SAND2020-XXXX
LDRD PROJECT NUMBER: 220719/04
LDRD PROJECT TITLE: Deactivation of SARS-CoV-2 by Boronic Acid-Functionalized Polymer
PROJECT TEAM MEMBERS: George D. Bachand, Brooke N. Harmon, Brad H. Jones, Philip R. Miller, James B. Ricken, Maxwell Stefan

ABSTRACT

Boronic acid-modified polymers (BAMPs) can interact with glycoproteins and other glycosylated compounds through covalent binding of the boronic acid moieties to saccharide residues. As a first step toward evaluating the utility of BAMPs as SARS-CoV-2 antiviral agents, this COVID-19 rapid response LDRD was intended to examine the effect of BAMPs on SARS-CoV-2 spike glycoprotein and its subsequent binding with ACE2 receptor protein. Multiple different approaches were attempted in order to determine whether BAMPs based on poly(ethylene glycol) and poly(ethylenimine) bind the spike protein, but failed to produce a definitive answer. However, two different enzyme-linked immunosorbent assays clearly showed no discernable effect of boronic acid in inhibiting spike-ACE2 binding.

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EXECUTIVE SUMMARY

We hypothesized that boronic acid-modified polymers (BAMPs) could effectively crosslink or otherwise bind the spike glycoprotein of SARS-CoV-2 via its saccharide residues. Such binding could potentially inhibit the intrinsic spike dynamics and complexation with the host ACE2 receptor, a key recognition event at the advent of the SARS-CoV-2 infection process. If true, BAMPs would be of interest as antiviral materials, deployable in various ways to combat the spread of COVID-19: as a coating incorporated into existing personal protective equipment such as N95 masks, as a functional element of air filtration devices, or in decontamination sprays.

This LDRD was intended to probe the above hypothesis by studying the interactions between various BAMPs and SARS-CoV-2 spike protein. Specifically, we sought to determine (i) whether BAMPs can bind and/or aggregate the spike protein and, (ii) more importantly, whether BAMPs inhibit or otherwise affect the binding of the spike protein to ACE2. It was expected that positive confirmation of either of these phenomena would spur future research into the antiviral properties of BAMPs toward SARS-CoV-2 and other enveloped viruses.

We used two different BAMPs, derived from poly(ethylene glycol) (PEG) and poly(ethylenimine) (PEI). We screened several approaches to evaluate binding of BAMPs to SARS-CoV-2 spike, including functional gel tests, polyacrylamide gel electrophoresis, and ellipsometry of functionalized surfaces. We were unable to obtain definitive information as to whether BAMPs can bind and/or aggregate the spike protein. On the other hand, an enzyme-linked immunosorbent assay (ELISA) designed to probe the effect of BAMP on binding of ACE2 to surface-adsorbed spike protein showed no benefit from the presence of boronic acid (BA). This outcome was confirmed by a slightly different ELISA, although it was further discovered that (PEI), whether BA-functionalized or not, does have a significant inhibiting effect on spike-ACE2 binding.

We further tested the BAMPs as antiviral compounds against Dengue virus (DENV) and the bacteriophage Φ6. We were unable to obtain definitive information as to the effect of BAMPs on DENV. However, there was a strong effect of the BAMPs against Φ6, with BA-functionalized PEI completely inhibiting Φ6 infection. Due to critical differences in the surface chemistry of Φ6 compared to DENV or SARS-CoV-2, the mechanism of inhibition is unclear and, hence, the relevance of this outcome to SARS-CoV-2 is quite limited. In sum, our initial hypothesis, and the potential of BAMPs as antiviral materials, remain open questions.

SUMMARY OF APPROACH AND RESULTS

Boronic acids (BAs) are mild Lewis acids with unique utility in biological applications due to their ability to reversibly bind to molecules possessing 1,2-diol functionalities, of which all saccharides and many related compounds constitute an important subset.[1-10] The covalent equilibrium between free BA and 1,2-diol versus the corresponding bound species – a cyclic boronate ester – is shown in Figure 1. The equilibrium constant K depends on the choice of BA

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and diol, as well as the pH.[11,12] In general, the boronate ester is favored at high pH, while the free, unbound species are favored at low pH. The pH over which this reversibility occurs is a complex function of several factors, but can be tuned over a broad range including physiological pH.

