Combined therapeutic/prophylactic hepatitis B VLP vaccine prototype

Andris Dishlers, Ivars Petrovskis, Dace Skrastina, Ieva Berza, Ilva Lieknina, Juris Jansons, Inara Akopjana, Jelena Zakova, Velta Ose, Irina Sominskaya

Latvian Biomedical Research and Study Centre, Ratsupites Str 1, Riga 1067, Latvia

Riga 2022

Hepatitis B

Hepatitis B remains a major global health problem, thus it can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. An effective prophylactic vaccine that offers 98% to 100% protection against hepatitis is available for 30 years, but is ineffective against chronic infection.

WHO

296 million people are living with chronic hepatitis B infection

1.5 million new infections each year

820 000 deaths, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer) each year

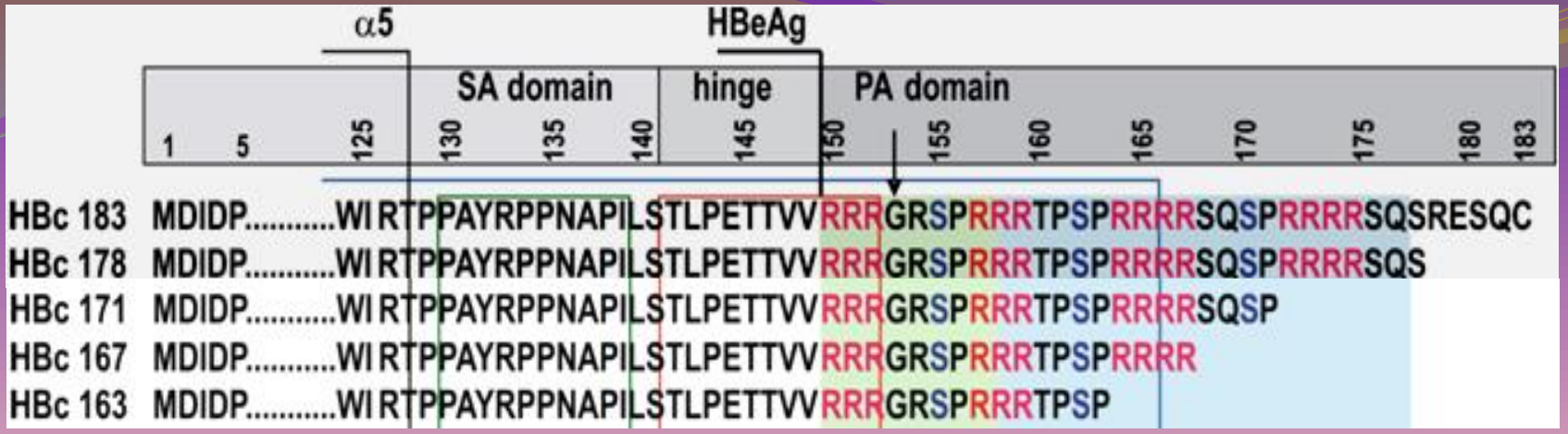
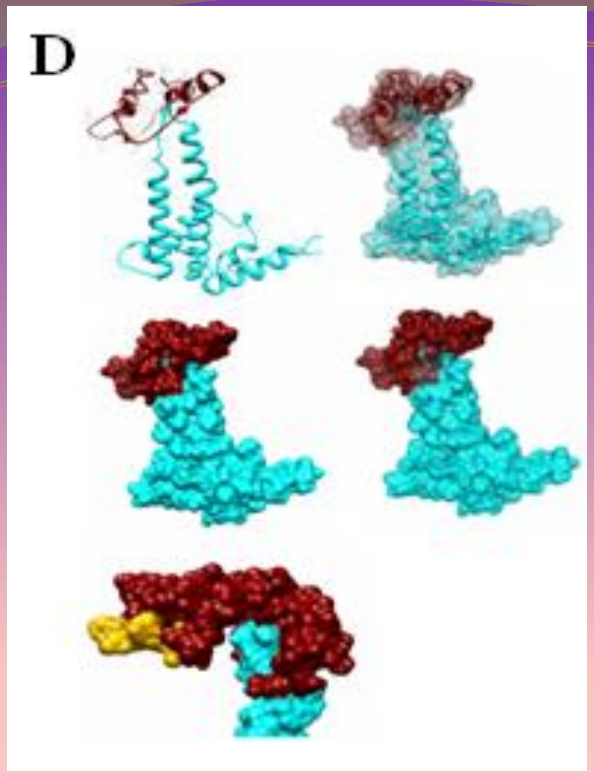
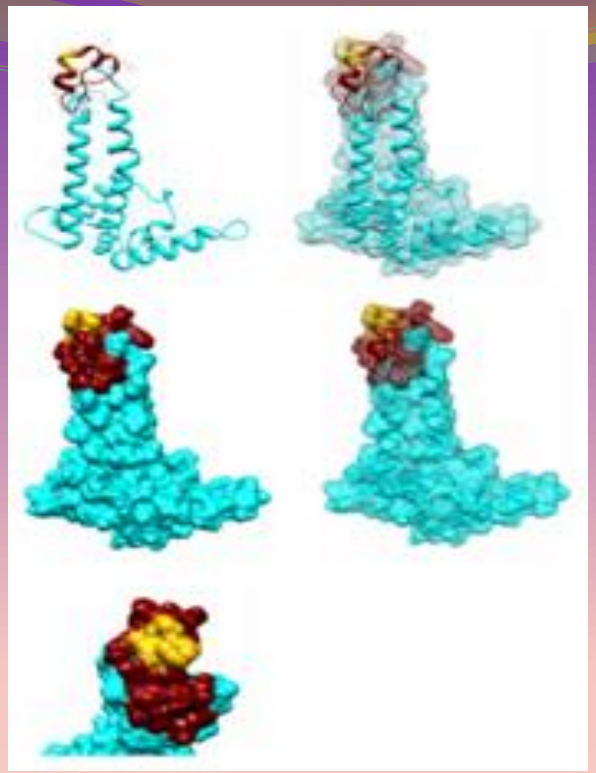


