Mathematical Simulation Tools in Bioanalytical Assay Development:

The Question of Free vs. Total Drug Quantification

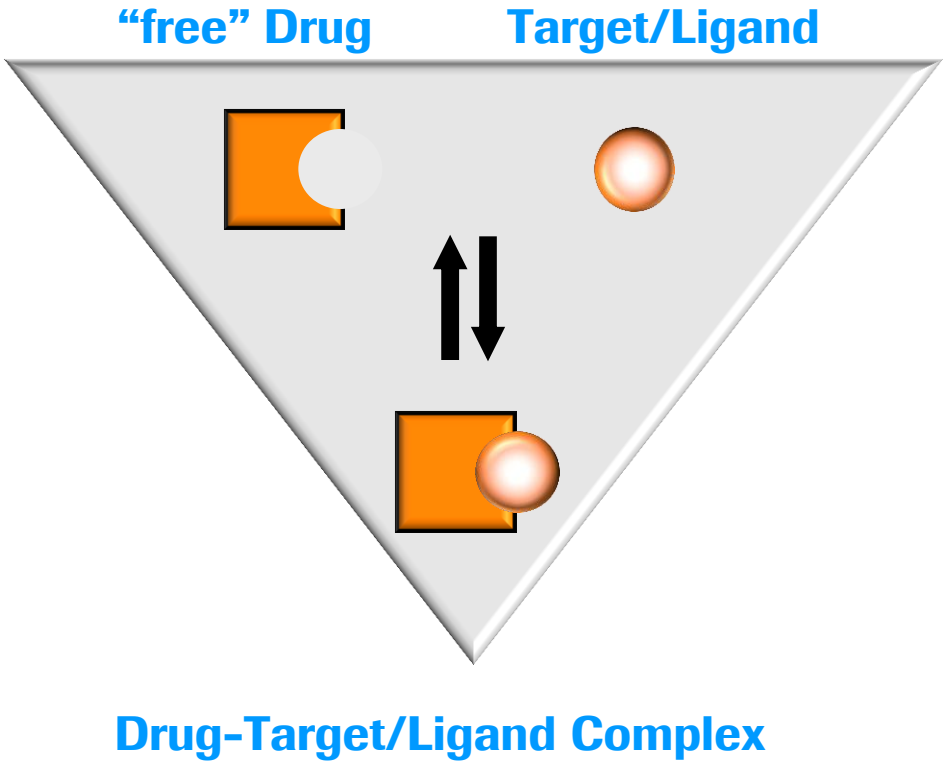
Roland Staack, Gregor Jordan, Julia Heinrich

Pharma Research & Early Development, Roche Penzberg, Germany

EBF Open Symposium , November 16-18, 2011

Free vs. Total Drug

When do we need to differentiate?

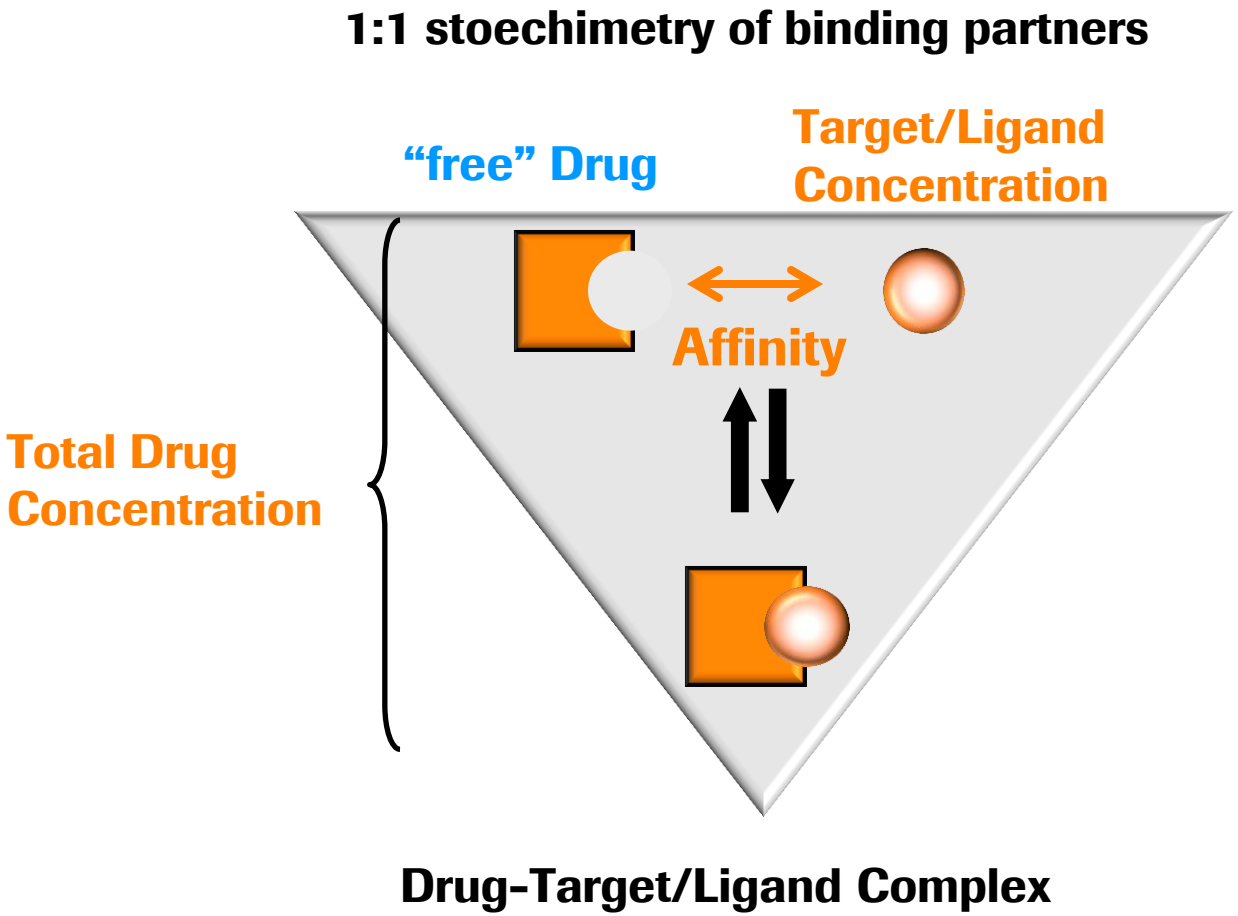


Questions

- What target/ligand concentration has an significant impact on free drug fraction?
  - Bioanalytical Strategy
- Accurate free drug quantification?
  - Bioanalytical Challenges

Free vs. Total Drug

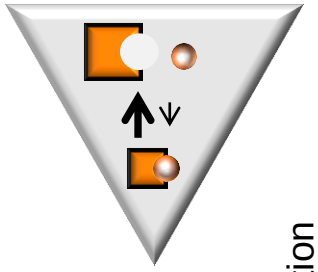
Variables for Mathematical Simulations

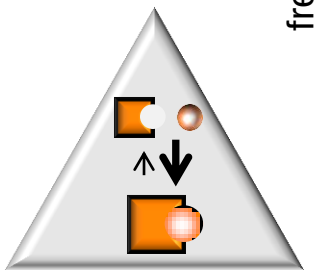


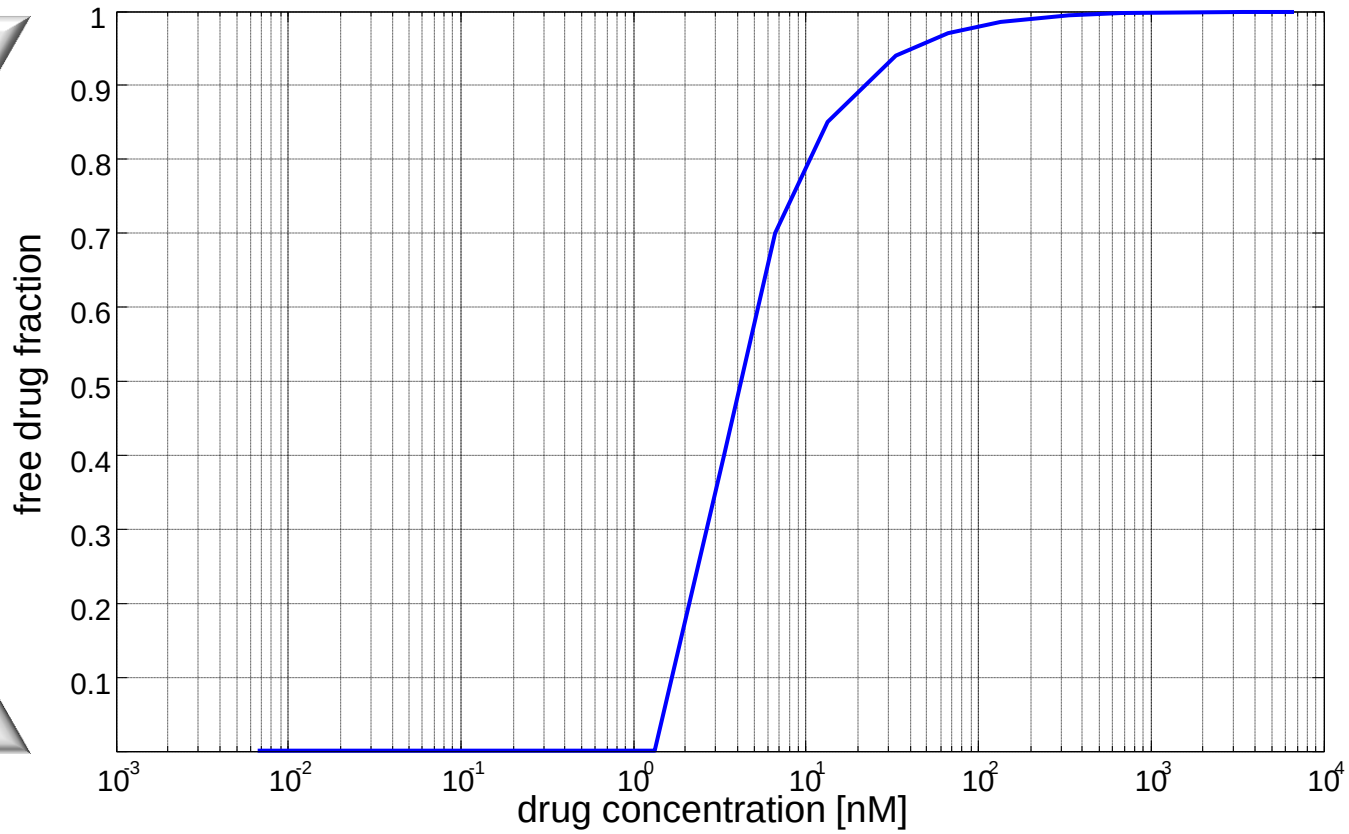
Influence of soluble Target on Free Drug Fraction

