

NGC Chromatography System Connection Guide for Wyatt Instrumentation Protocol

Protocol

Protein Purification

Bulletin 7208

Introduction

Multiangle light scattering (MALS) and refractive index (RI) are commonly used in chromatography to determine sample molecular weight, oligomerization state, and uniformity. This guide describes how to connect the NGC Chromatography System with the Wyatt TREOS MALS and T-rEX RI instruments. This connection was achieved with the help of Dr. Sharp and Dr. Mobley at the University of Mississippi Department of BioMolecular Sciences.

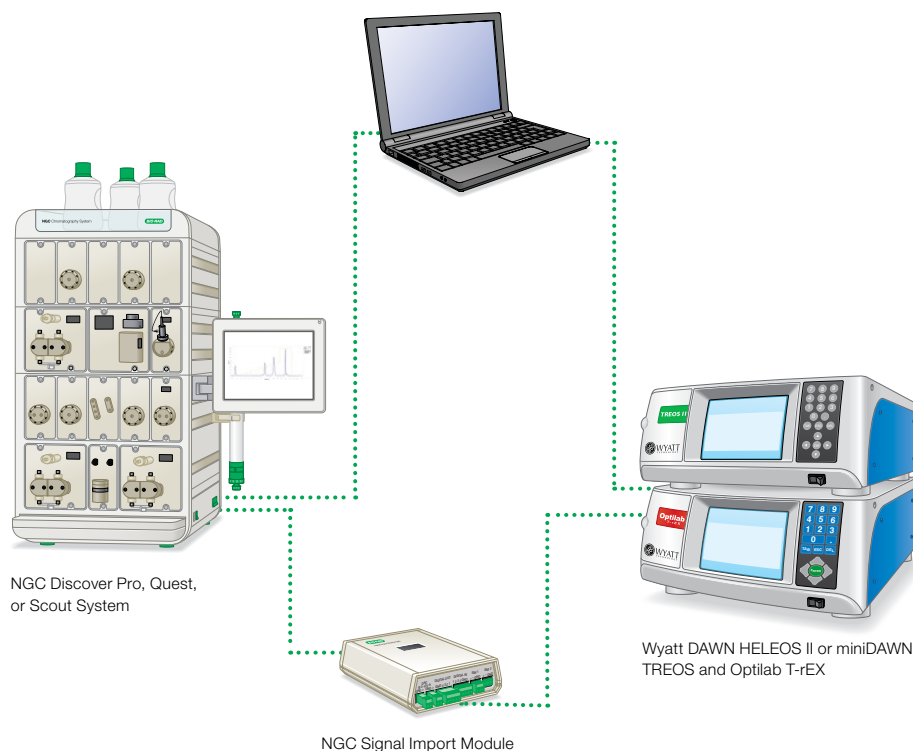
Materials

Bio-Rad Laboratories

- NGC Chromatography System with ChromLab Software, version 4.0 or higher (catalog #7880001)
- Flathead screwdriver (provided in NGC Fittings Kit, catalog #7884017)
- NGC Signal Import Module (SIM; catalog #7884016)

Wyatt Technology

- Wyatt Light Scattering Detector (HELEOS II or TREOS)
- Wyatt Refractive Index Detector (T-rEX)
- Astra Software, version 7
- RJ-12 Wires with Flying Leads, 3 total (catalog #P4045-19)



BIO-RAD

Procedure

Warning: Power off all devices prior to proceeding.

1. Hardware Connections — Connecting the NGC SIM to the Wyatt MALS and RI

1.1. Using one RJ-12 cable, connect DAC A of NGC SIM to AUX In 1 & 2 of Wyatt MALS detector.

- Remove the green plug from DAC A on NGC SIM and loosen screws using the flathead screwdriver provided in the NGC Systems Fittings Kit
- Using the flying leads end of the RJ-12 cable, insert the black wire into the GND position of the connector. Tighten the screw such that a firm and secure contact with the connector is made
- Using the same RJ-12 cable, insert the white flying lead into the + position of the connector. Tighten the screw such that a firm and secure contact with the connector is made
- Insert the green plug into the NGC SIM DAC A position
- Insert the plug end of the RJ-12 into the AUX In 1 & 2 position of the MALS instrument

1.2. Using a second RJ-12 cable, connect DIGITAL OUT of NGC SIM to Auto Inject In of Wyatt MALS detector.

- Remove the green plug from DIGITAL OUT of NGC SIM and loosen the screws of the GND and 2 positions using the flathead screwdriver provided in the NGC Systems Fittings Kit
- Using the flying leads end of the second RJ-12 cable, insert the green wire into the GND position of the connector. Tighten the screw such that a firm and secure contact with the connector is made
- Using the flying leads end of the second RJ-12 cable, insert the red wire into the 2 position of the connector. Tighten the screw such that a firm and secure contact with the connector is made
- Insert the green plug into the NGC SIM DIGITAL OUT position
- Insert the plug end of the RJ-12 into the Auto Inject In position of the MALS instrument

