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Isolation of Peptide Ligands for the HIV Capsid Protein p24 by Phage-Display

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Abstract

Purpose Isolate renewable and cost-efficient affinity reagents that will facilitate the detection of p24, the capsid protein of Human Immunodeficiency Virus (HIV), by screening phage-displayed combinatorial peptide libraries and identifying peptide ligands.

Method Four in-house combinatorial peptide libraries were screened for binders in three progressive rounds against monomeric p24 protein. Peptide binders were characterized by ELISA and Surface Plasmon Resonance (SPR) and one peptide sequence was evaluated in a lateral flow assay (LFA).

Result We identified 26 unique peptide sequences that exhibit varying phage ELISA signals above background for p24. We subsequently validated the binding of one linear and two cyclized peptide sequences with synthetic peptides. Alanine-scanning identified several residues critical to binding in the linear peptide. The linear peptide could be used for p24 detection in ELISA and LFAs.

Conclusion Phage-displayed combinatorial peptide libraries are suitable for isolation of binders against p24 and potentially other targets. Upon identification of a minimal binding sequence, the subsequent characterization and future optimization of it can lead to a variety of diagnostic assays.

Keywords Combinatorial peptides · HIV · p24 capsid protein · Phage display

Introduction

Phage display, pioneered by George Smith (Smith 1985), involves the expression of coding regions fused in-frame with a capsid protein of a virus, with the resulting recombinant virions displaying a foreign peptide or protein. From libraries of displayed peptides, cDNA segments, enzymes, and antibody fragments, researchers can select valuable reagents for use in research, industry, and medicine (Smith 2019). For example, from libraries containing up to 10^9 phage-displayed combinatorial peptides, peptide ligands can be readily identified that bind at the active sites of enzymes (Kay and Hamilton 2001) or hot spots of protein-protein interactions (Hertveldt et al. 2009). Such peptides can then be used in displacement assays for drug screening and target validation in cells (Tao et al. 2000), or as the starting point for drug delivery systems (Sioud 2019). Other display technologies, such as mRNA (Huang et al. 2019) and ribosome display (Zahnd et al. 2007) have been employed similarly for such purposes.

The utility of a peptide binder against a protein ligand could be extended to multiple applications, such as a drug delivery system like a peptide drug conjugate (PDC) such as ^{177}Lu-Dotatate (Ortega et al. 2021), or as a therapeutic itself by acting as an agonist/antagonist of the target such as semaglutide (Mahapatra et al. 2022). One novel application of peptide ligands isolated through phage-display is their use in diagnostics (reviewed in Pandey et al. 2021). As peptide ligands can be chemically synthesized, are highly renewable, production is scalable and they are amenable to

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incorporation of non-canonical amino acids and chemical conjugation, which are attractive attributes for reformatting reagents into analytical assays. For example, peptide ligands that have been identified through phage-display against cholera toxin (Lim et al. 2018), the receptor binding domain (RBD) of SARS-CoV-2 (Yang et al. 2022) and calprotectin (Díaz-Perlas et al. 2023) have been implemented into diagnostic tests.

In this study, we report the isolation of a series of peptide ligands to the capsid protein, p24, of Human Immunodeficiency Virus (HIV). With its high abundance (approximately 1500 copies per viral particle) and importance in the lifecycle of the virus (Ganser-Pornillos et al. 2008), p24 is the principal biomarker used in early detection before seroconversion of the virus (Gray et al. 2018). We describe the discovery of 26 unique linear and cyclic peptides that bind p24 and dissect the binding properties of one dominant peptide, named TBP121. We also report the applicability of TBP121 in a proof-of-concept ELISA and lateral flow assay for HIV p24 detection.

Materials and Methods

Escherichia coli cells are grown in 2xYT medium (per liter: 16 g tryptone, 10 g yeast extract, and 5 g NaCl) medium, supplemented with either carbenicillin (50 µg/mL), kanamycin (50 µg/mL) or tetracycline (10 µg/mL). All cells and phage stocks are maintained in -80 °C as glycerol stocks. All oligonucleotides are ordered from IDT (Coralville, IA) and synthetic peptides were synthesized by LifeTein (Somerset, NJ) at >90% purity or Mimotopes (Victoria, Australia) at 70% purity. For biotinylation of the peptide on the N-terminus end, aminohexanoic acid was used as a spacer arm between the biotin and peptide, while for biotinylation on the C-terminus end, Fmoc-Lys(Biotin)-OH was incorporated during peptide synthesis.

Molecular Cloning

For the bacterial expression of HIV p24, we have constructed and deposited at Addgene, (Plasmid #212195) a pET28a-HIV p24 plasmid with a N-terminus six-histidine tag and ordered it from Twist Bioscience (South San Francisco, CA). The sequenced-verified plasmid was transformed into *E. coli* LOBSTR (*LO*w *B*ackground *STR*ain) cells.

For the cloning of the 59H10 nanobody for phage display, a gBlock encoding the DNA sequence of the nanobody (Gray et al. 2017) was ordered from IDT. The DNA segment included BsaI restriction enzyme sites at its termini, which allowed Golden Gate assembly (Pryor et al. 2020) into a type 3 phage-display vector, mTB3 (Grahn et al. 2023). The final construct was confirmed by whole plasmid DNA sequencing.

