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Competitive Antigen Panning for Selection of HIV-1 Neutralizing Human Monoclonal Antibodies Specific for gp41

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Abstract

HIV envelope glycoprotein (Env) is composed of two non-covalently associated subunits: gp120 and gp41. Panning of phage-displayed antibody libraries against gp140 (covalently linked gp120 and the extracellular portion of gp41) has resulted mostly in selection of anti-gp120 antibodies. Native gp41 in the absence of gp120 is unstable. The use of gp41 fragments as antigens has resulted in selection of antibodies with only relatively modest neutralizing activity. To enhance selection of antibodies specific for gp41 in the context of the whole Env we developed a methodology termed competitive antigen panning (CAP). Using CAP, we identified a panel of gp41-specific human monoclonal antibodies from an HIV-1 immune library derived from long-term nonprogressors. These antibodies recognize conformational epitopes in gp41 and exhibited, to various extents, neutralization activity in assays based on spreading infection in peripheral blood mononuclear cells. The CAP methodology is generally applicable for selection of antibodies specific for any epitope that is not a dominant epitope in the antigen. It is superior to a traditional pre-depletion method in avoiding potential loss of target-specific antibodies.

Keywords

Phage display; antibody; HIV; envelop; gp41; methodology

1. Introduction

Antibody phage display has been widely used for selection of antibodies specific for targets of interest (1–4). HIV envelope glycoprotein (Env) is composed of two non-covalently associated subunits: gp120 and gp41. Isolated HIV transmembrane subunit gp41 ectodomain cannot be used as a screening antigen because it is unstable and aggregates. Its conformation, even if stabilized, in the absence of gp120 could be different from that in the envelope (Env). To enhance the selection of gp41-specific antibodies we developed an approach termed competitive antigen panning (CAP) based on outcompeting gp120-specific antibodies with an excess of gp120 (5, 6). CAP facilitates the selection of gp41-specific clones. Using CAP, we isolated a panel of gp41-specific antibodies, one of which would otherwise not be selected using the conventional pre-depletion methodology (5).

The CAP methodology may also be effective in the selection of antibodies to domains of other whole proteins that are unstable when isolated. The CAP methodology can also be applied to selection of antibodies against epitopes that are genetically (e.g., mutants) or biologically modified (e.g., phosphorylation, sulfation, etc.). CAP is superior to a traditional pre-depletion method in avoiding potential loss of target-specific antibodies. The selected novel gp41-specific antibody has nM affinity for most of the recombinant gp140s from

different HIV-1 isolates and may have potential for prevention of HIV-1 infection, and their epitopes may have implications for HIV-1 vaccine design.

2. Materials

2.1. Biotinylation of Antigens

1. *PBS*: 9.0 g/l NaCl, 144 mg/l KH_2PO_4 , 795 mg/l Na_2HPO_4 , pH 7.4.
2. *1 M NaHCO₃*: 84.01 g/l NaHCO_3 , pH 8.6.
3. *2 M glycine*: 150.14 g/l glycine.
4. *H₂O*: Molecular biology grade.
5. *Freshly prepared 10 mM biotin stock*: Weigh 2.2 mg of EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) (MW: 556.59), dissolve it in 400 μl H_2O (see Note 1).
6. *Microcon YM-10*: Regenerated cellulose 10,000 MWCO (Millipore) (see Note 2).

2.2. Preparation of Phage Antibody Library

1. *2YT medium*: 16 g/l bacto-tryptone, 10 g/l bacto-yeast extract, 10 g/l NaCl, pH 7.0; Autoclaved for 20 min at 15 psi on liquid cycle.
2. *E. coli TGI*: Suppressor strain (K12, D(lac-pro), supE, thi, hsdD5/FtraD36, proA +B+, lacIq, lacZDM15) for propagation of phage particles.
3. M13KO7 helper phage (Invitrogen, Carlsbad, CA).
4. *Ampicillin*: 200 mg/ml stock, filter-sterilized by 0.2- μm filter.
5. *Kanamycin*: 70 mg/ml stock, filter-sterilized by 0.2- μm filter.
6. *20% glucose*: Filter-sterilized by 0.2- μm filter
7. *PEG/HCl*: 20% polyethylene glycol 6000, 117 g/l NaCl; Autoclaved for 20 min at 15 psi on liquid cycle (see Note 3).
8. *2YTAG agar plates*: 1.5% agar in 2YT medium, autoclaved for 20 min at 15 psi on liquid cycle, cool down the medium (about 42–45°C) and supplement it with a final concentration of 200 $\mu\text{g/ml}$ ampicillin and 2% glucose, pour the medium onto 9-cm petri dishes. Keep the plates at 4°C when agar is solidified.
9. *Glycerol*: Autoclaved for 20 min at 15 psi on liquid cycle.

