Structure and function of carnitine acyltransferases

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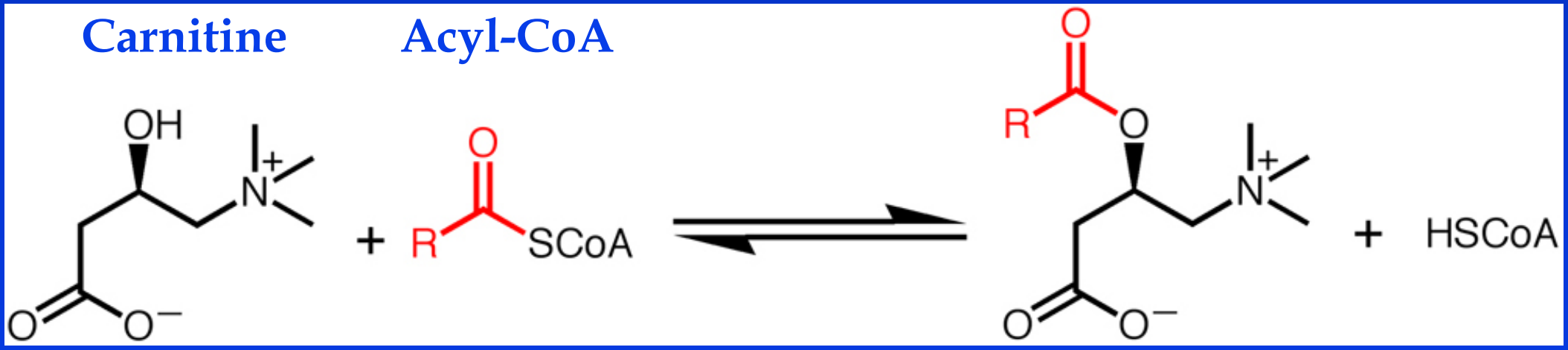
Structural studies of carnitine acetyltransferase (CRAT)

- G. Jogl & L. Tong, *Cell*, 112, 113-122, (2003)

Wu, *et al. J. Biol. Chem.* 278, 13159, (2003)

Reaction catalyzed by carnitine acyltransferases



Acylcarnitines are activated acyl groups

Carnitine acyltransferases

- Carnitine palmitoyltransferases (CPTs)
  - Specific for long-chain fatty acids
  - L-CPT-I (CPT-1a), M-CPT-I (CPT-1b), and CPT-1c, associated with the outer membrane of mitochondria
  - CPT-II, in the mitochondrial matrix
  - The activities of CPT-Is are controlled exquisitely by malonyl-CoA
- Carnitine octanoyltransferase (COT)
  - Specific for medium-chain fatty acids
- Carnitine acetyltransferases (CAT, CRAT)
  - Specific for short-chain fatty acids

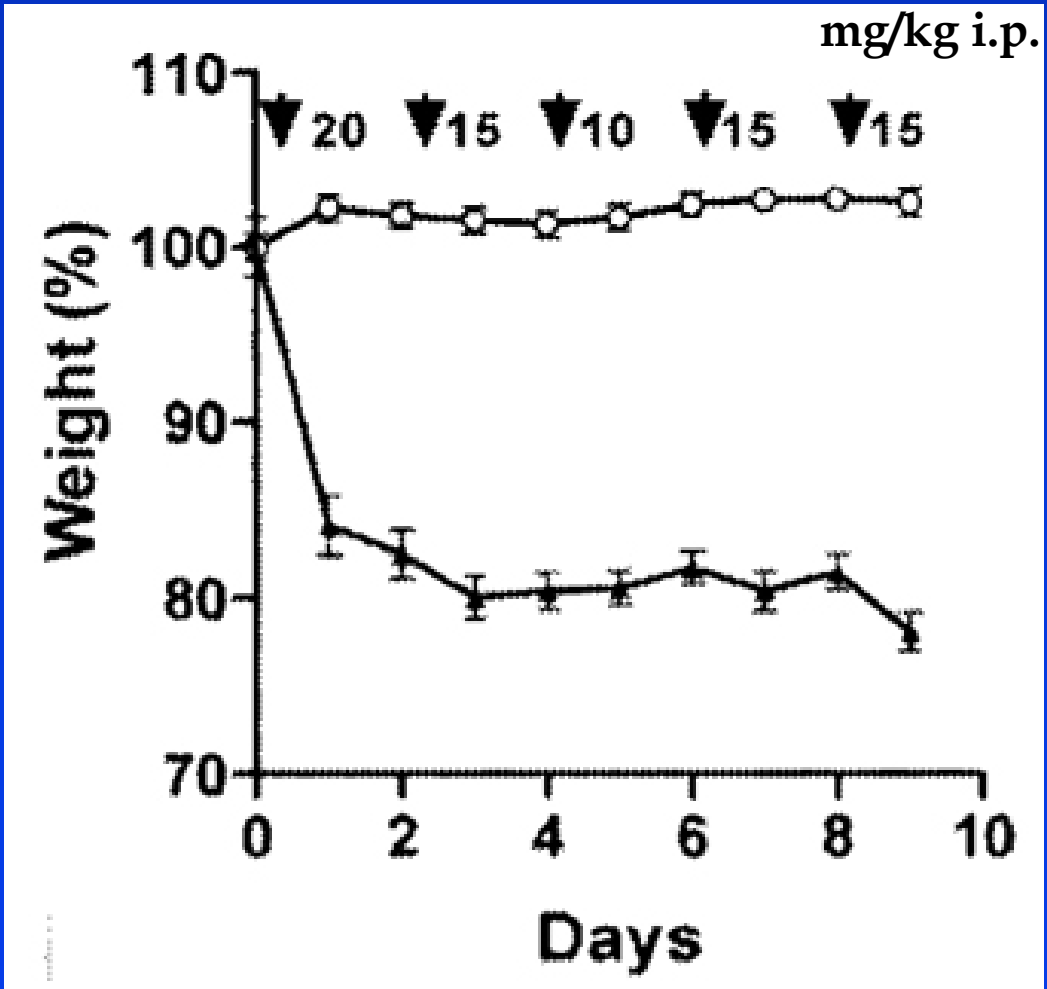
Carnitine acyltransferases and human diseases

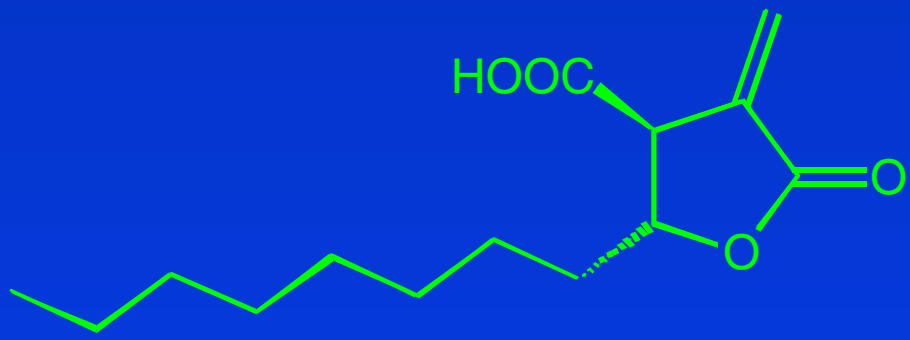
- Inherited recessive mutations of CPT-I and CPT-II are linked to hypoglycemia
- CPT-II deficiency is the most common cause of abnormal lipid metabolism in skeletal muscle
- Inherited deficiency in CRAT activity is linked to neurological and heart problems
- Alzheimer's patients also have reduced CRAT activity

Carnitine acyltransferases and drug discovery

- L-CPT-I is a target for drug development against NIDDM (type 2 diabetes)
- A covalent inhibitor of L-CPT-I, etomoxir, can lower blood glucose levels in diabetic animals and humans
- Clinical use limited by the toxic side effects

An agonist of CPT-I can lower body weight





C75

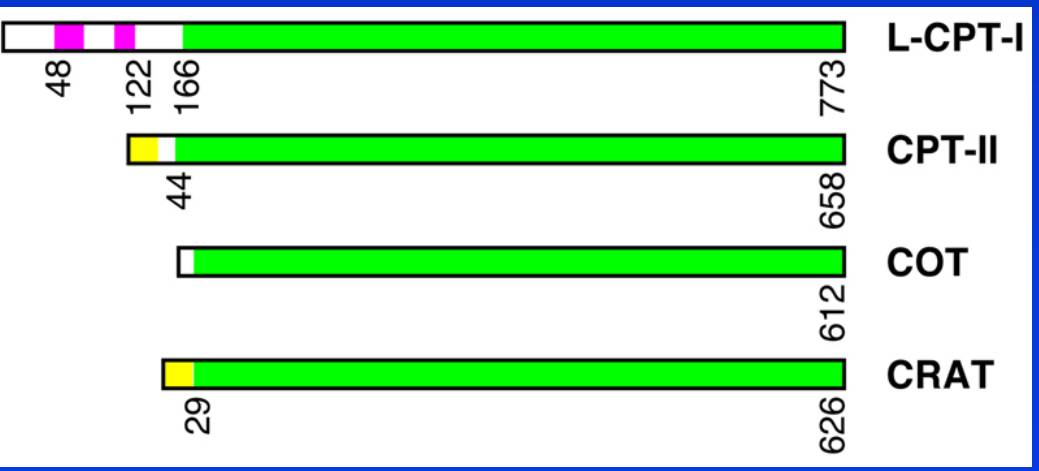
Originally developed as an inhibitor of FAS

