



4th International Electronic Conference on Medicinal Chemistry

1-30 November 2018

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by



pharmaceuticals

Conformational changes of secondary and tertiary structures of interferon α -2b under the influence of oligoribonucleotides- based drugs

**Roman Nikolaiev^{1*}, Maryna Vivcharyk¹, Svitlana Levchenko¹, Svitlana Chernykh¹,
Nataliya Obernikhina², Zenovii Tkachuk¹**

¹ Institute of Molecular Biology and Genetics of NASU, 150, Ac. Zabolotnogo St., Kyiv, Ukraine, 03680

² Bogomolets National Medical University, 34, Peremogy Ave., Kyiv, Ukraine, 03055

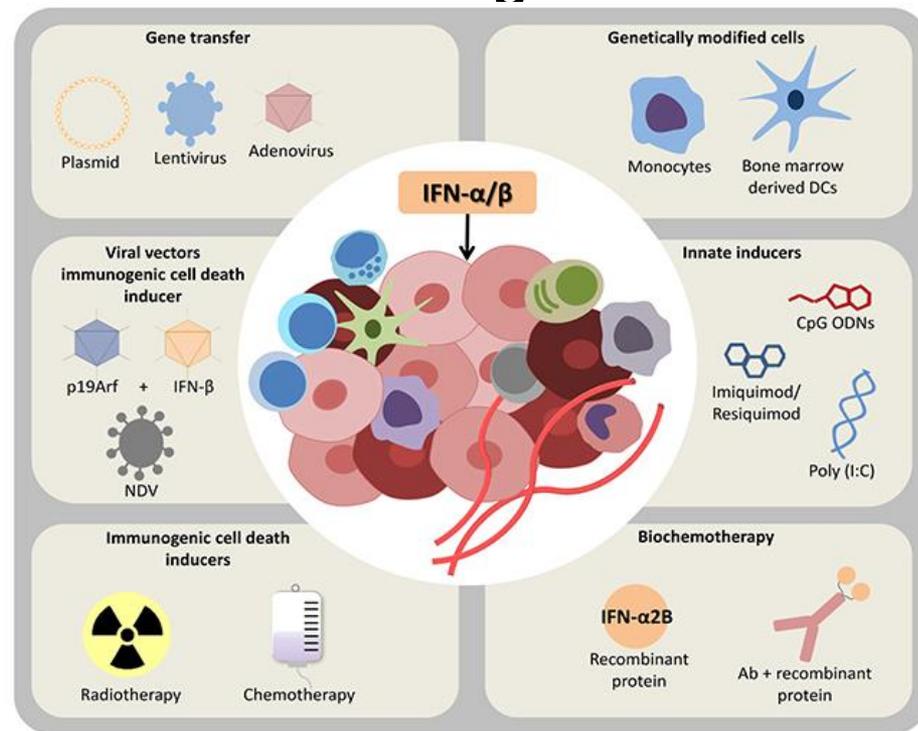


* romanfromukrain@gmail.com

Conformational changes of secondary and tertiary structures of interferon α -2b under the influence of oligoribonucleotides-based drugs

ORNs and ORNs-D-mannitol complexes led to an increase of thermal stabilization of interferon, to a decrease of α -helix components in the protein structure, and to an increase of antiparallel β -strand, β -turn, and random coil components.

The results of this research demonstrated that the addition of oligoribonucleotides-D-mannitol changed the architecture of the protein from the 2-layer sandwich to alpha-beta complex and that ORNs-D-mannitol complexes with interferon had more binding energy than ORNs complexes.



Reference: <https://doi.org/10.18632/oncotarget.19531>



Abstract: At this stage of our investigation, we studied the ability of oligoribonucleotides from total yeast RNA (ORNs) and oligoribonucleotides-D-mannitol complexes to affect the conformation and stability of interferon (IFN) α -2b – a key protein of the antiviral cell defense mechanism.