2.3. Panning of Phage Library

1. *5% and 3% MPBS*: 5% and 3% skim milk in PBS.
2. *Freshly prepared 100 mM triethanolamine*: 700 μl triethanolamine (7.18 M) in 50 ml H_2O , diluted prior to use.
3. *1 M Tris-HCl, pH 7.5*: 121.14 g/l Tris base, adjust pH to 7.5 using 1 N HCl.
4. *PBST*: 0.1% Tween-20 in PBS.

¹Sulfo-NHS-Biotin reagents are moisture-sensitive. Store the vial of biotin reagent at –20°C with desiccant. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening.

²The molecule cut-off of Microcon used for removing free biotin and changing buffer to PBS depends on the size of the protein to be biotinylated. For recombinant gp140s, both YM-30 and YM-10 can be used.

³Mix it well after autoclaving; keep the solution at room temperature.

5. Dynabeads M-280 streptavidin (Invitrogen).
6. *Magnetic separation apparatus*: Dynal magnetic stand from Invitrogen.
7. Head-to-tail rotator.
8. *Bioassay plates*: Petri Dish, Bioassay, 245 mm (VWR).
9. *Recombinant gp140/120_{89.6}*: Produced by recombinant vaccinia viruses and purified by a combination of lentil lectin affinity chromatography using Sepharose 4B (Pierce) followed by size-exclusion chromatography using HiLoad 16/60 Superdex 200 prep grade column (Amersham Pharmacia Biotech, Piscataway, NJ).
10. *HIV-1 immune antibody Fab library*: Constructed using pComb3H phagemid vector and 30 cc of bone marrow obtained from three long-term nonprogressors whose sera exhibited the broadest and most potent HIV-1 neutralization among 37 HIV-infected individuals.

2.4. Polyclonal Phage ELISA

1. *Coating buffer*: 8.401 g/l NaHCO₃, 29.25 g/l NaCl, pH 8.3.
2. *3% MPBS*: 3% skim milk in PBS.
3. Horseradish peroxidase (HRP)-conjugated monoclonal mouse anti-M13 antibody (GE Science).
4. ABTS (Roche).
5. *96-well ELISA plates*: Nunc-Immuno MaxiSorp surface plates (Nalge Nunc International).

2.5. Preparation of Monoclonal Phage

1. *96-well tissue culture plates*: Sterile, U-bottom (VWR).
2. *2YT medium*: The same as described in **Section 2.2**.
3. M13KO7 helper phage (Invitrogen, Carlsbad, CA).
4. Ampicillin and kanamycin: The same as described in **Section 2.2**.

2.6. Monoclonal phage ELISA

The same as described in **Section 2.4**.

2.7. Expression and purification of soluble Fab fragments

1. *SB medium*: 10 g/l MOPS (Sigma), 30 g/l bacto-tryptone, 20 g/l bacto-yeast extract, pH 7.0. Autoclaved for 20 min at 15 psi on liquid cycle.
2. *E. coli HB 2151*: Non-suppressor strain (K12, ara, D(lac-pro), thi/FproA+B+, lacIqZDM15) for expression of soluble antibodies.
3. *2 M MgCl₂*: 190.42 g/l magnesium chloride, anhydrous.
4. *IPTG, 1 M stock*: Freshly prepared or kept at -20°C.

2.8. Binding of Soluble Fab to Recombinant gp140/120_{89.6}

1. *96-well ELISA plates*: Nunc-Immuno MaxiSorp surface plates (Nalge Nunc International).

