Zhang *et al.*, Supplemental data

Fig. S1. **TolQ interacts with TolR and forms multimers.** (A) *In vivo* formaldehyde (FA) cross-linking of TPS13 (*tolQR*) cells producing the indicated TolR variants (TolR-P37A and TolR deleted of its C-terminal region, TolR-ΔC, described in ref.42) and TolQ proteins. TolR-ΔC is lacking the C-terminal 25 residues, whereas TolR-P37A displays a mobility defect. 0.4×10^8 cells treated by FA were loaded on 12.5%-acrylamide SDS-PAGE and proteins were immunodetected using anti-HA mAb. The TolQR (QR), TolQ dimer (2Q), a and b (see fig. 1) complexes are indicated.

Fig. S2. **Comparative phenotypic analyses of the *tolQ* TMH2-3 point mutants.** (A) Phenotypes of mutations affecting small and polar residues of TolQ TMH-2 and -3 (extracted from Ref. 18). The substituent residue is indicated outside the helical wheel. (B) Phenotypes of cysteine mutants of TolQ TMH-2 and -3. Phenotypes are reported by a color code: WT phenotype (blue, colicin sensitive and DOC resistant), *tol* phenotype (red, colicin resistant and DOC sensitive) and discriminative phenotype (cyan, colicin and DOC sensitive). On the 17 common residues targeted, 7 gave comparable results whether it is substituted by Cys or another residue. Among the 10 that differ, 8 can be explained by the difference of characters (charge, length,...) of the side chain: the P138C substitution led to the presence of a nucleophilic side chain that is not present when P138 is replaced by Val; T145C kept the nucleophilic character of the Thr residue (lost by the T145A substitution); the replacement of A152 by a charged Glu residue is stronger than the A152C mutation (note that a A152L mutant displayed a WT phenotype; 18); the side chain length differences between the G181A and G181C substitutions, and between the A184I and A184C substitutions might explain the differences (the larger substitution having stronger negative effects); the A185 residue [which contact the TolR-D23 residue; 18] is probably more sensitive to replacement by Asp than by Cys; although G148C and A177C are indicated with a discriminative phenotype in panel (B), they present a turbid phenotype for colicin sensitivity (see Table 1) and have therefore an intermediate phenotype between *tol* and discriminative. The differences obtained for the G157 and E173 substitutions can not be explained by the differences of character of the side chains.

Fig. S3. **Cysteine scanning of TolQ TMHs.** NEM-treated membrane extracts from 0.4×10^8 TPS13 (*tolQR*) cells producing TolR and the $TolQ_{HA}$ single cysteine variants were loaded on a 12% SDS-PAGE and analyzed by western-blot immunodetections using anti-HA mAb. The position targeted is indicated below each corresponding lane. Pre-stained molecular mass markers (in kDa) are indicated.

Fig. S4. **Tandem cysteine scanning of TolQ TMHs.** NEM-treated membrane extracts from 0.4 × 10^8 TPS13 (*tolQR*) cells producing TolR and the $TolQ_{HA}$ tandem cysteine variants were loaded on a 12% SDS-PAGE and analyzed by western-blot immunodetections using anti-HA mAb. The positions targeted are indicated below each corresponding lane. Pre-stained moleculer mass markers (in kDa) are indicated.

