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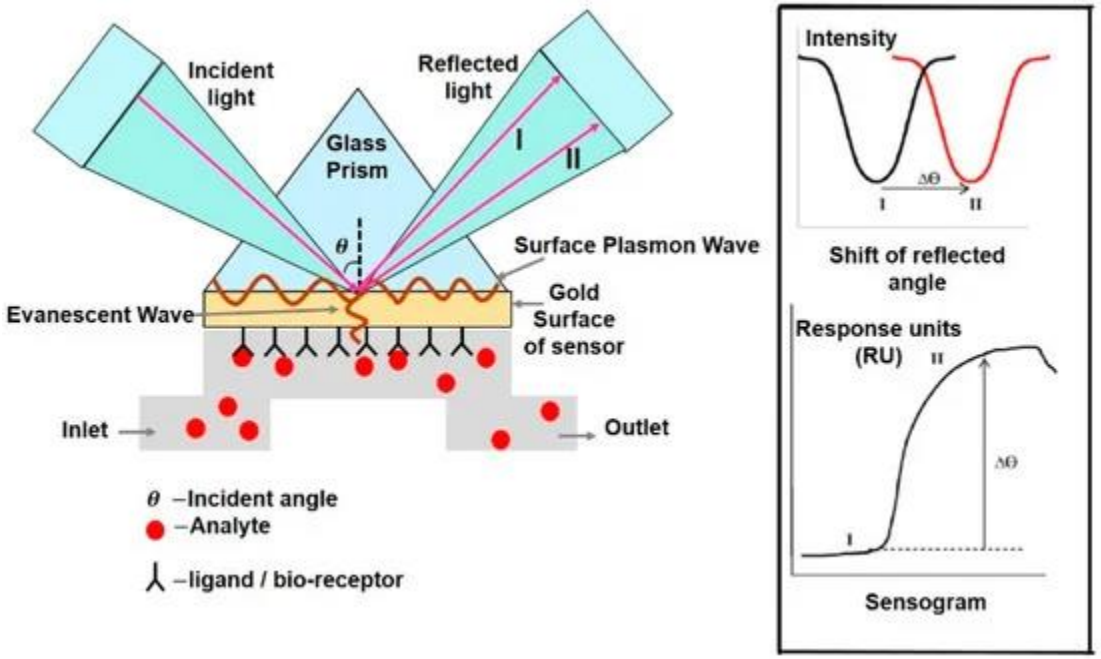
SPR FOR BIOSIMILARS

WHAT IS SPR?

- Surface Plasmon Resonance allows for the real time detection of mass binding to a surface – hence the analysis of molecular binding
- Typically one interaction partner is immobilized or captured on the surface of the sensor chip (ligand), the other interaction partner is injected in solution into the flow channel (analyte)
- Based on the binding characteristics of the interaction, the flow rate and the analyte concentration binding is observed (sensorgram) and can be fit using various mathematic models to determine binding metrics
- Alternatively, for the comparison of two differernt analytes, binding curves can be compared directly to assess similarity
- SPR is a very versatile tool for the analysis of molecular interactions



Mehrotra, P.; Chatterjee, B.; Sen, S. EM-Wave Biosensors: A Review of RF, Microwave, mm-Wave and Optical Sensing. *Sensors* **2019**, *19*, 1013. https://doi.org/10.3390/s19051013

WHAT CAN SPR DO FOR YOU?

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- Excellent comparative analysis of binding behaviour
- Analysis of all relevant binding sites (e.g. FcR binding + target binding for antibodies etc.)
- Determination of active concentration for individual binding sites
- Competitive binding analysis
- High throughput screening (e.g. for clone screening)

WHY SPR OVER E.G. ELISA?

