

Conformational change of mammalian serine racemase upon inhibitor binding

Myron Smith, Volker Mack, Andreas Ebnet, Isabel Moraes, Brunella Felicetti, Michael Wood, Dorian Schonfeld, Owen Mather, Andrea Cesura and John Barker

Abstract

Serine racemase is responsible for the synthesis of D-serine, an endogenous co-agonist for N-methyl-D-aspartate receptor-type glutamate receptors (NMDARs). This pyridoxal 5'-phosphate-dependent enzyme is involved both in the reversible conversion of L- to D-serine and serine catabolism by α,β -elimination of water, thereby regulating D-serine levels. Since D-serine affects NMDAR signalling throughout the brain, serine racemase is a promising target for the treatment of disorders related to NMDAR dysfunction. To provide a molecular basis for rational drug design the X-ray crystal structures of human and rat serine racemase were determined at 1.5 Å and 2.1 Å resolution respectively, and in the presence and absence of the orthosteric inhibitor malonate. The structures revealed a fold typical of β -family PLP-enzymes, with both a large domain and a flexible small domain associated into a symmetric dimer, and indicated a ligand-induced rearrangement of the small domain that organises the active site for specific turnover of the substrate.

Serine racemase background

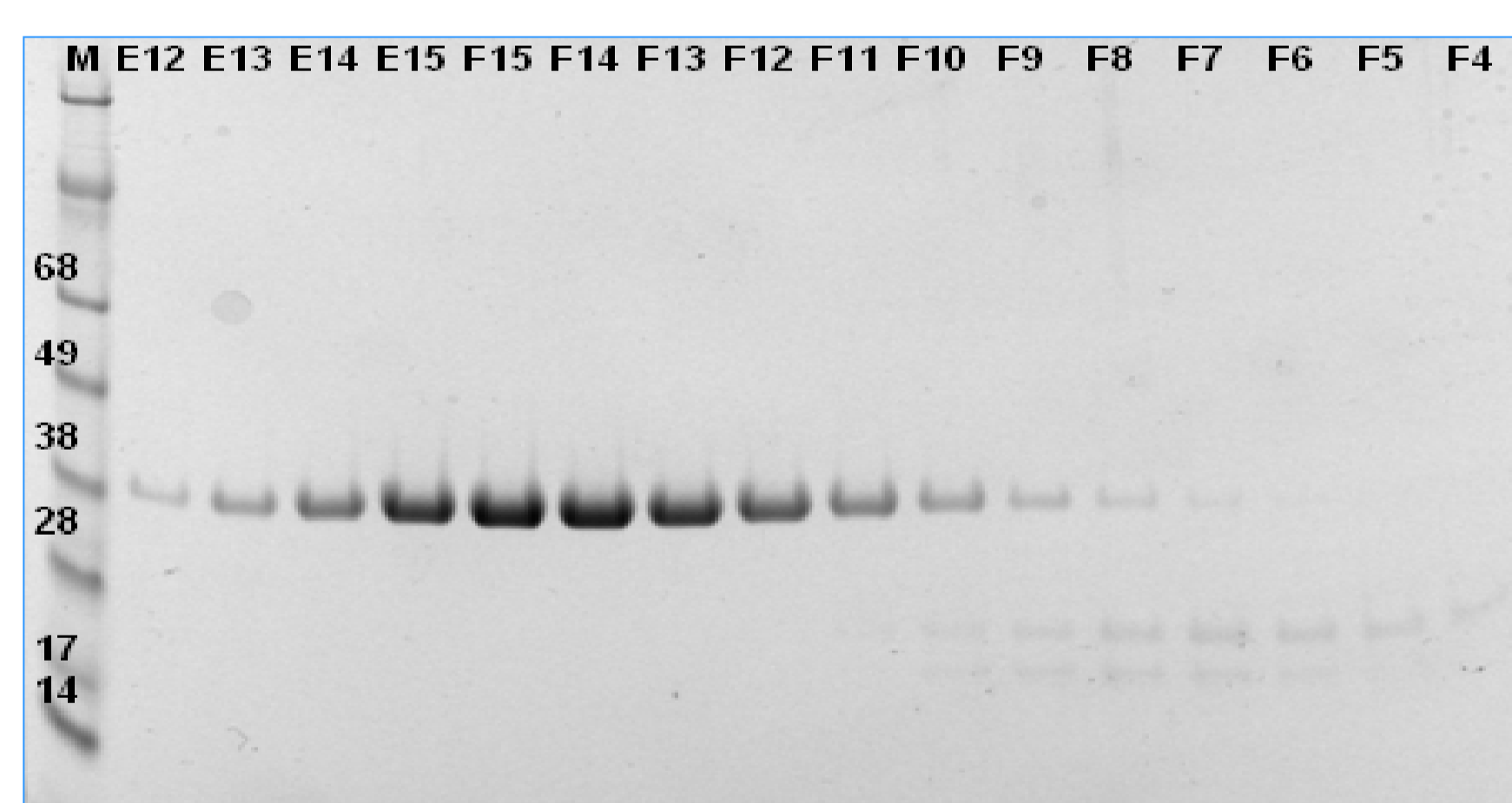
Serine racemase (SR) is expressed in glial cells and neurons, and constitutes the sole endogenous source for D-serine in mammals.⁽¹⁾ D-serine exerts its function predominantly in cortico-limbic brain structures as one of the two co-agonists to the NMDAR glycine modulatory site, and is produced by enzymatic conversion by SR of L- to D-serine mediated by pyridoxal-5'-phosphate (PLP). In addition to serine isomerisation, the enzyme catalyses the α,β -elimination of water from L- and D-serine to produce pyruvate and ammonia. SR can therefore not only elevate, but can also reduce the level of D-serine, a role that previously has mainly been attributed to D-amino acid oxidase.⁽²⁾

