HKU Breakthrough in Influenza Research: New Target New Drugs

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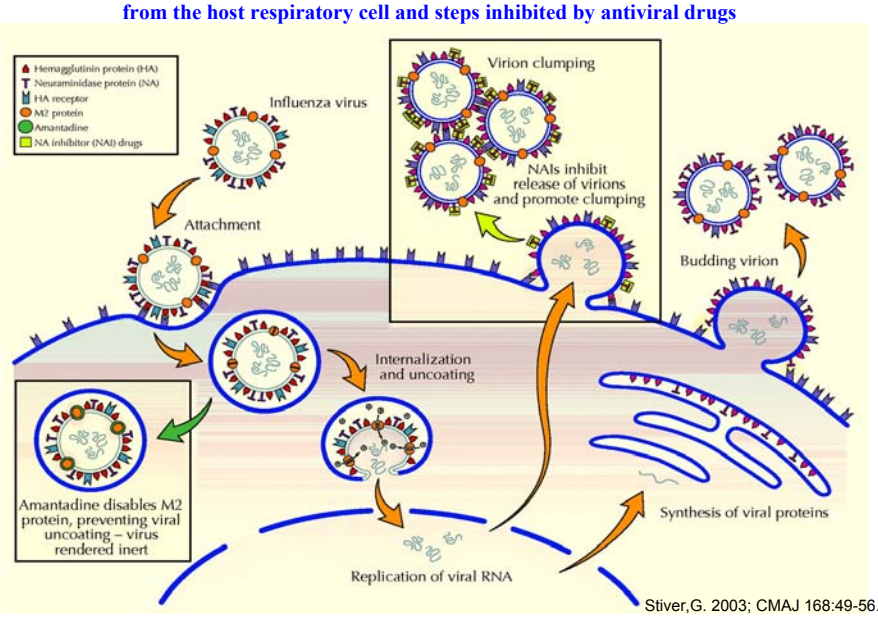
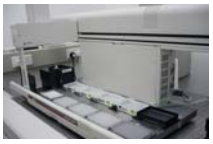
Schematic representation of influenza virus attachment, internalization, replication and exit from the host respiratory cell and steps inhibited by antiviral drugs



Stiver,G. 2003; CMAJ 168:49-56.

Automated High-throughput Screening platform from Chemical Genetics Unit, Research Center of Infection and Immunity, LKS Faculty of Medicine, The University of Hong Kong

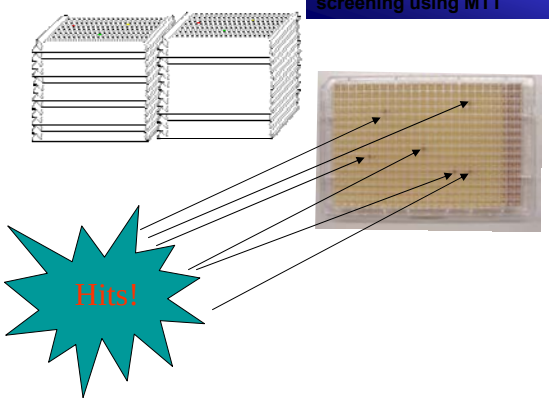
- Fully automated robotics screening platform
- Optimized for cell-based screening assays
- The only 384-well formatted chemical genetics screening facility in Hong Kong



