Optimizing Solution-Phase Epitope Mapping with ALFA-Tagged Peptides and FIDA

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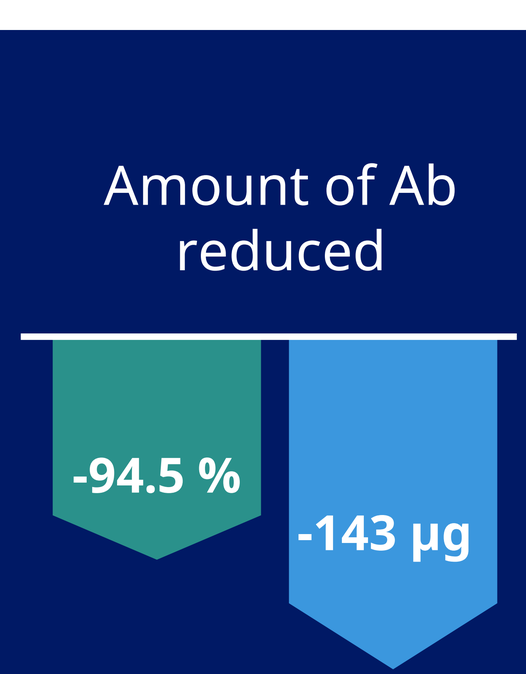
Abstract

Accurate epitope identification is essential for reliable ligand-binding assay performance and for preventing misquantification of peptide therapeutics after metabolism. We present a solution-phase workflow using ALFA-tagged peptide variant libraries and an Alexa-labelled anti-ALFA VHH to measure antibody-dependent changes in complex hydrodynamic size, avoiding artefacts from surface immobilization. For this poster, we outline initial optimization of temperature, determination of optimal peptide concentration [30 nM] by binding-curve analysis, and mapping of antibody interactions across human GIP to delineate likely epitopes and assess assay robustness. Using an updated FIDA measurement approach, we demonstrate the value of a solution-phase strategy for rapid epitope mapping and assay development.



Aim & Methods

The aim is to develop a low-consumption, plug-based Flow-Induced Dispersion Analysis (FIDA) workflow for rapid solution-phase epitope mapping.

- Development strategy: Sequential optimization of temperature, antibody concentration, and injection method to enable rapid epitope mapping and affinity assessment with minimal material.
- Temperature: Assay runs were performed at 5 °C (vs 25 °C) to prevent condensation in the wells.
- Concentration optimization: Binding-curve analyses guided selection of VHH, peptide and antibody concentrations (Figure 2) to ensure measurable dispersion shifts without excess reagent use.
- Injection method: A discrete antibody plug was injected into the capillary at low pressure of 400 mbar for 10 s (Figure 1), instead of filling the capillary, reducing sample consumption while preserving signal. Typical pressure/time steps used during development are summarized in Table 1 & 2.

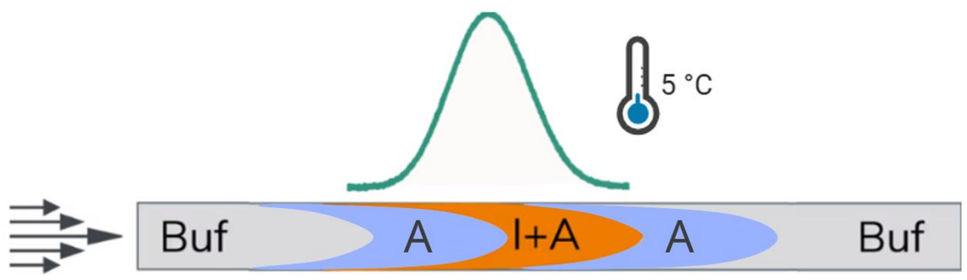


Figure 1: Indicator (I: VHH + peptide) and analyte antibody (A) migrate in OA buffer at 5 °C. The orange region represents formation of the I + A complex; blue regions show free A.

Table 1: *Original method (25 ℃)*

Step	Sample	Flow (mbar/s)
1. Injection	Analyte	3500/30
2. Injection	Indicator	50/10
3. Injection	Analyte	400/180

Table 2*: Plug-based method (5 ℃)*

Step	Sample	Flow (mbar/s)
1. Injection	OA buffer	3500/30
2. Injection	Analyte	400/10
3. Injection	Indicator	50/10
4. Injection	Analyte	400/10
5. Injection	OA buffer	400/180