Obtained thermal denaturation profiles of IFN α -2b alone and in the presence of ORNs and ORNs-D-mannitol complexes show that the addition of these ligands led to an increase of thermal stabilization of protein of 2 and 1.8 °C respectively. The dissociation constant between INF and total yeast ORNs was $K_d = 2.88 \pm 1.14 \mu\text{M}$ and between INF and ORNs with D-mannitol – $K_d = 0,92 \pm 0.23 \mu\text{M}$.

The analysis of IFN secondary structure changes by Bestsel shows that addition of ORNs and ORNs-D-mannitol complexes led to a decrease of α -helix components in the protein structure and to an increase of antiparallel β -stand, β -turn, and random coil components. At the same time, the analysis of the tertiary structure shows that adding ORNs-D-mannitol changes the architecture of the protein from the 2-layer sandwich to alpha-beta complex. On the other hand, adding ORNs did not cause any change in the tertiary structure.

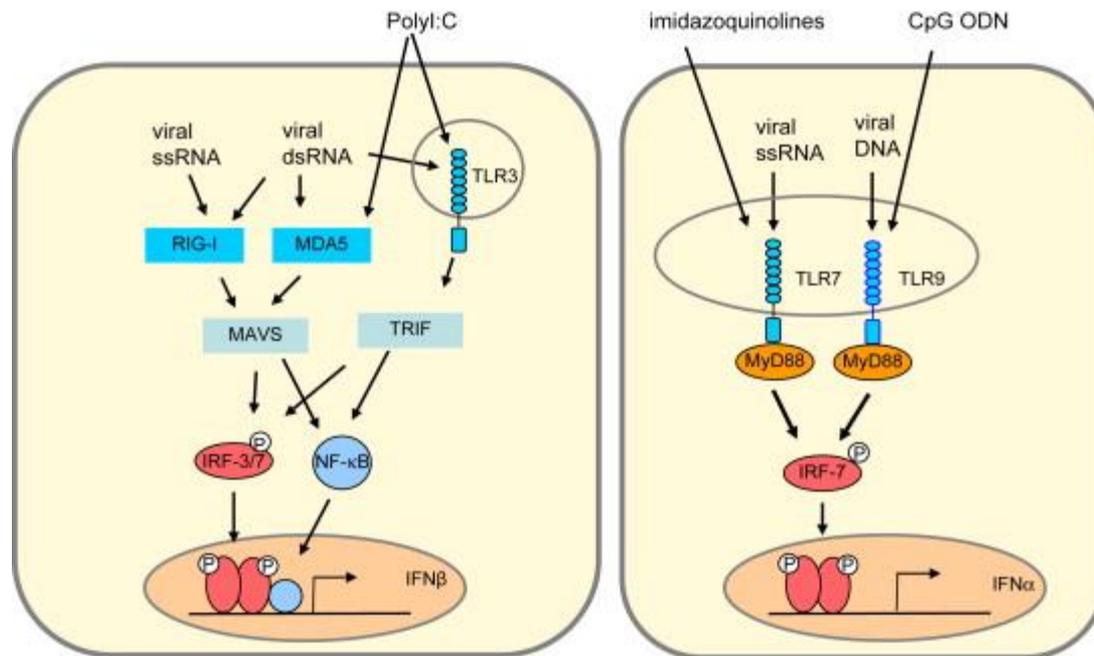
Keywords: Oligonucleotides; interferon; mannitol; secondary structure



Introduction

Reference:

