

Supporting Figures

SpyAvidin Hubs Enable Precise and Ultrastable Orthogonal Nanoassembly

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A

>Tre

MAEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAEGDYVLTGRYDSAPATDGSGTALGWTVAWKNNYRN
 AHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASEEEEEE

>DTag

MAEAGITGTWYAQLGDTFIVTAGADGALTGTYEAAVGNAESRYVLTGRYDSAPATDGSGTALGWTVAWKNNYRN
 AHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASSGSGGAHIVMVDAYKPT

B

>Tr

MAEAGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAEGDYVLTGRYDSAPATDGSGTALGWTVAWKNNYRN
 AHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAAS

>DCatch

MAEAGITGTWYAQLGDTFIVTAGADGALTGTYEAAVGDDDGDDDGDDGAESRYVLTGRYDSAPATDGSGTALG
 WTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLLTSGTTEANAWKSTLVGHDTFTKVKPSAASSGSGDSAT
HIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQQQ
VTVNGKATKGAHI

C

Streptavidin subunit orientation

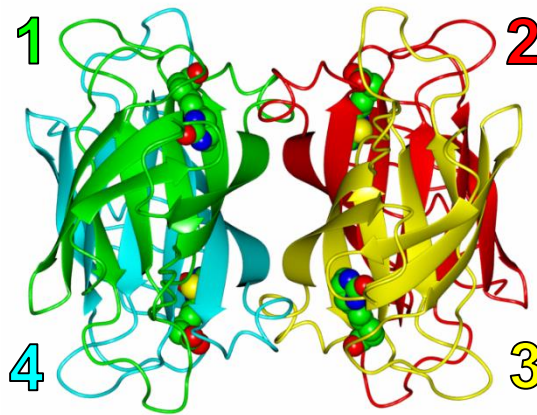


Figure S1. Streptavidin Variant Sequences and Subunit Orientation. (A) Amino acid sequence of chains to make Tre/DTag tetramers. For Tre: S52G R53D mutation in green and C-terminal hexaglutamate tag in red. For DTag, mutations to block biotin binding in pink, spacer in beige, and C-terminal SpyTag in cyan. (B) Amino acid sequence of chains to make Tr/DCatch tetramers. For Tr: S52G R53D mutation in green. For DCatch, mutations to block biotin binding in pink, negatively charged D-loop in red, spacer in beige, and C-terminal SpyCatcher in dark blue. (C) Subunit orientation in the streptavidin tetramer, with each subunit numbered and shown in a different color in ribbon format, while biotin is in space-fill, from PDB 3RY2.

