cytiva

Applications of SPR analysis in primary and secondary screening

Real-time analysis of molecular interactions

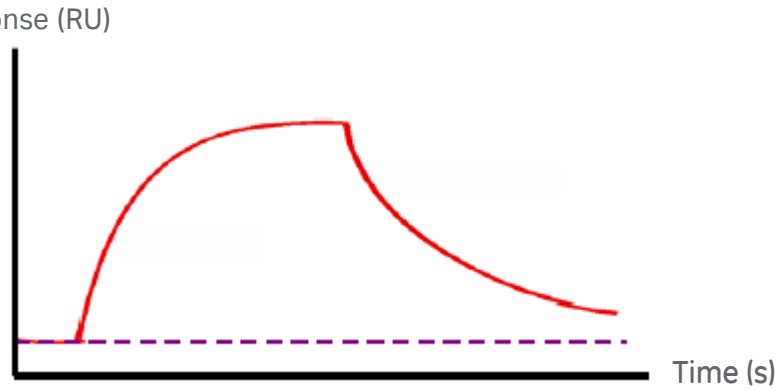
A second interaction partner flows over the sensor surface: **the Analyte**

Interaction on sensor surface

One interaction partner immobilized (attached) on sensor surface: **the Ligand**

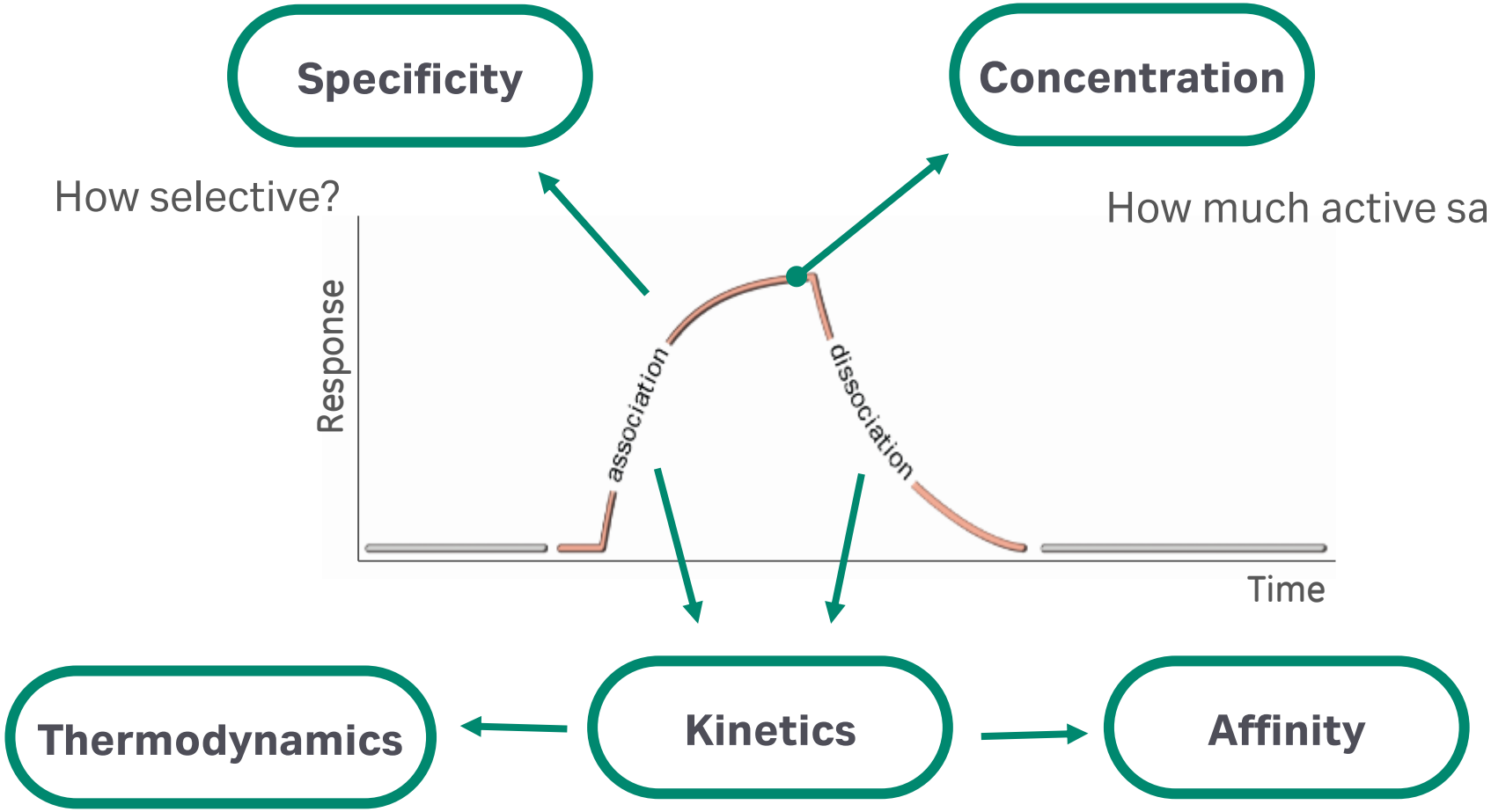
Change in mass concentration and refractive index

Detected in real-time using phenomenon of surface plasmon resonance (SPR)



Continuous display of RU over time gives the sensorgram

What do Biacore systems measure?

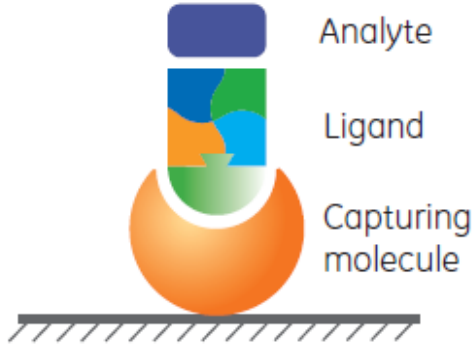


Functionality: How strong? How fast? Why?

