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.