To test the importance of the residue at position 1 of the 12-mer TBP121 peptide, a small library of amino acid replacements was constructed. A forward primer, 5'-*TNN* CAACAATTTTTTCGCCAAATATCAGAGCTCT-3' was ordered, with the TNN codon potentially encoding six different amino acids (i.e., C, L, F, S, W and Y). A reverse oligonucleotide, 5'-ACTAGACGCCGATGCGCTAAACGCT AAAACTAAACC-3', was used to amplify a recombinant plasmid, pTB208-TBP121 by inverted polymerase chain reaction (PCR) with RepliQa DNA polymerase (QuantaBio, Beverly, MA). After verifying successful production of the amplicon by agarose gel electrophoresis, the sample was cleaned up with the Monarch PCR and DNA cleanup kit (New England Biolabs, Ipswich, MA) and eluted into 50 µL of 10 mM Tris-HCl, 1 mM EDTA (pH 7.5). One µL of the recovered DNA was added to a 10 µL reaction with T4 polynucleotide kinase, T4 DNA ligase, and Dpn I and incubated at room temperature for one hour. Two microliters of the reaction were used to transform Mix-and-Go JM109 cells (Zymo Research, Irvine, CA) and spread onto 2xYT supplemented with carbenicillin plates. Several colonies were selected at random for DNA sequencing. Clones carrying F, Y, S, and W at position 1 of the TBP121 sequence were identified, grown overnight in 2xYT supplemented with carbenicillin at 37 °C, and used to start a second culture with K07 helper virus present to yield virions for phage ELISA.

Expression and Purification of Recombinant p24

E. coli LOBSTR cells, which harbored the p24 expression construct, were grown overnight in 2 mL 2xYT supplemented with kanamycin. The next day, the cells were refreshed 100-fold in fresh 2xYT medium supplemented with kanamycin and incubated at 37 °C with shaking (200 rpm). Upon reaching an OD_{600nm} value of 0.8, expression of p24 protein was induced by supplementing the medium with isopropyl β-d-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Induction was allowed to proceed for 2 h at 37 °C with shaking, after which the cell pellet was harvested through centrifugation. The retrieved cell pellet was flash frozen at -80 °C overnight. The next day, the cell pellet was resuspended in 25 mL of lysis buffer (50 mM sodium phosphate, 300 mM sodium chloride, 0.15% β-mercaptoethanol and EDTA-free protease inhibitor cocktail (Roche) and cells were lysed via sonication. The suspension was subsequently mixed with His60 Ni Superflow Resin (Takara, San Jose, CA), resuspended in a filter column and washed thrice with wash buffer (50 mM sodium phosphate, 300 mM sodium chloride and 10 mM imidazole). The protein was subsequently eluted via the

addition of elution buffer (50 mM sodium phosphate, 300 mM sodium chloride and 300 mM imidazole). The eluted protein samples were checked for purity using SDS-PAGE and subsequently buffer exchanged through a desalting column with a molecular cutoff of 10 kDa. Protein concentration was determined with Bradford assay (Bradford 1976). Protein samples were resuspended in PBS-50% glycerol at a concentration of 1 mg/mL and stored at -80 °C.

Affinity Selection

Phage-displayed libraries displaying linear combinatorial peptides of either 12-, 10-, or 8 amino acids (aa) or cyclic combinatorial peptides of 10 aa, XCX_6CX, were screened against recombinant p24 through three rounds of affinity selection (Fig. 1). Briefly, biotinylated p24 proteins (20 µg) were immobilized on Streptavidin MagneSphere Paramagnetic particles (Promega, Madison, WI) (400 µL) and after 1 h incubation, non-specific binding sites were blocked by incubation with 2% Non-Fat Dry Milk (NFDM), supplemented with 10 µM biotin, for ≥1 h. The p24-bound magnetic particles were then washed with PBST (Phosphate Buffered Saline with 0.1% Tween 20) once, and 2×10^{12} PFU (Plaque Forming Units) from the appropriate phage-display library was mixed with the magnetic particles and incubated at room temperature (RT) for 1 h. After incubation, the magnetic particles were washed three times with 800 µL of PBST, followed by 800 µL of PBS three times. To elute the target-bound phage from the magnetic particles, 200 µL of 50 mM glycine (pH 2) was added and the suspension was incubated (with frequent shaking) for 10 min at RT. The pH of the recovered liquid was neutralized to pH 7 by adding 12 µL of 2 M Tris (pH 12). An aliquot of the neutralized eluate was used to infect mid-logarithmic phase XL-1 Blue cells for overnight amplification of M13 virions. The same procedure described above was also applied for subsequent affinity selections, where instead of the phage-display library, the amplified phage population from the previous round was used as input for the affinity selection. In addition, the amount of target used for selections were reduced to 10 µg and 5 µg for rounds 2 and 3, respectively. After three rounds of affinity selection, the amplified phage population was serially diluted to obtain single plaques. Ninety-five individual plaques were randomly selected and cultured in fresh 2xYT liquid media to obtain phage virions that will be used in an Enzyme-Linked Immunosorbent Assay (ELISA) to identify potential p24 binders. The isolates demonstrating high signal in ELISA, indicating affinity for p24, were submitted for Sanger dideoxy DNA sequencing (Genewiz, Indianapolis, IN).

