Some fundamentals of immunogenicity assessment and a case study

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Bioassays and Bioanalytical Method Development - IIR

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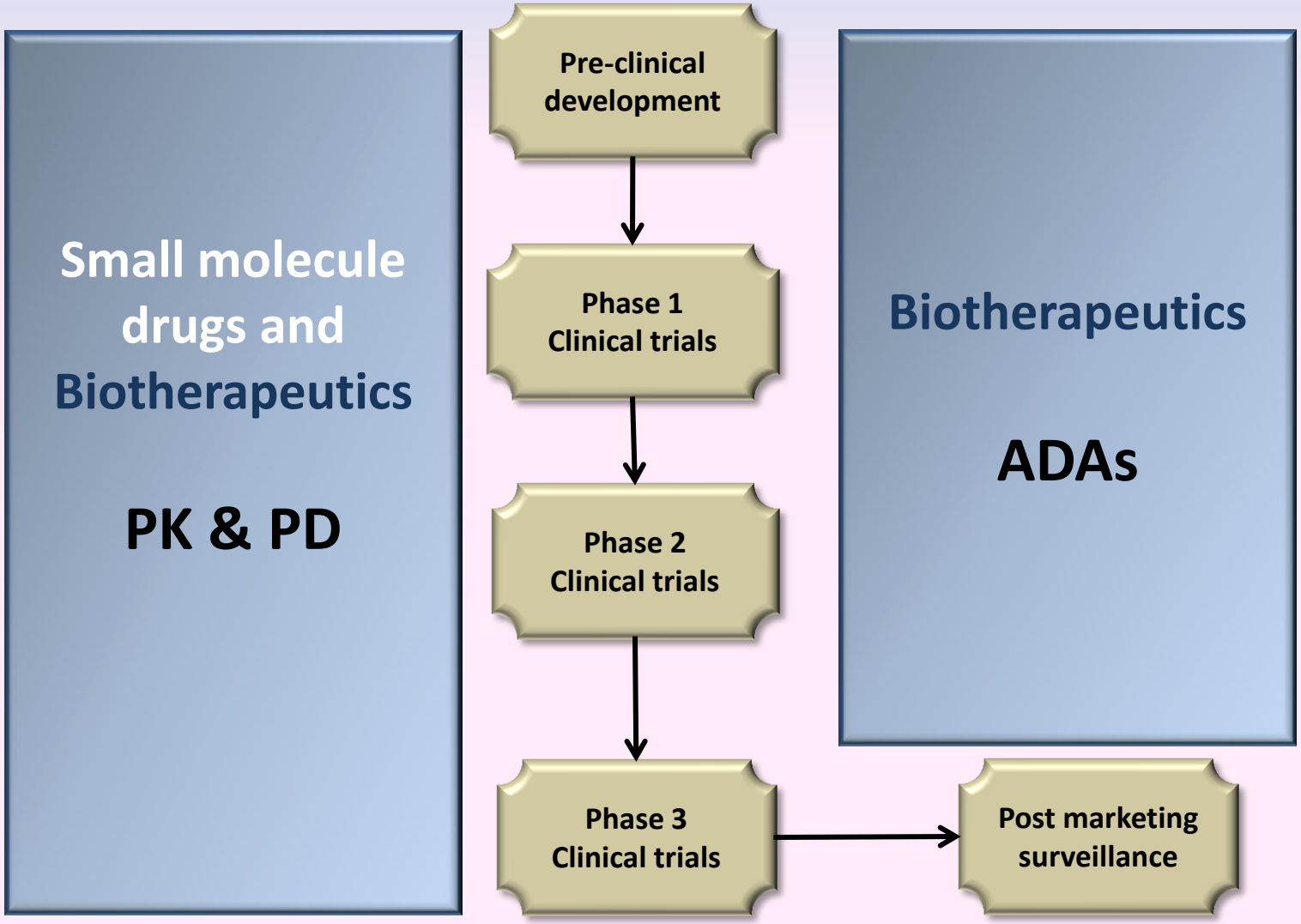
Outline

- ❖ Some fundamentals of immunogenicity assessment
  - ✓ Introduction – basic concepts
    - ✓ Humoral
  - ✓ The antibody detection pyramid
  - ✓ Assay selection: “fit for purpose”
  - ✓ EMA Annex 2 (2007)
  - ✓ Drug inhibition of antibody assays
- ❖ Case study: Addressing drug tolerance of surface plasmon resonance assays for the detection of antibodies against a bispecific therapeutic protein
- ❖ Conclusions
- ❖ Reference list
- ❖ Discussions

Time for acronyms

- ADA = anti-drug antibody
  - ✓ drug = biotherapeutic = therapeutic protein
  - ✓ Immunoglobulins
- Ig = immunoglobulin
  - ✓ IgG, IgM, IgE, IgD and IgA
- NAb = neutralizing antibody
- PK = pharmacokinetics
  - ✓ “what the body does to the drug”
- PD = pharmacodynamics
  - ✓ “what the drug does to the body”
- HLA = human leukocyte antigen
- SPR = surface plasmon resonance
- Gdn = guanidine

Development of small molecule drugs and biotherapeutics



ADA responses and assays

- Animal models or patients dosed with a biotherapeutic may develop ADAs
  - ✓ Immunoglobulins of various affinities for the drug
  - ✓ Antibody affinity is a measure of the strength of the bond between a protein epitope and the antibody binding site
- Immunoglobulins (antibodies) are bifunctional molecules
  - ✓ *Basic* unit of 2 light chains and 2 heavy chains

- ADA assays
  - ✓ Detection of ADAs (polyclonal antibodies) in plasma or serum samples
  - ✓ Qualitative/semi-quantitative assays

ADA responses: variable consequences

- ADAs may affect safety and/or efficacy of the drug

  (Clinical safety concerns may vary from life-threatening situations to less severe infusion reactions)

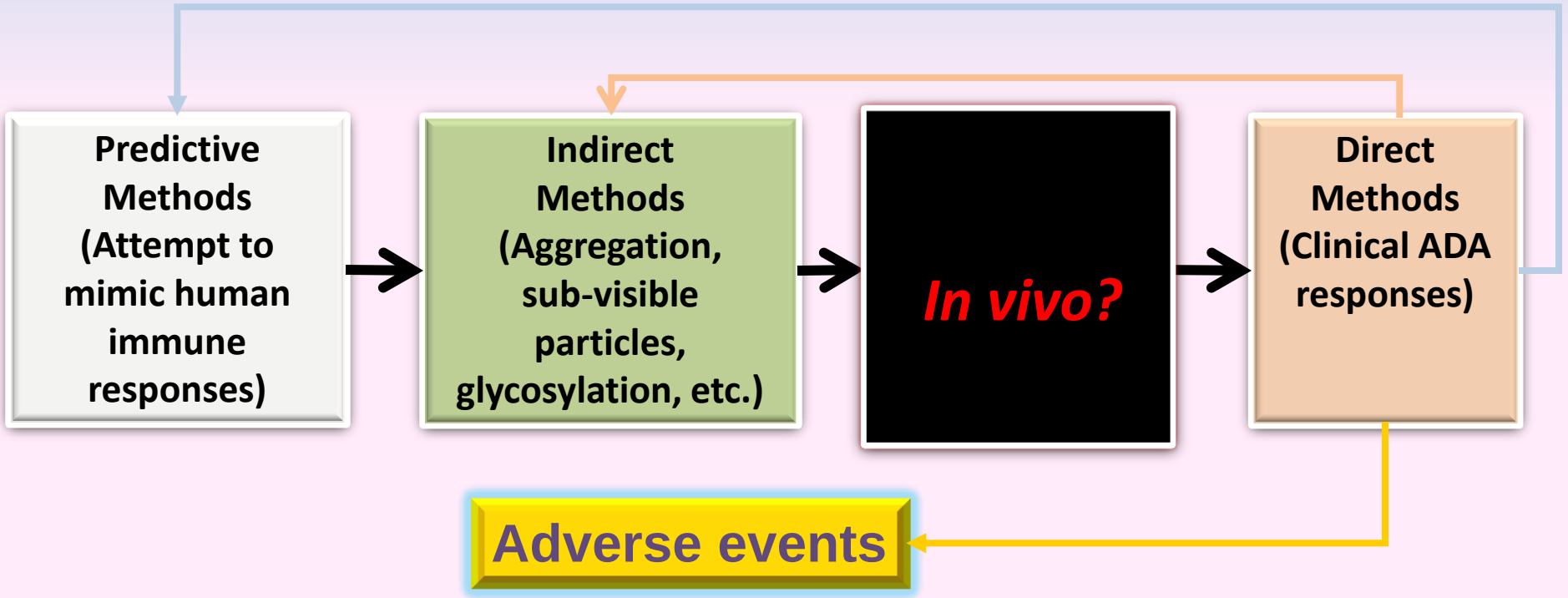
  - Hypersensitivity responses
  - Binding ADAs
  - Neutralizing ADAs
    - ✓ May bind and neutralize the pharmacological activity of the drug and the endogenous protein counterpart

Rosenberg, A. S. 2003. Immunogenicity of biological therapeutics: a hierarchy of concerns. Dev. Biol. (Basel). 112: 1521.

