SUPPLEMENTARY INFORMATION: IMPACT OF MACROMOLECULAR CROWDING ON DNA REPLICATION.

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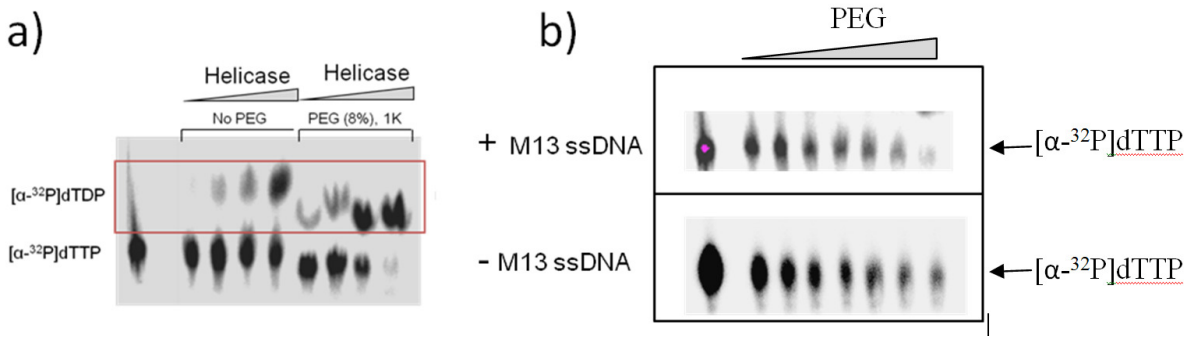
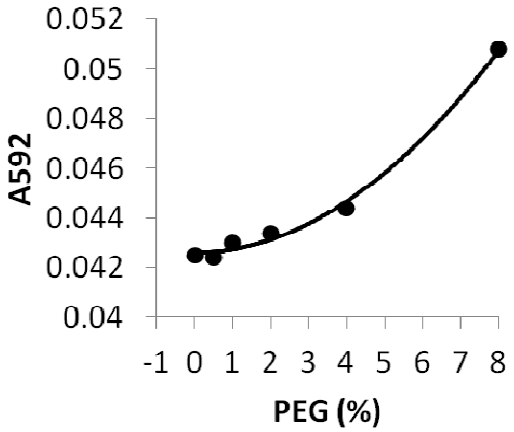
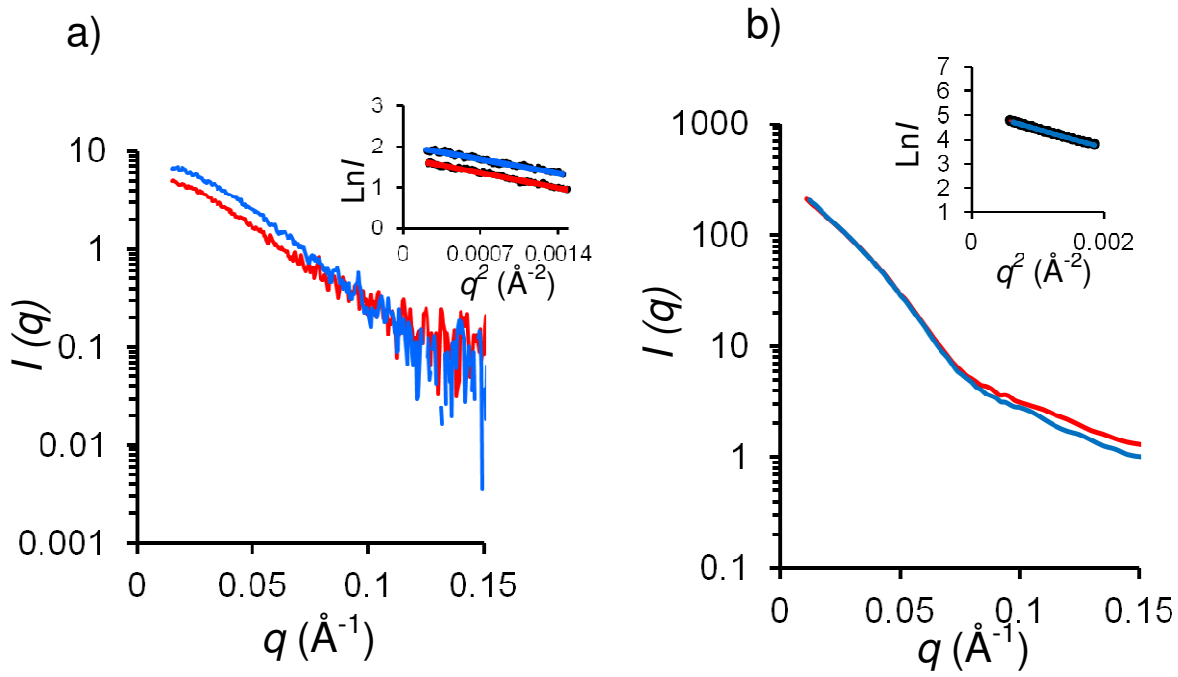


