

# **New types of potential edible and intranasal vaccines based on engineered polypeptides containing neutralizing epitopes of influenza virus haemagglutinin**

**Department of Virology (Moscow State University) and  
Research Institute of Influenza**

## **PLAPROVA - Plant produced vaccines**

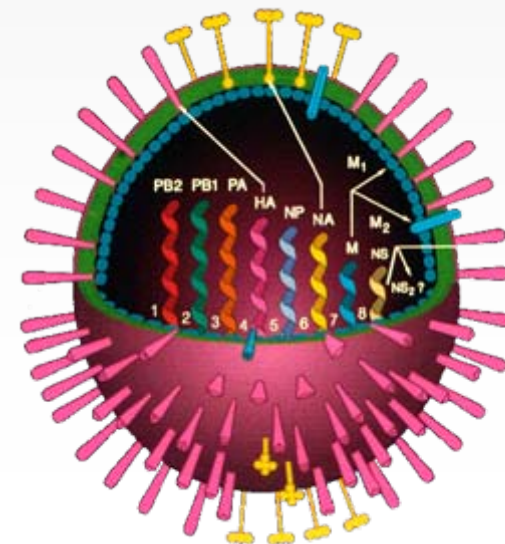
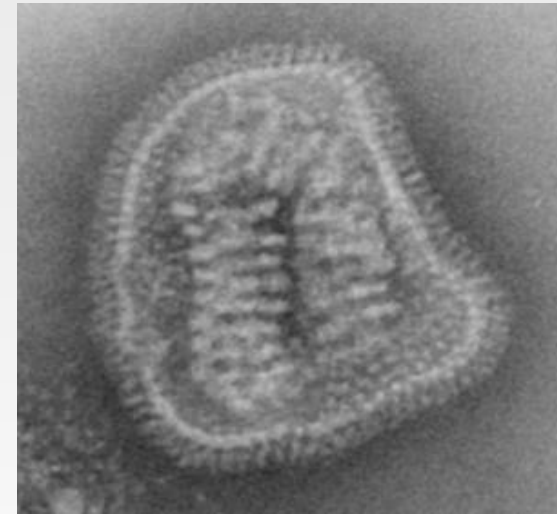
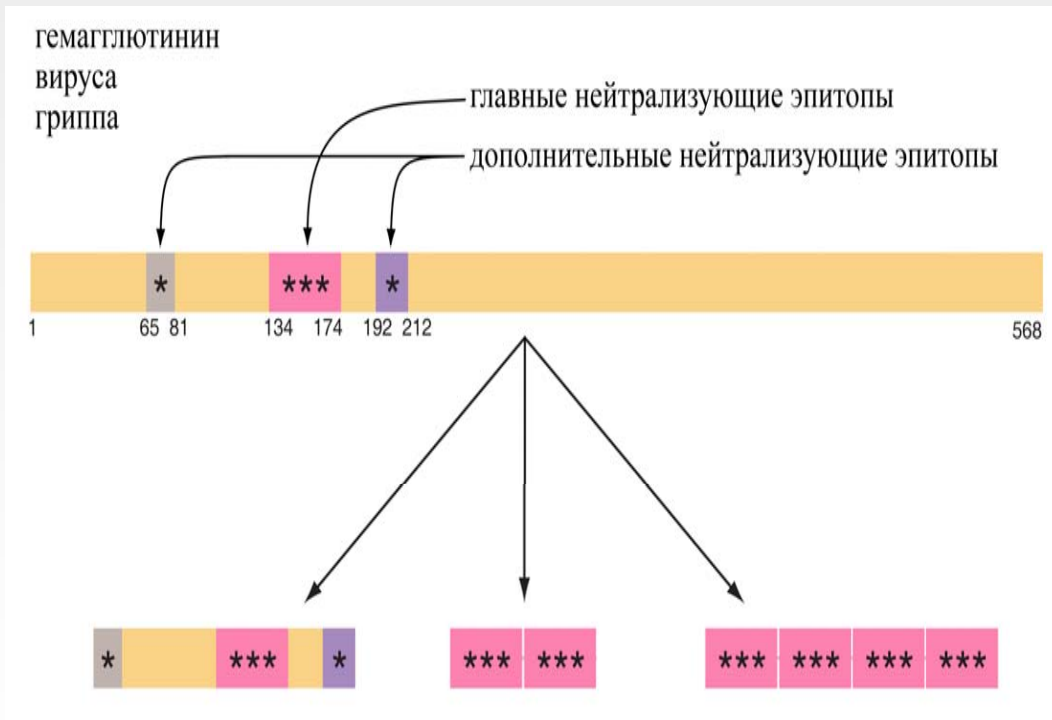
EU-FP7 project [PLAPROVA](#) is a collaborative project between the EU and Russia with participation of South Africa that aims to develop a rapid plant-based system to produce and assess the capacity of different proteins to act as vaccines against important diseases of livestock such as avian influenza and blue tongue.

