

SUPPLEMENTARY INFORMATION

Structural variability of EspG chaperones from mycobacterial ESX-1, ESX-3 and ESX-5 type VII secretion systems

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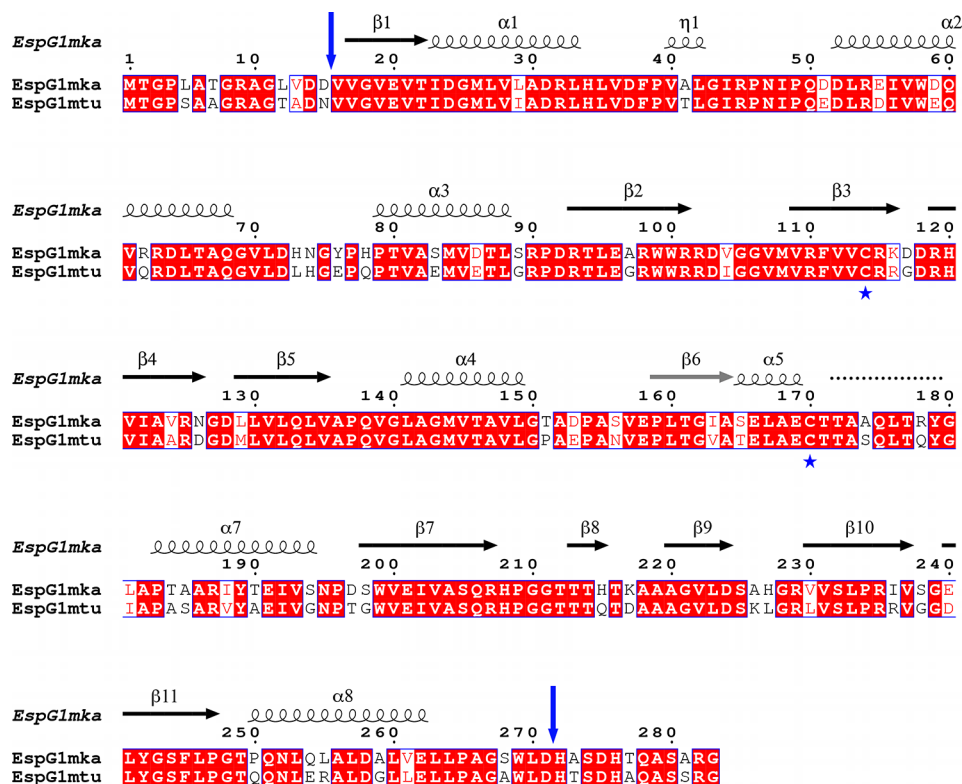


Figure S1. Sequence alignment of EspG₁ from *M. tuberculosis* (EspG_{1mtu}) and *M. kansasii* (EspG_{1mka}). Secondary structure elements corresponding to the EspG_{1mka} structure are shown above the alignment. Vertical blue arrows indicate the beginning and the end of the truncated construct used for crystallization. Blue stars indicate the positions of cysteine to alanine mutations in the crystallization construct. The residues corresponding to β 6 strand (grey arrow) do not satisfy strict criteria for a β -strand [1]. Residues corresponding to the α 6 helix display poor electron density and were not modeled (dashed line).

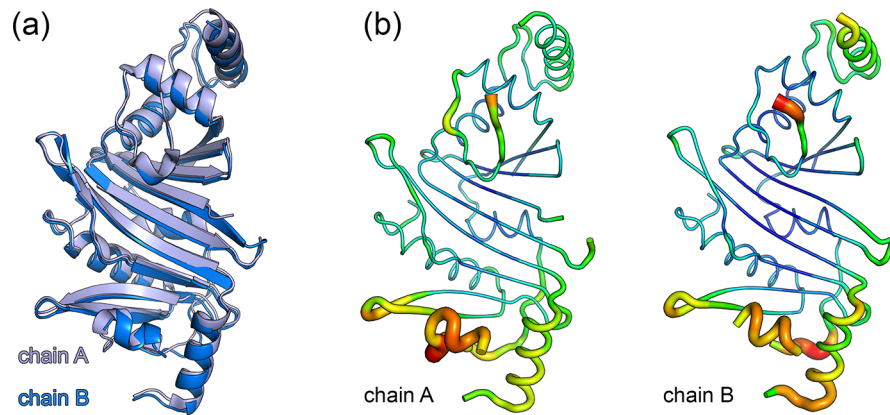


Figure S2. Structural superposition of the two copies of EspG_{1mka} in the asymmetric unit.

