Chapter 2

Developing a PCC Agent Cocktail for the Detection of Anti-HIV Antibodies

2.1 INTRODUCTION

Detecting the immune response to an infectious agent can provide a useful *in vitro* diagnostic surrogate relative to direct pathogen detection [1]. Such assays are commonly used for detecting HIV infection because of its characteristic immunopathology [2]. Direct detection of HIV viral RNA and p24 antigen is only effective at an early stage of infection, approximately 2-6 weeks of initial exposure [3,4]. Antibodies against HIV envelope proteins emerge in patients' blood around 3-4 weeks of infection [2,5] as the viral RNA and p24 levels decline as a result of immunocomplex formation [6]. The high serum level of anti-HIV IgG is maintained throughout the course of clinical latency (2-20+ years), during which time viral antigens are under detection limits until the onset of acquired immunodeficiency syndrome (AIDS) [2,5]. Typically in these assays, immunogenic and conserved antigens from the HIV are expressed as regions of a single chimeric protein. That chimeric protein is then used to capture specific antibodies from the body fluid (e.g. blood, saliva or urine) of potentially infected patients; a positive assay result implies infection. However, the polyclonal diversity of antibodies across a patient population can translate into large variations in assay performance from patient to patient. In addition, the chimeric recombinant proteins are biological reagents, and so may have limitations related to shelf life and batch-to-batch variability. These limitations can adversely influence the performance of a diagnostic test [3,5,7], especially one that is deployed in harsh physical environments.

Previous work in the Heath group has demonstrated the ability to selectively target biologically relevant proteins with PCC Agents [8,9]. Targeting anti-HIV antibodies requires selective detection of anti-HIV antibodies over other IgG and serum proteins while maintaining sensitivity to variations in immune response across patients. This chapter reports on the discovery, synthesis, and characterization of a cocktail of capture agents that selectively target different anti-HIV antibodies to increase the target capture probability while minimizing cross reactivity with other proteins in sera.

2.2 *IN SITU* CLICK

The development of a PCC Agent against a protein target utilizes the target itself to promote the 1,3-dipolar cycloaddition between an acetylene and an azide group to form a triazole linkage (the *in situ* 'click' reaction) [10]. The protein effectively plays the role of an extremely selective, but much less efficient, variant of the Cu(I) catalyst that is commonly used for such couplings [11,12]. For the present work, the two reacting species are peptides – one peptide (the anchor) is a chemically modified variant of a conserved, immunogenic epitope on the HIV-1 gp41 protein, and the second peptide is selected via an *in situ* click screen from a large (10^6 element) one-bead-one-compound (OBOC) [13] peptide library. The protein targets are human monoclonal antibodies raised against variants of the gp41 epitope represented by the anchor peptide.

2.3 ANCHOR SELECTION

The PCC Agents developed here were designed to capture antibodies that are selective for residues 600-612 (IWCGSGKLICTTA) of gp41. Previous studies have shown that a large fraction of HIV-1-positive patients develop antibodies against this epitope [14,15]. Our strategy for sampling the polyclonal diversity of such antibodies was to develop PCC Agents that exhibited both differential, as well as similar avidities for human monoclonal antibodies (mAbs) raised against different parts of this epitope. To select the anchor peptides, we modified the polypeptide fragment corresponding to residues 600-612 of gp41 with artificial amino acids at multiple locations, and tested the ability of these modified cyclic peptides to detect two different monoclonal anti-gp41 antibodies 3D6 and 4B3 (Polymun, Klosterneuburg, Austria). The 3D6 mAb was raised against the epitope SGKLIC, whereas the 4B3 mAb was raised against SGKLICTTA. One anchor peptide, A21, was synthesized by adding a propargyl glycine (Pra) residue at the C-terminus of the residues 600-612 of gp41. This anchor peptide was also N-terminally tagged with a polyethylene glycol (PEG) oligomer bridge and a biotin label. For a second anchor peptide (A22), Leu-607 was substituted with Pra. A22 also included an N-terminal PEG-biotin label. A21 equally detected 3D6 and 4B3 with an estimated dissociation constant (K_d) of 1-50 nM, while A22 differentially detected 3D6 (K_d >10 μM) and 4B3 (K_d = 1-50 nM) (Figure 2.1). A21 and A22 were then separately developed into PCC agents against 3D6 and 4B3, respectively.

2.4 SCREENING

The *in situ* click screens are illustrated in Scheme 2.1. The target IgG is incubated with an excess of the selected anchor peptide and a large OBOC library at 4^{o}C overnight (see Materials and Methods). The OBOC library is synthesized on TentaGel resin (Rapp Polymere, Tuebingen, Germany), and is a comprehensive library of 5-mers with a 6th amino acid at the N-terminus presenting an azide functionality. To help ensure chemical and biochemical stability, the OBOC library is comprised of non-natural (D) stereoisomers of the 20 natural amino acids, excluding cysteine and methionine. The *in situ* screen is designed to identify a secondary (2^{o}) peptide that, when coupled to the anchor, forms a biligand with increased selectivity and/or affinity for the target IgG.

