HIV-1 Dual-Epitope Targeting Immunogens to Enhance CD4bs and Apex Recognition

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Structure-guided immunogen design has become as a promising strategy for eliciting broadly neutralizing antibodies (bnAbs) against HIV-1. Several conserved HIV-1 Env epitopes have been explored as targets for vaccine design, including CD4-binding site (CD4bs), V1V2 Apex, V3-glycan site, and others. Currently, most immunogen designs focus on targeting individual bnAb epitopes, limiting the breadth of elicited immune responses and motivating the development of multi-epitope designs. Here, we engineered a novel dual epitopes-targeting immunogen, Q23.ACE, derived from Q23.MD39, which is Q23.17 Env with prefusion stabilized mutations introduced. To simultaneously induce immune responses against both CD4bs and V1V2 Apex, we engineered both epitopes by glycan deletion and residue modification. *In-vitro* expression demonstrated a high protein yield and predominant well-folded trimer by size exclusion chromatography, suggesting its potential to be suitable for multiple delivery platforms for future *in-vivo* investigation. Q23.ACE exhibited strong binding to HIV-bnAbs with minimal binding to non-neutralizing antibodies by characterizing its antigenic profile, confirming its stabilized Env trimer features. Furthermore, Q23.ACE demonstrated an enhanced antigenicity to CD4bs and V1V2 Apex, when comparing to Q23.MD39 and our other engineered immunogens, by showing improved binding and affinity to a larger antibody panel targeting CD4bs and V1V2 Apex, including bnAbs, intermediates and UCAs, with SPR and ELISA. To minimize off-target response against the Env trimer base, we also designed and produced a membrane-anchored Q23.ACE construct, which recapitulated the enhanced recognition by CD4bs- and Apex-directed antibodies, meanwhile maintaining the closed native-like trimer conformation by detection of its antigenic profile using a cell surface-display assay by flow cytometry. Together, these results identified Q23.ACE as a promising immunogen candidate achieving enhanced dual epitope antigenicity and supported its future *in-vivo* evaluation for immunogenicity.