PREDICTING FUTURE MUTATIONS FOR ESCAPE-RESISTANT VACCINES

The continuing mutation of COVID-19 (delta, lambda, omicron) during COVID-19 pandemic has shown the need for new type of vaccine designs - one that is as dynamic and nimble as the virus it plans to protect against. Periodic reformulations similar to seasonal flu vaccines, is problematic for COVID-19 given its current rapid rate of mutation coupled with a high transmissibility, infectious asymptomatic patients, vaccine hesitancy, and potentially high mortality. This situation is not unique: emergent viruses experience diverse selection pressures fostering adaptations via new mutations. The current state of knowledge has no reliable tools to preempt such viruses: we do not know when or how new mutants will arise, and how to protect against them [1–7]. Thus, there is need of revolutionary conceptual breakthroughs to predict how a viral strain mutates in the wild under realistic selection, allowing the design and testing of hundreds of vaccines before the viral strain emerges.

Unique Aspects, Personnel: To achieve this goal, we formulated the methodological foundations to elicit a deep understanding of the evolutionary dynamics in the sequence/strain space. Our overarching vision, backed by pilot studies over the past year with limited intramural funding from the UChicago Big Ideas incubator, is to computationally interrogate evolutionary patterns underlying the current pandemic and beyond. Since each viral strain is but a single point in a $\approx \times 10^4$ dimensional space (SARS-CoV-2 genome $\approx 3 \times 10^4$ bases), we can never comprehensively explore the state space. But we don't need to. We reduce the number of combinations by accounting for only the combinations that occur along evolutionary trajectories – making calculation possible on high-performance computing clusters.

We have to-date predicted new mutations on the SARS-CoV-2 spike protein, and shown in in-vitro experiments that these computationally predicted variants express correctly, are functional (bind to the human ACE2 receptor), and some are more resistant to antibody binding assays compared to the wild type strain. Using data from early 2020 when the pandemic was in its infancy, we could preempt mutations that eventually arose in the delta variant. Testing the idea along a longer time-frame, we applied the same concept to Influenza drift. Here, this approach consistently out-perform WHO/CDC predictions for vaccine components with respect to how far removed the predictions were from the dominant strain in the future season [8].

Thus, via a cross-disciplinary collaboration between Prof. Ishanu Chattopadhyay [9–12] (mathematical modeling, information theory, machine learning) and Prof. Aaron Esser-Kahn [12–14] (immunology, vaccine science), we envision a radically approach to escape-resistant vaccine design. Beyond predicting likely future mutations in circulating strains, the goal of this proposal will be to build a platform technology which can be developed/tested toward (1) predicting mutations within a single individual as a potential source of novel variant emergence, (2) develop a rank-ordering of sampled strains in animal reservoirs by risk of emergence (a capability well-beyond the state of the art). Such methods would form the nucleus of a burgeoning field of precision interventions in the animal reservoir to preemptively neutralize threats, *before the first human infection*.

#1. More details on what we are going to do?

Justifying Keck Support: Our vision entails risks; we are challenging a prevailing dogma, that future mutations, and variants, of a pathogen are intrinsically random and hence unpredictable. We have sufficient evidence to the contrary, and need Keck's support to validate our tool in a well-vetted test/design/test loop ultimately fostering a paradigm shift in how we combat pandemics in future. While we have been turned down recently by NIH (FOA: AI21-035, Application id: 1 R21 AI169352-01), this study can fundamentally change the game, with high future interest from stakeholders.

Budget, Timeline: Conducted over a period of three years costing 1.15M USD, supporting study personnel (PI time + Postdoctoral time $\approx 33\%$, computational costs ($\approx 10\%$) and experimental costs ($\approx 50\%$).

PROJECT DESCRIPTION

Overview: The COVID-19 pandemic, despite multiple vaccines, continues to be an ongoing challenge as new variants and potential escape mutants emerge. The current practice has no tools to predict, let alone preempt such emergence: we do not know when new mutants will arise, and how these mutants will differ in terms of pathogenicity, transmissibility and resistance to current vaccines. A key conceptual barrier is the missing ability to numerically estimate the likelihood of specific future mutations. Currently this likelihood is equated to sequence similarity, which is measured by how many mutations it takes to change one strain to another (the edit distance). In reality, the odds of one sequence mutating to another is a function of not just how many mutations they are apart to begin with, but also how specific mutations incrementally affect fitness. Ignoring the constraints needed to conserve function makes any assessment of the mutation likelihood suspect. In this study we plan to computationally learn these complex and hitherto unknown evolutionary constraints from large sequence databases, enabling us to the chart trajectories of wild pathogens at scale. We propose to experimentally validate our approach in binding and neutralization assays, allowing us to leverage sequence and structural annotation databases, to predict when and how new strains are expected to appear, along with their impact on pathogenicity, and the potential for vaccine escape.

