EBF view and future perspective of free/total large molecule drug quantification

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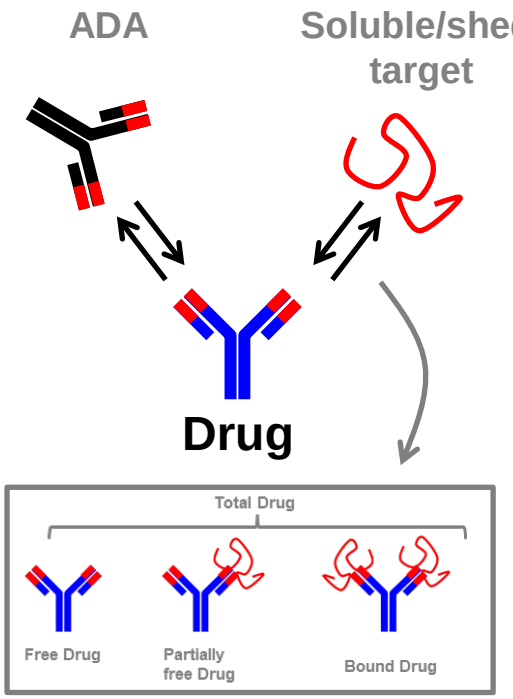
on behalf of the European Bioanalysis Forum (EBF)

9th JBF Symposium
February 6-8, 2018
Tokyo

What is it all about?

(neutralizing) soluble binding partner(s)



EBF Activities:

Topic Team 20

Daniela Stoellner (Novartis – TT lead)
Margarete Brudny-Kloeppel (Bayer)
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Eva Vieser (Amgen)

10th EBF Open Meeting, Barcelona 2017

- **Mini Workshop on „free/total“ drug quantification**
  Daniela Stoellner, Roland Staack, Joanne Goodmann, Marianne Scheel Fjording
- **WS-9 Calibration concepts in LBA**

To what extend do we (bioanalytical scientists) need to consider soluble binding partner(s) when defining a bioanalytica assay strategy

Why do we need „free/active“ drug data?

→ Literature statements

- The possible **influence of plasma binding proteins and/or antibodies** in plasma/serum on the assay performance should be determined!!! (ICHS6 Guideline)
- The validity of a non-clinical safety study relies upon the demonstration of **active drug exposure** throughout the dosing phase of the study (Ponce *et al., Regul.Toxicol.Pharmacol.* 54(2), 164-182 (2009))
  - by an appropriate PD marker (Ponce *et al., Regul.Toxicol.Pharmacol.* 54(2), 164-182 (2009)
  - by appropriate Bioanalytical Methods to show “active drug exposure → if an appropriate PD Marker is not available
- The **interference** of **soluble targets**, extracellular domain of the target **receptors**, or **ADA** on the PK assay need to be considered in the design of the sampling strategy by **looking for free or total drug**. (Chirmule et al. *AAPS.J.* 14(2), 296-302 (2012))
- The success of the PK–PD modeling effort depends on close communication between the PK–PD bioanalytical and clinical scientists: **the PK–PD model is only as good as the data provided for modeling**.(Roskos *et al., Bioanalysis.* 3(6), 659-675 (2011))

”Ignition” of the discussion within the bioanalytical community

AAPS white paper entitled

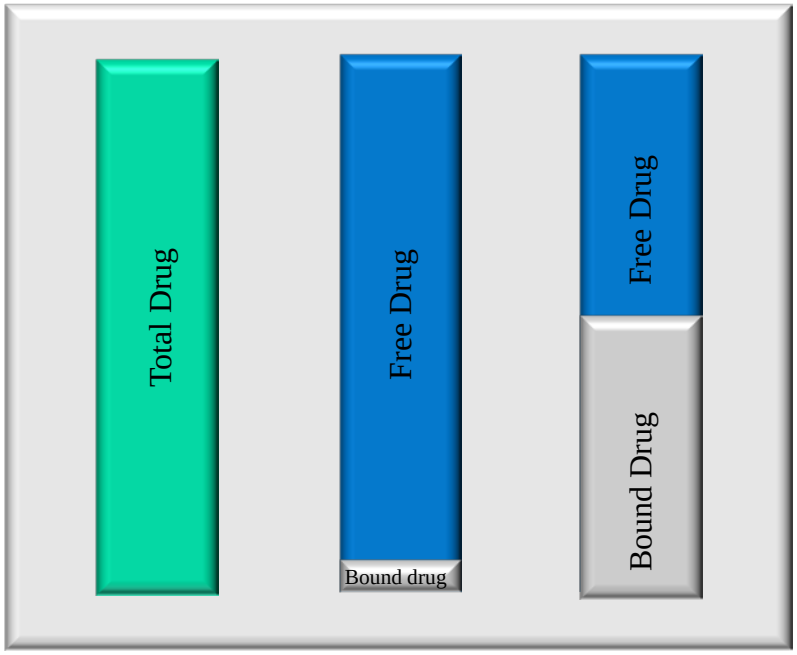
Bioanalytical approaches to quantify “total” and “free” therapeutic antibodies and their targets: technical challenges and PK/PD applications over the course o drug development.

