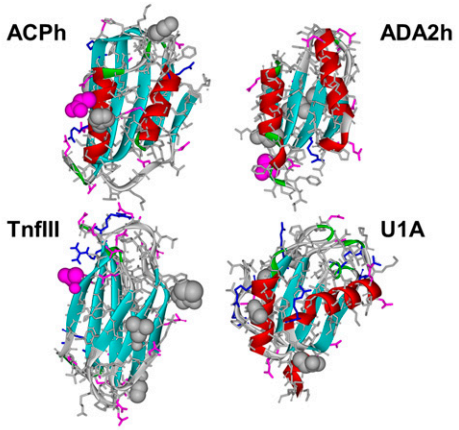
Supporting Information

Tzul et al. 10.1073/pnas.1410424112




```
ACPh-wt     MAEGNTLISVDYEIFGKVQGVFFRKHTQAEGKKLGLVGWVQNTDRGTVQGQLQGPISKVRHMQEWLETRGSPKSHIDKANFNNEKVILKLDYSDFQIVK
ACPh-des    MAEGNTLISVDYEIFGKVQGVFFRKHTQAEGKKLGLVGWVQNTDRGTVQGKLQGPISKVREMQKWLETRGSPESHIDKANFKNEKVILKLDYSDFQIVK

ADA2h-wt    MDQVLEIVPSNEEQIKNLLQLEAQEHLQLDFWKSPTTPGETAHVRVPFVNVQAVKVFLESQGIAYSIMIEDVQ
ADA2h-des   MDEVLEIVPSNEEQIKNLLQLEAQEHLQLDFWKSPTTPGETAEVRVPFVNVQAVKVFLESQGIAYKIKIEKVQ

TnfII-wt    MRLDAPSQIEVKDVTDTTALITWFKPLAEIDGIELTFGIKDVPGDRTTIDLTEDENQFSIGNLKPDTEFEVSLISRRGDMSSNPAKETFTTGA
TnfII-des   MRLDAPSKIEVKDVTDTTAKITWFKPLAEIDGIELTFGIKDVPGDRTTIKLTEDENQFSIGNLKPDTEFEVSLISRRGDMSSNPAKETFKTGA

U1A-wt      MAVPETRPNHTIYINNLNEKIKKDELKKSLHAIFSRFGQILDILVSRSLKMRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRIQYAKTDSDIIAKM
U1A-des     MAVPETRPNHTIYIENLNEKIKKDELKKSLHAIFSRFGEILDILVSRSLKMRGQAFVIFKEVSSATEALRSMQGFPFYDKPMRIKYAKTDSDIIAKM
```

Fig. S1. Cartoon structure showing different topologies of four studied proteins and the sequence comparison for WT and *des* protein pairs. ACPh—PDB ID code: 2ACY (1), ADA2h—PDB ID code: 1AYE (2), TnfIII—PDB ID code: 1TEN (3), U1A—PDB ID code: 1URN (4). Charged residues are color coded: magenta—acidic residues, blue—basic residues. The positions that were substituted in the des variants are shown in space-filling representation. The sequence alignment of the WT and *des* proteins shows substituted residues in red font.

1. Thunnissen MMGM, Taddei N, Liguri G, Ramponi G, Nordlund P (1997) Crystal structure of common type acylphosphatase from bovine testis. *Structure* 5(1):69–79.
2. García-Sáez I, Reverter D, Vendrell J, Avilés FX, Coll M (1997) The three-dimensional structure of human procarboxypeptidase A2. Deciphering the basis of the inhibition, activation and intrinsic activity of the zymogen. *EMBO J* 16(23):6906–6913.
3. Leahy DJ, Hendrickson WA, Aukhil I, Erickson HP (1992) Structure of a fibronectin type III domain from tenascin phased by MAD analysis of the selenomethionyl protein. *Science* 258(5084):987–991.
4. Oubridge C, Ito N, Evans PR, Teo CH, Nagai K (1994) Crystal structure at 1.92 A resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. *Nature* 372(6505):432–438.