<https://doi.org/10.1016/j.fsi.2018.02.004>

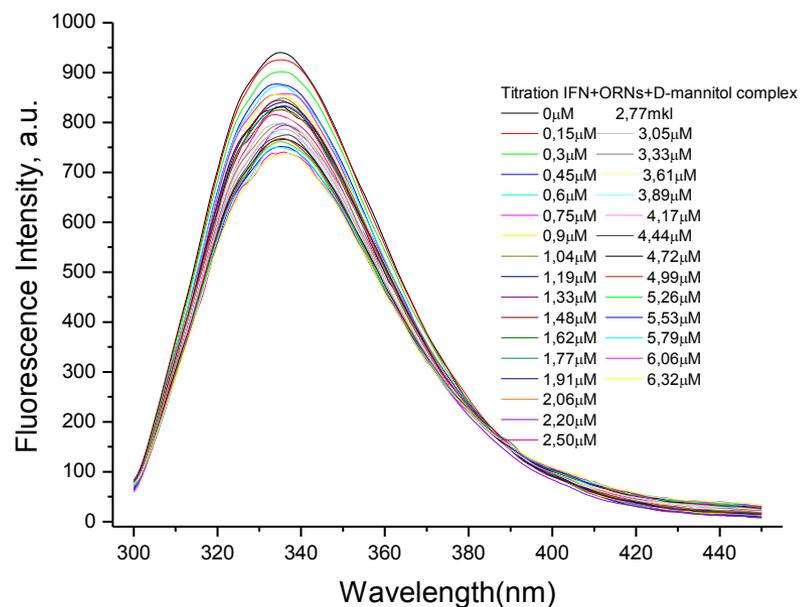
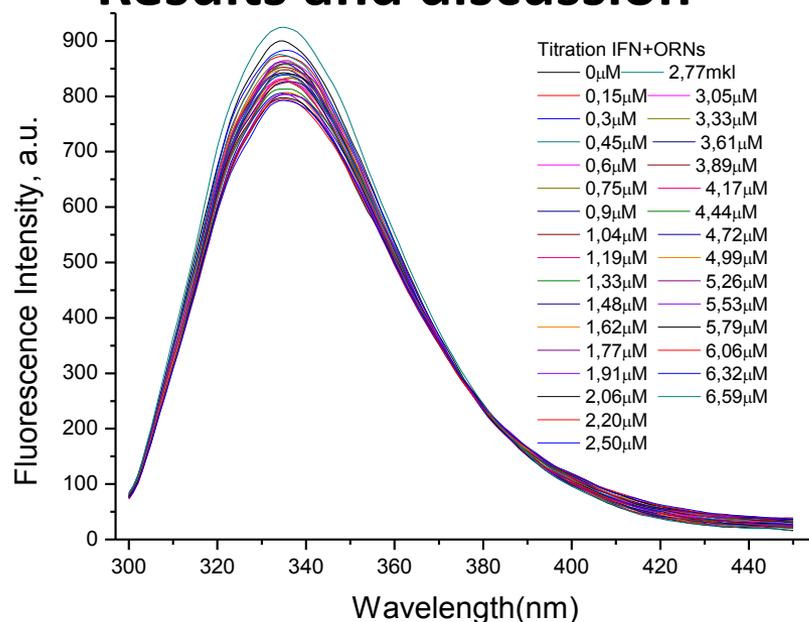


Oligonucleotides antiviral drugs have been actively implemented in medicine during the last decades nevertheless the molecular mechanism of their action is still unclear. As it was shown in our previous work, the combination of oligonucleotides with alcohol sugar D-mannitol leads to changes in their biological activity and efficiency.

It is known that ORNs-based drug increases interferon production and stimulates non-specific antiviral protection but the molecular mechanism of its action is still unclear. In our research we studied the ability of ORNs and complex of ORNs with alcohol sugar – D-mannitol to affect the conformation of interferon α -2b – a key protein of the antiviral cell defense mechanism.