Surface Preparation - Immobilization

What is immobilization?

Coupling of ligand or capture molecule to the sensor surface

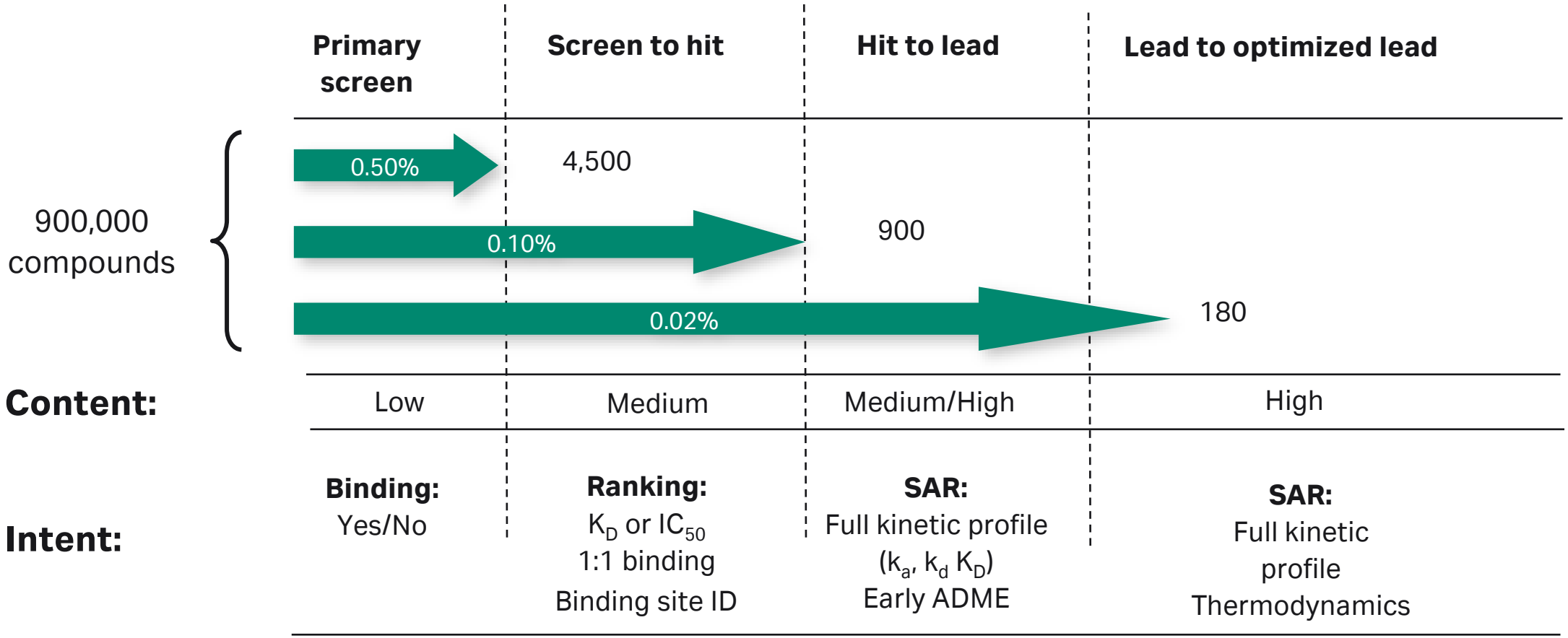


Direct immobilization
Covalent coupling of ligand to surface

Capture approach
Covalent coupling of capture molecule
Capture of ligand during each cycle via a high-affinity interaction

Traditional HTS workflows

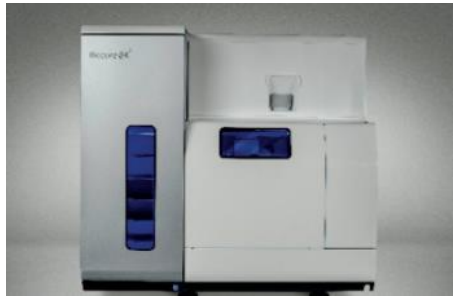
Very large libraries, compound throughput paramount



Biacore™ 8K series

Discover more, more efficiently with maximized capacity

- Screening of **2300 molecules in a day**
- High-quality kinetic characterization of **64 interactions in 4 h**
- Exceptional sample capacity of up to **12 × 384-well microplates**
- Up to **72 h unattended run time** with queueing abilities and rapid multi-run evaluation



Main benefits of label-free interaction analysis in screen-to-hits

- Similar to those for primary screening
  → Selectivity may be more important here

- Label-free analysis reduces risks of "validating" false positives
  → Current validation of hits often uses compound concentration series with same basic assay as HTS
  → Risks repeating same artifacts

- Affinity ranking & binding site data from label-free analysis are also of value at this stage

