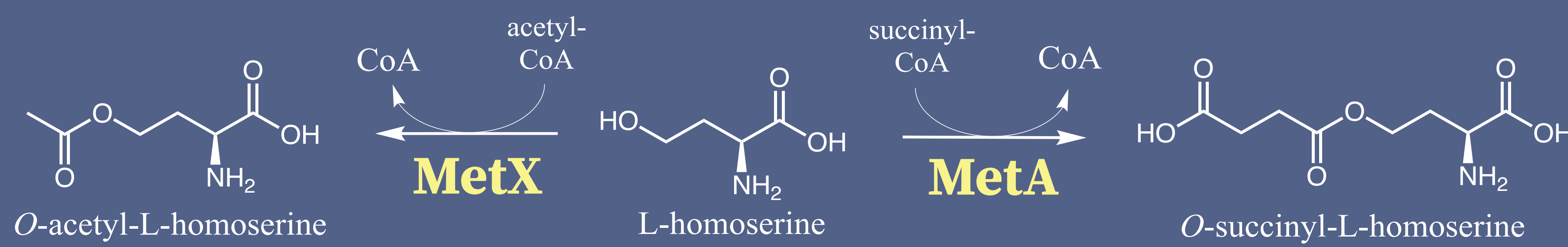


CO₂ Fixation for L-Methionine Production – The Role of Acyl Transferases in the Biosynthetic Pathway

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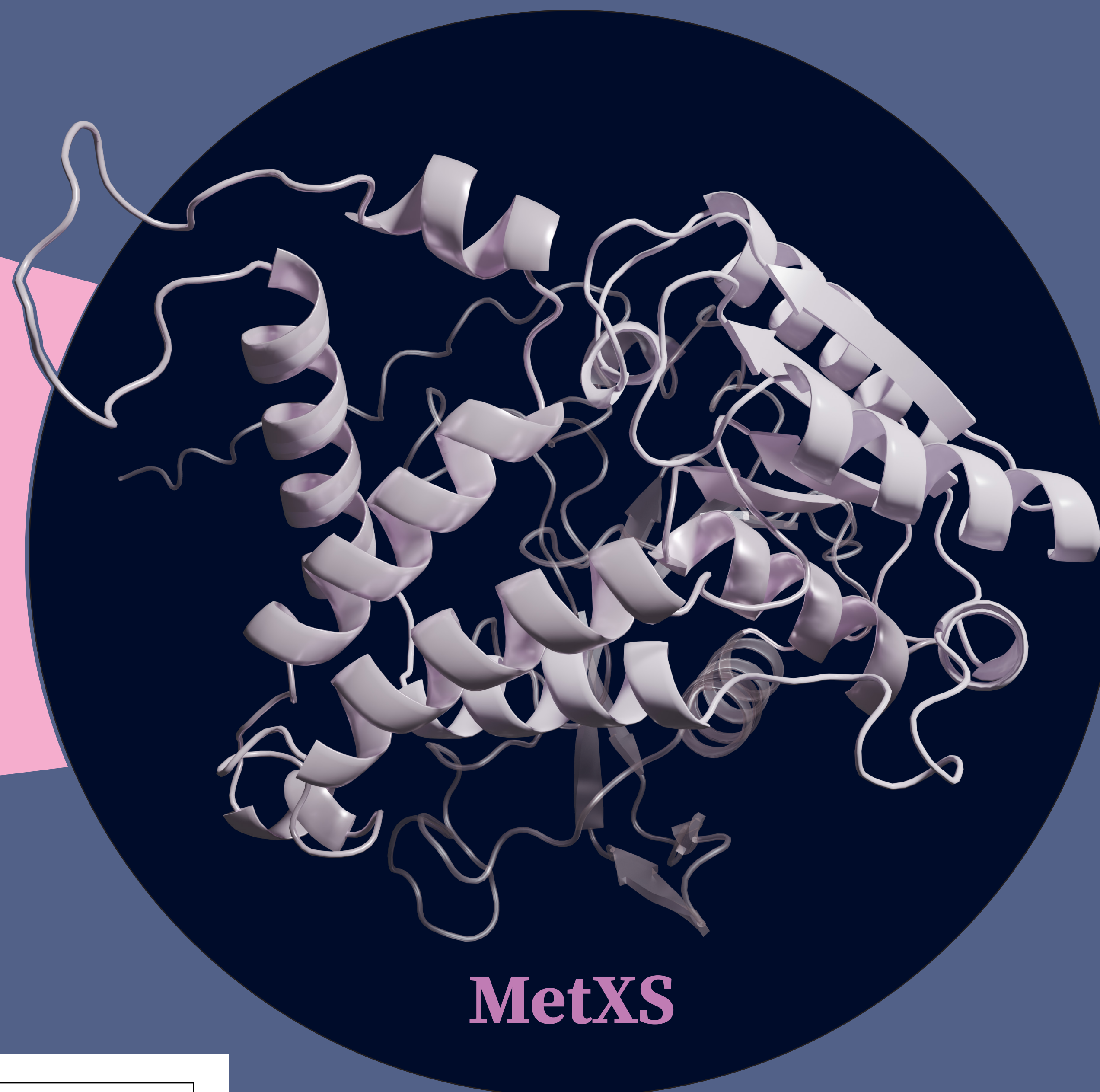