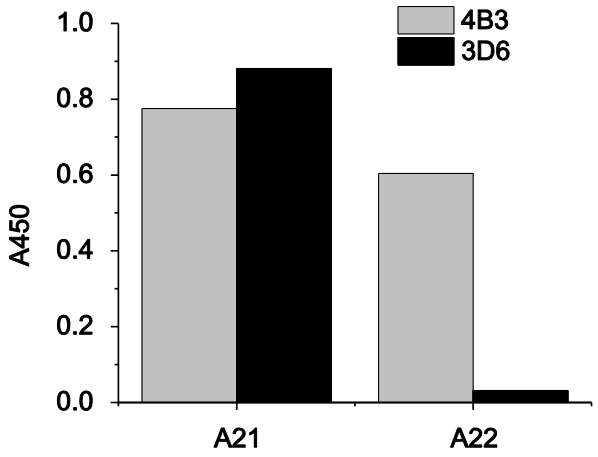
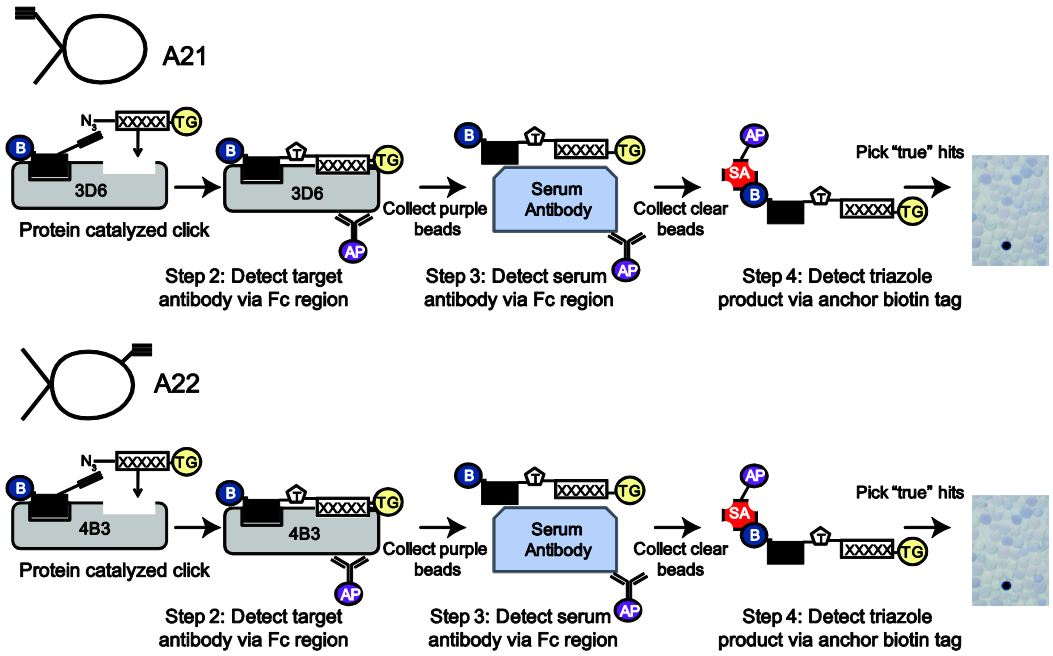


Figure 2.1. Differential detection of 3D6 and 4B3 by anchor ligands. Relative affinities of A21 and A22 for 3D6 and 4B3 were determined by sandwich ELISA. Biotinylated anchor ligands A21 and A22 were immobilized on streptavidin-coated 96-well plated at a concentration of 100 nM, and incubated with the solutions of target anti-HIV antibodies 3D6 and 4B3 at 100 nM in TBS. Captured antibody was detected by peroxidase-conjugated anti-human IgG antibody.

The screen proceeds stepwise. In the first step (not shown in Scheme 2.1) the OBOC library is cleared of beads that exhibit non-specific binding to alkaline phosphatase-conjugated streptavidin (SA-AP), which is used as a detection reagent in a later step. Step 2 is a target screen, and so is designed to detect the presence of the bound IgG target to specific beads, and defines possible hits. The step 3 screen is designed to remove those beads from the pool of potential hits that also exhibit binding to off-target serum proteins. Step 4 is called a product screen, and is unique to sequential *in situ* click screens [16]. This screen is designed to detect for the presence of *in situ* clicked reaction products, which are those hit beads containing the triazole-linked anchor peptide. Typically, Step 2 yields a few hundred hits (~0.05% of the OBOC library). Step 3 reduces that pool by a factor of 2 or 3 to about 100 hits, and Step 4 further reduces the number of hits to around 10. This is a manageable number, meaning that each hit can be separately synthesized as a biligand using Cu(I) catalyzed click chemistry to couple the anchor and 2^{o} peptides. A complete list of the hits for the A21/3D6 and A22/4B3 screens is given in Table 2.1.



