

ABSTRACT

Development of a successful drug candidate requires extensive analysis of the product. Analytical tests fall into broad categories of characterization and comparability assays. This work describes the application of static and dynamic light scattering techniques for the characterization of a recombinant protein as a drug candidate.

The size exclusion chromatography (SEC) profile of the recombinant protein was analyzed using a Wyatt on-line light scattering instrument with quasi-elastic option (MALLS-QELS). The presence of monomer, dimer and degradants was confirmed. Determination of the hydrodynamic radius of monomer helped to explain its early elution from the column.

Use of a DynaPro Plate Reader as a tool to detect the formation of protein aggregates in different formulations was explored and the results were compared to the SEC-MALLS-QELS measurements. The obtained results were found to be in good agreement. The DynaPro Plate Reader offers higher throughput capabilities than the SEC and experiments are performed under equilibrium conditions.

BACKGROUND

- The protein consists of 532 amino acid residues (Mw 56 264 Da) folded into three structural domains (N1, N2, N3).
- Appropriate analytical tests must be designed to achieve detailed understanding of the product.
- Dynamic and static light scattering in combination with size exclusion chromatography are examined as tools to provide critical data toward the determination of recombinant protein purity and stability.

METHODS

- Size exclusion chromatography with on-line light scattering detection**

Agilent 1100 HPLC System with diode array detector (UV) was connected to DAWN HELEOS light scattering instrument with internal QELS and to Optilab rEX differential refractometer (Wyatt Technology, Santa Barbara, CA). The detectors were connected in the following order: UV-LS-RI.

TSK-Gel G3000SWxl 7.8mm x 30cm (TOSOH Corporation) was used with a mobile phase of Dulbecco's phosphate buffered saline (6mM sodium phosphate, 1mM potassium phosphate, 150mM NaCl, pH 7.4) delivered at a flow rate of 0.5mL/min.

Astra V software version 5.1.9.1 (Wyatt Technology, Santa Barbara, CA) was used to collect and process experimental data.

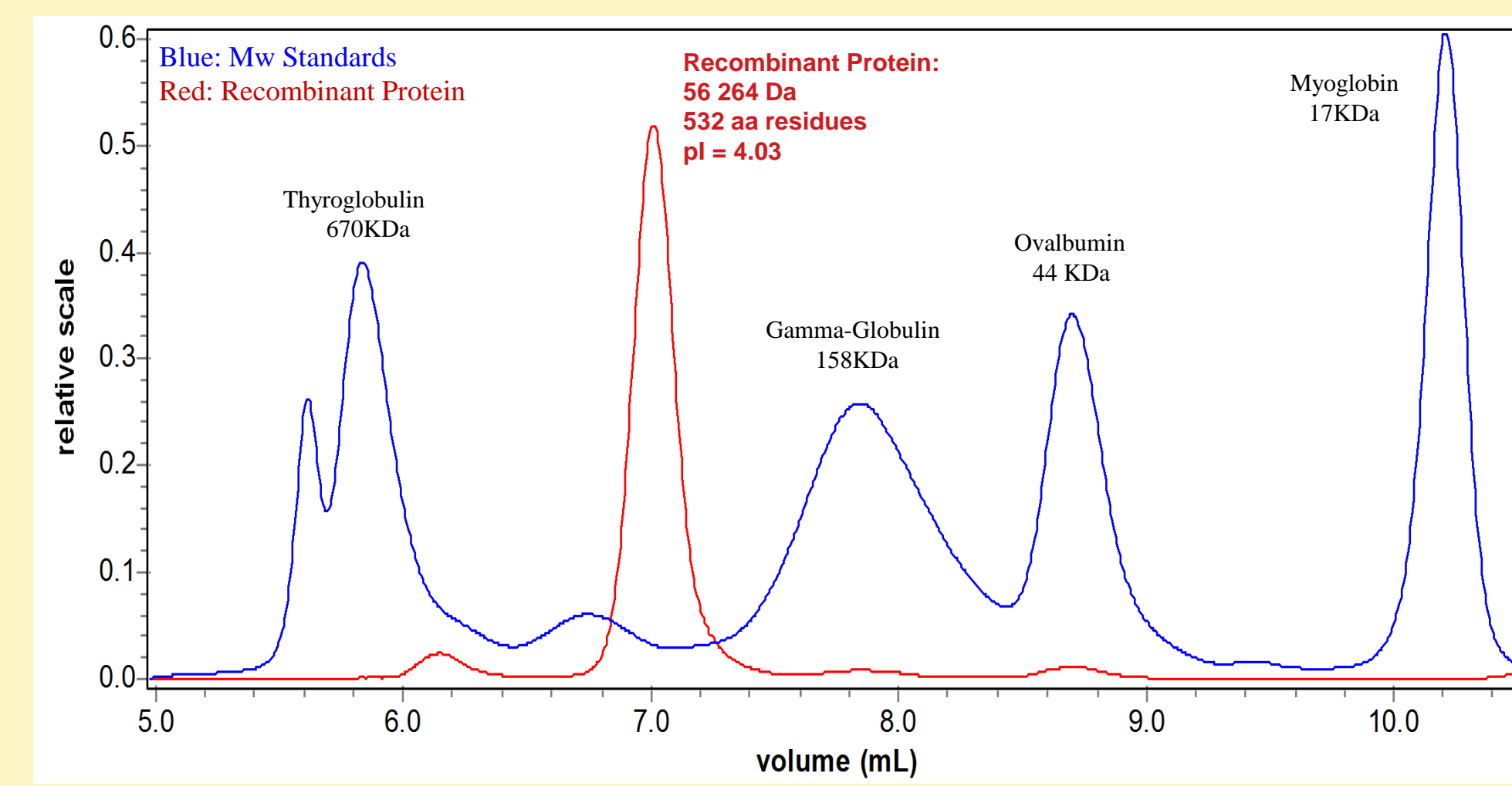
- Dynamic light scattering analysis**

Dynamic light scattering measurements were performed on a DynaPro Titan instrument with Plate Reader (Wyatt Technology, Santa Barbara, CA) using 384-well plates (Thermo Electron Corporation, Cat. No: 95040000). Where indicated, samples in Eppendorf® tubes were placed for 2 hours in the incubator (Thermo Electron Corporation, Model: 2EG, 3520) set to 37 °C, and then transferred into the well plate. Sample volume per well was 55µL. All DLS measurements were performed at ambient temperature.

