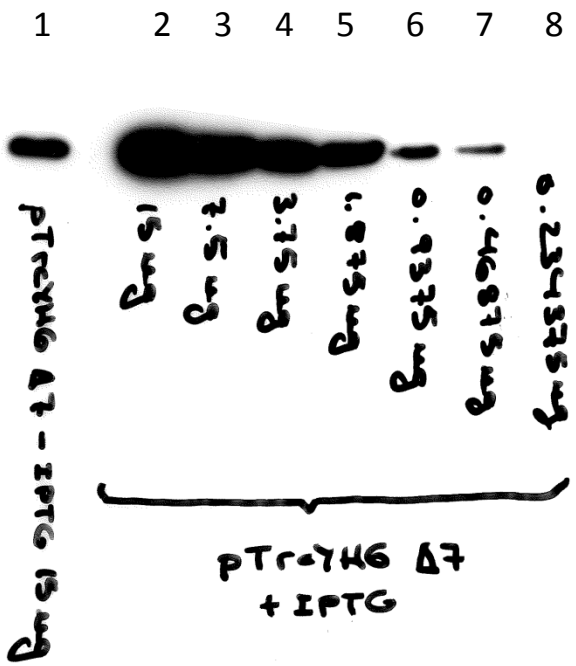

Figure S1. Detection levels of MscS in total membrane preparations. The plasmid pTrcYH$_6$ expressing C-terminally His-tagged MscS was transformed into MJF641 (Δ7) and total membrane proteins were harvested from cells grown in a minimal citrate-phosphate medium pH7. An overnight culture was grown with 0.04% glucose and in the next day supplemented with 0.2% glucose until OD_{650} = 0.4. A subsequent 100x dilution was performed and growth continued until cells reached OD_{650} = 0.4. The culture was split and cells one portion of cells was induced for expression of MscS by addition of 1 mM IPTG for 30 min prior to harvest. No growth inhibition resulted from expression and the two portions of culture (induced and uninduced) were harvested in parallel. Total membrane proteins were prepared as previously described in EXPERIMENTAL PROCEDURES. Lane 1: 15µg of uninduced MscS. Lanes 2 to 8: 2-fold serial dilutions (15 µg to 0.23 µg protein) of IPTG-induced samples in PBS. Volumes loaded per lane were kept constant (15µl). Samples were separated by SDS/PAGE 12% acrylamide gels and the MscS protein recognized by anti-His HRP conjugated antibody and the ECL substrate Supersignal® West Dura Extended Duration. Figure shown is from a single gel/blot/film after 1 min exposure time. The approximate mass of MscS was 20 kDa.

