APPLICATION NOTE

No. AN53429

Using NanoDrop Spectrophotometers in NMR, XRC and Cryo-EM workflows for structural biology

Introduction

Structural biology methods such as nuclear magnetic resonance (NMR), X-ray crystallography (XRC), and cryogenic electron microscopy (cryo-EM) have been staples for drug discovery, virus research, and many more applications. Determining protein structure via structural biology methods grants a better understanding of molecular mechanisms as protein functionality can be related to structure.[1]

The workflows for NMR, XRC, and cryo-EM are extensive and require the protein sample to be pure and concentrated enough to produce valuable data. The Thermo Scientific™ NanoDrop™ One/One^C Microvolume UV/Vis Spectrophotometer determines protein absorbance at a 280 nm wavelength and calculates the protein concentration, making it a useful tool in structural biology workflows. The use of the NanoDrop One/One^C instrument in NMR, XRC, and cryo-EM methods allows users to verify that the protein sample remains pure and concentrated throughout the various steps.

Application of NanoDrop Instruments to NMR, XRC, and Cryo-EM

The NanoDrop One/One^C spectrophotometer can be easily implemented alongside NMR, XRC, and cryo-EM workflows. In under 8 seconds, the NanoDrop One/One^C spectrophotometer determines concentration and purity of a protein sample using a cuvette or the microvolume pedestal. The microvolume pedestal uses 1 – 2 µL measurements, allowing for precious sample conservation.

The Thermo Scientific™ Acclaro™ Sample Intelligence Technology integrated into the NanoDrop One/One^C software identifies DNA contamination in protein preparations using the Protein A280 application (Figure 1). If DNA is detected in a protein sample, the Acclaro technology will identify the absorbance of the contaminating molecule and report a corrected protein concentration. Along with the Acclaro technology, the software will report an A260/A280 purity ratio—considered to be about 0.5 – 0.6 for pure protein—as well as the UV spectrum from 220 nm to 350 nm, allowing the user to identify additional contaminants present in the spectrum.

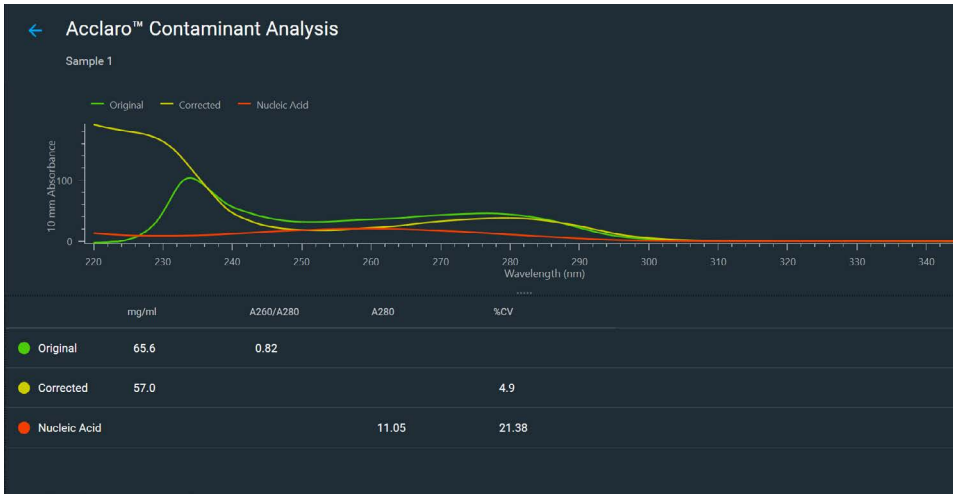

Figure 1: The Acclaro technology in the NanoDrop One/One^C software identifies DNA contaminants in protein preparations in the Protein A280 application. The corrected spectrum is reported in yellow, the original spectrum is reported in green, and the contaminant spectrum is in orange.

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NMR workflow and NanoDrop Spectrophotometer integration

The functionality of NMR is based on the interaction of atoms with a magnetic field. For some nuclei, they may exhibit a spin that creates a magnetic field, depending on whether the nucleus has unpaired protons or neutrons. When an external magnetic field is applied to the atoms, the nuclei will either reside in a low or high energy state, absorbing or emitting energy, respectively, as it travels to the opposite energy state. This absorption or emission of energy is ultimately shown as a peak in the NMR spectrum.[2] The analysis of nuclei with NMR provides specific structural details for the protein of interest.

The NMR workflow begins with producing the protein of interest through recombinant expression (Figure 2). The appropriate DNA vector for expressing the protein of interest should first be created by polymerase chain reaction (PCR) plasmid cloning following the protocol outlined by Hoseini and Sauer (2015). The target sequence is first usually amplified by PCR, and the PCR product is purified, where the purified product is digested with restriction enzymes.[3] After digestion, the DNA is isolated via gel electrophoresis, subsequently extracted from the gel, and the A260/A280 purity ratio and concentration of the isolated DNA product is determined with the NanoDrop One/One^{C} instrument, where the purity ratio of pure DNA is considered to be ~1.8. Following the purity and concentration assessment, the DNA is ligated into the recipient plasmid and is then transformed into cells.[3] *Escherichia coli* (*E. coli*) is a common expression system to utilize for the transformation of the DNA plasmid.[4] The cells are then cultured to translate the target protein, and the optical density at 600 nm (OD_{600}) is determined.

