Is correct quantification of free/active drug concentrations by hybrid LC-MS possible? An evaluation applying the “free analyte QC concept”

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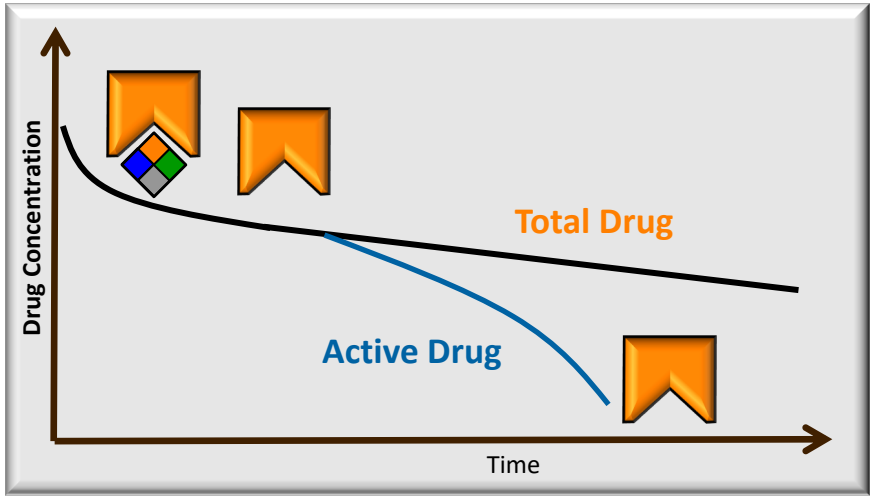
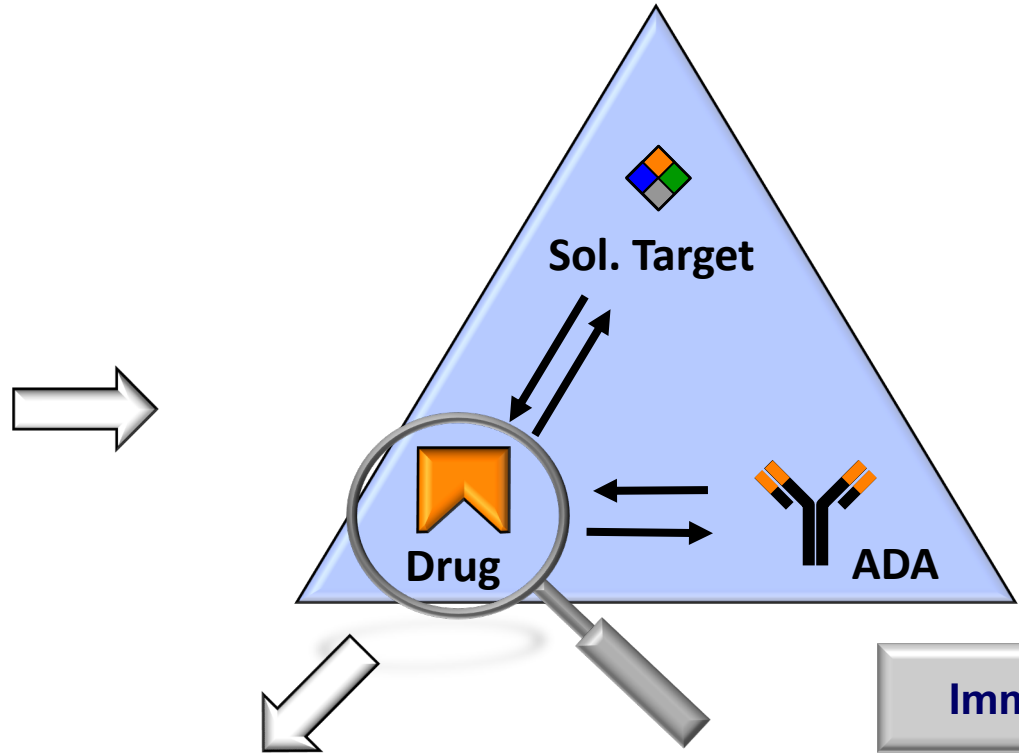
EBF spotlight on ProteinMS, virtual , June 17+18 , 2021

Roche *pRED*

“Free” vs. “Total” Drug Quantification

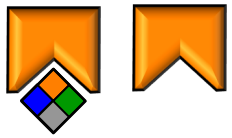
…when do we need to Differentiate?





Immunogenicity

- **Immunogenicity testing**
- **Neutralizing ADA**



- Total drug assay
- Free drug assay

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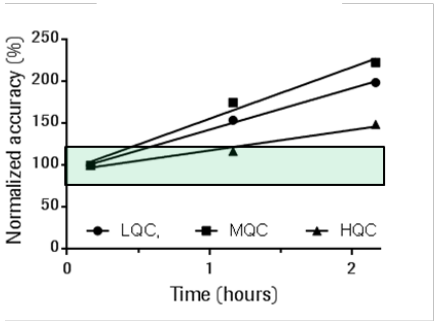
Interaction with the target is needed