2. HRP-conjugated polyclonal anti-human IgG, F(ab')₂ antibodies (Jackson ImmunoResearch, Westgrove, PA).

3. Methods

3.1. Biotinylation of the Antigens

1. Take out the vial of biotin reagent from −20°C freezer and equilibrate it to room temperature.
2. *Prepare antigens:* Adjust the antigen (recombinant gp140s) concentration to 0.1–1 mg/ml in PBS (no Tris, no glycine). If the original buffer contains Tris or glycine, change the buffer to PBS or other suitable buffer before biotinylation.
3. Add 1/10 (v/v) of 1 M NaHCO₃ to the antigen.
4. *Prepare 10 mM biotin stock:* Gently open the biotin bottle, weigh 2.2 mg biotin, and dissolve it in 400 µl of molecular biology grade water.
5. Add 20 × molar excess of biotin to the antigens
Calculate the volume (µl) of 10 mM biotin needed for biotinylation
For example, to biotinylate 250 µg of recombinant gp140 (MW = 140 kDa)
$$V (\mu\text{l}) = 20 \times 250 / (140,000 \times 0.01) = 250 / 70 = 3.57 \mu\text{l}.$$
6. Incubate at room temperature for 30 min.
7. Add 1/20 (v/v) of 2 M glycine to the mixture to stop the reaction.
8. Dialyze the mixture against PBS (change buffer 3–4 times) or use Microcon YM-10 to remove free biotin if dealing with a small volume.
9. Check the biotinylation efficiency by dot blot.

3.2. Preparation of Phage Antibody Library

1. Inoculate 500 µl of TG1 glycerol stock of HIV-1 immune human antibody Fab library (*see Note 4*) in 500 ml 2YT medium supplemented with 200 µg/ml ampicillin and 2% glucose, grow at 37°C for about 3 h with shaking at 250 rpm till the optical density (OD) at 600 nm reaches 0.6.
2. Take 25 ml culture into a 50-ml falcon tube (*see Note 5*), add 4 ml M13KO7 (>1 × 10¹¹ cfu/ml) to the culture and gently mix them by inverting the tube twice (*see Note 6*). Keep the tube still at 37°C for 30 min. Keep growing the rest of 475 ml culture for 3 h, centrifuge at 3300 *g* for 20 min, resuspend the cell pellet in 2YT medium containing 15% glycerol, and keep the glycerol stock at −80°C.
3. Centrifuge the culture at 3300 *g* for 10 min, and resuspend the cell pellet in 500 ml 2YT medium supplemented with 200 µg/ml ampicillin and 35 µg/ml kanamycin (2YTAK medium) (*see Note 7*); grow the cells at 30°C for 16–20 h with shaking at 250 rpm.

⁴The OD at 600 nm should be below 0.1 after inoculation. The minimum cell number of the initial inoculum should be equal to the size of the library. For example, if the library size is 1 × 10¹⁰, the inoculum should contain at least 1 × 10¹⁰ TG1 cells.

⁵Try to use disposable containers if possible when handling phage particles. 1 OD_{600 nm} TG1 culture = 8 × 10⁸ TG1 cells per milliliter.

⁶The ratio of phage to bacteria for infection is 20:1.

⁷The cell pellet can be resuspended in 10 ml 2YTAK medium and then transferred to a 2-l flask containing 490 ml 2YTAK medium.

4. Spin down the cells at 11,000 *g* for 10 min (*see* Note 8), transfer the supernatant to two 500-ml centrifuge bottles, 250 ml each, add 1/5 (v/v) of PEG/HCl (50 ml) to each bottle, mix them well. Keep the bottle at 4°C for 1 h or more.
5. Centrifuge the solution at 11,000 *g* for 30 min, remove the supernatant (*see* Note 9), resuspend the phage pellet in each bottle in 20 ml sterile H₂O and combine them. Add 1/5 (v/v) PEG/HCl (8 ml) to the phage suspension, mix them well, keep the mixture at 4°C for 20 min or more.
6. Centrifuge the phage solution at 11,000 *g* for 10 min, remove the supernatant, recentrifuge for 2 min, and remove the residue supernatant.
7. Resuspend the pellet in 5 ml PBS in 14-ml falcon tubes, centrifuge it at 11,600 *g* for 10 min to remove bacterial debris. Transfer the supernatant containing phage particles to a new tube.
8. *Titer the phage library.* Make serial phage dilutions in PBS, take 1 µl of each: 10⁻⁶, 10⁻⁷, and 10⁻⁸ dilutions to infect 1 ml log-phase TG1 cells by incubating at 37°C for 30 min without shaking, spread 50 µl cells of each infection onto 2YTAG plates, incubate the plates at 30 µl overnight, count colonies and calculate the phage titer (*see* Note 10). Alternatively, the phage titer can be estimated by measuring the OD at 280 nm (1 OD_{280 nm} = 2.33 × 10¹² cfu/ml)
9. Keep the phage library at 4°C for short-term storage or add 15% glycerol and keep it at -80°C for long-term storage (over a year).