The orientation is the same as in Figure 1b. (a) Superposed structures of chain A and B in ribbon representation. (b) Tube representation of chain A and chain B of EspG_{1mka}. The tube thickness is proportional to *B* factors with thinner tubes corresponding to lower *B* factors and thicker tubes corresponding to higher *B* factors. The rainbow colors are from lower *B* factors (blue) to higher *B* factors (red).

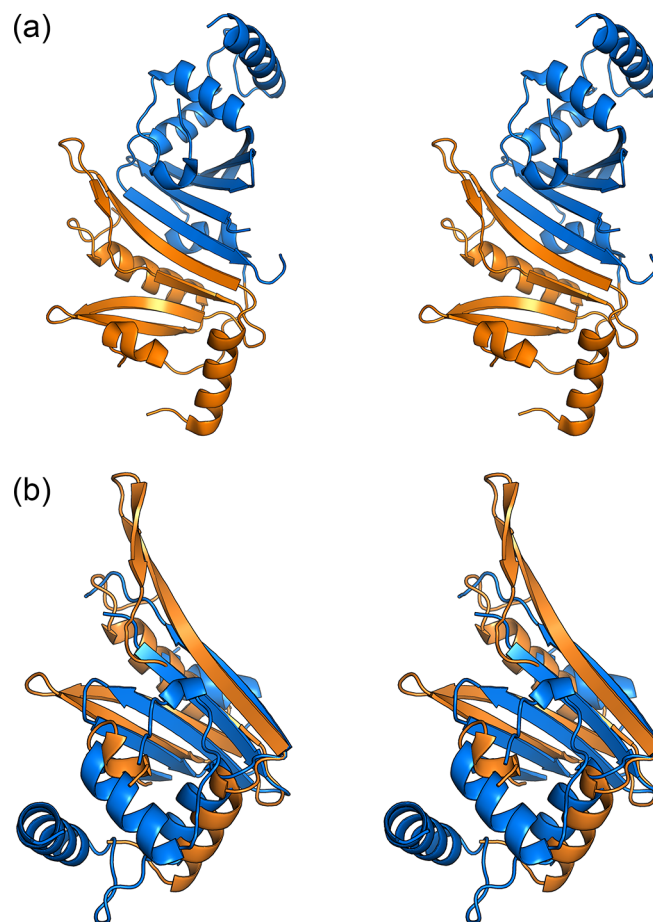


Figure S3. The N- and C-terminal subdomains of EspG₁ are related by quasi two-fold symmetry. (a) Stereoview of EspG_{1mka} in ribbon representation with the N-terminal subdomain colored in blue and the C-terminal subdomain colored in orange. (b) Stereo view of the structural superposition of two subdomains of EspG_{1mka}. The orientation of the C-terminal subdomain is similar to panel (a).