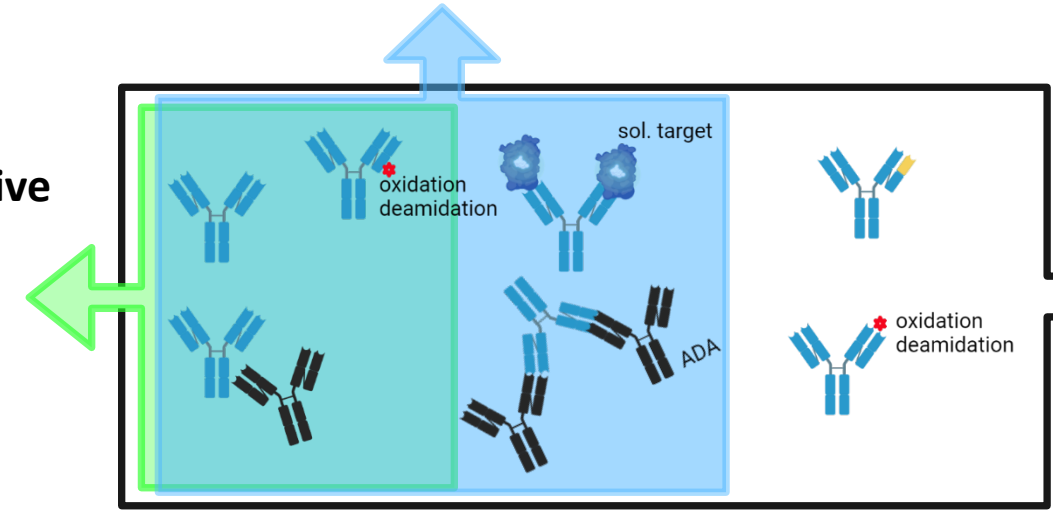
… to enable binding specific extraction

Target binding competent

Free analyte QC samples

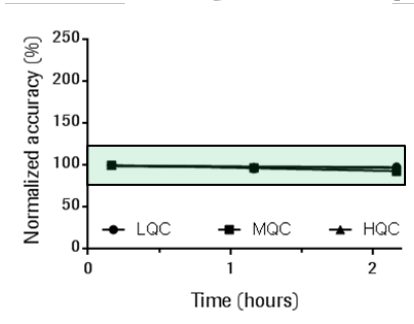


Active



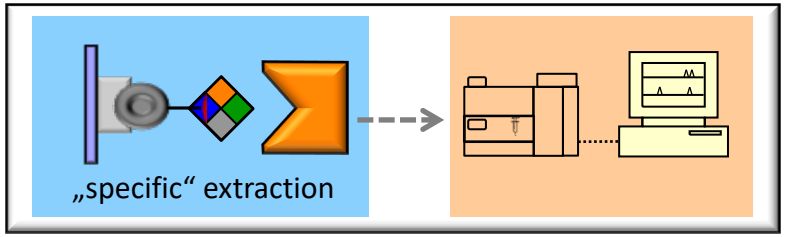
Total

Total analyte QC samples



Target Capture Hybrid LC-MS……

…is a Ligand Binding Assay with a MS detection!



…is a Mass Spec. method with a bit of sample preparation!

➢ **Clear understanding of the „hybrid LC-MS“ requires both LBA and LC-MS expertise!**

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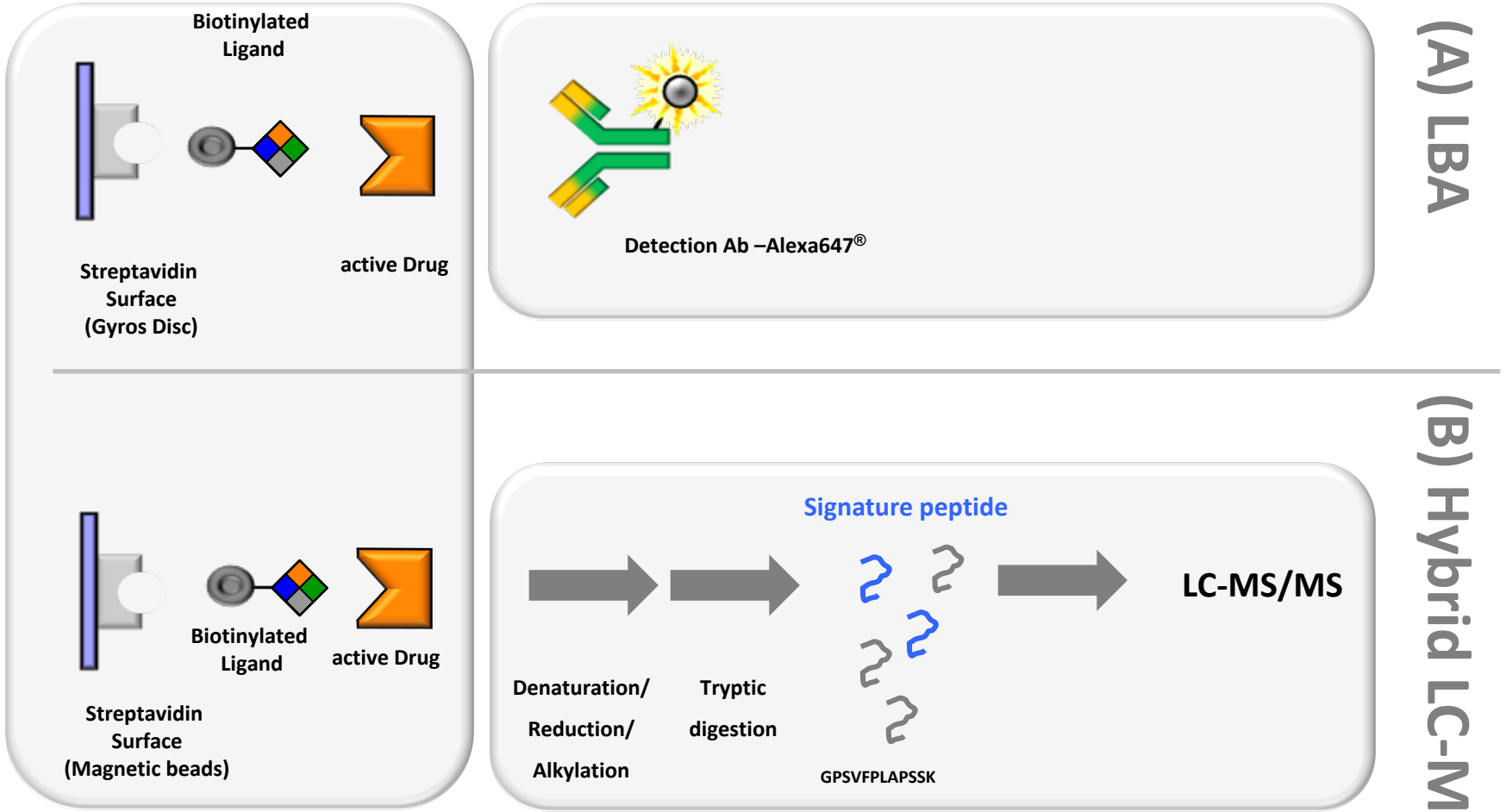
Free Drug can be analyzed independently of the chosen technology