Figure S1

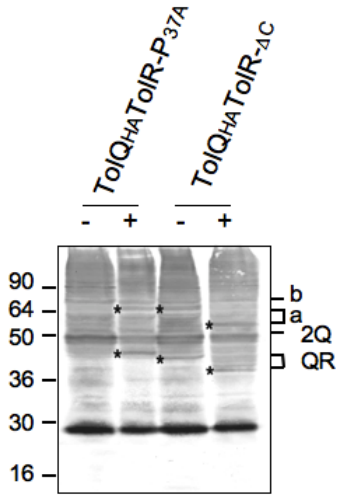

Figure S2

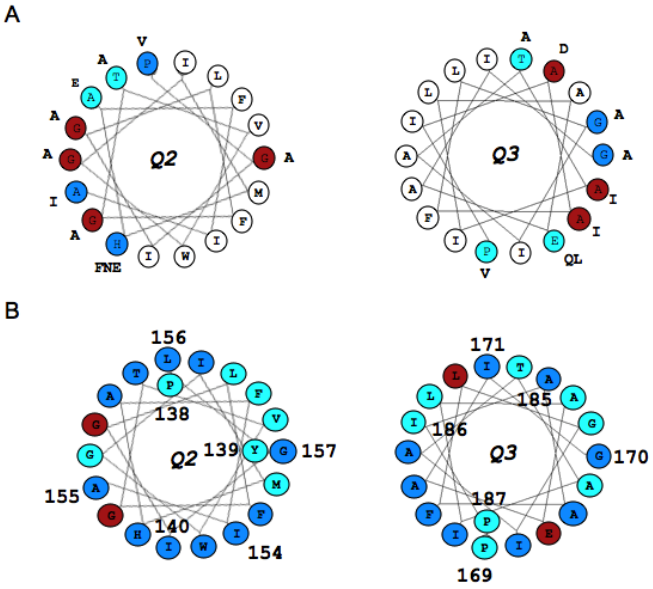

Figure S3

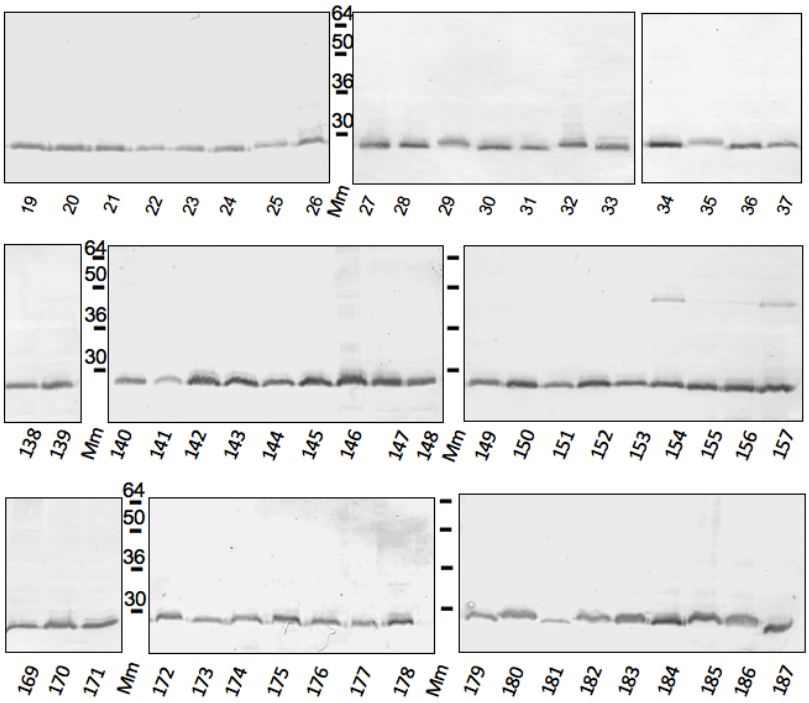
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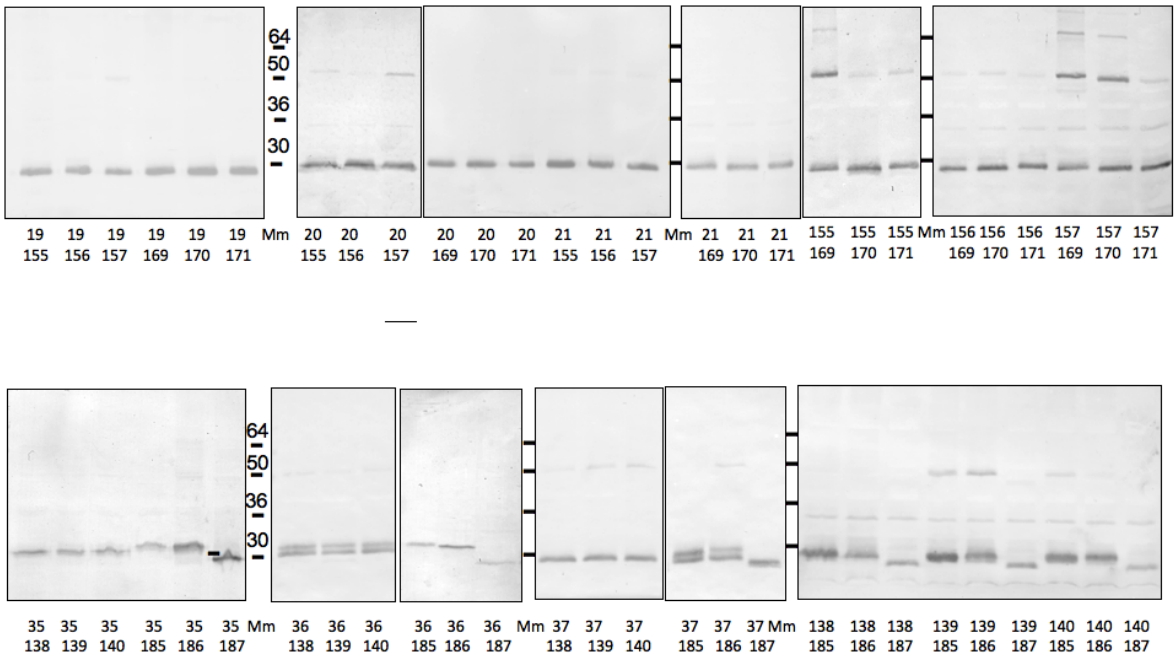
Figure S4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