Lee JW, Kelley M, King LE, Yang J, Salimi-Moosavi H, Tang MT, Lu JF, Kamerud J, Ahene A, Myler H, Rogers C.

APPS J. 2011 Mar;13(1):99-110.

Do we always need to differentiate between „free/active“ vs „total“ drug?

- **Answer: NO!** → highly dependent on the project/biology!



- **Are there soluble binding partners?**
- **Are soluble binding partner present at „*relevant*“ amounts?**

„*relevant*“ depends on concentrations of drug & binding partner

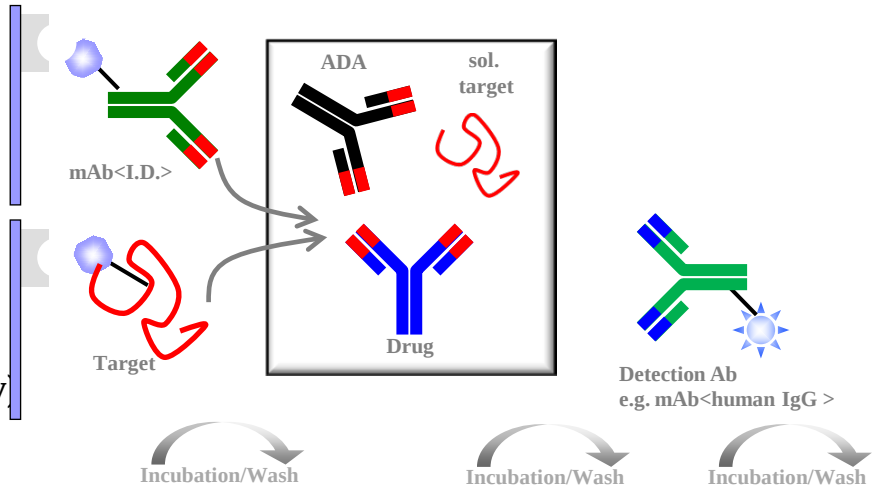
If „free/active“ drug data is important → appropriate assay development is required

What makes an assay an „free/active“ drug assay?

General assumption:
„drug-target interaction“ = surrogate for activity

Assay Reagents?

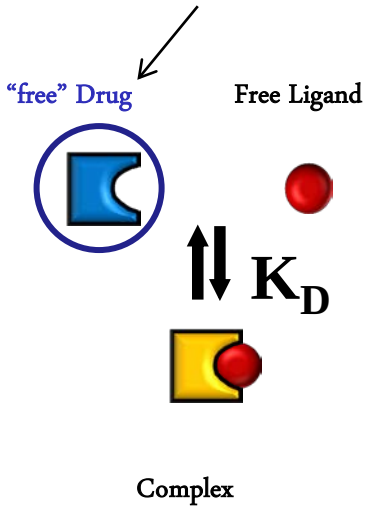
Rec. target or specific Ab?
(e.g. Anti-idiotypic Antibody)



Assay Procedure? → **Impact of assay procedure in case of (neutralizing)soluble binding partners?**

Challenges of active drug determination in the presence of soluble binding partners

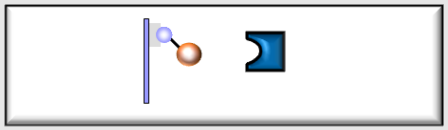
Quantification of one analyte-form out of an equilibrium!