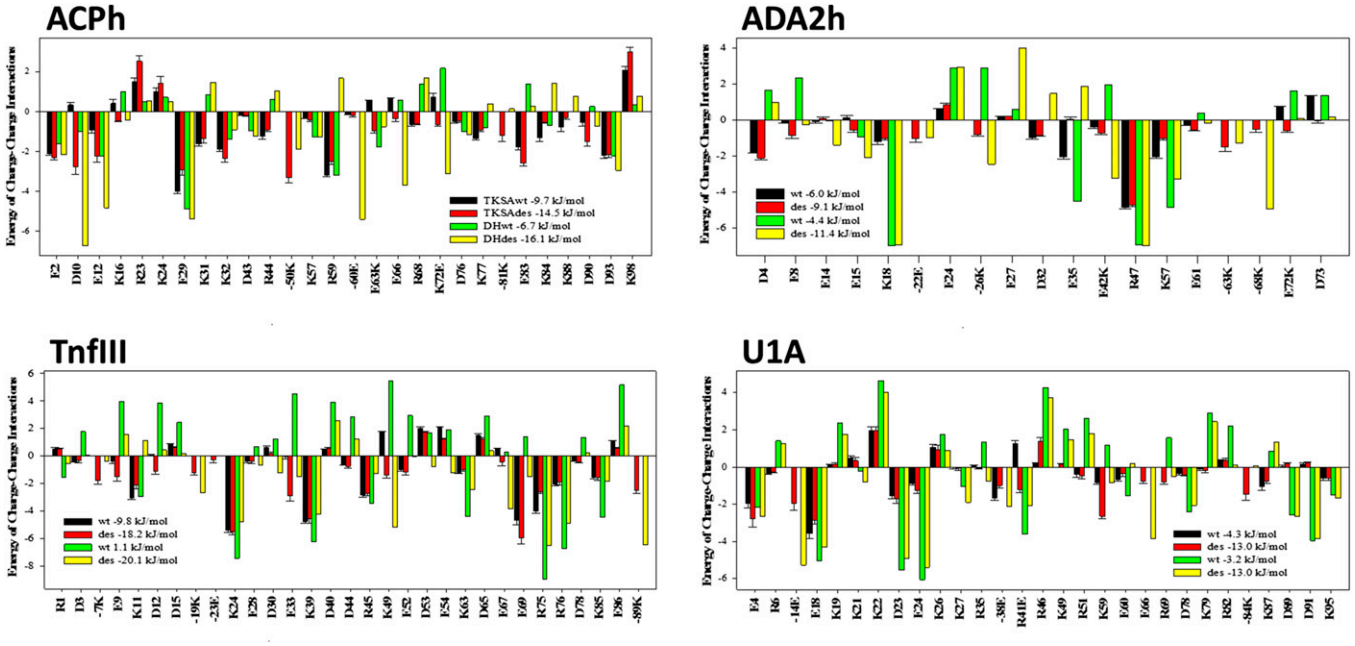


Fig. S2. Comparison of the energies of charge–charge interactions in the WT and *des* sequences ACPh, ADA2h, TnfIII, and U1A in the native state. Each bar corresponds to the total energy of charge–charge interactions of that residue with all other ionizable residues in the protein. Black and green bars are for the Wt proteins, and red and yellow bars are for the *des* variants. The black and red bars show the original TKSA (Tanford–Kirkwood model with solvent accessibility) energies calculated using all-atom models (1, 2). The green and yellow bars show the Debye-Hückel (DH; see Eq. **5** of the main text) energies calculated from the Cα-SBM ensemble. The numbers on each plot represent the sum of all charge-charge interactions and clearly show that whether TKSA or DH energy is used, the *des* proteins have more optimized energy than the corresponding *wt* proteins.

1. Gribenko AV, et al. (2009) Rational stabilization of enzymes by computational redesign of surface charge-charge interactions. *Proc Natl Acad Sci USA* 106(8):2601–2606.
2. Strickler SS, et al. (2006) Protein stability and surface electrostatics: A charged relationship. *Biochemistry* 45(9):2761–2766.