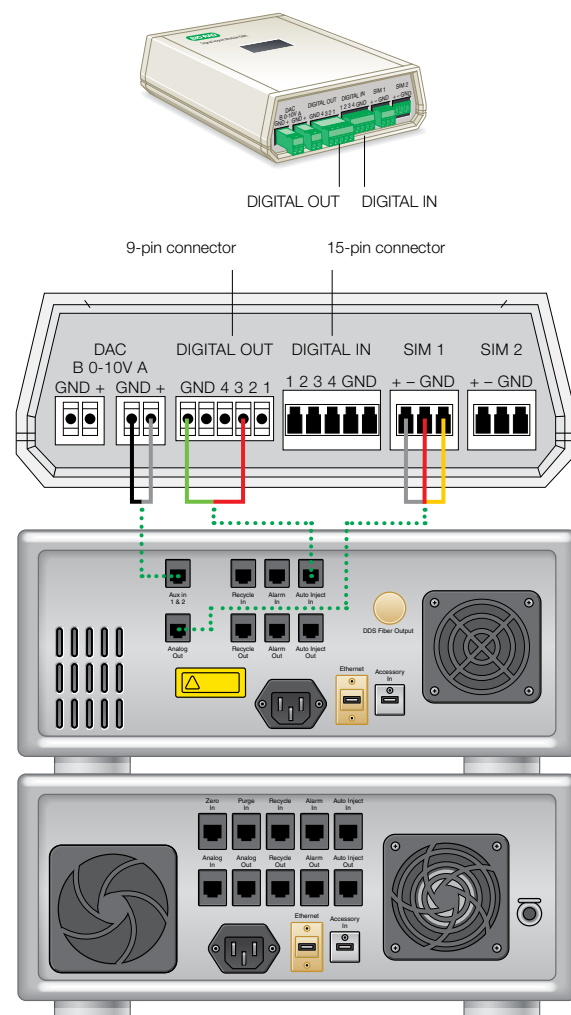
1.3. Using the third RJ-12 cable, connect SIM 1 of NGC SIM to Analog Out of Wyatt MALS.

- Remove the green plug from SIM 1 of NGC SIM and loosen the screws using the flathead screwdriver provided in the NGC Systems Fittings Kit
- Using the flying leads of the third RJ-12 cable, insert the yellow wire into the GND position of the connector. Tighten the screw such that a firm and secure contact with the connector is made

- Using the flying leads of the third RJ-12 cable, insert the white wire into the + position of the connector. Tighten the screw such that a firm and secure contact with the connector is made
- Using the flying leads of the third RJ-12 cable, insert the red wire into the – position of the connector. Tighten the screw such that a firm and secure contact with the connector is made
- Insert the green plug into the NGC SIM at the SIM 1 position
- Insert the plug end of the RJ-12 into the Analog Out position of the MALS instrument

1.4. Plug the NGC SIM into the peripheral port on the base of the NGC System.

1.5. Turn on the computer, fraction collector, NGC System, and both Wyatt MALS and RI instruments.



Wired connections between the NGC SIM and Wyatt TREOS and T-REX.

2. Networking

This section will require IT support if the communication is to go through pre-existing network infrastructure. Moreover, IT support may be required to change some of the network settings on the computer controlling the hardware. This section focuses on a minimal connection between the hardware and computer; however, the steps can be modified by IT personnel to adapt to your network.

All instruments communicate via Ethernet network connection. The communication from each of the three instruments is consolidated to a single connection to the controlling computer using a network switch. Setting a static IP address on all the instruments is recommended to ensure reliable communication.

