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Supporting information for article:

BioSAXS on the SWING beamline at Synchrotron SOLEIL

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S1. BioSAXS High-Level Control

S1.1. SEC-SAXS

S1.1.1. SetupHPLC.moml

For each SEC column (hence for each circuit), the user has to edit the column name, the total elution volume, the requested flow rate, the maximum allowed pressure, the optional equilibration time (pre-elution) and the optional additional elution time (post-elution). If set, the equilibration time specifies a time delay before the first sample is injected, and during which both columns are getting equilibrated. Three of the UV-monitors wavelengths are fixed, 214, 260 and 280nm, the others can be chosen arbitrarily.

When all parameters are set, the user is requested to click on the “execute” button. As schematized in Figure 5, the PasserelleIDE macro then creates all the corresponding HPLC injection/elution, equilibration and purge method files, further interpreted by the HPLC system. The time needed to rinse the injection loop when switching between two circuits (part of the purge methods) cannot be changed by the user and is set to two minutes at 2.5 ml/min.

S1.1.2. RunHPLC_Eiger.moml

For each sample, five main parameters must be specified: sample acronym, position in the rack, volume to be injected, circuit to be used and After_Injection_Start_Delay value (AISD). The AISD parameter defines at which time after the sample injection both the X-ray irradiation and SAXS data collection should start. The data recording then stops at the end of the elution time defined in the first PasserelleIDE macro. The AISD parameter was added to the general workflow to circumvent the possible and avoidable fouling of the capillary by sample aggregates. Hence our recommendation is to start the sample measurement after the larger species have eluted, that is to say at least some time after the void volume of the columns. Each of these parameters can be inserted into a list of arguments to , making a string of values with a comma as separator, instead of a single scalar value: this is how several sample injections can be planned at once. An additional parameter, the PI name, is used to store the data in the appropriate files and folders. It is first checked to be part of the Soleil Users Database declared PIs. It is used as the root name to further name the raw data files. The files are then automatically stored in a dedicated folder with appropriate access rights. In principle, a list of PI names could also be specified, but in practice, this possibility is very rarely used.

Concerning the buffer scattering data, a large number of frames (typically two hundreds) should be recorded after injection has occurred and before the void volume is reached. To this purpose, and for

each of the two circuits, the starting time and the duration for buffer data collection must be also specified by the user.

Other options, such as, inserting an initial equilibration step, stopping the pump flow after the last sample was eluted, changing the SAXS individual frame exposure time, cleaning the capillary with the cleaning robot, or choosing the capillary illumination light color are also editable.

S1.2. BATCH

Other parameters can be optionally edited, such as the exposure time per frame, the precise circuit volume between the automated auto sampler exit and the position of the X-ray beam along the SAXS capillary, the flow rates for pipetting, injecting and measuring, the incubation time (time during which the sample is maintained in the capillary to reach the desired temperature before it is flown and measured), the additional volume to scan (punctually used to include measurement of air and water for data normalization to absolute units) and the capillary illumination color, sometimes useful for light sensitive proteins.

S2. : Data File management

Each proposal is assigned a dedicated folder, separated in two main branches. The first branch is write-protected and contains the raw NeXus files, which include the raw SAXS and in situ UV-Vis absorption data and the contextual data (metadata) as well. The second branch, not write-protected, contains the reduced and analyzed data files, directly saved by the users and/or by the automated data reduction workflows. As the same proposal may have several sessions attributed during the year, a subfolder is automatically created for each new session of the proposal in each of the two branches. Moreover, since several PIs may share the same proposal and the same session, as is the case for a BAG proposal, their data are stored each in their own subfolder, again in each of the two branches. To obtain a copy of their raw and analyzed data, the users can then opt between two possibilities. The staff recommends to bring a FAT32 or NTFS formatted external hard drive. A synchronization script automatically updates the user's hard drive every minute. The other choice is to download the data a posteriori through the "Soleil Data Retrieval" portal, which is accessible via the SOLEIL User Network webpage. The average disk space used by a Batch set of measurements (4 buffers and 3 samples) and a SEC-SAXS measurement (elution time of 15min) are respectively 1.3 GB and 3.4 GB.

