Dynamics Direct Specificity of Effector Caspases

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The caspase family of cysteine proteases plays a key role in programmed cell death and inflammation, turning caspases into interesting drug targets [1]. Unfortunately, specific ligand binding to one particular caspase isoform is difficult to achieve, as substrate specificities of caspases are highly similar.

In an effort to rationalize subtle differences in substrate specificity of two closely related caspases [2], we investigated the substrate promiscuity of the effector caspases 3 and 7 by data mining [3] and by molecular dynamics simulations. We found a strong correlation between binding site rigidity and substrate readout for individual caspase subpockets explaining more strigent substrate readout of caspase 7 via its narrower conformational space. Caspase 3 subpockets S3 and S4 show elevated flexibility explaining the more unspecific substrate readout of this isoform in comparison to caspase 7. We show by *in silico* exchange mutations in the S3 pocket of the proteases, that a proline residue in caspase 7 contributes to the narrowed conformational space of the binding site.

These findings explain substrate specificities of caspases via a mechanism of conformational selection [4] and highlight the crucial importance of conformational dynamics in substrate recognition of proteases. The ensemble perspective of substrate specificity is proposed to be extended to general protein-protein-interfaces. Hence, we hypothesize that molecular dynamics simulations could lead the way to identify specific anchor points for targeting this challenging target class.

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