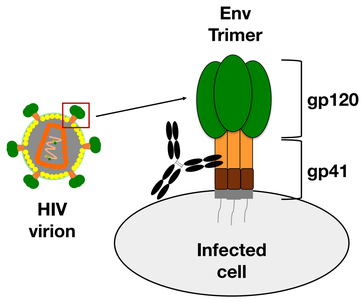
A NOVEL PHAGE DISPLAY APPROACH MAPS LINEAR EPITOPES OF GP41-SPECIFIC MONOCLONAL ANTIBODIES THAT MEDIATE ADCC

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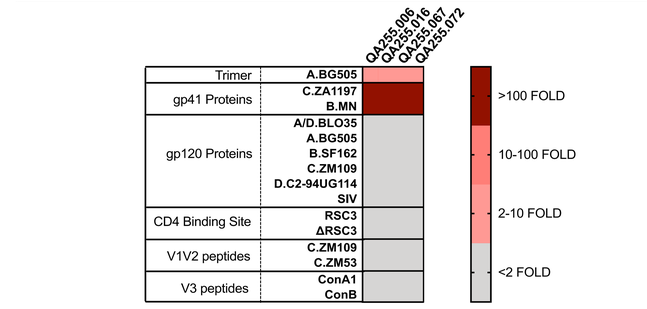
Introduction



- Antibodies that mediate killing of HIV-infected cells through antibody-dependent cellular cytotoxicity (ADCC) have been implicated in protection from HIV infection and disease progression.
- We currently lack a high-throughput, inexpensive, rapid way to map the epitope of ADCC-mediating mAbs.
- Here we describe a novel epitope mapping strategy we applied to mAbs isolated from an HIV infected individual (QA255) from Kenya.

Preliminary epitope mapping

Binding Antibody Multiplex Assay (BAMA) shows that four mAbs from QA255 bind specifically to gp41

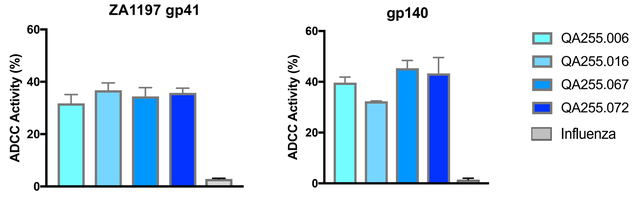


Competition ELISAs using mAbs with defined epitopes show **QA255.006** and **QA255.016** target epitopes that include FPPR and CHR, **QA255.067** and **QA255.072** target the C-C loop

ADCC activity of mAbs

All four mAbs have ADCC activity against gp41 and gp140 protein antigens in RF-ADCC assay

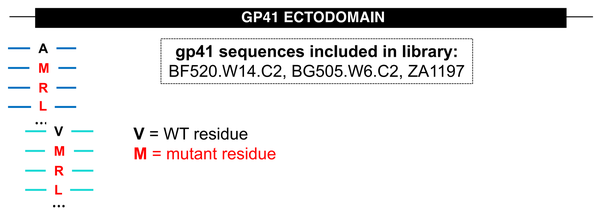
Target cells were coated with Clade C ZA1197 gp41 and Clade A Q461.d1 gp140. Results from RF-ADCC assay with one representative PBMC donor are shown.



Phage/DMS: Epitope mapping using a deep mutational scanning phage display library

Creating a DMS phage display library that contains peptides sampling every possible single-amino acid

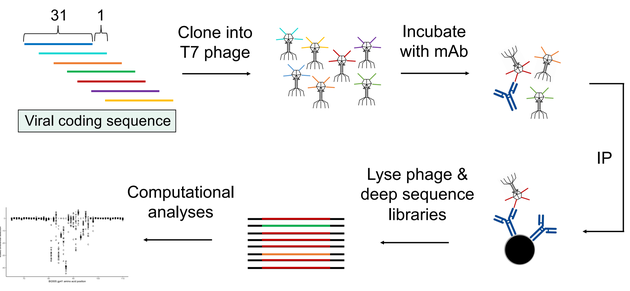
Deep mutational scanning (DMS) is a massively parallel method of interrogating the role of each amino acid in protein-protein binding interactions. Here we generated a library of tiled peptides 31 amino acids in length, with either a **wildtype** or **mutant** residue at the central amino acid, across the ectodomain of gp41.



