Stronger Together: Multivalent Phage Capsids Inhibit Virus Entry

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Antivirals are now more important than ever. To efficiently inhibit virus replication, antiviral multivalent strategies need sufficient affinity to overcome the excellent matching between the virus and its receptor. This report highlights a phage capsid scaffold strategy that can be used to precisely position sialic acid moieties to inhibit influenza A virus replication.

Human infection by emergent viruses such as Ebola, dengue, Zika, HIV, influenza A virus, or the current SARS-CoV-2 is a serious global health problem. Even for extensively studied pathogens, such as influenza or HIV, and despite the enormous progress of the last decades, optimal strategies for treatment and prevention are still unavailable.[1] In this context, a relevant question arises: What can chemistry do in the search for new strategies and approaches to solve this emergent problem?

Reversible carbohydrate-receptor interactions have been widely implicated in many physiological and pathological processes, ranging from cell-cell communication or tumor metastasis to viral infections.[2,3] Due to the usually weak interaction between a protein and a carbohydrate, typically a multivalent effect takes place. A multivalent effect occurs when the binding affinity between the receptor and the multivalent ligand is drastically increased with respect to that found for the monovalent ligand.[2] As in the case of carbohydrate-protein interactions, multivalent interactions at biological interfaces are frequent in nature and mediate recognition and interactions in essential physiological processes. In recent years, different glycosylated platforms or glycoconjugates have been developed as antivirals to block the carbohydrate recognizing domain of viruses, revealing new therapeutic opportunities.[2,4] These multivalent carbohydrate structures prevent the virus from attaching to its target on the cell surface, and therefore, inhibit virus replication at the entry step. However, to act as effective competitors for virus and cell carbohydrates interaction, they have to improve an already multivalent interaction.

For this, a variety of platforms have been evaluated as useful scaffolds for conjugation of carbohydrates to create different multivalent systems where the size, shape, and valency (number of carbohydrates) are perfectly controlled.[4,5] Initial compounds were based on the repetition of the sugar motif on a polymeric linear scaffold, such as polyacrylamide,[2] focusing mostly on the number of carbohydrates and less on the control of their disposition. Since then, many multivalent systems have been employed as scaffolds functionalized with different ligands for a variety of biological applications. For instance, polypropylenimine dendrimer cores decorated with globotriose or 3′-sialyllactose are able to inhibit *in vitro* HIV infection.[6] Similarly, dendrimers derived from multivalent alkynes and a pseudo-disaccharide acted against HIV and dengue virus infection in cell culture.[7] More complex tridecafullerenes, carbohydrate multivalent systems based on well-defined hexakis adducts of [60]fullerene with octahedral geometry and globular shape, have been used to block the entry of viruses pseudotyped with the spike proteins of Ebola,[8,9] Zika and dengue[10] viruses.

But perhaps it is influenza A viruses, with their well-defined interaction between the hemagglutinin trimer and the cell surface sialic acids, which have attracted the most attention.[2–4,11] Polymers,[2] dendrimers,[12] flexible nanogels[13] or liposomes[14] are just a few examples of the structures investigated for sialic acid anchoring and hemagglutinin attachment inhibition.

The recent report by Lauster et al.[15] is of great interest because it illustrates the importance of the defined arrangement and particle size to obtain a successful multivalent binder to prevent influenza viral infection. The binding of an influenza virus to a target cell occurs through multiple simultaneous interactions between hemagglutinin (HA) and sialic acid (SA; Figure 1). In this work, the authors have selected the icosahedral capsid of the bacteriophage Qβ as the rigid scaffold for the attachment of the sialic acid to achieve a structurally defined presentation of the ligands that matches the binding sites of the trimeric hemagglutinin. The authors identified a residue on the capsid protein that is spaced about 5–6 nm on the particle surface, quite close to the predicted distance between the SA binding sites of HA (4.7 nm). By expression in *Escherichia coli* in the presence of L-homopropargylglycine, they introduced at that position a propargyl group for the anchoring of the SA ligands by azide-alkyne cycloaddition using different linkers. The importance of the perfect positioning of the SA moieties is highlighted by the dramatic drop in affinity caused by small changes in the linker length, as determined by hemagglutination inhibition assays and microscale thermophoresis. This dependence is also reflected in the different binding affinities when using HA of different influenza strains, with small differences in the HA structure.