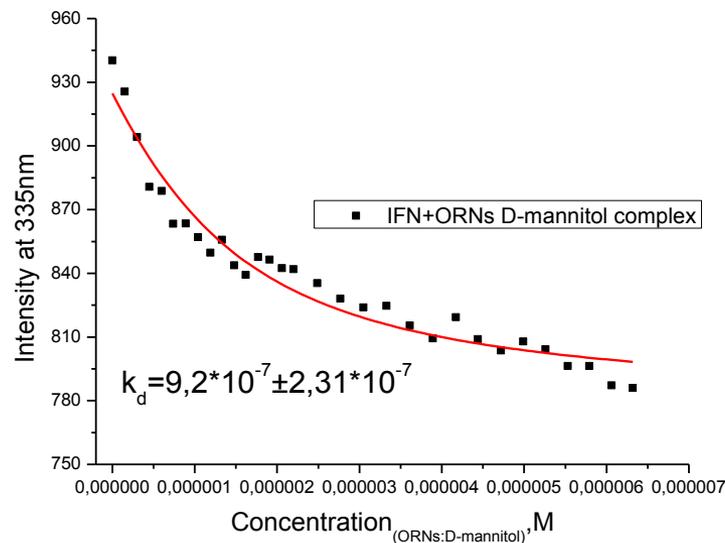
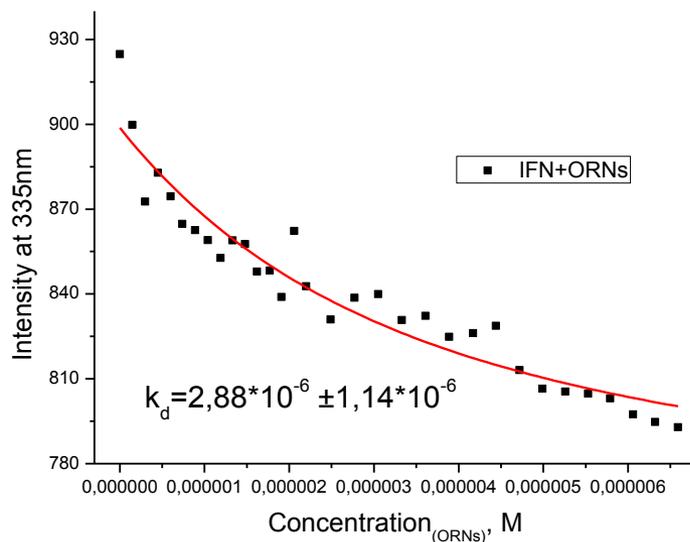
Results and discussion



To study the effects of ORNs on conformation and protein activity, we used the fluorescence spectroscopy method. On this slide the spectrum of interferon (upper black line) and spectra obtained by titration of Interferon by ORNs and ORNs complexed with D-mannitol are displayed. The fluorescence spectrum of the protein exhibits one peak at 336 nm, which is a feature of the proteins of this type. Initially, the spectra of the interferon IFN and ORNs or ORNs:D-mannitol complex were subtracted from the spectra of ORNs or ORNs:D-mannitol, with the same concentrations in the buffer.



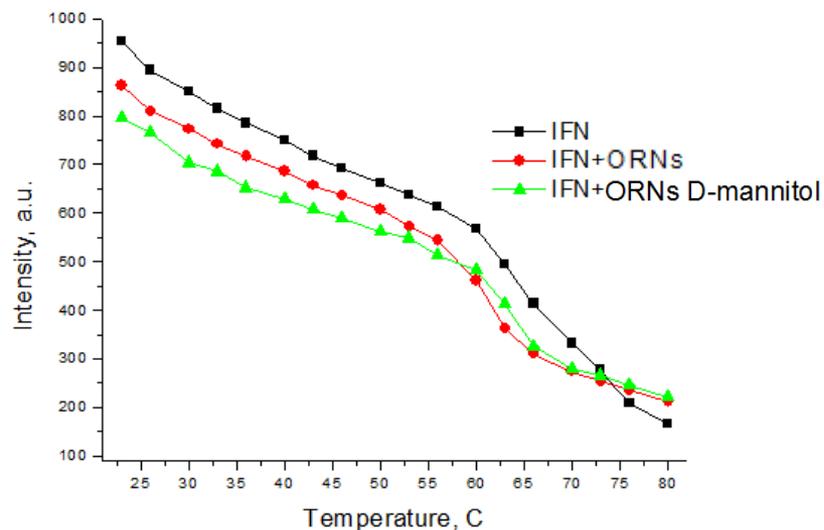
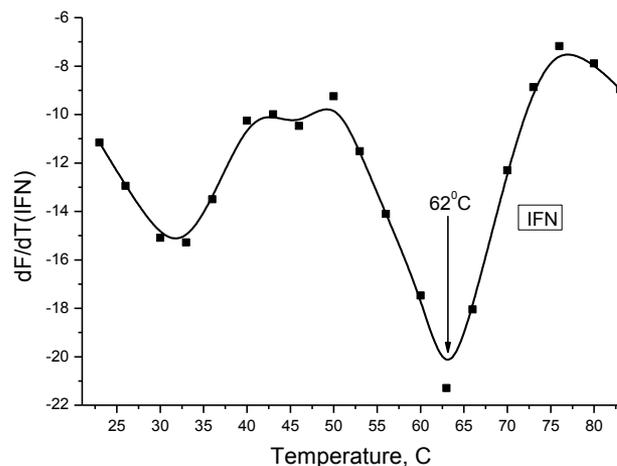
Results and discussion