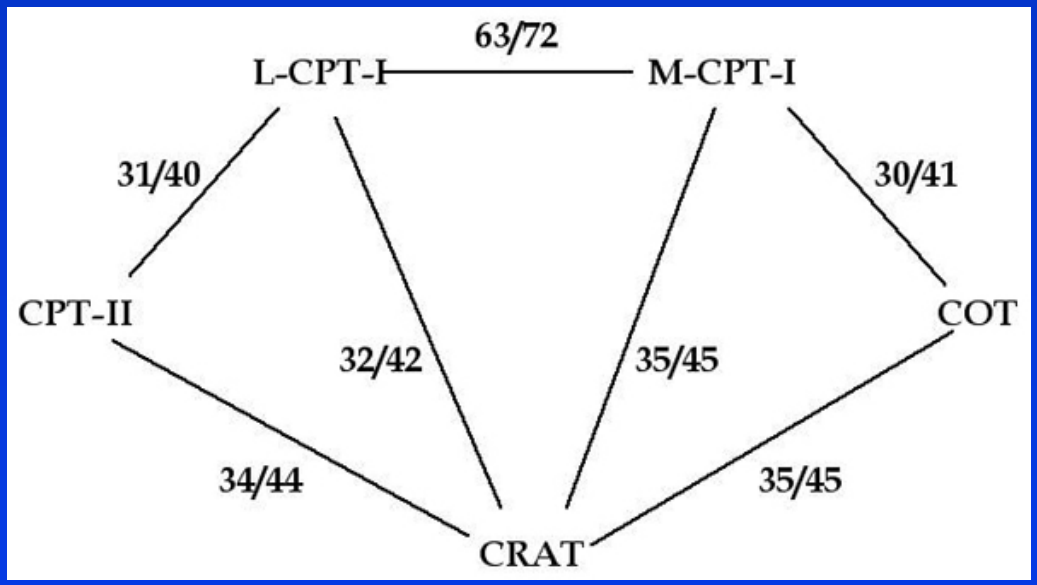
Thupari *et al. PNAS*, 99, 9498, (2002)

Carnitine acyltransferases

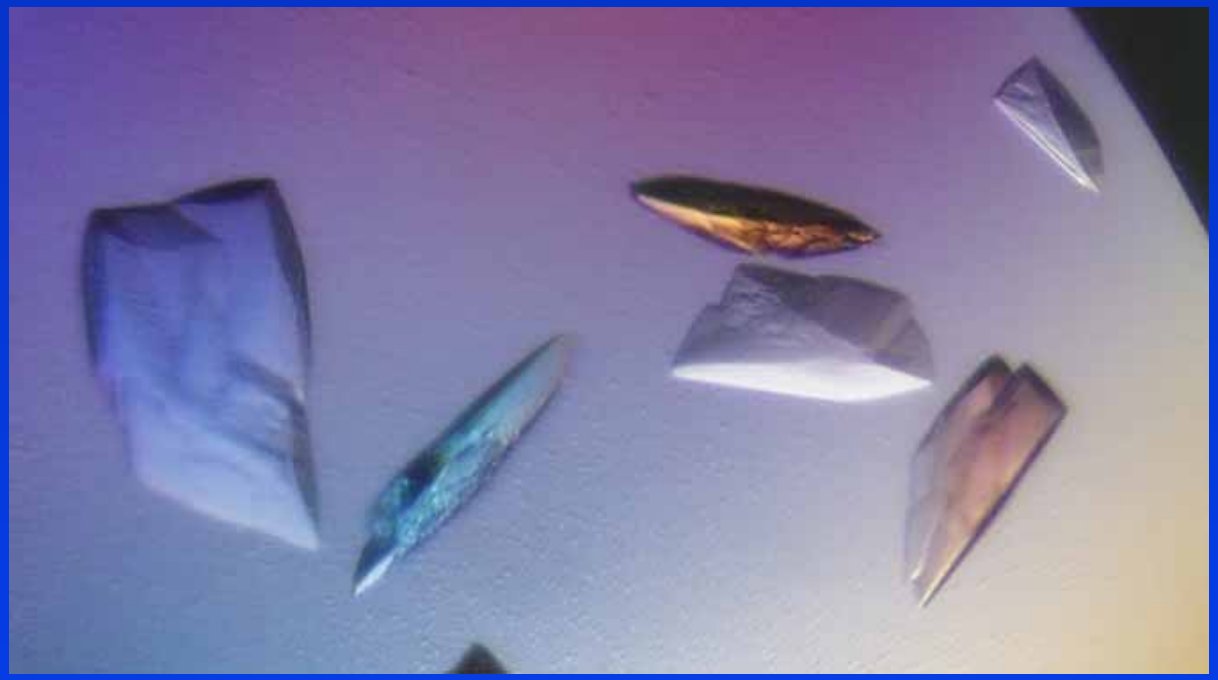
- Contains about 600 to 700 amino acid residues, 70kD
- Strongly conserved among various living organisms
- About 35% sequence identity between CPT-I and CRAT
- No detectable sequence homology to other proteins in the database
- No structural information

Sequence conservation of carnitine acyltransferases





Crystals of mouse CRAT



*C*2
a=158.9 Å
b=89.6 Å
c=119.4 Å
β=127.5 °

Z=2

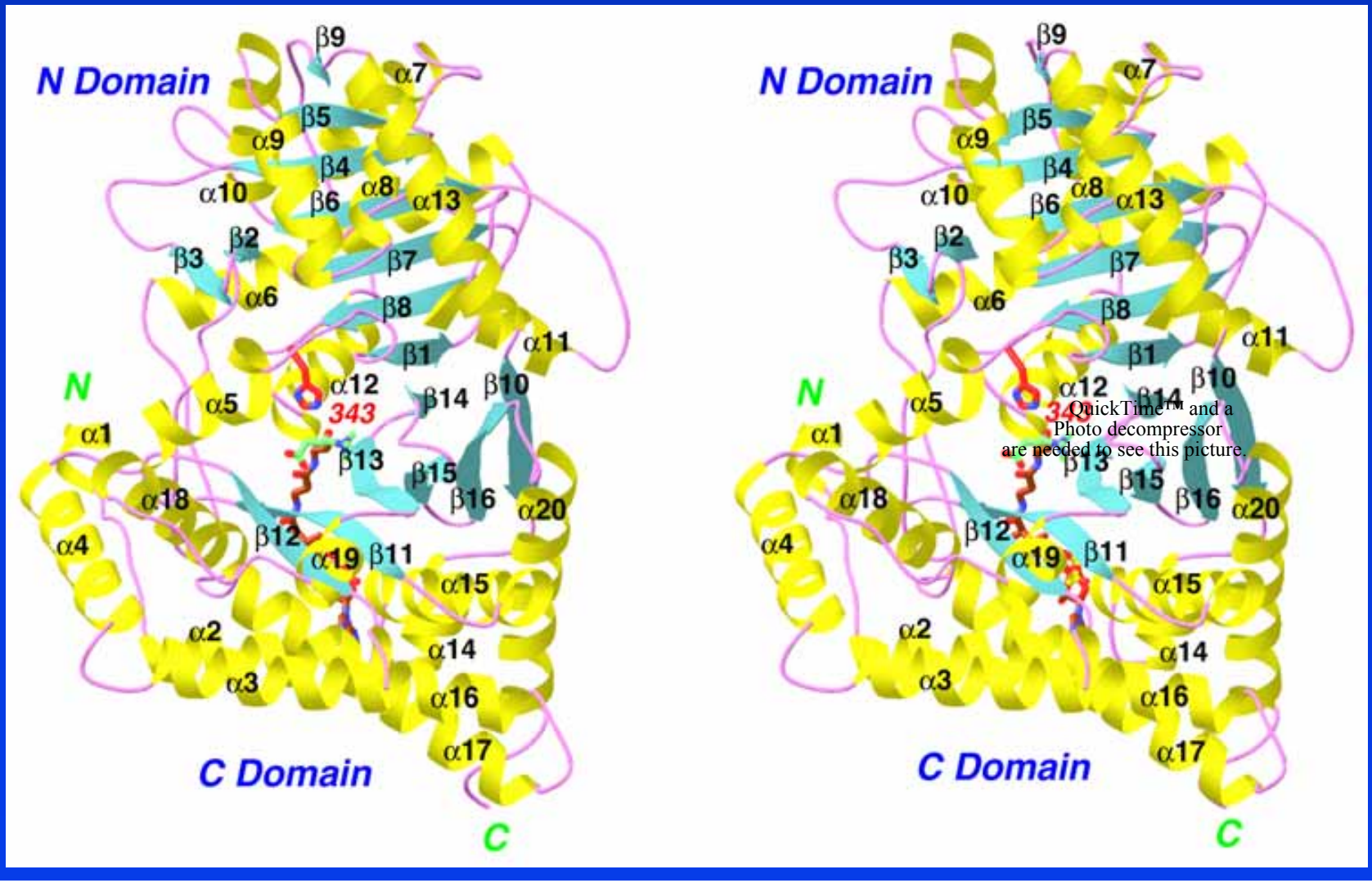
Structure determined by Se-Met SAD phasing (40 Se sites)

