Supplementary Material

EMSA with the cross-linked dimeric forms of TraA and $TraAN_{246}$

The dimeric forms of the proteins were prepared by chemical cross-linking. Reaction volumes of 100 µl were used, containing 100 µg of protein, 100 mM Bicine, pH 7.5, 300 mM NaCl, 1 mM dithiothreitol and 0.1% (v/v) glutaraldehyde. The reaction was stopped after 15 min by adding 1 M glycine, pH 8.0, to a final concentration of 140 mM. The samples were incubated for another 5 min, and stored at 4°C.

The amount of cross-linked proteins was examined by SDS/PAGE. The TraA solution consisted of ~70% dimeric form and 30% higher-order oligomers, whereas the $TraAN_{246}$ solution was composed of ~30% monomeric and ~70% dimeric forms.

The [^{32}P]ATP-labelled 42-mer was subjected to EMSA with the cross-linked dimeric forms and native forms of both proteins. The reaction mixtures were prepared in the same way as the samples used for the K_D determination of the complexes (see Figure 7 of main paper) and loaded on to a 10% (w/v) native polyacrylamide gel. The final concentration of the native protein was 1.25 µM. The same amounts of native and cross-linked dimeric protein were used for the experiment.

Supplementary Figure 1 Dimers of TraA and $TraAN_{246}$ bind the *oriT* DNA

A concentration of 0.5 nM 42-mer was incubated with 1.25 µM native protein or 625 nM dimeric protein at 42°C for 30 min. Lane 1, free DNA; lane 2, 42-mer + native $TraAN_{246}$; lane 3, 42-mer + dimeric $TraAN_{246}$; lane 4, free DNA; lane 5, 42-mer + native TraA; lane 6, 42-mer + dimeric TraA.