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**Supporting information for article:**

**Crystal structure of the catalytic subunit of bovine pyruvate  
dehydrogenase phosphatase**

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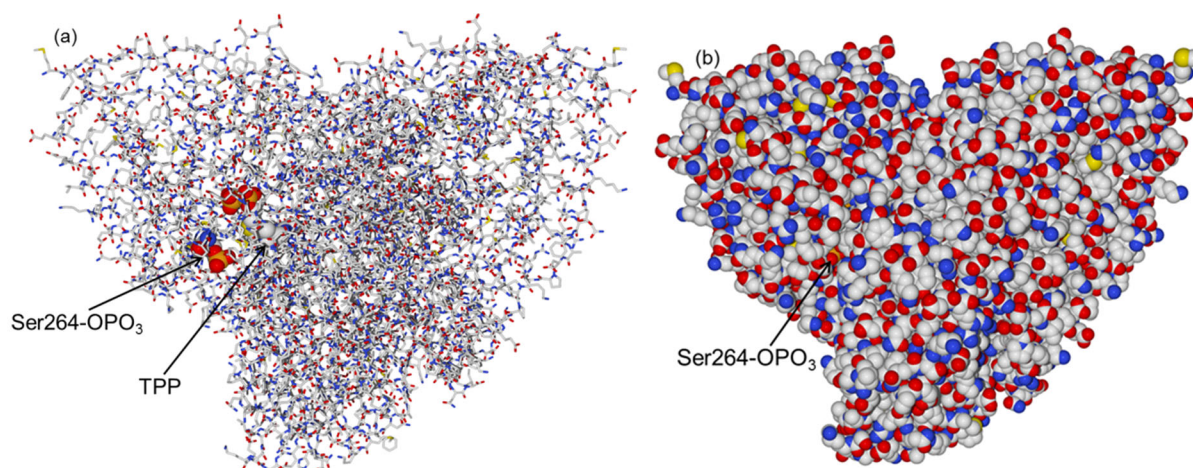


Figure S1

Phosphorylated-E1. Panel (a) shows phosphorylated Ser264 (site 1) (Kato *et al.*, 2005) which is at the front of the channel that leads to thiamine pyrophosphate (TPP) that participates in the decarboxylation of pyruvate followed by the reductive acetylation of an oxidized lipoyl group delivered on a lipoyl domain of the E2 or E3BP component. E1 has high specificity for the lipoyl domains of the pyruvate dehydrogenase complex (Gong, *et al.*, 2000, Liu, *et al.*, 2001) and will not use lipoyl domains of the  $\alpha$ -ketoglutarate dehydrogenase complex or branched-chain  $\alpha$ -ketoacid dehydrogenase complex. Panel (b) shows a space-filled model exhibiting how with the best view the phosphate group on Ser264 is barely visible. Ser264 is on a loop and this is thought to loop out to be more available both during the phosphorylation reaction by PDK and dephosphorylation by PDP.