3.3. CAP of Phage Library

1. Take 5 × 10¹² to 1 × 10¹³ cfu phage particles from phage library to a sterile 1.5-ml eppendorf, add 500 µl 5% MPBS and 100 µl pre-washed streptavidin-M280-Dynabeads; make the final volume to 1 ml by adding PBS. Incubate the solution at room temperature for 1 h with gentle mixing on a head-to-tail rotator (*see* Fig. 9.1).
2. Separate the Dynabeads from the solution by letting the tube sit in a magnetic separator for a few minutes; transfer the solution to a new 1.5-ml eppendorf tube.
3. Add biotinylated recombinant gp140_{89,6} to 1 ml phage solution to a final concentration of 50 nM, add non-biotinylated recombinant gp120_{89,6} to a final concentration of 250 nM, and incubate the mixture at room temperature for 2 h with gentle mixing on a head-to-tail rotator.
4. Add 100 µl pre-washed streptavidin-M280-Dynabeads to the mixture, and keep rotating for 30 min.
5. Separate the Dynabeads from the solution as described in Step 2; wash the beads ten times with PBST and ten times with PBS.
6. Elute bound phage from Dynabeads by adding 1 ml of freshly prepared 100 mM TEA and incubating the beads at room temperature for 10 min with gentle shaking on a head-to-tail rotator; separate the beads from solution, transfer the eluted phage to a 50-ml falcon tube containing 0.5 ml 1 M Tris-HCl, pH 7.5, vortex the eluted phage for 10 s.

⁸All centrifuge steps should be done at 4°C except indicated.

⁹All waste solutions from phage preparation need to be decontaminated by adding 10% clorox (final concentration) to the solutions and incubating for at least 2 h before dumping them.

¹⁰The phage library should have a titer of 10¹³–10¹⁴ cfu/ml. If the titer is too high or too low, repeat the preparation of phage library.

7. Add 10 ml log-phase TG1 culture to the eluted phage, keep the tube still and incubate the phage/cell mixture at 37°C for 30 min.
8. Centrifuge the mixture at 3300 *g* for 10 min, remove the supernatant, resuspend the cell pellet in 1 ml 2YT medium; titer the first round panned library by spreading 50 μ l of 10^{-3} , 10^{-4} , and 10^{-5} dilutions onto 2YTAG plates and incubating the plates at 30°C overnight. Spread the rest of the cells onto a bioassay plate with 2YTAG agar; incubate the bioassay plate at 30°C overnight.
9. Prepare phage library for the second round of panning: follow the method 3.2, but with decreased scale and a simplified procedure. Briefly, add 5–6 ml 2YT medium containing 15% glycerol to the bioassay plate and scrap off the colonies. Inoculate 100 μ l glycerol stock in 100 ml 2TAG medium (*see* Note 11), grow to log-phase ($OD_{600\text{ nm}}=0.5-0.6$) (about 2 h). Take 10 ml culture; infect it with 1.6 ml M13KO7 helper phage. Centrifuge the cells, resuspend the cell pellet in 100 ml 2YTAK, and incubate at 30°C overnight. Centrifuge the culture, precipitate phage particles in the supernatant by adding 1/5 (v/v) PEG/HCl and centrifuging at 11,000 *g* for 10 min, resuspend the phage in 2 ml PBS and centrifuge at 11,600 *g* for 10 min to remove bacterial debris. Keep the phage library at 4°C.
10. Second round of panning against 10 nM biotinylated gp140_{89,6} (*see* Note 12): Repeat Step 1–8. The input phage can be decreased to 1×10^{11} to 1×10^{12} cfu. The biotinylated gp140_{89,6} concentration is decreased to 10 nM and non-biotinylated gp120_{89,6} concentration decreased proportionally to 50 nM. In addition, the washing stringency is increased to 20 times with PBST and 10 times with PBS.
11. Prepare phage library for the third round of panning: Repeat Step 9.
12. Third round of panning against 2 nM biotinylated gp140_{89,6}: Repeat Step 1–8. Input phage can be kept the same as in the second round of panning. The biotinylated gp140_{89,6} concentration is decreased to 2 nM and non-biotinylated gp120_{89,6} concentration decreased proportionally to 10 nM.
13. Prepare phage library for the fourth round of panning: Repeat Step 9 (*see* Note 13).

