Supporting Information

Molecular crowding enhanced ATPase activity of the RNA helicase eIF4A correlates with compaction of its quaternary structure and association with eIF4G

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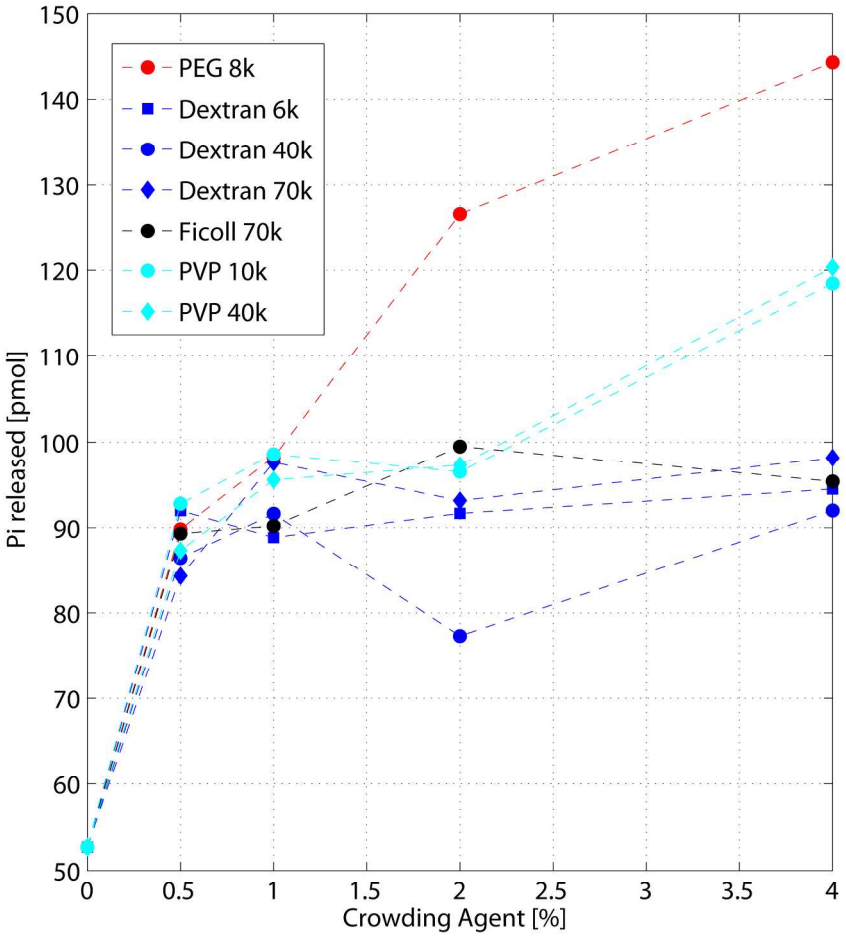

Figure S1: ATP hydrolysis activity of eIF4A using different types and sizes of crowding agents determined using a Norit assay The reaction mixture (20 µl) contained 10 mM Na-phosphate buffer (pH 7), 80 mM KCl, 3 mM $MgCI_2$, 50 mM EDTA, 2 mM DTT, 50 µM [γ-^{32}P]-ATP (30 cpm/pmol), 3 µM eIF4A and one of the crowding agents at concentrations ranging from) to 4%.

The standard assay for ATPase measures the formation of Norit-adsorbable nucleoside arising from the hydrolysis of [γ-32P]-ATP. Samples were incubated for 60 minutes at 37 °C. The reaction was stopped by the addition of 200 µL of a solution consisting of 0.6 ml of 1 M HCl, 0.1 M $Na_4P_2O_7$, 0.02 M KH_2PO_4, 0.2 ml of a Norit A suspension (20% packed volume), and 0.1 ml of bovine serum albumin (5 mg/ml). The solutions were mixed and allowed to sit for 5 min at 0 °C. After centrifugation, 4 µL of the supernatant was spotted on a filter paper and the radioactivity was determined.