2.1. Connect all Ethernet cords to a common network switch.

2.2. Set the IP address on each instrument to a static IP.

- Set the NGC System static IP
 - On the NGC System touch screen, tap the ChromLab Software icon in the upper left corner
 - Tap **System Information**
 - Scroll to System IP Address and tap **Configure**
 - Under Configure IP Address, select **Use the following IP address**. Set IP Address to **192.168.254.100** and Subnet Mask to **255.255.255.0**
 - Click **OK**
- Set the Computer IP address (Windows 10)
 - Click **Start**, then **Settings**
 - Click **Network and Internet**
 - Click **Change Adaptor Options**
 - Right click the wired connection that will communicate with the hardware and click **Properties**
 - Select **Internet Protocol Version 4** and click **Properties**
 - Select **Use the following IP address**. Set IP address to **192.168.254.101** and Subnet mask to **255.255.255.0**
 - Click **OK**

- Set the IP address on the Wyatt MALS
 - On the top of the screen tap **COMM**
 - Under Configure Ethernet, select **Use the following IP address**. Set IP Address to **192.168.254.102** and Subnet Mask to **255.255.255.0**
 - Tap **Apply**
- Set the IP Address on the Wyatt RI detector
 - On the top of the screen tap **COMM**
 - Under Configure Ethernet, select **Use the following IP address**. Set IP Address to **192.168.254.103** and Subnet Mask to **255.255.255.0**
 - Tap **Apply**

2.3. Restart all systems.

- Unplug the network switch
- Turn off the computer, fraction collector, NGC System, and both Wyatt MALS and RI instruments
- Plug in the network switch
- Turn on the computer, fraction collector, NGC System, and both Wyatt MALS and RI instruments

3. Software Connections and Setup

3.1. Set up the ChromLab Software to recognize the addition of the NGC SIM hardware and adjust settings to input/output data.

- Add the NGC SIM module to the fluidic scheme. Modify your fluidic scheme according to the instructions in chapter 3 of the NGC Chromatography Systems and ChromLab Software User Guide (#10000049092)
- Set the delay volume for the instrument
 - Click **System Control**
 - Go to File, then click **System Settings**
 - Click **Delay Volume** and select **Synchronize with detector**
 - Enter the appropriate information
 - For further instructions about setting the delay volume, see chapter 3 of the NGC Chromatography Systems and ChromLab Software User Guide (#10000049092)

System Settings - NGC

Device Output	Air Sensors	Email Notifications	System Flush	System Name
Delay Volume	Control Flow	Remote Access	Trace Settings	Device Input

☒ Synchronize with detector

Detector to Fraction Collector Drophead

Fraction Collector:

Tubing inner diameter: 0.02 in.

Tubing length: 116 cm

Divert Valve: 67 µl

Additional volume: 200 µl

Inline Devices

☒ Analytical 5mm UV Flow Cell 16 µl

☒ Conductivity Cell 6 µl

☒ Backpressure regulator (20 or 40 psi) volume 130 µl

FC1: NGC FC

Total delay volume: 454 µl

Help OK Cancel

- Configure the NGC SIM to export the ultraviolet (UV) absorbance signal to the Wyatt MALS
 - Click **System Control**
 - Go to File, then click **System Settings**
 - Click **Device Output**
 - Under Signal Output A, click **Enabled**
 - Set the Signal as λ for a single wavelength detector. For a multi-wavelength detector, set the signal as λ_1 , λ_2 , λ_3 , or λ_4 , depending on which wavelength is designated to be 280 nm
 - Set the Scale appropriately. For a single wavelength detector, set to **Min = 0, Max = 3000**. For a multi-wavelength detector, set to **Min = 0, Max = 4000**
 - Set the Range to **Min = 0, Max = 10**

System Settings - NGC

Delay Volume	Control Flow	Remote Access	Trace Settings	Device Input
Device Output	Air Sensors	Email Notifications	System Flush	System Name

Signal Output A

☒ Enabled Signal: λ

Scale (mAU) Min 0.00 Max 3000.00

Range (0 - 10) Volt Min 0.00 Max 10.00

Help OK Cancel

- Configure the NGC SIM to import the MALS signal from the Wyatt detector
 - In System Settings, click **Device Input**
 - Under SIM 1, click **Enabled**
 - Name the Trace appropriately (for example, Wyatt MALS)
 - Set the Range to **Min = 0.0, Max = 1.0**
 - In the Trace Unit field, enter **mV**
 - Set the Scale to **Min = 0, Max = 1000**
 - Set the Delay Volume caused by the instrument. This will dictate where the trace from the MALS is graphed

System Settings - NGC

Device Output | Air Sensors | Email Notifications | System Flush | System Name

Delay Volume | Control Flow | Remote Access | Trace Settings | **Device Input**

SIM 1

☒ Enabled

Trace Name: Wyatt MALS

Range (Volt) : Min 0.00 Max 1.00

Trace Unit : mV

Scale (mV) : Min 0.0000 Max 1000.0000

Delay Volume : 150 µl

SIM 2

☐ Enabled

Trace Name: External Detector 2

Range (Volt) : Min -2.50 Max 2.50

Trace Unit : Units

Scale (Units) : Min -9999.0000 Max 9999.0000

Delay Volume : 0 µl

Help OK Cancel

3.2. Set up the Wyatt DAWN HELEOS II or miniDAWN TREOS output. Set up the Wyatt detector to export the signal from 90 degrees on the hardware.