Scheme 2.1. Screening strategy for selecting capture agents against anti-HIV antibodies 3D6 and 4B3. The flow chart represents the use of the A21 and A22 cyclic peptides as anchor ligands for separate in situ click screens against a large OBOC azide-presenting peptide library.

	X_1	X_2	X_3	X_4	X_5
Az4	d	k	G	l	p
Az4	v	a	d	p	a
Az4	n	i	d	n	G
Az4	p	G	v	t	f
Az4	s	r	r	r	s
Az4	d	q/e	G	a	f
Az4	y	w	d	y	n
Az4	h	l	l	y	q
Az4	s	G	a	q	s
Az4	d	d	w	a	i
Az4	q	i	d	l	r
Az4	t	f	L	q	s
Az4	h	n	p	f	k
Az4	w	G	e	h	p
Az4	q	n	d	w	K
Az4	l	t	s	r	Y
Az4	d	i	k	s	p
Az4	e	i	h	n	y
Az4	q	p	i	d	q
Az4	k	i	d	r	v
Az4	q	s	p/d	w	l
Az4	e	q	t	f	d

Table 2.1. Biligand screen results. List of pentapeptide "hits" from OBOC biligand screens performed with A21/3D6 and A22/4B3. The selected secondary ligands corresponding to **(i)**, **(ii)**, and **(iii)** are shown in red, blue, and green respectively (shown in Figure 2.2).

2.5 BILIGAND CHARACTERIZATION

The performance of these biligands is then characterized using immunoprecipitation (pulldown) assays detected by Western blotting from spiked serum samples for specificity (data not shown), and sandwich ELISAs and surface plasmon resonance (SPR) assays for affinity estimations (Figure 2.3-2.4). This approach yielded two equivalently performing biligands against 3D6, and one biligand against 4B3. These three PCC agents (Figure 2.2) were combined, in equal parts, to form a capture agent cocktail. The cocktail slightly outperformed both the standard commercial chimeric antigen and A21, when tested against healthy human serum spiked with both 3D6 and 4B3 (Figure 2.5). A21 is the equivalent of the original antigenic epitope of gp41.

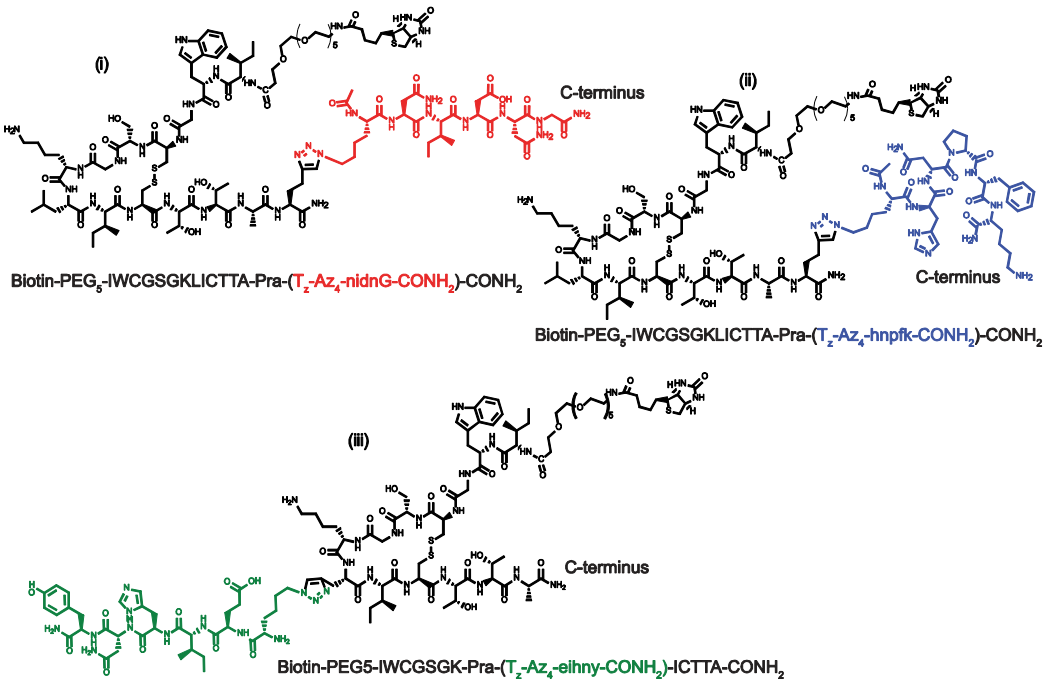


Figure 2.2. Structures of peptide ligands in PCC Agent cocktail. Acetylene-presenting anchor peptides (black) were derived from the immunogenic epitope of HIV-1 gp41 (residues 600-612). A22-nindG (**i**) and A21-hnpfk (**ii**) were evolved from the original epitope appended with Pra at the C-terminus whereas A22-eihny (**iii**) utilizes the "substituted" anchor where residue Leu-607 is replaced with Pra. Secondary ligand branches (colored) were identified from the *in situ* click screen of a 5-mer OBOC library presenting an azide functionality. Biligands (**i**) and (**ii**) were raised against the target anti-HIV antibody 3D6, and the biligand (**iii**) was raised against the antibody 4B3.