Alternative screening paradigms

Traditional HTS approach

– Very large libraries, compound throughput paramount

Structure-based biophysical assays

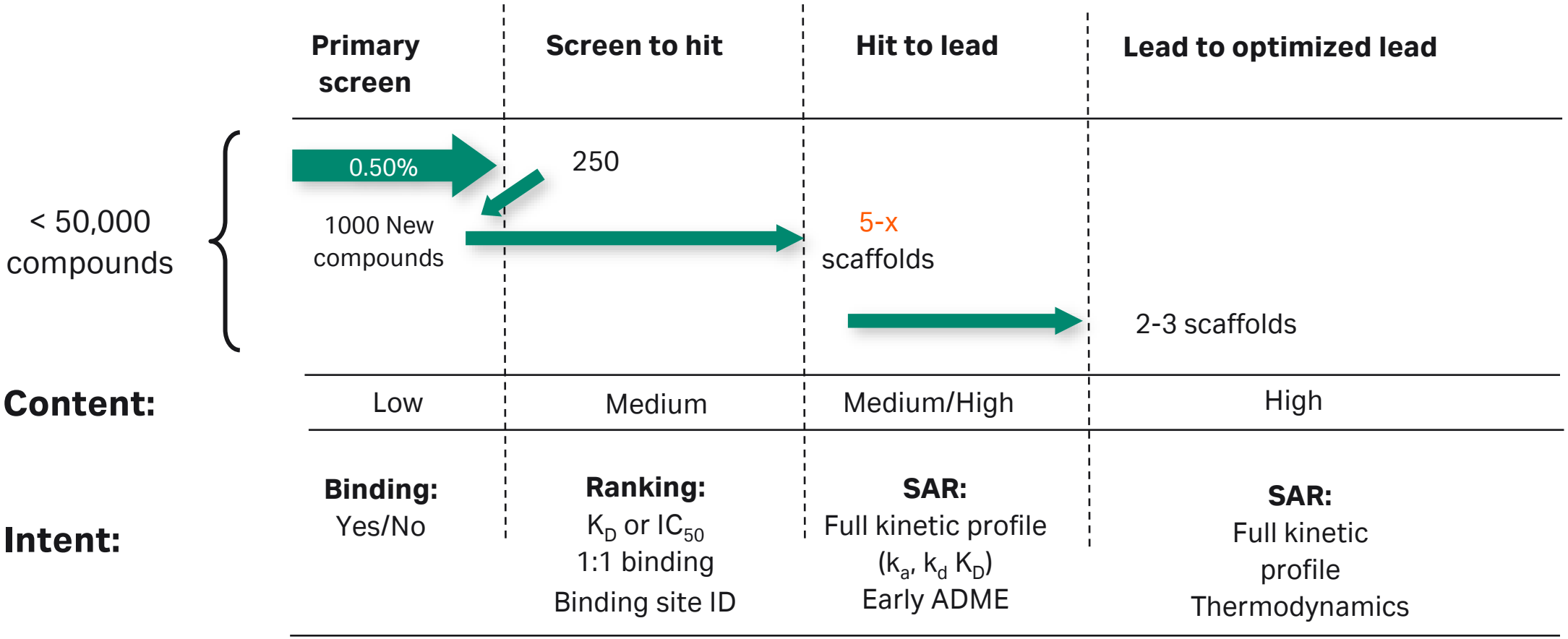
– A new paradigm: cyclic process, based on binding site data

– Number of compounds screened much less
  ⟶ Focused/ fragment/ directed libraries, hits from HTS or *in silico* screens

– Drug design based on structure of the target binding site
  ⟶ X-ray crystallography & NMR

– Direct binding screening methods are essential
  ⟶ Used in combination with *in silico* techniques

Workflows in a structural/biophysical approach



Technologies available:

Label-free interaction analysis

In silico

X-ray/NMR

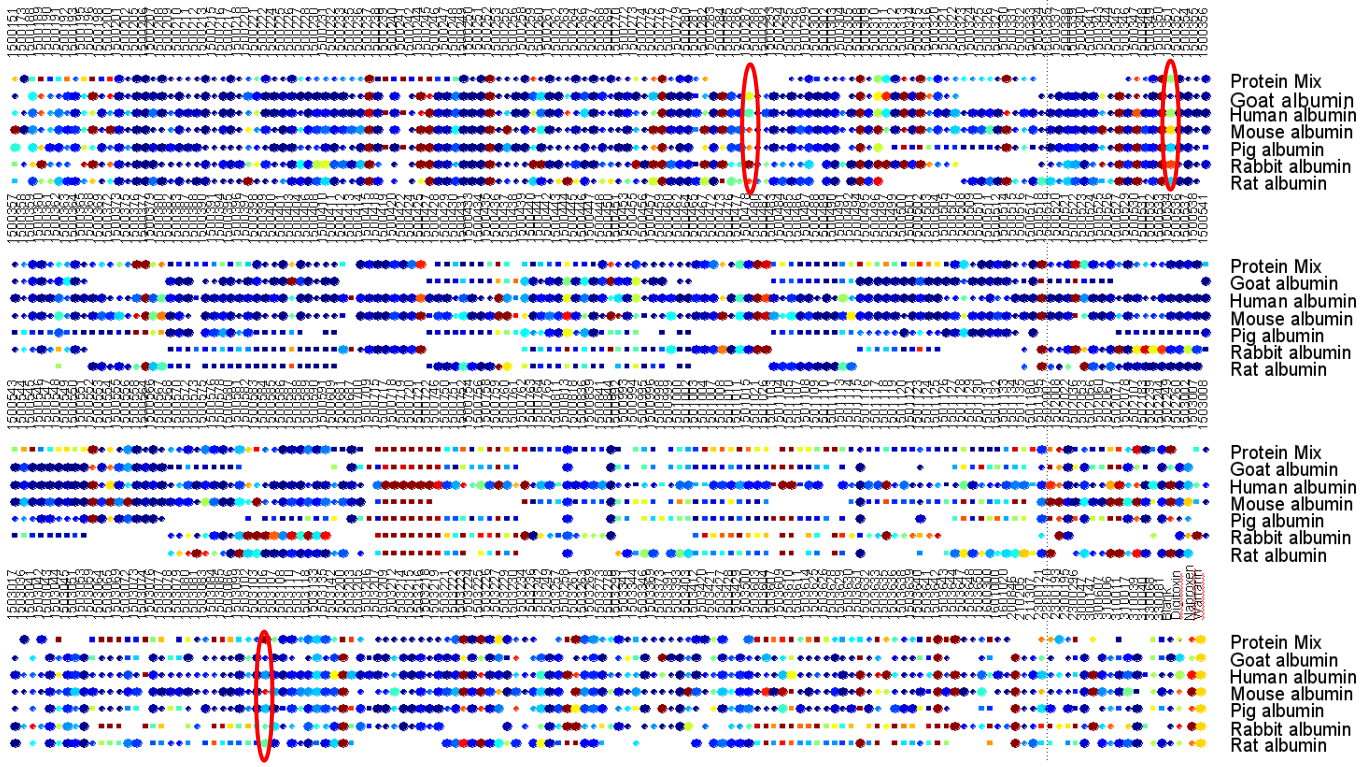
High-productivity screening applications

