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Development and optimization of the assay for screening the compounds inhibiting endonuclease activity and disrupting cap-binding of influenza A polymerase

Content

Influenza virus A circulates in birds and mammals and causes severe infectious disease with potential fatal outcomes. Virus circulates worldwide and triggers annual epidemics that affect from 3 to 5 million people each year (WHO, 2014). There are two classes of anti-influenza drugs available: neuraminidase and M2 channel inhibitors. Increasing of resistance against these two types of inhibitors along with potential emergence of new viral strains emphasize an unmet need for new inhibitors.

Therefore, we aim to develop a high-throughput assay for screening of compounds targeting Influenza RNA polymerase, particularly, its cap binding and endonuclease domains. The screening methods are planned to be based on AlphaScreen technology and recently published DIANA assay (Navrátil et al., 2016).

In our laboratory, we have expressed and purified recombinant cap binding domain of PB2 subunit with C-terminal His-tag and endonuclease domain of PA subunit with N-terminal GST fusion, both from pandemic isolate A/California/07/2009 H1N1. For AlphaScreen assay we designed a biotinylated probe based on published nanomolar endonuclease inhibitor. Binding properties of several probes with different types of linker connecting inhibitor and biotin molecules were tested by surface plasmon resonance. To find an optimal screening condition, we tested several conditions with different probe/protein ratio in presence of Mn2+, Mg2+ and reducing agent. We also designed a PB2-cap binding inhibitor probe for DIANA assay which is currently in preparation.

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