DARPins as Alternative to HIV-1 broadly Neutralizing Antibodies

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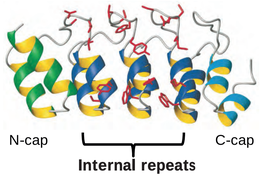
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Background

Development of drugs that share the properties of broadly HIV-1 neutralizing antibodies (bnAbs) is highly desirable. Here we utilize the **Designed Ankyrin Repeat Protein (DARPin)** technology to generate HIV-1 inhibitors with broad neutralization activity directed against the **V3 loop and the MPER domain**. DARPins are small synthetic binding proteins (~20 kDa) with high target affinities and specificities that recognize their target in a structure dependent manner and are selected *in vitro* from DARPin libraries by ribosome display (*Plückthun A.*, Annu. Rev. Pharmacol. Toxicol., 2015). In a recent proof of principle study we showed that DARPins have interesting properties as anti-HIV inhibitors (*Mann et al.*, J. Virol., 2013). As the initial developed gp120 DARPin inhibitors were subtype specific we sought here to evolve novel broadly neutralizing DARPin molecules recognizing the V3 loop and the MPER domain. Using structure arrested mimetics of the V3 loop and the MPER domain as targets in combination with a **2nd generation DARPin library** with higher diversity and stabilized DARPin structures we successfully selected V3 and MPER specific DARPins that compare in **breadth to the best bnAbs** targeting these epitopes.

DARPins and their selection



Structural overview of an N3C DARPin
(*Binz et al.*, Nature Biot., 2004)

DARPin features

- DARPins exhibit high target specificity and affinity and can be used as alternative to antibodies
- Monovalent binding
- small (~20 kDa) compared to antibodies (~160 kDa)
- full *in vitro* selection process
- Low-cost high yield bacterial production

Selection of HIV-1 Specific DARPins

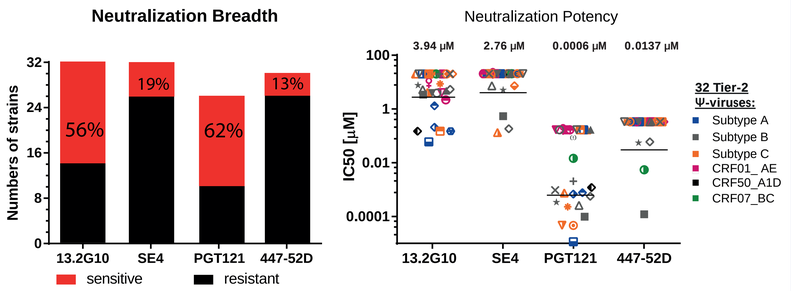
- **Selection** of DARPins by ribosome display from high diversity DARPin libraries (1st and 2nd generation) against HIV specific targets
- **Screening:** ELISA reactivity against panning target, neutralization activity against a panel of 3-5 HIV-1 pseudoviruses in a TZM-bl neutralization assay

Successful selection of broadly neutralizing V3 specific DARPin

DARPin Library	Panning strategy	Numbers of neutralizing/binding DARPins (n=190)	Selected, Representative DARPins
1st generation	V3 IY MN mimetic alternating with CD4 complexed JR-FLgp120	7 / 62	SE4
2nd generation		21 / 175	13.2G10

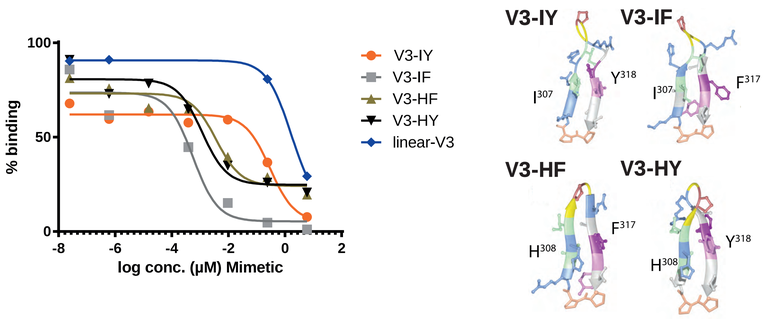
Overview of applied panning strategies to obtain V3 specific DARPins

V3 DARPin 13.2G10 reaches breadth of bnAb PGT121



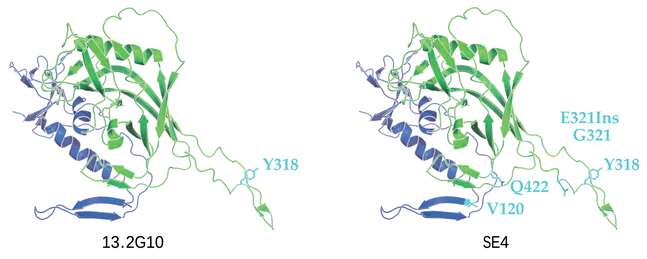
Neutralization breadth and potency analysis. Neutralization activity against 32 env pseudotyped viruses was determined in the TZM-bl assays. Breadth: the number of sensitive (red) and resistant strains (black) are indicated and % breadth depicted. Potency: Median IC_{50} (µM) (black line, and numbers above) was determined for viruses neutralized at IC_{50} <20 µM for DARPins, <0.166 µM for PGT and <0.333 µM for 447-52D and is depicted. Both V3 specific DARPins had a significantly higher median IC_{50} than the probed V3 mAbs.

