



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Original Research Article

Spiky nanostructures for virus inhibition and infection prevention

Chuanxiong Nie^a, Lang Ma^b, Hongrong Luo^{c, **}, Jinku Bao^d, Chong Cheng^{a, *}^a College of Polymer Science and Engineering, Sichuan University, No.24 South Section 1, Yihuan Road, Chengdu, 610065, China^b Department of Ultrasound, Laboratory of Ultrasound Imaging Drug, West China Hospital, Sichuan University, Chengdu, 610041, China^c National Engineering Research Center for Biomaterials, Sichuan University, Chengdu, 610064, China^d School of Life Sciences and Key Laboratory of Bio-resources and Eco-environment, Ministry of Education, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy, Chengdu, 610064, China

ARTICLE INFO

Keywords:

Spiky nanostructures
Virus binding
Inhibitor
Infection prevention
Public health

ABSTRACT

The outbreak of a novel highly infectious virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has aroused people's concern about public health. The lack of ready-to-use vaccines and therapeutics makes the fight with these pathogens extremely difficult. To this point, rationally designed virus entry inhibitors that block the viral interaction with its receptor can be novel strategies to prevent virus infection. For ideal inhibition of the virus, the virus-inhibitor interaction has to outperform the virus-host interaction. In our view, the morphology of the inhibitor should be carefully designed to benefit virus-inhibitor binding, especially that the surfaces of viruses are mostly rough due to the existence of surface proteins for receptor-binding. In this perspective article, we would like to discuss the recent progress of designing inhibitors with spiky topography to maximize the interactions between viruses and inhibitors. We also would like to share our idea for the future study of inhibitors to prevent virus infection.

Viruses have long been a threat to public health, especially the unexpected outbreak of highly infectious viruses. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak in 2020 has caused millions of infections and ~500,000 fatalities [1,2]. Besides coronaviruses, influenza A virus (IAV) is also a highly infectious respiratory virus that can be equally devastating for the seasonal outbreaks. The world has experienced several IAV pandemics, including the 'Spanish flu' in 1918, the 'H2N2' outbreak in 1957, and the recent 'H1N1' outbreak in 2009, each of them has caused massive damage to the human society [3]. Flu vaccines have been developed to prevent the infection, but the high error rate of RNA polymerase enables IAV to mutate with unpredictable antigen shifts and drifts, which makes it challenging to prepare the correct vaccine for the coming flu outbreak [4]. Therefore, inhibitors that prevent virus infection are urgently needed.

The infection cycle of IAV starts with binding to the sialic acid receptors on the host cell surface [5]. Blocking the virus binding with a decoy has been realized as an effective strategy to inhibit the virus infection at the beginning [6–11]. To design such an inhibitor, maximizing the inhibitor-virus interaction to compete virus-cell interaction is the key to achieve potent virus inhibition. To this point, functional group valency, inhibitor size, and scaffold stiffness are reported to be the main

parameters to match the receptor binding sites of targeted virions [12, 13]. Additionally, the morphological matching of two subjects also benefits the binding, e. g. most of the plant weeds have evolved a spiky surface to adhere to a host for long-distance spreading [14]. As the virus-inhibitor binding is the key for inhibition, this morphological matching principle can be considered for the design of inhibitors to robustly bind to the virions and compete with the virus-host binding as shown in Scheme 1.

In a recent report by Nie et al. [15], the principle of morphology-matching for IAV inhibition was introduced. They firstly obtained the morphological details from cryogenic transmission electron microscopy (cryo-TEM) images as shown in Fig. 1. Despite the difference between spherical and filament morphologies, the virions of IAV exhibit similar surface nanostructure. The hemagglutinin (HA) and neuraminidase (NA) generate a rough surface as shown in Fig. 1a. From the re-construction of the geometry models, they found a gap around 10 nm for the surface proteins of IAV. In order to match the surface nanostructure of IAV virion, geometry models of spiky nanoparticles are generated with similar size but different spiky length and space as shown in Fig. 1b,c. Compared with smooth nanoparticles, these spikes increase the interface area by inserting into the gap of the proteins as shown in

* Corresponding author.

** Corresponding author.

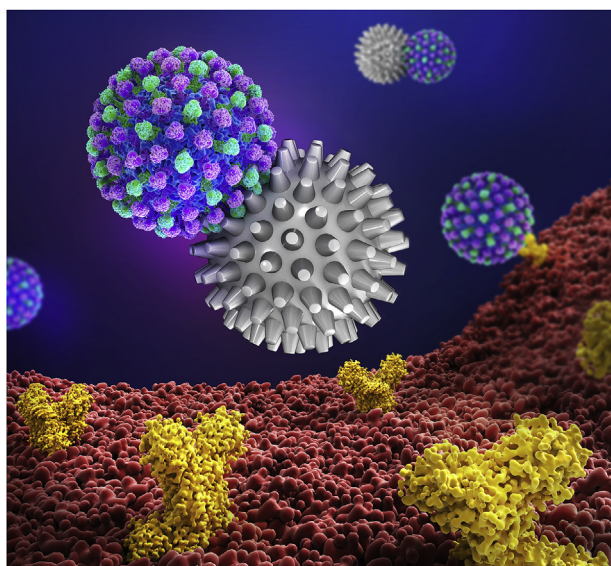
E-mail addresses: hluo@scu.edu.cn (H. Luo), chong.cheng@scu.edu.cn (C. Cheng).<https://doi.org/10.1016/j.smaim.2020.07.004>

Received 29 June 2020; Received in revised form 8 July 2020; Accepted 9 July 2020

Available online 16 July 2020

2590-1834/© 2020 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND

license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



Scheme 1. The general concepts of using spiky nanostructures for inhibiting virus binding to cell membranes.