Overactivation of NMDARs is involved in acute and progressive neurodegenerative diseases such as stroke, ALS, Huntington's, Alzheimer's, and Parkinson's disease.⁽³⁾ In addition, considerable evidence was found for enhanced NMDAR-mediated transmission and plasticity as a potential cause for pain conditions, e.g. neuropathic pain.⁽⁴⁾ The evidence highlights the importance of SR as a pharmacological target to treat neuropathological conditions associated with dysfunction in NMDAR neurotransmission.

Pharmacological intervention with SR-activity constitutes an innovative strategy to treat neurological disorders. To pave the way for rational drug design by means of fragment-based screening and related *in silico* approaches the X-ray crystal structure of mammalian SR was determined. This information will be important for the rational design of novel drugs for the treatment of diverse neurological diseases.

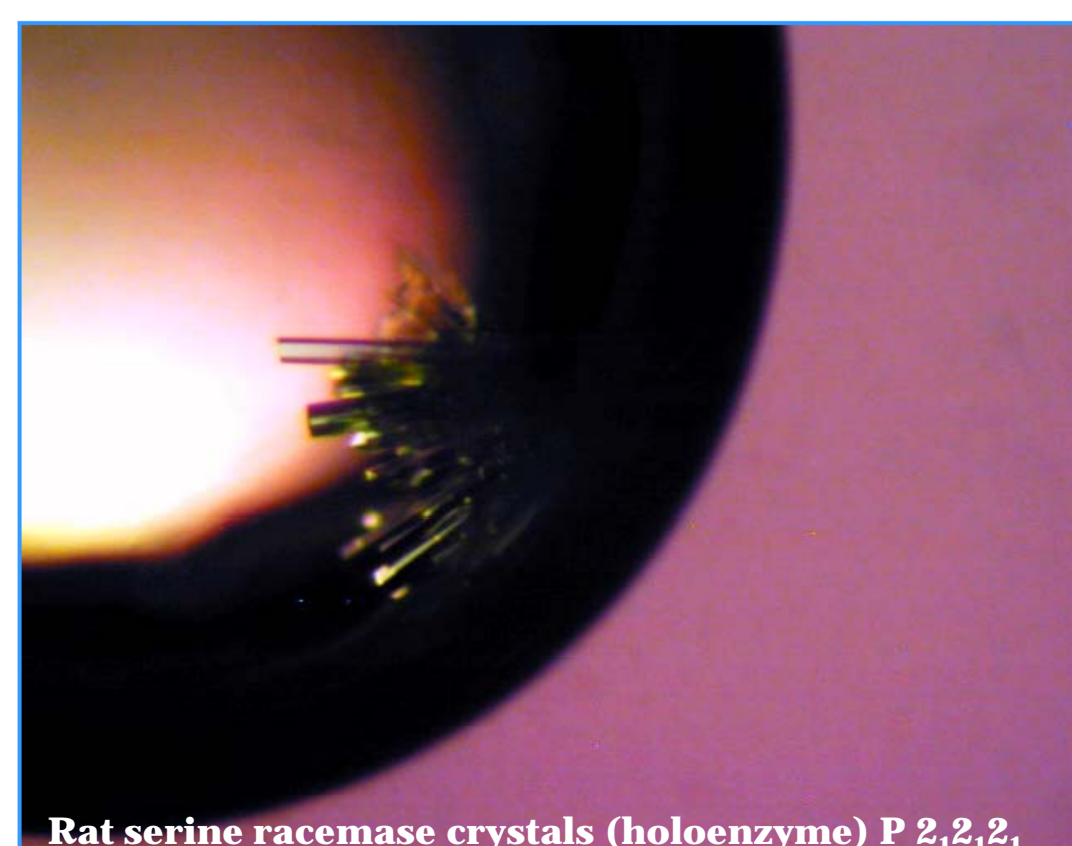
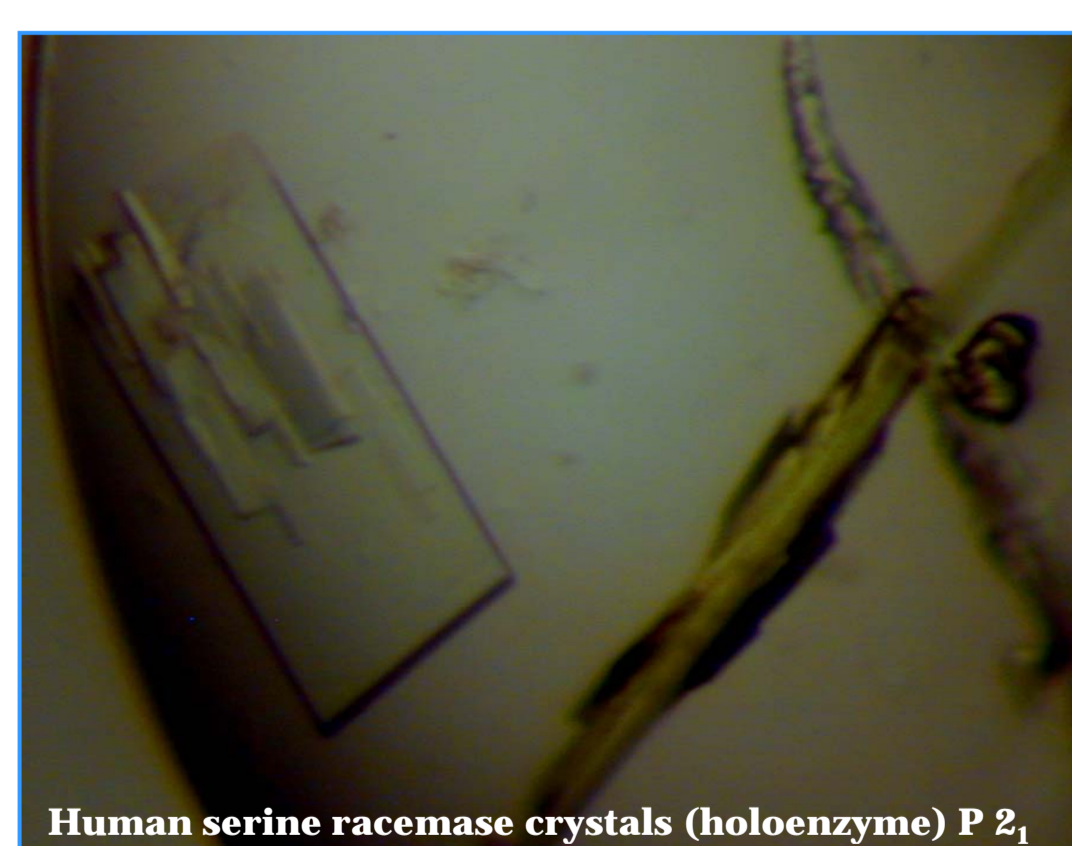
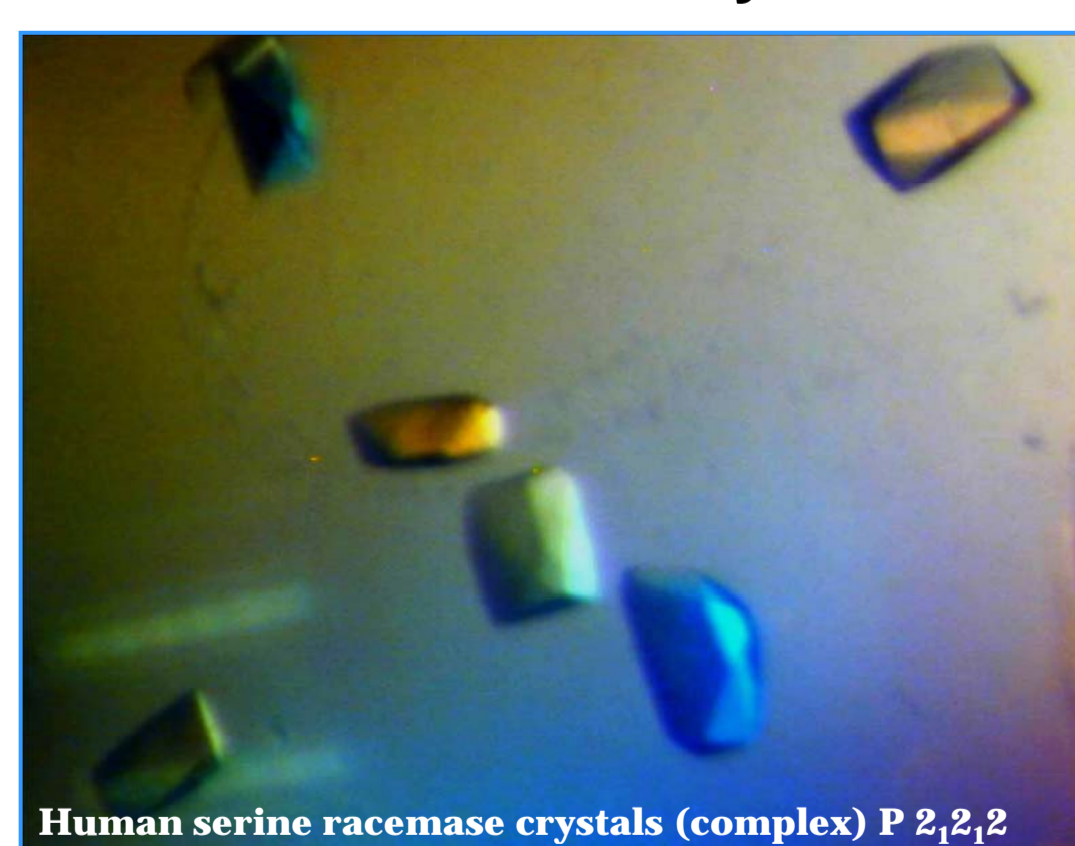
Obtaining pure soluble protein

