A new approach to discovering surface epitopes with potential antigenic properties using molecular imprinting

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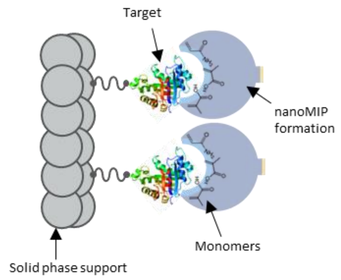
Background

The surface epitopes of a biomarker are key to the development of effective *in-vitro* diagnostic assays, since this is where antibody binding occurs. Mapping these surface epitopes using traditional techniques such as x-ray crystallography and oligopeptide scanning can be time-consuming and expensive, plus success is not guaranteed. These techniques are more often utilized for the analysis of therapeutic antibodies as opposed to diagnostic antibodies, which are typically developed against a whole protein, or a peptide sequence already identified within the literature. A new rapid and cost-effective method for identifying surface epitopes with potential antigenic properties prior to binder synthesis has been developed. In this method, molecular imprinting is used to identify multiple surface epitopes, and the peptides are then sequenced using mass spectrometry.

MIP Diagnostics' nanoMIP™ technology

nanoMIPs™ are synthesized using proprietary methodology, whereby a template (target) (small molecule, peptide etc.) is immobilised on a solid phase, monomers and cross-linker are added, controlled polymerisation is initiated and, ultimately, nanoMIPs™ with high affinity for the target are eluted. The affinity of the nanoMIPs™ is typically comparable to that of a monoclonal antibody, but with incredible thermal stability and robustness.

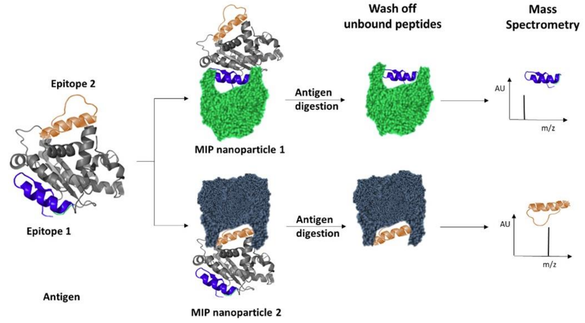


MIP production

nanoMIP imprinting demonstrating the polymerisation step of the production process.

Epitope discovery methodology

The target protein was brought into contact with a selection of monomers, which were polymerized to create a protein-polymer complex. The protein was then digested to leave a peptide-polymer complex in which the identified epitope(s) are 'captured' within the polymer pocket. The peptide-polymer complexes were then isolated, the peptide was removed, and later sequenced using mass spectrometry. The peptide sequence(s) identified correspond to potential binding sites for a binding partner such as an antibody, aptamer, affimer or nanoMIP™. The total process was completed for each antigen in 1 week.

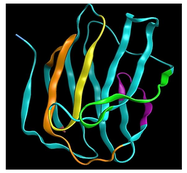


Results

Human Galectin-1

The galectins are a family of beta-galactoside binding proteins implicated in a number of conditions. Most recently, and perhaps most significantly, Galectin-1 levels have been linked to the progression of sepsis (University of Connecticut, 2021). It was therefore a good target for the epitope discovery process.

Known epitopes (Ab)	Epitopes found by our process
	SFVLNLGK^
	DGGAWGTEQR#
LPDGYEFKFP	LPDGYEFKF
NLEAIN	LNLEAINYMAADGDFK



The antibody ab112525 recognizes a sequence near the C-terminus of human galectin 1 (NLEAIN) and antibody Gal1-mAb3 recognizes a sequence slightly nearer the middle of the molecule (LPDGYEFKFP) – these two sequences, and two others were clearly picked up the Epitope Discovery process. The two others^# may prove excellent pairing candidates for either of the known antibodies in assay development.

Gliadin (wheat)

Gliadins are classified in 4 groups (α-, β-, γ-, and ω-gliadins). PFPQPQL is one of the most abundant celiac disease (CD) epitopes (from alpha-gliadins), and it is present within residues 57-89 (33-mer peptide)

Known epitopes (Ab)	Epitopes found by our process
QPQLPY	QLQPFPQPQLP (alpha)
PFPQPQL	
KLQPFPQPELPYPQPQ*	
QLPYPQP	not found
-	QQPQQQYPSGQGSF (alpha)
-	QQQQQQQL (alpha)

The antibody G12 recognizes the QPQLPY sequence, Abcam antibody 1D5 is specific for the de-amidated form (*) targeting LQPFPQPELPYPQPQ and antibody A1 recognizes QLPYPQP. The epitopes found by the epitope discovery process may constitute good pairing candidates with the known epitopes for use in gliadin assay development. NB: a number of epitopes from beta, gamma and omega gliadins were also identified, given the crude antigen preparation employed, indicating the power of the process in identifying suitable surface epitopes even under more complex conditions.

Conclusions

This new method for epitope discovery has been shown to identify previously unknown surface epitopes on the target biomarkers, which can then be utilized to create an anti-peptide antibody, apatamer, affimer or molecularly imprinted polymer for diagnostic applications. Other potential applications include the preliminary screening of therapeutic antibodies and to support vaccine design.

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^ Subsequent to publication at AACC2021, SFVLNLGK was identified as the known epitope of a commercial monoclonal antibody
# DGGAWGTEQR includes amino acids associated with the carbohydrate-binding region

To discuss collaboration opportunities, please contact Alan Thomson at enquiries@mip-dx.com