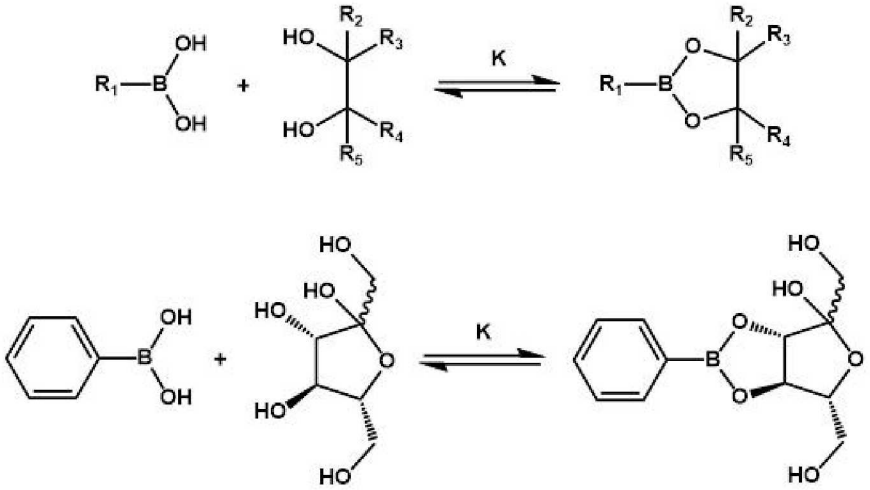

Figure 1. Reversible binding of 1,2-diols, including saccharides, to BAs. The top scheme shows a general equilibrium between the bound and unbound forms of arbitrary BAs and 1,2-diols. The bottom scheme shows this specific equilibrium for phenylboronic acid and fructose. Note that the BAs and boronate esters can exist in both trigonal and tetrahedral forms (uncharged and charged, respectively), with significant implications for the binding equilibrium, but only the former are shown.

We, and others, have shown that BA-modified polymers (BAMPs) can be used to capture and aggregate cells via BA binding with saccharide residues of glycoproteins residing within the cell membrane.[13-16] More recently, we have shown that the BAMPs can be utilized as functional adhesives, for example, to bind articles to saccharide-rich materials such as cellulose paper or plant leaves.[17] At the advent of the infection process, enveloped viruses such as SARS-CoV-2 bind to host cells (*e.g.*, respiratory epithelial cells) via a transmembrane spike protein rich in glycans (polysaccharides).[18,19] Conformational changes in the S1 and S2 subunits of the spike protein are necessary for successful binding with the host receptor and viral uptake, making this protein an ideal target for rapid viral inactivation.[20] We hypothesized that BAMPs could effectively crosslink or otherwise bind the spike protein via its saccharide residues, inhibiting the intrinsic subunit dynamics and complexation with the host ACE2 receptor. Of particular note, recent published work has shown excellent antiviral activity against human coronavirus HCoV-229E in carbon quantum dots functionalized with BAs.[21]

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positive confirmation of either of these phenomena would spur future research into the antiviral properties of BAMPs toward SARS-CoV-2 and other enveloped viruses.

Two different BAMPs were used in this work (Figure 2), a BA-functionalized poly(ethylene glycol) (PEG-BA) and a BA-functionalized poly(ethylenimine) (PEI-BA). These BAMPs were prepared as previously described.[15] Briefly, an 8-arm star, amine end-functionalized PEG of number-averaged molecular weight $M_n \sim 40$ kDa and a branched PEI of $M_n \sim 10$ kDa were used as starting materials. Phenylboronic acid was grafted to amine functional groups on both polymers via reductive amination of 4-formylphenylboronic acid using sodium cyanoborohydride as a reducing agent. The resulting PEG-BA and PEI-BA possessed 7.3 and 5.6 BA units per polymer chain, respectively, as determined by ^{1}H nuclear magnetic resonance spectroscopy.[15] Furthermore, the successful introduction of BA units was confirmed through a simple gel test.[15] The BAMPs were dissolved in deionized water at 50 mg/mL and mixed with equal volumes of aqueous 50 mg/mL solution of a linear PEG end-functionalized with catechol (an aromatic diol that strongly binds BA[11]), designated hereafter as PEG-CAT. Immediately after mixing, a self-supporting hydrogel forms, confirming the successful generation of a cross-linked polymer network through BA-diol interaction.[22]