Dynamics software, version 6.7.6 (Wyatt Technology, Santa Barbara, CA) was used to collect and process experimental data.

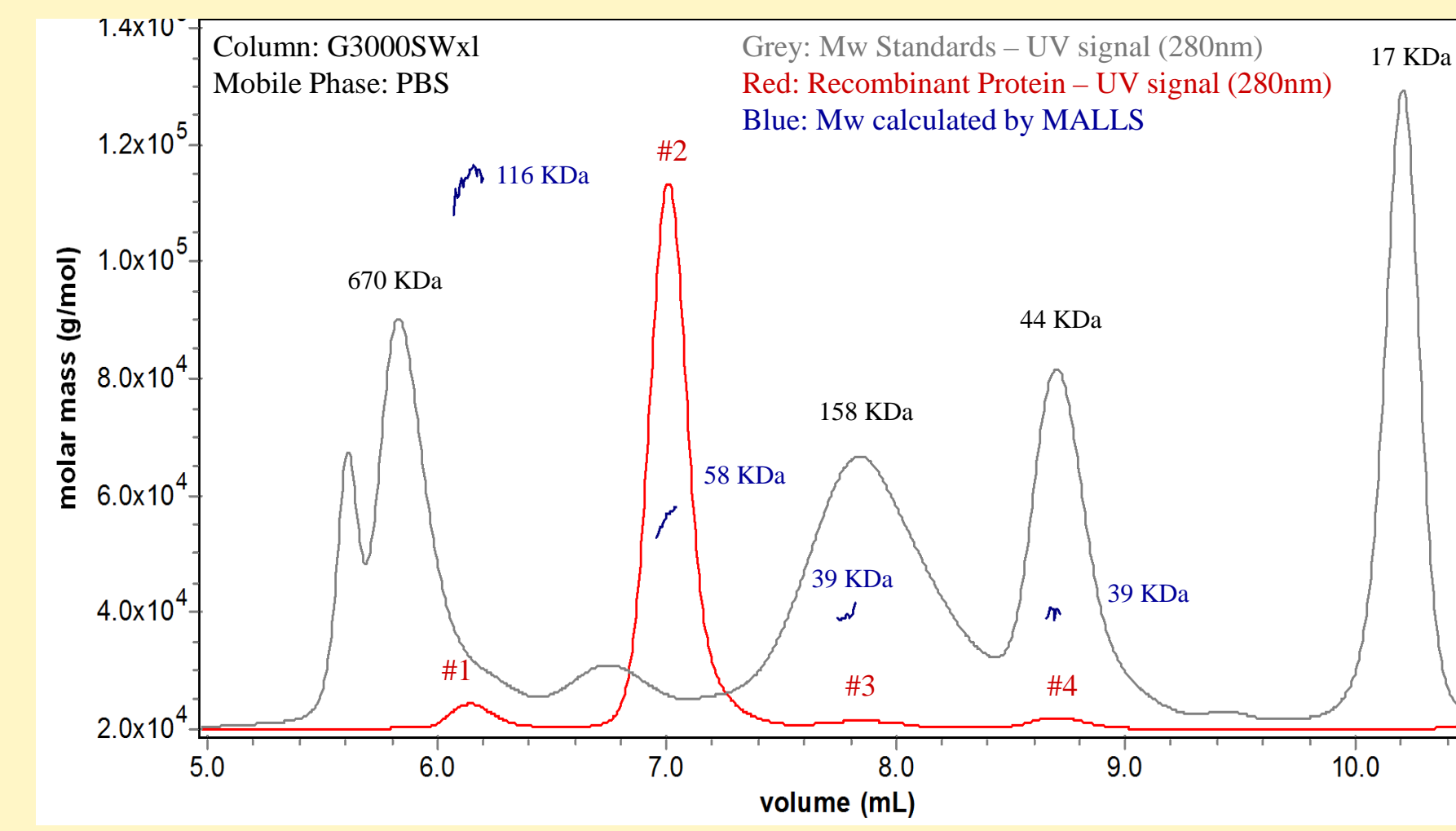
RESULTS

Recombinant Protein and Protein Standards on SEC
(Column: G3000SWxl in PBS)



