Protein Quantification by LBA or LC-MS: Key Criteria for the Definition of the Bioanalytical Strategy

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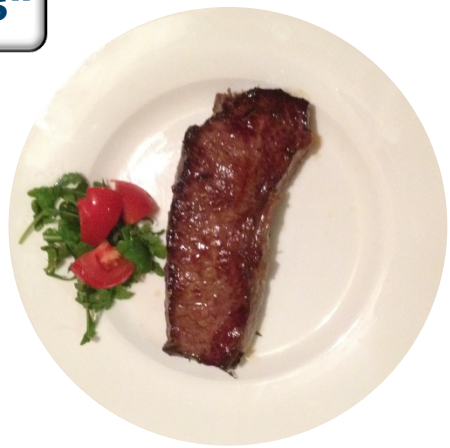
What are we Discussing?
„Fork vs. Spoon“ or „Steak vs. Soup“

„Analytes“



Shouldn‘t the tool selection be based on the „analyte“?

Tools

Features:
- High Selectivity
- Low Interference
- Complex „Production“

„Sample Preparation“

Features:
- High Throughput
- Broad Applicability
- Simple „Production“
- Potential Interferences

LBA or LC-MS „Tool Discussion“
The Customary Arguments

LBA offers high sensitivity

LBA is not selective

LC-MS is highly selective

LC-MS is not sensitive

LC-MS assay development time is much shorter than LBA

LBA provides info on proteins structural stability/integrity

LBA enable high throughput

LBA is prone to „ADA Interference“ & matrix effects

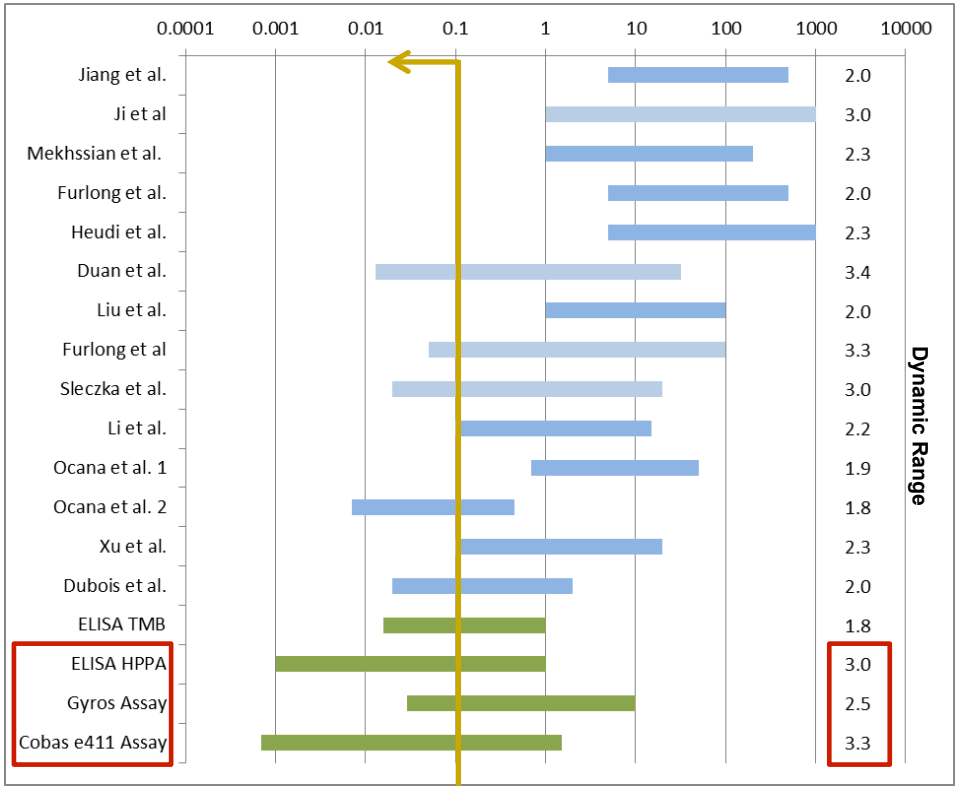
LBA only have a limited dynamic range

The Reality
Sensitivity, Dynamic Range, Sample Volume

Publication	Dyn. Range [µg/mL]	Extraction Proc.	Vol. [µL]
Jiang et al. Anal chem; 2013;85(20):9859-67	5 – 500	No	25
Ji et al Analchem; 2009,81(22):9321-8	1-1000	No	10
Mekhssian et al. Bioanalysis; 2014 (13):1767-79	1-200	No	50
Furlong et al. Bioanalysis; 2014 (13):1747-58	5 – 500	No	25
Heudi et al. Anal chem; 2008;(11):4200-7	5 – 1000	No	50
Duan et al. J chrom A; 2012 ;1251:63-73	0,013 – 32	No	2
Liu et al. Anal biochem 2011 ;414(1):147-53	1 – 100	Prot A	50
Furlong et al. Bioanalysis; 2013 (11):1363-76	0.05 – 100	Prot. A	25
