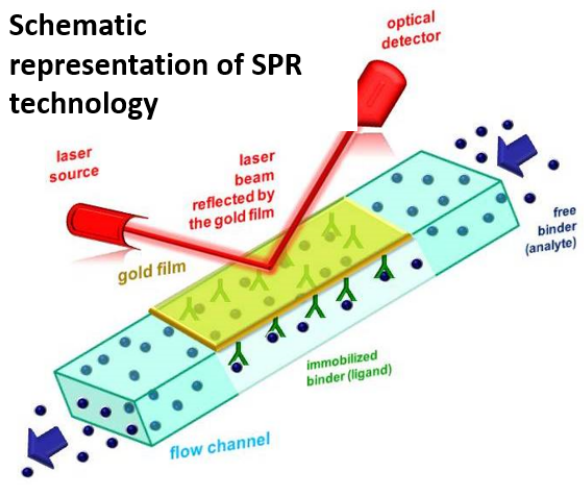
INTERACTION ANALYSIS METHODS

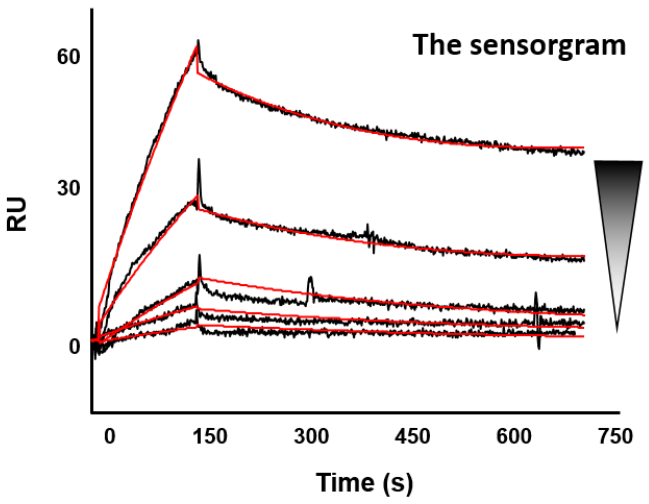
Unfortunately, the most common procedures for **interaction analysis** are rather lengthy, complex and demanding, allowing only qualitative results (i.e. immunofluorescence or immunoprecipitation), being affected by limitations related to the use of large amounts of purified proteins (i.e microcalorimetry), involving the labeling of the interactants (i.e radioisotope or fluorochrome tags), or using secondary antibodies or enzymes to generate a signal (i.e ELISA assay).

To meet the intricacy of the interactome(s) and to make its study easier, in the last years some new technologies have been developed, among which **surface plasmon resonance** and **microscale thermophoresis**.

SURFACE PLASMON RESONANCE

Surface plasmon resonance (**SPR**) is a handy, reliable and high-throughput optical technique to evaluate biomolecular interactions. Launched less than 20 years ago, its use has seen tremendous growth and this trend is predicted to continue as the technology becomes more accessible and its applications more diverse.
Briefly, a polarized beam of visible monochromatic light passes through a prism fitted with a glass slide coated with a gold film.
Once reflected off the gold surface, the intensity of the beam is detected at the specular angle. When the light hits the glass, an electric field (evanescent wave) is generated and absorbed by the free electron clouds in the gold layer, causing a decrease of the intensity of the reflected beam that depends on the refractive index (RI) of the material present within 300 nm from the gold surface.
In a SPR assay, a molecule (ligand) is immobilized onto the gold film and exposed to a sample containing a binder (analyte). The interaction of the analyte with the ligand causes a change of the RI at the gold surface resulting in the shift of the resonance angle and thus in the label-free transduction of the binding reaction that is presented as a real-time graph of the response units (RU) against time (sensorgram).

