Antiviral Research 216 (2023) 105663

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Structural basis for RNA-cap recognition and methylation by the mpox methyltransferase VP39

Petr Skvara, Dominika Chalupska, Martin Klima, Jan Kozic, Jan Silhan, Evzen Boura *

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, V.v.i, Flemingovo nám. 2, 16610, Prague 6, Czech Republic

A B S T R A C T

Mpox is a zoonotic disease caused by the mpox virus (MPXV), which has gained attention due to its rapid and widespread transmission, with reports from more than 100 countries. The virus belongs to the Orthopoxvirus genus, which also includes variola virus and vaccinia virus. In poxviruses, the RNA cap is crucial for the translation and stability of viral mRNAs and also for immune evasion. This study presents the crystal structure of the mpox 2′-O-methyltransfarase VP39 in complex with a short cap-0 RNA. The RNA substrate binds to the protein without causing any significant changes to its overall fold and is held in place by a combination of electrostatic interactions, π-π stacking and hydrogen bonding. The structure also explains the mpox VP39 preference for a guanine base at the first position; it reveals that guanine forms a hydrogen bond that an adenine would not be able to form.

Mpox is a zoonotic disease caused by the mpox virus (MPXV), which presents as a milder form of smallpox with a previously estimated fatality rate of 2%–7% (Li et al., 2023). In recent years, an outbreak of mpox disease has gained attention from the World Health Organization and Centers for Disease Control and Prevention due to its rapid and widespread transmission. Mpox has now been reported in more than 100 countries, prompting concerns about its potential for a pandemic (Kmiec and Kirchhoff, 2022; Yang, 2022). However, the fatality rate of the current outbreak appears to be much lower, estimated at around 0.1% (Saghazadeh and Rezaei, 2023; (CDC) C. f. D.C.a.P. Mpox, 2022).

Mpox virus is a member of the *Orthopoxvirus* genus, which also includes variola virus and vaccinia virus (Shchelkunova and Shchelkunov, 2022). The virus consists of enveloped virion with an encapsulated dsDNA genome, which replicates in the host cytoplasm. This replication strategy involves the synthesis of viral proteins that are analogous to host nuclear proteins, such as DNA-dependent DNA polymerase, DNA-dependent RNA polymerase, and RNA processing machinery. In fact, RNA capping was discovered, at least in part, thanks to antiviral research (Furuichi et al., 1975; Shuman and Hurwitz, 1981). In poxviruses, the RNA cap, specifically the m7GpppN cap (cap-0), is crucial for the translation and stability of viral mRNAs (Shuman, 2015; Meade et al., 2019; Grimm et al., 2022; Decroly et al., 2011). Moreover, the formation of cap-1 via 2′-O-methylation allows poxviruses to evade innate immunity (Meade et al., 2019), as non-methylated RNAs are detected by cellular RIG-I and IFIT sensory proteins (Zust et al., 2011; Hyde and Diamond, 2015; Daffis et al., 2010; Russ et al., 2022). Understanding the molecular mechanisms of poxvirus replication and evasion of host immune responses is essential for developing effective therapies and vaccines.

In a recent study, we structurally characterized the mpox virus 2′-O-methyltransfarase (MTase) VP39 in complex with the natural pan-MTase inhibitor sinefungin, and we developed sub-micromolar inhibitors of this enzyme (Silhan et al., 2023). This MTase, akin to the majority of MTases, uses S-adenosylmethionine (SAM) as the methyl group donor. It catalyzes the transfer of the methyl group from SAM to the 2′-OH group of the ribose ring of cap-0, thereby generating cap-1. Consequently, SAM undergoes conversion to S-adenosylhomocysteine (SAH) as a byproduct of the reaction. In this study, we focused on the interaction of VP39 with cap-0 RNA, which plays a crucial role in viral replication and immune evasion. To study this interaction, we prepared a short RNA substrate (sequence m7GpppGAAAAA, detailed in the SI) and used it for crystallization trials in complex with recombinant VP39 at a molar ratio of 2:1, supplemented with 2 mM S-adenosylhomocysteine (SAH). We successfully obtained crystals that belonged to the orthorhombic space group $P2_12_12_1$ and diffracted to a resolution of 2.1 Å. The structure was solved by molecular replacement using the structure of SAH-bound VP39 as a search model. The crystals contained two VP39 molecules in the asymmetric unit and the RNA was well visible in one of them (SI Fig. 1). The structure was refined to good R-factors (R_{work} = 21.5% and R_{free} = 25.2%) and geometry (SI Table 1) and deposited in the Protein Data Band under PDB accession code: 8OIV.