- The yield of soluble wild-type enzyme was too low for crystallographic studies
- Homology modelling suggested two N-terminal cysteines were solvent exposed
- A construct with a C2D and C6D double mutation considerably improved yield and retained activity, however the protein suffered from C-terminal degradation
- Relocating the His-tag to the C-terminal improved the crystal quality and abrogated protein degradation
- The final yield improved to 1-2 mg/L of protein at better than 95% purity as assessed by SDS-PAGE and has been optimised to 3.5 mg/L



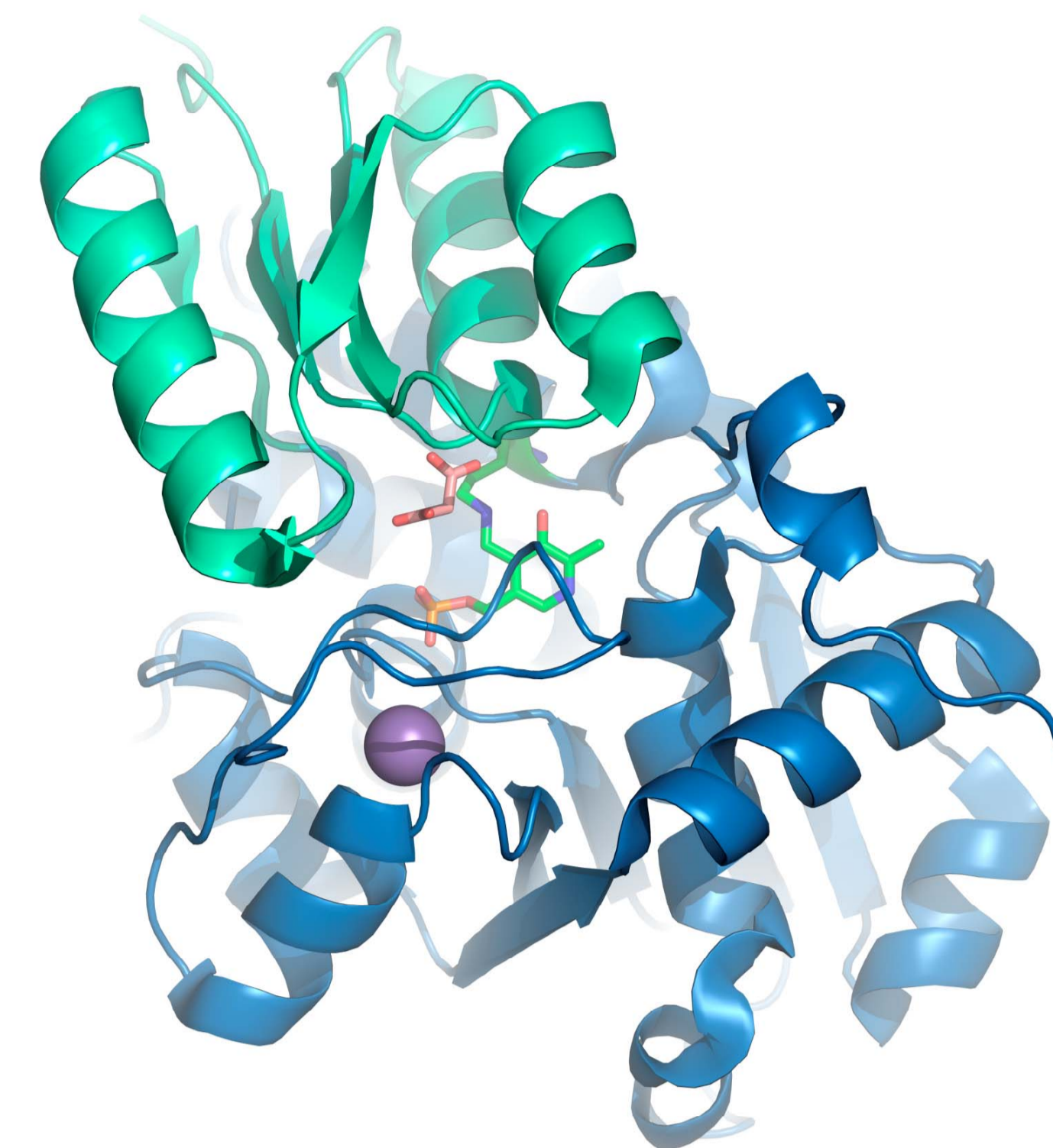
SDS-PAGE of human serine racemase after gel filtration

