

# Overcoming drug & target interference in ADA and nAb assays

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# Presentation Outline

- **Case Study 1**

- Drug & target interference in a clinical anti-drug antibody (ADA) assay

- **Case Study 2**

- Drug & target interference in a clinical neutralizing antibody (nAb) assay

# Case Study 1

## Drug & target interference in a clinical anti-drug antibody (ADA) assay

### ■ Background

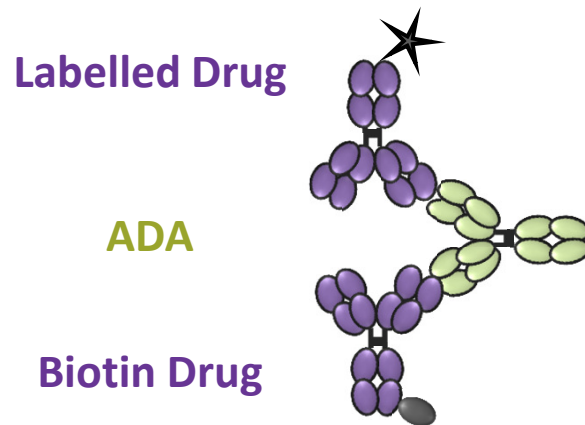
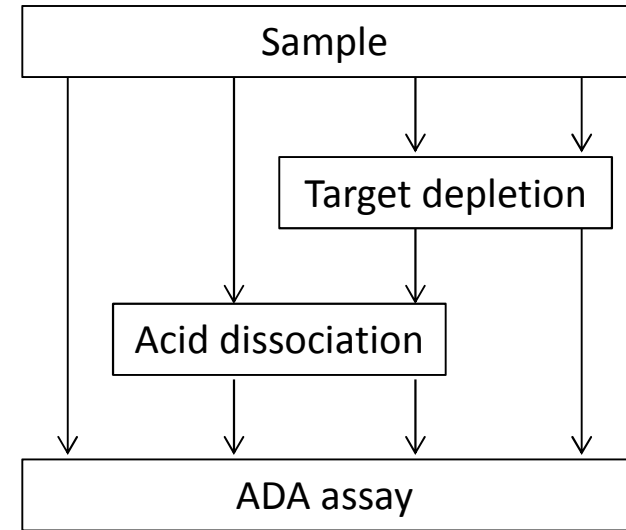
- Monoclonal antibody (mAb) therapeutic with a target in inflammatory disease
- Dosing levels in mg/kg range
- Dimeric soluble target

# Case Study 1: ADA Assay Considerations

- Anticipate **drug** levels based on dosing regimen
- Predict **target** levels
  - Access to healthy volunteer samples
  - Limited access to patient samples
  - **Accumulation of target in presence of drug**
    - Literature
    - Exploratory data
    - PK-PD modelling

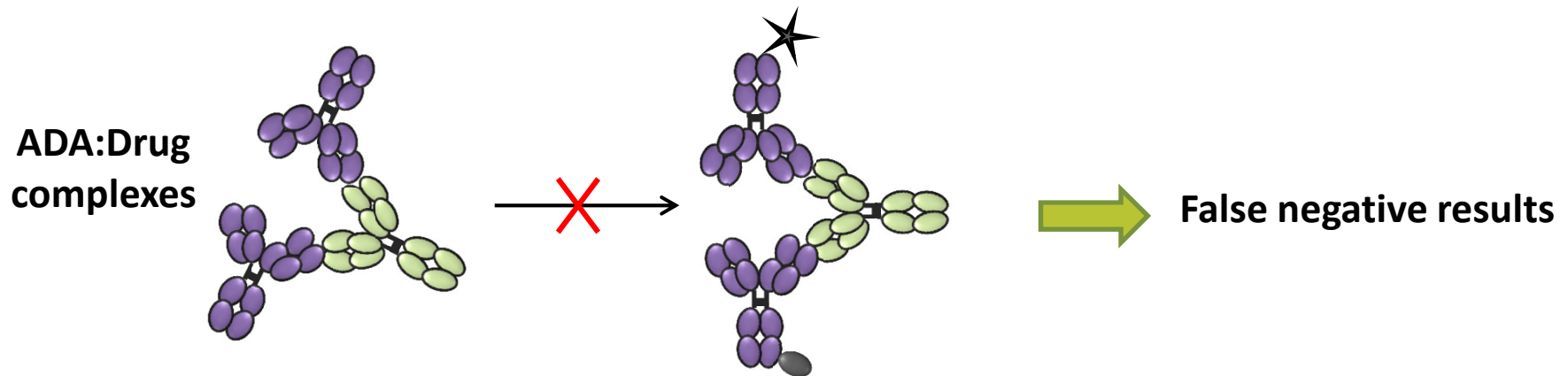
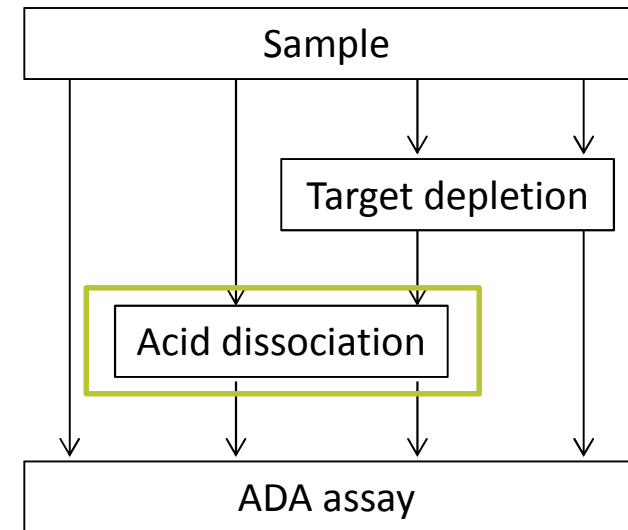
# Case Study 1: ADA Assay Development