Sleczka et al. Bioanalysis; 2014 (13):1795-811	0,02 – 20	pAb <hFc>	25
Li et al. AAPS journal; 2013;15(2):337-46	0.1 -15	<hFc>	25
Ocana et al. Anal chem; 2012; 17;84(14):5959-67	0.7 – 50 0.007 – 0.45	Prot. G Anti-ID.	100
Xu et al. Bioanalysis; 2014 (13):1781-94	0.1 – 20	Anti-ID	25
Dubois et al. Anal chem; 2008, 1;80(5):1737-45	0.02 - 2	Target (sEGFR)	500

Sensitivity:

- MS-Based Methods can reach „appropriate“ Sensitivities



Sample Volume/Preclinical Support:

- Required Volumes generally high
  - Benefit of „fast“ development??

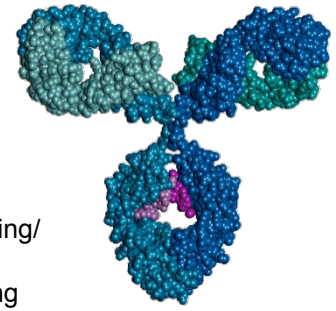
Dynamic Range:

- MS-Based Methods do not necesarily show „large“ dynamic ranges
- Appropriate **LBAs** also enable ranges of **> 3 orders of magnitude**

The Reality II
Selectivity

PHYSICOCHEMICAL CHARACTERISTICS

- Deamidation
- Oxidation
- N-term Pyro-Glu
- Glycosylation
- Glycation
- Acetylation
- C-term Lys
- Di-sulfide bond shuffling/ cleavage
- Fragmentation/clipping



Variant	#	Comb
Met	3	2^3 = 8
Cys (inter-HC)	4	2*2 = 4
Deamidation	7	3^7 = 2187
HC-N-term.	3	3
Glycosylation	7	7
Fucose	1	2
Bisect. GlcNAc	1	2

Possible modfications which may occur in the IgG1 HC and LC

(*) Not all possible modifications which may be found
during IPC- and release anaylsis and extended characterization are listed

Antibodies like all biologics are heterogenous molecules.
Assuming that all modifications listed in the table(*) are independent a total of $5.8*10^6$ variants for each IgG1-half-antibody (1 HC + 1 LC) may occur resulting in a total of **$3.5*10^{13}$ different variants** of the mAb.

How can we quantify „active“ isoforms??