The binding of RNA to the protein did not result in any significant changes to its overall fold (Fig. 1A) and the position of the RNA observed in our study was consistent with what was previously reported for the

* Corresponding author.
E-mail address: boura@uochb.cas.cz (E. Boura).

https://doi.org/10.1016/j.antiviral.2023.105663
Received 27 April 2023; Received in revised form 30 June 2023; Accepted 3 July 2023
Available online 7 July 2023

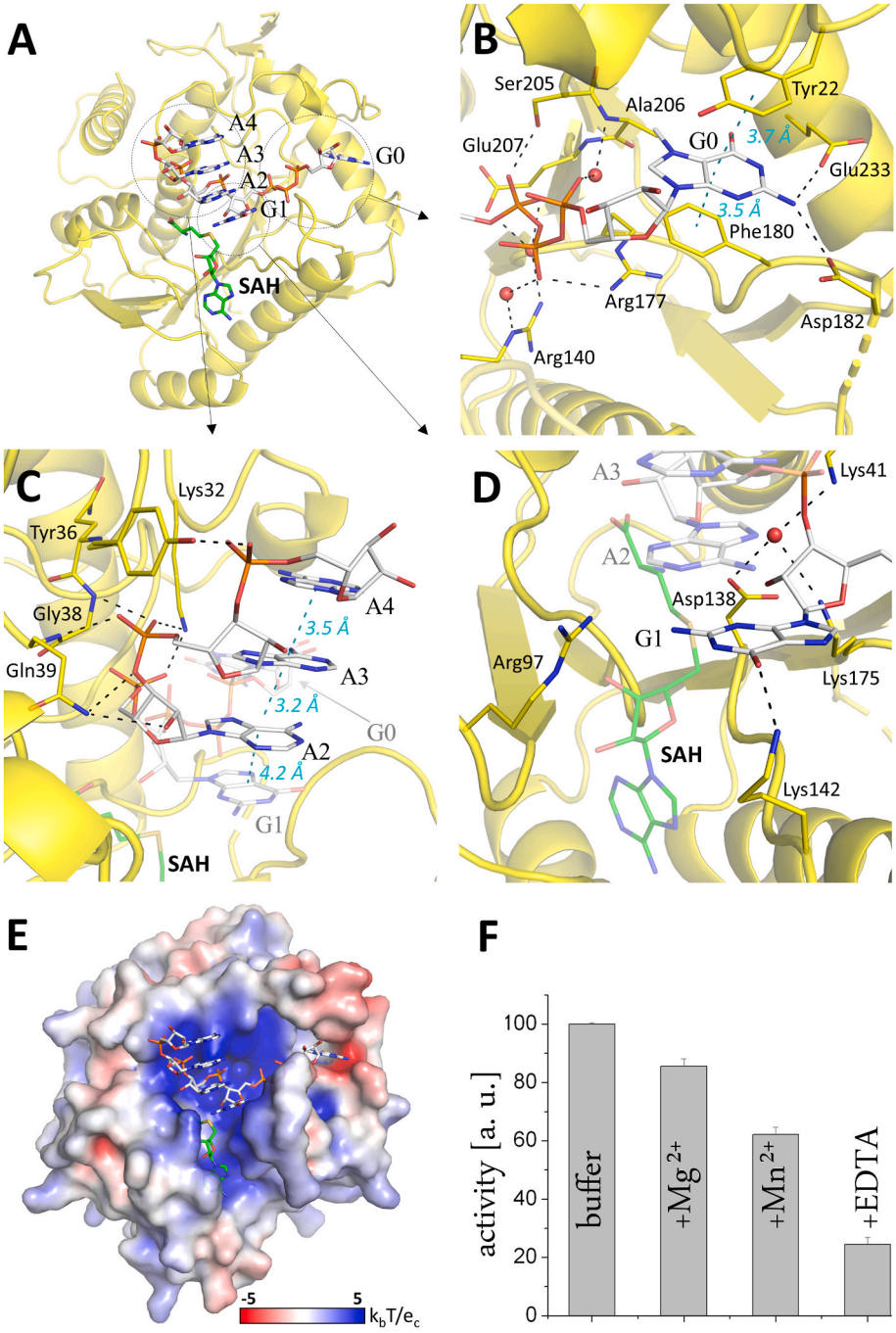

Fig. 1. Structure of mpox VP39 in complex with cap-0 RNA A) The overall structure shows VP39 in yellow cartoon, SAH and RNA in stick representation, with all atoms colored as usual except for SAH carbons, which are in green, and RNA carbons, which are in white. B-D) Zoomed views of important regions of the capped RNA molecule are shown. Selected water molecules are shown as red spheres, hydrogen bonds as black dashed lines, and distances between bases as teal dashed lines. E) Surface representation of VP39 colored according to its electrostatic potential. F) Enzymatic activity of the recombinant VP39 comparing the effects of Mg^{2+}, Mn^{2+} and EDTA.

vaccinia virus VP39 (Hodel et al., 1998). The RNA substrate was positioned such that the very 5′ end of the RNA, the base of the cap (m7G, referred to as G0), was sandwiched between two aromatic side chains, Tyr22 and Phe180. These two residues stabilized G0 base through a face-to-face π-π stacking interaction (Fig. 1B). The usual distance for π-π stacking is between 3.3 and 3.8 Å (Janiak, 2000) and the observed distance here is 3.5 Å. The amino