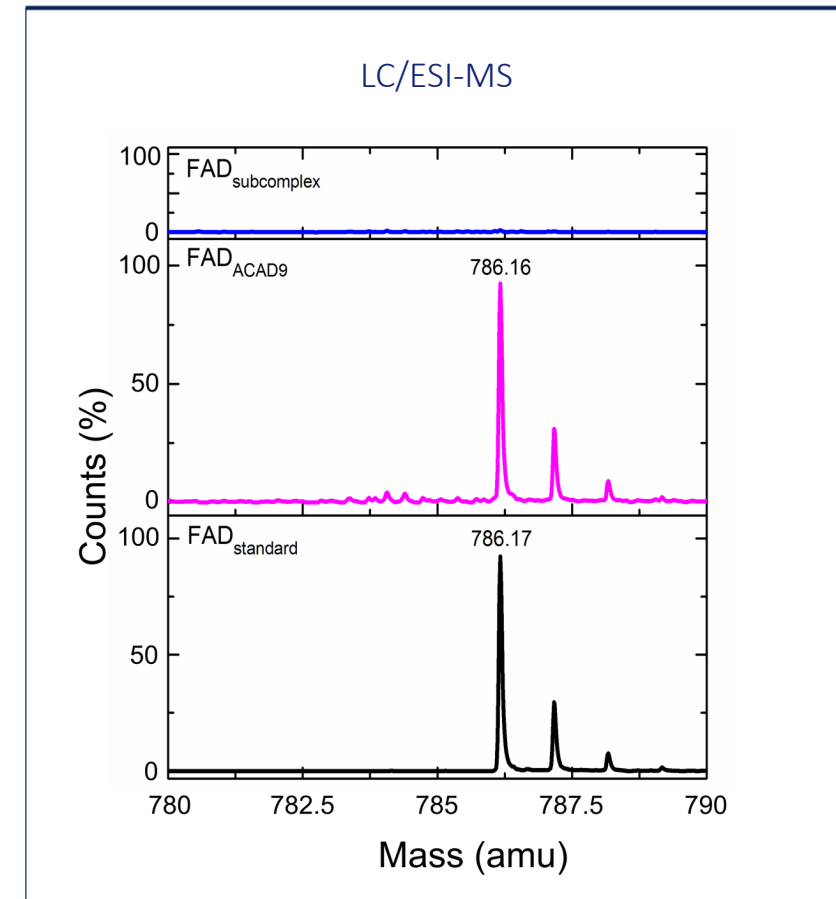
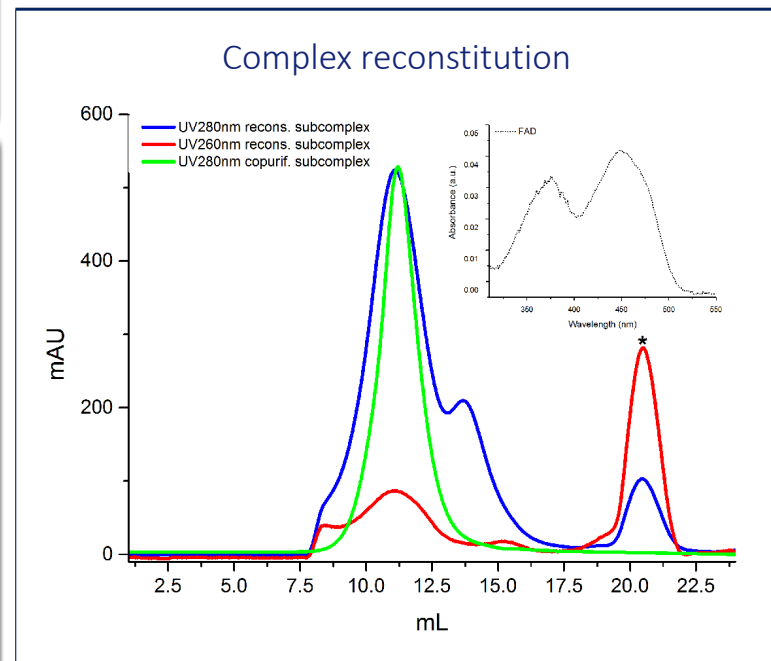
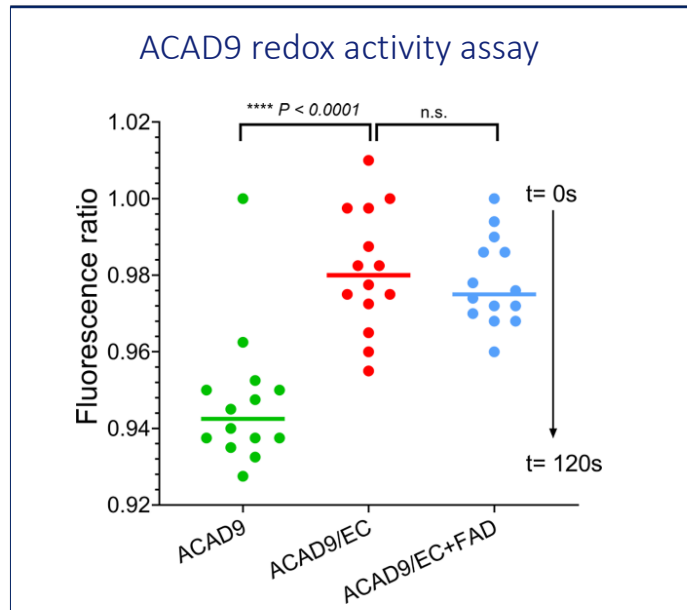
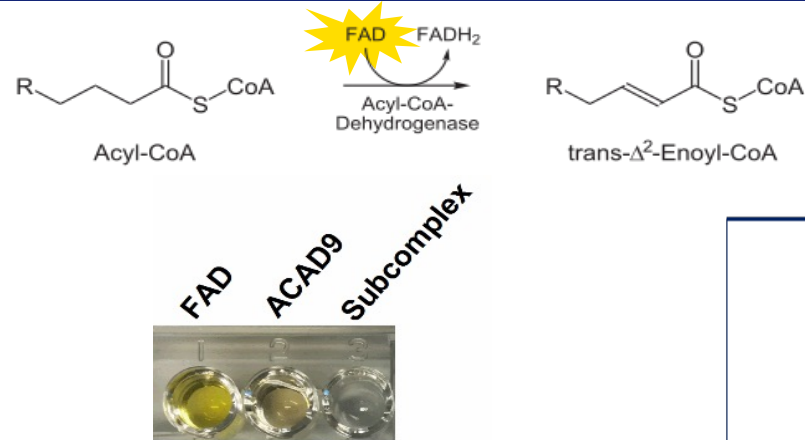
