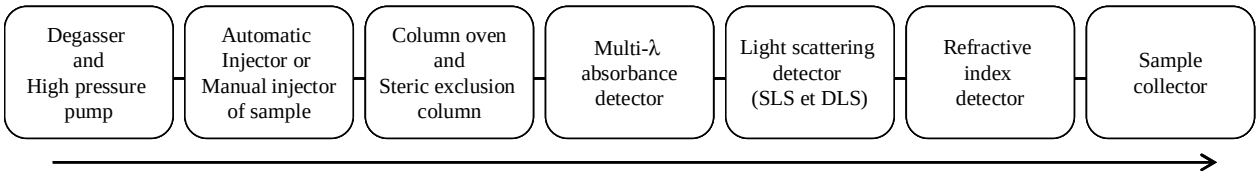
Description of PAOL (*Protein Analysis On Line*)

The characterization platform PAOL (*Protein Analysis On Line*) comprise several components:



The **degasser "SHIMADZU DGU 20A"** enables to degas the -previously filtered at 0.1µm- column solvents.

The **pump** "**SHIMADZU LC-20AD"** enables to generate the solvent flow through the different components of PAOL. The flow is characterized by a constant flow rate (typically 0.5 ml/min) and very low pulsations; because detectors are sensitive to any flow fluctuations.

The automatic sample injector "**SHIMADZU SIL-20AC HT"** enables to store up to 70 samples at a controlled temperature in the range 4 - 40°C, and injects them. The usual injection loop has a volume of 100µl and can be coupled to a 2 ml loop; the typical injection volume is 20µl.

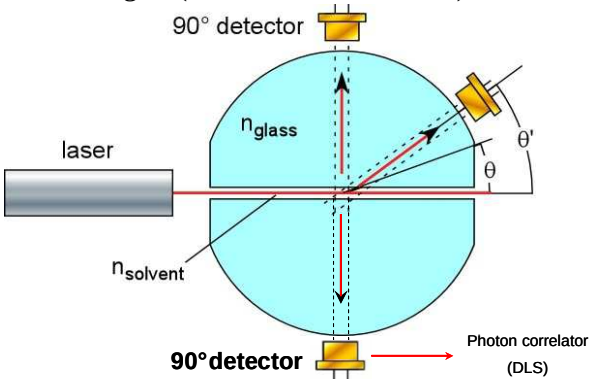
Size exclusion chromatography is used to separate physically the different macromolecular species. A small molecule that can penetrate every corner of the pore system of the stationary phase "sees" the entire pore volume and will elute later that the large molecule that cannot penetrate the pore system: there is separation. The -dedicated to PAOL- **columns** are: **Protein KW-804, Protein KW-803 and Protein KW-802.5**, with, as guard column, Protein KW-G **(Shodex)**, **WTC 050N5G**, with, as guard column, WTC 050N5 **(Wyatt)** and **Superdex 200 10/300 GL (GE Healthcare).** The matrix is silica or polymer; the columns have different separation scale up to 5 000 kDa.

The **column oven "XL T-01" (WynSep)** maintains the column at a temperature in the range 4 - 30°C.

The **multi-λ absorbance detector "SHIMADZU SPD-M20A"** measures the absorbance of the eluate between 190 and 700 nm. The signal for three wavelengths is recorded for the ASTRA software for combined analysis of the absorbance, SLS and RI signal. λ = 280 nm is typically used to determine the protein concentration, with: $A_{280nm} = \varepsilon_{280nm} \times l \times c$

The **static light scattering detector (SLS: *Static Light Scattering* = MALLS: *Multi Angle Laser Light Scattering*) "WYATT mini DAWN TREOS"**: The solution goes through the measurement cell illuminated by a laser beam (λ = 658 nm). The scattered light is measured at 3 different angles (41, 9°; 90° and 138, 1°).

Fig. 1: Schema of the MALLS detector (coupled to the QELS detector)



According to the Rayleigh-Debye-Gans model, the intensity I of the light scattered by the solution is directly proportional to the total concentration c (g/ml) and to the average molar mass Mw (g/mol) of the macromolecules in the solution: $I = k \times c \times Mw \times \left(\frac{dn}{dc}\right)^2$. $k = \frac{4\pi n_0^2}{\lambda^4 N_A}$ depends on the refractive index n_0 of the solvent, λ and the Avogadro's number N_A. (dn/dc) means the variation of the refractive index of the solution with the concentration. The concentration is determined independently from the RI detector (see below). It is thus possible to determine Mw from the intensity of the scattered light.