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- Label-free (No altered binding due to conjugated molecules)
- Real-time (No end-Point measurement, comprehensive binding data from a single injection)
- Double-referenced (Minimized matrix effects)
- Ideal for comparative analysis (e.g. Analysis of two analytes on the exact same surface with immobilized target)
- Simple competitive binding analysis in real time

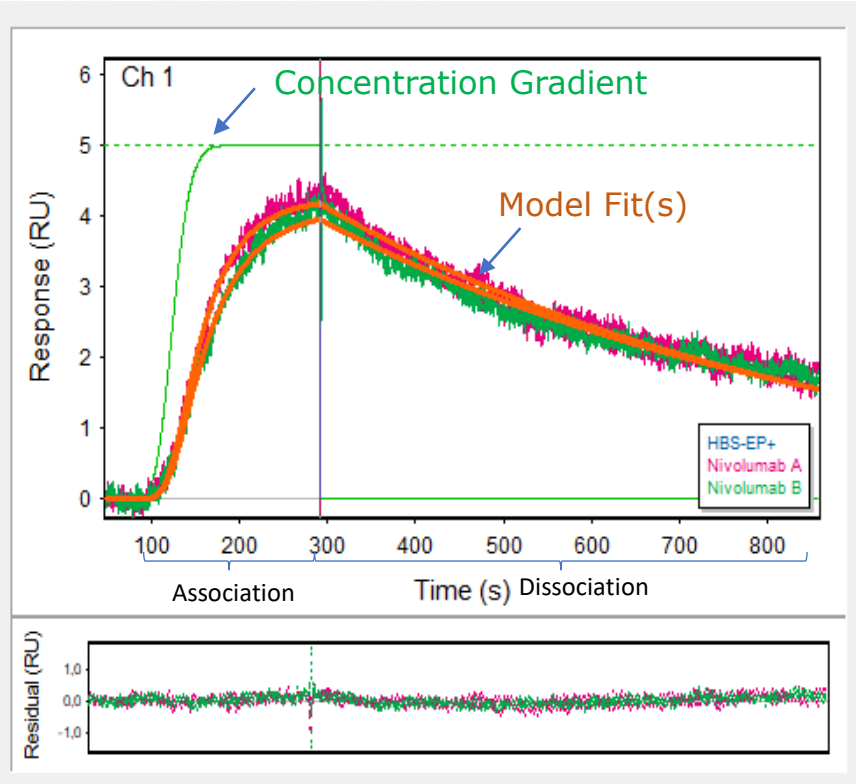
SPR AT REFERENCE ANALYTICS

- The Octet SF3 currently is the most innovative cutting edge technology on the market
- The device is capable of all standard SPR applications – and more
- Utilizing the unique dispersion loops for OneStep and NextStep injections, binding analysis and competitive binding assays can be performed better and faster than with conventional devices
- Combined with years of experience in SPR at RA this technology allows for innovative and cost-efficient solutions to analytical challenges in establishing a portfolio for biosimilar analytics

