Synergistic effects in DNA condensation

Supervision: Assoc. Prof. Rita Dias (phone: 73593422, rita.dias@ntnu.no)

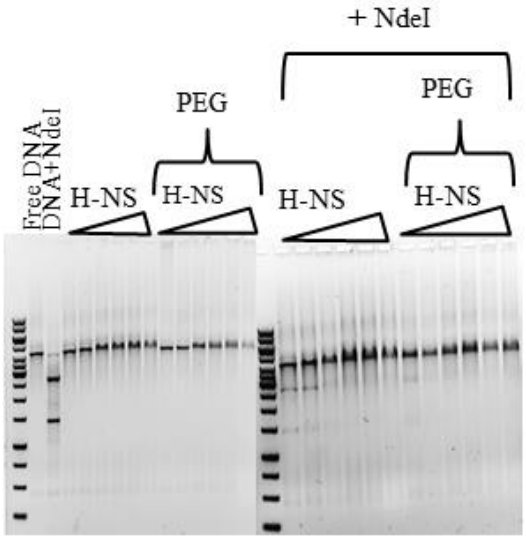
The genome of E. coli has about 6.4 million base pairs, corresponding to a circumference of 1.6 mm. In order to fit inside the small volume of the cell (ca. 0.65 μm^3), the DNA needs to be folded. In eukaryotic (e.g. humans), DNA condensation is done by proteins called histones. This type of proteins does not exist in bacteria. Instead, there are a few different types of proteins, such as H-NS proteins, that modulate the genome. In addition, and contrary to eukaryotic cells, bacteria have no nuclear membrane, and the fact that the cytoplasm has a very large number of macromolecules (RNA and proteins) is believed to help DNA condensation, by the so-called molecular crowding effect.

Despite the extensive study in this area during the past years, the mechanistic details and their relative contribution to DNA condensation is still unclear.

We have found, using competitive DNA foot printing and DNA digestion assays, that H-NS protein binds preferentially to the more flexible AT tracks of the DNA, and that the presence of crowding molecules, such as PEG (a neutral polymer) enhances DNA–H-NS binding.

In this project we assess the characteristics of the crowding environment (charge and topology) on DNA condensation and protection towards DNase digestion.

We use a range of biophysical techniques, including fluorescence spectroscopy, fluorescent correlation spectroscopy, and gel electrophoresis, to evaluate the binding of various DNA binding agents to DNA and DNA condensation.



DNA foot printing assay. Left-hand side: Agarose gel electrophoresis of linear DNA with increasing H-NS concentrations in the absence and presence of PEG. Right-hand side: same samples as in the first gel but now in the presence of NdeI, a restriction enzyme for AT-rich regions. We can see that sufficiently large concentrations of H-NS hinder the DNA digestion, and that the presence of PEG lowers the concentration of H-NS needed to protect DNA towards enzyme activity. Experiments and figure by Sravani Keerthi Ramisetty (PhD student)

Students that have worked or are currently working in this project:

Šárka Sovová, Master student from Brno University of Technology, Czech Republic