**Supplementary File 1.­­**

**Supplementary File 1a. Summary of SEC-SAXS parameters.** Lactoferrin (Lf) has a calculated molecular weight of 76.3 kDa, lactoferricin (Lfcn) 1.4 kDa, *Ng*LbpB 78.4 kDa, and *Nm*LbpB 79.5 kDa.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Lf** | ***Ng*LbpB** | ***Nm*LbpB** |
|  |  |  | **+Lf** | **+Lfcn** |  | **+Lf** | **+Lfcn** | **+Lf+Lfcn** |
| Rg | 33.2 ± 0.3 | 37.8 ± 0.1 | 45.9 ± 0.1 | 37.7 ± 0.2 | 35.9 ± 0.1 | 44.6 ± 0.2 | 34.9 ± 0.2 | 44.6 ± 0.1 |
| Vc MW | 75.8 | 88.8 | 168.0 | 66.8 | 88.9 | 155.3 | 83.7 | 171.3 |
| Vp MW | 88.1 | 106.3 | 190.9 | 86.3 | 104.6 | 178.8 | 98.2 | 208.7 |
| Bayes MW | 80.8 | 94.2 | 169.6 | 74.3 | 94.2 | 157.1 | 85.7 | 185.8 |
| Shape/Size MW | 81.4 | 102.2 | 181.9 | 89.9 | 98.3 | 150.0 | 86.8 | 202.2 |
| Dmax | 110 | 114 | 139 | 119 | 116 | 134 | 117 | 135 |

**Supplementary File 1b. Data collection and refinement statistics for the *Nm*LbpB-Lf X-ray crystal structure.**

|  |  |
| --- | --- |
| **Data Collection** | ***Nm*LbpB-Lf** |
| λ (Å) | 1.0 |
| Space group | P 43212 |
| a, b, c (Å) | 120.39, 120.39, 207.38 |
| α, β, γ (º) | 90, 90, 90 |
| Resolution (Å)\* | 50 – 2.85 (2.95 – 2.85) |
| Completeness (%)\* | 99.9 (100) |
| Redundancy\* | 8.9 (8.7) |
| Rsym †\* | 0.263 (2.93) |
| I / σ (I)\* | 14 (1.33) |
| CC1/2 | 0.985 (0.405) |
| **Refinement** |  |
| Resolution (Å) | 47.62 – 2.85 (2.95 – 2.85) |
| No. reflections | 36,134 (3516) |
| R§/Rfree¶ | 0.20/0.25 |
| **r.m.s. deviations** |  |
| Bonds (Å) | 0.002 |
| Angles (º) | 0.57 |
| No. Protein atoms | 9,459 |
| No. Ligand atoms | 75 |
| No. Waters | 13 |
| **B-factors** (Å2) |  |
| Wilson B | 77.46 |
| Protein | 87.41 |
| Ligands | 133.38 |
| Waters | 60.17 |
| **Ramachandran Analysis¥** |  |
| Favored (%) | 92.58 |
| Allowed (%) | 7.18 |
| Outliers (%) | 0.24 |
| PDB ID | 7JRD |

†*R*sym = Σ*hkl,j* (|Ihkl-<Ihkl>|) / Σ*hkl,j* Ihkl, where <Ihkl> is the average intensity for a set of j symmetry related reflections and Ihkl is the value of the intensity for a single reflection within a set of symmetry-related reflections.

§ *R* factor = Σ*hkl* (||Fo| - |Fc||) / Σ*hkl*|Fo| where Fo is the observed structure factor amplitude and Fc is the calculated structure factor amplitude.

¶ *R*free = Σ*hkl,T*(||Fo| - |*F*c||) / Σ*hkl,T*|*F*o|, where a test set, T (5% of the data), is omitted from the refinement.

¥ Performed using Molprobity within PHENIX.

\* Indicates statistics for last resolution shell shown in parenthesis.

