
Supplementary information

Hierarchical design of pseudosymmetric protein nanocages

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Hierarchical design of pseudosymmetric protein nanocages

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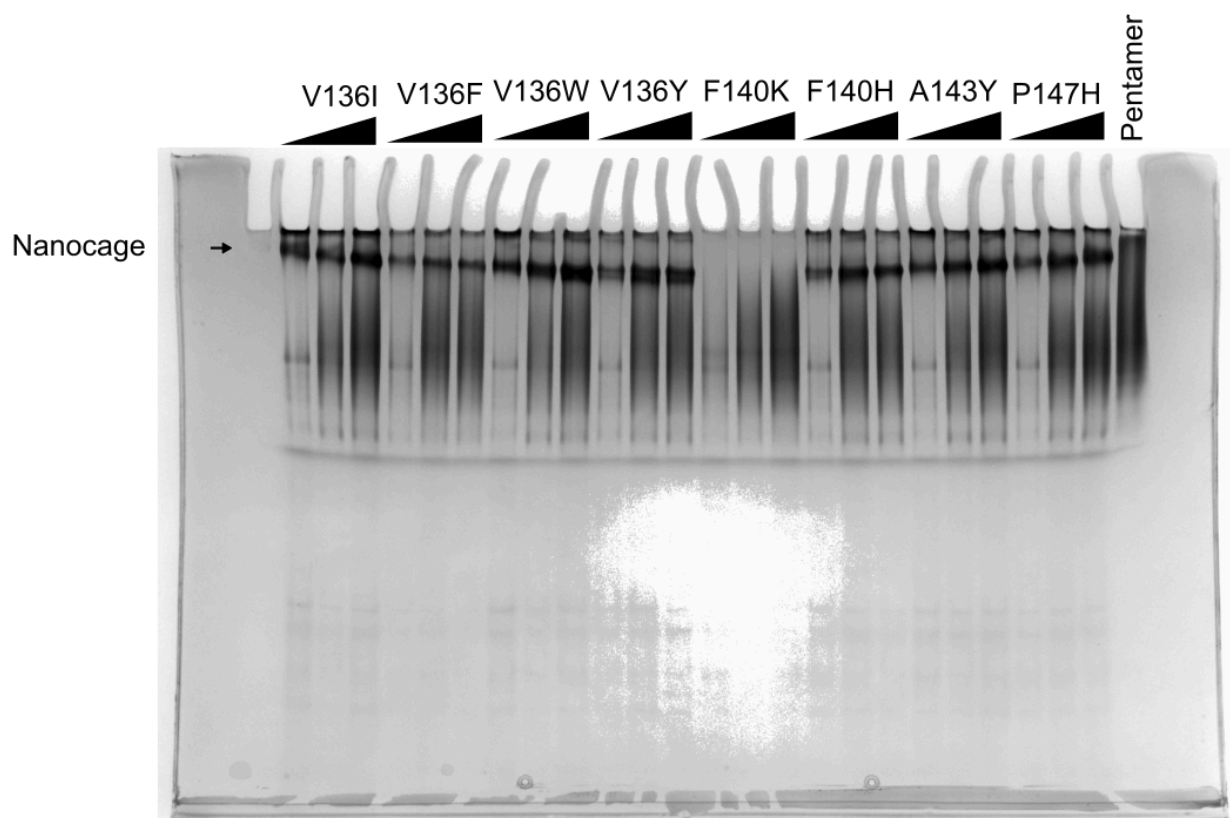
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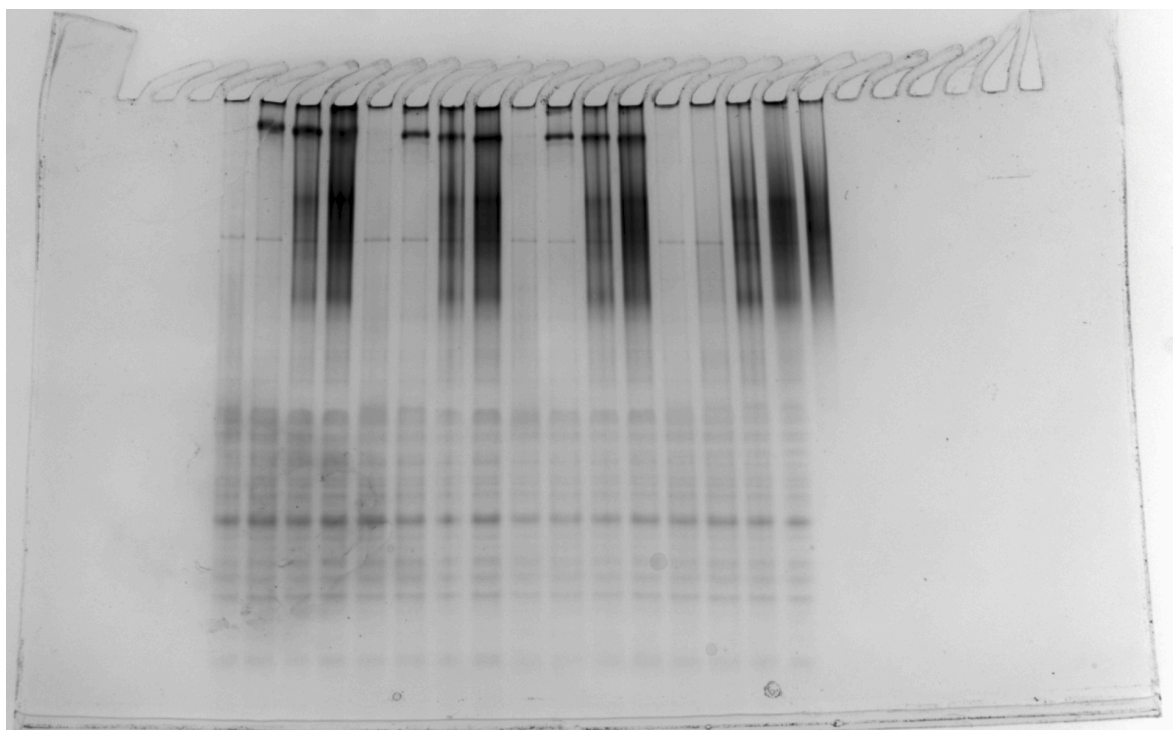
