A new structural approach to study lipid-protein interactions within a viral envelope

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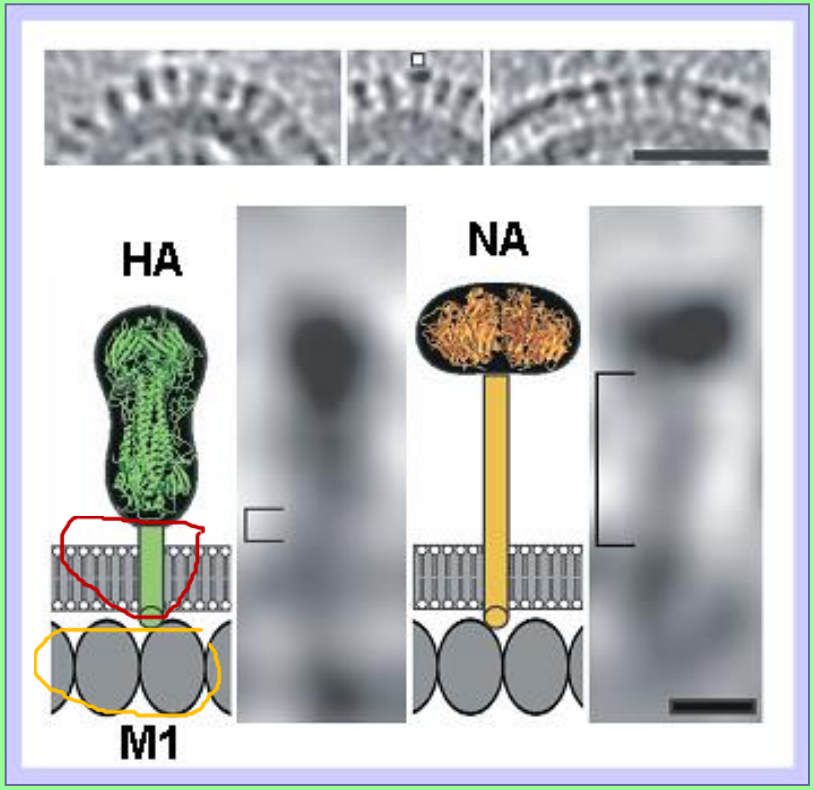
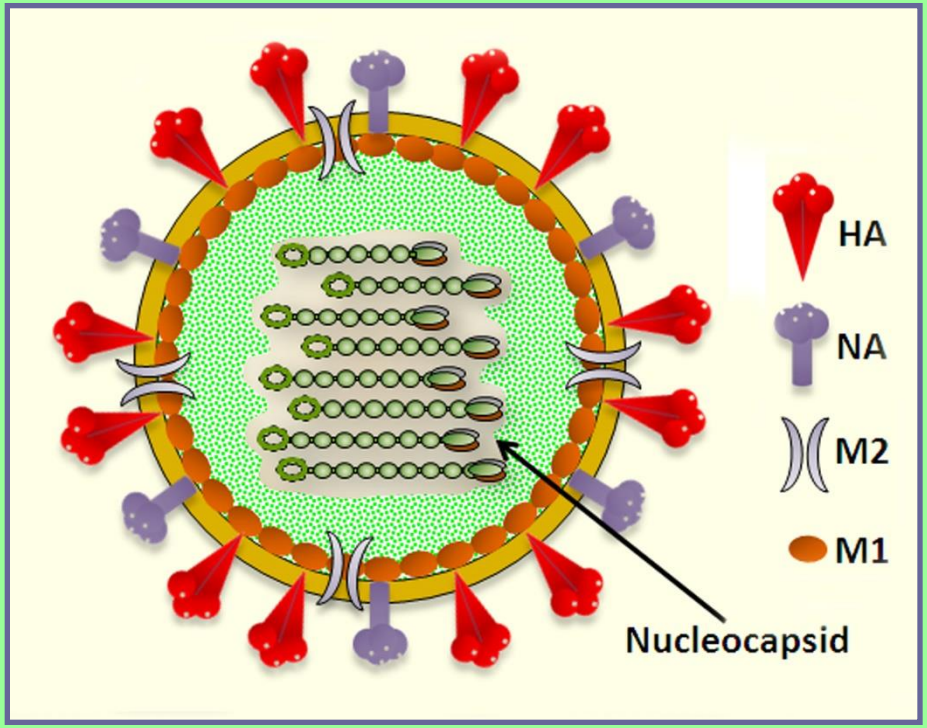
Orthomyxoviridae. Genus Influenzavirus A

HA: Trimer

NA: Tetramer

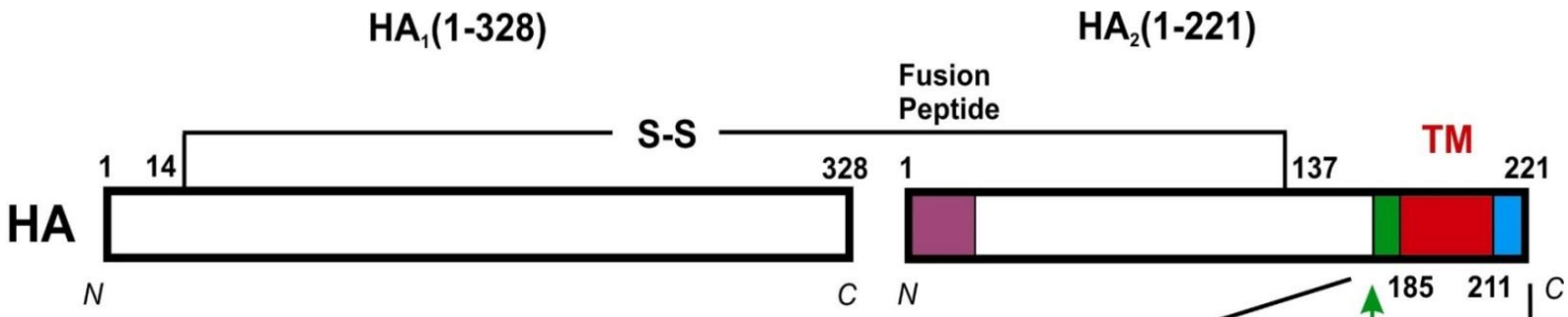


Harris et al., *PNAS*, 2006, 103: 19123

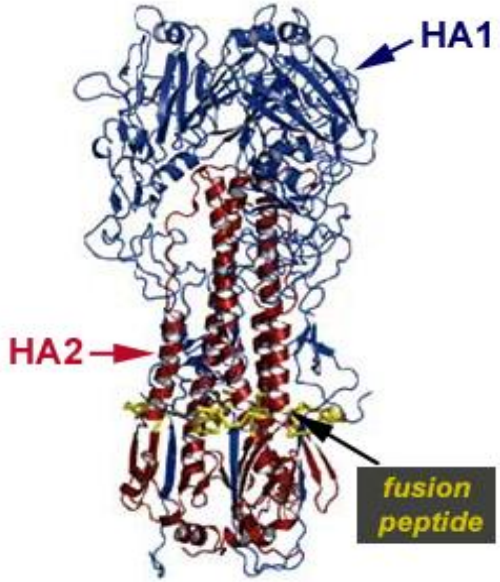


- Mechanisms of HA and NA interactions with a layer of M1 matr
lipid membrane are poo
- Such interactions are
particles assembly and b

Structure of influenza hemagglutinin (HA)



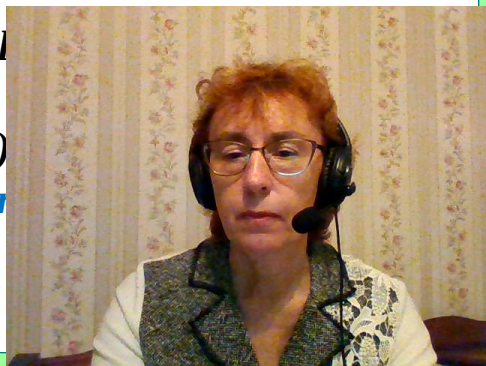
Ectodomain (Homotrimer)



