MAPPING THE EPITOPE:
DEFINING THE STRUCTURE OF THE HIGHLY IMMUNOGENIC ENV-CD4 COMPLEX

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INTRODUCTION

Despite recent advances in the design of HIV-1 Env-based vaccine immunogens, such immunogens have not yet elicited broadly cross-reactive neutralizing antibodies against circulating primary HIV (Fig. 1). A major research aim of the HIV Pathogenesis Research Unit (HPRU) at the University of the Witwatersrand in South Africa is the evaluation of vaccine design strategies to identify Envelope(HIV)-CD4(human host) (Fig. 2) vaccine immunogens capable of inducing potent, durable, and broadly protective neutralizing antibodies responses against clinically relevant HIV-1 subtype C.

The HPRU has focused on the development of an effective prophylactic HIV vaccine which utilizes a novel immunogen called Env-2dCD4^{S60C} that consists of a human two domain CD4 with an S60C mutation (2dCD4^{S60C}) covalently bound to monomeric gp120 (Fig. 3). We have designed, expressed, and purified sufficient quantities of the recombinant gp120 monomers, the 2dCD4^{S60C} capable of forming a covalent interaction with Env, and have subsequently generated, isolated, and performed functional analyses on the novel covalent complex (reported in [1]).

In several independent rabbit immunogenicity studies conducted at the HPRU, Env-2dCD4^{S60C} elicited potent broadly neutralising antibodies against HIV-1 Tiers 1, 2, 3, HIV-2 and SIV pseudoviruses (Fig. 4). This preclinical testing showed that the Env-2dCD4^{S60C} complexes act as **"super immunogens"**, and that the epitopes on the Env-2dCD4^{S60C} complexes are accessible and highly immunogenic in rabbits. These observations strongly indicate that Env-2dCD4^{S60C} complexes could provide similar effects in humans, and should be considered as a viable candidate for an effective preventative and/or therapeutic vaccine against HIV-1. Prior to initiating further preclinical development of Env-2dCD4^{S60C} subunit vaccine immunogens in rhesus macaques however, it would be critical to characterize and define the structures of the protective HIV-1 Env-2dCD4^{S60C} epitopes, and ultimately fine map the specificities of the potent, broadly neutralizing antibodies elicited by the Env-2dCD4^{S60C} complexes in rabbits.

This is achievable with the current collaboration between the HPRU and the ESRF/ILL. **The broad aim of this study is therefore to characterize the structures of 2dCD4^{S60C} and 2dCD4^{WT} liganded and unliganded gp120 monomers, and to define the structures of the Env-2dCD4^{S60C} complex epitopes which correlate to potent viral neutralization.** We will thus gain insights into the underlying mechanisms of the antiviral activity that will guide strategies for optimizing CD4-based immunogens that will likely provide protection from infection.

HIV and its interaction with CD4

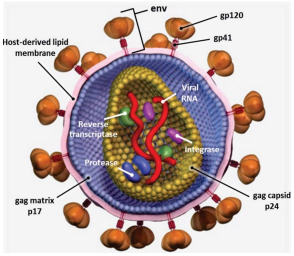


Fig. 1: A model representation of the structural organization of the HIV-1 virion and its associated proteins [2].

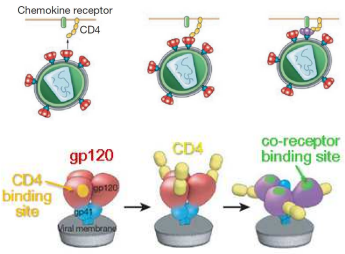


Fig. 2: Conformational changes in the gp120 trimeric spike induced by CD4 binding and the associated consequences for viral attachment to the target cell [3].

Novel *gp120-CD4* covalent complex

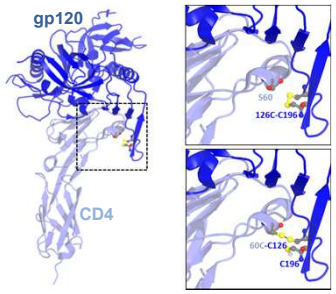


Fig 3: Structure of the gp120-CD4 complex. Modelling of the CD4 S60C variant calculates the rotomeric positioning of the Cys60 side-chain thiol to within 2Å of the target gp120 disulphide and predicts the formation of the interchain CD4 Cys60–Cys126 gp120 disulphide.