"Generic" Approach

Assumption: KD= fixed (1x10^{-18} M), L = fixed, Drug = variable



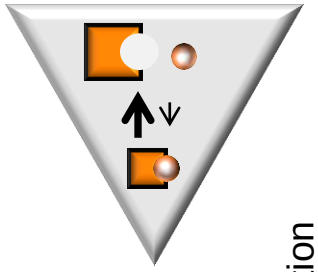


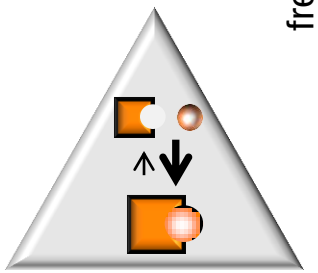


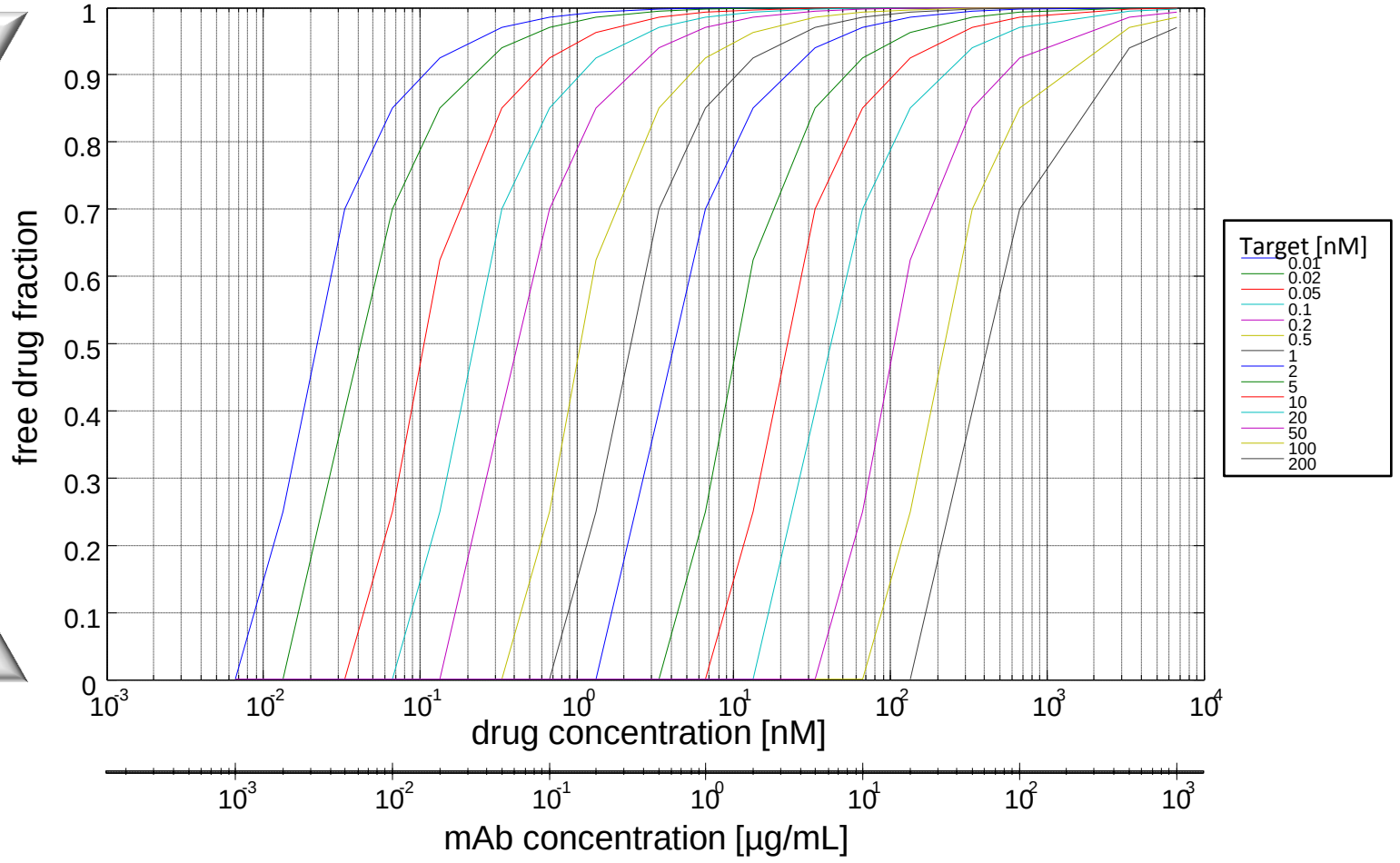
Influence of soluble Target on Free Drug Fraction