Challenges of Correct „Free Drug" Quantification

❶ Perturbation of equilibrium

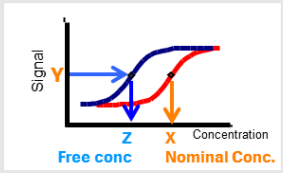
➢ **Sample Dilution**



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

Free Drug Concentration

❷ "Biased" Quantification

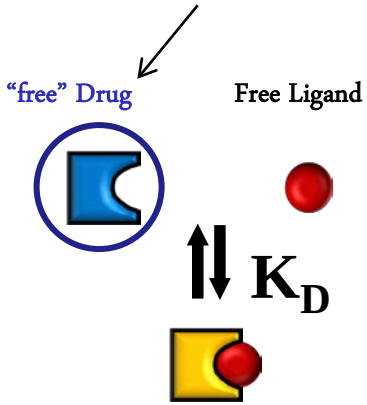


➢ **Calibration Concept**
- ➡ "Ligand-free" Calibration required

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

Challenges of active drug determination in the presence of soluble binding partners

Quantification of one analyte-form out of an equilibrium!



Relvant for LBA* & hybrid LC-MS!**

Challenges of Correct „Free Drug" Quantification

❶ **Perturbation of equilibrium**

➢ **Sample Dilution**

➢ **Appropriate „Capturing Step"**
- ➡ Capturing ***Reagent***
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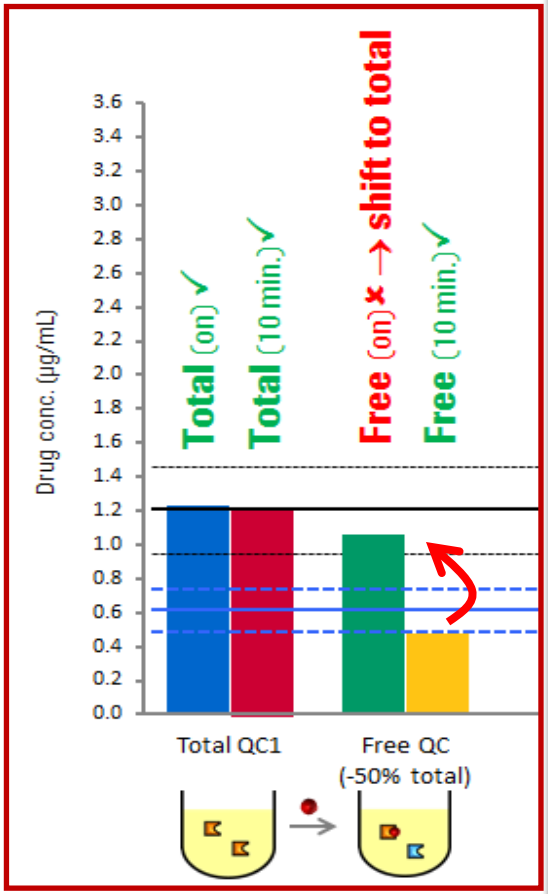


*Staack, Jordan, Heinrich, Bioanalysis 2012, Feb;4(4):381-95

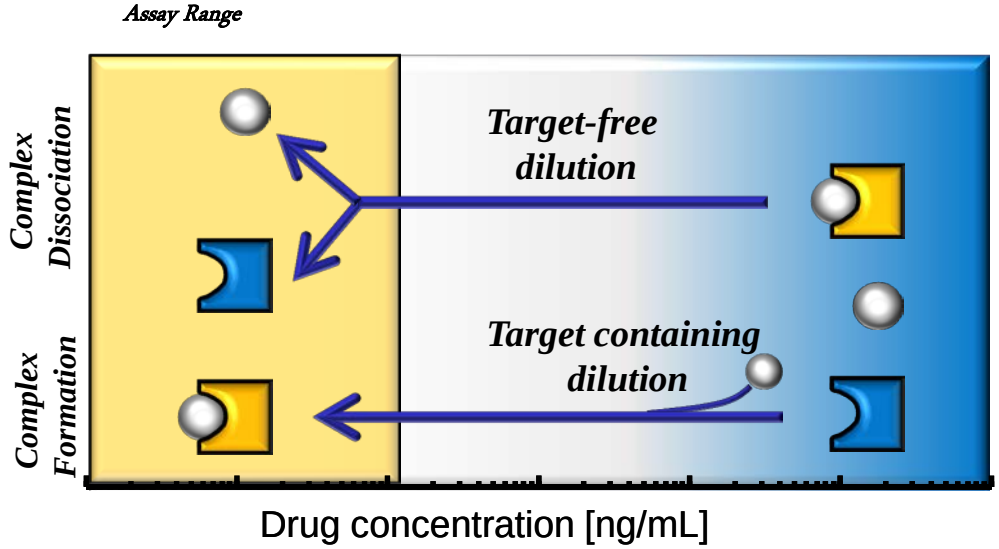
** Jordan, Onami, Heinrich, Staack, Bioanalysis. 2017 Nov;9(21):1705-1717

