Developing new tools for rapid quantification of viral vectors – a proof of concept

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Background

Lentiviral vectors have become essential tools during the manufacture of gene-modified cell therapies and are likely to become widely used for *in vivo* cell therapy, once non-integrating Lentiviruses become better characterized. Accurate and reliable quantification is a critical step during the development and manufacture of viral vectors, yet accuracy and consistency between operators remains a challenge.

Synthetic affinity reagents are smart polymers, designed and built around modelled epitopes to create a functional binding pocket. Through rational design and chemical manufacture, smart polymers are robust, stable and readily functionalized affinity reagents which exceed the limitations of antibodies.

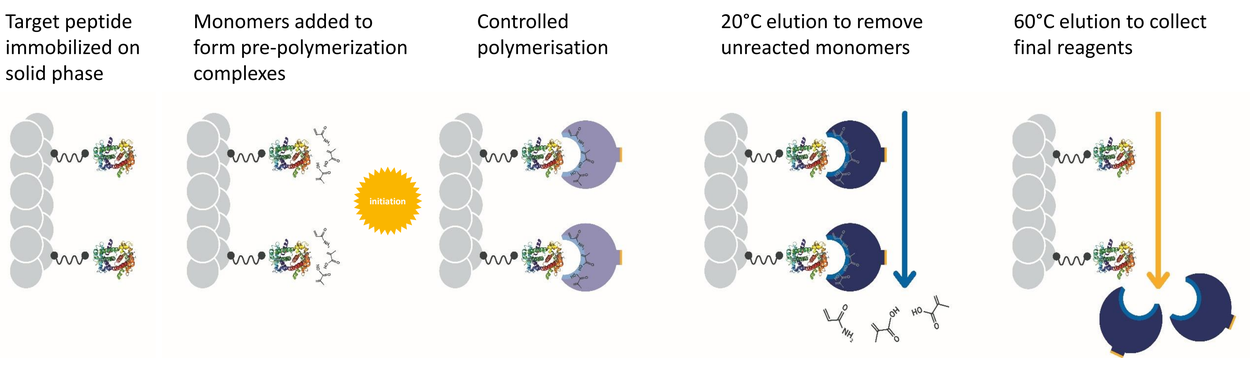
In this poster, we introduce a process to design, develop and manufacture smart polymers, exemplified with gp120 (HIV virus envelope glycoprotein) in collaboration with the Bill and Melinda Gates Foundation. This proof of concept outlines our ongoing work to develop better tools for the quantification, characterization and purification of viral vectors to support cell and gene therapies.

Methodology

Smart polymers targeting HIV gp120 protein were designed using *in silico* molecular modelling. Loops, beta sheets and alpha-helical structures were assessed for solvent accessibility and hydrophobicity. Here, target epitopes were identified, then a library of monomers (the building blocks of smart polymers) was challenged against each selected epitope (synthetized peptide) via HPLC and further modelling. Results provided optimal monomer compositions for polymer manufacture.

During smart polymer manufacture, the optimal monomer combinations were challenged to each immobilized epitope and fused via a controlled free radical polymerization reaction. A series of elution steps remove unreacted monomers and low affinity reagents to yield our smart polymers (Scheme 1). Once released, the highest affinity reagents were identified through flow induced dispersion analysis (FIDA) and surface plasmon resonance (SPR) screening.

Smart polymers are selected or further tuned for specific applications, such as ELISA, BLI, lateral flow and magnetic/Sepharose resins for purification.



Scheme 1. Visual representation of the synthetic affinity reagent polymerization process.

Results

Four surface epitopes were identified during *in silico* epitope discovery, (Figure 1). Sixteen monomer compositions were utilized to create affinity reagents, four compositions per epitope (Figure 2). Screening using FIDA identified six lead candidates (Figure 3). Four were assessed using SPR, and the top candidate demonstrated an affinity of 0.11 nM (Figure 4). Finally, the leading reagents were evaluated using a lateral flow concept set-up and an electro sensor device, screening against recombinant HIV gp120 protein (Figure 5).

Conclusions

We exemplify a novel process to design, develop and manufacture smart polymers. Using *in silico* modelling and imprinted polymer technology, we 'design recognition' and can develop highly specific synthetic affinity reagents with unique and tunable properties, for use in various analytical and bioprocessing applications.

With this process, Tozaro are developing new tools for viral vector detection, characterization and separation.

NEW!