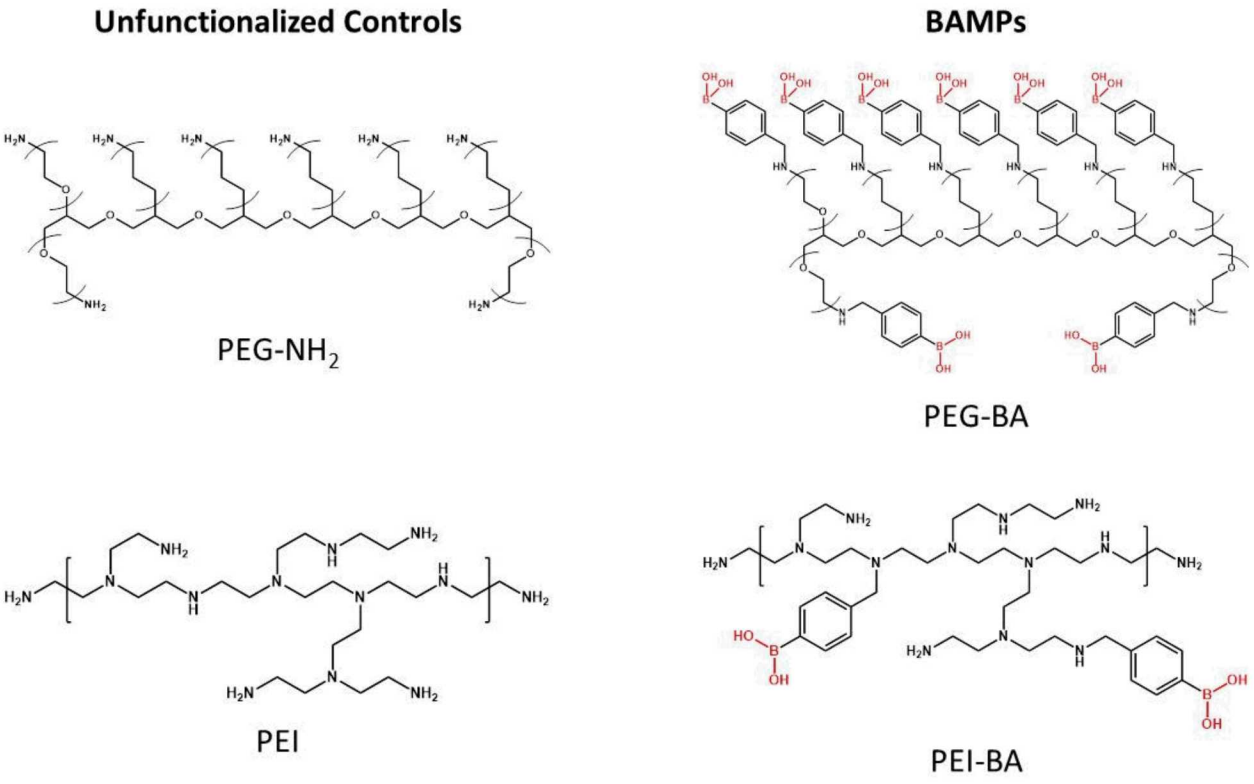

Figure 2. Starting polymers (unfunctionalized controls) and corresponding BAMPs used in this work. The BA functionalities are shown in red.

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For a simple initial test of potential interactions between BAMPs and SARS-CoV-2 spike protein, buffered solutions of the two were mixed in a similar manner as the gel test described above. In a representative example, 47 µg recombinant SARS-CoV-2 S1 subunit protein (RayBioTech, Peachtree Corners, GA) was dissolved in 6 µL pH 7.4 phosphate buffered saline (PBS) for a concentration of 7.83 mg/mL. Separately, PEG-BA was dissolved in pH 7.4 PBS at 94 mg/mL. 0.5 µL volumes of the PEG-BA solution were added to the spike protein solution until a total of 2 µL PEG-BA solution was added (final concentrations 5.9 mg/mL spike protein, 23.5 mg/mL PEG-BA). The resulting solution did not exhibit any visual indication of gelation, nor the formation of a precipitate, and remained readily pipettable with each addition of PEG-BA. However, it is important to note that the model system of PEG-BA and PEG-CAT combined at 10 mg/mL also failed to produce a gel. Indeed, in these types of tests, macroscopic gelation generally requires relatively high concentrations of the species responsible for crosslinking, whereas dilute solutions typically yield soluble microgels.[23] Unfortunately, due to the high cost and limited availability of the spike protein (we obtained ~ 650 µg from RayBioTech and other sources), we were unable to perform a gel test at higher concentrations.

We further sought to employ gel electrophoresis to probe potential interactions between BAMPs and SARS-CoV-2 spike protein. Gel electrophoresis separates proteins and other macromolecules on the basis of their molecular size and charge. We conjectured that any binding of BAMP to saccharide residues of the spike would necessarily alter the molecular size of the BAMP-spike conjugate relative to the neat spike protein and, thus, be revealed in analysis by gel electrophoresis. We chose to use native polyacrylamide gel electrophoresis (native-PAGE), rather than the more common sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as the latter involves denaturation of the protein analyte, whereas the former preserves the protein's inherent structure. Unfortunately, our attempts to obtain a reproducible band for even the neat spike protein were plagued by issues related to the use of old polyacrylamide gels. In addition, we avoided the use of the standard glycerol-based loading buffer typically used in native-PAGE, instead using low molecular weight PEG-based buffers, so as to avoid any interference of glycerol in BA-saccharide interaction. We suspect that the use of an unconventional buffer may have further contributed to our inability to reproducibly characterize the neat spike protein.

As a third approach to investigate potential BAMP-spike interaction, we attempted to functionalize surfaces with BAs, expose such functionalized surfaces to the spike protein, and indirectly evaluate BA-spike binding by determining the thickness of the surface via ellipsometry. Various methods for surface functionalization were attempted. Au surfaces were exposed to 4-mercaptophenylboronic acid, in order to graft the BA via the well-known Au-thiol interaction.[24] Alternatively, Si surfaces were exposed to an epoxide-functional silane, (3-glycidyloxypropyl)trimethoxysilane, after which the grafted epoxide was reacted with 3-aminophenylboronic acid. To illustrate the challenges associated with this general surface functionalization approach, Figure 3 shows the thickness (height) of the grafted BA for four replicates of Si surfaces identically functionalized by the latter method. The error bars indicate the standard deviation from several measurements in different locations across the same sample.

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The thickness of the grafted BA cannot be evaluated by ellipsometry within an accuracy of *ca.* 1 nm, which is on the order of the size of the spike protein.[25] Therefore, it was unsurprising to find that measurements next performed after subsequent exposure of these surfaces to the spike protein did not produce an increase in thickness within the error of the measurement. Thus, this method was insufficient to evaluate whether or not the spike protein binds to BAs.

