A Set of Activity-Based Probes to Visualize Human (Immuno)proteasome Activities

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Proteasomes are therapeutic targets for various cancers and autoimmune diseases. Constitutively expressed proteasomes have three active sites, β1c, β2c, and β5c. Lymphoid tissues also express the immunoproteasome subunits β1i, β2i, and β5i. Rapid and simultaneous measurement of the activity of these catalytic subunits would assist in the discovery of new inhibitors, improve analysis of proteasome inhibitors in clinical trials, and simplify analysis of subunit expression. In this work, we present a cocktail of activity-based probes that enables simultaneous gel-based detection of all six catalytic human proteasome subunits. We used this cocktail to develop specific inhibitors for β1c, β2c, β5c, and β2i, to compare the active-site specificity of clinical proteasome inhibitors, and to demonstrate that many hematologic malignancies predominantly express immunoproteasomes. Furthermore, we show that selective and complete inhibition of β5i and β1i is cytotoxic to primary cells from acute lymphocytic leukemia (ALL) patients.