Title:	Investigations of DNA-binding and secretion patterns of HU using Molecular, Biochemical and Biophysical Methods
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Abstract:	Histone-like DNA binding protein also commonly known as HU belongs to the class of Nucleoid associated proteins (NAPs) [6]. It is widely present in Gram-negative bacteria [2]. This protein has a known DNA-binding disordered region or loop, rich in positively charged residues which helps it to bind to negatively-charged DNA [10, 11, 12]. It has also been reported that there are Lysine residues at position 83 and 86 and Phenylalanine at position 47 that are suspected to be non-canonical DNA-binding sites of HU [20, 21]. Therefore, several variants of HU were designed where lysine to alanine substitutions were engineered at the 83 rd and 86 th (non-canonical sites) residues in the background of loop- replaced HU (canonical site). Using biophysical and biochemical experiments, we observed that even after the removal of both the Lysine residues and the replacement of the disordered region, HU retains its three-dimensional structure, but completely loses the DNA-binding ability. Our group (from Bhishem's work) has also observed that HU provides cell-cell adhesion because of its LPS-binding ability (due to the structural similarity in the negatively-charged phosphate in Lipid A backbone of LPS and DNA) which is an indication of its possible role in maintaining the integrity of biofilms [32]. Hence, we were also interested in studying the ability of HU to be secreted from the cell into the extracellular matrix and looked at the localisation of HU in the periplasm. Since previous reports had confirmed the presence of HU in outer membrane vesicles [25], we also tried to investigate the link between the LPS-binding nature of HU with its presence in the OMVs extracted from the cells towards stationary phase.
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