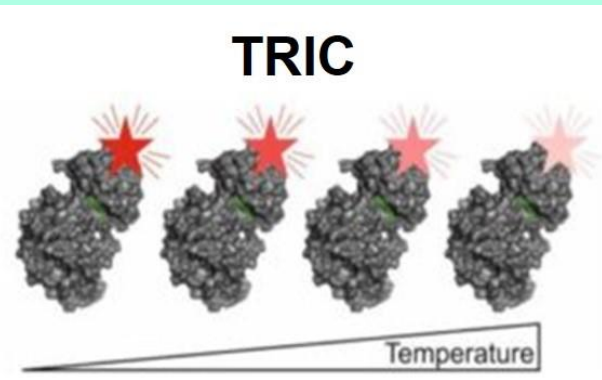


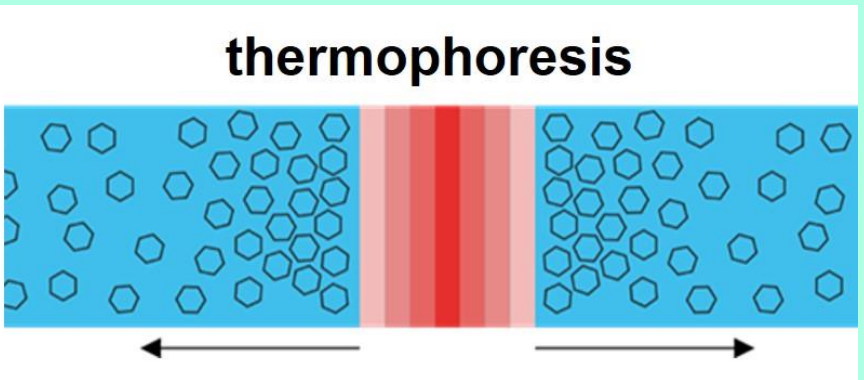


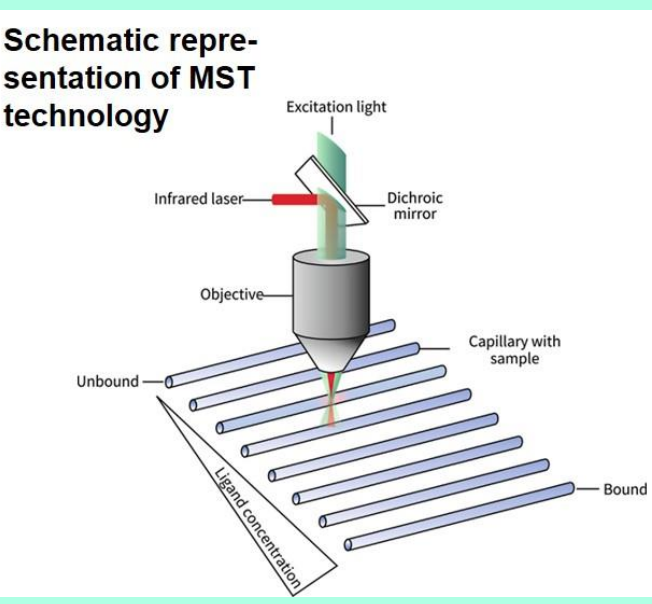
MICROSCALE THERMOPHORESIS

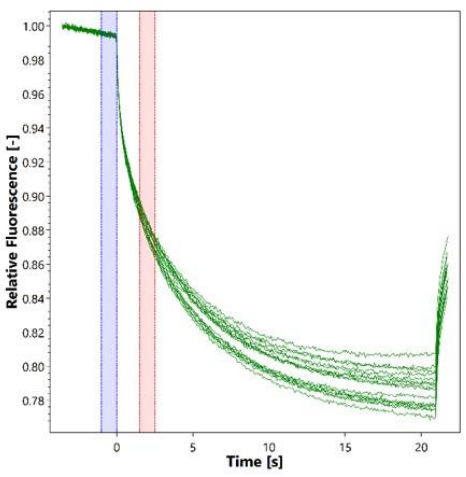
Microscale thermophoresis (MST) is a handy, reliable and contact-free optical technique to evaluate biomolecular **interactions**. It allows the study of interaction of a wide range of molecules, from ions to liposomes.
Briefly, **MST** signal is composed of two components:

- **TRIC** (temperature related intensity change), consisting in a decrease of fluorescence with increasing temperature, in turn dependent on the fluorophore environment (i.e. its interaction with a binder).
- **Thermophoresis**: Target molecule is fluorescent, allowing the evaluation of its changes in concentration. Molecules mobility depends on size, charge, and hydration. An interaction event leads to the change of one of these parameters and thus to an altered thermophoretic movement









The graph shows the fluorescence intensity of each capillary over time, reflecting the MST signal of the fluorescent target molecule in presence of increasing ligand concentrations.