Supplementary Information for:

Effect of arginine on oligomerization and stability of N-acetylglutamate synthase

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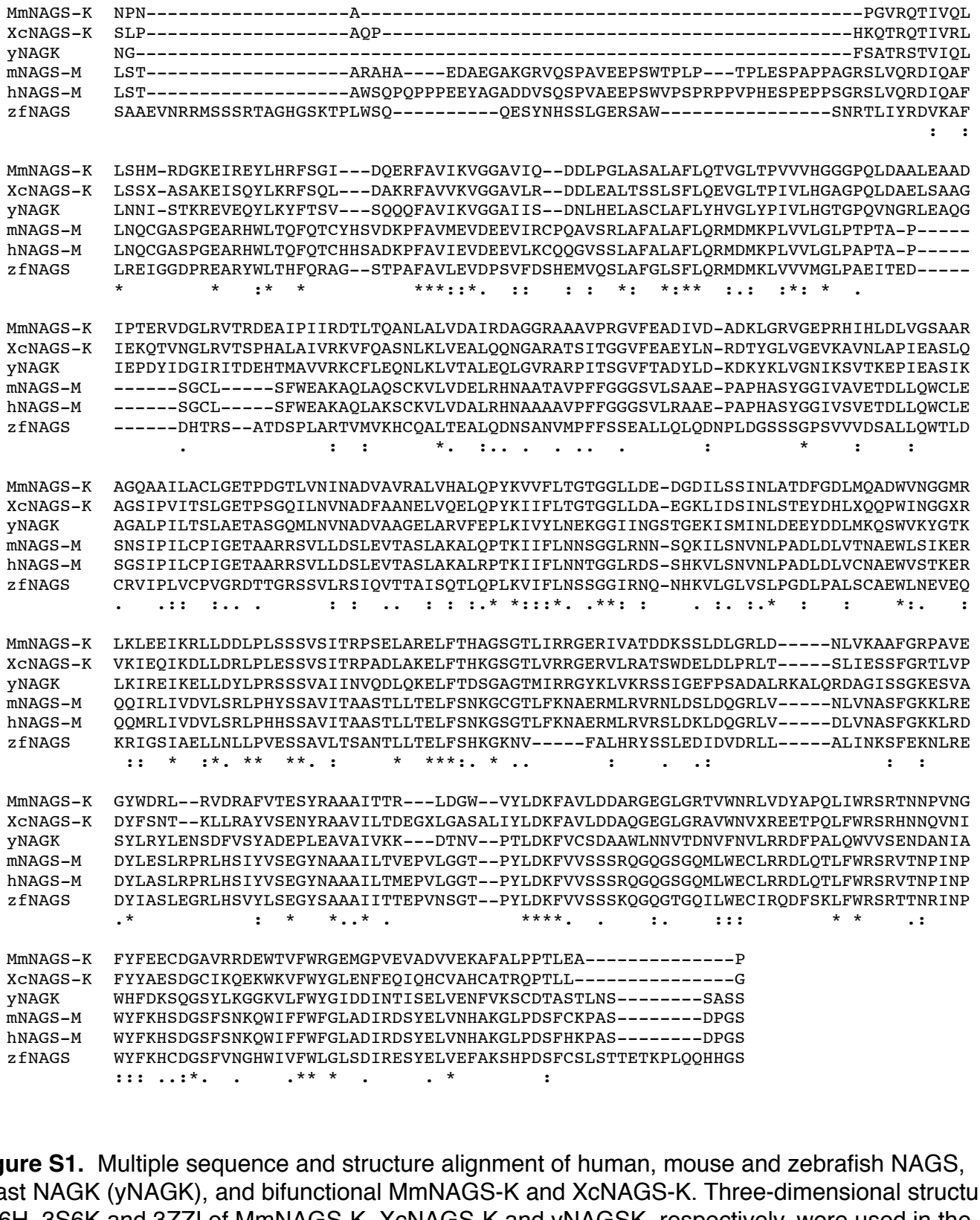

Figure S1. Multiple sequence and structure alignment of human, mouse and zebrafish NAGS, yeast NAGK (yNAGK), and bifunctional MmNAGS-K and XcNAGS-K. Three-dimensional structures 3S6H, 3S6K and 3ZZI of MmNAGS-K, XcNAGS-K and yNAGSK, respectively, were used in the alignment after manual removal of the polyhistidine affinity tag sequences. Reference sequences of human. mouse and zebrafsih NAGS (accession numbers: NP_694551, NP_665828 and XP_685919, respoctively) were used in the alighnment after manual removal of predicted mitochondrial targeting sequences. Proteins were aligned using Expresso structural alignment algorithm (www.tcoffee.org).