Crystals of serine racemase

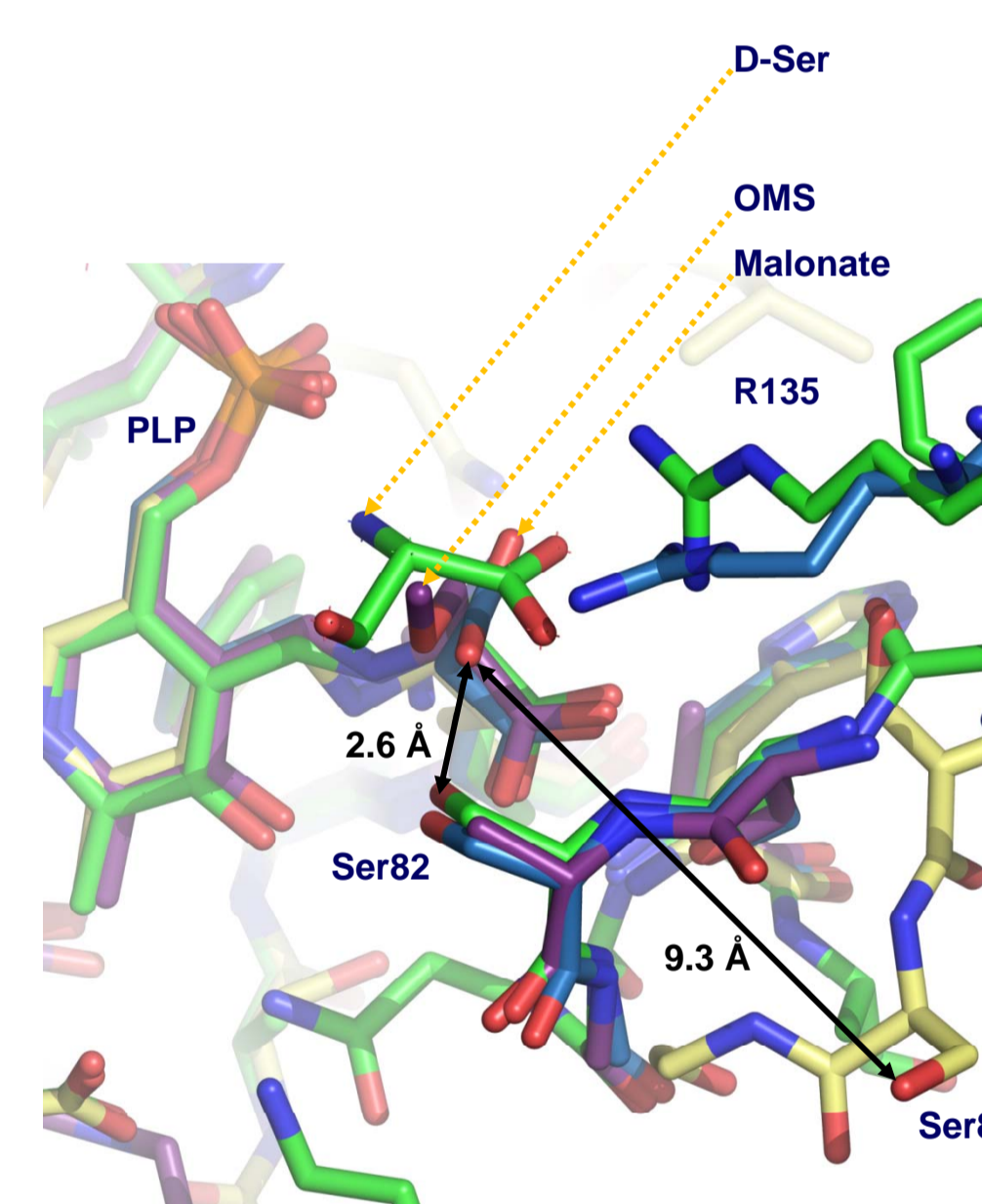
