Supporting Information

Schütz *et al.* 10.1073/pnas.0800418105

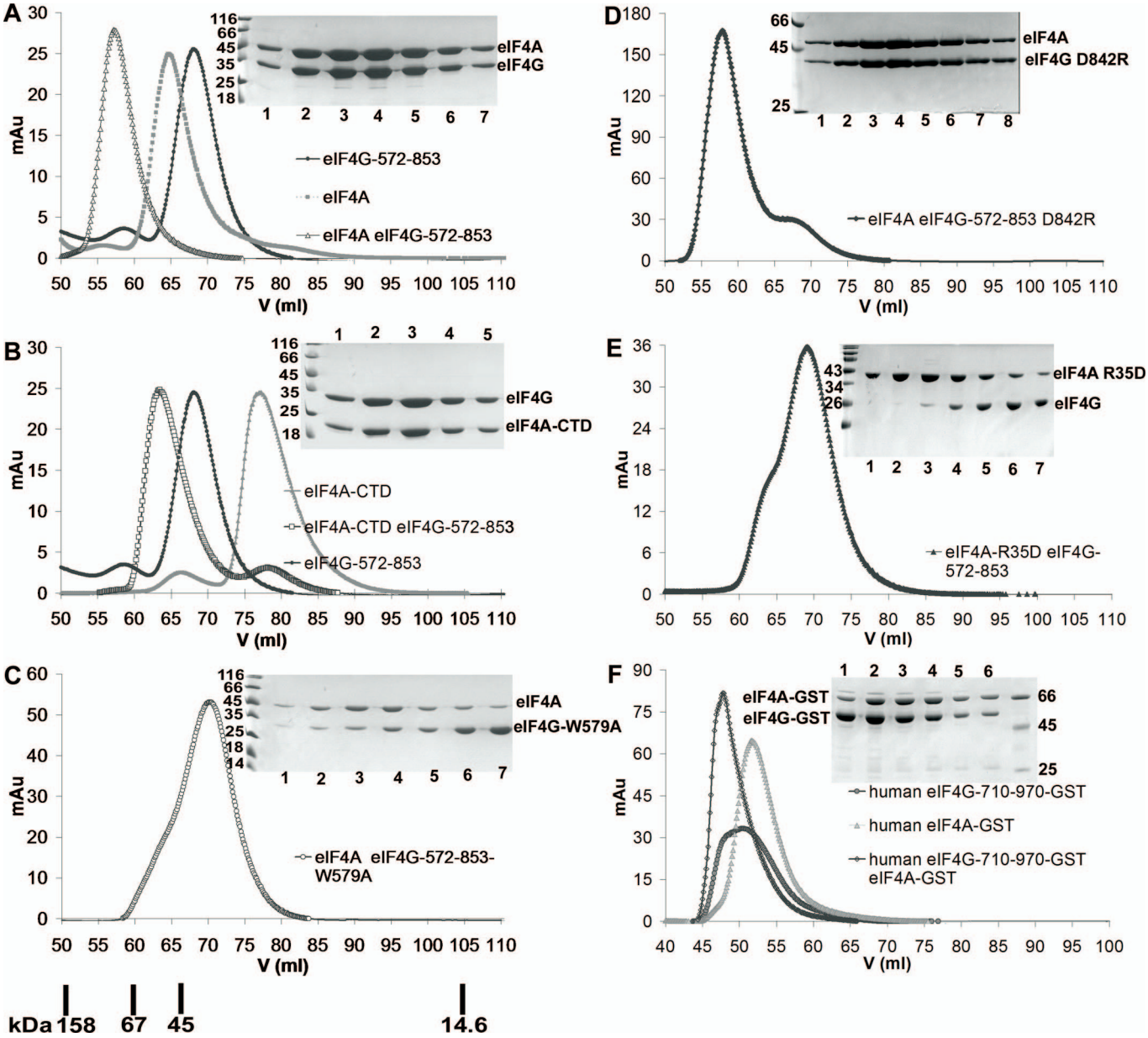

Fig. S1. Gel filtration profiles of eIF4G–eIF4A complexes. eIF4A (2–10 mg/ml) and eIF4G (2–10 mg/ml) were mixed in equimolar amounts and subjected to chromatographic separation on a S75 gel filtration column (Amersham Pharmacia; equilibrated with a buffer containing 20 mM Tris·HCl, pH 7.5, 100 mM NaCl). 1.5-ml fractions of relevant peaks were analyzed by SDS/PAGE. *A* to *E* show the elution profiles of yeast proteins, *F* the elution profile of human GST-fusion proteins. *D* to *F* illustrate the effects of mutations or deletions in the contact area of eIF4A-NTD. (*A*) Full-length eIF4A (gray squares), eIF4G (572–853) (black diamonds), and the complex (open triangles). SDS/PAGE shows fractions from 53.5 to 64.0 ml elution volume. (*B*) eIF4A-CTD (gray triangles), eIF4G (572–853) (black diamonds), complex (open squares). Fractions from 61 to 68.5 ml elution volume were analyzed by SDS/PAGE. (*C*) Elution profile of full-length eIF4A and eIF4G (572–853)(W579A). Fractions from 59.5 to 70.0 ml were analyzed by SDS/PAGE. (*D*) Full-length eIF4A and eIF4G (572–853)(D842R) do still form a complex. SDS/PAGE shows fractions from 52 to 64 ml elution volume. (*E*) eIF4A(R35D) and eIF4G (572–853) do not form a complex. SDS/PAGE shows fractions from 61 to 71.5 ml elution volume. (*F*) Human GST-eIF4A (gray triangles) and GST-eIF4GI (710–970) (dark circles) form a stable complex (open diamonds). Fractions from 44.5 to 53.5 ml were submitted to SDS/PAGE analysis.