3.4. Polyclonal Phage ELISA

1. *Coating*: Prepare 1 μ g/ml recombinant gp140_{89,6} in coating buffer, coat 100 μ l per well on MaxiSorp plates (*see* Note 14). Incubate the plates at 4°C overnight.
2. *Blocking*: Wash the plates four times with PBST, add 200 μ l per well 3% MPBS and incubate the plates at 37°C for 1 h.
3. *Polyclonal phage*: Dilute phage libraries after each round of panning in 3% MPBS; remove the blocking buffer in the plates, add 1×10^9 cfu per well in 100 μ l each diluted phage library, incubate the plates at 37°C for 2 h.
4. *Second antibody*: Remove the phage solutions, wash the plates four times with PBST, add 100 μ l per well HRP conjugated to anti-M13 monoclonal antibody (1:5000), and incubate the plates at 37°C for 1 h.

¹¹Keep the rest of the glycerol stock at –80°C.

¹²Decreased antigen concentration used in panning facilitates the selection of high-affinity clones. The concentration of antigen used in the solution-phase panning is almost the range of affinity of selected clones.

¹³Keep the titrating plates after third round of panning. They will be used to prepare monoclonal phages for screening. Perform polyclonal phage ELISA after third round of panning. If there is no enrichment of gp140-specific clones after the third round of panning, perform fourth round of panning by repeating the third round of panning.

¹⁴MaxiSorp plates enhance the coating of gp140s/120s since they are envelope glycoproteins. Standard ELISA plates may be used for other antigens.

5. *Substrate*: Remove the second antibody solution, wash the plates four times with PBST, add 100 μ l per well ABTS, measure the OD at 405 nm after color development at room temperature for 10–20 min.

3.5. Preparation of Monoclonal Phage (see Note 15)

1. Inoculate well-isolated single colonies from titrating plates in 100 μ l 2YTAG medium in a sterile 96-well plate (seed plate), grow at 37°C overnight with shaking at 220 rpm.
2. Transfer 20 μ l overnight culture from each well to 180 μ l 2YTAG medium containing 10^9 cfu/ml $M_{13}KO_7$ helper phage in a sterile U-bottom 96-well plate (see Note 16), grow at 37°C for 2 h to reach the log-phase with shaking at 220 rpm; keep the seed plate at 4°C for short-term storage (less than a month) or –80°C after adding 15–20% glycerol for long-term storage.
3. Spin down the bacteria in the U-bottom plate at 1800 g for 10 min, pipette off the supernatant, add 200 μ l 2YTAK medium to each well and resuspend the bacteria pellets.
4. Grow the bacteria at 30°C overnight with shaking at 200 rpm.
5. Spin down the bacteria in the U-bottom plate at 1800 g for 10 min, the supernatant can be directly used in monoclonal phage ELISA (Step 3 in Section 3.6).

3.6. Monoclonal Phage ELISA

1. *Coating*: Dilute recombinant gp140_{89,6} and gp120_{R2} (see Note 17) to 1 μ g/ml in coating buffer, coat 100 μ l per well on MaxiSorp plates. Incubate the plates at 4°C overnight.
2. *Blocking*: Wash the plates four times with PBST, add 200 μ l per well 3% MPBS, and incubate the plates at 37°C for 1 h.
3. *Monoclonal phage*: Remove the blocking buffer, add 50 μ l per well 3% MPBS to the plates, transfer 50 μ l per well monoclonal phage-containing culture supernatant, incubate the plates at 37°C for 2 h.
4. *Second antibody*: Remove monoclonal phage solutions, wash the plates four times with PBST, add 100 μ l per well HRP conjugated to anti-M13 monoclonal antibody (1:5000), and incubate the plates at 37°C for 1 h.
5. *Substrate*: Remove the second antibody solution, wash the plates four times with PBST, add 100 μ l per well ABTS, measure the OD at 405 nm after color development at room temperature for 10–20 min (see Note 18).

3.7. Expression and Purification of Soluble Fab Fragments

1. Transform electroporation-competent HB2151 cells with phagemid DNA of selected clones by electroporation. Plate the transformed cells onto 2YTAG agar plates and incubate at 37°C overnight.

¹⁵Prepare monoclonal phage after third and/or fourth rounds of panning if they show enrichment in polyclonal phage ELISA for recombinant gp140_{89,6}.

¹⁶To avoid phage cross-contamination, U-bottom plates are recommended.

