# 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

<table>
<thead>
<tr>
<th>VHH Ratio</th>
<th>VHH Shuffle</th>
<th>VHH hShuffle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific oligo pools model the natural VHH repertoire</td>
<td>Natural llama CDR sequences in the context of a llama consensus framework</td>
<td>Natural llama CDR sequences in the context of a partially humanized VHH framework</td>
</tr>
</tbody>
</table>

### VHH Ratio

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

### Three Single Domain Antibody Libraries

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<td>1239 unique CDR1s</td>
<td>1600 unique CDR2s</td>
<td>1608 unique CDR3s</td>
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### 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

- **5 successive rounds against protein target**
- **Phage ELISA screen**
- **NGS clonal enrichment monitored at each round**
- **All unique ELISA positive clones synthesized as VHH-Fc**
- **VHH-Fc affinities determined using Carterra LSA platform**
- **VHH-Fc developability assessed using a panel of assays**

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

<table>
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<tr>
<th>Library</th>
<th>Round 3</th>
<th>Round 4</th>
<th>Round 5</th>
<th>Uniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHH Ratio</td>
<td>58</td>
<td>85</td>
<td>188</td>
<td>47</td>
</tr>
<tr>
<td>VHH Shuffle</td>
<td>128</td>
<td>211</td>
<td>287</td>
<td>58</td>
</tr>
<tr>
<td>VHH hShuffle</td>
<td>82</td>
<td>222</td>
<td>255</td>
<td>56</td>
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**CDRH3 length distributions**

**Array SPR Binding Analysis**

- 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

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