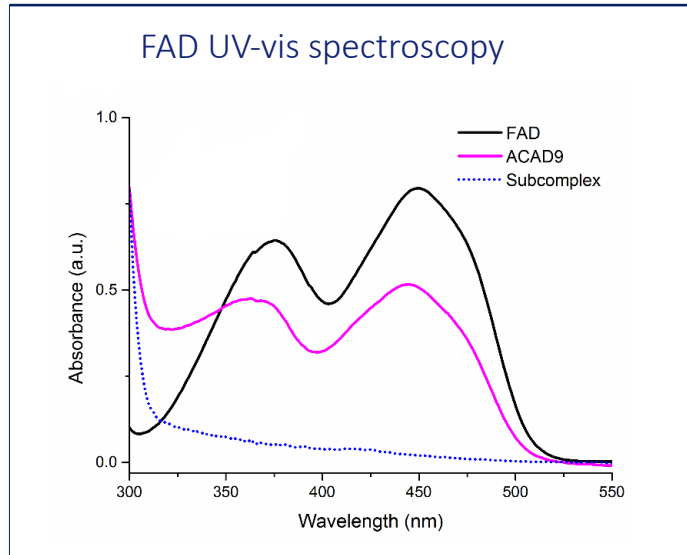
# BIOPHYSICAL CHARACTERISATION OF MCIA SUBCOMPLEX: STOICHIOMETRY & MOLECULAR FOLDING