“Mass“ Selectivity

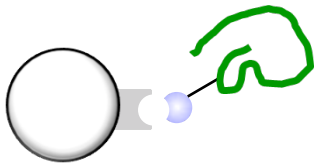
Mass Spec.:
- **Bottom-up** : „ ***selectivity not relevant“***
  - “total“ drug only
- **Top –down**: ***„limited selectivity“***
  - Isobaric isoforms
  - Sensitivity

↔

“Functional“ Selectivity

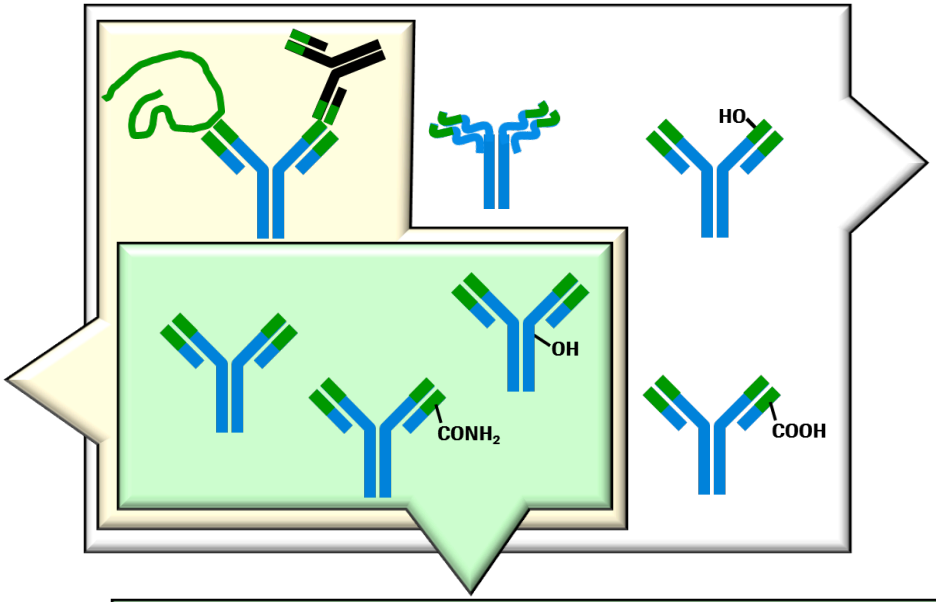
- **LBA:**
  - **Target Capture Assay *: „highly selective“***
  - **(„Total Drug Assays“: *„no selectivity“*)**
- **Mass Spec.:**
  - **„Hybrid Approach“: *????????***

What is the Analyte?
…..there is more than one Result!



Target-binding competent Drug

- *LBA*:
  - „Target Capture“ Assay
- *LC-MS*
  - „Hybrid“ Assays (Peptide)



Total Drug

- *LC-MS*
  - Sign. Peptide Assays
- *LBA*
  - Platform Assay (<hIgG>)

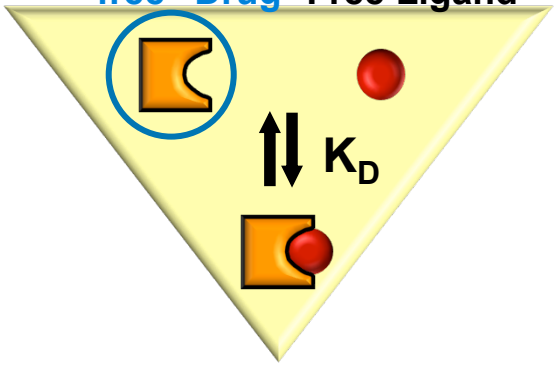
„Free“ (Active?) Drug

Correct Quantification of „free“ drug = challenging!!!

Challenges of “Free” Drug Quantification

Quantification of one analyte-form out of an equilibrium!