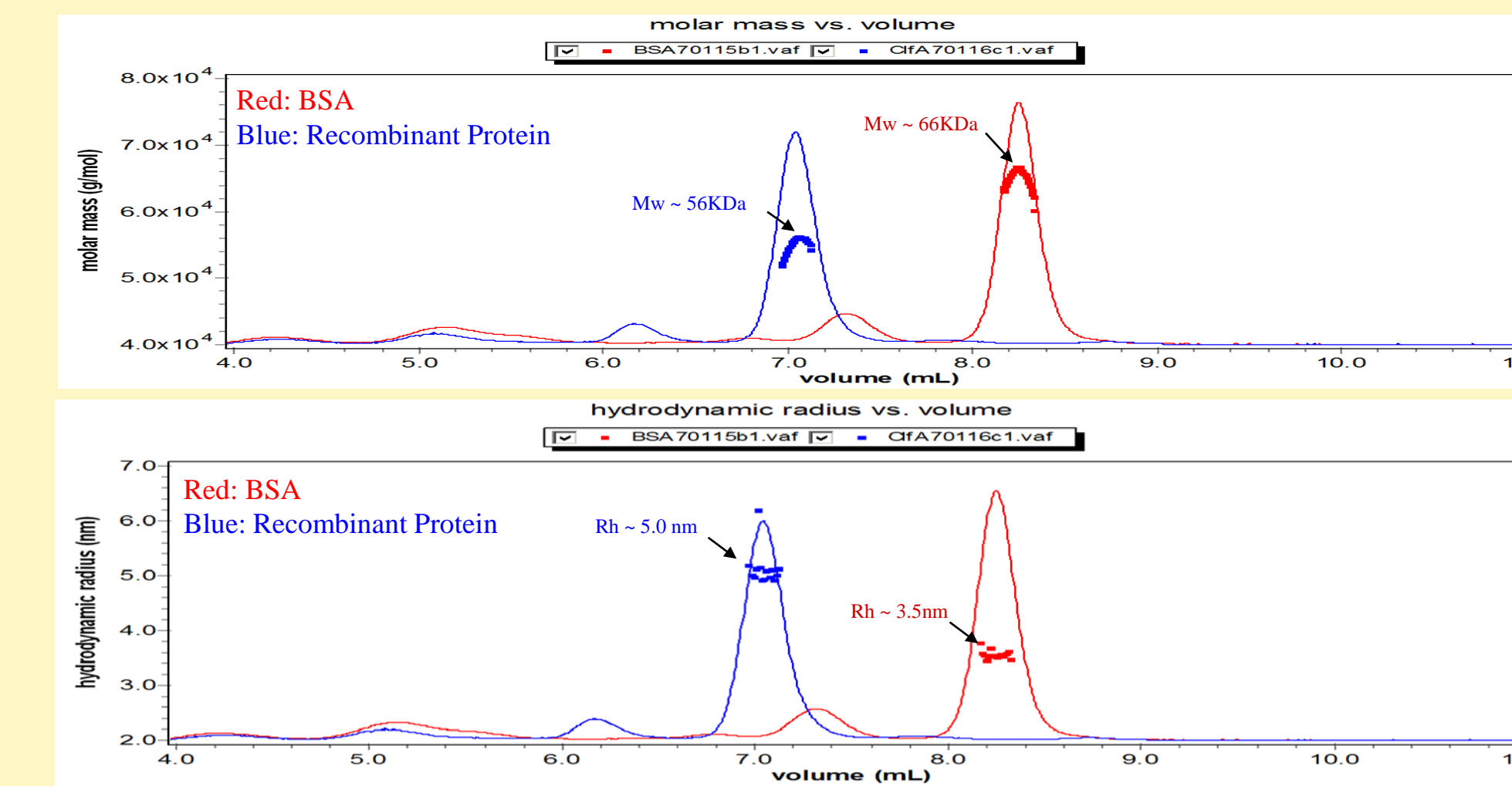
- Early elution of recombinant protein indicates either oligomerization or nonglobular shape of the molecule.

Recombinant Protein on SEC-MALLS
(Column: G3000SWxl in PBS)



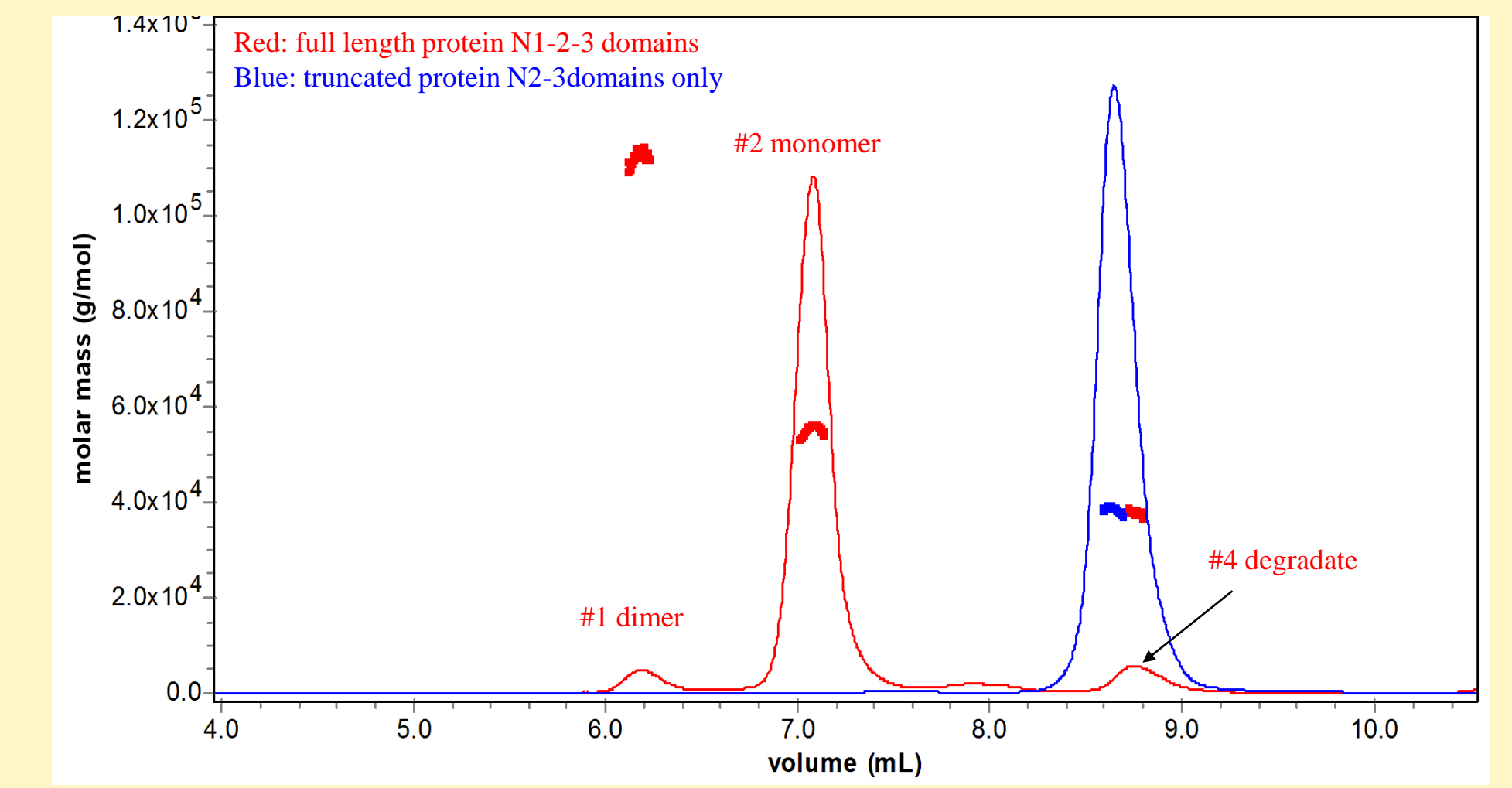
- SEC-MALLS confirms that the majority of recombinant protein is in monomeric form (peak#2) under the given conditions.
- Peak#1 is a dimer, peaks #3 and #4 appear to be degradants.

