The first-in-class peptide binder to the SARS-CoV-2 spike protein

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Abstract

Coronavirus disease 19 (COVID-19) is an emerging global health crisis. With over 200,000 confirmed cases to date, this pandemic continues to expand, spurring research to discover vaccines and therapies. SARS-CoV-2 is the novel coronavirus responsible for this disease. It initiates entry into human cells by binding to angiotensin-converting enzyme 2 (ACE2) via the receptor binding domain (RBD) of its spike protein (S). Disrupting the SARS-CoV-2-RBD binding to ACE2 with designer drugs has the potential to inhibit the virus from entering human cells, presenting a new modality for therapeutic intervention. Peptide-based binders are an attractive solution to inhibit the RBD-ACE2 interaction by adequately covering the extended protein contact interface. Using molecular dynamics simulations based on the recently solved ACE2 and SARS-CoV-2-RBD co-crystal structure, we observed that the ACE2 peptidase domain (PD) α1 helix is important for binding SARS-CoV-2-RBD. Using automated fast-flow peptide synthesis, we chemically synthesized a 23-mer peptide fragment of the ACE2 PD α1 helix composed entirely of proteinogenic amino acids. Chemical synthesis of this human derived sequence was complete in 1.5 hours and after work up and isolation >20 milligrams of pure material was obtained. Bio-layer interferometry revealed that this peptide specifically associates with the SARS-CoV-2-RBD with low nanomolar affinity. This peptide binder to SARS-CoV-2-RBD provides new avenues for COVID-19 treatment and diagnostic modalities by blocking the SARS-CoV-2 spike protein interaction with ACE2 and thus precluding virus entry into human cells.

Key words: SARS-CoV-2, peptide binder, protein-protein interaction inhibitor, coronavirus, COVID-19, rapid response, peptide therapeutic, MD simulation, automated flow peptide synthesis

1. Introduction

A novel coronavirus (SARS-CoV-2) from Wuhan, China, has caused 207,855 confirmed cases and 8,648 deaths globally, according to the COVID-19 situation report from WHO on Mar 19, 2020 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/), and the number is continually growing. Similar to the SARS-CoV outbreak in 2002, SARS-CoV-2 causes severe respiratory problems. Coughing, fever, difficulties in breathing and/or shortage of breath are the common symptoms. Aged patients with pre-existing medical conditions are at most risk with a mortality rate ~1.5% or even higher in some regions. Moreover, human-to-human transmission can occur rapidly by close contact. To slow this pandemic and treat infected patients, rapid development of specific antiviral drugs is of the highest urgency.

The closely-related SARS-CoV coronavirus invades host cells by binding the angiotensin-converting enzyme 2 (ACE2) receptor on human cell surface through its viral spike protein (S) [1-4]. It was recently established that SARS-CoV-2 uses the same receptor for host cell entry [5, 6]. Recent crystallographic studies of the SARS-CoV-2-S receptor binding domain (RBD) and full-length human ACE2 receptor revealed key amino acid residues at the contact interface between the two proteins and provide valuable structural information that can be leveraged for the development of disruptors specific for the SARS-CoV-2/ACE2 protein-protein interaction (PPI) [7, 8]. Small-molecule inhibitors are often less effective at disrupting extended protein binding interfaces [9]. Peptides, on the other hand, offer a synthetically accessible solution to disrupt PPIs by binding at interface regions containing multiple contact “hot spots” [10].

We hypothesize that disruption of the viral SARS-CoV-2-RBD-host ACE2 interaction with peptide-based binders will prevent virus entry into human cells, offering a novel opportunity for therapeutic intervention. Toward this aim, we launched a campaign to rapidly discover peptide binders to SARS-CoV-2-RBD. Analyzing the RBD-ACE2 co-crystal structure, we found that SARS-CoV-2-RBD/ACE2 interface spans a large elongated surface area, as is common for PPIs.