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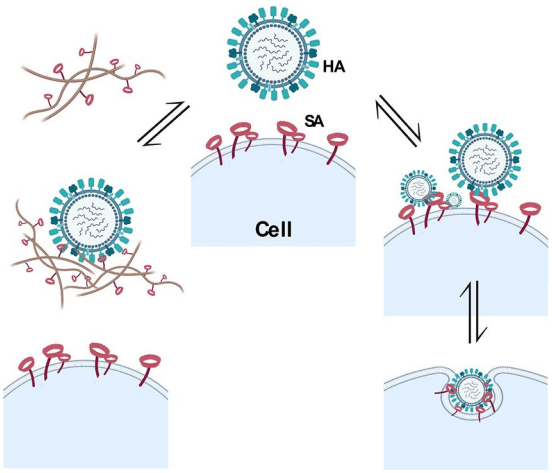

Figure 1. The influenza virus attaches to cells by the interaction of the hemagglutinin trimer (HA) with sialic acid (SA), which triggers internalization of the virus within the cell. The binding can be effectively blocked by an inhibitor that is itself polyvalent. Figure created with BioRender.

When co-incubated with influenza virus, Qβ capsids cover most of the virion surface, as visualized by cryo-electron tomography. The relatively large size of Qβ capsids, of around 25 nm diameter, suggests that not only the higher affinity, but also a steric shielding may be involved in the inhibition of the interaction with the cells. They demonstrate that these sialylated Qβ particles are potent inhibitors of viral infection *in vitro*, both in single round infections and in multicyclic replication. Of note, they observe an even better activity of the sialylated Qβ nanoparticles than the small-molecule antiviral oseltamivir in the latter assay with multiple rounds of replication, even when oseltamivir is added at higher concentrations.

To investigate a more relevant model, they studied viral replication in *ex vivo* cultures of human lungs and observed a significant reduction in viral titer, even when the nanoparticles are added after virus adsorption to the cell surface. Finally, they evaluated their model *in vivo* in mice infected with influenza virus preincubated with the sialylated capsids. The preincubation of the nanoparticles reduced the body weight loss of the infected mice, indicative of a milder disease.

All these findings demonstrate that these phage capsid nanoparticles can be employed as efficient inhibitors of influenza A virus and may be the first step towards the development of a new group of antivirals based on protein scaffolds for the treatment of influenza. Importantly, these nanoparticles were able to reduce viral infection at nanomolar concentrations, confirming the importance of the ligands positioning as an important factor for the activity of these multivalent systems. However, the design of new antiviral strategies is complex, and some important challenges will need to be considered. Despite the promising lack of inflammatory cytokines in lung tissue reported by the authors, it will also be important to determine the immunogenicity of the protein capsid scaffold after repeated administration, a potential scenario for a virus that causes yearly outbreaks. Also, the *in vivo* effectivity when administered after the onset of symptoms, the moment when patients seek treatment, will determine the therapeutic or prophylactic antiviral potential of this attractive new conceptual approach.

Seasonal and pandemic flu are still responsible for many hospitalizations and deaths. Without an effective universal vaccine, and with the delay necessary to produce a vaccine each time a new virus strain appears, the treatment of severe infections and the prevention in exposed individuals has to rely on the use of antivirals, such as oseltamivir or amantadine.[1] Unfortunately, their application is challenged by the rapid development of antiviral resistance by the virus. Virus evasion could also be challenging for multivalent systems, as effective inhibition would require an excellent match between the sialic acid orientation and the particular hemagglutinin. Nevertheless, multivalent inhibitors of cell adhesion could be employed together with other antivirals, enabling combination therapies which slow resistance acquisition.[11] Importantly, optimization of the sialic acid presentation for different strains could also be faster than the development and scaling-up of a new vaccine. It should also be mentioned that this elegant approach might be potentially extended to different viral capsids such as MS2 or PP7.[16] In this regard, the controlled presentation of peptides fragments that would be multivalently recognized by viral proteins could also lead to potential viral inhibition. This intriguing possibility also opens new potential therapeutic strategies towards different pathogens, including the current or other coronavirus species or even broader applications such as prevention of cancer metastasis.