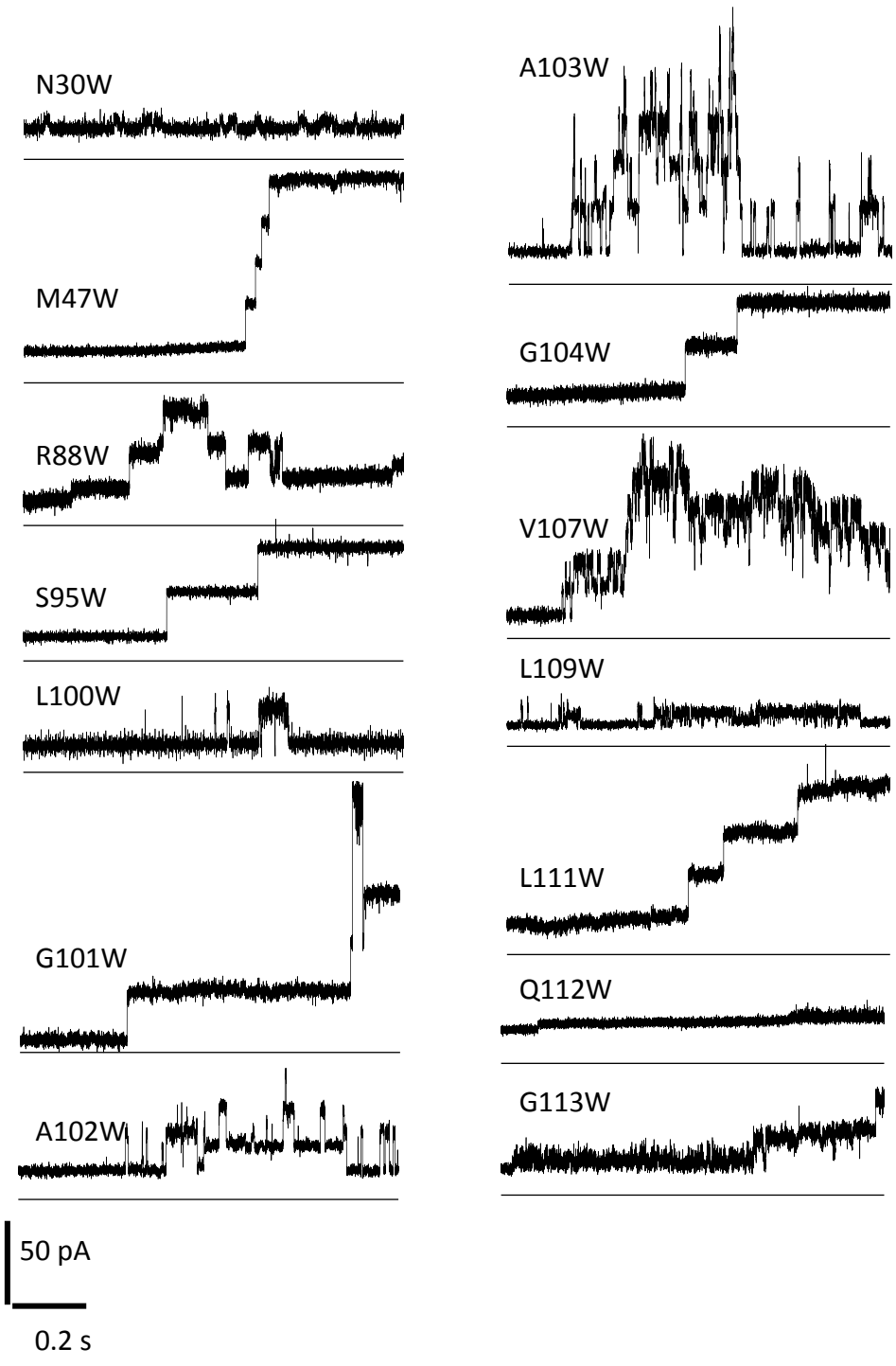

Figure S2. The figure shows typical traces for the Trp mutants created in this study but not shown in the main body of the paper. Details as described in Experimental Procedures.