Results & Conclusion

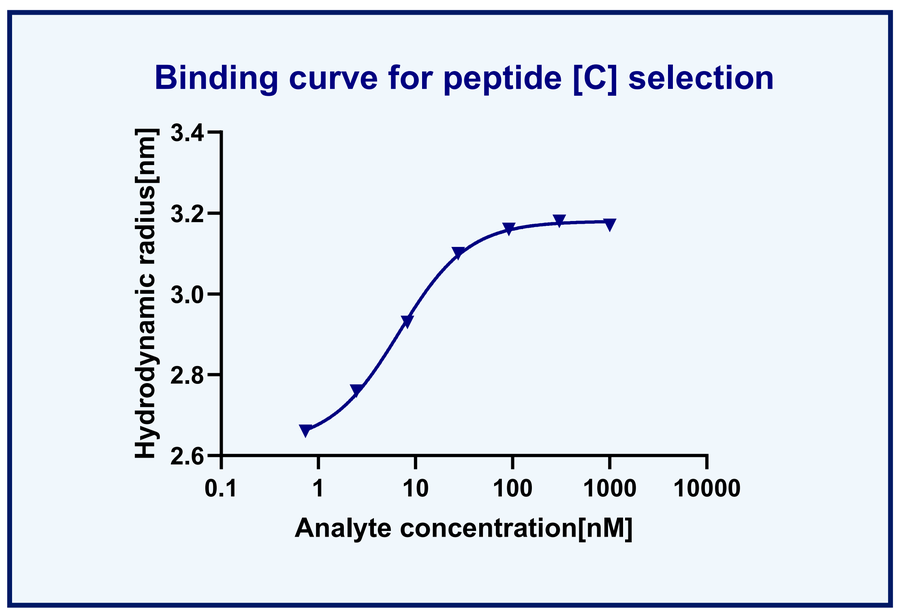


Figure 2: An antibody binding titration curve was generated to determine the optimal working concentration using Capmix, in which ALFA-tagged hGIP and an Alexa-labelled anti-ALFA VHH are combined and mixed directly within the capillary.

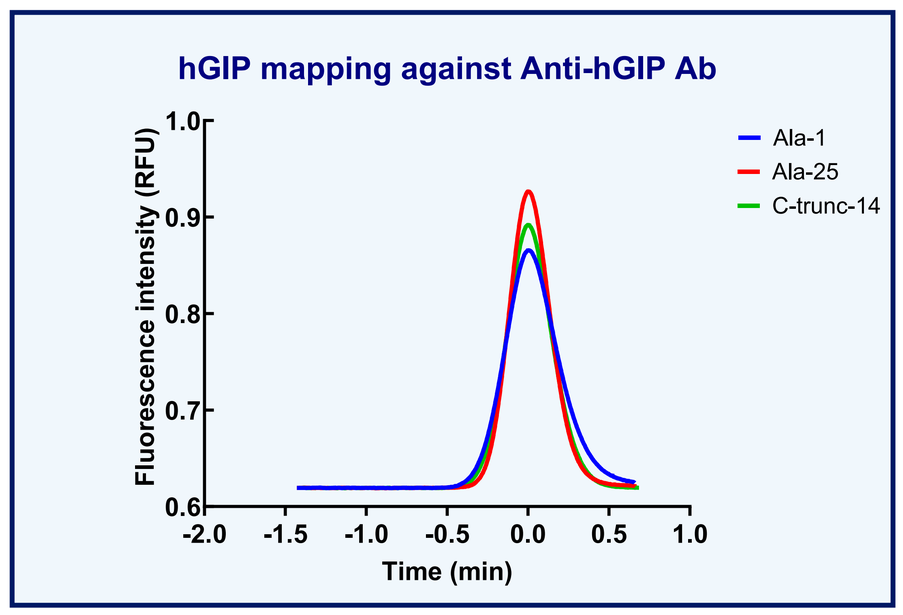


Figure 3. hGIP epitope mapping for antibody Ab0236. Normalized RFU traces for Ala-1 (blue), Ala-25 (red) and Trunc-14 (green) from C-terminal. Ala-25 shows a marked reduction in peak amplitude, indicating residue 25 is critical for Ab0236 recognition.

- We established an optimized assay setup that efficiently characterizes antibody–hGIP interactions while using minimal antibody quantities.
- Orthogonal validation using a LOCI assay confirmed the observed interaction patterns, demonstrating agreement between the two assay formats.
- The plug-based method dramatically reduces reagent and sample consumption (antibody mass reduced by 94.5 %), enabling cost-effective assays (Table 3).

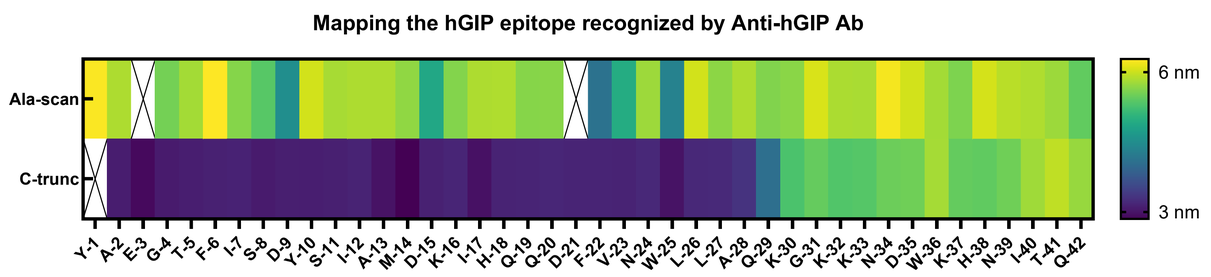


Figure 4. Epitope mapping of Ab0236 on an hGIP peptide panel. Heatmap shows change in dispersion (nm); teal→yellow = stronger binding. Includes alanine-scan and C-terminal truncation panels.

Table 3: *Comparison of reagent and sample consumption*

	Mass (µg)	Volume (µL)
Original method	149	24.3
Plug-based method	6.75	1.09