Fig. 1b. The binding patterns for the spiky nanoparticles are also different for different spike structures as shown in **Fig. 1c**. If the spikes are too long, the virion interacts only with the tip, resulting in decreased area. When the spikes are too dense, they no longer fit the gap, which decreases the interface area. At a suitable spike length and spacing (10 nm length, 10 nm spacing), the nanoparticle can tightly bind to IAV virion with a maximized contact area.

As an experimental proof to this idea, spiky nanostructures (SNS) with similar morphologies with bioinert SiO₂ cores are synthesized as shown in **Fig. 2a**. Nanoparticles with a ~120 nm core and spike length of 0–30 nm are synthesized. The virion-binding abilities are compared via a centrifuge-western blot assay as shown in **Fig. 2b**. It is clear that the spikes enhance the binding between the nanoparticle and IAV virion. When the spikes are 5–10 nm, maximized virus binding is achieved, which is in agreement with the geometry simulation results in **Fig. 1c**. To check the binding patterns between the spiky nanoparticles and IAV virion, cryo-TEM images are also acquired (**Fig. 2c**). For the smooth nanoparticle (SNS-0), the virion interacts with the nanoparticle with a very limited area. As expected, for SNS with 10 nm spikes (SNS-10), the spikes insert into the gap of the protein, forming a conjunction to benefit the binding. However, for SNS with 30 nm spikes (SNS-30), tip-to-tip interactions are noticed, for which the interaction area is smaller than