"Generic" Approach

Assumption: KD= fixed (1x10^{-18} M), L = fixed, Drug = variable







Influence of soluble Target on Free Drug Fraction

"Generic" Considerations

Molar
Drug:Target ratio

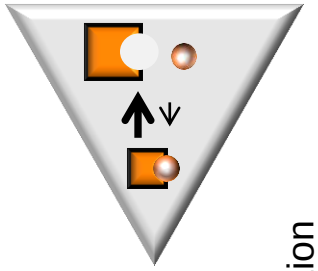
≤5

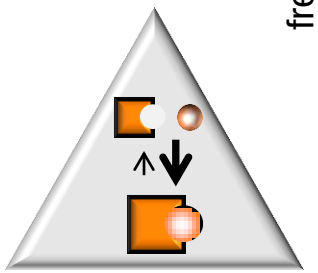
↑

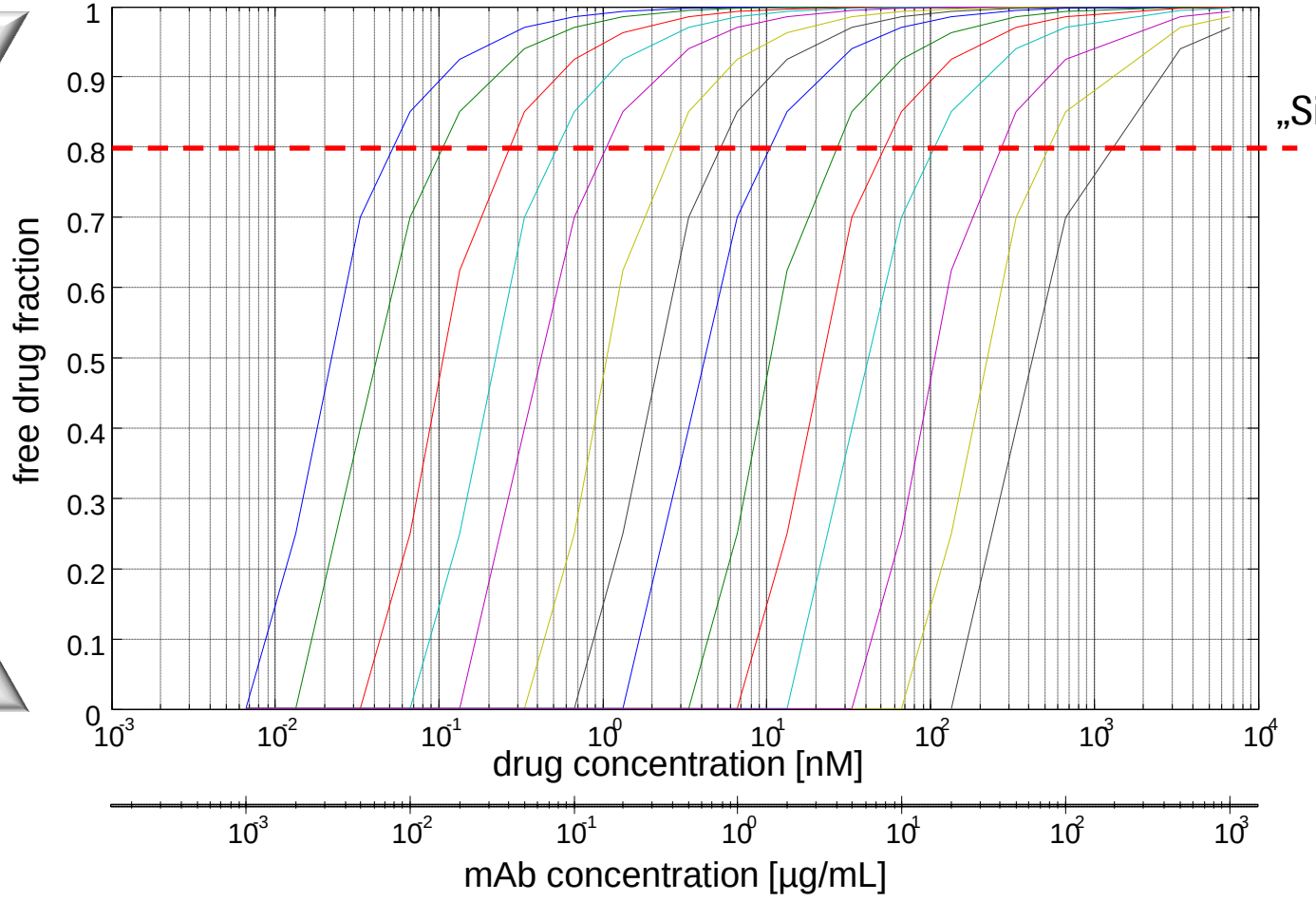
„Significant" Influence:

80%

Assumption: KD= fixed (1x10^{-18} M), L = fixed, Drug = variable







Influence of soluble Target on Free Drug Fraction

"Generic" Considerations

Molar
Drug:Target ratio

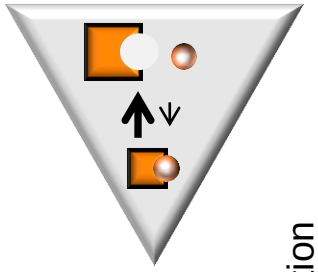
≤5

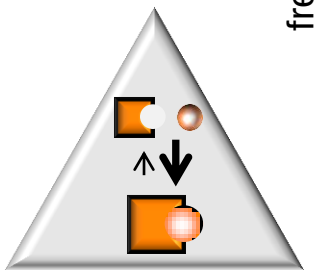
↑

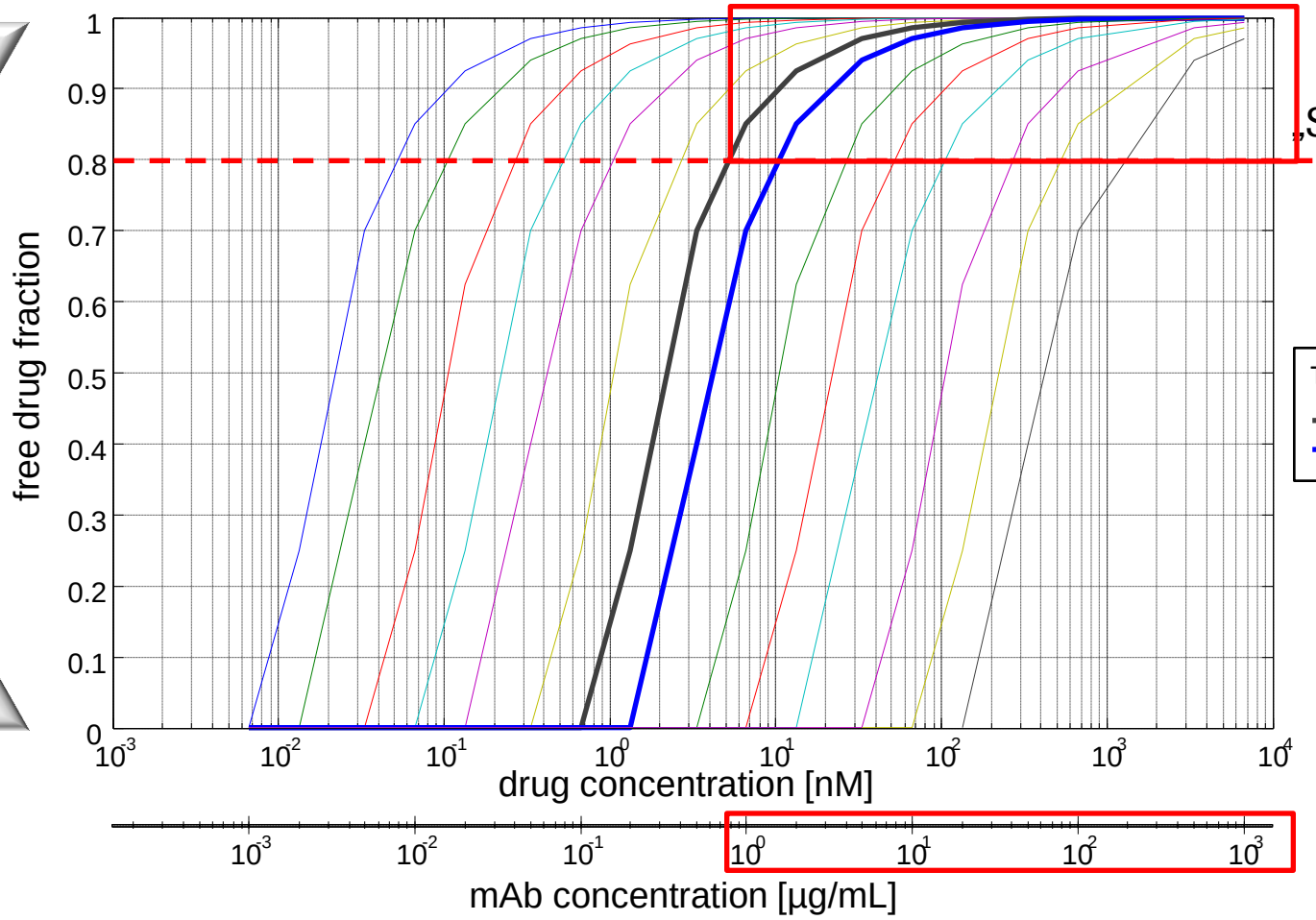
„Significant" Influence:

80%

Assumption: KD= fixed (1x10^{-18} M), L = fixed, Drug = variable





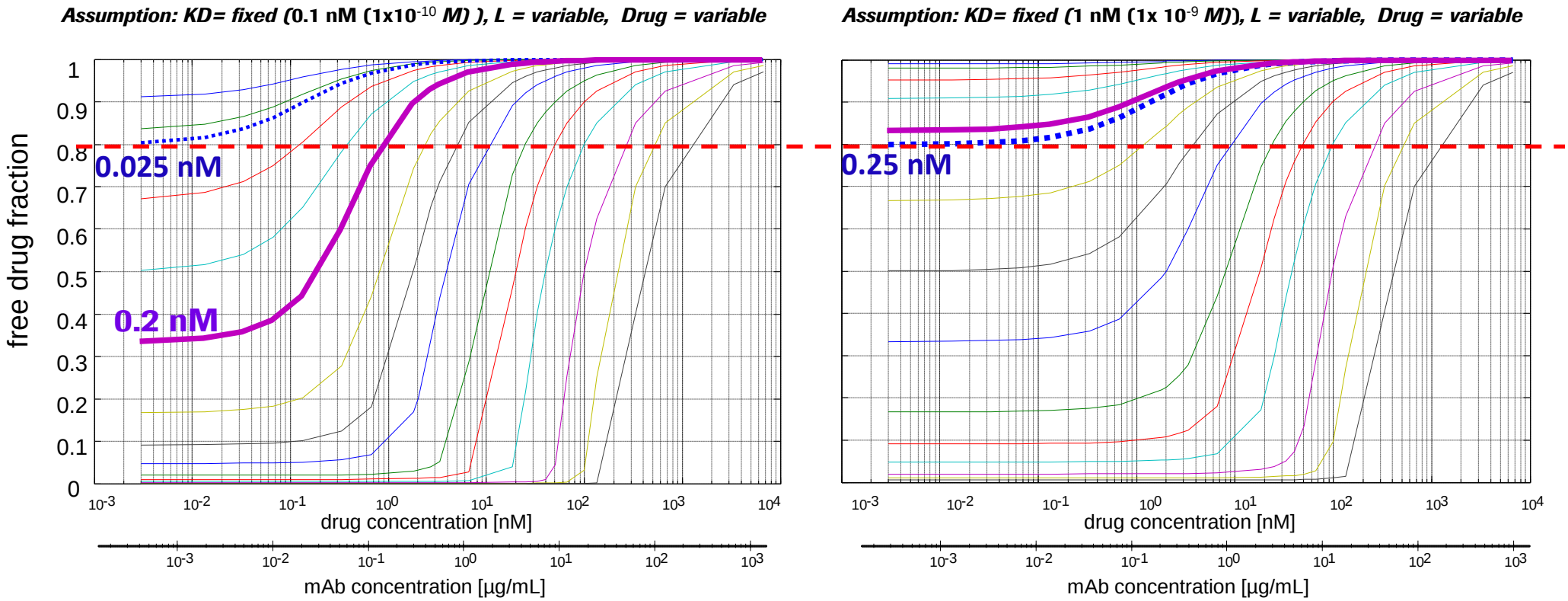


mAb drugs:

„µg/mL- range"

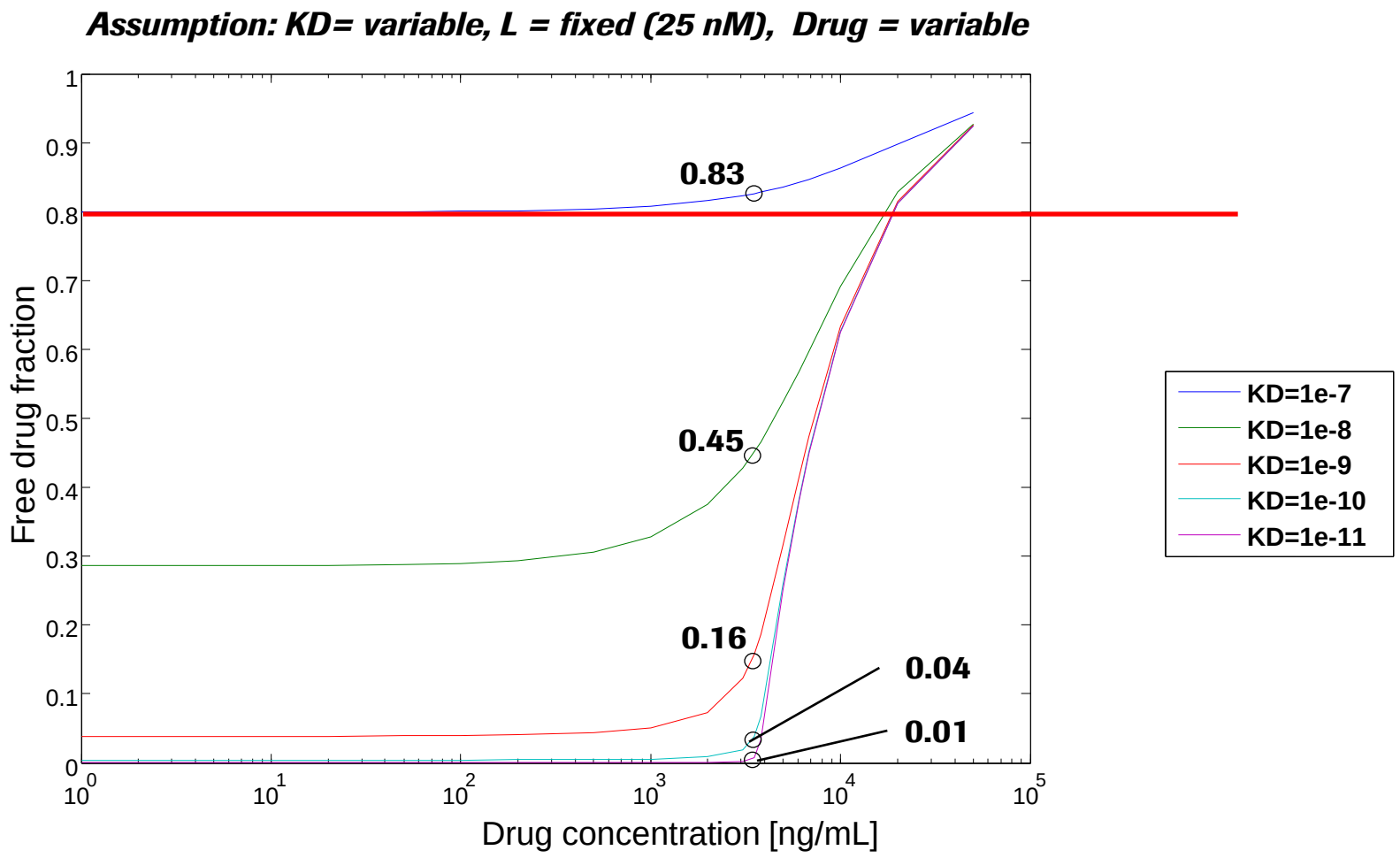
Influence of soluble Target on Free Drug Fraction

"Realistic" Conditions



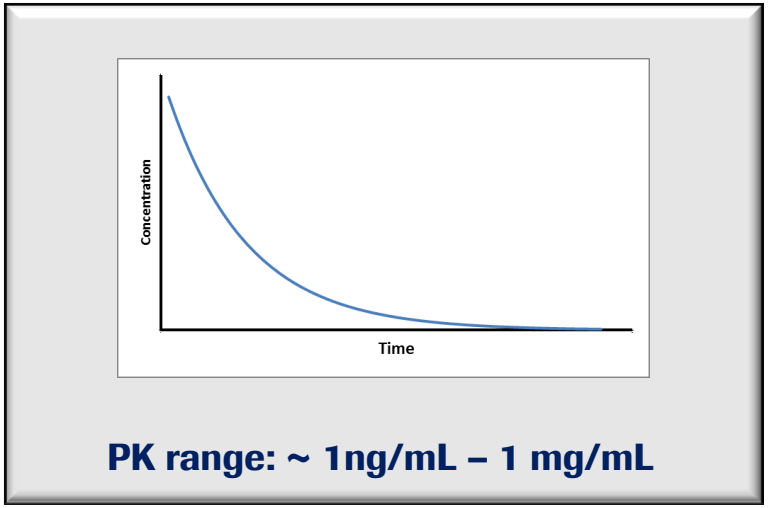
Influence of soluble Target on Free Drug Fraction

Influence of Drug:Target Affinity



Bioanalytical Challenges

Sample Dilution



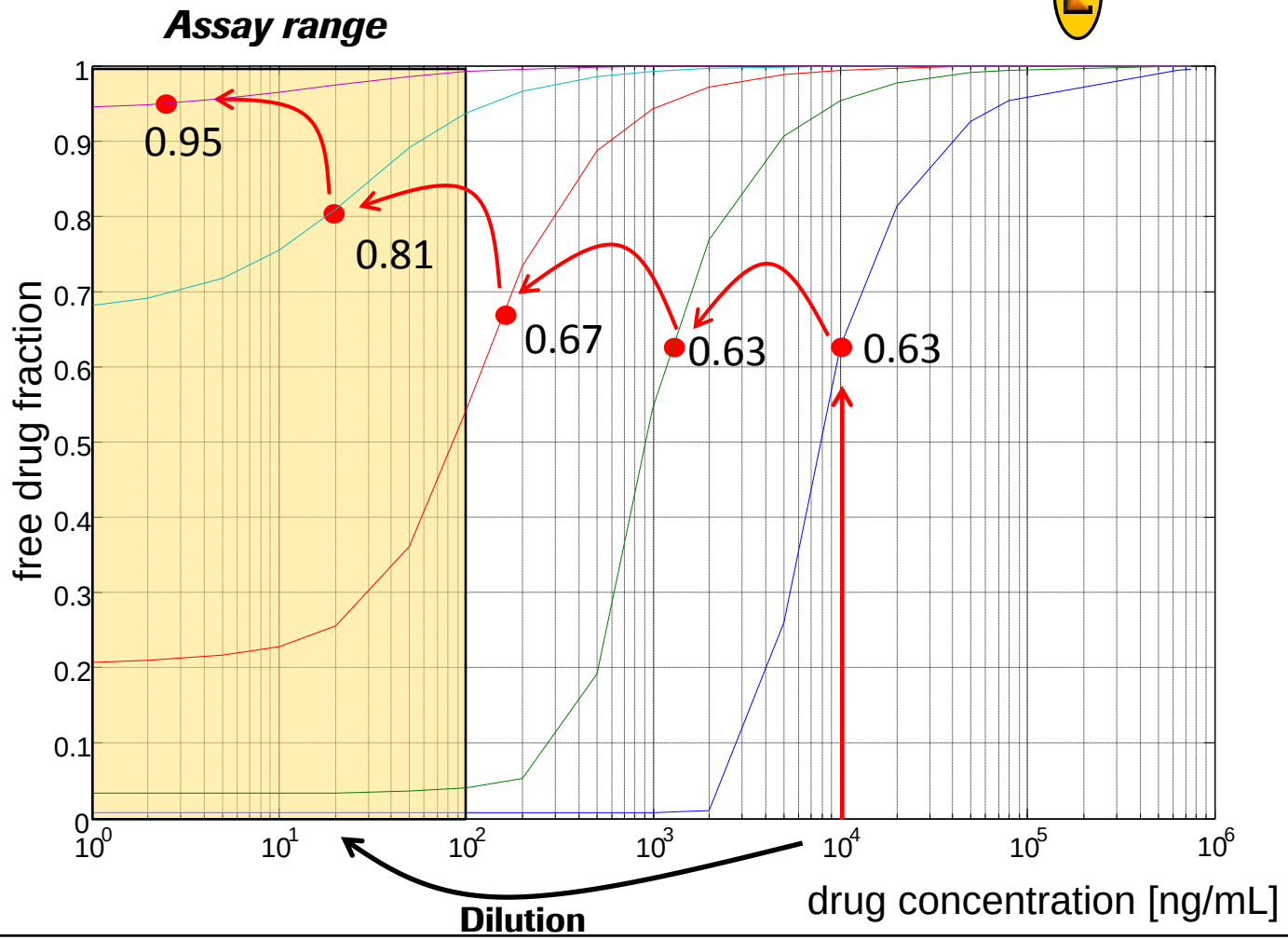
- **Impact of Sample dilution on free drug fraction?**