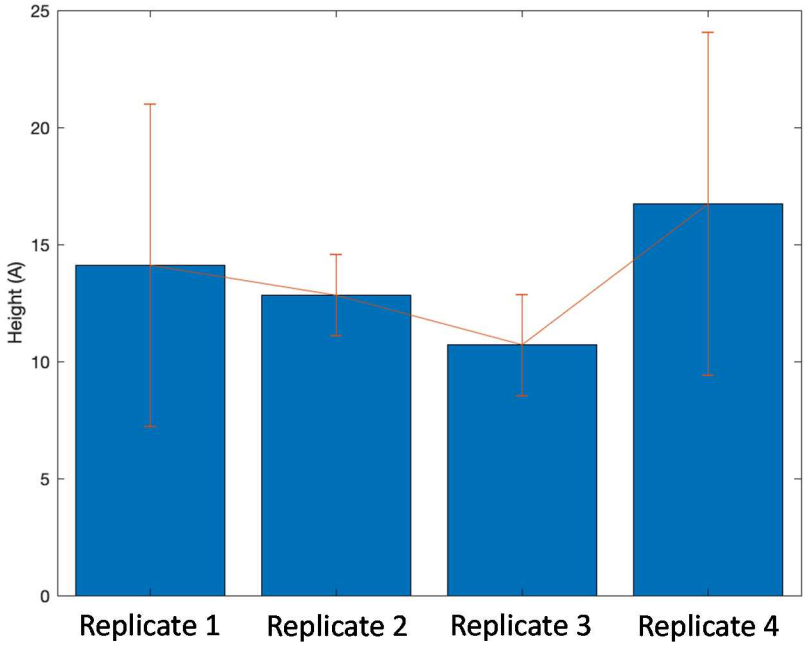

Figure 3. Representative example of thicknesses of BA-functionalized Si surfaces determined by ellipsometry.

While we were unable to obtain definitive information as to whether BAMPs can bind and/or aggregate SARS-CoV-2 spike protein, we were successfully able to evaluate, in part, the effect of BAMPs on the key spike-ACE2 interaction through the use of enzyme-linked immunosorbent assays (ELISAs). Figures 4 and 5 show the results of ELISAs performed on SARS-CoV-1 and SARS-CoV-2 spike proteins. The spike is adsorbed on polycarbonate surfaces in a 96 well plate, exposed and incubated to a solution of the polymer of interest, then exposed to ACE2, followed by a secondary antibody and an appropriate substrate to convert the amount of bound ACE2 to a quantitative optical readout.

The control curves in Figure 4(a) and Figure 5(a) clearly show that ACE2 binds to both spike proteins; increasing ACE2 concentration yields a larger absolute amount of bound ACE2, as evidenced by the increased absorbance of the sample. In the remaining panels, the polymer (or antibody, in the case of Figure 4(b) and Figure 5(b)) concentration is varied, while the subsequent ACE2 concentration is held constant. The positive control curves in Figure 4(b) and

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Figure 5(b) illustrate the outcome of the ELISA in a scenario where spike-ACE2 is effectively inhibited. The antibody m396 is known to inhibit spike-ACE2 binding for SARS-CoV-1, but not SARS-CoV-2. Confirming this notion, increasing concentration of m396 produced no significant change in the amount of ACE2 bound by SARS-CoV-2 spike, whereas increasing concentration of m396 clearly decreased the amount of ACE2 bound by SARS-CoV-1 spike.

Curiously, for both spike proteins, the addition of PEI and PEI-BA resulted in increasing amounts of ACE2 bound with increasing polymer concentration (Figure 4(c)-(d) and Figure 5(c)-(d)). Both PEI and PEI-BA are strong polyelectrolytes, possessing a high density of protonated amines in the buffered conditions (PBS) used in this ELISA. We suspect that a significant amount of PEI became attached to the surface-bound spike through electrostatic interactions, in turn enhancing the amount of ACE2 deposited through the same mechanism. This outcome precludes (for the specific case of PEI) any determination of the effect of the BA on spike-ACE2 binding through this particular ELISA. On the other hand, Figure 4(e)-(f) and Figure 5(e)-(f) clearly show that both PEG-NH_2 and PEG-BA had no effect on the amount of ACE2 bound by either SARS-CoV-1 or SARS-CoV-2 spike. More explicitly, these data indicate that the presence of BA in PEG-BA did not inhibit spike-ACE2 binding in either case.

