Temperature-induced reorganization of influenza A nucleoprotein complex

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FRM II
Forschungs-Neutronenquelle
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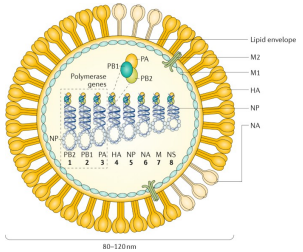


Influenza A virus [5]

Drugs

M2 inhibitors

Neuraminidase inhibitors

Polymerase inhibitors

Vaccination

1918	**Spanish Flu caused by H1N1**
1933	Isolation of influenza virus[36]
1935-1941	Early vaccination studies identified the importance of dose and matching strains[36]
1942-45	Trials with concentrated and inactivated vaccines[36]
1945	First commercial influenza vaccine available in the US[36]
1947	Global surveillance initiated by WHO
1957	**"Asian flu" caused by H2N2**
1960s	Attempts to generate attenuated viruses
1968	**Pandemic caused by H3N2**
1976-1977	Subunit influenza vaccine was developed and found to be less reactogenic than inactivated whole virus vaccines[47-49]
1976-1977	Swine flu (H1N1) outbreak in Fort Dix, US, prompting a short-lived mass vaccination campaign
1977-1978	**"Russian flu" outbreak (H1N1)**
1980s	Russians developed cold-adapted attenuated vaccine strains[64,65]
1997	**Outbreak of highly pathogenic H5N1 in Hong Kong**
1990s	Reverse-genetics system developed, leading to attenuated H5 vaccine strains[78,79]
2003	FluMist, intranasal LAIV licensed by FDA for adults*
2003-2004	**Outbreak of highly pathogenic H5N1 in Asia**
2007	H5 vaccine from Sanofi-Pastuer approved by FDA*
2007	Optaflu, MDCK-cell derived vaccine approved for use in Europe^
2009	**Swine Flu pandemic (swine origin H1N1)**
2009	FluZone High Dose licensed and recommeded by ACIP for elderly#
2009	Adjuvanted vaccines against 2009 swine flu strain approved under exceptional circumstance for use in Europe^
2010	ACIP recommends National Influenza vaccination for all ages 6 months and older#
2011	FluZone Intradermal licensed by FDA*
2012	Vepacel, Vero-cell derived influenza vaccine by GSK, licensed in Europe^, Flucelvax, MDCK-cell derived vaccine, Fluarix (quadrivalent TIV) and FluMist Quadrivalent, approved by FDA*
2013	FluBlok (baculovirus-derived) approved by FDA*

From [3]

Two types of influenza vaccine are widely available: inactivated influenza vaccines (IIV) and live attenuated influenza vaccines (LAIV) + subunit vaccines [1]

Gene shift => drug resistance, escape from immunity

"The influenza polymerase has no proofreading activity, resulting in a high gene mutation rate of approximately one error per replicated genome, so each cell can produce 10,000 new viral mutants to infect neighboring cells" [2]

Development of cold-adapted vaccines [4]

1. https://www.euro.who.int/en/health-topics/communicable-diseases/influenza/vaccination/types-of-seasonal-influenza-vaccine

2. Boivin S, Cusack S, Ruigrok RW, Hart DJ. Influenza A virus polymerase: structural insights into replication and host adaptation mechanisms. J Biol Chem. 2010 Sep 10;285(37):28411-7. doi: 10.1074/jbc.R110.117531. Epub 2010 Jun 10. PMID: 20538599; PMCID: PMC2937865.

3. Wong SS, Webby RJ. Traditional and new influenza vaccines. Clin Microbiol Rev. 2013 Jul;26(3):476-92. doi: 10.1128/CMR.00097-12. PMID: 23824369; PMCID: PMC3719499.

4.Polezhaev FI, Garmashova LM, Polyakov YM, Golubev DB, Aleksandrova GI. Conditions for production of thermosensitive attenuated influenza virus recombinants. Acta Virol. 1978 Jul;22(4):263-9. PMID: 29464.