Several factors may be involved in immunogenicity of biotherapeutics

- Human genetics
- Amino acid sequence
- Protein aggregation
- Impurities
- Route, dosage and frequency of administration
- Other factors

Immunogenicity assessment and mitigation

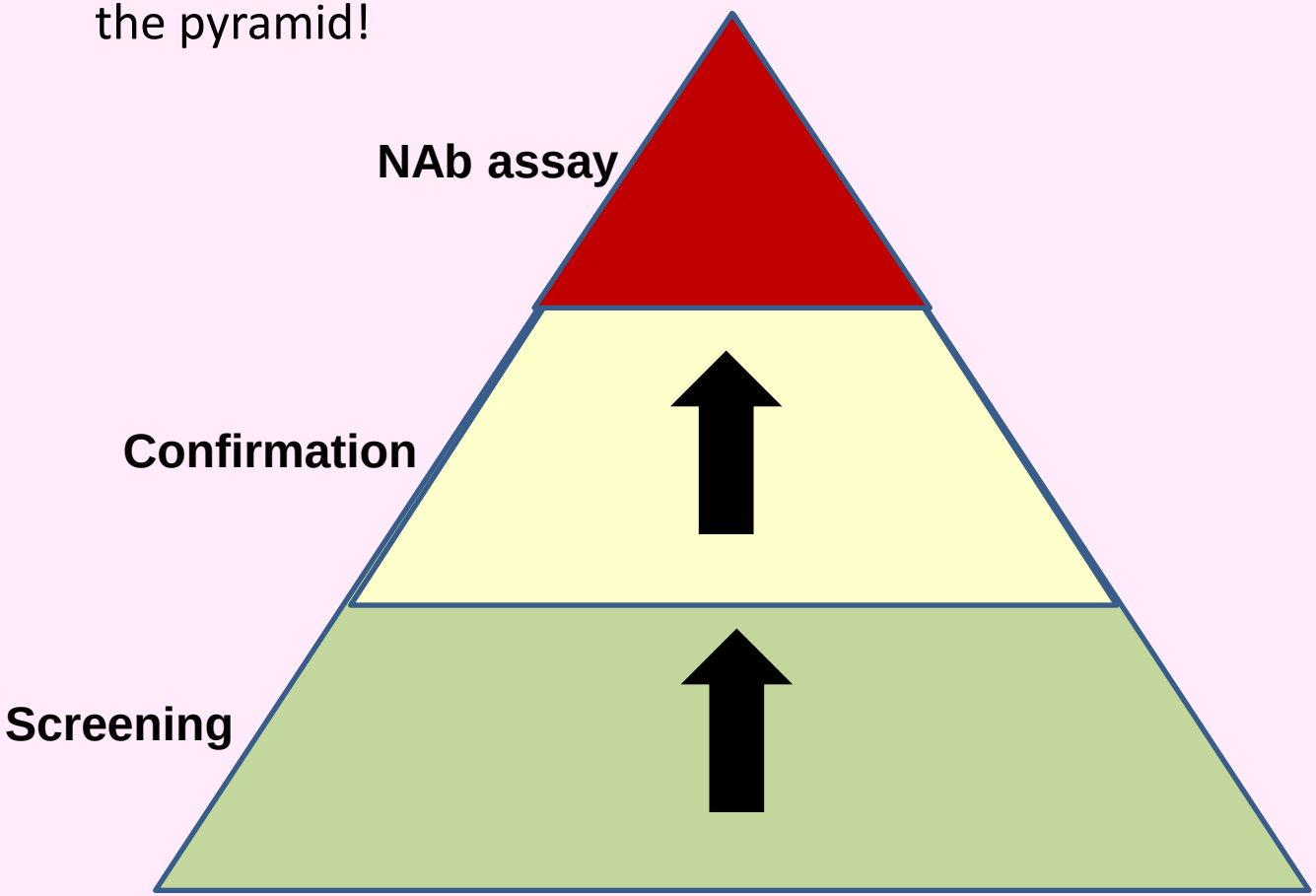


Accurate and sensitive ADA assays are required for proper assessment of antibody responses against biotherapeutics (including biosimilars and biobetters)

From: Barbosa, M.D.F.S. Audio slide presentation in ScienceDirect. Anal. Biochem. (2013) 441: 174-179

The ADA testing pyramid

- ADA testing: three tier
  - ✓ False negatives during screening will **not** be detected at the top of the pyramid!



Selection of ADA assay: “fit for purpose”

- Several screening ADA assay formats available
  - ✓ Radio immuno-precipitation, surface plasmon resonance (SPR), Immunoassay (electrochemiluminescence, ELISA), etc.
- Safety considerations
  - ✓ Screening assay should be able to detect all antibody isotypes, particularly IgM and the different IgG isotypes
  - ✓ IgE
- Assessment of associations between patient genetics and IgG responses
  - ✓ Post-marketing; Interfereon-β assays capable of detecting IgG
  - ✓ Ref.: Barbosa et al. 2006. Clinical link between MHC class II haplotype and IFN-β immunogenicity. Clinical Immunol. 118: 42-50
  - ✓ Hoffmann et al. 2008. HLA-DRB1*0401 and HLA-DRB1*0408 are strongly associated with the development of antibodies against interferon-beta therapy in multiple sclerosis.. Am. J. Hum. Genet. 83, 219-227

