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Supplemental Information

A Single Protein Disruption Site Results in Efficient Reassembly by Multiple Engineering Methods

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WT RBP:
KEGKTIGLVISTLNNPFFVTLKNGAEEKAKELGYKIIVEDSQNDSSKELSNVEDLIQQKVDVLLINPVDSD
AVVTAIKEANSKNIPVITIDRSANGGDVVSHIASDNVKGGEMAAEFIAKALKGKGNVVELEGIPGASAARD
RGKGFDEAIAKYPDIKIVAKQAADFDRSKGLSVMENILQAQPKIDAVFAQNDEMALGAIKAIEAANRQGII
VVGFDGTEDALKAIKEGKMAATIAQQPALMGSLGVEMADKYLKGEKIPNFIPAELKLITKENVQNLEHHHH
HHHH

RBP$^{1-96}$:
WGKEGKTIGLVISTLNNPFFVTLKNGAEEKAKELGYKIIVEDSQNDSSKELSNVEDLIQQKVDVLLINPVD
SDAVVTAIKEANSKNIPVITIDRSANG

RBP$^{97-277}$:
GSSHHHHHHSQDPNSSSGDVVSHIASDNVKGGEMAAEFIAKALKGKGNVVELEGIPGASAARDRGKGFDEA
IAKYPDIKIVAKQAADFDRSKGLSVMENILQAQPKIDAVFAQNDEMALGAIKAIEAANRQGIIVVGFDGTE
DALKAIKEGKMAATIAQQPALMGSLGVEMADKYLKGEKIPNFIPAELKLITKENVQW

CyPet-RBP$^{1-96}$:
GSKGEELFGGIVPILVELEGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTWGVQCF
SRYPDHMKQHDFFKSVMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLE
YNYISHNVYITADKQKNGIKANFKARHNITDGSVQLADHYQQNTPIGDGPVILPDNHYLSTQSALSKDPNE
KRDHMVLLEFVTAAGITHGMDELYKGGASGKEGKTIGLVISTLNNPFFVTLKNGAEEKAKELGYKIIVEDS
QNDSSKELSNVEDLIQQKVDVLLINPVDSDAVVTAIKEANSKNIPVITIDRSANGLEHHHHHHHH

RBP$^{97-277}$-YPet:
GSSHHHHHHSQDPNSSSGDVVSHIASDNVKGGEMAAEFIAKALKGKGNVVELEGIPGASAARDRGKGFDEA
IAKYPDIKIVAKQAADFDRSKGLSVMENILQAQPKIDAVFAQNDEMALGAIKAIEAANRQGIIVVGFDGTE
DALKAIKEGKMAATIAQQPALMGSLGVEMADKYLKGEKIPNFIPAELKLITKENVQASGGTGMSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLLCTTGKLPVPWPTLVTTLGYGVQCFARYPDHMKQ
HDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVY
ITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRDHMVLLE
FLTAAGITEGMNELYK

CP97:
GDVVSHIASDNVKGGEMAAEFIAKALKGKGNVVELEGIPGASAARDRGKGFDEAIAKYPDIKIVAKQAADF
DRSKGLSVMENILQAQPKIDAVFAQNDEMALGAIKAIEAANRQGIIVVGASGTEDALKAIKEGKMAATIAQ
QPALMGSLGVEMADKYLKGEKIPNFIPAELKLITKENVQGGAASGGAAGGSSAAASSGAGAAGGSGAGGKE
GKTIGLVISTLNNPFFVTLKNGAEEKAKELGYKIIVEDSQNDSSKELSNVEDLIQQKVDVLLINPVDSDAV
VTAIKEANSKNIPVITIDRSANG

RU97:
MKEGKTIGLVISTLNNPFFVTLKNGAEEKAKELGYKIIVEDSQNDSSKELSNVEDLIQQKVDVLLINPVDS
DAVVTAIKEANSKNIPVITIDRSANGMQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIF
AGKQLEDGRTLSDYNIQKESTLHLVLRLRGGGDVVSHIASDNVKGGEMAAEFIAKALKGKGNVVELEGIPG
ASAARDRGKGFDEAIAKYPDIKIVAKQAADFDRSKGLSVMENILQAQPKIDAVFAQNDEMALGAIKAIEAA
NRQGIIVVGFDGTEDALKAIKEGKMAATIAQQPALMGSLGVEMADKYLKGEKIPNFIPAELKLITK
ENVQNLEHHHHHH

Fig. S1. Amino acid sequences of representative constructs used in this study. His-tag purification sequences are shown in orange, the extra Trp residue (added for concentration determination) in purple, CyPet in cyan, YPet in green, CP linker in red, and ubiquitin underlined.