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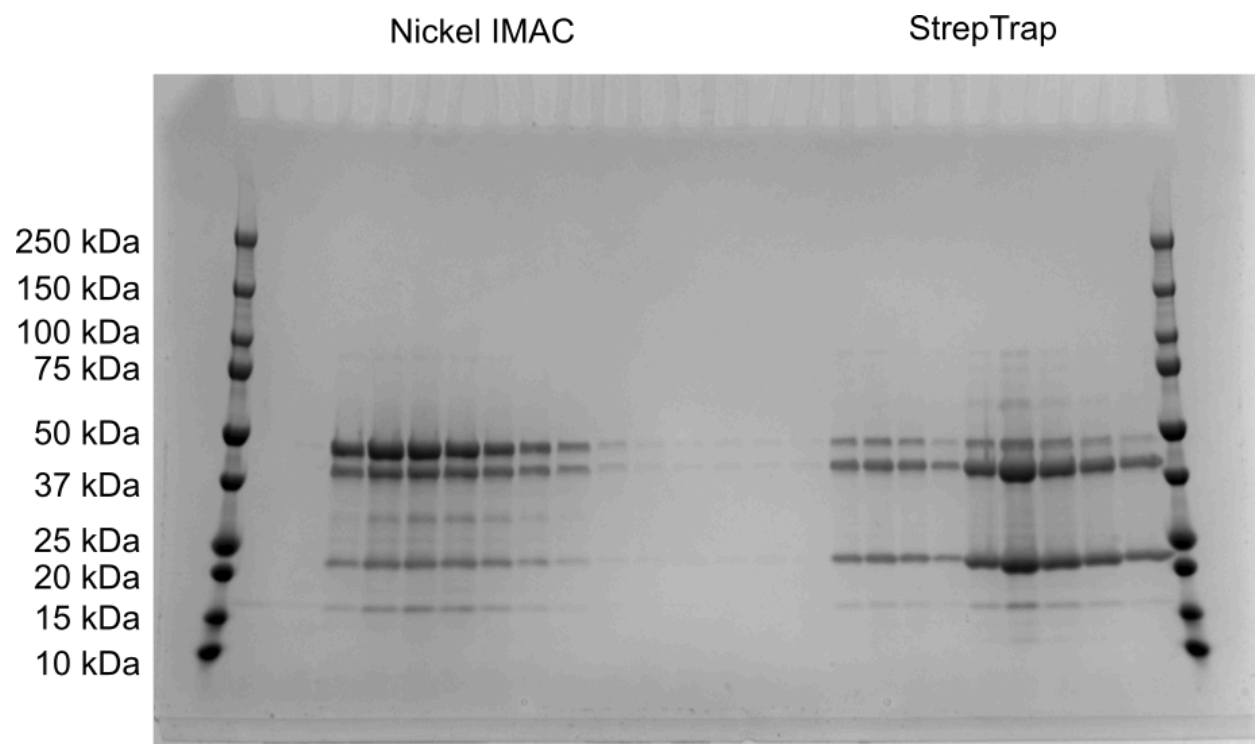
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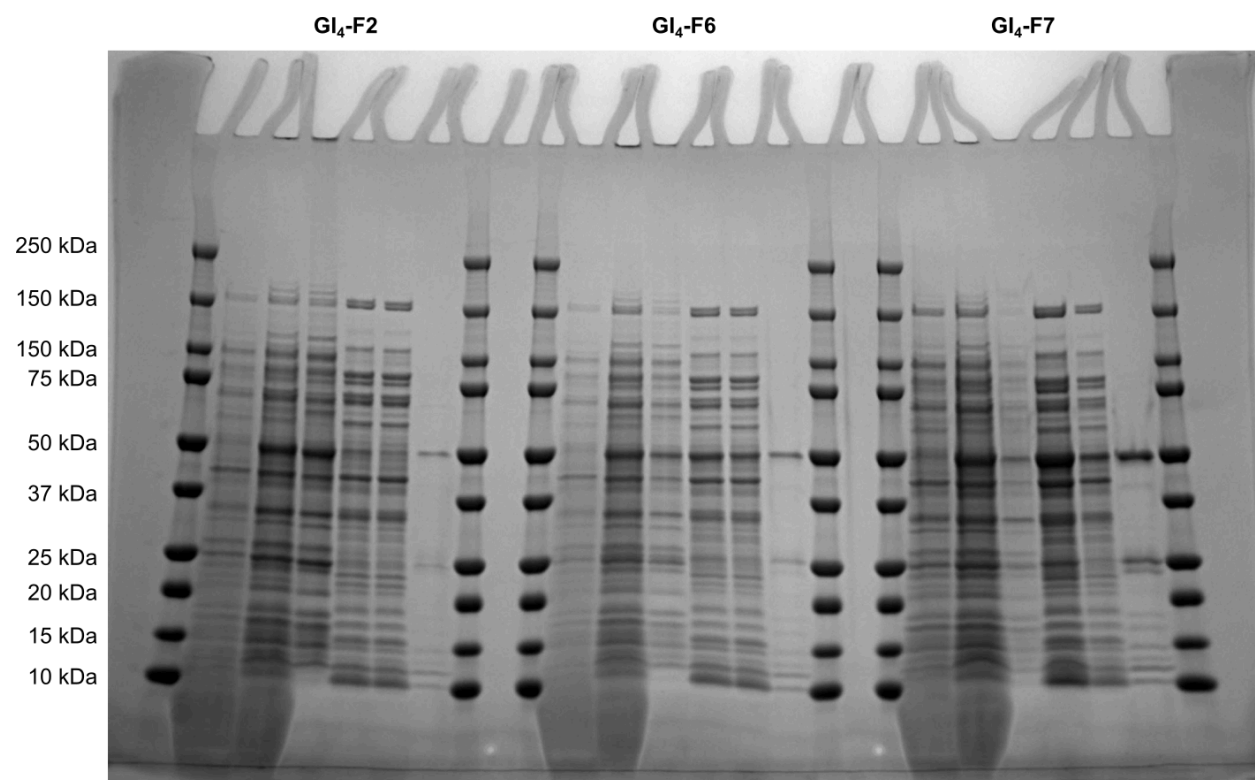
Supplementary Fig. 1: Uncropped Native-PAGE image of representative single-mutant assemblies from Main Text Fig. 1.