Enzyme-Link ImmunoSorbent Assay (ELISA)

Costar half-area 96 well plates (Corning, Corning, NY) were coated with 50 µL per well of either p24 (12 µg/mL), or NeutrAvidin (NA; 12 µg/mL) overnight at 4°C. Separate wells that had been coated with 0.01% gelatin or Bovine Serum Albumin (BSA) (12 µg/mL) served as a negative control. After incubation, wells were washed with PBST and blocked with 2% NFDM (supplemented with 10 µM biotin if necessary) for at least 1 h. After blocking, the wells were washed thrice with PBST and 50 µL of phage suspension or peptide suspension and 25 µL of PBST were added into the appropriate wells and incubated at RT for at least 1 hour. The supernatant was removed, and the wells were washed three times with 100 µL of PBST. To detect for p24-bound phage virions, anti-M13-HRP (Horseradish Peroxidase; Sino Biological) was diluted 1:5000 in PBST and 50 µL was added into the wells. To detect p24-bound biotinylated peptides, streptavidin-conjugated HRP (Jackson ImmunoResearch, West Grove, PA) was diluted 1:5000 in PBST and 50 µL was added into the appropriate wells.

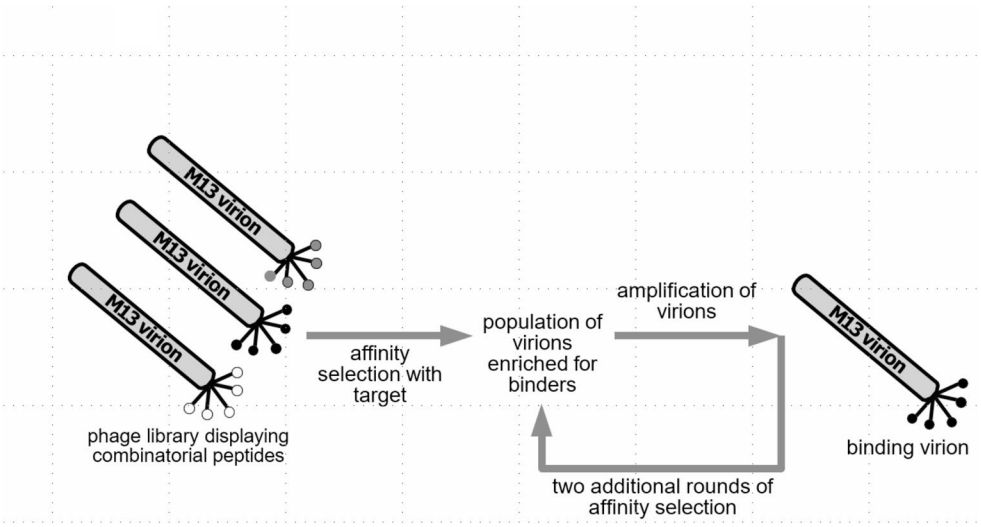

Fig. 1 Schematic showing the affinity selection process in phage display. A target is incubated with virions and target-virion complexes are recovered. After each round, potential binders were eluted and amplified by infecting bacterial cells. The amplified phage population undergoes two more rounds of selection and individual phage clones are isolated for further assessment of their binding capacity on the target using phage ELISA

After incubation of at least 1 h, the wells were washed three times with 100 µL of PBST. To detect HRP-linked antibody retained in the wells, 50 µL of 3,3',5,5'-Tetramethylbenzidine (TMB) were added to wells and incubated at RT for 5 min, followed by the addition of 0.1 N HCl to terminate the reaction. The absorbance of the wells was measured with a plate reader at 450 nm (Abs_{450}). The data analyses were performed with Prism version 10 application (GraphPad Inc. Boston, MA).

Surface-Plasma Resonance (SPR)

SPR analysis was performed on a Biacore T200 instrument at the Biophysics Core, in the Research Resource Center at the University of Illinois Chicago. The C-terminus biotinylated form of the TBP121 peptide was immobilized on the streptavidin chip (SAD200L) at a concentration of 5 ng/mL. The chip was equilibrated with PBST buffer at a rate of 30 µL per min for 2 h until the change in RU per minute was less than 1. The analyte, purified recombinant p24 protein, was two-fold serially diluted from 2000 nM to 125 nM, in PBS buffer. The analyte was flown through the immobilized ligand in increasing concentrations at a rate of 25 µL per min. One of the flow channels on the chip was used as a reference control. A single-cycle kinetics method was performed, and Biacore Insight evaluation software was used to analyze the data. The resulting sensograms were fitted to a 1:1 binding model and the steady state affinity K_D was calculated.

LFA Prototype

Anti-p24 LFA strips were manufactured on CN95 nitrocellulose membrane (Sartorius; #1UN95ER100025NTB) and assembled on a 30-cm custom backing adhesive card (MDI Diagnostics) along with an absorbent pad (Ahlstrom; #440) and a sample pad (Ahlstrom; #8964). Mouse anti-p24 antibodies (Medix Biochemica; #MM084) diluted to a concentration of 2 mg/mL in 1× PBS/10% sucrose was striped using a XYZ3060 BioDot dispenser. Antibody-printed cards were processed as previously described (Brosamer et al. 2023). Lastly, the card was cut into 5 mm LFA strips and stored at 20 °C, under desiccation, until use.

