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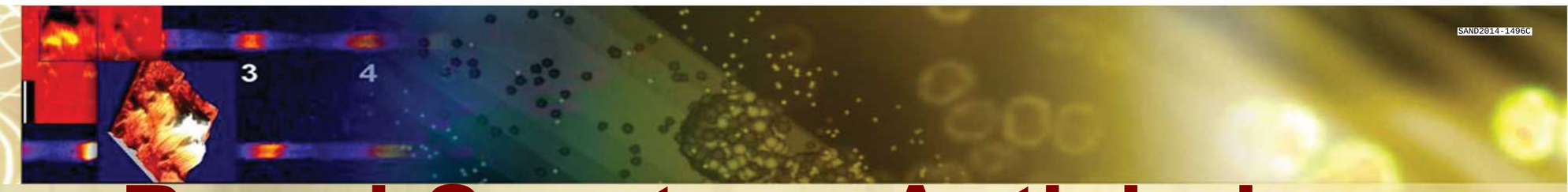

SAND2014-1496C

Designing Broad-Spectrum Antivirals

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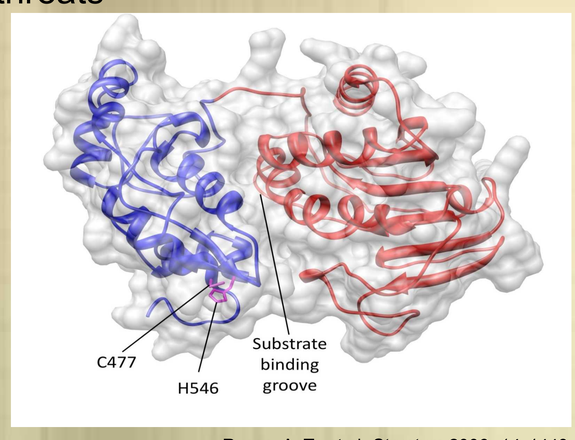
New-World Alpha Viruses

- Biodefense, agriculture and public health threats
- No current therapies
- RNA viruses with considerable sequence diversity
- **GOAL**: Identify lead compounds toward a broad-spectrum inhibitor for:
  - Venezuelan Equine Encephalitis virus (VEEV)
  - Eastern Equine Encephalitis virus (EEEV)
  - Western Equine Encephalitis virus (WEEV)



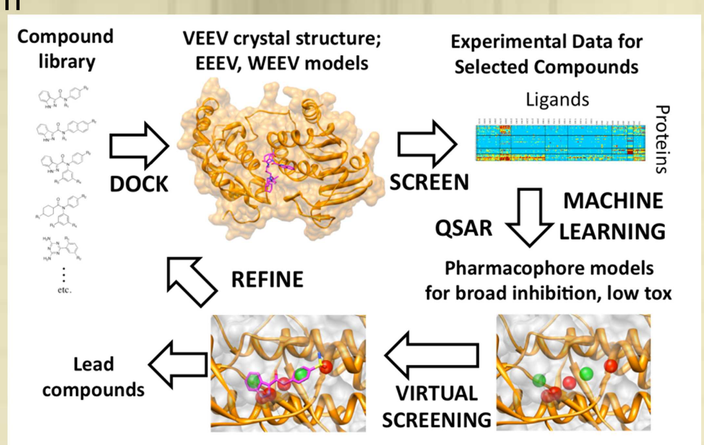
Russo, A. T.; et al. *Structure* **2006**, *14*, 1449.

