Table S1

buffer A	50 mM Tris-HCl pH 7.3, 100 mM NaCl, 50 mM $(NH_4)_2SO_4$, 2 mM $CaCl_2$, 1mM DTE
buffer G	200 mM NaCl, 50 mM NaH_2PO_4/Na_2HPO_4 pH 7.4, 1 mM TCEP
buffer H	10 mM Tris-HCL pH 7.0, 2% (w/v) SDS, 0.02 % (w/v) bromphenol blue, 0.2 % (v/v) β-mercaptoethanol, 2.8 % (w/v) DTE, 2% (v/v) glycerol
buffer B	50 mM Tris-HCl pH 8.0, 100 mM NaCl and 1 mM DTE
buffer C	50 mM Tris-HCl pH 8.0, 1000 mM NaCl and 1 mM DTE
buffer D	200 mM NaCl, 50 mM Hepes pH 7.5, 1 mM DTE
buffer E	300 mM NaCl, 50 mM Hepes pH 7.5 and 10 mM TCEP
buffer I	150 mM KCl, 5% glycerol and 50 mM Hepes pH 7.5
buffer J	200 mM NaCl, 50 mM Hepes pH 7.5, 0.5 mM TCEP

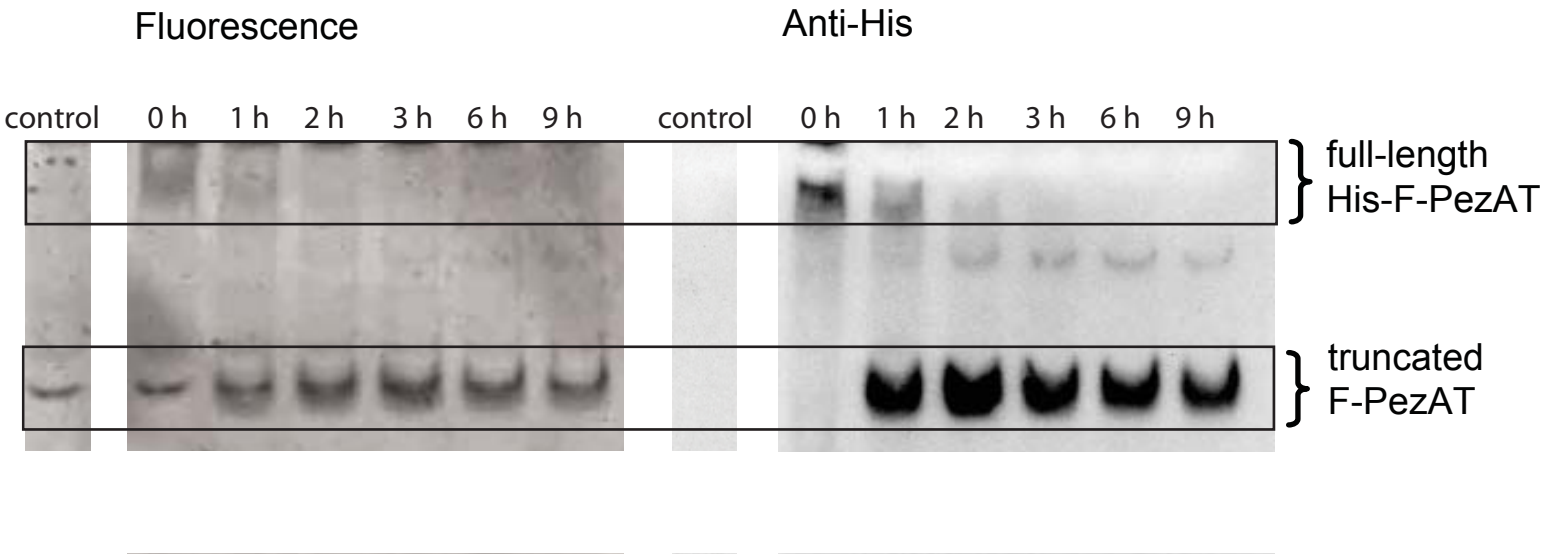


Figure S1 – Native PAGE of His-F-PezA/PezT(D66T) complex processing in *B. subtilis* raw extract. Protein incubation and visualization procedures were the same as described for SDS-PAGE in the experimental procedures section. Native-PAGE was performed in 15 % polyacrylamide gels in 25 mM Tris pH 9.0 and 0.2 M glycine. Note that full-length His-F-PezAT entered the gel only poorly leading to decreased fluorescence and chemiluminescence. The truncated PezAT core complex could be visualized by IAEDANS fluorescence indicating the presence of truncated PezA and Anti-His-HRP chemiluminescence indicating the presence of PezT(D66T). The controls containing lysate only showed a fluorescent band in absence of either PezT(D66T) and His-F-PezA.

