Contents:

1) General terms

- Only non-pathogenic biological samples are accepted. Decree of the July 18, 1994 establishing the list of biological pathogens, amended by Decrees of April 17, 1997 and June 30, 1998 (Decrees in French). The list of biological pathogens is available on the website of the IPBS.
- The platform is open to academics, which usually will be trained for data analysis. It's also open industrials and academics for carrying out studies and analysis by the platform, depending on the availability of the equipment and staff.
- Users can be trained to use of the equipment by themselves. Each applicant aiming to use the equipment must be trained, the obligatory training been renewed every 3 months. The applicant has to follow and respect the instructions (part 2). In case of failure, the user can be excluded.
- The applicant has the responsibility of his samples, which will not be stored (unless explicit request).
- PAOL is connecting to the IBS internal network, so as to make easy printing and safeguarding the results. Generated data are safeguarded during 3 years. Past the delay, data will be deleted without notice.
- The platform doesn't guarantee the confidentiality of the data (unless explicit request).
- The user should acknowledge the platform in the publications: "This work used the platforms of the Grenoble Instruct centre (ISBG; UMS 3518 CNRS-CEA-UJF-EMBL) with support from FRISBI (ANR-10-INSB-05-02) and GRAL (ANR-10-LABX-49-01) within the Grenoble Partnership for Structural Biology (PSB). We thank Aline Le Roy, and/or Michel Thépaut and/or Christine Ebel, for assistance and/or access to the Protein Analysis On Line (PAOL) platform" and to communicate the reference of the article to the responsible of the platform.
- The personal of the platform is a co-author if he participates to the redaction of the article.

Instructions for use

Procedures

- Each applicant contacts the platform and fills the file "request of analysis". After discussion, the experimental conditions and the schedule are decided. The platform doesn't operate during the weekend.
- Commitments for samples, buffers, columns … are described in part 3 and must be checked for each experiment.
- If something crops up, you must inform the local contact as soon as possible and fill an anomaly form.
- For each experiment, a PAOL data acquisition form must be filled in:
  - Note the details of the experiment.
  - Note daily check values.
  - Note the anomaly, in case.

Documents

- Protocols for PAOL are available on the internal IBS network and in the ring binder nearby PAOL.
- The reference manuals of the software (LC Solution, ASTRA and Dynamics) can be consulted on PAOL computer.

Training

- The procedures described below are a part of those given during the **obligatory training.** Documentation is free available on the internal IBS network and in the ring binder nearby PAOL.
- At the end of each experiment, you must:
  - Equilibrate the columns in H_2O, 0.3g/L NaN_3.
  - Make sure that the person who will properly stop the PAOL system is designed: you or the local contact?
  - Save your data in the space of storage of the platform (partages). It is possible to copy the data on your USB key.

2) **Commitment on PAOL** (Copy of the protocol « Columns »)

- The available columns with the PAOL system are:

Columns	Providers	Separation scale (kDa)	Composition and size of particules	Column dimension DxL (mm)	Buffer pH	Injec-tion µl	Storage buffer
PROTEIN KW-804	Shodex	until 1 000	Silice 7 µm	8.0 x 300	3 – 7.5	20	H_2O, 0.3g/L NaN_3
PROTEIN KW-803	Shodex	until 700	Silice 5 µm	8.0 x 300	3 – 7.5	20	H_2O, 0.3g/L NaN_3
PROTEIN KW-802.5	Shodex	until 150	Silice 5 µm	8.0 x 300	3 – 7.5	20	H_2O, 0.3g/L NaN_3
PROTEIN KW-G	Shodex	Guard column		6.0 x 50			H_2O, 0.3g/L NaN_3
Superdex 200 10/300 GL	GE Healthcare	10 – 600	cross-linked agarose and dextran 13 µm	10 x 300	3 – 12	100	20% Ethanol
Superose 6 10/300 GL	GE Healthcare	5 – 5000	cross-linked agarose 13 µm	10 x 300	3 – 12	100	20% Ethanol
Superdex 75 10/300 GL	GE Healthcare	3 – 70	cross-linked agarose and dextran 13 µm	10 x 300	3 – 12	100	20% Ethanol
WTC 050N5	Wyatt	15 - 5 000	Silice 5 µm	4.6 x 300		30	20% Ethanol
WTC 050N5G	Wyatt	Guard column		6.0 x 50			20% Ethanol

- The precautions of the columns are:
  - A flow rate between 0.2 and 0.5 ml/min (typically: 0.5ml/min).
  - Any changes in flow rate (*e.g.* stop the pump) must be done gradually.
  - The columns are always preceded of a guard column except the columns superdex and superose.
  - The elution buffer must:
    - Have a pH adapted to the used column (see the table above).
    - Contain 0.3 g/L NaN_3 (life = 8 days) **WARNING : NaN_3 = POISON=>protective devices !**
    - Contain at least 100 mM salt.
    - Be filtred at 0.1 µm

      Our standard buffer = PBS: 30mM Na Phosphate pH 6.8, 100 mM NaCl, 0.3 g/L NaN_3

- A new silica column must be eluted during 1 week, with H_2O Buffer (H_2O, 0.3 g/L NaN_3), at 0.5 ml/min.

- Equilibrate with the H_2O Buffer all the columns, before and after each use (because possible reaction between alcohol and salts)

- The samples must be centrifuge during 5-10 min with the Airfuge (Cf. protocol: Airfuge) - eventually, for heat-unstable samples, at 13 000xg during 30 min-, or filtered at 0.1µm.

- For membrane proteins, the elution buffer contains detergent: equilibrate the column with the elution buffer without detergent during 1 to 2 days, then with detergent during 3 hours, before injecting the samples.

- General description of the columns according to providers:

DEVICE RESPONSIBLE'S DATE AND VISA: 2013/05/13 ; ALINE LE ROY AND C. EBEL