…but we need to understand what we do…



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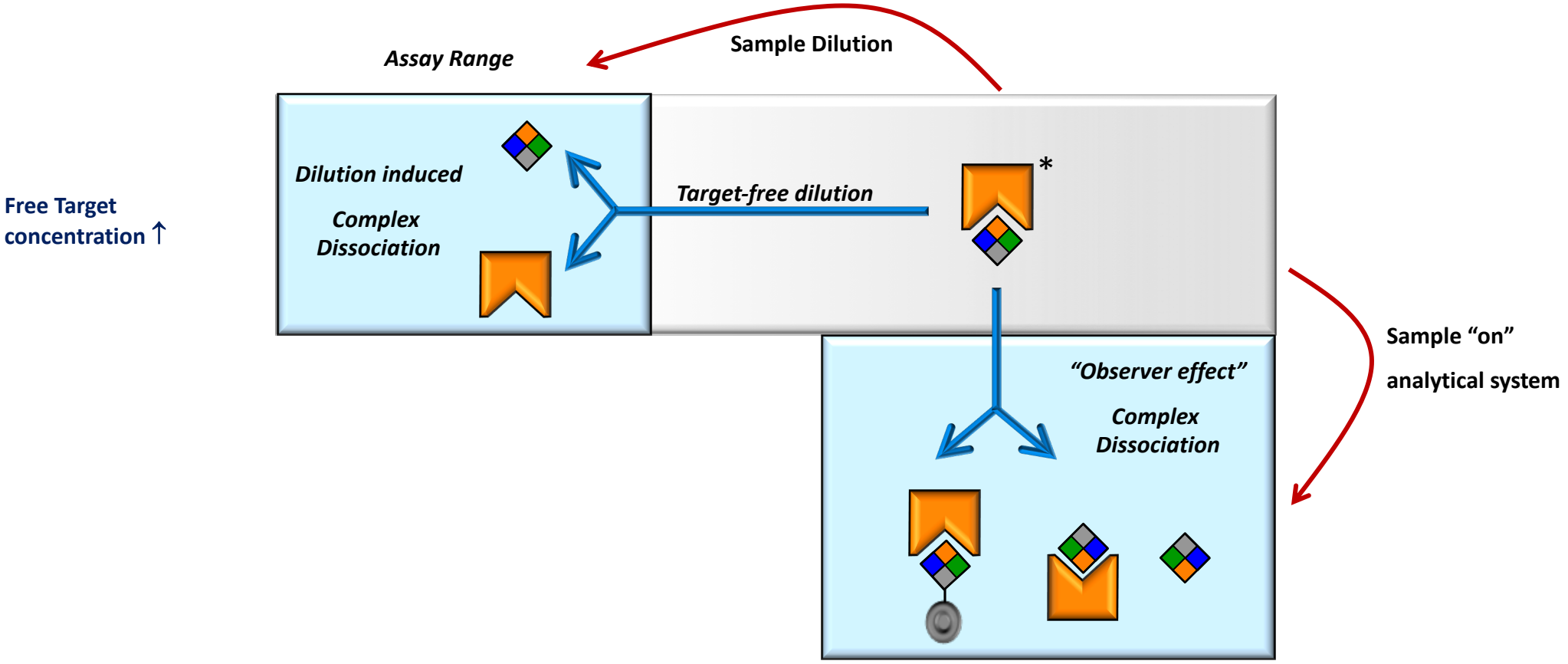
Manipulation of sample might result in complex dissociation

…either due to dilution of the sample or "during analysis"