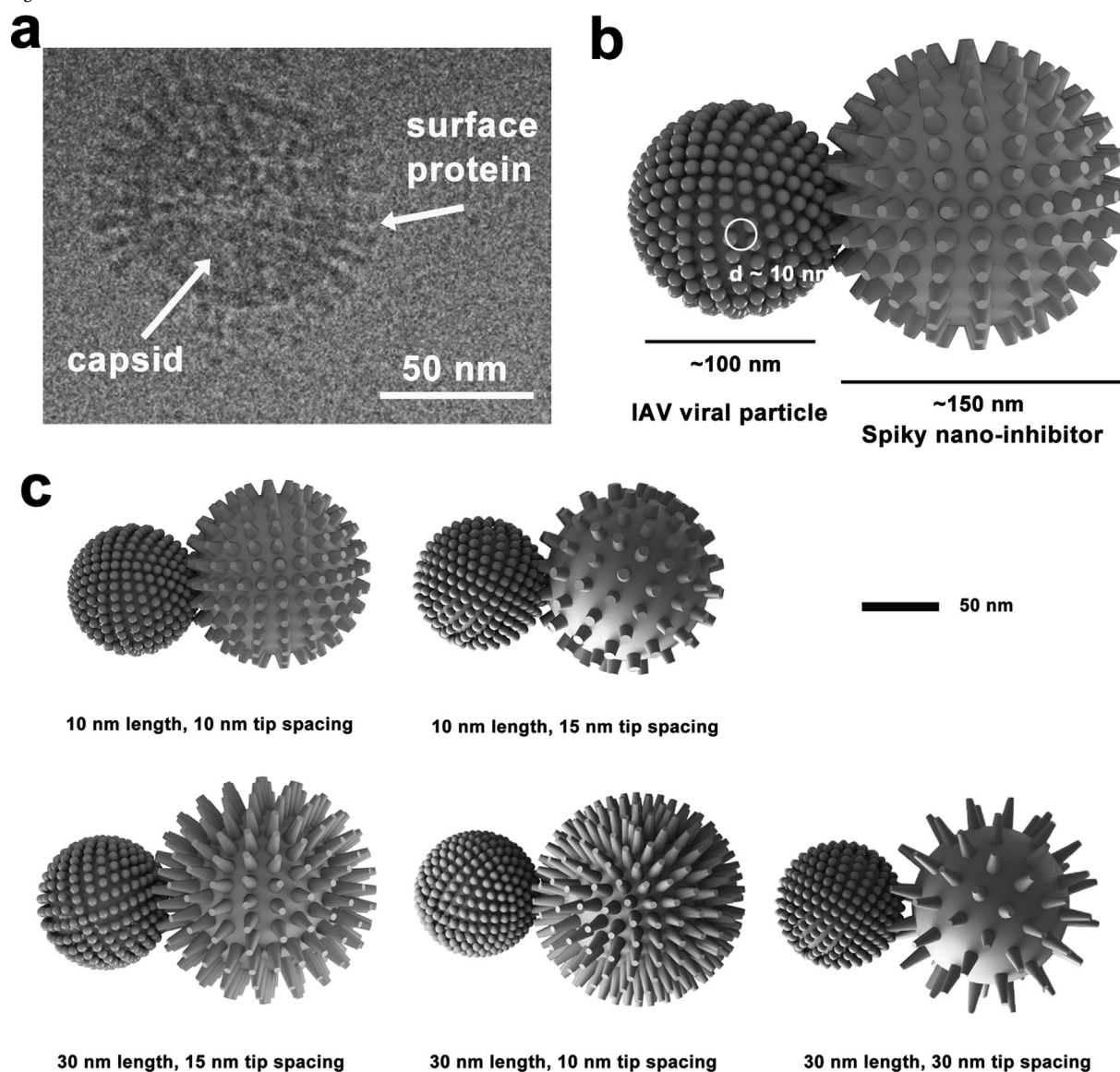


Fig. 1. (a) Typical cryo-EM image for an IAV virion. Scale bar: 50 nm. (b) Morphological illustration of a spiky nanoparticle interacting with an IAV virion. (c) Interaction between IAV virion and spiky nanoparticles with different geometry parameters. Reprinted with permission from Ref. [15]. Copyright (2020) American Chemical Society.

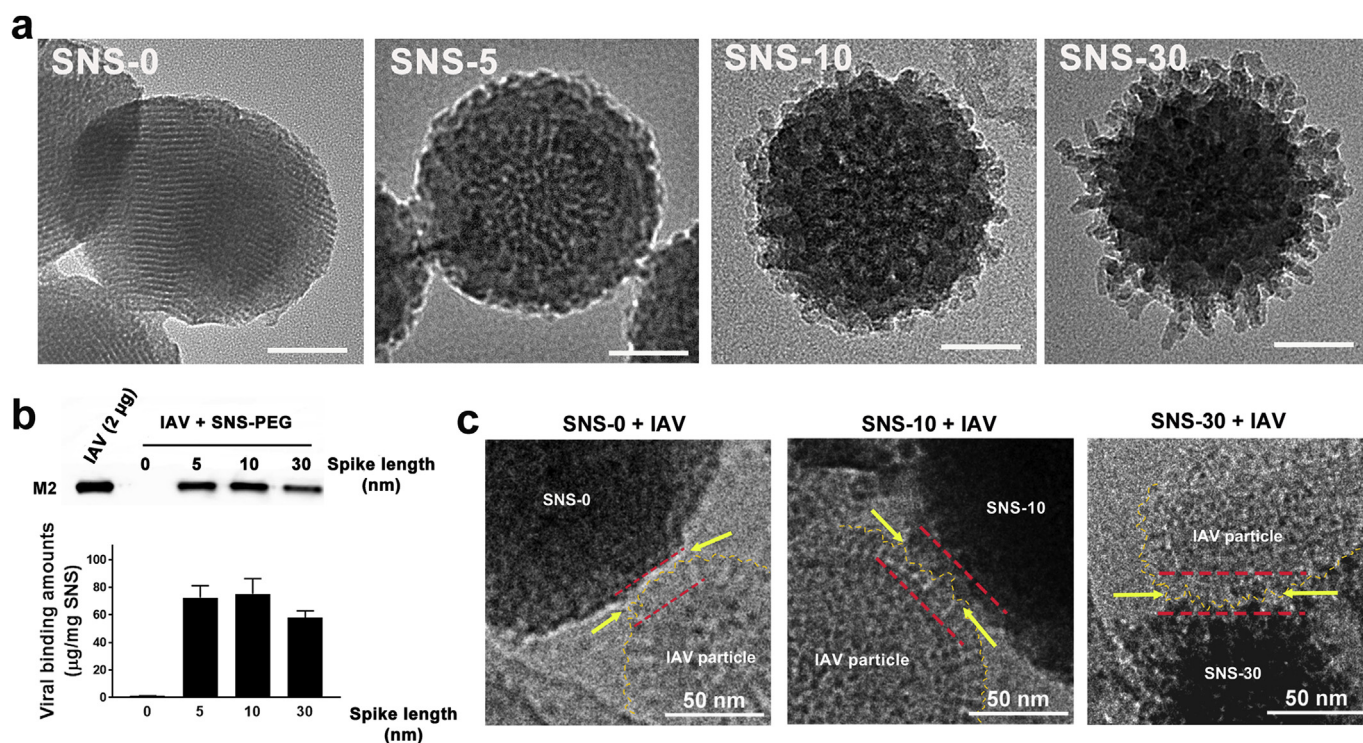


Fig. 2. (a) High-resolution TEM images for the synthesized nanoparticles with different surface morphologies. Scale bar: 50 nm. The number in the nomenclature reveals the length of the spike. (b) A western blot based assay for the virus binding the nanoparticles. (c) Cryo-TEM images for the IAV virion binding to the spiky nanostructures. Scale bar: 50 nm. Reprinted with permission from Ref. [15]. Copyright (2020) American Chemical Society.