MetX and **MetA** are non-homologous isofunctional enzymes and prone to incorrect annotation. Both catalyse the **transfer of an acyl group to homoserine** in the biosynthetic pathway of L-methionine in bacteria.

Acetyl-CoA is considered to be the original sole substrate of these isofunctional enzymes which have then evolved to use exclusively succinyl-CoA.

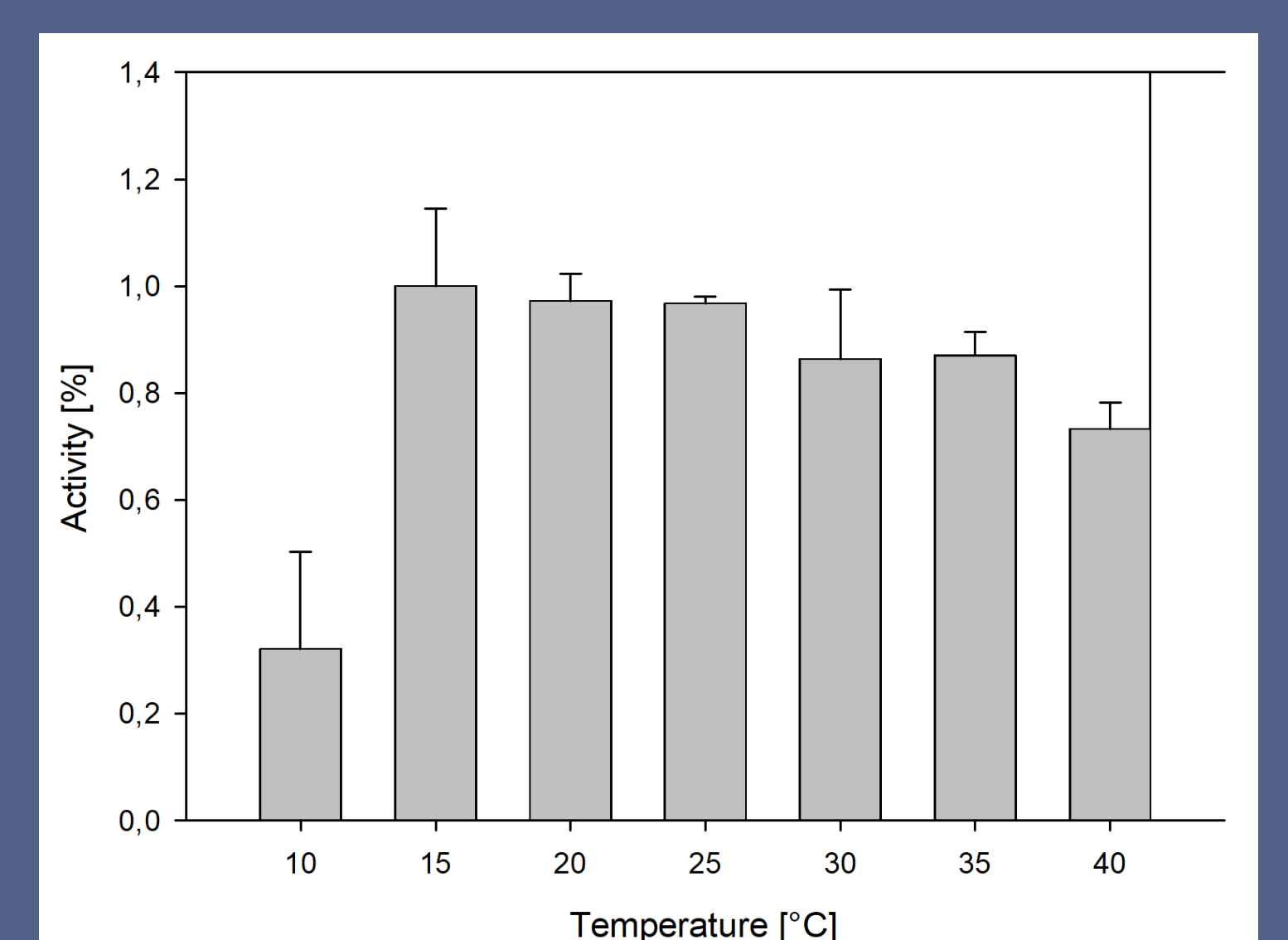
Objectives

- Experimental determination of the reaction catalysed by MetXS
- Biochemical characterization
 - Enzyme kinetics
 - Temperature/pH optimum
 - Monomer/multimer?
 - Binding of the substrates