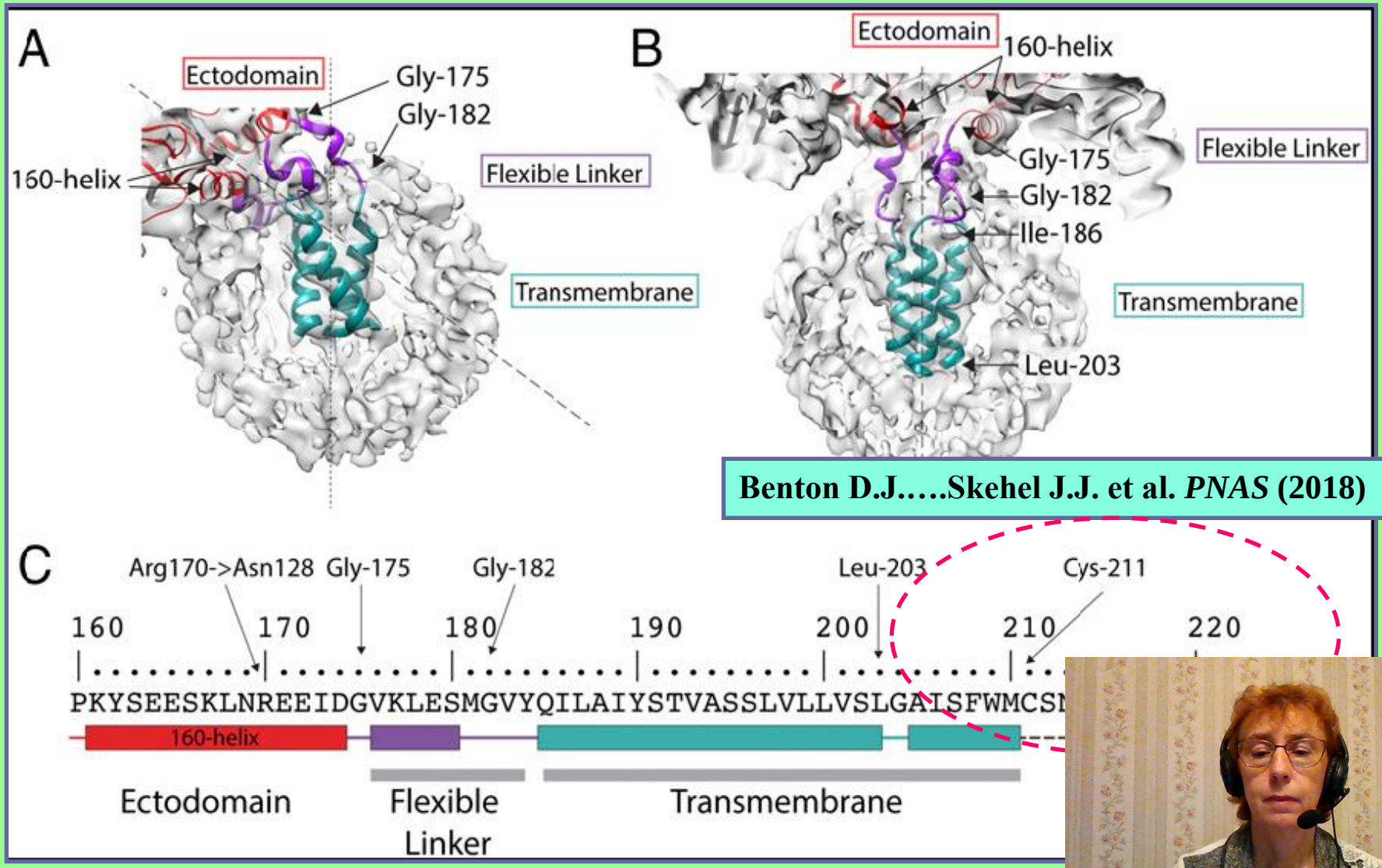
?

Anchoring segment

- *HA monomer contains HA1 and HA2 chains l*
- *X-ray data are available for the homotrimeric*
- *Anchoring segment (~45 amino acids)*
  transmembrane domain + cytoplasmic tail (intr
- *Several cysteine residues in C-terminus*
  modified with long saturated fatty acids bound



Cryo-EM: the N-terminal part of HA membrane anchor is resolved

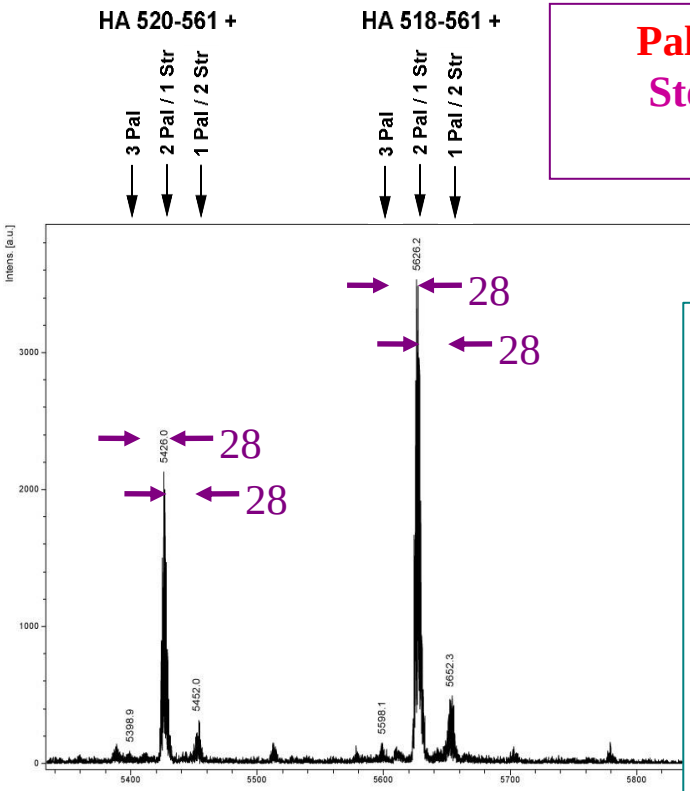


Benton D.J.….Skehel J.J. et al. *PNAS* (2018)

