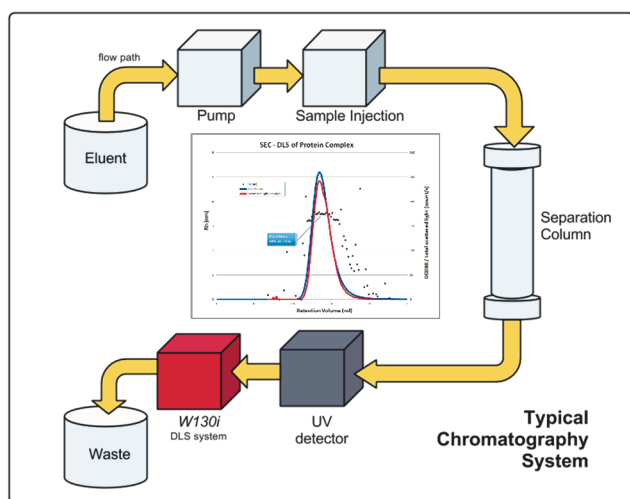


# Online Hydrodynamic Size Measurements Using the pUNK

## Background

The speed and sensitivity of dynamic light scattering lends itself to online applications such as chromatography of proteins. Size Exclusion Chromatography (SEC) separation systems are routinely used for molecular characterization and purification. The pUNK DLS system from Unchained Labs can be fitted with a low volume flow cell and simply connected to any chromatography system to provide instant online measurement of hydrodynamic radius (Rh) and scattering intensity as well as for detection of aggregates.



SEC systems use UV detection to determine the elution times of each size component and therefore provide an indication of the size distribution and aggregate content. The addition of (static) light scattering provides molecular weight measurement capability, however this requires calibration with standards. DLS does not require calibration to measure Rh. In addition to directly measuring Rh and intensity, the molecular weights of globular species may be reliably estimated using the proprietary algorithm built into Unchained Labs' i-Size 2.0 software.

## Method

The pUNK DLS system flow cell tubing is connected inline after the separation column or UV detector before going to fraction collection or waste. As soon as the sample is injected or once the UV peak begins to appear, DLS acquisitions are initiated until all peaks pass through.

The collected DLS and UV data is exported directly to a spreadsheet application for further analysis and correction for inter-detector volume if desired.

### Typical Samples:

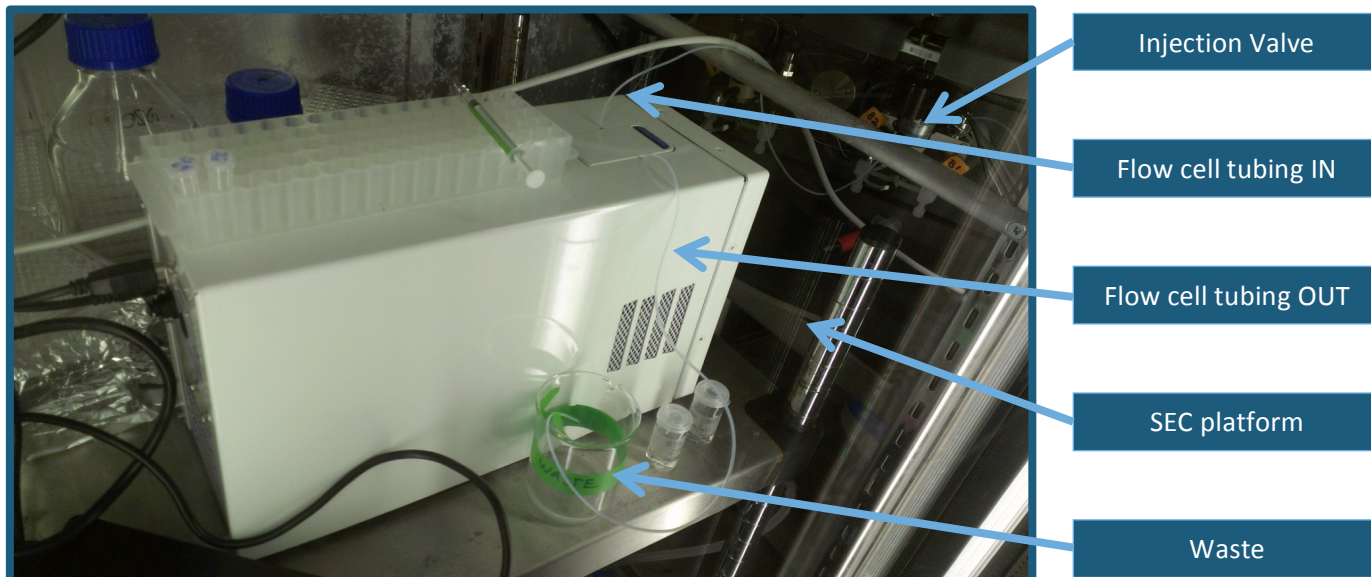
25kDa@5mg/ml  
 100kDa@1mg/ml  
 Injection Volume : 50uL

### Typical Column :

2.4 ml GE Superdex 200  
 Flow rate : 500uL/min SEC  
 Temp : 15 C

### DLS System:

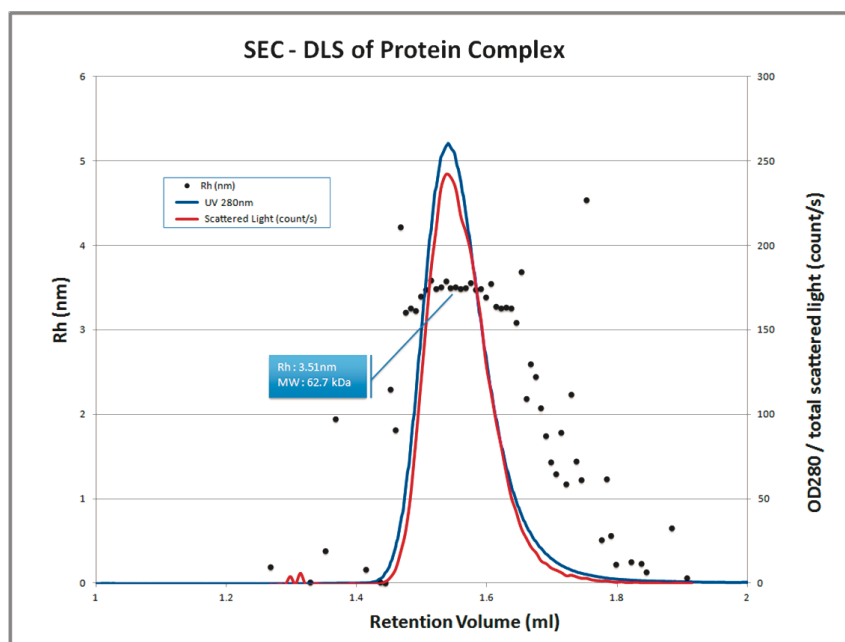
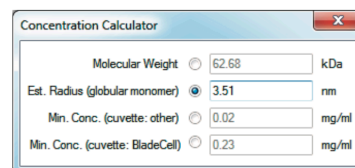
Acq. Time : 5sec  
 # of repeats : 180 (15 mins)  
 Laser output : 100%  
 DLS Cell Temp : 15 C



Compact size of pUNk system makes hardware placement within cold cabinet possible

## Results

Directly measuring Rh and scattering intensity every few seconds provides a plot, which can be easily overlaid with the UV chromatogram. The Rh measurements can be averaged across each peak and molecular weight estimate determined by using the Concentration Calculator utility (right) built into i-Size 2.0 software.



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