Affinity enrichment and femtomole detection of spike protein using smart polymers

LC-MS Detection of SARS-CoV-2 Spike Protein Using Smart Polymers

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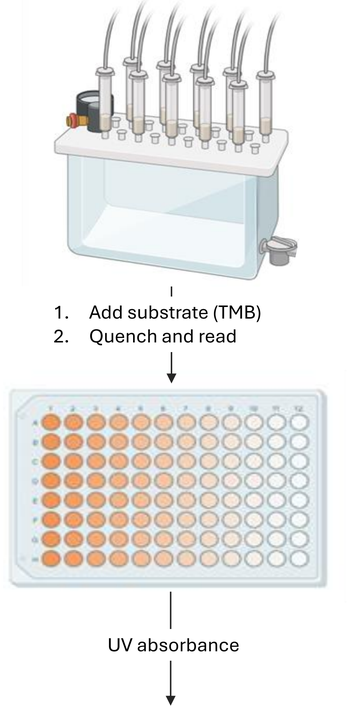
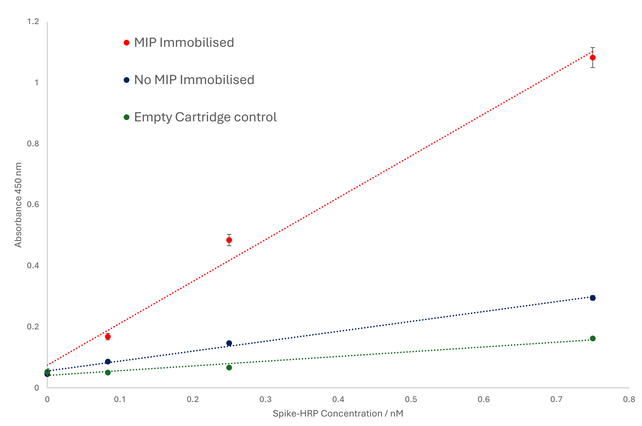
Introduction

Smart polymers targeting the SARS-CoV-2 spike protein have been developed and tested using surface plasmon resonance (SPR), demonstrating a binding affinity in the picomolar range. Exploiting this exceptional affinity, these smart polymers were then integrated into chromatography-based protocols for the purpose of matrix purification. To enable this, the polymers were immobilised on a silica glass bead support, and successfully integrated into a Liquid Chromatography – Mass Spectrometry (LC-MS) sample preparation workflow.

Methodology

The smart polymer was coupled to amine coated glass beads via glutaraldehyde chemistry, and then blocked with ethanolamine. The beads were subsequently washed and dried, then – for the purpose of benchtop development – they were incubated with an HRP-conjugated spike protein in the presence of Tween 20. The beads were subsequently washed in a vacuum manifold and the spike-HRP quantified (Figure 1). Later, direct quantification of native spike was achieved by LC-MS, by first allowing the spike protein to be captured by the smart polymers on the solid support, and then proceeding with the enzymatic digestion of the captured spike protein (Figure 2). Finally, the supernatant was retrieved, and then injected and analysed via Data Independent Acquisition (DIA) LC-MS (Figure 3 & 4).





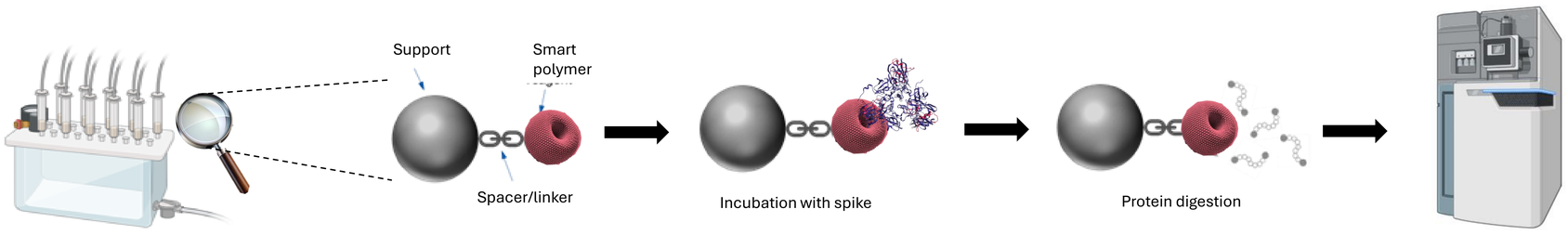


Figure 2. Representation of the process in which smart polymers are immobilized on a solid-support and used to capture and quantify the spike protein via LC-MS.

