Assessing Immunogenicity Barriers Of The HIV-1 Envelope Trimer

308

Nikolas Friedrich[1*], Liridona Maliqi[1*], Matthias Glögl[1], Peter Rusert[1], Maryam Zaheri[1], Chloé Pasin[1], Stefan Schmutz[1], Irene A. Abela[1], Roger D. Kouyos[1,2], Huldrych F. Günthard[1,2], Marit J. van Gils[3], Rogier W. Sanders[3], Ralf Wagner[4], Andreas Plückthun[5], Alexandra Trkola[1]

[1] Institute of Medical Virology, University of Zurich (UZH), Zurich, Switzerland, [2] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich (USZ), Zurich, Switzerland, [3] Department of Medical Microbiology and Infection Prevention, University of Amsterdam, Amsterdam Institute for Infection and Immunity, Amsterdam, the Netherlands, [4] Institute of Clinical Microbiology and Hygiene, University Hospital, Regensburg, Germany, [5] Department of Biochemistry, University of Zurich (UZH), Zurich, Switzerland. * These authors contributed equally.

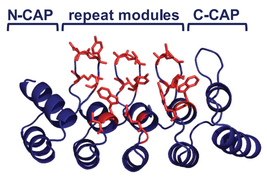
We developed DANA, an in vitro screening tool, to probe antigenicity of Env immunogens. DANA mimics in vivo antibody selection processes and provides clues for the selection of immunogens for prime-boost regimen.

- We utilized the Designed Ankyrin Repeat protein (DARPin) technology to devise **DARPin An**tigenicity **A**nalysis (DANA)
- DANA is a purely in-vitro screening tool that provides information of antigenic properties of Env immunogens
- DANA recapitulates antigenicity features of candidate Env immunogens defined in vivo
- As observed for bnAbs in vivo, trimer immunogens select for highly mutated DARPins in DANA
- DANA indicates that a balance between open and closed Env features is needed to evoke neutralization activity

Background: DARPin characteristics

- In silico designed small binding proteins (14-20 kDa) (Binz et al., J. Mol. Biol., 2003; Binz et al., Nat. Biotechnol., 2004; Plückthun, Annu. Rev. Pharmacol. Toxicol., 2015)
- Target-specific DARPins can be selected from a DARPin library (10^{12}) by ribosome display
- DARPins have a conformation dependent binding mode

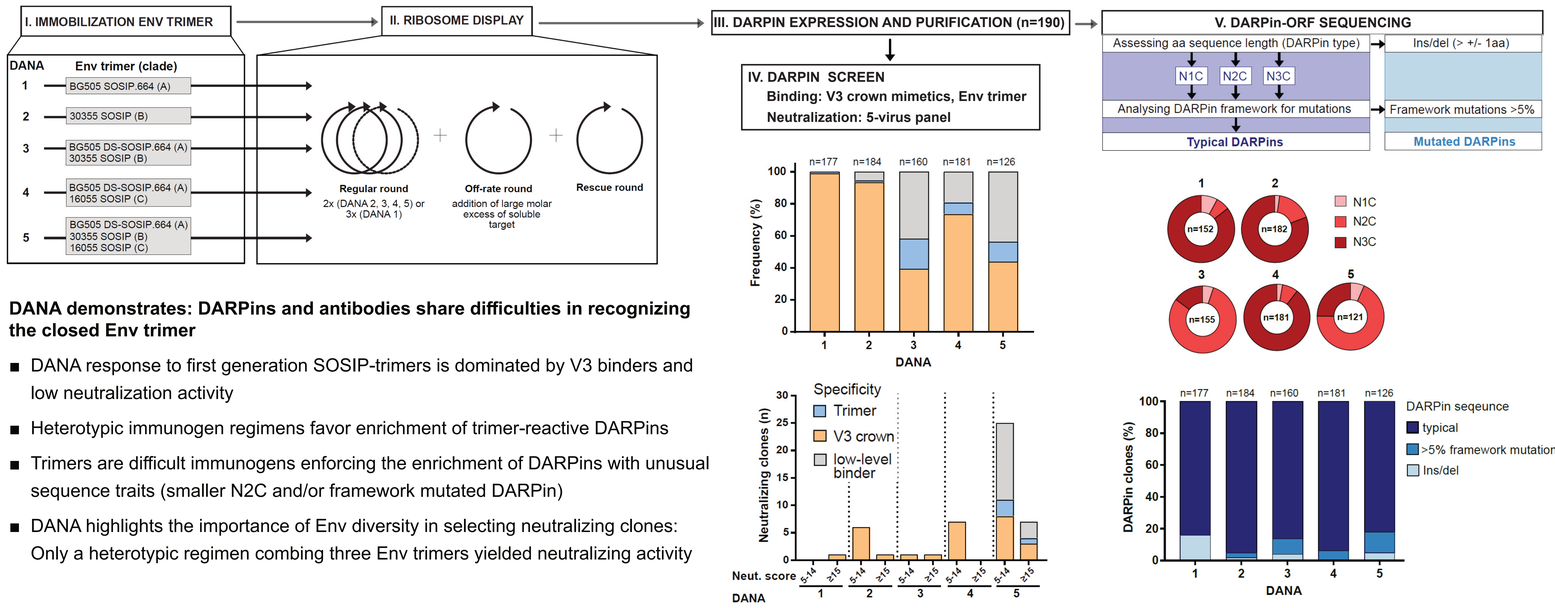
→ **DARPin system is an attractive tool for defining distinct epitope configurations**



Randomized surface-exposed residues
→ Target interaction
(diversity DARPin library:10^{12})

- We recently developed **broadly neutralizing DARPins (bnDs)** targeting the V3 loop and defining several neutralization relevant V3 conformations (Friedrich et al., Nat. Commun., 2021; Glögl et al., in revision).