- **Bridging format**
  - Gyrolab platform (Bioaffy 200 nL CD)



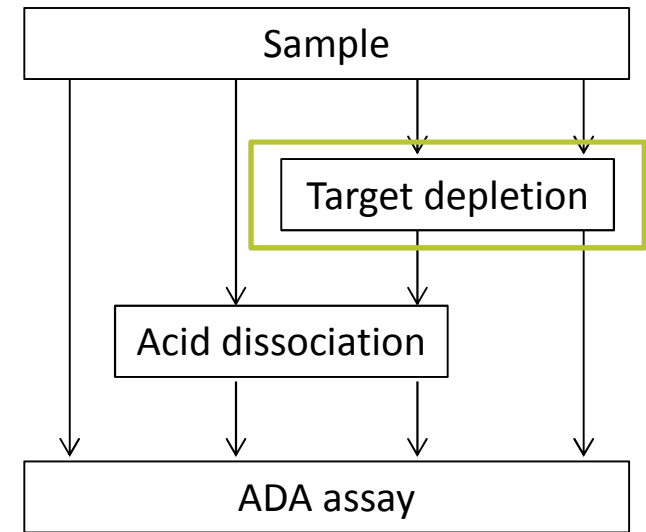
# Case Study 1: ADA Assay Development

- **Bridging format**
  - Gyrolab platform (Bioaffy 200 nL CD)
- **Acid dissociation**
  - 300 mM acetic acid
  - Drug tolerance of 20 µg/mL



# Case Study 1: ADA Assay Development

- **Bridging format**
  - Gyrolab platform (Bioaffy 200 nL CD)
- **Acid dissociation**
  - 300 mM acetic acid
  - Drug tolerance of 20 µg/mL
- **Target Depletion**
  - Target tolerance of 50 ng/mL



Labeled Drug:Target complexes



➔ False positive results

# For further details see:

Hindawi Publishing Corporation  
Journal of Immunology Research  
Volume 2016, Article ID 5069678, 15 pages  
<http://dx.doi.org/10.1155/2016/5069678>



*Research Article*

## **Evaluation of Multiple Immunoassay Technology Platforms to Select the Anti-Drug Antibody Assay Exhibiting the Most Appropriate Drug and Target Tolerance**

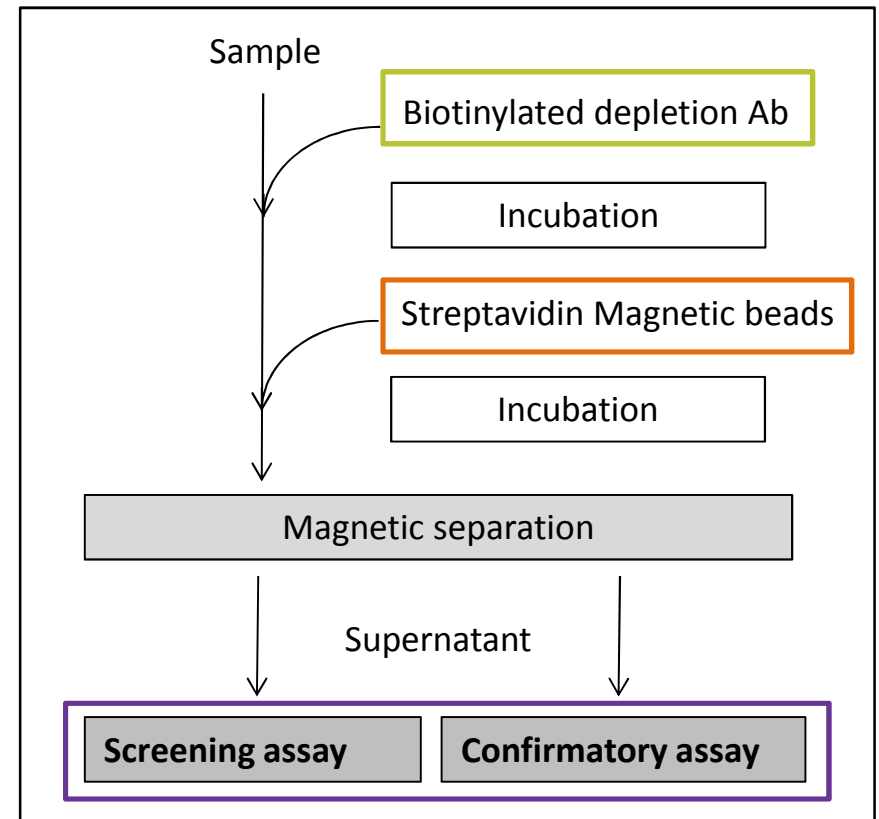
**Justine Collet-Brose, Pierre-Jean Couble, Maureen R. Deehan, Robert J. Nelson, Walter G. Ferlin, and Sabrina Lory**

