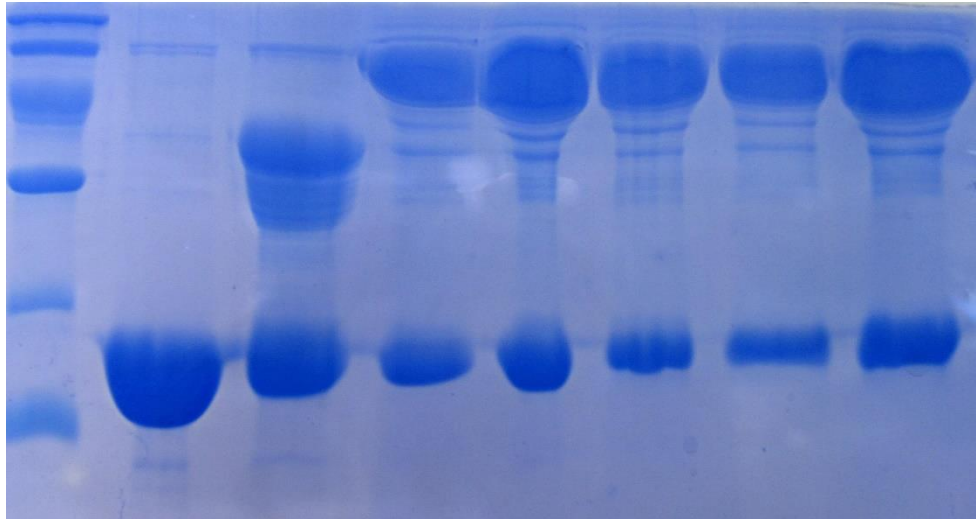# *Supplementary Material*

**Supplementary Figures and Tables**

1. **Supplementary Figures**



**Supplementary Figure 1.** SDS-PAGE analysis of the samples used in the SAXS experiments after returning to the home laboratory. From left to right: 1) LMW marker (GE Healthcare): 98 kDa, 69 kDa, 45 kDa, 30 kDa, 20.1 kDa, 14.4 kDa, 2) IpgC, 3) IpgC with IpaB Nter, 4 – 8) IpgC with full length IpaB.