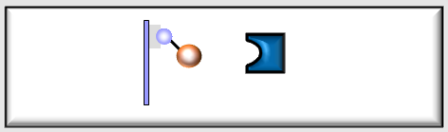
“free” Drug Free Ligand



Challenges of Correct „Free Drug“ Quantification

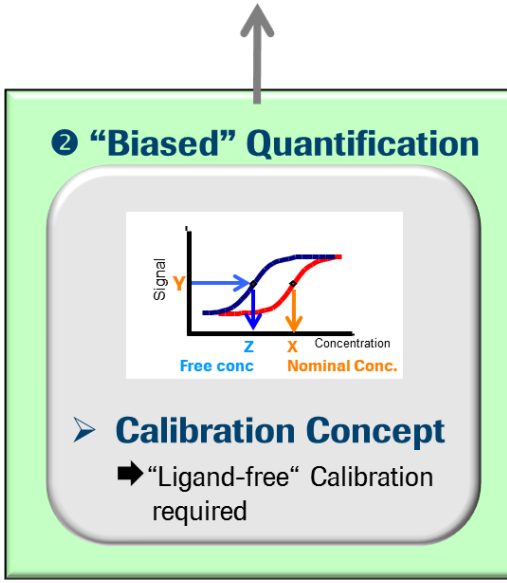
❶ Perturbation of equilibrium

➢ Sample Dilution



➢ Appropriate „Capturing Step“
- ➡ Capturing ***Reagent***
- ➡ Capturing Reagent ***Conc.***
- ➡ Capturing ***Time***

Free Drug Concentration



❷ “Biased” Quantification

➢ Calibration Concept
- ➡“Ligand-free“ Calibration required

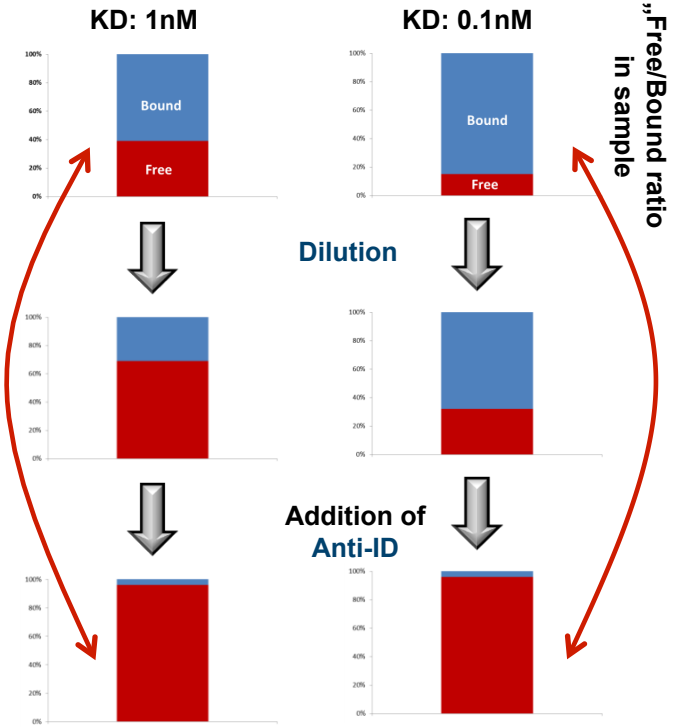
Staack, Jordan, Heinrich, Bioanalysis 2012, Feb;4(4):381-95