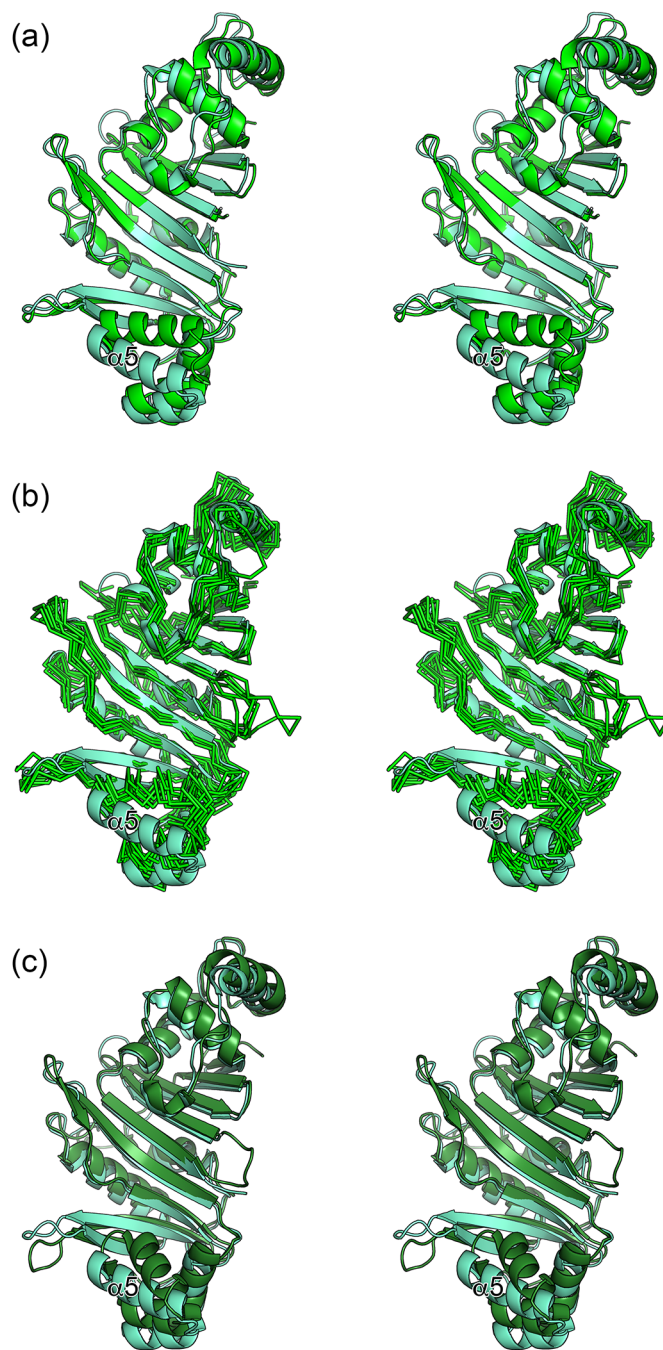


Figure S4. Comparison of EspG₃ crystal structures. (a) Structural superposition of EspG_{3mma} (aquamarine) and EspG_{3msm} (PDB ID 4L4W) (green). (b) Structural superposition of EspG_{3mma} (aquamarine) with all EspG₃ structures that display a closed conformation of the C-terminal α -helical bundle (green). EspG_{3msm} (PDB ID 4L4W), EspG_{3msm} (PDB ID 4RCL) (this work), and EspG_{3mtu}, EspG_{3msm} (PDB ID 4W4J) [2] are shown as C α atom traces. (c) Structural superposition of EspG_{3mma} (aquamarine) and EspG_{3msm} (PDB ID 5SXL) (green).

