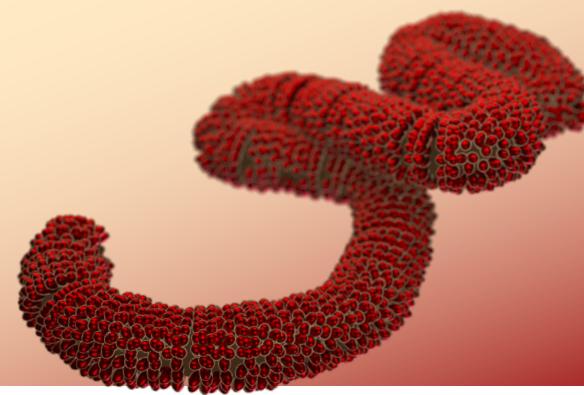


Using Ligand Express™ to understand an anti-viral's mechanism-of-action

Identifying the mechanism-of-action for the anti-depressant sertraline (Zoloft®) in attenuating Ebola virulence



PROBLEM

Phenotypic screens rapidly identify drug candidates, but provides insufficient data on the underlying mechanism-of-action to support validation studies and drug development

TECHNOLOGY

Ligand Express™ tools:
PROBE_x Proteome Docking

SOLUTION

Sertraline, which exhibited anti-Ebola activity, was examined using PROBE_x proteome-wide screening to uncover the protein targets responsible for its anti-viral activity

INTRODUCTION

Ebola virus disease is a severe, often fatal, illness in humans caused by the pathogen *Ebolavirus*, a genus in the family *Filoviridae*¹. It is highly contagious and presents as a hemorrhagic fever that is fatal in 50% of cases¹. The most recent global outbreak of Ebola began December 2013 and ended two and half years later in June 2016. The outbreak, which spread from West Africa and into the developing world, highlighted the need for an approved treatment for the deadly virus. Several laboratories, including the U.S. Army Medical Research Institute of Infectious Diseases, investigated small molecule, FDA-approved drugs for their ability to attenuate Ebola virulence to quickly bridge this therapeutic gap. A preliminary *in vitro* fluorescence-based screening assay, using engineered Ebola virus expressing green fluorescent protein (GFP), rapidly identified potential anti-viral agents. An Ebola virus-like particle (VLP) host-entry assay was used in conjunction with the GFP experiments. Candidates capable of impeding viral protein expression were identified in the GFP imaging assay, including the anti-depressant sertraline (Zoloft®), a selective serotonin reuptake inhibitor (Figure 1)². Sertraline also prevented VLP entry in a follow-up assay². While results were promising, sertraline's underlying target(s)-of-action could not be elucidated solely from these preliminary phenotypic experiments nor from deep learning approaches.

To understand the mechanism underlying sertraline's activity, knowledge of a virus' life cycle is required. Many viruses, including filoviruses like Ebola, first gain entry into a cell and then release genetic material for replication. Once internalized, the virus 'hijacks' the biological machinery of their host to drive the assembly of new viruses. Given a virus' dependence on both viral and host proteins, therapeutics that target either can serve as anti-virals. Cyclica's PROBE_x

proteome-wide screening technology is uniquely suited to predict critical viral and human proteins likely to interact with a small molecule and halt Ebola progression. Furthermore, PROBE_x expands the search of protein space by discovering novel binding sites, including undocumented allosteric sites and protein-protein interfaces. In early 2015, PROBE_x identified sertraline's plausible targets-of-action that could explain its observed activity in anti-Ebola phenotypic screens.

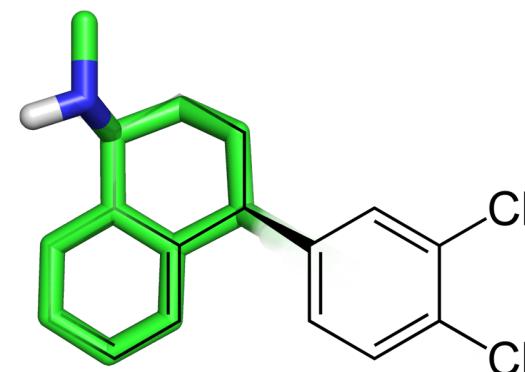


Figure 1. A fused 3D-2D Structure of sertraline.

METHODOLOGY

The Ligand Express™ PROBE_x proteome-wide screening technology examines protein structures from all species, including twenty structurally characterized Ebola-specific proteins. The PROBE_x search space includes proteins assembled in their anticipated oligomeric state, resulting in an expanded collection of interfaces for exploration. PROBE_x also generates binding modes at atomic resolution for any predicted ligand-protein interactions. Putative ligand-protein

interactions were ranked based on their docking scores and then filtered based on the protein's parent species. Ebola protein hits were examined for their functional role in viral infection. Additionally, human proteins were cross-referenced against virus-host protein interaction networks³ to rapidly identify proteins with established roles in virology.

RESULTS

PROBE_x screening identified a glycoprotein (GP) trimer as the top Ebola-specific protein hit likely to interact with sertraline, which ranked in the 96th percentile of all proteins screened. Interestingly, sertraline bound a completely novel protein-protein interface, present only in the trimeric GP interface (**Figure 2**). Given GP's critical role in cellular uptake, this result suggests that sertraline is disrupting viral entry via its interaction with the GP trimer, in agreement with *in vitro* VLP entry studies².

