

# Multifunctional Enzymes

## Substrate Channeling in Multifunctional Enzymes

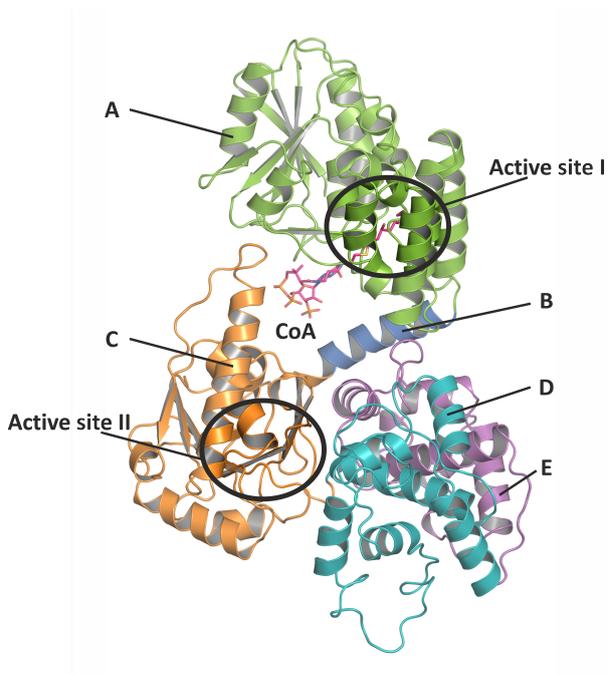
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### In Short

- Substrate Channeling
- Dynamical Properties
- Substrate Specificity

The metabolism of fatty acids is one of many important biological processes. Metabolism is done in many organisms with the help of the multifunctional enzyme type-1 (MFE-1) (**Figure 1**).

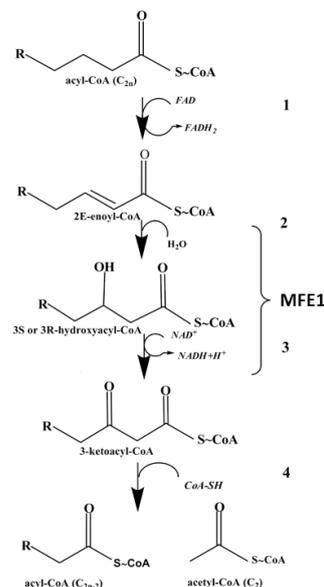
This enzyme consists of five domains (A-E) and has two active sites: hydratase and dehydrogenase active site.



**Figure 1:** Cartoon representation of MFE1. Domain A is colored green, domain B blue, domain C orange, domain D cyan and domain E violet. The ligand is represented in pink sticks. Hydratase active site I and dehydrogenase active site II are highlighted.

It plays a crucial role in the beta-oxidation pathway and catalyzes three reactions (**Figure 2**). First, the isomerization of 3Z-enoyl-CoA to 3E-enoyl-CoA following the hydration at the first active site with the help of water.

Then the fatty acid substrate is released from the hydratase active site and diffuses to the dehydrogenase active site where the third (oxidation) reaction takes place with the help of NAD<sup>+</sup>.



**Figure 2:** The four steps of the beta-oxidation pathway are shown (1) FAD dependent 2,3-dehydrogenation, (2) water mediated hydration, (3) NAD<sup>+</sup> dependent 3-hydroxy dehydrogenation, (4) thiolitic cleavage. The reactions catalyzed by MFE1 (hydratase and dehydrogenase) are shown in brackets. Each of these reactions concerns thioester chemistry. In general, thioester chemistry is of great importance in many enzyme catalyzed reactions.

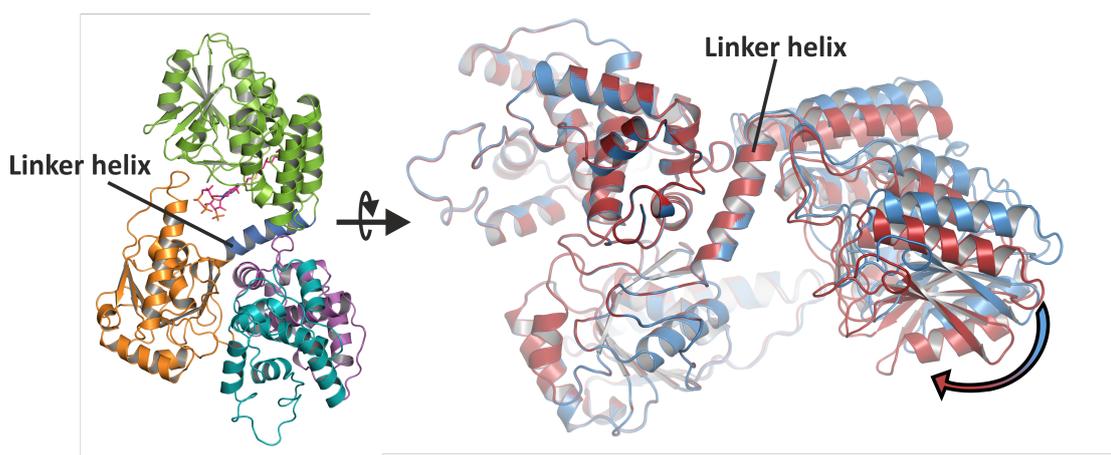
Unfortunately, the mechanism of the substrate channeling and the substrate specificity at the first active site has been investigated so far.

Therefore, this project addresses these two issues on an atomic level using classical molecular dynamics, accelerated molecular dynamics and hybrid QM/MM as well.

Classical molecular dynamics is a well-known computational approach and is used for studying the physical movements of atoms and molecules. The trajectories of these molecules are calculated by solving Newton's equation of motion for interacting particles. In this case forces between particles and also their potential energies are calculated using molecular mechanics force fields (here CHARMM). Because of the huge molecular size of the MFE-1 it is necessary to capture those dynamics on a higher timescale. For this, we use accelerated molecular dynamics (aMD).

This approach modifies the potential by adding a boost potential. This potential raises the energy so that it improves the conformational space sampling that is not accessible via conventional molecular dynamics.

Conventional molecular dynamics and accelerated molecular dynamics are fully covered and included



**Figure 3:** **Left panel:** Overview of the overall structure of MFE1. **Right panel:** Rotated cartoon representation of MFE1. Superposition of open (blue) and close (red) structure of MFE1. The blue-red arrow indicates the motion of domain A towards domain C. The motion is highlighted by the color code starting at blue shifting to red.

in the package NAMD on the HLRN architecture. We will sample the structural and dynamical properties of MFE-1 using these computational methods. Before addressing the substrate channeling between the two active sites, we investigate in more detail the dynamical properties depending on substrate and NAD<sup>+</sup>/NADH-binding.

It is suggested that two of the five domains (here domain A and C) are crucial for the substrate channeling assuming that they perform a so-called open-close movement (**Figure 3**). For this, to reveal and quantify these motions we use the dynamical cross correlation method (DCC).

#### WWW

<http://www.biomodelling.tu-berlin.de/>

#### More Information

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- [2] Kasaragod, P., Schmitz, W., Hiltunen, J.K., Wierenga, R. *FEBS J.* **280**, 3160-3175 (2013).
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#### Project Partners

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