*Novimmune SA, 14 Chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland*



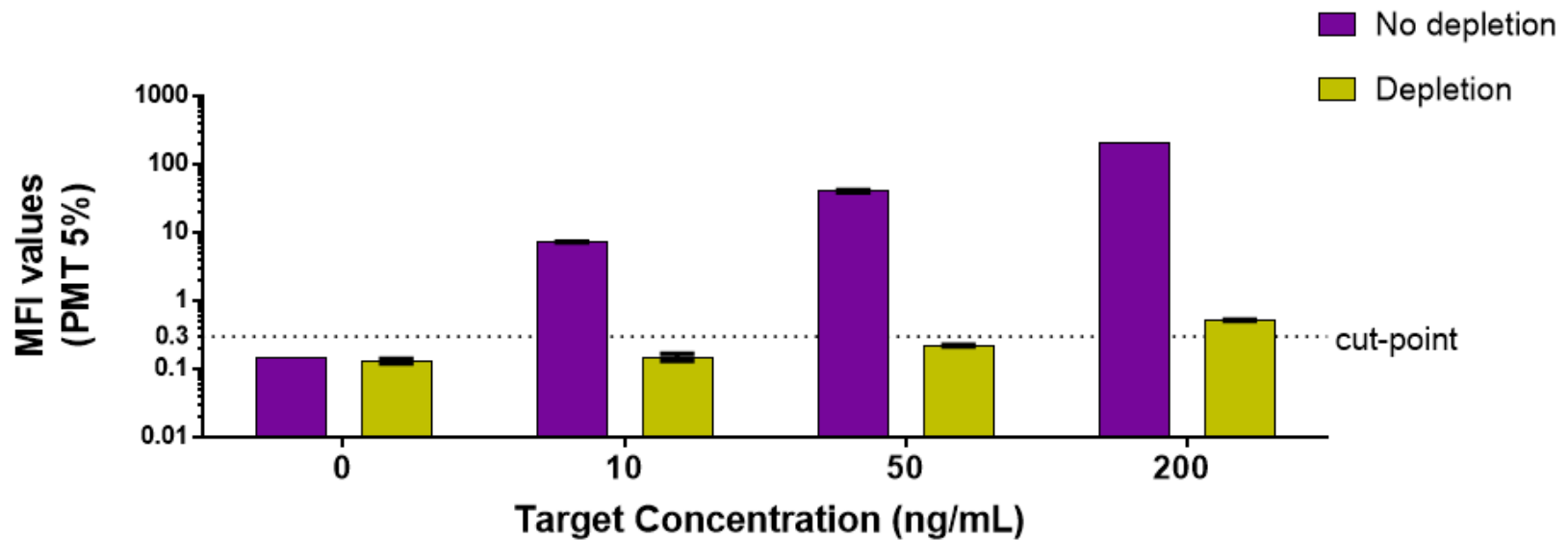
# Case Study 1: ADA Assay Development

- **Target Depletion Protocol**
  - Anti-target antibody **non-competitive** with drug
  - **Solid phase extraction (SPE)** with magnetic beads
  - Anti-target Ab **competitive** with drug added to capture-detection mixture



# Case Study 1: ADA Assay Development

- **Target Depletion Protocol**
  - Target interference addressed effectively



# Case Study 2

## Drug & target interference in a clinical neutralizing antibody (nAb) assay

### ■ **Background**

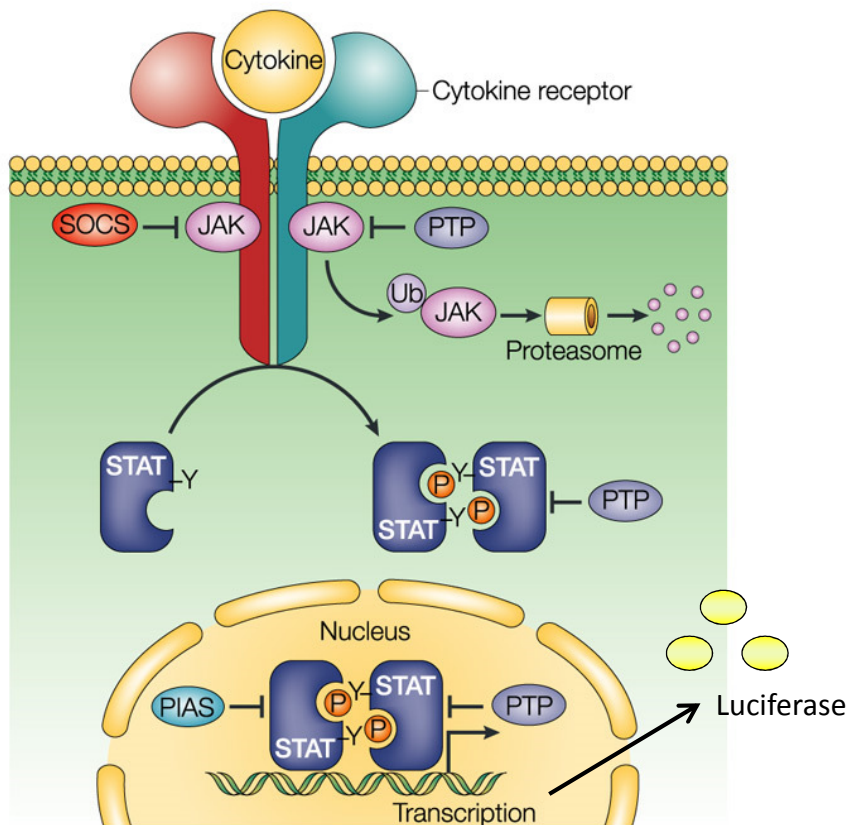
- Monoclonal antibody (mAb) therapeutic with a target in inflammatory disease
- Dosing levels in mg/kg range
- Soluble target