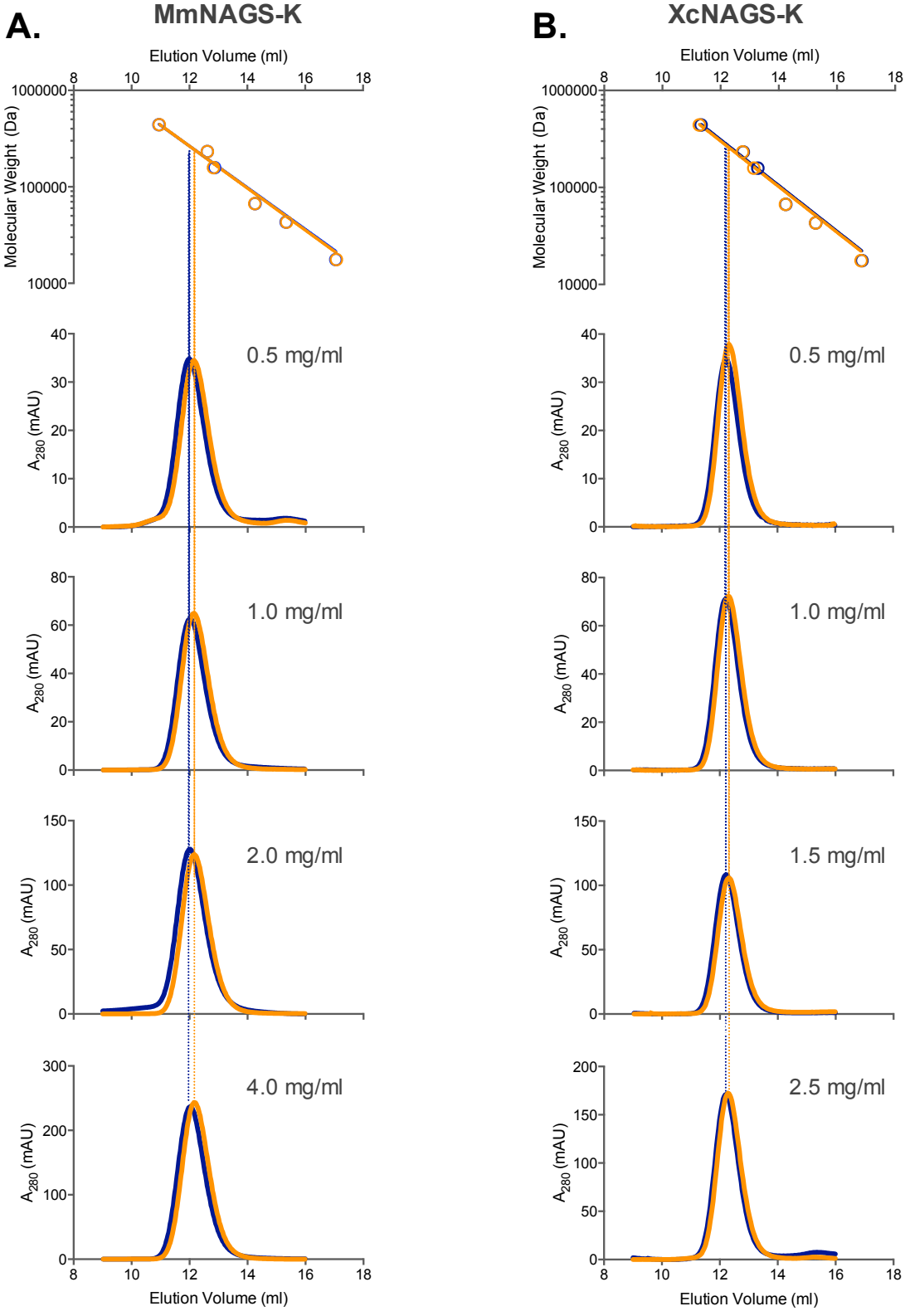

Figure S2. Analytical gel chromatography of MmNAGS-K (A) and XcNAGS-K (B) with and without L-arginine. The top panels show a semi-logarithmic plot of molecular mass vs. elution volume. Open circles correspond to elution volumes of ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), and myoglobin (16 kDa). Lower panels show absorption at 280 nm as a function of elution volume and concentration of each protein loaded on the column. Blue - elution profiles in the absence of L-arginine. Orange – elution profiles in the presence of 1 mM L-arginine.