Recombinant Protein and BSA on SEC-MALLS-QELS
(Column: G3000SWxl in PBS)



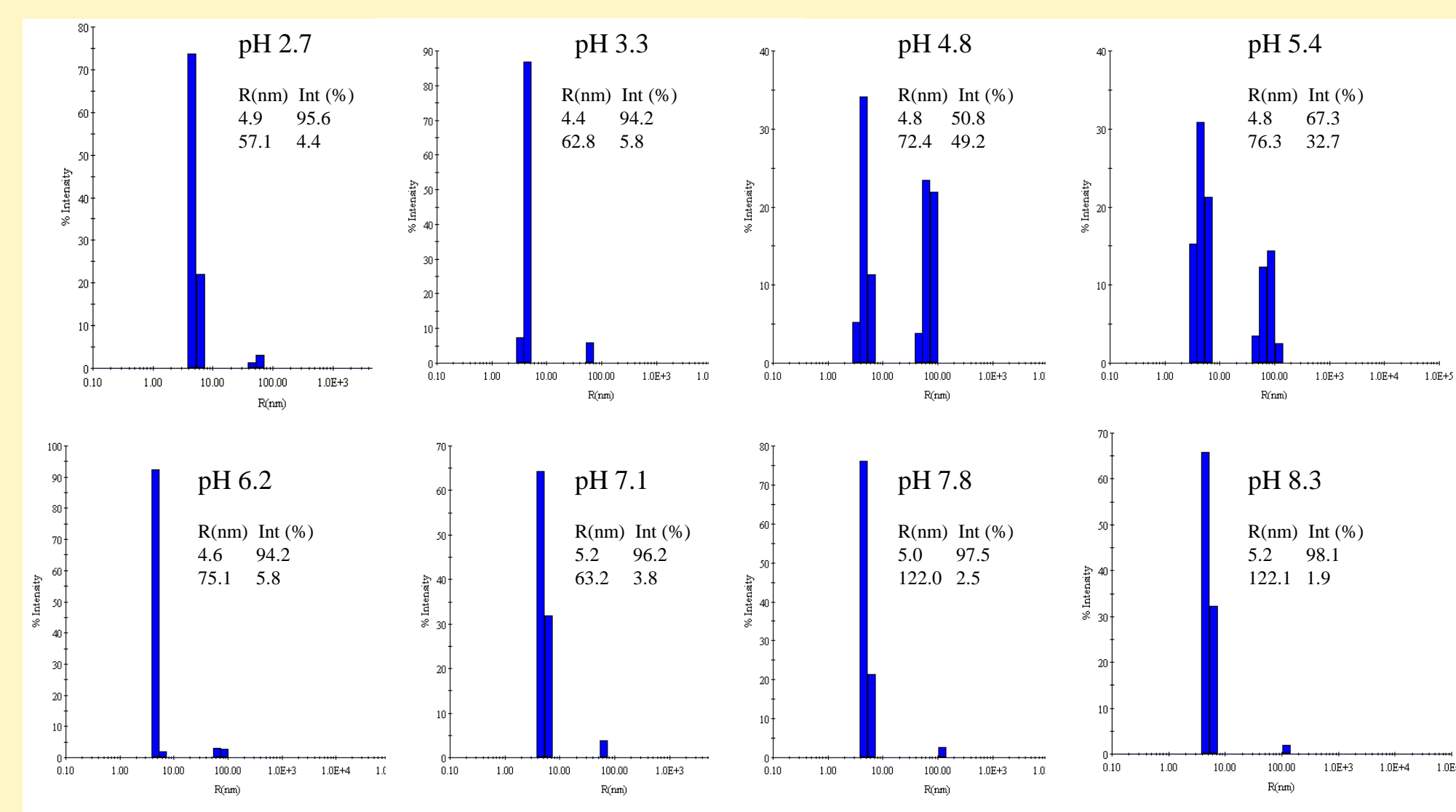
- Hydrodynamic radius of recombinant protein monomer is higher than hydrodynamic radius of globular protein of similar molar mass.