This new approach from the Hackenberger group and collaborators represents a promising example of how controlled protein assemblies can be used as precise multivalent scaffolds in the development of innovative antiviral therapeutics. In these times of the COVID-19 pandemic, we all encourage the efforts from worldwide researchers that improve our response against viral and pathogen infections.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] F. Krammer, G. J. D. Smith, R. A. M. Fouchier, M. Peiris, K. Kedzierska, P. C. Doherty, P. Palese, M. L. Shaw, J. Treanor, R. G. Webster, et al., *Nat. Rev. Dis. Prim.* **2018**, *4*, 3.
[2] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794; *Angew. Chem.* **1998**, *110*, 2908–2953.
[3] W. Lu, R. J. Pieters, *Expert Opin. Drug Discovery* **2019**, *14*, 387–395.
[4] S. Bhatia, L. C. Camacho, R. Haag, *J. Am. Chem. Soc.* **2016**, *138*, 8654–8666.
[5] S. Behren, U. Westerlind, *Molecules* **2019**, *24*, 1004.
[6] A. Rosa Borges, L. Wieczorek, B. Johnson, A. J. Benesi, B. K. Brown, R. D. Kensinger, F. C. Krebs, B. Wigdahl, R. Blumenthal, A. Puri, et al., *Virology* **2010**, *408*, 80–88.
[7] N. Varga, I. Sutkeviciute, R. Ribeiro-Viana, A. Berzi, R. Ramdasi, A. Daghetti, G. Vettoretti, A. Amara, M. Clerici, J. Rojo, et al., *Biomaterials* **2014**, *35*, 4175–4184.
[8] B. M. Illescas, J. Rojo, R. Delgado, N. Martín, *J. Am. Chem. Soc.* **2017**, *139*, 6018–6025.
[9] A. Muñoz, D. Sigwalt, B. M. Illescas, J. Luczkowiak, L. Rodríguez-Pérez, I. Nierengarten, M. Holler, J.-S. Remy, K. Buffet, S. P. Vincent, et al., *Nat. Chem.* **2016**, *8*, 50–57.
[10] J. Ramos-Soriano, J. J. Reina, B. M. Illescas, N. de la Cruz, L. Rodríguez-Pérez, F. Lasala, J. Rojo, R. Delgado, N. Martín, *J. Am. Chem. Soc.* **2019**, *141*, 15403–15412.
[11] N. Sriwilaijaroen, Y. Suzuki, *Lectin Purification, Analysis: Methods and Protocols* (Ed.: J. Hirabayashi), Humana, Totowa **2020**, pp. 547–565.
[12] S. J. Kwon, D. H. Na, J. H. Kwak, M. Douaisi, F. Zhang, E. J. Park, J. H. Park, H. Youn, C. S. Song, R. S. Kane, et al., *Nat. Nanotechnol.* **2017**, *12*, 48–54.
[13] S. Bhatia, M. Hilsch, J. L. Cuellar-Camacho, K. Ludwig, C. Nie, B. Parshad, M. Wallert, S. Block, D. Lauster, C. Böttcher, A. Hermann, R. Haig, *Angew. Chem. Int. Ed.* **2020**, *59*, 12417–12422.
[14] C.-T. Guo, X.-L. Sun, O. Kanie, K. F. Shortridge, T. Suzuki, D. Miyamoto, K. I.-P. J. Hidari, C.-H. Wong, Y. Suzuki, *Glycobiology* **2002**, *12*, 183–190.
[15] D. Lauster, S. Klenk, K. Ludwig, S. Nojoumi, S. Behren, L. Adam, M. Stadtmüller, S. Saenger, S. Zimmler, K. Hönzke, et al., *Nat. Nanotechnol.* **2020**, *15*, 373–379.
[16] L. Zhao, M. Kopylov, C. S. Potter, B. Carragher, M. G. Finn, *ACS Nano* **2019**, *13*, 4443–4454.

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