SNS-10.

However, it is noticed that even the spiky nanoparticles show viral binding abilities, they are not able to inhibit the infection of IAV. This is probably because the virus-inhibitor binding is not strong enough to compete with the virus-host binding. To achieve potent virus inhibition, the surface of the nanoparticles should be functionalized with binding motifs towards IAV. Erythrocyte has a natural display of sialic acid as the binding target for several subgroups of IAV (Fig. 3a). As a proof of concept, the authors coat the spiky nanostructures with erythrocyte membrane (EM) as a targeting shell towards IAV virion. During the coating process, all the membrane components are transferred to the surface of spiky nanoparticles. With EM coating, the viral binding is further enhanced, and an inhibitor that can effectively reduce the viral binding to the host cells is obtained. In the cellular infection assay, all the inhibitors show a reduced number of infected cells as shown in Fig. 3b, c. SNS-10 is the best inhibitor, by which >90% of cellular infection is inhibited. They also check if the inhibitors can prevent virus replication by plaque assay (Fig. 3d, e). 99.9% inhibition is achieved with 1 mg/mL dose of the inhibitor. Be co-used with NA inhibitor, more potent (>99.99%) inhibition of virus replication is achieved.

HA and NA regulate IAV binding in mucus and to host cells. Briefly speaking, HA interacts with sialic acid to trigger virus entry, and NA cleaves the HA-sialic acid-binding for virus release after the replication [16]. To achieve a better inhibition with sialic acid-based compounds, the negative effects of NA on the binding should be minimized. In another report by this group, a heteromultivalent IAV inhibitor based on the spiky nanoparticles is developed. The surface of the spiky nanoparticle is functionalized covalently with sialyllactose (SAL) and zanamivir (Zanamivir), via a linear polyglycerol linker as shown in Fig. 4a, b [17]. With zanamivir on the surface, the activity of NA is inhibited (Fig. 4e), the virus-inhibitor binding is further enhanced (Fig. 4c, d), which results in nearly 100% blocking of virion interaction with the host cells. In a cellular infection assay, it is noticed that in the presence of the

spiky inhibitors, there are no infected cells. Even being used after the first cycle of infection, the inhibitor, VLNP-SAL/Zan, still shows a >99.9999% inhibition of virus titre (Fig. 4f). They also report that the inhibitor is active against three human IAV strains, which are A/X31 (H3N2), A/PR/8/34 (H1N1) and A/Panama/2007/1999 (H3N2), as shown in Fig. 4g.

As most of the virus starts the replication with the binding to the receptors and most of the virus exhibit similar spiky morphology as IAV, it is envisioned that such a strategy can also be used for other viral strains, e. g. coronaviruses. For a potent virus inhibition, the concept of geometry matching can be incorporated with different antiviral strategies. The nanostructures in these studies are hollow mesoporous structures, which are capable of loading antiviral cargos as delivery systems to further increase the virus inhibitor performance. On the other hand, enzyme-mimicking nanostructures have also been developed as a powerful tool to combat pathogen infections. These nanostructures exhibit the ability to produce highly ‘toxic’ reactive oxygen species, including OH^\cdot , O_2^\cdot , and etc., which show the ability to prevent wound infections [18–20]. These enzyme-mimic nanostructures have been fabricated into a self-disinfection mouth mask for the combating of the respiratory pathogen infections [21]. It is envisioned that combining functional materials cores with the spiky surfaces, a more potent virus inhibitor with multiple modes of action can be produced.

However, the drawback of such spiky inhibitors is also clear: the non-specific interaction with the biological molecules will facilitate the uptake and clearance by immune systems [22]. Therefore, the surface of the inhibitor should be also be functionalized with highly antifouling or bio-stealth groups to avoid the rapid clearance after the intaking. Cellular membrane coating can be a good solution, which enables the nanoparticle to bypass the immune system for long-term circulation. In such a system, the source of host cells needs to be carefully selected to avoid the side effects of cellular membrane antigens [23–25]. Another solution can be smart nanostructures that are able to change the morphology upon

