

## Introduction and aim of the study:

The quaternary structure of enzymes is an important aspect in understanding their activity and regulation. Multi-subunit enzymes frequently display cooperative or anti-cooperative properties with respect to substrate binding or allosteric inhibition. N-Acetyl-L-Glutamate Kinase (NAGK) is a key enzyme in the biosynthesis of ornithine and arginine. Crystal structure analysis of NAGK from cyanobacteria and plants revealed a homo-hexameric quaternary structure, in which three homodimers form a toroid (ring-like structure with a central hole). Non-denaturing gel electrophoresis of the NAGK enzyme from the mutant cyanobacterial strain *Synechococcus* WH5701 indicated that the hexameric structure may be unstable.

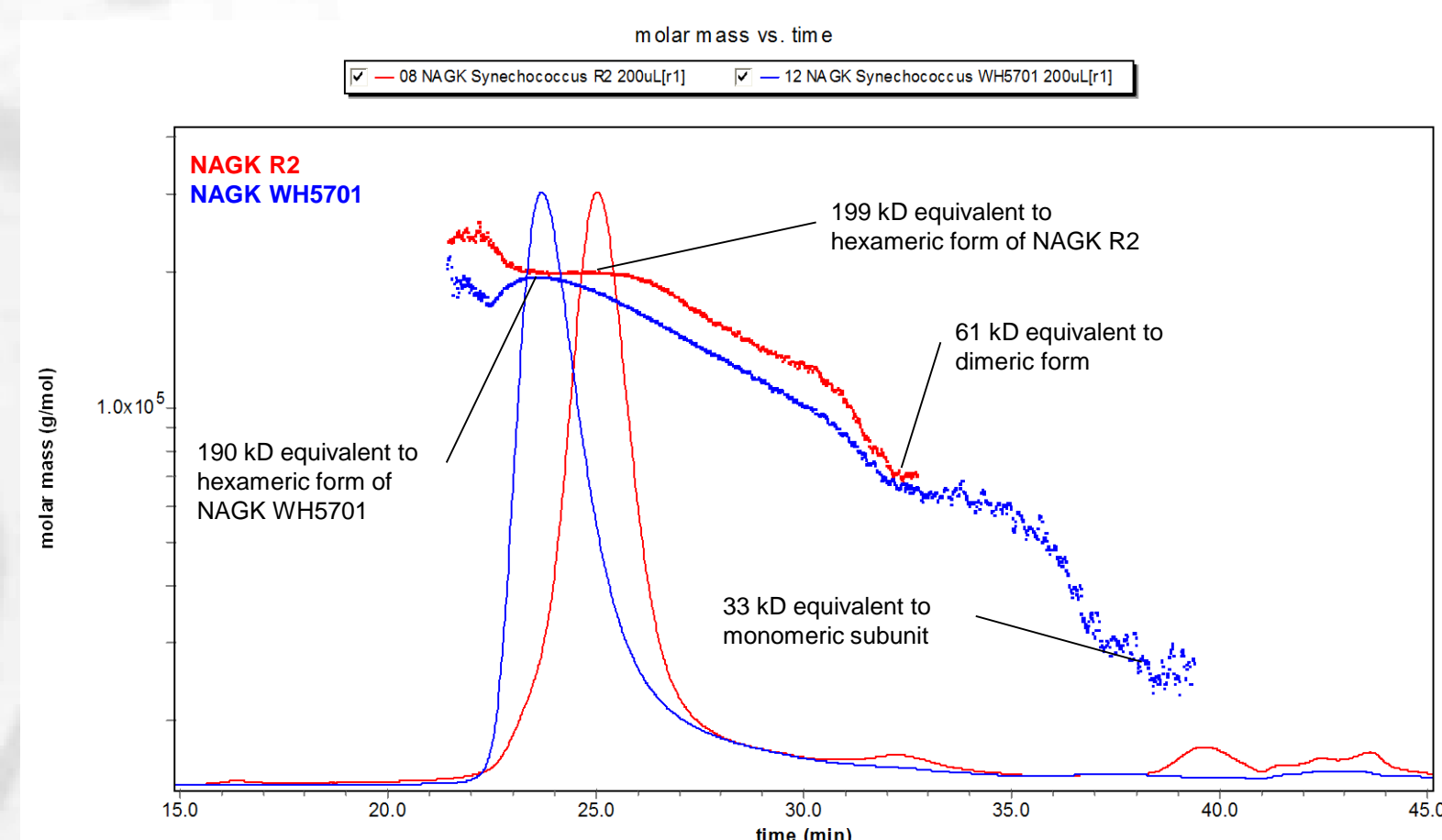
To gain deeper insight into the enzymatic structures, the oligomeric state of NAGK from strain WH5701 was compared to the well-studied enzyme from *Synechococcus* PCC7942 (R2).