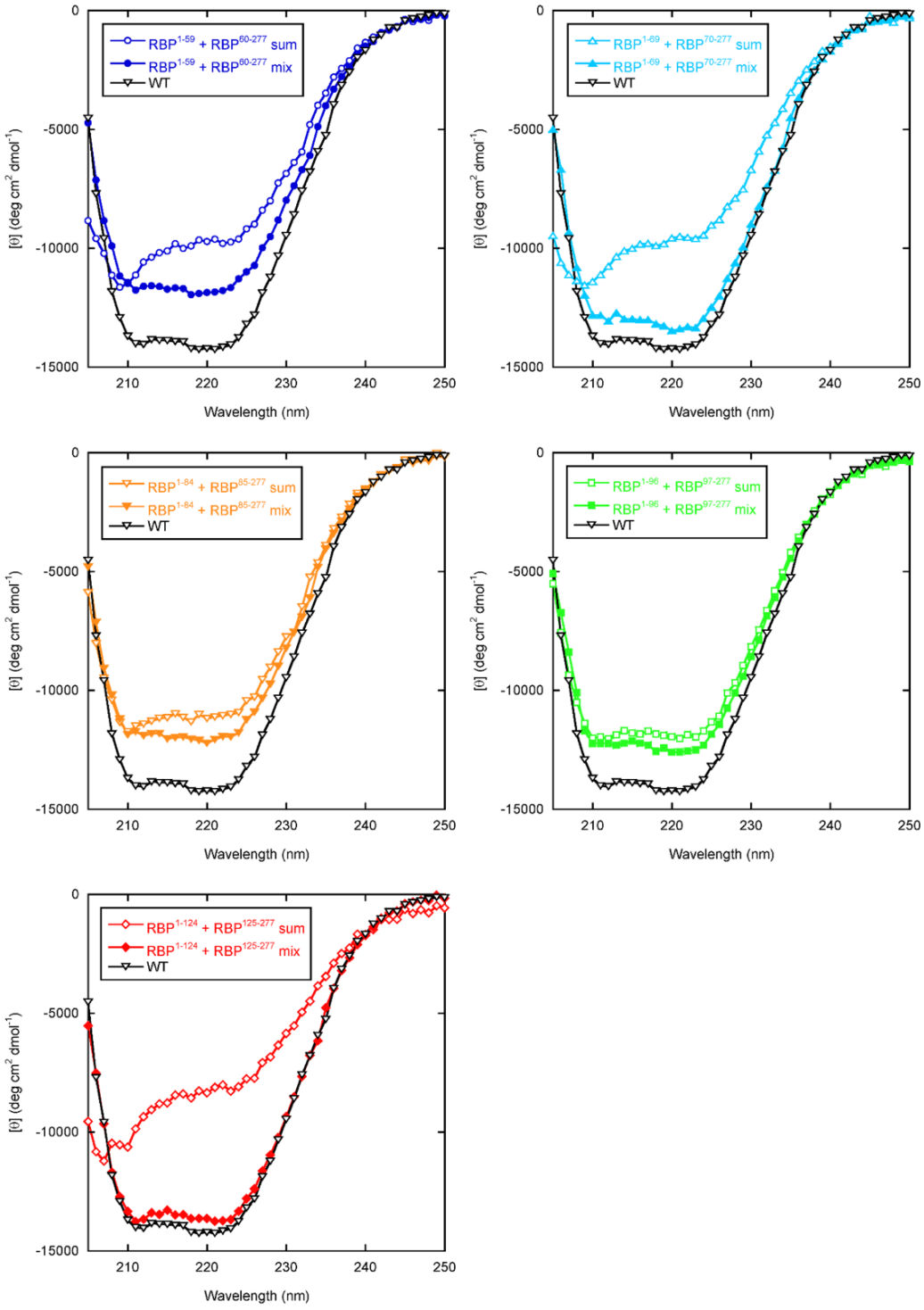

Fig. S2. Binding and refolding of homo fragments monitored by CD. Open symbols are the summed scans of the individual N- and C-terminal fragments shown in Fig. 2A and Fig. 2B of the text, respectively. Closed symbols correspond to the physical mixture of the N- and C-fragments (2 μM each). Buffer conditions are as described in *Materials and Methods*.

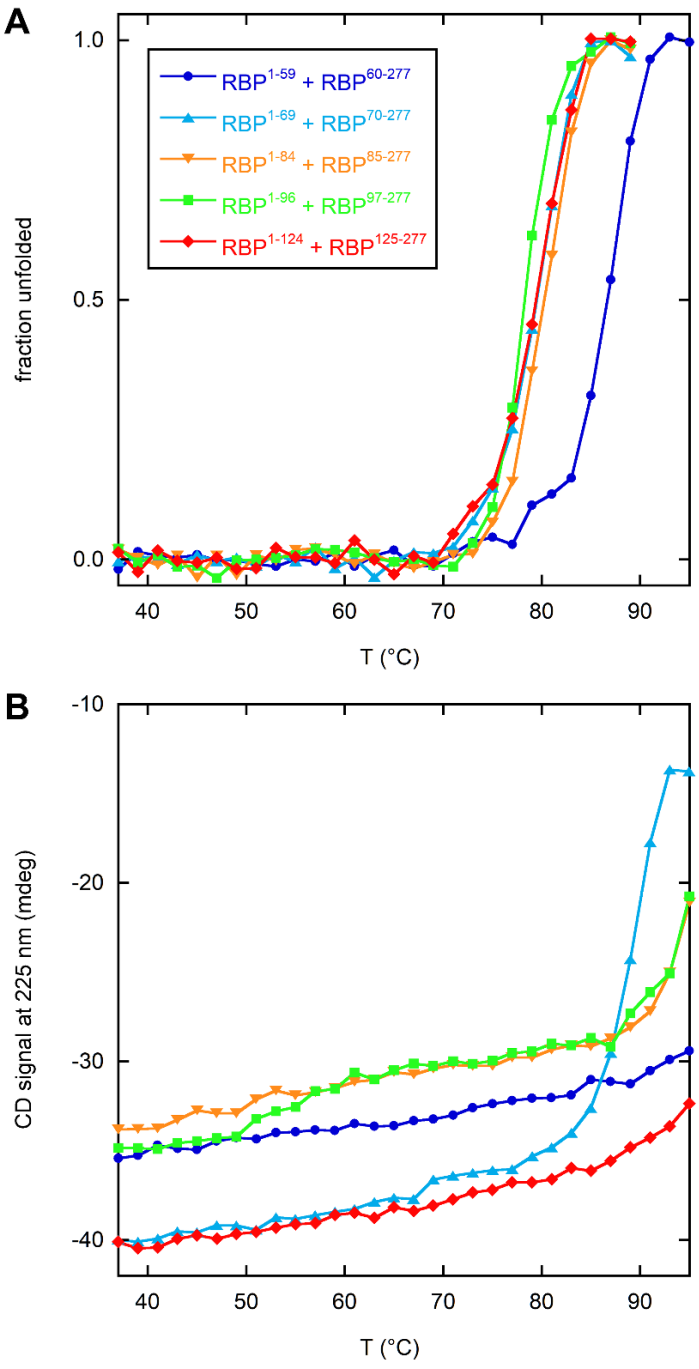

Fig. S3. Thermal stabilities of homo complexes in the absence (A) and presence (B) of ribose. In panel A, ellipticity values were converted to fraction unfolded assuming a two-state unfolding model (lines are meant to guide the eye only). Symbols in panel B are identical to those in panel A. Circular dichroism data were recorded at 225 nm in a 1 cm path length cuvette using a heating rate of 10 °C/m. Sample conditions are 1 μM protein, 1 mM ribose, 10 mM sodium phosphate (pH 7.0), 0.15 M NaCl, 0.1 mM EDTA.