Bioanalytical Challenges: Sample Dilution

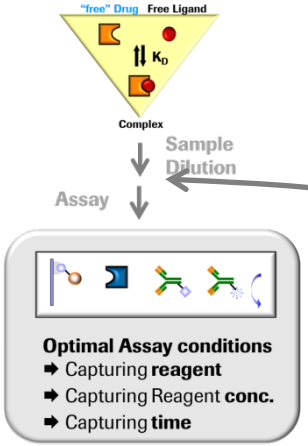
- **Theoretical Problem → „Dilution Matrix“***

Free Drug concentration ↑

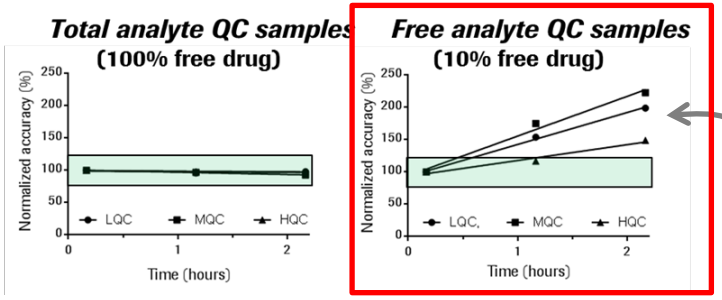
Free Drug concentration ↓



- **…which might be relevant!!**



Optimize Assay „timing“ → time between end of dilution and assay conduct



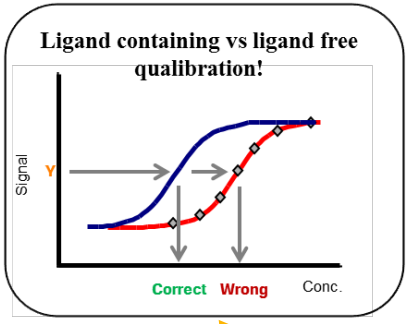
Time-dependent increase of „free drug“ due to complex dissociation

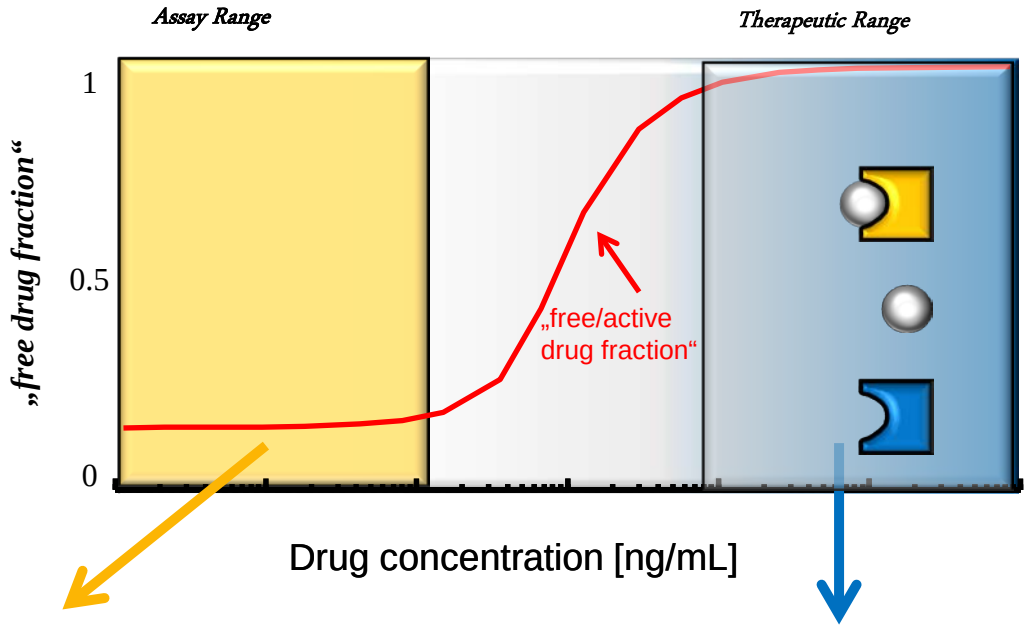
*Staack, Jordan, Heinrich, Bioanalysis, Feb;4(4):381-95 (2012)

**Schick, Staack, Haak, Jordan, Dahl, Heinrich, Birnboek, Papadimitriou; Bioanalysis; 8(24):2537-2549 (2016)

“Project relevance”vs “analytical relevance”

Potential impact of calibration matrix on assay result!