- **Impact of dilution solvent: buffer vs. matrix?**

Bioanalytical Challenges: Sample Dilution

Buffer Dilution

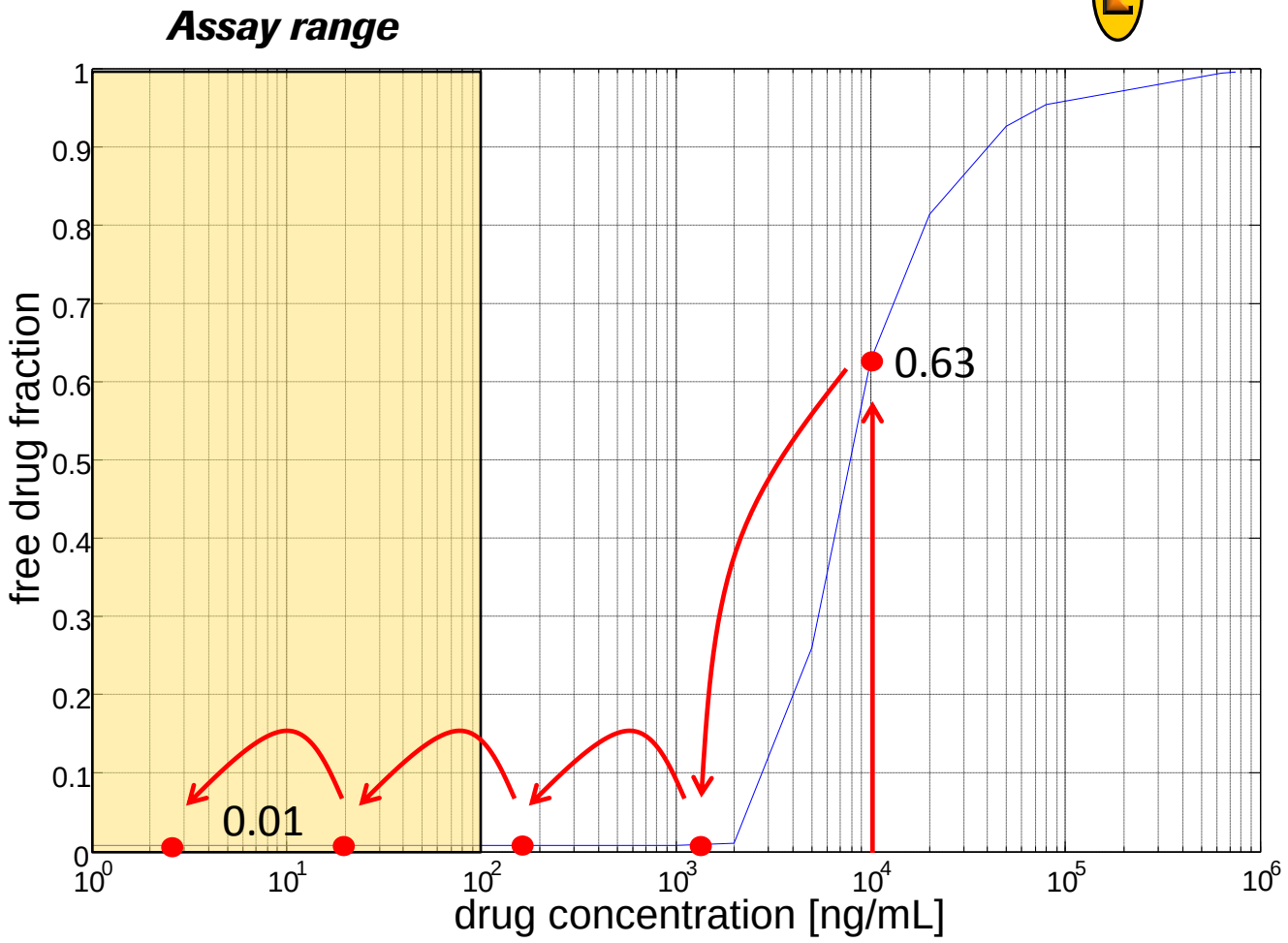
Target: 25 nM
KD: 1x10^{-10} M



Bioanalytical Challenges: Sample Dilution

Matrix (target-containing) Dilution

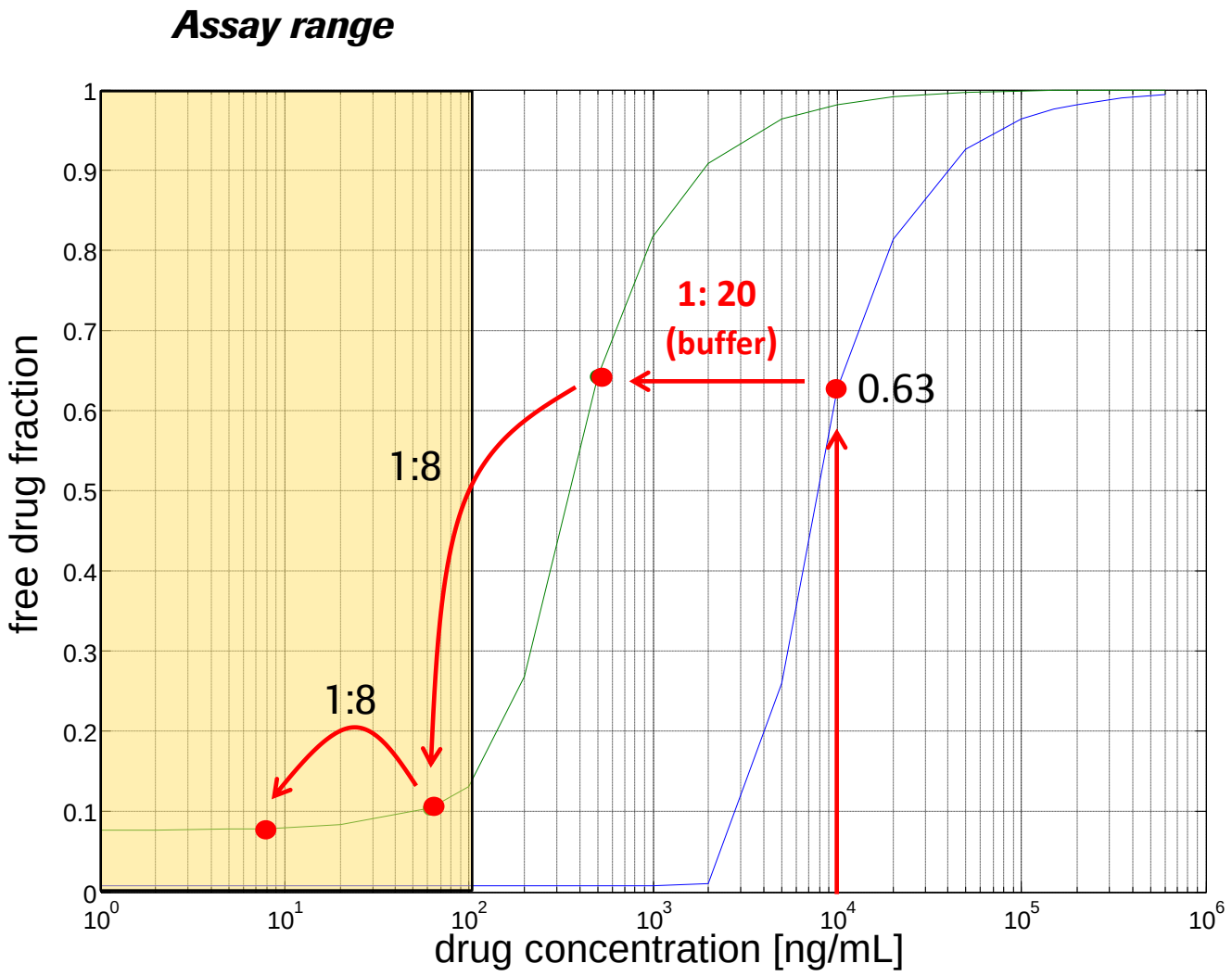
Target: 25 nM
KD: 1x10^{-10} M



Bioanalytical Challenges: Sample Dilution

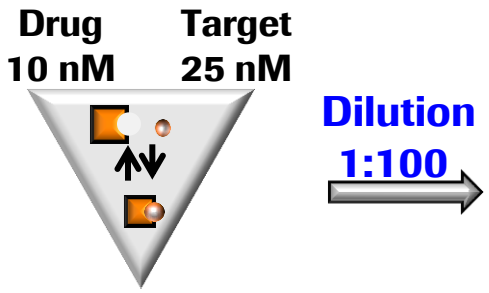
Typical Dilution Scheme