BUT: ***The C-terminal part of TMD and the CT including S-acylated c***

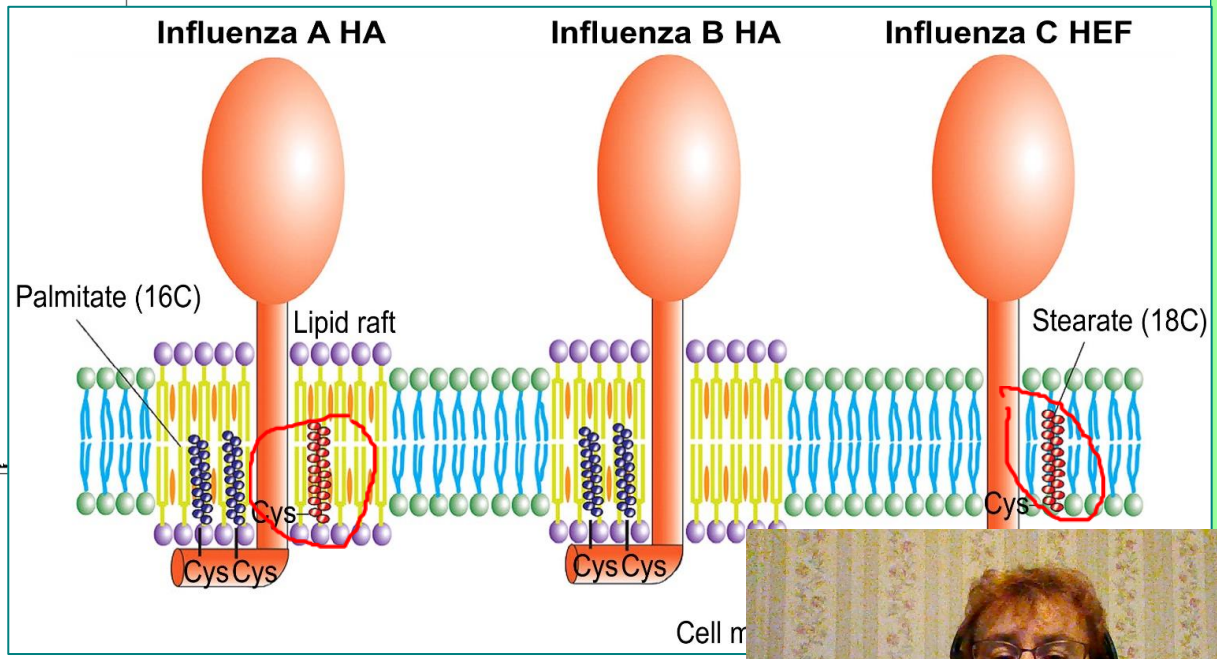
MALDI-TOF MS to study S-acylation of HA

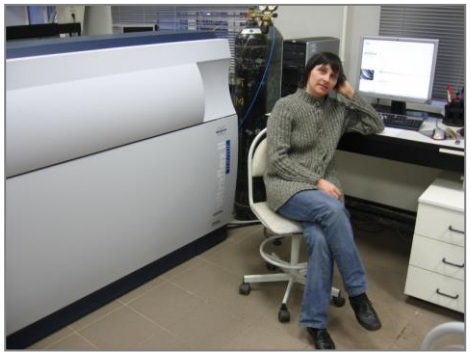
A/chicken/Germany/N/49 (H10N7)



