

A**Nanobody Constructs****Construct** **Description**

MT991 Nb 1-1

MT992 Nb 2-2

MT993 Nb 5-5

MT994 Nb 5-1

MT995 Nb 5-2

MT996 Nb 5-5-5

MT997 Nb 5-5 long "LL"

MT1032 Nb 5-5-cys (short)

MT1033 5-5-cys (long1)

MT1034 2-2-cys (long2)

MT1035 5-5-cys(long2)

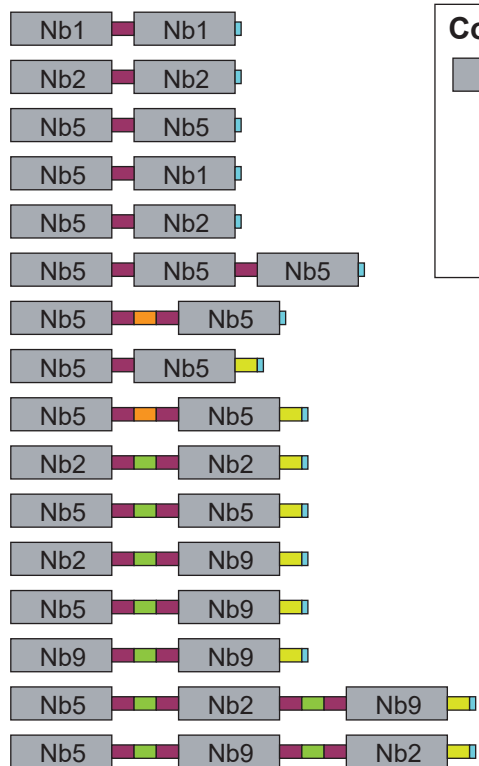
MT1036 2-9-cys(long2)

MT1037 5-9-cys(long2)

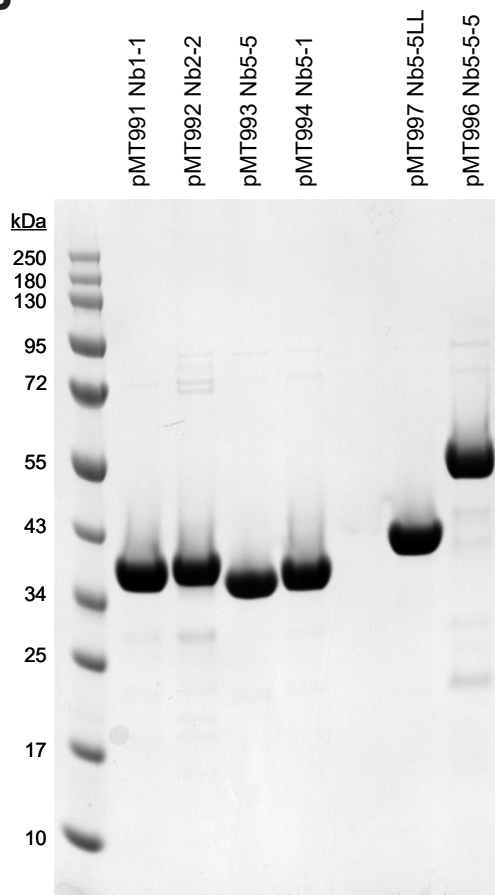
MT1038 9-9-cys(long2)

MT1039 5-2-9-cys

MT1040 5-9-2-cys

**Components**

- Nb X Camelid Nanobody
- (GGGGS)₄ linker
- His6 Tag
- (EAAAK)₃ linker
- (DAAAR)₃ linker
- 5xCys Tag

B

Supplementary Figure 11. Engineered Nanobody constructs. (A) Schematic of design of engineered dimeric and trimeric nanobody constructs, with flexible (GGGGS × 4) and rigid helical (EAAAK × 3 or DAAAR × 3) linker. 5xCys tag sequence is CGSGRCGSGRCGSGRCGSGRC. (B), Representative preparation of engineered nanobody constructs, Coomassie stain.