Recombinant Protein on SEC-MALLS
(Full length protein N1-2-3 domains and N2-3 domains only)



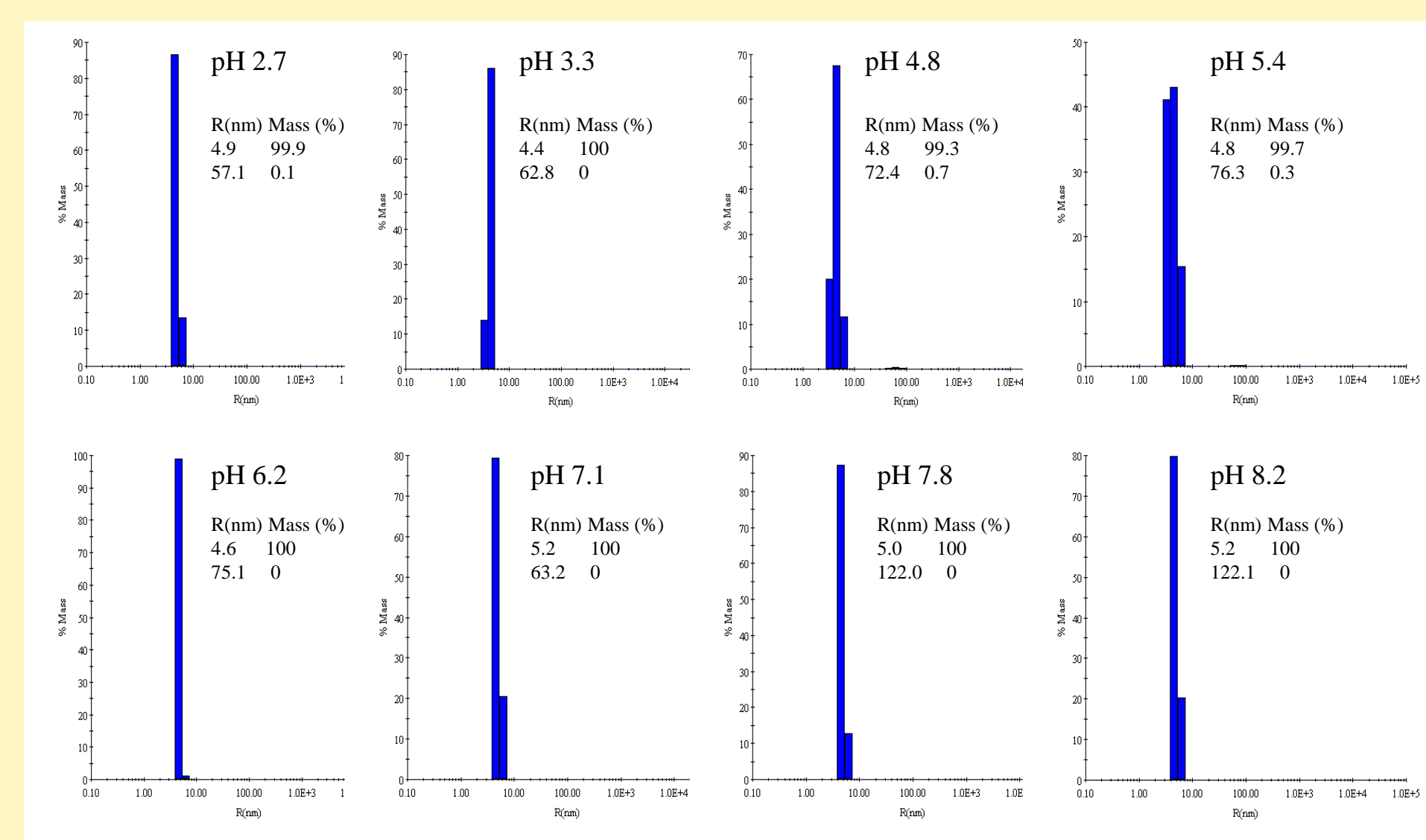
- Data suggests that peak#4 can be the N2-3 degradate.
- Identity of peak#4 as N2-3 domain was confirmed by LC/MS analysis.

Recombinant Protein on DynaPro – Scattering Intensity Distributions
(Protein at 0.9mg/mL in 50mM Phosphate, 50mM NaCl at R.T.)