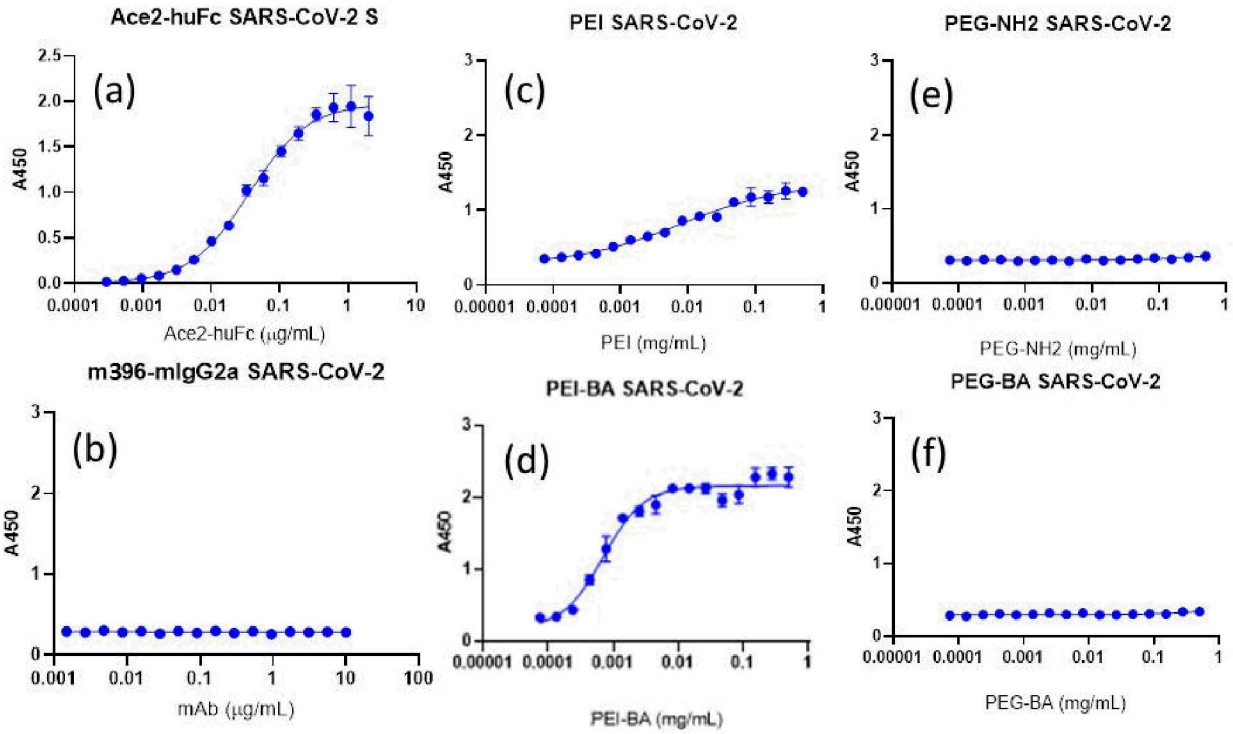

Figure 4. ELISA results for SARS-CoV-2 full trimeric spike protein attached to surface and exposed to **(a)** ACE2 (control curve), **(b)** m396 antibody, **(c)** PEI, **(d)** PEI-BA, **(e)** PEG-NH_2, and **(f)** PEG-BA. For **(b)-(f)**, the ACE2 concentration used was 0.01 μg/mL.

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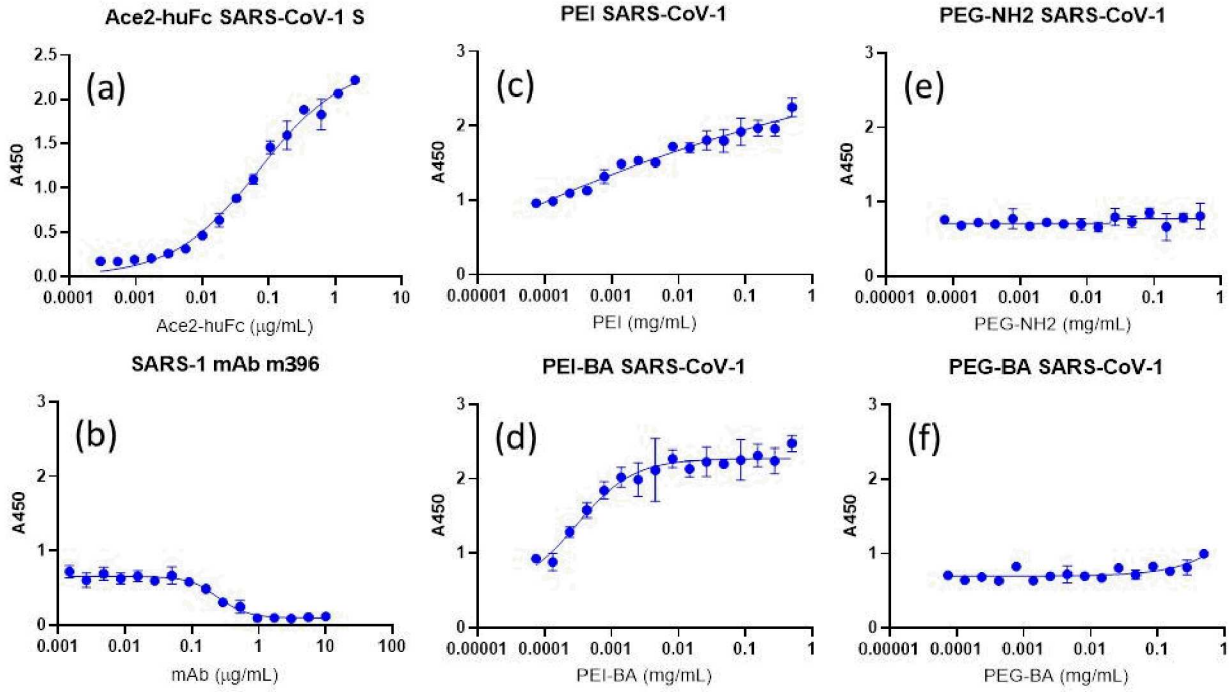