During the course of this study, we calculated the protein binding constant of the ligands. It has been established that the binding constant for interferon α -2b with ORN is in an order of magnitude different from the binding constant in the interaction of interferon with the ORNs:D-mannitol complex, $K_d = 2,88 \cdot 10^{-6} \pm 1,14 \cdot 10^{-6}$ in the case of Interferon α -2b-ORNs and $K_d = 9,2 \cdot 10^{-7} \pm 2,31 \cdot 10^{-7}$ in the case of Interferon α -2b-ORNs:D-mannitol complex. The obtained results can indicate the interaction between ORNs / ORNs:D-mannitol and the protein. This assumption is confirmed by the calculation of the dissociation constants.



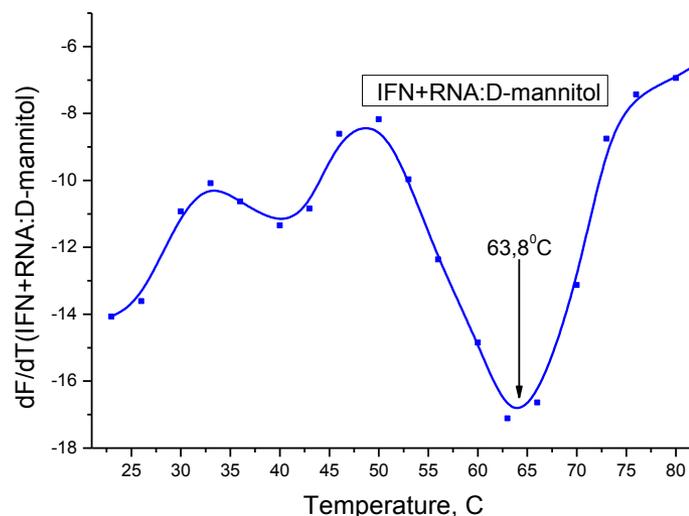
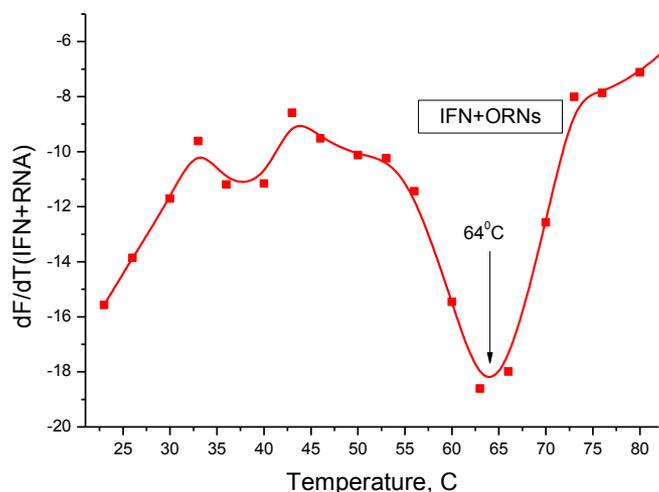
Results and discussion



To confirm the effect of ORNs-based preparations on the conformation and stability of the protein, we analyzed the thermal stability of the protein. Interferon fluorescence spectra of ORNs and interferon with ORNs: D-mannitol were measured in the temperature range of 23-80°C, increasing the temperature every 3 °C.