Figure 1. A library of HBc proteins by gradually truncating the full-length HBcAg of HBV, genotype D1, subtype ayw2.

Figure 2. Insertion sites within MIR and possible structure of HBc protein. Asymmetric units of HBc were downloaded from the VIPER data base [Reddy, 2001]. Positions for the preS1 insertions within the asymmetric unit are marked by arrows.



preS1phil
12-60+89-119 aa



pS1(20-47)
^{20}NPLGFFPDHQL**DPAFR**ANTANPDWDFN^{47}

Figure 3. Structure modeling of HBc183pS1phil and **HBc183pS1(20-47),** monomer **D**, HBc vector – cyan. Inserts - dark red, DPAFRA - yellow. Top rows - elements of the secondary structure, middle rows - surface, bottom rows – insertion in an enlarged form and in optimal orientation (to highlight the position of the DPAFR within insert on the surface).

DPAFR – major epitope of anti-preS1 monoclonal antibody MA18/7.

The production level and VLP formation were compared in *E. coli* thus *E. coli* remains the most attractive system when efficient expression and high yields of recombinant proteins are necessary. The VLP formation was observed in case of all constructs, but the production level for HBc-preS1 itself and the VLP yield for different in length HBc-preS1 proteins varied.

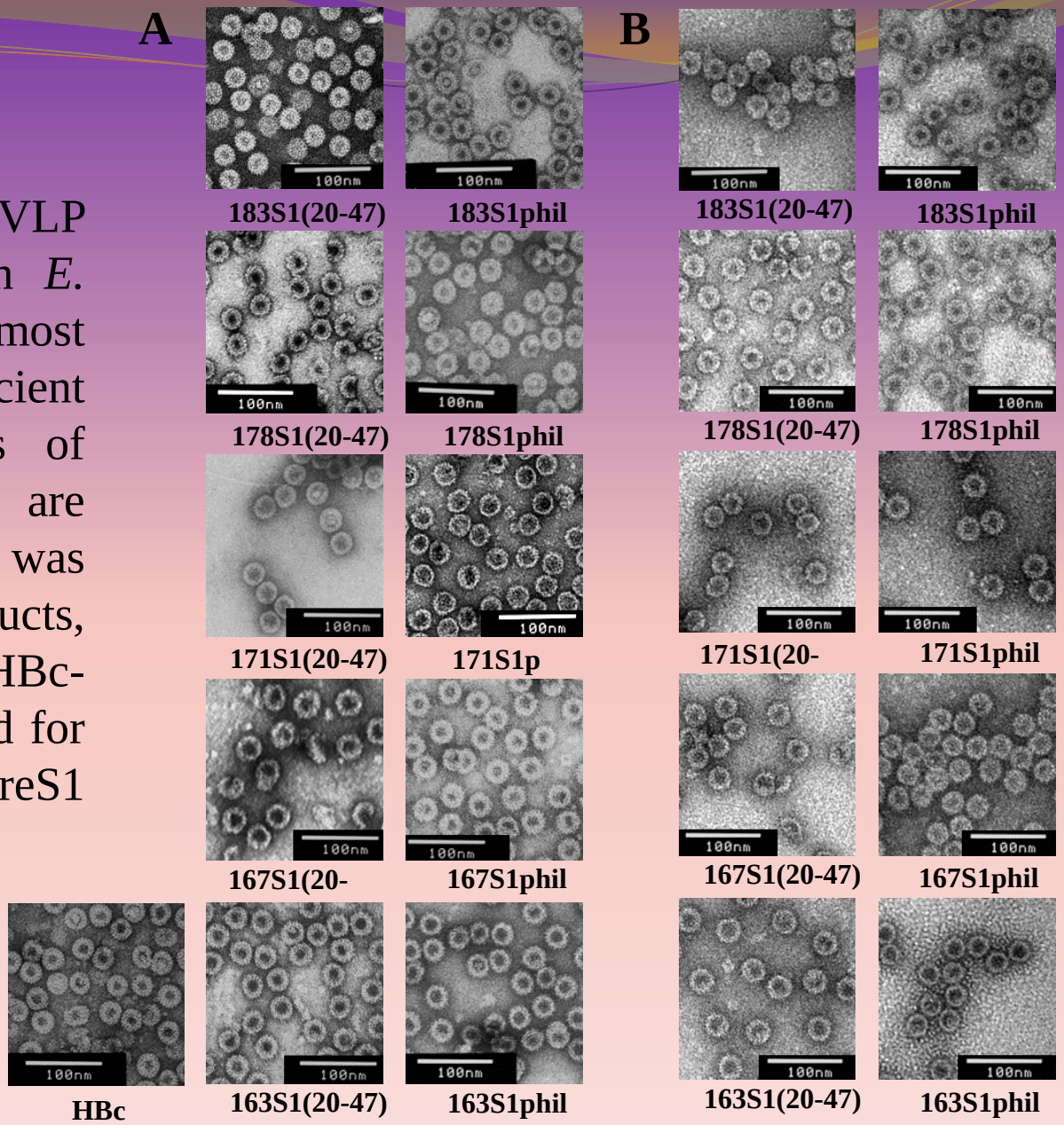


Figure 4. EM of HBc-preS1 preparations: **A**. 2011, **B**. 2021. Scale bar, 100 nm.

DLS was used to characterize the size homogeneity of VLP preparations.