EXAMPLE DATA - INTRO

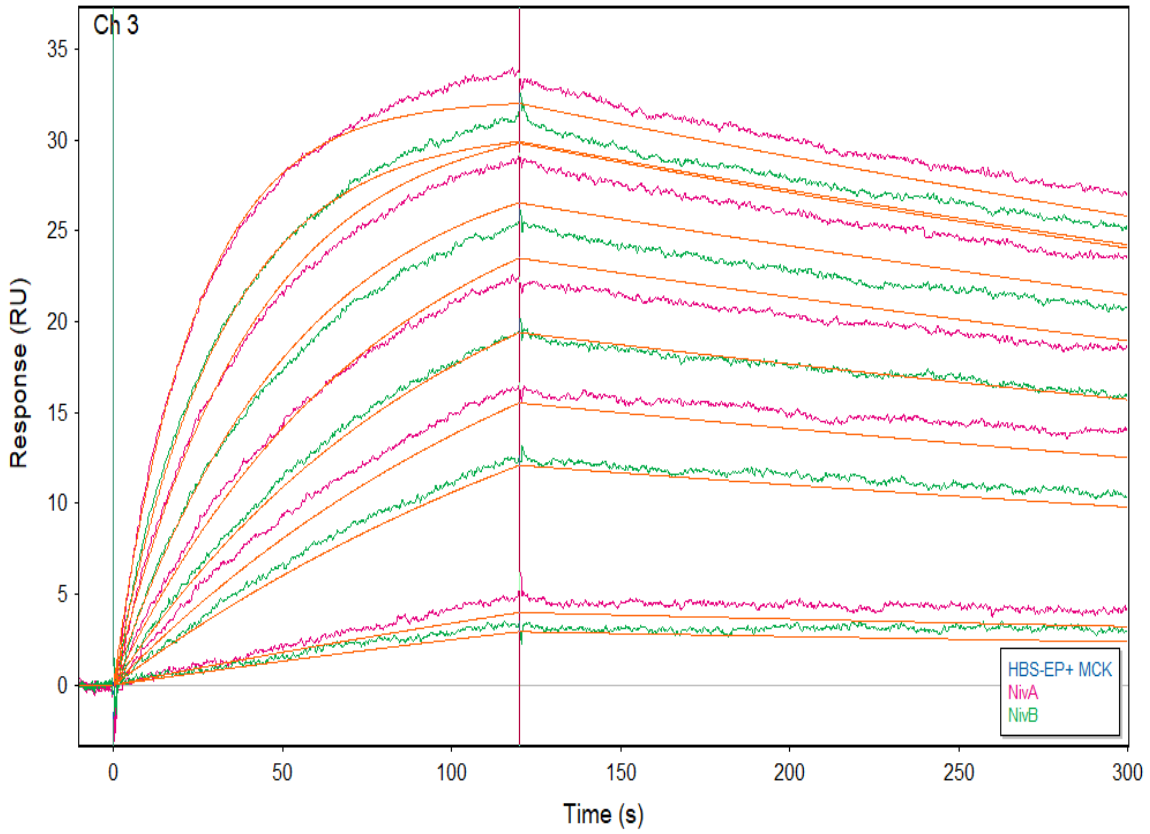
- Aim: Direct comparison of two anti-PD01 antibodies (two Nivolumab batches Exp.2018/Exp.2025)
- Recombinant PD01 (Nivolumab‘s target) was covalently immobilized on 2 flow cells of a sensor chip via amine-coupling
- One flow cell was immobilized at a very low level, the other at a moderate level
- Very low levels of ligand (PD01) are better for „clean“ 1-to-1 interactions, higher levels improve the signal-to-noise ratio
- The interaction was analysed via OneStep analysis, unique to the Octet SF3) and multicycle kinetic analysis

EXAMPLE DATA - ONESTEP



- In OneStep analysis a concentration gradient (achieved by diffusion) is injected over the sensor chip surface (low immob.)
- Exclusive feature of the Octet SF3
- The example shows the evaluation via kinetic fit
- This data is also highly suitable for curve comparison – a hallmark comparability tool for biosimilarity
- Binding information in the association phase of the interaction is limited by the analyte concentration used
- A single OneStep injection contains unprecedented data-density, as the analyte concentration is gradually increased over the entire association phase
- The dissociation phase needs to be sufficiently long to allow for a precise estimation of the dissociation rate constant (kd) – needing only one injection increases time efficiency immensly

EXAMPLE DATA MULTICYCLE KINETICS



- In multicycle kinetic analysis multiple fixed concentrations of analyte are injected over the sensor chip surface (moderate immob.)
- This is the classical approach for binding analysis/comparison
- More time-consuming (1 concentration = 1 curve, minimum 5 conc., the more the better)
- Effectively less binding information than 1 OneStep curve

CONCLUSIONS

- SPR is ideal for comparative analysis
- SPR is not optimal for the generation of absolute values (e.g. ka, kd, KD) to be compared between individual analyses due to potentially high day-to-day, chip-to-chip, buffer-to-buffer variability etc.
- Interaction between any two molecules can be analyzed, as long as one of them can be captured/immobilized
- Limited utility for very small molecules due to the signal being proportional to mass
- Very flexible analytic system that can be tailored to specific analytical challenges