Figure 5. ELISA results for SARS-CoV-1 full trimeric spike protein attached to surface and exposed to **(a)** ACE2 (control curve), **(b)** m396 antibody, **(c)** PEI, **(d)** PEI-BA, **(e)** PEG-NH_2, and **(f)** PEG-BA. For **(b)-(f)**, the ACE2 concentration used was 0.033 μg/mL. The higher ACE2 concentration used here, relative to the SARS-CoV-2 ELISA was intended to more closely match the inflection point in the control curve.

We also tested the same BAMPs in a commercial, ELISA-based SARS-CoV-2 inhibition kit. This test differed slightly from the ELISA depicted by Figures 4 and 5 in that the spike protein and polymer of interest were incubated together and simultaneously exposed to surface-bound ACE2. The results are shown in Figure 6. Note that, in this case, the data are expressed as a percent inhibition; in other words, 100% inhibition corresponds to no bound spike protein.

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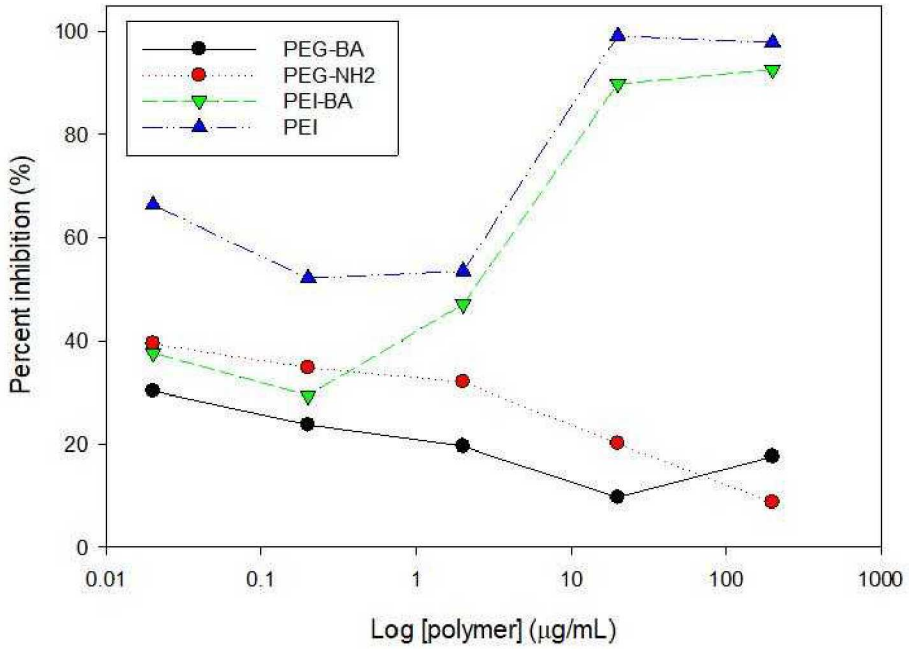

Figure 6. Inhibition of SARS-CoV-2 receptor binding domain recognition by surface-bound ACE2 after incubation of the former with BAMPs or unfunctionalized controls.

In this test, both PEI and PEI-BA strongly inhibited spike-ACE2 binding. We conjecture again that this outcome is derived from the polyelectrolyte nature of PEI. Electrostatic interactions may potentially disrupt the activity or structure of the spike, thereby reducing the efficacy of binding with ACE2. Nevertheless, the presence of BA does not impart any improvement in inhibition for PEI-BA relative to PEI. In contrast, PEG-NH_2 and PEG-BA both exhibited a marginal capacity to inhibit spike-ACE2 binding. More importantly, however, the presence of BA again does not impart any improvement in inhibition for PEG-BA relative to PEG-NH_2.

It is important to emphasize that the ELISAs are intended to interrogate only the spike-ACE2 binding event. The data presented here provide evidence that, contrary to our intended purposes, BAMPs **do not** interfere with this binding event. Lacking definitive information regarding the interaction of our BAMPs with the spike itself, two possible scenarios must be considered. On one hand, the BA moieties present in the BAMPs may simply have failed to form the desired boronate esters with spike saccharide residues under the conditions used for these ELISAs. In particular, the accessible residues in the spike protein, as well as the particular BA chemistry chosen, may perhaps require a higher working pH for appropriate binding. Alternatively, it may be possible that the BA moieties are, in fact, binding the spike protein via saccharide residues, yet the spike can still efficiently bind ACE2 in such a modified form.

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Despite the fact that the presence of BA did not inhibit or otherwise affect spike-ACE2 binding in the ELISAs, we remained curious as to whether the presence of BA could disrupt other key, downstream processes associated with viral infection, such as membrane fusion and viral entry. Therefore, two viral inhibition tests were further pursued, using Dengue virus (DENV) and bacteriophage Φ6 as surrogates in lieu of SARS-CoV-2. DENV is, of course, a mosquito-borne, enveloped virus that, like SARS-CoV-2, is amply decorated with glycoproteins.[26] Our test protocol with DENV involved infecting Vero cells with DENV; unfortunately, however, we were unable to obtain a proper control condition for successful infection of the Vero cells over the short duration of the project.