Liquid handling system

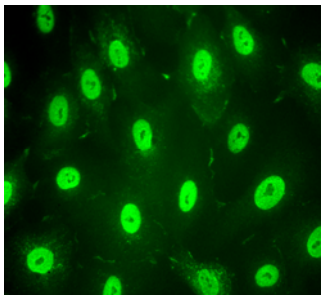
automated plate reader

incubator with carousel

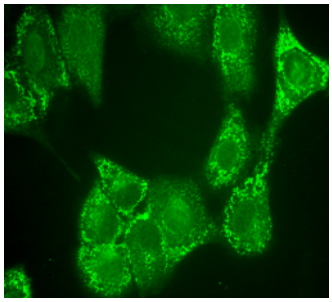


Influenza A nucleoprotein is a very important protein for the virus

Early: 6 hours post infection - RNP predominately nuclear

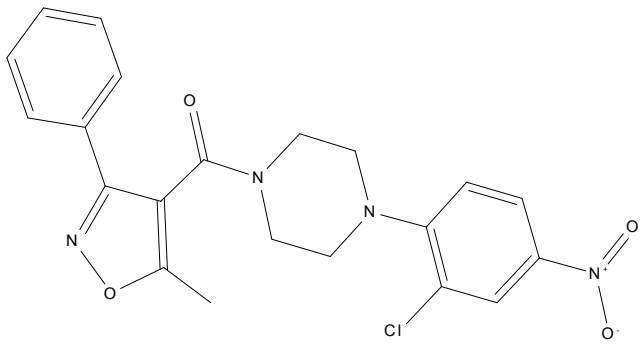


Late: 24 hours post infection - RNP exclusively cytosolic



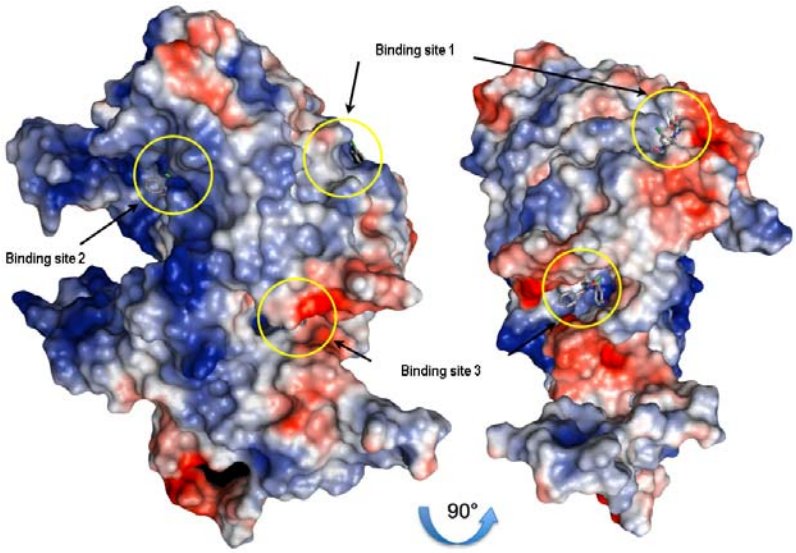
It has been well documented that the influenza A nucleoprotein (NP) accumulates in the nucleus of the infected cells during early infection for viral replication and assembling ribonucleoprotein particles (RNPs), but becomes exclusively cytosolic during late infection for viral maturation and assembly of infectious viral particles. NP thus is a very important protein for the virus.

Discovery of a potent anti-influenza drug: Nucleozin

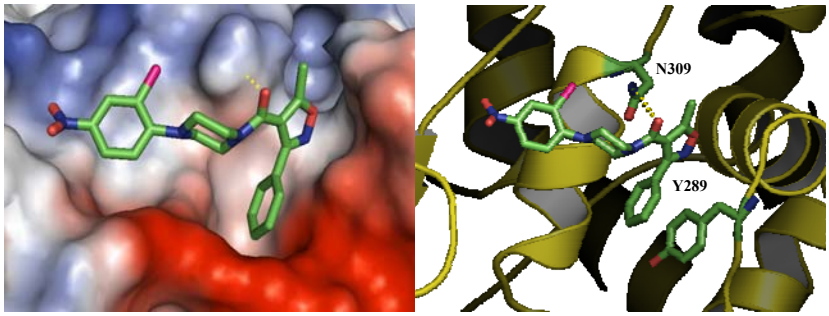


[4-(2-Chloro-4-nitro-phenyl)-piperazin-1-yl]-(5-methyl-3-phenyl-isoxazol-4-yl)-methanone

Predicted binding sites of nucleozin in NP

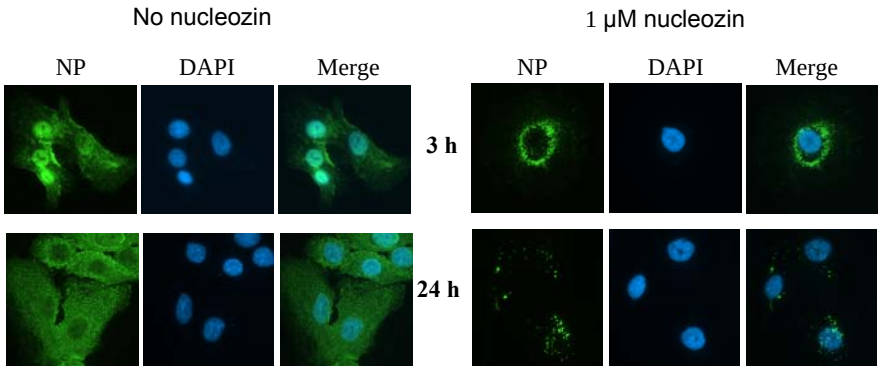


Predicted binding conformation of nucleozin to binding site 1 in NP at higher magnification