• Giachin et al. Angew. Chem. Int. Ed. 2021, 60, 4689–4697



# FUNCTIONAL ACTIVITY OF ACAD9 UPON MCIA COMPLEX ASSEMBLY

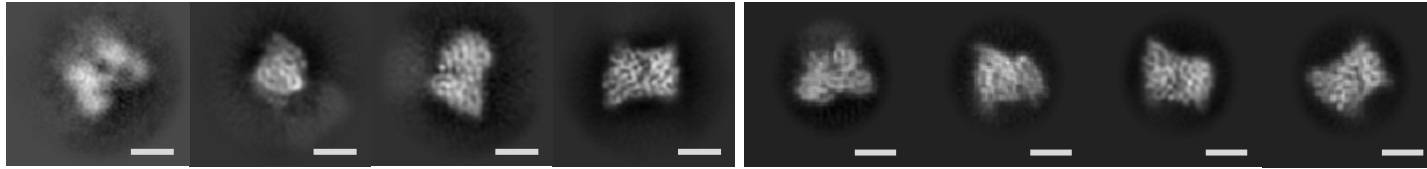


ECSIT ejects FAD and shuts down the dehydrogenase activity of ACAD9

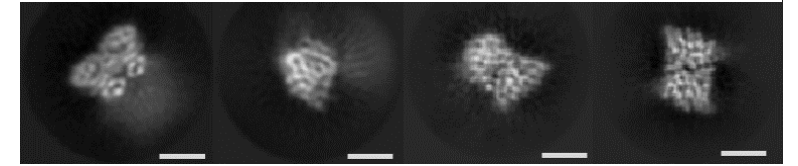
# STRUCTURAL CHARACTERIZATION OF ACAD9 AND MCIA SUBCOMPLEX @ HIGH RESOLUTION

in collaboration with Irina Gutsche (IBS)

Reconstruction ACAD9 homodimer @ 4 Å (anisotropic resolution)

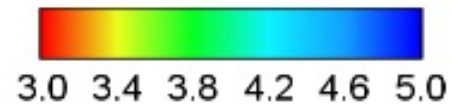
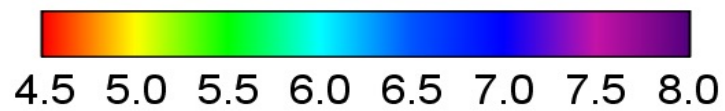
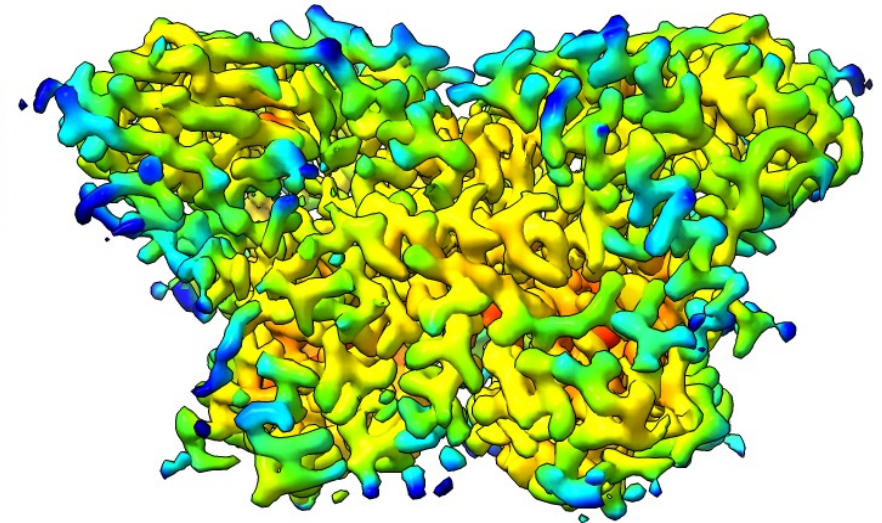