# Case Study 2: nAb Assay Considerations

- **Cell-based nAb assay**
  - Most appropriate format based on mechanism of action
  - Matrix interference
- **Drug interference**
  - May be complexed with nAb (false negative)
  - May cause shift in cell-based assay parameters
- **Target interference**
  - May cause shift in cell-based assay parameters

# Case Study 2: nAb Assay Considerations

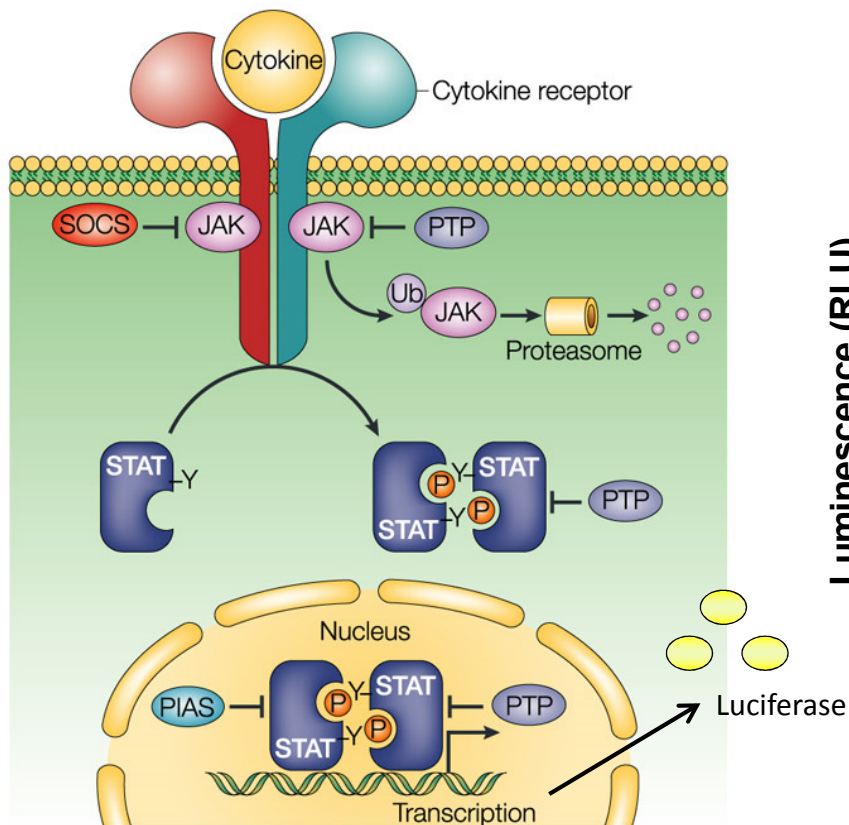
- Cell-based nAb assay



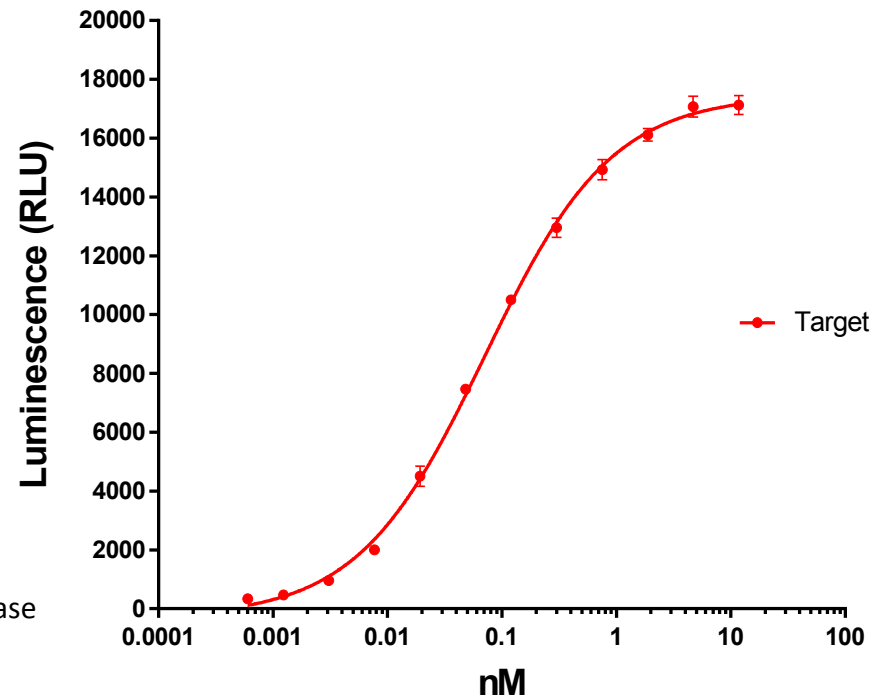
Adapted from Shuai & Liu, Nature Reviews Immunology 3, 900-911 (2003)