Template alignment | Template 3D Structure | PDBe

**3WXX_B** AopB; Translocator, Membrane protein-Chaperone complex; HET: MSE; 2.7A {Aeromonas hydrophila}

Probability: 99.51%,  E-value: 3e-12,  Score: 127.37,  Aligned cols: 154,  Identities: 19%,  Similarity: 0.25,  Template Neff: 5.9

```
Q ss_pred          HHHHHHHHHHhhchHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHhHHHHHHHHHHHHHHHHHHHHh--
Q Q_1957385   250  TQLMATFIQLVGKNNEESLKNDLALFQSLQESRKTEMERKSDEYAAEVRKAEELNRVMGCVGKILGALLTIVSVVAAA--  327 (580)
Q Consensus   250  ~~Lma~l~~l~~~~~~~~l~~~e~~~~~~~~a~~~~~~~kk~eE~~~e~~~~KAe~~~K~~GcigKIlG~i~~avsvvaav--  327 (580)
                   ..+...|..+.++..+.+.+...+.++..++.++++++++.++++|..+|+++++|.  |||+|||||||..++++|+++
T Consensus    58  ~~l~~~L~~L~~kl~~~q~~~~~~~I~~~~~~~~~~~~~~e~~~Ki~Ea~ek~~eAkK~-G~~~KIfgwI~a~~svV~gav~  136 (219)
T 3WXX_B        58  EEFEIELSKMTSELEQTQKKLKLADLERIRAENLKKIDENQTKMKEASEAADKAKKS-GLASKIFGWISAIASMVIGAIL  136 (219)
T ss_dssp          CCCCC---------------CHHHHHHHHHHTHHHHHHHHHHHHHHHHHHHHHHHS-SCHHHHSTTSCHHHHHHHHHHH
T ss_pred          HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHc-cHHHHHHHHHHHHHHHHHHHHH


Q ss_pred          -ccHH--HHHHHHHHHHHHHHHHHHHHHHHHhCCCHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHCCCCHHHHHHHHHHHH
Q Q_1957385   328  -FSGG--ASLALAAVGLALMVTDAIVQAATGNSFMEQALNPIMKAVIEPLIKLLSDAFTKMLEGLGVDSKKAKMIGSILG  404 (580)
Q Consensus   328  -fTGG--aslalAavglal~vaD~I~~a~TG~s~~~~~m~PlM~~v~~pl~~~i~~~i~~~L~~~Gvd~~~A~~ig~I~~   404 (580)
                   +|||  ++-++...+.++++++.++++++|.++                               ++++..+.++.+..
T Consensus   137  vatG~ga~Aga~liA~gvi~vv~~iv~~a~~~g~--------------------------------i~~~~~k~~G~v~~  184 (219)
T 3WXX_B       137  IATGVGAAVGAMMIVGGAVGVANMAIQQAAADGR-------------------------------ISPETMKVLGPIMI  184 (219)
T ss_dssp          HHHHHHHHHHHHHHCTTTHHHHHHCCC--------------------------------------CCCCSHHHHHH
T ss_pred          HHccchHHHHHHHHHHHHHHHHHHHHHHHhcccc-------------------------------CCHHHHHHhhHHHHH


Q ss_pred          HHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
Q Q_1957385   405  AIAGALVLVAAVVLVATVGKQAAAKLAENIGK    436 (580)
Q Consensus   405  av~~ava~va~~~~~~~~~~aa~~~~~~~a~    436 (580)
                   ++-.+++++.+++..+....+.+++..+..++
T Consensus   185  ~~~I~~avv~~~~s~g~~aa~~~~~~~k~a~~    216 (219)
T 3WXX_B       185  AAEILVAIVSIAVTFGASAASTAMKAVKFATQ    216 (219)
T ss_dssp          HHHHHHHHHHHHHHSCHHHHHHHHHHHHHHHH
T ss_pred          HHHHHHHHHHHHHHhHHHHHHHHHHHHHHHh
```

**Supplementary Figure 2.** Remote protein homology between AopB and the IpaB domain following the long Coiled Coil domain of IpaB is detected with various fold recognition servers. Here, *Aeromonas hydrophila* AopB sequence (PDB id 3WXX, chain B) (Nguyen et al., 2015) is shown in pair-wise alignment with the domain of IpaB (residues 250 to 435) as an output of the HHpred prediction server (Zimmermann et al., 2018). The IpaB sequence can be modeled after the AopB structural template with a 99.51% confidence despite the low sequence identity (19%).