Reconstruction MCIA subcomplex @ 3 Å



WT

Single point mutant

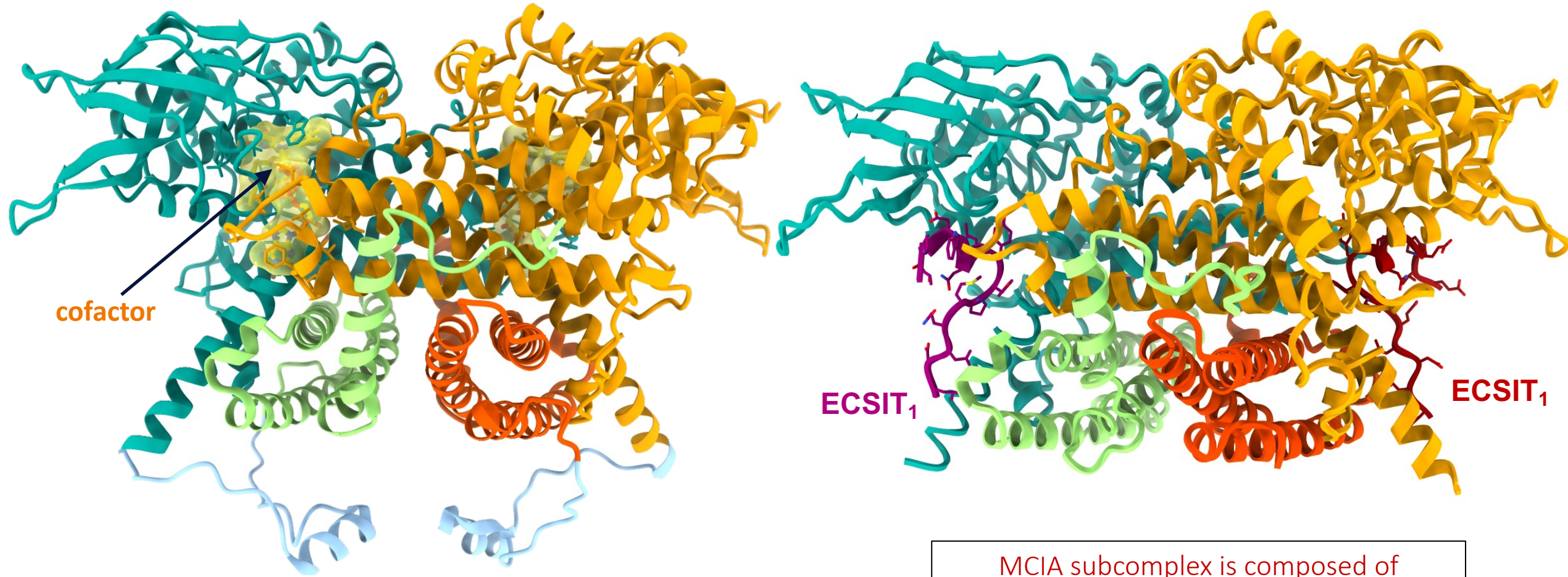




# STRUCTURE OF ACAD9 ALONE VS IN COMPLEX WITH AN ECSIT FRAGMENT

ACAD9 homodimer