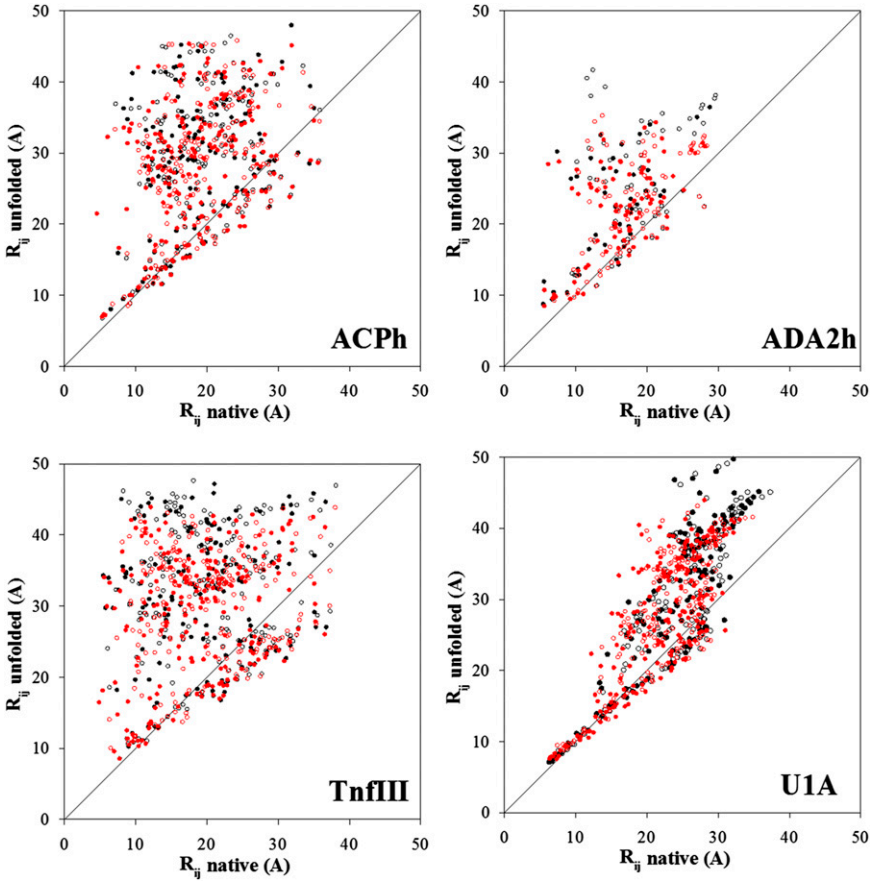


Fig. S3. Plots of distances between pairs of charged residues in the native state ensemble, R_{ij} native, versus distances in the unfolded state ensemble, R_{ij} unfolded. Black symbols are for the WT proteins and red symbols for the *des* proteins. Filled symbols are for the oppositely charged pairs whereas open symbols are for pairs with the same charge. The majority of data points are above the diagonal, suggesting that the distances between charged pairs increase in the unfolded state relative to the native state. Those data points that are below the diagonal are at the distances over 15 Å. The 15 Å in the Cα-SBM can be considered as maximal possible distance for the potential salt-bridge formation, based on the calculated maximum distance between the Cα atoms of longest side chains forming a salt bridge (e.g., Glu and Lys) in all-atom representation. This comes to about ∼15 Å: 4 Å for a salt bridge plus 5 Å distance from Cα to carboxyl carbon of Glu side chain plus 6 Å distance from Cα to nitrogen of Lys side chain. These data support the notion that there are no stable native or nonnative "salt bridges" in the unfolded state ensemble.

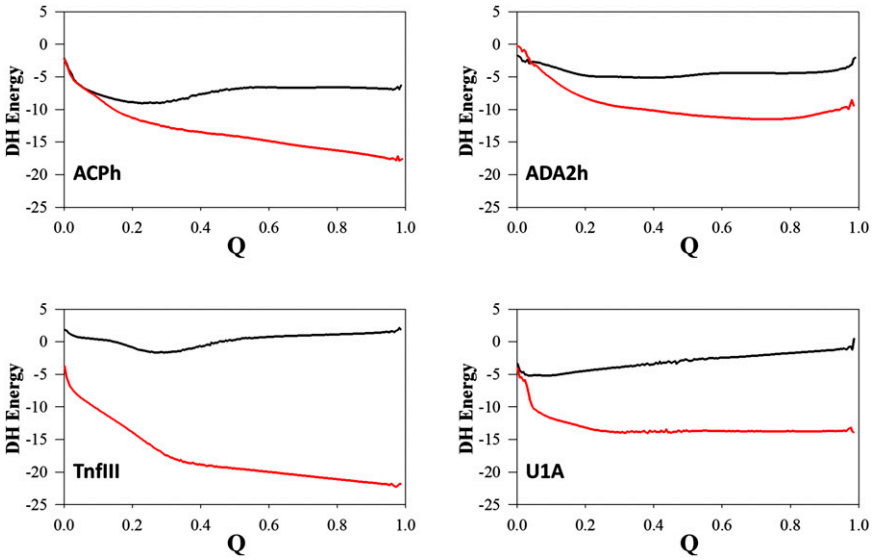


Fig. S4. Dependence of the Debye–Hückel (see Eq. **5** of the main text) energies on Q for the four studied proteins pairs. Colors are the same as in Fig. 3 of the main text. These dependencies provide clear indication that the *des* sequences are stabilized through long-range charge–charge interactions relative to the WT proteins.

