BioF:GREAT Glycoscience Research Education and Training

External User: Project Proposal

Project Title: Assessing the impact of HIV-Env inter-clade glycoform conservation on broadly neutralizing antibody recognition
BioF:GREAT Code: FA061325F
PI/Co-PI Name(s): Fikri Avci/Trevor Adams
BioF:GREAT Project Liaison Name: Lance Wells, PhD
Submission Date: 06.13.25

Section I: Project Summary (1 page)

1. Project Overview and Goals

a. Motivation for the proposed work: Human immunodeficient virus (HIV), the agent responsible for acquired immune deficiency syndrome (AIDS), is one of the leading sources of morbidity in the world. Roughly 40 million people are currently living with HIV, and over a million new infections occur each year. There is significant interest in finding new avenues for HIV vaccine targets that promote a broadly neutralizing and protective immune response. We postulate that HIV-Env N-glycosites associated with conserved glycan distributions are key determinants of antibody binding breadth and may serve as the foundation of a broadly neutralizing immune response.

b. Desired aims:

Specific Aim 1: *Establish a scalable glycoform similarity comparison metric using glycoproteomics and statistical modeling and characterize site-specific glycosylation on inter-clade HIV-Env glycoproteins to identify broadly conserved N-glycan microheterogeneity regions.*

Specific Aim 2: *Identify determinants of glycoform specificity in bnAb binding across clades. Glycoengineer antigenic regions on HIV-Env to rescue bnAb binding in otherwise poorly binding clinical strains of HIV-1*.

2. Relevant to Glycoanlytics, Glycoenzymes and Bioinformatics, the proposed research activities are expected to advance:

a. *Tools and methods*: This research will advance glycoanalytics and bioinformatics due to our approach utilizing stringent statistical quality control to overcome stochastic noise that frequently muddles glycoproteomics experiments.

b. *Knowledge*: Increased understanding of N-glycoforms across a highly diverse and clinically important retrovirus

c. *Technologies*: Glycoproteomics, glycoanalytics, structural biology, and molecular modeling

d. *Training of next-generation glycoscience researchers*: This will further the training of Trevor Adams, a postdoctoral researcher that has attended multiple glycobiology conferences and centers his work around mass spectrometry and glycobiology.

3. Intellectual Merit: The proposed work will advance and provide a reproducible framework for confident N-glycoproteomics analysis across the glycobiology community. It will also inform on the constraints of viral evolution with regards to glycan shielding.

4. Broader Impacts:

a. Outcomes to be shared with BioF:GREAT community: All manuscripts will be shared with and will acknowledge the BioF:GREAT community.

b. Material(s), data or model(s) to be made available to BioF:GREAT databases: All mass spectrometry datasets will be made available freely on public repositories (e.g. PRIDE), as well as any BioF:GREAT databases.

c. Planned collaborations within and outside of BioF:GREAT: We will collaborate with the CCRC analytical core and mass spectrometry experts at the University of Georgia

Section II: Research Plan (1-2 pages)

1. User Research Team

Dr. Lance Wells: Wells will oversee the mass portion of the proposal and provide expertise if needed.

Dr. Fikri Avci: Avci will oversee the project in its entirety and will be the corresponding author on all manuscript published

Dr. Trevor Adams: Adams will perform all sample preparations for mass spectrometry and analyze the raw data for manuscript preparation

Travel to BioF:GREAT facility:

Trevor Adams will drive to the facility from Atlanta (~90min drive)

2. Background:

For over four decades, the acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) has been a major threat to human health. By 2023, ~40 million people were living with HIV (PLWH), with >1 million new infections every year[1]. Despite the success of antiretroviral therapy (ART) in managing HIV infection, there is no cure nor a broadly effective vaccine. PLWH must maintain an ART regimen for life, and any interruption in access to care risks viral rebound within weeks[2]. Therefore, there is still an urgent need for an HIV vaccine that can generate a broadly neutralizing immune response.

The sole antigenic target of HIV is the envelope glycoprotein on the surface of the virion (HIV Env). Current approaches in generating HIV Env vaccines include reverse-engineering of existing broadly neutralizing antibodies (bnAbs) by targeting rare B-cell populations, serial vaccinations with differing antigens to guide bnAb development, and engineering immunogens to expose potential sites of vulnerability. The sites of vulnerability targeted roughly span the entire surface of the trimer, making it difficult to rationalize which specific regions may be the most promising to target. Our approach presents a new avenue in HIV vaccine development by identifying novel glycoepitopes that are conserved across clades of HIV. The five most common clades globally by population are C, A, CRF01_AE, B, and D, with class C being of particular interest due to its high proportion of all infections (~23%) and unique transmission dynamics in sub-Saharan Africa.