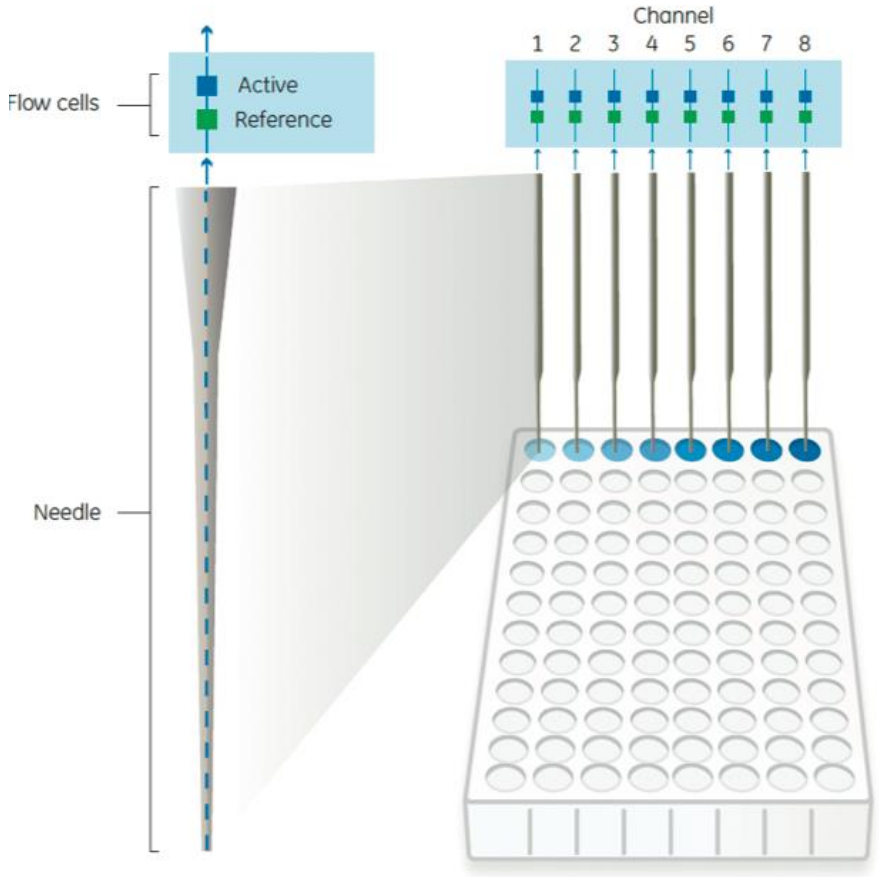
Parallel targets screened, appropriate throughput, good repeatibility

Compound binding to HSA from multiple species (Biacore 8K Series)

➡ 30 000 interactions analyzed in 2 weeks

560 compounds - Replicate screens - Multiple targets in parallel





Response level (% warfarin)

0 50 100 150

large dot = s.d <20

square = single measurement

Cytiva = examples with marked selectivity for species-specific HSA binding

Solving a problem where HTS did not work!

Previous HTS assays failed to identify suitable compounds

→ **Aimed against a complex drug target**

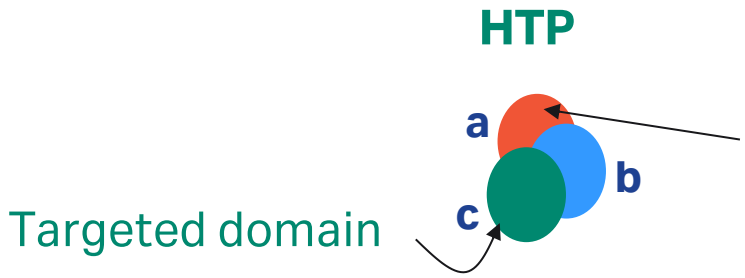
Need: make confident choices based on binding selectivity

→ **High quality compounds with therapeutically relevant binding properties**

Identification of selective binders using informative protein panels

Aim: find allosteric agonists for a complex protein target (HTP)

Problems: selectivity complications/ non-availibility of some target subunits → HTS approach had previously failed



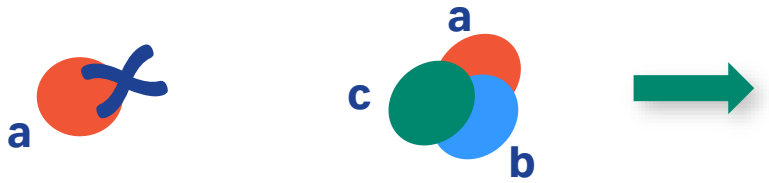
a-subunit binds similar natural ligands to targeted domain – selectivity issues