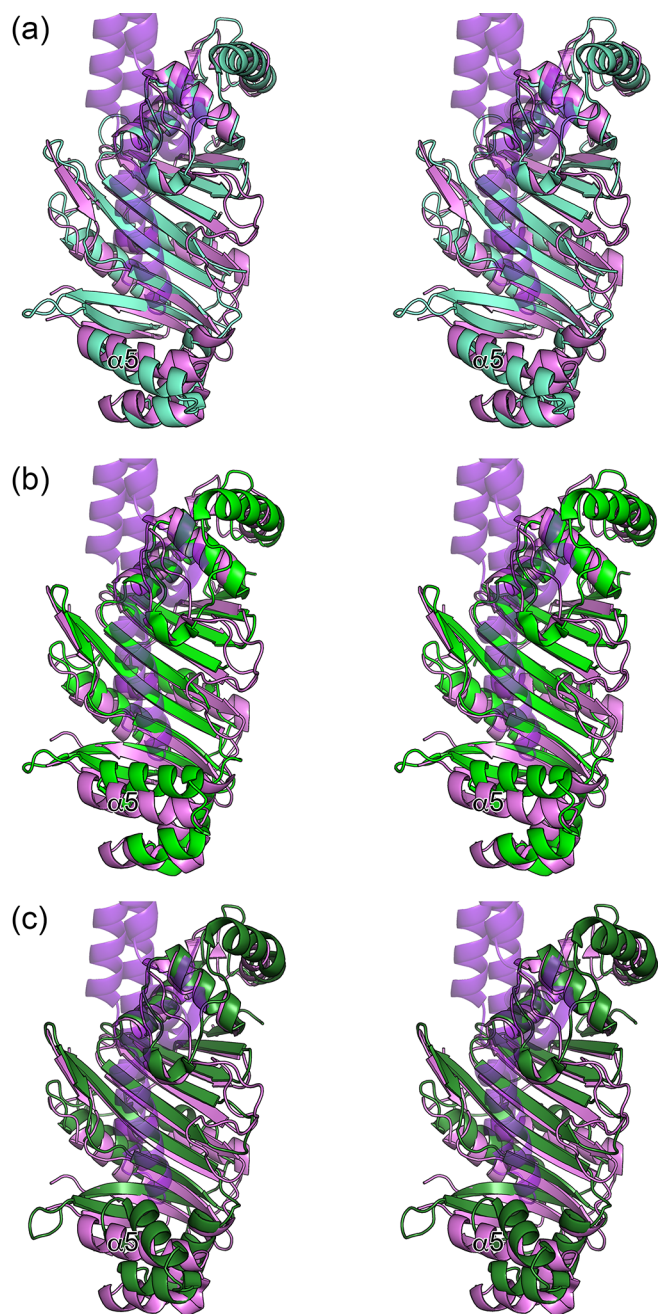


Figure S5. Comparison of crystal structures of EspG₃ and EspG₅. Structural superpositions of (a) EspG_{3mma} (aquamarine), (b) EspG_{3msm} (PDB ID 4L4W, light green), and (c) EspG_{3msm} (PDB ID 5SXL, dark green) with EspG_{5mtu} (PDB ID 4W4I [2], violet) from the PE25-PPE41-EspG_{5mtu} trimer crystal structure (PDB ID 4KXR [3]). PPE41 (purple) is shown in semi-transparent ribbon representation; PE25 is not in contact with EspG_{5mtu} and is omitted for clarity.