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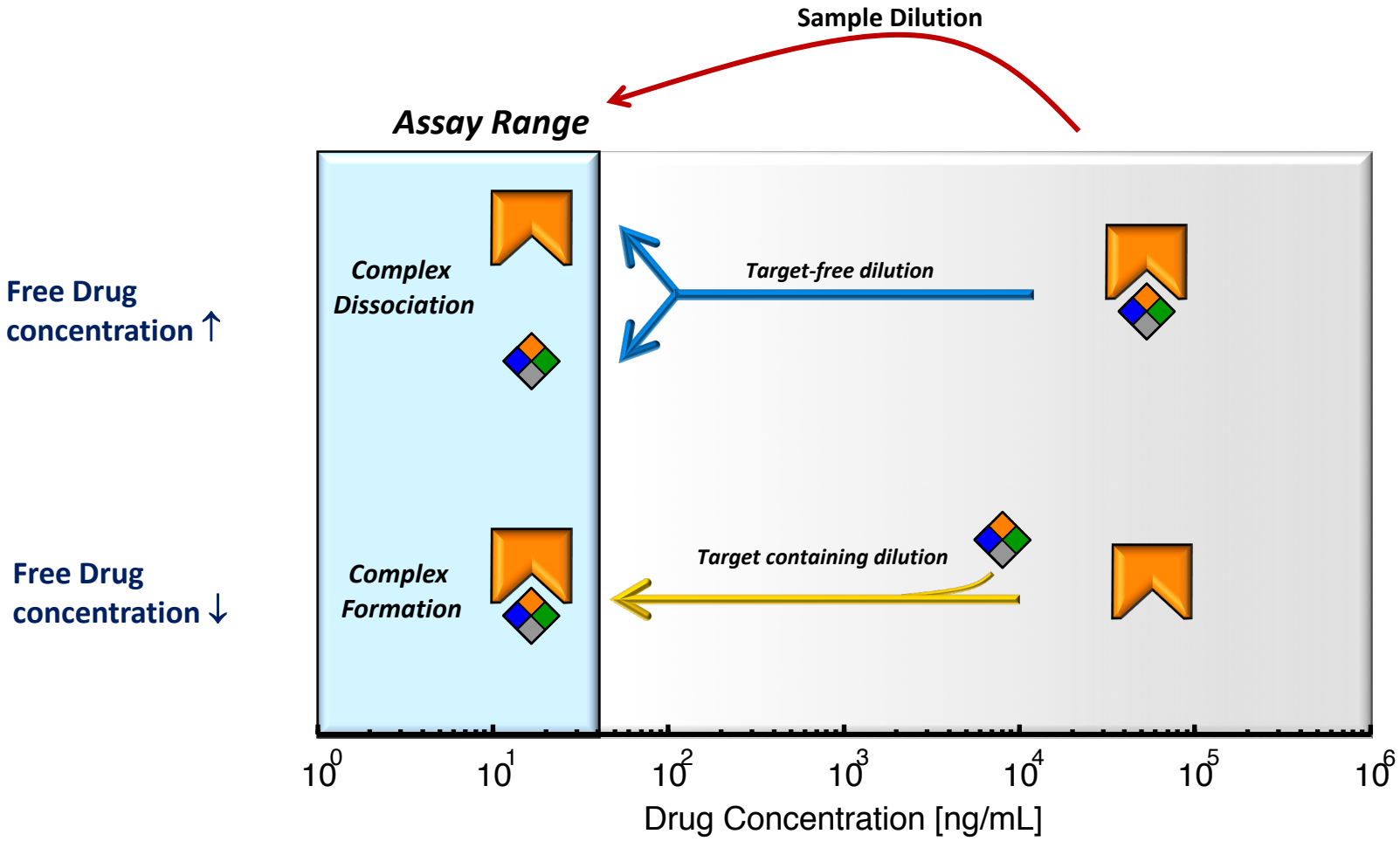
* Mathematical simulations for bioanalytical assay development; Staack, Jordan; Bioanalysis 2012

A cross reactive surrogate matrix might interfere as well

… used for constructing the calibration curve as well as for sample dilution



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

Bioanalytical Challenges: Example of Sample Dilution

… is the test on dilution linearity the appropriate test for active assays?

Assumptions:
- Drug: 500 µg/mL
- Ligand: 20 nM
- KD: $1x10^{-10}$ M

* At equilibrium

Target-containing dilution

	500 µg/mL Sample				
Dilution	total drug in diluted sample [µg/mL]	free drug fraction	free drug concentration* [µg/mL]	back calculated concentration [µg/mL]	Recovery based on undiluted sample
1	500	0.994	497.000	497	100%
10	50	0.994	49.700	497	100%
1386	0.361	0.277	0.100	139	28%
1781	0.281	0.178	0.050	89	18%
2353	0.212	0.118	0.025	59	12%
3846	0.130	0.077	0.010	38	8%
6250	0.080	0.063	0.005	31	6%
11024	0.045	0.055	0.0025	28	6%
25316	0.020	0.051	0.0010	25	5%
49128	0.010	0.049	0.0005	25	5%
96749	0.005	0.048	0.00025	24	5%
239606	0.002	0.048	0.00010	24	5%
477702	0.001	0.048	0.00005	24	5%

Assay range:
1-100 ng/mL

CV=72 %
back calculated
average = 58 µg/mL

Assay range:
0.1-10 ng/mL

CV=19 %
back calculated
average = 28 µg/mL

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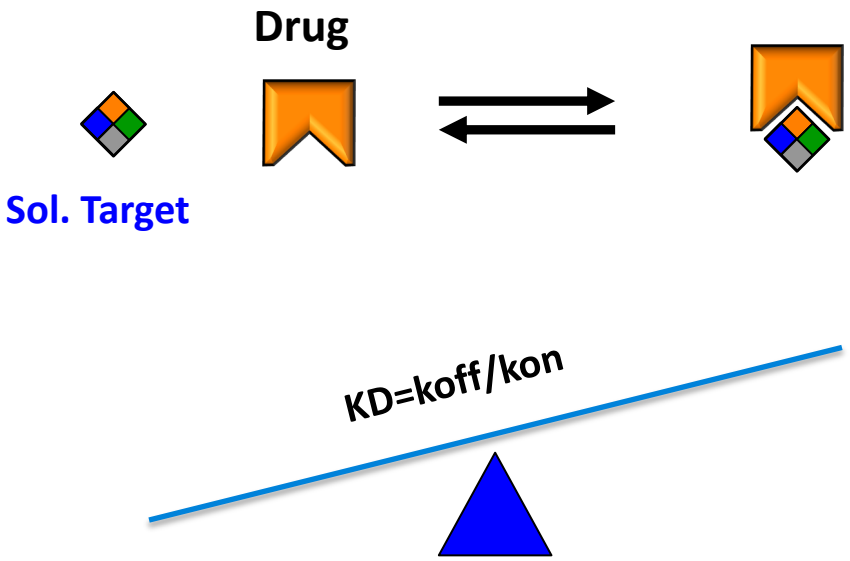
* Mathematical simulations for bioanalytical assay development; Staack, Jordan; Bioanalysis 2012