**The coordinator of the project is Professor George Lomonossoff (John Innes Centre, Norwich, UK).**

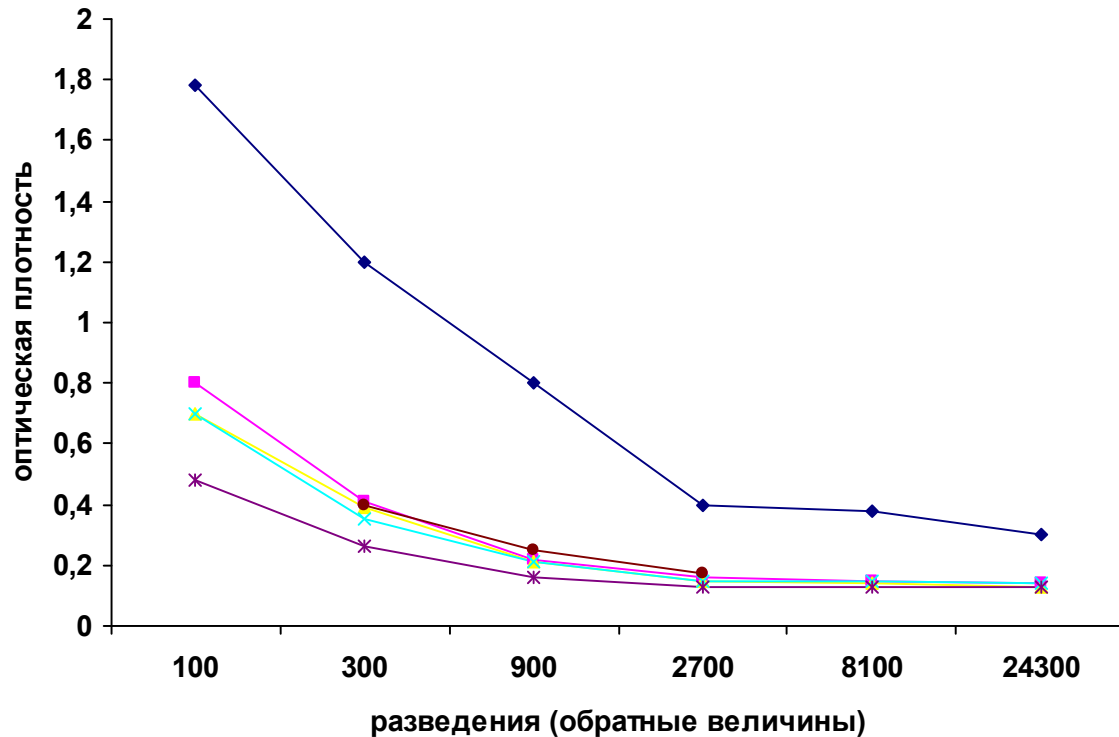
# **Main aims according to project objectives for the first year**

- 1. Selection of HA epitope region(s) producing antibodies capable of interaction with whole influenza virus particles
- 2. Finding a method for most efficient antibody production. Namely, design of scaffold(s) for epitope presentation

# Localization of epitopes used for design of vaccine HA-based polypeptides



# Testing of polyclonal antibodies (ELISA) for interaction with whole purified influenza virus particles (A/Vietnam\1194\2004)(mice immunized intranasally)