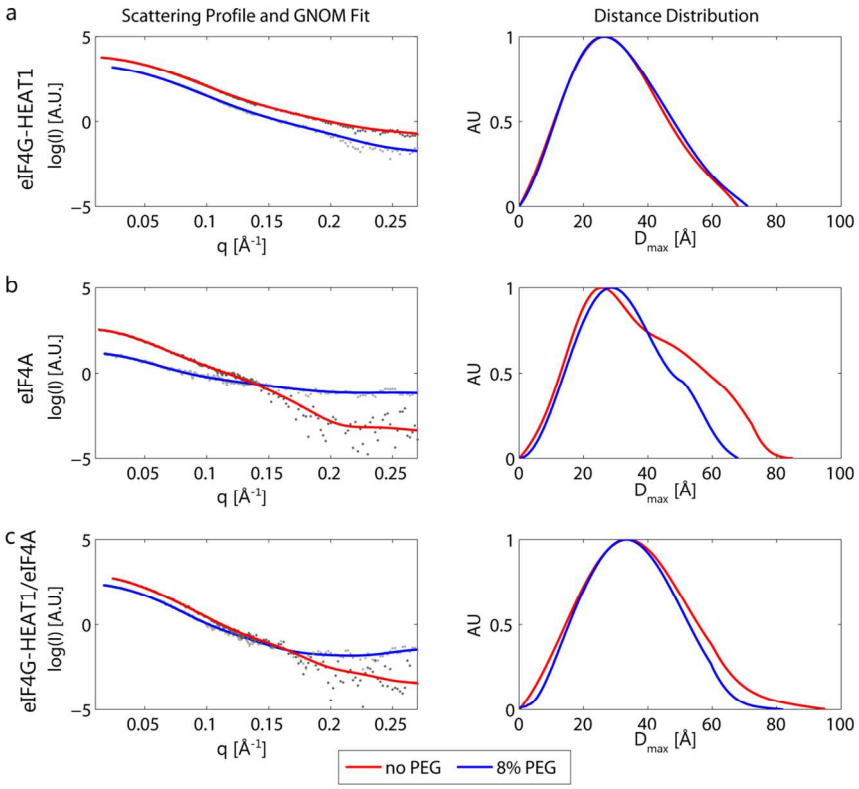

Figure S2: GNOM Fit and Distance Distribution Functions for eIF4G-HEAT 1 (a), eIF4A (b) and eIF4G-HEAT1/eIF4A (c).

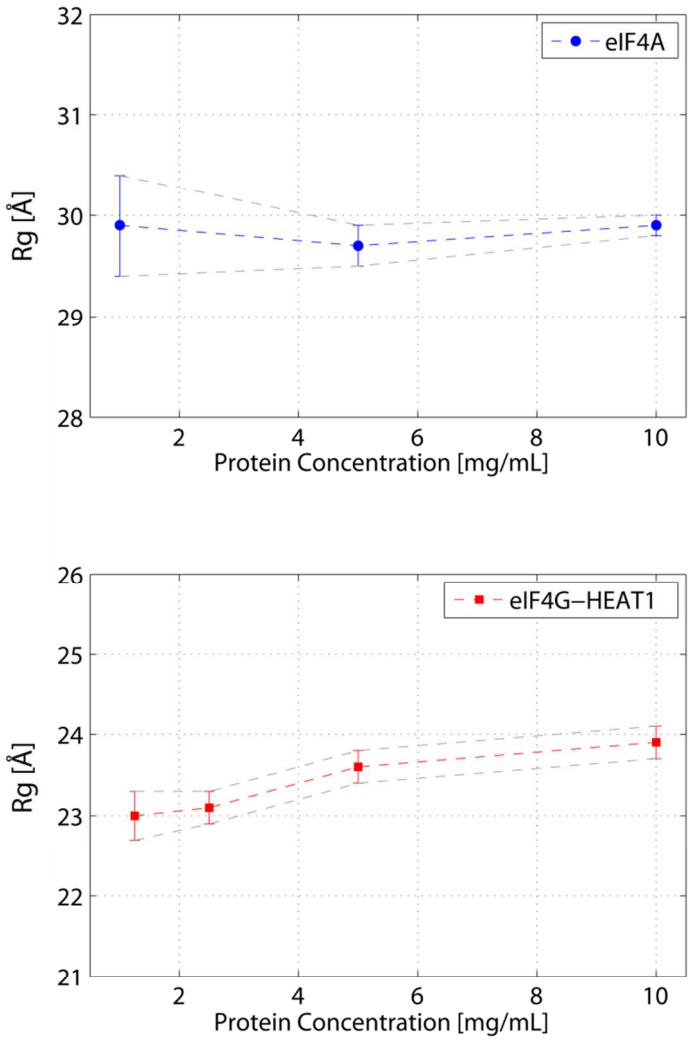

Figure S3: Concentration dependence of the radius of gyration for eIF4G-HEAT1 (red) and eIF4A (Blue). Rg values were determined from the Guinier plots of scattering profiles of samples with concentrations between 1 and 10 mg/mL.