- **High-resolution crystal structure of VEEV nsP2pro protease enables rational inhibitor design of New-World Alpha viruses via a multipronged experimental and computational approach**

An Integrated Experimental-Computational Approach

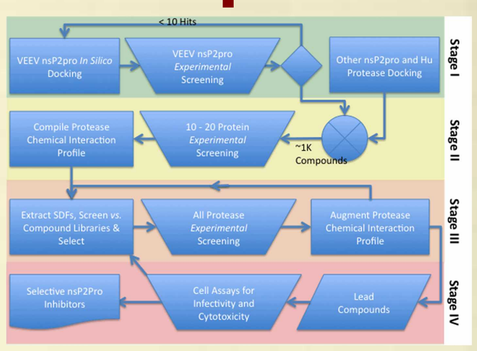
- Start with experimental structures and binding data from target protein variants and off-target host receptors.
- Model all protein-ligand complexes.
- Machine learning extracts predictive ligand-protein interactions

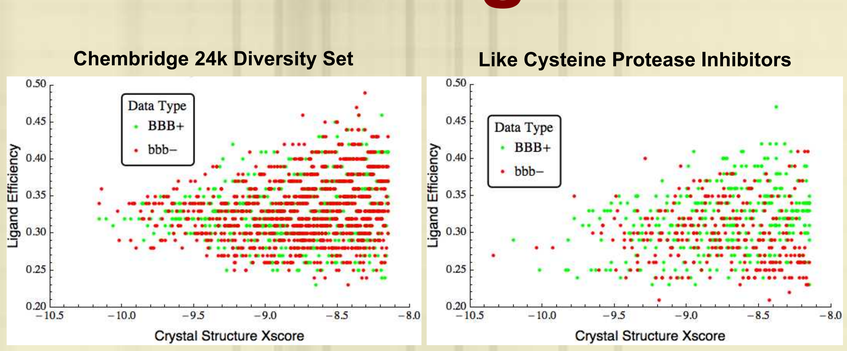
  (↑Affinity & ↑Specificity)!
- Identify ligand features that prevent off-target binding!!!



Anderson, P. C.; et al. *J. Med. Chem.* **2012**, *55*, 1926.

Computational Screening

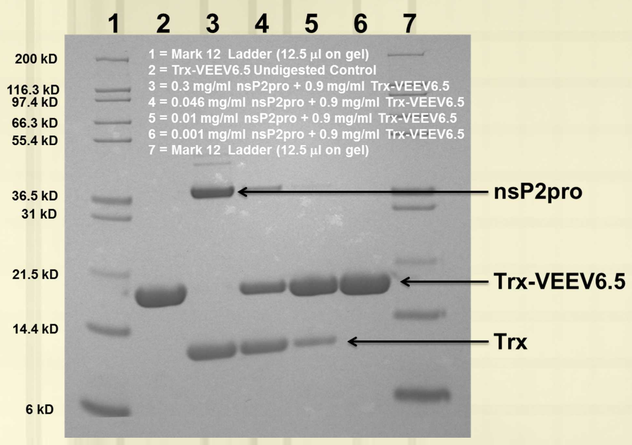




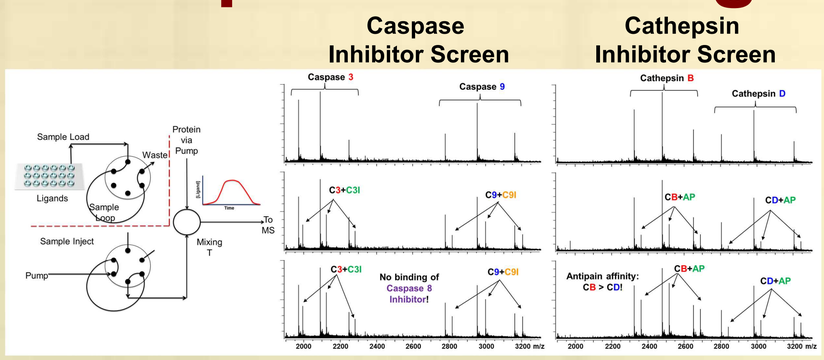
- Staged experiment model identifies initial candidates and aids design of secondary screens.
- *In silico* screening has identified compounds in available libraries that should bind nsP2Pro and possess good blood-brain barrier permeability.