◆ AC Flu 1-3

■ AC Flu 2x

▲ AC Flu 4x

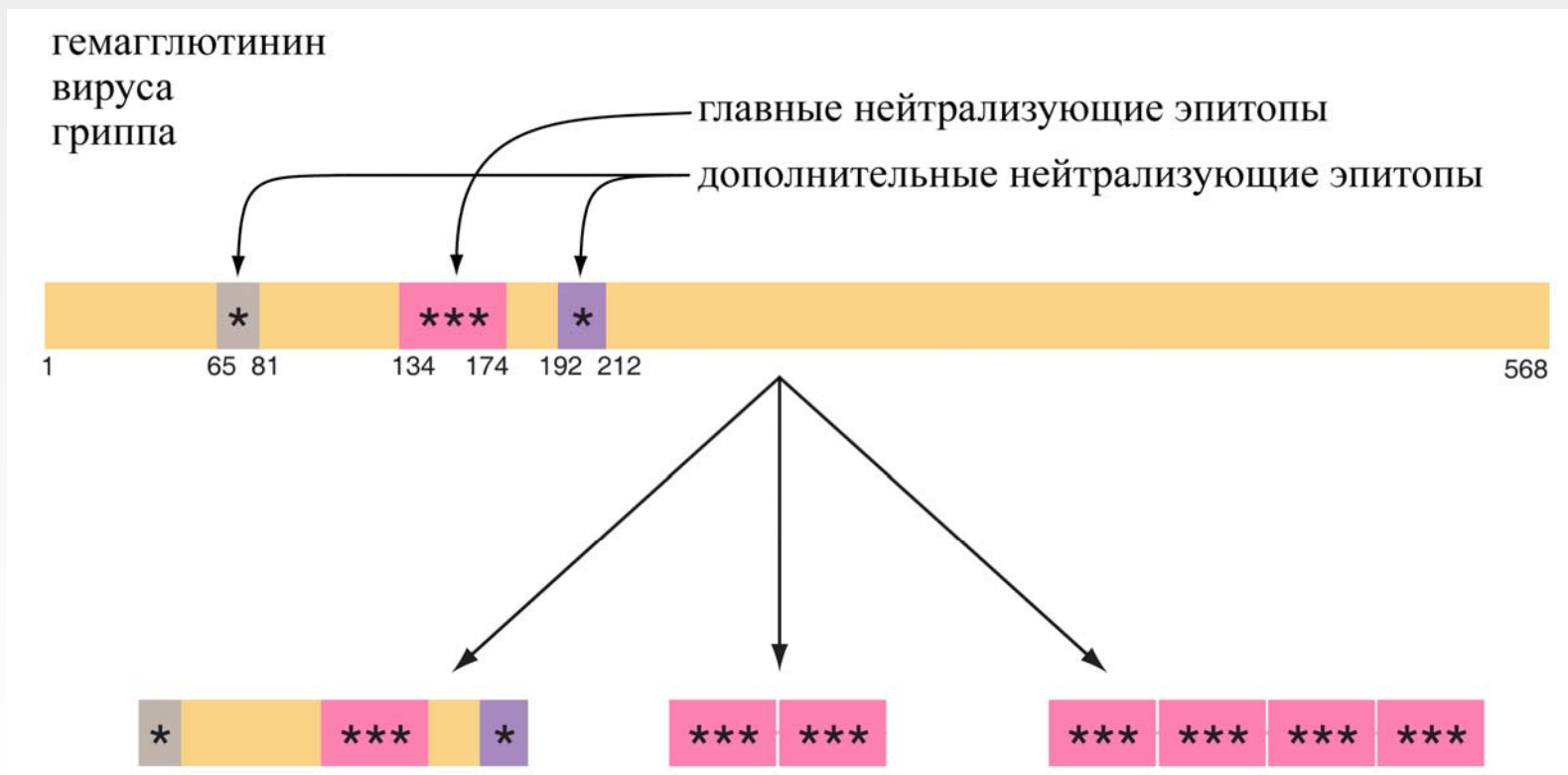
✦ AC M2E

✧ AC M2E-DHFR

● контрольная сыворотка

# Design of vaccine HA-based polypeptides based on HA precursor gene (A/Kurgan/5/05)

(uninterrupted HA fragment possessing all main and minor neutralizing epitopes is most efficient)

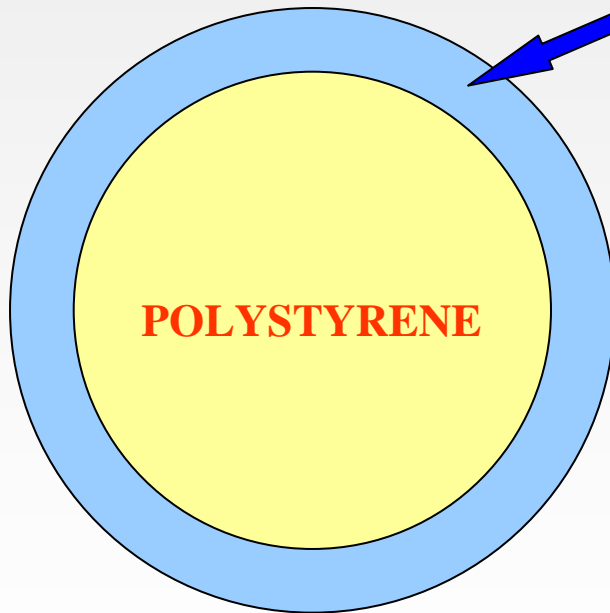


## **Design of scaffold for epitope presentation**

- **Display of peptides or proteins in an ordered, repetitive array, such as on the surface of virions and virus-like particles, is known to induce an enhanced immune response relative to vaccination with the "free" protein antigen.**