5.Krammer, F., Smith, G.J.D., Fouchier, R.A.M. *et al.* Influenza. *Nat Rev Dis Primers* **4,** 3 (2018). https://doi.org/10.1038/s41572-018-0002-y

Cold-Adapted Vaccines

ATTENUATION

Virus growth in chicken embryos

Cold-Adaptation

Incubation at temperature, lower than optimal. Spontaneus mutations.

Adaptation.

”The influenza polymerase has no proofreading activity”

Temperature sensitive strains selection

Negative selection: Growth @ 37°C

Cold-Adapted strains can grow at conditions suitable for manufacturing

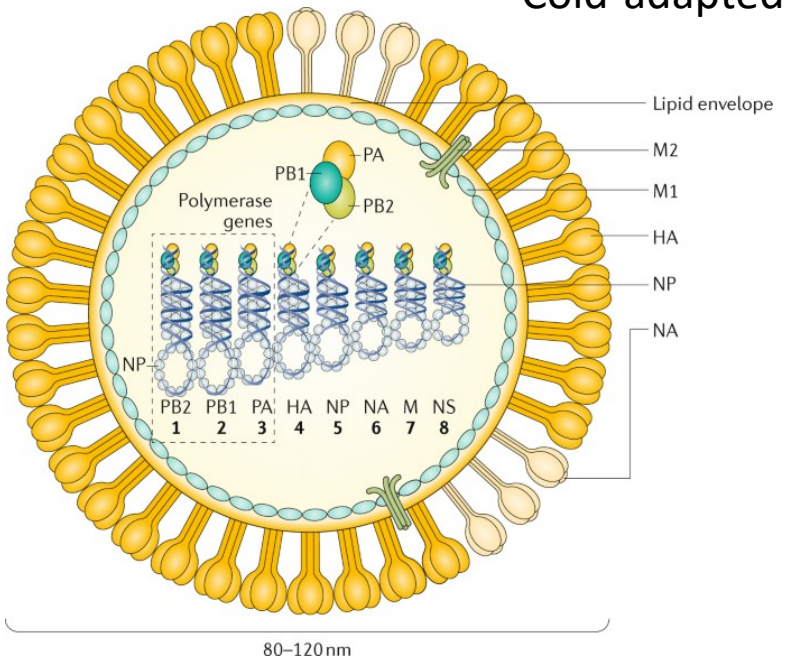
Temperature-sensitive strains cannot replicate at human organism temperature

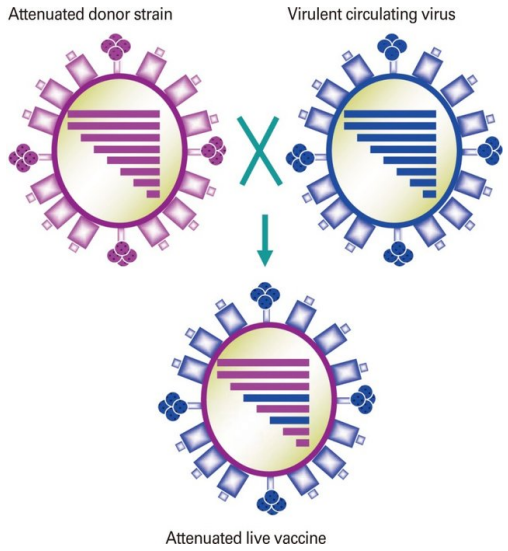
Stability Test

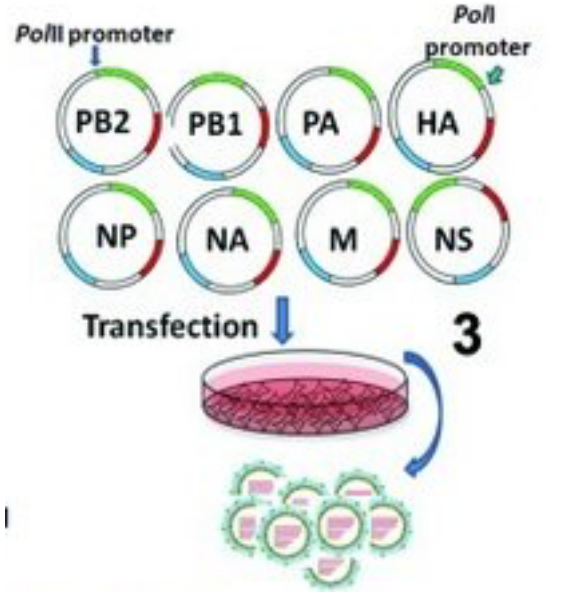
Cold-adapted temperature-sensitive strain