Palmitate (C16:0) = +238 m/z
Stearate (C18:0) = +266 m/z
266-238=28 m/z

PD Dr. Michael Veit
Free University Berlin



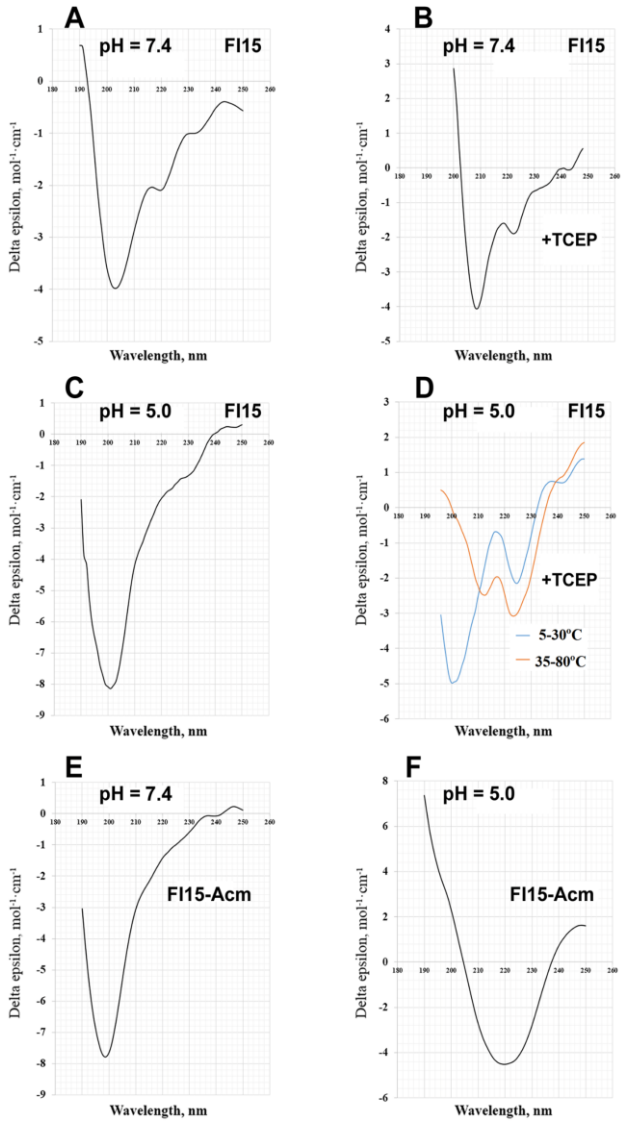


Dr. Marina Serebryakova
Lomonosov Moscow State University

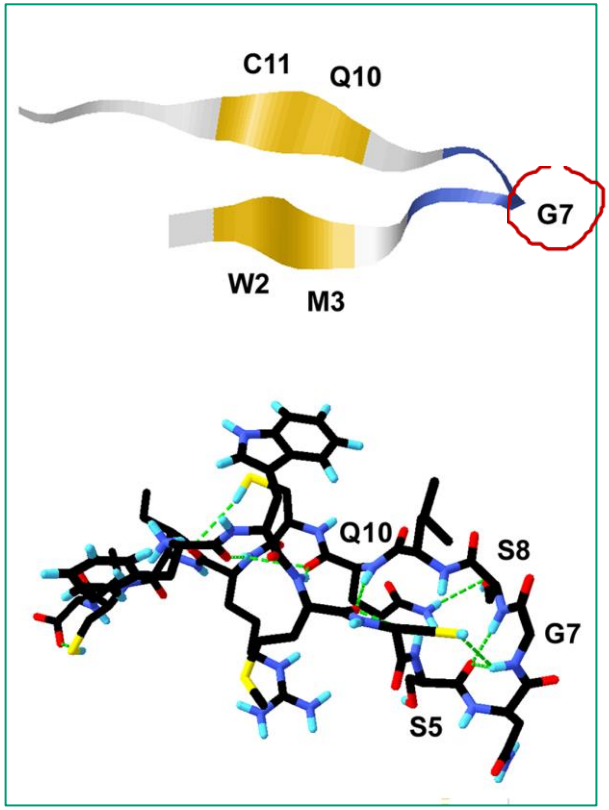
Glyco
envel
differ

Cytoplasmic tail of HA is β-structural

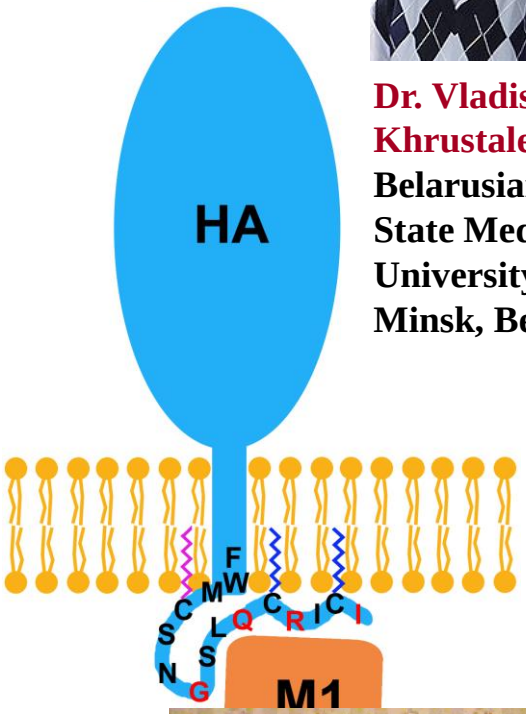
Circular dichroism analysis



Molecular modeling



Homology-based 3D models of the FI15 peptide



Dr. Vladislav V. Khrustalev
Belarusian State Medical University, Minsk, Belarus

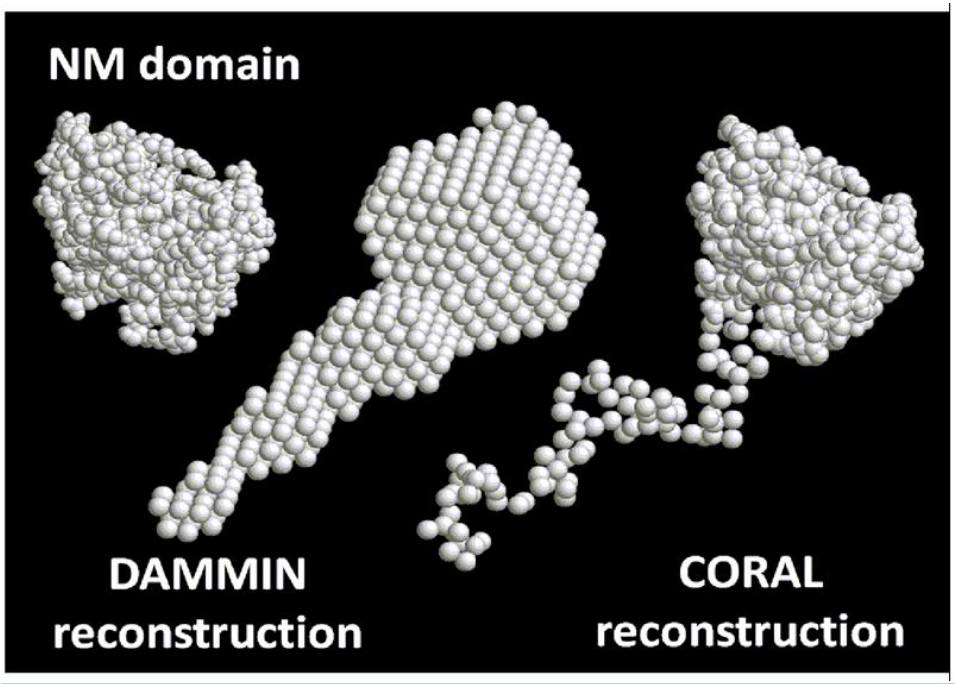
FI15: NH_2-FWMCSNGSLQCRICI-*COO*

FI15-Acm: NH_2-FWMC*SNGSLQC*RI

***acetaminomethylated –SH groups of cy**

Small Angular X-ray Scattering (SAXS) to analyze protein structure in solution

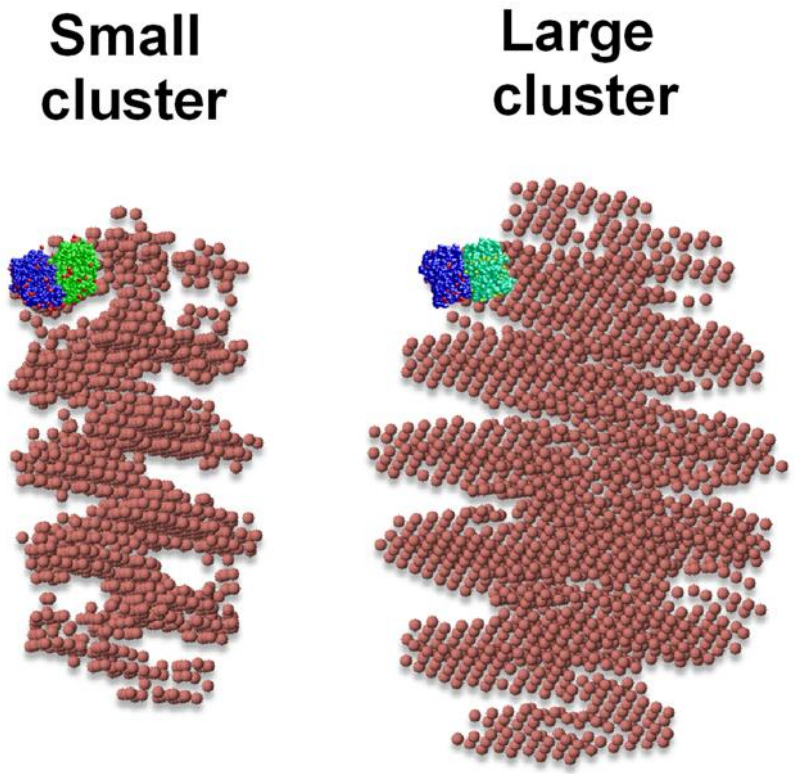
SAXS analysis:
full-length M1 model with globular NM-domain and flexible C-domain



Shtykova E.V. et al. PLoS One (2013) 8(12):e82431;

Shtykova E.V. et al. Sci. Rep. (2017) 7(1):16793.

EMBL, Hamburg Outstation,
"Deutsche Synchrotrone" (DESY), Germany

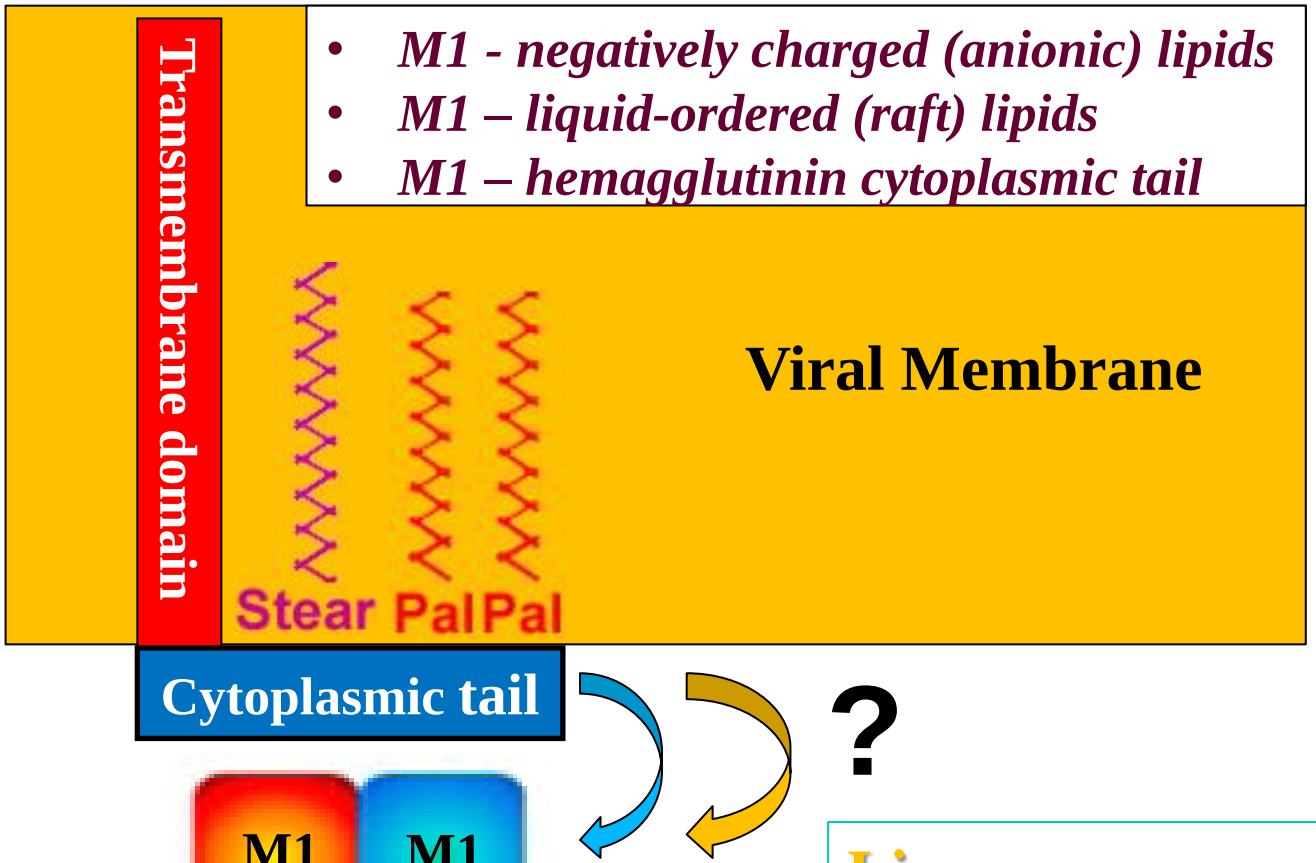


Acidic pH
NM-domain
1AA7 PDB

M1-M1 associatio
(association increas

SAXS to model M1-lipid and M1-HA interactions?