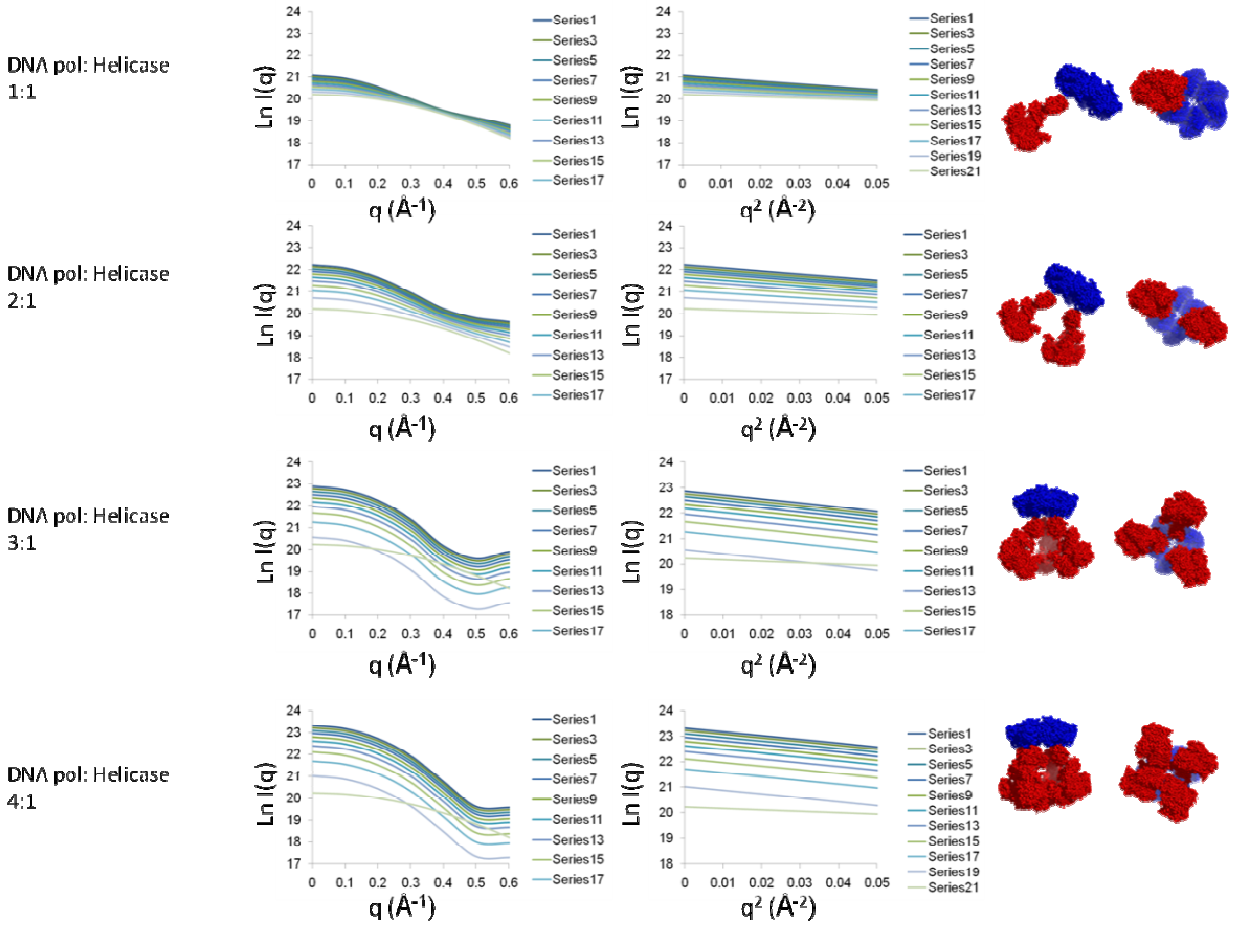
Supplementary Figure S1: The effect of PEG on the dTTPase activity of gp4. (a) Raw results for the experiments presented in Fig. 4(c). Reaction mixture contained 1 nM M13 ssDNA, 5 mM dTTP, 0.1 mCi of [α–32P] dTTP, and increasing amounts of gp4B (0-200 nM) and 8% PEG. After incubation for 10 minutes at 37 °C the reaction was halted with 40 mM EDTA and 0.4 µL of the reaction mixture was spotted onto PEI cellulose TLC plate and developed using 0.5 M LiCl, 0.5 M sodium formate. Spots on TLC plate were visualized by autoradiography. (b) Comparison between the amount of [α–32P] dTTP remain at the end of dTTP hydrolysis in DNA dependent or independent manner by gp4. Reaction mixture contained 5 mM dTTP, 0.1 mCi of [α–32P] dTTP, gp4B (monomeric, 200 nM), and increasing amounts of PEG(0-8%) in the presence or absence of 1 nM M13 ssDNA. After incubation for 10 minutes at 37 °C the reaction was halted with 40 mM EDTA and 0.4 µL of the reaction mixture was spotted onto ESI cellulose TLC plate and developed using 0.5 M LiCl, 0.5 M Na–formate. TLC spots were analyzed using autoradiography. The decrease in the remaining [α–32P] dTTP at the end of both reactions indicates that PEG linearly increases the DNA-dependent dTTPase activity of gp4.