The figure displays two side-by-side screenshots of the Passerelle software interface for the SEC-SAXS Mode.

Left Window: SEC elution configuration macro

Bin Circuit Setup

- BinColumnName: value: BioSec3-300
- Bin Flow (mL/min): value: 0.3
- Bin Max Pressure (bars): value: 150
- Bin Elution Time (min): value: 15
- Bin Additional Elution Time (min): value: 5
- Bin Additional Equilibration Time (min): value: 5

Cap Circuit Setup

- CapColumnName: value: S200 increase 5/150
- Cap Flow (mL/min): value: 0.3
- Cap Max Pressure (bars): value: 50
- Cap Elution Time (min): value: 15
- Cap Additional Elution Time (min): value: 5
- Cap Additional Equilibration Time (min): value: 5

Additional MWD wavelength

- UVSignalD (nm): value: 290
- UVSignalE (nm): value: 320
- Column Temperature (Celsius): value: 15

Additional DAD wavelength

- UVSignalD (nm): value: 290
- UVSignalE (nm): value: 320
- UVSignalF (nm): value: 550
- UVSignalG (nm): value: 600
- UVSignalH (nm): value: 650

Right Window: injection/acquisition process macro

Model Parameters

- ListNxRootName_SubFolder: swing
- ListAcquisitionName: prot1_prot2_prot3
- ListPositions: A1, A2, B1
- ListVolumes_uL: 5.0, 45.0
- ListStartDelay: 7
- ListComments: example of injection

Exposure Parameters and Questions to Users

- ExposureTime (ms): value: 990
- DeadTime (ms): value: 10
- Do you want to stop the flow after the last sample ? (Yes/No): value: No
- Capillary cleaning after end of elution ? (Yes/No): value: Yes
- Do you want to illuminate the capillary ? (Yes/No): value: Yes
- Do you want to perform a pre-equilibration before the first injection ? (Yes/No): value: Yes

Buffer Start Time Parameters

- Bin BufferStartDelay (min): value: 2
- Bin BufferElutionDuration (min): value: 3
- Cap BufferStartDelay (min): value: 2
- Cap BufferElutionDuration (min): value: 3

Fig. S1. Views of the UIs for the SEC-SAXS Mode. Left: SEC elution configuration macro. Right: injection/acquisition process macro.

The figure displays a screenshot of the Passerelle software interface for the Batch Mode.

Passerelle - file:/usr/Local/configFiles/passarelle/models/RobotPIERRE_Eiger.moml (sur srv4.swing.rcl)

Parameters

- Find: Find Next Find Previous

Model Parameters

- ListNxRootName_SubFolder: swing
- ListAcquisitionName: buffA_prot1_buffA_prot2_bufferA_prot4_buffA
- ListPositions: A1,A2,A1,A3,A1,A4,A1
- ListVolumes: 40
- ListBufferSample_BorS: B,S,B,S,B,S,B
- ListConcentration_mgml: 0,1,0,2,0,4,0
- ListComments: example of injections
- DryingDelay: 30
- ListIncubationTime_sec: 0

Parameters

- ExposureTime (ms): value: 990
- DeadTime (ms): value: 10
- Offset (uL): value: 175

ExpertParameters

ExpertParameters.FlowRate

- Recording Speed (uL/min): value: 75
- Pipetting Speed (??L/min): value: 250
- Injection Speed (??L/min): value: 800

ExpertParameters.Others

- Incubation Time (s): value: 0
- Additional Volume to scan (uL): value: 10
- Do you want to illuminate the capillary ? (Yes/No): value: Yes

Log

Fig. S2. View of the UI for the Batch Mode.