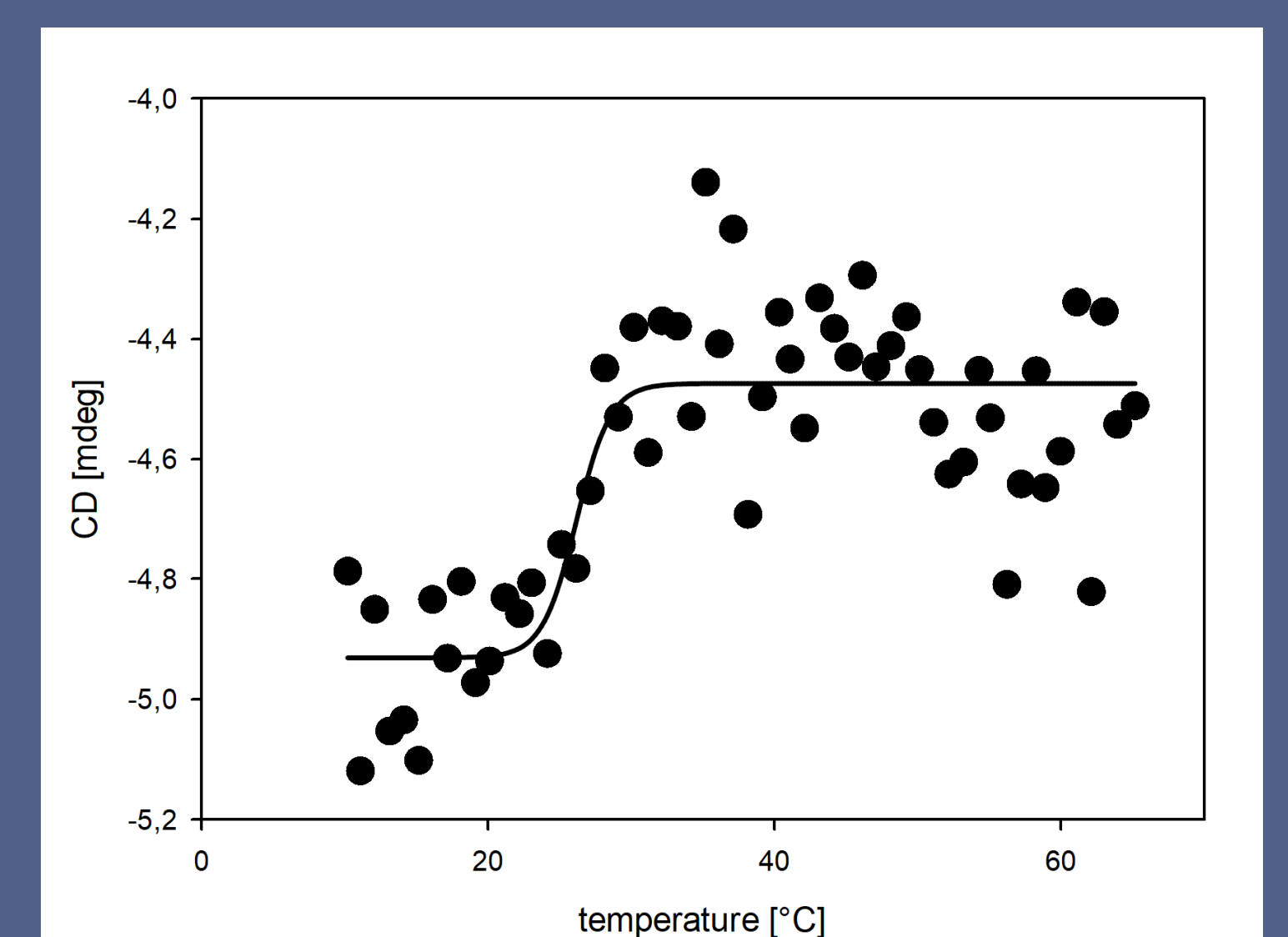


MetXS

- Transferase involved in the biosynthesis of L-methionine in *C. necator*
- SEC suggests mainly the formation of a homodimer, a significant amount is in a higher oligomeric state.