Protease Targets and Off-Targets

- nsP2Pro is a cysteine protease.
- Many human cysteine proteases (caspace and cathepsin families) are essential for cellular function and are sometimes involved in viral infection.
- Inhibition of the host proteins results in false positives in cell-based assays.
- We have expressed nsP2pro and purchased a panel of relevant off-target proteases.



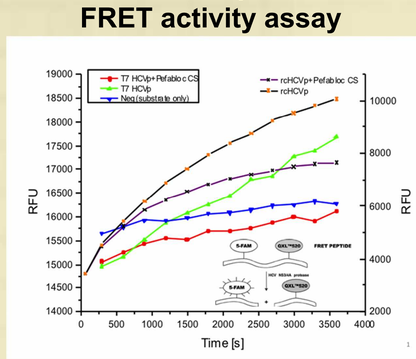
High-Throughput Mass Spec Screening



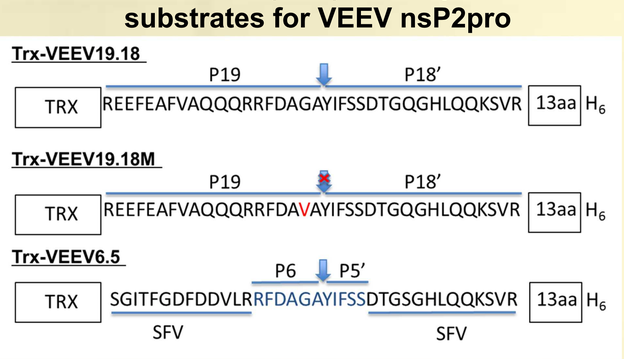
- Microfluidics and mass spectrometry (MS) provide ideal method for rapid measuring of dissociation constants and stoichiometry of ligand-protein complexes.

High-Throughput Activity Screening

HCV NS3/4A protease FRET activity assay



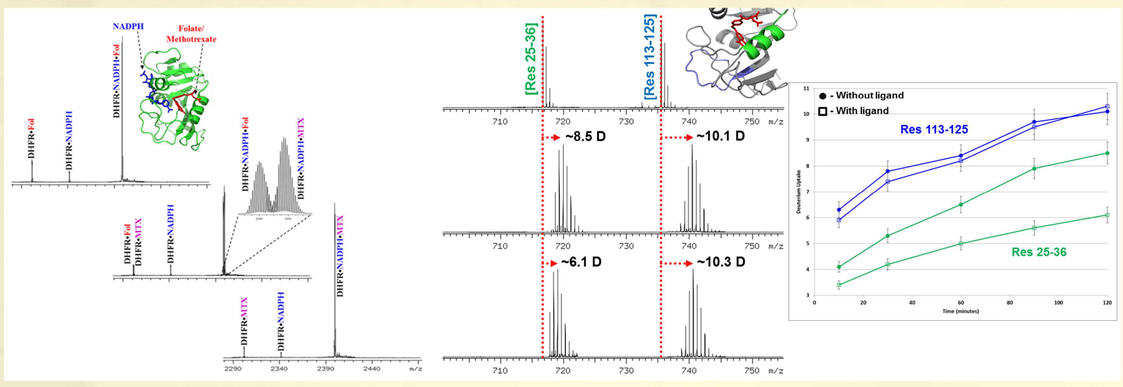
Thioredoxin (TRX) fusion protein substrates for VEEV nsP2pro



Zhang, D. et al. *Protein Expres. Purif.* **2009**, 64, 89

- Fluorescence dequenching assays for viral protease activity screens in microwell-plate formats enable robotic screening at university centers.

Structural Characterization



- Mapping ligand binding sites through competitive inhibition (Left) and hydrogen-deuterium exchange (HDX) with MS (Right).

Current Status

- Expressed active nsP2Pro.
- Demonstrated conditions to allow MS- based activity and affinity screening.
- Currently selecting ligands for purchase for first-round screening.
- Development of high-throughput assay is underway.

Acknowledgements

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000. This work was funded by the Defense Threat Reduction Agency Basic and Supporting Sciences Division of Chem-Bio Technologies Directorate (B114458I).

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