Supplemental Material

FUNCTIONAL AND STRUCTURE-BASED MUTATIONAL DISSECTION ANALYSIS OF THE *SALMONELLA* EFFECTOR SIFA

Lautaro Diacovich, Audrey Dumont, Daniel Lafitte, Elodie Soprano, Aude-agnes Guilhon, Christophe Bignon, Jean-Pierre Gorvel, Yves Bourne and Stéphane Méresse

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Stoichiometry of the SifA and PH complex- Molecular mass was estimated by size exclusion chromatography using an ÄKTA basic high-performance liquid chromatograph (Amersham). Samples containing 0.4 mg of SKIP(PH), 0.4 mg of SifA, or 1 mg of a mixture of both proteins were loaded onto a Superdex S75 column (Amersham) pre-equilibrated in 10 mM Tris, pH 7.6, 150 mM NaCl, and 1 mM DTT and eluted with the same buffer. The column was calibrated with the following molecular mass standards: blue dextran: 2,000 kDa; bovine serum albumin: 67 kDa; Ovalbumin: 43 kDa; chymotrypsin: 25 kDa; and ribonuclease A: 13,7 kDa. Fractions were analyzed by SDS-PAGE.

Isothermal titration calorimetry- The binding isotherm was obtained at 25 °C using the MCS-ITC instrument (Microcal, Northampton, MA). Successive amounts of 10 µl of SKIP(PH) (310 µM) were injected, using a 250 µl syringe, in the calorimetric cell containing SifA (30 µM) or SifA orthologs until complete saturation. Purified proteins were in the same buffer (10 mM Tris pH 7.6, 150 mM NaCl). Experiments were repeated three times. The same experiment was performed without the protein in the calorimetric cell to subtract the background noise. The experimental data were fitted using the original ITC software assuming one set of site. The binding enthalpy (ΔH) and association constant *Ka* were then directly obtained from the isotherm binding. ΔG and ΔS were calculated using the standard equation $\Delta G = - R.T.\ln Ka$ and $\Delta H = \Delta G - T.\Delta S$.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. *The N-terminal domain of SifA interacts with the PH domain of SKIP-* (A) SKIP(PH) and SifA(1-141) bind *in vitro*. Purified $(His)^6$::SifA(1-141), $(His)^6$::SKIP(PH) or a mixture of both proteins were analyzed by size exclusion chromatography. The protein profiles are shown (A_{280} in milli-absorbance units). (B) A SDS-PAGE analysis of the fraction corresponding to the SifA(1-141)/SKIP(PH) complex is shown. (C and D) Stoichiometry of the SifA-SKIP(PH) complex. Purified $(His)^6$::SifA, $(His)^6$::SKIP(PH) or a mixture of both proteins were analyzed by size exclusion chromatography. The protein profiles (A_{280} in milli-absorbance units, mAU) and molecular masses of protein standards used for calibration are shown. When the two partners were mixed prior to injection, a single peak was observed with an estimated mass of 47.8 kDa, nearly corresponding to the molecular mass of a 1:1 SifA-SKIP(PH) complex. (D) A fraction corresponding to the complex was analyzed by SDS-PAGE. (E) Binding affinity measured by isothermal titration calorimetry. $(His)^6$::SifA (30 µM) was titrated with $(His)^6$::SKIP(PH) (310 µM) at 25°C. The top panel shows the baseline corrected data from 27 automatic injections of 10 µl of SKIP(PH) into the SifA-containing cell. The bottom panel shows molar enthalpy change as a function of the molar ratio. The solid line represents the line of best fit for the binding isotherm based on a single-site binding model. The energy of interaction was strongly driven by the change in enthalpy since the free energy of binding (ΔG value of -29 kJ/mol) had an enthalpy contribution, ΔH, of -43 kJ/mol counterbalanced by a strong and unfavorable entropy contribution, TΔS, of -14 kJ/mol, indicating that van der Waals contacts and hydrogen bonds contributed to this interaction. Dissociation constants for interaction SKIP(PH)/SifA, /SifA(s2983) and /SifA(s3015) were calculated to be 5.7×10^{-6} M, 4.6×10^{-6} M and 9.4×10^{-6} M, respectively.

Fig. S2. *Amino acid sequence alignment of SifA orthologs and SifB-* Sequence alignment of SifA from *Salmonella thyphimurium*, *Salmonella enterica* s3015, *Salmonella enterica* s2983 and SifB from *Salmonella thyphimurium*; NCBI accession numbers NP_460194.1, AAL38949.1, AAL38931.1, and

AAF82084.1, respectively. Identical amino acids are shown by black boxes and bars below the alignment indicate the two conserved motifs. SifA residues buried at the complex interface WITH SKIP(PH) are indicated by a filled circle. Secondary structure elements of SifA are shown above the alignment. Alignment was generated using MUSCLE (1) and Figure drawn with ESPript (2,3).

