

## Characterization of Heparin Binding to Tau Protein *via* CG-MALS

**T**au protein is a neuronal microtubule-associated protein. Apart from its physiological function, tau “tangles”, consisting of insoluble fibers, are found in Alzheimer’s disease brain tissue. Heparin is a highly sulfated, and hence highly negatively charged, glycosaminoglycan occurring naturally in mast cells, and is commonly used as a potent anticoagulant. Polyanionic cofactors such as heparin induce tau filament formation in vitro, and serve as models for the study of mechanisms which can lead to tau polymerization in vivo. The strength and stoichiometry of interaction between inducer substances (such as heparin) and tau strongly influence the rate and amount of filament formation.

We used the Wyatt Calypso system to determine the stoichiometry and equilibrium association constant for heteroassociation of a tau fragment (Tau<sub>244-372</sub>) and a low molecular weight heparin fraction. Solutions of tau and heparin were prepared by extensive dialysis against a buffer containing 10 mM HEPES, 100 mM NaCl and 1 mM DTT, at pH 7.4.

A composition gradient was created using the software-controlled Calypso multiple syringe pump system, by means of which the mole fraction of tau in a solution mixture is decreased from 1 to 0 and the mole fraction of heparin is simultaneously increased from 0 to 1 (Figure 1). Solution mixtures of varying composition are sequentially pumped into parallel flow cells for simultaneous measurement of the static light scattering intensity (DAWN-EOS) and refractive index (Optilab rEX), the latter quantity being a measure of solution composition.

The results of the experiment, summarized in Figure 2A, were analyzed using the Calypso modeling software, which takes into account the difference between the specific refractive increment of tau protein (0.185 cm<sup>3</sup>/g) and heparin (0.126 cm<sup>3</sup>/g). The experimentally determined dependence of the scaled scattering intensity ( $R/K_{\text{opt}}$ ) upon solution composition was modeled according to the general scheme  $nA + mB \leftrightarrow A_nB_m$ , where A denotes tau and B heparin. The best fit of the model to the experimental data (Figure 2B) was obtained with  $n = m = 1$ ,  $M_A = 13790$ ,  $M_B = 7089$ , and  $\log_{10} K_{AB} (M^{-1}) = 6.56$ .

This result demonstrates that CG-MALS, as implemented with the Calypso system, provides a simple and rapid way to characterize interactions between proteins and other classes of macromolecule.

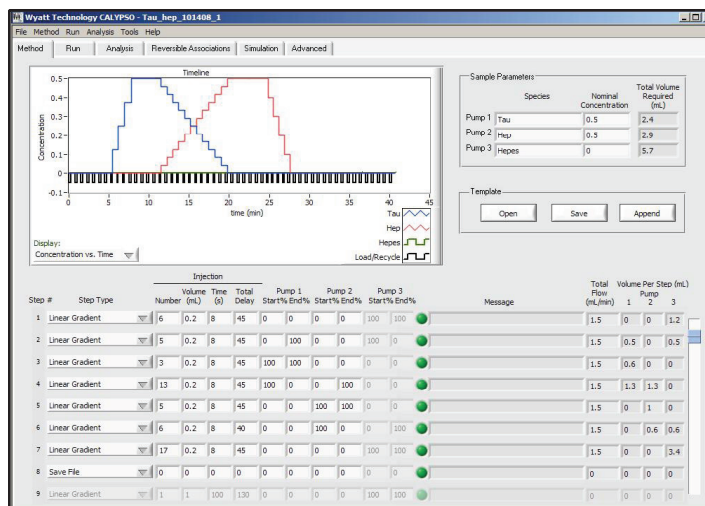


Figure 1. Method for the characterization of tau and heparin interaction, consisting of (i) a rapid increase in tau concentration (minutes 6-8), (ii) a simultaneous gradual decrease in tau concentration and increase in heparin concentration (minutes 12-20) and (iii) a rapid decrease in heparin concentration (minutes 25-28).

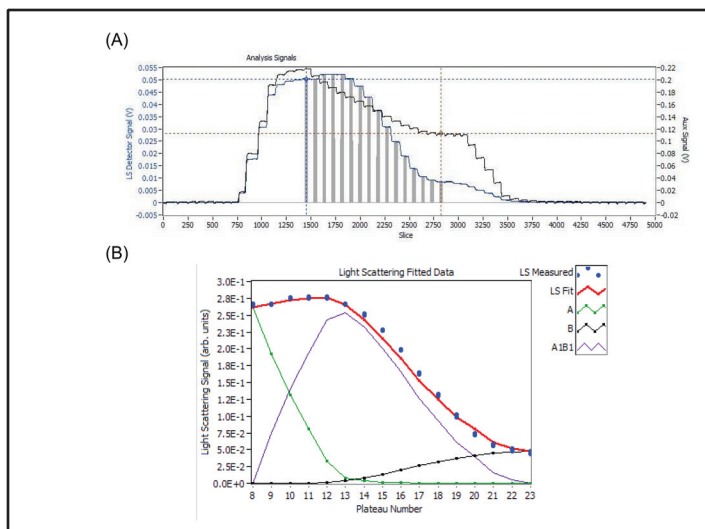
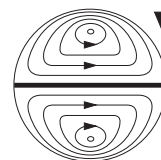


Figure 2. (A) Composition gradient static light scattering data for tau and heparin in 10 mM HEPES, 100 mM NaCl, 1 mM DTT, at pH 7.4. Differential refractive index signal in black, 90° light scattering intensity in blue. The vertical grey bars represent regions of each plateau selected for the modeling analysis. (B) Graphical representation of the results of modeling by a 1:1 equilibrium association model. Blue dots, processed scattering intensity corresponding to the mean of each region selected in Fig 2A. Red line: best fit of model to the full composition-dependent scattering intensity profile. Green line: calculated contribution of free (unbound) tau to total intensity. Black line: calculated contribution of free (unbound) heparin to total scattering intensity. Purple line: calculated contribution of 1:1 heterocomplex to total scattering intensity.

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