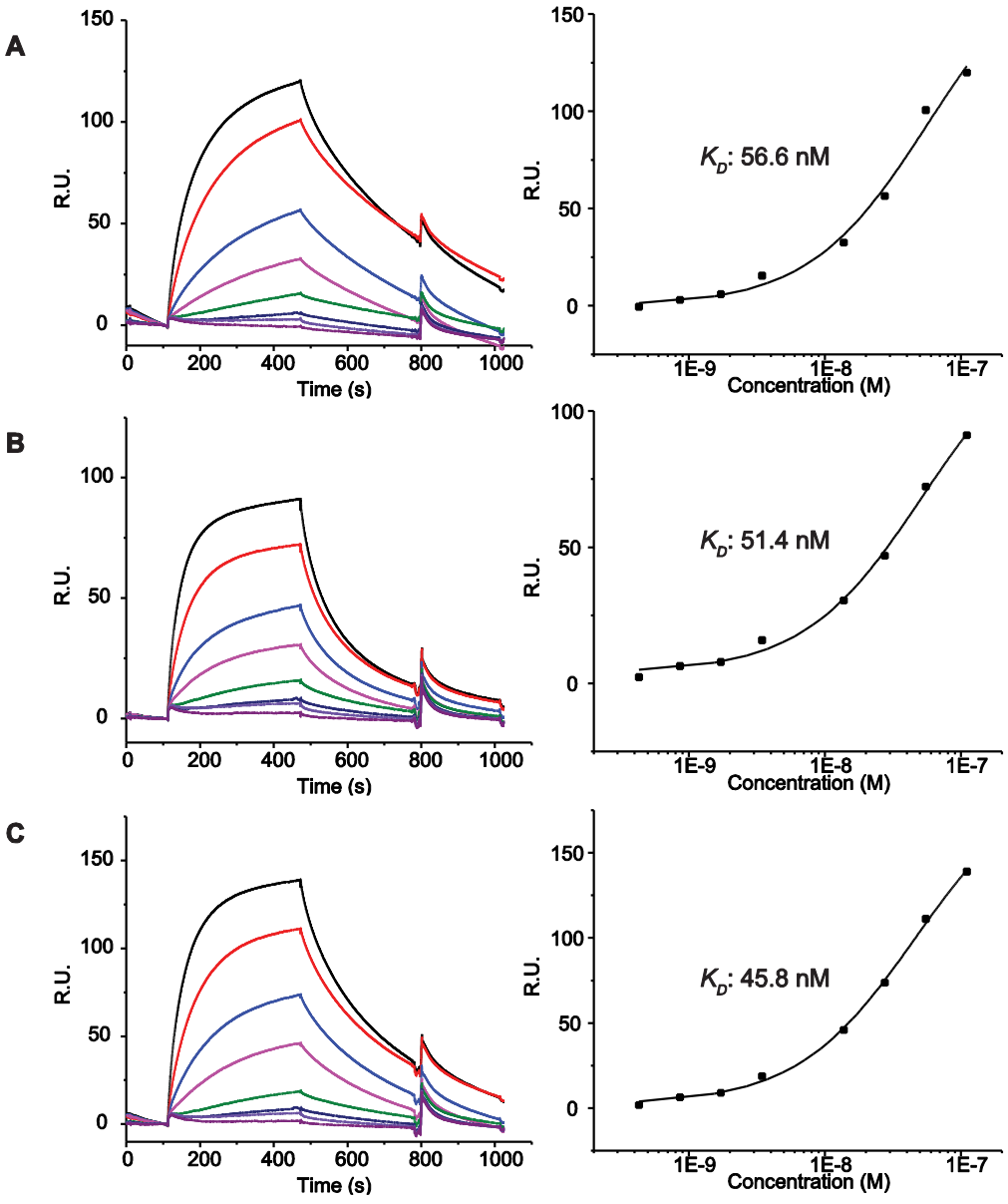


Figure 2.3. Apparent affinity of A21 and biligands directed against 3D6 as determined by SPR. **A.** Sensorgram and 1st order Hill fit to affinity data for A21. **B.** Sensorgram and 1st order Hill fit to affinity data for A21-nidnG **(i)**. **C.** Sensorgram and 1st order Hill fit to affinity data for A21-hnpfk **(ii)**.

