P 10 Cloning, expression, purification, and crystallisation of *B. burgdorferi* outer surface protein *BbA69* for structural and functional studies

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Borrelia burgdorferi is the causative agent of Lyme disease – the most prevalent arthropod-borne infectious disease. When *B. burgdorferi* is transmitted from ticks to mammals there are several outer surface proteins that are up-regulated or down-regulated by temperature or mammalian host-specific signals. One of these proteins which is up-regulated by temperature shift from 25°C to 37°C, but 1.8-fold down-regulated by host-specific signals is BbA69, and we are interested to study the protein as there is little known about the protein functional role in *B. burgdorferi*, and accordingly there are no data on protein structure.

In this study, a gene coding for *B. burgdorferi* outer surface protein BbA69 was amplified from *B. burgdorferi* strain B31. Cloning and expression of the protein in *E. coli* C2566(DE3) was carried out using pET expression systems vector with integrated histidine tag and TEV protease cleavage site (EMBL). After cell lysis by sonication, the recombinant protein was purified using various chromatographic methods – affinity chromatography using Ni-NTA agarose (Qiagen), ion-exchange chromatography, and size-exclusion chromatography to obtain the recombinant protein of sufficient purity. The purified protein was crystallised using sparse-matrix screen with vapour diffusion method in sitting drops by screening 200 initial conditions followed by the optimization of crystallisation conditions to get the most suitable crystals for diffraction analysis.

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