V3 DARPin 13.2G10 has preference for specific V3 structures



Competition ELISA. DARPin 13.2G10 binding to V1V2 deleted BG505 gp120 in presence of increasing doses of structural V3 mimetics (IY, IF, HF, HY; *Riedel et al.*, CHEMBIOCHEM, 2011) and linear V3 peptide.

Presence of Tyrosine 318 in V3 is critical for 13.2G10 activity



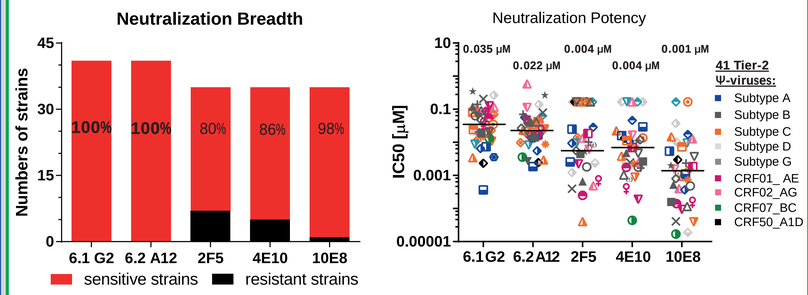
Alanine scanning using JR-CSF Env alanine point mutants (*Pantophlet et al.*, J. Virol., 2003) and readout by TZM-bl based neutralization assay. Resistance conferring mutations are indicated in blue on the structure of the JR-FLgp120core+V3 in complex with sCD4 and FabX5 (*Huang et al.*, Science, 2005).

Successful selection of broadly neutralizing MPER specific DARPins

DARPin Library	Panning strategy	Number of neutralizing/binding DARPins (n=190)	Selected, Representative DARPins
2nd generation	MPER mimetic as single target	17 / 144	6.1G2 6.2A12

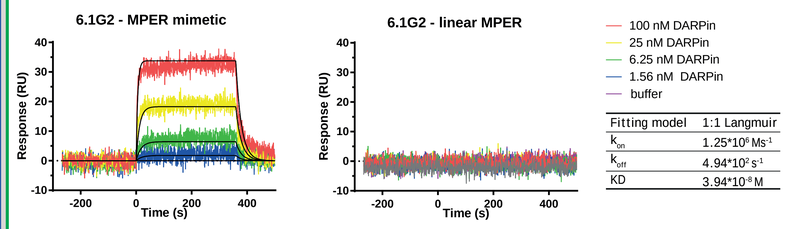
Overview of applied panning strategies to obtain MPER specific DARPins

MPER DARPins show neutralization breadth comparable to bnAb 2F5 and 4E10



Neutralization activity was determined against 35-41 env pseudotyped viruses in the TZM-bl assays. Breadth: the number of sensitive (red) and resistant strains (black) are indicated and % breath depicted. Potency: Median IC_{50} (µM) (listed on top and line) was determined for viruses neutralized at IC_{50} <10 µM for DARPins and >0.166 µM for MPER bnAb. Median IC_{50} of the monovalent MPER DARPin was significantly higher than the IC_{50} of MPER bnAbs but within a 4-8 fold range of bnAbs 2F5 and 4E10.

MPER specific DARPins bind in the nanomolar range and show structure dependent binding



Fitting model	1:1 Langmuir
k_{on}	$1.25*10^6$ Ms^{-1}
k_{off}	$4.94*10^2$ s^{-1}
KD	$3.94*10^{-8}$ M

SPR sensograms of DARPin 6.1G2 and binding affinities against MPER mimetic and linear MPER peptides

Summary

- V3 specific **DARPin 13.2G10** preferentially binds distinct V3 conformations and shows **PGT121 like neutralization breadth**
- MPER specific **DARPins** 6.1G2 and 6.2A12 have **MPER bnAb** like neutralization **breadth** reach **potency close to MPER bnAbs** despite monovalent binding
- MPER DARPins 6.1G2 and 6.2A12 show a structure dependent binding mechanism and fail to recognize linear MPER peptide
- Resolution of the structures of MPER and V3 mimetics with the broadly neutralizing DARPins is ongoing to derive further information on the preferred structures recognized to obtain guidance for V3 and MPER immunogen design

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