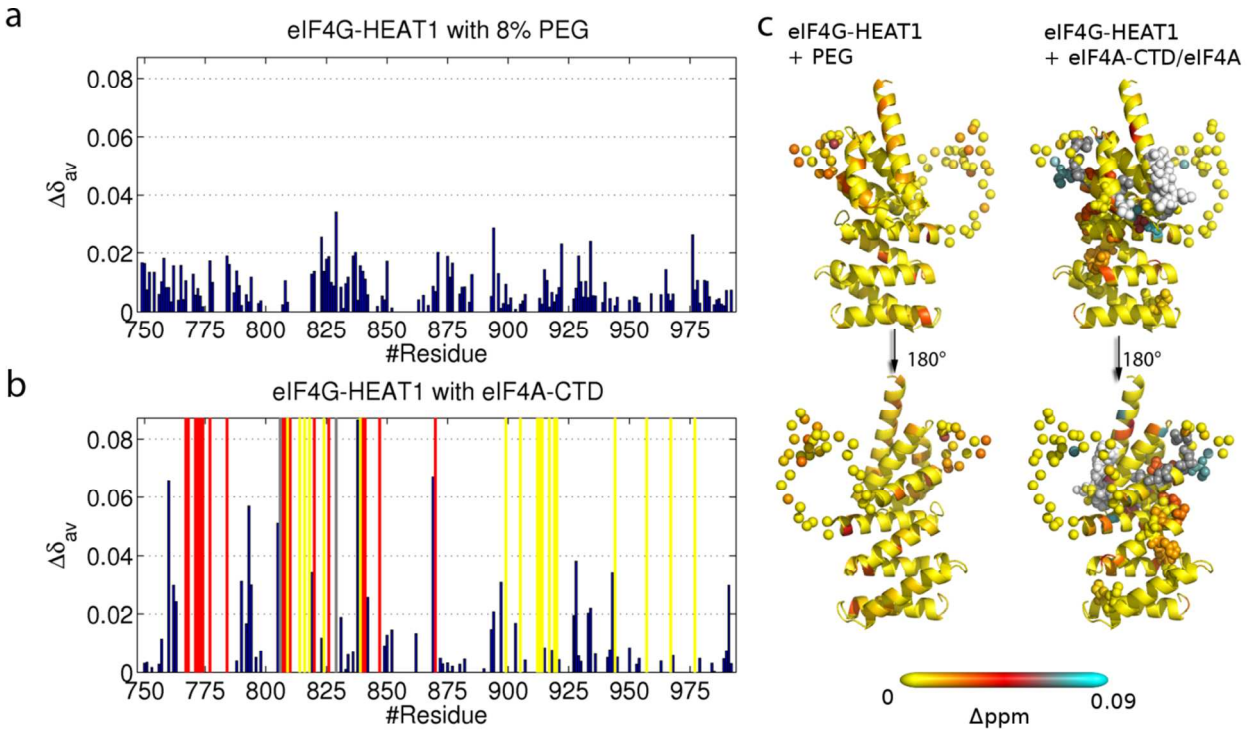

Figure S4: Chemical shift changes on eIF4G-HEAT1 upon addition of 8% PEG (a) and eIF4A-CTD (b). Chemical shift changes were calculated based on the formula $\Delta ppm=\sqrt{(\Delta H)^2+\left(\frac{\Delta N}{5}\right)^2}$ (equation S1). Red lines indicate residues that disappeared due to line broadening after addition of eIF4A-CTD. Yellow lines indicate residues that disappeared in addition after addition of full length eIF4A. Panel c shows the mapping of the chemical shift changes on the model of eIF4G-HEAT1. Residues that disappeared after addition of eIF4A-CTD or full length eIF4A are represented as spheres.