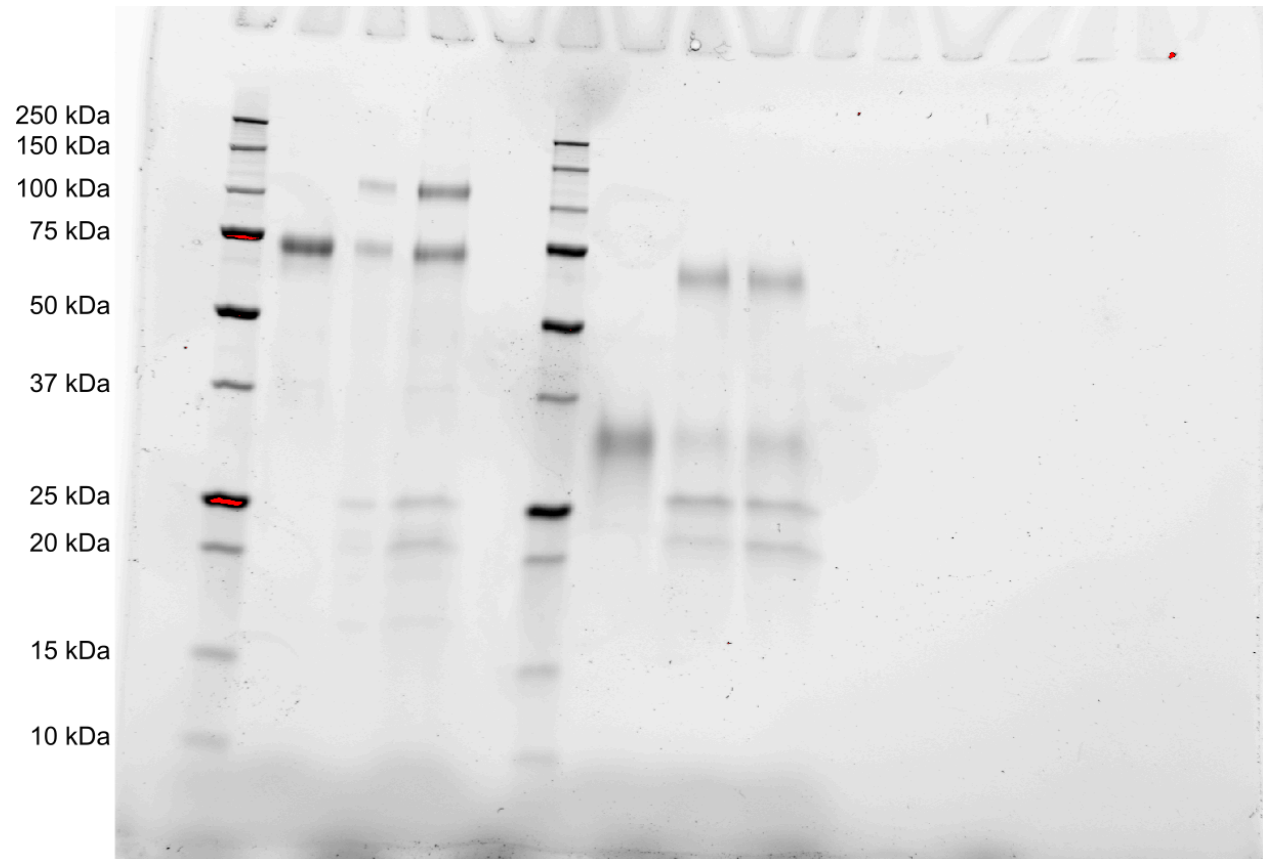
Supplementary Fig. 2: Uncropped Native-PAGE image of representative double-mutant assemblies from Extended Data Fig. 1.



