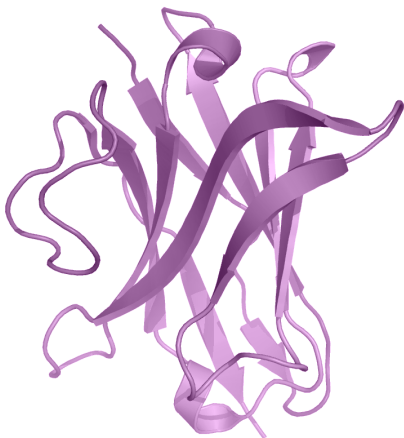


# Single Domain Antibody Libraries

Explore VHH sequence space with three new single domain antibody libraries

## BENEFITS OF SYNTHETIC SINGLE DOMAIN ANTIBODY LIBRARIES

- Small & modular antibodies
- Stable & robust
- Full antigen binding capacity
- Easier to engineer & manufacture
- Access to epitopes usually sterically hindered by an IgG
- Create building blocks for bispecific antibodies
- Faster than traditional approaches – no immunization required



## Three Single Domain Antibody Libraries

### VHH Ratio

Specific oligo pools model the natural VHH repertoire

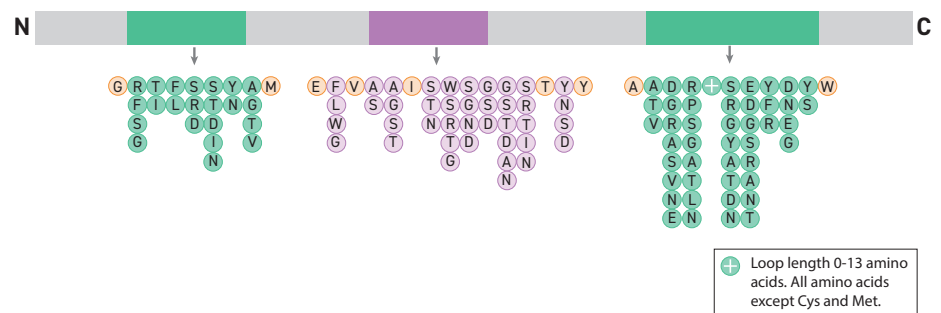
### VHH Shuffle

Natural llama CDR sequences in the context of a llama consensus framework

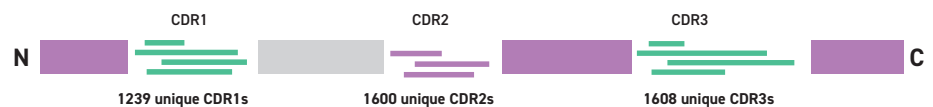
### VHH hShuffle

Natural llama CDR sequences in the context of a partially humanized VHH framework

### VHH Ratio



- 2391 CDR sequences analyzed for position-specific variation
- Controlled CDR diversity introduced in the library
- Consensus llama framework