Knowledge of the kinetic of the binding partner plays a fundamental role

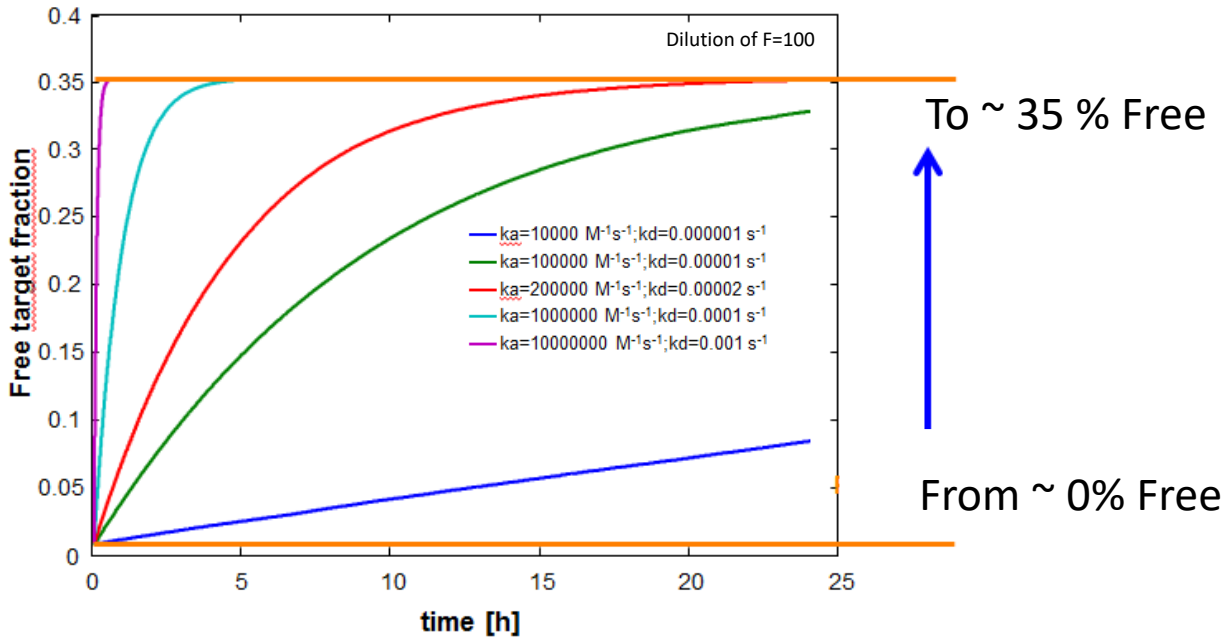
… when setting up an active assay



KD describing the position of the balance

kon/koff responsible for the dynamic

Simulation of perturbed samples with constant KD value but varying rate constant *



Estimation of the maximal analysis time allowed after a perturbation of the sample without affecting significant the concentrations of the binding partner

➢ Forming new equilibrium from **minutes** to several **hours**

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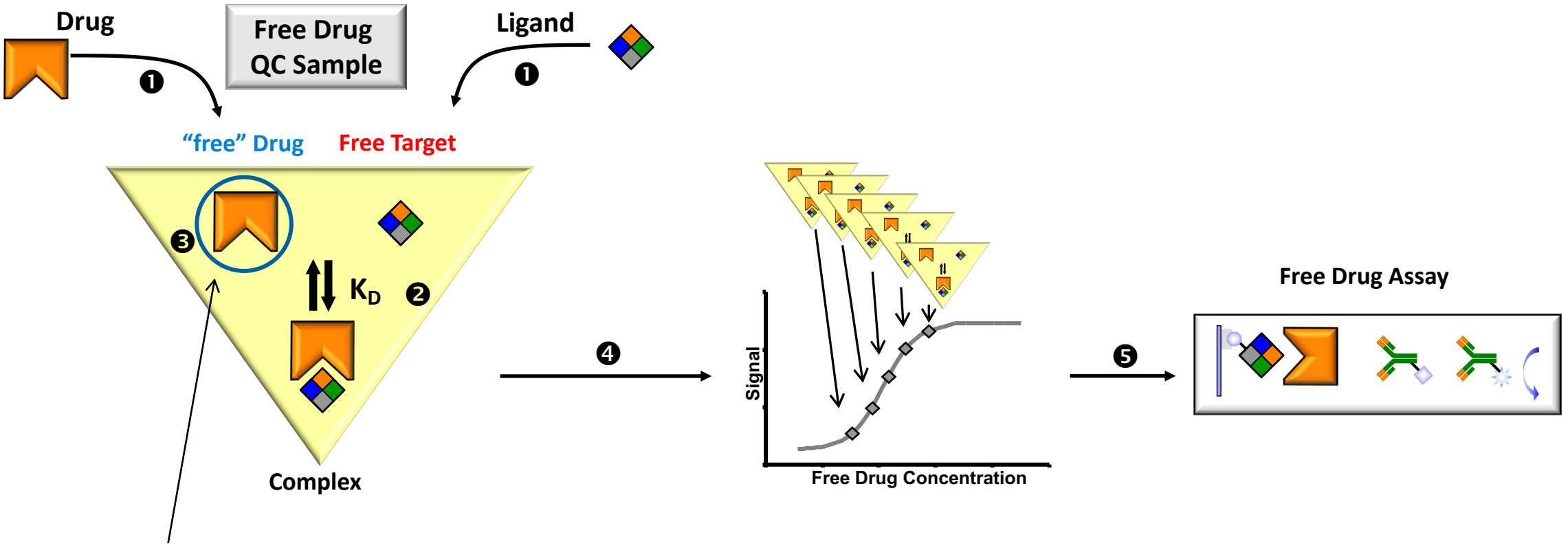
* Mathematical simulations for bioanalytical assay development; Staack RF, Jordan G - DOI: 10.4155/bio.11.321

„Free Analyte QCs“

…enable monitoring of all critical steps



Quantification of one analyte → out of an equilibrium!