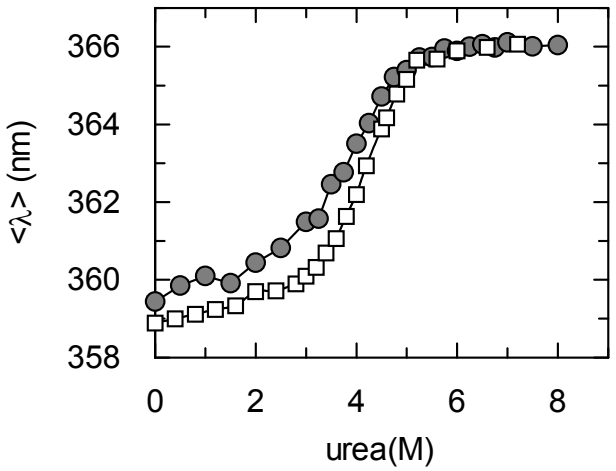
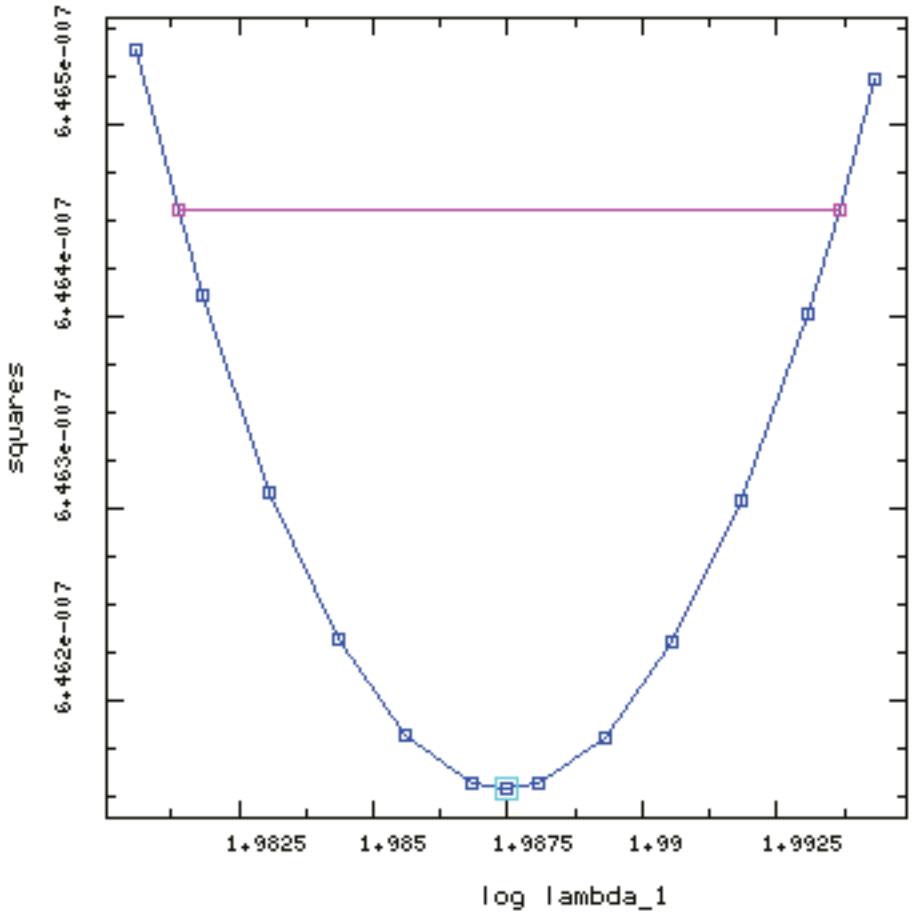


Figure S2 – Concentration dependency of the red-shift of tryptophan emission during urea induced PezA denaturation using urea. 3 μM PezA (grey) shows a slightly broader transition mid-point shifted to lower urea concentrations compared to 5 μM PezA (white). This indicates that a part of the overall signal changes originates from a urea induced pertubation of the PezA homodimer equilibrium prior to general PezA unfolding.