Figure 1. Response from the HRP-spike bound to the immobilized smart polymers.

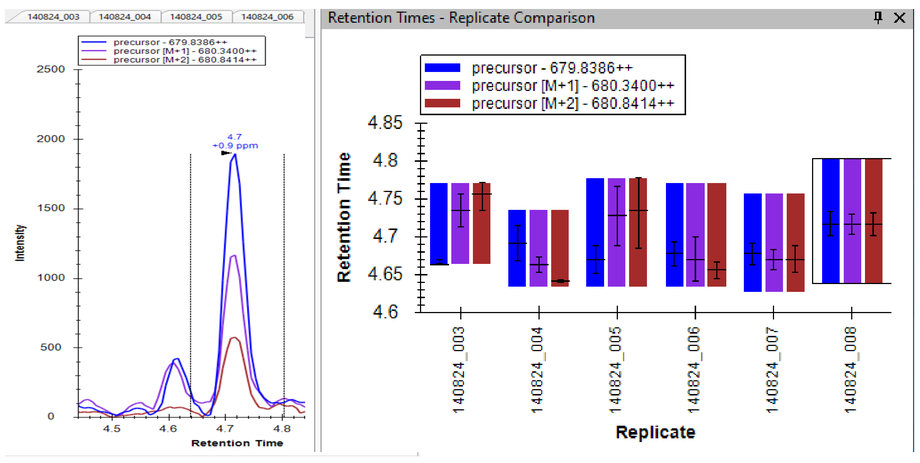


Figure 3 (left). Extracted mass chromatograms and retention time reproducibility for one of the spike peptide fragments (VYSTGSNVFQTR) following protein digestion on polymer-functionalised beads.

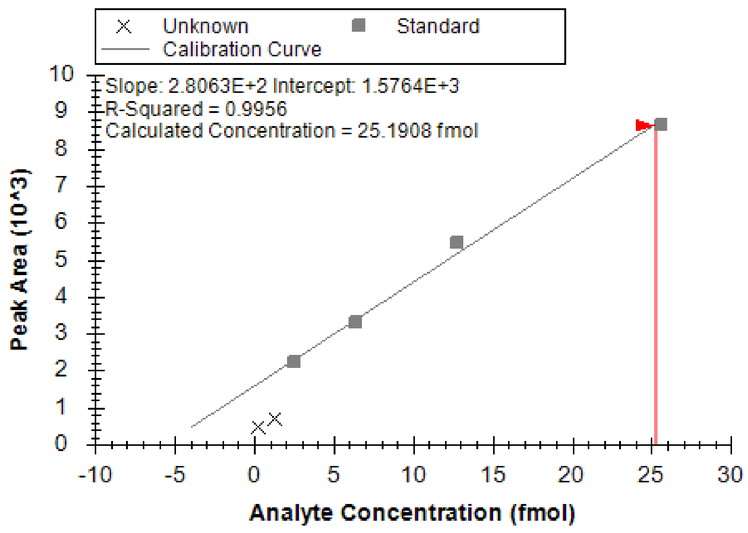


Figure 4 (right). Quantitative response for one of the tryptic peptide fragments (VYSTGSNVFQTR) detected using LC-MS following digestion on polymer-functionalised beads.

Results

The polymer-modified solid-phase was successful in capturing the spike protein and withstanding the digestion protocol, in a process that could be completed in just 20 min. The resulting LC-MS analysis produced a signal at **2 femtomoles** using regular flow chromatography and DIA LC-MS acquisition, demonstrating excellent sensitivity.

The use of non-biological affinity reagents (such as smart polymers) together with a non-protein-based blocking solution (ethanolamine) allows the protein digestion to go ahead with no concerns of other peptides contaminating the solution. In addition, the smart polymer-functionalized beads demonstrated they can maintain full functionality for at least 5 months storage at 5°C. This work emphasizes the possibility of utilising smart polymers for viral purification, and work is now being carried out to expand on this application with viral vectors in the CGT space.

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