

Determination of Stoichiometry of Protein-Protein Interactions

Domain antibodies (dAbs, 12-14kDa) are small molecules comprising Vh or Vk domains of human IgG molecules, which are capable of binding to various antigens. The aim of this experiment was to assess the stoichiometry of interaction between dAbs and their corresponding antigen—in this instance, human serum albumin (HSA), using SEC MALS.

dAbs and HSA alone were run on SEC MALS at the concentration of 80 μ M (\approx 1mg/ml). A molar ratio of dAb:HSA (1:1 and 3:1) was injected onto a column after pre-incubation of 1h. dAbs with different K_D s for HSA binding were used (ranging from 34-200nM) and different in-solution states (monomers and dimers, both typical solution states for dAbs). As a control, non-HSA binding dAb (HEL4) was used. PBS, pH 7.4 and citrate buffer, pH 5 were used as a mobile phase for comparison, as we are interested in the stability of the dAb-HSA complexes at both pHs.

The molar masses of free dAbs were calculated as expected at 13kDa (monomer) and 26 kDa (dimer), respectively. HSA alone elutes with two peaks corresponding to monomer (68kDa) and dimer (135kDa). From calculations of the molar masses of the dAb:HSA complexes it was possible to infer the stoichiometry of dAb to HSA, which was either 2 dAbs per HSA (7h8-HSA), or 1 dAb per HSA (7h14-HSA).

SEC MALS provides a valuable and rapid method for the determination of stoichiometry of protein-protein interactions, which can be readily applied to a wide variety of molecules. The binding of the molecules can be tested easily in a variety of conditions such as pH, salt and protein concentrations and relative ratios of interacting proteins.

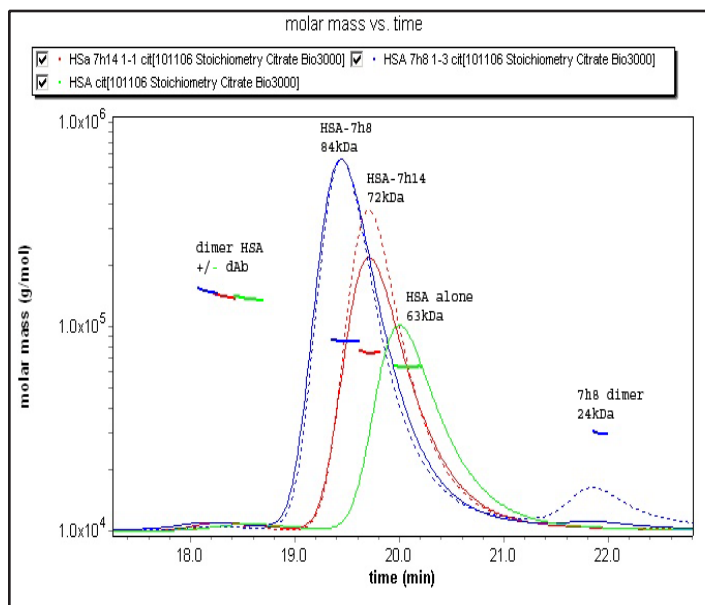


Figure 1. Binding of dAbs to HSA in PBS buffer. 7h8:HSA(3:1 ratio), 7h14:HSA (1:1) and HSA alone. Higher ratio of dAb:HSA is shown for 7h8:HSA (3:1) complex to account for 2:1 dAb:HSA stoichiometry of binding.

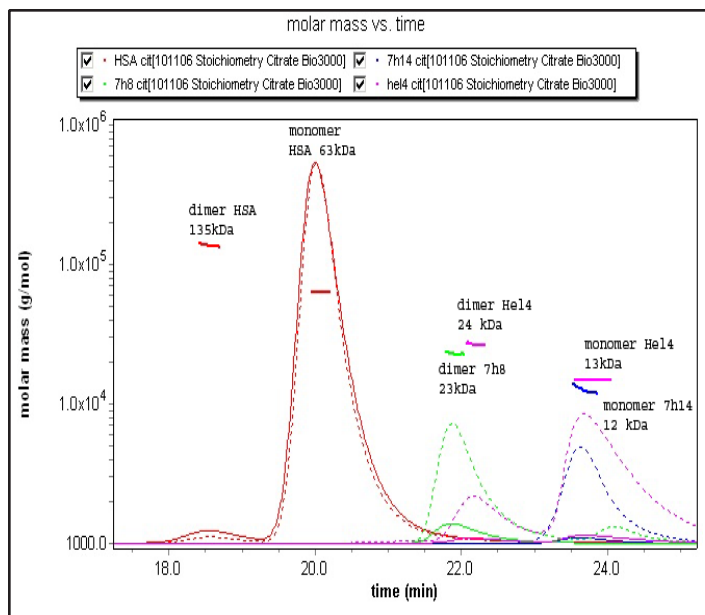


Figure 2. Control injections – HSA alone, Hel4 alone, 7h8 and 7h14 alone. HSA has two peaks (monomer and dimer), 7h8 is a dimer, 7h14 is a monomer and Hel 4 has two peaks (monomer and dimer).