**Supplementary File 1c. Data collection and refinement statistics for the *Ng*LbpB-Lf cryoEM structure.**

|  |  |
| --- | --- |
|  | *Ng*LbpB-Lf |
| **Data Collection and Processing** |
| Magnification | 81,000 |
| Voltage (kV) | 300 |
| Electron exposure (e-/Å2) | 53.68 |
| Defocus range (µm) | -1 to -2.5 |
| Pixel size (Å) | 0.54 |
| Symmetry imposed | C1 |
| Initial particle projections (no.) | 3,330,059 |
| Final particle projections (no.) | 127,832 |
| Map resolution (Å) | 3.65 |
|  FSC threshold  | 0.143 |
| **Refinement** |
| Model Resolution (Å) | 3.9 |
|  FSC threshold | 0.5 |
| Map-model CC |  |
|  CC\_mask | 0.72 |
|  CC\_box | 0.74 |
|  CC\_peaks | 0.67 |
|  CC\_volume | 0.72 |
| Model Composition |  |
|  Non-hydrogen atoms | 9405 |
|  Protein residues | 1220 |
|  Ligands | 4 |
| B factors (Å2) |  |
|  Protein | 72.19 |
|  Ligand | 82.43 |
| R.M.S. deviations  |  |
|  Bond lengths (Å) | 0.008 |
|  Bond angles (°) | 1.020 |
| Validation |  |
|  MolProbity Score | 2.70 |
|  Clashscore | 35.26 |
|  Rotamer outliers (%) | 0.81 |
| Ramachandran Plot |  |
|  Favored (%) | 78.39 |
|  Allowed (%) | 21.36 |
|  Outliers (%) | 0.25 |
| PDB  | 7N88 |
| EMDB | EMD-24233 |

**Supplementary File 1d. Summary of the intermolecular interactions between *Nm*LbpB and Lf.** The information about interacting residues was obtained by QtPISA analysis.

|  |  |  |
| --- | --- | --- |
| ***Nm*LbpB** | **Lf** | **Distance** |
| **Hydrogen bonds** |
| R135 | R357 | 2.8 |
| R135 | Q513 | 2.9 |
| A136 | R357 | 2.9 |
| K139 | R357 | 2.6 |
| N141 | R357 | 3.5 |
| N154 | N360 | 3.6 |
| Y158 | E353 | 2.2 |
| R193 | D630 | 2.7 |
| S201 | S637 | 2.9 |
| S201 | T639 | 3.9 |
| T203 | K640 | 3.6 |
| D204 | D561 | 3.5 |
| Y210 | Q354 | 3.0 |
| Y210 | T639 | 3.9 |
| N213 | E638 | 2.8 |
| Y220 | Q513 | 2.5 |
| K230 | E512 | 3.5 |
| Y253 | E538 | 2.6 |
| Q255 | A537 | 3.4 |
| K257 | E538 | 3.6 |
| S258 | N539 | 3.0 |

|  |  |  |
| --- | --- | --- |
| ***Nm*LbpB** | **Lf** | **Distance** |
|  | **Salt bridges** |  |
| K117 [NZ] | E515 [OE1] | 3.4 |
| R135 [NE] | E353 [OE1] | 3.7 |
| R135 [NH1] | D511 [OD2] | 3.6 |
| R135 [NH2] | D511 [OD1] | 4.0 |
| R135 [NH2] | D511 [OD2] | 3.8 |
| D204 [OD1} | K640 [NZ] | 3.0 |
| D204 [OD2] | K640 [NZ] | 3.5 |
| R223 [NE] | D561 [OD2] | 3.9 |
| R223 [NH2] | D561 [OD2] | 3.6 |
| D227 [OD1] | R525 [NH1] | 3.5 |
| D227 [OD2] | R525 [NH1] | 2.8 |
| D227 [OD2] | R525 [NH2] | 3.4 |
| K230 [NZ] | E512 [OE1] | 2.9 |
| K257 [NZ] | E538 [OE1] | 3.1 |
| K257 [NZ] | E538 [OE2] | 3.5 |

**Supplementary File 1e. Intermolecular interactions between *Ng*LbpB and Lf.** The information about interacting residues was obtained by QtPISA analysis.

