Asymmetric flexibility of a homodimeric enzyme as shown by molecular dynamics computations.

A case study of the cold-active Vibrio alkaline phosphatase.

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Introduction
Multiple all-atom explicit solvent molecular dynamics simulations were employed in conjunction with different metrics to analyze the dynamics patterns and the paths of intra- and intermolecular communication in a cold-active alkaline phosphatase (VAP). Asymmetric dynamics have been suggested to play a part in the catalytic cycle in homodimeric alkaline phosphatases. A conformational change might be the rate-limiting step since the chemical transformations are much faster than κ2. Asymmetric protein dynamics would influence protein function and stability by modulating conformational changes consistent with half-of-the-sites mechanism2,4. Here, we wanted to see if the symmetric crystal structure of VAP would become asymmetrical in solution, a good predictor of half-of-the-sites mechanism.

Results
Fig. 1 - Dynamic patterns of the two subunits had a different distribution of intramolecular interactions and correlated motions (rmsf).

Fig. 2 - VAP displayed a low number of intersubunit interactions. Coupled motions between the two halves were also few.

Fig. 3 - Numerous salt-bridge clusters were observed with asymmetric distribution in the two subunits.

Fig. 4 - Hub residues (those that link 3 or more other residues) were nonsymmetrically distributed. Several were located in area specific for VAP, i.e. the large loop (insert II).

Conclusions
• Dynamic patterns of the two VAP subunits were asymmetric.
• VAP subunits had a different distribution of intramolecular interactions and correlated motions.
• VAP displayed a low number of intersubunit interactions. Coupled motions between the two halves were also few.

Our results provide a structural rationale to support the half-of-the-sites mechanism for VAP. This will help us to understand the reaction mechanism of this metallo-phosphatase better and open the door for studying how cold-adaptation has generated a cold-active variant by mutagenesis.

References
(3) Akalin, A., and Tashman, L. (2005) The Protein Structure Network analyzes of the interatomic distance distribution on the 3D structure of subunit A and B, respectively. The four inserts (i) to (iv) not conserved in the warm-adapted counterparts are in green. R129 and S65 are shown as sticks.

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Asymmetric flexibility patterns