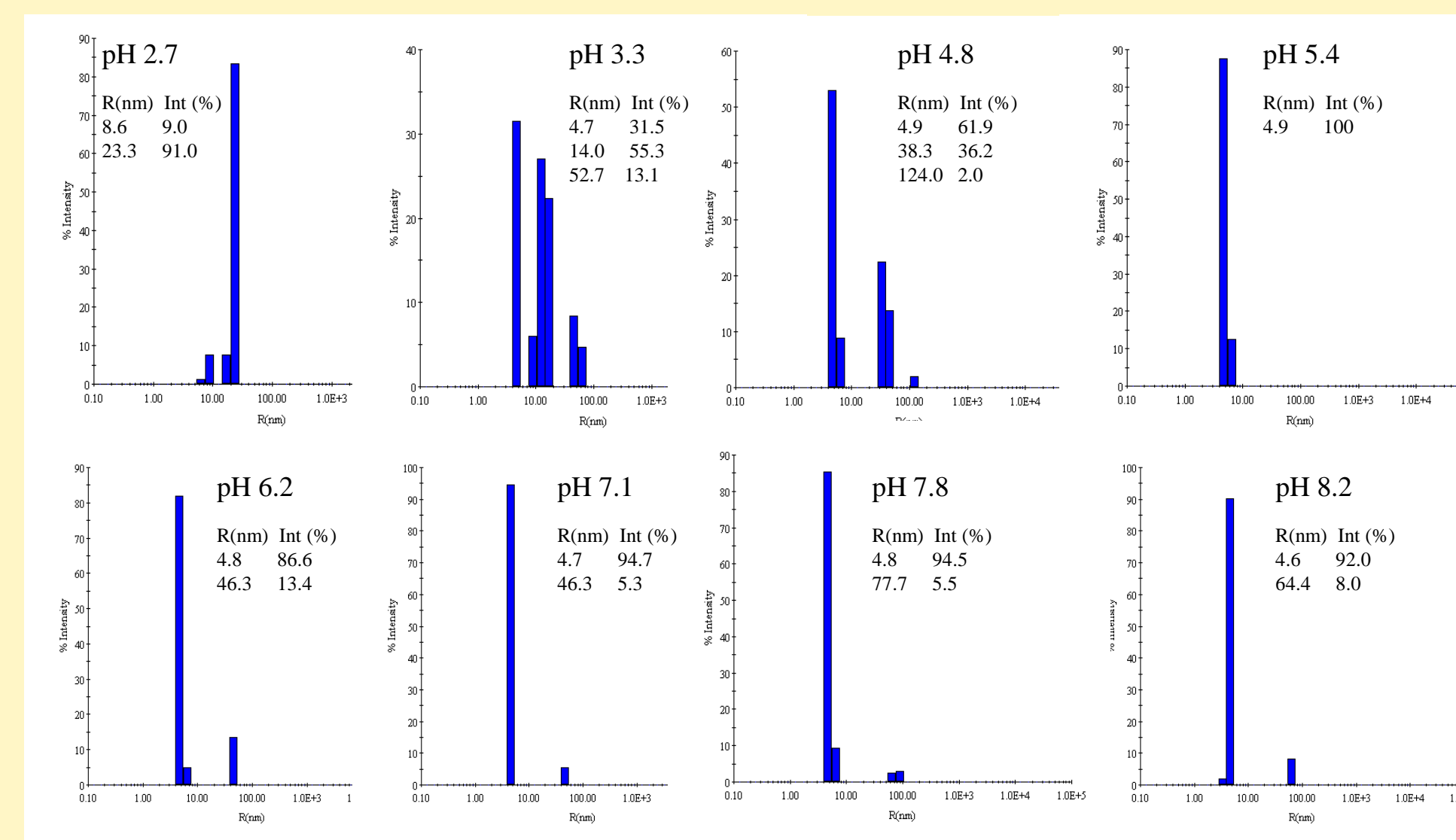
- Intensity distributions indicate formation of some very high Mw aggregates (Rh > 70nm) at pH 4.8 and 5.4.

Recombinant Protein on DynaPro – Mass Distributions
(Protein at 0.9mg/mL in 50mM Phosphate, 50mM NaCl at R.T.)



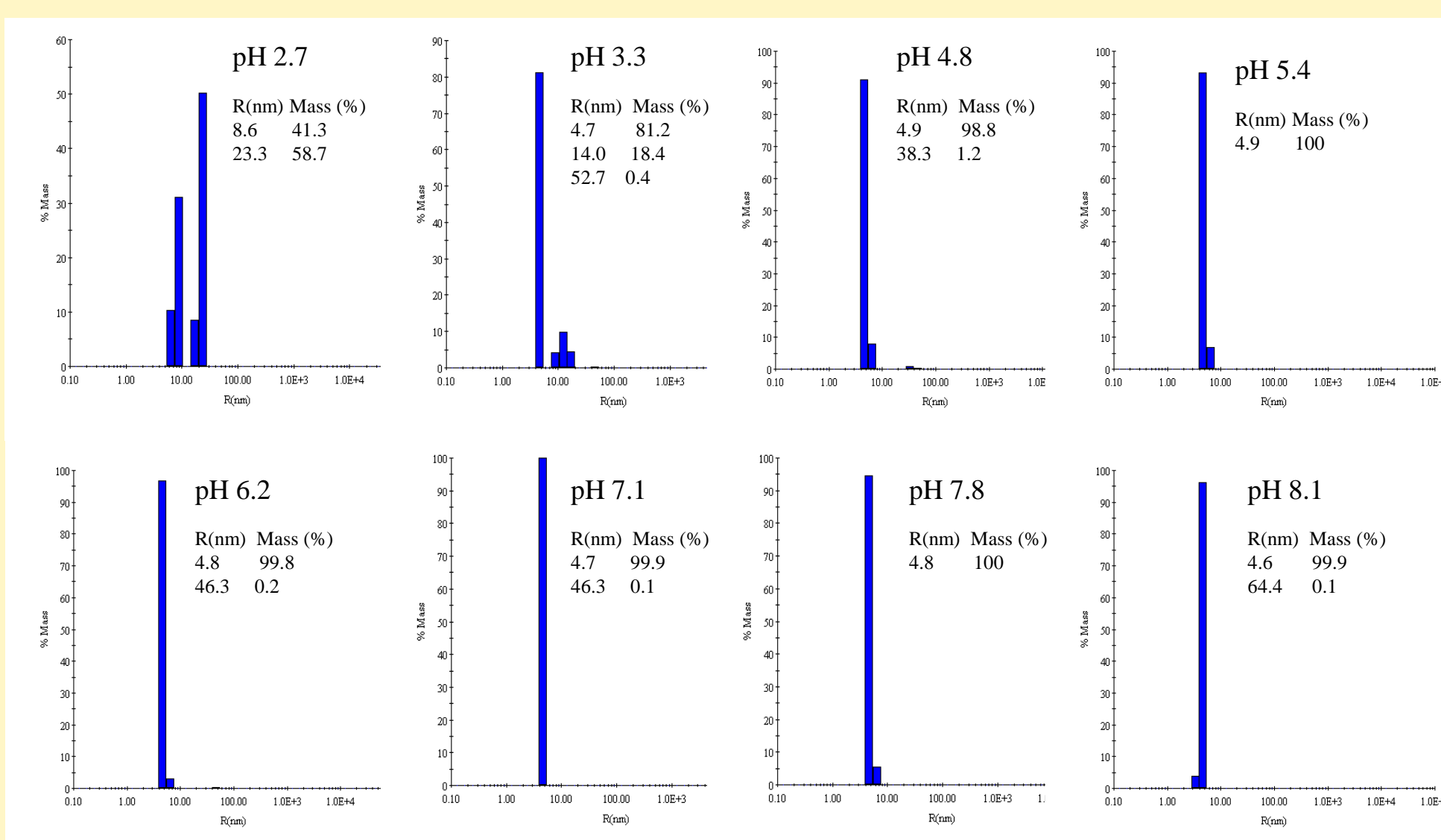
- Mass distributions show that over 99% of recombinant protein is in monomeric state in all tested buffer conditions.