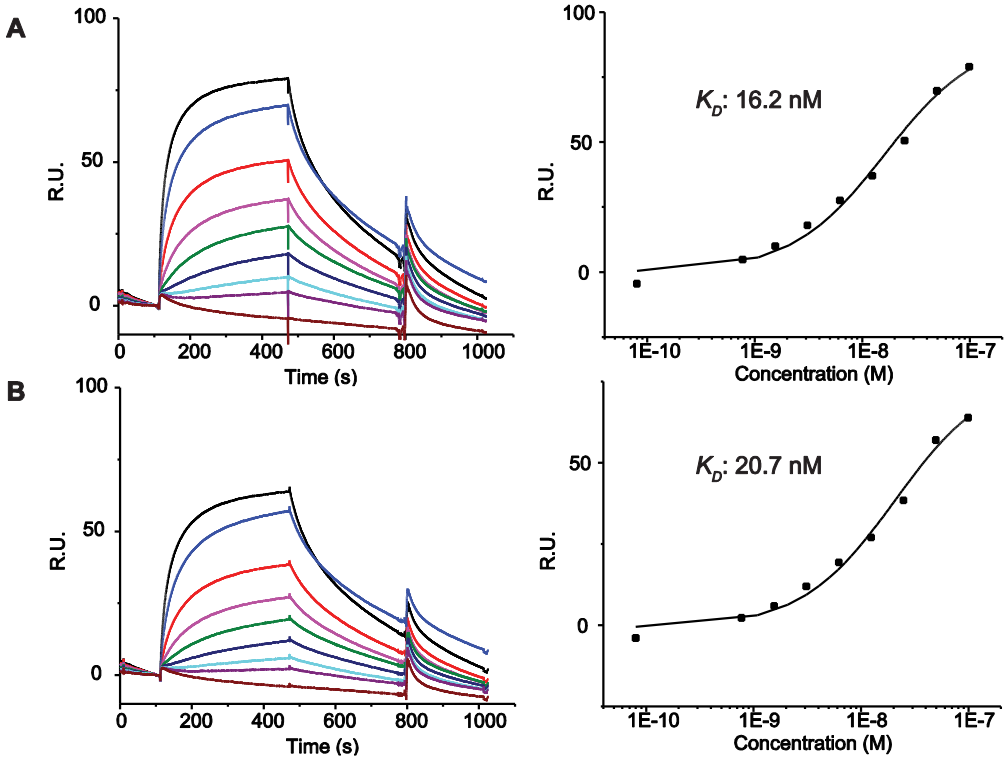


Figure 2.4. Apparent affinity of A22 and biligand directed against 4B3 as determined by SPR. **A.** Sensorgram and 1st order Hill fit to affinity data for A22. **B.** Sensorgram and 1st order Hill fit to affinity data for A22-eihny **(iii)**.

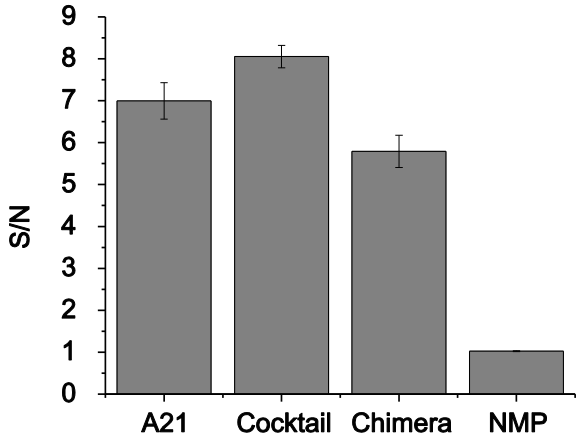


Figure 2.5. Performance of PCC agent cocktail to detect 3D6 and 4B3 from human serum. Comparative performance of the PCC Agent cocktail versus the original gp41 epitope A21 and the commercial chimeric protein antigen was tested by a sandwich ELISA. Target antibodies 3D6 and 4B3 (4 nM each) were both spiked into diluted, HIV-free human serum (1% v/v in TBS), and captured antibody was detected by peroxidase-conjugated anti-human IgG antibody.