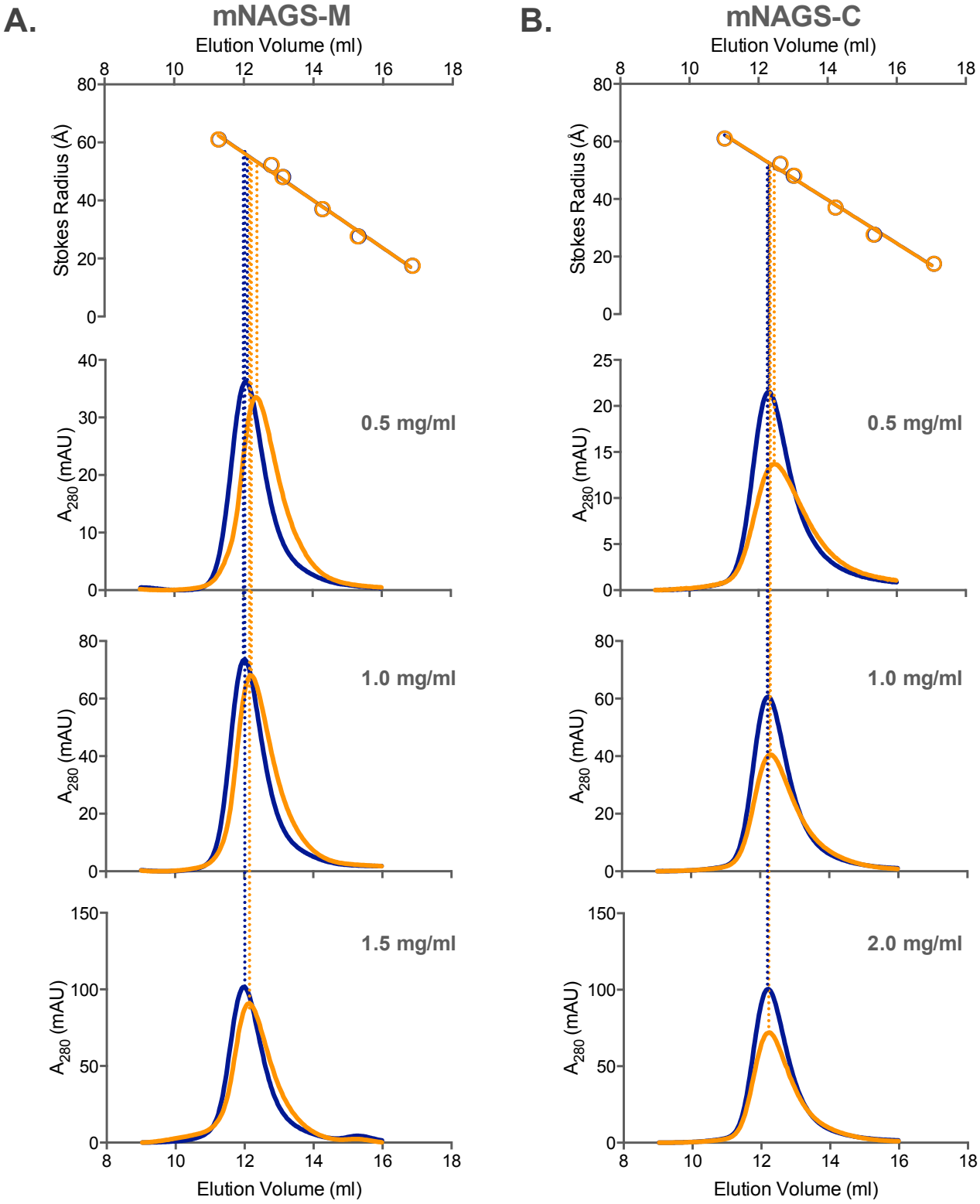

Figure S3. Analytical gel chromatography of mNAGS-M (A) and mNAGS-C (B) with and without L-arginine. The top panels show plots of Stokes' radii vs. elution volume. Open circles correspond to elution volumes of ferritin (61.0 Å), catalase (52.2 Å), aldolase (48.1 Å), bovine serum albumin (37.0 Å), ovalbumin (27.6 Å), and myoglobin (17.5 Å). Lower panels show absorption at 280 nm as a function of elution volume and concentration of each protein loaded on the column. Blue - elution profiles of mNAGS-M and mNAGS-C without arginine. Orange – elution profiles of mNAGS-M and mNAGS-C in the presence of 1 mM arginine.

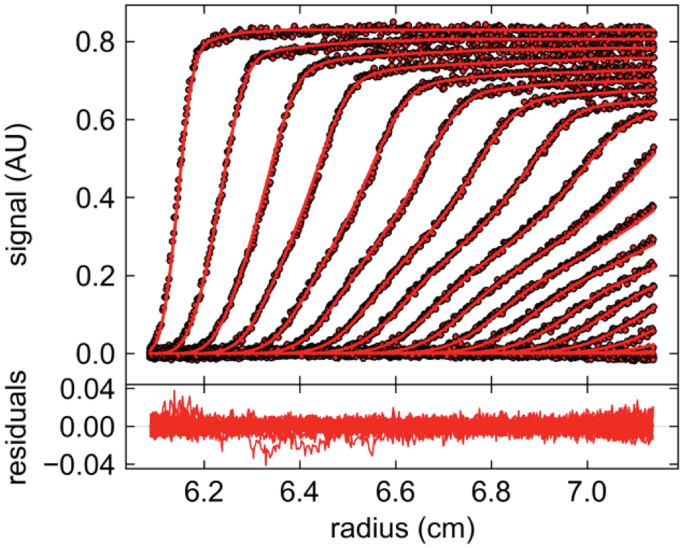