34-49 nm

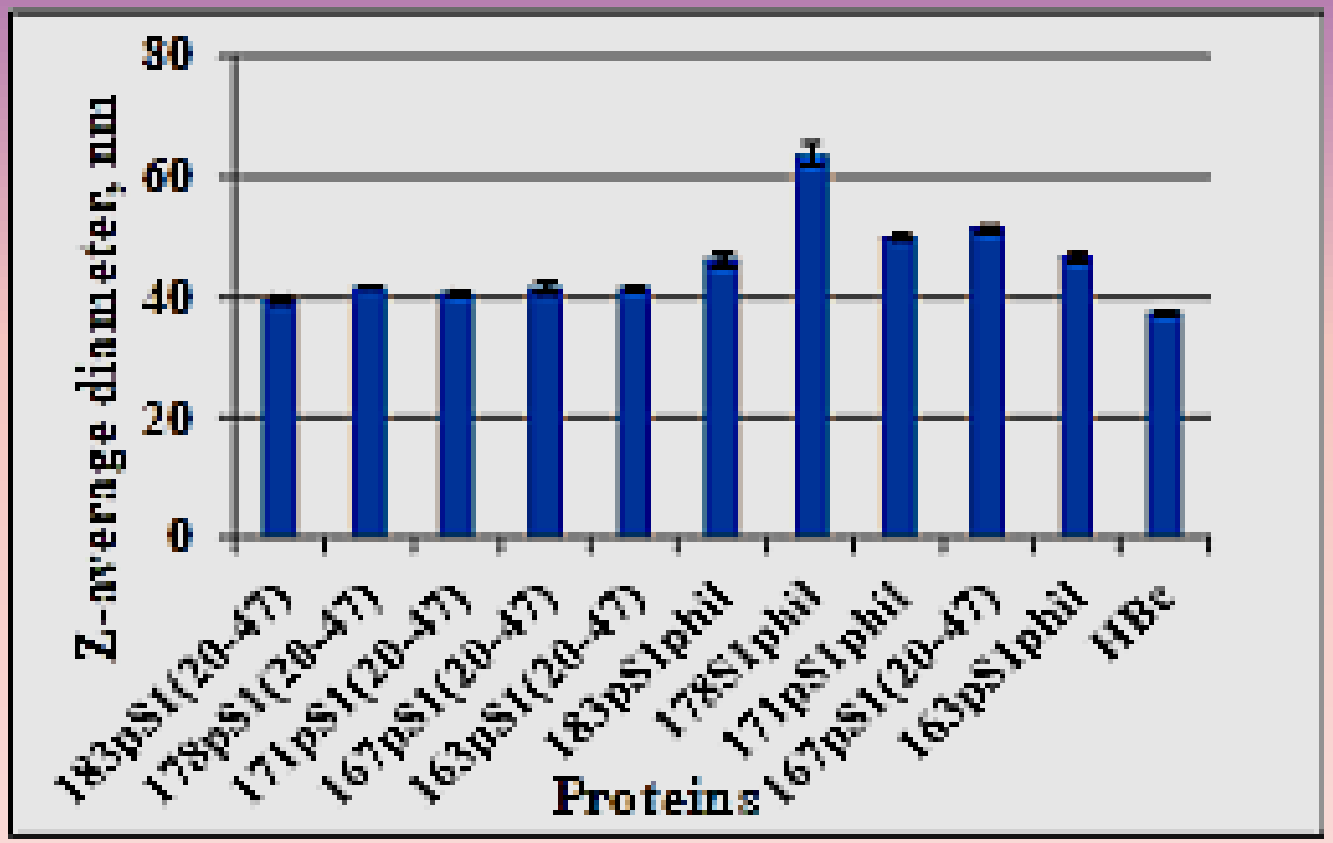


Figure 7. DLS analysis of the preparations of HBc-preS1 VLPs.