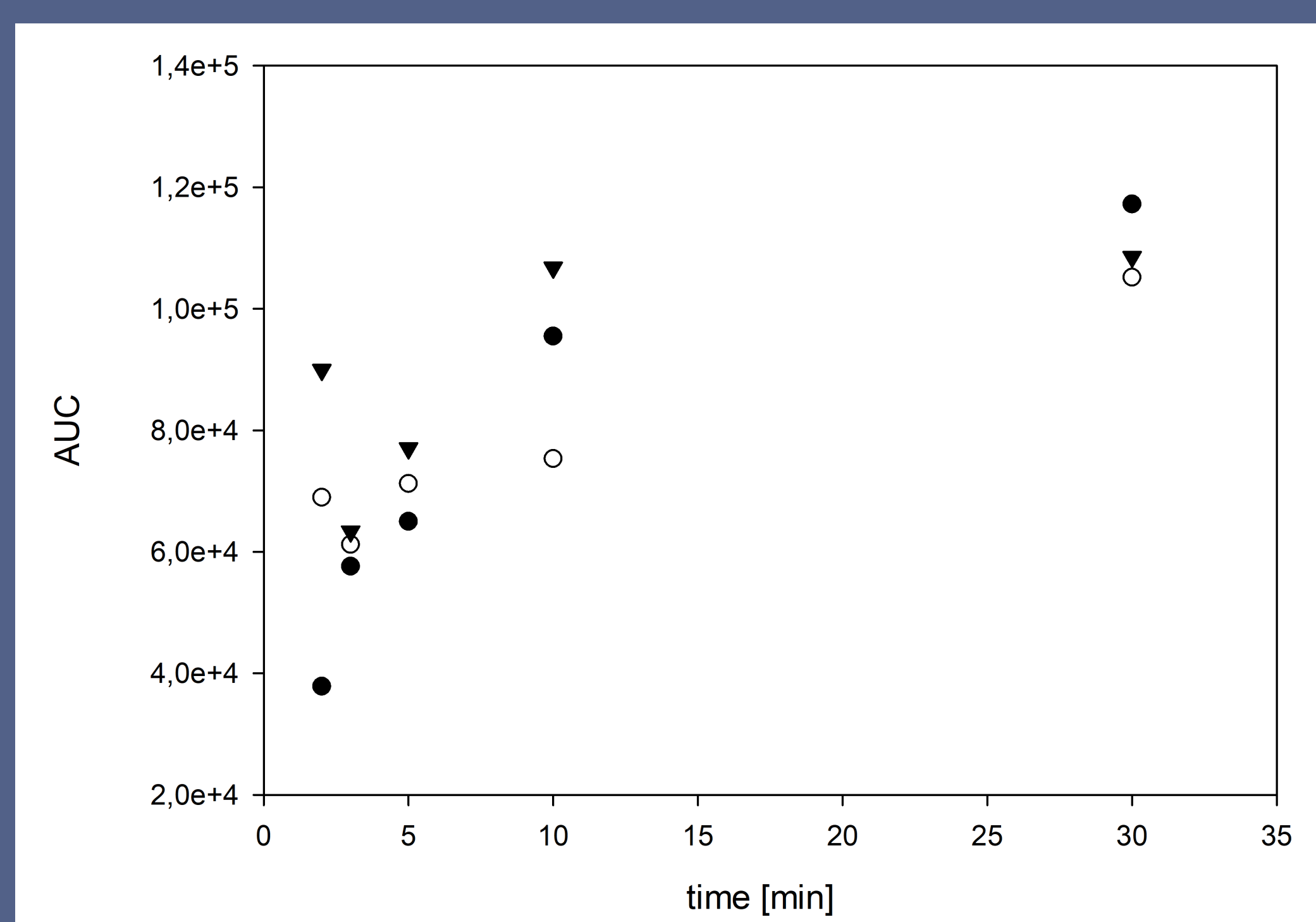


Determination of the temperature optimum.



CD spectroscopy (0.1 mg/mL).

