Abstract

Influenza virus A circulates in birds and mammals and causes severe infectious disease that affects from 3 to 5 million people each year. There are two classes of anti-influenza drugs currently available: neuraminidase and M2 channel inhibitors. However, increasing resistance against these two types of inhibitors along with the potential emergence of new viral strains and unpredictability of pandemic outbreaks emphasize an unmet need for new types of inhibitors.

RNA-dependent influenza polymerase serves as a novel promising target for the development of anti-influenza medications. The aim of this master thesis is to develop in vitro high-throughput assays for screening of compounds targeting influenza RNA polymerase, particularly, its cap binding and endonuclease domains. For cap-binding domain the screening is based on DIANA (DNA-linked Inhibitor ANtibody Assay) method that was recently developed in our laboratory; for endonuclease domain, the method is based on AlphaScreen technology.

For the purposes of the methods development, recombinant cap binding domain of PB2 subunit and N-terminal endonuclease domain of PA subunit of influenza polymerase were expressed with appropriate fusion tags and purified using affinity and gel permeation chromatography. The probes for the screening assays were designed based on already known inhibitors; their binding properties were tested. Both methods were evaluated, and the first set of AlphaScreen-based screening was performed.

The methods developed would allow rapid quantitative *in vitro* screening for inhibitors of two activities within influenza RNA polymerase that are considered potential therapeutic targets, and, therefore, may bring advances to development of new anti-influenza medications.