Use of the Affinity Module on the Gyrolab Platform to Inform and Assess Critical Reagent Selection during Method Development

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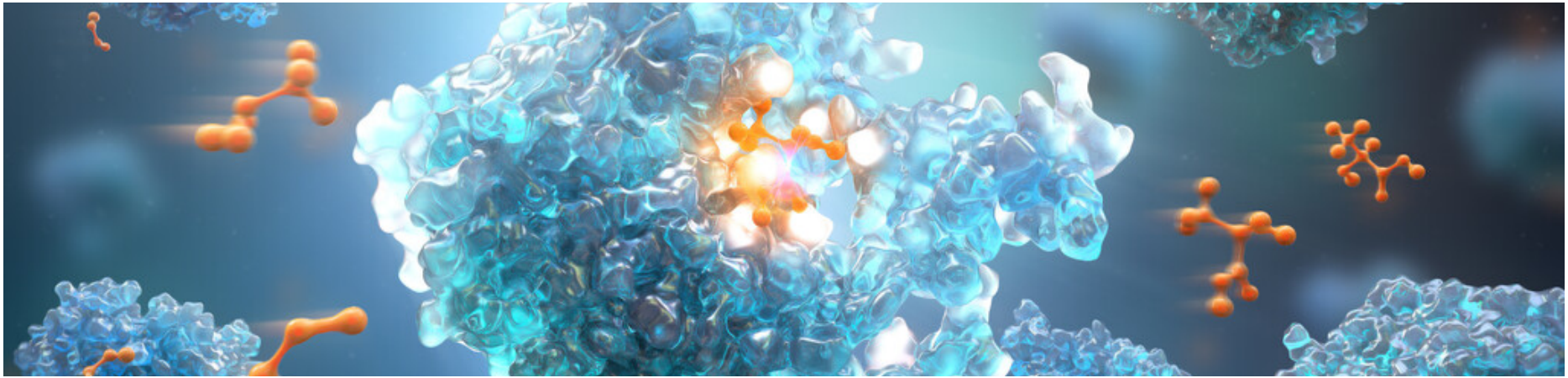


Overview

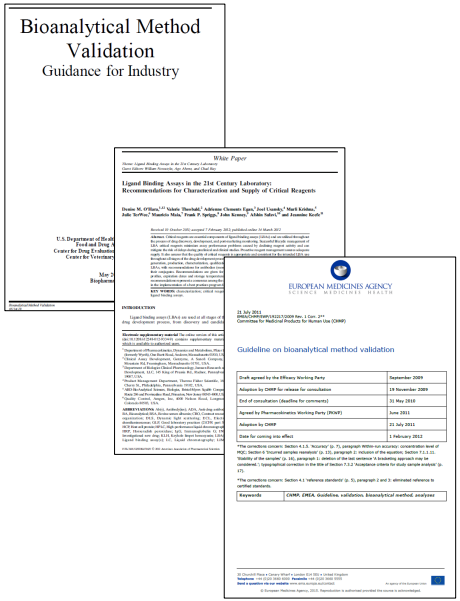
- Introduction – critical reagent selection
- Affinity assessment
- Use of Gyrolab Affinity Module
- Case Study

Critical reagent selection is crucial for development of successful ligand-binding assays

"Assays rely on binding properties of the reagents to quantify the analyte"

EBF recommendation on practical management of critical reagents for PK ligand-binding assays. Pihl *et al.* Bioanalysis (2018)

- Literature on critical reagents typically address the full spectrum of life-cycle management
  - Bioanalytical method validation guidance documents are focussed further down the assay life cycle
- Decisions made in early assay development are incredibly far-reaching
  - From immediate validation through to long-term application of a method
- Importance of selection and appraisal of critical reagents in early method development should not be under-stated



A successful ligand-binding assay requires reagents that meet multiple criteria

- A successful LBA requires antibodies that are:

Specific

- *"The ability of the method to assess, unequivocally, the analyte in the presence of other components that are expected to be present"* FDA BMV 2018

High affinity

- The strength of interaction between the antigenic determinant and the antigen impacts multiple assay parameters - sensitivity, robustness, accuracy and reproducibility

Complementary

- Antibodies competing for the region of the antigen will not work in combination