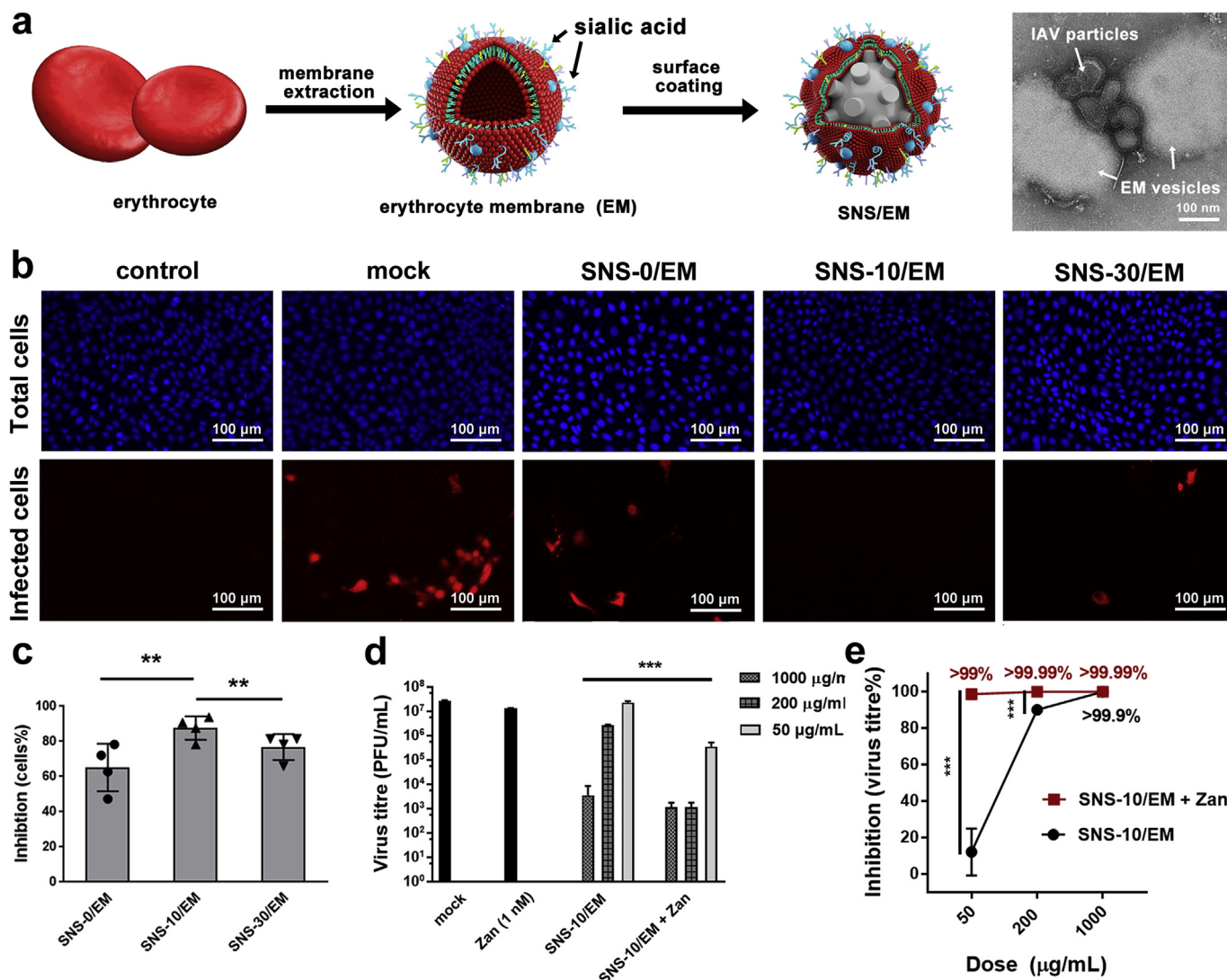


Fig. 3. (a) Preparation of the EM coated spiky nanoparticles for virus inhibition and IAV virion binding the EM vesicles. Scale bar: 100 nm. (b, c) Immunofluorescent staining for the virus infection in the presence of the inhibitors. (d–e) Inhibition of virus replication in the presence of the inhibitors. Reprinted with permission from Refs. [15]. Copyright (2020) American Chemical Society.