group of G0 base forms hydrogen bonds with the sidechains of Asp182 and Glu233 while sidechains of Arg177 and Ser205 interact directly with the triphosphate bridge, while Ala206, Arg140 and Glu207 form water bridges with this triphosphate bridge (Fig. 1B).

We observed clear electron density for the next four nucleotides (hereafter referred to as G1, A2, A3 and A4) (SI Fig. 1). The individual bases are positioned above each other with near-perfect distances to enable π-π stacking interaction (Fig. 1C). Additionally, the phosphate backbone is stabilized by hydrogen bonding with Lys32, Tyr36, Gly38, and Gln39 (Fig. 1C). Notably, the bases do not form hydrogen bonds with the protein, explaining the sequence unspecificity of poxviral VP39 enzymes (Hodel et al., 1998). However, G1 stands out as its oxygen atom, situated on the purine ring, functions as an acceptor of hydrogen from the adjacentLys142 (Fig. 1D). This explains why mpox VP39 shows a preference for a guanine base at this position (Silhan et al., 2023); adenine would not be able to serve as hydrogen acceptor here. The 2′-OH group of the G1 ribose ring, which would be methylated by VP39 if SAM were present, is 2.8 Å away from a water molecule that most probably represents the catalytic water. It is approximately in the center of the catalytic tetrad, forming hydrogen bonds with Lys41, Asp138 and Lys175 but not with Glu207 (Fig. 1D). Interestingly, this catalytic tetrad is conserved even among unrelated viruses such as are the *Poxviridae*, *Flaviviridae* and *Coronaviridae* (Nencka et al., 2022; Hercik et al., 2017;