# Carboxylated Polystyrene Latex (CPL)

ANIONIC COPOLYMER OF  
STYRENE WITH  
METHACRYLIC ACID



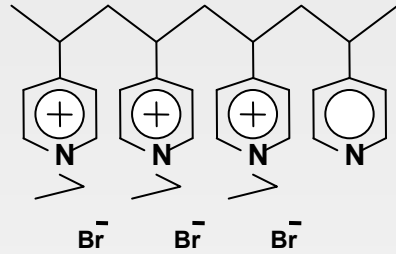
$$D_e = 0.51 \mu\text{m}$$

$$S_{\text{COOH}} = 0.2 \text{ nm}^2$$

$$\text{pK}_0 = 6.8$$

$$\text{EPM}_{\text{pH}=9; I=0.01} = -4.3 (\mu\text{m/s})/(\text{V/cm})$$

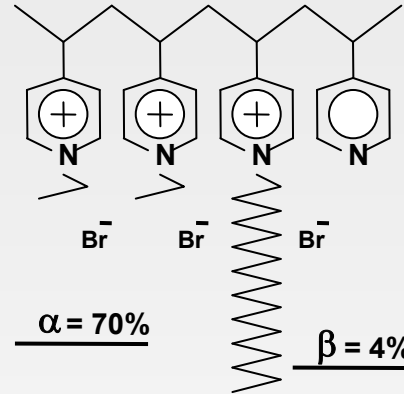
# Cationic Polyelectrolytes



$\alpha = 90-95\%$

