APPLICATION NOTE

Title:
Optimizing buffers for protein purification using Dynamic Light Scattering

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Summary: A proprietary material, ProteinT, failed to crystallize in the initial preparation and test buffer. The formulation was subsequently changed and improved conditions were determined with the aid of dynamic light scattering. The resultant buffer choice was selected for overnight dialysis and resulted in improved protein purification.

Introduction

Detailed information about protein molecules can be obtained from their molecular structure. To date the most successful method of obtaining this information is the use of protein crystallography. However, the crystallization step remains a significant bottleneck in structural biology: producing crystals of the quality needed for diffraction studies is still one of the most time-consuming steps. These crystals are essential for elucidating the high-resolution information required for understanding proteins and their potential interactions with other molecules.

Before starting any crystallization trial, sufficient protein material must be available. Thus protein purification and yield are important prerequisites for protein crystallography. This application note presents an example of the use of light scattering to optimize buffer conditions for a proprietary protein known as 'ProteinT'.

Methods

Protein preparation

ProteinT was prepared initially in a typical test buffer (20 mM Tris.HCl pH 8, 150 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 5% glycerol). We were not able to crystallize the protein from this buffer and in order to produce enough material for further screens, it was necessary to optimize the purification procedure. We started by considering the addition of different additives in varying ratios. Following a 2 x 30 min dialysis at room temperature the protein was assessed in the different buffers. All samples were measured using the Zetasizer Nano (Malvern Instruments) in a low volume quartz cuvette (12 µL).

Dynamic Light Scattering (DLS)

The size of molecules can be measured easily using dynamic light scattering (DLS). Here, the fluctuations in the scattered light are analyzed to detect diffusion of the molecules and deduct their hydrodynamic size. We used the Zetasizer Nano S for protein size measurement to evaluate the polydispersity of purified proteins. Results were analyzed using the Z-average and the Pdi parameters as well as the "Size Distribution by Intensity" and the "Raw Correlation Data" curves.

The Z-average is the overall average size of all scattering objects in the sample, and includes aggregates, oligomers, and fragments. The Pdi parameter describes the width of this overall average distribution, and provides an indication of the homogeneity of the sample (small=monodisperse, homogeneous). Data can also be inverted to determine the complete size distribution of species in solution.

Results

In seeking to optimize buffers, we look for a monomodal peak (> 90% intensity) around the expected size and a good polydispersity index (Pdi =0.3). DLS measurement of the protein in the initial buffer produced a main peak at a diameter of 87 nm. This was far higher than expected, indicating that the protein had a tendency to aggregate under these conditions. The results for different buffer formulations and preparations are listed in Table 1.

All data showed more than one peak in the size distribution. Tests #1 to #10 were performed using dialysis of 2 x 30 minutes, at room temperature. Buffer conditions #3, #4 and #8 showed relatively high contributions (in %intensity of the main peak) from a size in the expected protein size range (less than 20 nm). We then selected an improved dialysis routine for the most promising candidate.

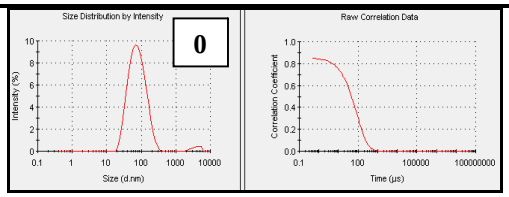
Test #11 used the same buffer as Test #3 but the dialysis was performed overnight at a temperature of 4ºC. The contribution from the protein peak remained above 90% and the size was reduced, indicating that fewer oligomers were present under these conditions. Adding Chaps or octoglucoside did not improve the distributions further, but instead introduced a third distribution peak in the size analysis. We now use condition #11 for standard ProteinT purification. In summary, this work allowed us to determine the buffer most suitable for this protein, where appropriate protein size and Pdi were found.

