Exploring the Multi-Stage Catalytic Cycle of Cezanne-1 through MD Simulations

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Deubiquitylating enzymes (DUBs) catalyze the removal of ubiquitin (Ub) moieties attached to proteins by cleaving the iso-peptide bond between distal and proximal Ubs. Thus, DUBs play a pivotal role in the regulation of various cellular processes. The DUB Cezanne-1 selectively cleaves K11-linked polyUb chains. Although crystal structures of Cezanne-1 are available at different enzymatic stages, they do not reveal dynamic conformational changes. Crystal structures of diUb- and monoUb Cezanne-1 were resolved using activity-based probes that covalently attach to the catalytic cysteine residue, thus potentially altering the active site and substrate recognition [1].

Here, we employed full-atomistic molecular dynamics simulations to explore conformational changes during Cezanne-1 activation and its proteolytic activity by reconstituting the native substrate. [2] Our MD simulations reveal that the Ub-free Cezanne-1 shuttles between catalytically active and inactive states. Only the catalytically active, substrate-free Cezanne-1 allows the substrate access to the catalytic center. This, in turn, favors the catalytical activation of Cezanne-1 in a substrate-assisted activation process. Upon cleavage of the diUb's iso-peptide bond, the proximal Ub is prone to dissociate first from Cezanne-1, which leads to the catalytically inactive monoUb-bound state. This is followed by the release of the C-terminus of the distal Ub from the catalytic center and recovery of the Ub-free state.

To sum up, the activation and catalytic turnover of the DUB Cezanne-1 is a complex multi-stage cycle with various dynamic transitions that are unable to be deciphered based on static and non-native protein crystal structures.

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[2] M. Ilter, E. Schulze-Niemand, M. Naumann, M. Stein, *J. Chem. Inf. Model.*, **2023**, 63, 2084–2094