MCIA subcomplex



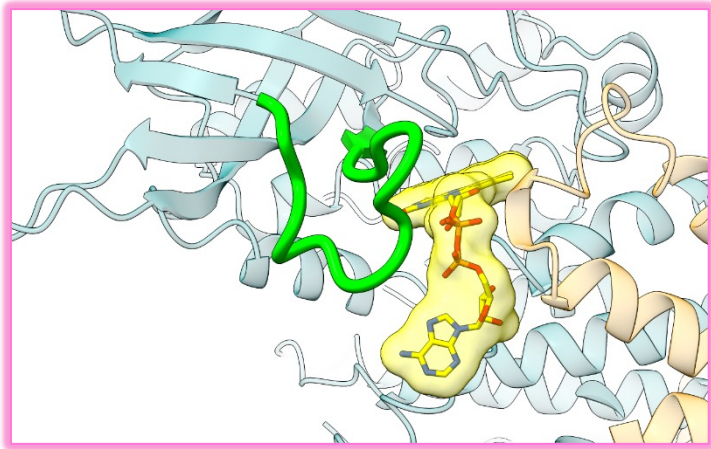
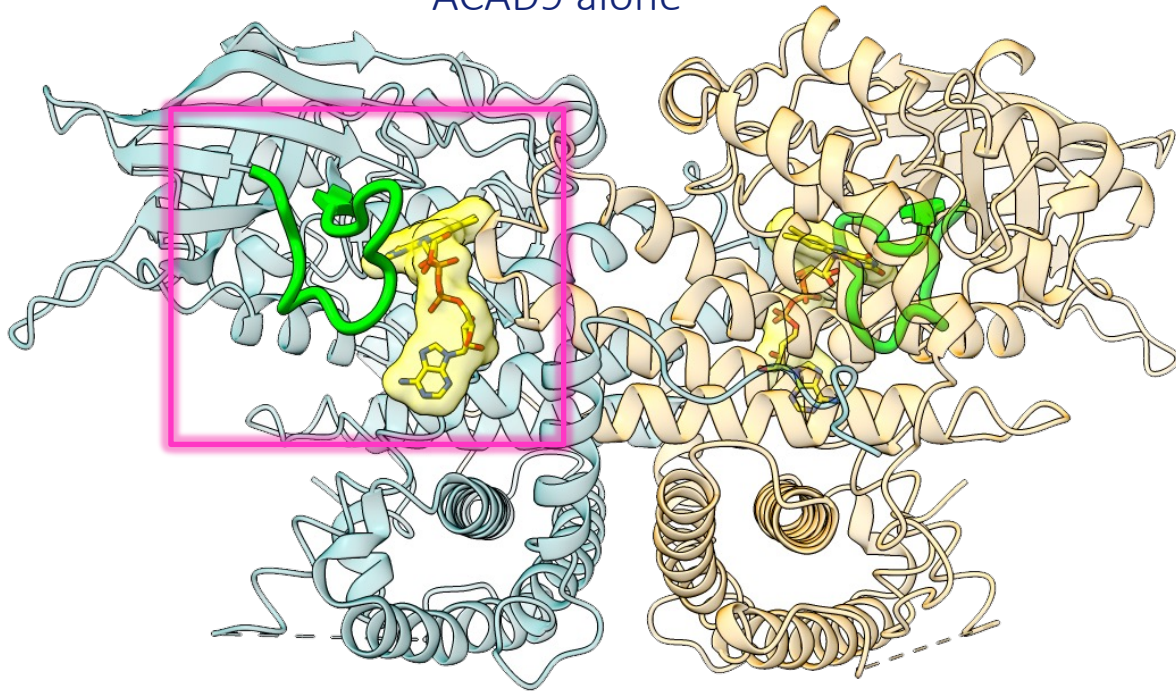
ACAD9 alone has a bound cofactor and flexible helices

