Combination of Affinity Selection Mass Spectrometry with Biophysical approaches to identify and characterize biomolecules binders

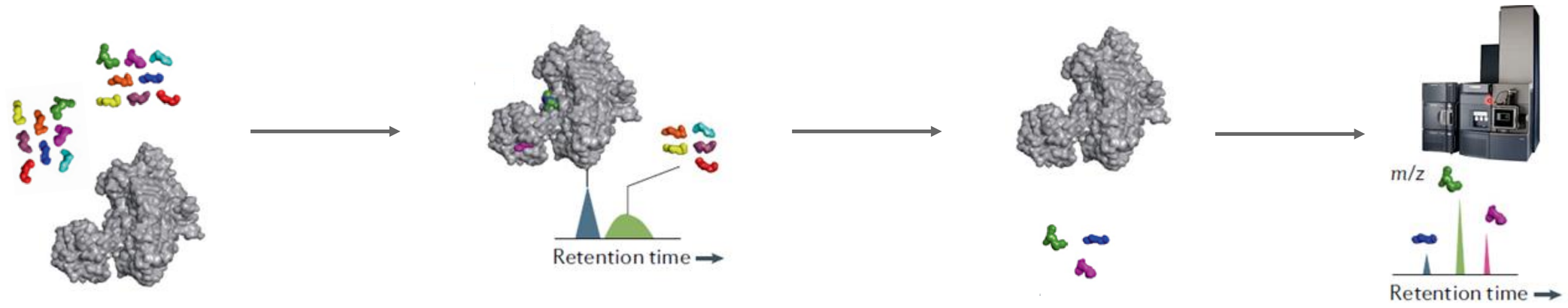
Julien Parra[1], Delphine Parrot[1], Julie Saulières[1], Jacqueline Blouin[1] , Viviane Liziard[1], Julia Soullier[1], Mohammed Kebdani[1], Pierre-Jean Rockstroh[1], Ghislaine Marchand[1]

[1]Evotec (France) SAS – Campus Curie 195 route d'Espagne, 31036 Toulouse CEDEX, (France)

The Emerging of Affinity Selection Mass Spectrometry (ASMS) in drug discovery

- Affinity Selection Mass Spectrometry (ASMS) is a high-throughput screening (HTS) technique enables rapid screening of large collections of compounds
- As a binding assay, it allows to identify ligands for a specific biomolecular target.
- Gaining more and more interest in the HTS community due to its ability to identify ligands, notably for some undruggable targets.
- In solution and label-free approach that is used for many years to identify ligands of proteins, but also of RNAs in a more recent past.
- Use of mixtures of compounds with different MWs allows to screen very large libraries in a small amount of time.
- Dedicated software solution was developed in strong collaboration with Virscidian

- **Evotec presents a strong expertise since several years into ASMS screens, and is now equipped with 4 different platforms (3 in Toulouse, 1 in Princeton (US))**



1-Incubation

Target of interest is incubated with mixture of compounds, allowing binding of potential ligands. At Evotec, classical workflow is using mixtures of ~600cpds/wells for the in-house compound libraries.

2-Size Exclusion Chromatography

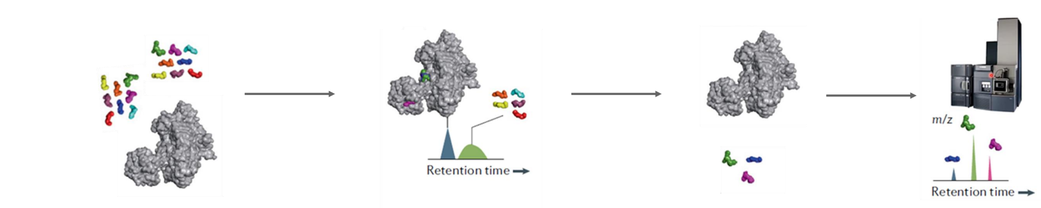
Complexes created between target and ligands are isolated from unbound molecules by using a fast SEC. The aim is to get large complexes eluted as fast as possible, in order to save binders with fast koff. In the current Evotec method, those are eluted in less than 20s.