- Chequerboard experiments are typically the first development assessments performed
  - Require labelling of multiple reagents
  - Can become complex with multiple combinations to assess
- Seek to enhance development through more targeted appraisal of reagents, targeting affinity

Affinity is the expression of the interaction between the antibody paratope and its corresponding epitope

$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AB$$

Where:

- A represents free antigen
- B represents free antibody
- AB represents antigen/antibody complex
- K_{on} is the rate of association
- K_{off} is the rate of disassociation

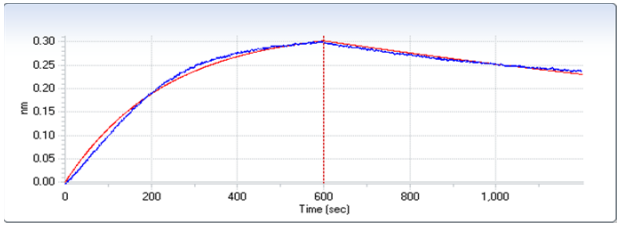
- K_D is the equilibrium constant and is the ratio of the rate of dissociation (k_{off}) and rate of association (k_{on})

$$K_D = k_{off}/k_{on}$$

There are principally two approaches to understanding antibody interactions

Solid-phase methods

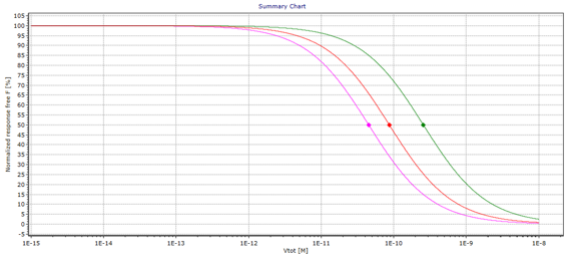
- Affinity is calculated based on the kinetic properties K_{on} and K_{off}



- Key advantage – informs as to individual kinetics

Solution phase methods

- Concentration of interactants are measured in equilibrium in solution
- Affinity is calculated based upon curve fit



- Key advantage – K_D determined from free interactant in an unperturbed equilibrium

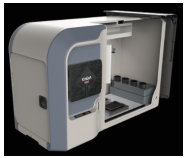
Multiple platforms are marketed for determining antibody affinity and kinetics

Solid-phase platforms

- Biacore
- Surface Plasmon Resonance

- Octet
- Bio-Layer Interferometry

Solution-phase platforms



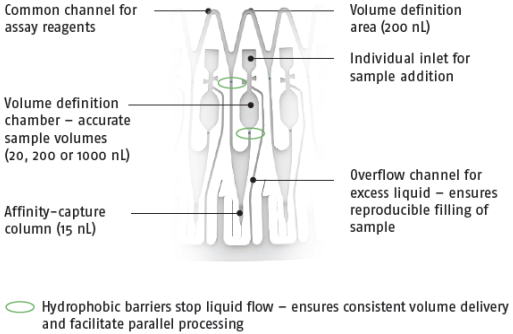
- KinExA
- Kinetic Exclusion Assay

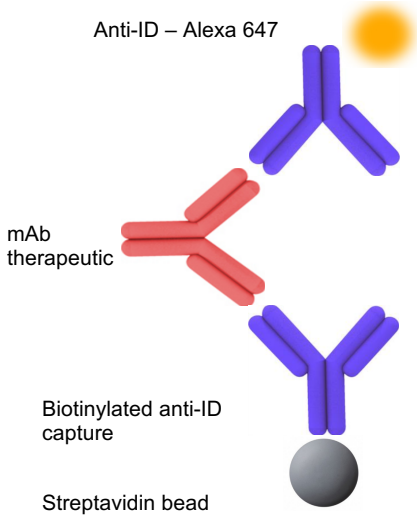
- Gyrolab

- MSD

- ELISA

The Gyrolab has been the platform of choice for PK assays at MedImmune/AZ for over a decade

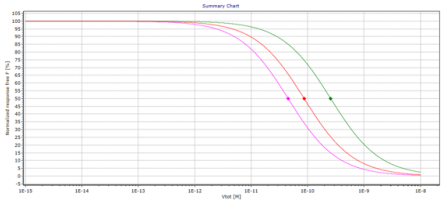




- Capabilities for quantitative methods are well understood
- There are advantages in applying the same platform to selection of critical reagents

