We have studied the phase behavior of model globular protein, human serum albumin (HSA) in solution in the presence of yttrium chloride. The protein solutions show a reentrant condensation phase behavior [1], i.e. a phase-separated regime occurs between two critical salt concentration $c^* < c < c^{**}$. The condensed phase is a meta-stable liquid-liquid phase separation (LLPS). The effective protein-protein interactions have been studied within and outside the LLPS by static light scattering and small-angle X-ray scattering (SAXS). The second virial coefficient, $A_2$, has been determined as a function of salt concentration (FIG.1).

It is shown that below $c^*$, $A_2 > 0$, the effective interactions are repulsive, and $A_2$ decreases quickly with increasing salt concentration. In the range of $c^* < c < c^{**}$, $A_2$ are negative with a minimum. Further increasing salt concentration above $c^{**}$, $A_2$ becomes positive again, but only slightly larger than zero, and increases slowly with salt concentration. SAXS measurements confirms that screened Coulomb repulsion dominates the protein interactions at $c < c^*$, and a short-ranged attraction dominates at $c^* < c < c^{**}$. Above $c^{**}$, SAXS results indicate that clustering effect contribute significantly in the low q region. The effective interaction between clusters becomes repulsive which is consistent to the $A_2$ measurements.

FIG.1. (left) Plots of $Kc_p/R$ vs $c_p$ for SLS measurements of HSA solutions in the presence of YCl$_3$. Non-linear relationship is due to the cation (Y$^{3+}$) binding to protein surface. (right) Using an ultrafiltration method, $A_2$ was successfully determined.