Carboxylate-modified polystyrene particles containing encapsulated europium chelate (diameter: 200 nm; Excitation 365 nm/ Emission 610 nm; Bangs Laboratories, #FCU002) were functionalized with NeutrAvidin protein (Thermo; #31000) using standard EDC-NHS chemical activation, as previously described (Brosamer et al. 2023). Conjugated particles were diluted at 0.5% solids in PBS-1% BSA and stored at 4 °C until use.

Fifty µL of p24 protein (50 ng/mL) was mixed with 2 µL of biotinylated TBP-121 (25 µM) in LFA Running Buffer (PBS, pH 7.4, 1% w/v BSA, 0.25% v/v Tween-20, 0.3% w/v PEG MW 3350) and applied onto the sample pad of the LFA strip. After 5 min, 30 µL of LFA running buffer was added onto the strips. After 5 min, 30 µL of neutravidin-conjugated particles (ca. 0.1% solids in LFA running buffer) were applied on to the LFA strips followed by 20 µL of the running buffer after 5 min. The wash step was repeated twice. After 10 min from the last wash, the strips were imaged on a portable time-resolved fluorescence LFA analyzer (LTRIC-600, Lumigenex). Intensity profiles along the length of the LFA strips were recorded and the integrated area under the curve for each peak was calculated using LReader, the analyzer's proprietary software.

Results and Discussion

With a goal of isolating peptide ligands for the HIV p24 protein, four different phage-displayed combinatorial peptide libraries were screened. The libraries consisted of either 8–, 10– or 12–mer linear peptides or cyclic XCX_6CX peptides. In all four libraries, the combinatorial peptide sequence is generated via NNK degenerate codons. The expected frequency of amino acids encoded by 32 resulting possible codon sequences is once for C, D, E, F, H, I, K, M, N, Q, W, Y, stop (TAG), twice for A, G, P, V, T, and three times for L, R, S. These peptides are expressed as N-terminal fusions to the mature form of the minor capsid protein, protein III, of M13 bacteriophage, and are displayed on the surface of phage particles (i.e., virions) in a pentavalent fashion. The estimated sequence diversity of each library is $\geq 10^9$ peptide sequences.

The four combinatorial peptide libraries were individually screened by incubating phage virions from each library with biotinylated p24 target and capturing virion-p24 complexes with streptavidin-coated magnetic beads. After three successive rounds of affinity selection, 26 unique peptide sequences were identified that bind to p24 via an enzyme-linked immunosorbent assay (ELISA). Based on their ELISA signals, peptides were arbitrarily grouped into high, moderate, and low signals. Table 1 lists sequences, frequency of isolation, and the ELISA signal values of the 12 strongest binders among the 26 identified sequences.

To verify the binding of the phage named TBP121, which exhibited the highest ELISA during the screening process, two peptides were synthesized with biotin attached at their N- or C-terminus. These two peptides were used in an ELISA (Fig. 2a), and it was observed that the C-terminus biotinylated TBP121 bound p24, whereas the N-terminus biotinylated TBP121 did not (Fig. 2b). This result suggests

Table 1 List of p24 peptide binders that are sequenced and grouped based on their ELISA signal

High				Moderate				Low			
Name	Isolated frequency	Sequence	Signal threshold*	Name	Isolated frequency	Sequence	Signal threshold	Name	Isolated frequency	Sequence	Signal threshold
TBP121	8	WQQF-FAKY-QSSQ	24–54	–	8	LSL-APRFD	12.9–26	–	6	LIRFHYTT-PAEL	3.5–15
–	1	LRFTY-AEH	36	–	1	WNQ-FLDTTP-SEL	23	–	1	ARWLVNNT	6.63
				TBPC61#	1	LCALPK-SKCL	9.2	–	1	VSRIE-IPYRK	6.2
				TBPC62	1	LCTITN-RYCK	8.6	–	1	ATYL-SKVNHP (+8 other 10-mers)	<5.9
				TBPC63	1	FCHNIF-GRCA	6.94	–	1	SCNLSK-RACK (+6 others cyclized)	<4.5

* Derived from the ELISA signal readout

Cysteine pairs are underlined to indicate cyclization of peptide

that the N-terminus of TBP121 is crucial for binding to p24; based on this observation, all subsequent peptides in this study were synthesized with biotin at the C-terminus. A total of two (TBPC62 and TBPC63) of the three cyclic peptides tested as synthetic peptide exhibited strong binding to p24 (Fig. 2b).

