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Supporting information for article:

Crystal structure of FhuD at 1.6 Å resolution: a ferrichrome-binding protein from the animal and human pathogen *Staphylococcus pseudintermedius*

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S1. Materials and Methods

S1.1. Analytical size-exclusion chromatography

Purified FhuD samples at concentrations of 0.5 mg/mL, 0.75 mg/mL and 1.5 mg/mL (i.e. up to approximately 50 μM concentration) were loaded as 20 μl samples onto a Superdex-75 resin, 5/150 analytical SEC column (GE Healthcare) equilibrated at room temperature (18-26 °C) in buffer containing 20 mM Tris-HCl, 150 mM NaCl, pH 8.0, with flow rate 0.15 mL/min. At all three concentrations tested, the elution profile revealed a single symmetrical peak, with a maximum at 11.1 min. The elution time was used to obtain an apparent molecular weight by running standard proteins (Bio-Rad markers ranging from 1.35-670 kDa) on the same column and under the same conditions as used for FhuD.

S1.2. Differential scanning calorimetry

The thermal stability of FhuD was assessed by differential scanning calorimetry (DSC) using a MicroCal VP-Capillary DSC instrument (GE Healthcare). Purified FhuD was prepared at 10 μM concentration in phosphate buffered saline pH 7.4 (PBS). DSC experiments were performed in the presence or absence of 200 μM ferrichrome ($C_{27}H_{42}O_{12}N_9Fe$; MW 740; obtained from EMC microcollections GmbH). The DSC temperature scan ranged from 10 °C to 110 °C, with a thermal ramping rate of 200 °C per hour and a 4 second filter period. Data were analyzed by subtraction of the reference data for a sample containing only buffer (e.g. PBS only, or PBS + 200 μM ferrichrome), using the Origin 7 software.

S1.3. Surface plasmon resonance

The binding of ferrichrome to FhuD was assessed by surface plasmon resonance (SPR) using a Biacore T200 instrument equilibrated at 25 °C in HBS-EP running buffer (GE Healthcare). Experiments were performed using a CM5 sensor chip with covalent immobilization via amine coupling of FhuD (prepared at 50 μg/mL in 10 mM sodium acetate buffer pH 4.5, reaching a surface density of approximately 2000 RU), essentially as described previously (Mariotti *et al.*, 2013). The analytes were tested using injections at 1 μM concentration.