Solution properties of DmpR – A phenol sensing, sigma-54 dependent transcription co-activator and member of the AAA+ superfamily of proteins

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Our overall objective is to understand the molecular mechanisms by which bacteria sense, respond to, and degrade aromatic compounds. At the top of the hierarchy of events that allow bacteria to utilize aromatic compounds as carbon sources lie complex regulatory systems that control the expression of the specialized catabolic enzymes.
Pseudomonads are bacteria that are able to clean wastes from sewage water at water treatment plants. They are able to grow on pollutants such as phenols and creosols as sole carbon sources. These compounds are degraded by enzymes encoded by the dimethylphenol operon that harbors the fifteen structural genes required to encode the nine catabolic enzymes.

DmpR, which regulates the *dmp*-operon in a (methyl)phenol dependent way in *Pseudomonas putida*, belongs to the AAA^+ superfamily of ATPases and more specific to the family of σ^{54}-dependent transcriptional regulators that control a multitude of physiological processes in response to environmental signals. The domain structure of DmpR is typical for σ^{54}-dependent regulators, where each of the four domains mediates a specific function such as signal reception, A-domain, multimerization, B- & C-domains, interaction with σ^{54} RNA-polymerase and ATP triggered activation, C-domain, and binding to DNA, D-domain.
Inactive DmpR forms homodimers of 70 kDa. Upon activation by dimethylphenols and ATP, DmpR undergoes structural rearrangements that lead to the formation of most likely hexameric rings. For reviews see [1, 2] and references therein.

We have initiated the solution structure determination of DmpR, using small angle X-ray scattering, SAXS, in collaboration with Dr. Dimitri Svergun at EMBL-Hamburg,. For this purpose we use an N-terminally truncated version, ΔDmpR that forms stable dimers in the absence of ATP. Addition of ATP triggers a dimmer-to-hexamer transition. This is in analogy to the full length protein, where dimers correspond to the effector free or effector bound form and where hexamers can only be induce when the effector loaded protein also binds ATP.

Although the DmpR protein is a dimer during purification, we were only able to observe much larger particles during the SAXS measurements. The data obtained are compatible with a model where two hexameric rings of DmpR pack back to back.

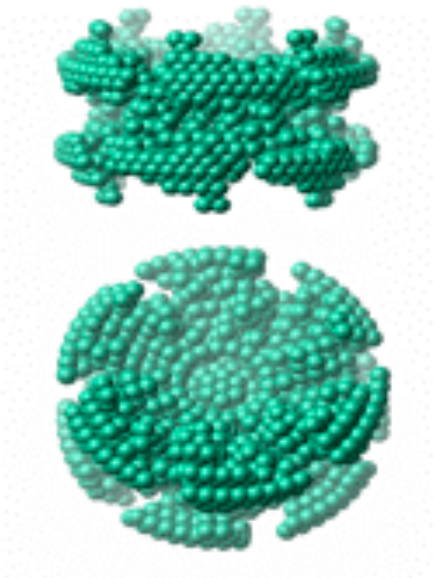

Figure 1. The "dummy sphere" model of the dodecamer sandwich structure as modelled from SAXS data.

In addition of the SAXS measurements we have also attempted to crystallize DmpR. Crystals diffracting to about 2 Å were obtained as a result of the EMBL-Hamburg Crystallization Workshop held November 2005. Interestingly, we have crystallized DmpR in its dimeric form, as determined from size exclusion chromatography. The crystal structure determination is under way.

The structural dissection of DmpR, using small angle solution X-ray scattering and crystallographic techniques will hopefully provide sufficient information on how aromatic compounds as well as intra- and inter-molecular interactions directly regulate the activity of DmpR. In particular we would like to be able to derive a general mechanism for the transition from the inactive dimeric to the active hexameric state of DmpR and the other members of this important family of of σ^{54}-dependent transcriptional activators.

References:

1. Shingler V: **Signal sensing by sigma 54-dependent regulators: derepression as a control mechanism.** *Mol Microbiol* 1996, **19:**409-416.
2. Schumacher J, Joly N, Rappas M, Zhang X, Buck M: **Structures and organisation of AAA+ enhancer binding proteins in transcriptional activation.** *J Struct Biol* 2006, **156:**190-199.