We are using our rapid and rational design process to develop new affinity reagents in the cell and gene therapy space. Arrange a call to learn more.

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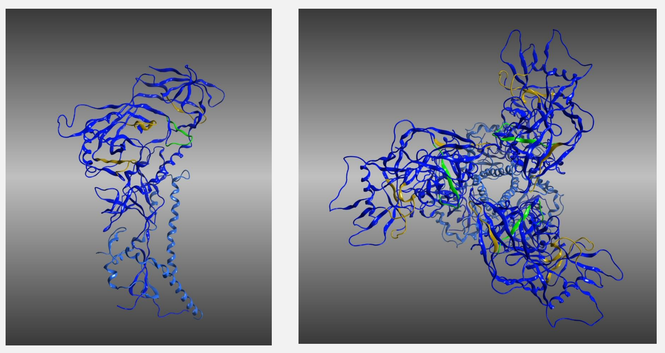
in silico epitope selection 1



Identified epitopes highlighted in yellow, and the eventual leading epitope highlighted in green.

Figure 1. Molecular modelling of the HIV gp120 protein (monomer & trimer) with identified epitopes for reagent development highlighted.

Monomer selection 2

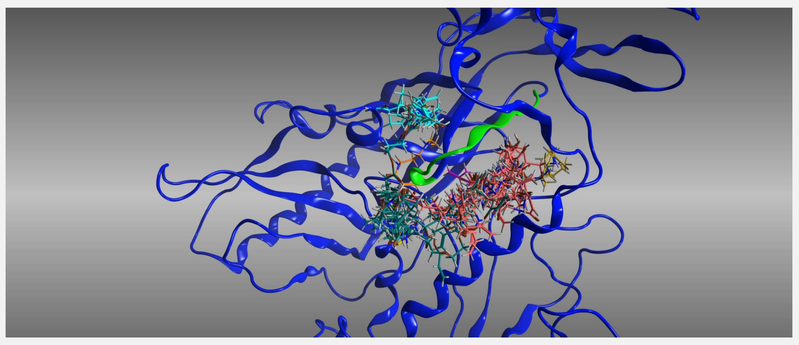


Figure 2. Monomer screening against the leading target epitope, shown here in green.

FIDA Results 3

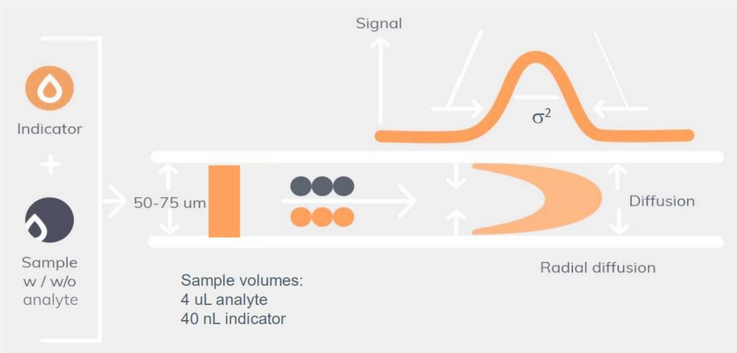
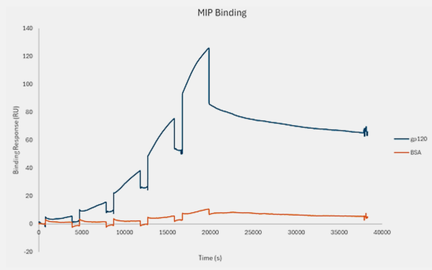


Figure 3. FIDA technology concept. Indicator showed an increase in hydrodynamic radius from 0.93nm to 1.32nm upon binding of the reagent.

SPR Results 4



LIGAND	K_D (nM)	K_{ON}	K_{OFF}
Polymer	0.11	$2.7x10^6$	$3.1x10^{-4}$
PAb	0.19	$1.4x10^6$	$2.6x10^{-4}$
MAb	0.027	$3.8x10^5$	$1.04x10^{-5}$

Figure 4. SPR analysis using a Biarcore T200, demonstrating an affinity constant (K_D) of 0.11 nM.

Dipstick Results 5

Control 1
Control 2
Affinity reagent Composition 1
Affinity reagent Composition 2
Affinity reagent Composition 3
Affinity reagent Composition 4

Figure 5. Dipstick screening test with synthetic affinity reagent immobilized on membranes and tested against gp120-gold conjugate.

To discuss collaboration opportunities, please contact our team at enquiries@tozaro.com