QUATERNIZED  
POLY(4-VINYLPYRIDINE),  
CARRYING ETHYL  
PENDANT RADICALS

**P(2)**

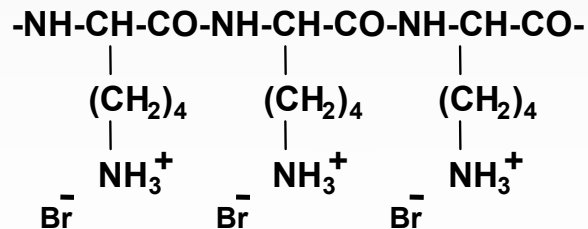


$\alpha = 70\%$

$\beta = 4\%$

QUATERNIZED  
POLY(4-VINYLPYRIDINE),  
CARRYING ETHYL and CETYL  
PENDANT RADICALS

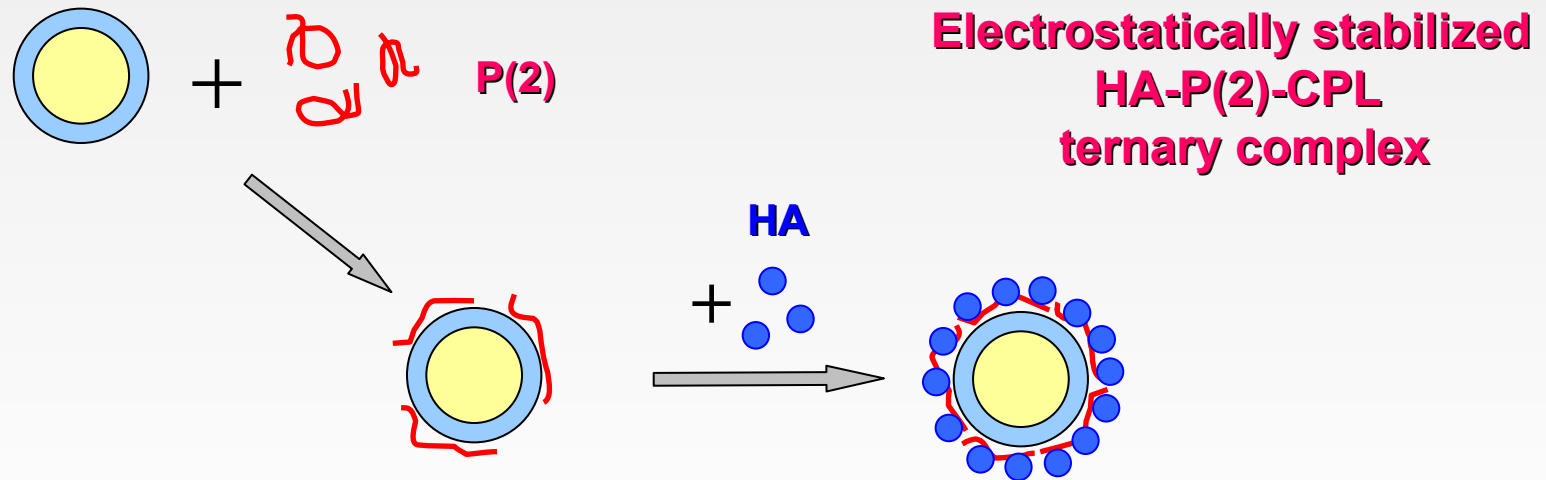
**P(2,16)**



POLYLYSINE HYDROBROMIDE

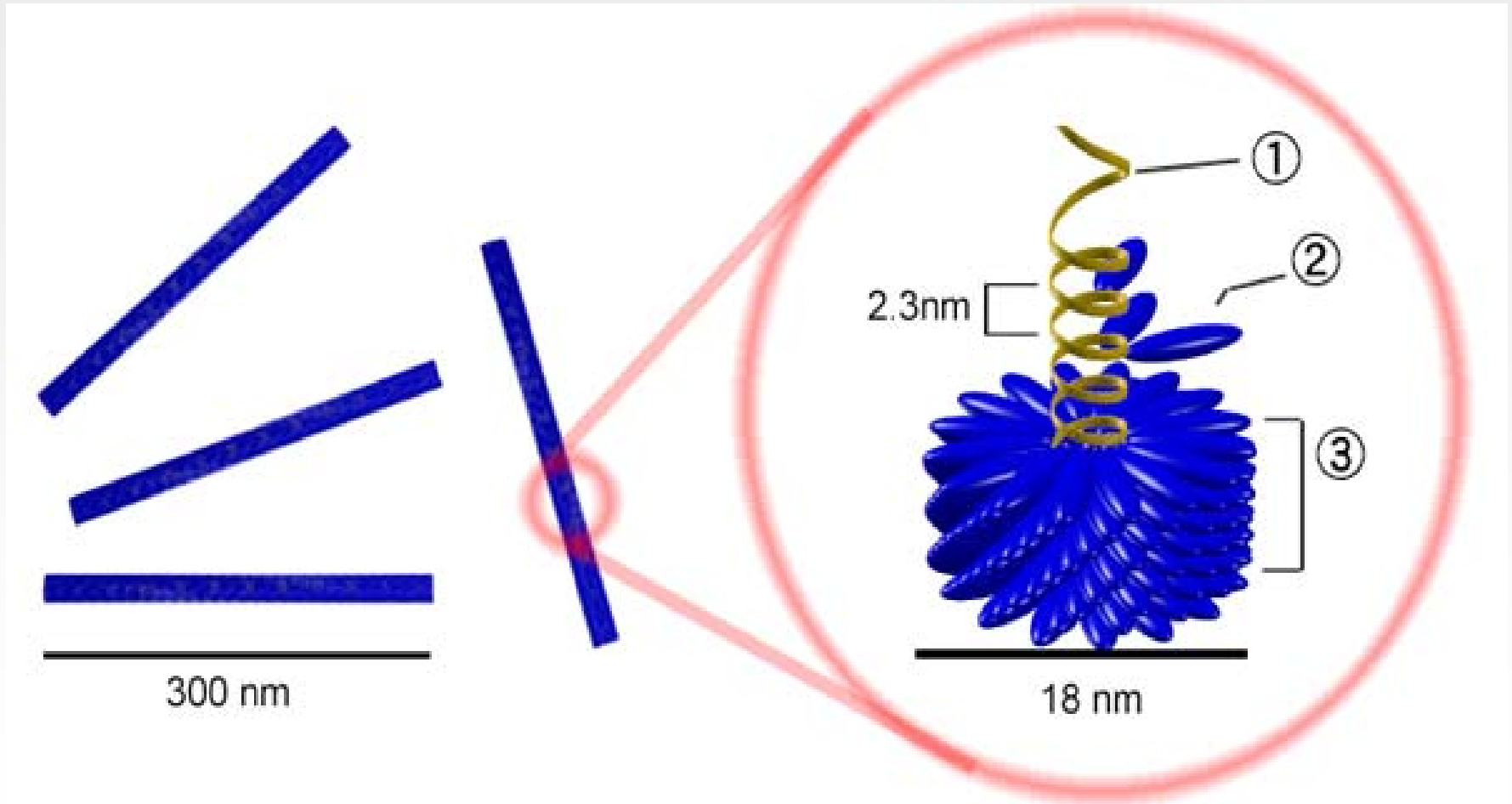