The presence of host nucleic acids in VLPs

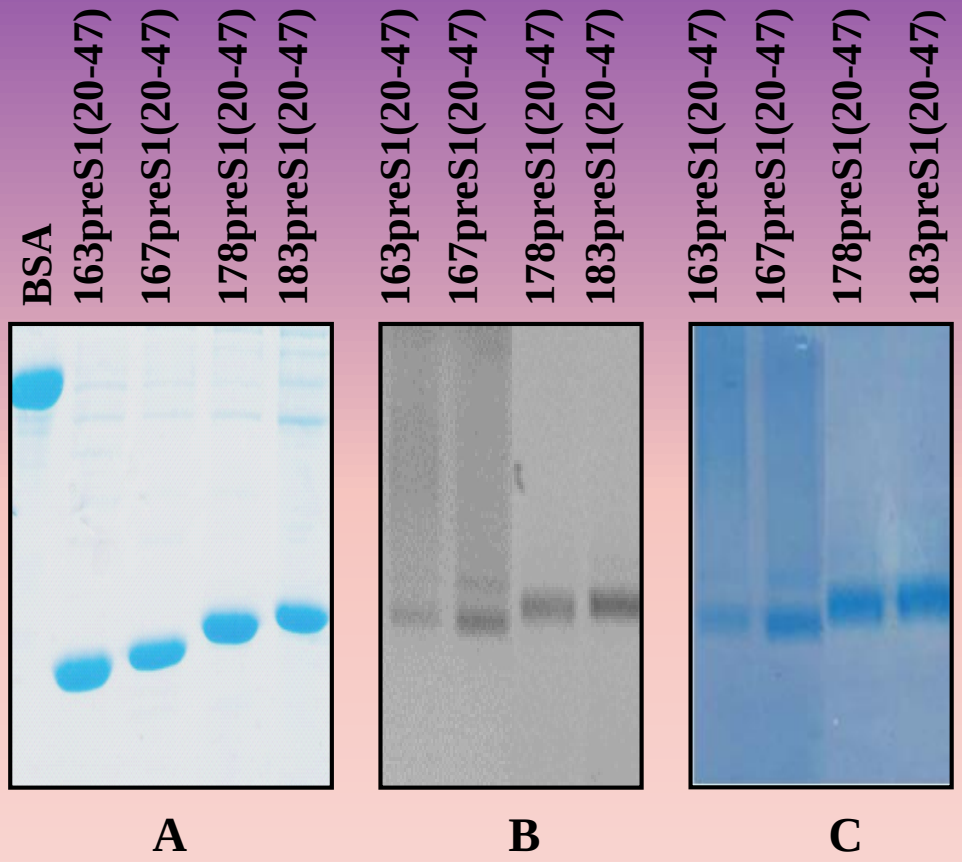
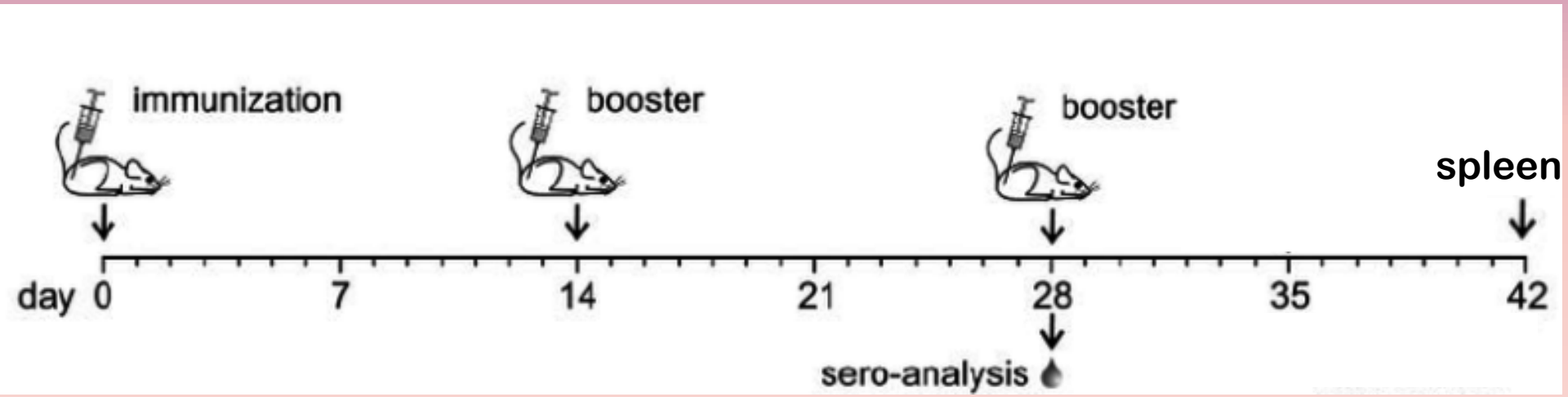


Figure 5. SDS-PAGE and NAGE of HBc-preS1 (20-47) preparations. (A) - 15% SDS-PAGE of purified HBc-preS1 proteins stained with Coomassie Brilliant Blue R-250. (**B**), (**C**), - 1 % NAGE in TBE buffer: (**B**) stained with EtBr (**C**) – stained with Coomassie Brilliant Blue R-250.

BALB/c mice immunization sheme



BALB/c mice were subcutaneously immunized on days 0, 14, and 28 with 25 μg of protein in PBS formulated with 250 μg Alhydrogel in a total volume of 0.2 mL per mouse.