## Materials:

- Size-exclusion chromatography SEC (GE Healthcare Superdex 200 300/10, 1.0 mL/min)
- UV detection (Agilent G1314 UV detector operated at 280 nm)
- MALS (Wyatt Technology DAWN HELEOS 18 angle light scattering detector + online QELS)
- DRI detection (Wyatt Technology Optilab rEX differential refractometer)

## Results:

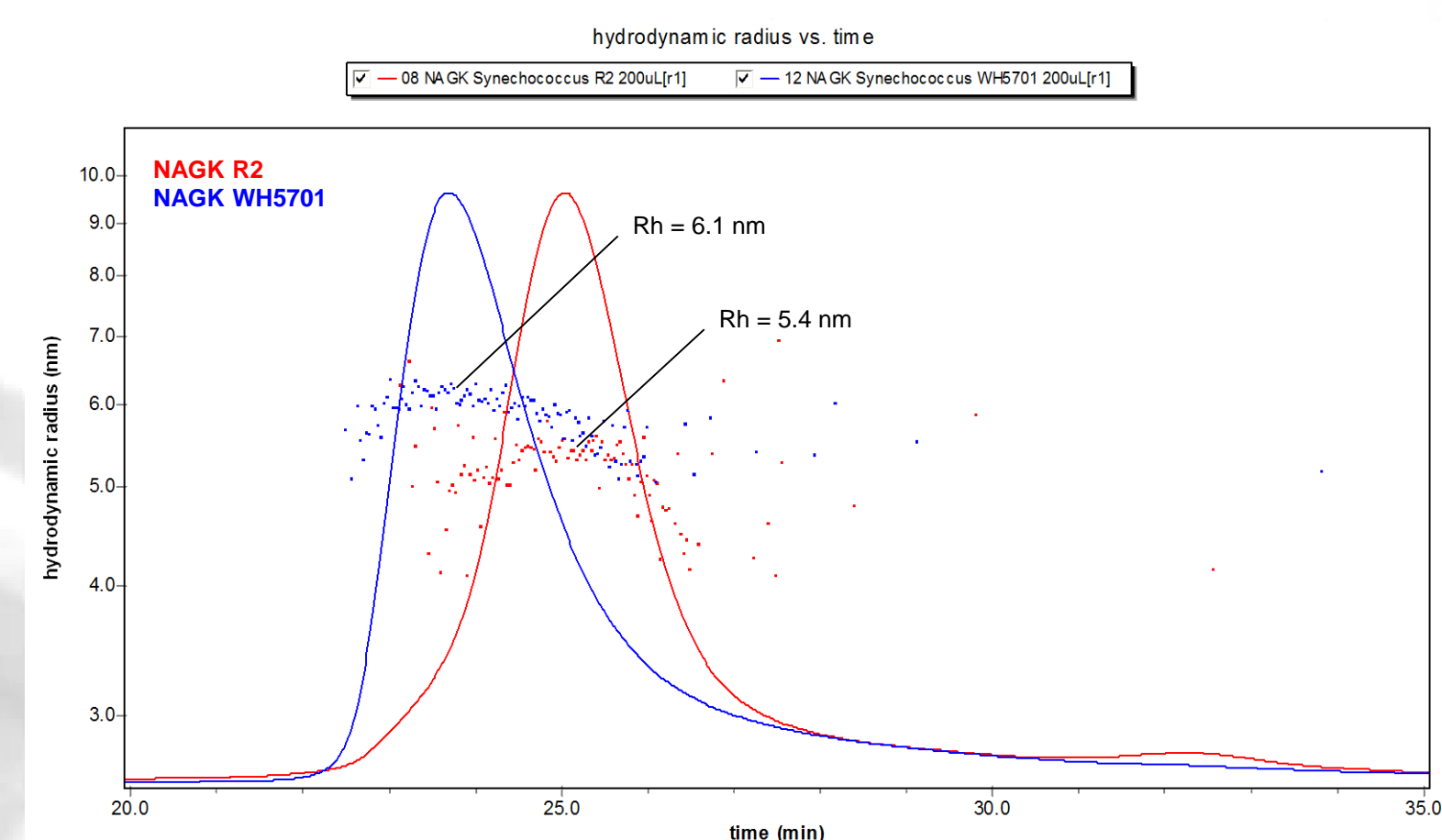
### Comparison of molar masses of NAGK derived from strains R2 and WH5701