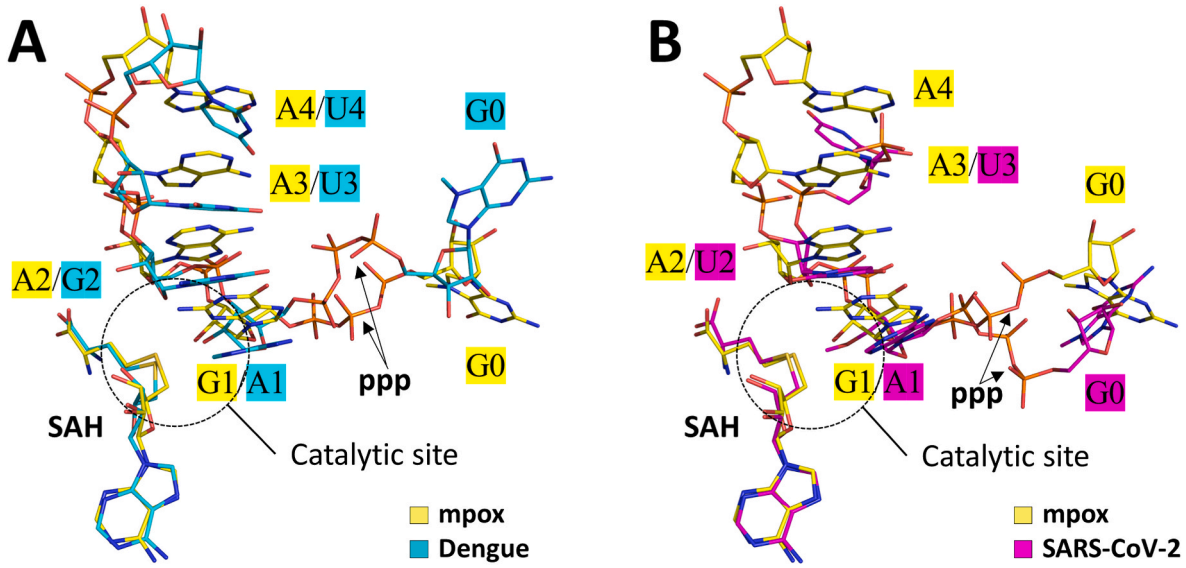

Fig. 2. A structural comparison of capped RNA molecules crystallized with their respective 2′-O-MTase. A) Comparison of mpox and Dengue. B) Comparison of mpox and SARS-CoV-2. Carbon atoms are colored according to their virus: mpox (PDB code 8OIV) in yellow, Dengue in cyan (PDB code 5DTO), and SARS-CoV-2 in purple (PDB code 7L6T).

Krafcikova et al., 2020).

Apart from these specific interactions, the RNA is also held in place by electrostatic interactions. The capped RNA binding site and the SAM binding pocket are both highly positively charged, as evident from the surface coloring of VP39 by electrostatic potential (Fig. 1E). We compared the overall structure of the mpox capped RNA with the structure of RNA from other viruses, including Dengue and SARS-CoV-2, which were crystallized with the respective 2′-O-MTases (Zhao et al., 2015; Minasov et al., 2021). The alignment showed that, although the 2′-OH group of the ribose ring that is methylated must be in close proximity to the methyl donor SAM (Fig. 2), the position of the 7 mG base differed in each case. Surprisingly, the positions of the subsequent nucleotides were very similar for mpox and Dengue, but not for SARS-CoV-2. Another significant difference is that SARS-CoV-2 nsp16 2′-O-MTase requires a metal atom (Mg^{2+} or Mn^{2+}) (Minasov et al., 2021) to position the RNA correctly, whereas we could not locate any metal in our structure. However, the addition of EDTA results in a significant (~75%) inhibition of the activity of the recombinant protein (measured as previously (Silhan et al., 2023)). This finding suggests that metals are not essential for the MTase reaction catalyzed by VP39, but they do play a helpful role (Fig. 1F). Furthermore, the addition of metals (2 mM Mg^{2+} or Mn^{2+}) also demonstrates an inhibitory effect on the enzyme. This observation indicates that the recombinant enzyme retains a small amount of Mg^{2+} from bacteria, which appears to be sufficient for its function, whereas higher concentrations of Mg^{2+} are somewhat inhibitory.

Recently, especially during the COVID-19 pandemic, viral MTases attracted considerable scientific attention (Krafcikova et al., 2020; Minasov et al., 2021; Rosas-Lemus et al., 2020; Wilamowski et al., 2021; Dostalik et al., 2021; Aggarwal and Kottur, 2022; Benoni et al., 2021). These enzymes are relatively unexplored targets for antivirals, as no antiviral drug, currently on the market, targets an MTase. However, inhibitors are rapidly being developed against SARS-CoV-2 (Otava et al., 2021; Devkota et al., 2021; Bobileva et al., 2021; Klima et al., 2022), and we have recently reported the first inhibitors of mpox MTase VP39 (Silhan et al., 2023). Nevertheless, much work remains to establish whether MTases are valid targets for antivirals and to define their precise role during viral life-cycle, which could differ somewhat for each virus. Further research is needed to fully elucidate the intricacies of the virus-host interaction and to develop strategies to prevent and control the spread of mpox.

Author contribution

P.S. D.C. and J.K. performed experiments. P.S., M.K. and J.S. analyzed data. E.B. conceived the project and wrote the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available in the PDB database under the access code 8OIV.

Acknowledgment

We thank the Helmholtz-Zentrum Berlin für Materialien und Energie for the allocation of synchrotron radiation beamtime. This research was funded by the project the National Institute of Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU. RVO: 61388963 is also acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2023.105663.

References

Aggarwal, A., Kottur, J., 2022. High Resolution Structures of the SARS-CoV-2 N7-Methyltransferase Inform Therapeutic Development. Res Sq.