Solution: Two-stage selectivity screen using Biacore 8K

2 targets
1280 compounds

Screen 1

Rapidly eliminate unwanted binders to a-subunit



Screen 2

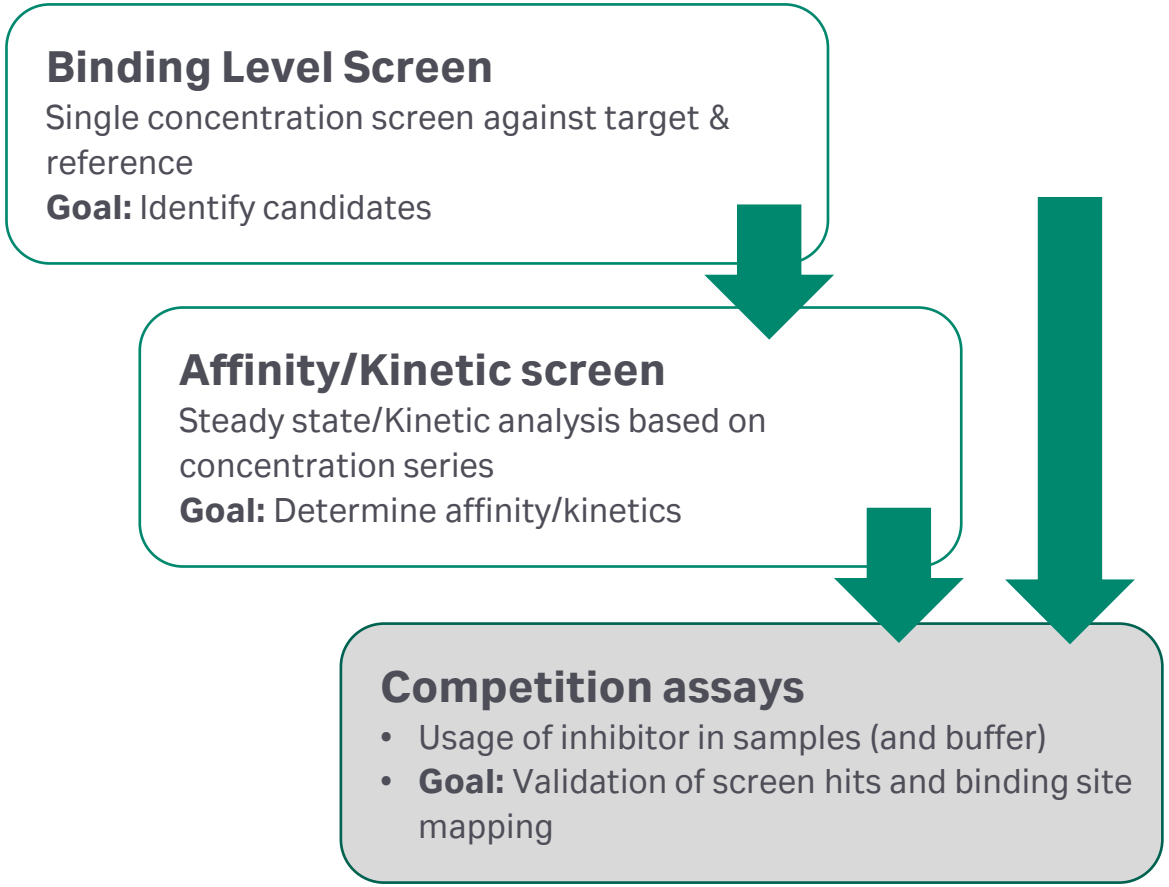
Cytiva

Data courtesy of Hoffman La-Roche

Small molecule screen setup

Flow rate	30 µL/min
Contact time	30 s to 60 s
Dissociation time	60 s
Sample concentration	Recommended starting value 30 µM May need to be adjusted according to the expected binding affinity. Use the same order of magnitude as the expected affinity (for example, if µM affinities are expected, use µM concentrations)
Regeneration	Not used
Carry-over injection	Included to detect “sticky” compounds
Extra wash	Optional (50% DMSO recommended) Extra wash does not pass over the sensor surface
Molecular weights	Required for adjusting response levels for molecular size
Startup cycles	Include 1 to 3 startup cycles to equilibrate the system before injecting the first sample
Solvent correction	Repeated at regular intervals. The recommended interval differs in different systems. See the system-specific documentation for details.

Small molecule screening with Biacore
Overview



Fragment-related challenges for interaction analysis

Requires high-sensitivity instrumentation

Low molecular weight (80 -300 Da)

- Low signals in mass-dependent detection such as SPR analysis
- Even lower signals if target only partially active, which potentially limits targets to be studied

Low affinities (0.1 – 10 mM)

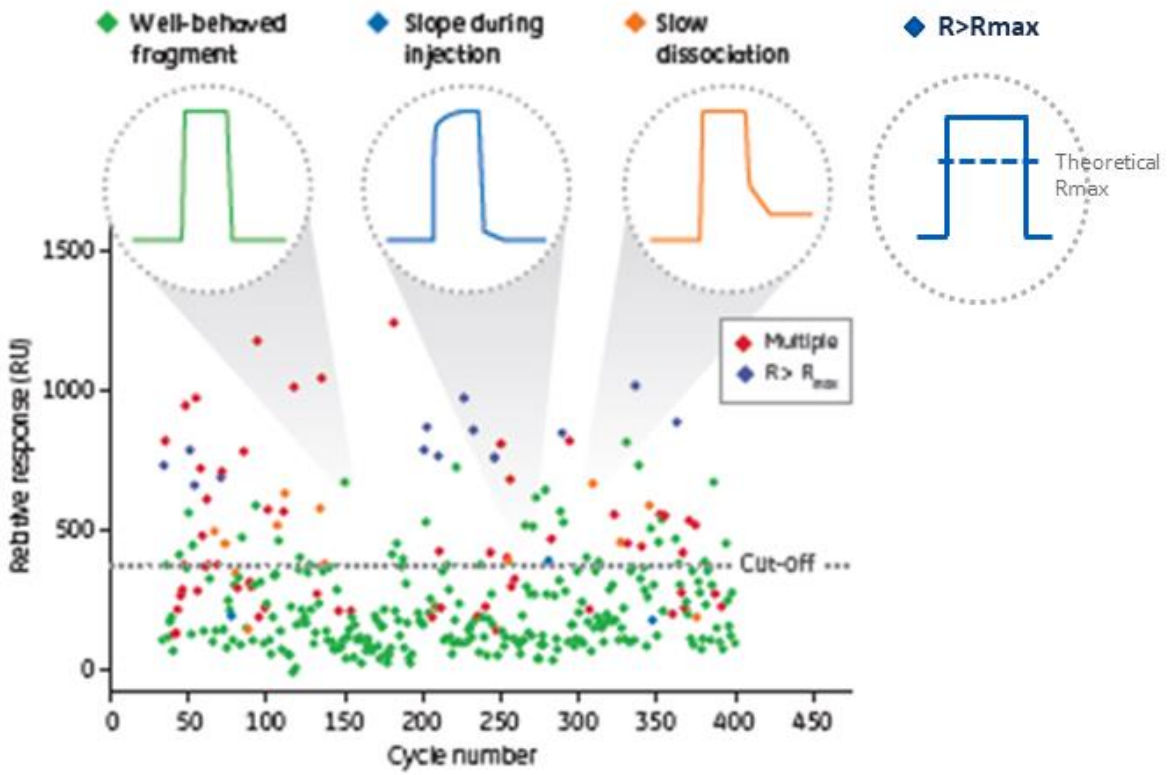
- High sample concentrations, typically mM range, required for high binding site occupancy
- Solubility limitations force the use of suboptimal concentrations