1.8 Å

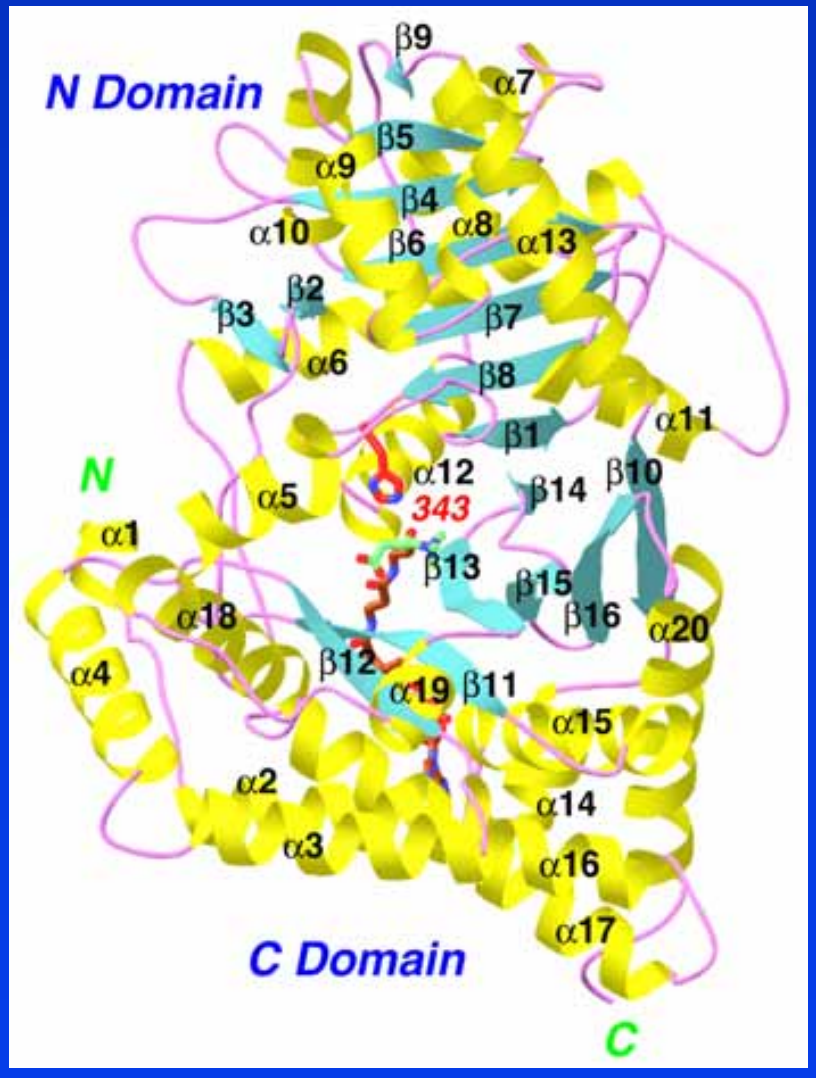
Structures of mouse CRAT

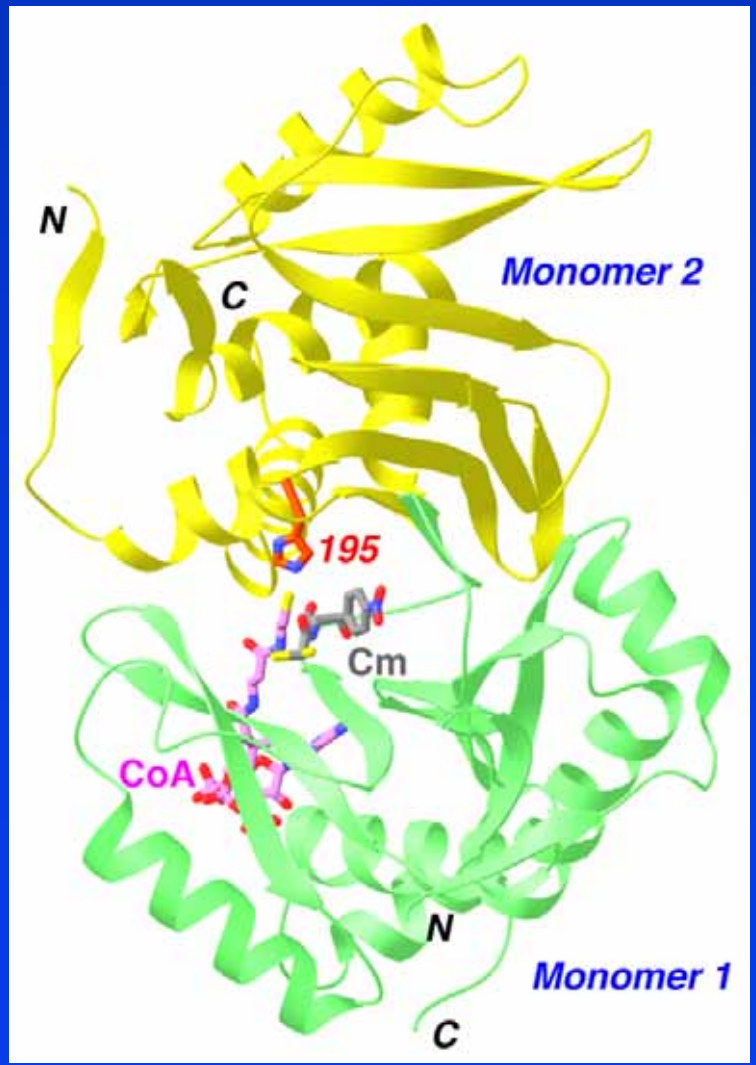
Complex	Free enzyme	Carnitine	CoA
Resolution (Å)	1.8	1.9	2.3
R factor (%)	18.8	20.1	27.0
Free R factor (%)	21.1	24.7	36.1

