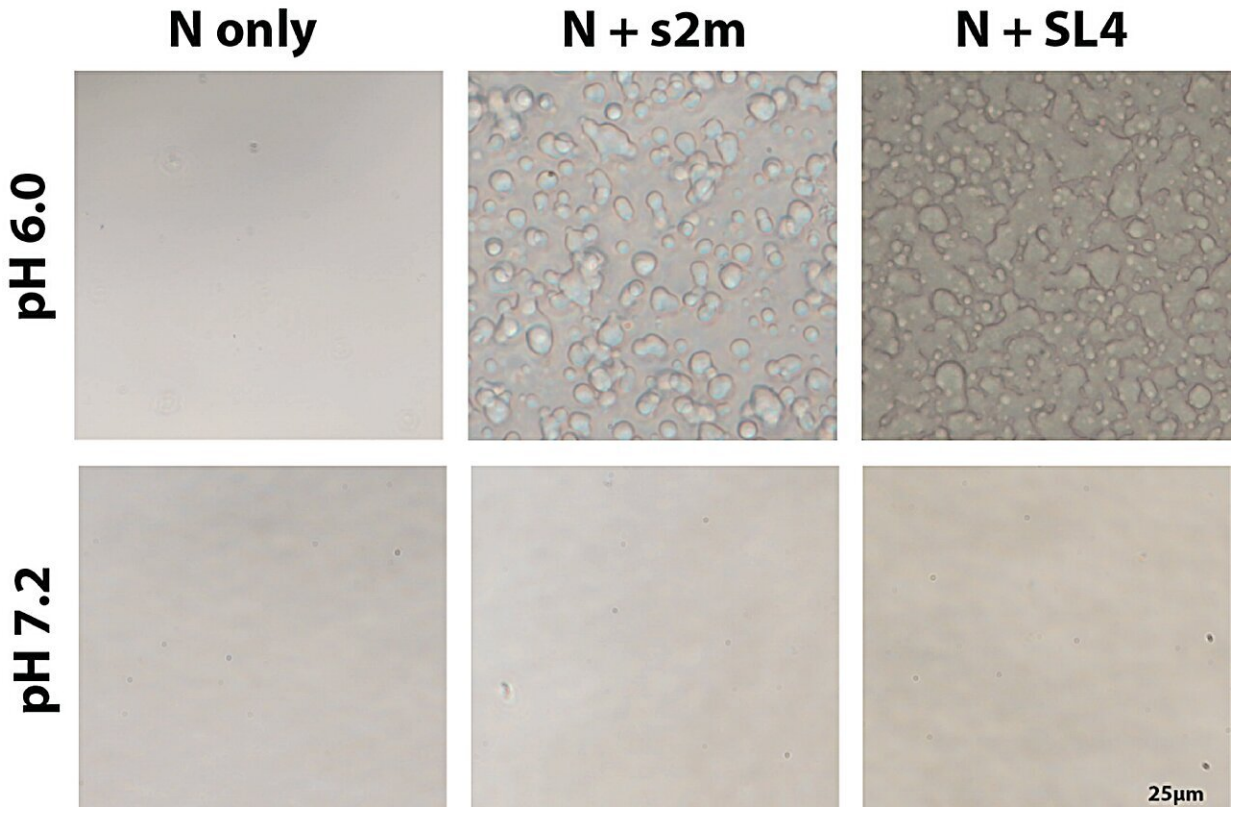
Scientists report that buffer and pH strongly affect the phase separation of SARS-CoV-2 N protein

April 8 2024, by Dominic Dähler



Credit: *Molecular Biology of the Cell* (2024). DOI: 10.1091/mbc.E23-12-0500

In a new paper published in *Molecular Biology of the Cell*, the Allain lab (IBC) reported that the phase separation of the SARS-CoV-2 N protein strongly depends on the chosen buffer and pH. For example, the protonation of a single histidine side chain makes the difference if the protein phase separates or not.

The SARS-CoV-2 structural nucleocapsid (N) protein is involved in viral genome packaging and was reported in the literature to undergo phase separation in the presence of RNA. Using a phosphate buffer not used in the published experiments, the researchers were puzzled that they could not reproduce phase separation at pH 7.2 but only at non-physiological pH 6.

They showed that the difference in phase separation behavior at these two pH values in phosphate buffer is due to the protonation state of a single histidine side chain.

They then extended their experiments to buffers used in the published literature describing phase separation of the N protein. They found that some buffers directly interact with the N protein and that the choice of buffer affects the protein's net charge at pH 7.2, also providing a rationale as to why different RNA amounts were needed to affect phase separation behavior at this pH.

The results show that buffer and pH choice must be considered together, especially when drawing conclusions from in vitro experiments regarding the situation in the cell. Regarding the initial experiment, where a lower pH was required to induce phase separation, it is interesting to note that viral infections can lead to a decrease in cellular pH, which might help then viral replication by facilitating phase separation of the N protein.

More information: Nina C. Kathe et al, Buffer Choice and pH Strongly Influence Phase Separation of SARS-CoV-2 Nucleocapsid with RNA, *Molecular Biology of the Cell* (2024). DOI: 10.1091/mbc.E23-12-0500

Provided by ETH Zurich