Target: 25 nM
KD: 1x10^{-10} M



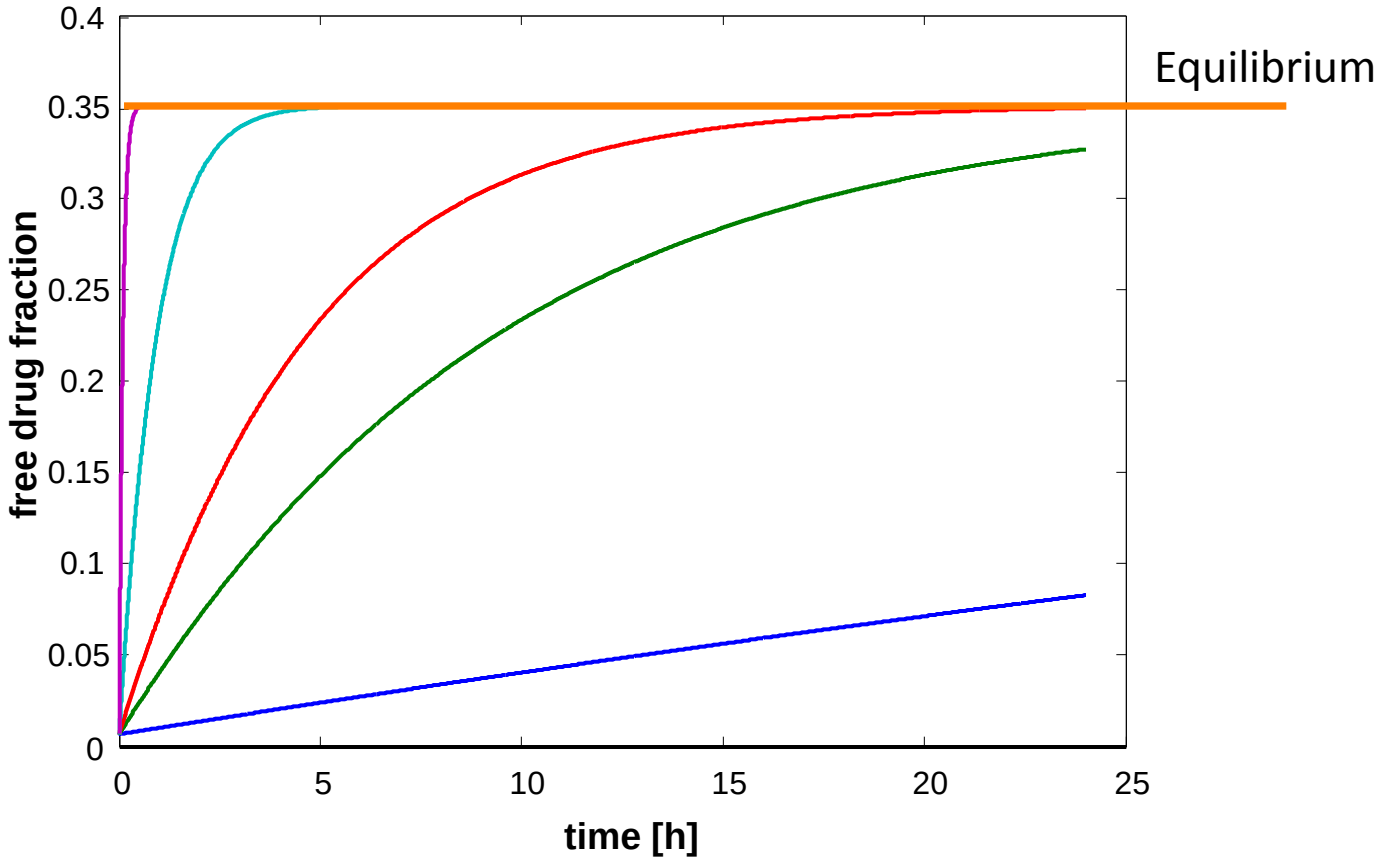
Bioanalytical Challenges: Sample Dilution

Impact of Binding Kinetics



Dilution 1:100

KD
1 x 10^{-10} M

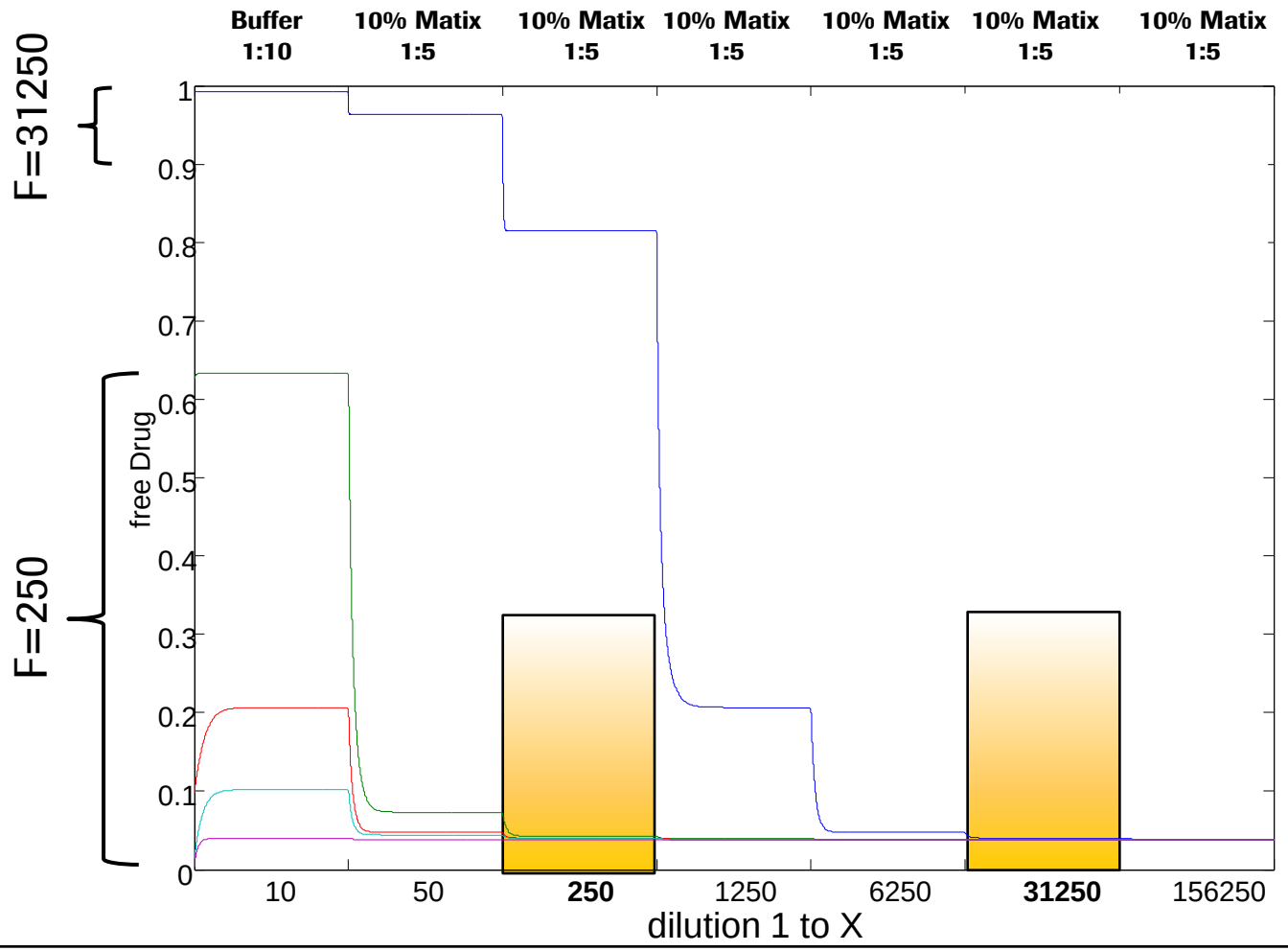


- ka=10000 $M^{-1}s^{-1}$;kd=0.000001 s^{-1}
- ka=100000 $M^{-1}s^{-1}$;kd=0.00001 s^{-1}
- ka=200000 $M^{-1}s^{-1}$;kd=0.00002 s^{-1}
- ka=1000000 $M^{-1}s^{-1}$;kd=0.0001 s^{-1}
- ka=10000000 $M^{-1}s^{-1}$;kd=0.001 s^{-1}