Structure of mouse CRAT: two domains with the same topology

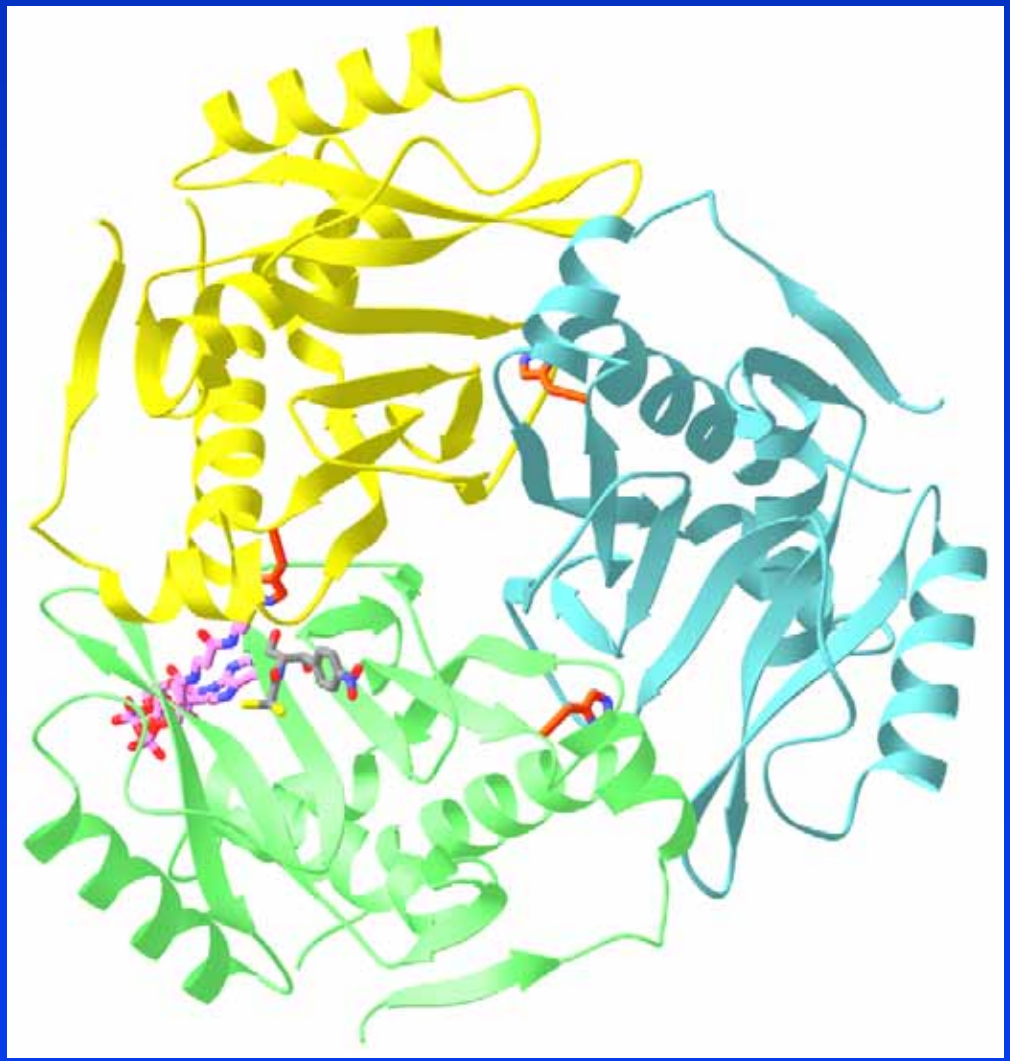


The two domains of CRAT are arranged similar to two subunits of CAT





CAT is a trimer



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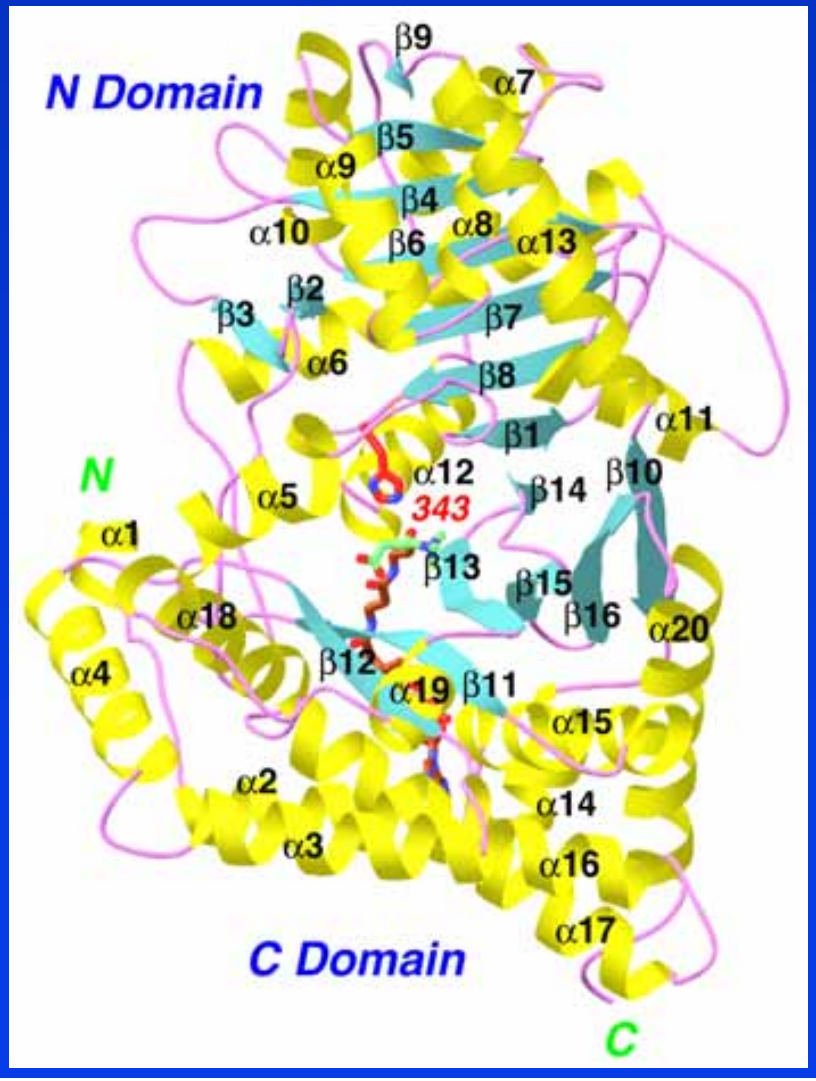
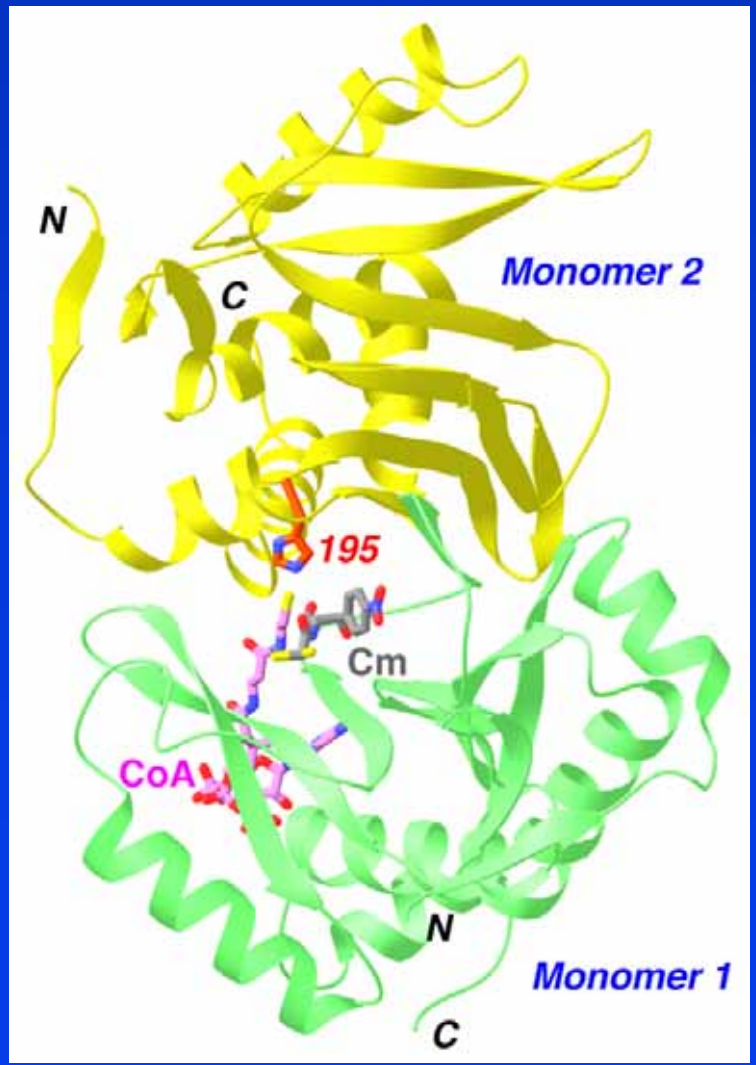
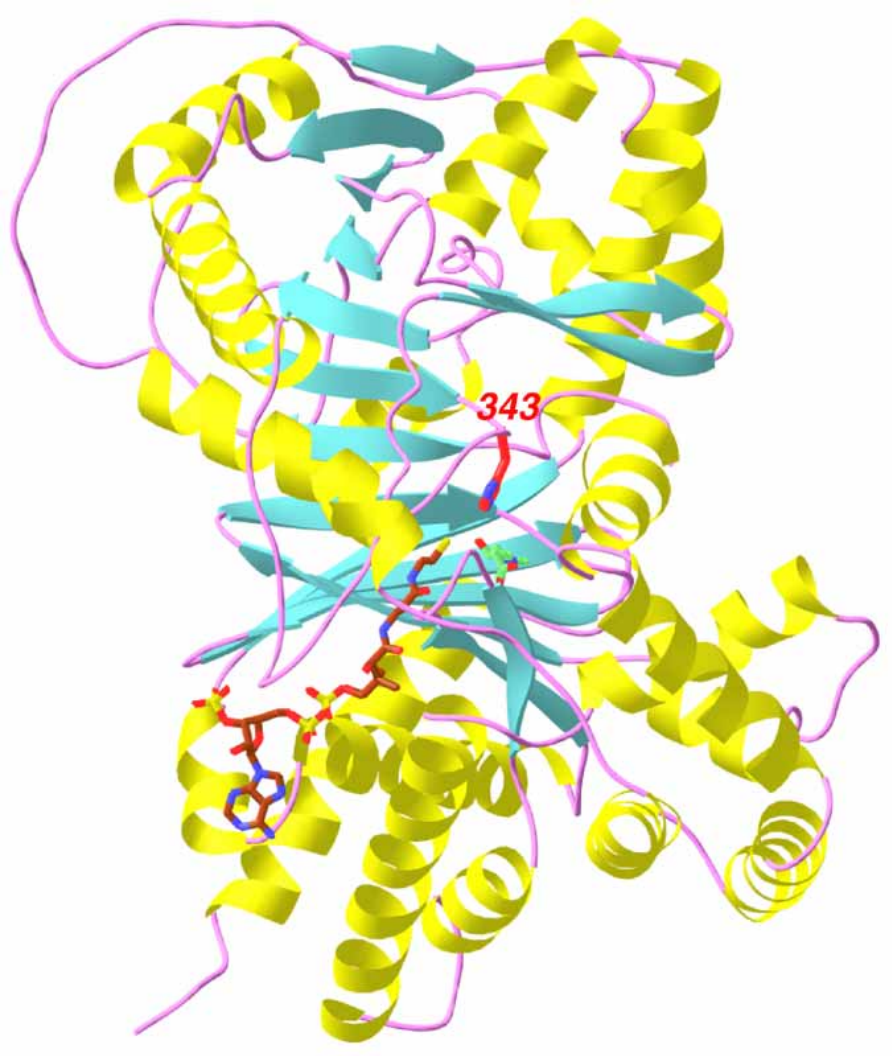
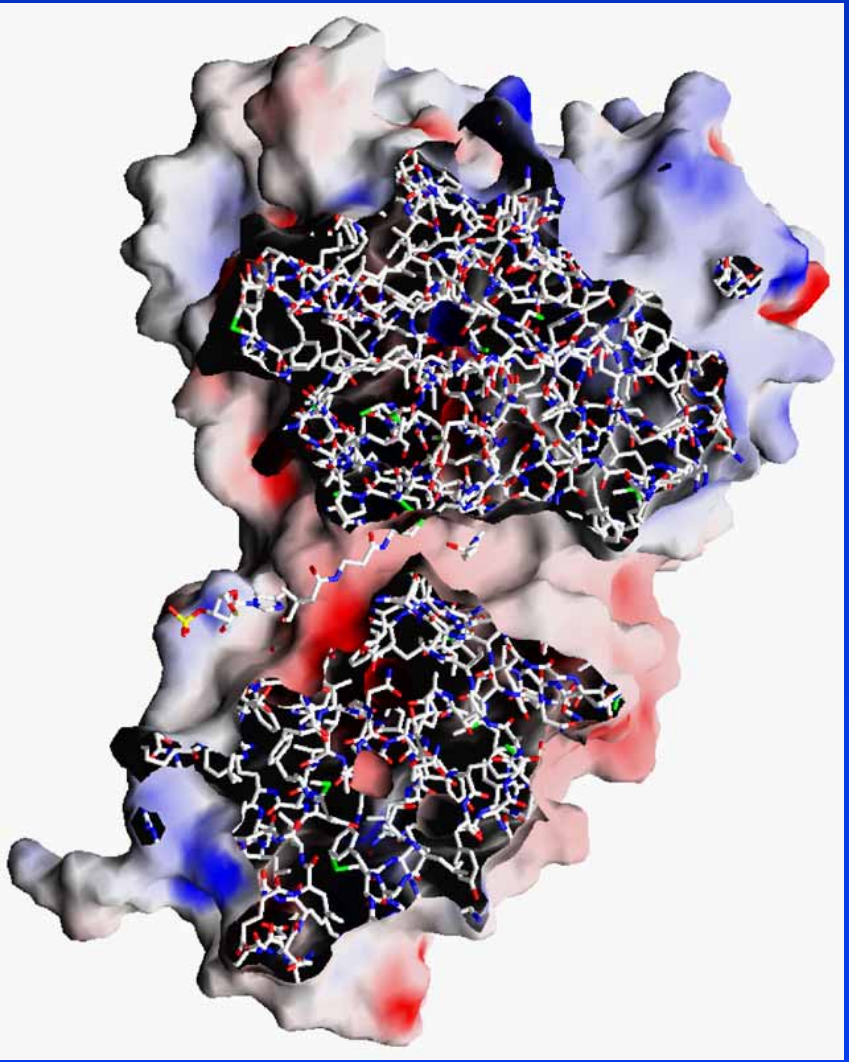