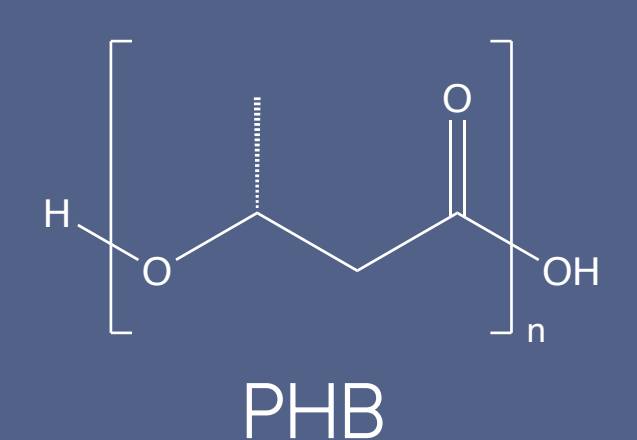
Initial activity assay



1 mM homoserine, 2 mM acetyl-CoA
20 mM KPi buffer pH 7.0, 30 °C

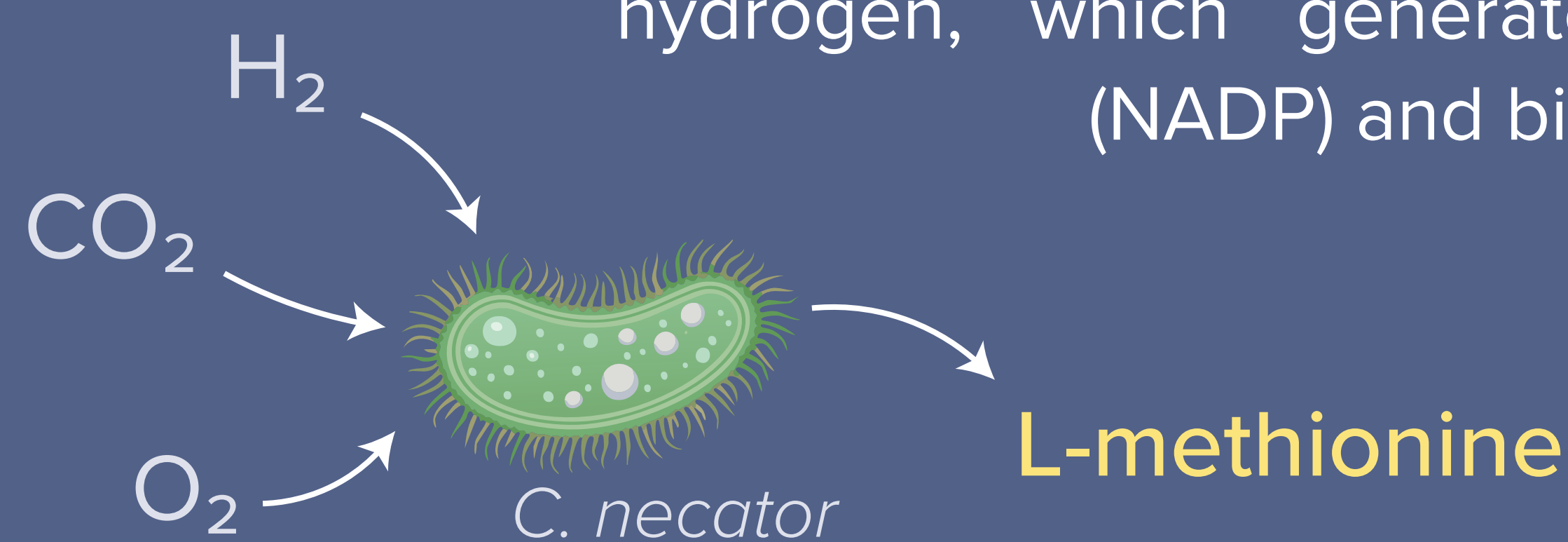
- Full conversion after 10 minutes
- Acetyl-CoA is a substrate of MetXS

Cupriavidus necator is a chemolithoautotrophic bacterium well-known for producing PHB under unfavourable conditions.



CO₂ can be used as the sole carbon source for the cultivation of *C. necator*.

The chemolithoautotrophic bacterium can generate the necessary energy to fix CO₂ by oxidising hydrogen, which generates reducing agents (NADP) and biological energy (ATP).



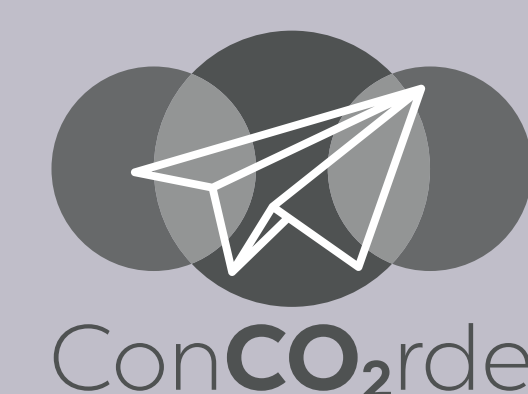
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