2.6 MATERIALS AND METHODS

2.6.1 Anchor Synthesis

The anchor peptides A21 and A22 were synthesized C → N on 200-300 mgs of Rink amide resin (AnaSpec, Fremont, CA) with a Titan peptide synthesizer using standard Fmoc chemistry [17]. The synthesis used Fmoc and side chain protected L amino acids (Aaptec, Louisville, KY), Fmoc protected propargylglycine (Pra) (Aaptec, Louisville, KY), PEG_5 (EMD Millipore,Germany), and biotin (Sigma-Aldrich, St. Louis, MO). The finished peptides were side-chain deprotected and cleaved from resin in a mixture of 95% v/v trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO), 5% v/v dH_2O, and 5% triethylsilane (TES) (Sigma-Aldrich, St. Louis, MO), and precipitated in diethyl either. $Cu(Phen)_3$ stock solution was prepared by mixing 150 mM Cu(II) sulfate pentahydrate (Sigma-Aldrich, St. Louis, MO) and 500 mM 1,10-phenanthroline (Sigma-Aldrich, St. Louis, MO) in 4:1 H_2O:Ethanol. The ether-precipitated ligands were cyclized by stirring the cleaved peptides dissolved in ~5 mL acetonitrile (ACN) overnight with 1 mL of the $Cu(Phen)_3$ stock solution. The cyclization mixtures were lyophilized, and the final peptide products were purified using reverse phase (RP) HPLC on a Kinetex 5µ XB-C18 250 x 4.6 mm prep column (Phenomenex, Torrance, CA). MALDI-TOF MS: A21: Expected mass $[M+H]^+ = 2006.96$, Observed mass $[M+H]^+ = 2006.24$. A22: Expected mass $[M+H]^+ = 1892.89$, Observed mass $[M+H]^+ = 1892.85$

2.6.2 OBOC Screens

All screens used naïve one-bead-one-compound (OBOC) D-pentapeptide libraries on TentaGel resin (TG) (Rapp Polymere, Tuebingen, Germany) of the form NH_2-Az_4-$X_1X_2X_3X_4X_5$-TG, excluding cysteine and methionine, where Az_4 is L-azidolycine. Libraries were synthesized on a Titan peptide synthesizer (Aaptec, Louisville, KY) using a split/mix method, and couplings were done using standard Fmoc SPPS chemistry in *N*-methylpyrrolidone (NMP).

A21 with 3D6

Screening against 3D6 was carried out in four main steps: pre-clear, target screen, anti-screen, and product screen. ***Step 1 preclear****:* 400 mg of OBOC library was blocked in 5% w/v dry milk in Tris buffered saline (TBS) (25 mM Tris-HCl, 150 mM NaCl, 10 mM $MgCl_2$, pH 7.6) for 2 hr at room temperature, and then incubated with 1:10,000 phosphatase-conjugated streptavidin (SA-AP, Promega, Madison, WI) in 0.5% w/v dry milk in TBS for 1 hr. The beads were washed in high salt buffer (25 mM Tris-HCl, 750 mM NaCl, 10 mM $MgCl_2$, pH 7.6) for 1 hr, and then developed with BCIP/NBT (Promega, Madison, WI) in BCIP buffer (100 mM Tris-HCl, 150 mM NaCl, 1 mM $MgCl_2$, pH 9). After 20 minutes, the reaction was quenched with conc. HCl, and the purple beads were discarded. The remaining library was decolorized in NMP, dried with methanol (MeOH) and dichloromethane (DCM), and then swelled and blocked in 5% w/v dry milk in TBS for 2 hr at room temperature. ***Step 2 target screen****:* 4 mL of a solution containing 11.4 µM A21 and 147 nM 3D6 in 0.5% w/v dry milk in TBS was added to the precleared OBOC

library, and the *in situ* click reaction was allowed to proceed overnight at 4°C. Bound target was probed with 1:10,000 AP-conjugated rabbit anti-human IgG Fc (Thermo Scientific, Rockford, IL) in 0.5% w/v dry milk in TBS for 1 hr at room temperature. The beads were then washed in high salt buffer for 1 hr at room temperature, and developed with BCIP/NBT in BCIP buffer. The reaction was quenched after 20 minutes with conc. HCl, and the purple beads were retained and decolorized in NMP and dried with MeOH and DCM. The hit beads were then stripped of all bound protein by washing with 7.5 M Guanidine-HCl (pH 2) for 2 hr at room temperature, rinsed 10 times with water, and then blocked in 5% w/v dry milk in TBS for 2 hr at room temperature. ***Step 3 anti-screen:*** A solution of 1% v/v human serum (Omega Scientific, Tarzana, CA) in TBS was incubated with the hit beads from Step 2 for 1 hr at room temperature. Off-target human antibodies bound to the beads were probed with 1:10,000 AP-conjugated rabbit anti-human IgG Fc in 0.5% w/v dry milk in TBS for 1 hr at room temperature. The beads were then washed in high salt buffer for 1 hr, and developed with BCIP/NBT in BCIP buffer. The reaction was quenched after 20 minutes with conc. HCl, and the purple beads were discarded. The remaining beads were decolorized in NMP and dried with MeOH and DCM. The anti-screened beads were swelled in water and washed with Guanidine-HCl for 2 hr at room temperature, rinsed 10 times with water, and then blocked in 5% w/v dry milk in TBS for 2 hr at room temperature. ***Step 4 product screen****:* The beads were incubated for 1 hr at room temperature with 1:10,000 SA-AP in 0.5% w/v dry milk in TBS, washed for 1 hr with high salt buffer, and then developed with BCIP/NBT in BCIP buffer for 20 minutes. The reaction was quenched with conc. HCl, and

the purple beads were retained and sequenced using a Procise CLC Edman Degradation Protein Sequencing System (Applied Biosystems, Kingston, RI).