For macromolecular sizes > 20 nm, the scattered intensity decreases with the diffusion angle θ and M_W is obtained by extrapolation of I at angle 0. This is why light scattering intensity is measured at 3 different angles.

The **dynamic light scattering detector (DLS : Dynamic Light Scattering = QELS : Quasi Elastic Light Scattering) "WYATT DynaPro Nanostar"** measures the fluctuation of the scattered light intensity at 90°. It can be measured upon chromatography, using a fiber optic connected to the mini DAWN TREOS detector (fig. 1). Measurements can also be done on a sample in an UVette or Quartz cuvette, with help of an independent lazer.

From the fluctuations of the light intensity of a function of time τ, the auto-correlation function $g^{(2)}(\tau)$ is determined. For a homogeneous solution, $g^{(2)}(\tau)=1+\beta\times e^{-2\times D_t\times q^2\times\tau}$ with D_t the diffusion coefficient and q the scattering vector $q=\frac{4\pi\times n_0}{\lambda_0}\times\sin\frac{\theta}{2}$.

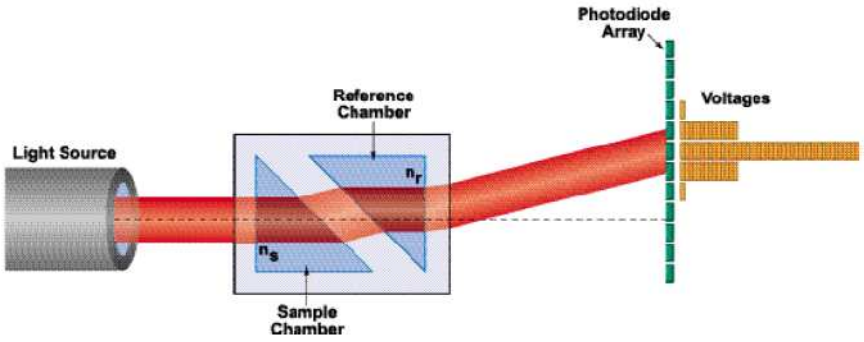
D_t is related to the hydrodynamic radius R_h: $D_t=\frac{kT}{6\pi\eta R_h}$, with k the Boltzmann constant, T the temperature (Kelvin) and η the solvent viscosity.

MALLS analyses the time averaged (1s) scattered light intensity *I*, and allows determining molar mass *Mw* (and radius of gyration R_g if > 20 nm).

QELS analyses the fluctuations of the scattered light intensity, *I*, as a function of time (10^{-8} – 10^{-9} s) and allows the determination of the diffusion coefficient D_t and thus hydrodynamic radius R_h.

The **differential refractive index detector « WYATT Optilab rEX » RI** (*Refractive Index*) measures the difference in the refractive indexes of the sample and reference.

Fig. 2: Scheme of RI detector:



The detector works with a laser at the same λ as MALLS, the beam crosses two cells of triangular section. The first cell is the sample chamber in which the column elute flows continually. The second cell contains the reference solution. When getting out of the sample chamber as well as the reference chamber, the beam undergoes a deviation. If the refractive indexes in the 2 chambers are identical, the second deviation cancels exactly the first, so the beam is not deviated but only shifted. An even little difference between the refractive index, n_s of the sample chamber and n_r, of the reference chamber, has for effect to deviate the beam direction. This deviation is converted into unit of difference of refractive index, Δn. Δn enables to determine the macromolecule concentration: $\Delta n=n_s-n_r=\left(\frac{dn}{dc}\right)\times c$.

For proteins, (*dn*/*dc*) is 0.186 ml/g; for membrane proteins: 0.187 ml/g; for detergent, sugar, lipids or RNA, the value of (dn/dc) differs but is in the same order of magnitude (0.08-0.2 ml/g).

The analysis of multicomponent systems –such as membrane proteins- uses the combination of absorbance, RI and LS signals for determining the particle composition and molar mass.

The **sample collector « SHIMADZU FRC-10A »** enables to collect and store fractions after elution, at a controlled temperature (4 to 35°C). It can be enslaved to the absorbance detection.

DEVICE RESPONSIBLE'S DATE AND VISA: 2011/02/13 ; ALINE LE ROY