Screening candidate Env trimer immunogens by DANA



DANA demonstrates: DARPins and antibodies share difficulties in recognizing the closed Env trimer

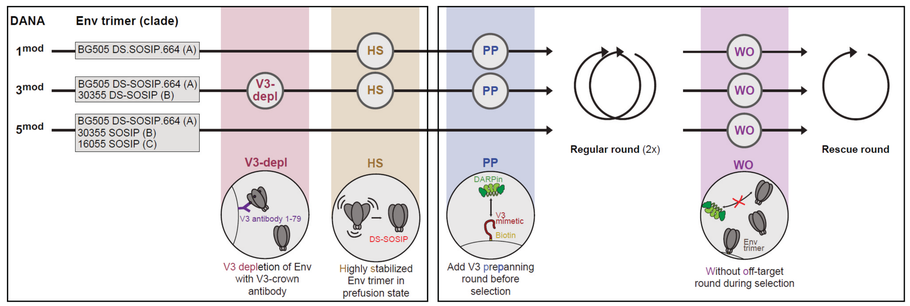
- DANA response to first generation SOSIP-trimers is dominated by V3 binders and low neutralization activity
- Heterotypic immunogen regimens favor enrichment of trimer-reactive DARPins
- Trimers are difficult immunogens enforcing the enrichment of DARPins with unusual sequence traits (smaller N2C and/or framework mutated DARPin)
- DANA highlights the importance of Env diversity in selecting neutralizing clones: Only a heterotypic regimen combing three Env trimers yielded neutralizing activity

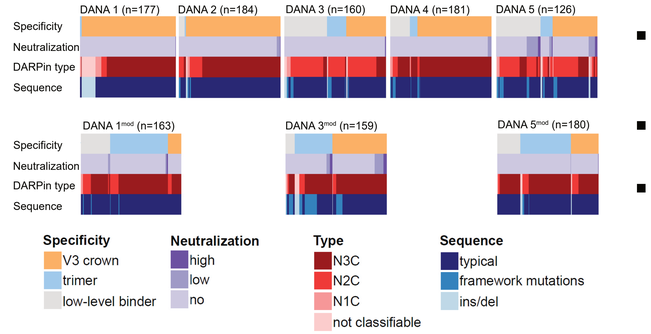
Analysis of B cell receptors (BCR) in bnAb inducers

- **Trimer-specific BCR have higher somatic hypermutation (SHM) rates than V3-specific BCR in the same individual**
- 10 x genomics BCR sequencing of B cells labelled with V3-loop peptides (JR-FL, BG505) or Env trimer probes (ConMSOSIP.v7, AMC011SOSIP.v4, Du422SOSIP.v4) in libra seq.
- PBMC derived memory B cells from HIV-1 bnAb inducers (n=19 individuals, 22 PBMC samples) identified in the Swiss 4.5K screen (Rusert et al., Nat. Med., 2016)



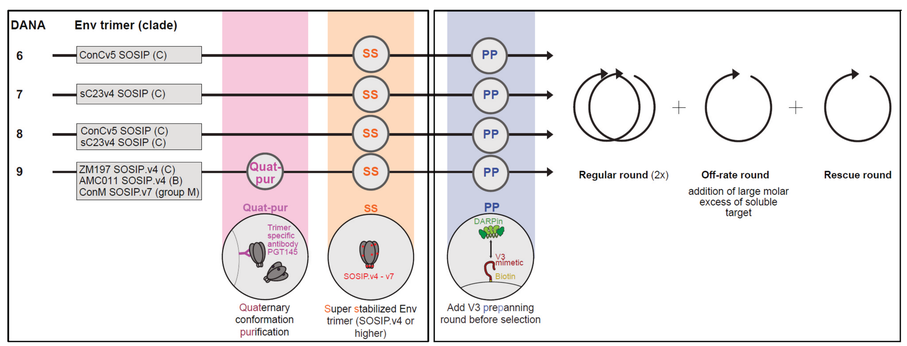
Reducing V3 dominance promotes the selection of trimer-specific DARPins but achieving neutralization activity remains difficult

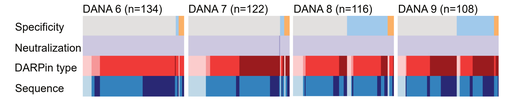




- DANA 5 versus 5^{mod} : Most trimer specific clones are of low affinity. Affinity maturation is required to achieve neutralizing activity.
- DANA 1 versus 1^{mod}: Selection of trimer-specific DARPins with neutralizing activity
- DANA 3 versus DANA 3^{mod} : Strong increase in both trimer- and V3-specific neutralization capacities. High yield of typical N3C DARPins indicates good accessibility.

Strongly stabilized trimers do not select for neutralizing activity





- New generation of strongly stabilized trimers shields V3 well
- Heterotypic regimens favor selection of trimer-specific DARPins
- DANA enriches for mutated clones which do not neutralize

→ **Improved accessibility of target epitope(s) is needed to initiate the generation of high affinity binders with neutralizing activity**

Contact

friedrich.nikolas@virology.uzh.ch



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