In contrast, the bacteriophage Φ6 is a well-known virus that infects *Pseudomonas* bacteria.[27] Φ6 is relatively unique among bacteriophages in that it possesses a lipid membrane, similar to DENV and SARS-CoV-2. However, the spike protein of Φ6 is not glycosylated, and the process by which Φ6 recognizes and infects *Pseudomonas* is much different from SARS-CoV-2 and its characteristic spike-ACE2 process. Nevertheless, and quite interestingly, we found a significant inhibitory effect of BAMPs to Φ6 infection. *Pseudomonas syringae* were cultured and exposed to samples of Φ6 containing *ca.* 10^7 viral particles per sample, previously incubated with BAMP at 0.05 mg/mL. After allowing infection to occur for 2 days, the amount of Φ6 was evaluated as shown in Table 1. Relative to the control experiment in the absence of any polymer, the presence of the unfunctionalized polymers PEI and PEG-NH_2 caused roughly an order of magnitude decrease in the quantity of Φ6 detected after infection. For PEG-BA, there was a further order of magnitude decrease in Φ6 relative to PEG-NH_2. For PEI-BA, the polymer completely inhibited the Φ6 infection process, as no Φ6 was ultimately detected. There was a clear effect of the BA functionality in inhibiting Φ6 infection, particularly in the case of PEI-BA. It is important to note that Table 1 shows the result of a single experiment, the only experiment with Φ6 successfully completed over the short duration of this project; hence, to increase confidence in the reproducibility and associated outcome, this result should ideally be confirmed through future repetition.

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Table 1. Φ6 counts after incubation with BAMPs for the indicated duration followed by infection of *Pseudomonas syringae*.

	PEI	PEI-BA	PEG-NH2	PEG-BA	Control
5 min	9e6	0	1e6	1.9e5	1.2e7
15 min	1.3e6	0	1.9e6	2.2e5	1.2e7
30 min	2.0e6	0	1.7e6	1.8e5	7e6
45 min	1.0e6	0	1e6	1.2e5	1.2e7

To reiterate, the spike protein of Φ6 is not glycosylated. Therefore, the inhibitory effect of the BA was somewhat surprising. It may be that the BA interacts with an alternative component of Φ6, such as its characteristic phospholipids. However, in truth, the mechanism by which the inhibitory effect of the BA is derived is wholly unclear at this point. If the BA targeted a component of Φ6 that is universally found in a wide variety of viruses, then these BAMPs would reasonably be expected to exhibit broad anti-viral behavior. Until additional information regarding the specific mechanism is uncovered, or inhibition tests are successfully completed with other viruses, such a notion is purely speculative.

CONCLUSIONS

It remains unclear from the experiments attempted as to whether BAMPs can effectively crosslink or otherwise bind SARS-CoV-2 spike protein. Regardless, initial results from two different ELISAs clearly show no appreciable effect of the BA functionality in inhibiting spike-ACE2 binding relative to unfunctionalized controls. In contrast, an initial test of the effect of BAMPs on Φ6 bacteriophage infection showed a marked inhibitory effect due to the BA functionality, although this outcome is of dubious relevance to SARS-CoV-2. We recommend that any future efforts to evaluate the fundamental nature of interaction between BAMPs and the spike proteins of enveloped viruses focus on the development and implementation of experimental protocols that enable determination of the appropriate conditions (*e.g.*, pH, BA selection) necessary to enforce BA-spike binding.

REFERENCES

(1) James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S. Saccharide Sensing with Molecular Receptors Based on Boronic Acid. *Angew. Chem. Int. Ed.* **1996**, *35*, 1910-1922.

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(2) Yang, W.; Gao, X.; Wang, B. Boronic Acid Compounds as Potential Pharmaceutical Agents. *Med. Res. Rev.* **2003**, *23*, 346-368.

(3) Bull, S. D.; Davidson, M. G.; van den Elsen, J. M. H.; Fossey, J. S.; Jenkins, A. T. A.; Jiang, Y.-B.; Kubo, Y.; Marken, F.; Sakurai, K.; Zhao, J.; James, T. D. Exploiting the Reversible Covalent Bonding of Boronic Acids: Recognition, Sensing, and Assembly. *Acc. Chem. Res.* **2013**, *46*, 312-326.

(4) Wu, X.; Li, Z.; Chen, X.-X.; Fossey, J. S.; James, T. D.; Jiang, Y.-B. Selective Sensing of Saccharides Using Simple Boronic Acids and Their Aggregates. *Chem. Soc. Rev.* **2013**, *42*, 8032-8048.

(5) Guan, Y.; Zhang, Y. Boronic acid-containing hydrogels: synthesis and their applications. *Chem. Soc. Rev.* **2013**, *42*, 8106-8121.