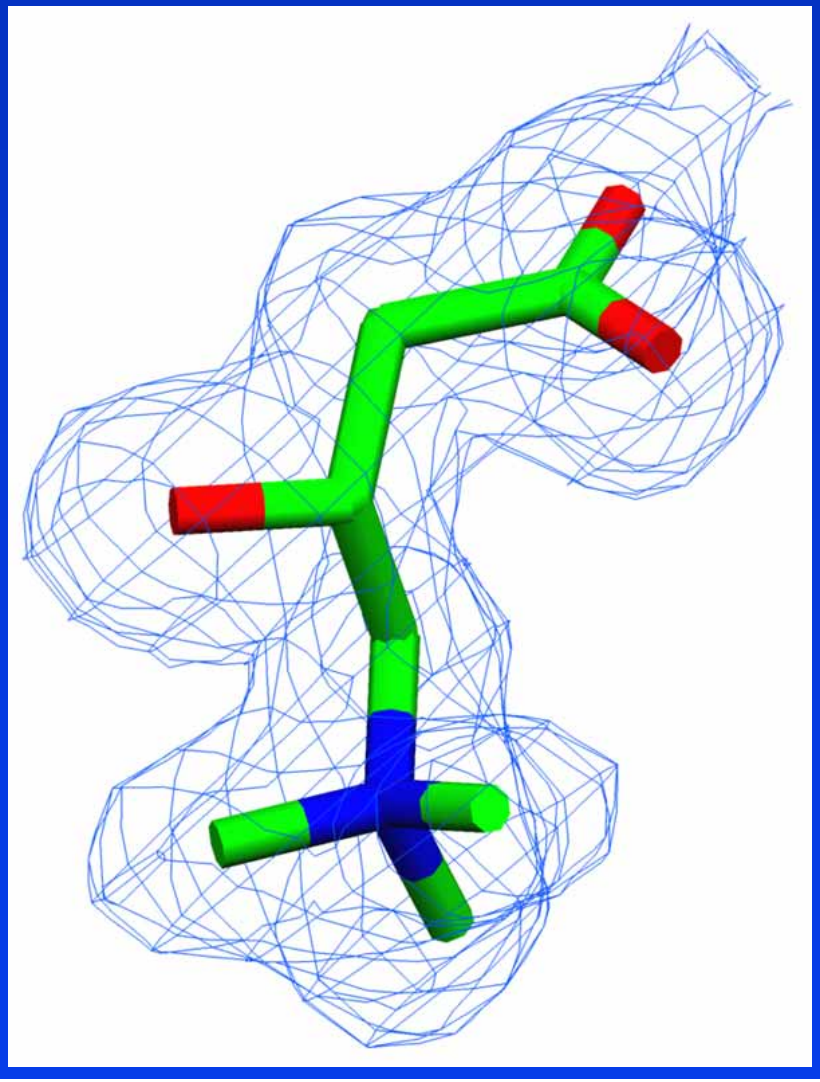


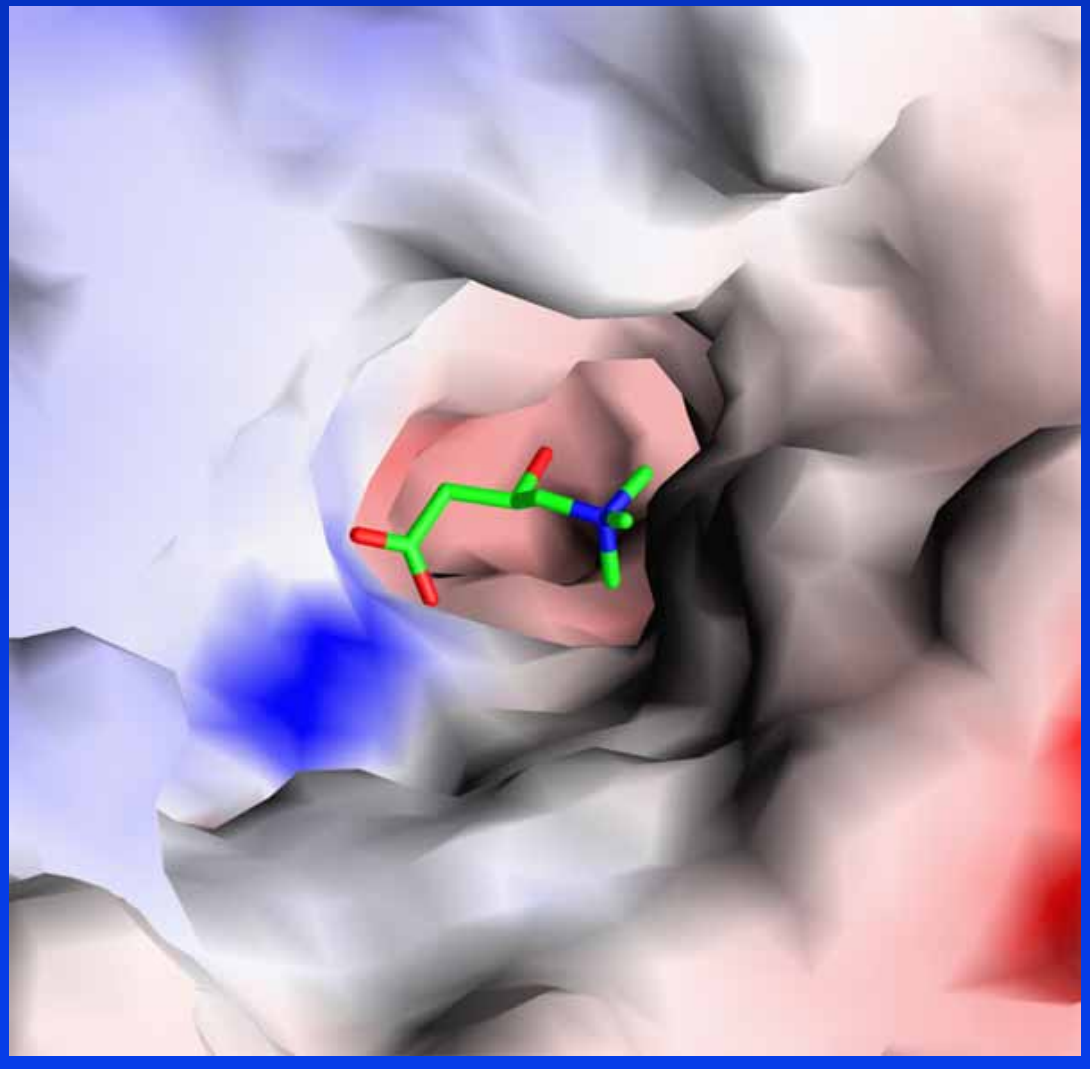
The substrate binding sites of CRAT



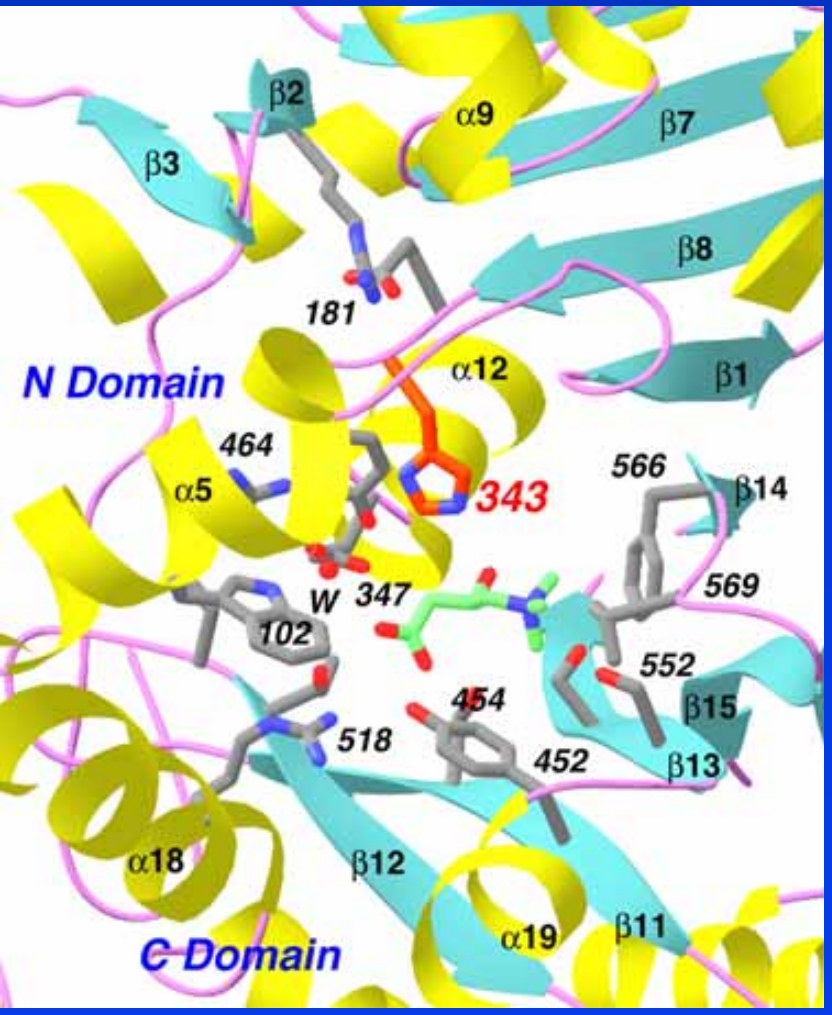


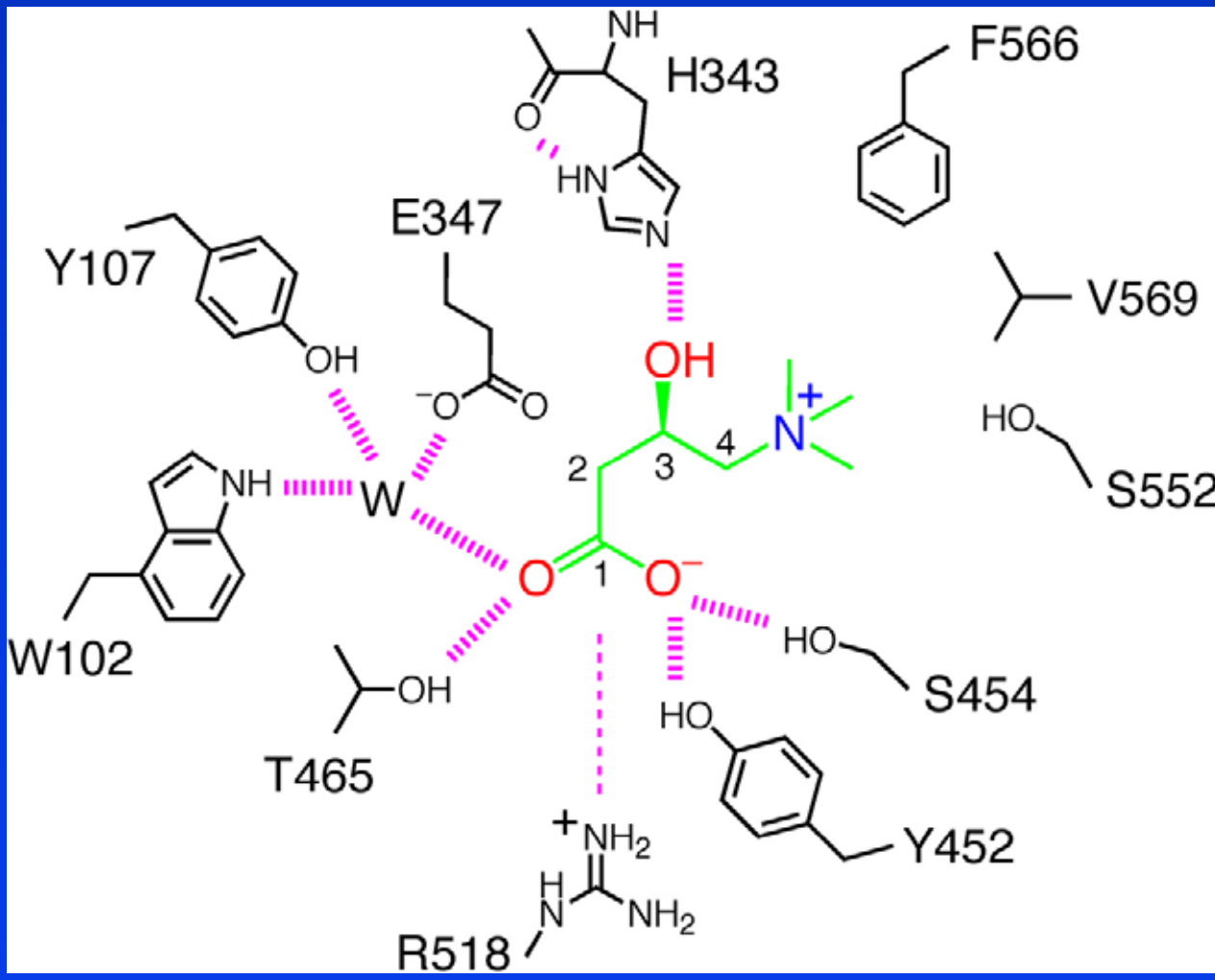