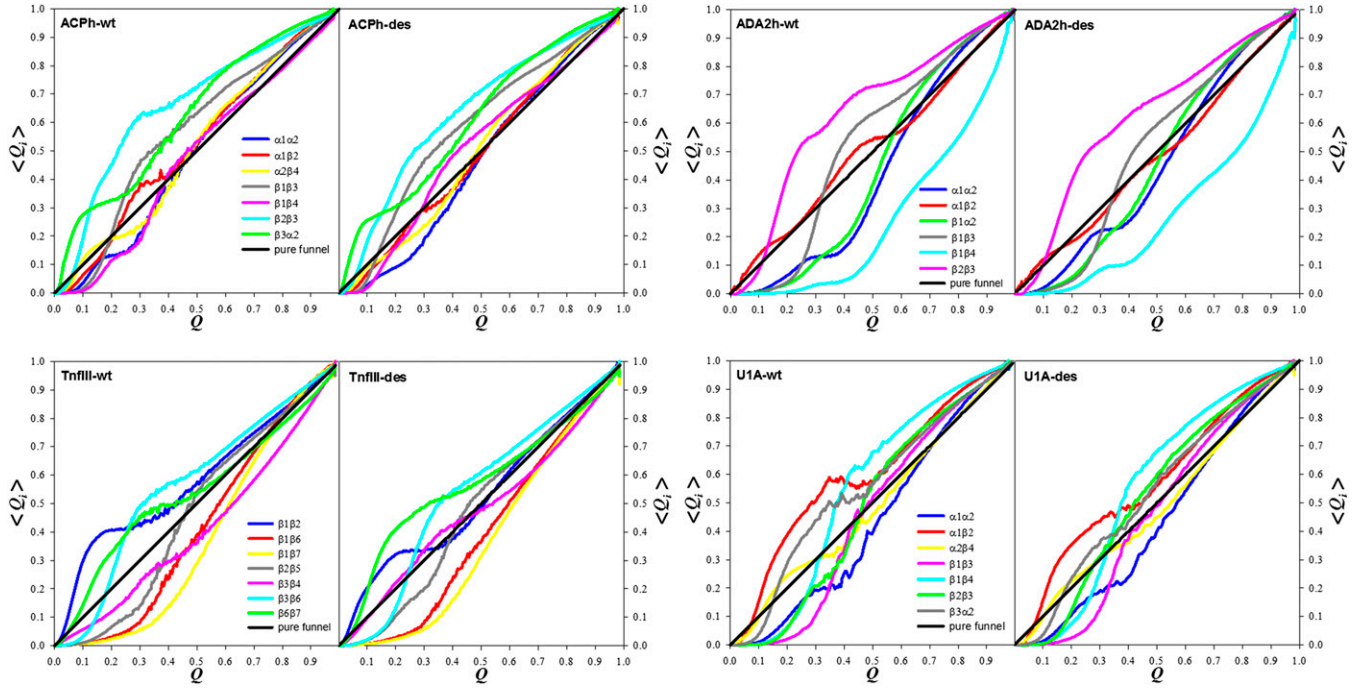


Fig. S5. Dependence of <Qi> on Q for the four studied proteins pairs. Colors are the same as in Fig. 3 of the main text.