A22 with 4B3

Screening against 4B3 was carried out similarly to that described for 3D6, with two exceptions. In ***Step 2***, the *in situ* click reaction solution contained 10 µM A22 and 430 nM 4B3 in 0.5% w/v dry milk in TBS. In ***Step 3***, the beads were anti-screened against 0.1% v/v human serum in TBS spiked with 430 nM 3D6.

2.6.3 Biligand Synthesis

Pra-OtBu

The C-terminally protected Pra molecule used in the synthesis of **(i)** and **(ii)** was made by refluxing Fmoc-L-Pra-OH (Aaptec, Louisville, KY) with tert-butyl-tricholroacetamide (Sigma-Aldrich, St. Louis, MO) at 50°C for 3 hr in DCM. The formed product was separated from unreacted species on a silica flash column in DCM.

Peptides (i) and (ii)

The secondary ligands including N-terminal Az_4 for **(i)** and **(ii)** were synthesized C → N on 200-300 mgs of Rink amide resin with a Titan peptide synthesizer using standard Fmoc chemistry. The synthesis used Fmoc and side chain protected D amino acids and L-Az_4 (Aaptec, Louisville, KY). Fmoc- and C-terminally protected Pra (Pra-OtBu) was clicked on-bead to the azide side chain of Az_4 by adding equal molar amounts of

Pra-OtBu, Cu(I) iodide (Sigma-Aldrich, St. Louis, MO), and L-ascorbic acid (Sigma-Aldrich, St. Louis, MO), in 2x excess to the azide on bead. The click mixture was agitated in 20% v/v piperidine in NMP for 3 hr at room temperature, and the copper was chelated by repeated washing with 10% w/v sodium diethylduthiocarbamate trihydrate (Sigma-Aldrich, St. Louis, MO) and 10% v/v *N,N*-diisopropylethylamine (DIEA) (Sigma-Aldrich, St. Louis, MO) in NMP until the beads were clear. The Fmoc deprotected Pra was then the starting point for the remainder of the anchor component of each ligand, including N-terminal PEG_5 and biotin. The anchor components were synthesized using standard Fmoc chemistry with protected L amino acids. The finished peptides were side-chain deprotected and cleaved from resin in 95:5:5 TFA:dH_2O:TES, then precipitated in diethyl either. The ligands were cyclized by stirring the cleaved peptides dissolved in ~5 mL ACN overnight with 1 mL of the $Cu(phen)_3$ stock solution. The cyclization mixtures were lyophilized, and the final peptide products were purified using RP-HPLC on a Kinetex 5µ XB-C18 250 x 4.6 mm prep column. MALDI-TOF MS: **(i)**: Expected mass $[M+H]^+$ = 2733.30, Observed mass $[M+H]^+$ = 2733.64. **(ii)**: Expected mass $[M+H]^+$ = 2843.40, Observed mass $[M+H]^+$ = 2843.60.

Peptide (iii)

A22 was synthesized, cyclized, and purified as described above. The secondary ligand component including N-terminal Az_4 for **(iii)** was synthesized C → N on Rink amide resin with a Titan peptide synthesizer using standard Fmoc chemistry. The synthesis used Fmoc and side chain protected D amino acids and L-Az_4. Resulting

secondary peptide was side-chain deprotected and cleaved from resin in 95:5:5 TFA:dH_2O:TES, precipitated in diethyl either, and purified using RP-HPLC on a Kinetex 5µ XB-C18 250 x 4.6 mm prep column. The purified secondary peptide was clicked to A22 in solution by mixing with 2x molar excess of A22, 10x Cu(I) iodide, 50x L-ascorbic acid, and 4x Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (Sigma-Aldrich, St. Louis, MO) in 5:1 NMP:dH_2O and stirring overnight at room temperature. The clicked biligand product was purified from the reaction mixture by RP-HPLC on a Kinetex 5µ XB-C18 250 x 4.6 mm prep column. Expected mass $[M+H]^+ = 2720.31$, observed mass $[M+H]^+ = 2720.5$.