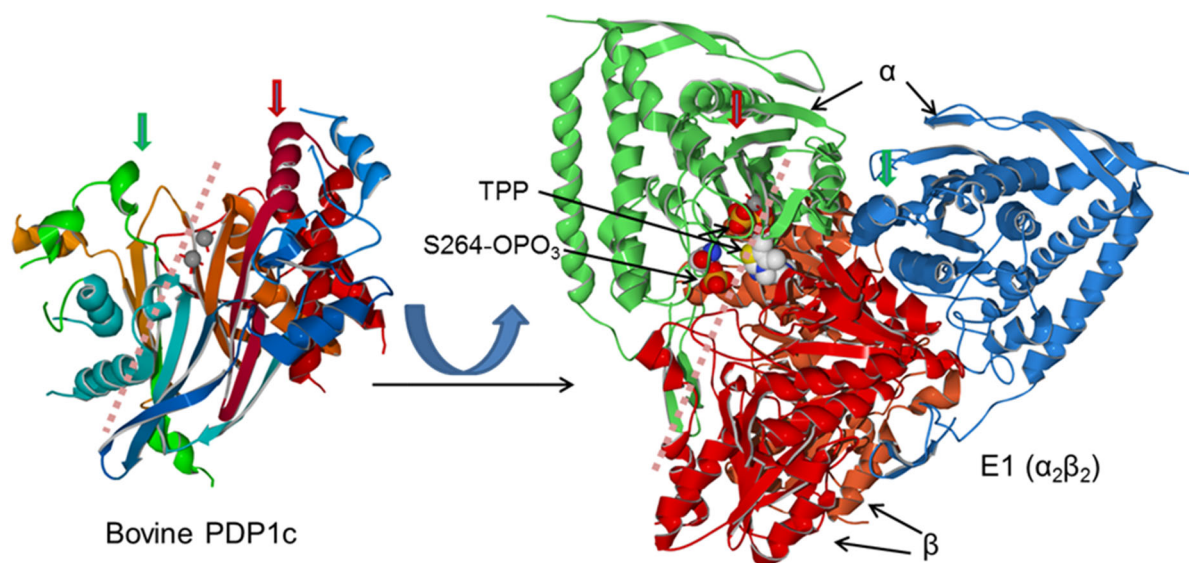


Figure S2

Proposed interaction of PDP1c with site 1 phosphorylated-E1. The ribbon structure for bovine PDP1c is the same as in Figure 1a. The subunits of E1 are each given a different color with much of the orange E1β subunit on the back side behind the red β subunit. With an ~180° rotation of PDP1c, the near joining of the dashed orange lines approximate the positioning for the interaction of the active site Mn<sup>2+</sup> adjacent to Ser264 phosphate ester.

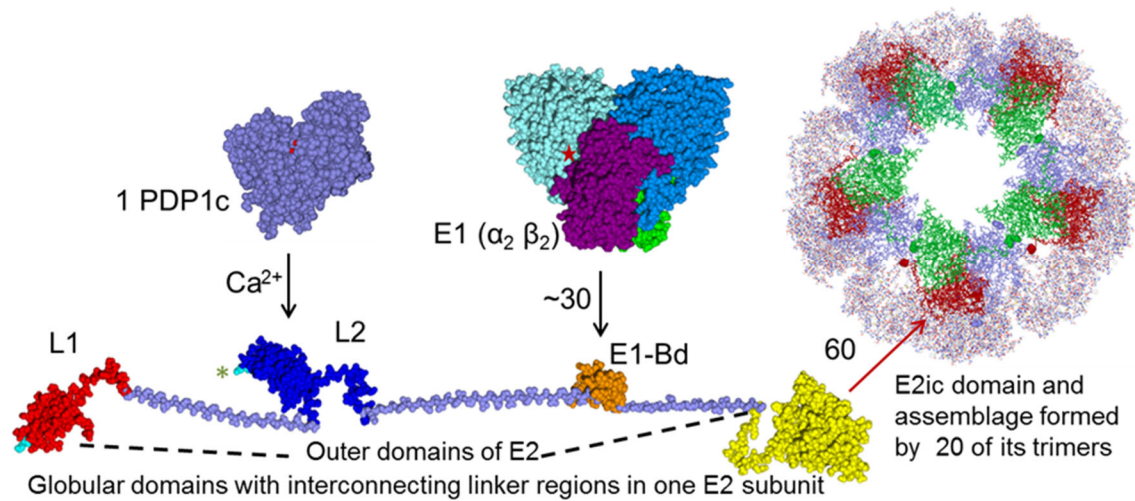


Figure S3

E2 domains, inner core assemblage and interactions of one PDP1c with the L2 -domain and many E1 with the Bd domain in the very mobile outer domain-structure of E2. A \* is placed next to the L2 lipoyl group; L2 is shown as found in its NMR-derived structure (Howard, *et al.*, 1998); in binding to PDK the C-terminal structure extending to right folded down on rest of the domain. In its known binding with PDP1c, E1, E2, or E3 this part of the domain may organize differently. The inner core is formed by the E2ic domain which catalyzes acetyl transfer from dihydrolipoyl group to CoA. The E2 assemblage is as formed by 60 E2ic in the absence of the E3BP which has a related structure to E2 including a C-terminal inner domain that integrates into the inner core (Hiromasa, *et al.*, 2004). Here we show set of five trimers with different colored subunits around one of the twelve faces of the dodecahedron-shaped inner core. With stick atom presentation of the inner core, for clarity only the front 40 E2ic trimers are shown.