Benoni, R., Krafcikova, P., Baranowski, M.R., Kowalska, J., Boura, E., Cahova, H., 2021. Substrate specificity of SARS-CoV-2 nsp10-nsp16 methyltransferase. Viruses 13 (9), 1722.

Bobileva, O., Bobrovs, R., Kanepe, I., Patetko, L., Kalnins, G., Sisovs, M., Bula, A.L., Gri Nberga, S., Boroduskis, M.R., Ramata-Stunda, A., Rostoks, N., Jirgensons, A., Ta Rs, K., Jaudzems, K., 2021. Potent SARS-CoV-2 mRNA cap methyltransferase inhibitors by bioisosteric replacement of methionine in SAM cosubstrate. ACS Med. Chem. Lett. 12 (7), 1102–1107.

(CDC), C.f.D.C.a.P. Mpox, 2022. Outbreak cases and data. https://www.cdc.gov/poxvirus/mpox/response/2022/index.html.

Daffis, S., Szretter, K.J., Schriewer, J., Li, J., Youn, S., Errett, J., Lin, T.Y., Schneller, S., Zust, R., Dong, H., Thiel, V., Sen, G.C., Fensterl, V., Klimstra, W.B., Pierson, T.C., Buller, R.M., Gale Jr., M., Shi, P.Y., Diamond, M.S., 2010. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature 468 (7322), 452–456.

Decroly, E., Ferron, F., Lescar, J., Canard, B., 2011. Conventional and unconventional mechanisms for capping viral mRNA. Nat. Rev. Microbiol. 10 (1), 51–65.

Devkota, K., Schapira, M., Perveen, S., Khalili Yazdi, A., Li, F., Chau, I., Ghiabi, P., Hajian, T., Loppnau, P., Bolotokova, A., Satchell, K.J.F., Wang, K., Li, D., Liu, J., Smil, D., Luo, M., Jin, J., Fish, P.V., Brown, P.J., Vedadi, M., 2021. Probing the SAM Binding Site of SARS-CoV-2 Nsp14 in Vitro Using SAM Competitive Inhibitors Guides Developing Selective Bisubstrate Inhibitors. SLAS Discov, 24725552211026261.

Dostalik, P., Krafcikova, P., Silhan, J., Kozic, J., Chalupska, D., Chalupsky, K., Boura, E., 2021. Structural analysis of the OC43 coronavirus 2'-O-RNA methyltransferase. J. Virol. 95 (15), e0046321.

Furuichi, Y., Muthukrishnan, S., Shatkin, A.J., 1975. 5'-Terminal m7g(5')Ppp(5')Gmp invivo - identification in reovirus genome rna. Proc. Natl. Acad. Sci. U.S.A. 72 (2), 742–745.

Grimm, C., Bartuli, J., Fischer, U., 2022. Cytoplasmic gene expression: lessons from poxviruses. Trends Biochem. Sci. 47 (10), 892–902.

Hercik, K., Brynda, J., Nencka, R., Boura, E., 2017. Structural basis of Zika virus methyltransferase inhibition by sinefungin. Arch. Virol. 162 (7), 2091–2096.

Hodel, A.E., Gershon, P.D., Quiocho, F.A., 1998. Structural basis for sequence-nonspecific recognition of 5'-capped mRNA by a cap-modifying enzyme. Mol. Cell. 1 (3), 443–447.

Hyde, J.L., Diamond, M.S., 2015. Innate immune restriction and antagonism of viral RNA lacking 2-O methylation. Virology 479–480, 66–74.

Janiak, C., 2000. A critical account on pi-pi stacking in metal complexes with aromatic nitrogen-containing ligands. J. Chem. Soc. Dalton Trans. (21), 3885–3896.

Klima, M., Khalili Yazdi, A., Li, F., Chau, I., Hajian, T., Bolotokova, A., Kaniskan, H.U., Han, Y., Wang, K., Li, D., Luo, M., Jin, J., Boura, E., Vedadi, M., 2022. Crystal structure of SARS-CoV-2 nsp10-nsp16 in complex with small molecule inhibitors, SS148 and WZ16. Protein Sci. 31 (9), e4395.

Kmiec, D., Kirchhoff, F., 2022. Monkeypox: a new threat? Int. J. Mol. Sci. 23 (14).

Krafcikova, P., Silhan, J., Nencka, R., Boura, E., 2020. Structural analysis of the SARS-CoV-2 methyltransferase complex involved in RNA cap creation bound to sinefungin. Nat. Commun. 11 (1), 3717.