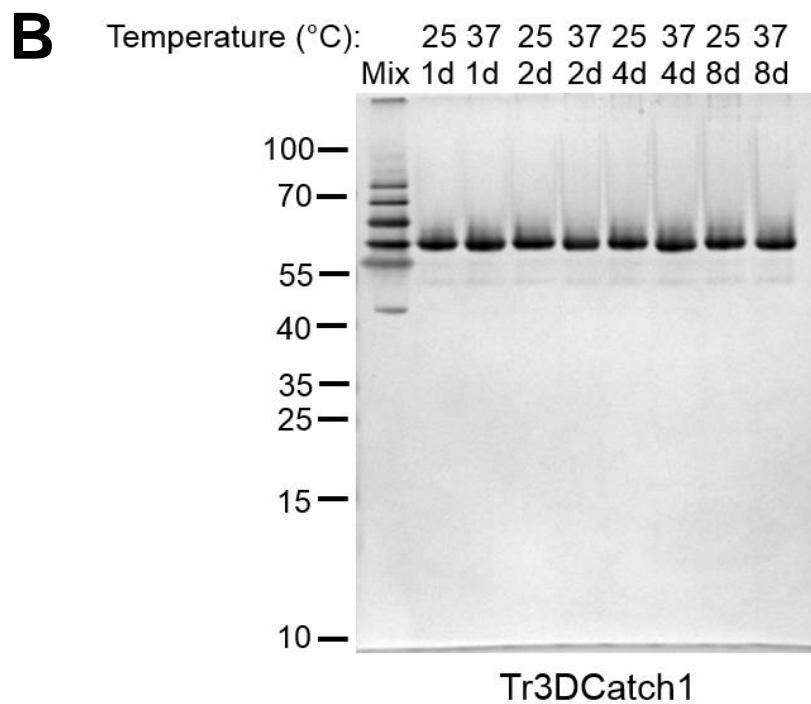
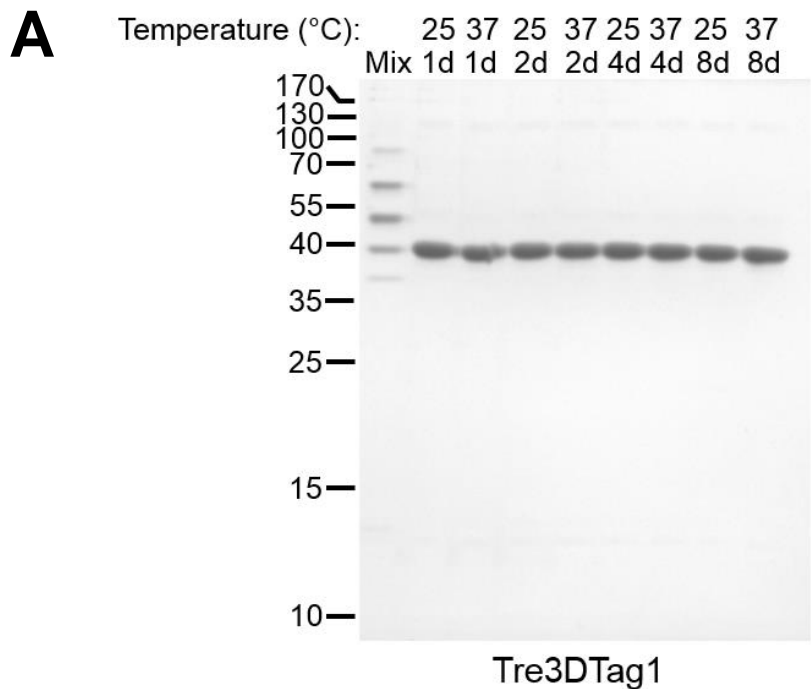


Figure S2. Stability of Chimeric Tetramers. Tre3DTag1 (A) or Tr3DCatch1 (B) was incubated at 25 or 37 °C for 1 - 8 days and analyzed on 10% SDS-PAGE with Coomassie staining, to check for degradation or tetramer rearrangement. Mix is the original mixture of refolded tetramers, to mark the mobility if Tre3DTag1 or Tr3DCatch1 subunits rearranged.