Relevant Efforts: The Big Ideas Generator (BIG) program at the University of Chicago has funded our initial work, with substantial interest going forward.

Peer Groups: Very recently, two articles have explored the possibility of predicting pathogenicity from genomic sequences (Mollentze[15]), and forecasting which amongst observed mutations will dominate the circulating population (Maher *et al.*[16]). While these questions overlap with our framework, our approach is distinct, and vastly more ambitious both intellectually and in scope. Mollentze uses classical sequence similarity; extended to include similarity to human housekeeping genes hoping to identify viruses evading the human immune system more easily, with poor performance (tagging incorrectly all SARS-related coronaviruses as potentially pathogenic), and un-actionable specificity. And, Maher outright assumes mutations to be independent, with SARS-CoV-2 specific manually selected features hacked together via machine learning. Importantly, these approaches only aim to predict point mutations, with the gargantuan complexity of tracking a more complete strain (or the RBD fragment) through an ultra-high-dimensional sequence space lying well beyond reach. Thus, even the question if a yet-to-be-seen strain is indeed a valid biological encoding of a virus (which is simpler to determining risk posed by such future variants) cannot answered by our peers, limiting such approaches to analyzing mutations already seen, or strains already collected. Additionally, generalizability and actionability is suspect, given that Maher's features are SARS-CoV-2 specific, and Mollentze's similarity to house-keeping genes might not be universal.

Goals & Methodology: We computationally infer a recursive forest of predictors (the Q-net) that maximally extracts dependency information between mutations & motifs, and can preempt complete strains that have never been seen before, but nevertheless represent a valid genomic sequence. Our framework is generalizable, we do not need to manually curate features that apply to individual viruses, resulting in unprecedented scalability. Our planned goals are:

#2. more details here?

□ **Aim 1: Validate a biologically meaningful metric (the q-distance) for comparison of genomic sequences. (6 months)** Combining novel machine learning, and information theory, we will characterize patterns of mutations from large sequence databases that constrain evolutionary trajectories, to inform a biologically relevant metric of similarity between genomic sequences. We hypothesize this q-distance to adapt to specific organisms, its background population, and realistic selection pressures. We plan to demonstrate these results in expression and functional assays, showing that smaller q-distance indicates similar phenotypes.

□ **Aim 2: Develop and validate algorithm for preempting possible future variants (18 month)** With tractable function-aware sampling (q-sampling) of the neighborhood of an observed strain in ultra-high-dimensional possibility space, we will preempt: 1) future likely mutations 2) probability of spontaneous jump via specific mutations, and 3) likely variants arising within specific time-frames in the wild. Validate that predicted mutations/strains are biologically plausible, expressing functional proteins – in silico and in laboratory assays, piloted with the spike protein for SARS-CoV-2 and Hemagglutinin (HA) for Influenza A.

□ **Aim 3. Preempt and characterize escape variants. (24 month)** Preempt escape variants, via characterizing future mutations that evade standard antibody neutralization assays, and thus are candidate escape mutants for SARS-CoV-2 and Influenza A.

□ **Aim 4. Evaluate the ability of the proposed tools to define the emergence edge identifying animal strains poised to emerge into humans with high transmissibility/pathogenicity. (24 month)** Piloted with emerging Influenza A strains, we will compare our predictions against CDC-developed Influenza Risk Assessment Tool (IRAT) scores [17] for influenza variants, and aim to characterize all Influenza A sequences (and predicted variants) in public databases. If validated, such a tool will vastly accelerate such risk assessment, cutting down the time required for individual strains from weeks/months to under a second.