SUPPLEMENTAL REFERENCES

1. Edgar, R. C. (2004) *Nucleic Acids Res* **32**, 1792-1797
2. Gouet, P., Courcelle, E., Stuart, D. I., and Metoz, F. (1999) *Bioinformatics* **15**, 305-308
3. Gouet, P., Robert, X., and Courcelle, E. (2003) *Nucleic Acids Res* **31**, 3320-3323

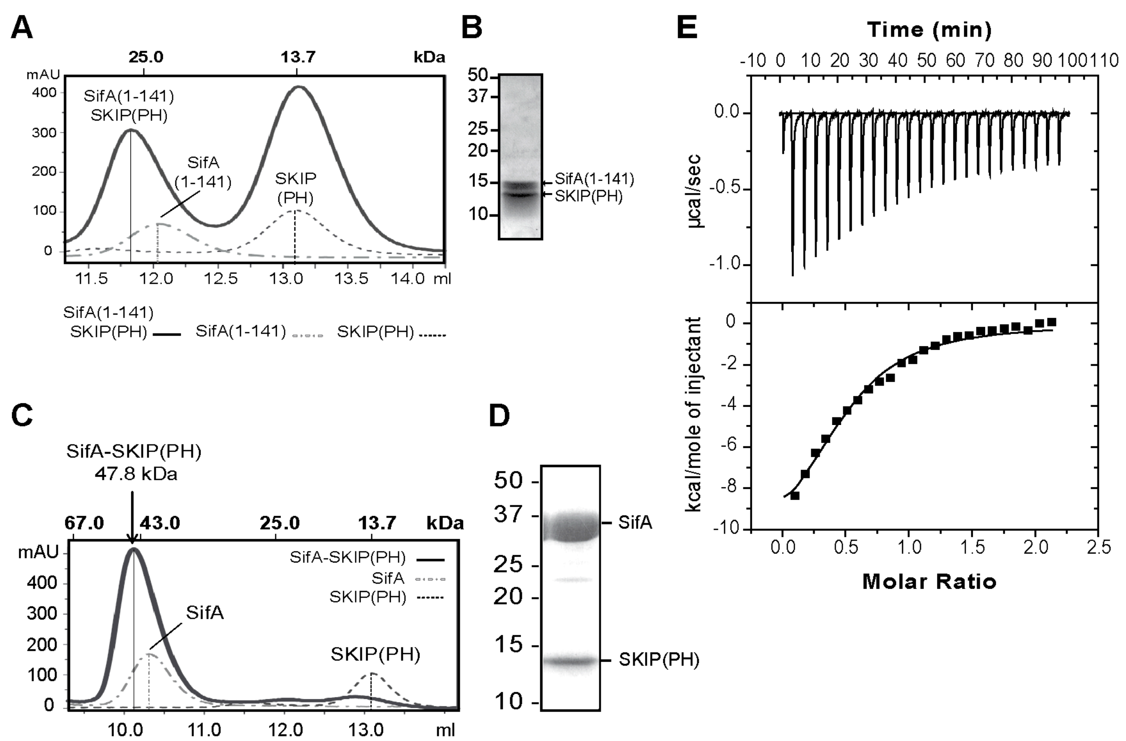

Fig. S1

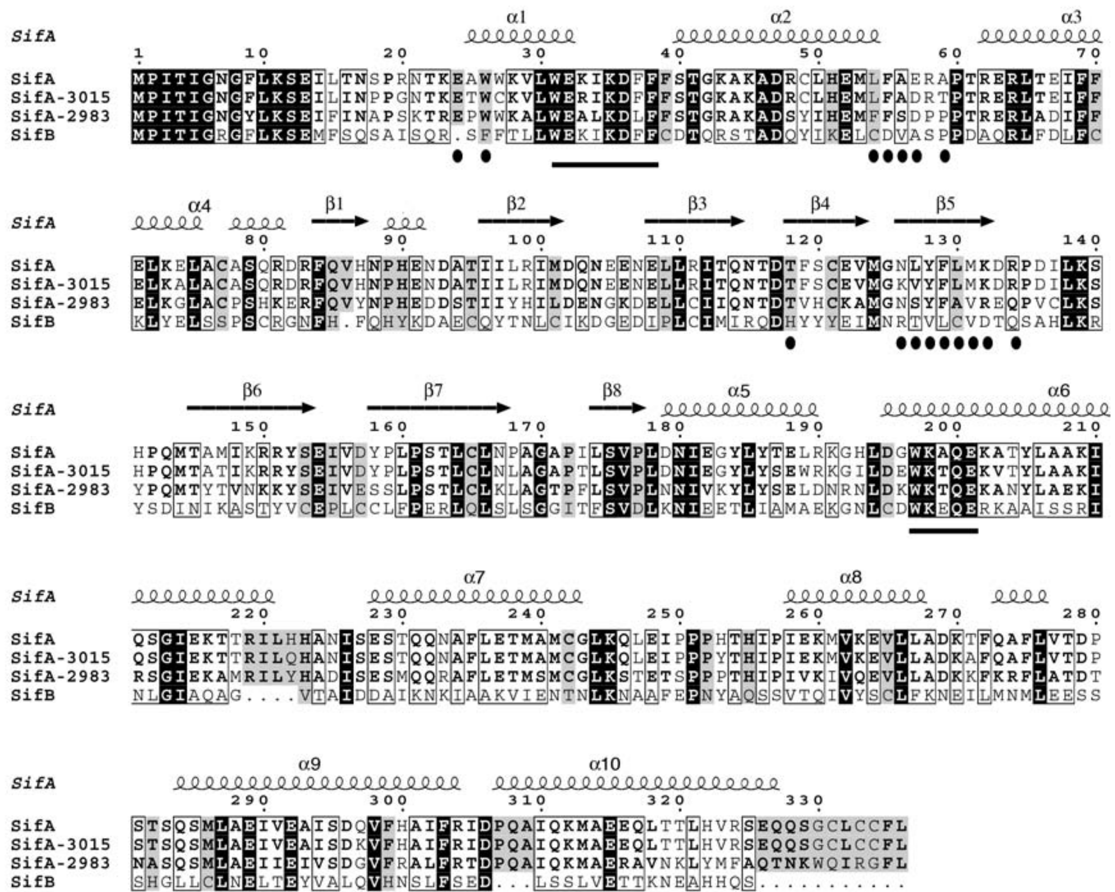

Fig. S2