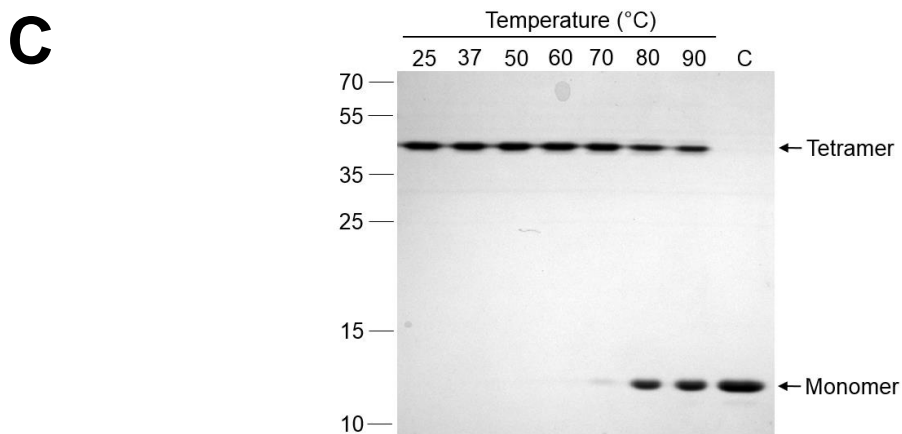
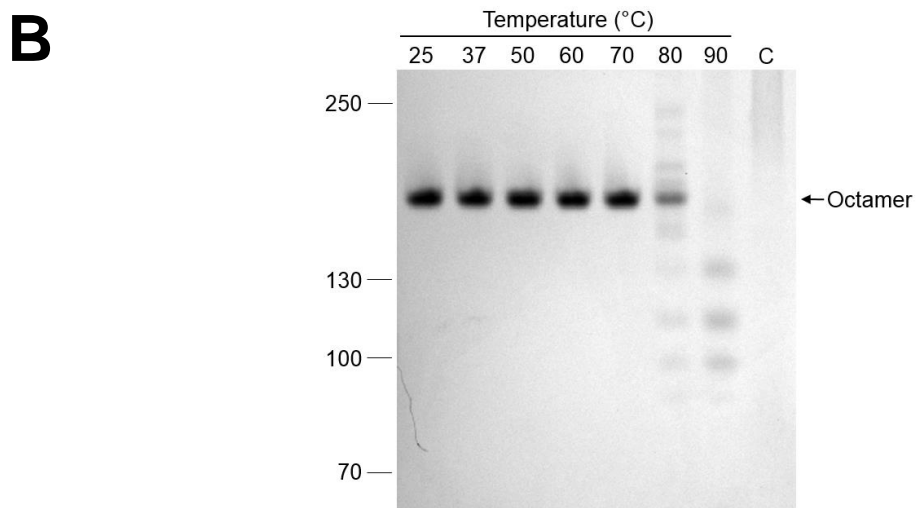
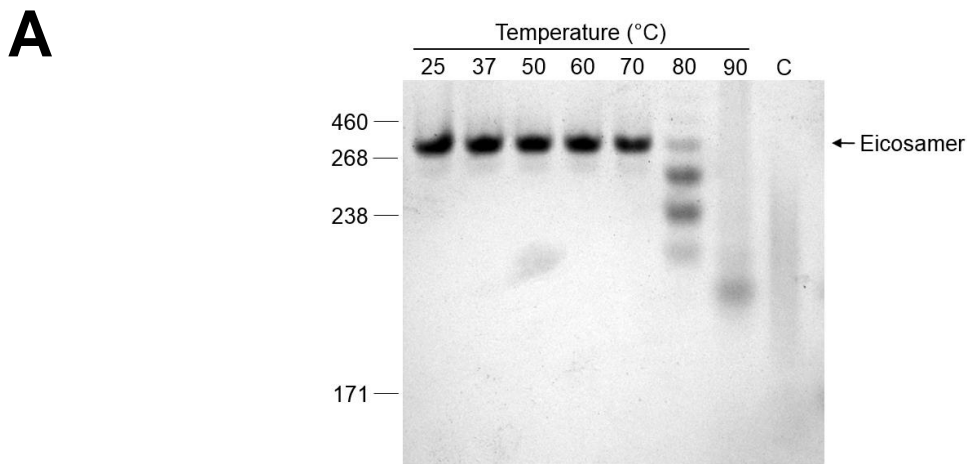


Figure S3. Thermostability of Octamer and Eicosamer Assemblies. Eicosamer (A), octamer (B), and streptavidin tetramer (SAe4) (C) were heated in PBS at the indicated temperature for 3 min before analysis of the integrity of the assembly by SDS-PAGE with Coomassie staining. C in the right lane of each gel refers to a control sample incubated in SDS loading buffer at 95 °C.