¹⁷Recombinant gp120_{89,6} is not suitable for coating, so here we use gp120_{R2} to check the specificity of monoclonal phage.

¹⁸Choose phage clones that cross-react with all the antigens tested in the monoclonal phage ELISA (OD_{405 nm} 1.0), prepare plasmid DNA using corresponding cultures in the seed plate as inoculums. Send the plasmid DNA for sequencing and analyze the sequences. Express clones with unique sequences as soluble Fab fragments.

2. Inoculate a single colony from fresh transformation plates in 3–5 ml of 2YTAG medium. Grow at 37°C for 6–8 h with shaking at 250 rpm.
3. Transfer the culture to SB medium (inoculation: 1–5 %) supplemented with 200 µg/ml ampicillin and 20 mM MgCl₂. Grow bacteria at 37°C with shaking at 250 rpm until OD_{600 nm} = 0.6–0.9.
4. Induce the culture by adding IPTG to a final concentration of 0.5 mM (range: 0.1–1.0 mM) and keep growing the bacteria at 30°C with shaking at 250 rpm for 12–20 h (*see* Note 19).
5. Centrifuge the culture at 3300 *g* for 15 min at 4°C. Resuspend the pellet in 10 ml PBS containing protease inhibitors.
6. Sonicate the bacteria on ice in a sonic disrupter for 180 s pulsing at 50% duty cycle, output control set at 5.
7. Pellet the cellular debris by centrifuging at about 48,000 *g* for 30 min at 4°C. Transfer the supernatant to a clean tube. The lysate can be stored for up to 1 month at –20°C.
8. Purify the Fab fragments by protein G affinity purification.

3.8. Binding of Soluble Fab to Recombinant gp140/120_{89,6}

1. *Coating*: Dilute recombinant gp140_{89,6} to 1 µg/ml in coating buffer, coat 100 µl per well on MaxiSorp plates. Incubate the Recombinant plates at 4°C overnight.
2. *Blocking*: Wash the plates four times with PBST, add 200 µl per well 3% BSA in PBS, and incubate the plates at 37°C for 1 h.
3. *First antibody*: Remove the blocking buffer, add 100 µl per well threefold serially diluted soluble Fab antibodies with a starting concentration of 20 µg/ml, incubate the plates at 37°C for 2 h.
4. *Second antibody*: Remove antibody solutions, wash the plates four times with PBST, add 100 µl per well HRP conjugated to goat anti-human IgG, F(ab')₂ polyclonal antibodies (1:2500), incubate the plates at 37°C for 1 h.
5. *Substrate*: Remove the second antibody solution, wash the plates four times with PBST, add 100 µL per well ABTS, measure the OD at 405 nm after color development at room temperature for 20–30 min.
6. Determine the 70% of maximum binding of each antibody from the titration, repeat the Steps 1–5 with the modification at Step 3 to determine antibody specificity (gp120- or gp41-specific)

First antibody (modified Step 3): Remove the blocking buffer, add 50 µl per well soluble Fab antibodies at a concentration that leads to 70% maximum binding (*see* Note 20). Simultaneously add 50 µl threefold serially diluted free gp120_{89,6} with a starting concentration of 20 µg/ml, incubate the plates at 37°C for 2 h (*see* Note 21).

¹⁹The final OD_{600 nm} before induction, IPTG concentrations, and time for induction can vary depending on the particular antibody and should be optimized. If the Fab is toxic, glucose should be used for suppression of Fab expression before induction. Glucose needs to be removed before adding IPTG by changing the medium.

²⁰Antibody concentration in 50 µl should be doubled, so that after adding another 50 µl of competitor (free gp120_{89,6}), the antibody concentration is the one that leads to 70% maximum binding.

²¹If antibody binds to gp120_{89,6}, free gp120_{89,6} competes with coated gp140_{89,6} for binding to the antibody and the OD at 405 nm will decrease. If antibody does not bind to gp120_{89,6}, antibody binding curve is a flat line.

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Fig. 9.1.

Schematic representation of the competitive antigen panning (CAP) methodology. CAP allows enhanced selection of antibodies to subunits of whole proteins. For example, gp41 is difficult to isolate in native state. To enhance selection of such antibodies against gp41 the library is panned against the whole protein, in this case soluble HIV Env, gp120-gp41 (gp140), in the presence of excess gp120, which binds most of the gp120-specific antibodies. Then isolation of the tagged whole protein with bound phage-displayed antibodies leads to enrichment of gp41-specific antibodies.