# Case Study 2: nAb Assay Considerations

## Cell-based nAb assay



### Concentration-Response Curve

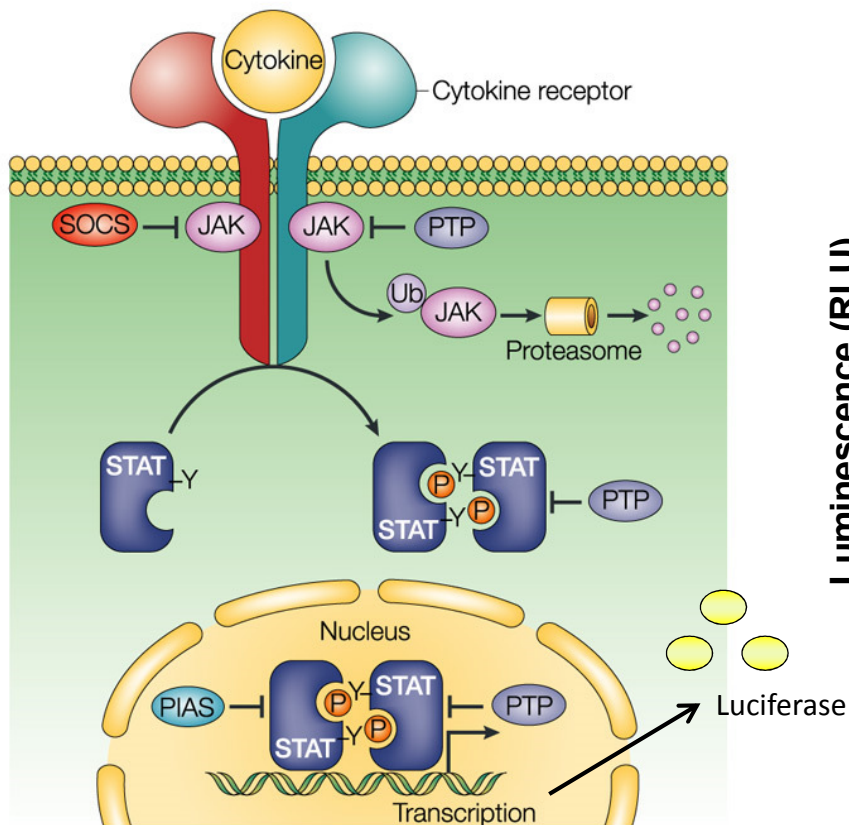


$EC_{50}$ : Approx. 0.1 nM (3.3 ng/mL)

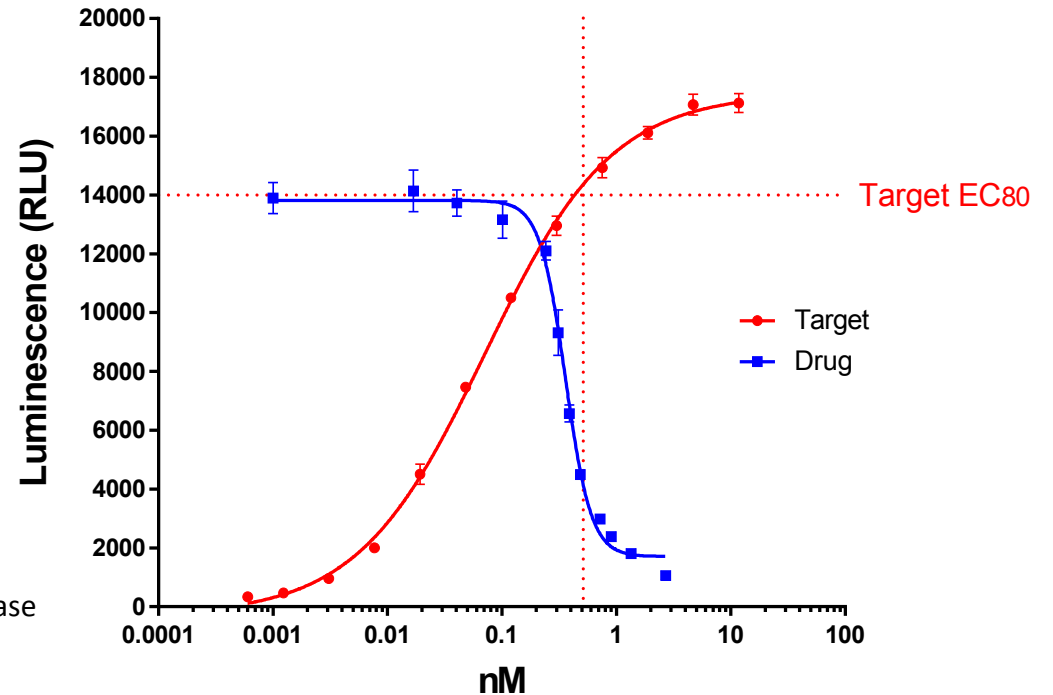
Adapted from Shuai & Liu, Nature Reviews Immunology 3, 900-911 (2003)