The 12-mer peptide TBP121 was selected for further characterization by several methods, as it was isolated with high frequency while demonstrating the highest ELISA signal value during the screening process, and the synthetic peptide was validated to be binder of p24. First, in SPR measurements, where C-terminus biotinylated TBP121 was anchored onto the surface of a streptavidin-coated chip, its K_D value was determined as 2.64 µM ± 0.55 µM. Second, the minimal length of the TBP121 peptide required to bind p24 was investigated. Two variant peptides were synthesized corresponding to TBP121 that truncate two residues at a time from the C-terminus and were tested for binding to p24 in ELISA. Both the 10-mer and 8-mer sequences of TBP121 bound p24 to a comparable degree as full-length TBP121 based on their calculated EC_{50} value from the ELISA (Fig. 2c), suggesting that the last four amino acids of TBP121 are not essential for binding p24. Third, to identify what residues in the N-terminal region of TBP121 are critical for binding p24, we performed alanine scanning (Cunningham and Wells 1989), in which each position (excluding the preexisting alanine at position 6) was replaced individually by alanine and the binding strength of each peptide was measured relative to the native TB121 (Fig. 3a). It was observed that both glutamine (Q) residues at positions 2 and 3 do not contribute to binding of p24, as substitution to alanine did not abolish the binding affinity of these two variant peptides. Instead, alanine substitution of the tryptophan (W) in position 1 or the pair of phenylalanines (F) at positions 4 or 5 resulted in an 80% reduction of binding capacity relative to the native TBP121. To a lesser extent, alanine substitution of lysine (K) at position 7 and tyrosine (Y) at position 8, showed 60% and 70% reduction in binding affinity, respectively, as compared to the TBP121 peptide. Thus, alanine-scanning experiments indicate that five positions in the 8-mer contribute to binding, with positions 1, 4, and 5 being the most critical.

Next, tryptophan was interrogated as the optimal amino acid at position 1 of TBP121 using phage-display. To facilitate the one-to-one interaction between a phage-displayed peptide and target, variants of TBP121 peptide were tested in a phagemid vector system that yields monovalent displaying virions. The binding of four different constructs displaying the peptide sequence, XQQFFAKYQSSQ, where X = W, F, Y, S was compared in an ELISA assay. As seen in Fig. 3b, peptides with Y or S were unable to bind, while the F substitution resulted in weak binding, suggesting that the optimal aromatic amino acid at position 1 is W. Thus, when optimizing the binding of TBP121 in the future, position 1 can be fixed as W (along with any other residues examined in a similar approach) in a secondary library and subsequently select for higher affinity binders.

To assess the utility of TBP121 as a diagnostic reagent, it was tested in a sandwich ELISA. The sandwich ELISA was assembled in a stepwise manner (Fig. 4a) in the presence of 10% human serum that included an immobilized capture reagent (C-terminus biotinylated TBP121), the target (p24), and the probe, which in this case, are virions displaying anti-p24 nanobody, 59H10 (Gray et al. 2017).

