## SUMMARY

# Structural and stability studies for the N-terminal region (1-57) of HP-NAP,
## Molecular dynamics simulations

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The **H**elicobater **P**ylori **N**eutrophil **A**ctivating **P**rotein (HP-NAP) (Evans et al. 1995) is a 180 kDa dodecameric protein of human pathogen *Helicobacter pylori* (Zanotti et al. 2002) which has been shown to upregulate adhesion molecules of the series on human neutrophils, increasing binding of these cells to the endothelium (Montecucco and de Bernard 2003). It is also known that interacts with neutrophils, leading them to an oxidative burst, which is accompanied by host tissue distraction (Montecucco and de Bernard 2003).

In order to identify the region of the protein which is responsible for the binding with neutrophils, its N-terminal region (fragment 1-57) has been cloned in pET11a overexpression after Polymerase Chain Reaction by using pET11a-HP-NAPwt DNA as template and the appropriate primers. The recombinant vector was introduced into “competent” E.coli BL21(DE3) cells for gene overexpression and 3 hours after IPTG induction the cells were harvested, suspended in a buffer containing 6M Urea, 20mM Tris-HCl pH 7.5, 0.5M NaCl and sonicated.

After centrifugation the clear cell lysate was subjected to $(NH_4)_2SO_4$ fractionation and the fraction containing the highest amount of the protein was used for the next steps. Dialysis of the fraction against low ionic strength aqueous solutions resulted only in co precipitation of the N-terminal part with the other proteins of the solution. Therefore, all purification procedures were carried out in buffers containing at least 2M urea. Several purification procedures as ion exchange chromatography were used for the purification of the fragment without success. This HPNAP region is consisted of two a-helixes that are connected with a loop (Zanotti et al. 2002). One a-helix, the hydrophilic, is exposed into the interior of the intact protein and is likely to be involved in the iron uptake and deposit process while the other one is hydrophobic and is protected by other structural elements within the protein. During the overproduction within the host the fragment is exhibited to several cell elements to which it could theoretically develop interactions or it is structurally reorganized. In

both cases the possible interactions lead to its accumulation with other proteins that did not allow its effective purification. The purification of this fragment was finally achieved by cloning its gene on a pET29c plasmid vector and overexpressing it as a chimeric protein (bound to a 6-His tagged “tail”). The purification procedure includes two steps, the implementation of a Ni-NTA affinity column and a subsequent $(NH_4)_2SO_4$ fractionation, where this protein fragment is successfully purified at the 40% fraction.

In order to understand the unique and unexpected behavior of the N-terminal region of the studied protein we studied its structural properties and stability by Molecular Dynamics techniques using the program NAMD (Kalé et al. 1999) with the CHARMM27 force field for proteins and nucleic acids. The fragment has been cut off from the crystal structure of HP-NAP (Zanotti et al. 2002) and was simulated in water at a temperature range from 300 to 340 K in order to understand its remarkable resistance to traditional purification methods. The structure of the molecule appears stable up to 320 K and is gradually denaturated from 320 to 340 K.

What appears to be concluded from these simulations is the fact that the fragment owes its stability to hydrophobic interactions between side chains situated at the central regions of the two helical regions as well as to a few hydrogen bonds between the two a-helices.