

Supplemental Data

The Crystal Structure of ORF-9b, a Lipid Binding Protein from the SARS Coronavirus

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Supplemental Experimental Procedures and Results

Is ORF-9b a Dimer in Solution? Analytical Ultracentrifugation (AUC)

To determine the oligomeric state of ORF-9b in solution, analytical ultracentrifugation was performed using a Beckman Opima XL-I (Palo Alto, CA) analytical ultracentrifuge. Samples at a number of concentrations were brought to equilibrium at 12,000 rpm, 15,000 rpm and 21,000 rpm and their distributions were measured using absorbance optics. Protein distributions at equilibrium were analysed using ULTRASPIN (Altamirano et al., 2001) with a single-species model. Any oligomerisation behaviour is then observed as a higher whole-cell weight-average molecular weight (Mw). Values for Mw over the concentration range used were plotted using ProFit (QuantumSoft, Uetikon am See, Switzerland).

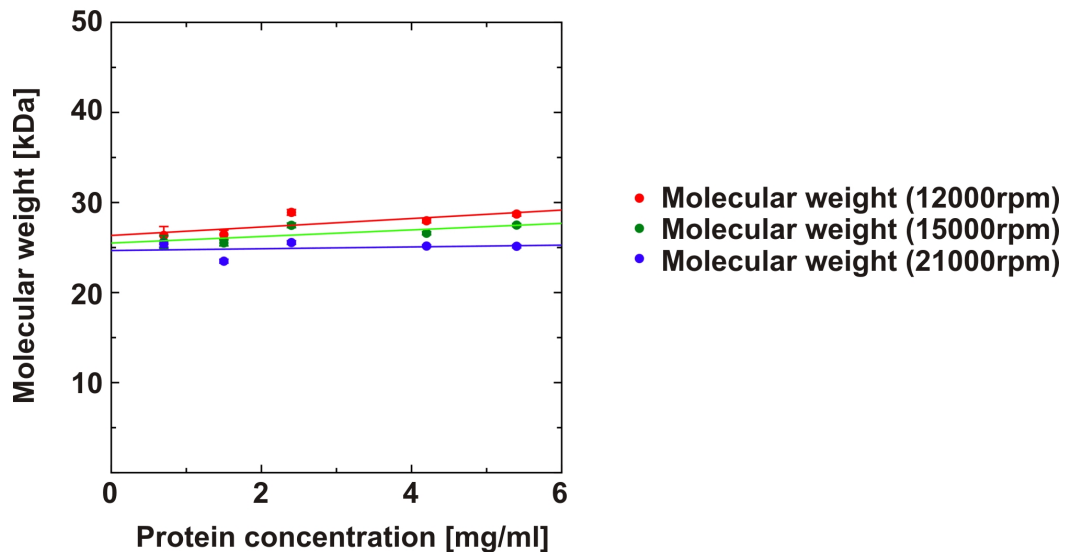


Figure S1: Analytical Ultracentrifugation Results. Equilibrium-mode analytical ultracentrifugation indicates that the protein is a dimer at all concentrations analysed, with a small amount of higher oligomers or interactions at higher concentrations and lower speeds. The mass at infinite dilution is calculated to be 24.67kDa, close to the expected mass of dimeric ORF-9b (23.25kDa).

ORF-9b Sequence Conservation in Different SARS Isolates

The sequence of the ORF-9b protein is well-conserved in the genomes of all human SARS known isolates (for example, 100% sequence identity in SARS isolates from Hong Kong, Taiwan, Shanghai). The sequence conservation with recently identified isolates from animals (Li et al., 2005; Wang et al., 2005) is slightly lower (see Figure S2, below), with most significant changes found in the poorly ordered regions of the crystal structure.

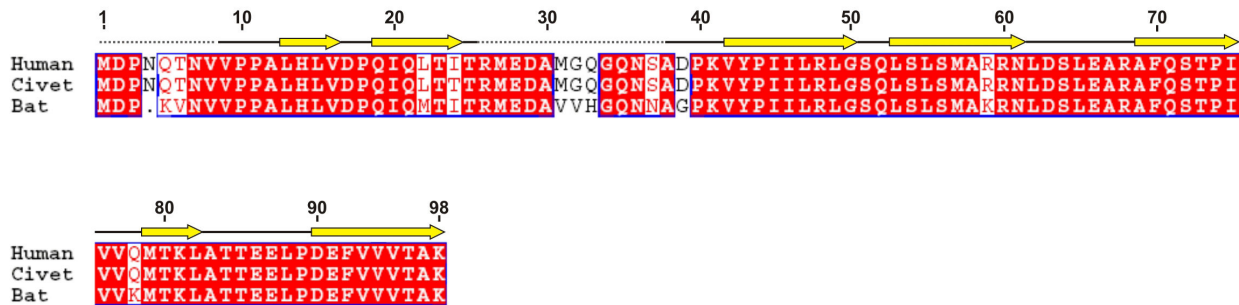


Figure S2: SARS-Coronavirus ORF-9b in Human and Animal Isolates (Li et al., 2005; Wang et al., 2005).

Residue numbering refers to the ORF-9b protein from human SARS-CoV. Protein secondary structure is represented as yellow arrows (β -strands), solid lines (random coil conformation) and dotted lines (disordered regions). Figure produced with MultAlin (Corpet, 1988) and ESPript (Gouet et al., 1999).

Supplemental References

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