When the cell culture is deemed to be at adequate growth, the cells are lysed to extract the target protein, and the protein is purified for NMR application. Protein purification methods typically include immobilized metal affinity, ion exchange, and size-exclusion chromatography.[5] The purified protein is then concentrated, and the final concentration can be measured on the NanoDrop One/One^{C} spectrophotometer to determine the accurate concentration for application to NMR. The NMR data collection provides the peaks and, typically, the more concentrated the protein, the better the peaks will appear on the spectrum. From the NMR spectrum, the sequence-specific resonance assignments may be determined, ultimately leading to the protein structure calculation.[2]

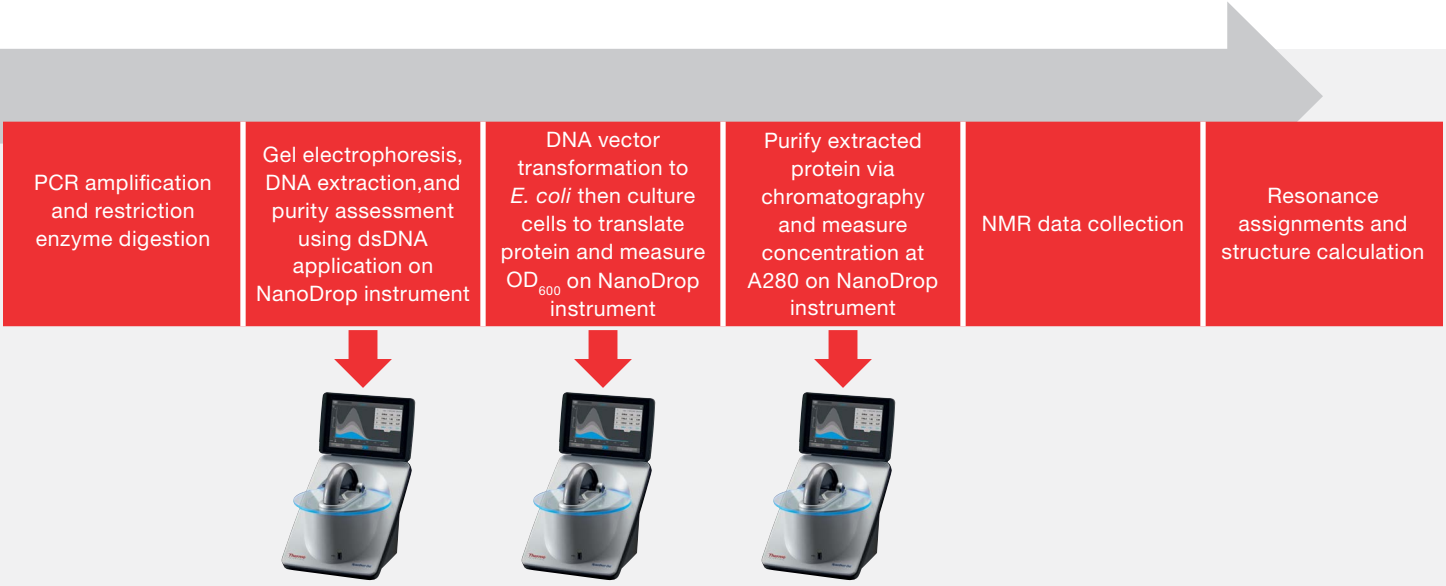

Figure 2: Workflow involving creation and purification of the DNA plasmid, measuring DNA concentration on the NanoDrop One/One^{C} spectrophotometer, transformation of DNA vector to *E. coli* cells, culturing cells, measuring OD_{600} on the NanoDrop instrument, and assessing purity and concentration after each purification method with the Protein A280 application and data is then collected via NMR.

XRC workflow and NanoDrop Spectrophotometer integration

The basis of XRC is the diffraction of X-rays by an atom's electron cloud in a crystalline lattice whose atoms are aligned in a repeating pattern.[6] Extensive mathematics based on geometry is then applied to determine the electron density map from the specific diffraction pattern. With the information in the electron density map, the protein's atomic model, including secondary structures, can be identified.[6]

As shown in Figure 3, the protein of interest must be produced via recombinant expression and purified prior to crystallization trials. To form the crystal, the crystallization solution, including the buffer, precipitation solution, and any additives, must be optimized to support the growth of the crystal. This may be performed in a 96-well plate with variable solution conditions. Another important factor for crystal formation is the initial protein sample concentration. If the sample is too dilute, nucleation may not occur, and if the sample is too concentrated, precipitate may form instead of a crystalline solid.[7] The concentration may be confirmed using the NanoDrop One/One^{C} instrument to ensure the optimal concentration for crystallization experiments (Figure 3).

Figure 3: X-ray crystallography workflow to include implementation of the NanoDrop One/One^{C} spectrophotometer for determining sample concentration.

