MX-578 X-ray diffraction of a viral-like capsid. Mutagenesis and chemical modification of lumazine synthase

Symmetry, size and availability make lumazine synthase an attractive model system to approach the use of viral capsids as nanoplatforms with applications in materials science and medicine. However its use in vivo implies to circumvent problems such as the interaction with the immune system or the engineering of a specificity to drive it to the target receptor via the modification of the surface. An attractive alternative to overcome many of the inconveniences of the biological approach (i.e. site directed mutagenesis, overexpression, purification and folding/assembling for each chimaera) is the chemical modifications of the surface.

The aim of this proposal is to gather structural information to extend our knowledge on reactivity and specificity of the complex surface of lumazine synthase as a necessary step to develop structure-based ligands that react with the surface of the capsid and modulate the interaction with other molecules (receptors at target organ or immune cells). To this end data sets were collected from three different samples:

1. Cys-free mutant. Lumazine synthase from Bacillus subtilis crystallizes in space group P6322 or C2. However, the quality of these crystals is poor and structural information is only available at 2.4 A resolution. As classical strategies for growing better diffracting crystals have so far failed, protein engineering has been employed in order to improve the overexpression and purification of the molecule as well as to obtain new crystal forms. The replacement of the cysteines forced a different molecular packing that yielded R3 crystals with unit-cell a = b = 313.02, c = 365.77 Å, $\alpha=\beta = 90.0$, $\gamma= 120$, and diffracted to 1.6 Å resolution. Results have been published in *Acta Cryst*. (2008). F**64**, 625-628. This is the best resolution ever achieved for lumazine synthase from B. subtilis and is on a par with the resolution obtained with the enzyme from the hyperthermophilic eubacterium Aquife eolicus. Work is currently in progress towards structure determination by molecular replacement and towards analysis of the crystal rotational order by transmission electron microscopy.

2. PEGylated sample. Full data set of the PEGylated capsid was collected at high resolution. Unit cell are similar to those for the Cys-Free mutant. Anomalous signal was also collected in the context of the beam time grated at BM16 by project 16-01 678.

3. Glycosylated samples. Crystals did not diffract at suitable resolution for structural purposes.