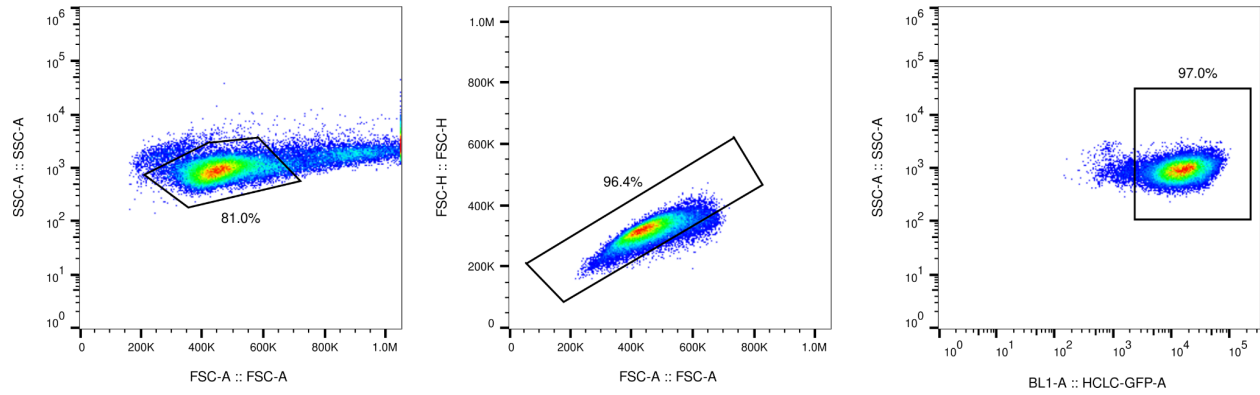
Supplementary Fig. 3: Uncropped SDS-PAGE image IMAC and StrepTrap purification gel from Extended Data Fig. 1.



