

Parent topic: Protein life cycle: from structure to function

Topic: Drug development

Expression and NMR characterization of labelled P-domains of emerging Norovirus

Siyu Lin^{* 1, 2}, Henry Flatau³, Ileana Zeravica¹, Rebecca Calamandrei^{1, 2}, Linda Cerofolini^{1, 2}, Jesús Angulo³, Cristina Nativi¹, Marco Fragai^{1, 2}

¹Department of Chemistry, University of Florence, ²Magnetic Resonance Center CERM, Florence, Italy, ³Consejo Superior de Investigaciones Científicas, Seville, Spain

Rationale: Noroviruses are members of the Caliciviridae family and are non-enveloped, single-stranded, positive-sense RNA viruses. Norovirus is the major cause of acute gastroenteritis, particularly affecting vulnerable populations such as children, the elderly, and immunocompromised individuals. With millions of illnesses and thousands of deaths yearly, norovirus poses a significant global health burden. The economic impact is equally staggering, with billions of dollars in costs annually. Additionally, new virus strains emerge every 2-5 years, making outbreaks difficult to contain. Among the various norovirus strains, the GII.4 variant is the most prevalent globally. The ability of norovirus to infect host cells depends on the interaction between the Protruding domain (P domain) of its capsid protein VP1 and Histo-Blood Group Antigens (HBGAs) on the surface of host cells. Furthermore, previous cell-based studies found, that Human Milk Oligosaccharides (HMOs) prevent norovirus infection to cells, by acting as a decoy receptor and blocking the interaction between HBGAs and human noroviruses. Therefore, understanding the structure of the P domain is essential for the development of effective antiviral drugs and vaccines.

Methods: In this study, the P domain of the GII.4. P16 norovirus strain was successfully expressed and purified from *E. coli*. To enhance NMR signal resolution, isotopically labeled samples (¹⁵N, ²H, and ¹³C) were prepared.

Multidimensional NMR experiments, along with chemical shift perturbation (CSP)-based NMR experiments, were conducted to investigate the binding of the P domain to HBGAs and antiviral compounds, including HMO analogs.

Results: The NMR experiments will facilitate the identification of key amino acid residues in the P domain that are critical for binding interactions with HBGAs and other antiviral agents. The insights gained from these studies are anticipated to enhance the understanding of norovirus infection mechanisms.

Conclusions: This research provides essential information about the interaction between norovirus and host cell receptors, which is important for developing new therapeutic strategies. The findings could lead to the identification of novel antiviral drugs and contribute to vaccines aimed at combating norovirus infections.