# Case Study 2: nAb Assay Considerations

## Cell-based nAb assay



### Concentration-Response Curve

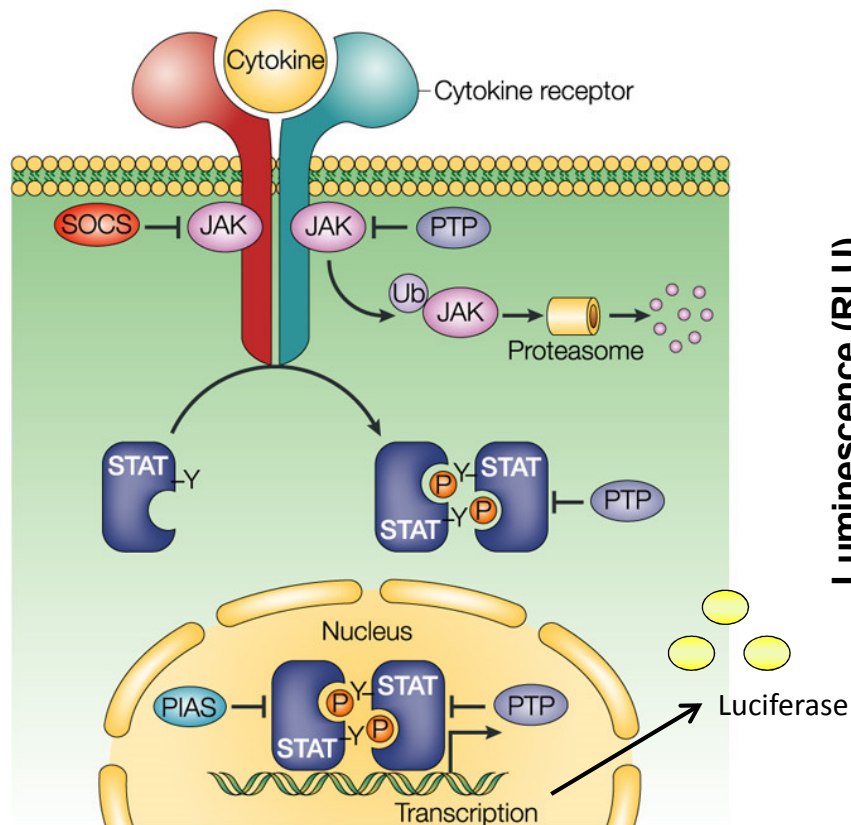


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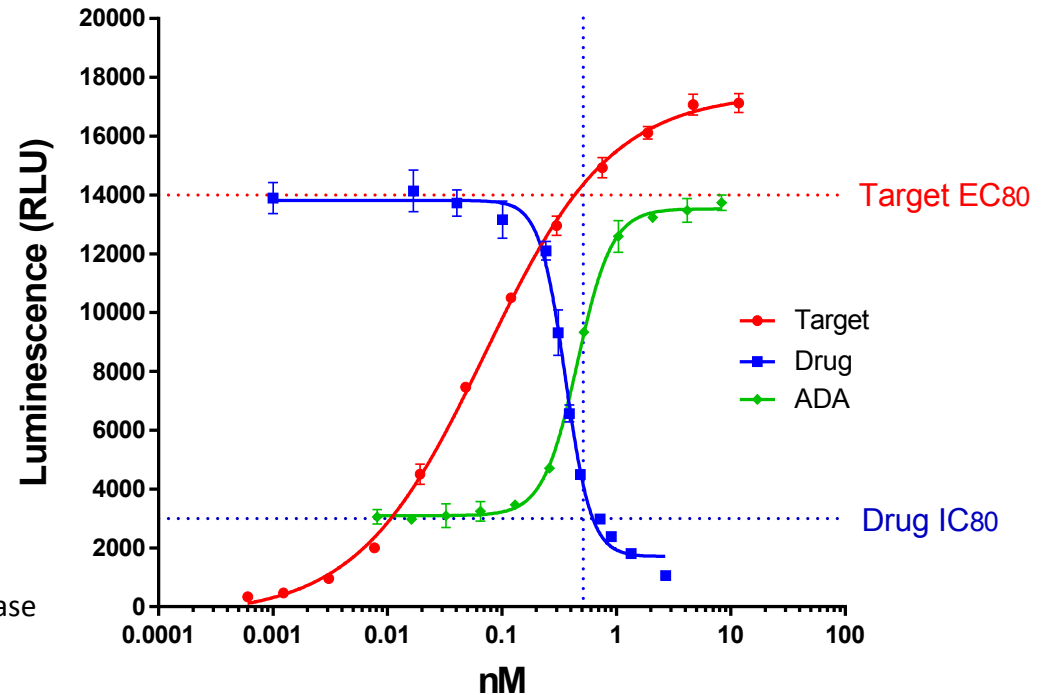
IC<sub>50</sub>: Approx. 0.4 nM (60 ng/mL)