**PL**

# Binding of HA epitope region to P(2)-covered CPL scaffold



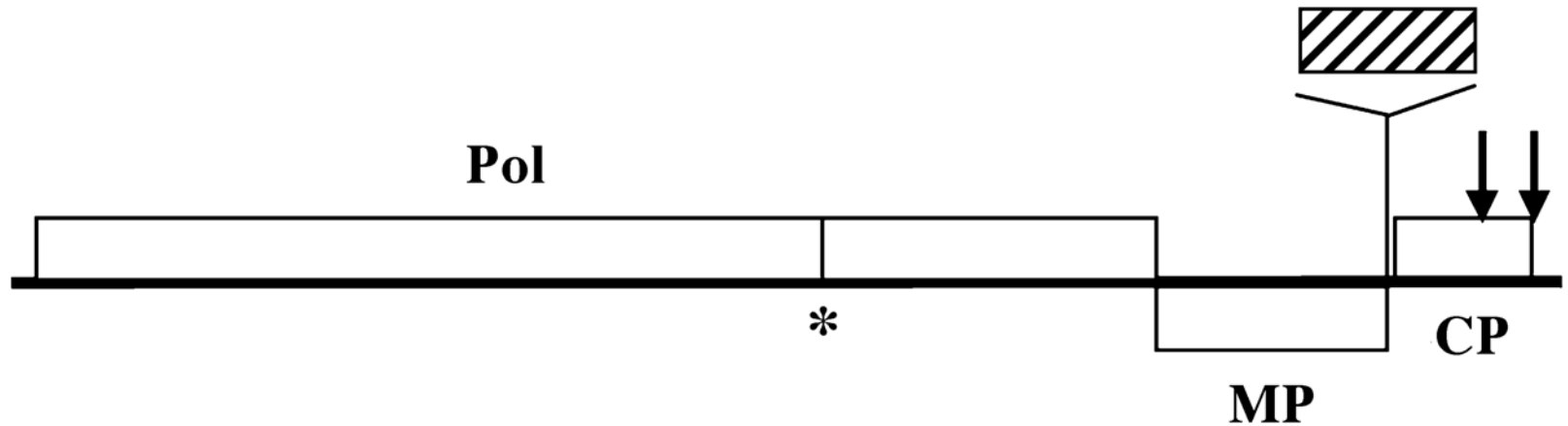
**TMV particles stimulate cellular immunity by interacting directly with immune cells ([McCormick](#) and [Palmer](#), 2008).**

## Structure of TMV particle

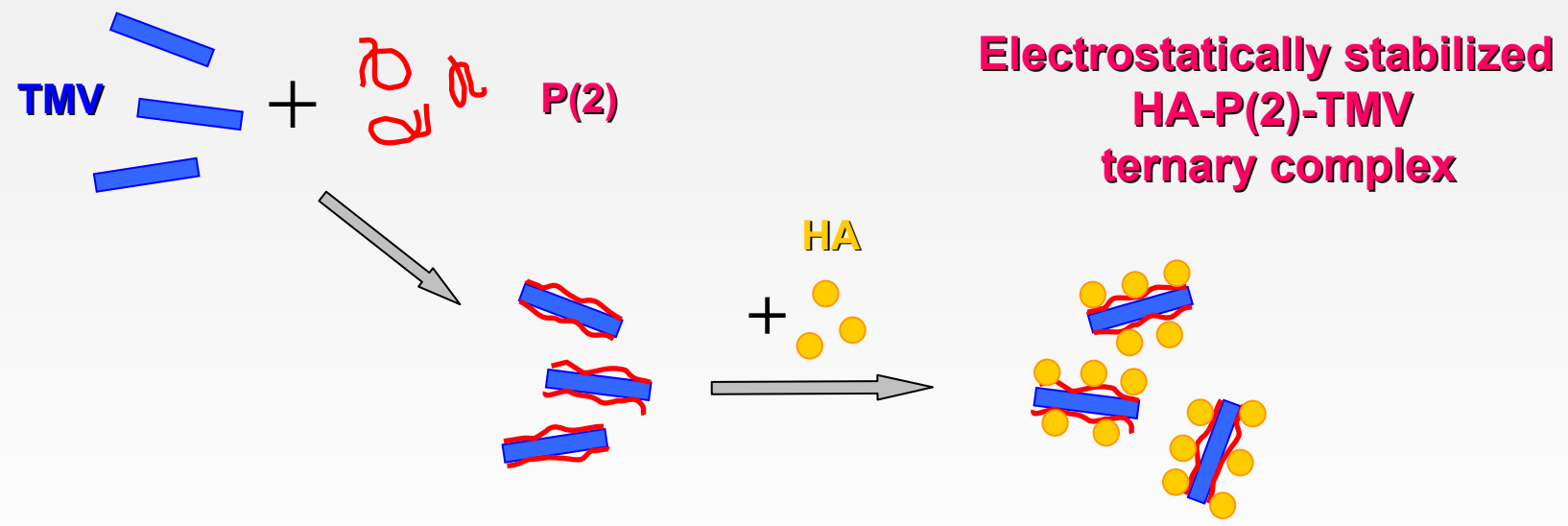


# Structure of tobacco mosaic virus genome (arrows indicate points for epitope insertions in genetically engineered construct)