3.3. Set up the Method in ChromLab Software to signal the Wyatt MALS when injecting.

- Open the Method to be run during the analytical run
- Click **Sample Application**
- Under Injection Point, select **SIM Output 2**
- Save the Method

3.4. Set up Astra Software. Refer to the ASTRA 7 User Guide and the ASTRA Quick Guide for assistance with selecting the appropriate ASTRA method and configuration for your particular detector configuration.

- Select **Default Astra Method** and **Instrument Configuration**

- Expand the tree under Experiments

- Click **Generic UV Instrument** and modify to match your NGC System

ASTRA - BSA run 2

File Edit View Experiment Sequenced Processing System Window Help

Method Builder Run Pause Stop

Default Method RUN DEFAULT

Experiments

- BSA run 2
 - Configuration (UV - LS - RI online)
 - Generic Pump (pump)
 - Injector
 - miniDAWN TREOS
 - Optical 1-4x (HE)
 - Generic UV Instrument (UV)
 - Aux Channel Connection (UV to LS)
 - Auto Inject Connection (untitled)
 - Basic Collection
 - Dispensing
 - Baselines
 - Peaks
 - Molar Mass & Radius from LS
 - Distribution Analysis

Configuration (UV - LS - RI online)

Generic Pump (pump)

Injector

miniDAWN TREOS

Optical 1-4x (HE)

Generic UV Instrument (UV)

Aux Channel Connection (UV to LS)

Auto Inject Connection (untitled)

Basic Collection

Dispensing

Baselines

Peaks

Molar Mass & Radius from LS

Distribution Analysis

Experiments

Sequences

Profiles

Instrument

Configuration Generic Pump (pump) Solvent Inlet(s) Injector Injector

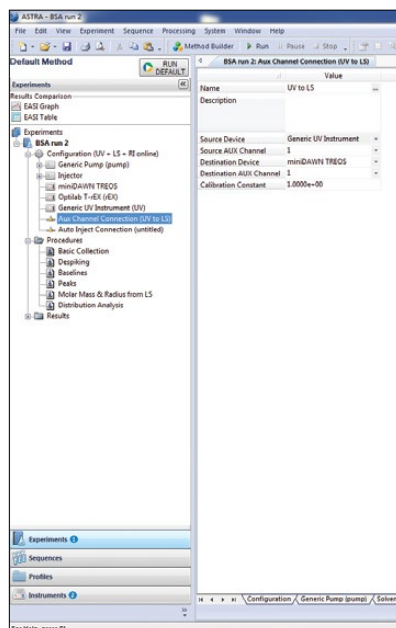
OK Cancel Apply

– Modify Cell Length to match the cell length indicated on top of the UV flow cell. The NGC System will be equipped with either 0.2, 0.5, or 1.0 cm

– Under Channels, select enable for Channel 1, set the wavelength to 280 nm, and set the UV response according to the detector equipped: Single wavelength: 0.3 or Multi-wavelength: 0.4

– Click **OK** (at bottom of screen) to save changes and exit this window

- In Experiments tree, click **Aux Channel Connection (UV to LS)**



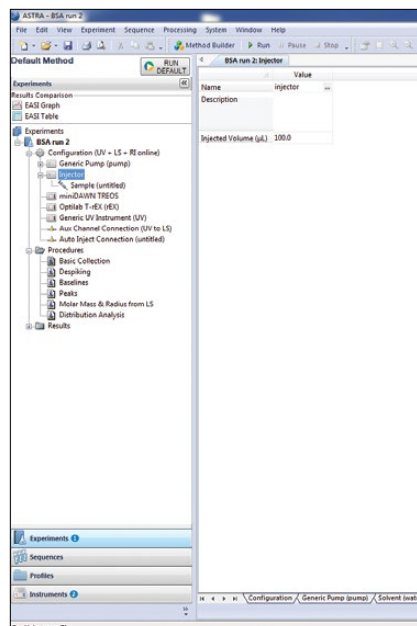
- Set the Source Device settings. Source Device should be left as default Generic UV Instrument. Source Aux Channel should be left as 1
- Set the Destination Device settings. Destination Device should be set to the MALS Instrument. Destination Aux channel should be left as 1
- Do not change the Calibration Constant; usually this is 1

Note: Changing this constant has an effect on calculated molecular weight.