* Free analyte QC concept, Staack, Jordan; Bioanalysis 2014

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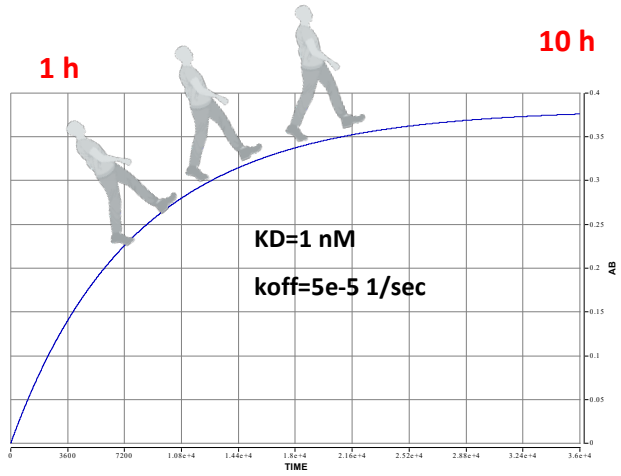
Why might the Free QC approach fail?

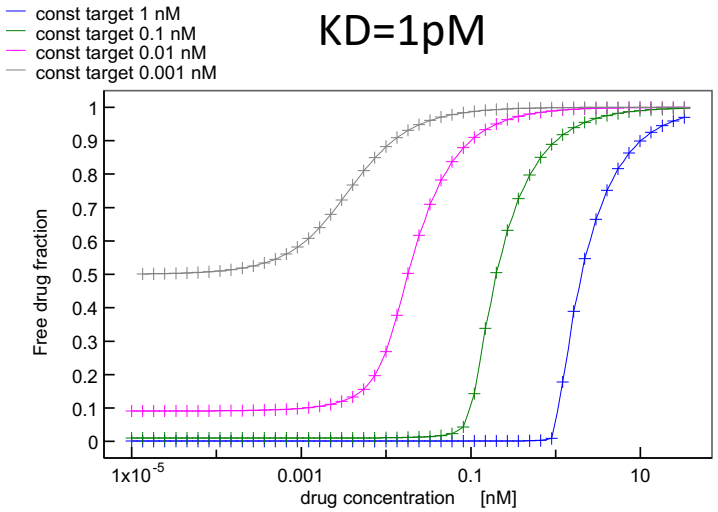
…KD determination is well understood but …

Surface plasmon technologies

In solution estimation

Kinetic consideration when preparing the samples

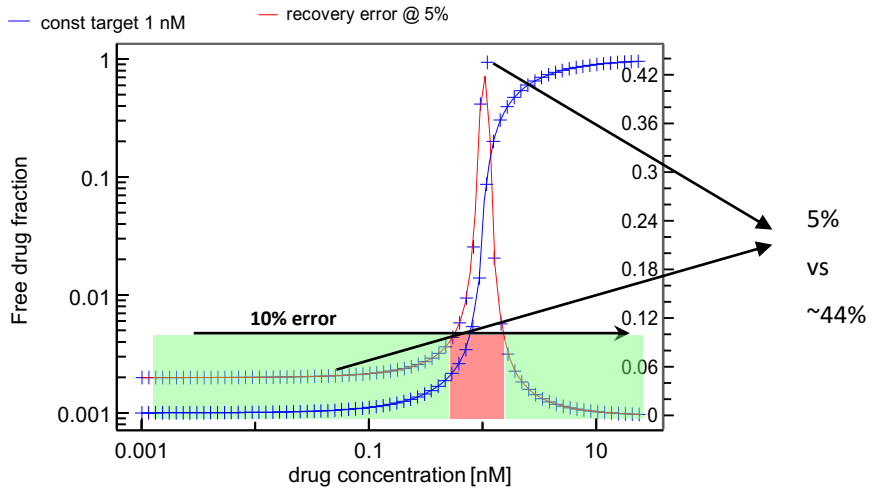




"QC preparation error"