Figure S4. Raw sedimentation velocity profile (black circles) for Xc-NAGS (0.64 mg/mL, which corresponds to 12 μM monomer concentration) in the absence of L-arginine in overlay with the best-fit curves resulting from the c(s) sedimentation coefficient distribution analysis (upper panel).
The residuals from the c(s) analysis with root-mean-square differences of 0.0063 OD (lower panel).

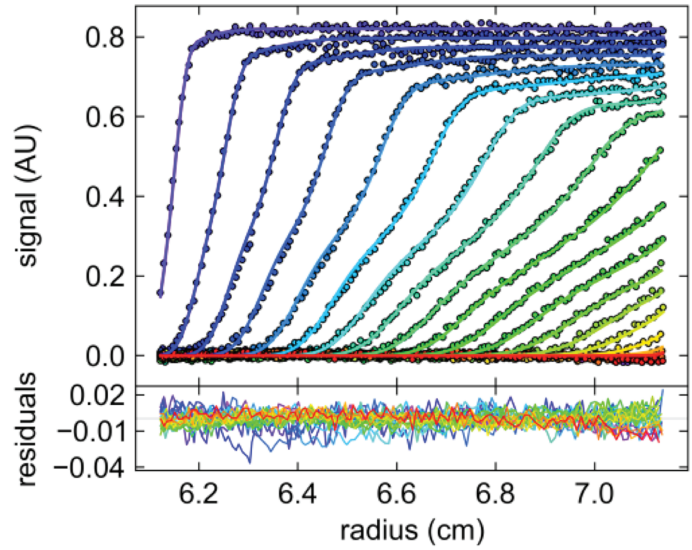

Figure S5. The raw sedimentation velocity profile for XcNAGS (0.64 mg/mL, which corresponds to 12 μM monomer concentration) in presence of 1 mM arginine (circles) overlayed by the best-fit curves resulting from a Global analysis of seven data sets at several loading concentrations (solid lines) fit to a tetramer-octomer association model using explicit Lamm Equation solutions with reaction kinetics. The best fit Kd is 2.6 μM [2.2 – 3.1].