research advances

April 2012 featured article

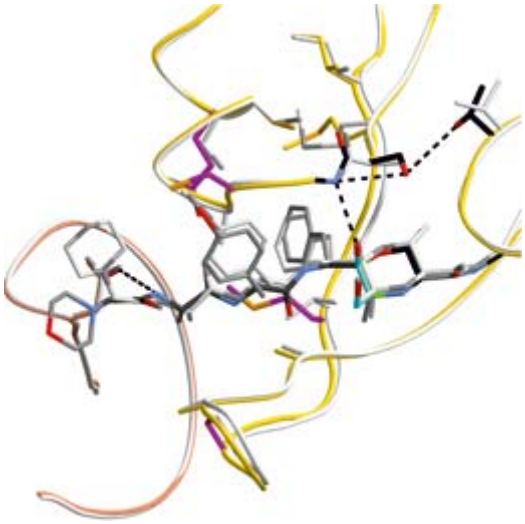
Substrate specificity sleuths

SBKB [doi:10.1038/sbkb.2011.71]

Crystal structures of constitutive and immunoproteasomes in the presence and absence of inhibitor reveal the structural basis for substrate and inhibitor specificity.

Circulating immune cells identify other cells as targets for destruction by reading out their overall protein content. This information is encoded on the cell surface as an array of peptides (epitopes) presented by major histocompatibility complex class I (MHC-I) molecules. Epitopes originate from self and foreign protein degradation executed by both constitutive (cCP) and immunoproteasomes (iCP), which contribute to peptide diversity by harboring different catalytic subunits. iCPs predominantly generate peptides with hydrophobic C-termini that anchor to MHC-I, making them attractive targets for blocking antigen and cytokine production.

In a rigorous crystallographic study, Groll, Groettrup and colleagues reveal the structural basis for enhanced epitope production by iCPs. While murine cCP (PDB **3UNE**) and iCP (PDB **3UNH**) share common architecture, subtle structural deviations modulate substrate specificity. Important differences were observed in subunits β5i and β5c (from iCP and cCP, respectively), which confer chymotrypsin-like activity. The β5i catalytic site comprises an extended hydrogen bonding network that attracts water molecules to enhance peptide bond hydrolysis and give β5i a dominant role in epitope production. The β5i-S1 recognition pocket is also distinct; a modest conformational change by highly conserved Met45 results in a larger hydrophobic pocket which can accommodate aromatic residues like Phe, Tyr and Trp, while the smaller β5c-S1 prefers Ala, Val and Leu. iCP also exhibits increased hydrophobicity at β1i-S1. Collectively, iCP is well-equipped to produce an array of hydrophobic C-terminal peptides optimized for presentation to MHC-I.



Superposition of β5i (yellow) and β5c (gray) subunits bound to PR-957 highlight the changes in β5c's binding pocket needed for PR-957 to bind. Figure courtesy of Michael Groll.

Can subtle differences between cCP and iCP be exploited for drug development? Crystal structures in complex with β5i-selective inhibitor PR-957 (PDB **3UNF** and **3UNB**) are elucidating. Ligand binding to β5c requires a demanding conformational change commencing with Met45, which fully rotates to accommodate the ligand's Phe moiety, triggering a ripple effect through ~40 flanking residues; this high penalty is reflected in PR-957's lower affinity for β5c relative to β5i. The higher affinity for β5i is consistent with its larger S1 pocket, which accommodates PR-957-Phe via minor conformational changes by Met45 alone.

Structural knowledge of iCP substrate and inhibitor selectivity opens the door for the rational design of drugs that block epitope production in immune and inflammatory diseases.

Barbara Potts

References:

1. E.M. Huber *et al.* Immuno- and Constitutive Proteasome crystal structures reveal differences in

substrate and inhibitor specificity.
Cell. 148, 727-738 (2012). **doi:10.1016/j.cell.2011.12.030**

PSI-Nature Structural Biology Knowledgebase ISSN 1758-1338