

Insights into SARS-CoV-2 nucleocapsid protein and its interaction with polyanions

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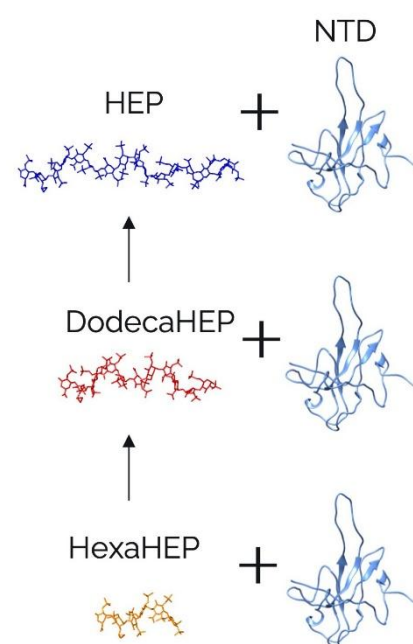
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The nucleocapsid (N) protein from SARS-CoV-2, a multidomain protein characterized by a flexible and heterogeneous structure, plays a central role in the virus life cycle. The protein is organized in two main globular domains: the N-terminal domain (NTD), which recognizes and binds RNA, and the C-terminal domain (CTD), which promotes dimer formation. These structured regions are flanked by three intrinsically disordered regions that enhance molecular flexibility.

In this work we decided to focus on the N-Terminal Domain (NTD) and to investigate the interaction with polyanionic ligands through NMR spectroscopy. Heparin, one of the most negatively charged molecules of natural origin, was selected as a possible ligand to interfere with the interaction with RNA. Moreover, recent studies demonstrated that the N protein can interact with heparan sulfate, naturally present on cell membranes. This interaction exacerbates COVID-19 damage on patients [1]. We investigated how the length and complexity of the heparin-based ligands influence the interactions with NTD. Interactions were examined through NMR titrations using different fragments of enoxaparin, starting from a small heparin fragment, such as hexamer (HexaHEP), progressing to the dodecamer (DodecaHEP), and then continuing with enoxaparin (HEP), which contains a mixture of heparins of varying lengths, with the main polymer consisting of sixteen repeating units. This allowed us to assess how the length and structural complexity of the ligand influences interaction and binding affinity at atomic level [2].

By exploring this aspect, we gain deeper insights into the mechanisms of N protein-ligand interactions.



[1] Fahoum J, Billan M, Varga JK, et al. Transfer of SARS-CoV-2 nucleocapsid protein to uninfected epithelial cells induces antibody-mediated complement deposition. *Cell Rep.* 2025;44(5):115512. [10.1016/j.celrep.2025.115512](https://doi.org/10.1016/j.celrep.2025.115512)

[2] Bolognesi T., Schiavina M., Ciabini C., Paraforiti M., Gardini C., Elli S., Guerrini M., Pierattelli R., Felli I.C., 2025. Exploring the role of structural and dynamic complexity in SARS-CoV-2 nucleocapsid protein-heparin interactions by NMR. *J. Mol. Biol.*, in press, 169437. <https://doi.org/10.1016/j.jmb.2025.169437>

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