3-Online RPLC-MS

Complexes eluted from SEC are isolated in a loop and then directly injected onto a reverse phase chromatography equipped with a C_{18} column. It will lead to the complex dissociation, releasing and separation of the binders that were carried out by the target if interest (either protein or RNA). MS detection will then allow to determine the m/z of those binders. A dedicated software (Analytical Studio, Virscidian®) performing data deconvolution and peak assignment allows to finally identify detected binders.

ASMS as a versatile tool in HTS campaign

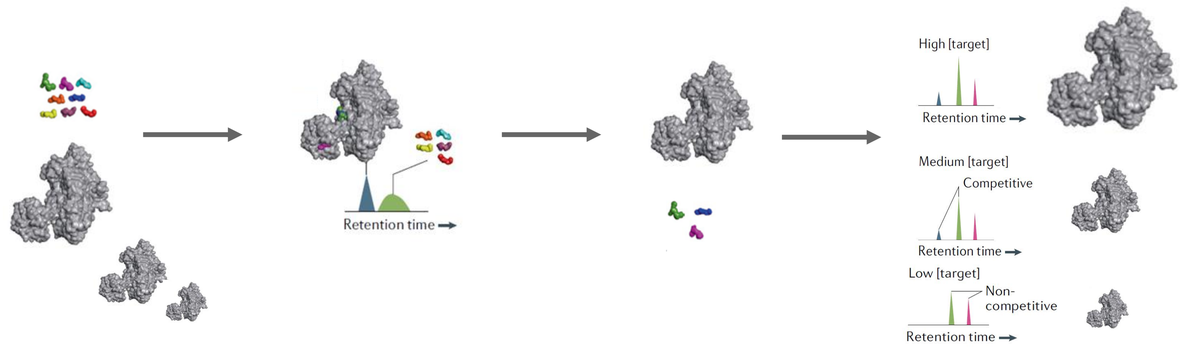
ASMS as a primary technique for affinity screen



- ASMS routinely used to perform screening campaigns either on proteins or on RNAs
- Dedicated pooled chemical libraries
  - Evotec library (300k compounds, 540 cpds/well)
  - Aptuit library (400k compounds, 640 cpds/well)
  - RNA focused library (7k compounds, 150 cpds/well)
  - Others from clients, small dedicated libraries….
- High throughput: technique; up to 60K compounds per day (in duplicate, *i.e*. 120.000 datapoints)
- Workflow: Between 2 and 3 months for the full process, depending on screened library

Pilot study (30k cpds)	Primary screen (up to 400k cpds)	Hit Confirmation
Part of the used library (in mixture) assessed in N=3. Confirmation in singletons for selected hits → **Reproducibility of the assay**	Full library (mixture of compounds, up to 640/well) assessed in N=2 → **Very high confidence Hit list**	Selected hits from primary screen assessed in singleton (1 compound/well) N=1 Including control without target to remove false positive → **Final binders list**

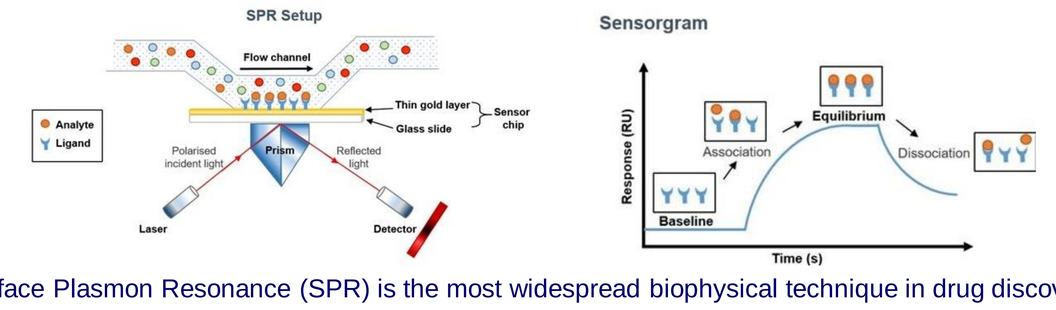
ASMS as a tool to compare and classify binders