Secondary interactions

- Interactions not related to the addressed binding site are common at high concentrations and disturb the screening evaluation
- Non-specific binding to target/reference
- Binding to secondary and/or tertiary sites
- Promiscuous binding aggregates

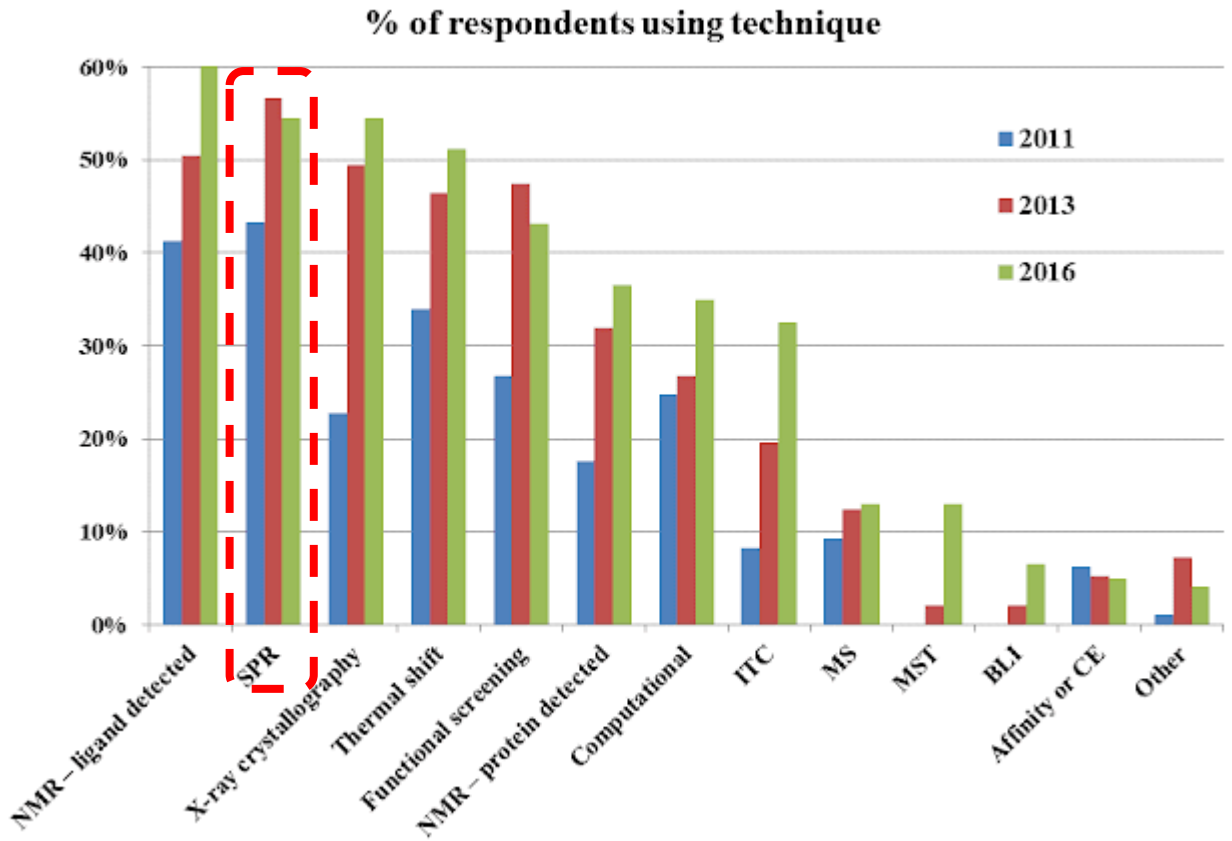
Why use Biacore systems in fragment-based drug discovery ?