Cryo-EM workflow and NanoDrop Spectrophotometer integration

A relatively new method for studying protein structure and function, cryo-EM, has caught the interest of many protein biologists due to eliminating the need for crystals. The Thermo Scientific™ Tundra™ Cryo-Transmission Electron Microscope is hailed as an easier-to-use, affordable, space-saving cryo-EM model. Cryo-EM utilizes a high-dose electron beam which passes through a sample, and the scattered electrons are focused by a lens series. To protect the sample from the damaging effects of electron radiation, the protein sample is vitrified, preserving the protein complex in a native state.[8]

The workflow for cryo-EM single-particle analysis begins the same as NMR and XRC with the recombinant expression of the desired protein and subsequent extraction and purification (Figure 4). Once the protein is purified, the sample undergoes negative staining to assess sample purity and further sample optimization for application to the cryo-EM grid. The cryo-EM grid is a mesh that supports the sample while images are obtained using a transmission electron microscope.[8]

Sample optimization for application to the grid includes determining the appropriate protein sample concentration. Shown in Figure 4, the NanoDrop One/One^{C} instrument can be used in conjunction with negative staining to determine protein purity as well as concentration for grid preparation. Some contaminating analytes in the protein sample may interfere with the 3D reconstruction of the protein so negative staining and making measurements on the NanoDrop spectrophotometer are useful to avoid wasting valuable time and resources.

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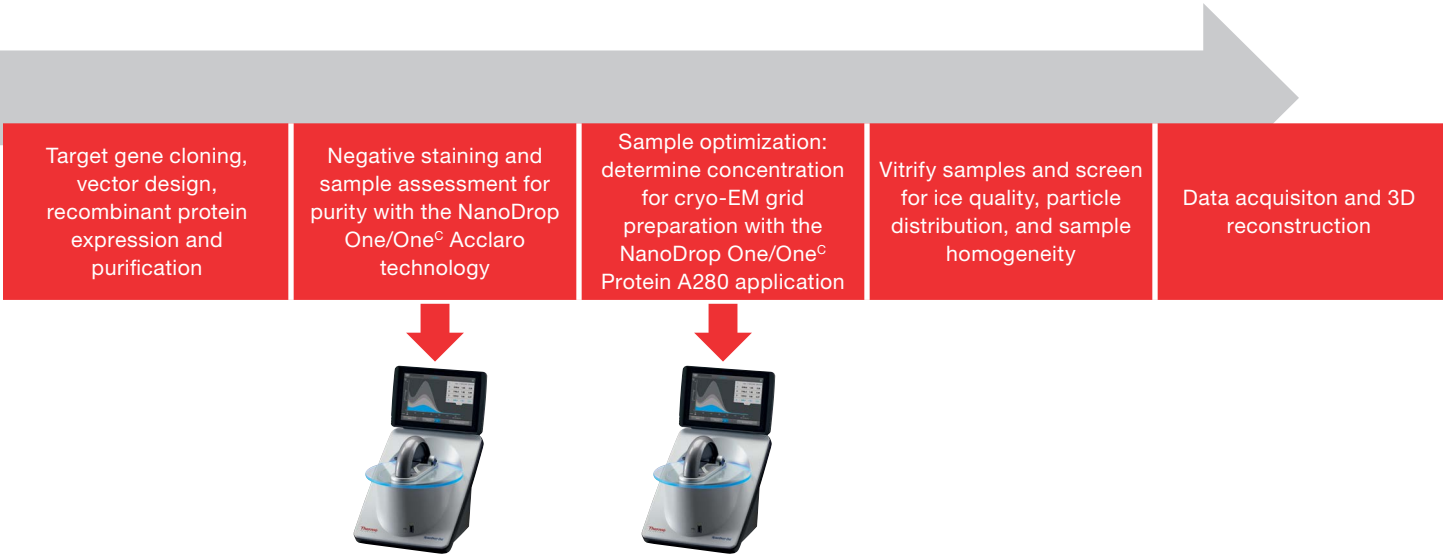

Figure 4: Cryo-EM workflow including the NanoDrop One/One^C spectrophotometer integration for determining protein purity and concentration prior to application to the EM grid.

Following optimization, the sample is vitrified to preserve it in the native state. Ice quality, particle distribution, and homogeneity are all screened to obtain the best grid for data acquisition and subsequent 3D reconstruction.[9]

Conclusion

The NanoDrop One/One^C spectrophotometer can be easily integrated into the workflow for NMR, XRC, or cryo-EM to determine an accurate protein concentration and purity assessment. The Acclaro technology built into the NanoDrop One/One^C software determines the amount of contaminating DNA present in a protein sample in the Protein A280 application and calculates a corrected protein concentration. Determining contaminants prior to NMR, XRC, and cryo-EM is important because contaminants may influence the structural imaging of the protein, thus wasting time and resources. The NanoDrop One/One^C spectrophotometer is an all-in-one, easy-to-use instrument that can be a valuable addition to the NMR, XRC, and cryo-EM workflows.

References

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