High-throughput analysis of protein formulations by DLS: thermal stability, colloidal stability and a thermal anomaly in the colloidal stability parameter D_1

Sophia Kenrick and Daniel Some, Wyatt Technology Corporation

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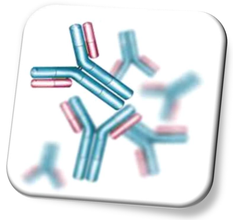
Molar Mass • Size • Interactions • Charge

1. Introduction

High-Throughput Screening by Dynamic Light Scattering (HTS-DLS) is a versatile tool for characterizing various aspects of protein stability *in parallel*:

- **Thermal / conformational stability (T_M, T_{onset})**
- **Colloidal stability (D_1 or k_D)***
- **Actual aggregation state**



D_1 undergoes a dramatic, previously unobserved modulation around the thermal transitions.

Changes to in D_1 are observable several degrees earlier than T_{onset}. We speculate that this reflects an unfolding transition.

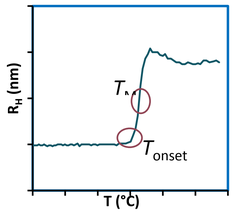
*we prefer 'D_1' to 'k_D' in order to avoid confusion with other 'K-D' meanings and to open a pathway to higher-order terms.

2. Model System

mAb1

- pI = 8.9 by electrophoretic light scattering (Möbius®, Wyatt Technology)
- R_H : 4.7-4.8 nm at 25°C, c→0
- pH conditions: 8.5 (Z_{DHH}=4), 9.5 (Z_{DHH}=-2)
- Concentrations: 0.47, 0.94, 1.88, 3.75, 7.5, 15 mg/mL
- 3 replicates per pH & concentration
- Wells capped with silicone oil
- Measured every 0.5°C over 25°C - 85°C

3. Analysis



T_M is the midpoint of a thermal transition in the hydrodynamic radius R_h (calculated from the mutual diffusion constant D_m under dilute conditions).

T_{onset} is the temperature at the onset of aggregation, determined from R_h.

D_1 is determined from the concentration dependence of D_m:

$$D_m = D_0(1 + D_1c + D_2c^2 + \ldots)$$

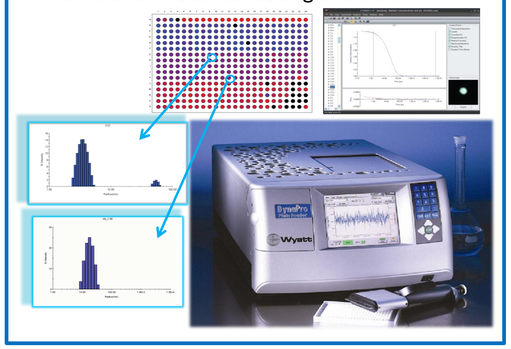
D_1 is closely related to the second virial coefficient A_2 (B_{22}) and is an indicator of colloidal interactions.

HTS-DLS of T_M/T_{onset}, D_1 and aggregation size distributions under multiple formulation buffer conditions, can be accomplished simultaneously *in situ* in standard low-volume microtiter plates with a DynaPro® Plate Reader II.

All parameters determined from cumulants.

4. DynaPro® Plate Reader II

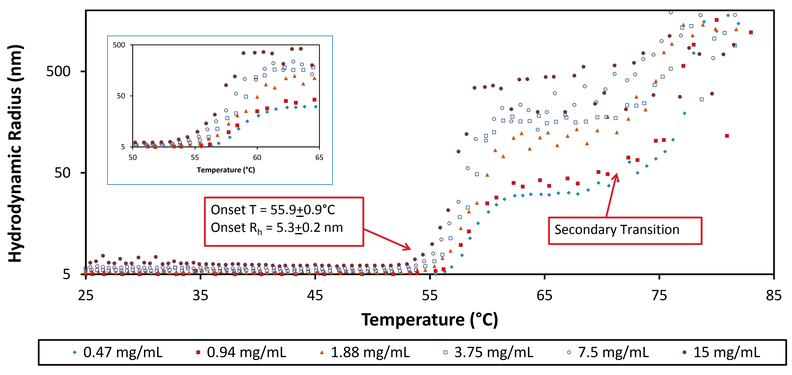
- Standard 96, 384 or 1536 microwell plates
- Sample stays in wells – no carryover
- Measurement time = 4-10 s/well, typical
- Temperature ramps 4-85C
- Camera for troubleshooting bad data



