Conners, Hill et al:  
Supplementary data 6: Details of Structural Studies

Determination of the UspA1\(^{(527-665)}\) crystal structure

Crystals of UspA1\(^{(527-665)}\) were grown by vapour diffusion from 12 % PEG 3350 and 0.2 M ammonium phosphate, to which 25 % glycerol was added before cooling to 100K. Diffraction data were collected on beamline BM14 (\(\lambda = 0.95350 \text{ Å}\)) at ESRF Grenoble, France, and at the Daresbury SRS. Data were integrated and scaled in HKL2000 (Otwinowski and Minor, 1997) and manipulated with the CCP4 suite of crystallographic software (1994). The structure was solved by a novel exploratory molecular replacement method using in excess of 20 different coiled-coil structures as search models in the program PHASER (McCoy et al., 2005). The coordinates of a single strand of the coiled coil of cortexellin (1D7M.pdb), which has no recognisable sequence identity to UspA1\(^{(527-665)}\), was eventually successful despite being from a dimeric coiled coil. Solutions from PHASER had Z scores of 6 and 13 after rotation and translation respectively to find the first molecule in the asymmetric unit, and 5 and 9 for the second molecule.

Initial phases were improved by mutation of the sequence to poly-Ala followed by “atoms update and refinement” mode of ARP/wARP (Perrakis et al., 2001). Symmetry operators were applied to the first monomer to create the two trimers. The structure was refined with iterative cycles of manual model-building using COOT (Emsley and Cowtan, 2004), restrained refinement with REFMAC5 (Murshudov et al., 1997) and density improvement with ARP/wARP as the correct sequence was gradually built into the improving electron density maps. Data collection and final refinement statistics are summarised in Table 1.

Small angle X-ray scattering experiments and data analysis

Small angle X-ray scattering (SAXS) data of UspA1\(^{(527-665)}\) and the N-CEACAM1/UspA1\(^{(527-665)}\) complex were collected on beamline X33 at the European Molecular Biology Laboratory (EMBL) of the Deutsches Elekronen Synchrotron (DESY, Hamburg, Germany) at a wavelength of \(\lambda = 0.15 \text{ nm}\). The sample to detector distance was 2.4 m, covering a scattering range of 0.16 nm\(^{-1}\) < q < 4.85 nm\(^{-1}\)(q = 4\(\pi\) sin\(\theta\)/\(\lambda\), where 2\(\theta\) is the scattering vector). Samples of the complex at concentrations of 2, 5 and 10 mg/ml were exposed for 60s. Images were integrated and corrected for background buffer scattering using in-house software at station X33 and then merged using PRIMUS (Konarev, 2003). The particle distance distribution function, \(p(r)\), and the radius of gyration, \(R_g\), were calculated using GNOM (Semenyuk and Svergun, 1991). The shape of the protein was evaluated using DAMMIN (Svergun, 1999). Twenty four reconstructions of the N-CEACAM1/UspA1\(^{(527-665)}\) complex were generated and the resulting structures were filtered and averaged in DAMAVER (Volkov and Svergun, 2003).

Molecular modelling

N-CEACAM1 was manually docked into its putative binding site (the hydrophobic patch identified from the crystal structure) on UspA1\(^{(527-665)}\) using guidance from the mutational data of both components and visual inspection of shape complementarity.
This model complex was then soaked with a 5 Å layer of water and relaxed with 2000 steps of conjugate gradient energy minimisation and the CVFF forcefield. Multiple 0.1 ns molecular dynamics simulations of UspA1 - with none, one and two N-CEACAM1s bound - were performed at 300 K under 12 different initial random velocities to investigate possible bending of the coiled-coil. A representative example of each is shown in Supplementary data 6. InsightII 2005/ Discover 2.98 (Accelrys) were used to view, manipulate molecules and for the molecular mechanics calculations.

Electron microscopy

Bacterial cells were disrupted with 0.5mm glass beads at 48000 oscillations per minute for 30 sec in a mini-bead beater™ (Biospec Products Inc. OK, USA). Unruptured bacteria were removed by centrifugation (2500g, 5 min), bacterial membranes were collected by ultracentrifugation (30,000g, 30 min at 4°C) and suspended in PBS. Samples (5 µl) of envelopes, either native or pre-incubated with N-CEACAM1 (2.5 ng/ml) were applied to glow-discharged collodion coated copper grids. The grids were then washed by placing face down on two consecutive drops of sterile filtered water and negatively stained using 1% methylamine tungstate. The grids were viewed using an FEI CM10 EM operated at 80KV. Images were recorded at 39,000 x magnification on Kodak 4489 film with the first zero of the contrast transfer function in the range 20 - 25Å.

References: