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Exploring aldehyde chain length selectivity in bacterial luciferases

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Bacterial luciferases are homologous enzymes that catalyze the light-emitting reaction, using myristyl aldehyde as one of the substrates. Other saturated aldehydes (C8-C16) can also participate in the reaction *in vitro*. Despite the high structural similarity among luciferases from different bacterial species, the reaction kinetics and light intensity vary with enzyme origin and aldehyde chain length. Although previous studies have characterized hydrocarbon tail interactions in the active center of *Vibrio harveyi* luciferase [1], the structural details of the aldehyde binding site for other species remain unclear.

Considering the divergence of bacterial luciferases into two structural groups, this study aimed to characterize their aldehyde interactions through molecular docking. Structural findings were integrated with evolutionary selection analysis and validated against experimental data on luciferase affinity for aldehydes of varying chain lengths.

Molecular docking was performed in AutoDock Vina. Two representative luciferases from distinct structural groups were selected: (1) *V. harveyi* luciferase (PDB ID: 3FGC) and (2) a model of *Photobacterium leiognathi* luciferase. Both protein structures contained the C4a-hydroperoxyflavin, relative to which the docking search space was defined. 28 luciferase sequences from the NCBI database were analyzed for dN/dS ratio using a sliding window approach in DnaSP. Bioluminescence kinetics were measured using stopped-flow technique with SX-20 analyzer (Applied Photophysics).

Molecular docking revealed mostly similar binding modes for the two studied luciferases, with the exception of a buried region containing Phe8 in *P. leiognathi*. dN/dS analysis identified strong purifying selection in regions encoding the active center. In contrast, the predicted aldehyde binding cavity showed positive selection at residues related to regions 6–8 and 191–195 of the amino acid sequence. The kinetics of bioluminescence were consistent with docking results: the affinity for aldehydes decreased with chain lengths shorter than tetradecanal.

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References

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