Application Note

Tsz Kin Martin Tsui[1], Claudius Mundoma[1], Hong Li[1,2]

pH Effects on Stability and Homogeneity of Protein Complex

I. Introduction

A heterodimeric protein complex (HPC) from Pyrococcus furiosus is identified for its functional role in binding RNA. HPC has a total molecular weight of 65 kDa and has a tendency to form fibril materials in vitro as observed by electron microscopy. By using the Wyatt DynaPro® dynamic light scattering (DLS) system, we characterized homogeneity and stability of HPC under various buffer conditions. We found that the pH of the buffer solutions influence the sample homogeneity and stability.

II. Experimental Methods & Results

DLS data were acquired at 20°C using Wyatt DynaProDLS system with Dynamics® V7 and analysis was carried out using Dynamics® V7.0.0.95. The intensity autocorrelation function (Fig. 1) provides a direct indicator of the size distribution of the sample. The polydispersity index provides a direct measure of the heterogeneity of the each molecular species in sample. A polydispersity index below 20% means that the molecular species is monodisperse, otherwise it is heterogeneous. Results show that HPC at pH 7.5 (Tris-Cl buffer, 3% NaCl) has a weighted average polydispersity (%Pd) of 19.5%, relatively less polydisperse than that at pH 3.0 (Glycine-HCl buffer, 3% NaCl), which has 27.2%. A total of 10 measurements were performed for each sample, HPC at pH 3.0 contains larger species than that at pH 7.5, which was indicated by the longer rate of decay in the autocorrelation function.

The histogram from the regularization fit of the autocorrelation function graph (Fig. 2) shows the size distribution of HPC at aforementioned conditions. As observed from the autocorrelation function, the size distribution histogram shows that HPC at pH 7.5 contributed one strong peak around a lower hydrodynamic radius of 4.0 nm, with an average peak %intensity (average %I) of 90.5 %; HPC at pH 3.0 yielded two peaks at a higher hydrodynamic radius, 13.0 nm (average %I = 32.8 %) and 112.4 nm (average %I = 64.0 %), respectively. Results

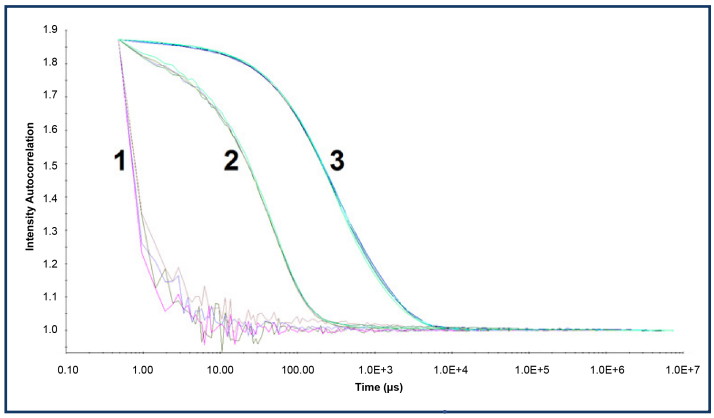

Figure 1. The autocorrelation function for HPC at pH 3.0 and pH 7.5. Data collected with 5 seconds acquisition time, 10 acquisitions per measurement at laser power 100% (buffer) and 90% (HPC samples). 1) Buffer at pH 3.0 and pH 7.5 (overlapped), 2) HPC at pH 7.5, and 3) HPC at pH 3.0. More HPC with a longer decay rate at pH 3.0 (up to ~1.0×10^4 µs) than that at pH 7.5 (up to ~2.0×10^2 µs).

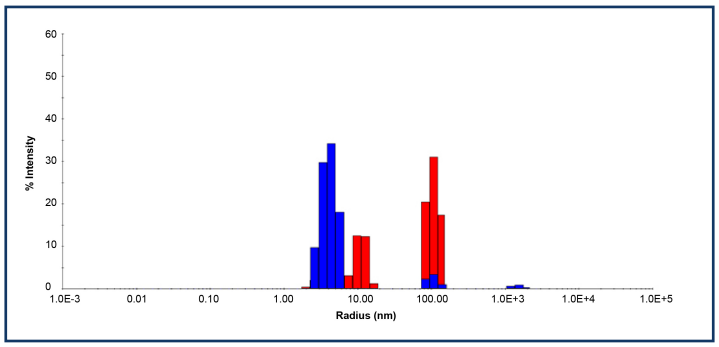

Figure 2. The regularization histograms for HPC at pH 3.0 and pH 7.5. HPC at pH 7.5 (blue) and at pH 3.0 (red) are shown in this histogram. The distribution suggested that possible aggregation of HPC occurred at pH 3.0. HPC at pH 7.5 is less polydisperse than that at pH 3.0, with fewer intensive peaks.

suggest that HPC at pH 7.5 is indeed more homogeneous and stable relative to HPC at pH 3.0, as the peak for HPC at pH 7.5 possibly splits into two peaks while those two peaks shift to higher hydrodynamic radii.

III. Discussion & Conclusion

The stability and homogeneity determined from polydispersity and autocorrelation function using DLS provide information on the biophysical property of HPC. Results from this study help verify the conditions where polymerization of HPC is thermodynamically more favorable, hence, improving the quality in sample preparation using appropriate buffer condition for biophysical studies, such as structural analysis.

Author Affiliations:
[1] Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306, USA

[2] Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, USA

WYATT TECHNOLOGY

6300 Hollister Avenue
Santa Barbara, CA 93117
Tel: **+1 (805) 681-9009**
Fax: **+1 (805) 681-0123**
Web: **www.wyatt.com**