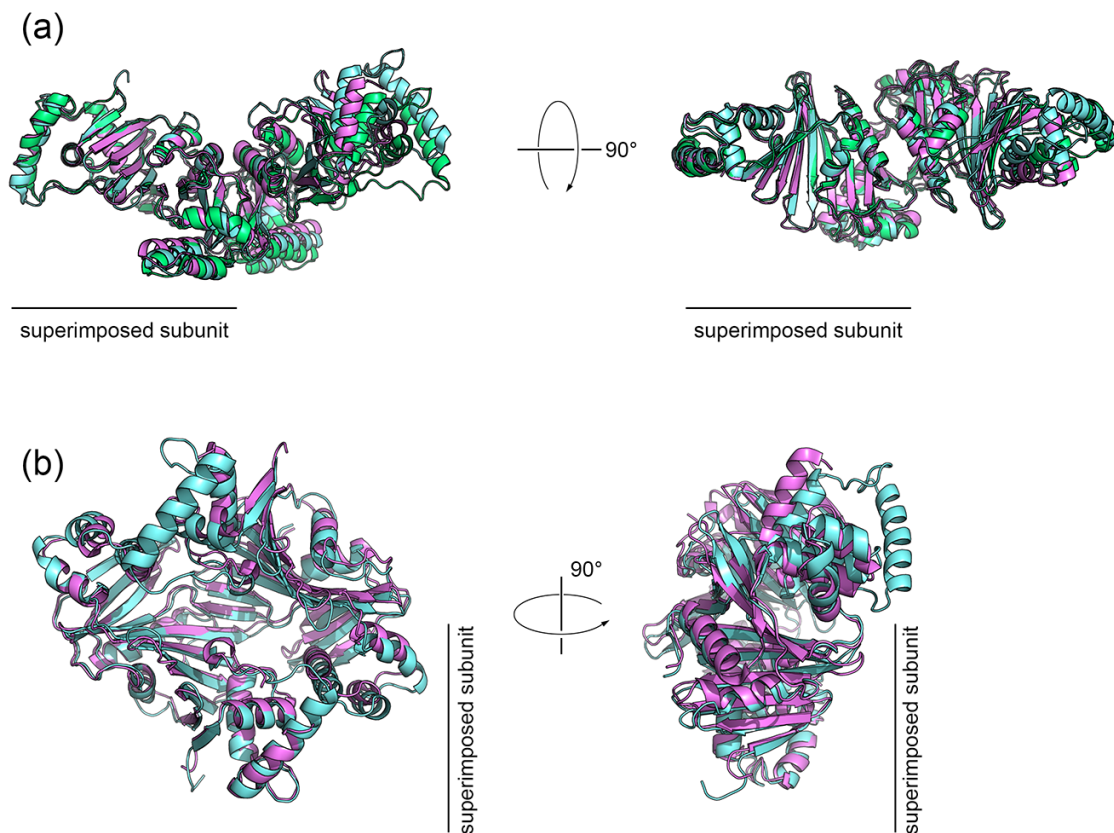
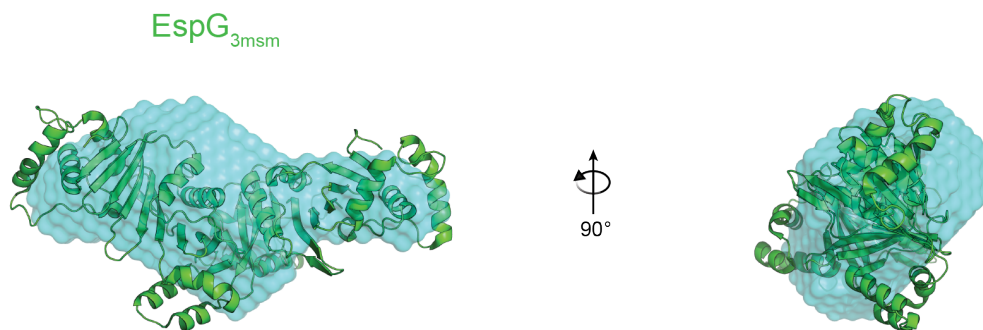


Figure S6. Comparison of common EspG₃ dimers. (a) Comparison of the wing-shaped dimers with superimposed subunits on the left. EspG_{3msm} (PDB ID 4L4W) is in green, EspG_{3msm} (PDB ID 4RCL) is in violet, EspG_{3msm} (PDB ID 4W4J) is in cyan. (b) Comparison of β 8-mediated dimers with superimposed subunits at the bottom.

(a)



(b)

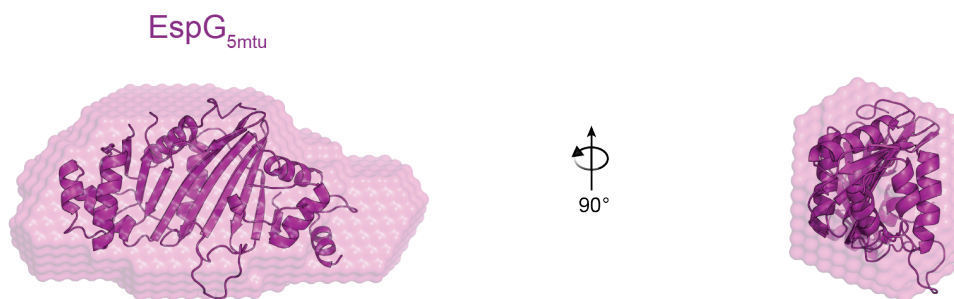


Figure S7. Comparison of *ab initio* SAXS models and crystal structures of EspG₃ and EspG₅. (a) Structural alignment of the *DAMMIF ab initio* model (turquoise surface) and the crystallographic structure of SeMet-EspG_{3msm} (PDB ID 4L4W) (green cartoon) (normalized spatial discrepancy (NSD) = 1.1). (b) The *DAMMIF ab initio* model of EspG_{5mtu} aligned with the EspG_{5mtu} crystal structure from the heterotrimeric EspG_{5mtu}-PE25-PPE41 crystal structure (PDB ID 4KXR, chain C [3]) (NSD 0.93).