Pseudovirus inhibition assays with immunised rabbit sera

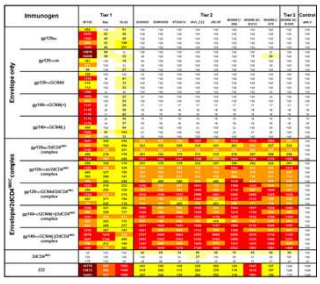
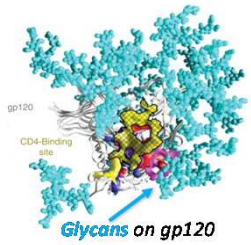
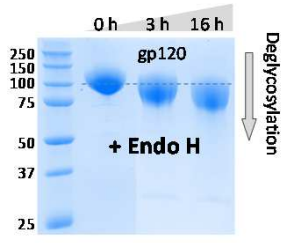


Fig. 4: Neutralizing antibody profile data of immunized rabbit sera against a panel of 12 HIV-1 pseudoviruses. Neutralization data is indicated as the reciprocal of the dilution required to achieve 50% inhibition.

PRELIMINARY RESULTS

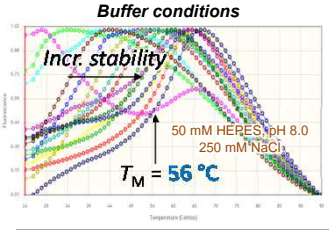
Deglycosylation = *decrease mobility & dynamics*



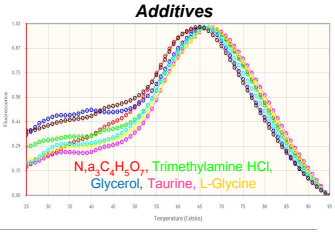


Crystallisation buffer optimisation = *increase stability*

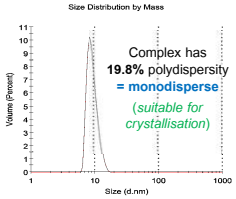
Buffer conditions



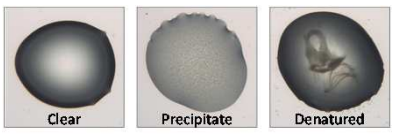
Additives



Purity analysis



Crystallisation screening

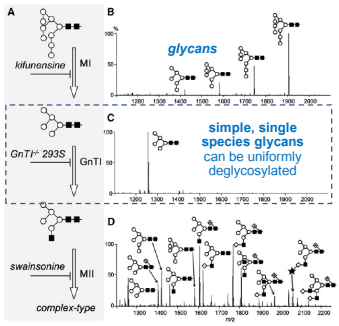


Examples of the outcomes of the high-throughput crystallisation trials, including no change in the liquor of the hanging drop (**clear**), as well as development of **precipitated** or **denatured** protein.

PERSPECTIVES & FUTURE WORK

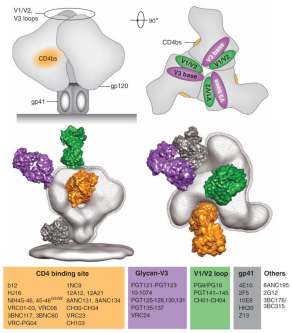
Attempting to establish the conditions for crystallising the monomeric gp120-2dCD4^{S60C} complexes using the **high-throughput crystallisation (HTX)** facilities available in the Partnership for Structural Biology (PSB) have been unsuccessful. Unfortunately, as of now, no conditions have yielded crystal growth, but as some protein crystals may take from weeks to months to grow, there is still a possibility for crystals to develop in the hanging drops that do not already have precipitated or denatured protein. However, **insufficient deglycosylation** of the complex glycans and the subsequent introduction of heterogeneity into the gp120 protein sample may be preventing its crystallisation. Steps are being taken to solve this problem.

Solving the Glycosylation problem



Manipulating the glycosylation pathway in HEK293 Cells [4].

Neutralising Abs against HIV



Antibody target sites on the HIV-1 envelope spike [5].

The structural work on this system will ultimately require a multi-faceted approach in terms of analytical techniques. Both low resolution (**SAXS**) and high resolution (**synchrotron X-ray crystallography**) techniques will be employed in the full knowledge of the likely difficulties in crystallising the flexible, highly glycosylated, gp120 protein. SAXS will provide important low resolution structural information on the monomeric gp120-2dCD4^{S60C} complex.

REFERENCES

1. Cerutti *et al.* (2010) Stabilization of HIV-1 gp120-CD4 receptor complex through targeted interchain disulfide exchange. *J Biol Chem*, **285** (33): 25743-52.
2. Unknown. Milestones in the AIDS pandemic: Viral Structure. *New Eng J Med* [March 20, 2014] http://www.nejm.org/doi/full/10.1056/NEJMra1201533.
3. Liu *et al.* (2008) Molecular architecture of native HIV-1 gp120 trimers. *Nature*, **455** (7209): 109-13.
4. Chang *et al.* (2007) Glycoprotein structural genomics: solving the glycosylation problem. *Structure*, **15** (3): 267-73.
5. Klein *et al.* (2013) Antibodies in HIV-1 vaccine development and therapy. *Science*, **34** (6151): 1999-204.