- ASMS is also a technique allowing to perform Affinity Ranking between binders (see above)
  - Decreasing the target concentration allows to create competition between binders in mixture
  - Binders with highest affinity will be detected even at the lowest target concentration
  - Binders can so be ranked based on their affinity for the target
- ASMS might also be used to perform **NanoSAR** (**Nano**scale **S**ynthesis and **A**ffinity **R**anking)
- ASMS is also used as a tool to perform some concentration response curves for identified binders
  - Provides "apparent Kds" instead of Kds, as it is using a flow system
  - Allows to triage some unspecific binders prior to other orthogonal techniques to determine Kds

Biophysical techniques used as orthogonal assays to validate binders

Surface Plasmon Resonance (SPR)



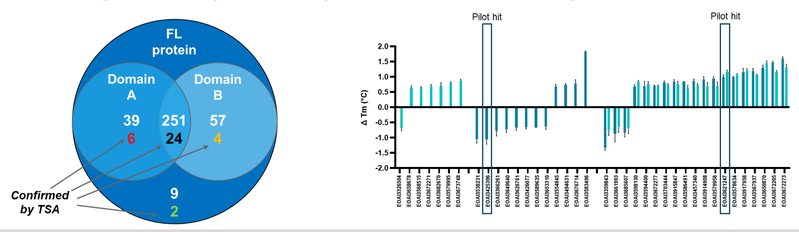
- Surface Plasmon Resonance (SPR) is the most widespread biophysical technique in drug discovery
  - Measures biomolecular interactions in real time in a label-free environment
  - Provides kinetics parameters (Kd, K_{on}, K_{off})
  - Needs to immobilize targets of interest onto a chip

135,149 cpds → ASMS screen → 630 hits → Confirmation → 198 hits → SPR

			Target T binding			Off-target X	Off-target Y
Analytes ID	MW (Da)	Top conc (µM)	Rmax Experimental	SA %	ss Kd (µM)	ss Kd (µM)	ss Kd (µM)
Compound A	279,341	30	20,0	43,6	**32**	>>30 (94)	-
Compound B	388,449	30	8,0	15,6	**29**	-	>>30 (86,5)
Compound C	510,718	30	30,0	41,7	**20**	22	15
Compound D	282,317	30	10,0	21,6	**6**	13	-
Compound E	286,337	30	7,5	16,0	**31**	-	-

Thermal Shift Assay by Differential Scanning Fluorimetry (DSF)

- Differential Scanning Fluorimetry (DSF) is a biophysical technique:
  - In solution, using a dye to get fluorescent signal
  - Providing melting temperature (Tm) of a protein in many conditions (+/- binders) very rapidly
  - Often used as a triage technique among lot of binders coming from ASMS screen



Combination of ASMS with other biophysical techniques to identify and characterize small molecules binders

- Evotec, as a CRO, has a strong expertise for several years in applying biophysical techniques in the drug discovery process. High throughput screening methodologies are diverse, and among them, ASMS is growing very fast. This approach has demonstrated a strong ability for the identification of binders from large small molecules libraries.
- ASMS is also a versatile technique that is usable as a screening strategy, but also as an approach to characterize binders, by either performing dose response experiments (providing "apparent Kds"), or doing Affinity ranking analysis (by decreasing target concentration with mixture of binders in order to rank them).
- Combination of ASMS with other biophysical techniques, such as SPR or DSF as illustrated here, but also ITC, NMR, MST, X-Ray-, Cryo-EM, are powerful approaches used from hit identification to deep characterization of binders. This enables a significant increase in the understanding of the chemical properties that are involved in small molecule binding on targets, either proteins or RNAs.