**Methanol /Chloroform extraction**

(method to remove salts and detergents)

This procedure is applied to fractions of a sucrose gradient (step gradient 5% -30% -40% sucrose in 25mM MES pH 6.5 + NaCl 0.15 M) to precipitate the proteins contained in the various fractions. The percentage of sucrose starts from 5% of the first fraction, and gradually increases up to 30% of the last fraction that we analyze.

The volume of each fraction is 375μl

* 4 volumes of methanol are added to the sample;
* mix well (vortex);
* add 1 volume of chloroform. Vortex;
* add 3 volumes of H2O. Vortex;
* centrifuge for 1 minute at 14,000 g;
* remove the upper aqueous phase (proteins are in the interphase);
* add 4 volumes of methanol. Vortex;
* centrifuge 2 minutes at 14,000g;
* remove all possible methanol (LIPIDS) without disturbing the pellets;
* dry the pellets (leave to dry in the air);
* resuspend in 2x sample buffer for PAGE;
* incubate at least 1 hour at 65 ° C to promote protein resuspension.