→ Assessment of affinity module conducted

The Gyrolab affinity module calculates the equilibrium constant (K_D) from an affinity curve



Solution phase methods

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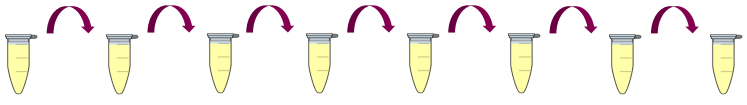
- Affinity is calculated based upon curve fit

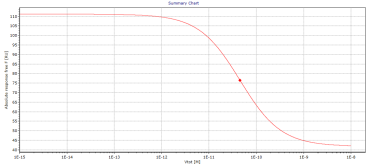
Generating an affinity series



Two interactants are required

- Interactant F is assessed at fixed concentration
- Interactant V is assessed at varying concentration

Equilibrium



Measure unbound component of interactant F and plot against molar concentration of V

K_D = [V][F]/[VF] at equilibrium

Calculate K_D

Key considerations for experimental set-up

- Identify which reagent to utilise as F and which as V
- Require an assay that can quantitatively measure the free component of F

Case study for assessment of anti-idiotypic reagents

Anti-ID production

Murine hybridoma

Variable interactant = drug

Fixed interactant = anti-ID

Develop a sandwich IA to detect anti-ID

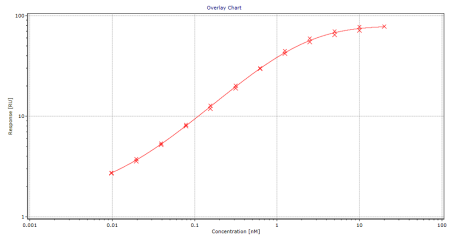