Hypothetical 5% error by target addition to sample

1.05nM added instead of 1 nM



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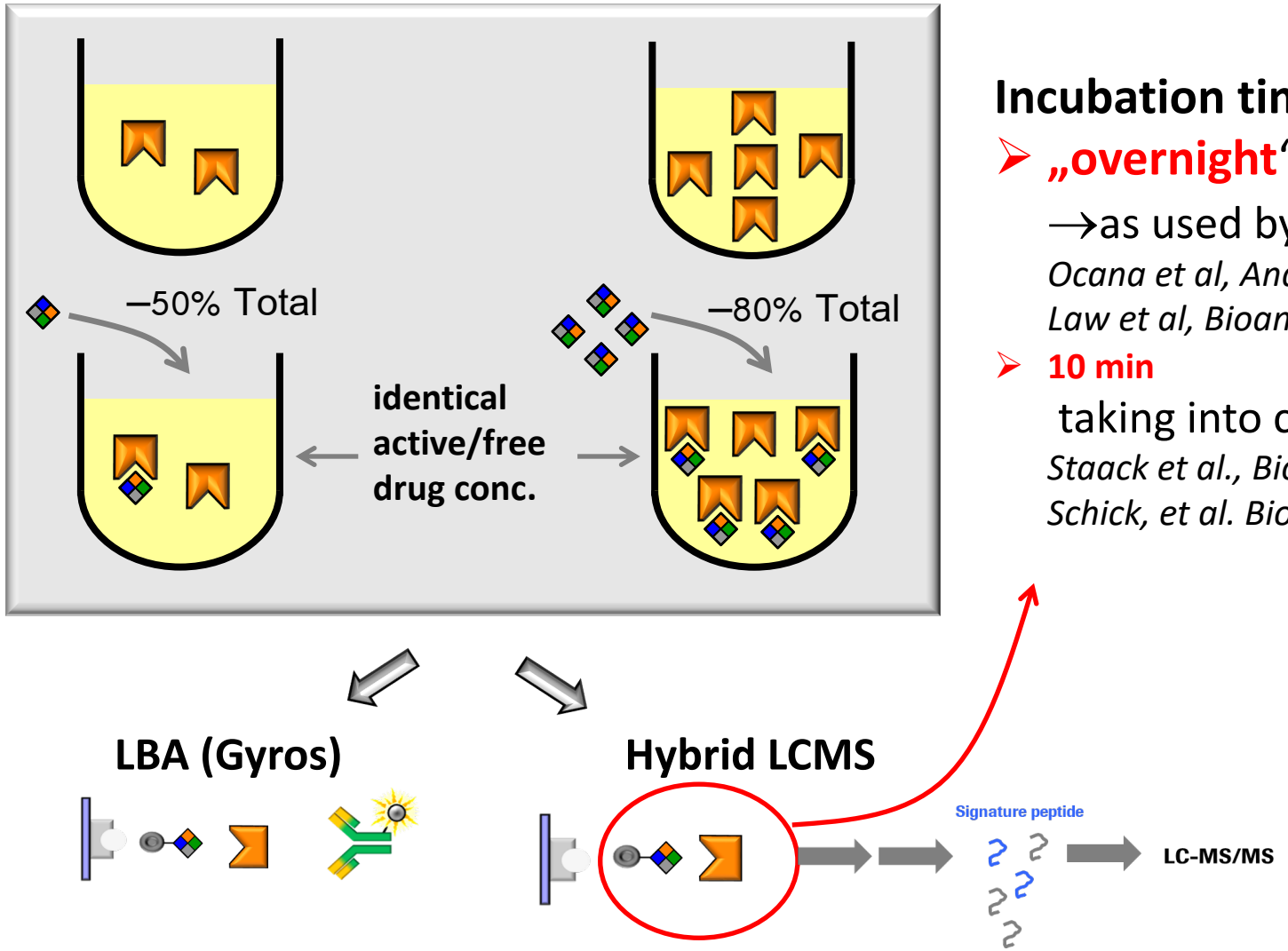
Evaluation of „hybrid LC-MS“ procedures using „free anaylte QCs“

…Study design



Incubation times:

- **„overnight“**

  →as used by described hybrid LC-MS methods

  Ocana et al, Anal. Chem. 84(14), 5959–67 (2012)

  Law et al, Bioanalysis. 6(23), 3225–35 (2014)

- **10 min**

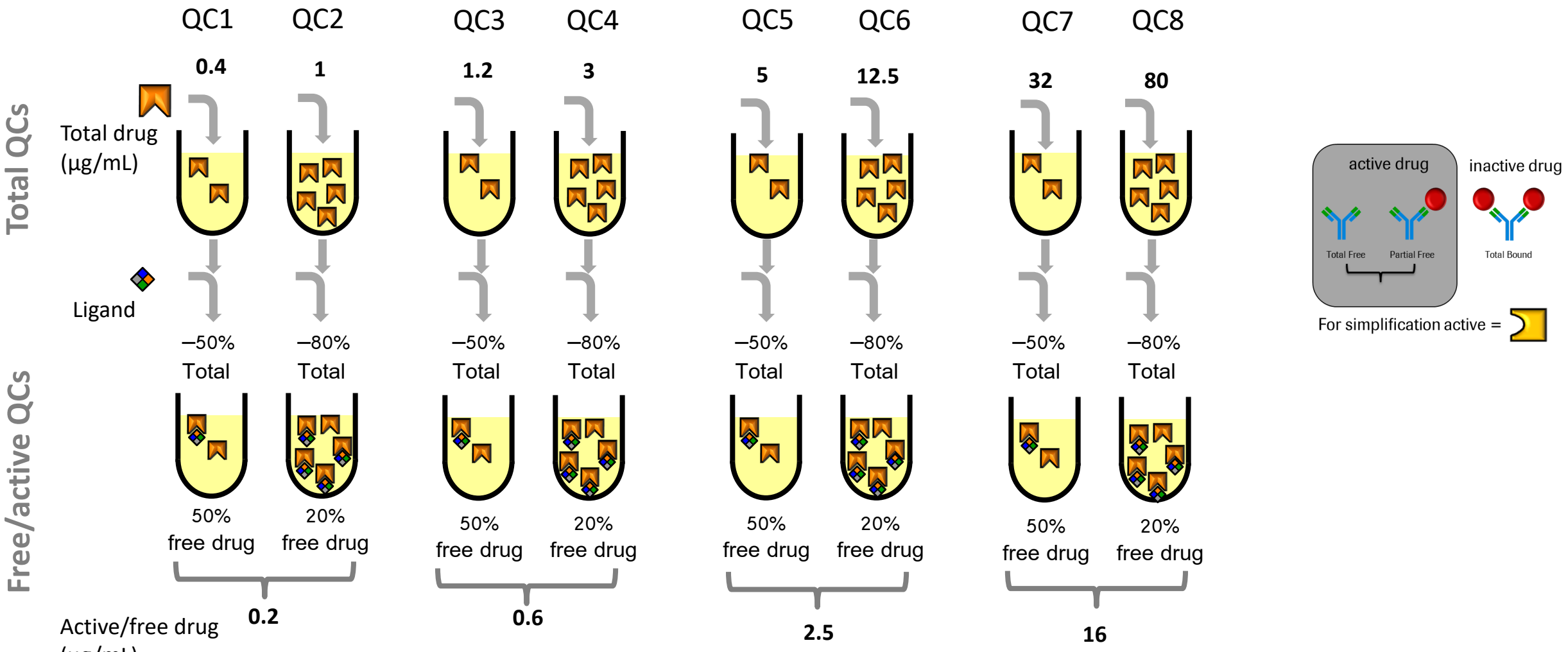
  taking into considerations the LBA experiences