Recombinant Protein on DynaPro – Scattering Intensity Distributions
(Protein at 0.9mg/mL in 50mM Phosphate, 50mM NaCl after 2 hours incubation at 37C.)



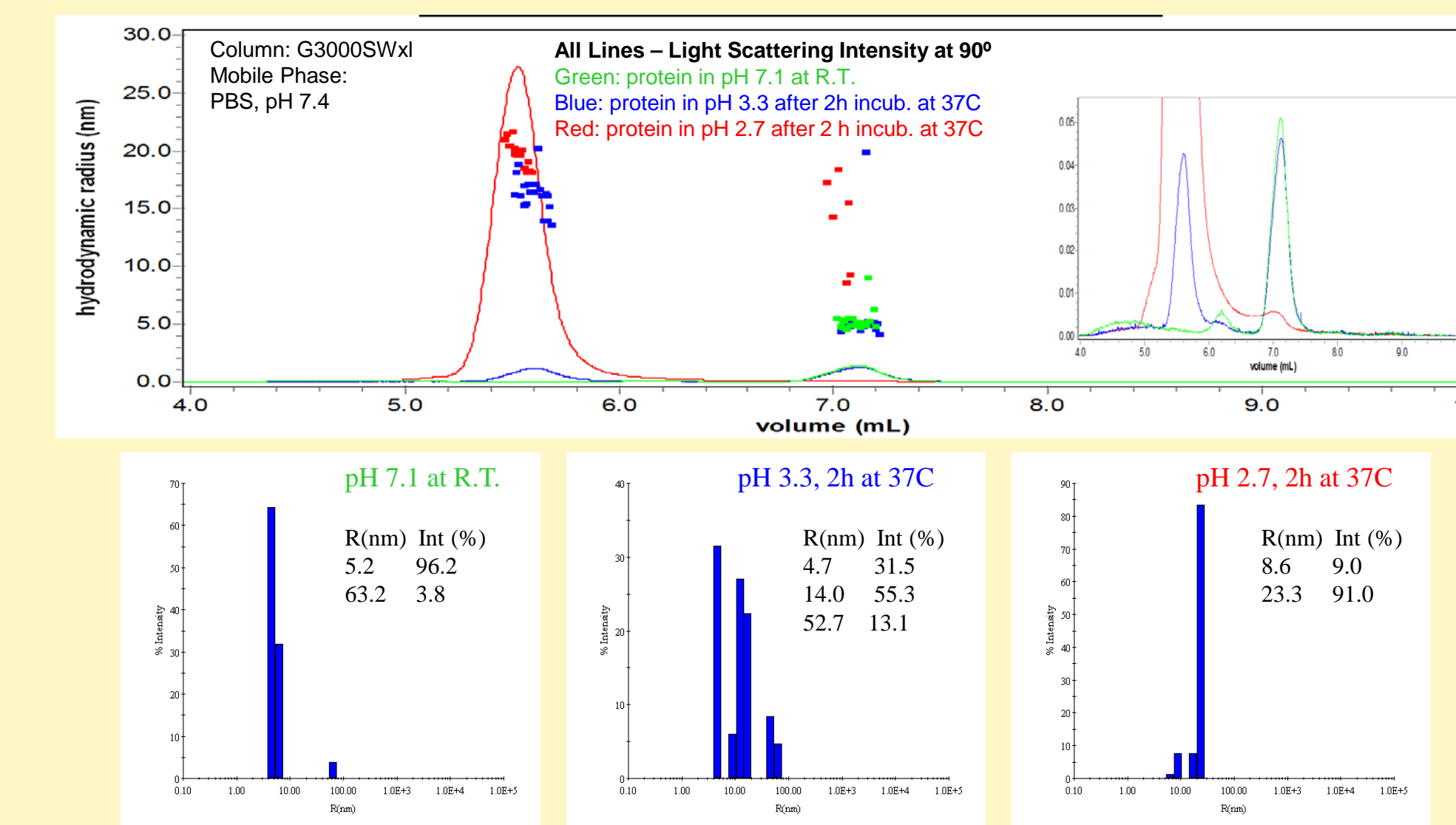
- Intensity distributions indicate the presence of significant amounts of aggregates at pH 2.7 (90% of Rh~23nm) and pH 3.3 (50% of Rh~14nm).