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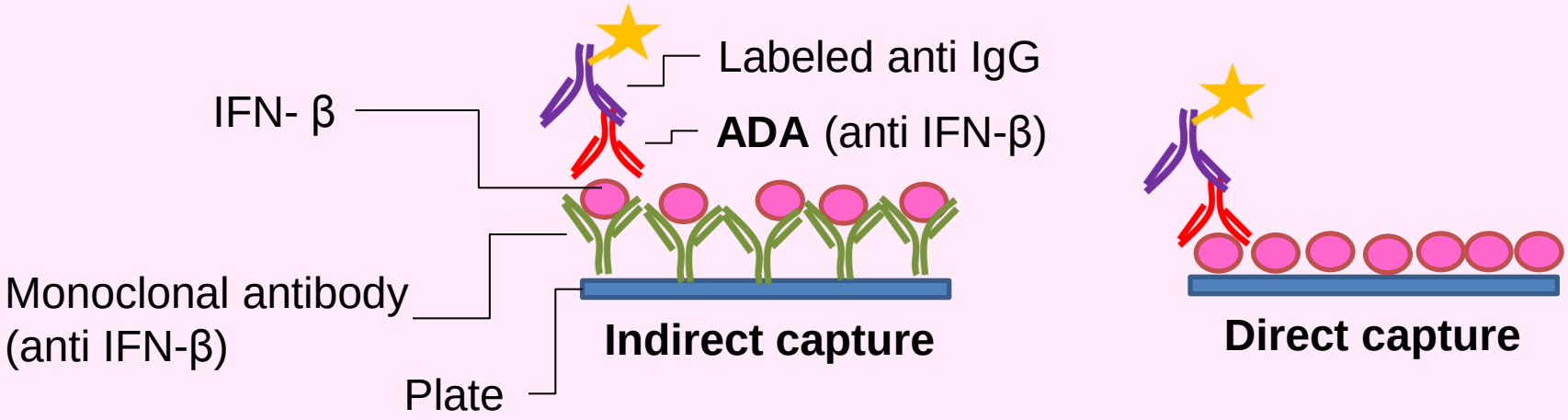
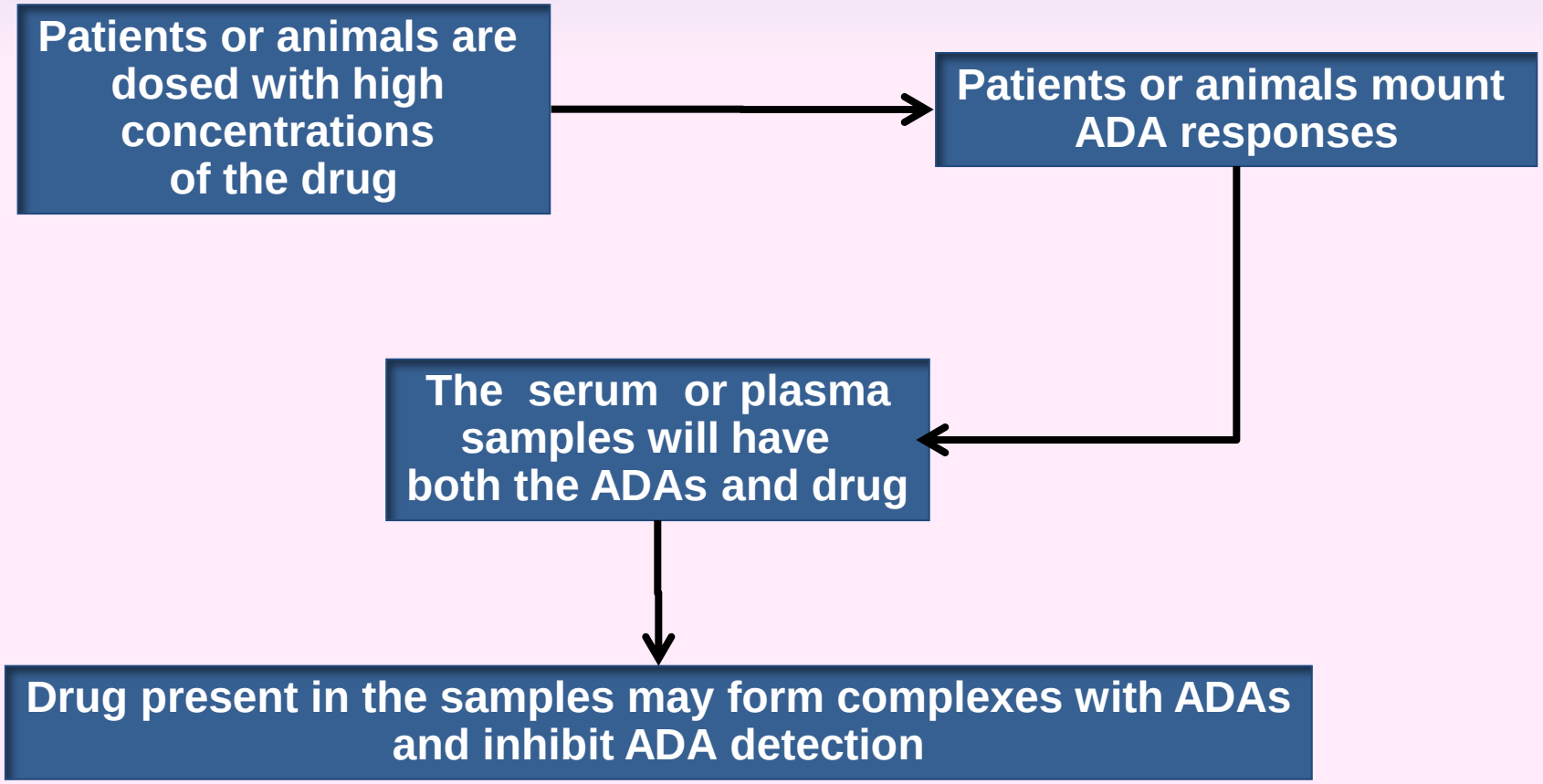
Different assays may detect distinct ADAs in the same sample

- **Example:** Detection of anti interferon-β (IFN- β) IgG to investigate ADA associations with patient HLA types
  - ✓ IgG detection; 2 screening assays used
  - ✓ Results varied with assay (direct or indirect capture of IFN- β)
  - ✓ 39 samples total ; only results for 32 samples agreed (they were both positive of both negative for the presence of IgG) -



Ref.: Barbosa et al. 2006. Clinical link between MHC class II haplotype and IFN-β immunogenicity. Clinical Immunol. 118: 42-50

High drug concentrations in samples may inhibit ADA assays



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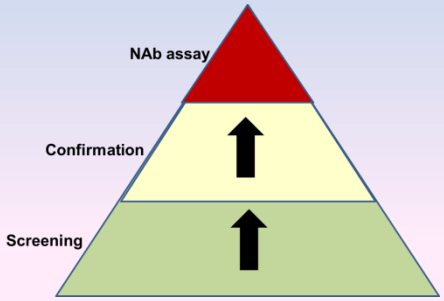


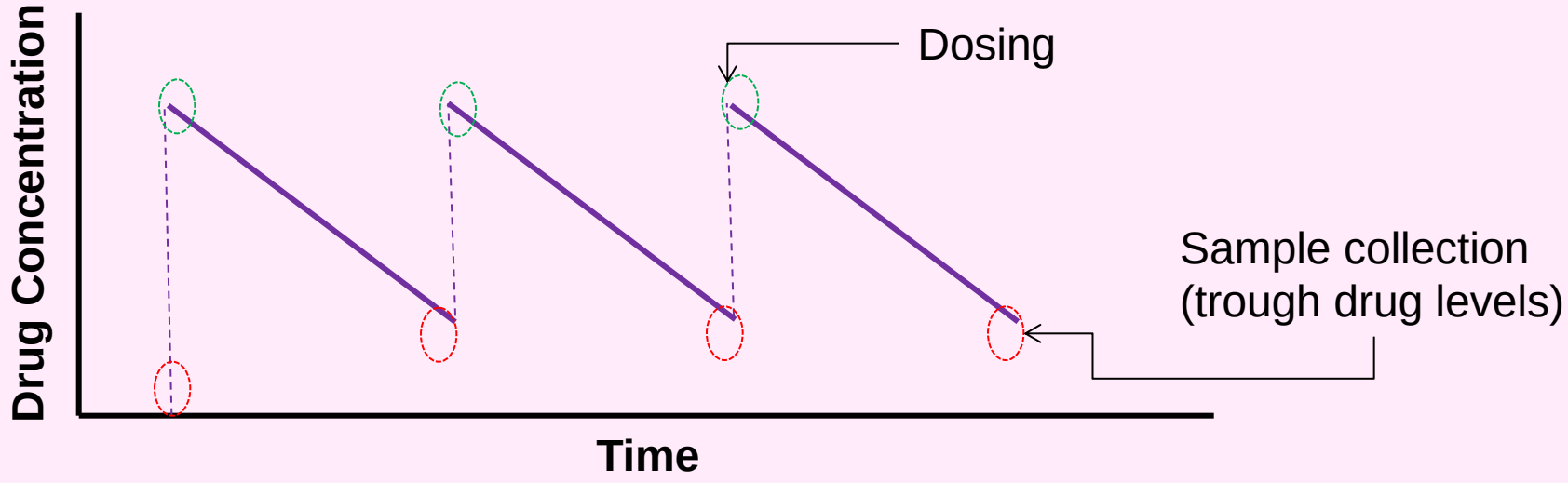
- False negatives

- ADA assay drug tolerance needs to be consistent with drug levels in the test samples
  - Ideally assay positive controls should reflect sample ADAs

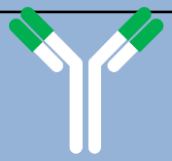
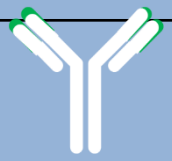
- Acid dissociation of drug-ADA complexes has been used to increase drug tolerance of ADA assays
  - Acid dissociation may denature ADAs
  - Might not dissociate all drug-ADA complexes

Sample collection should be planned to minimize drug present in ADA samples

- ADA assay drug inhibition is a particular concern for samples from patients or animals receiving high doses of biotherapeutics with long half-lives
- Sample collection prior to initiation of treatment and prior to dosing (and if appropriate after end of treatment)
- Case-by-case decisions on number and frequency of samples



Drug inhibition of ADA assays complicate evaluation of de-immunization strategies