Carnitine binding site



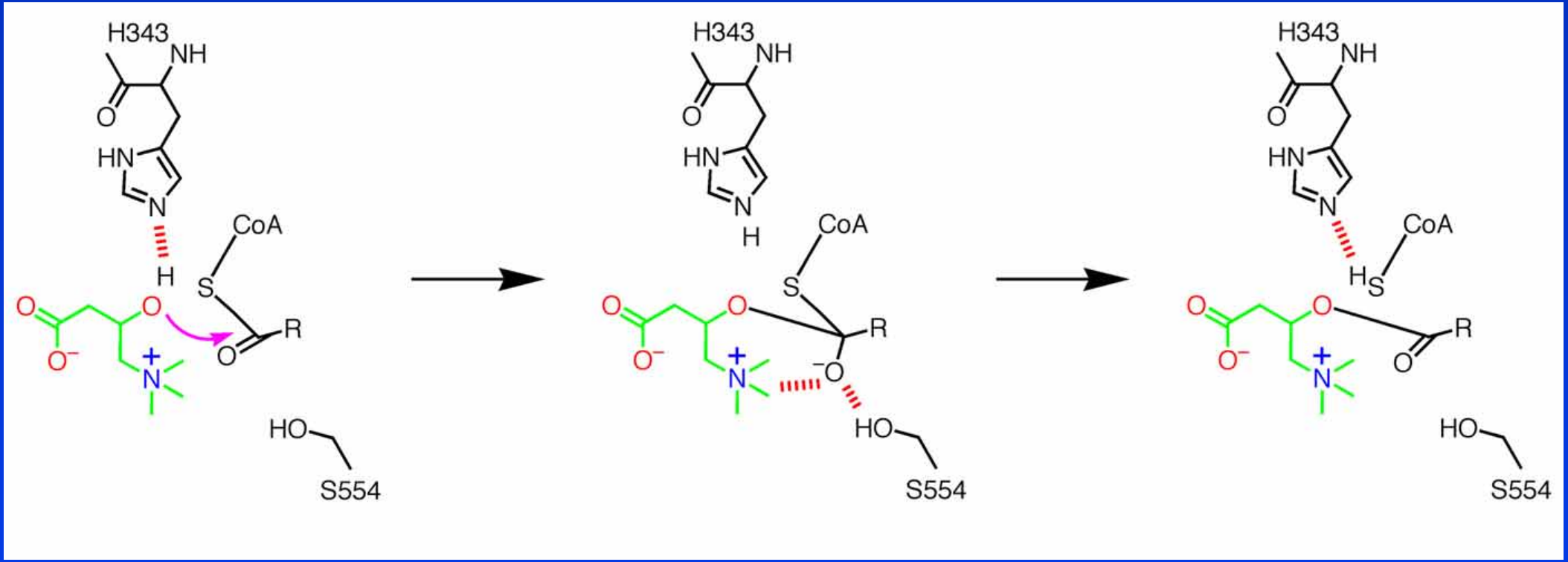


Carnitine binding mode



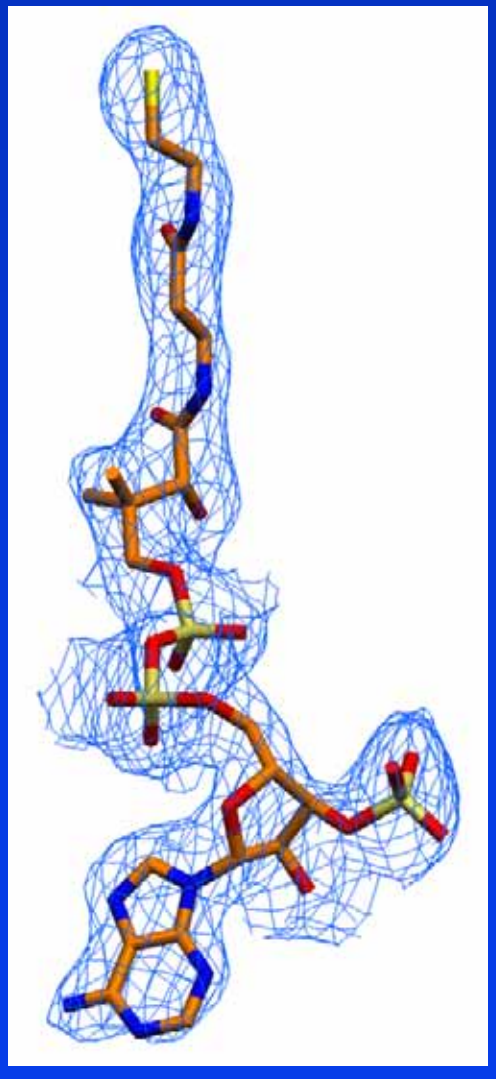


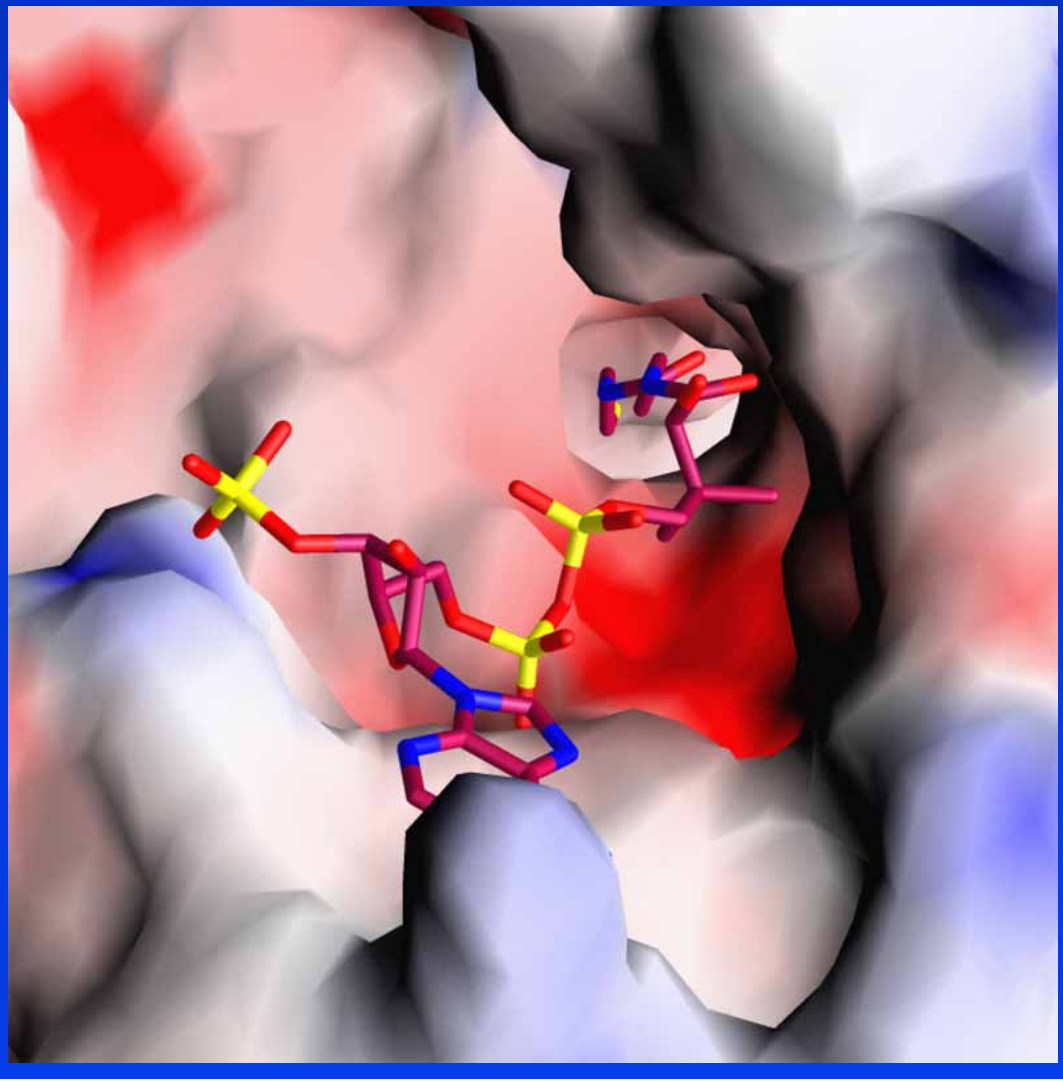
