Table 1: framework sequence variability (compared to consensus sequence) across stable and unstable nanobodies

**Unstable NBs**

extra disulfide

|  |  |  |  |
| --- | --- | --- | --- |
|  | IMGT # 11111-11111111111 |  |  |
|  | 11111111222222222-34444444444555555-666677777777778888888888999999999900000-11222222222 |  |  |
|  | 12345678901234567890123456-90123456789012345-678901234567890123456789012345678901234-89012345678 |  |  |
|  | **MAQVQLQESGG-GLVQAGGSLRLSCAAS-MGWFRQAPGKEREFVAA-TYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYC-WGQGTQVTVSS** | animal | CDR3 length |
| **3ZKQ** | - P - T V G W ST- T R L -R P | llama | 5 |
| **3K1K** | V -A P - R Y W G-SS E D R - | dromedary | 8 |
| **3K7U** | - T - -LF N R T - | llama | 10 |
| **3CFI** | - P - S V GL W SG- S TAP IL R -R | llama | 11 |
| **4LGP** | V T - P T G -I H WLVC- V V A L D GI - | alpaca | 11 |
| **4MQS** | - D -I Q G SC- I A S E V - | llama | 11 |
| **1ZV5** | D V - S E -I D G VF- Q S - | dromedary | 14 |
| **2P42** | V - - G - L KG D T - | dromedary | 14 |
| **3V0A** | V - P - S V EGF W SS-AWDG A T D L SN Q G - | llama | 14 |
| **4QKX** | - - Y Q L - N N A - | llama | 14 |
| **4WEU** | - - K Y - IA L D - | llama | 14 |
| **4X7C** | D V - P - Y Q L S- N G -R R | alpaca | 14 |
| **1KXV** | V - T P - S Y R G D SG- T V A QG A D D M - | dromedary | 15 |
| **4LHJ** | V - - Y L T-SN G S - | alpaca | 15 |
| **4OCL** | - P VD - A T- R - R | llama | 15 |
| **4WEM** | - E - Q GY-LN G F SN S G F - K | llama | 15 |
| **4WEN** | - P T - Y SK H- EF T D - K | llama | 15 |
| **5IV0** | V - P T T -I G SC- FMN D I - | alpaca | 15 |
| **1KXQ** | V - S S -V G - S L Q N GI - | dromedary | 16 |
| **4HEM** | V - - P T- RN NM - | llama | 16 |
| **4LAJ** | V - P -I G SC- -Y K | llama | 16 |
| **4C58** | - S G - A G C-S S R Q T AF L S I -A | llama | 17 |
| **4KML** | - P - G S-SD T M N T - | llama | 17 |
| **4KRM** | K E - S T T - SG- G D I - | llama | 17 |
| **2X6M** | G V - S - R G R- A D E I - | dromedary | 18 |
| **4W6X** | - S T - G C-S Q D F R I - | llama | 18 |
| **3K74** | - P - Y V R GL W SM- K E L TS -K | llama | 19 |
| **4GRWf** | E V - P -I G SC- ES - | llama | 19 |
| **4W6W** | - S - G C-VN Q S K L E L S - | llama | 19 |
| **4I13** | - A -I - V GE E I MN V R N - R | llama | 20 |
| **4S10** | - - S- R A K S DN N D - | llama | 20 |
| **1G6V** | V - S - G T- G Q I -R | dromedary | 21 |
| **1KXT** | VA - S - Y C LS R-AN A A D - | dromedary | 21 |
| **1RJC** | E A - S Q T - G V- A Q L L L M - | dromedary | 21 |
| **3JBC** | - S T - G G-A Q K I - | dromedary | 21 |
| **4I0C** | - S E - A G -P Q RM E M - | dromedary | 21 |
| **4LGS** | V - S - A S- L AL N - | alpaca | 22 |
| **4QGY** | V - -I G SC- P A S K M - K | llama | 22 |
| **4W6Y** | - S - A V G S- S T - | llama | 22 |