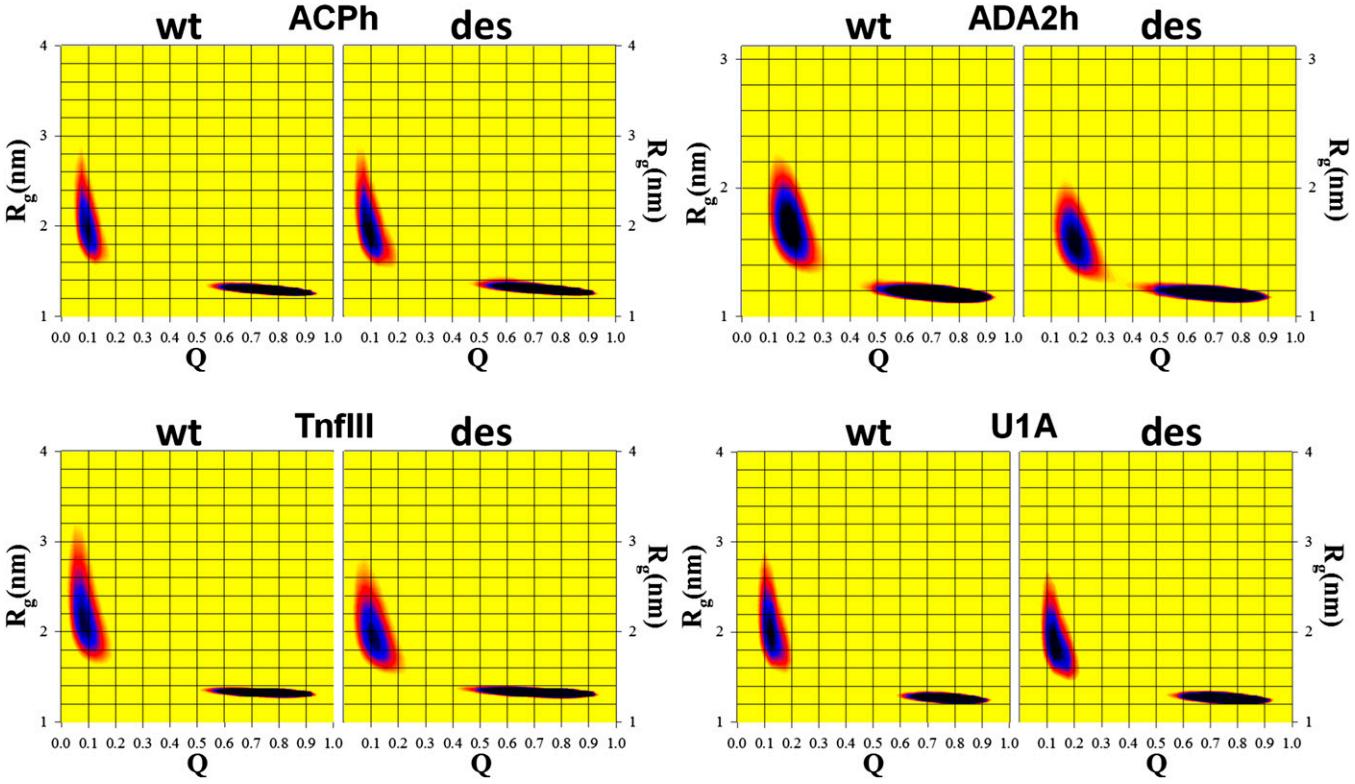


Fig. S6. Distribution of radii of gyration R_g as a function of fraction of native contacts Q shows that in the unfolded state the *des* proteins are more compact than the corresponding WT proteins. Protein identifiers are shown above each plot.

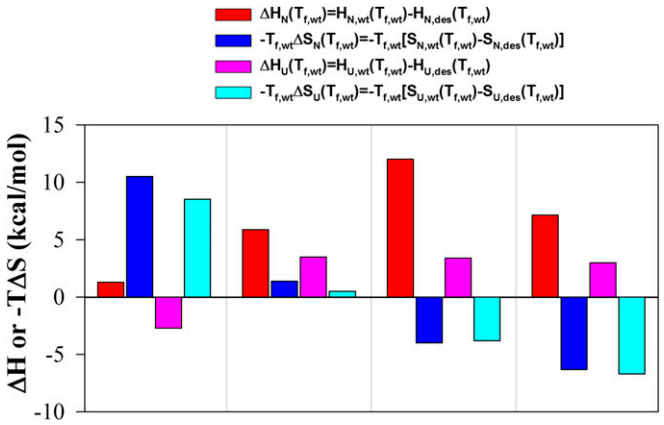


Fig. S7. Contribution of the enthalpy (ΔH) and entropy (−TΔS) change for the folded state and for the unfolded state of the *des* variants relative to the corresponding WT proteins. Enthalpies and entropies were calculated using WHAM by combining simulation data from different temperatures into single free-energy profiles (1). Comparison is done at the T_f values for the WT proteins. No common enthalpic or entropic stabilization signature for the four studied proteins is observed, which emphasizes that salt bridges in the native or unfolded states are not responsible for the observed stabilization.

1. Kumar S, Bouzida D, Swendsen RH, Kollman PA, Rosenberg JM (1992) The weighted histogram analysis method for free-energy calculations on biomolecules. 1. The method. *J Comput Chem* 13(8):1011–1021.