MCIA subcomplex is composed of an ACAD9 dimer and two ECSIT<sub>CTD</sub>

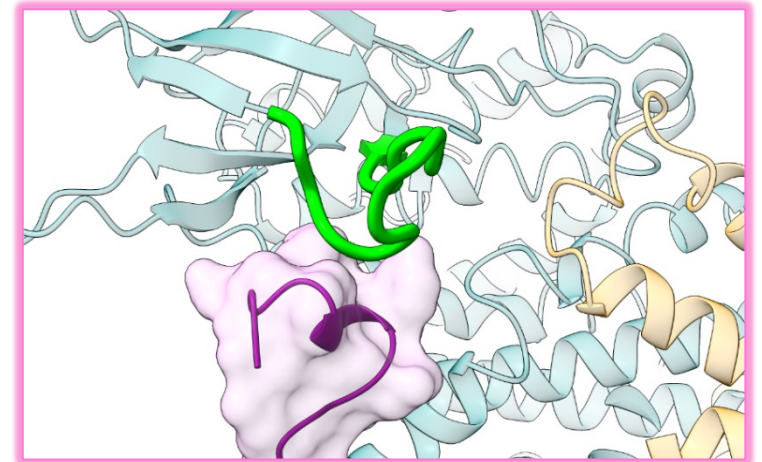
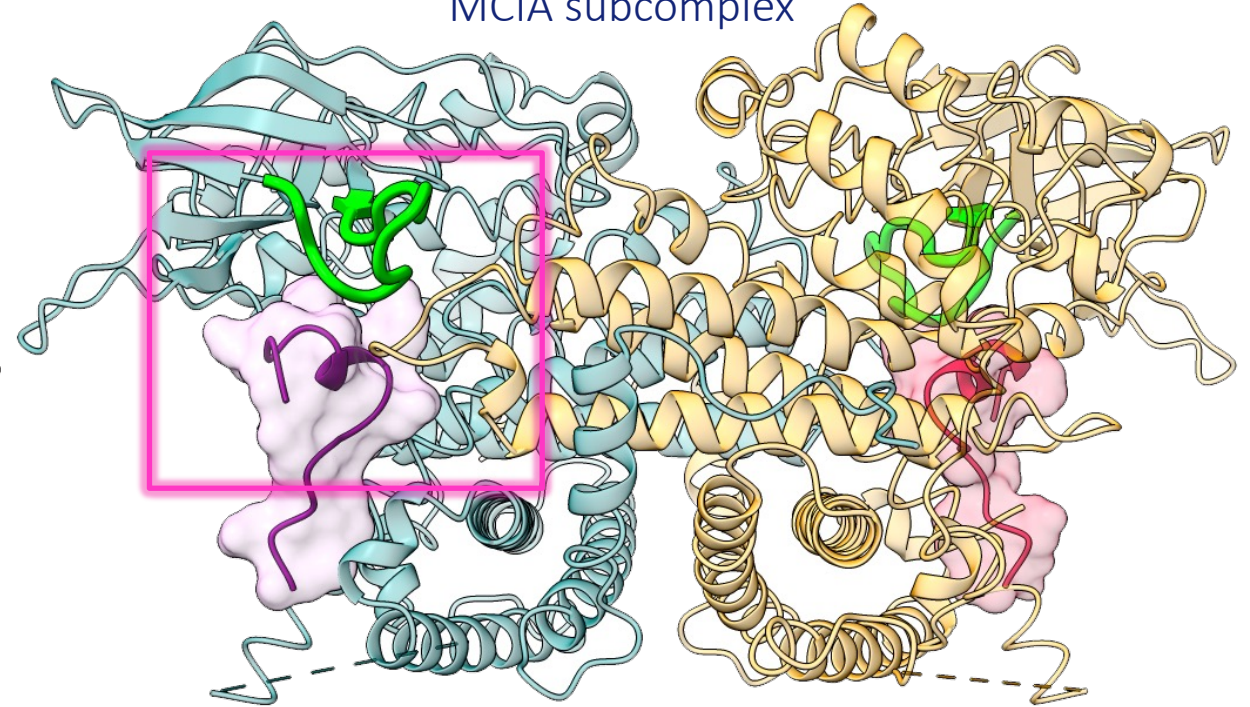