Supplementary Figure S2: The effect of PEG on the turbidity of gp5/trx. Gp5/trx (10 µM) was measured in buffer containing 40 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 50 mM potassium glutamate, and increasing amounts of PEG (0-8%). Turbidity assay measures the light that is scattered by the microscopic suspended particles. Increase in turbidity after 3 hours at 25 °C was measured and indicates on the formation of larger particles than the wavelength used due to protein precipitation.



Supplementary Figure S3: Scattering curves and Guinier plots for gp5/trx and gp4D
(a) Experimental SAXS data and the corresponded Guinier plots (inset) from gp5/trx in a buffer containing 20 mM Tris–HCl (pH 7.5), 50 mM potassium glutamate, 2 mM DTT, and 10% Glycerol, in the presence or the absence of PEG 1kDa 4%. The Guinier plots for gp5/trx are presented and are compared with gp5 lacking the thioredoxin-binding loop (gp5Δ69) measured in the absence of PEG. Red: +PEG, Blue: No PEG (b) SAXS results of gp4D in the presence (red) or the absence (blue) of PEG 4%. Sample buffer is the same as in (a).



Supplementary Figure S4: Theoretical scattering data for varying ratios of polymerase:helicase.
The theoretical small angle scattering curves were calculated from putative structural models of the T7 replisome with different arrangements of the polymerase vs. helicase hexameric ring using the software CRYSOL (Svergun, D.I., Barberato, C. & Koch, M.H.J., 1995, J. Appl. Cryst. 28, 768-73) and the available crystal structures of gp5/trx and gp4D.
Each spectrum represents the sum of scattering contributions from the complex (1-x) and free gp4D.