Recombinant Protein on DynaPro – Mass Distributions
(Protein at 0.9mg/mL in 50mM Phosphate, 50mM NaCl after 2 hours incubation at 37C.)



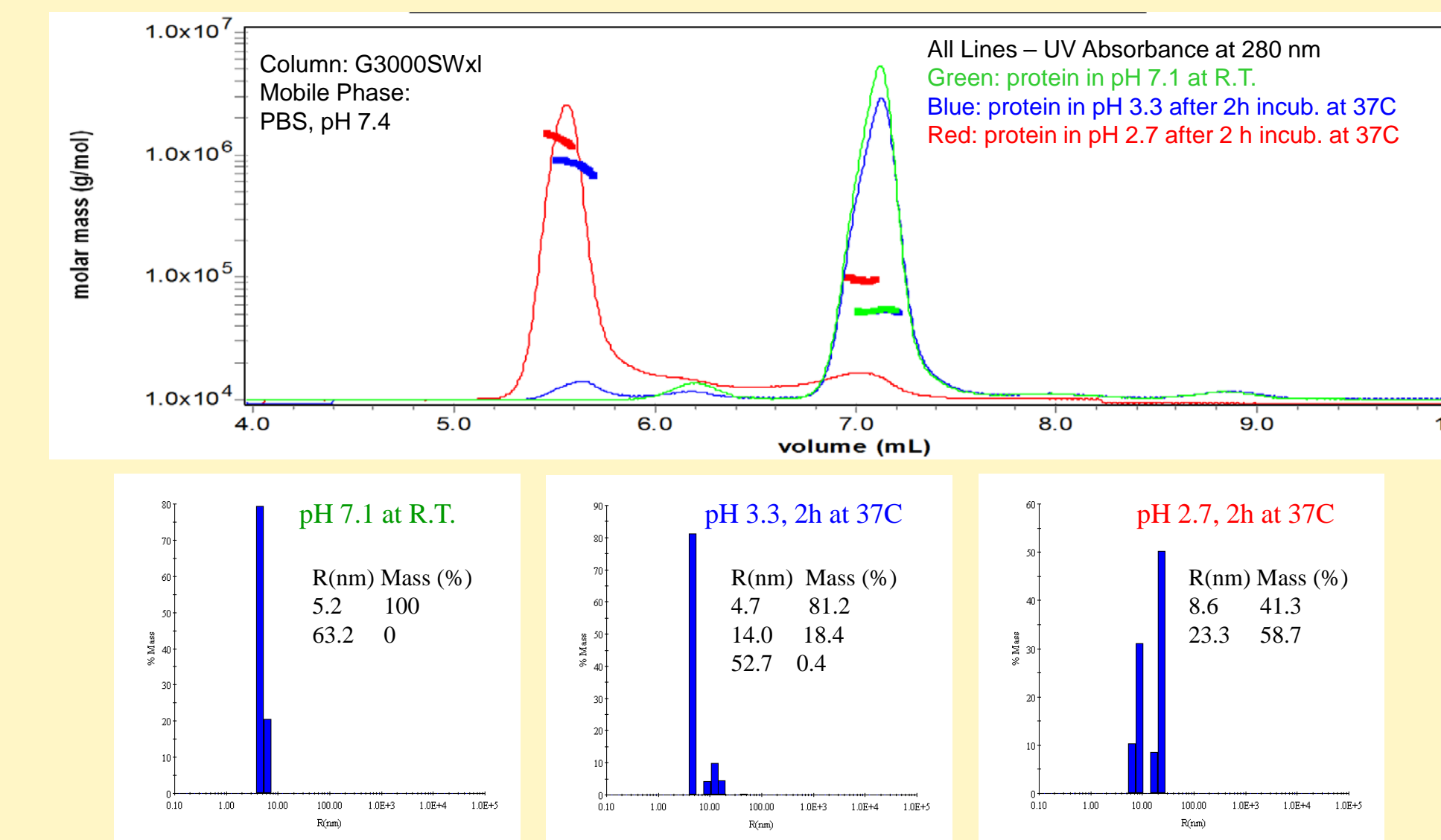
- Mass distributions show that ~60% of recombinant protein is aggregated at pH 2.7 (Rh~23nm) and ~20% is aggregated at pH 3.3 (Rh~14nm).

Recombinant Protein on SEC-MALLS and DynaPro
Scattering Intensity Distributions
(Protein at 0.9mg/mL in 50mM Phosphate, 50mM NaCl)



- The data on particle sizes and their distribution in given protein samples measured by DLS and SEC-MALLS are very close.

Recombinant Protein on SEC-MALLS and DynaPro
Mass Distributions
(Protein at 0.9mg/mL in 50mM Phosphate, 50mM NaCl)



- The data on determination of the amount of aggregates in given protein samples measured by DLS and SEC-MALLS are very close.

SUMMARY

- The presence of monomer, dimer and degradants was confirmed in the SEC profile of the recombinant protein by MALLS.
- The determined hydrodynamic radius of the recombinant protein monomer (5nm) is higher than the hydrodynamic radius of the globular protein of similar molar mass which can explain its early elution from the SEC column.
- DLS data indicates that at room temperature (~25C) the recombinant protein is in a stable monomeric state over a broad range of pH (2.7–8.0). Significant amounts of aggregates are formed when the protein is incubated for 2 hours at 37C at pH below its pI (4.03).
- Data on particle sizes and their distribution obtained by DLS DynaPro Plate Reader and SEC-MALLS-QELS were found to be in good agreement. DynaPro Plate Reader offers higher throughput screening capabilities for aggregate formation under equilibrium conditions than SEC-MALLS-QELS.

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