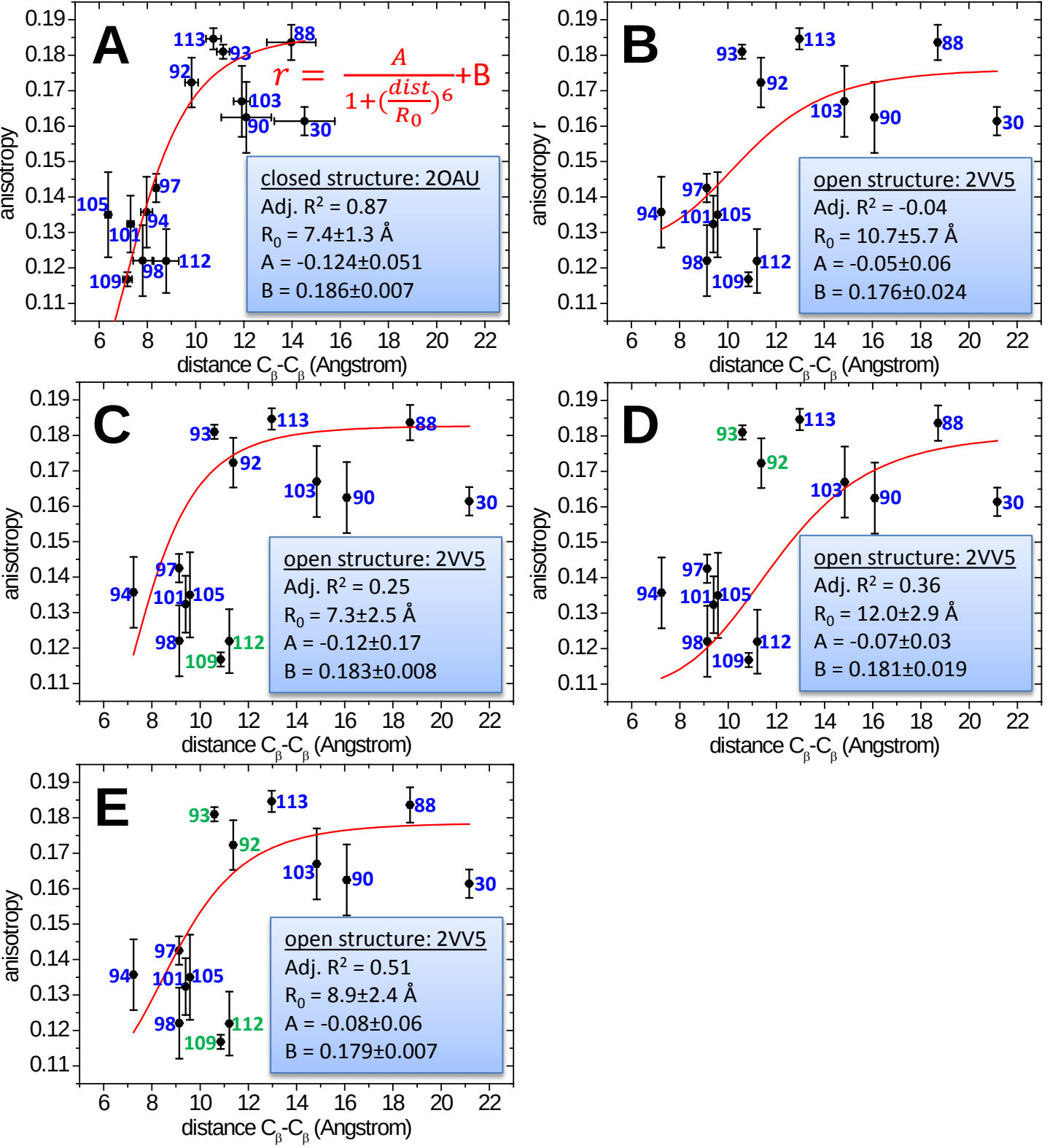

Figure S3: Steady state anisotropy of tryptophan plotted against the distance. Distances of side chain C_β-atoms between the same residue on neighbouring subunits were obtained from the closed (2OAU) and open (2VV5) crystal structures and plotted against the anisotropy r. The data (residue number in blue) were fitted assuming homoFRET between the tryptophan residues (red equation), where R_0 is the Förster distance while A and B are scaling factors. The goodness of fits are represented by the adjusted R^2 values and the fitted parameters are given with the standard errors of fitting. It can be seen that the fit for the closed structure (A) is better than for the open structure (B). Additional fit are shown for the open structure where the green points were not considered (C to E).