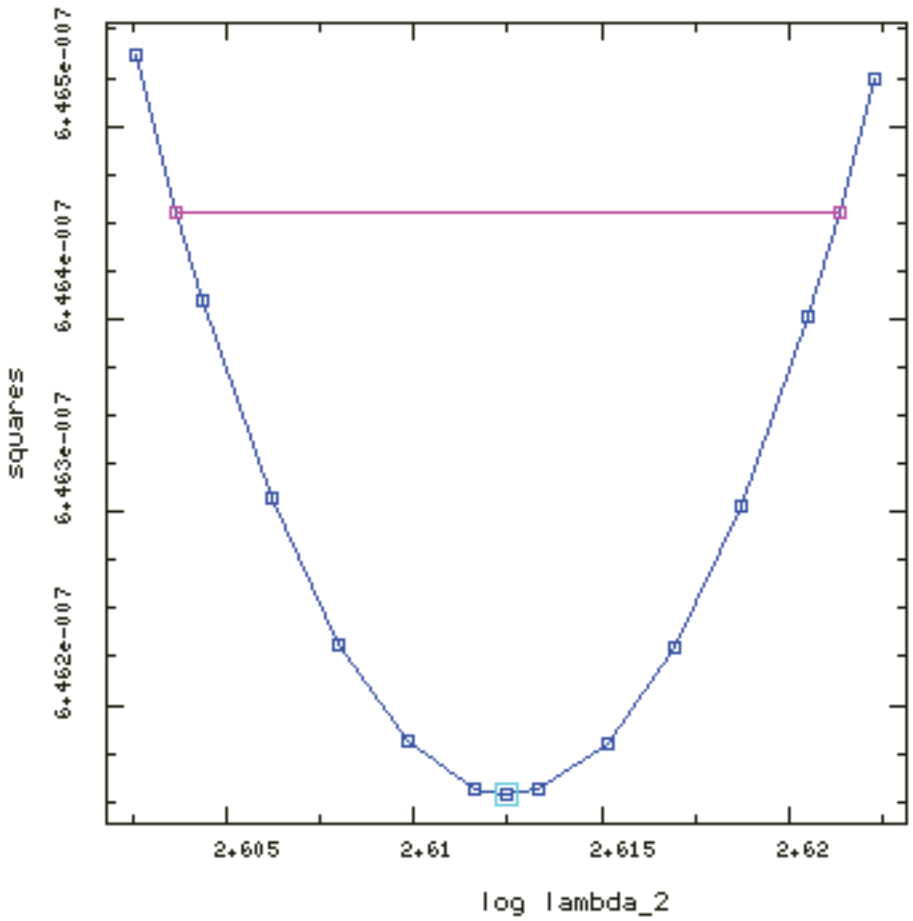


λ_1

best fit value:
97.2 μM^{-1} s^{-1}

standard error:
0.7 μM^{-1} s^{-1}

95% confidence interval:
[95.8; 98.6] μM^{-1} s^{-1}



λ_2

best fit value:
410 s^{-1}

standard error:
4 s^{-1}

95% confidence interval:
[401; 418] s^{-1}

Figure S3 – Confidence intervals and best fit characteristics of the apparent rates λ_1 and λ_2 obtained by the global fit of the simplified two-step model of PezAT association model using DynaFit 4.0 .

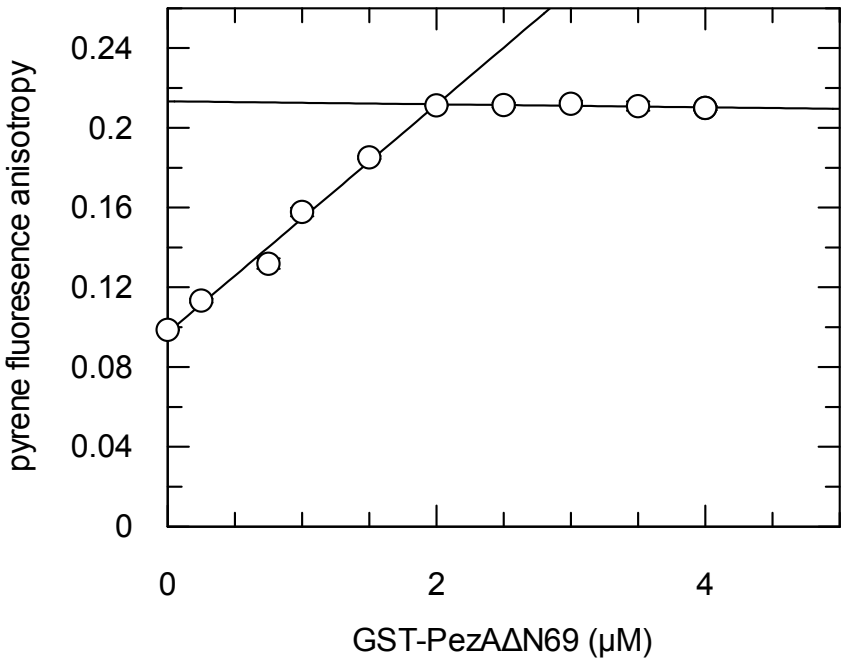


Figure S4 – Titration of GST-PezAΔN69 with PezT(D66T)$_{Pyr}$. PezT(D66T) was labeled at cysteine 209 with N-(1-pyrene)maleimide (Invitrogen) in a similar manner as described for Alexa488-maleimide. The PezA three helix bundle without the N-terminal repressor domain (PezAΔN69) was fused to a glutathion-S-transferase tag using the pGEX-4T-1 vector system and purified similarily as described for full length PezA with the expection that the initial purifications step was performed using a glutathion sepharose column (GE Healthcare) instead of a Ni-affinity column. The purified fusion construct was titrated to 2 μM of PezT(D66T)$_{Pyr}$ and pyrene fluorescence anisotropy recorded in a FluoroLog-3 Spectrofluorometer (HORIBA Scientific) at an excitation wavelength of 343 nm and an emission wavelength of 374 nm. The binding curve indicates a tight sub-nanomolar affinity of GST-PezAΔN69 to PezT(D66T)$_{Pyr}$ implying that the PezA three helix bundle is sufficient to establish strong binding to PezT.