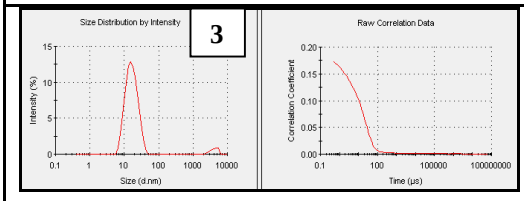
Conclusion

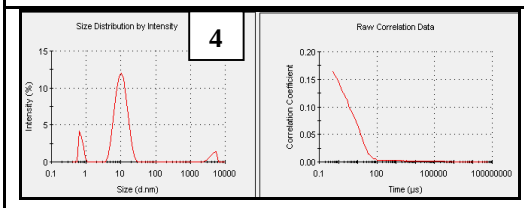
Light scattering is an easy-to-use tool that can help optimize buffer conditions for protein purification and crystallization. This rapid test allowed us to identify a buffer that was ideally suited to ProteinT. Using the buffer conditions in test #11, protein size and Pdi were found to be appropriate and this is now used as a standard protocol for the purification of this protein.

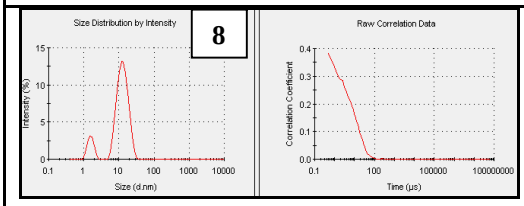
The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined dynamic, static, and electrophoretic light scattering measurements, providing the researcher with access to a wide range of sample properties, including size, molecular weight, and zeta potential. The system has been specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications, along with the high concentration requirements for colloidal applications.

Test #	Protein size measurement / DLS							Pdi	Peak	%	Size (nm)
0	**First Buffer: $T(pH8)_{20}$ N_{150} $G_{5\%}$ $EDTA_{0.1}$ DTT_5**							0.3	1	**97%**	**87**
									2	3%	4040
	Buffer Tests / Dialysis 2x 30mn at RT										
	Propane-diol (%)	**Tris-HCl pH7,5 (mM)**	**Tris HCl pH8,5 (mM)**	**$AcNH4^+$ (mM)**	**NaCl (mM)**	**TCEP (mM)**	**EDTA (mM)**				
1	5	20		150		1	1	0.95	1	67%	410
									2	33%	10.9
2		20		150		1	1	1	1	50%	1620
									2	42%	7.5
									3	8%	5440
3	5	20		300		1	1	0.23	1	**96%**	**17.3**
									2	4%	4220
4		20		300		1	1	0.08	1	**85%**	**10**
									2	10%	0.7
									3	5%	4580
5	5	20			300	1	1	0.99	1	95%	7.28
									2	5%	49
6	5		20	150		1	1	0.46	1	70%	10.9
									2	30%	324
7			20	150		1	1	0.83	1	61%	1240
									2	34%	7.3
8	5		20	300		1	1	0.26	1	**88%**	**13.4**
									2	12%	1.62
9			20	300		1	1	1	1	75%	275
									2	25%	7.9
10	5		20		300	1	1	0.97	1	52%	132
									2	26%	11
									3	22%	1230
11	**DIALYSIS O/N 4°C $T(pH7,5)_{20}$ $AcNH4_{300}$ $TCEP_1$ $EDTA_1$**							0.3	**1**	**93%**	**12.3**
									2	**7%**	**3910**
12	+ Chaps 1mM							0.69	1	53%	757
									2	29%	6.29
									3	12%	1.15
13	+ octo gluco 1%							0.87	1	61%	374
									2	39%	5.37
14	+ octo gluco 5%							1	1	81%	541
									2	19%	7.52









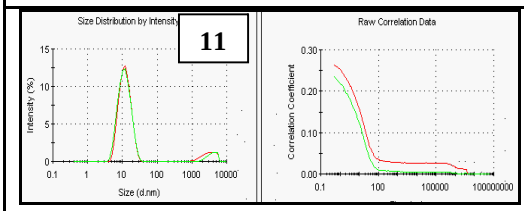


***Table 1**: Summary of light scattering results for ProteinT under various buffer conditions. The main peak size in the initial buffer in test #0 indicated significant aggregation of the protein. Based on initial buffer tests #1 to #10 improved conditions were identified and used in test #11. Here, the main size peak moved to the expected region (12.3 nm). The addition of two detergents (Chaps, octoglucoside) did not result in any further improvement*