

## Action Proposal: Developing an Inquiry Based Research Lab modeling actual research performed on HIV-1 Protease with Double and Triple Mutants

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- Abstract

Emerging Pathogens such as MRSA, H1N1, Dengue Fever, and HIV are real concerns in South Florida. Researchers have worked on HIV for years and are still finding it difficult to develop a cure. One of the most promising strategies was to block protease active site. Protease cuts the polypeptide chain forming the active virus. If the active site is blocked, the enzyme is unable to cut the polypeptide. RNA viruses mutate more frequently and HIV Protease averages one mutation for each division. Our goal was to sequence the double and triple HIV Protease mutants, get them to produce proteins, measure the enzyme activity and eventually fold the protein so that x ray diffraction can be used to construct a model. My proposal is to use this research model to challenge honors biology and marine science students with the challenge of inserting a plasmid, expressing it, and harvesting of protein.

- Rational

Real world problems such as HIV mutation and the drug resistance related to those mutations need scientists to design solutions. HIV is one of many Emerging Pathogens needing a prepared workforce. Students can be challenged with potential carriers

- Description of teaching unit or module(s), including expected outcomes

9<sup>th</sup> and 10<sup>th</sup> grade biology students

Following a unit on genetics, students will be introduced to biotechnology and will have the opportunity to conduct an experiment using Green Fluorescent Protein. They will transform E. coli cells by inserting plasmid DNA into the cell by heat shock, incubate to begin expression, lyse cell, and purify by protein chromatography.

Students will be introduced to emerging pathogens and the diseases they cause followed by Mission Biotech

- Data collection techniques and / or student assessments

Pre/Post Test, Virtual Lab Notebook, Actual Lab Notebook

- ICORE summer instate elements specifically included (UF connections)

Mission Biotech

HIV-1 Protease Lab – Ben Dunn PhD

BioRad pGLO Lab with protein purification

- Literature cited

Mission Biotech

AIDS The Biological Basis 4<sup>th</sup> Edition, Weeks, Benjamin S, Alcamo, I. Edward

- Budget and budget justification  
Request \$200 for BioRad pGLO with protein purification

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#### Outline

#### Background information

Introduce to HIV-1 protease mutation

Structure and function of HIV protease

Research into causes of drug resistance

HIV – a RNA virus

Reverse transcriptase; RNA → DNA → RNA → Amino acids → Polypeptides → Protein

#### Mutations

Results of wrong amino acids on:

- proteins ability to fold
- inhibitors ability to block active site

Protease is an enzyme which cuts polypeptides which then are folded into viral parts. i.e. capsids, ...

Inhibition of Protease

#### Model lab

pGLO Lab with protein gel, column separation

HIV protease insert from Dr. Dunn's lab

- Plasmid prep
- Double digest with BAMM HI, Nde I

Transformatin

- Insert into DH5α cell line
- Express with BL21(DE3) plysS

[www.statehealthfacts.org/comparetable.jsp](http://www.statehealthfacts.org/comparetable.jsp)

\$3,000,000,000 spent on HIV USA