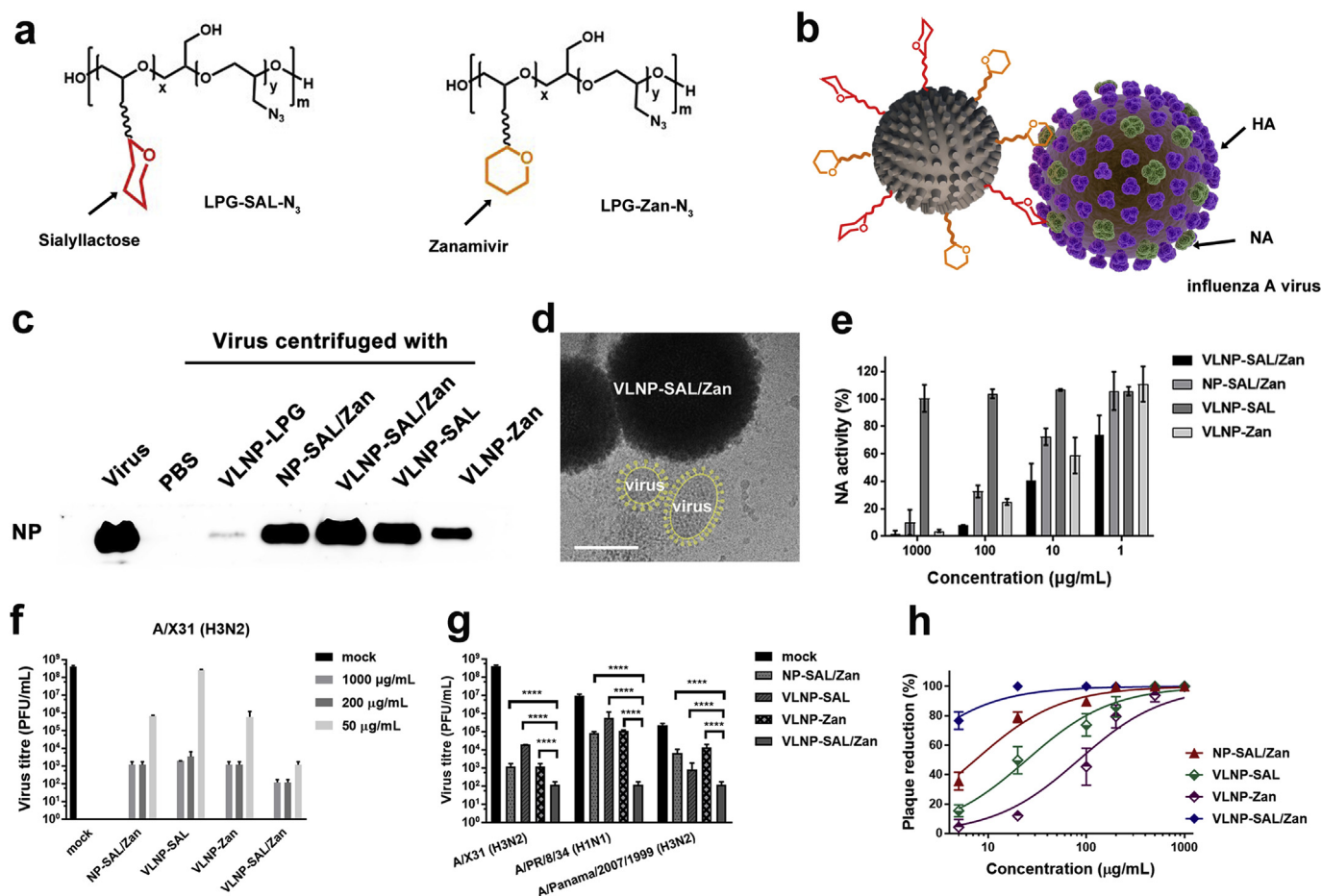


Fig. 4. (a) Molecules for the development of hetero-multivalent nano-inhibitor towards IAV. (b) Schematic illustration of the virus binding to the hetero-multivalent nano-inhibitor. (c) A western blot based assay for IAV binding to the nano-inhibitor. NP: smooth nanoparticle. VLNP: virus-like spiky nanoparticle. (d) Cryo-EM images for the virus binding the nano-inhibitor. Scale bar: 100 nm. (e) Inhibition of the NA activity for by the nano-inhibitors. (f) Inhibition of influenza A/X31 (H3N2) replication by the nano-inhibitors. (g) Inhibition of influenza A/X31 (H3N2), A/PR/8/34 (H1N1) and A/Panama/2007/1999 (H3N2) replication by the nano-inhibitors. (h) Plaque reduction efficiency for the nano-inhibitors. Reprinted with permission from Ref. [17]. Copyright (2020) Wiley.

stimuli [26].

Spiky nanostructures can also be obtained via other approaches, e. g. coating of a small particle onto an existing core material, which offers more possibilities to control the surface morphology and functionalization [27]. Nevertheless, the geometry matching can be a universal approach to benefit the binding between the two subjects. Not only for virus inhibition, this idea can also be used as a general approach for the combat with bacteria and tumors [28–31].

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Chuanxiong Nie: Writing - original draft. **Lang Ma:** Writing - original draft. **Hongrong Luo:** Supervision, Writing - review & editing. **Jinku Bao:** Writing - review & editing. **Chong Cheng:** Supervision, Writing - review & editing.

Acknowledgments

This work was financially supported by the National Key R&D Program of China (2019YFA0110600, 2019YFA0110601), National Natural Science Foundation of China (Nos. 51903178, 81971622, 51803134, and 51703141), Sichuan Science and Technology Program (Nos.

2019YFS0219, 2020YFH0087, and 2020YJ0055), the State Key Laboratory of Polymer Materials Engineering (No. sklpm2019-2-03), Fundamental Research Funds for the Central Universities, Special Funds for Prevention and Control of COVID-19 of Sichuan University (2020scunCoV-YJ-20005) and SKLFPM, Donghua University (YJ202005), the Thousand Youth Talents Plan, and the Post-Doctor Research Project, West China Hospital, Sichuan University (No. 2018HXBH077).

