Title:	HU-AB simulacrum: Fusion of HU-B and HU-A into HU-B-A, a functional analog of the Escherichia coli HU-AB heterodimer
Authors:	Arora, Kanika (/jspui/browse?type=author&value=Arora%2C+Kanika) Thakur, Bhishem (/jspui/browse?type=author&value=Thakur%2C+Bhishem) Gupta, Archit (/jspui/browse?type=author&value=Gupta%2C+Archit) Guptasarma, Purnananda (/jspui/browse?type=author&value=Guptasarma%2C+Purnananda)
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Abstract:	In enteric bacteria such as Escherichia coli, there are two homologs of the DNA-binding nucleoid associated protein (NAP) known as HU. The two homologs are known as HU-A and HU-B, and exist either in the form of homodimers (HU-AA, or HU-BB) or as heterodimers (HU-AB), with different propensities to form higher-order oligomers. The three different dimeric forms dominate different stages of bacterial growth, with the HU-AB heterodimer dominating cultures in the stationary phase. Due to similarities in their properties, and the facile equilibrium that exists between the dimeric forms, the dimers are difficult to purify away from each other. Although HU-AA and HU-BB can be purified through extensive ion-exchange chromatography, reestablishment of equilibrium interferes with the purification of the HU-AB heterodimer (which constitutes ~90% of any population with equal numbers of HU-B and HU-A chains). Here, we report the creation of a functional analog of HU-AB that does not appear to partition to generate any minority populations of HU-AA or HU-BB. The analog was constructed through genetic fusion of the HU-B and HU-A chains into a single polypeptide (HU–B-A) with a glycine/serine-rich linker of 11 amino acids separating HU-B from HU-A, and a histidine tag at the N-terminus of HU-B. HU-B-A folds to bind 4-way junction DNA, and displays a significant tendency to form dimers (i.e., analogs of HU tetramers), and a higher thermodynamic stability than HU-BB or HU-AA, thus explaining why it dominates mixtures of HU-B and HU-A chains.
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