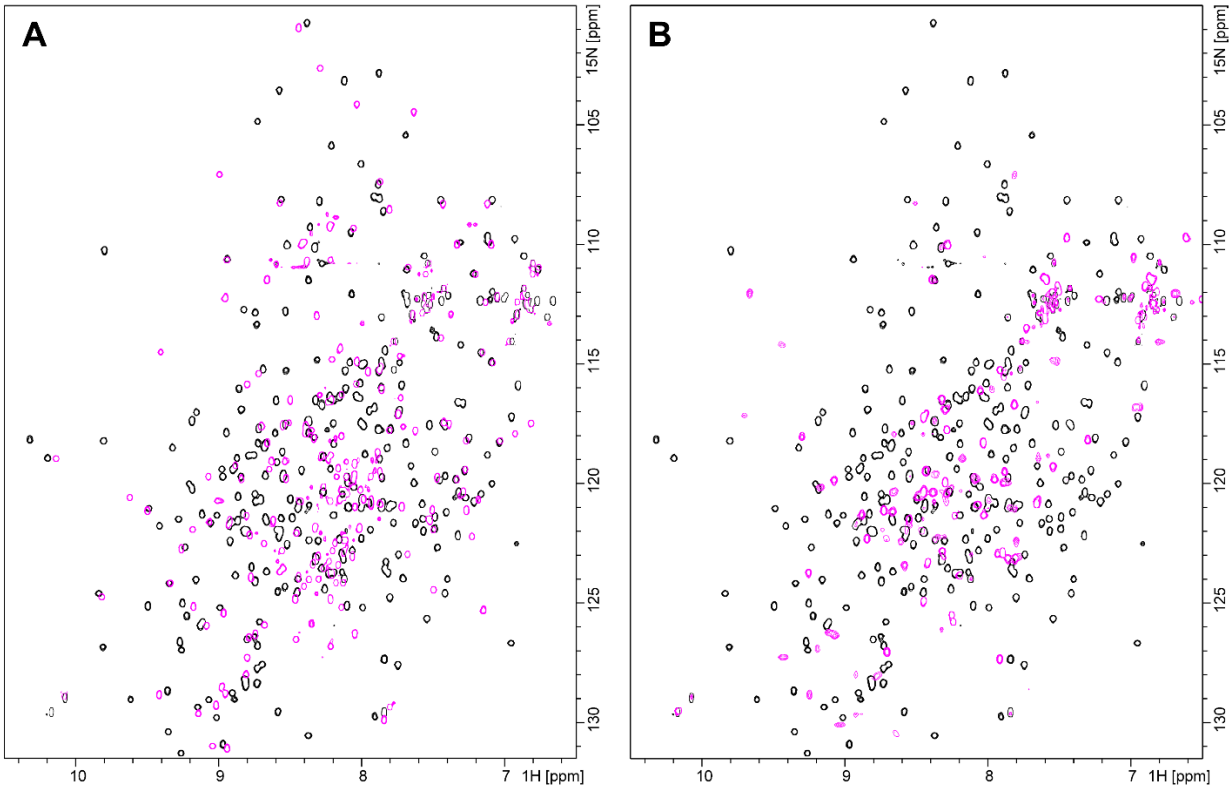

Fig. S4. 800 MHz ^{15}N-HSQC NMR spectra of (A) RBP$^{97-277}$ and (B) RBP$^{1-96}$. The individual fragments are shown in purple and the complex of RBP$^{1-96}$ + RBP$^{97-277}$ in black. Sample conditions are 1 mM RBP$^{1-96}$, 1 mM RBP$^{97-277}$, 0.6 mM RBP$^{1-96}$ + RBP$^{97-277}$, 20 mM sodium phosphate (pH 7.0), 0.1 M NaCl, 30 °C. The RBP$^{1-96}$ + RBP$^{97-277}$ sample contains 1 mM ribose.