DRUG	CHARACTERISTICS	ADA	TROUGH DRUG LEVELS	ASSAY
Erbitux (cetuximab)	Chimeric mAb 	49/1001 (5%)	41-85 µg/ml	*
Herceptin (trastuzumab)	Humanized mAb 	1/903 (<1%)	79 µg/ml	**
Vectibix (panitumumab)	Fully human mAb 	3/613 (<1%)	39 ± 14µg/ml	***Acid dissociation ELISA
Vectibix (panitumumab)	Fully human mAb 	28/613 (4.6%)	39 ± 14 µg/ml	***BIACORE®

Drug trough concentrations and ADA incidence extracted from the product labels

***ADA tested with either a double antigen radiometric assay or an ELISA assay**

****ADA detection method not indicated in the product label**

***** Lofgren, J.A. *et al*. 2007. *J Immunol 178, 7467-7472***

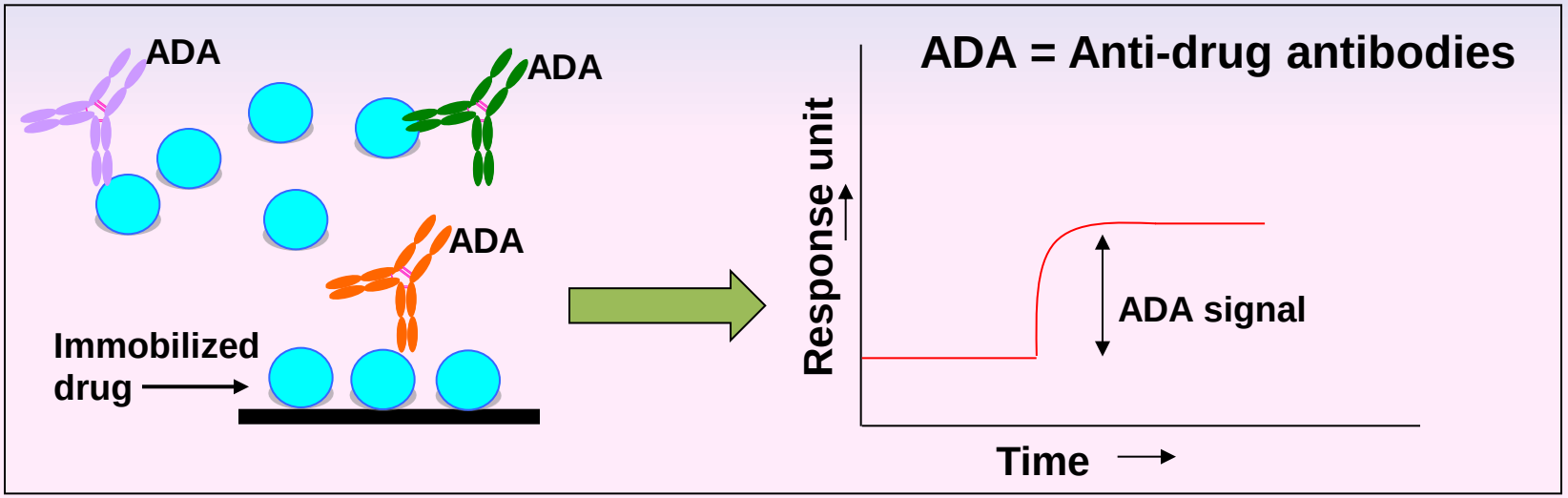
From: Barbosa et al. 2012. Drug Discovery Today. 17: 1282-1288.

CASE STUDY

Addressing drug tolerance of surface plasmon resonance assays for the detection of antibodies against a bispecific therapeutic protein

Reference: Barbosa, M.D.F.S., Gokemeijer, J., Martin, A.D., Bush, A. 2013. Analytical Biochemistry 441: 174-179

Surface Plasmon Resonance (SPR) ADA assays



✓SPR assays detect both low and high affinity of binding ADAs

✓In the presence of high drug concentrations SPR may preferentially detect ADAs with lower affinity for the drug

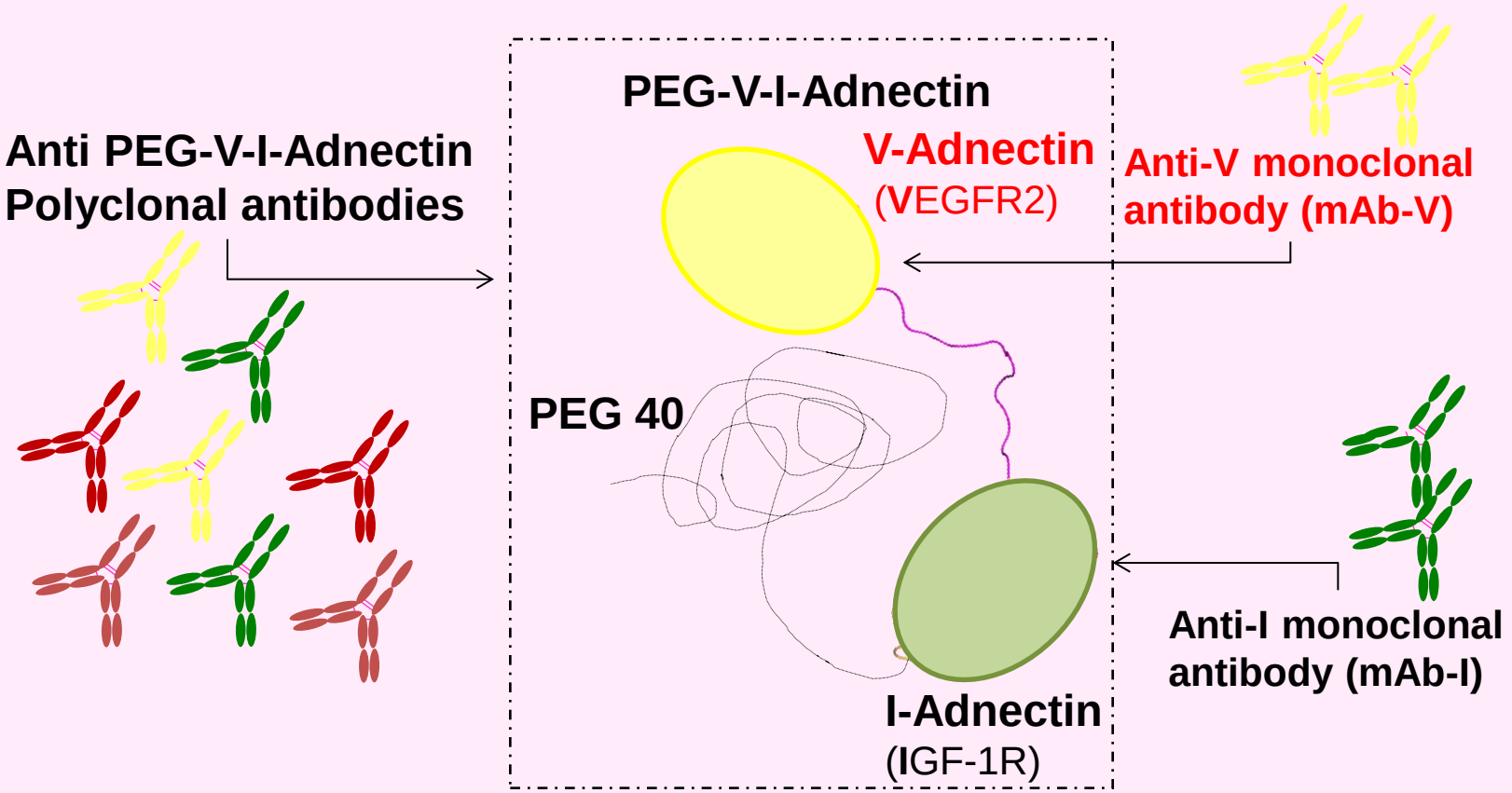
✓Alter drug tolerance of SPR ADA assays by decreasing the affinity of ADAs for the drug