Anti-murine polyclonal– Alexa 647

Bioaffy 1000

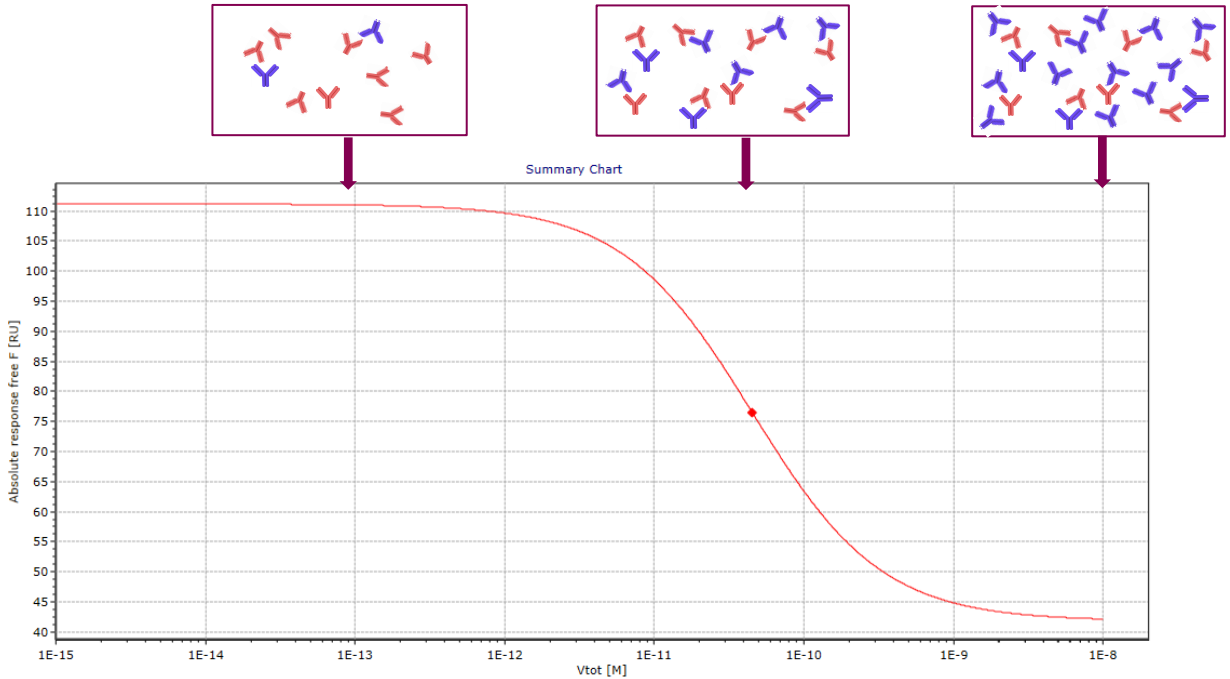
Anti-ID

Biotinylated drug capture

Streptavidin bead



Concentrations of F and V require optimisation depending upon expected affinity



- For accurate calculation of K_D require concentration of F to be less than K_D
  - Where $F \leq K_D$ the affinity curve is derived from the equilibrium
  - Where $F > K_D$ the curve becomes stoichiometric – the concentration of F in excess is measured

Case study

- Unexpected lack of robustness during PK validation
  - Variable precision and accuracy observed, concern method would fail

→ In 4 weeks method was redeveloped, validated and PK data were delivered to support study progression

→ Could the affinity module have aided initial investigation and reduced pressure on timelines?

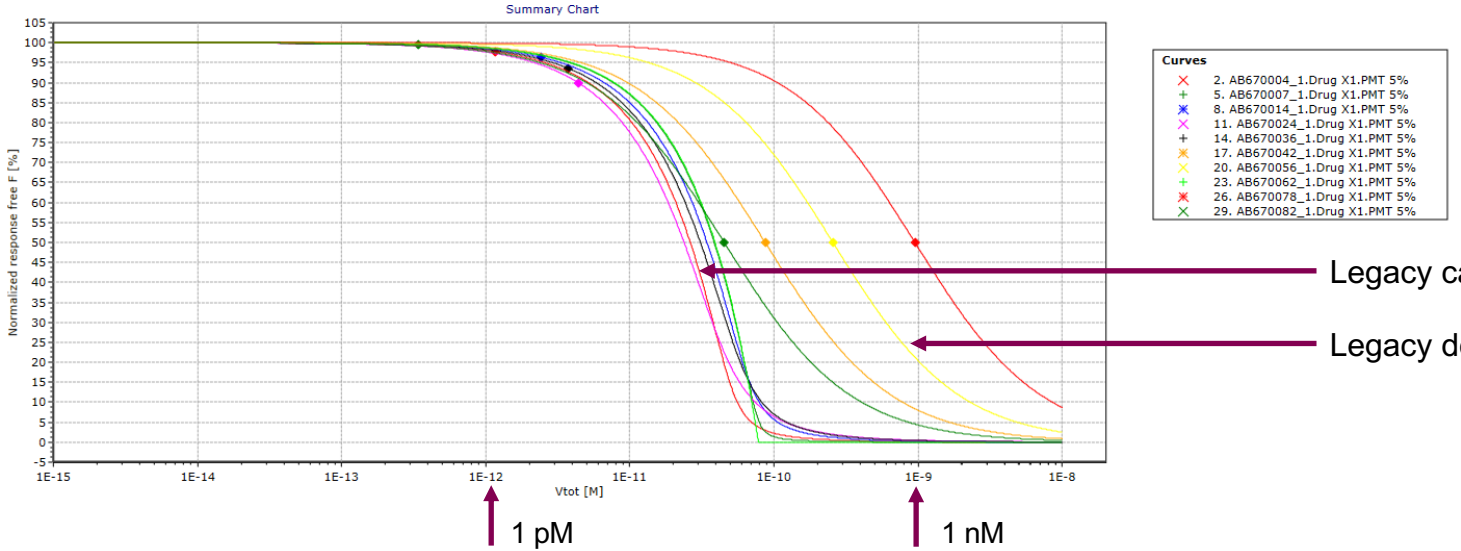
The affinity module was used to assess all available anti-idiotypic antibodies

- Each anti-ID run as interactant F at 0.05nM
- Drug run as interactant V in titration from 10 nM to 0.156 pM