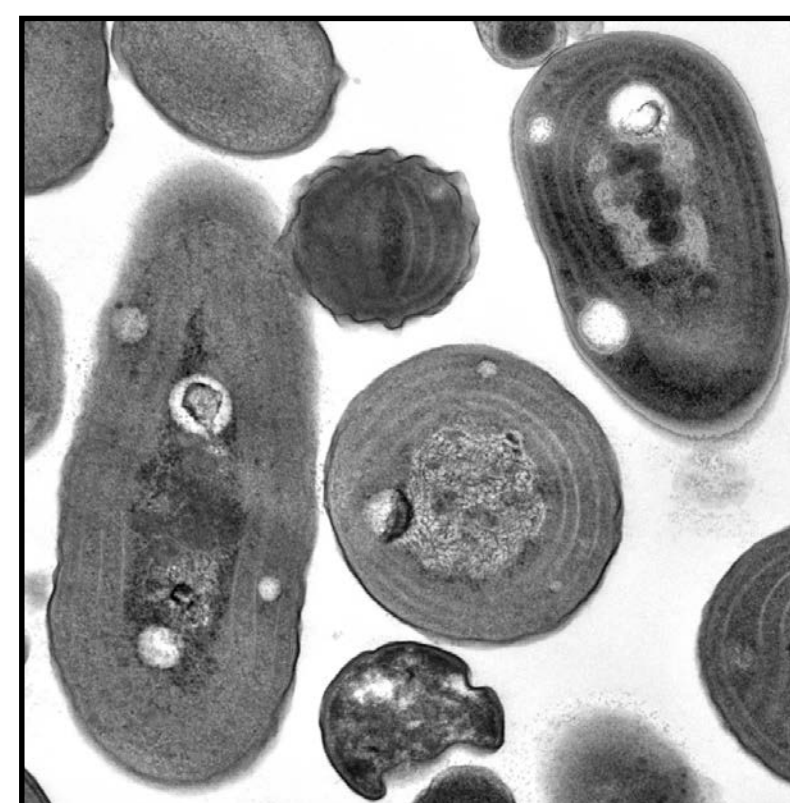
Plot of molar masses determined by DAWN light scattering versus elution time. The UV elution profile is plotted as an overlay. **Red:** NAGK from R2; **blue:** NAGK from strain WH5701.