# STRUCTURAL ANALYSIS OF ACAD9 ALONE VS IN COMPLEX WITH AND ECSIT FRAGMENT

ACAD9 alone

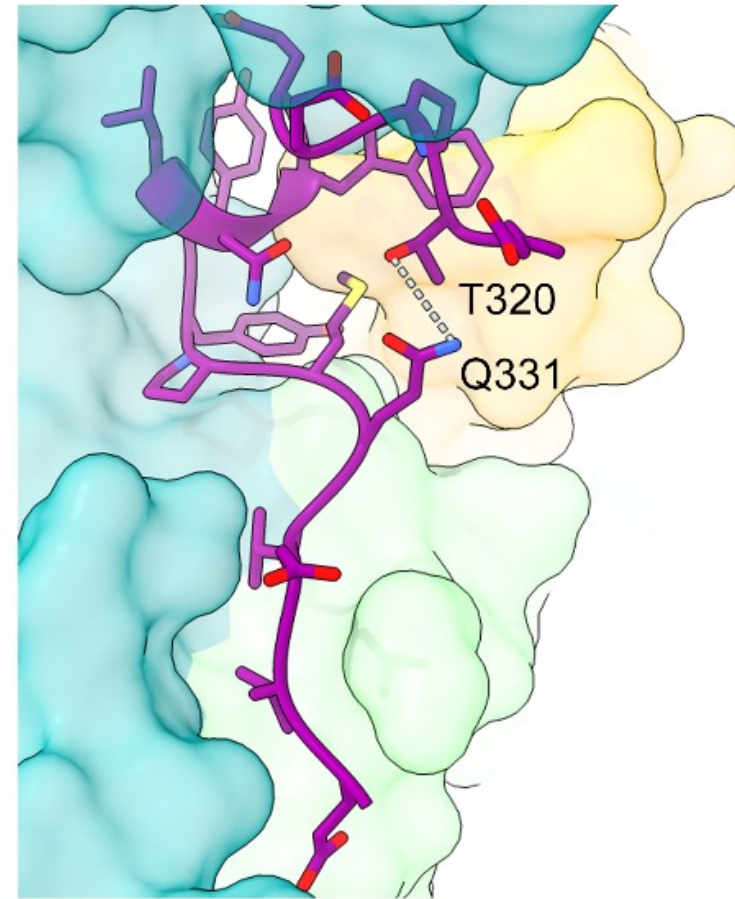
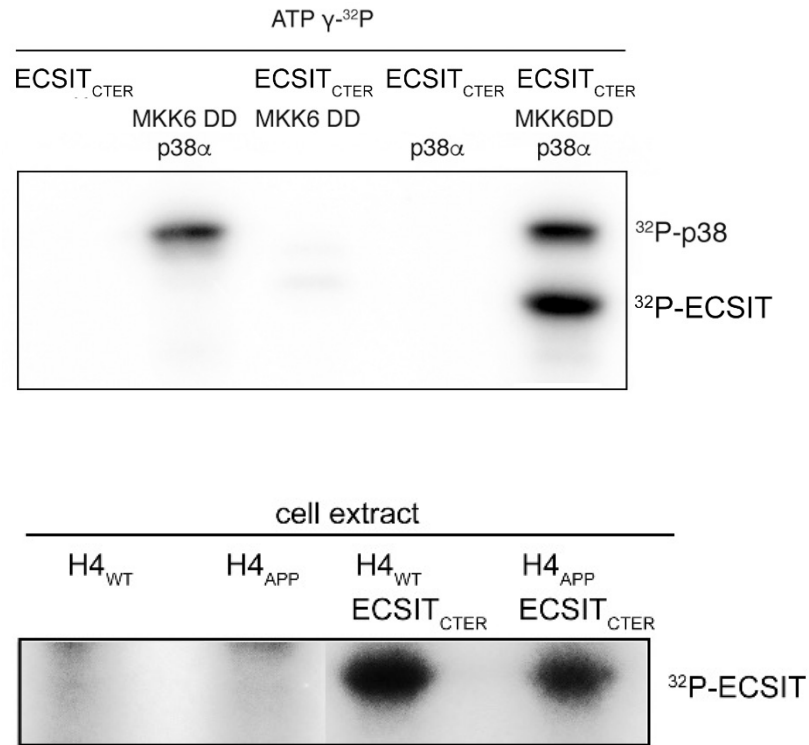


