## SUPPLEMENTARY MATERIAL AND METHODS

### Homology models

The modelling was started with the conformer 2, for which, firstly, chain A was constructed by extracting CT domain from the human PC structure (PDB ID 3BG3 chain A) (Xiang et al., 2008) and reconstructing missing residues based on homology with the PC structure of *Staphylococcus Aureus* (PDB ID 3BG5 chain A) (Xiang et al., 2008). The BC domain was built by homology to the 3BG5 chain A and chain B of the human acetyl-CoA carboxylase (PDB ID 5KKN chain B). The pyruvate carboxylase tetramerization (PT) and BCCP domains were directly extracted from the 3BG3 chain A and the missing 7 residue loop fragment in the BCCP domain was modelled by means of Rosetta kinematic loop modelling followed by a relaxation with the constraints on all the atoms, except for the reconstructed loop. For this and all the further Rosetta applications we used a Cartesian version of Rosetta version 2017.39.59729 with the Talaris2014 scoring function (except for the free energy calculation procedure where Talaris2014 and ref2015 (Alford et al., 2017) energy functions were used). In the final step, the 3BG5 chain A was superimposed onto the 3BG3 chain A, and subsequently the modelled CT, PT and BCCP domains were superimposed on the 3BG3 chain A, while the modelled BC domain was superimposed on the 3BG5 chain A. Having constructed chain A, chain B was assembled from the already modelled domains by symmetry to the chain A: superpositioning of the domains was performed accordingly to the procedure as for the chain A.

The CT domain for chain D of the conformer 2 was modelled by homology using 3BG3 chain D and 3BG5 chain D CT domains. For the BC domain, the 3BG5 chain C and 5KKN chain B BC domains were used. PT and BCCP domains were extracted from the chain D of 3BG3 and the missing loop was modelled in the same way as described previously. The final domain assembly was performed by means of superpositioning onto the 3BG3 and 3BG5 structure. The chain C was modelled by symmetry using the domains modelled for chain D.

For the conformer 1, chain A was modelled by using chain A of the structure 2QF7 (Maurice et al., 2007) and the BC domain of the modelled conformer 2 chain A as templates. Chain C was subsequently modelled by piecewise superposition of the modelled chain A domains onto the 2QF7 chain B. Chain B and chain D were reconstructed by symmetry by using the modelled chains A and C, respectively.

For both modelled conformers a covalently bound biotin moiety was constructed for every chain based on the 3BG3 structure by locally superpositioning residues in the vicinity of biotin. For the conformer 1, pyruvate molecules were added for every chain based on the 3BG5 structure and $Mn^{2+}$ ions were positioned in the place of $Zn^{2+}$ based on 2QF7. For every chain of conformer 1, ATP molecules were placed in the position of ATP analog resolved in the structure 2QF7. For the conformer 2, pyruvate and $Mn^{2+}$ ions for every chain were placed based on the 3BG3 structure; ATP molecules for every chain were placed according to the positions in the 3BG5 structure. In both conformers two partial structures of ethyl-CoA molecules were placed according to the positions in the 2QF7 structure.

**Energy minimization**

Subsequently, both modelled conformers were relaxed by means of Rosetta FastRelax protocol. During the relaxation, pyruvate, manganese, acetyl-CoA and ATP moieties were constrained. In addition, for the relaxation of the conformer 2, biotin coordinates were constrained as well. Afterwards, starting from the relaxed structure, each conformer was minimized 100 times by ramping down the constraint contribution. The conformers with the lowest energy were identified as the final model structures and used further in the analysis.

**Thermodynamic model**

For a heterozygous variant of a tetrameric protein there exist 16 combinations of mutated variants, when every monomer is considered to be distinguishable, i.e. labeled. In this scenario, the ΔΔG value can be calculated for each of the sixteen cases by means of Rosetta and an overall double free energy difference for a single conformation can be expressed:

$$\Delta\Delta G_j = \frac{\ln 16}{\beta} - \frac{1}{\beta}\ln\left(\sum_{i=1}^{15} e^{-\beta\Delta\Delta G_i} + 1\right)$$

where β is the inverse of thermodynamic temperature, $\Delta\Delta G_i$ is change in free energy of protein stability upon a mutation *i* (comprises the combinations of mutations in the monomers). $\Delta\Delta G_j$ is the overall change in stability of the enzyme for the conformer *j* (in our setup we consider two conformers).

For a homozygous case the double free energy simplifies to the ΔΔG value of the mutations occurring in four monomers at once, i.e. $\Delta\Delta G_j = \Delta\Delta G_{mut\_in\_4\_mon}$.

Assuming that the protein needs to be stable in both conformations to function, combination of the two conformers gives the final expression to the double free energy difference: $\Delta\Delta G = \Delta\Delta G_1 + \Delta\Delta G_2$, where $\Delta\Delta G_1$ and $\Delta\Delta G_2$ are calculated for the heterozygous or homozygous scenario, depending on the case considered. This ΔΔG value quantifies the overall change in protein's stability upon a mutation. The free energy calculations were performed using two scoring functions: Talaris2014 and ref2015. The final $\Delta\Delta G$ values are reported as a range covering the estimates from both energy functions, in turn quantifying the uncertainty associated with the computed values.