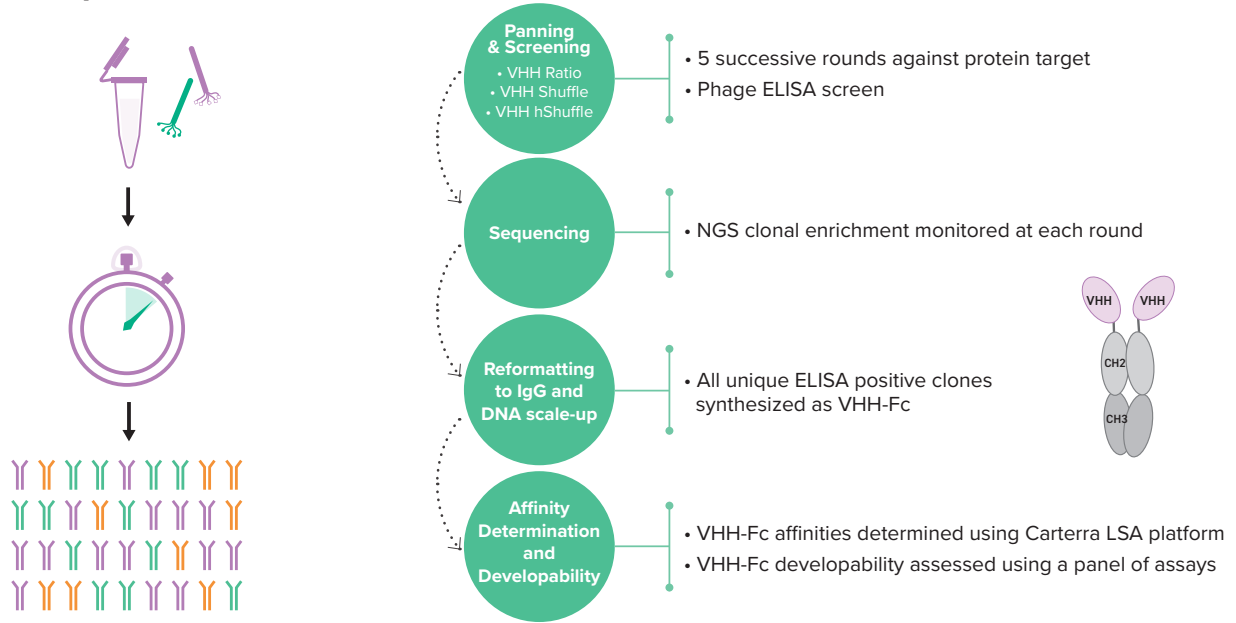
### VHH Shuffle

- Each unique CDR individually synthesized
- Shuffled in consensus llama framework
- Final diversity of library > theoretical diversity
- Theoretical library diversity of  $3.2 \times 10^9$

### VHH hShuffle

- Shuffled CDRs with theoretical library diversity of  $3.2 \times 10^9$
- Partially humanized framework: Framework 1, 3 and 4 were humanized using the human germline DP-47 framework.

**Proof of Concept Data**

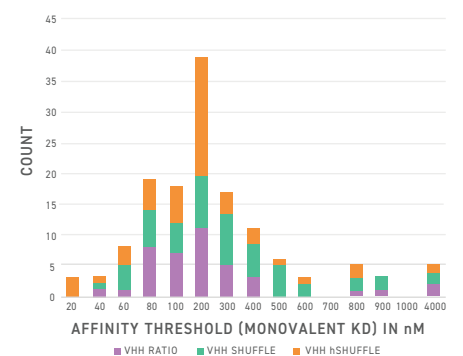


**ELISA+ colony count: 1 × 384 well plate picked per library per round**

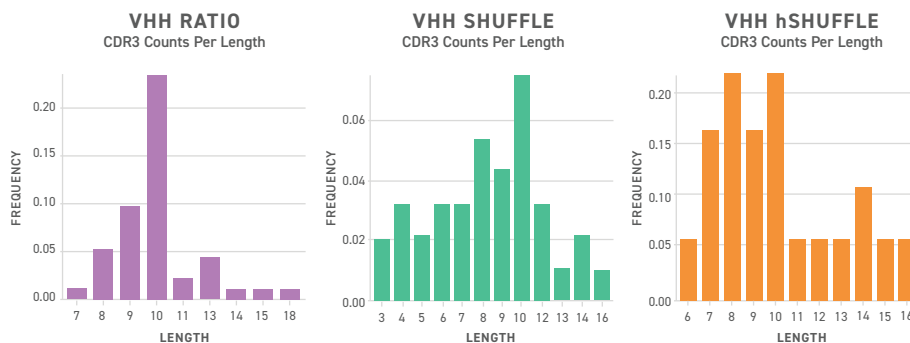
Library	Round 3	Round 4	Round 5	Uniques
VHH Ratio	58	85	188	47
VHH Shuffle	128	211	287	58
VHH hShuffle	82	222	255	56

**Array SPR Binding Analysis**

TIGIT AFFINITY DISTRIBUTION - VHH LIBRARIES



**CDRH3 length distributions**



Out of 140 VHH binders  
 • 51 variants < 100 nM  
 • 90 variants < 200 nM

**Anti-TIGIT clones from VHH libraries encompass a range of affinities and diversity**

**MORE INFORMATION**

Contact Twist Biopharma  
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 for more information.