| **4HEP** | D V - P E -I G SY- V T S L - K L | llama | 24 |
| **3G9A** | D - S - A C L SN- T G D VN S R - K | dromedary | 25 |
| **1JTP** | D A - S - G - Q L E I - | dromedary | 26 |
|  |  |  |  |
|  | IMGT # 11111-11111111111 | **Stable NBs** |  |
|  | 11111111222222222-34444444444555555-666677777777778888888888999999999900000-11222222222 |  |  |
|  | 12345678901234567890123456-90123456789012345-678901234567890123456789012345678901234-89012345678 |  |  |
|  | **MAQVQLQESGG-GLVQAGGSLRLSCAAS-MGWFRQAPGKEREFVAA-TYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYC-WGQGTQVTVSS** | animal | CDR3 length |
| **2XT1** | V - - A Y A LI - V D T DD IL D M - | alpaca | 6 |
| **4X7F** | D V - P - A Y G EQ L V- D M L SN R - | alpaca | 7 |
| **4ORZ** | - - Y Q L F- D P V - S | llama | 10 |
| **4EIG** | - K - T Y L L- MTV VQ E N - | llama | 11 |
| **4CDG** | - - A Y T Q RI I- N V D M I - R | llama | 13 |
| **4LGR** | V - P H -TC Y GT Q L - ID - | alpaca | 13 |
| **4M3K** | - P - Y D G L L- T G E S - | llama | 14 |
| **3P0G** | - - Y Q L - N N A - | llama | 15 |
| **4C57** | - P S - S V RV GL W G-AH R ML S SD GL -SS | llama | 15 |
| **4WGV** | - -AN Y P MQ L T-AN R R G - | llama | 15 |
| **2BSE** | - T - LA P L V - V SG I - | llama | 16 |
| **4GRWh** | E V - -V -P D R L - | llama | 16 |
| **4IOS** | V - D V -I - GR M CAA A L - | llama | 16 |
| **4NBX** | V - A - A P - - | llama | 16 |
| **4NC2** | V - - -PN S Q - | llama | 16 |
| **4P2C** | - V - Y Q S- N R - | llama | 16 |
| **4QO1** | - - R L Q- E D T E N NAD GI F - | llama | 16 |
| **1OP9** | - S S -S G - Q M - E | dromedary | 17 |
| **3EBA** | V - S S -S GL W - Q M - | dromedary | 17 |
| **4AQ1** | - - G- GA M G A - | llama | 17 |
| **4GFT** | - T P K S A- Q G-Q M A V A - | llama | 17 |
| **4NBZ** | K E - -VA A V- V N R F - | llama | 17 |
| **1ZVH** | D V - S -L G -P V L E L - | dromedary | 18 |
| **4GRWe** | E V - P -IA G SG-A S R - K L | llama | 18 |
| **1ZVY** | D V - S - T A G - T Q K MA R D V S I T - | dromedary | 20 |
| **3RJQ** | - T - - D A K A - | llama | 20 |
| **4DK3** | - - - T E - | llama | 20 |
| **4N1H** | - A K - - K A D W R - | llama | 20 |
| **4TVS** | V - -V L T- H - | alpaca | 20 |
| **4EIZ** | - T - T - L M - | llama | 21 |
| **4FHB** | - E - D V- A I - | llama | 22 |
| **4LHQ** | V T - T T S - - R V HL L A - | alpaca | 22 |
| **4KRO** | - P - Q - T T - | llama | 23 |

\*all nanobodies contain a conserved set of cysteines that normally forms a disulfide bond through the hydrophobic core of the nanobody (23Cys and 104Cys). Highlighted in yellow, “extra disulfide bond” refers to nanobodies with an additional pair of cysteines, one of which is always located in CDR3, that normally forms a disulfide bond that impacts CDR3 conformation.