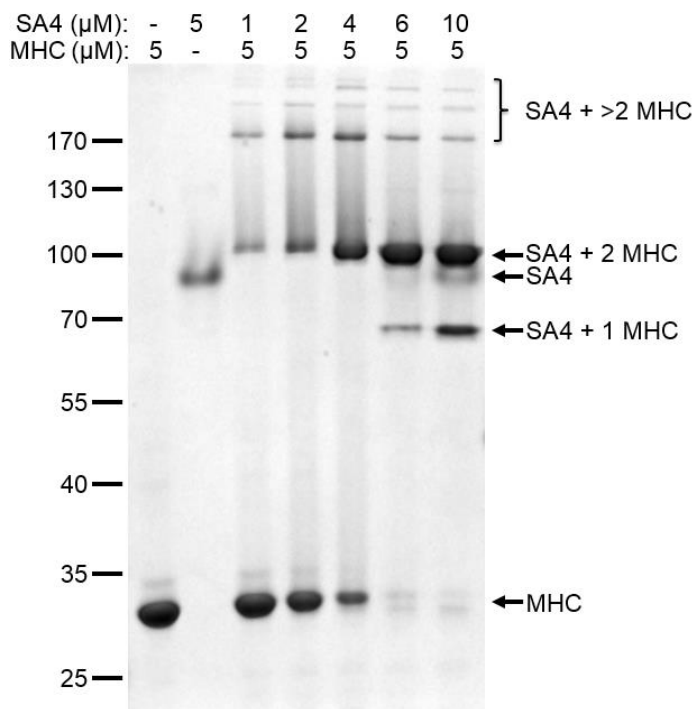
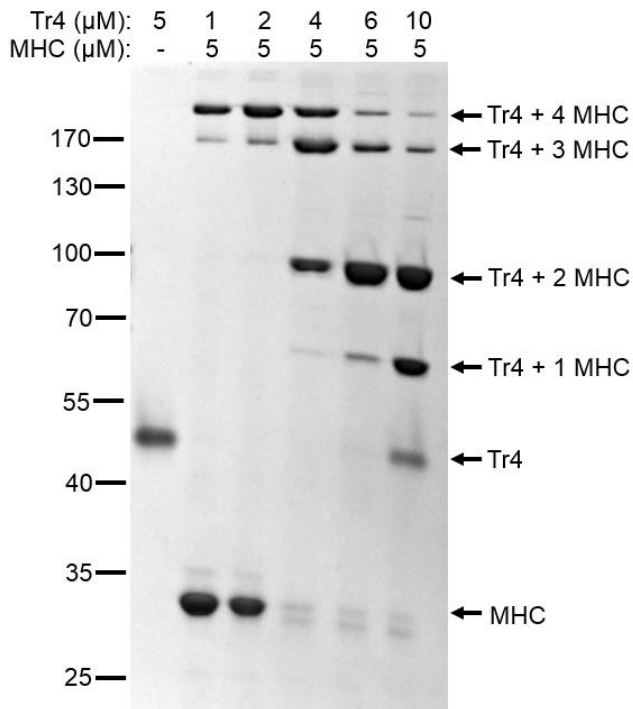
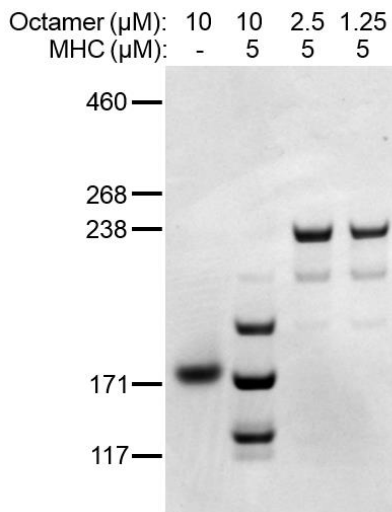
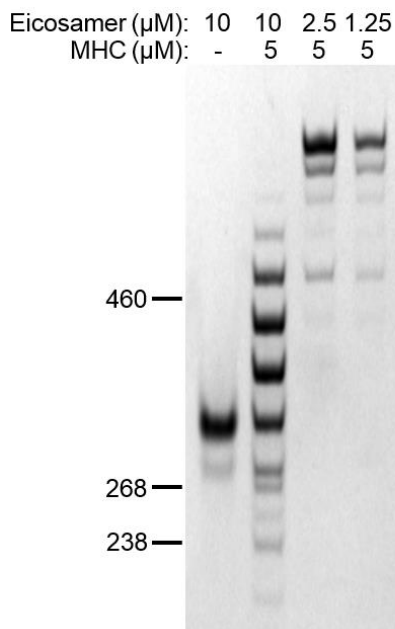
A**B****C****D**

Figure S4. Binding of MHC Class I by Streptavidin Variants. Streptavidin (A), traptavidin (B), octamer (C) or eicosamer (D) at the indicated concentrations were incubated together with MHC class I-cognate peptide complexes overnight at room temperature in PBS and then analyzed by SDS-PAGE with Coomassie staining. MHC arrows refer to heavy chain, since interactions with $\beta_2\text{m}$ and peptide are lost in the presence of SDS.