  Staack et al., Bioanalysis. 6(4), 485-496 (2014)

  Schick, et al. Bioanalysis; 8(24):2537-2549 (2016)

Jordan, Ohnami, Staack; Bioanalysis 2017

QC covering broad concentration range



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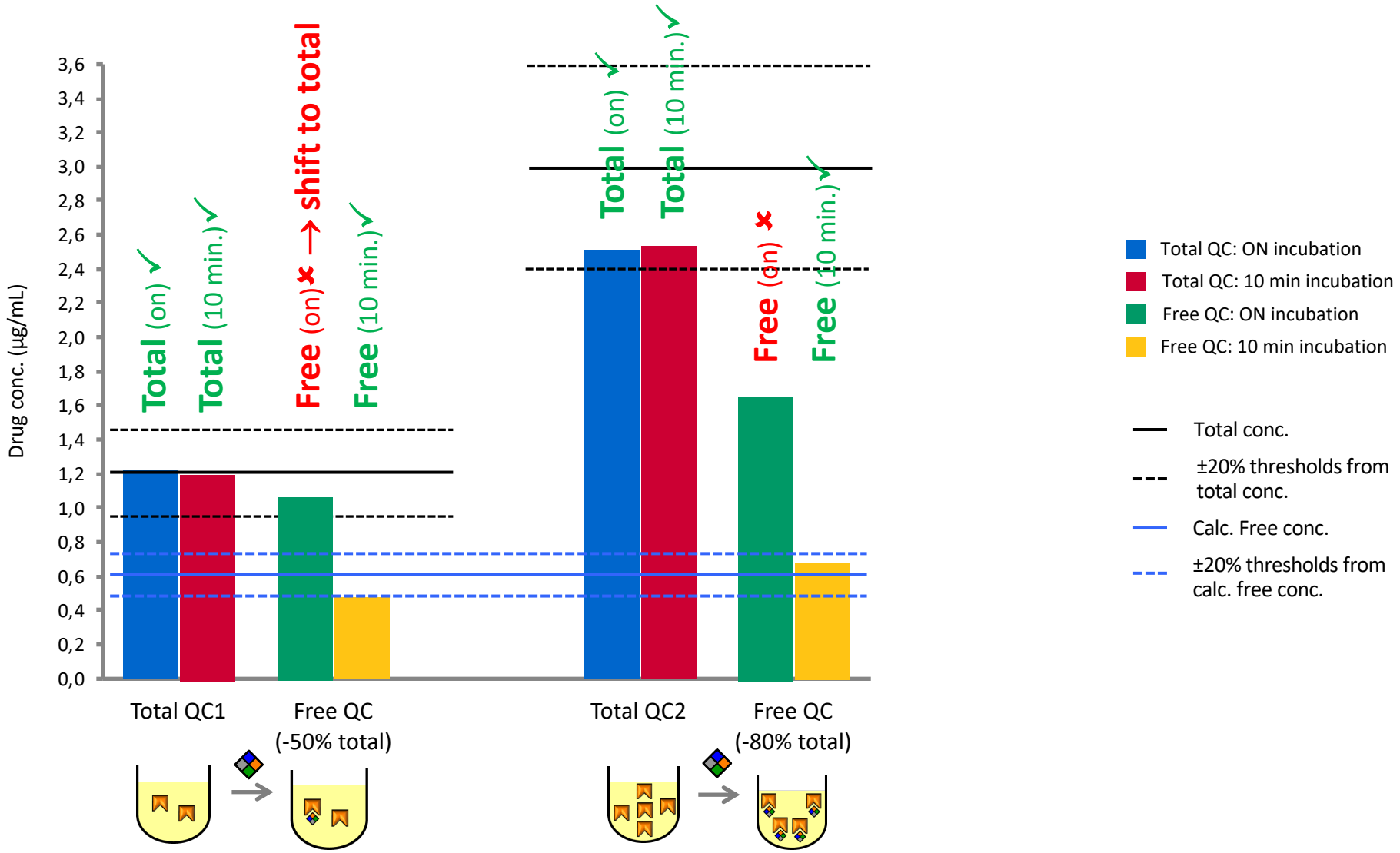
Evaluation of „hybrid LC-MS“ procedures using „free anaylte QCs“

…Importance of optimal incubation time



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Summary and conclusion

- **Active exposure** data is a crucial information in the drug development process
- **KD** and **preparation of QC samples** has do be **accurate**
- **Challenges** of accurate free drug quantification are **independent of the applied technology** (LBA or (hybrid) LC-MS)
  - **Shift of** the drug-binding partner **equilibrium** resulting in erroneous results due to:
    - Sample preparation
    - Selected matrix (even if the target concentration is very low)
    - QC preparation
- **„Free Analyte QC Approach“** enables appropriate assay development/validation

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Thank you to:

Roland Staack

Julia Heinrich

Ichio Ohnami

Doing now what patients need next

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