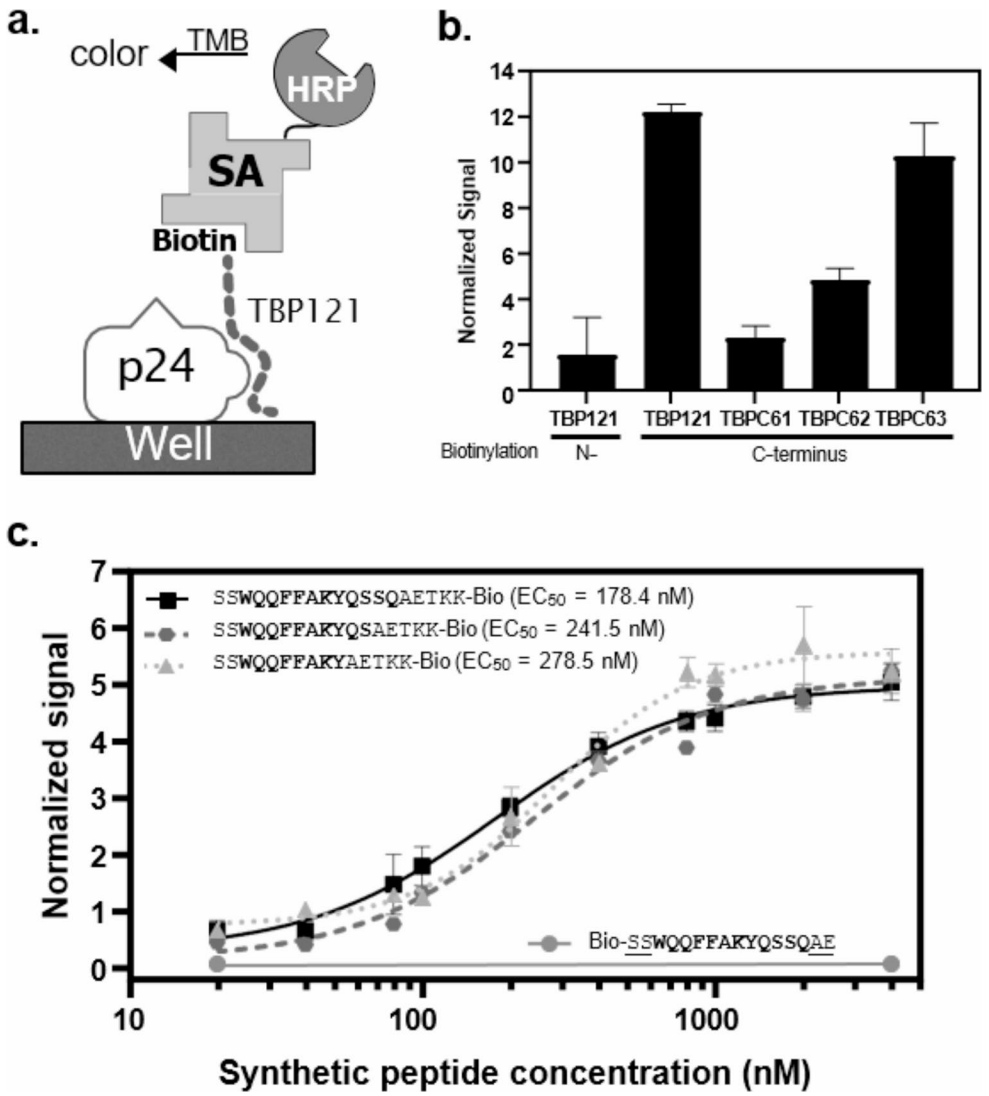

Fig. 2 ELISA setup to validate the binding of the synthetic peptides against p24. (**A**) Schematic illustrating the format of the ELISA and its assembly: wells coated with p24, incubated with biotinylated peptide, and bound peptide detected with streptavidin (SA)-conjugated HRP and TMB. The resulting chemical reaction provides a chromogenic signal that can be read as absorbance at 450 nm wavelength. (**B**) Relative signals of various biotinylated peptides binding to wells coated with p24. The *y*-axis value is calculated based on the chromogenic signal read at 450 nm wavelength for p24 (target) that is normalized against the same signal value for gelatin, the negative control. (**C**) Comparison of the concentration-dependent binding of 8-, 10- and 12-mer versions of the TBP121 peptide. The normalized signal value on *y*-axis refers to the chromogenic signal read at 450 nm wavelength for p24 that is normalized against the corresponding peptide sample's signal value for gelatin, the negative control. The TBP121 peptide sequence in bold are flanked by dipeptide sequences derived from the pIII protein of M13 phage. The addition of T and K residues help improve solubility and the biotin labelling is achieved through the addition of modified lysine. All results are the average of triplicate wells where error bars indicate the standard deviation of the averaged results

Anti-M13-IgG-HRP was then used to detect virions retained in the wells. As shown in Fig. 4b, a high signal was observed only when all the components of the sandwich were present, indicating that the TBP121 peptide and the nanobody can bind different epitopes on p24 simultaneously.

The utility of TBP121 as a diagnostic reagent was further evaluated using a LFA for the detection of p24. Similar to the layout of the ELISA sandwich assay described in the previous section, the successful detection of p24 relies on the assembly of a series of reagents as illustrated in Fig. 5a. Europium-based fluorescent reporters were used to detect captured p24 on the LFA strip (Fig. 5b-c), corroborating the previous observation that the phage-displayed identified TBP121 peptide ligand is a suitable reagent for future LFA development and optimization.