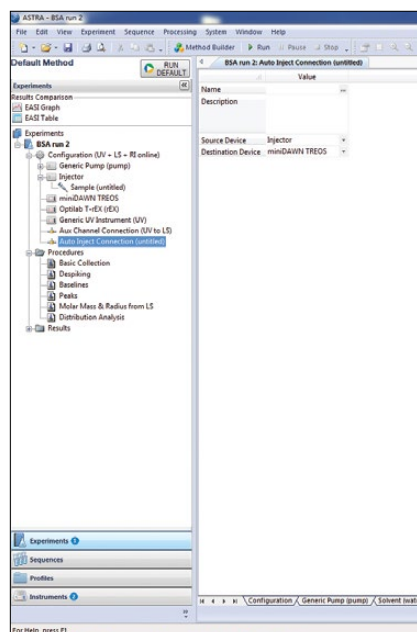
- Click **OK** (at bottom of screen) to save changes and exit this window

- Click **Injector**. Set Injected Volume (μL) to match the volume the NGC System will inject, then click **OK** (at bottom of screen)

Note: This will not control the BioRad NGC System hardware and is used only for ASTRA Software calculation of Recovered Masses and associated Mass Recovery percentages.

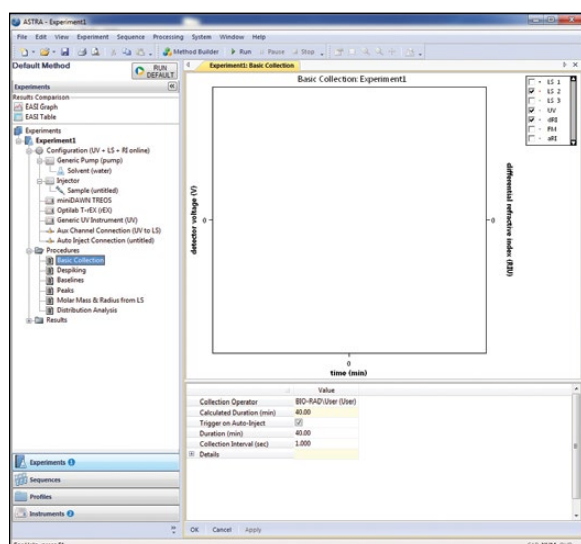


- Click **Auto Inject Connection**



- Set the Source Device. Leave Injector as the default
- Set the Destination Device as DAWN HELEOS or miniDAWN TREOS
- Click **OK** (at bottom of the screen)

- Set up the collection procedures



- Click **Procedures**, then click **Basic Collection**
- Set the Calculated Duration based on the column run time in ChromLab Software

Note: This will dictate the ASTRA Collection Interval, that is, the MALS and RI data collection period. We recommend setting a time that will exceed the total column run time.

- Check the box next to Trigger on Auto-Inject
- Click **Apply**

4. Starting a Method

4.1. Open both ChromLab and Astra Software.

- In ChromLab Software, open the Method to be run
- In Astra Software, open the Method to be run
- Verify that the Collection Run Time set in Astra exceeds the time listed in ChromLab Software after injection
- Ensure the Sample Volume in ChromLab Software matches the Injected volume in Astra

4.2. In Astra Software, click **Run** at the top of the screen.

4.3. In ChromLab Software, click **Start Run** at the top of the screen.

5. Appendix

5.1. Apply alignment, band broadening, and normalization procedures (performed only on normalization standard, for example bovine serum albumin [BSA]).

5.2. Align signals.

- Right click **Configuration**
- Select **Alignment**
- Highlight the peak, for example, BSA monomer peak
- Click **Align Signals** (in top left corner)
- Click **OK** (in bottom left corner), then close the window

5.3. Apply band broadening.

- Right click **Configuration**
- Select **Band Broadening**
- Reset the broadening terms in the table at the bottom
- Select a region in the center of the peak (not the entire peak)
- Click **Perform Fit** (in top left corner)
- Use the Broadened button to toggle the broadening on and off. If the match between the peaks is not good, repeat the fit
- Click **OK** to save the band broadening correction

5.4. Normalize detectors.

- Right click **Configuration**
- Select **Normalize**
- Choose which peak to use for normalization. Default is peak 1
- Click **Normalize**. Software will prompt peak selection if one hasn't been selected
- Click **OK** (in bottom left corner), then close the window
- Right click on the name of the experiment and select **Save** to save this experiment with the above changes

5.5. Save the system parameters.

- To save the normalization, alignment, and band broadening parameters as an ASTRA method, click on the name of the experiment, right click, and select **Save as Method**
- Give this method a new name
- Use this method to collect ASTRA data for all future experiments with these parameters

Visit bio-rad.com/NGC-WyattSetup for more information.

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