- *M1 - negatively charged (anionic) lipids*
- *M1 – liquid-ordered (raft) lipids*
- *M1 – hemagglutinin cytoplasmic tail*



SAXS analysis of lipid vesicles mixtures

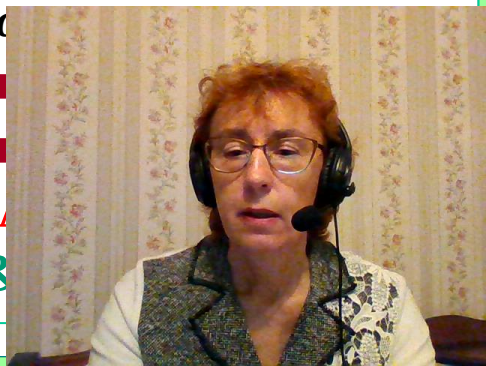
↑

M1
from A/PR8/8/34
(acid solubilization)
Adjusted to pH = 6.8
+

Liposomes:

Extrusion through 100 nm po

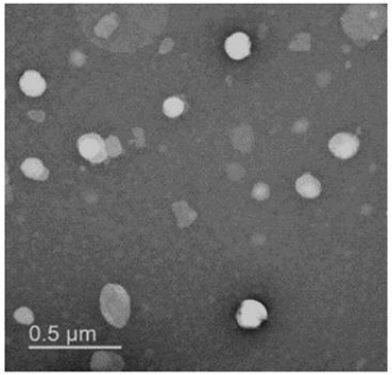
(1) Synthetic - with anion
(2) Synthetic - with anion
(3) Native (viral) lipids (A
(4) Native (viral) lipids &



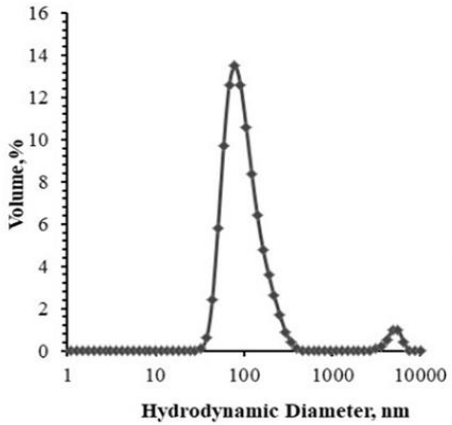
EM and DLS characterization of liposomes

Liposomes extruded through 100 nm pores

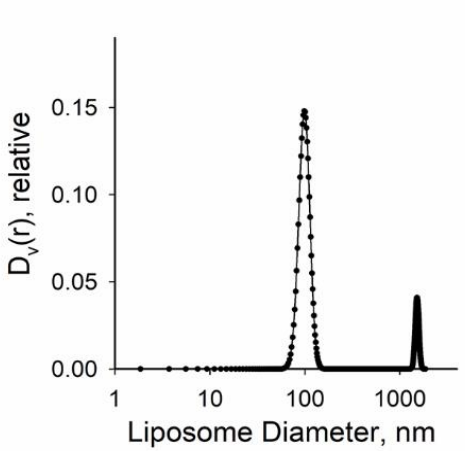
10 mol.% DOPS
90 mol.% DOPC



(a)

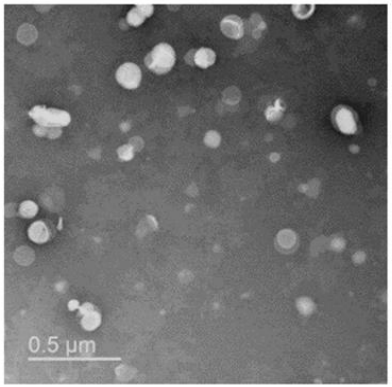


(b)

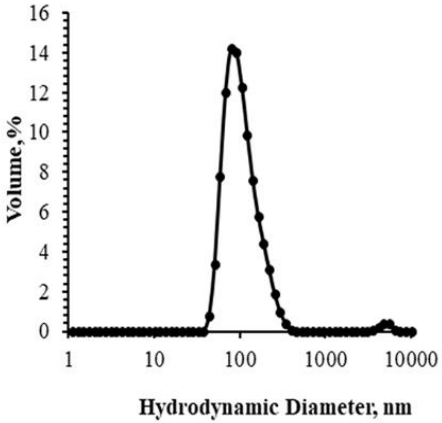


(c)

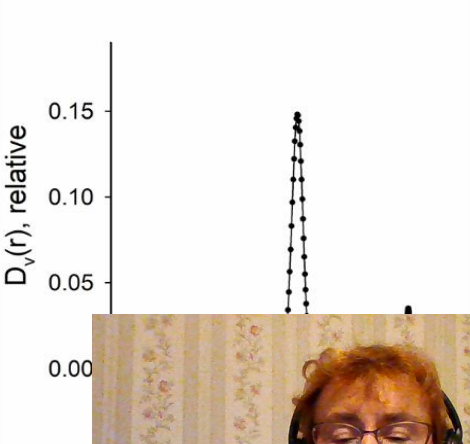
30 mol.% DOPS
70 mol.% DOPC



(d)

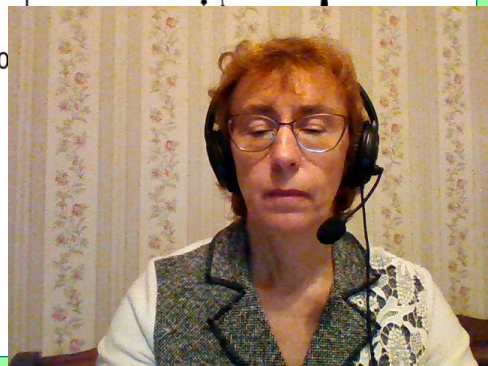


(e)

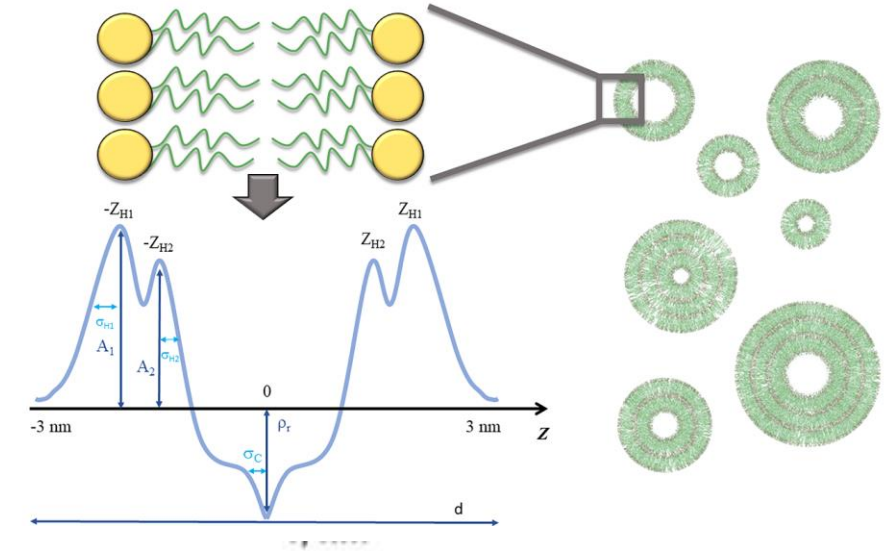


Electron Microscopy (negative contrasting)