The catalytic mechanism: substrate-assisted catalysis



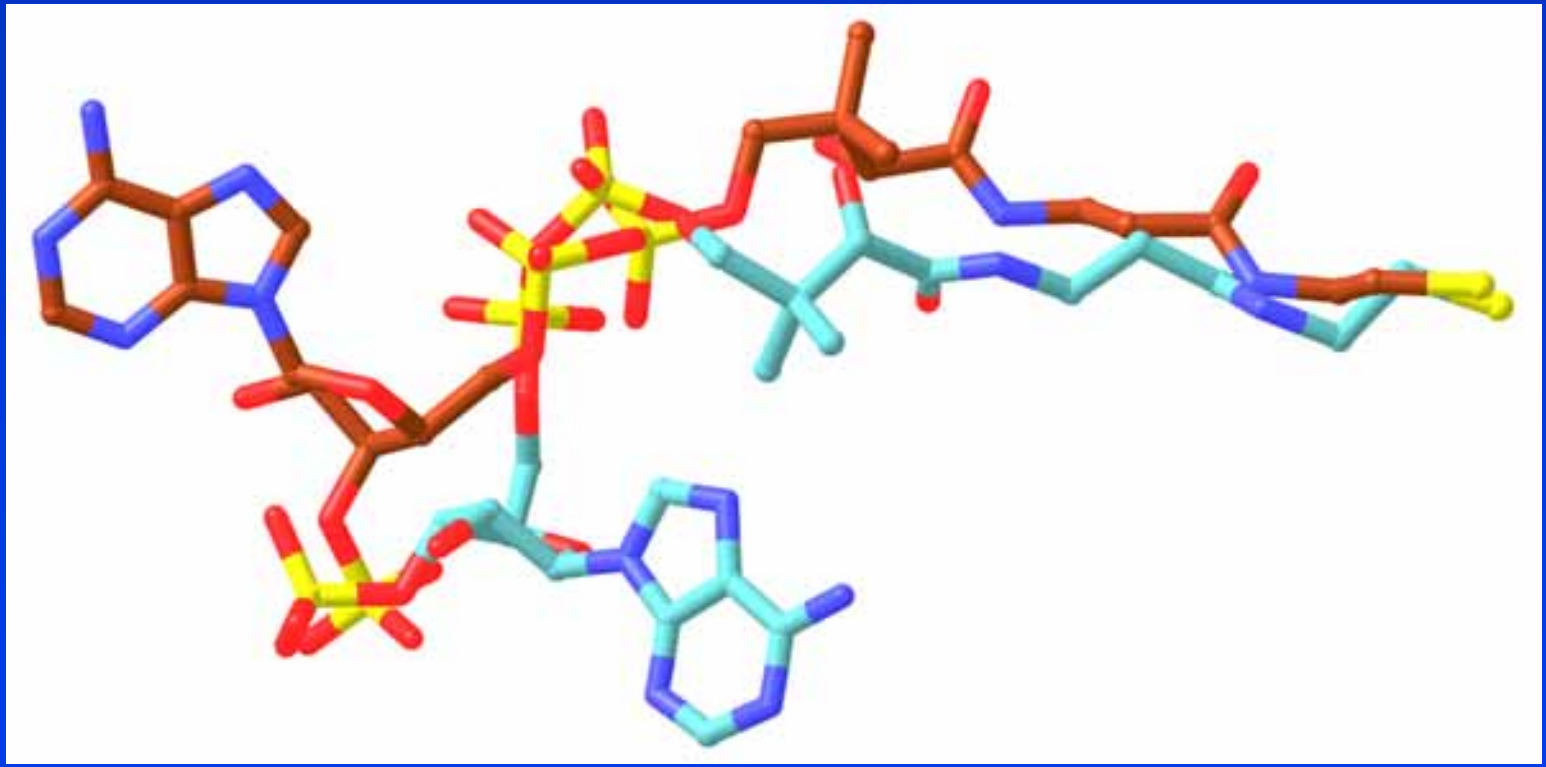
The positive charge is not required for binding, but is required for catalysis.