Hybrid Immunoaffinity MS

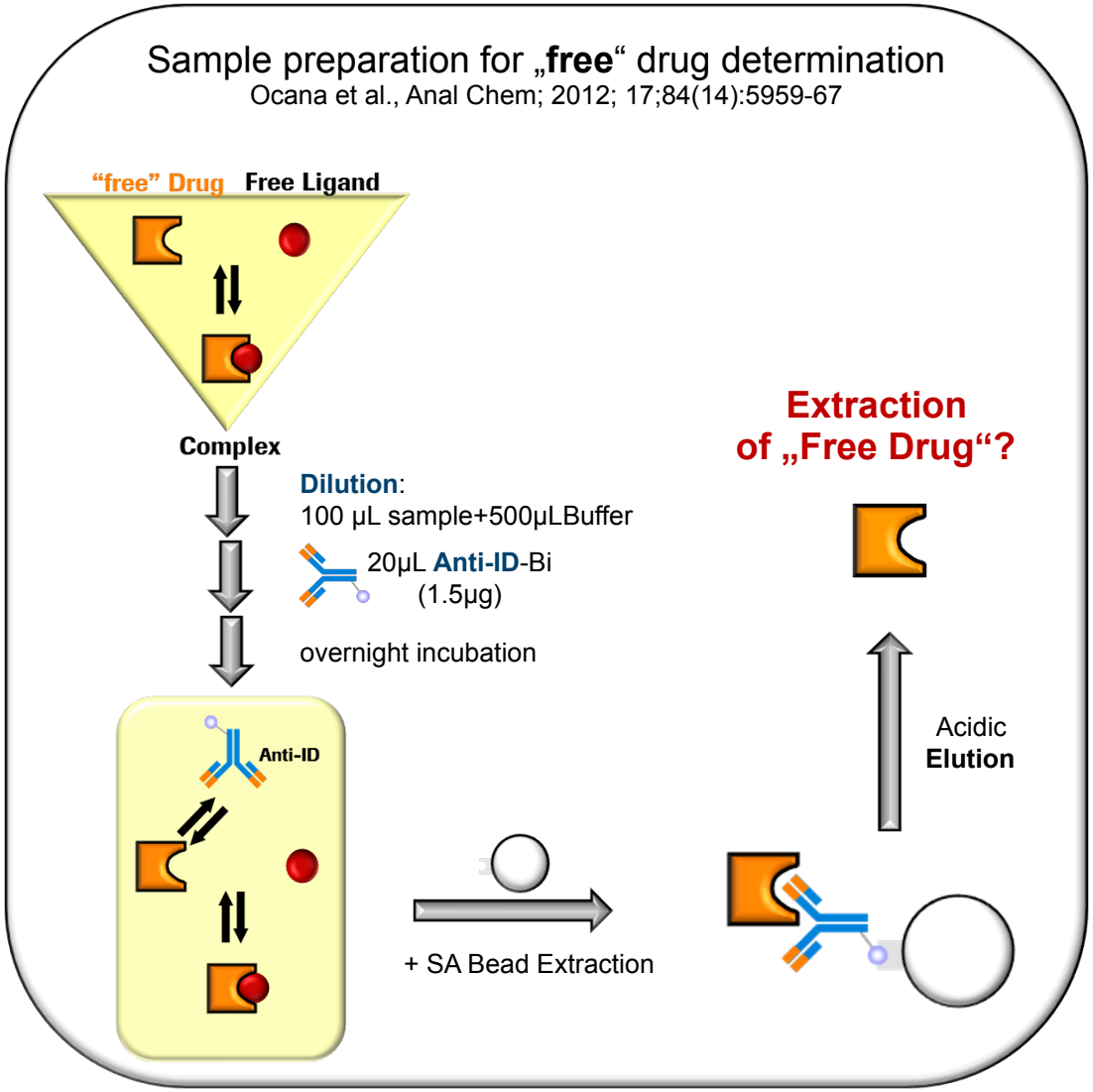
….the Way to MS-based „Free Drug Quantification“?

Assumptions:

- Target: 237 ng/mL
  Leung et al. *Immunology and Cell Biology* (2004) **82**,400–409
- Drug: 600 ng/mL
- 1:1 binding
- KD: 1 nM + 0,1 nM
- Anti-ID: True surrogate & same affinity as target
- Equilibrium (overnight incubation!)