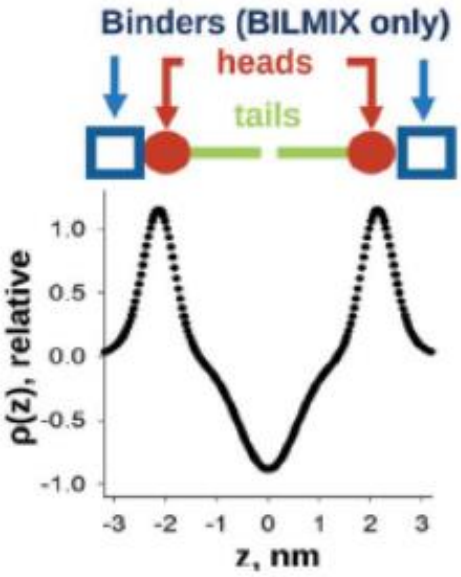
Dynamic Light Scattering



Mixtures of lipid vesicles: SAXS data analysis



Bilayer electron density



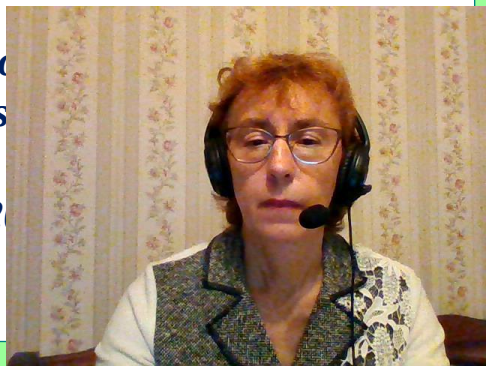
+ protein

Mixtures of lipid vesicles, SAXS analysis

Program BILMIX* (BIlayer Lipid MIXtures) restores the electron density of a lipid bilayer and simultaneously generates the size distribution of the unilamellar lipid vesicles (using either spherical or ellipsoidal models)

BILMIX allows also the modelling of asymmetric electron-density profiles, e.g. proteins associated with the inner or outer leaflets of the liposome

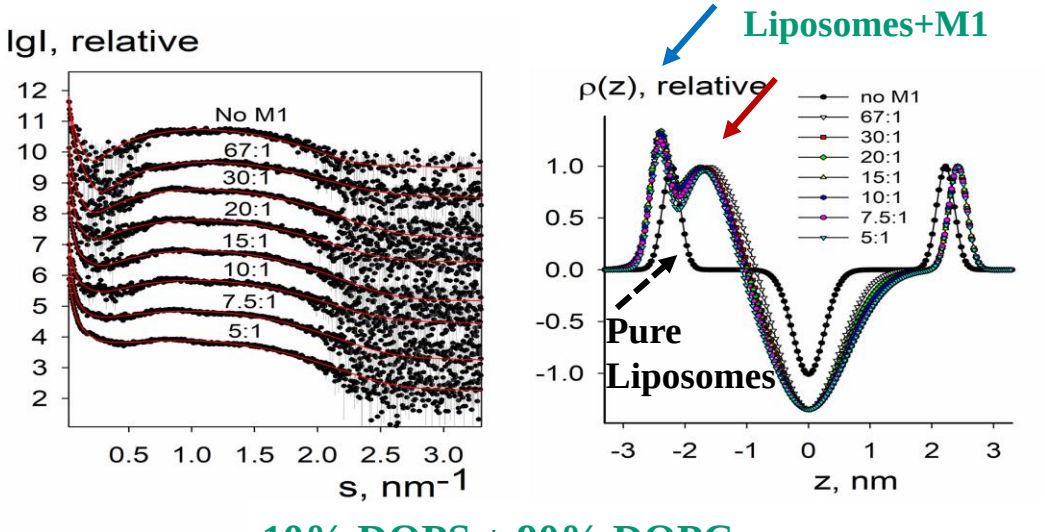
** Konarev P.V., Petoukho*
Fedorova N.V., Volyns
Batishchev O.V.
J.Appl. Cryst. (2



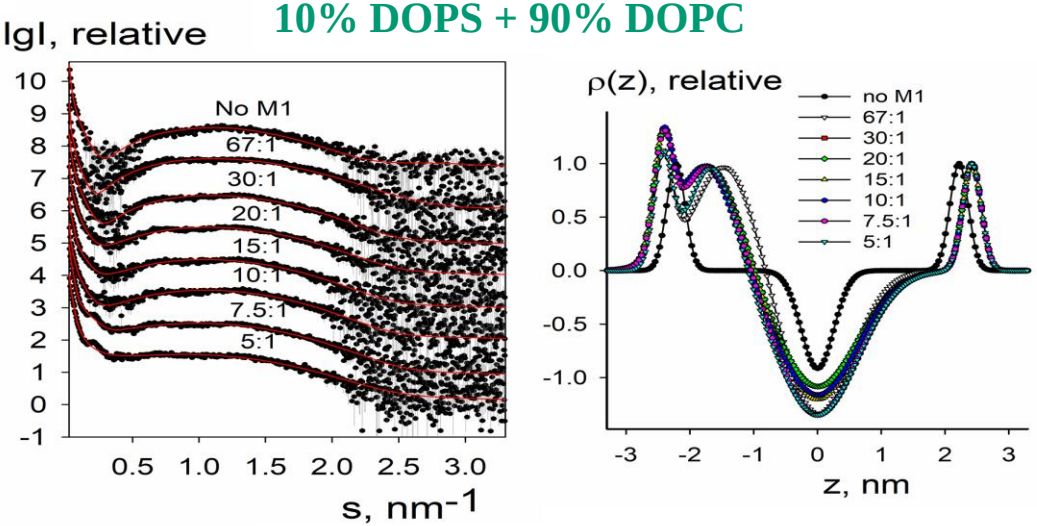
SAXS to model M1 - anionic lipid interactions

SAXS experimental curves and BILMIX calculations

Restored electron density profiles



10% DOPS + 90% DOPC



30% DOPS + 70% DOPC

In <u>two-component liposomes</u>, the left maximum splits into two sub-peaks upon loading liposomes with M1: the left (M1-associated) sub-peak with increased intensity implies charged lipid condensation. The right sub-peak indicates outer monolayer bending inside