We leveraged molecular dynamic simulations and automated fast-flow peptide synthesis [11] to prepare a 23-mer peptide binder (SBP1) to SARS-CoV-2-RBD, the sequence of which was derived from the ACE2 α1 helix. Using bio-layer interferometry, we determined that SBP1 binds SARS-CoV-2-RBD with low nanomolar affinity (dissociation constant, K_D = 47 nM). SBP1 is a first-in-class peptide binder to SARS-CoV-2-RBD, potentially inhibiting entry of the virus into human cells. In addition, the human protein-derived sequence of SBP1 is fully proteinogenic, and is not expected to be immunogenic. Taken together, computational simulations coupled with automated flow peptide synthesis technology enable an accelerated discovery loop from design to experimental validation, and rapidly delivered SBP1 as a promising pre-clinical drug lead. Antiviral activity studies in mammalian cell cultures are in progress in order to evaluate efficacy of the current and future optimized peptide binders.

2. Results

Molecular dynamic simulations guide peptide binder design

Using the Amber force field [12], a helical peptide sequence (spike-binding peptide 1, SBP1) derived from the α1 helix of ACE2 peptidase domain (ACE2-PD) in complex with SARS-CoV-2-RBD was simulated under TIP3P explicit water conditions. Analyzing the simulation trajectory after 200 ns, we found that SBP1 remains on the spike RBD protein surface in a stable conformation (Fig. 2B) with overall residue fluctuations smaller than 0.8 nm compared with their starting coordinates (Fig. 2A). Per-residue analysis along the 200 ns trajectory showed that the middle residues of SBP1, a sequence we termed SBP2, have significantly reduced fluctuations (Fig. 2C, 2D), indicating key interactions. The results of this MD simulation suggest that SBP1 and SBP2 peptides derived from the ACE-PD α1 helix may alone potentially bind the SARS-CoV-2 spike RBD protein with sufficient affinity to disrupt the associated PPI.

Automated fast-flow peptide synthesis yielded >95% pure compound

The two biotinylated peptides, SBP1 and SBP2, derived from the α1 helix were prepared by automated fast-flow peptide synthesis [11, 13] with a total synthesis time of 1.5 h for a total of 35 couplings. After cleavage from resin, global deprotection, and subsequent C18 solid-phase extraction, the purity of the crude peptides was estimated to be >95% for both biotinylated SBP1 and SBP2 based on LC-MS TIC chromatograms (supplemental Fig. 1A and 1B). We assessed this purity as acceptable for direct downstream biological characterizations.

SBP1 peptide specifically binds SARS-CoV-2-RBD with low nanomolar affinity

Bio-layer interferometry was employed to measure the affinity of synthesized peptides (e.g., SBP1) to glycosylated SARS-CoV-2-RBD or the human protein menin as a negative control. Biotinylated peptide was immobilized onto streptavidin (SA) biosensors. After fitting the association and dissociation curves from serial dilutions of protein, the dissociation constant (K_D) of SBP1 to the RBD was determined to be 47 nM with the average $K_{on} = 4.69 \times 10^4$ M^{-1} s^{-1} and $K_{off} = 2.2 \times 10^{-3}$ s^{-1} (Fig. 2E). However, SBP2 (a truncate of SBP1) did not associate with the spike RBD protein (Fig. 2F). SBP1 had no observable binding to the negative control human protein menin (Fig. 2G). Together, these data indicate that SBP1 specifically binds SARS-CoV-2-RBD with low nanomolar affinity.

3. Discussion

Recently published cryo-EM and co-crystal structures of the RBD of SARS-CoV-2 with human ACE2 have identified this PPI as key step for the entry of SARS-CoV-2 to human cells. Blocking this binding interface represents a highly promising therapeutic strategy, as it could potentially hinder SARS-CoV-2 from entering cells and replicating.

Drugging PPIs is a longstanding challenge in traditional drug discovery and peptide-based approaches might help to solve this problem. Small molecule compounds are unlikely to bind large protein surfaces that do not have distinct binding pockets. Peptides, on the other hand, display a larger surface area and chemical functionalities that can mimic and disrupt the native PPI, as is the case for the clinically approved HIV peptide drug Fuzeon [14, 15].