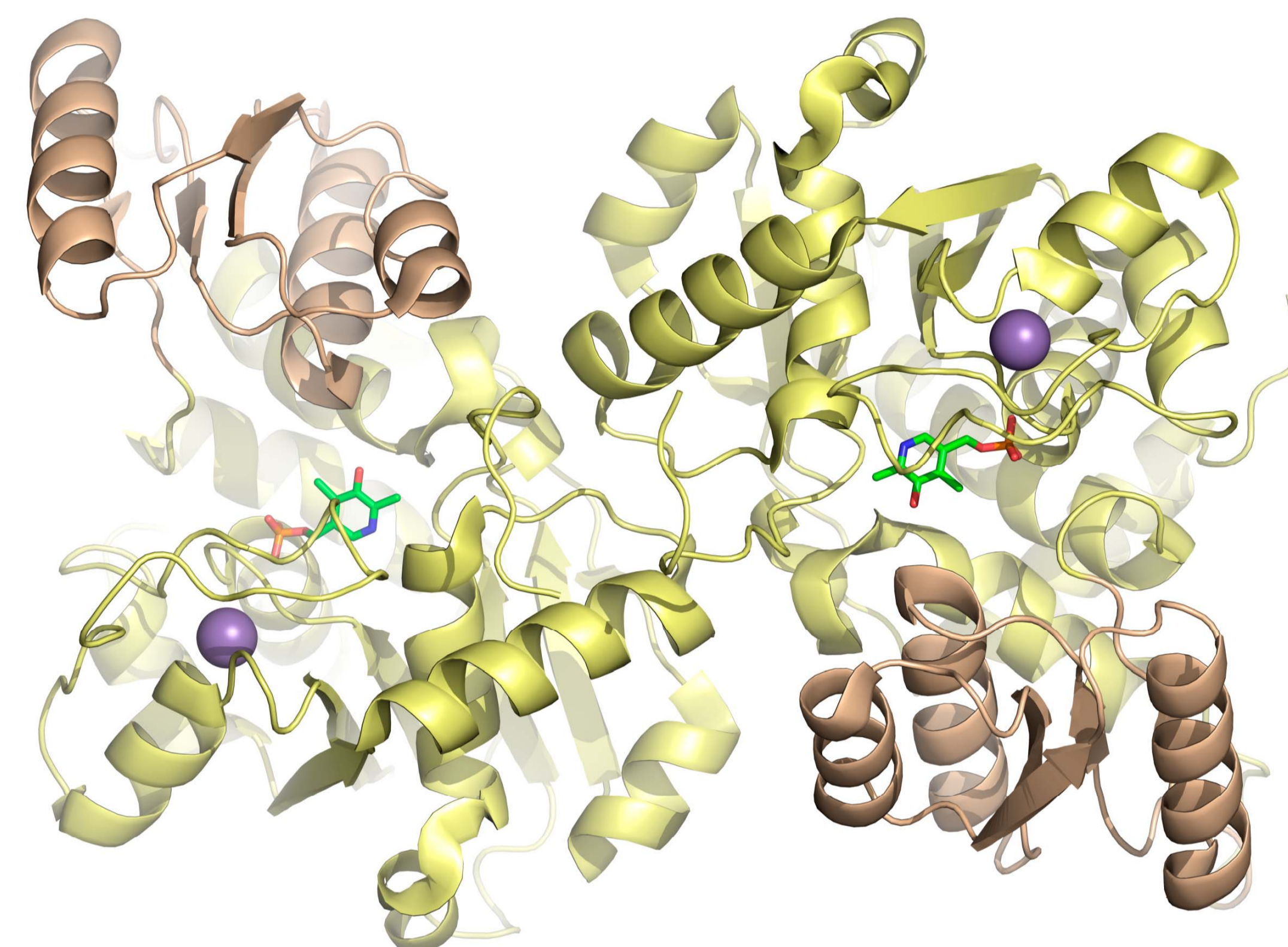