P12 beam
synchrotron
III (DES

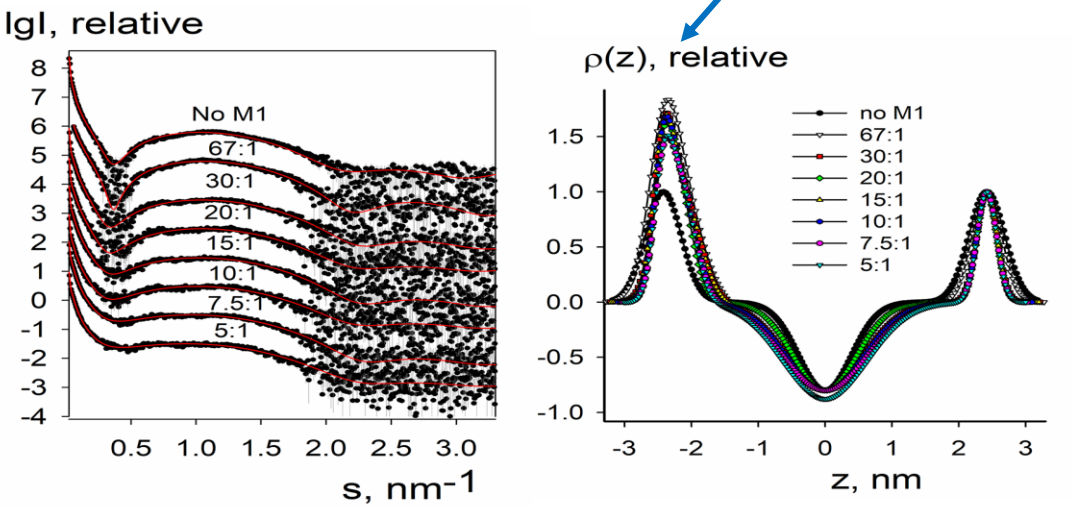
ATSA

BILM

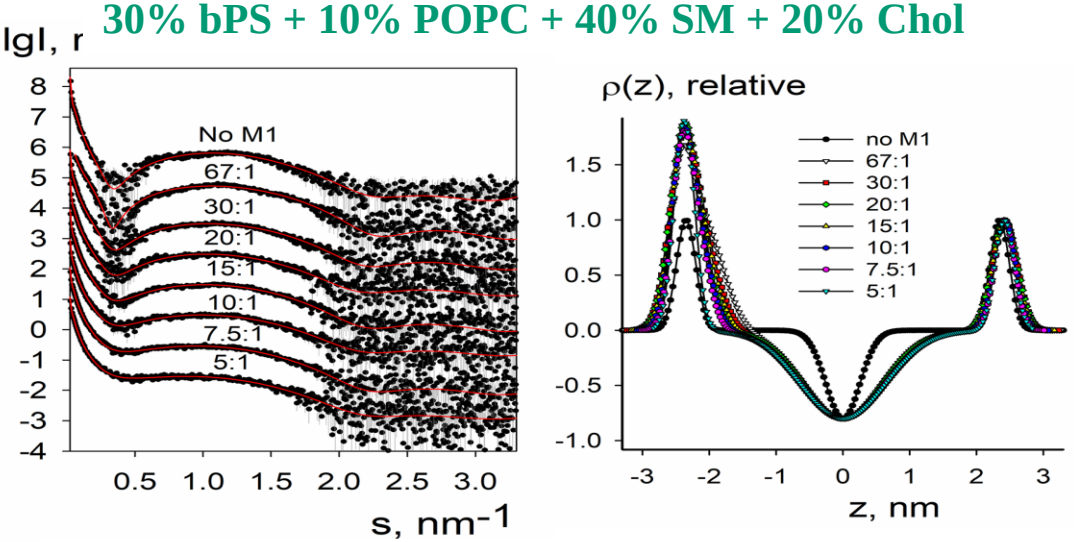
SAXS to model M1 – raft lipid interactions

SAXS experimental curves and BILMIX calculations

Restored electron density profiles



30% bPS + 10% POPC + 40% SM + 20% Chol

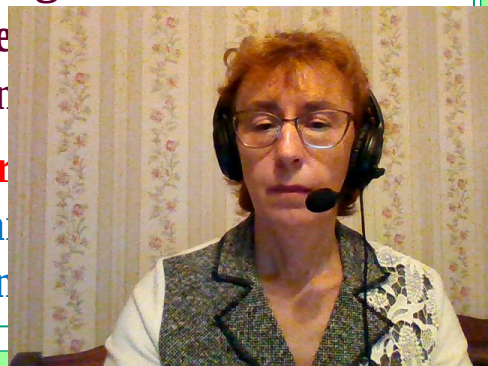


20% bPS + 13.3% POPC + 33.3% SM + 33.3% Chol

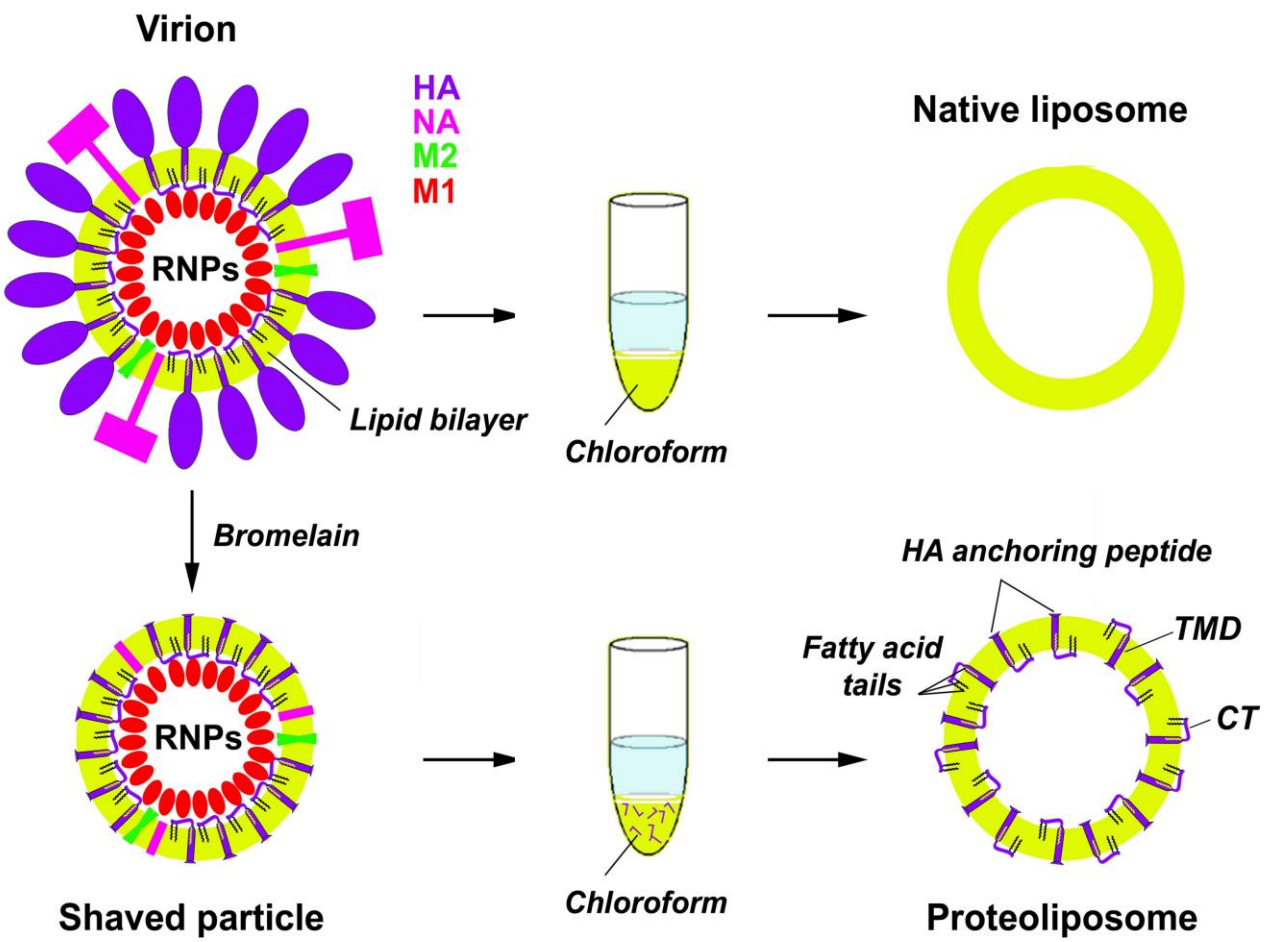
In <u>four-component liposomes</u>, we do not observe a separated protein-associated peak after loading with M1; the intensity of the left maximum increases. We may suggest that M1 introduces its helices into the lipid bilayer.