Long Development Cycle

Cold-adapted temperature-sensitive strain as a donor of attenuation [6]







Reverse genetics system [7]

Cold-adapted temperature-sensitive strain

Initial (wild-type) strain

Genome sequencing

List of mutations

Genotype-phenotype analysis

5.Krammer, F., Smith, G.J.D., Fouchier, R.A.M. *et al.* Influenza. *Nat Rev Dis Primers* **4,** 3 (2018). https://doi.org/10.1038/s41572-018-0002-y

6.Jang YH, Seong BL. Principles underlying rational design of live attenuated influenza vaccines. Clin Exp Vaccine Res. 2012 Jul;1(1):35-49. doi: 10.7774/cevr.2012.1.1.35. Epub 2012 Jul 31. PMID: 23596576; PMCID: PMC3623510.

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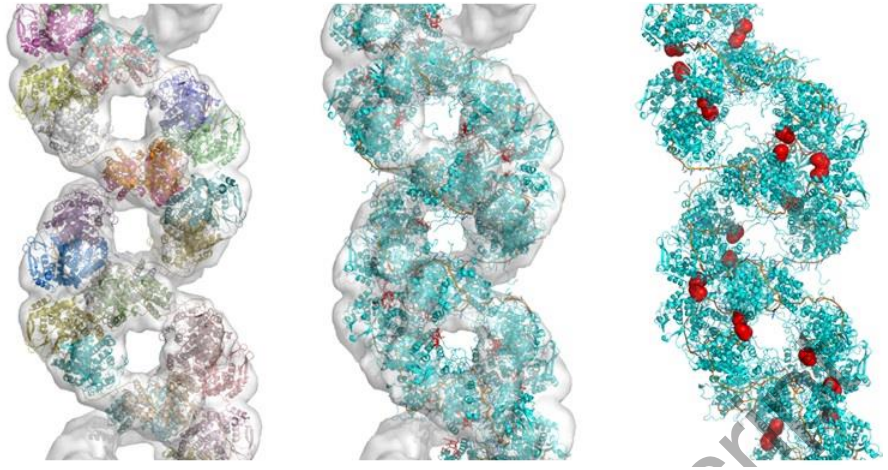
E292G mutation in NP is correlated with cold-adaptivity [8]



MW – around 100*56 kDa + RNA

MD simulations: 24*56 kDa + RNA
around 2 months @ SPbPU

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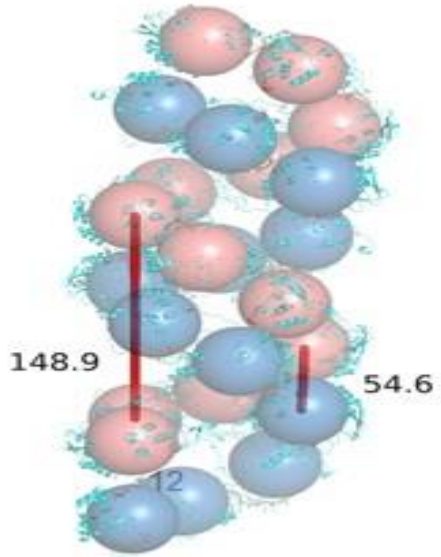
WT NP 299K 312K
E292G NP 299K 312K

Cryo-EM based structure. Residues 292 are designated as red balls



8. Pulkina AA, Sergeeva MV, Petrov SV, Fadeev AV, Komissarov AB, Romanovskaya-Romanko EA, Potapchuk MV, Tsybalova LM. [Impact of mutations in nucleoprotein on replication of influenza virus A/Hong Kong/1/68/162/35 reassortants at different temperatures]. Mol Biol (Mosk). 2017 Mar-Apr;51(2):378-383. Russian. doi: 10.7868/S0026898417010141. PMID: 28537245.

