A. **Project Title – Pan-Covid-19 MultiValent Binders to Block Virus Entry**

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D. **Budget** –

EHRA Salary	38,625
SHRA Salary	0
Grad Student	28,000
Temps	32,751
Fringe Pool	16,420
Non-Personnel Expenses	61,776
Total	**177,572**

E. **Specific problem** (i) No specific anti-Covid-19 therapeutics or prophylactic exist for treatment or prevention of COVID-19 infection. (ii) A number of nonspecific antiviral drugs, including IFN, lopinavir-ritonavir (HIV protease inhibitors), chloroquine, favipiravir (T-705) and remdesivir (GS-5734), have been used in clinics to treat covid-19 infection but their efficacies still require further confirmation. Also, ***their potential use for treatment of infection by other coronaviruses and emerging coronaviruses in the future is unclear***. (iii) While spike proteins are the main target of monoclonal antibody (mAb) development, single site binding will not fully inhibit entry, nor provide protection from emerging Covs. The spike has multiple binding sites enabling adhesion in a multivalent manner to host cells, and the dense packing of spike on the virion enable tight attachment. *For an effective immune prophylaxis in humans, broad coverage of different strains of CoVs and control of potential neutralization escape variants is required. Combinations of virus-neutralizing, noncompeting peptide binders onto one scaffold or fused to mAbs may have these properties.*

F. **Goals and objectives over 6-Month:** Our approach to prevent infection is the design of synthetic or semisynthetic multivalent binders that block viral entry by recognizing different epitopes on the receptor-binding domain (RBD) – Figure 1. We will synthesize peptides or peptoids analogues (Freeman) (for excess stability) mimetic of the angiotensin-converting enzyme 2 (ACE2) peptidase domain (green) and fuse them (Freeman & Kuhlman) to mAbs CR3022 (blue). We will vary the spatial arrangement of epitopes and optimize it using computational approaches (Kuhlman & Forest) to match the geometry of binding sites of the spike protein. These binders will cover the entire virus envelope, thus preventing its binding to the host cells. Such approach presents an alternative to strategies that target virus entry by spike-inhibiting mAbs or that address late steps of the viral replication cycle.

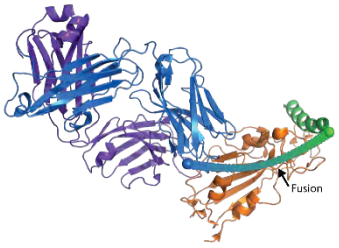

Figure 1 superimposed crystal structures of the antibody CR3022 and ACE2-mimetic peptide binding to the COVID-19 RBD. The two binding sites do not overlap allowing fusing of peptides to the terminal of CD3022 for enhanced avidity.

G. **Outcome:** Anti-covid-19 multivalent binders can be used for treatment and prevention of infection by not only COVID-19, but also other corona viruses. We are one of very few working on peptide drugs. **At the end of 6 months we will**: (i) generate a library of peptide-antibody fusions introducing peptide variants for induced binding. (ii) Test peptide versus peptoid binding (ii) perform computational

simulations to predict the interactions between the various peptide variants and the spike protein. (iv) scale up peptide and peptoid productions (using high throughout microwave synthesizer)

Multivalent binders show exceptional promise as antiviral therapeutic for current and future CoVs. The Freeman lab received funding through the Research Corporation for Science Advancement ($110K) and would leverage these funds to expand the activity with additional collaborators at UNC with the support of the NC Collaboratory.