Supplementary Fig. 4: Uncropped SDS-PAGE image of nanocage IMAC screening gel from Extended Data Fig. 2.



Supplementary Fig. 5: Uncropped SDS-PAGE image of antigen-nanocage conjugation from Main Text Fig. 3.



Supplementary Fig. 6: Flow cytometry gating strategy for COVA2-15 IgG RAMOS cells. Cells were gated on size, singlets, and GFP reporter for IgG transduced cells before analysis of FuraRed signals.

Supplementary Table 1: Amino acid sequences for novel proteins used in this study.

>Pseudosymmetric I53-50A A Chain

MGHHHHHHHHHGSLQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAK
RQKEMDSLRFLYDGIRIQADQAPEDLDMEDNDIIEAHREQIGGSEKAAKAEAAARKMEELFKKHKIVA
VLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEF
IVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHDILKLFPGEVVGPQFVKAMKGPFPPNVKFVP
TGGVNLDNVCKWFKAGVLAVGVGKALVKGKPDEVREKAKKFVKKIRGCTE

>Pseudosymmetric I53-50A B Chain

MKMEELFKEHKIVAVLRANSVEEAISKALAVFAGGVHLIEITFTVPDADQVIKELEFLKEAGAIIGAGTVTS
VEQCREAVESGAEFIVSFHLDEEISQFCKEEGVFYMPGVMTPTELVKAMKLGHTILKLVPGEVVGPQFV
EAMKGPFPPNVKFVPTGGVNLDNVCEWFEAGVLAVGVGSALVEGEPAEVAELAIRFVEKIRGCTEGS
WSHPQFEK