Analytical Relevance

Calibration

- In a range with relevant target concentrations?
- **Calibration – dependent bias of assay result?**

Project Relevance

- Drug in excess over target
- **„Free“ drug ~ Total drug**
  → Differentiation not relevant?

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

EBF views:

- **Assay development**
  - Appropriate assay development technically challenging & requires:
    - Appropriate assay reagents
    - Understanding of drug mode of action
    - Understanding of drug-ligand binding kinetics

Success factor: implementation of BA scientist into project team!

How the bioanalytical scientist plays a key role in interdisciplinary project teams in the development of biotherapeutics – a reflection of the EBF

Dudal S, Staack RF, Stoellner D, Fjording MS, Vieser E, Pascual MH, Brudny-Kloeppel M, Golob M. Bioanalysis. 2014 May;6(10):1339-48.

- **Assay „characterization“**
  - Experiments should be performed to undertstand the result of the bioanalytical method (→ different approaches are reported)

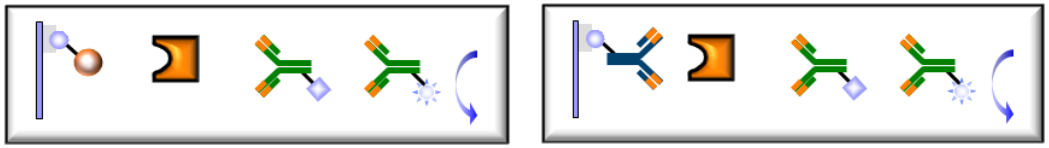
Critical factor: Communication!

- **Define name of analyte species and deliver assay with short description on what it actually measures considering all interferences and factors impacting binding equilibrium**

What makes an assay a „free drug assay?

Reported Approaches

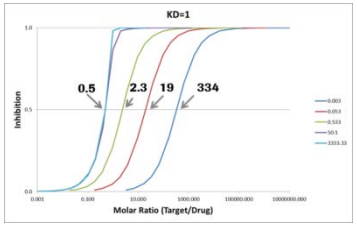
- **Assay Format** → *Target-Capture Assay*



- **„Interference“ Testing**

- **„IC50“ Approach***

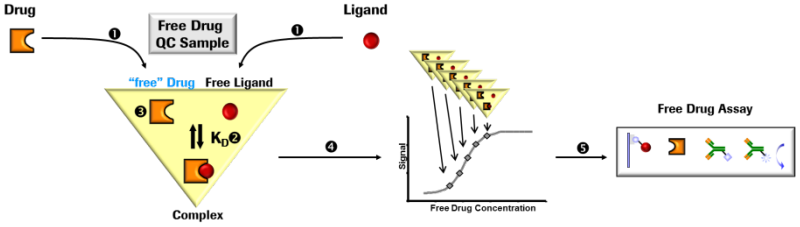
* Lee JW et al., *AAPS.J.* 13(1), 99-110 (2011)



Assumption 1:1 Bindung

- **„Free Analyte QC“****

*** Staack, Jordan, Dahl, Heinrich, Bioanalysis. 6(4), 485-496 (2014)*



EBF views…..and outlook

- **Matrix selection for calibration/QCs**
  EBF Discussion: WS-9 Calibration concepts in LBA @ EBF 2017
  - Impact of specific matrix effect (target interference) versus non-specific matrix effect needs to be evaluated
  - Potential bias of the assay result due to use of „ligand-containing“ matrix shoud be assessed!

- **Bioanalytical Strategy**
  EBF Discussion: Mini Workshop on „free/total“ drug @ EBF 2017

  If multiple PK assays are required, e.g. to differentiate between „free & total“ drug or to monitor different domains of multi-domain biologics,

  - differentiate between ***„investigative bioanalysis“*** (e.g. assays to monitor drug integrity)
  - „**bioanalysis to assess drug exposure**“
    - *Pivotal studies: aim for a „**lean assay panel**“*

Outlook:

Highly potent (→ low dosed) multi-domain biologics will significantly increase the complexity of large molecule bioanalysis.

- **EBF will address these challenges**

Acknowledgement

All EBF colleagues!

- *Daniela Stoellner*
- Margarete Brudny-Kloeppel
- Sherri Dudal
- Marianne Scheel Fjording
- Marie-Hélène Pascual
- Michaela Golob
- Eva Vieser
- Joanne Goodmann

- Philip Timmermann

All colleagues of the BA departments of the different companies which were represented in the Topic Teams

All colleagues who participated and contributed to the EBF workshops at the Open Meeting 2017