- main fraction of both proteins corresponds to a mass of 199 kD (R2) or 190 kD (WH5701)
  - equivalent to hexamer of 34 kD subunits
- minor peak for R2 NAGK, corresponding to the dimeric form of 61 kD
- gradient of molar masses between dimeric and hexameric state
  - various mixtures of dimeric and hexameric forms
- WH5701 shows monodisperse species at 33 kD, corresponding to the monomeric subunit
  - higher proportion of subunit decomposition in WH5701 than in R2 NAGK

### R2 and WH5701 DLS measurements of hydrodynamic radius Rh



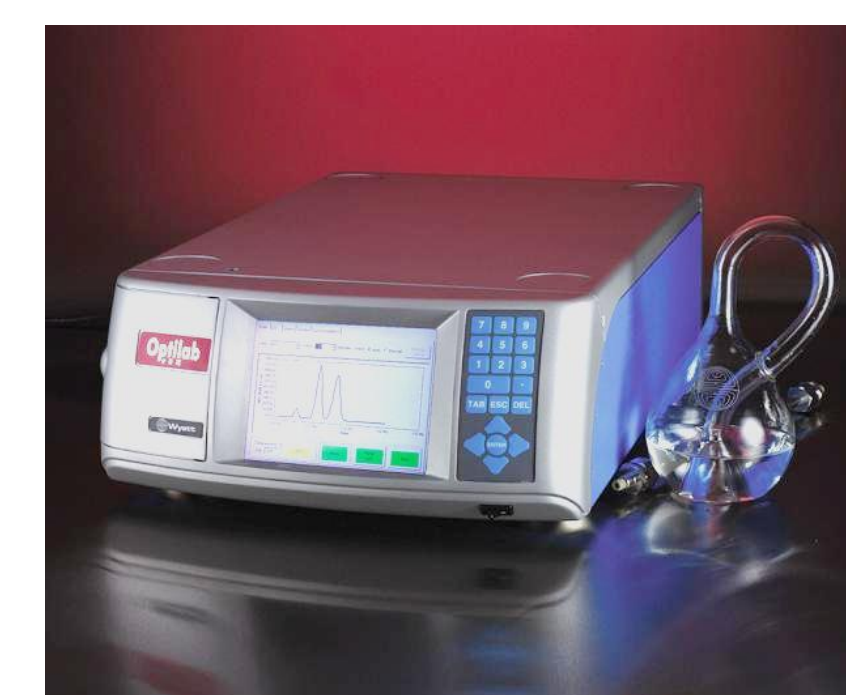
- Rh of the WH5701 NAGK toroid is larger than R2
  - WH5701 toroid is less tightly packed
  - looser association of enzymatic subunits



The oceanic cyanobacterium *Synechococcus* SP. (Transmission electron micrograph by Wenche Eikrem and Jahn Thronsen, University of Oslo)