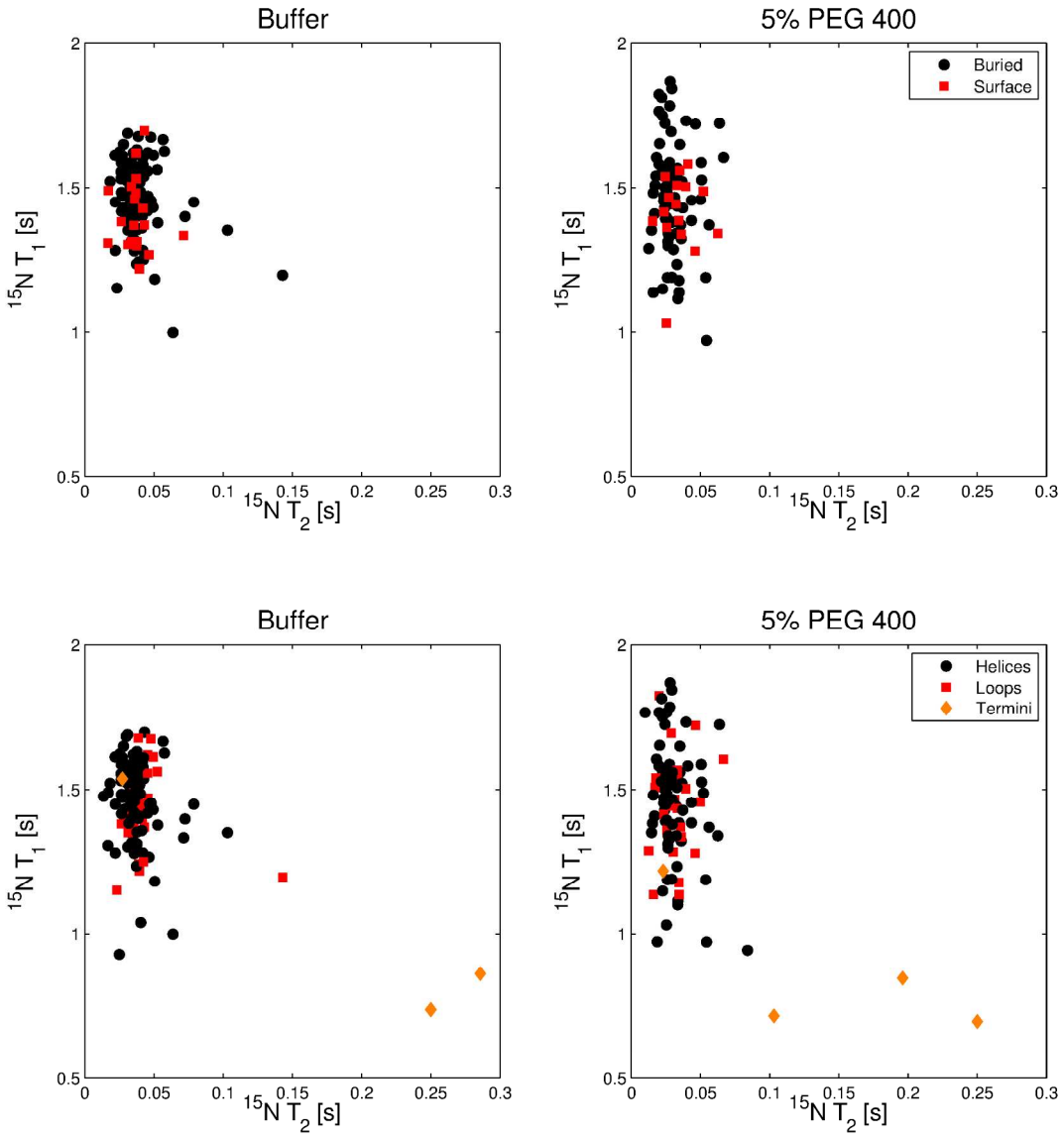

Figure S5: ^{15}N-T$_1$/^{15}N-T$_2$ plots for eIF4G-HEAT1 in buffer (left panels) and with addition of 5% PEG 400 (right panels). Color coding for the top row is according to surface accessibility (black: surface accessibility < 40%, red > 40%). Termini and the loop from 844-881 were not taken into account. Color coding in the bottom row was done according to secondary structure (black: helices, red: loops, orange: termini).