Scaling up (iii)

The secondary ligands including N-terminally Boc-protected Az_4 for **(iii)** was synthesized C → N on Rink amide resin with a Titan peptide synthesizer using standard Fmoc chemistry. The synthesis used Fmoc and side chain protected D amino acids and L-Az_4 (AnaSpec, Fremont, CA). Truncated A22 (Pra-ICTTA) was synthesized C → N on Sieber amide resin (ChemPep, Wellington, FL) with a Liberty 1 microwave peptide synthesizer (CEM, Matthews, NC) using standard Fmoc chemistry. The synthesis used Fmoc and side chain protected L amino acids and Fmoc-L-Pra. Fully protected truncated A22 was cleaved from the resin with 1% v/v TFA in DCM, and neutralized with DIEA. Solvent was removed using a rotary vacuum evaporator and the protected peptide was purified by RP-HPLC on a Kinetex 5µ XB-C18 250 x 4.6 mm prep column. Resulting truncated A22 was clicked on-bead to the secondary peptide through the azide side

chain of Boc-Az_4 by adding equal molar amount of the protected truncated A22, 2x molar excess of Cu(I) iodide, and 2x excess of L-ascorbic acid to the azide on bead. The click mixture was agitated in 20% v/v piperidine in NMP overnight at room temperature, and then the copper was chelated by repeated washing with 10% w/v sodium diethyldithiocarbamate trihydrate and 10% v/v DIEA in NMP until the beads were clear. The Fmoc deprotected Pra was then the starting point for the remainder of the anchor component, including N-terminal PEG_5 and biotin (Biotin-PEG_5-IWGCSGK). The anchor components were synthesized using standard Fmoc chemistry with protected L amino acids. The finished peptide was N-terminal and side-chain deprotected and cleaved from resin in 95:5:5 TFA:dH_2O:TES, then precipitated in diethyl either. The ligand was cyclized by stirring the cleaved peptide dissolved in ~5 mL ACN overnight with 1 mL of the $Cu(phen)_3$ stock solution. The cyclization mixtures were lyophilized, and the final peptide products were purified using RP-HPLC on a Kinetex 5µ XB-C18 250 x 4.6 mm prep column.

2.6.4 Assays

Anchor binding ELISA

Biotinylated anchor peptide was immobilized on streptavidin-coated 96-well plates (Thermo Scientific Pierce, Rockford, IL) at a concentration of 100 nM in TBS. The plates were blocked with 5% w/v dry milk in TBS, and then the anchor peptides were incubated with either 100 nM 3D6 or 4B3 in 0.5% w/v dry milk in TBS at room temperature for 1 hr. Bound antibody was probed with horseradish peroxidase (HRP)-

conjugated mouse monoclonal antibody to human IgG Fc (Abcam, Cambridge, MA), diluted 1:10,000 in 0.5% w/v dry milk in TBS for 1 hr at room temperature. The colorimetric assay was developed with TMB substrate (KPL, Gaithersburg, MD), then quenched with 1 M H_2SO_4 and read at 450 nm.

Surface Plasmon Resonance

SPR experiments were performed on a Biacore T200 instrument. Biotinylated peptides were immobilized on streptavidin-coated sensor chips (GE Healthcare Biosciences, Pittsburg, PA) at R.U. values ranging from 7-12. 4B3 or 3D6 was flowed at various concentrations in 1x HBS-EP buffer (GE Healthcare Biosciences, Pittsburgh, PA), and affinity curves were fit using the default settings of the Biacore evaluation software.

Cocktail binding ELISA

Recombinant multi-epitope chimeric HIV antigen ("chimera") (BioLink International, Lisle, IL) was chemically biotinylated using ChromaLink Biotin Labeling Kit (Solulink, San Diego, CA) according to the manufacturer's instructions, using 10x molar excess of the ChromaLink biotinylation reagent to the buffer-exchanged protein. Streptavidin-coated 96-well plates were saturated with the biotinylated biligand cocktail or chimera using 1 uM solutions in TBS. The plates were blocked with 5% w/v dry milk in TBS, and then the capture reagents were incubated with either 1% v/v human serum or a mixture of 4 nM each 3D6 and 4B3 spiked in 1% v/v human serum in TBS at room temperature for 1 hr. Bound antibody was probed with HRP-conjugated mouse

monoclonal antibody to human IgG Fc, diluted 1:15,000 in 0.5% w/v dry milk in TBS for 1 hr at room temperature. The colorimetric assay was developed with TMB substrate, then quenched with 1 M H_2SO_4 and read at 450 nm (A450). The A450 for each sample is normalized against the A450 for the serum control, to yield a measurement of the signal-to-noise ratio of the assay.

2.7 CONCLUSION

Described in this chapter is a method for developing a PCC Agent cocktail to capture the diversity of human antibodies generated in response to an infectious agent, with specific application to HIV. The components of the cocktail were raised against 4B3 and 3D6, two representative anti-HIV antibodies, and individual and collective binding of the capture agents to the target antibodies was characterized. The cocktail displayed a moderately enhanced performance compared to the original gp 41 epitope (A21) and the gold standard chimeric antigen when used to detect 4B3 and 3D6 from human serum.

2.8 ACKNOWLEDGEMENTS

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