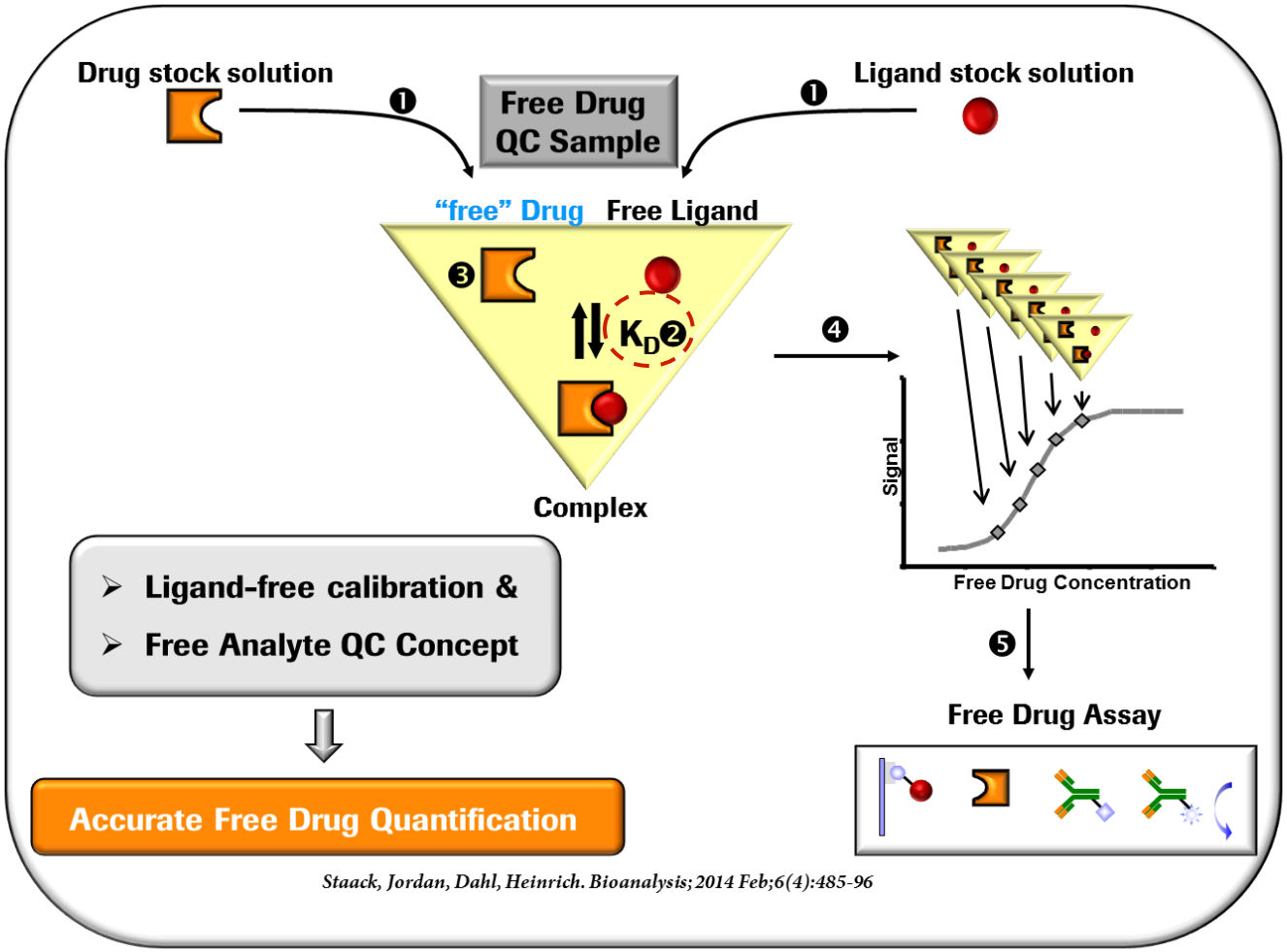
Sample preparation can significantly influence the assay result!



New Idea: „Free Analyte QC Approach“

For Development & Validation of Accurate „Free Drug“ Assay

The **free analyte (QC) concept** is based on QC samples containing **defined amounts of free drug in equilibrium with the respective ligand/**drug–ligand complexes, which **enables evaluation of all potential influencing factors on the equilibrium**.