# Case Study 2: nAb Assay Considerations

## Cell-based nAb assay



### Concentration-Response Curve

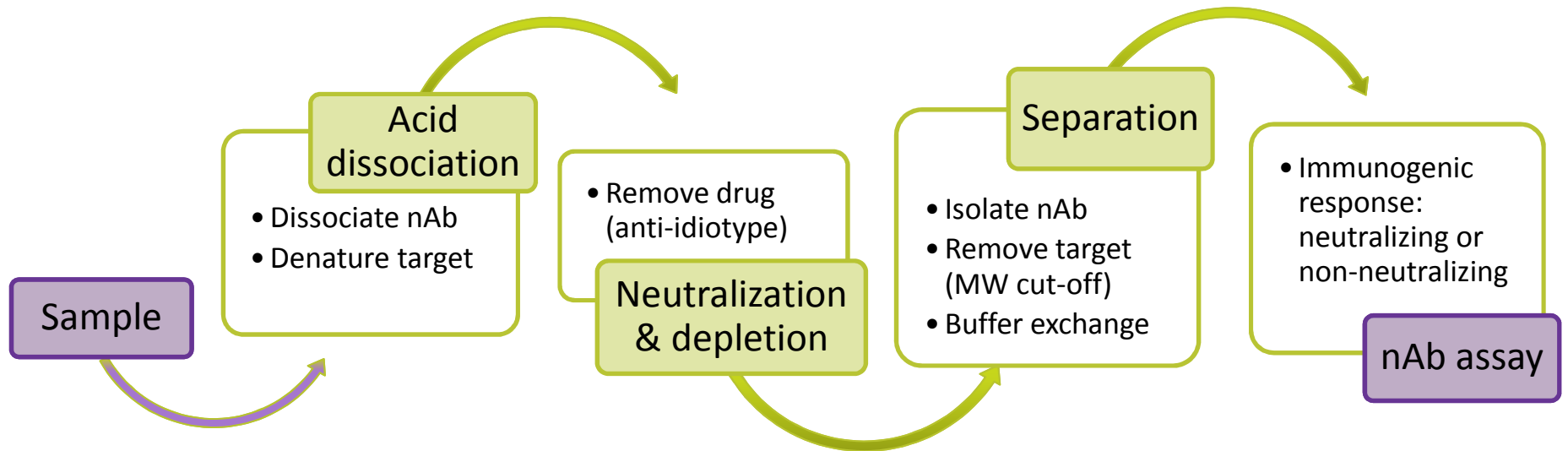


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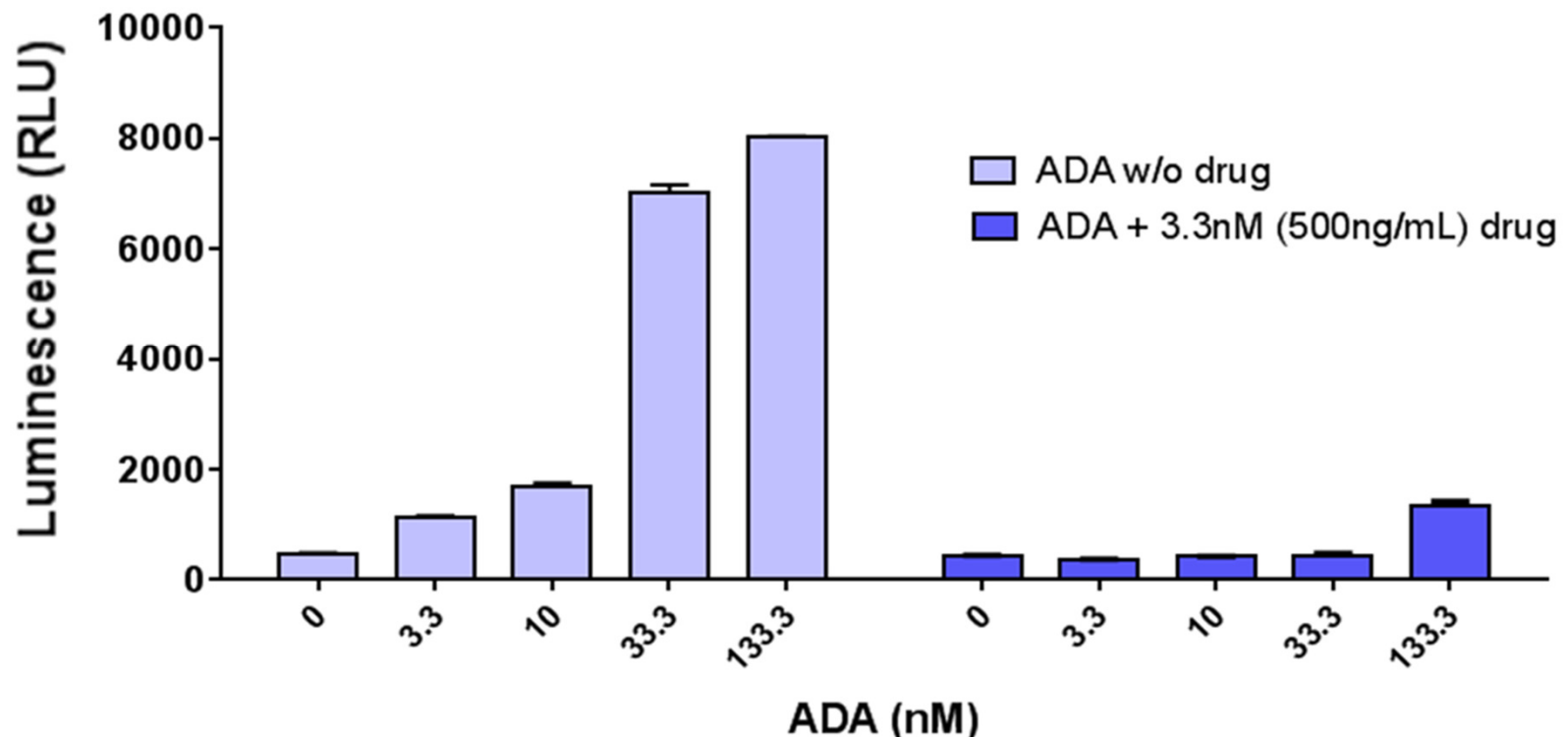
# Case Study 2: nAb Assay Development