Crystal structures

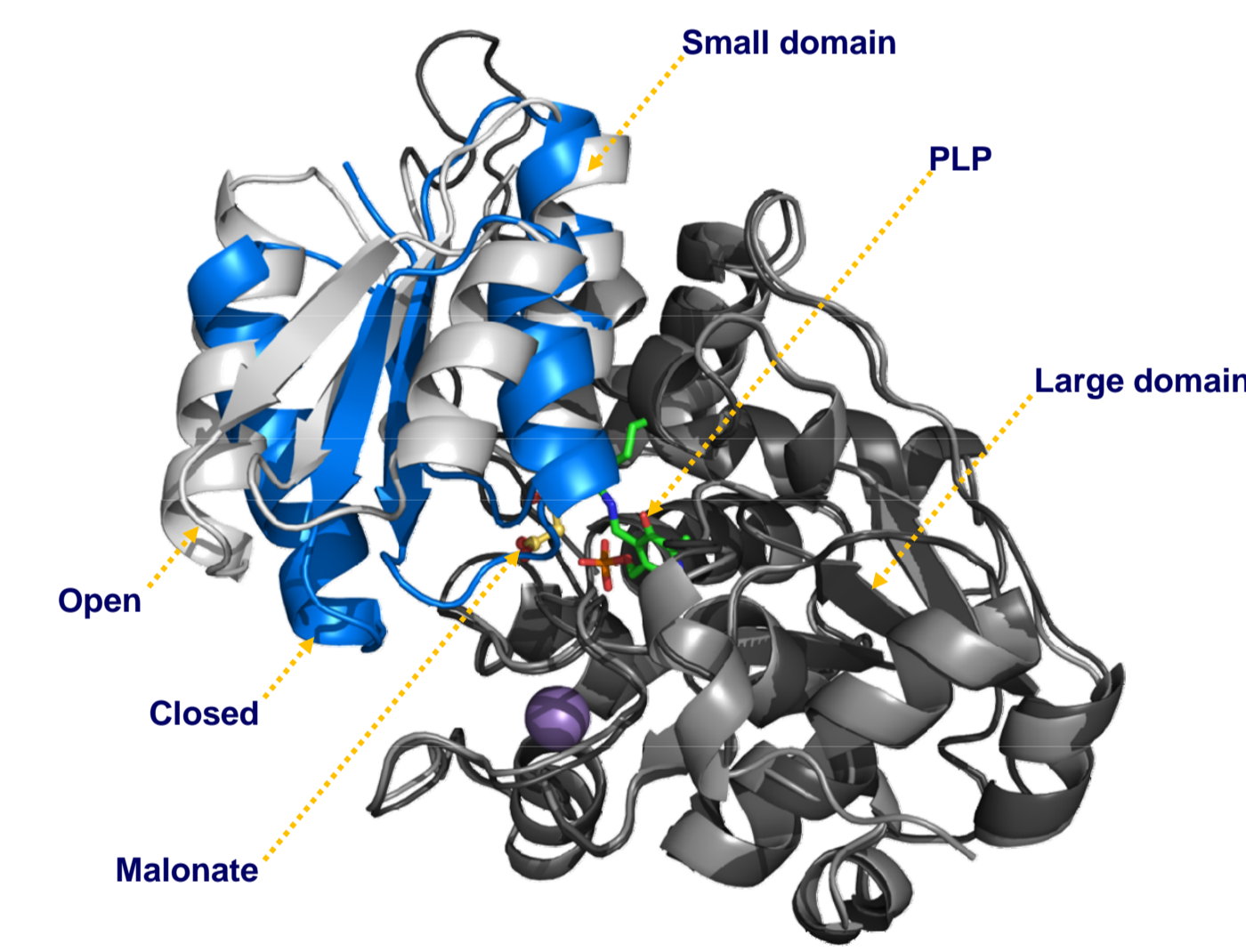
1.5 Å resolution structure of the closed conformation human SR-malonate complex: The small domain is displayed in light blue, the catalytic domain in dark blue, malonate (pink) and manganese ion (purple). The human dimer is generated by a crystallographic axis of symmetry (3L6B).



2.1 Å resolution structure of the open conformation rat SR-malonate complex: A symmetric dimer with PLP (green) at the dimer interface. The large domain is coloured yellow and the small domain is brown. The manganese ion (purple) is thought to play a structural role (3HMK).

