Proteins that cross-link and polymerize on DNA, studied by physical chemical techniques

PhD-fellow: C.H.J.M. Wintraecken, MSc (Kathelijne) (Laboratory for Physical Chemistry and Colloid Science, WUR)
Kathelijne.Wintraecken@wur.nl

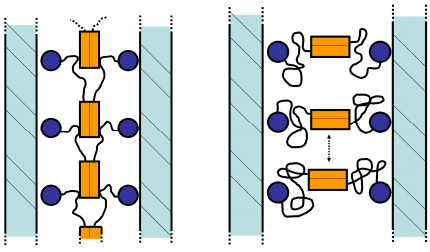
Supervisors: Dr. R. de Vries
Prof. Dr. M.A. Cohen Stuart

Sponsors: Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)

H-NS is a global regulator of many environmentally sensitive genes, particularly virulence genes. This bacterial nucleiod protein acts as a repressor by polymerising on DNA, crosslinking two helices. H-NS is a small dimer protein consisting of a DNA-binding domain, coupled to a dimerization domain by a long flexible linker. The dimerization domain may have different conformations dependent on temperature (fig1). Genetic data suggests that higher order H-NS self-assembly is due to interaction between a few residues close to the N-terminal and some unidentified residues in the flexible linker region of H-NS. Very little is known about how H-NS polymerization on DNA depends on the environmental conditions such as temperature, osmolarity etc. to which it responds in the cell.

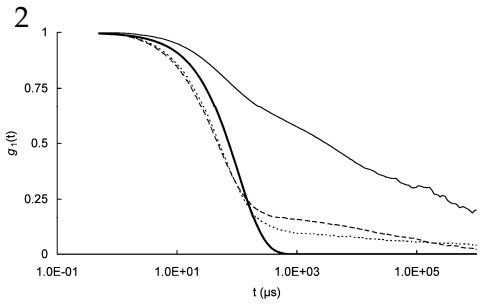
Our aim is to: 1. elucidate whether DNA topology or H-NS self-assembly triggers polymerization/repression, 2. investigate H-NS polymerization and its dependence on environmental factors, using DLS.

1



Low T High T

2



Our autocorrelation data shows H-NS polymerizes without DNA present, and forms network-like structures (fig2). Large self-assembled H-NS structures that exist at temperatures below 28°C, and fall apart at higher temperatures, in range of *in vivo* virulence activation. H-NS polymerization is also dependent on salt concentration, strongest at salt concentrations similar to *in vivo* values. Data form our mutants indicates involvement of the linker and helix 3 in temperature dependent switching behaviour and oligomerization. These results show clearly that H-NS is capable of higher-order self-association independent of DNA, and it also shows a strong temperature- and salt dependence of this phenomenon in line with *in vivo* observations. Furthermore, we have identified a patch in the linker that is strongly involved in this behaviour.

References

Wintraecken, K., Spurio, R., Cohen Stuart, M. and R. de Vries. 2009. submitted