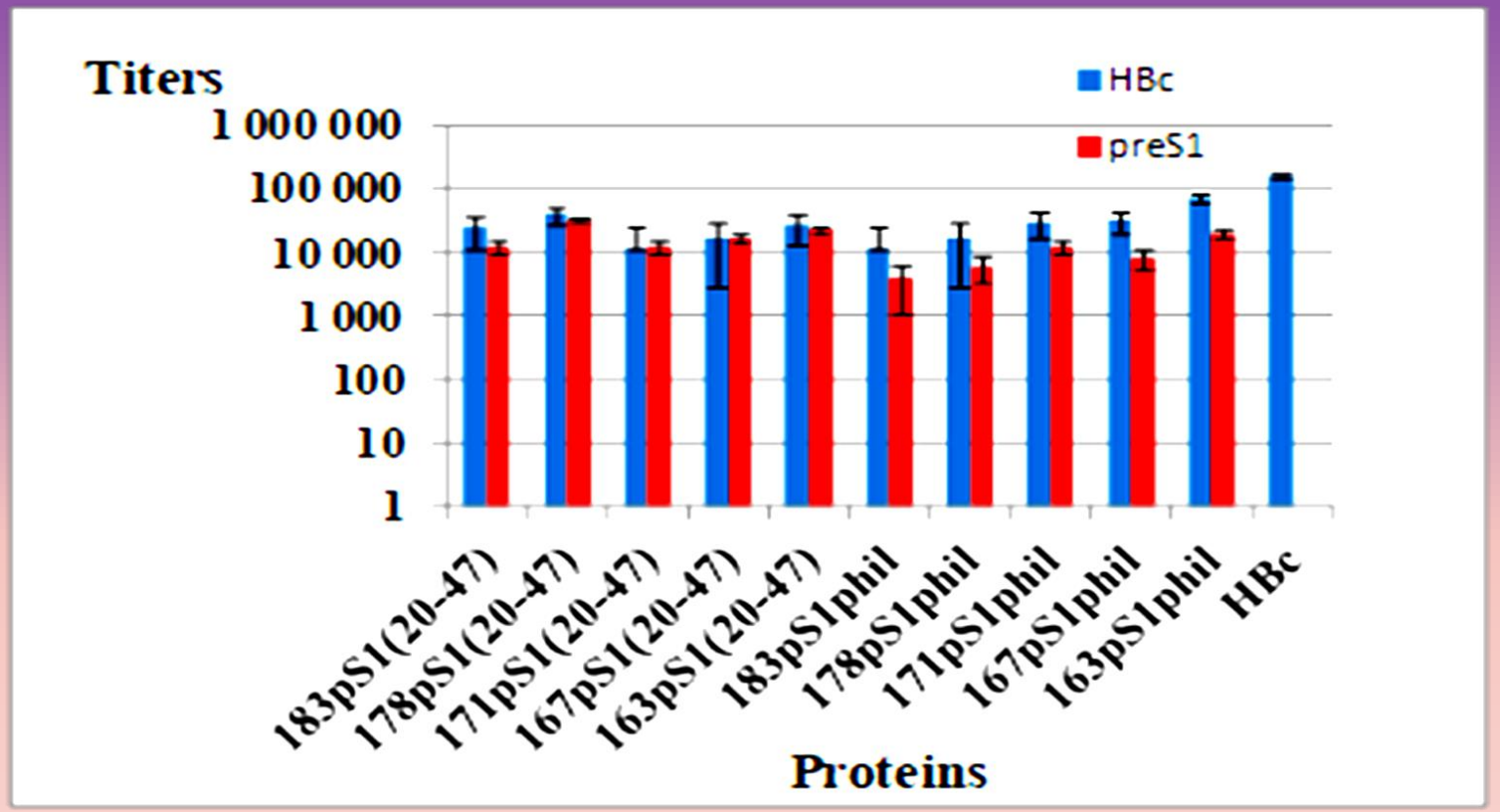


Figure 6. Anti-HBc and anti-preS1 levels in BALB/c mice immunized with HBc-preS1 VLPs designed in the study. Antibody titers average for the sera from five animals are shown. The recombinant full-length HBc183 protein (blue columns) and synthetic preS1 peptide (20-47) (red columns) were used to coat the solid phase.