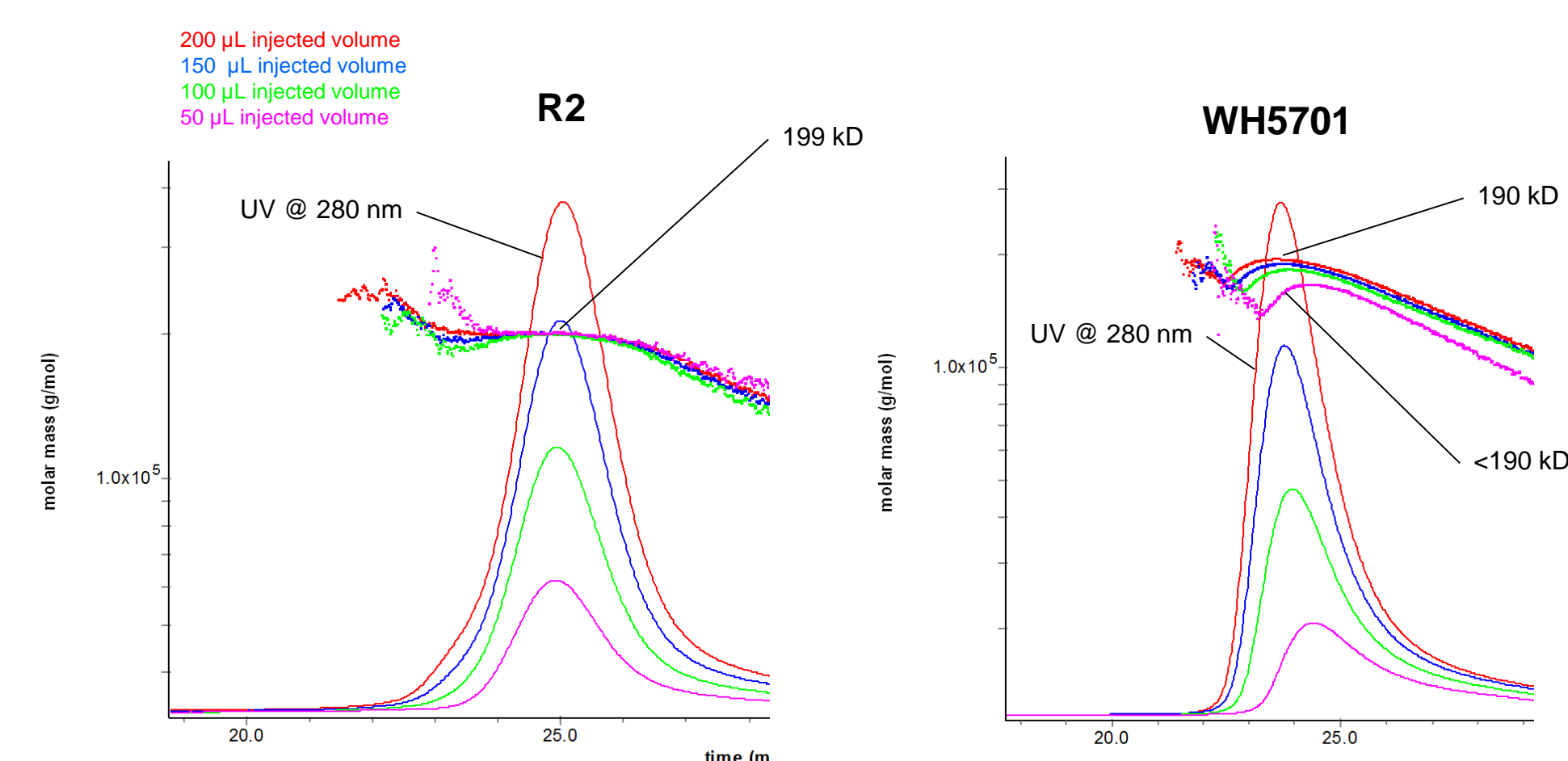


N-Acetyl-L-Glutamate Kinase (NAGK)



High-end equipment for macromolecular characterization: Wyatt's DAWN Heleos II (left) and Optilab rEX

### Comparison of complex stability between R2 and WH5701



Plot of molar masses using different injection volumes. Whereas the Mw for the R2 strain is constant for all volumes, WH5701 shows reduced Mw with low volume (<190 kD).

→ WH5701 enzyme complex is less stable than R2

## Summary:

- NAGK R2 and NAGK WH5701 were characterized using SEC/MALS.
- Hexameric, dimeric, and monomeric fractions were identified.
- The Rh of the hexamer was measured to be 5.4 and 6.1 nm, respectively.
- The larger radius of the WH5701 toroid compared to R2 indicates that its subunits are less tightly packed, showing a significant extent of subunit decomposition.

The present analysis shows for the first time that the hexameric structure of cyanobacterial NAGK enzymes, depending on the strain investigated, is constituted in a dynamic equilibrium of mono- and dimeric forms and that the mutant type WH5701 enzyme complex is less stable than R2.

The data presented in this poster were graciously submitted by Prof. Dr. Karl Forchhammer, Lehrstuhl für Mikrobiologie/Organismische Interaktionen, University of Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany