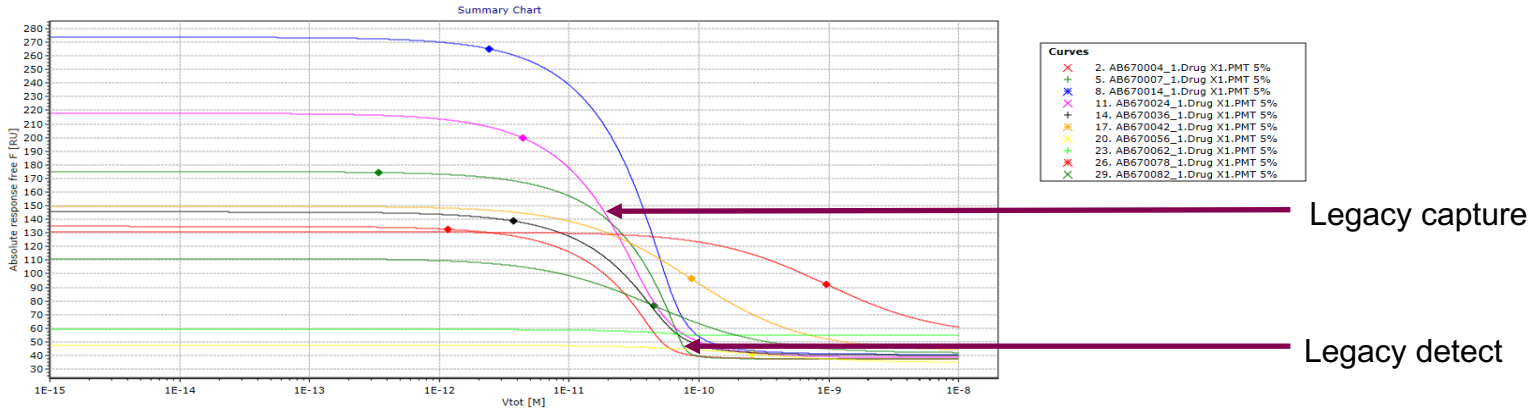
AB670004
AB670007
AB670014
AB670014
AB670024
AB670036
AB670042
AB670056
AB670062
AB670078
AB670082

Legacy capture

Legacy detect

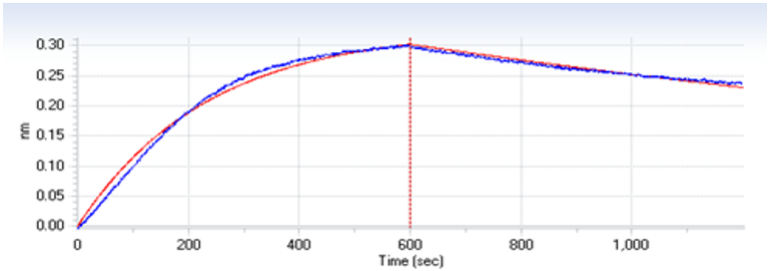


Viewing non-normalised data adds insight….



As does comparison with data from the Octet

AB670024



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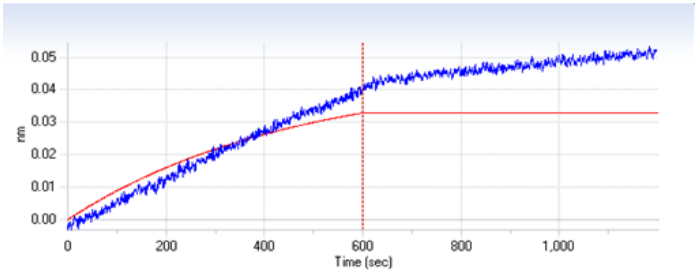
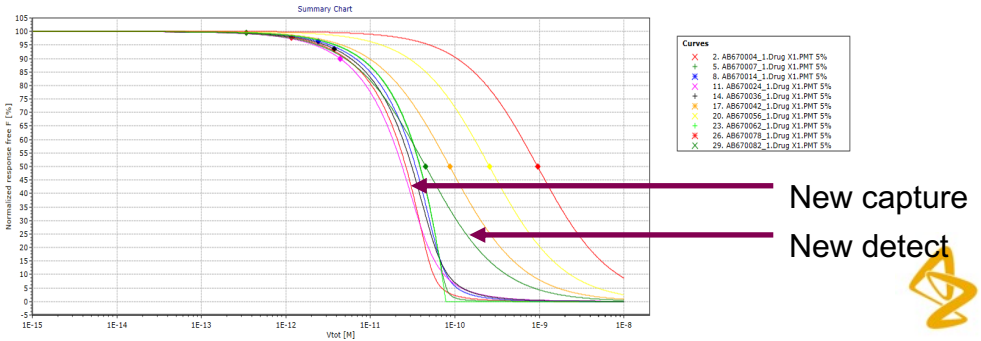