- Temperature / chaotropic agents

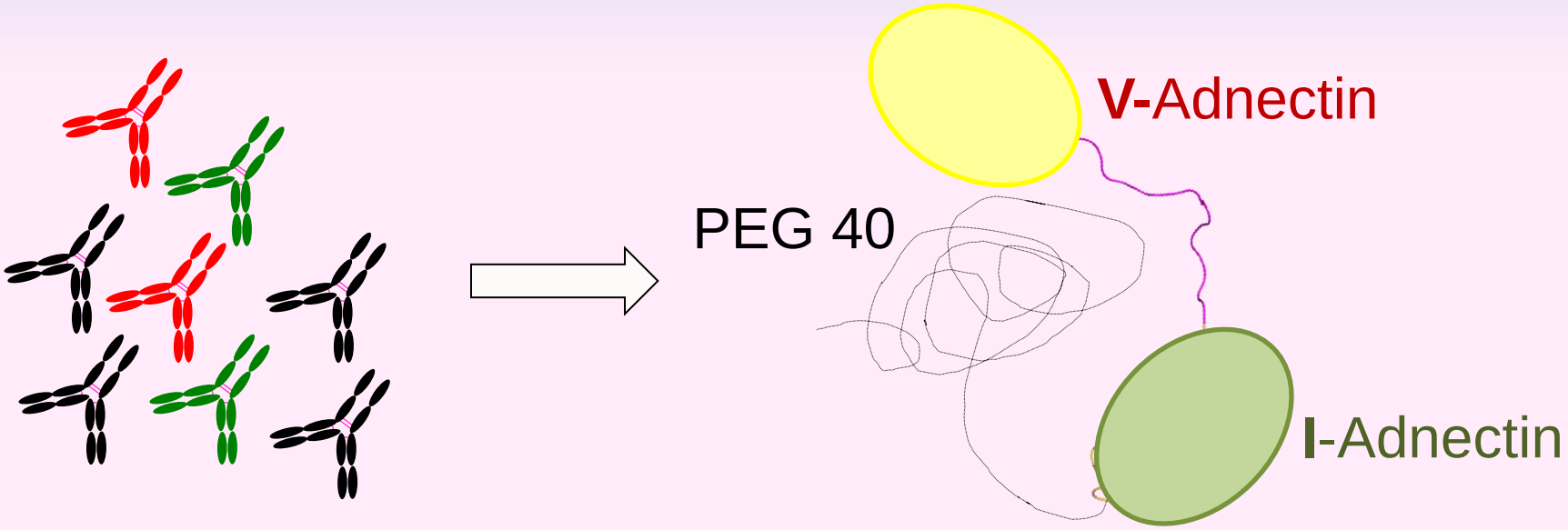
Test system: The drug, SPR assay and ADAs

DRUG: **Bispecific tandem Adnectin (V-I-Adnectin; +/-PEG)**

SPR ASSAY: **V-I-Adnectin or PEG-V-I-Adnectin immobilized**

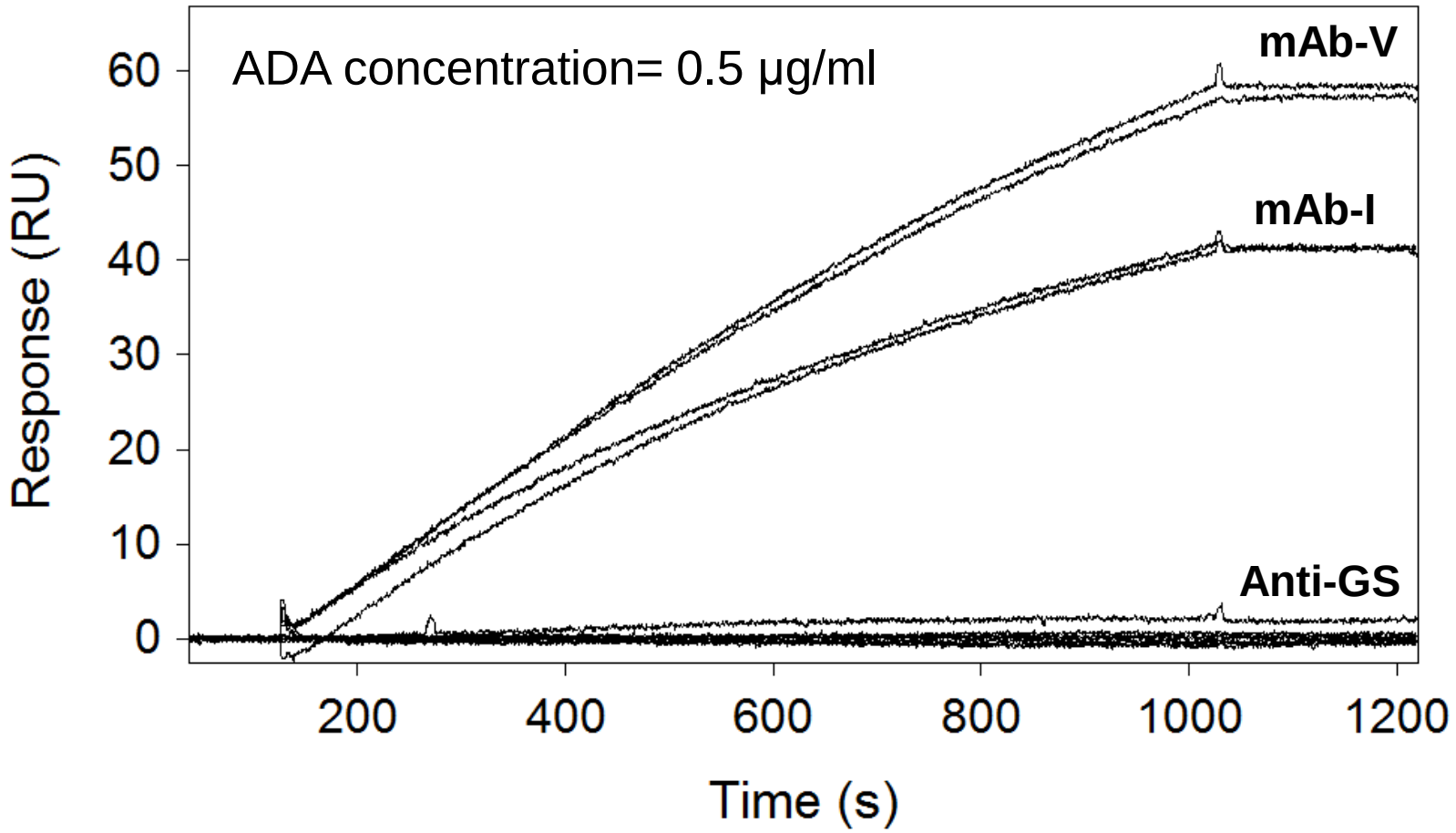


Anti-PEG-V-I-Adnectin polyclonal ADAs: samples from pre-clinical study



- Cyno polyclonal anti-PEG-V-I-Adnectin ADAs (**pAb-V-I**)
  - Pooled plasma samples
- Monkey study plasma samples (PEG-V-I-Adnectin study)
  - **H**, **M**, **L** and **BLQ**

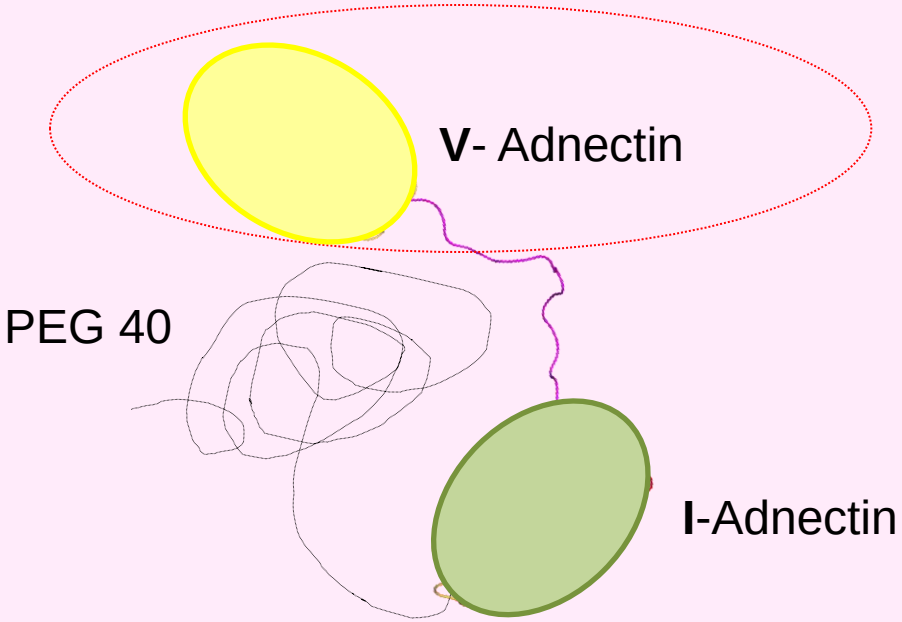
Binding of anti-V-I-Adnectin antibodies to immobilized PEG-V-I-Adnectin