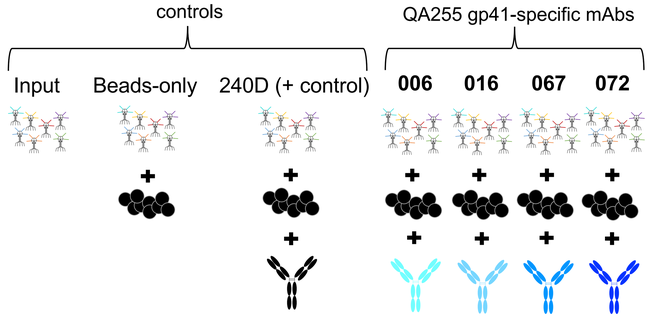
gp41 sequences included in library:
BF520.W14.C2, BG505.W6.C2, ZA1197

V = WT residue
M = mutant residue

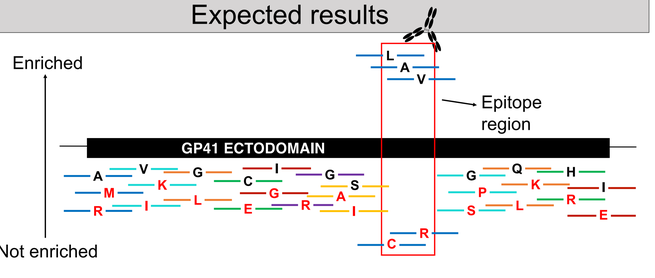
Phage Immunoprecipitation - Sequencing method



Setup of a phage/DMS experiment with the newly identified gp41 mAbs and a positive control (240D) with a defined gp41 epitope

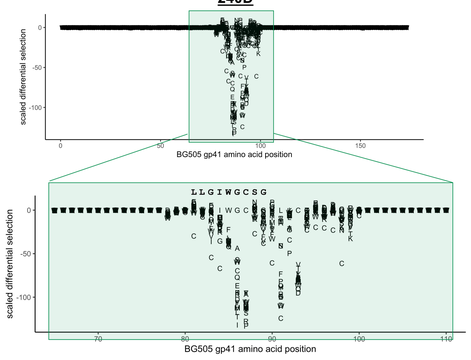


Expected results

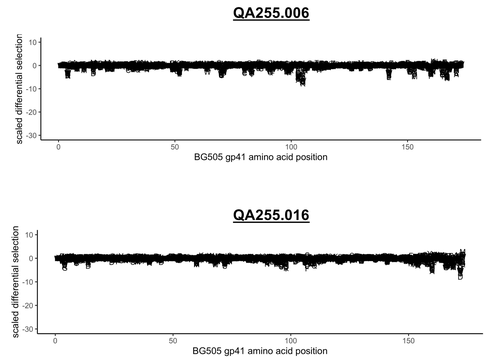


RESULTS: Phage/DMS defines the linear epitopes of gp41-specific antibodies

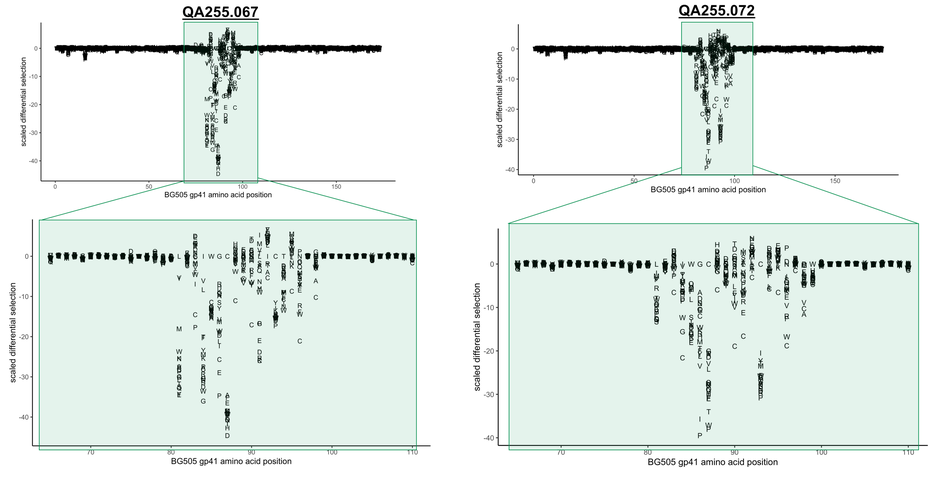
Positive control mAb 240D bound to peptides with the expected amino acids as defined by prior studies (displayed above the plot)



QA255.006 and **QA255.016** did not significantly enrich for any peptides above background



Both **QA255.067** and **QA255.072** significantly enriched for peptides spanning the immunodominant C-C loop region of gp41, with certain mutations in this region abolishing mAb binding, indicating they disrupt a residue critical to the epitope.



Conclusions

- ✓ We have identified four distinct gp41-specific mAbs in a single individual that mediate ADCC activity.
- ✓ We have finely mapped the epitope of two of these mAbs (**QA255.067** and **QA255.072**) using a novel DMS phage display approach, which reveals specific amino acids critical for binding within and just outside the C-C loop of gp41.
- ✓ Two of the mAbs (**QA255.006** and **QA255.016**) recognize a discontinuous epitope on gp41 consisting of the FPPR and CHR, as indicated by competition ELISA experiments, and could not be further mapped using this DMS phage display approach.
- ✓ Overall, this DMS phage display method provides a rapid, high throughput way of mapping the linear epitopes of antibodies down to the single amino acid level.

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