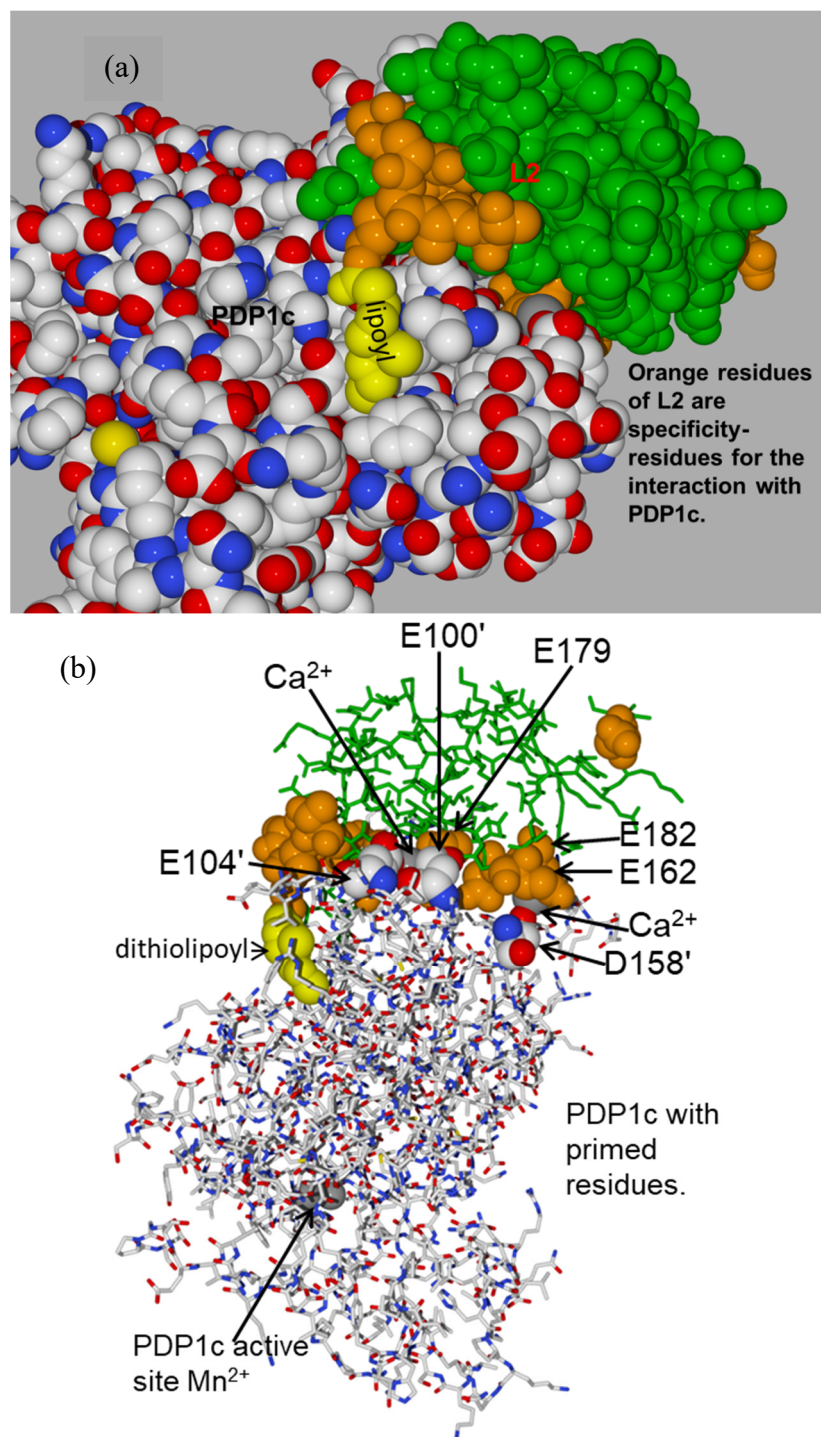


Figure S4

PDP1c-L2 interaction (Vassilyev & Symersky, 2007). Panel (a) displays space-filled residues and the region of PDP1c that interacts with the L2 domain. In panel (a) and (b), most residues of the L2 domain are shown in green (as stick structures in (b)), but the specificity-residues of L2, that are required for effective binding to PDP1c (Turkan, *et al.* 2002), are shown in orange and space filled in both panels. In both panels, the dithiolipoyl group of L2 is shown in yellow and all residues of PDP1c are shown with CPK colors. Panel (a) exhibits how the lipoyl group sits mainly exposed

at the surface of PDP1c. Panel (b) exhibits how two  $\text{Ca}^{2+}$  ions are sandwiched between L2 and PDP1c with residues of L2 (specificity residues E162, E179, E182) and of PDP1c (E100', E104' and D158') engaged in binding these  $\text{Ca}^{2+}$ . These PDP1c residues are the only ones shown space-filled in this panel. Several orange-colored specificity residues of L2 are not directly involved in this putative placement of L2 in binding to PDP1c. Mutations at these residues, leading to L2 that did not bind well to PDP1c (Turkan, *et al.* 2002), still produced stable, lipoylated L2 domains (Liu, *et al.* 1995). L2 is shown with its C-terminal region folded onto the folded domain as found in PDK3-L2 complex (Kato, *et al.*, 2005). The C-terminal region (residues 208 to 229) of L2 were not folded onto the domain in an NMR-structure (Howard *et al.*, 1998). That study was conducted at a reduced pH; the same unfolding was found in NMR studies conducted at pH 7.0 (T. Peng, O. Prakash, and T.E. Roche, unpublished). It is not certain that this region folds in this same manner in its many interactions with other components including PDP1c (see main text).

## References:

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