(6) Brooks, W. L. A.; Sumerlin, B. S. Synthesis and Applications of Boronic Acid-Containing Polymers: From Materials to Medicine. *Chem. Rev.* **2016**, *116*, 1375-1397.

(7) Akgun, B.; Hall, D. G. Boronic Acids as Bioorthogonal Probes for Site-Selective Labeling of Proteins. *Angew. Chem. Int. Ed.* **2018**, *57*, 13028-13044.

(8) Ryu, J. H.; Lee, G. J.; Shih, Y.-R. V.; Kim, T.-I.; Varghese, S. Phenylboronic Acid-polymers for Biomedical Applications. *Curr. Med. Chem.* **2019**, *26*, 6797-6816.

(9) Antonio, J. P. M.; Russo, R.; Carvalho, C. P.; Cal, P. M. S. D.; Gois, P. M. P. Boronic acids as building blocks for the construction of therapeutically useful bioconjugates. *Chem. Soc. Rev.* **2019**, *48*, 3513-3536.

(10) Stubelius, A.; Lee, S.; Almutairi, A. The Chemistry of Boronic Acids in Nanomaterials for Drug Delivery. *Acc. Chem. Res.* **2019**, *52*, 3108-3119.

(11) Springsteen, G.; Wang, B. A detailed examination of boronic acid–diol complexation. *Tetrahedron* **2002**, *58*, 5291-5300.

(12) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols—it is not as simple as it appears. *Tetrahedron* **2004**, *60*, 11205-11209.

(13) Amaral, A. J. R.; Pasparakis, G. Macromolecular cell surface engineering for accelerated and reversible cellular aggregation. *Chem. Commun.* **2015**, *51*, 17556-17559.

(14) Amaral, A. J. R.; Pasparakis, G. Rapid Formation of Cell Aggregates and Spheroids Induced by a "Smart" Boronic Acid Copolymer. *ACS Appl. Mater. Interf.* **2016**, *8*, 22930-22941.

(15) Bachand, G. D.; Greene, A.; Jones, B. H.; Ko, R. Cell Gels – Building Reversible Networks from Boronic Acid and Cell Membrane Chemistry. SAND2017-10056R, Sandia National Laboratories, **2017**.

(16) Amaral, A. J. R.; Pasparakis, G. Cell Membrane Engineering with Synthetic Materials: Applications in Cell Spheroids, Cellular Glues and Microtissue Formation. *Acta Biomater.* **2019**, *90*, 21-36.

(17) Miller, P. R.; Jones, B. H. Reversible Binding Tissue Adhesive. SD # 15288, Sandia National Laboratories, **2020**.

(18) Watanabe, Y.; Allen, J. D.; Wrapp, D.; McLellan, J. S.; Crispin, M. Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* **2020**, DOI: 10.1126/science.abb9983.

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(19) Shajahan, A.; Supekar, N. T.; Gleinich, A. S.; Azadi, P. Deducing the N- and O-glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2. *Glycobiology* **2020**, DOI: 10.1093/glycob/cwaa042.

(20) Shang, J.; Wan, Y.; Luo, C.; Ye, G.; Geng, Q.; Auerbach, A.; Li, F. Cell entry mechanisms of SARS-CoV-2. *Proc. Nat. Acad. Sci.* **2020**, DOI:10.1073/pnas.2003138117.

(21) Łoczechin, A.; Séron, K.; Barras, A.; Giovanelli, E.; Belouzard, S.; Chen, Y.-T.; Metzler-Nolte, N.; Boukherroub, R.; Dubuisson, J.; Szunerits, S. Functional Carbon Quantum Dots as Medical Countermeasures to Human Coronavirus. *ACS Appl. Mater. Interf.* **2019**, *11*, 42964-42974.

(22) He, L.; Fullenkamp, D. E.; Rivera, J. G.; Messersmith, P. B. pH responsive self-healing hydrogels formed by boronate–catechol complexation. *Chem. Commun.* **2011**, *47*, 7497-7499.

(23) Thorne, J. B.; Vine, G. J.; Snowden, M. J. Microgel applications and commercial considerations. *Colloid Polym. Sci.* **2011**, *289*, 625-646.

(24) Pensa, E.; Cortes, E.; Corthey, G.; Carro, P.; Vericat, C.; Fonticelli, M. H.; Benitez, G.; Rubert, A. A.; Salvarezza, R. C. The Chemistry of the Sulfur-Gold Interface: In Search of a Unified Model. *Acc. Chem. Res.* **2012**, *45*, 1183-1192.

(25) Wrapp, D.; Wang, N.; Corbett, K. S.; Goldsmith, J. A.; Hsieh, C.-L.; Abiona, O.; Graham, B. S.; McLellan, J. S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **2020**, *367*, 1260-1263.

(26) Rey, F. A. Dengue virus envelope glycoprotein structure: New insight into its interactions during viral entry. *Proc. Nat. Acad. Sci.* **2003**, *100*, 6899-6901.

(27) Phillpotts, R. J.; Thomas, R. J.; Beedham, R. J.; Platt, S. D.; Vale, C. A. The *Cystovirus* phi6 as a simulant for Venezuelan equine encephalitis virus. *Aerobiologia* **2010**, *26*, 301-309.

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