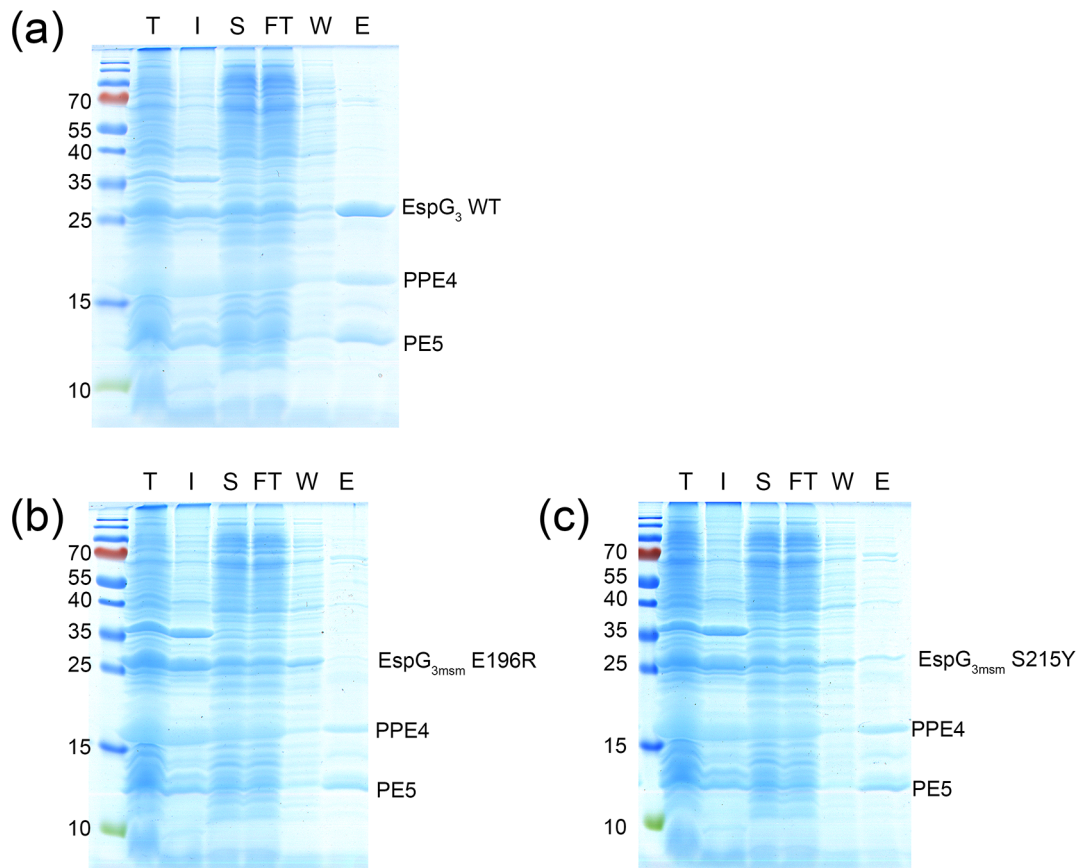


Figure S8. Co-purification of PE5–PPE4 dimers with wild-type and mutant variants of *EspG*_{3msm}. PE5–PPE4 dimers were co-expressed in *E. coli* together with either wild type *EspG*_{3msm} (a) or point mutants of *EspG*_{3msm}, namely E196R (b) and S215Y (c). Proteins were purified using immobilized Ni²⁺-affinity chromatography via the His₆-tag present on PE5. Only wild type *EspG*_{3msm} co-purifies with the PPE4/PE5 complex. Mutations of *EspG*_{3msm} did not affect solubility, with mutant proteins clearly present in the flow-through and wash fractions. T – total fraction after cell lysis, I – insoluble fraction, S – soluble fraction, FT – flow through fraction, W – wash fraction, E – elution fraction.