Testing treatments to weaken affinity of the drug-ADA interactions

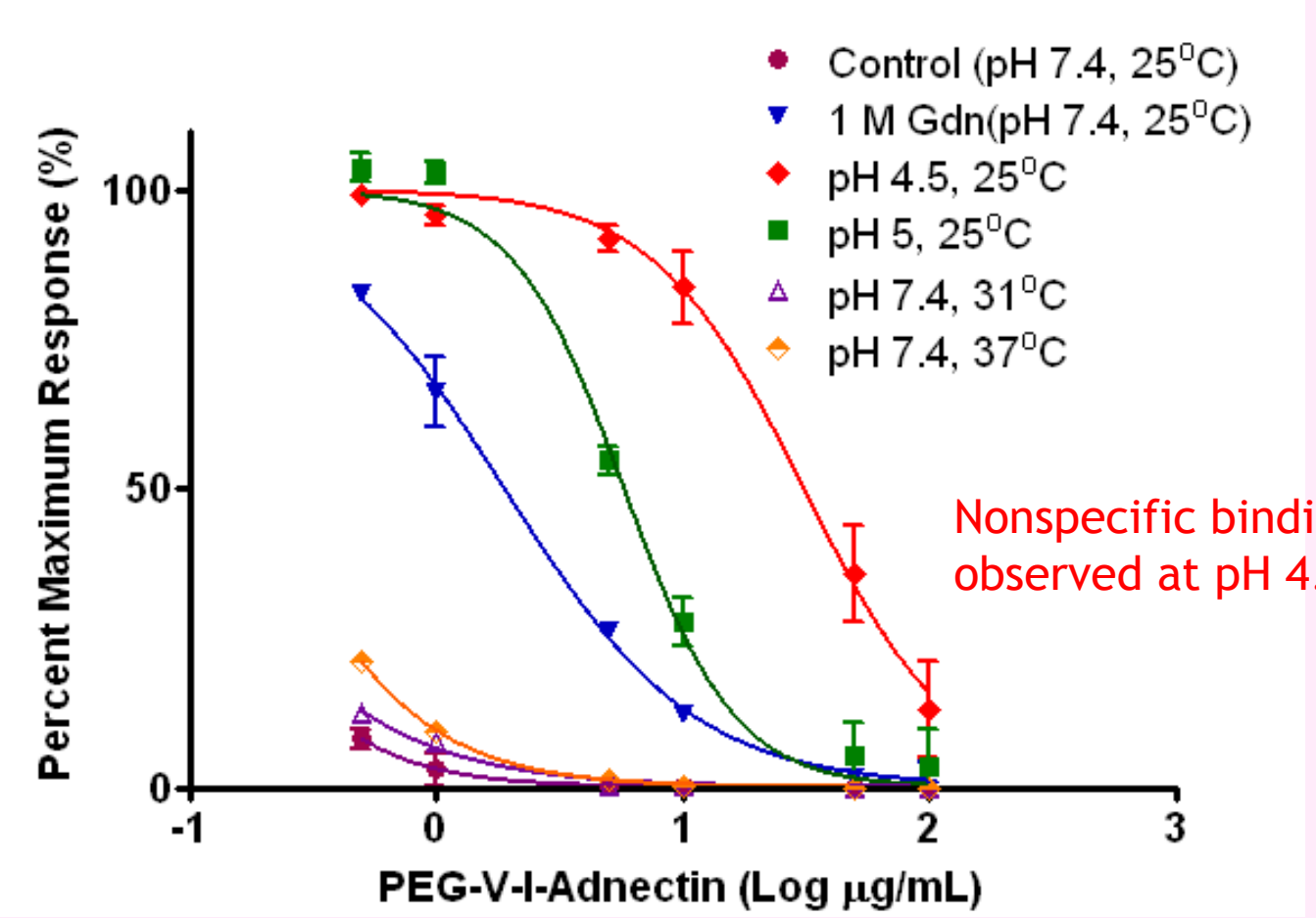
mAb-V ; PEG-V-I-Adnectin immobilized

- Low pH (4.5, 5.0)
- Increase of temperature (30^{o}C, 37^{o}C)
- Guanidine hydrochloride (Gdn)



Increased drug tolerance of an ADA SPR assay with Gdn

0.5 µg/ml mAb-V; PEG-V-I-Adnectin immobilized
100 % Maximum Response = Control (pH 7.4, 25°C) without drug added



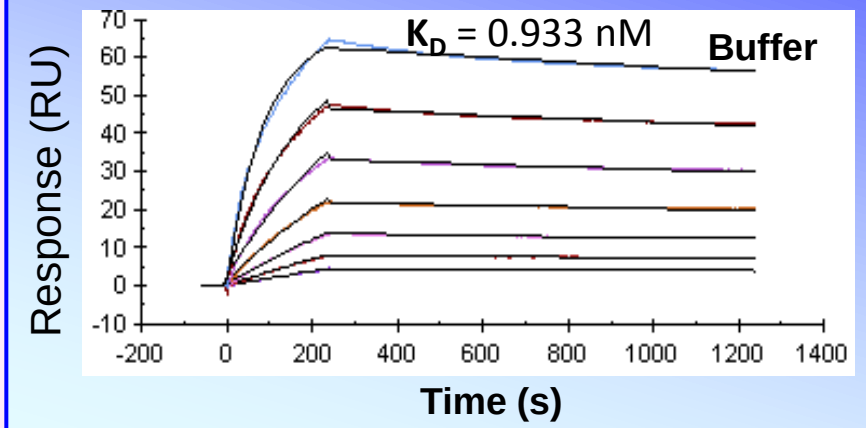
Nonspecific binding observed at pH 4.5 and 5

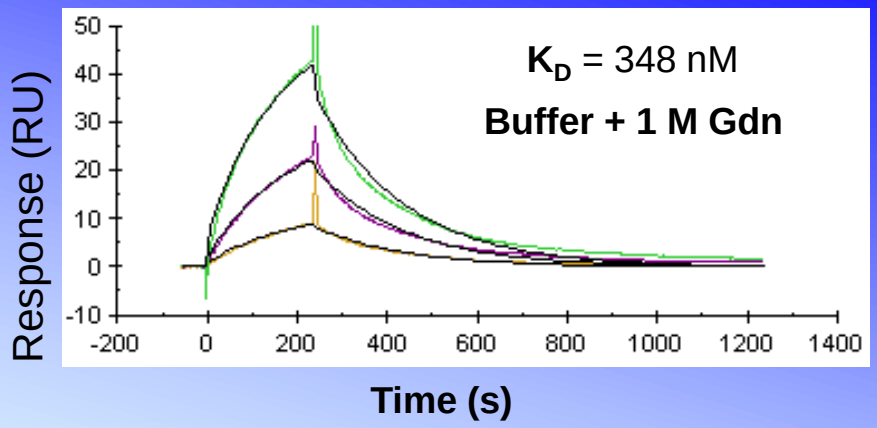
Binding affinity (K_D) of anti-V-I-Adnectin antibodies to PEG-V-I-Adnectin

	25°C	32°C	37°C
mAb-V	0.93 nM	1.06 nM	1.19 nM

- PEG-V-I-Adnectin immobilized
- mAb-V concentrations ranged from 0.23 to 15 µg/ml