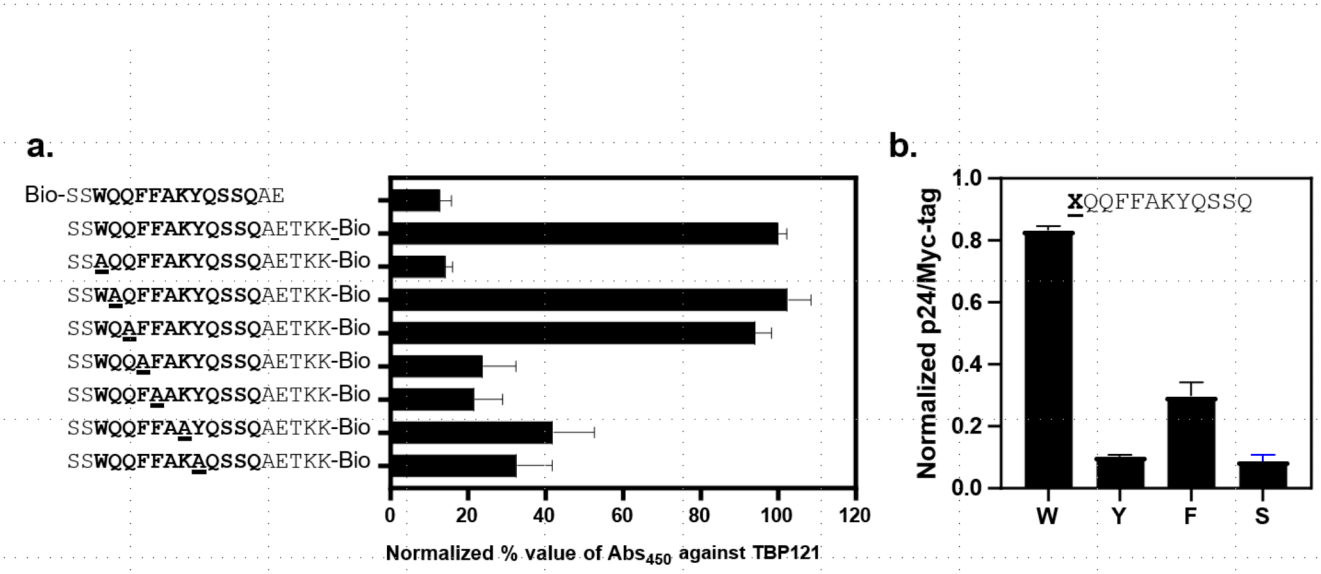

Fig. 3 Detailed characterization of TBP121 peptide ligand. A. Alanine-scanning across the TBP121 peptide sequence. The replacement alanine (**A**) for the corresponding position is underlined, whereas unchanged residues are in black. Biotin is attached at either the N- or C-terminus of the peptide. Binding strength of each peptide was detected with streptavidin (SA)-conjugated HRP and TMB. The resulting chemical reaction provides a chromogenic signal that can be read as absorbance at 450 nm wavelength (Abs_{450}). The binding of each variant peptide has been normalized as a percentage to that of TBP121. (**B**). Binding of virions displaying the TBP121 peptide sequence with W, Y, F, and S at the X position. The ELISA values have been normalized against the display of the c-Myc tag, which is adjacent to the displayed TBP121 peptide and its variants. All results are the average of triplicate wells where error bars indicate the standard deviation of the averaged results

Conclusion

From phage display libraries used in this study, linear and cyclic peptide ligands of p24 were isolated and three out of four of these candidate peptides were validated for binding to p24 as synthetic peptides. Focusing on TBP121, truncation, alanine-scanning and amino acid replacement revealed several positions that were essential for binding, paving the way for further optimization studies that could improve its affinity to p24. Moreover, this peptide was evaluated as a diagnostic reagent through its utility to serve as a capture or probe in a sandwich assay format for ELISA and LFA applications. Future work will focus on the characterization and optimization of other peptide binders of p24, such as TBPC63 as another potential reagent to be used as a pair with TBP121 or even as a tandem affinity reagent (Gorman et al. 2017). In the future, the performance metrics of the LFA prototype will be evaluated, determining the limit of detection of p24 as well as its ability to detect p24 in a blood sample.