#3. Need to discuss the methods

Impact: Tools for adhoc quantification of genomic similarity [18–23] are **not inherently biologically meaningful** – a smaller edit distance between two strains does not necessarily imply that a feasible wild trajectory exists from one to the other. Our algorithms are a first to learn the **appropriate metric of comparison from data**, without assuming any model of DNA/RNA or amino acid substitution, or a genealogical tree a priori, and are designed to be aware of the impact of the host environment and background epidemiology. This study, if successful, will have profound impact on biosurveillance strategies, and what we do with the products of such efforts. With risk-wise rank-ordering of newly collected strains, we can 1) better judge pandemic risks, 2) quantify the odds of a particular strain spilling to humans, and 3) estimate its potential to lead to a global pandemic. And for strains already circulating in the human population, we can potentially 4) preempt variants, 5) their timeline of emergence, 6) their odds of escaping current vaccines, and ultimately 7) design escape-resistant vaccines.

#4. need to have figure?

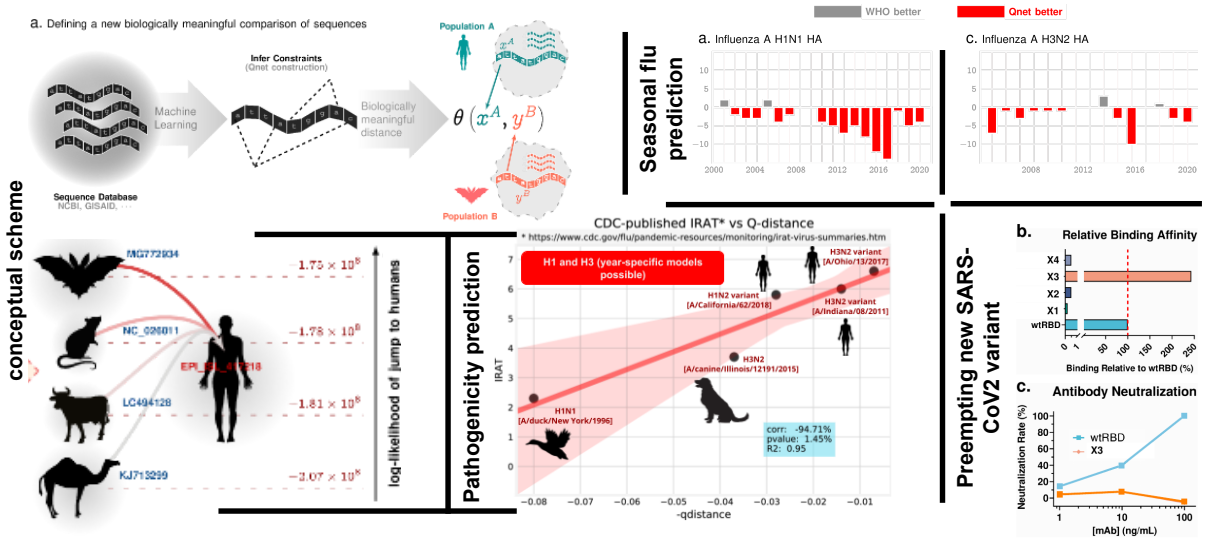

Fig. 1. Conceptual framework and current results

Fundraising: To date ≈100K has been allocated, including BIG funding + development funding from Chattopadhyay Lab. We have pending proposals at NSF (PIPP program, 1M USD, summer 2022) and NIH (NIAID R21, 400K, Fall 2022).

#5. Need 5, please suggest

KNOWLEDGEABLE EXPERTS IN THE FIELD

1. Peter Hraber
Theoretical Biology & Biophysics Group, T-6
Theoretical Division
Los Alamos National Laboratory
PO Box 1663, MS K710
Los Alamos, NM 87545
phone: 505 665 7491
email: phraber@lanl.gov

Dr. Hraber is an expert in theoretical biology and biophysics, focusing in computational immunology, evolution, and statistical genetics, and is well-suited to evaluate the interplay of mathematical modeling, evolutionary dynamics and immunological aspects of the proposed project.

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2. Patrick Wilson
Assistant Professor
Drukier Institute for Children's Health
Weill Cornell Medicine
1300 York Avenue
New York, NY 10065
phone: 212 746 4111
email: pcw4001@med.cornell.edu

Prof. Wilson is an immunologist with extensive experience in characterization of human immune responses, with definitive work in influenza vaccine designs and B cell biology.

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3. Balaji Manicassamy
Associate Professor of Microbiology and Immunology
Iowa State University
3-430 Bowen Science Building
51 Newton Rd
Iowa City, IA 52242
phone: 319 335 7590
email: balaji-manicassamy@uiowa.edu

Prof. Manicassamy is an expert in influenza viruses and respiratory pathogens, with extensive experience in reverse genetics and pathogenesis.

4.

5.

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