## ■ Drug and Target Depletion Protocol



## Case Study 2: nAb Assay Development

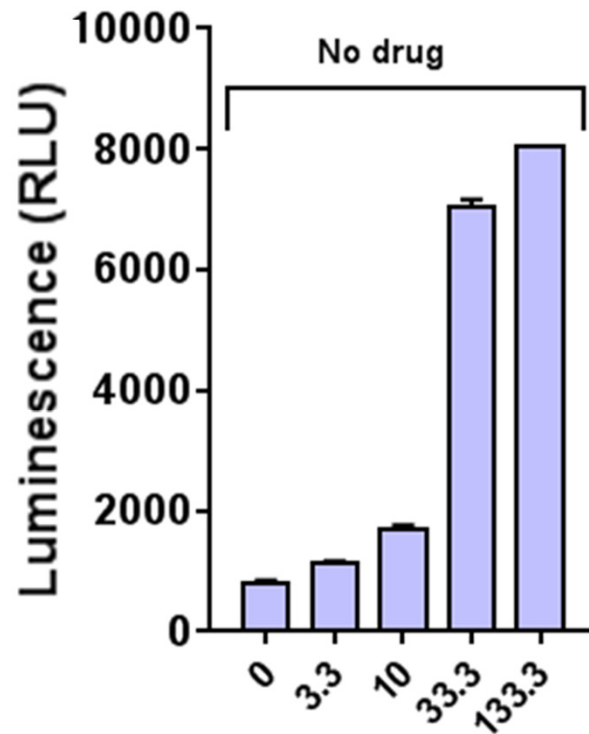
- **Without Drug and Target Depletion Protocol**
  - Low levels of drug interfere with nAb detection



3.3 nM = 500 ng/mL, 10 nM = 1500 ng/mL, 33.3 nM = 5,000 ng/mL, 133.3 nM = 20,000 ng/mL

# Case Study 2: nAb Assay Development

- **With Drug and Target Depletion Protocol**
  - Sensitivity of assay retained

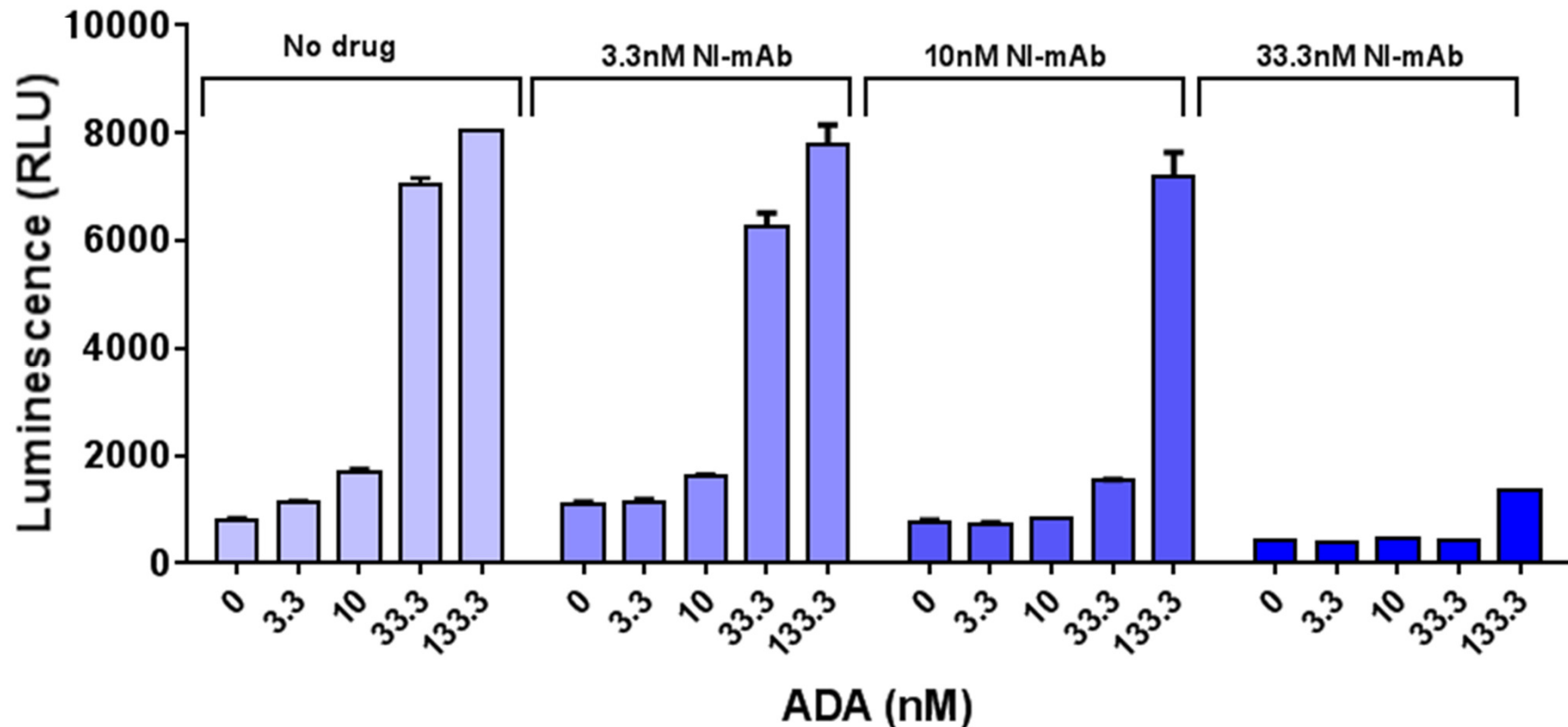


ADA (nM)

3.3 nM = 500 ng/mL, 10 nM = 1500 ng/mL, 33.3 nM = 5,000 ng/mL, 133.3 nM = 20,000 ng/mL

# Case Study 2: nAb Assay Development

- **With Drug and Target Depletion Protocol**
  - Drug interference greatly reduced



3.3 nM = 500 ng/mL, 10 nM = 1500 ng/mL, 33.3 nM = 5,000 ng/mL, 133.3 nM = 20,000 ng/mL

# Thank you for your attention!

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Image source: FreImages.com/Jonathan Woodcock