## 2. Supplementary Tables

| Calc. Mass | Obsrv. Mass | ± da | ± ppm | Start Seq. | End Seq. | Sequence | Ion Score | C. I. % |
|---|---|---|---|---|---|---|---|---|
| 872,4948 | 872,5002 | 0,0054 | 6 | 158 | 164 | INQIQTR | | |
| 1167,5641 | 1167,569 | 0,0045 | 4 | 290 | 299 | SDEYAAEVRK | | |
| 1196,6633 | 1196,604 | -0,0591 | -49 | 425 | 436 | QAAAKLAENIGK | | |
| 1329,7737 | 1329,772 | -0,0018 | -1 | 178 | 188 | LSREEIQLTIK | 51 | 99,765 |
| 1329,7737 | 1329,772 | -0,0018 | -1 | 178 | 188 | LSREEIQLTIK | | |
| 1446,7799 | 1446,779 | -0,0012 | -1 | 123 | 135 | INTLLSETEGLTR | 109 | 100 |
| 1446,7799 | 1446,779 | -0,0012 | -1 | 123 | 135 | INTLLSETEGLTR | | |
| 1520,7704 | 1520,768 | -0,0022 | -1 | 270 | 282 | NDLALFQSLQESR | 134 | 100 |
| 1520,7704 | 1520,768 | -0,0022 | -1 | 270 | 282 | NDLALFQSLQESR | | |
| 1604,8101 | 1604,799 | -0,0113 | -7 | 1 | 15 | MHNVSTTTTGFPLAK | | |
| 2160,0779 | 2160,068 | -0,0098 | -5 | 16 | 36 | ILTSTELGDNTIQAANDAANK | 135 | 100 |
| 2160,0779 | 2160,068 | -0,0098 | -5 | 16 | 36 | ILTSTELGDNTIQAANDAANK | | |
| 2280,1719 | 2280,182 | 0,0102 | 4 | 116 | 135 | NLEFSDKINTLLSETEGLTR | 149 | 100 |
| 2280,1719 | 2280,182 | 0,0102 | 4 | 116 | 135 | NLEFSDKINTLLSETEGLTR | | |
| 2335,1523 | 2335,164 | 0,0118 | 5 | 263 | 282 | NNEESLKNDLALFQSLQESR | 205 | 100 |
| 2335,1523 | 2335,164 | 0,0118 | 5 | 263 | 282 | NNEESLKNDLALFQSLQESR | | |
| 2371,1047 | 2371,132 | 0,0269 | 11 | 220 | 241 | EIDSFSAFSNTASAEQLSTQQK | | |
| 2389,1155 | 2389,108 | -0,0071 | -3 | 220 | 241 | EIDSFSAFSNTASAEQLSTQQK | | |
| 2392,1926 | 2392,162 | -0,031 | -13 | 546 | 567 | FGQLQEVIADLLASMSNSQANR | 11 | 0 |
| 2392,1926 | 2392,162 | -0,031 | -13 | 546 | 567 | FGQLQEVIADLLASMSNSQANR | | |

**Supplementary Table 1.** Mass spectrometry analysis performed on the IpaB-N band isolated from the SDS-PAGE gel of Supplementary Figure 1, lane 3. Information of the peptides detected is presented. The analysis was performed by the Mass Spec facility (ProteoPole) of Institut Pasteur.

| Model | Molecule | PDB template | PDB template residue numbers | Model residue numbers* |
|---|---|---|---|---|
| *Asymmetric IpgC* | IpgC | 3GZ1 | 8-151 | 8-151 |
| *Symmetric IpgC* | IpgC | 3KS2 | 18-154 | 18-154 |
| *IpgC-IpaB dimer* | IpgC (AcrH) | 3KS2 (3WXX) | 30-154 (10-159) | 30-154 (13-154) |
| | IpaB | 3GZ1 | 60-72 | 60-72 |
| | IpaB | 5WKQ | 74-239 | 74-239 |
| | IpaB (AopB) | 3WXX | 71-263 | 246-438 |

**Supplementary Table 2**. PDB models used for the modeling

*The numbering of the wild type protein is employed in the table but the IpgC construct we used contains the N-terminal tag "MGSSHHHHHHSSGLVPRG" (+17 residues).

### 3. Supplementary Video

**Supplementary Video 1.** Three-dimensional view of the IpgC/IpaB complex SAXS model.

### 4. References

Nguyen, V. S., Jobichen, C., Tan, K. W., Tan, Y. W., Chan, S. L., Ramesh, K., et al. (2015). Structure of AcrH-AopB Chaperone-Translocator Complex Reveals a Role for Membrane Hairpins in Type III Secretion System Translocon Assembly. *Structure* 23, 2022–2031. doi:10.1016/j.str.2015.08.014.

Zimmermann, L., Stephens, A., Nam, S. Z., Rau, D., Kübler, J., Lozajic, M., et al. (2018). A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. *J. Mol. Biol.* 430, 2237–2243. doi:10.1016/j.jmb.2017.12.007.