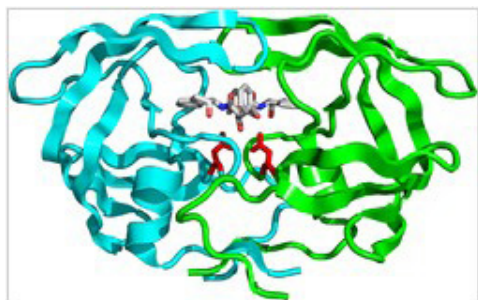




A T-CELL-BASED HIGH-THROUGHPUT ASSAY TO MONITOR THE PROTEOLYTIC ACTIVITY OF HIV-1 PROTEASE

A growing need exists to develop a simple, rapid and straightforward method for monitoring HIV-protease activity in order to facilitate the search for novel inhibitors/competitors of HIV protease that could lead to new therapeutics against HIV.

Due to the cyto-toxic effects of HIV protease when expressed in mammalian cells, the majority of assays has been designed in vitro, in either bacterial or yeast cells. More recent approaches designed for use in mammalian cells may not provide accurate evaluation of inhibitory effects because they are not performed in the natural environment of HIV infection, T-cells. In addition, they are not adaptable for efficient high-throughput screening.



The Wolkowicz lab at SDSU has developed a T-cell-based assay to monitor the proteolytic activity of the HIV-1 protease. The assay is based on an inducible Gal4 HIV-1 protease fusion which binds to upstream activation sequences and activates a reporter gene only in the presence of HIV protease inhibitor. The assay was developed through retroviral technology in T-cells to mimic the natural environment of HIV infection. Every element of the assay has been constructed in retroviral vectors for their stable expression in mammalian cells. It is based on selected clones which, when activated, express eGFP as a biosensor of HIV protease activity.

The assay has a robust and reliable readout that relies on green fluorescence, making it ideal for high-throughput screening. It will greatly facilitate the search for novel peptide- and chemical-compound-based protease inhibitors in T-cells.

Benefits

- Optimized sensitivity, readout appears only in presence of inhibition of HIV-1 protease
- Avoids the cyto-toxic effect of HIV-1 protease to the cell line through an inducible fusion protein system
- High-throughput screening for viable protease inhibitors
- Highly stable mammalian cell line; easy to propagate
- Utilizes a T-cell line that mimics the natural environment of HIV-1
- Turnkey assay; optimized and ready for commercial sales
- Provisional Patent pending

Applications

- Screen for novel and existing HIV-1 therapeutics
- Drug development

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For more information, please contact:

Michael Rondelli, Director

SDSURF - Technology Transfer Office

mroundelli@foundation.sdsu.edu, 619-594-3336



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