9. A.V. Shvetsov, D.V. Lebedev, Y.A. Zabrodskaya, A.A. Shaldzhyan, M.A. Egorova, D.S. Vinogradova, A.L. Konevega, A.N. Gorshkov, E.S. Ramsay, A. Radulescu, M.V. Sergeeva, M.A. Plotnikova, A.B. Komissarov, A.S. Taraskin, K.I. Lebedev, Yu.P. Garmay, V.V. Kuznetsov, V.V. Isaev-Ivanov, A.V. Vasin, L.M. Tsybalova & V.V. Egorov (2020) Cold and distant: structural features of the nucleoprotein complex of a cold-adapted influenza A virus strain, Journal of Biomolecular Structure and Dynamics, DOI: 10.1080/07391102.2020.1776636

Changing distances: E292G @299K had the opened structure like Wild type @312K [9].

NP SANS

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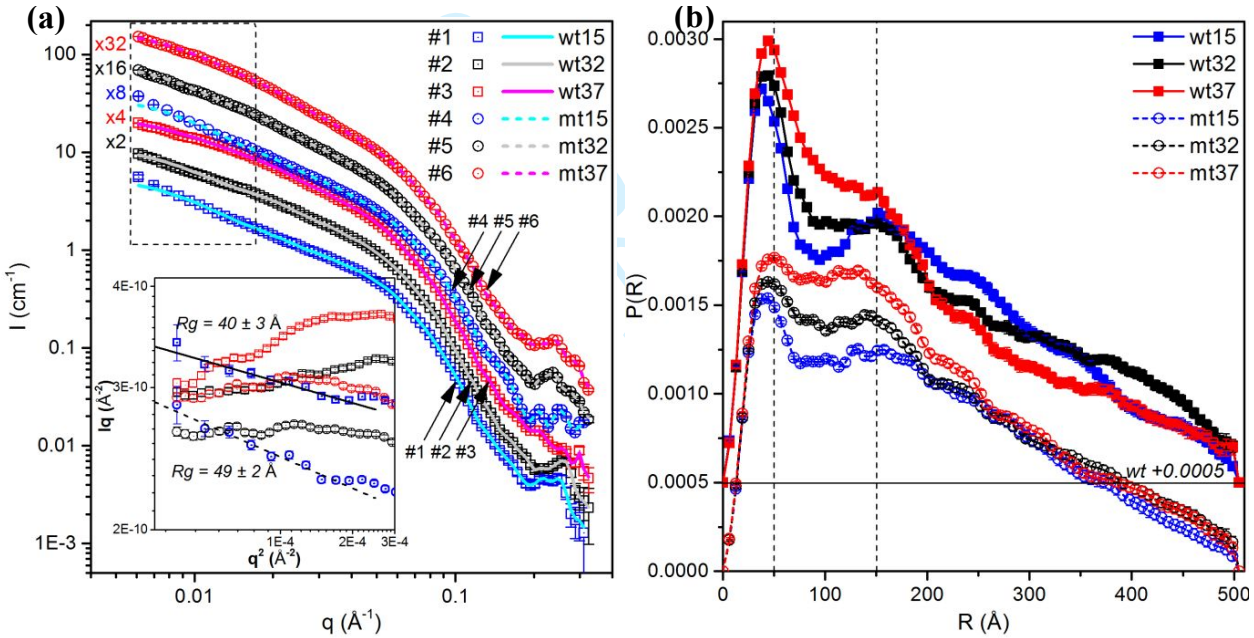
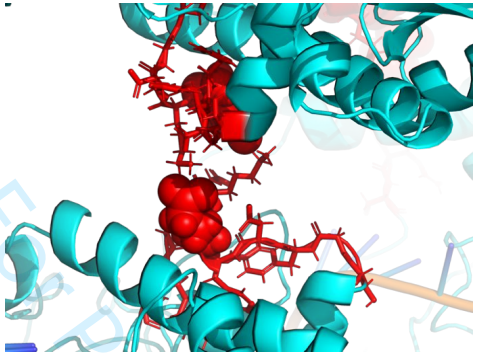