Case study conclusions

- Affinity data indicated two anti-IDs that were not viable reagents
- Data indicate a number of high affinity antibodies (low pM) for which assay sensitivity is insufficient to accurately determine. Data remain informative
- Anti-IDs were ranked based on Gyrolab and Octet data. Data broadly correlate

Gyrolab	Octet
AB670007	AB670036
AB670004	AB670004
AB670014	AB670007
AB670036	AB670024
AB670024	AB670082
AB670082	AB670042
AB670042	AB670078
AB670056	AB670014
AB670078	AB670056
AB670062	AB670062

- Data confirmed new reagent selections
- Assay validated successfully



Conclusions

- Making the right decisions about critical reagent selection in assay development is vital
- Understanding affinity can be a great aid in development. Platforms routinely used for regulated bioanalysis can be utilised for affinity assessment
- The Gyrolab Affinity Module offers intuitive package that can manage experimental and computational needs
- The affinity module was successfully applied troubleshooting challenging development programs

Acknowledgements

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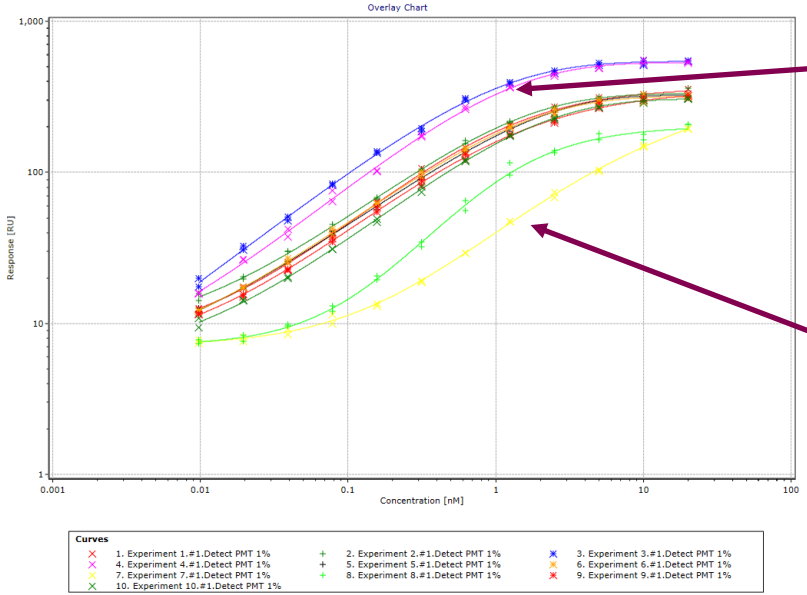
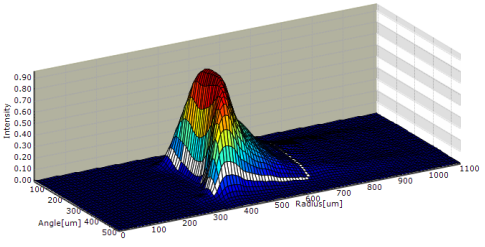
Confidentiality Notice

Back-up: Running the anti-murine SIA method alone is also insightful





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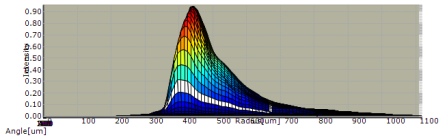

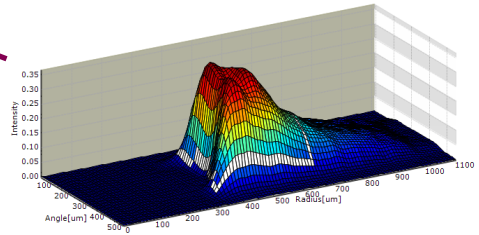

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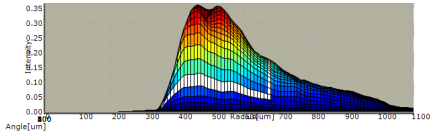

- Whilst not as informative as affinity data Gyrolab Viewer can also inform antibody selection