CoA binding site

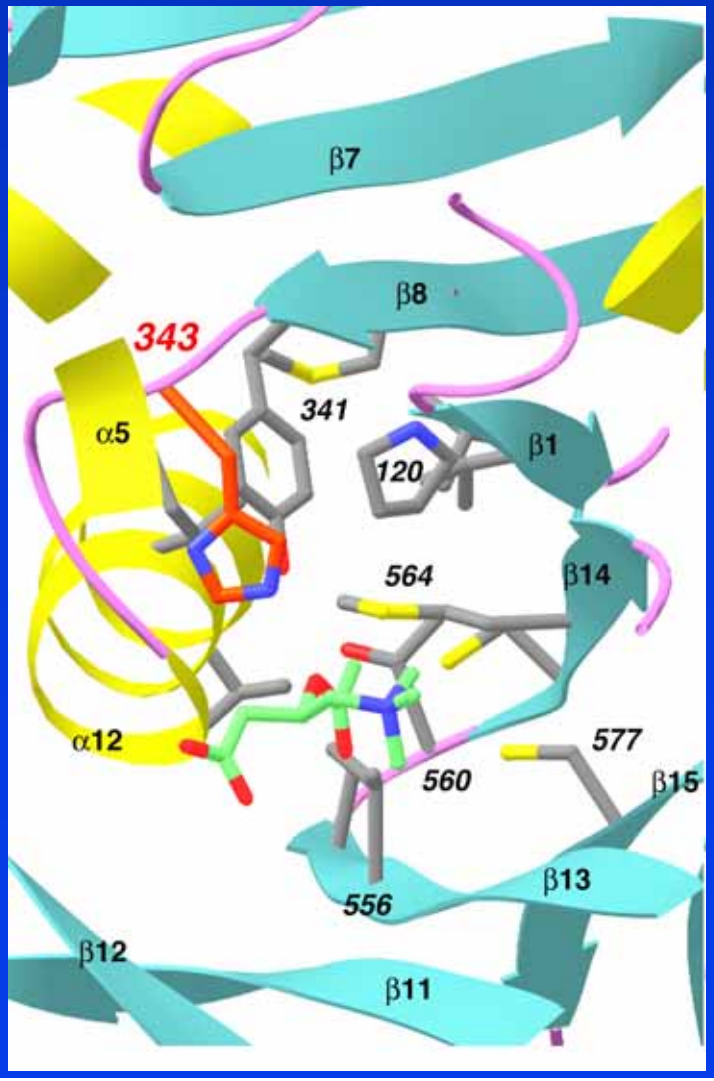




Binding mode of CoA to CRAT is different from that to CAT



Possible binding site for long-chain acyl-CoAs

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                5 560   5 570   5
  Human CRAT  QVPAKTDCVMFFGPVVPDGYG
  Mouse CRAT  QVPAKTDCVMFFGPVVPDGYG

 Human L-CPT-I NNPEYVSSGGGFGPVADDGYG
 Mouse L-CPT-I KYPDYVSCGGGFGPVADDGYG

 Human M-CPT-I QHPNHLGAGGGFGPVADDGYG
 Mouse M-CPT-I QYPNHLGAGGGFGPVADDGYG
Drosophila CPT-I KHPNCISAGGGFGPVADDGYG

 Human CPT-II TLSSPAVNLGGFAPVVSDGFG
 Mouse CPT-II TLSSPAVSLGGFAPVVPDGFG

  Human COT  SLVGYLRVQGVVVPMVHNGYG
  Bovine COT  SLVGYLRVQGVMVPMVHNGYG
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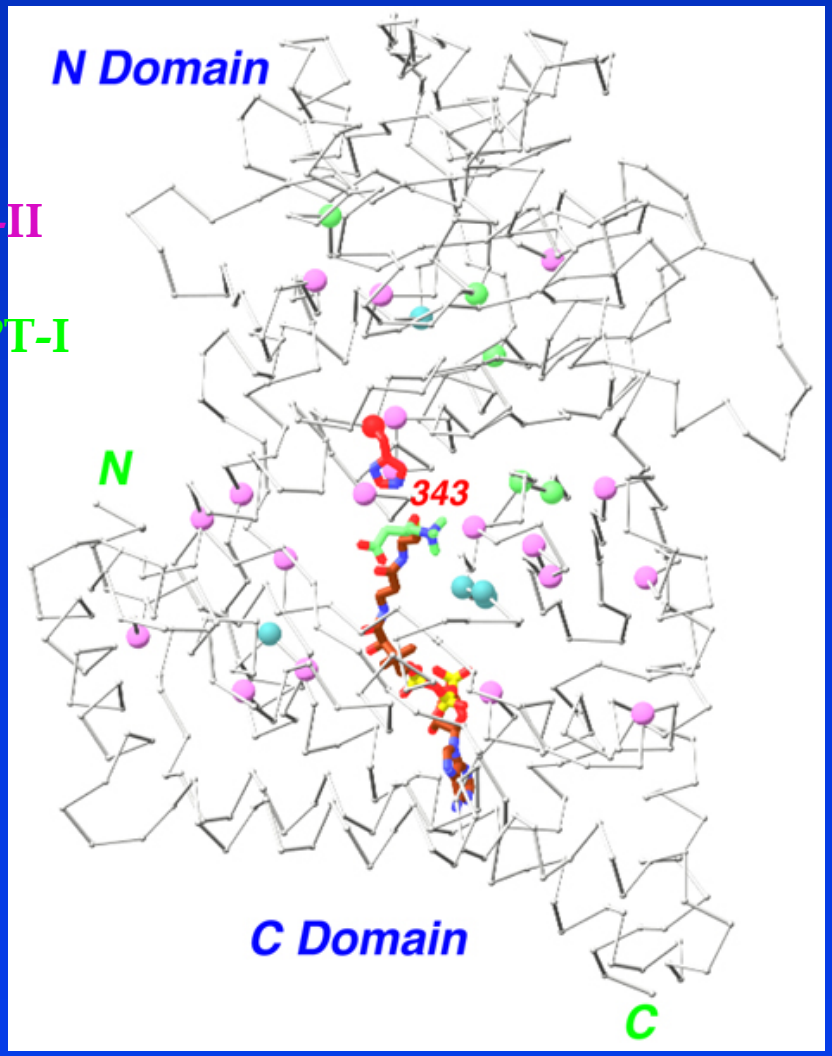
Disease-causing mutations reduce the activity of the enzymes

CPT-II

L-CPT-I

COT



QuickTime™ and a
Photo decompressor
are needed to see this picture.

Future research directions

- Determine the binding mode of the acyl groups to the enzyme
- Structural studies of other carnitine acyltransferases (CPT-I, CPT-II, COT)
- Understand the molecular basis for the malonyl-CoA inhibition of CPT-I
- Understand the molecular basis for the disease-causing mutations in CPT-I and CPT-II
- Identify inhibitors against L-CPT-I
- Identify agonists for L-CPT-I

Summary

- Carnitine acyltransferases have the same backbone fold as CAT
- The active site is at the interface of two domains of the enzyme
- The substrate binding channel extends through the middle of the enzyme
- The carboxylate of carnitine is bound tightly by the enzyme
- Carnitine helps the catalysis by the enzyme

Acknowledgements

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