- Can deal with the smallest fragments - **high sensitivity** instrument
- **Lower target consumption** than other biophysical methods
- Evaluation tools for **short time to results**
- Screening based on binding characteristics:
  - **Identifies promiscuous binders** with sensorgram shape
- **Confident selection** of candidates and validation of hits, to prioritize for structural determinations



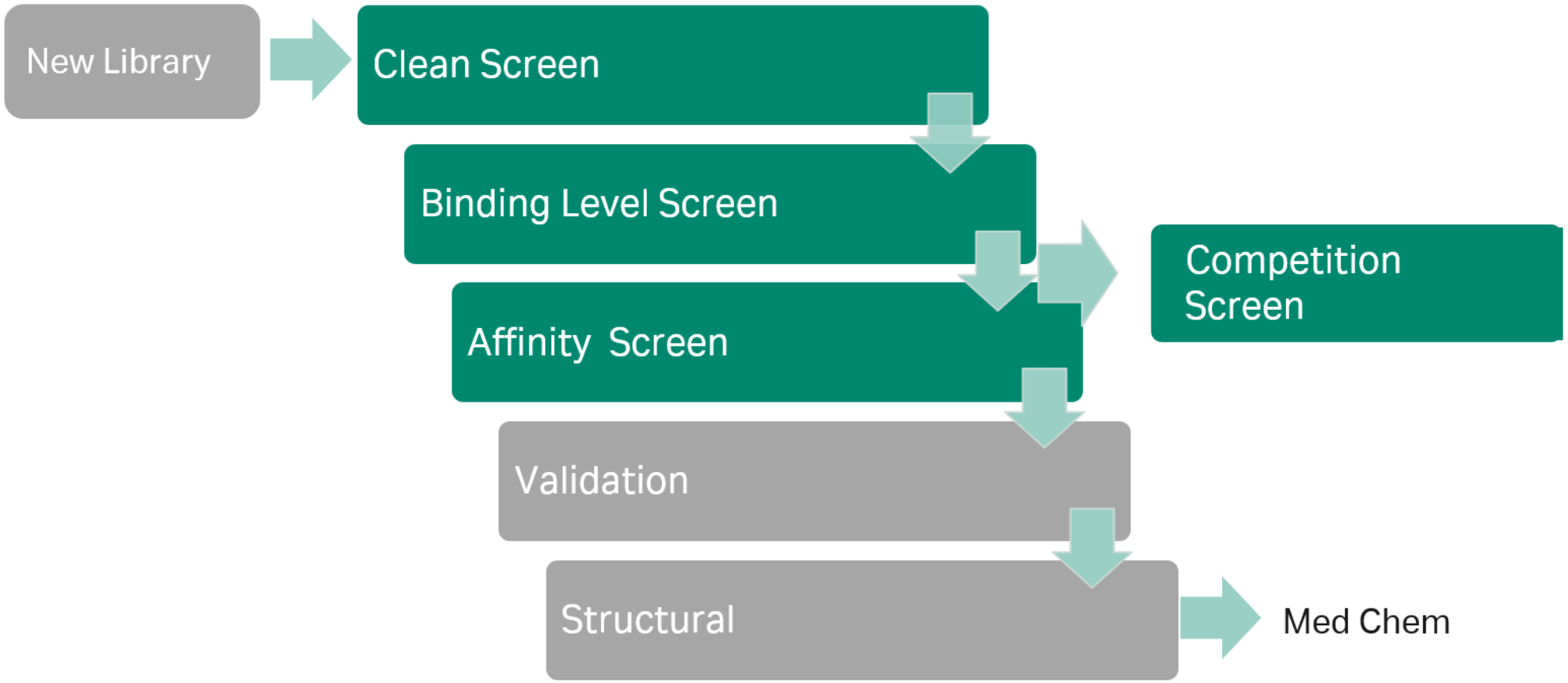
The usage of SPR in FBDD is growing

- SPR-based biosensors are established as powerful tools in FBDD and lead finding
- High sensitivity is required to observe low-affinity binding of LMW compounds to target proteins
  → More complex targets increase the need for sensitivity even further
- Shorter time to conclusive results are required to meet tight time lines
  → Cross correlation of high-quality data from several biophysical techniques to increase confidence in hit selection



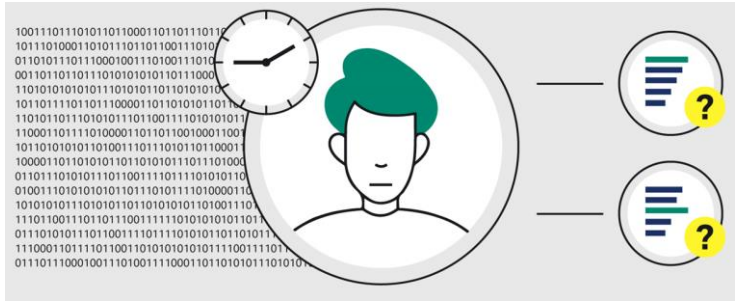
Fragment based drug discovery (FBDD)