The identification of a suitable starting point for drug discovery campaigns can be time-intensive. During a pandemic such as this one, therapeutic interventions are urgently needed. To rapidly identify potential peptide binders to the SARS-CoV-2 spike protein, we used molecular dynamics (MD) simulation on peptides extracted from the human ACE2 sequence. The starting point of the binding simulations was the cryo-electron microscopy model of SARS-CoV-2 spike protein and several peptides derived from the SARS-CoV-2-spike binding domain of human ACE2 protein. Our MD simulation (200 ns trajectory) indicated that the SBP1 peptide, corresponding to the *N*-terminal ACE2 α1 helix, stably bound to SARS-CoV-2-RBD. The overall peptide fluctuations were smaller than 0.8 nm from starting coordinates (Fig. 2A). These results indicated the potential of identifying a SARS-CoV-2-RBD-binding peptide derived from the human ACE2 α1 helix.

A 23-mer peptide sequence (SBP1) was synthesized by automated flow peptide synthesis. The 23 residues selected from the ACE2 α1 helix sequence (IEEQAKTFLDKFNHEAEDLFYQS) showed low fluctuations along the MD simulation trajectory and several important interactions with the spike protein were observed consistently with multiple lines of published data [8, 16]. We decided to use this peptide (SBP1) as an experimental starting point for the development of a SARS-CoV-2 spike protein binder. Our rapid automated flow peptide synthesizer [11, 13] enabled the synthesis of tens of milligrams of SBP1 peptide within minutes. The crude purity was determined to be >95% and therefore sufficient for binding validation by bio-layer interferometry (BLI).

The interaction between SBP1 and the RBD of SARS-CoV-2 spike protein was validated by bio-layer interferometry. The K_D, derived from protein association and dissociation kinetics, was found to be 47 nM after averaging the fitting values at different protein concentrations (Fig. 2E). This binding affinity is comparable with that of full length ACE2 binding to SARS-CoV-2-RBD (14.7 nM) [7]. Excess of SBP1 therefore, could potentially cover spike proteins on SARS-CoV-2 surface and outcompete the binding for ACE2. Further optimization of the sequence length and amino acid composition are in progress, in order produce binders with higher PPI inhibitory activity.

SBP1 is a “fully human,” endogenous peptide and therefore likely to be well tolerated by the human immune system. The amino acid sequence of SBP1 is entirely derived from human ACE2 and should be recognized as endogenous by the human immune system. This feature could be highly beneficial in later stages of pre-clinical development. Challenges associated with peptide drugs, such as proteolytic degradation and rapid renal elimination, will be addressed in the near future by chemical modifications of the sequence.

In conclusion, a peptide sequence derived from human ACE2 was found to bind the SARS-CoV-2 spike protein RBD with low nanomolar affinity. We believe disruption of the SARS-CoV-2-RBD/ACE2 binding interface with high-affinity peptides represents a promising strategy for preventing virus entry in human cells and paves the way for new COVID-19 treatment and diagnostic modalities. Upon request, we will make available our peptide binder and all upcoming optimized variants to research facilities testing and developing potential COVID-19 treatments. Rapid discovery, synthesis, and testing of potential drug leads should help the global scientific and healthcare communities efficiently address the ongoing crisis.

4. Experimental Materials and Methods

GPU-accelerated molecular dynamic simulation

The co-crystal structure of SARS-CoV-2-RBD with ACE2-B^0AT1 (PDB: 6M17) was chosen as the initial structure, which was explicitly solvated in an 87 $Å^3$ box, to perform a 200 ns molecular dynamical (MD) simulation using NAMD on MIT's supercomputing clusters (GPU node). Amber force field was used to model the protein and peptide. The MD simulation system was equilibrated at 300 K for 2 ns. Periodic boundary conditions were used and long-range electrostatic interactions were calculated with particle mesh Ewald method, with non-bonded cutoff set to 12.0 Å. SHAKE algorithm was used to constrain bonds involving hydrogen atoms. Time step is 2 fs and the trajectories were recorded every 10 ps. After simulation production runs, trajectory files were loaded into the VMD software for further analysis.