Table S1. Comparison of available EspG crystal structures.

	EspG _{1mka} 5VBA A	EspG _{1mka} 5VBA B	EspG _{3mma} 5DLB	EspG _{3msm} 4L4W A	EspG _{3msm} 4L4W B	EspG _{3msm} 4RCL A	EspG _{3msm} 4RCL B	EspG _{3msm} 5SXL	EspG _{3msm} 4W4J A	EspG _{3msm} 4W4J B	EspG _{3mtu} 4W4I	EspG _{5mtu} 4KXR	EspG _{5mtu} 4W4L	EspG _{5mtu} 5XFS
EspG _{1mka} 5VBA A		1.0 / 231 / 95	2.4 / 234 / 28	2.3 / 232 / 29	2.7 / 237 / 28	2.5 / 233 / 28	2.6 / 211 / 29	2.5 / 237 / 28	2.4 / 237 / 28	2.3 / 237 / 28	2.5 / 233 / 28	2.4 / 224 / 24	2.6 / 233 / 25	2.5 / 219 / 25
EspG _{1mka} 5VBA B	1.0 / 231 / 95		2.3 / 228 / 29	2.3 / 226 / 30	2.5 / 230 / 29	2.6 / 230 / 29	2.6 / 208 / 30	2.5 / 230 / 30	2.3 / 230 / 29	2.3 / 230 / 29	2.5 / 229 / 28	2.4 / 222 / 24	2.5 / 228 / 25	2.5 / 217 / 24
EspG _{3mma} 5DLB	2.4 / 234 / 28	2.3 / 228 / 29		2.3 / 258 / 61	2.6 / 260 / 60	3.0 / 253 / 60	2.8 / 230 / 61	2.2 / 260 / 61	2.3 / 260 / 60	2.4 / 260 / 60	2.5 / 256 / 84	2.4 / 238 / 18	3.1 / 251 / 19	2.4 / 234 / 18
EspG _{3msm} 4L4W A	2.3 / 232 / 29	2.3 / 226 / 30	2.3 / 258 / 61		1.4 / 262 / 100	1.3 / 252 / 98	1.1 / 230 / 99	1.7 / 260 / 93	1.3 / 259 / 100	1.2 / 258 / 100	1.4 / 253 / 62	2.6 / 239 / 18	3.0 / 255 / 18	2.8 / 239 / 18
EspG _{3msm} 4L4W B	2.7 / 237 / 28	2.5 / 230 / 29	2.6 / 260 / 60	1.4 / 262 / 100		1.1 / 257 / 98	1.0 / 235 / 99	2.3 / 266 / 94	1.6 / 264 / 100	1.5 / 263 / 100	1.6 / 258 / 61	2.8 / 245 / 19	3.1 / 257 / 18	2.5 / 241 / 17
EspG _{3msm} 4RCL A	2.5 / 233 / 28	2.6 / 230 / 29	3.0 / 253 / 60	1.3 / 252 / 98	1.1 / 257 / 98		0.7 / 235 / 100	2.4 / 256 / 100	1.5 / 257 / 98	1.3 / 256 / 98	1.3 / 251 / 61	2.6 / 244 / 18	2.7 / 254 / 19	2.6 / 240 / 18
EspG _{3msm} 4RCL B	2.6 / 211 / 29	2.6 / 208 / 30	2.8 / 230 / 61	1.1 / 230 / 99	1.0 / 235 / 99	0.7 / 235 / 100		1.8 / 230 / 97	1.5 / 235 / 99	1.3 / 234 / 99	1.3 / 229 / 63	2.7 / 227 / 19	2.5 / 232 / 17	2.5 / 228 / 18
EspG _{3msm} 5SXL	2.5 / 237 / 28	2.5 / 230 / 30	2.2 / 260 / 61	1.7 / 260 / 93	2.3 / 266 / 94	2.4 / 256 / 100	1.8 / 230 / 97		2.1 / 264 / 98	2.1 / 263 / 98	2.0 / 258 / 62	2.5 / 243 / 19	3.0 / 255 / 18	2.7 / 241 / 17