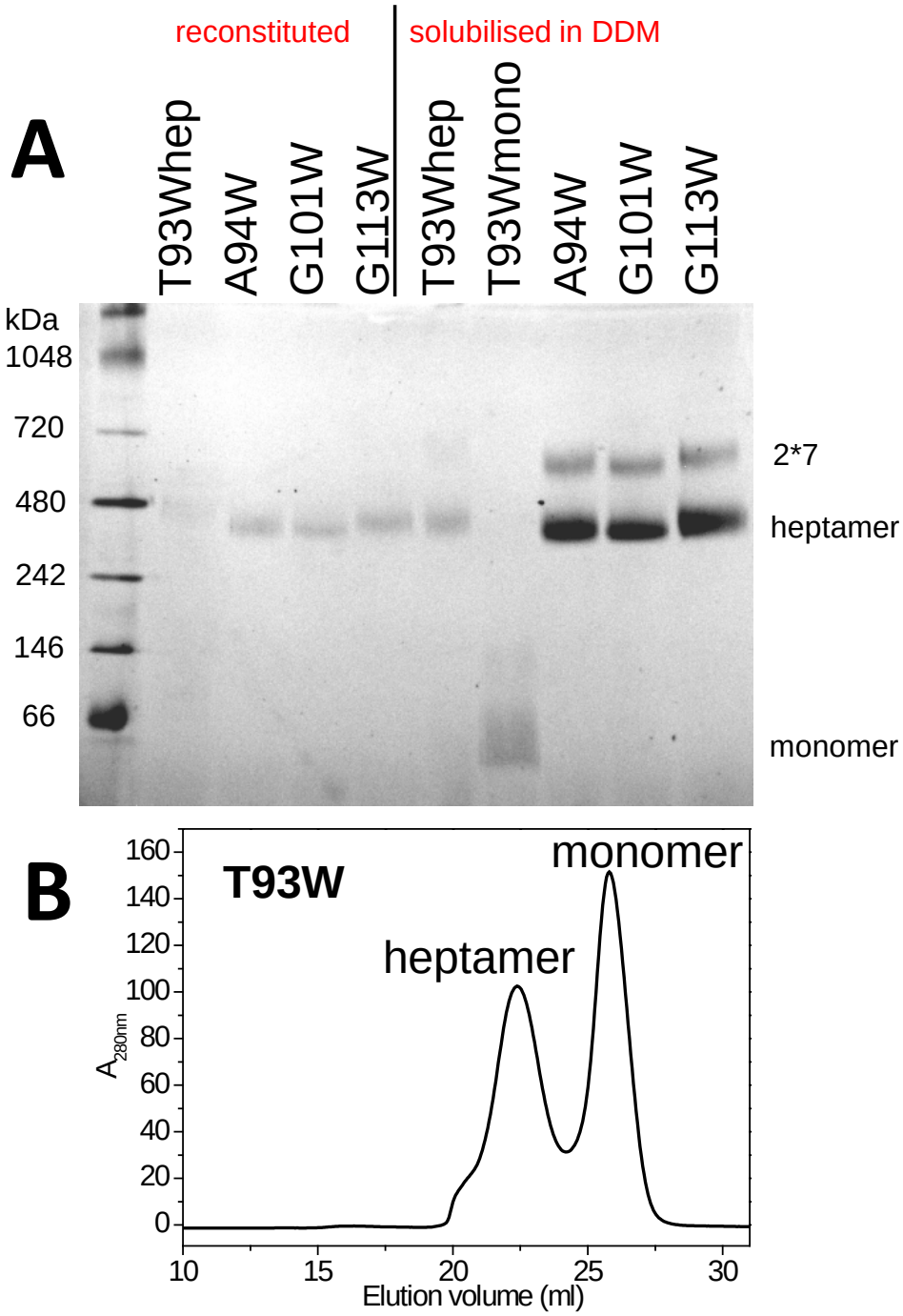

Figure S4: Blue native PAGE of selected MscS mutants. (A) MscS samples of selected mutants were directly taken from peak fraction after SEC chromatography (right side of gel), For the mutant T93W, samples for the heptameric complex as well as dissociated monomers were taken (see B). MscS was reconstituted and fluorescence spectra were measured. Afterwards the samples were concentrated with Microcon YM-10 spin filters (Millipore), re-solubilised in DDM and taken as samples for the BN gel (left side of the gel) which showed still heptameric complex composition. A NativePAGE Novex 4-16% gel (LifeTechnologies) was used as described by the manufacturer and further coomassie-stained afterwards (GelCode Blue, Thermo Scientific). MscS complex masses can be estimated with the help of the soluble marker proteins (first lane; NativeMark, Life Technologies) and a correction factor of 1.8 for bound dye and detergent (Rasmussen et al., 2007; Heuberger et al., 2002). (B) MscS YFF T93W was separated on a Superdex200 10/300 GL column.

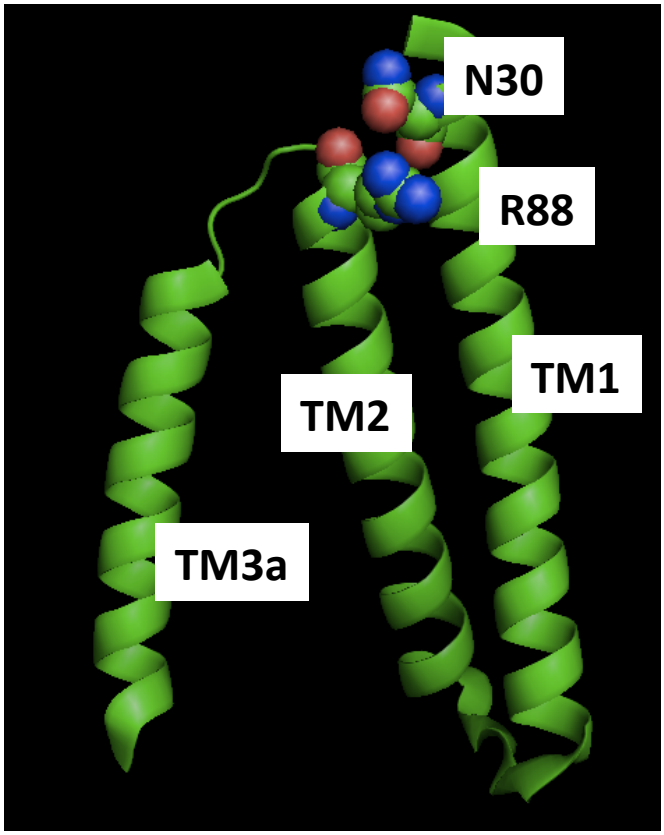

Figure S5. Tryptophan scanning mutagenesis of *E. coli* MscS. The image (created in Pymol) illustrates the proximity of N30 and R88 on TM1 and TM2, respectively.