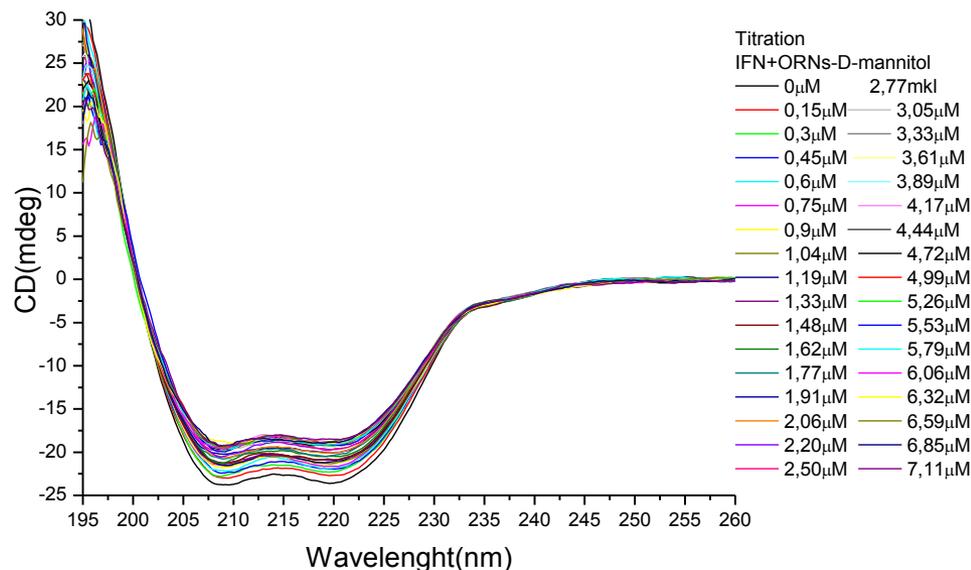
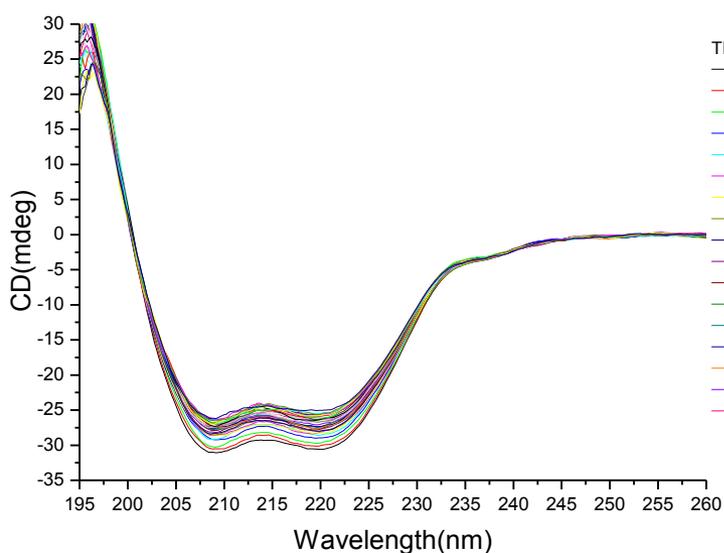
Results and discussion



During the work, it was found that the melting point of the protein, when added to the titrants (as ORNs and the ORNs:D-mannitol complex), is shifted towards higher temperatures of 64 and 63.8°C, in contrast to the melting point of the protein itself of 62°C, as evidenced by a slight stabilization of the protein. This may serve as another proof that the corresponding ligands bind to the protein and affect its conformation and activity.