References

- [1] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Müller, D. Niemeyer, T.C. Jones, P. Vollmar, C. Rothe, M. Hoelscher, T. Bleicker, S. Brünink, J. Schneider, R. Ehmann, K. Zwirgmaier, C. Drosten, C. Wendtner, Virological assessment of hospitalized patients with COVID-2019, *Nature* 581 (7809) (2020) 465–469.
- [2] Y. Bai, L. Yao, T. Wei, F. Tian, D.-Y. Jin, L. Chen, M. Wang, Presumed asymptomatic carrier transmission of COVID-19, *J. Am. Med. Assoc.* 323 (14) (2020) 1406–1407, <https://doi.org/10.1001/jama.2020.2565>.
- [3] D.J. Jamieson, M.A. Honein, S.A. Rasmussen, J.L. Williams, D.L. Swerdlow, M.S. Biggerstaff, S. Lindstrom, J.K. Louie, C.M. Christ, S.R. Bohm, V.P. Fonseca, K.A. Ritger, D.J. Kuhles, P. Eggers, H. Bruce, H.A. Davidson, E. Lutterloh, M.L. Harris, C. Burke, N. Cocoros, L. Finelli, K.F. MacFarlane, B. Shu, S.J. Olsen, H1N1 2009 influenza virus infection during pregnancy in the USA, *Lancet* 374 (9688) (2009) 451–458.
- [4] F. Wang, G. Chen, Y. Zhao, Biomimetic nanoparticles as universal influenza vaccine, *Smart Materials in Medicine* 1 (2020) 21–23.
- [5] J.K. Taubenberger, D.M. Morens, The pathology of influenza virus infections, *Annu. Rev. Pathol.* 3 (1) (2008) 499–522.

