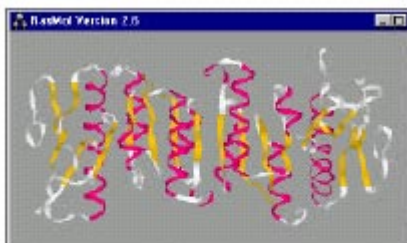


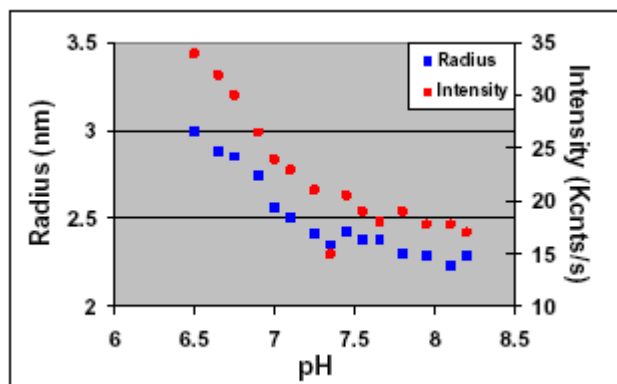
# pH Dependent Monomer-Dimer Equilibrium Constants for GART

Glycinamide ribonucleotide transformylase (GART) catalyzes formyl group transfer in the de novo purine biosynthetic pathway in cells. Because of the dependence of cancer cell growth on purine, the chemo-therapeutic inhibition of GART and other transformylase enzymes is a potential method for combating cancer. The crystallographic dimeric structure of GART at pH 6.5 is shown below.



*Figure 203-1: Crystallographic ribbon structure of dimeric glycinamide ribonucleotide transformylase.*

The pH of optimum activity for GART is circa 7.0. Below this pH, a decrease in activity is observed, and this decrease has been attributed to a pH dependent shift in the monomer-dimer equilibrium. A DynaPro 801 was used to monitor the size of GART as a function of pH. The radius and total intensity results are shown in Figure 203-2. As seen in this figure, the average particle size varies by 30% between pH 6.5 and 8.2. Since the weight concentration is fixed, the change in intensity ( $I \propto C \cdot MW$ ) suggests that the decrease in size with increasing pH is due to particle dissociation, rather than a shift in structural conformation.



*Figure 203-2: Apparent radius and total intensity data showing pH dependent dissociation of GART.*

Using a MW vs. RH calibration curve developed from a series of protein standards, the molecular weight for GART at pH

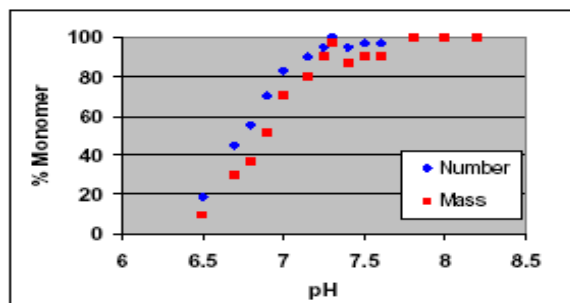
7.9 and 6.5 was estimated as 23 and 44 kDa respectively. These MW values are consistent with the reported monomer and dimer values of 23 and 46 kDa, suggesting that the shift from monomer to dimer occurs completely within the pH range of 6.5 to 7.9.

Because of polydispersity effects, the resolution in dynamic light scattering studies of mixtures is limited to particles with sizes differing by a factor of 2. However, for a mixture of particles of known size and mass, the average hydrodynamic radius can be used to estimate the number and mass fraction of each component in the mixture. The procedure is incorporated into the **DYNAMICS** software package in the Apparent Fraction Calculator.

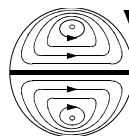


*Figure 203-3: Apparent Fraction Calculator in DYNAMICS software package.*

The pH dependent number and mass % monomer data for the GART study are shown in Figure 203-4. Since the number % is directly proportional to the molar concentration, the number % data in this figure is representative of the pH dependent binding constant for the monomer-dimer equilibrium.



*Figure 203-4: pH dependent Number and mass % monomer for GART monomer-dimer equilibrium.*



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