EspG _{3msm} 4W4J A	2.4 / 237 / 28	2.3 / 230 / 29	2.3 / 260 / 60	1.3 / 259 / 100	1.6 / 264 / 100	1.5 / 257 / 98	1.5 / 235 / 99	2.1 / 264 / 98		0.5 / 263 / 100	1.4 / 258 / 61	2.6 / 242 / 18	3.1 / 256 / 18	2.5 / 238 / 18
EspG _{3msm} 4W4J B	2.3 / 237 / 28	2.3 / 230 / 29	2.4 / 260 / 60	1.2 / 258 / 100	1.5 / 263 / 100	1.3 / 256 / 98	1.3 / 234 / 99	2.1 / 263 / 98	0.5 / 263 / 100		1.4 / 258 / 61	2.5 / 242 / 18	3.2 / 255 / 18	2.5 / 238 / 18
EspG _{3mtu} 4W4I	2.5 / 233 / 28	2.5 / 229 / 28	2.5 / 256 / 84	1.4 / 253 / 62	1.6 / 258 / 61	1.3 / 251 / 61	1.3 / 229 / 63	2.0 / 258 / 62	1.4 / 258 / 61	1.4 / 258 / 61		2.8 / 245 / 21	3.2 / 257 / 21	2.8 / 240 / 21
EspG _{5mtu} 4KXR	2.4 / 224 / 24	2.4 / 222 / 24	2.4 / 238 / 18	2.6 / 239 / 18	2.8 / 245 / 19	2.6 / 244 / 18	2.7 / 227 / 19	2.5 / 243 / 19	2.6 / 242 / 18	2.5 / 242 / 18	2.8 / 245 / 21		0.4 / 276 / 100	0.5 / 271 / 100
EspG _{5mtu} 4W4L	2.6 / 233 / 25	2.5 / 228 / 25	3.1 / 251 / 19	3.0 / 255 / 18	3.1 / 257 / 18	2.7 / 254 / 19	2.5 / 232 / 17	3.0 / 255 / 18	3.1 / 256 / 18	3.2 / 255 / 18	3.2 / 257 / 21	0.4 / 276 / 100		0.5 / 275 / 100
EspG _{5mtu} 5XFS	2.5 / 219 / 25	2.5 / 217 / 24	2.4 / 234 / 18	2.8 / 239 / 18	2.5 / 241 / 17	2.6 / 240 / 18	2.5 / 228 / 18	2.7 / 241 / 17	2.5 / 238 / 18	2.5 / 238 / 18	2.8 / 240 / 21	0.5 / 271 / 100	0.5 / 275 / 100	

Results of pairwise structural superpositions are shown in the format: rmsd (Å) / number of superimposed C α atoms / sequence identity (%) as reported by the Dali server [4].

References

- [1] Kabsch W, Sander C. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*. 1983;22:2577-637.
- [2] Ekiert DC, Cox JS. Structure of a PE-PPE-EspG complex from *Mycobacterium tuberculosis* reveals molecular specificity of ESX protein secretion. *Proc Natl Acad Sci U S A*. 2014;111:14758-63.
- [3] Korotkova N, Freire D, Phan TH, Ummels R, Creekmore CC, Evans TJ, et al. Structure of the *Mycobacterium tuberculosis* type VII secretion system chaperone EspG5 in complex with PE25-PPE41 dimer. *Mol Microbiol*. 2014;94:367-82.
- [4] Holm L, Laakso LM. Dali server update. *Nucleic Acids Res*. 2016;44:W351-5.