Automated fast-flow peptide synthesis

SBP1 and SBP2 sequences were synthesized at 90 °C on Rink Amide-ChemMatrix resin with HATU activation using a fully automatic flow-based peptide synthesizer. Amide bond formation was performed in 8 seconds, and Fmoc groups were removed in 8 seconds with 40% (v/v) piperidine in DMF. Overall cycle times were about 120 seconds. After completion of fast-flow synthesis, the resins were washed with DMF (3 x) and then incubated with HATU-activated biotin-PEG_4-propionic acid (CAS# 721431-18-1) at room temperature for 1.0 h for biotinylation on the peptide *N*-terminus.

Peptide cleavage and deprotection

After peptide synthesis, the peptidyl resin was rinsed with dichloromethane briefly and then dried in a vacuum chamber overnight. Next day, approximately 5 mL of cleavage solution (94% TFA, 1% TIPS, 2.5% EDT, 2.5% water) was added into the syringe containing the resin. The syringe was kept at room temperature for 2 h before injecting the cleavage solution into a 50 mL conical

tube. Dry-ice cold diethyl ether (~50 mL) was added to the cleavage mixture and the precipitate was collected by centrifugation and triturated twice with cold diethyl ether (50 mL). The supernatant was discarded. Residual ether was allowed to evaporate and the peptide was dissolved in water with 0.1% TFA for solid-phase extraction.

Solid-phase extraction (SPE)

After peptide cleavage, peptide precipitates were dissolved in water with 0.1% TFA. Agilent Mega BE C18 column (Part No: 12256130) was conditioned with 5 mL of 100% acetonitrile with 0.1% TFA, and then equilibrated with 15 mL of water with 0.1% TFA. Peptides were loaded onto the column for binding, followed by washing with 15 mL of water with 0.1% TFA, and finally, eluted with 5 mL of 30/70 water/acetonitrile (v/v) with 0.1% TFA.

Liquid chromatography-mass spectrometry (LC-MS)

Peptides were dissolved in water with 0.1% TFA followed by LC-MS analysis on an Agilent 6550 ESI-Q-TOF instrument using an Agilent Jupiter C4 reverse-phase column (2.1 mm × 150 mm, 5 µm particle size). Mobile phases were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Linear gradients of 1 to 61% solvent B over 15 minutes (flow rate: 0.5 mL/min) were used to acquire LC-MS chromatograms.

Kinetic binding assay using bio-layer interferometry (BLI)

A ForteBio Octet® RED96 Bio-Layer Interferometry system (Octet RED96, ForteBio, CA) was used to characterize the *in vitro* peptide-protein binding at 30 °C and 1000 rpm. Briefly, streptavidin (SA) tips were dipped in 200 µL of biotinylated peptide solution (2.5 µM in 1x kinetic buffer: 1x PBS with 0.1% BSA and 0.05% tween) for the loading step. The tips loaded with peptide were then sampled with SARS-CoV-2-RBD or menin protein at various concentrations in 1x kinetic buffer to obtain the association curve. Peptide only was used as reference for background subtraction. After association, the tips were dipped back into 1x kinetic buffer to obtain the

dissociation curve. The association and dissociation curves were fitted with ForteBio Biosystems using four experimental conditions (n = 4, global fitting algorithm, binding model 1:1) to obtain the dissociation constant K_D.

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Competing interests

B.L.P. is a founder of Resolute Bio and Amide Technologies.

Figure captions:

Figure 1. MD-guided target selection for rapid flow synthesis of a SARS-CoV-2-RBD-binding peptide. Fragments of ACE2-PD domain are docked against SARS-CoV-2 receptor-binding domain (PDB: 6M17). Low RMSD peptides are rapidly synthesized by fully automated flow peptide synthesis, and binding to glycosylated SARS-CoV-2-RBD is determined by BLI.