>Pseudosymmetric I53-50A C Chain

MKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLFICTTGKLPVPWPTLVTTLTYGVQC
FARYPDHMKQHDFFKSAMPEGYVQERTISFKDDGYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGH
KLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYSTQSVLS
KDPNEKRDHMLLEFVTAAGITHGMDELYKGGSGGSGGKMEELFKKHKIVAVLRANSVEEAIEKAVAVF
AGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPILDEEISQFCKEK
GVFYMPGVMTFTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGAFPPNVKFVPTGGVNLDNVCEWFKAG
VLAVGVGSALVKGTPDEVREKAKAFVEKIRGCTEEGSLNDIFEAQKIEWHE

>Pseudosymmetric Gl_T A Chain

MGSHHHHHHGSEKAAKAEAAARKMEELFKEHKIVAVLRANSVEEAKKALAVFLGGVHLIEITFTVPDA
DTVIKELSFLKEMGAIIGAGTVTSVEQCREAVESGAEFIVSPHLDEEISQFCKEEGVFYMPGVMTPTEL
KAMKLGHTILKLFPGEVVGPQFVEAMKGPFPPNVKFVPTGGVNLDNVCEWFEAGVLAVGVGSALVEGT
PVEVAEKAKAFVEKIEGCTE

>Pseudosymmetric Gl_T B Chain

MKMEELFKEHKIVAVLRANSVEEAISKALAVFAGGVHLIEITFTVPDADQVIKELEFLKEAGAIIGAGTVTS
VEQCREAVESGAEFIVSFHLDEEISQFCKEEGVFYMPGVMTPTELVKAMKLGHTILKLVPGEVVGPQFV
EAMKGPFPPNVKFVPTGGVNLDNVCEWFEAGVLAVGVGSALVEGEPAEVAELAIRFVEKIRGCTE

>Homotrimeric Gl_T BBB

MKMEELFKEHKIVAVLRANSVEEAISKALAVFAGGVHLIEITFTVPDADQVIKELEFLKEAGAIIGAGTVTS
VEQCREAVESGAEFIVSPHLDEEISQFCKEEGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFV
EAMKGPFPPNVKFVPTGGVNLDNVCEWFEAGVLAVGVGSALVEGEPAEVAELAIRFVEKIRGCTELEHH
HHHH

>Homotrimeric Gl_T CCC

MKMEELFKEHKIVAVLRANSREEAIEIALAVFAGGVHLIEITFTVPDADEVIKRLEMLKRAGAIIGAGTVTS
VEQCREAVESGAEFIVSPHLDEEISQFCKEEGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFV
EAMKGPFPPNVKFVPTGGVNLDNVCEWFEAGVLAVGVGSALVEGPKPSEVAEKARRFVKKIRGCTEGS
EHHHHHH

>RBD-SpyTag

MGILPSPGMPALLSLVSLLSVLLMGCVAETGTRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADF
SVLYNSASFSTFKCYGVSPTKLNDLCWTNIYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIA
WNSNNLDSKVGGNYNYLYRLFRKSNLKPFRDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGV
GYQPYRVVLSFELLHAPATVCGPKKSTGGSGGSGSGSGSGSGSAHIVMVDAYKPTKGGSGGSHHH
HHH

>Homotrimeric Gl_T CCC-SpyCatcher

MEELFKEHKIVAVLRANSREEAIEIALAVFAGGVHLIEITFTVPDADEVIKRLEMLKRAGAIIGAGTVTSVEQ
CREAVESGAEFIVSPHLDEEISQFCKEEGVFYMPGVMTPTLVKAMKLGHTILKLFPGEVVGPQFVEAM
KGPFPNVKFVPTGGVNLDNVCEWFEAGVLAVGVGSALVEGKPSEVAEKARRFVKKIRGCTEGSGGSG
GSGGSGAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISD
GQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHILEHHHHHH

Appended sequences including SUMO, GFP, SpyTag-, SpyCatcher, and deca- or hexa-histidine, avi-, and strep-tags are underlined.

Pseudosymmetrizing mutations are bold.