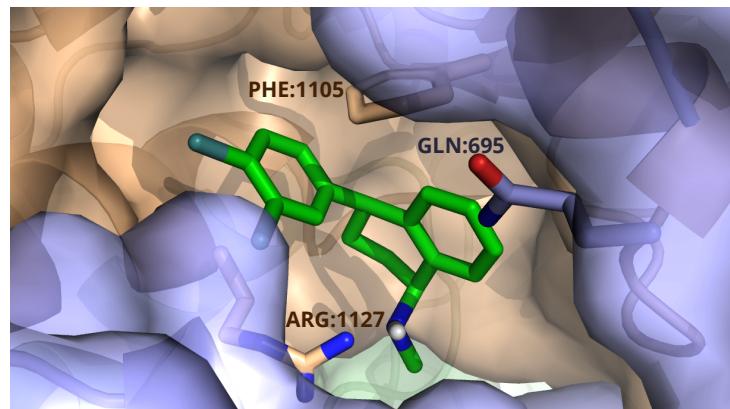


Figure 2. Binding mode of sertraline in the novel glycoprotein-trimer interface. Each monomer is surfaced and coloured independently (blue, cream, and cyan). Three residues in close contact with sertraline are represented by sticks (two residues from the cream monomer and one residue from the blue monomer).

Several human proteins identified by PROBE_x showed strong connectivity with processes linked to viral infection, including the protein chaperone Hsp90, which ranked in the 99.8th percentile (**Figure 3**). Hsp90 is a host-protein that assists virus replication by stabilizing viral proteins within the host cell. Inhibition of Hsp90 has been previously shown to result in anti-viral activity for a range of viral models⁴. A 2016 study reported that sertraline inhibited Hsp-related complexes *in vitro*, supporting the PROBE_x prediction⁵.

SUMMARY

The Ligand Express™ PROBE_x proteome wide-screening technology provides a short-list of testable ligand-protein interactions that are likely driving observed biological activity of a given small molecule modulator. In drug repurposing efforts, Cyclica's proteome-wide screening and associated tools can provide key insights into the mechanistic details necessary to facilitate drug development. Screening sertraline using this method yields two leading hypotheses for its

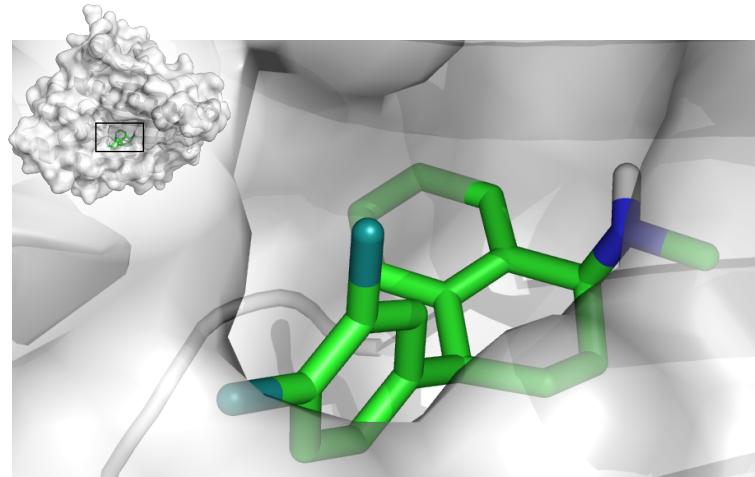


Figure 3. The novel interaction between sertraline (green) and Hsp90 (grey) as identified by PROBE_x proteome-wide screening.

anti-infective activity against Ebola which are independently supported by the scientific literature. These ligand-protein interactions represent radically different targets (i.e. viral and human proteins) and mechanisms by which sertraline may impede Ebola infection. Thus, sertraline may have synergistic, dual mechanisms-of-action that results in enhanced anti-infective activity *in vivo* compared to agents capable of singular disruption through either mechanism. Cyclica's Ligand Express™ accelerates key elements of discovery by directing researchers towards robust experiments supported by *in silico* predictions and informed by systems biology analyses.

RESOURCES

1. "Ebola virus disease," last modified January, 2016, <http://www.who.int/mediacentre/factsheets/fs103/en/>
2. Johansen, L. M. et al. A screen of approved drugs and molecular probes identifies therapeutics with anti – Ebola virus activity. *Sci. Transl. Med.* **7**, 1–14 (2015).
3. Navratil, V. et al. VirHostNet: A knowledge base for the management and the analysis of proteome-wide virus-host interaction networks. *Nucleic Acids Res.* **37**, 661–668 (2009).
4. Geller, R., Taguwa, S. & Frydman, J. Broad action of Hsp90 as a host chaperone required for viral replication. *Biochim. Biophys. Acta – Mol. Cell Res.* **1823**, 698–706 (2012).
5. Dias, J. M. et al. A shared structural solution for neutralizing ebolaviruses. *Nat. Struct. Mol. Biol.* **18**, 1424–1427 (2011).

In support of ongoing research at:



CYCLICA INC.

18 King St East, Suite 801
Toronto, Ontario, M5H 1A1, Canada
1-416-304-9201