|  |  |  |
| --- | --- | --- |
| ***Ng*LbpB** | **Lf** | **Distance** |
| **Hydrogen bonds** |
| E118 [OE1] | Q513 [NE2] | 3.8 |
| E118 [OE2] | Q513 [NE2] | 3.9 |
| Y131 [OH] | Q513 [NE2] | 3.2 |
| G137 [O] | R357 [NH1] | 2.3 |
| D139 [OD2] | N360 [ND2] | 2.3 |
| R191 [NH2] | D630 [O] | 2.7 |
| S200 [N] | E638 [O] | 3.4 |
| D202 [OD1] | N560 [N] | 3.7 |

|  |  |  |
| --- | --- | --- |
| ***Ng*LbpB** | **Lf** | **Distance** |
| **Salt bridges** |
| R113 [NH1] | D511 [OD2] | 3.9 |
| R113 [NH1] | E515 [OE1] | 3.5 |
| R113 [NH1] | E515 [OE2] | 3.8 |
| K117 [NZ] | E515 [OE2] | 3.7 |
| K228 [NZ] | E512 [OE1] | 3.4 |

**Supplementary File 1f. Summary of ITC parameters for lactoferrin binding to *Nm*LbpB mutants.** These experiments were performed using a MicroCal iTC200 ITC calorimeter (Malvern Panalytical).

*NmLbpB interface mutants*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Protein | Kd (μM) | ΔH (kcal/mol) | ΔS (kcal/mol/deg) | n |
| Wt | 0.1 ± 0.1 | -15.8 ± 2.5 | -0.02 ± 0.01 | 0.8 ± 0.1 |
| R193E | 2.0 ± 0.2 | -11.8 ± 0.3 | -0.014 ± 0.01 | 0.75 ± 0.04 |
| D227K | 1.9 ± 0.6 | -5.4 ± 0.3 | 0.008 ± 0.002 | 0.7 ± 0.1 |
| R135E | 11.5 ± 1.3 | -9.7 ± 1.1 | -0.02 ± 0.01 | 0.9 ± 0.1 |
| D140K | 0.9 ± 0.01 | -9.5 ± 0.1 | -0.004 ± 0.0004 | 0.6 ± 0.01 |
| K143E | 0.3 ± 0.2 | -16.7 ± 0.2 | -0.03 ± 0.01 | 0.6 ± 0.04 |

**Supplementary File 1g. Summary of ITC parameters for lactoferricin binding to *Nm*LbpB loop deletions.** These experiments were performed using a Nano ITC calorimeter (TA Instruments).

*NmLbpB loop deletions*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Construct | Kd (µM) | ΔH (kcal/mol) | ΔS (cal/mol.K) | n |
| WT | 3.7 | -11.4 ± 0.5 | -15.5 | 1.3 ± 0.03 |
| Δ372-383 | 4.9 | -9.9 ± 1.0 | -10.8 | 1.4 ± 0.1 |
| Δ416-418 | 5.9 | -9.1 ± 0.6 | -8.1 | 1.6 ± 0.1 |
| Δ445-526 | 9.1 | -9.6 ± 2.0 | -10.9 | 0.9 ± 0.1 |
| Δ561-565 | 4.1 | -10.1 ± 0.5 | -11.1 | 1.3 ± 0.04 |
| Δ594-599 | 4.8 | -9.7 ± 0.8 | -10.0 | 1.6 ± 0.1 |
| Δ665-698 | 5.6 | -10.7 ± 0.7 | -13.8 | 1.5 ± 0.1 |
| Δ445-526\_ Δ665-698 | insufficient binding to determine accurately |

**Supplementary File 1h. Summary of ITC parameters for lactoferrin and lactoferricin binding to *Nm*LbpB.** These experiments were performed using a Nano ITC calorimeter (TA Instruments).

*Titration with Lactoferrin (Lf)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Constructs | Kd (µM) | ΔH (kcal/mol) | ΔS (cal/mol.K) | n |
| *Nm*LbpB | 0.45 | -15.1 ± 0.3 | -21.7 | 0.7 ± 0.01 |
| *Nm*LbpB-Lfcn | 0.45 | -14.6 ± 0.4 | -20.0 | 0.8 ± 0.01 |

*Titration with lactoferricin (Lfcn)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Construct | Kd (µM) | ΔH (kcal/mol) | ΔS (cal/mol.K) | n |
| *Nm*LbpB-Lf | 7.29 | -10.6 ± 1.3 | -14.0 | 1.4 ± 0.1 |