5. Thermal Stability

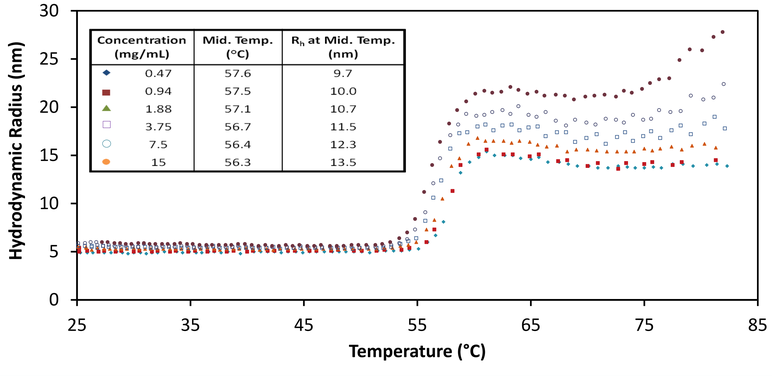
pH 8.5

- T_{onset} (c):
  - ~56°C (0.47 mg/mL)
  - ~53°C (15 mg/mL)
- Degree of aggregation depends on concentration
- Quasi-stable state between 60°C-70°C



pH 9.5

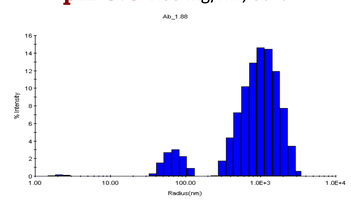
- T_{onset}(c):
  - ~55C (0.47 mg/mL)
  - ~52C (15 mg/mL)
- Degree of aggregation depends on concentration
- Stable state above ~ 61C



6. Aggregation States

- HTS-DLS indicates vastly different aggregation states, not expected to be identified by DSC or DSF signal loss
- Does form of D_1(T) point to aggregation pathway?

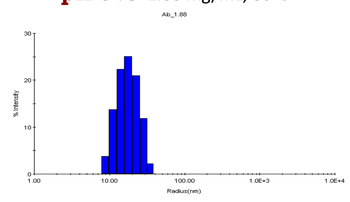
pH 8.5 1.88 mg/mL, 80°C



Partially crashes out of solution

May be detected by DSC as aggregating

pH 9.5 1.88 mg/mL, 80°C



Does not crash out of solution

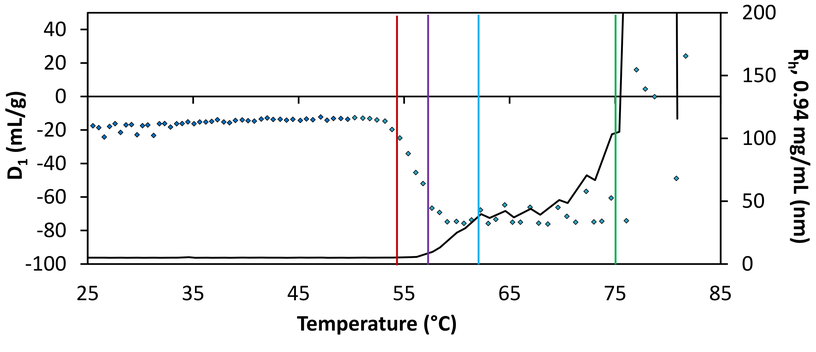
Probably not detected by DSC or DSF as aggregating

8. Colloidal Parameter D_1

pH 8.5

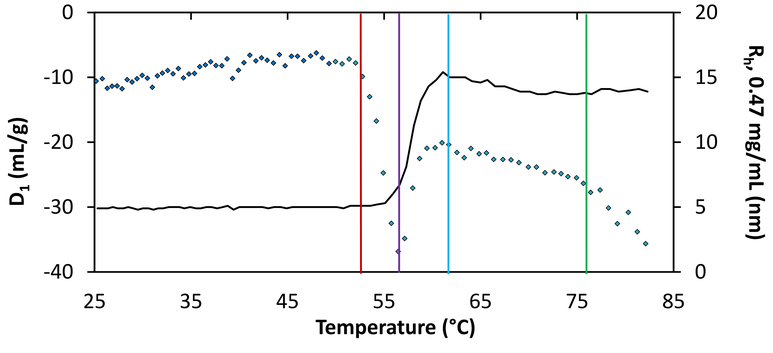
- '$D_1(T)$' – transition midpoint at T_{onset};
- 'Stabilizes' with R_h
- May not have much meaning

T>T_{onset} (new value just a consequence of earlier concentration-dependent aggregation?)



pH 9.5

- $D_1(T)$ – negative peak at T_{onset}!
- 'Stabilizes' with R_h, 2nd transition ~75°C
- After transition – true interaction between aggregates or consequence of earlier aggregation?



9. Conclusions & Future Studies

High-throughput analysis by the DynaPro DLS plate reader provides parallel analysis of *multiple* stability-indicating parameters for **screening of developability and optimal formulations**.

A novel indicator, $D_1(T)$, provides new insights into the **interplay of colloidal and conformational stability**. The form of $D_1(T)$ may point to aggregation mechanisms and rate-limiting parameters.

Better understanding of the significance of $D_1(T)$ will require further study including tests of reversibility.

The nature of $D_1(T)$ above the transition may be further elucidated by a dilution series performed on aggregated samples in the context of HTS-DLS.