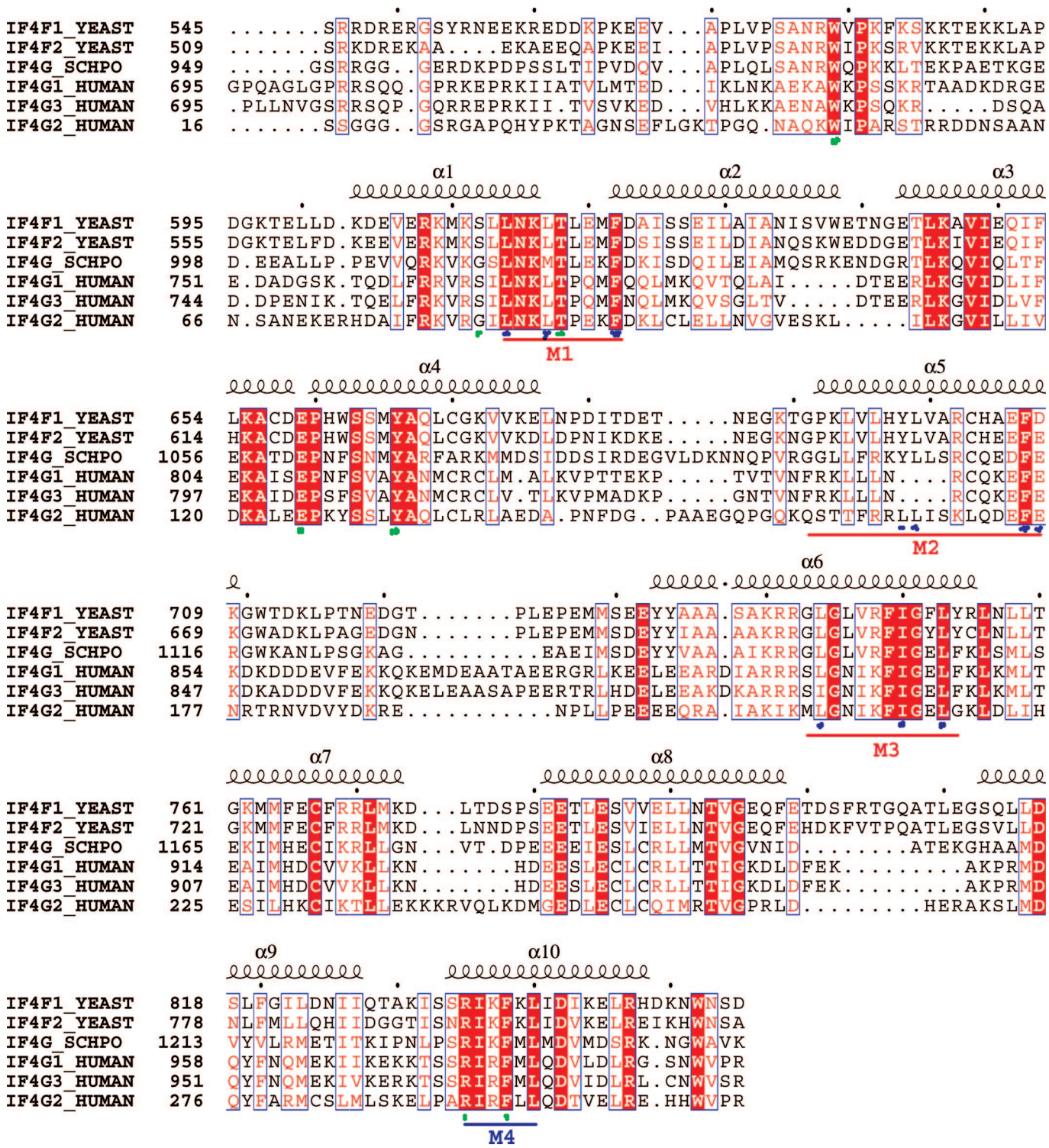

Fig. S2. eIF4G middle domain sequence alignment. Mutants with disrupted eIF4A binding are marked by dots. Blue dots indicate mutations in the hydrophobic core, and green dots are residues on the surface interacting with eIF4A. The positions of the α-helices are marked. The figure was prepared with ESPRIPT (2).

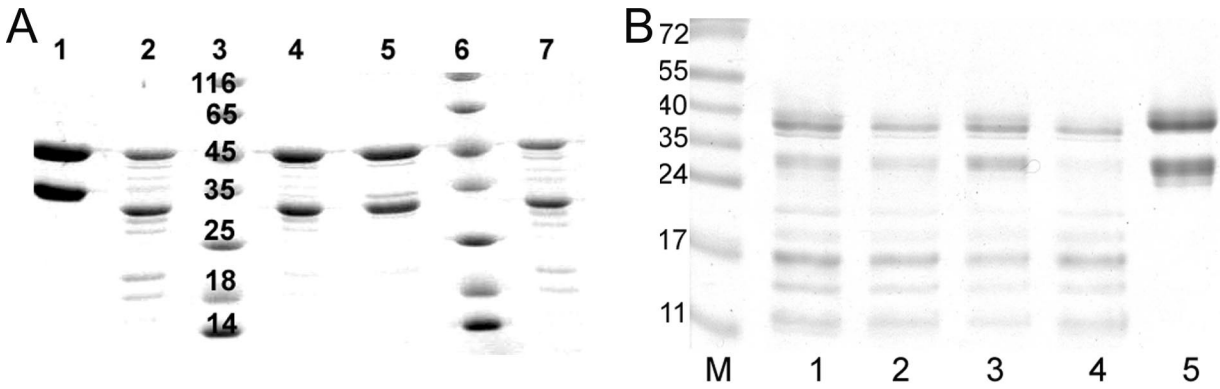

Fig. S3. Limited proteolysis of the eIF4A–eIF4G complex in the presence of different nucleotides. (*A*) Tryptic digest. 5 μg of eIF4A–eIF4G complex were digested for 30 min at room temperature in 20 mM Tris·HCl (pH 7.5), 100 mM NaCl, and 1 mM magnesium acetate. Lane 1, no protease; lane 2, apo; lane 3, marker; lane 4, 1 mM AMP; lane 5, 1 mM ADP; lane 6, marker; lane 7, 1 mM AMPPNP + 1 μg poly(U)-RNA. (*B*) Staphylococcus V8 digest. 5 μg of complex plus 1 μg of poly(U)-RNA were digested for 2 h at 37°C in the same buffer as described in *A*. Lane M, marker; lane 1, apo; lane 2, AMP; lane 3, ADP; lane 4, AMPPNP; lane 5, input.