Hypothesis

- **Adsorption of M1 protein at the liposomes containing phosphatidylserine leads to condensation of lipids underneath;**
- **Imbalance in the area of lipid monolayers occurs, leading to the bending of the membrane inside the**
- **Cholester process a membran**



Liposomes from viral lipids +/- HA LI45 peptide



Native liposomes and proteoliposomes prepared from Influenza A/H1N1 virus

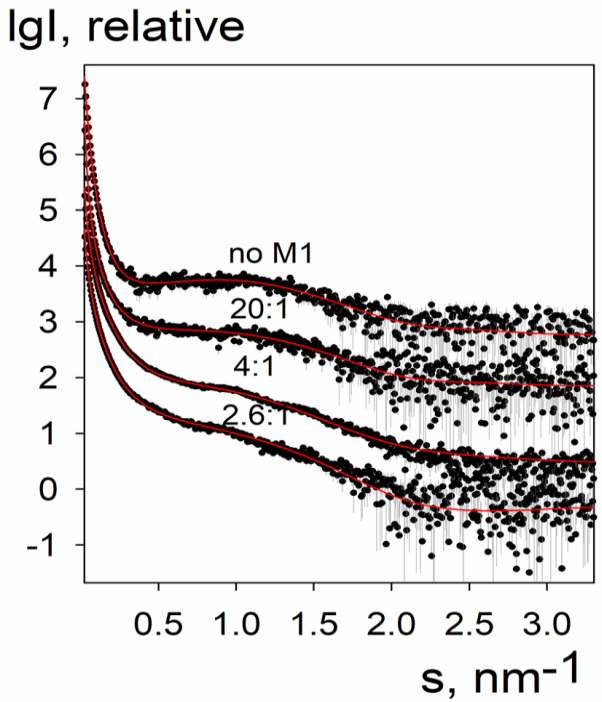
HA C-terminal peptide of H1N1 virus: "LI45" (45 amino

NH2-LESMGIYQILAIYSTVASSLVLLVSLGAISFWMCSNGSL

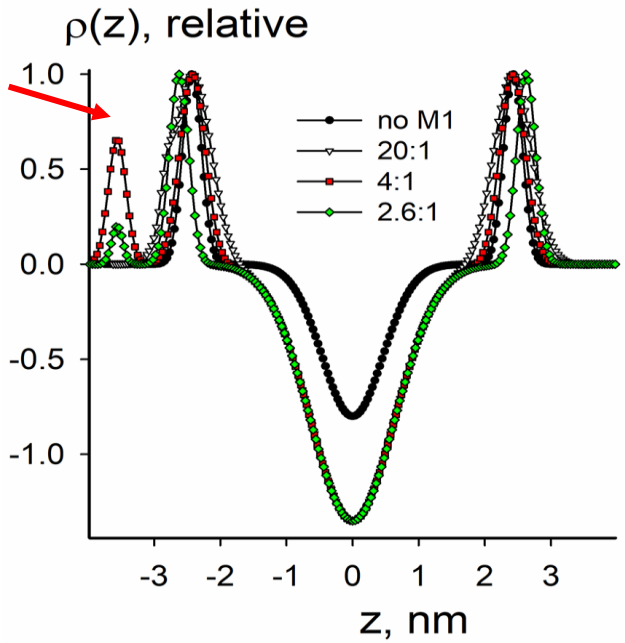
triply palmitoylated at three conserved cysteine resi

SAXS to model M1 - viral lipid interactions

SAXS experimental curves and BILMIX calculations



Restored electron density profiles

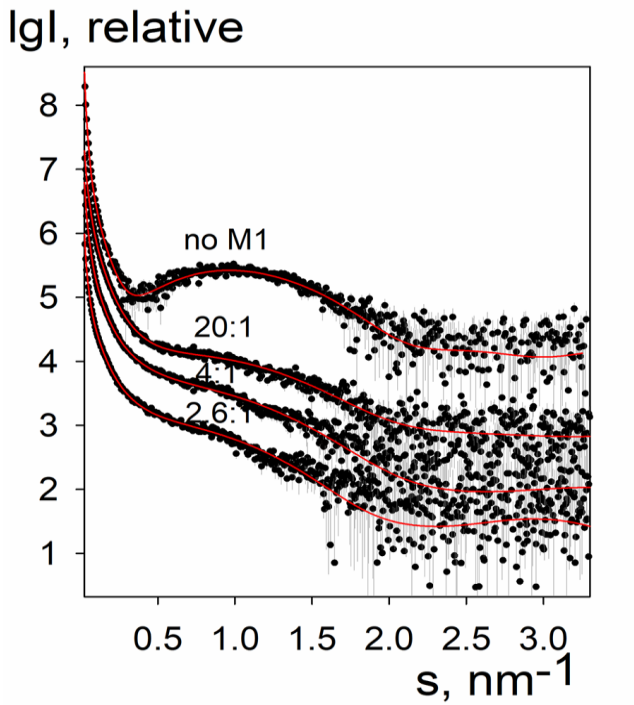


Native liposomes

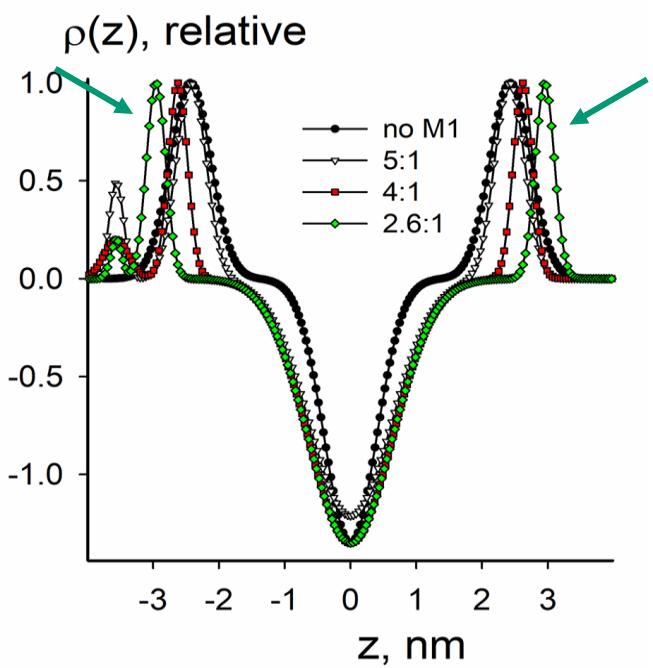
1. **A drop of the electron density at the intermonolayer surface is observed upon adsorption of the M1 protein only (not in the case of pure liposomes);**
2. **The M1-associated peak appears that is completely separated from the second, lipid-associated peak;**
3. **We do not see shifting of the lipid-associated sub-peak closer to the bilaye center. Thus, membrane tubular invaginations are not formed in complex mixtures of viral lipids in contrast to synthetic charged vesicles.**

SAXS to model M1 - HA interactions

SAXS experimental curves and BILMIX calculations

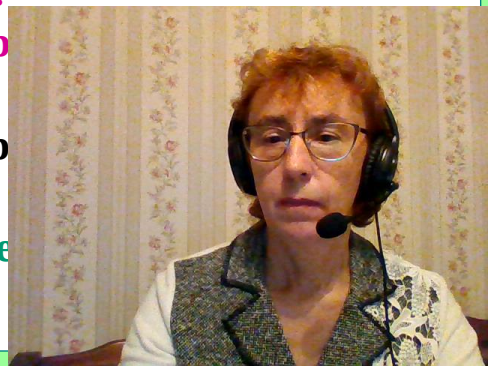


Restored electron density profiles



Proteoliposomes

1. **In contrast to native liposomes, a drop of the electron density at the intermonolayer surface is observed even before loading with M1 protein.**
2. **This result indicates that triply palmitoylated HA LI45 peptides make lip bilayer more ordered, and viral membrane becomes raft-like;**
3. **Two positive maxima move to the periphery from the bilayer centrum up adsorption with M1. This effect is more pronounced in proteoliposomes compared to native liposomes: the whole width of their lipid bilayer incre by 2 nm (1 nm at each side).**



CONCLUSIONS

- **M1 interaction with phosphatidylserine leads to condensation of the lipid in the protein-contacting monolayer thus resulting in formation of lipid tubules;**
- **This effect vanishes in the presence of the raft-forming constituents (sphingomyelin and cholesterol);**
- **Hemagglutinin anchoring peptides bearing three fatty acid residues demonstrate a specific role in ordering of viral lipid membrane into the raft-like one;**
- **Hemagglutinin anchoring peptides may stimulate the oligomerization of M1 on the lipid mem form a viral scaffold for subsequent buddi virion from the plasma membrane of the infec**

MAIN PARTICIPANTS:

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SAXS analysis

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Biophysics of lipid membranes

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Moscow State

Andrei Moi
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Moscow State

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Thank you for your attention!