MCIA subcomplex



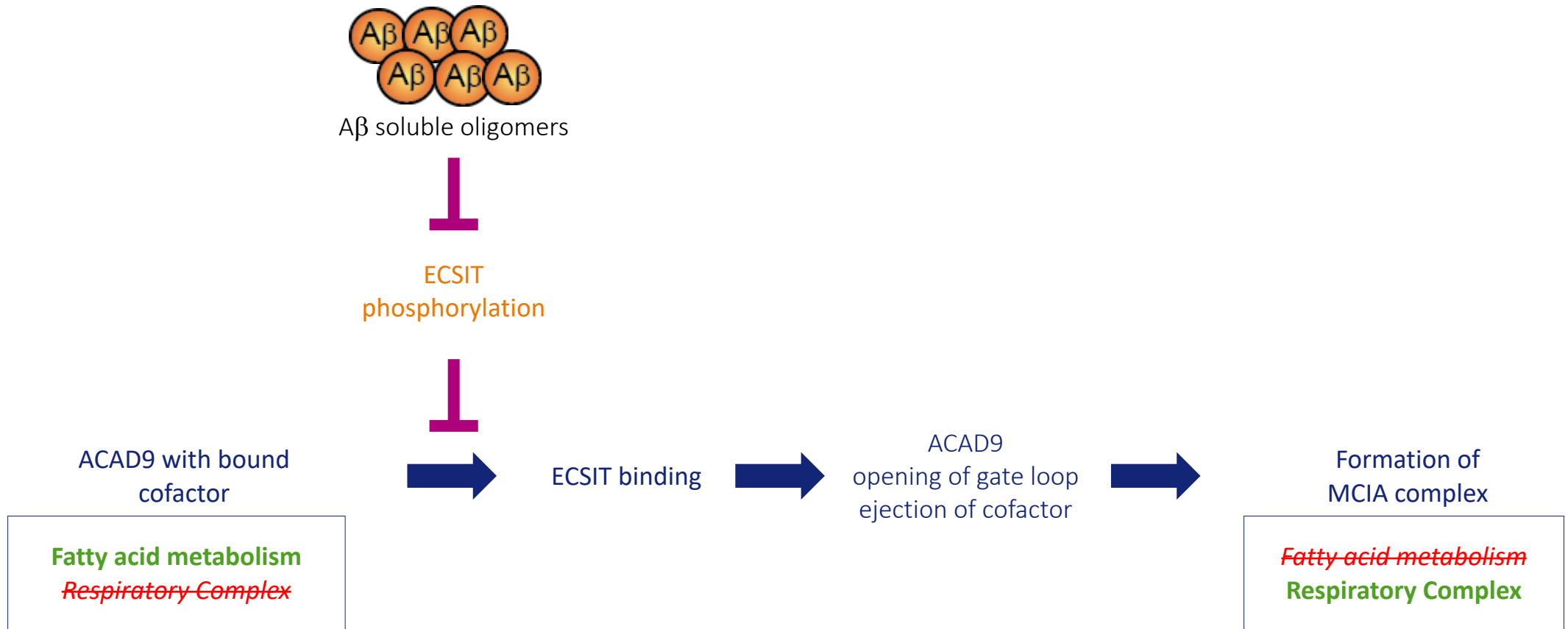


# LOOKING FOR MCIA ASSEMBLY TRIGGERS: ECSIT UNDERGOES PHOSPHORYLATION



ECSIT phosphorylation perturbs the assembly with ACAD9

# THE MOLECULAR MECHANISM OF THE MCIA COMPLEX ASSEMBLY, LINK WITH AMYLOID TOXICITY





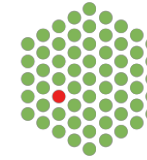
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Patrick Aloy



Alain Buisson



Sylvain Prévost  
Trevor Forsyth



Partnership For Structural Biology



### X-ray Macromolecular crystallography

With EMBL  
European Molecular Biology Laboratory

- **ID30A-1:** fully automated 12.8 keV, 20-100  $\mu\text{m}$
- **ID30B:** } tunable 6-20 keV (2.0-0.62  $\text{\AA}$ ), 20-50  $\mu\text{m}$
- **ID23-1:** }
- **ID30A-3:** minifocus 12.9 keV (0.96  $\text{\AA}$ ), 15  $\mu\text{m}$
- **ID23-2:** microfocus 14.2 keV (0.87  $\text{\AA}$ ), 5  $\mu\text{m}$
- **EBSL8 (ID29):** serial, nanofocus 10-25 keV (1.24- 0.5  $\text{\AA}$ ), 0.5 $\mu\text{m}$

With ibs  
Institut de Biologie Structurale

- **BM07/FIP2:** tunable 7-15 keV, 50  $\mu\text{m}$  – 250  $\mu\text{m}$

### Small angle X-ray scattering

- **BM29:** 7-15 keV, 50  $\mu\text{m}$  – 1.0 mm  
high-throughput, online size exclusion purification

### Cryo-Electron Microscopy

With ibs EMBL ILL  
Institut de Biologie Structurale European Molecular Biology Laboratory NEUTRONS FOR SOCIETY

- **CM01:** 300 kV, single-molecule
- **CM02:** 300kV, single-molecule/tomography

### Complementary methods

With ibs  
Institut de Biologie Structurale

- **icOS:** *In crystallo* optical spectroscopy: UV/Vis absorption, fluorescence, Raman

With cea  
GRENOBLE

- **HPMX:** high-pressure crystal freezing 200-2000 bar, cryo-protectant free cooling, introduction of gases





*In vitro* Sample production

*In vivo*

crystalline

solution

cells

**MX crystallography**

**SAXS**

**Single particle EM**

**Biophysics**

**Functional assays**

- ✓ Standard
- ✓ RT (dehydration)
- ✓ In situ plate
- ✓ Automated
- ✓ SSX
- ✓ MDX
- ✓ Spectroscopy (kinetic MX)
- ✓ Time resolved (TR) ~1ms
- ✓ High pressure cooling

- ✓ TR (ms to s)
- ✓ Online FPLC
- ✓ DLS
- ✓ UV
- ✓ Microfluidics

- ✓ **Negative stain EM**
- ✓ Vitrification & screening
- ✓ Automated, serial EM
- ✓ Tilted data collection
- ✓ Phase plate (small particles)
- ✓ Medium resolution data
- ✓ High resolution data
- ✓ Image processing

- ✓ SEC-MALS
- ✓ ESPRIT fragment library
- ✓ Calorimetry
- ✓ ESI/LC mass spectrometry
- ✓ Native mass spectrometry
- ✓ UV-vis spectroscopy
- ✓ MST, fluorescence assays
- ✓ NMR
- ✓ SANS
- ✓ Mass photometry
- ✓ ...

- ✓ Yeast two-hybrid
- ✓ Immunofluorescence
- ✓ BiFC
- ✓ Functional assays
- ✓ ...