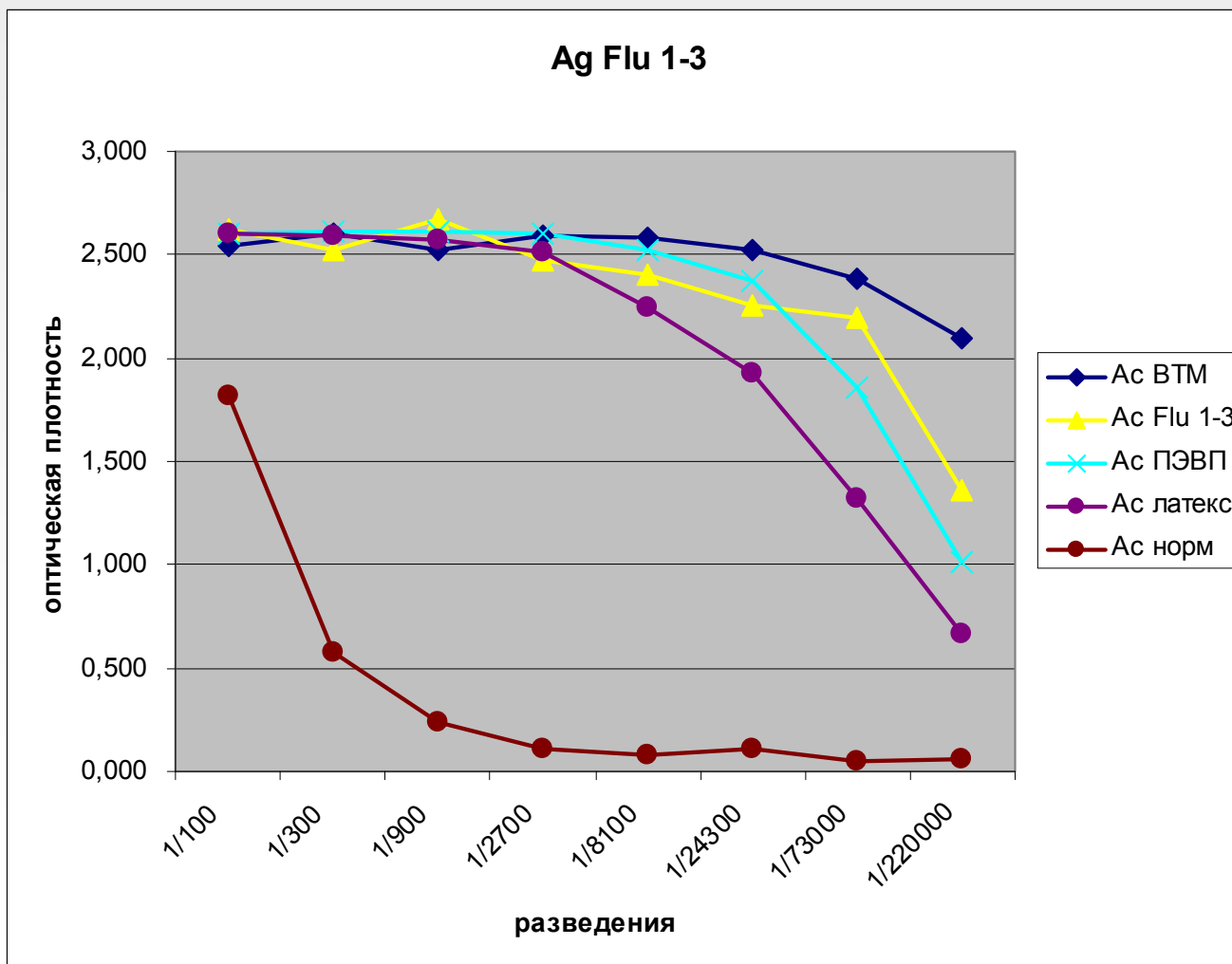
TMV



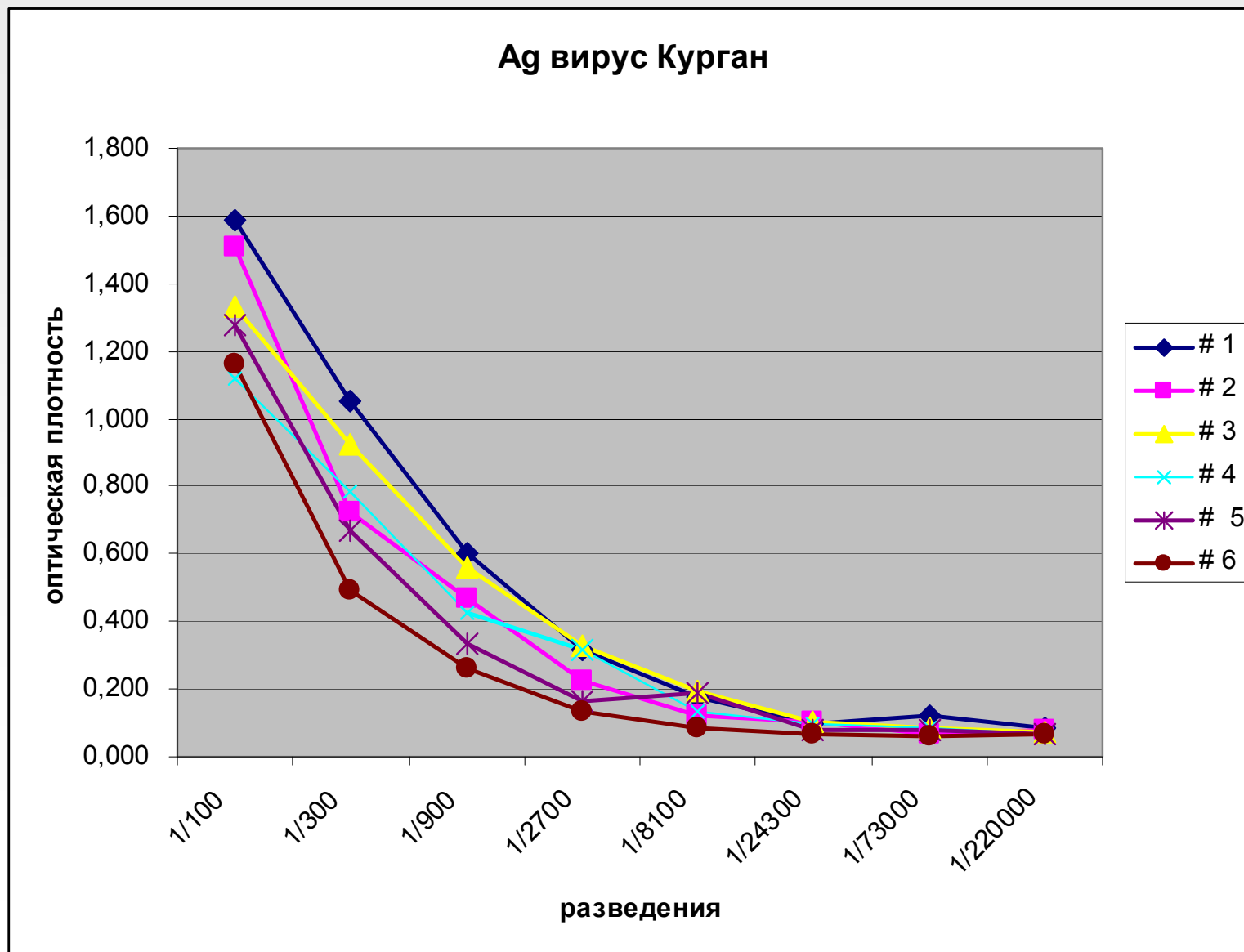
# New scheme for non-covalent binding of HA epitope region to P(2)-covered TMV



# Testing of polyclonal antibodies against different coated scaffolds for interaction with HA fragment (A/Kurgan/5/05) used for immunization



# Testing of polyclonal antibodies for interaction with whole influenza virus particles (A/Kurgan/5/05)



# CONCLUSIONS

- **1. HA region capable of producing antibodies interacting with whole influenza virus particles corresponds to the uninterrupted HA fragment possessing all main and minor neutralizing epitopes**
- **2. Display of engineered HA fragments on the surface of TMV virions (as non-covalently bound scaffolds) was shown to induce most efficient antibody production**

# Team members

- **D.V. Rakitina, A.D. Leschiner, A.G. Solovyev, S.Yu. Morozov, I.B. Kaplan, A.A. Yaroslavov and J.G. Atabekov** (*Moscow State University*)
- **T.N. Erokhina** (*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry*)
- **L.A. Stepanova and O.I. Kiselev** (*Research Institute of Influenza*)