Typical workflow on Biacore systems

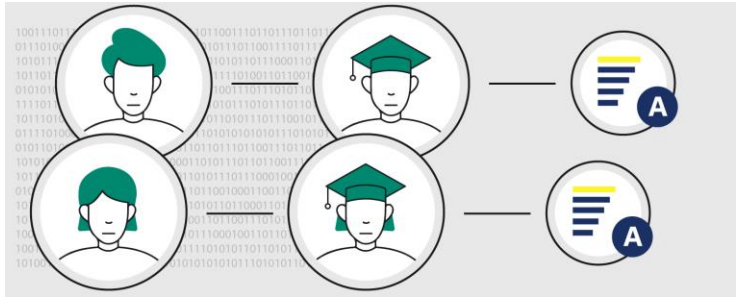


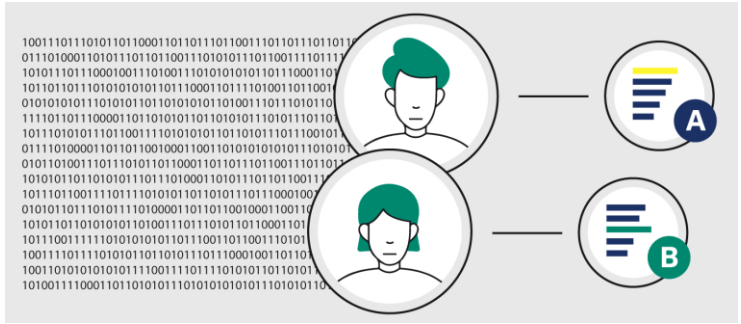
Data analysis bottleneck

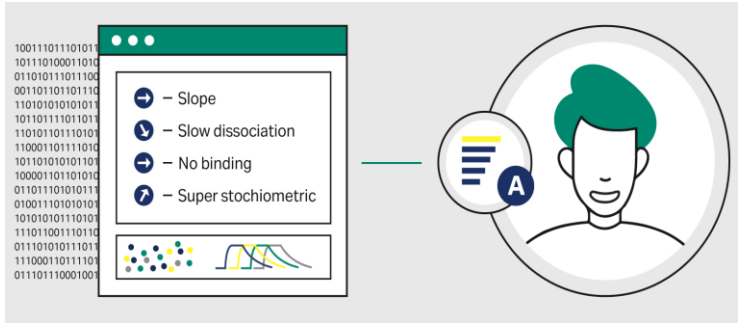
Today, manual assessment is the major part of data analysis



Biases and inconsistencies when analyzing large data sets





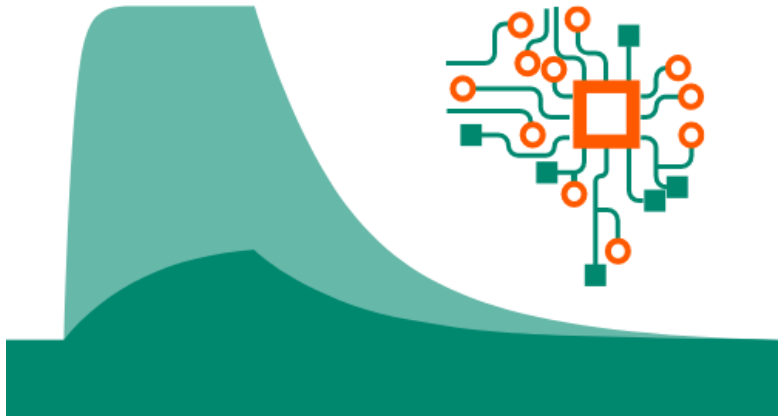


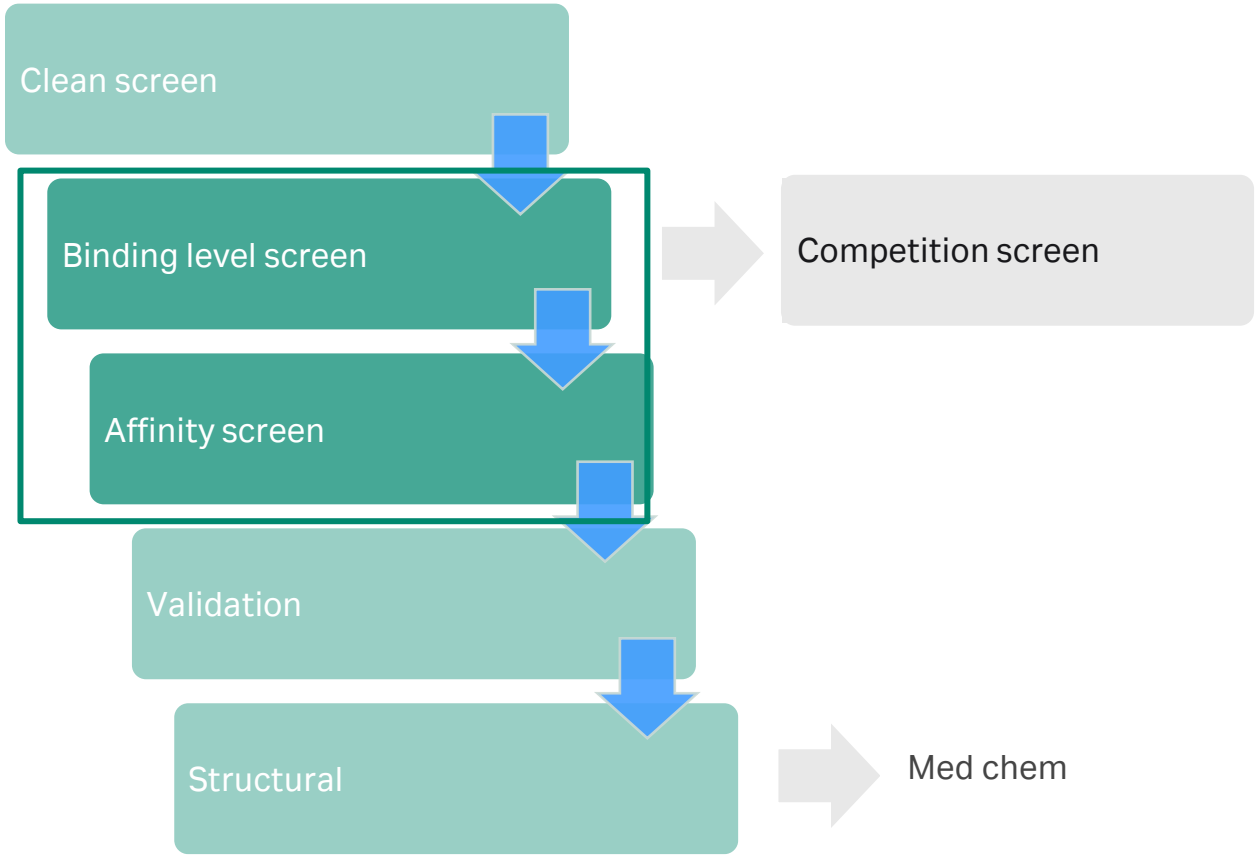
The expertise challenge

Introducing Biacore Intelligent Analysis™

For Biacore™ Insight Evaluation Software

Biacore Intelligent Analysis™





Automated and transparent evaluation of critical applications in FBDD drug discovery using machine learning