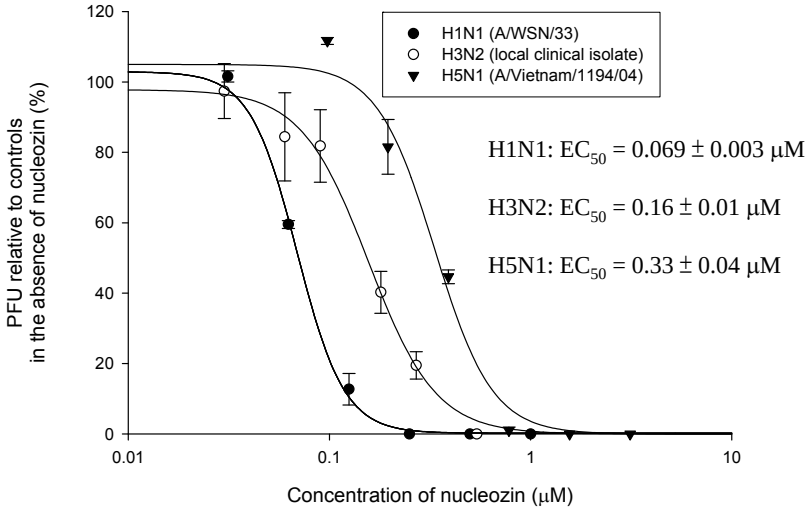
In this conformation nucleozin is interacting with residue N309 of influenza A nucleoprotein by hydrogen bond (yellow dash line) and Y289 by hydrophobic interaction, where the phenyl ring of nucleozin is paralleling with phenyl ring of Y289 and the distances between these two rings are between 3.2~4.3Å. (Sticks- green:carbon, blue:nitrogen, red:oxygen, pink:chlorine)

Human alveolar basal epithelial (A549) cells infected with influenza A/WSN/33 H1N1



After adding nucleozin, the viral neucleoprotein (NP) aggregates and forms a "halo" around the perinuclear region, preventing the viral neucleoprotein from entering the cell neucleus.

Nucleozin is active against H1N1, H3N2, and H5N1 viruses



nature biotechnology

Identification of influenza A nucleoprotein as an antiviral target

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Influenza A remains a significant public health challenge because of the emergence of antigenically shifted or highly virulent strains[1–5]. Antiviral resistance to available drugs such as adamantanes or neuraminidase inhibitors has appeared rapidly[6–9], creating a need for new antiviral targets and new drugs for influenza virus infections. Using forward chemical genetics, we have identified influenza A nucleoprotein (NP) as a druggable target and found a small-molecule compound, nucleozin, that triggers the aggregation of NP and inhibits its nuclear accumulation. Nucleozin impeded influenza A virus replication *in vitro* with a nanomolar median effective concentration (EC_{50}) and protected mice challenged with lethal doses of avian influenza A H5N1. Our results demonstrate that viral NP is a valid target for the development of small-molecule therapies.

in the nucleus in the early phases of infection and is exclusively distributed in cytoplasm later during viral assembly and maturation[12–15]. We examined the effects of the 39 compounds on NP nuclear trafficking by fluorescence microscopy and identified 5 compounds that blocked the nuclear accumulation of NP. Compound FA-1 showed the best potency with $EC_{50} < 1$ μM in a plaque reduction assay (PRA) on MDCK cells infected with influenza A/WSN/33 (H1N1) virus. The schematic representation of the procedures and results of the primary, secondary, and subsequent fluorescence microscopy screens are summarized in **Supplementary Figure 1**.

Based on the structural information of compound FA-1, four structurally similar analogs (**Fig. 1a**) obtained from commercial sources were shown to have EC_{50} against influenza A/WSN/33 virus at submicromolar levels in PRA. We selected compound FA-4 (nucleozin) for further characterization based on its better solubility in aqueous solutions (unpublished observations) and potent antiviral activities.

About *Nature Biotechnology* ……

Nature Biotechnology is an international monthly journal of the Nature Publishing Group, covering the science and business of biotechnology. According to the 2008 Journal Citation Report, the impact factor of Nature Biotechnology is as high as 22.297, reflecting that it is one of the most frequently cited and influential research journals in the field.

This is the first time that the research work from HKSAR led by local researchers is published in *Nature Biotechnology*, demonstrating the capability and potential of Hong Kong as a key contributor in biotechnology and drug discovery.

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Granting Agents :

HKU Seed Grant for Basic Research

HKU Seed Funding for Strategic Research Theme

Hong Kong RGC Area of Excellence (AoE/M-12/06)

Research Fund for the Control of Infectious Diseases

Hong Kong Sanatorium Hospital Doctors' Donation Fund

Q&A