- [6] S. Bhatia, L.C. Camacho, R. Haag, Pathogen inhibition by multivalent ligand architectures, *J. Am. Chem. Soc.* 138 (28) (2016) 8654–8666.
- [7] D. Lauster, S. Klenk, K. Ludwig, S. Nojoumi, S. Behren, L. Adam, M. Stadtmüller, S. Saenger, S. Zimmmer, K. Hönzke, L. Yao, U. Hoffmann, M. Bardua, A. Hamann, M. Witzenth, L.E. Sander, T. Wolff, A.C. Hocke, S. Hippenstiel, S. De Carlo, J. Neudecker, K. Osterrieder, N. Budisa, R.R. Netz, C. Böttcher, S. Liese, A. Herrmann, C.P.R. Hackenberger, Phage capsid nanoparticles with defined ligand arrangement block influenza virus entry, *Nat. Nanotechnol.* 15 (5) (2020) 373–379.
- [8] 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, J.S. Dordick, K.B. Lee, R.J. Linhardt, Nanostructured glycan architecture is important in the inhibition of influenza A virus infection, *Nat. Nanotechnol.* 12 (2016) 48.
- [9] S. Bhatia, D. Lauster, M. Bardua, K. Ludwig, S. Angioletti-Uberti, N. Popp, U. Hoffmann, F. Paulus, M. Budt, M. Stadtmüller, T. Wolff, A. Hamann, C. Böttcher, A. Herrmann, R. Haag, Linear polysialoside outperforms dendritic analogs for inhibition of influenza virus infection in vitro and in vivo, *Biomaterials* 138 (2017) 22–34.
- [10] S. Tang, W.B. Puryear, B.M. Seifried, X. Dong, J.A. Runstadler, K. Ribbeck, B.D. Olsen, Antiviral agents from multivalent presentation of sialyl oligosaccharides on brush polymers, *ACS Macro Lett.* 5 (3) (2016) 413–418.
- [11] M. Yamabe, K. Kaihatsu, Y. Ebara, Sialyllactose-modified three-way junction DNA as binding inhibitor of influenza virus hemagglutinin, *Bioconjugate Chem.* 29 (5) (2018) 1490–1494.
- [12] B. Ziem, H. Thien, K. Achazi, C. Yue, D. Stern, K. Silberreis, M.F. Gholami, F. Beckert, D. Gröger, R. Mülhaupt, J.P. Rabe, A. Nitsche, R. Haag, Highly efficient multivalent 2D nanosystems for inhibition of orthopoxvirus particles, *Advanced Healthcare Materials* 5 (22) (2016) 2922–2930.
- [13] P. Dey, T. Bergmann, J.L. Cuellar-Camacho, S. Ehrmann, M.S. Chowdhury, M. Zhang, I. Dahmani, R. Haag, W. Azab, Multivalent flexible nanogels exhibit broad-spectrum antiviral activity by blocking virus entry, *ACS Nano* 12 (7) (2018) 6429–6442.
- [14] S.E. Weaver, M.J. Lechowicz, The biology OF CANADIAN weeds.: 56. *Xanthium strumarium* L, *Can. J. Plant Sci.* 63 (1) (1983) 211–225.
- [15] C. Nie, M. Stadtmüller, H. Yang, Y. Xia, T. Wolff, C. Cheng, R. Haag, Spiky nanostructures with geometry-matching topography for virus inhibition, *Nano Lett.* 20 (7) (2020) 5367–5375.
- [16] E. de Vries, W. Du, H. Guo, C.A.M. de Haan, Influenza A virus hemagglutinin–neuraminidase–receptor balance: preserving virus motility, *Trends Microbiol.* 28 (1) (2020) 57–67.
- [17] C. Nie, B. Parshad, S. Bhatia, C. Cheng, M. Stadtmüller, A. Oehrl, Y. Kerkhoff, T. Wolff, R. Haag, Reverse design of an influenza neutralizing spiky nano-inhibitor with a dual mode of action, *Angew. Chem. Int. Ed.* (2020), <https://doi.org/10.1002/anie.202004832>.
- [18] W. Shao, C. He, M. Zhou, C. Yang, Y. Gao, S. Li, L. Ma, L. Qiu, C. Cheng, C. Zhao, Core-shell-structured MOF-derived 2D hierarchical nanocatalysts with enhanced Fenton-like activities, *J. Mater. Chem.* 8 (6) (2020) 3168–3179.
- [19] C. Yang, M. Zhou, C. He, Y. Gao, S. Li, X. Fan, Y. Lin, F. Cheng, P. Zhu, C. Cheng, Augmenting intrinsic fenton-like activities of MOF-derived catalysts via N-Molecule-Assisted self-catalyzed carbonization, *Nano-Micro Lett.* 11 (1) (2019) 87.
- [20] X. Fan, F. Yang, J. Huang, Y. Yang, C. Nie, W. Zhao, L. Ma, C. Cheng, C. Zhao, R. Haag, Metal-Organic-framework-Derived 2D carbon nanosheets for localized multiple bacterial eradication and augmented anti-infective therapy, *Nano Lett.* 19 (9) (2019) 5885–5896.
- [21] P. Li, J. Li, X. Feng, J. Li, Y. Hao, J. Zhang, H. Wang, A. Yin, J. Zhou, X. Ma, B. Wang, Metal-organic frameworks with photocatalytic bactericidal activity for integrated air cleaning, *Nat. Commun.* 10 (1) (2019) 2177.
- [22] W. Wang, P. Wang, X. Tang, A.A. Elzatahry, S. Wang, D. Al-Dahyan, M. Zhao, C. Yao, C.-T. Hung, X. Zhu, T. Zhao, X. Li, F. Zhang, D. Zhao, Facile synthesis of uniform virus-like mesoporous silica nanoparticles for enhanced cellular internalization, *ACS Cent. Sci.* 3 (8) (2017) 839–846.
- [23] R.H. Fang, A.V. Kroll, W. Gao, L. Zhang, Cell membrane coating nanotechnology, *Adv. Mater.* 30 (23) (2018) 1706759.
- [24] R.H. Fang, Y. Jiang, J.C. Fang, L. Zhang, Cell membrane-derived nanomaterials for biomedical applications, *Biomaterials* 128 (2017) 69–83.
- [25] Q. Liu, C. Bi, J. Li, X. Liu, R. Peng, C. Jin, Y. Sun, Y. Lyu, H. Liu, H. Wang, C. Luo, W. Tan, Generating giant membrane vesicles from live cells with preserved cellular properties, *Research* 2019 (2019) 9.
- [26] Y. Yang, L. Ma, C. Cheng, Y. Deng, J. Huang, X. Fan, C. Nie, W. Zhao, C. Zhao, Nonchemotherapeutic and robust dual-responsive nanoagents with on-demand bacterial trapping, ablation, and release for efficient wound disinfection, *Adv. Funct. Mater.* 28 (21) (2018) 1705708.
- [27] Y. Xiao, W. Wang, X. Tian, X. Tan, T. Yang, P. Gao, K. Xiong, Q. Tu, M. Wang, M.F. Maitz, N. Huang, G. Pan, Z. Yang, A versatile surface bioengineering strategy based on mussel-inspired and bioclickable peptide mimic, *Research* 2020 (2020) 12.
- [28] H. Song, Y. Ahmad Nor, M. Yu, Y. Yang, J. Zhang, H. Zhang, C. Xu, N. Mitter, C. Yu, Silica nanopollens enhance adhesion for long-term bacterial inhibition, *J. Am. Chem. Soc.* 138 (20) (2016) 6455–6462.
- [29] J. Wang, H.-J. Chen, T. Hang, Y. Yu, G. Liu, G. He, S. Xiao, B.-r. Yang, C. Yang, F. Liu, J. Tao, M.X. Wu, X. Xie, Physical activation of innate immunity by spiky particles, *Nat. Nanotechnol.* 13 (11) (2018) 1078–1086.
- [30] J. Wang, L. Fang, P. Li, L. Ma, W. Na, C. Cheng, Y. Gu, D. Deng, Inorganic nanozyme with combined self-oxygenation/degradable capabilities for sensitized cancer immunotherapy, *Nano-Micro Lett.* 11 (1) (2019) 74.
- [31] L. Wang, B. Zhu, J. Huang, X. Xiang, Y. Tang, L. Ma, F. Yan, C. Cheng, L. Qiu, Ultrasound-targeted microbubble destruction augmented synergistic therapy of rheumatoid arthritis via targeted liposomes, *J. Mater. Chem. B* 8 (24) (2020) 5245–5256.