Gdn affects binding and dissociation kinetics of mAb-V at 25°C

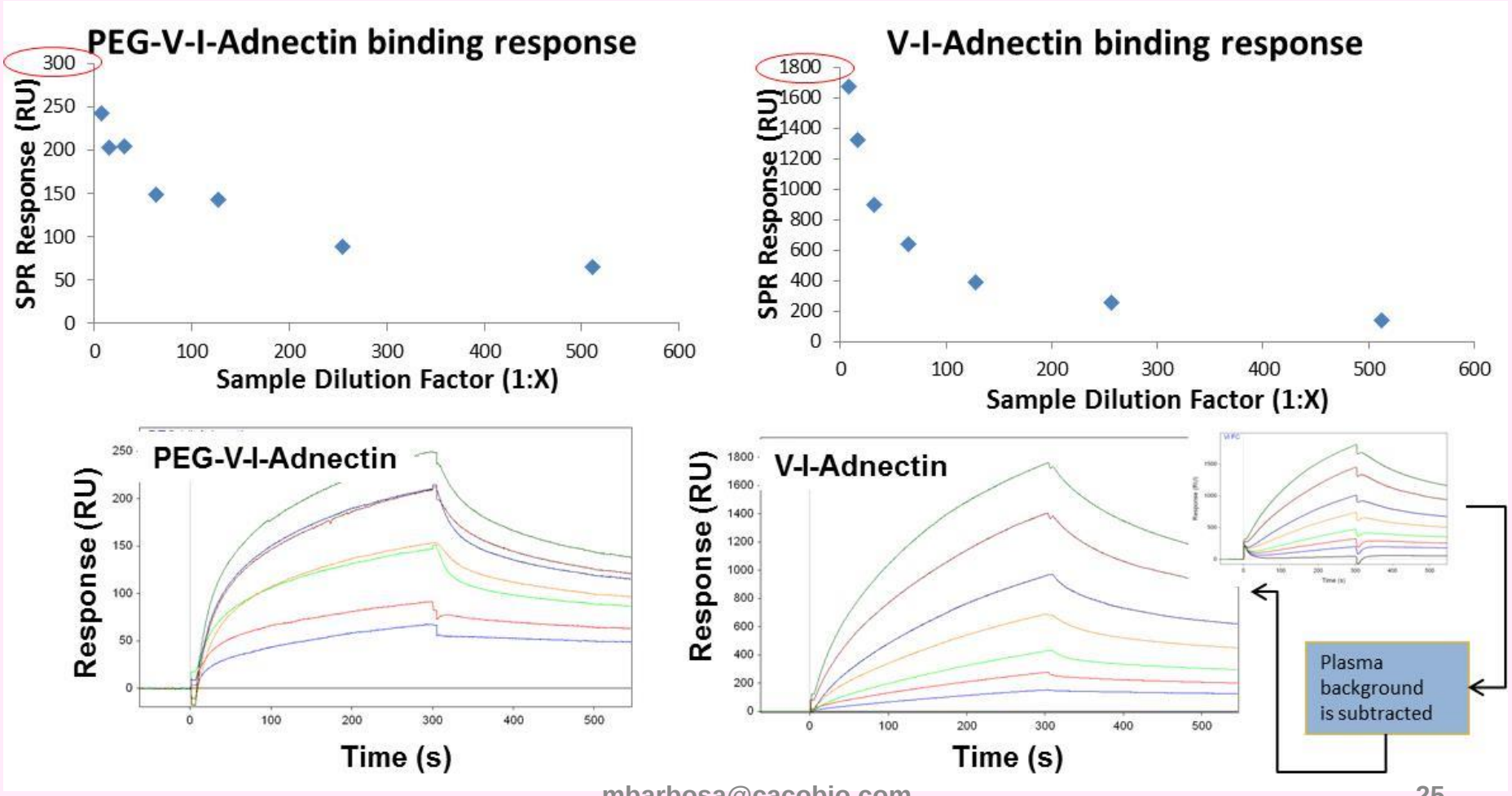




- PEG-V-I-Adnectin immobilized
- mAb-V concentrations ranged from 0.23 to 15 µg/ml

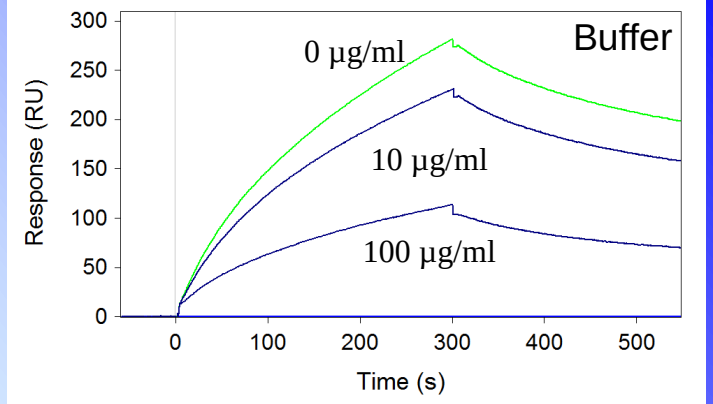
Working dilution determination and background subtraction for pAb-V-I

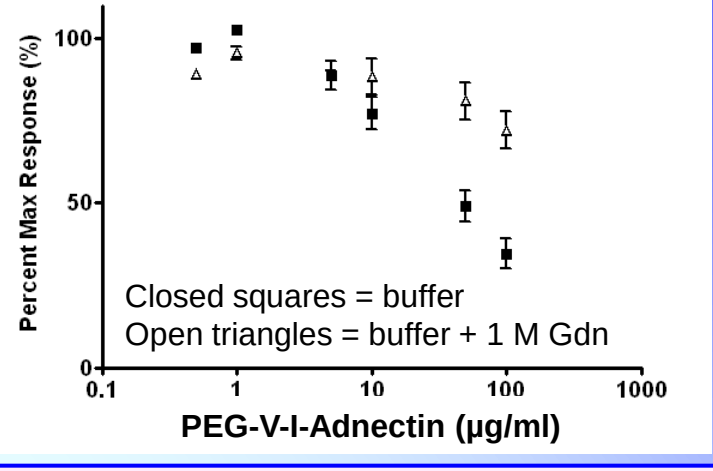
Cyno polyclonal anti-PEG-VI-Adnecting antibody (pAb-V-I) = pooled plasma samples; Same final plasma concentration for all dilutions



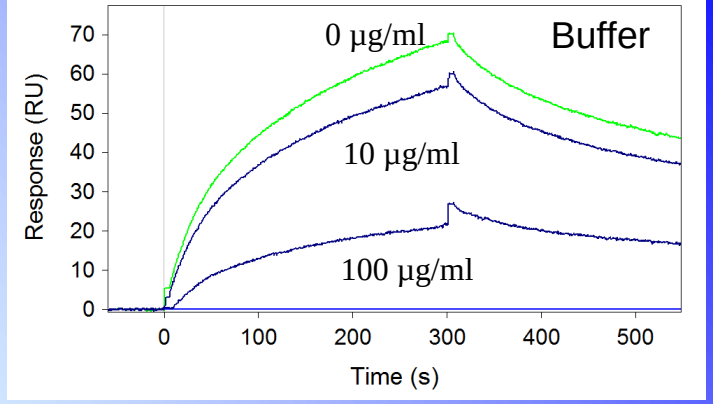
Drug tolerance of SPR assay with pAb-V-I