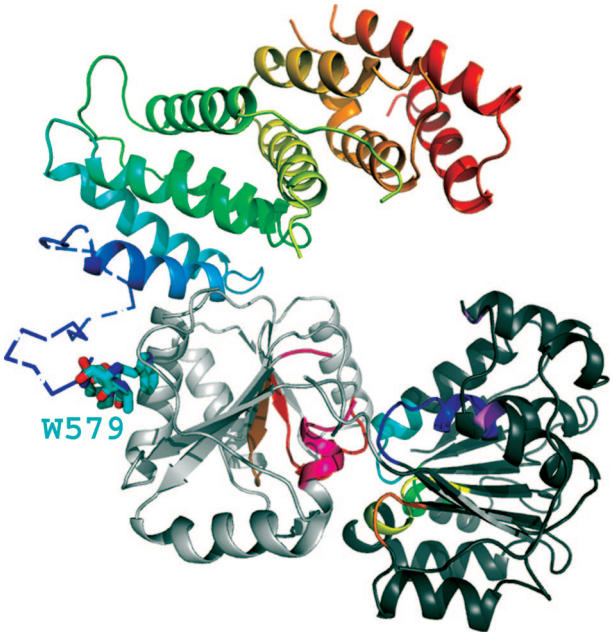

Fig. S4. Modeled closed conformation of eIF4A-AMPPNP-RNA-eIF4G. The orientation is similar to the orientation in Fig. 4*A*. Only eIF4A-NTD (dark gray) was moved to correspond to the arrangement observed in the VASA complex. eIF4A-CTD (light gray) and eIF4G (rainbow colored from N terminus blue to C terminus red) were kept as in the crystal structure. The DEAD-box motifs in eIF4A are color coded as in Fig. 4.

1. Kleywegt G J, Jones TA (1996) Phi/psi-chology: Ramachandran revisited. *Structure* 4:1395–1400.
2. Gouet P, Robert X, Courcelle E (2003) ESPript/ENDscript: Extracting and rendering sequence and 3D information from atomic structures of proteins. *Nucleic Acids Res* 31:3320–3323.

Table S1. Data collection and refinement statistics

Crystal	P1	$P2_1$
Unit cell		
a, *b*, *c* (Å)	69.26, 69.80, 101.97	68.92, 111.36, 111.28
α, β, γ (deg)	91.70, 102.0, 115.3	90.0, 99.71, 90.0
Data collection		
Beamline	ID29, ESRF Grenoble	in-house
Wavelength, Å	0.987	1.542
Resolution range, Å	50- 2.60 (2.75–2.60)	50- 2.80 (2.90–2.80)
No. unique reflections	48,104	40,598
Redundancy	1.8	3.4
Completeness, %	93.0 (91.6)	99.8 (98.6)
R_{sym}, %	3.4 (19.3)	9.3 (70.2)
I/σ (I)	14.6 (3.5)	11.5 (1.6)
Refinement		
Resolution range, Å	50- 2.60	50- 2.80
No. working/test set	46,752/1,302	38,907/1,668
No. atoms total	9,748	9,748
Water molecules	0	0
AMP	2	2
R/R_{free}, %	21.7/26.7	25.3/28.7
rms bonds, Å	0.011	0.003
rms angles, °	1.37	0.68
Ramachandran outliers,* %	3.1	3.1

*Ramachandran outliers are defined as by Kleywegt and Jones (1). According to this definition, a well refined 2.0-Å resolution structure has ≤5% outliers.