Small angle neutron scattering of wild-type (wt) and E292G mutant (mt) RNP solutions at 15oC, 32oC, and 37oC. (a) Representation in double logarithmic scale *I* vs *q*, where *I* – scattering intensity, *q* – magnitude of the momentum transfer; inset – Guinier coordinates (*Iq* vs *q2*). The data for *I* vs *q* plot were multiplied by 2 (x2), 4 (x4) etc for better representation. (b) Distance distribution function *P(R)*, calculated for SANS spectra of wild- type RNP and E292G mutant; dash lines mark *R* = 50 and 150 Å. The zero of wt P(R) plots were moved up to 0.0005 for better representation [9].

Temperature-dependent NP structure changes depend on interchain interaction interface

In Details

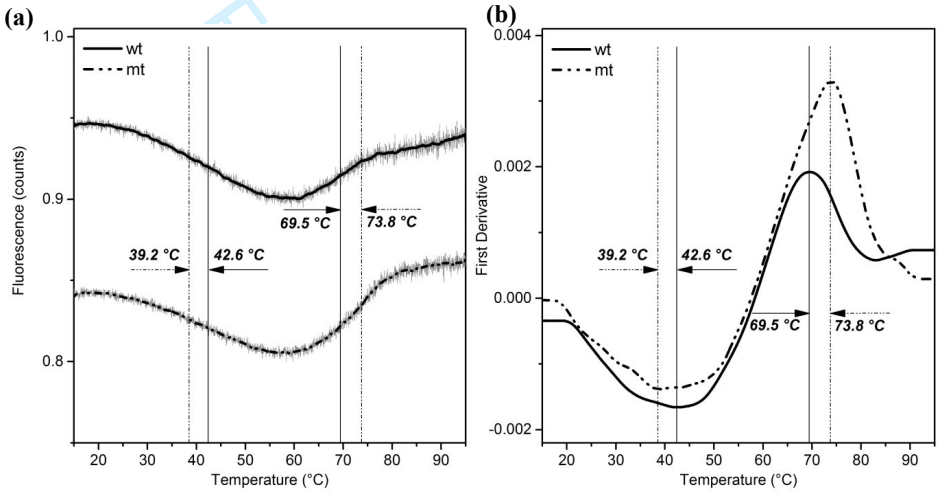
Peptide model



Two residues #292 from different NP helixes

	k_{a1} (1/Ms)	k_{d1} (1/s)	k_{a2} (1/s)	k_{d2} (1/s)	K_D (M)
SGYDF**E**REGYS	71.48	0.0799	0.0027	**1.54E-06**	6.32E-07
SGYDF**G**REGYS	17.09	0.2899	0.0032	**0.0076**	0.0115

Interaction analysis of WT and E292G peptide analogues with WT NP by SPR



Differential scanning fluorimetry (DSF): (a) intrinsic fluorescence (350/330 nm ratio) of tryptophan as a function of temperature in samples containing wild-type (wt) or E292G mutant (mt) RNP; (b) first derivative of (a).

- One amino acid substitution in the influenza A virus NP protein (E292G) can lead to global change in protein conformational mobility
- Such a substitution leads to influenza cold-adaptivity, which is essential for development of cold-adapted strains for live attenuated vaccines
- Conformational mobility can be predicted (!) by molecular dynamics simulation and demonstrated by SANS

Acknowelgements

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Mechanisms of action for the supramolecular drugs: neutron study

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ОИЯИ

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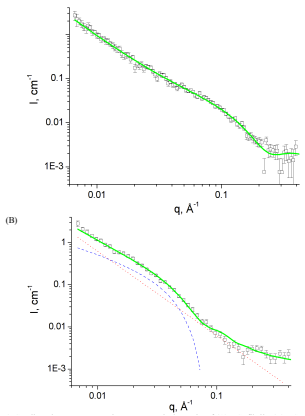
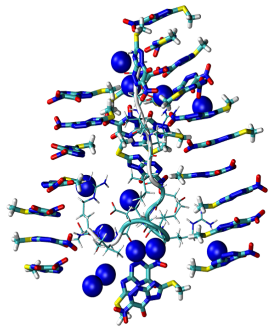
1. Low molecular weight compounds Many low molecular weight compounds and peptides are capable of forming supramolecular complexes. In the form of such complexes, the molecules are capable of multicenter cooperative binding to target proteins. It is advisable to study these complexes using small-angle scattering methods in combination with molecular dynamics modeling in the free diffusion approach.