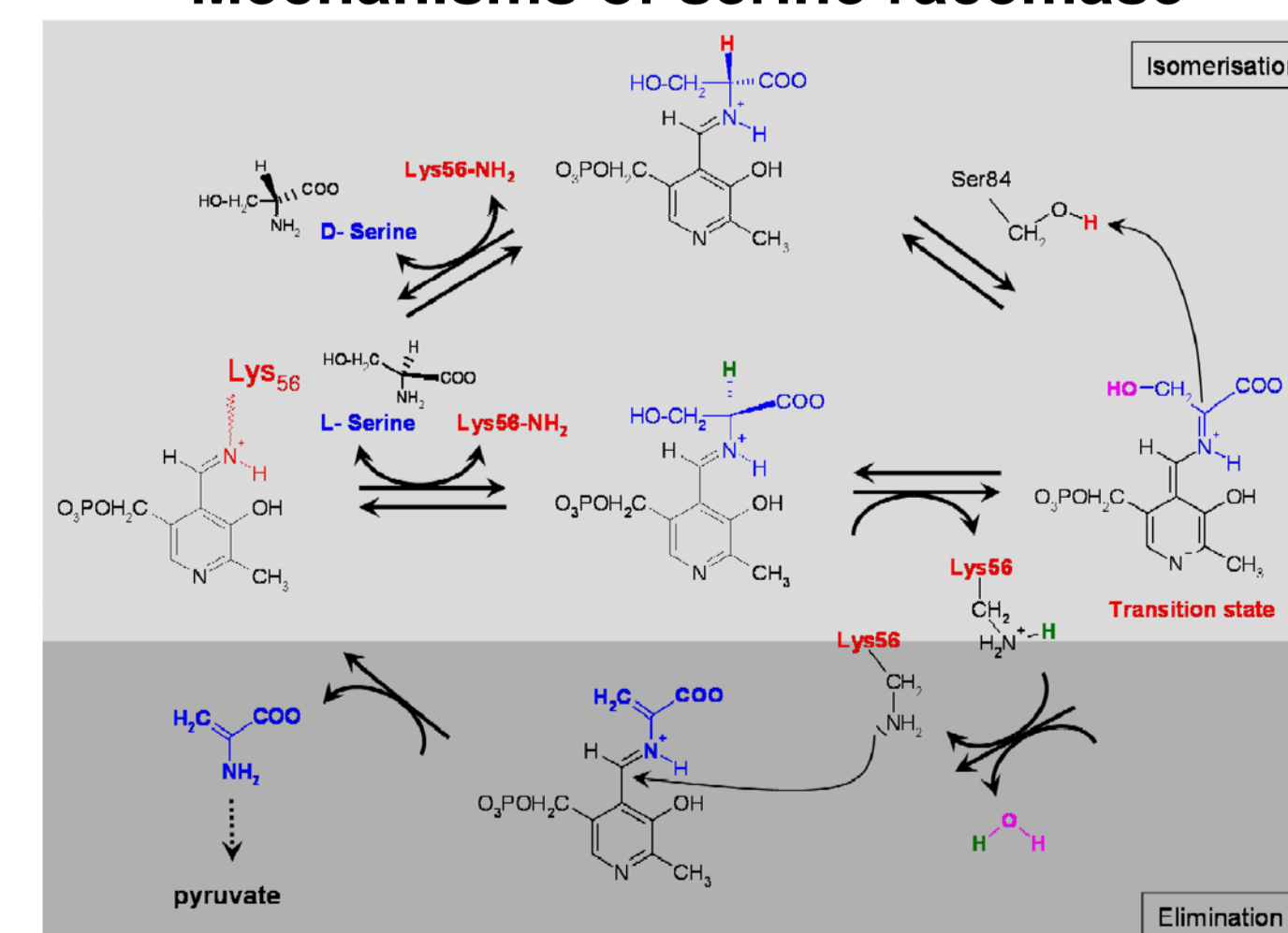


Overlay of the PLP site of human SR (blue), holo rat SR (yellow), yeast SR (green, 2ZR8)⁽⁵⁾, and rat serine dehydratase (purple, 1PWH): The catalytic serine (S82 yellow) in rat SR is too far from the ligands for any reactions to occur in its open conformation. O-methyl-serine (OMS) and malonate have similar orientations, D-serine in yeast is shown released after racemisation has occurred.



Structural alignment of rat holo (grey) and complexed human (blue) serine racemase: The small domain of each molecule has a different orientation with respect to the large domain (grey). Curved arrow at the base of the helix indicate the direction of movement from unbound (open) to ligand bound (closed) conformation.

Mechanisms of serine racemase



Conclusion

The multiple X-ray crystal structures have revealed that serine racemase is a dynamic system in which the binding of substrate induces a significant conformational change. The orthosteric inhibitor, malonate, adopts the binding pose of the cognate substrate, serine, in the active site of the closed structures. The racemisation reaction is critically dependent on the proximity of the catalytic Ser82 to the substrate as shown in the closed ligand bound structures. The structures of serine racemase reported here represent starting points for rational drug design and suggest the possibility of modulating the enzymatic activity possibly by blocking the movement of the small domain.

References

- 1) Basu, et al. (2008) Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. *Molecular Psychiatry* 14, 719-727
- 2) Nagata, Y. (1992) Involvement of D-amino acid oxidase in elimination of D-serine in mouse brain. *Experientia* 48, 753-755
- 3) Chaffey, H., and Chazot, P. L. (2008) NMDA receptor subtypes: Structure, function and therapeutics. *Current Anaesthesia & Critical Care* 19, 183-201
- 4) Brown, D. G. and Krupp, J. J. (2006) N-Methyl-D-Aspartate receptor (NMDA) antagonists as potential pain therapeutics. *Current Topics in Medicinal Chemistry* 6, 749-770
- 5) Masaru Goto, M., Yamauchi, T., Kamiya, N., Miyahara, I., Yoshimura, T., Mihara, H., Kurihara, T., Hirotsu, K. and Esaki, N. (2009) Crystal Structure of a Homolog of Mammalian Serine Racemase from *Schizosaccharomyces pombe*. *J. Biol. Chem.* 284, 25944-25952
- 6) Smith, M.A., Barker, J., Mack, V., Ebnet, A., Moraes, I., Felicetti, B., Cesura, A. (2010) The structure of mammalian serine racemase: evidence for conformational changes upon inhibitor binding. *J. Biol. Chem.* 285, 12873-12881