Bioanalytical Challenges: Sample Dilution

Normalization

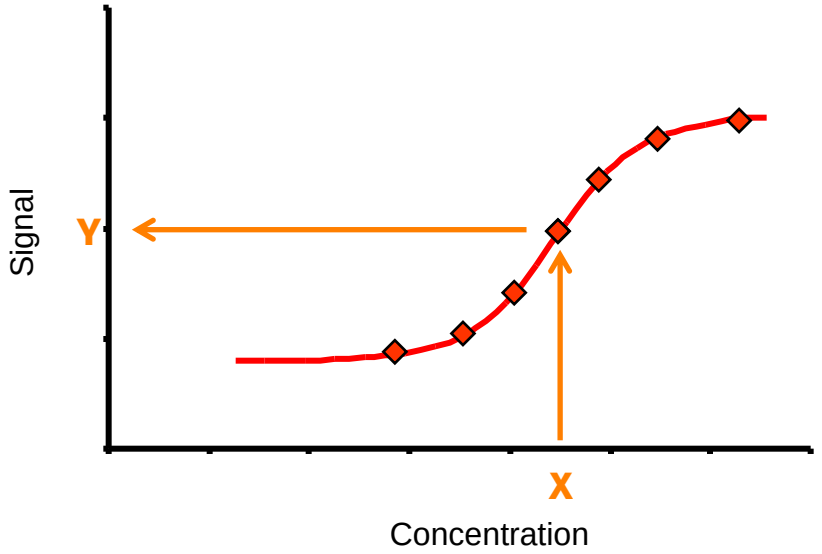
Target: 25 nM
KD = 0,1nM
Drug:
- 500 µg/mL mAb in 100% Serum
- 10 µg/mL mAb in 100% Serum
- 4 µg/mL mAb in 100% Serum
- 2.7 µg/mL mAb in 100% Serum
- 0.1 µg/mL mAb in 100% Serum