When studying the mechanism of interaction of a triazavirin drug with polypeptides by neutron small angle scattering methods in combination with molecular dynamics, it was shown that the drug molecules are capable of forming linear supramolecular complexes and altering the quaternary structure of proteins [1]–[3].

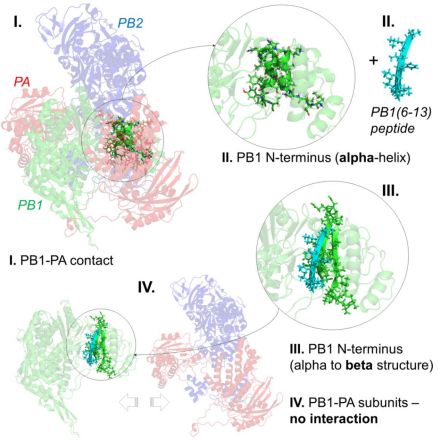


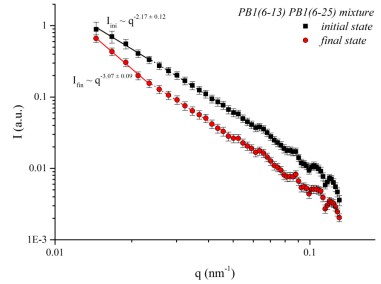
Small-angle neutron scattering curves analysis results of (A) – SI fibrils; (B) – SI fibrils with triazavirine. Data fitted to (A) worm-like model with fibril radius of 1.40 ± 0.04 nm and Kuhn length of 12.0 ± 0.7 nm ($\chi 2 = 0.83$, solid green line in Panel A) and (B) linear combination of random coil model (dotted red line) and long cylinders with the radii of 4.66 ± 0.14 nm (dashed blue line), $\chi 2 = 1.2$, shown in solid green line in Panel B.

Interactions between SI and TZV supramolecular complexes: MD simulation



2. Supramolecular amyloid-like peptide complexes are capable of specific effects on the secondary structure of the protein, which can be used to create a new class of antiviral drugs, as was shown using small-angle neutron scattering and time-resolved x-ray scattering [4]–[6].





The PB1(6-13) and PB1(6-25) peptide mixture system initial (t = 0) and final (t = ∞) states spectra, reconstructed on the basis of a change in the singular decomposition zero and first components fro TR-SAXS SVD analysis

- **Some drugs act only in the form of supramolecular complexes that are in dynamic equilibrium**
- **Existing of such complexes cannot be detected using traditional methods - chromatography or microscopy**
- **Only methods of light scattering, neutron scattering and X-ray scattering can be used in determination of its mechanism of action**

3. The interaction of supramolecular complexes formed in lipid membranes with receptors can be used to modulate cell signaling, including the creation of immunomodulating drugs that affect T cells. The effect of complexes on the chromatin structure can be used to create a new class of drugs - epigenetic regulators that affect gene expression [7].

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[5] Y. A. Zabrodskaya *et al.*, "The amyloidogenicity of the influenza virus PB1-derived peptide sheds light on its antiviral activity," *Biophys. Chem.*, vol. 234, no. January, pp. 16–23, 2018, doi: 10.1016/j.bpc.2018.01.001.

[6] O. V Matusevich *et al.*, "Synthesis and antiviral activity of PB1 component of the influenza A RNA polymerase peptide fragments.," *Antiviral Res.*, vol. 113, pp. 4–10, Jan. 2015, doi: 10.1016/j.antiviral.2014.10.015.

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