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Prospects of novel bio-antibiotics to inhibit the BAM complex

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Multi-drug resistance is rapidly spreading among bacteria and has become a severe threat to human health. The development of new antibiotics against Gram-negative bacteria is extremely challenging, mainly since the bacteria possess two membranes to protect against drug penetration. The essential surface-exposed outer-membrane protein β-barrel assembly machinery (BAM) complex is a promising target for new antibiotics, directly accessible from the extracellular side. Several BAM inhibitors have been discovered, namely Darobactin (1,2), Dynobactin (3) and BamA-OMPTA (4,5). Further efforts were made to target BAM with novel bio-antibiotics. Storek *et al.* (6) found an antibody (MAB1), which is binding and inhibiting BAM. However, the antibody is not able to bind in the cellular context of lipopolysaccharide (LPS), making it of limited use for clinical approaches. The smaller nanobodies are a promising alternative to circumvent the steric hindrance by LPS.

Here, we provide a detailed structural investigation of three nanobodies that bind BAM in living wild-type bacteria. The nanobodies were obtained by alpaca immunization and the deep screening method NestLink (7). We analyzed them with the structural methods cryo-EM, X-ray crystallography and NMR spectroscopy. The nanobodies bind a surface-exposed epitope, formed by three extracellular loops L4, L6 and L7 of the BAM complex. The nanobodies thus bind BAM in an almost perpendicular manner to the top of the β-barrel. This epitope is accessible in living wild-type bacteria, allowing to bind in context of LPS. Furthermore, the structures show that the discovered epitope is preserved among all observed conformations of BAM and that the characterized nanobodies therefore do not exhibit conformational selectivity. It is thus not surprising that they do not display antibacterial activity. The study reveals how BAM can be targeted *in vivo* with novel biomolecular compounds and highlights structural features of the epitope required for antibacterial activity. The findings provide a next step towards the development of bio-antibiotics targeting BAM.

References:

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