Figure 2. Human ACE2-PD domain α-helix 1-derived SBP1 binds SARS-CoV-2-RBD. (A) RMSD for SBP1 docked to SARS-CoV-2-RBD during 200 ns MD simulation. (B) Binding interface between SARS-CoV-2-RBD and SBP1 after 200 ns simulation. Individual RMSD (C) and average RMSD (D) values for SBP1 residues over the course of the 200 ns simulation. Arrows indicate residues contributing key hydrogen bonding interactions (determined using UCSF Chimera, Version 1.12). Individual residues with RMSD below 5 Å arbitrarily colored green. Binding affinity of SBP1 and SBP2 to glycosylated SARS-CoV-2-RBD (E, F), and affinity of SBP1 to negative control human protein menin (G), as determined by bio-layer interferometry.

Supplemental Figure 1. ACE2-derived peptides were prepared by solid-phase peptide synthesis. Total ion current chromatograms (TIC) of purified biotinylated SBP1 peptide (A), purified biotinylated SBP2 peptide (B), crude SBP1 peptide (C), and crude SBP2 peptide (D).

Figures:

Figure 1

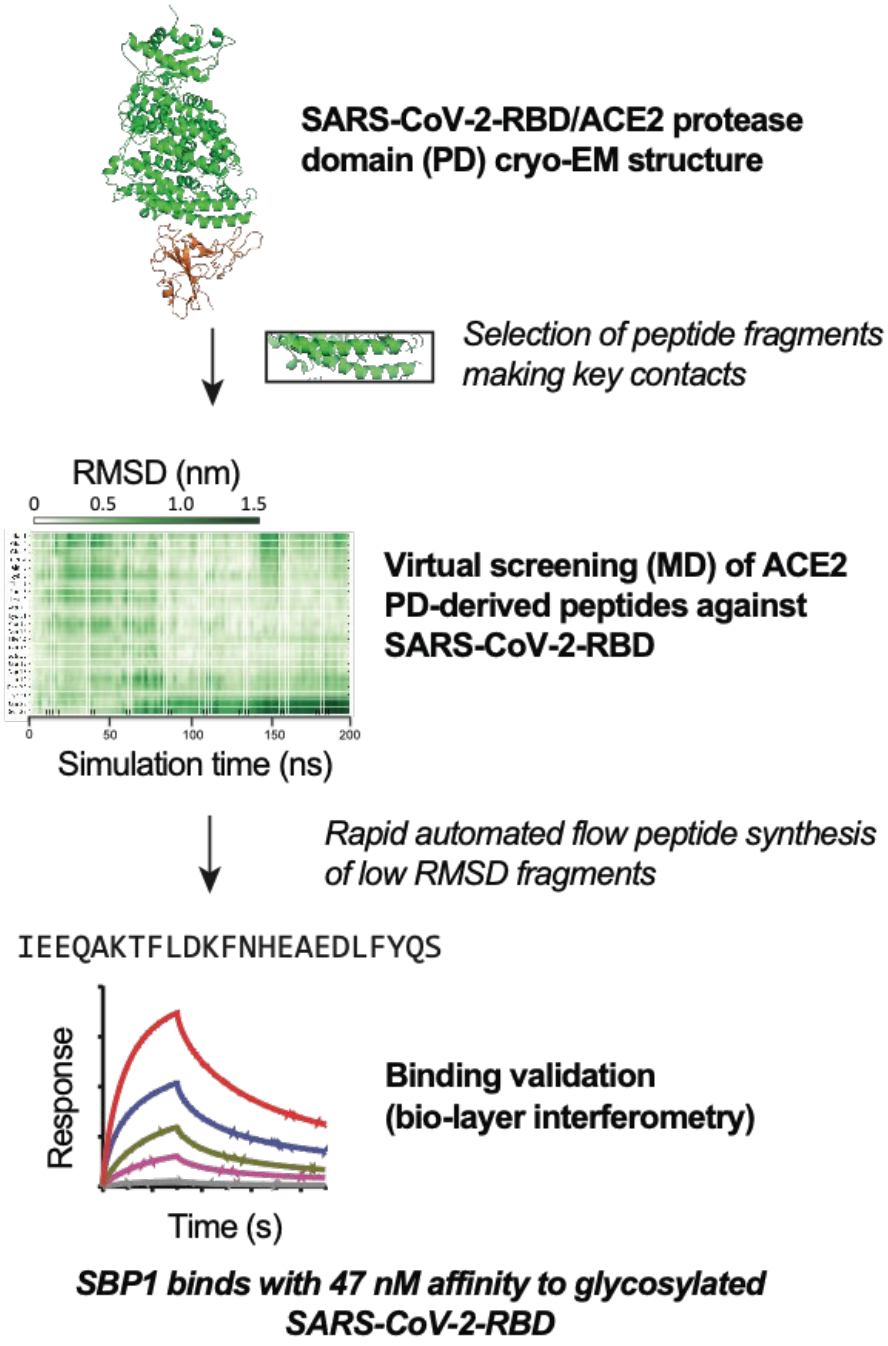
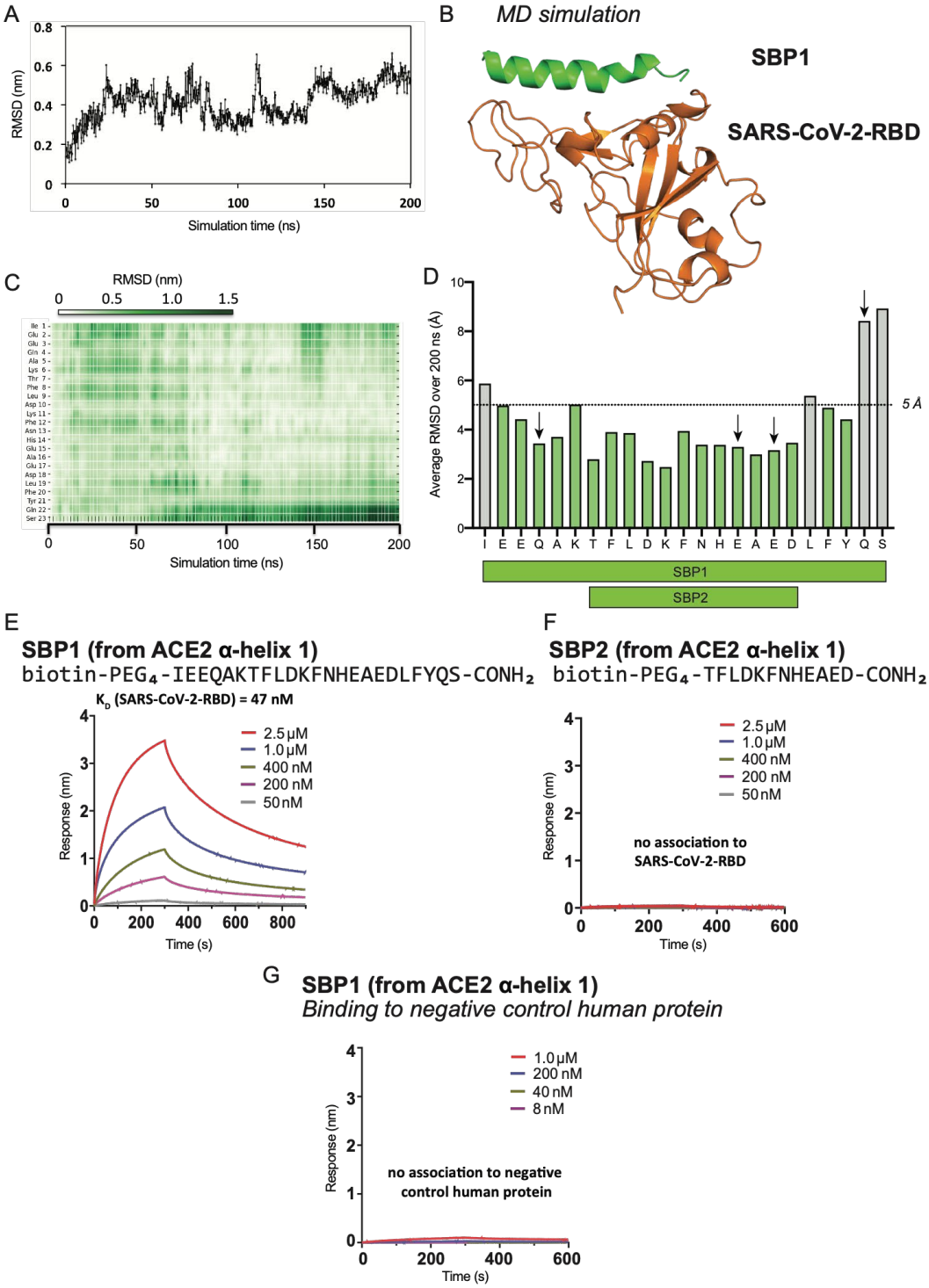
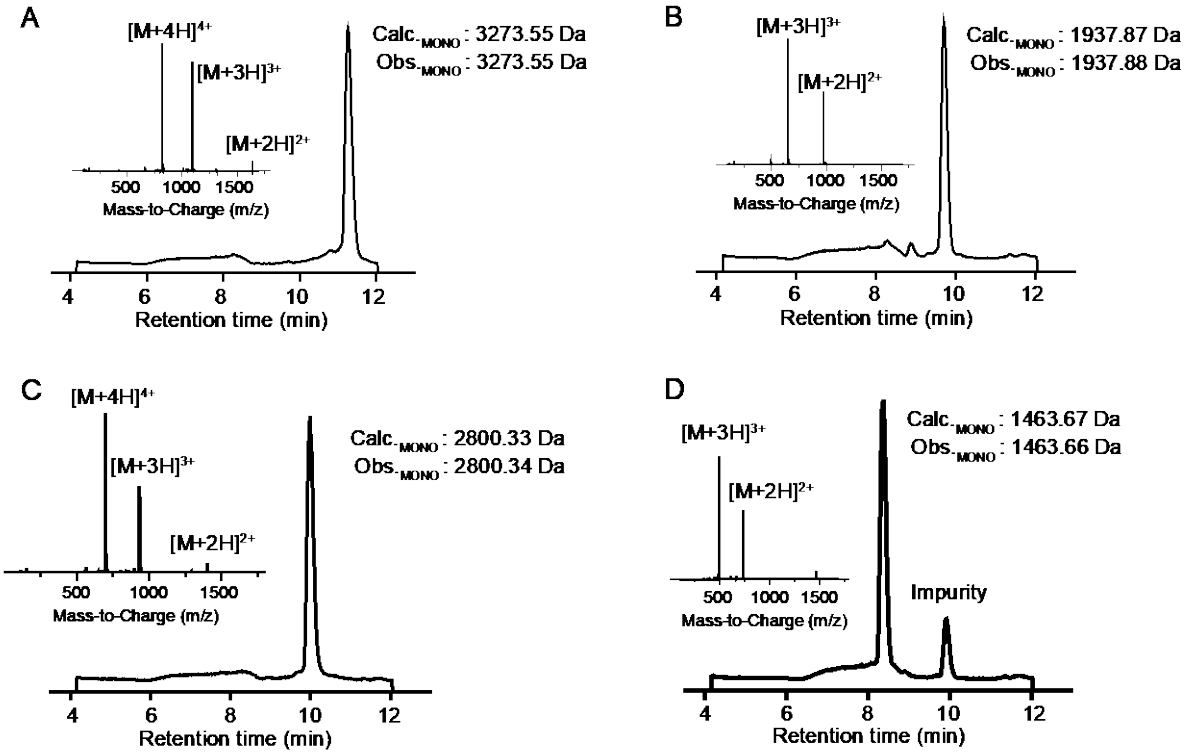


Figure 2



260 **Supplemental Figure 1**



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263

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