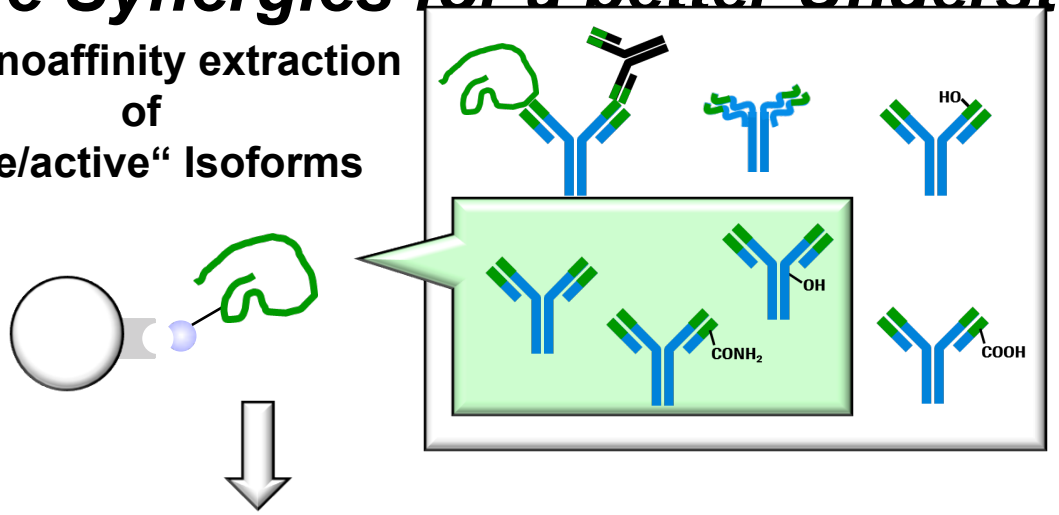
Staack, Jordan, Dahl, Heinrich. Bioanalysis; 2014 Feb;6(4):485-96

QC: Quality Control
KD: Dissociation Constant

„Hybrid“ Immunoaffinity – Top Down MS Approach

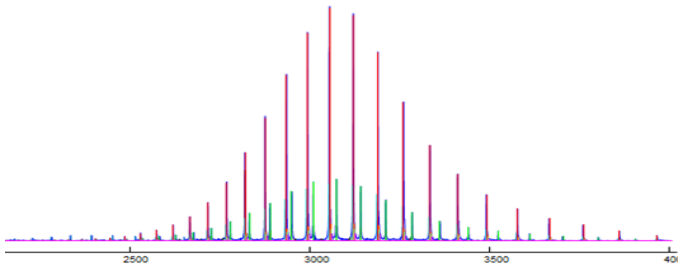
True Synergies for a better Understanding?

Immunoaffinity extraction of „free/active“ Isoforms



„Functional“ Selectivity

„Top-down“ MS-based structural Characterization



„Mass“ Selectivity

Knowledge about Structure-Function Relation

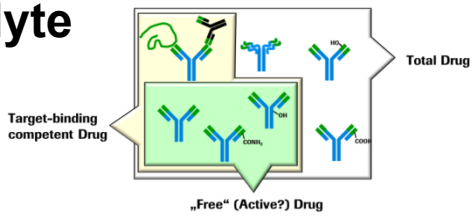
Papadimitriou, Bansal, Heinrich, Staack, Bioanalysis; 2014;6(13):1735-7

Definition of the Bioanalytical Strategy
The Biology drives the Technology Selection!

❶ Selection of the **„relevant“ Analyte**

⇩

Multifunctional Project Team*



❷ Selection of the **appropriate Bioanalytical Technology**

⇩

Bioanalyst

Influencing Factors:

- Biological Question
- Project Phase
- Expertise in & Availability of Technologie
- Suitability for routine use
- Costs

* EBF „TT 20“:
Dudal, Staack, Stoellner, Fjording, Vieser, Pascual, Brudny-Kloeppel, Golob, Bioanalysis; 2014 May;6(10):1339-48

Summary and Conclusion

- Therapeutic Proteins are **complex analytes** due to
  - Microheterogeneity
  - In-vivo Binding partners (soluble target, anti-drug antibodies etc.)
- A general technology comparison not helpful without **clear definition of the analyte**
- **Biological question drives the technology selection**
  (total drug – target-binding competent drug – free/active drug)
- Accurate Free Drug quantification = challenging task!!
  - **Sample preparation** might significantly **influence the free drug result**
- Future Outlook: **„Hybrid“** approach to **combine „functional“** selectivity with **„mass“ selectivity** to enable a profound characterization of active isoforms

Acknowledgement

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Doing now what patients need next