Li, P., Li, J., Ayada, I., Avan, A., Zheng, Q., Peppelenbosch, M.P., de Vries, A.C., Pan, Q., 2023. Clinical features, antiviral treatment and patient outcomes: a systematic review and comparative analysis of the previous and the 2022 mpox outbreaks. J. Infect. Dis..jiad034. doi: 10.1093/infdis/jiad034. Online ahead of print.

Meade, N., DiGiuseppe, S., Walsh, D., 2019. Translational control during poxvirus infection. Wiley Interdiscip Rev RNA 10 (2), e1515.

Minasov, G., Rosas-Lemus, M., Shuvalova, L., Inniss, N.L., Brunzelle, J.S., Daczkowski, C. M., Hoover, P., Mesecar, A.D., Satchell, K.J.F., 2021. Mn2+ coordinates Cap-0-RNA to align substrates for efficient 2 '-O-methyl transfer by SARS-CoV-2 nsp16. Sci. Signal. 14 (689).

Nencka, R., Silhan, J., Klima, M., Otava, T., Kocek, H., Krafcikova, P., Boura, E., 2022. Coronaviral RNA-methyltransferases: function, structure and inhibition. Nucleic Acids Res. 50 (2), 635–650.

Otava, T., Sala, M., Li, F., Fanfrlik, J., Devkota, K., Perveen, S., Chau, I., Pakarian, P., Hobza, P., Vedadi, M., Boura, E., Nencka, R., 2021. The structure-based design of SARS-CoV-2 nsp14 methyltransferase ligands yields nanomolar inhibitors. ACS Infect. Dis. 7 (8), 2214–2220.

Rosas-Lemus, M., Minasov, G., Shuvalova, L., Inniss, N.L., Kiryukhina, O., Brunzelle, J., Satchell, K.J.F., 2020. High-resolution structures of the SARS-CoV-2 2'-O-methyltransferase reveal strategies for structure-based inhibitor design. Sci. Signal. 13 (651).

Russ, A., Wittmann, S., Tsukamoto, Y., Herrmann, A., Deutschmann, J., Lagisquet, J., Ensser, A., Kato, H., Gramberg, T., 2022. Nsp16 shields SARS-CoV-2 from efficient MDA5 sensing and IFIT1-mediated restriction. EMBO Rep., e55648

Saghazadeh, A., Rezaei, N., 2023. Insights on Mpox virus infection immunopathogenesis. Rev. Med. Virol. 33 (2), e2426.

Shchelkunova, G.A., Shchelkunov, S.N., 2022. Smallpox, monkeypox and other human Orthopoxvirus infections. Viruses 15 (1).

Shuman, S., 2015. RNA capping: progress and prospects. RNA 21 (4), 735–737.

Shuman, S., Hurwitz, J., 1981. Mechanism of messenger-rna capping by vaccinia virus guanylyltransferase - characterization of an enzyme-guanylate intermediate. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences 78 (1), 187–191.

Silhan, J., Klima, M., Otava, T., Skvara, P., Chalupska, D., Chalupsky, K., Kozic, J., Nencka, R., Boura, E., 2023. Discovery and structural characterization of monkeypox virus methyltransferase VP39 inhibitors reveal similarities to SARS-CoV-2 nsp14 methyltransferase. Nat. Commun. 14 (1), 2259.

Wilamowski, M., Sherrell, D.A., Minasov, G., Kim, Y., Shuvalova, L., Lavens, A., Chard, R., Maltseva, N., Jedrzejczak, R., Rosas-Lemus, M., Saint, N., Foster, I.T., Michalska, K., Satchell, K.J.F., Joachimiak, A., 2021. 2'-O methylation of RNA cap in SARS-CoV-2 captured by serial crystallography. Proc. Natl. Acad. Sci. U. S. A. 118 (21).

Yang, Z.L., 2022. Monkeypox: a potential global threat? J. Med. Virol. 94 (9), 4034–4036.

Zhao, Y., Soh, T.S., Lim, S.P., Chung, K.Y., Swaminathan, K., Vasudevan, S.G., Shi, P.Y., Lescar, J., Luo, D., 2015. Molecular basis for specific viral RNA recognition and 2'-O-ribose methylation by the dengue virus nonstructural protein 5 (NS5). Proc. Natl. Acad. Sci. U. S. A. 112 (48), 14834–14839.

Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2 '-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nat. Immunol. 12 (2), 137-U46.