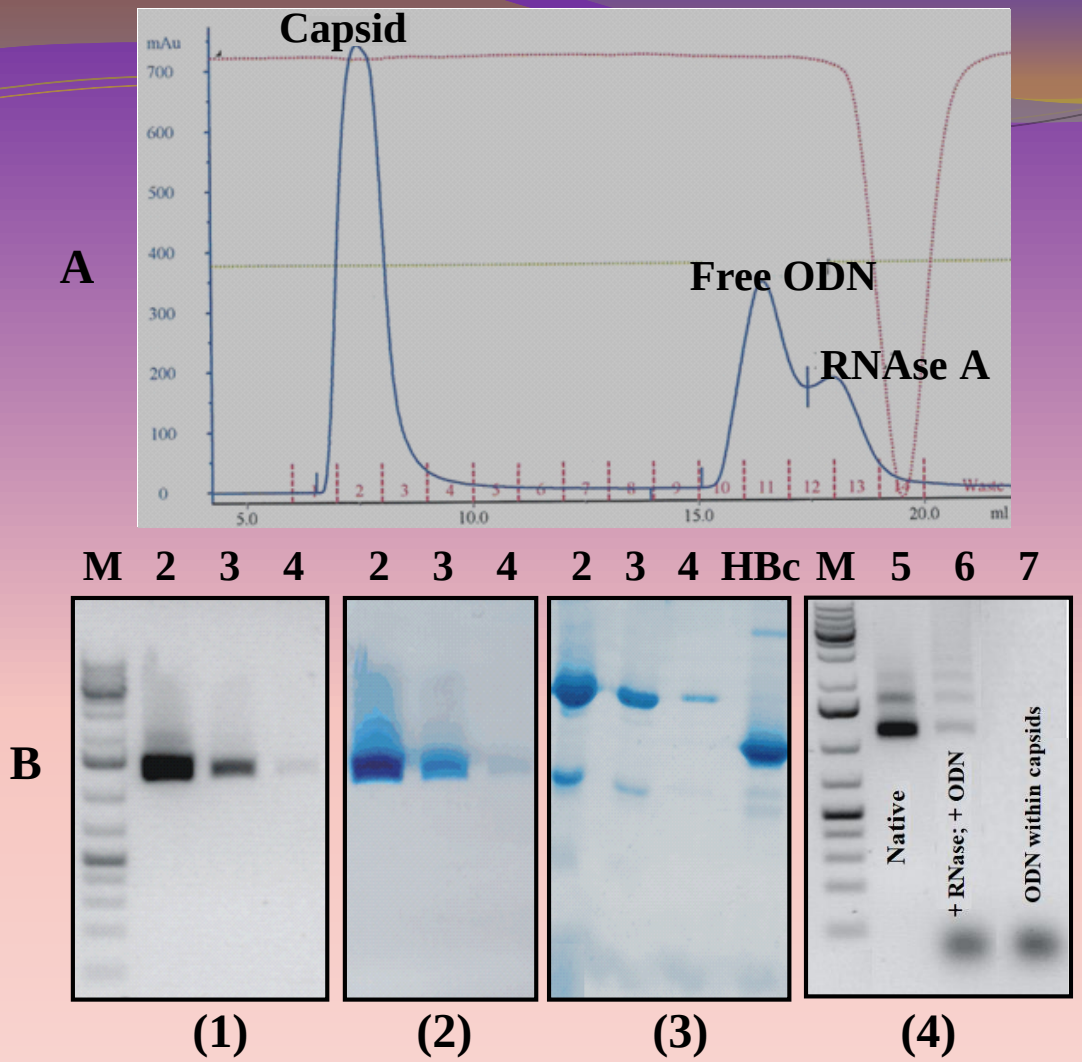


Figure 7. **ODN 1668 packaging into 183pS1(20-47) VLPs.** — GF analysis of 183pS1(20-47) VLPs after RNaseA treatment and incubation with ODN 1668; **(b)** — **1, 2** – Analysis of capsid-containing fractions (2, 3 and 4) by NAGE **1** – stained with EtBr; **2** – stained with Coomassie Brilliant Blue G250; **3** – SDS-PAGE of capsid-containing fractions stained with Coomassie Brilliant Blue G250. **4** – NAGE, stained with EtBr: line **5** – VLPs after purification, line **6** – VLPs after RNase-A treatment and in the presence of ODN 1668, line **7** – VLPs with packaged ODN 1668, 1**M** – GeneRuller 1 kb DNA Ladder (SM1331, Thermo Scientific™).

Conclusion

In summary, this study shows that HBc-preS1 VLPs can be regarded as prototypes for a long-awaited prophylactic/therapeutic hepatitis B vaccine because they can induce antibodies and package foreign substances.

The paper “Characterization of bacterially expressed chimeric HBc-preS1 VLPs” by A. Dishlers, I. Petrovskis, D. Skrastina, I. Berza, I. Lieknina , J. Jansons, I. Akopjana, J. Zakova, V. Ose, I. Sominskaya was submitted to *Microorganisms*.

Thank you for attention!