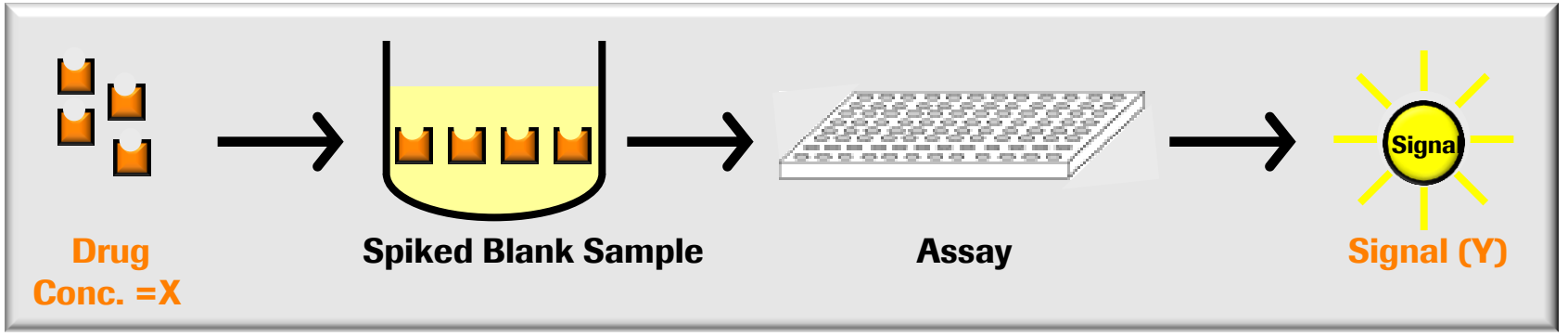


Bioanalytical Challenges: Accurate Quantification

Calibration

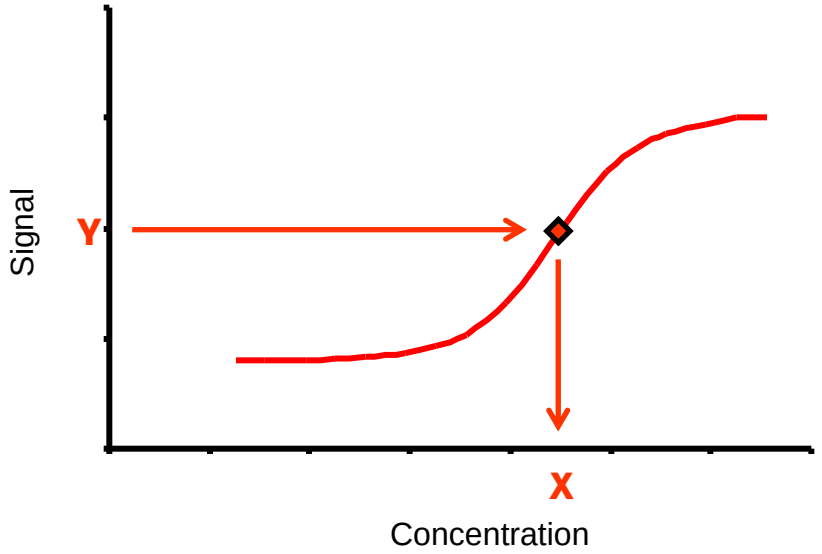
"Standard" Calibration

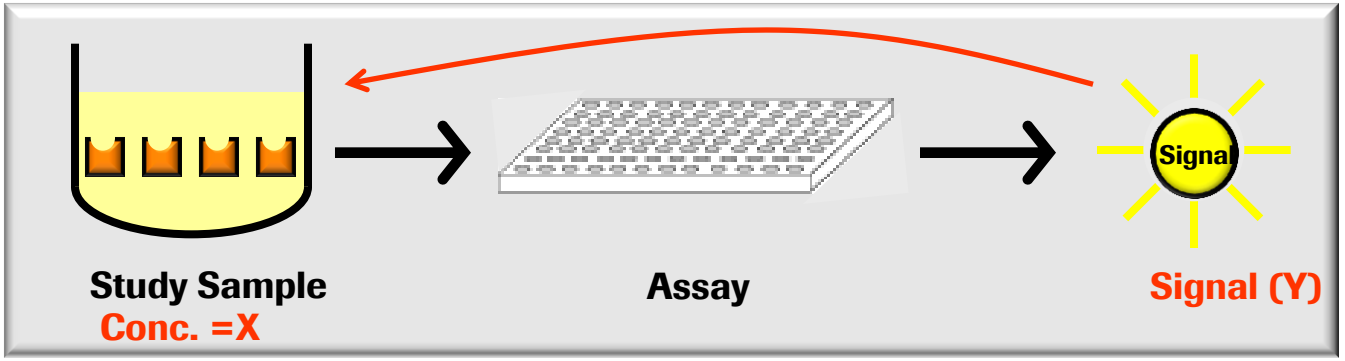




Bioanalytical Challenges: Accurate Quantification

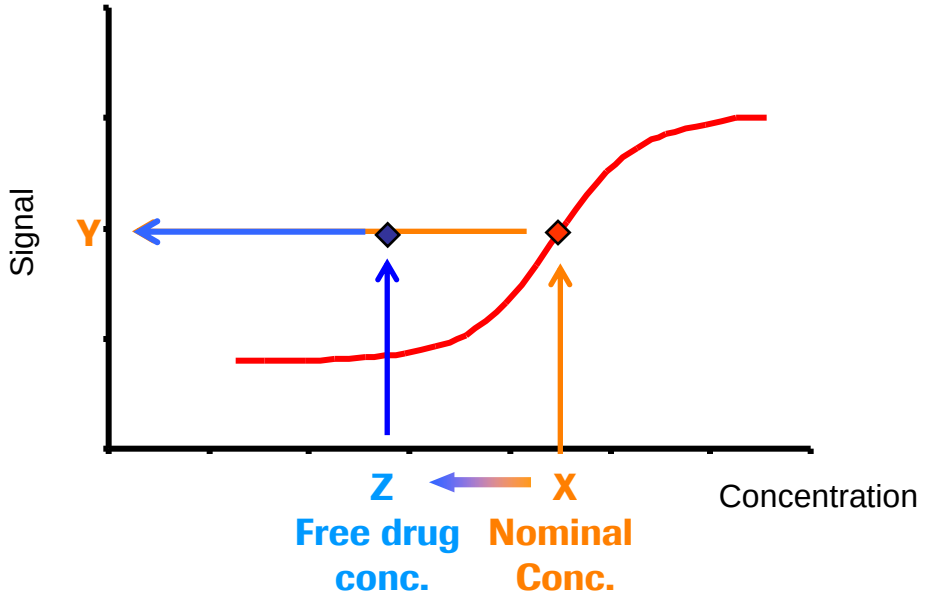
Quantification

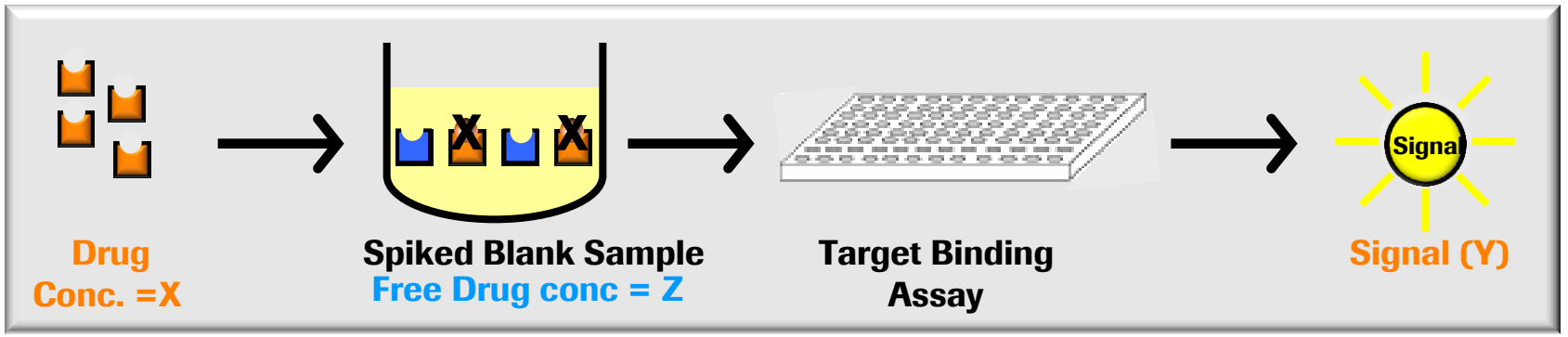




Bioanalytical Challenges: Accurate Quantification

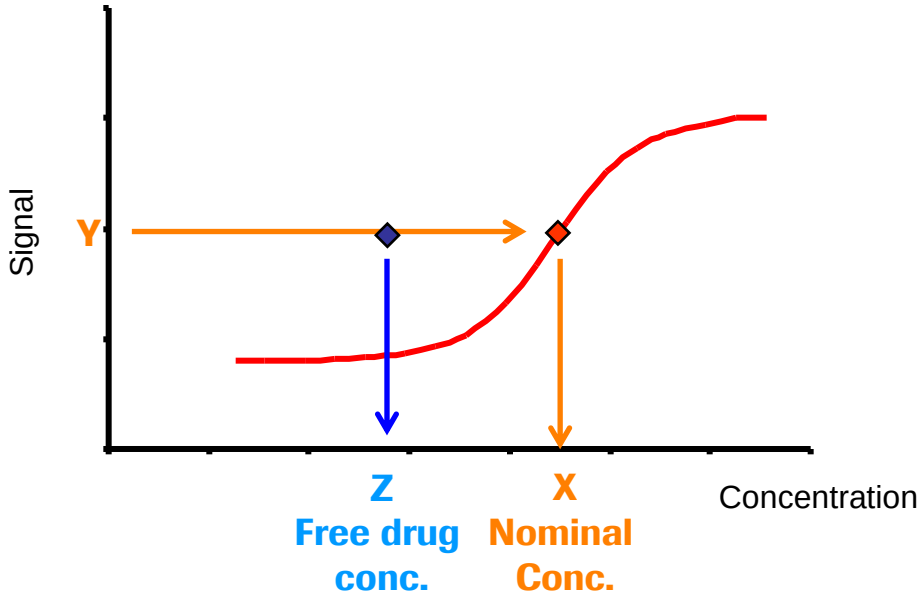
Calibration: Target containing Matrix

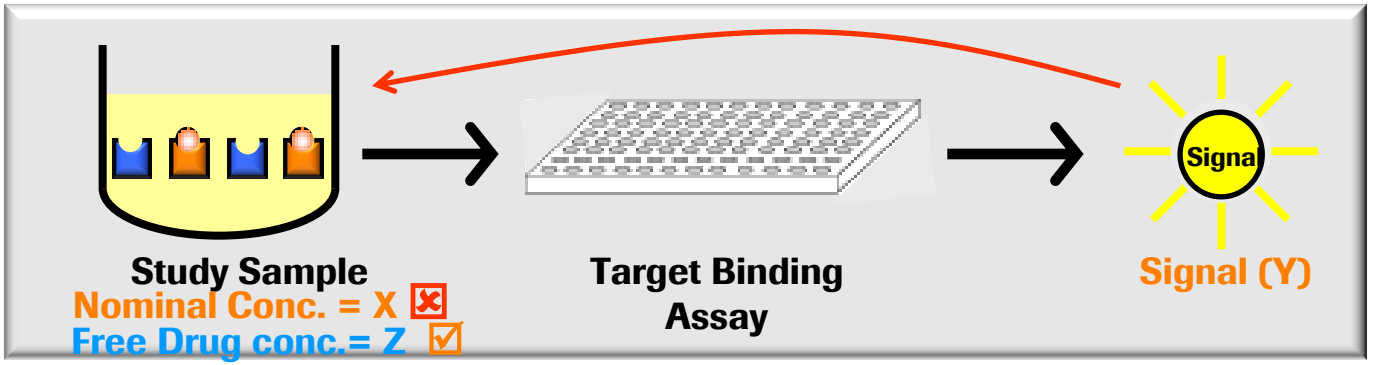




Bioanalytical Challenges: Accurate Quantification

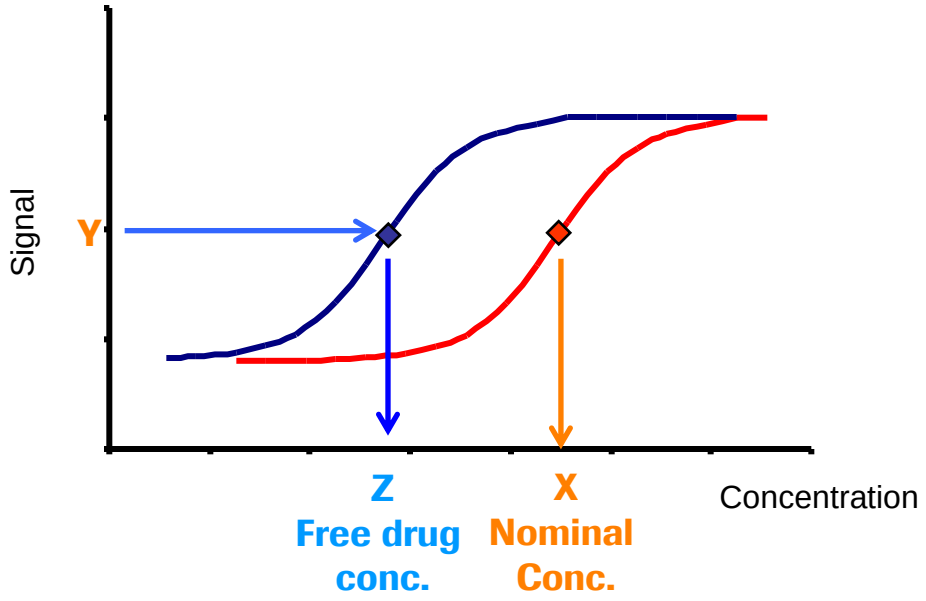
Quantification: Calibration in Target containing Matrix

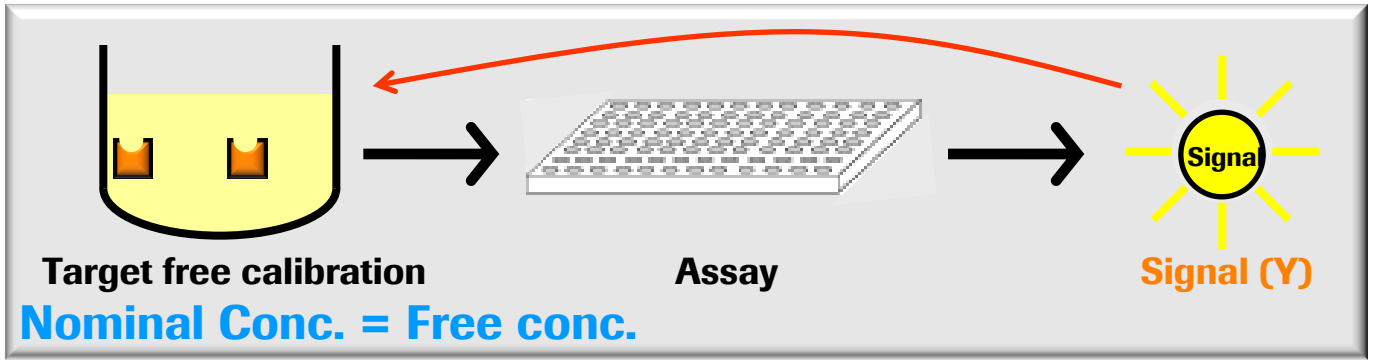




Bioanalytical Challenges: Accurate Quantification

→ *Target free Calibration required*





Summary & Conclusion

- Definition of an appropriate **bioanalytical strategy** requires considerations of
  - target biology – (expected) drug concentrations – **Drug:Target binding affinity/kinetics**
  - **Bioanalytical challenges**: accurate free drug quantification
  - Prerequisite: suitable assay format (e.g. target capture assay)
  - Impact of sample processing needs to be considered
  - Appropriate calibration concept required
  - **Mathematical simulation tools**
  - Valuable tool for a rational evaluation of the bioanalytical challenges
    - Definition of the bioanalytical assay strategy
    - Assessment of capabilities and limitations of a given assay
    - Guidance during assay development

Acknowledgement

Protein
Analytics & Bioanalytics Development
Apollon Papadimitriou

Uwe Dahl
Miriam Moheysen-Zadeh
Alexander Pöhler

We Innovate Healthcare