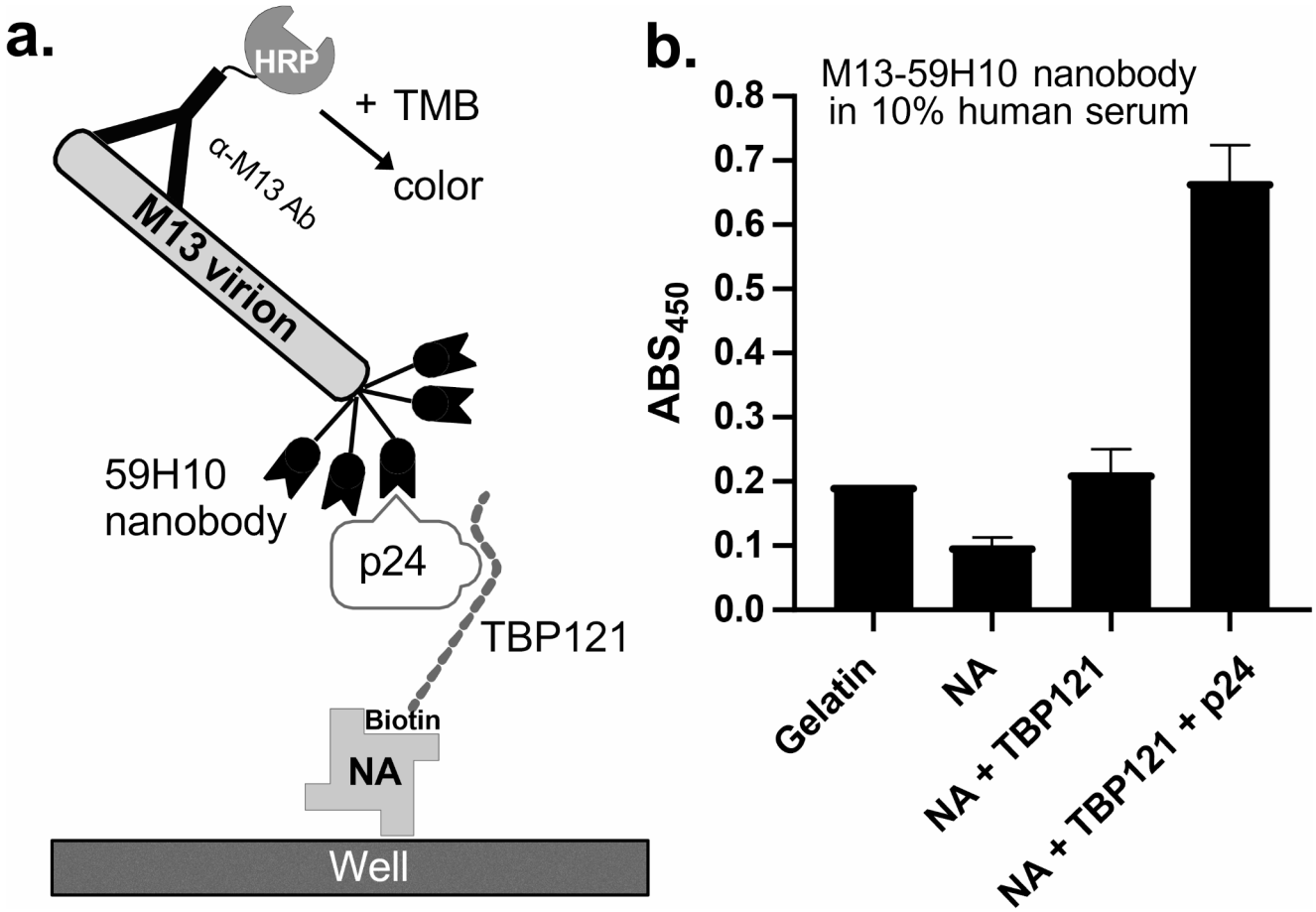

Fig. 4 TBP121 can be used in a sandwich assay. **A**. Schematic illustrating the format of the sandwich ELISA: wells coated with NeutrAvidin (NA) that capture the C-terminal biotinylated TBP121 peptide, incubated sequentially with p24, M13 phage displaying the anti-p24 nanobody (59H10), α-M13 Ab-HRP, and TMB. **B**. Absorbance values of triplicate wells incubated with anti-p24 nanobody and α-M13 Ab-HRP, with or without p24 and biotinylated TBP121 peptide in the presence of 10% human serum. Error bars indicate the standard deviation of the averaged results.

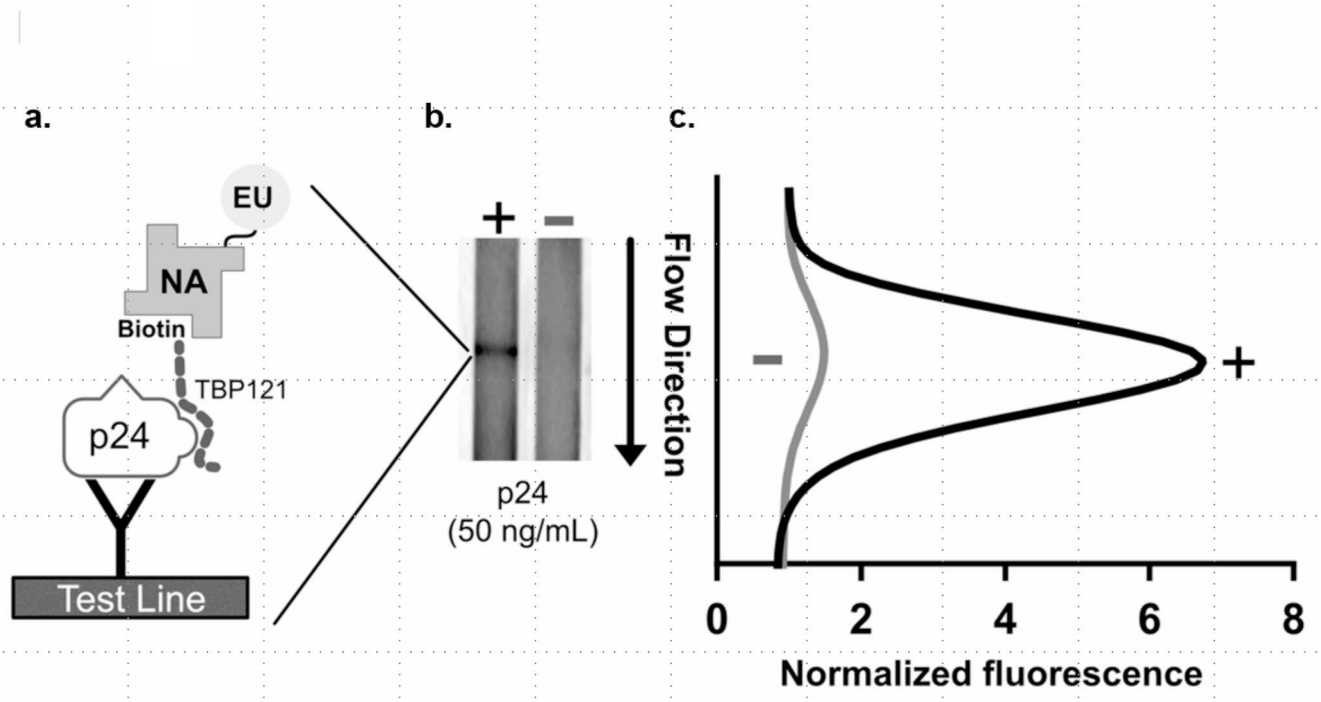

Fig. 5 TBP121 for the detection of p24 in a lateral flow assay (LFA) prototype. **A**. Schematic showing biotinylated anti-p24 TBP peptide bound to p24 and captured by anti-p24 monoclonal antibodies on the LFA test line. The assembled complex is detected using NeutrAvidin (NA)-functionalized europium (EU) chelate particle reporters. **B**. The LFA strips for the detection of p24 protein (+, 50 µg/mL) and no-analyte control (-) were excited and imaged using a FluorChem camera. **C**. The fluorescent intensity observed on the LFA strips test lines was also analyzed using a Lumigenex LTRIC-600 LFA europium reader. The calculated integrated area under the curve (AUC) was normalized to the background signal upstream of the test line.

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Author Contributions J. W, E. O, S. T, M. C and K. K. performed the experiments. J. W., K. K and B. K wrote the main manuscript text. J. W and B. K prepared the figures. R.W and B.K conceptualized the manuscript. All authors reviewed the manuscript.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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