While the number and location of N-glycosylation sites vary between HIV subtypes, many sites are mostly conserved, and elimination of these sites can expose points of vulnerability that are

otherwise camouflaged[3]. At some sites, the glycan coat is so dense that glycan processing is inhibited, leading to a buildup of high-mannose structures that sterically lock each other into place. This phenomenon has been observed across disparate viruses, perhaps best illustrated by the bnAb 2G12, which is cross-reactive to mannose patches both on HIV-Env[4,5] and H3N2 influenza hemagglutinin (HA)[6]. There is significant potential for exploiting glycan conservation across clades to reveal new vulnerabilities in a virus that has shown unprecedented resistance.

Numerous glycoproteomic profiles of HIV have been published over the last decade[7-18]. While these studies have been instrumental in characterizing broad trends in Env-trimer glycosylation, they often lack the rigor required for conclusive site-specific comparisons between HIV subtypes. Limitations include inadequate coverage, profiling of monomeric rather than trimeric Env, insufficient cross-clade coverage, overly broad classification of glycan microheterogeneity, enrichment with biased glycoform-specific antibodies, and a lack of replicates to enable robust statistical analysis. A high degree of data quality control and standardization in the analysis is essential to confidently make comparisons both within and between studies. This includes reporting intra-sample quality checks to ensure the reproducibility of analysis data and conclusions, rather than just reporting means and standard deviations for simplified glycan categorization.

Regarding bnAb binding, a substantial body of literature exists on the localization, breadth, and determinants of HIV-Env antibodies[19]. New glycan determinants are continually being found, and specific glycan antennae and terminal features appear critical for the breadth of antibody binding[20]. We aim to integrate our findings with this existing knowledge by leveraging binding data to generate hypotheses about the glycan dependency of bnAb breadth, informed by cryo-EM studies, computational predictions, ELISAs, and neutralization panels[19,21,22].

3. Approach and Proposed Activities

a. Scientific Approach:

Aim 1: *Assessing cross-clade HIV glycosimilarity with glycoproteomics and statistical modeling*

Hypothesis: I hypothesize that regions of HIV-Env with conserved glycan occupancy and topology among HIV clades will effectively bind to a broad range of glycan/glycopeptide binding bnAbs.

1a: *Establish a scalable glycoform similarity comparison metric using glycoproteomics and statistical modeling.*

1b: *Characterize site-specific glycosylation on inter-clade HIV-Env glycoproteins to identify broadly conserved N-glycan microheterogeneity regions.*

Aim 2: *Elucidating the relationship between glycoform conservation and bnAb breadth*

Hypothesis: I hypothesize that the conservation of glycoforms at antigenic epitopes is a key determinant of bnAb binding breadth.

2a: *Identify determinants of glycoform specificity in bnAb binding across clades.*

2b: *Glycoengineer antigenic regions on HIV-Env to rescue bnAb binding in otherwise poorly binding clinical strains of HIV-1*.

b. Need for use of the BioF:GREAT User Facility, including anticipated deliverables.

i. Sample details, quantity (as appropriate):

Digested intact glycopeptides derived from HIV-Env trimers in at microgram quantities.

ii. Expected analysis, data, and data format:

Analysis will be performed using Thermo Orbitrap Tribrid instrumentation. We will analyze the mass spectrometry RAW files using search engines such as MSFragger and pGlyco. The resulting data will then be processed by custom in-house scripts as well as the RAMZIS bioinformatics package.

c. Project Deliverables and Timeline:

Project Deliverable/Milestone	**Expected Timeline (months)**
N-glycoproteomics	36 months

Budget Expenditures	**Proposed Budget (USD)**
Travel Expenses for Dr. Trevor Adams (3 trips from Atlana to Athens and back)	$500
Total expected budget	**$500**

ESTIMATED budget expenditure of BioF:GREAT PI Resources:

Budget Expenditures	**Proposed Budget (USD)**
Payment of personnel (Rob Bridger and Dr. Linda Peng) 80 hours	$4,000
Consumables	$1,000
Mass spec time (20 injections @ $250 per injection) Wells Lab	$5,000
Total expected budget	**$10,000**

Certification and agreement of proposed work by User:

User Name (print): Trevor Adams

User Signature: Trevor Adams

Date: 06/13/2025

Section III: References:

1 World Health Organization *HIV statistics, globally and by WHO region, 2024*, <https://cdn.who.int/media/docs/default-source/hq-hiv-hepatitis-and-stis-library/j0482-who-ias-hiv-statistics_aw-1_final_ys.pdf> (2024).

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Section IV: PI/Co-PI Biosketch(es).