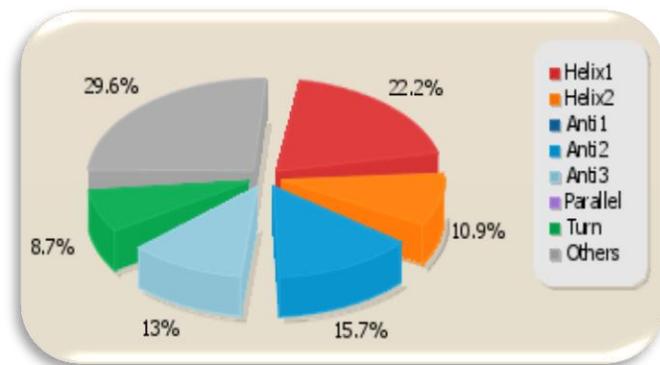
Results and discussion



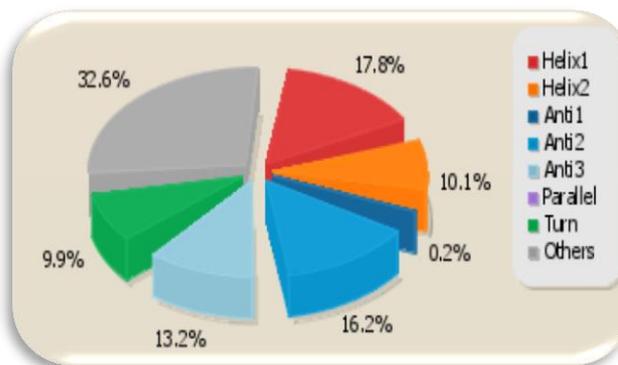
From the spectra of IFN + ORNs / ORNs:D-Mannitol in the buffer, we subtracted the ORNs / ORNs:D-mannitol spectra. Next, units of the CD spectra [mdeg] were recalculated in the units of molecular ellipticity, taking into account the concentration and the path length (1 cm).



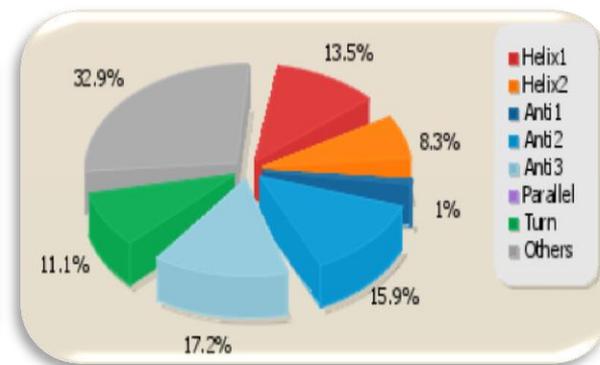
Results and discussion



INF



INF+ORNs



INF+ORNs+D-mannitol

Note: Helix1 - regular; Helix2 - distorted; Anti1 - left-twisted β -stand; Anti2 - relaxed; Anti3 - right-twisted

The analysis of IFN secondary structure changes by Bestsel shows that addition of ORNs and ORNs:D-mannitol complex led to a decrease of α -helix components in the protein structure and an increase of antiparallel β -stand, β -turn, and random coil components. At the same time, the analysis of the tertiary structure shows that adding ORNs:D-mannitol changes the architecture of the protein from the 2-layer sandwich to alpha-beta complex. On the other hand, adding ORNs did not cause any change in the tertiary structure.

References: Micsonai et al. Nucleic Acids Res. 46:W315-22 (2018), Micsonai et al. PNAS 112:E3095-103 (2015)



Conclusions

1. ORNs and ORNs:D-mannitol complexes increase the thermal stabilization of interferon.
2. ORNs:D-mannitol complexes with interferon have more binding energy than ORNs complexes.
3. ORNs and ORNs:D-mannitol complexes led to a decrease of α -helix components in the protein structure and an increase of antiparallel β -stand, β -turn, and random coil components.
4. The addition ORNs:D-mannitol, in contrast to ORNs. Changes the architecture of the tertiary structure of the INF from the 2-layer sandwich to alpha-beta complex.