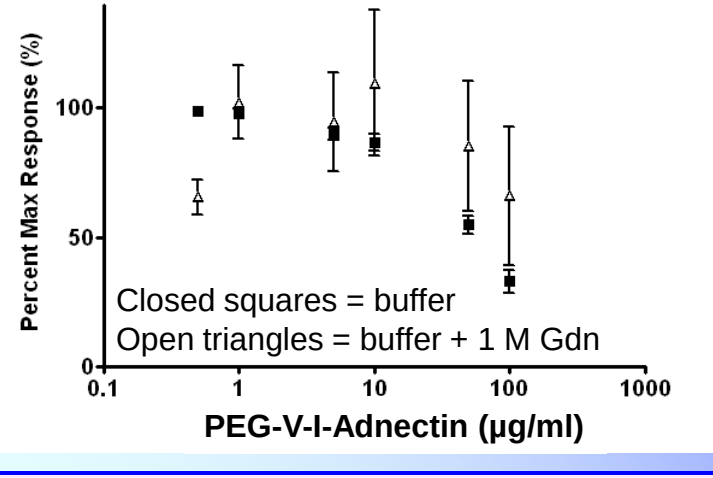
V-I-Adnectin immobilized



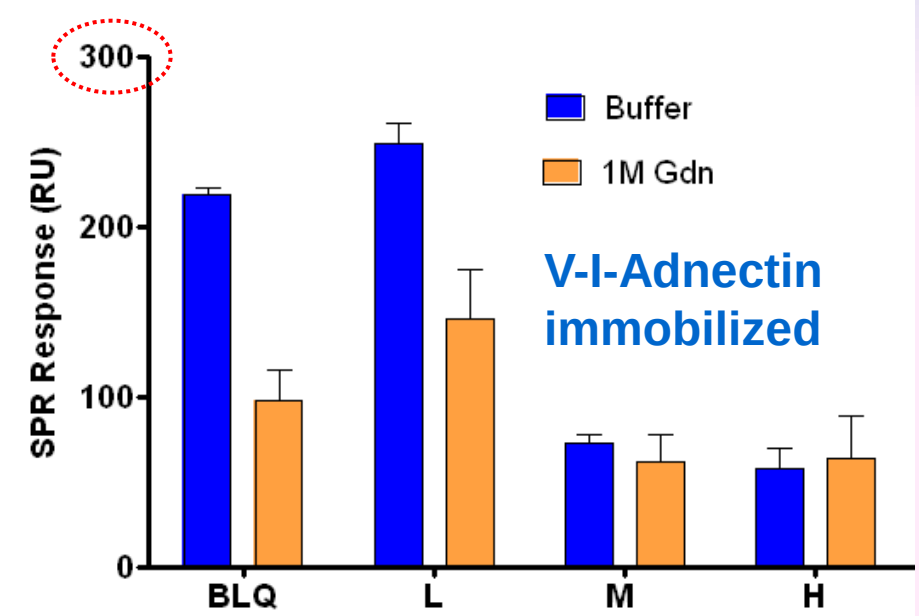


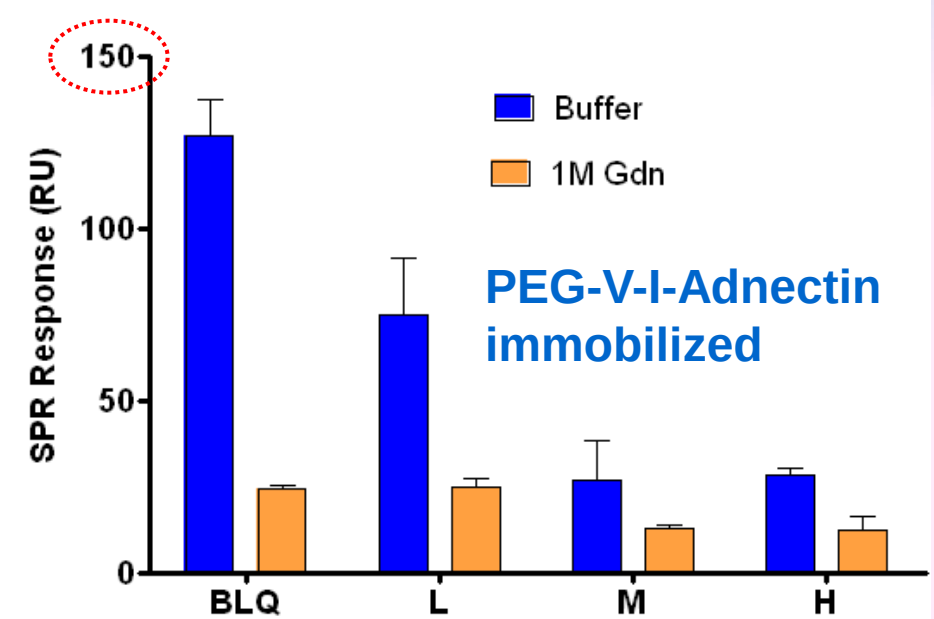
PEG-V-I-Adnectin immobilized





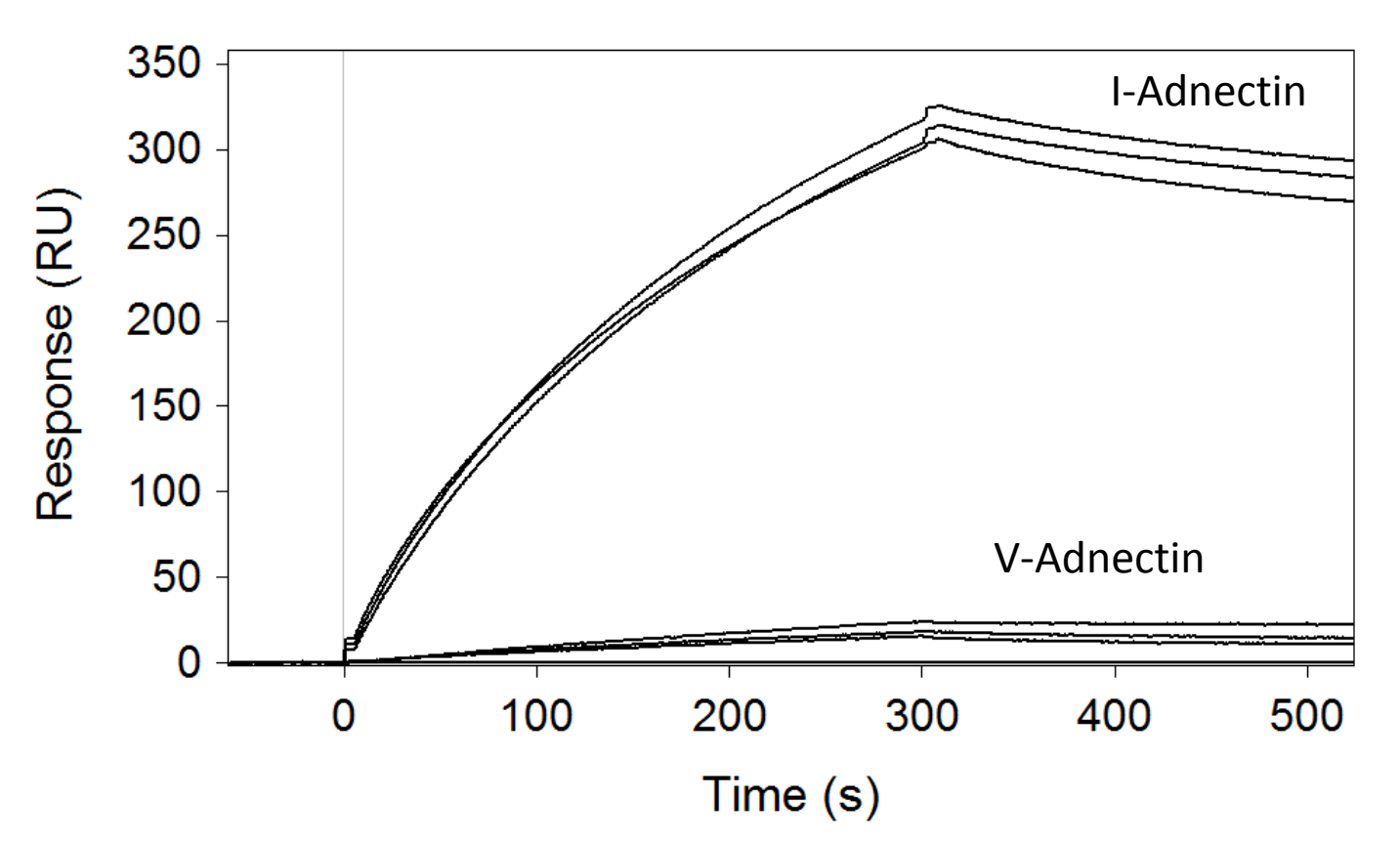
Effect of guanidine (Gdn) on ADA-positive pre-clinical study samples





Samples	PEG-V-I-Adnectin (µg/ml)	Ab titer	Dilution
BLQ	0	10,000-100,000	1:60
L: Low drug	11	10,000-100,000	1:60
M: Medium drug	60	10,000-100,000	1:60
H: High drug	719	10,000-100,000	1:60

Pre-clinical study sample (BLQ) ADAs preferentially bind to I-Adnectin



<u>BLQ:</u> Plasma sample containing cyno polyclonal anti-PEG-V-I-Adnectin antibodies; BLQ has PEG-V-I-Adnectin concentration below limit of quantification

Concluding remarks

- A higher SPR assay signal was observed with a non-PEGylated V-I-Adnectin immobilized than with a PEG-V-I-Adnectin
  - ❖ Higher V-I-Adnectin density on the chip
- Gdn increased the drug tolerance of a SPR ADA assay using an anti-V-Adnectin high affinity antibody
  - ❖ Binding kinetics showed that Gdn weakened affinity of drug-antibody interaction
- The drug-antibody complexes responded differently to Gdn
  - ❖ While it may be effective to weaken drug interaction with some antibodies, it may have deleterious effects on others
- In many situations a combination of approaches may be needed for accurate ADA detection

References

(Web sites last accessed on August 2013)

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