# Identification of Materials Binding Peptide Sequences guided by a MALDI ToF-MS depletion assay

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# Supplementary Information (SI)

**Content:**

## S1. Different MALDI-MS preparation parameters.

To test whether different sample preparation methods could reduce the variability associated with the MALDI-MS peak area measurements, we have carried out a series of test experiments, in which the peak areas associated with a pure solution of the peptide SBP has been collected in 9 spectra. The standard deviation of the 9 measurements (for each test) has been taken as a measure of the data variability. The tests include, among other conditions (see Fig. S1), the following changes with respect to the standard procedure described in the Methods Section of the main manuscript text:

1) the mixing of matrix and peptide solution has been performed before spotting on MALDI sample plate and larger volumes of peptide solutions have been used;
2) the matrix/peptide ratio was changed to 1:4 (instead of 1:1);
3) The number of accumulated spectra per spot has been doubled;
4) The concentration of the $NH_4HCO_3$ buffer has been changed from our standard 400 mM (as employed in the lysozyme digestion assay) to 100, 50, and 25 mM.

The results show that our standard procedure leads to variability comparable or better than the other tested preparation methods. We note that all analysed spectra did not suffer from a too low signal to noise ratio, so there was no need to discard any spectrum from the analysis. Moreover, we have adjusted the MALDI parameters specifically to remain far from the saturation limit of the detector, so that also in this case no spectra needed to be discarded.

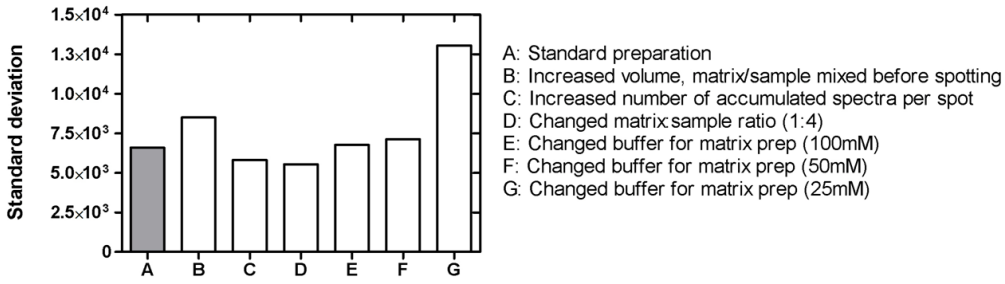


**Fig. S1:** Influence of different preparation methods for MALDI ToF-MS on the standard deviations associated with the peak area value of a solution of the SBP peptide (1,467 Da) in series of nine measurements for each preparation method. Our standard procedure (grey bar) leads to a standard deviation comparable or even smaller than other preparation methods. Variations from standard protocol (A) are as follows: B: matrix and sample were mixed before spotting using an increased volume to minimize pipette errors (50 µl of sample and matrix, respectively). C: Increased amount of accumulated spectra (10 instead of 5). D: Matrix/Sample ratio was adjusted to 1:4 (before: 1:1). E-G: Molarity of buffer ($NH_4HCO_3$) that was used for sample dissolving was decreased to 100, 50 and 25 mM instead of 400 mM.

## S2. Adhesion on tube walls.

We have performed control experiments to explore the possible adhesion of the peptides on the walls of the LoBind tubes (Eppendorf AG, Hamburg, Germany) used in our experiments (see Fig. S2). The results of these control experiments show that, apart from small deviations from the initial values well within the intrinsic error bar of the MALDI method, the peak areas of peptides placed in contact with tubes in the absence of $SiO_2$ nanoparticles remain stable up to 24 hours. In principles, it is possible to use the control experiment to correct the data in the presence of nanoparticles (after subtraction of the control values). However, whether or not such correction is performed does not alter the final conclusion. We believe that performing the binding experiments with nanoparticles is important to this respect, given the much larger surface area displayed by the particle suspensions compared to the surface area of the tube alone.

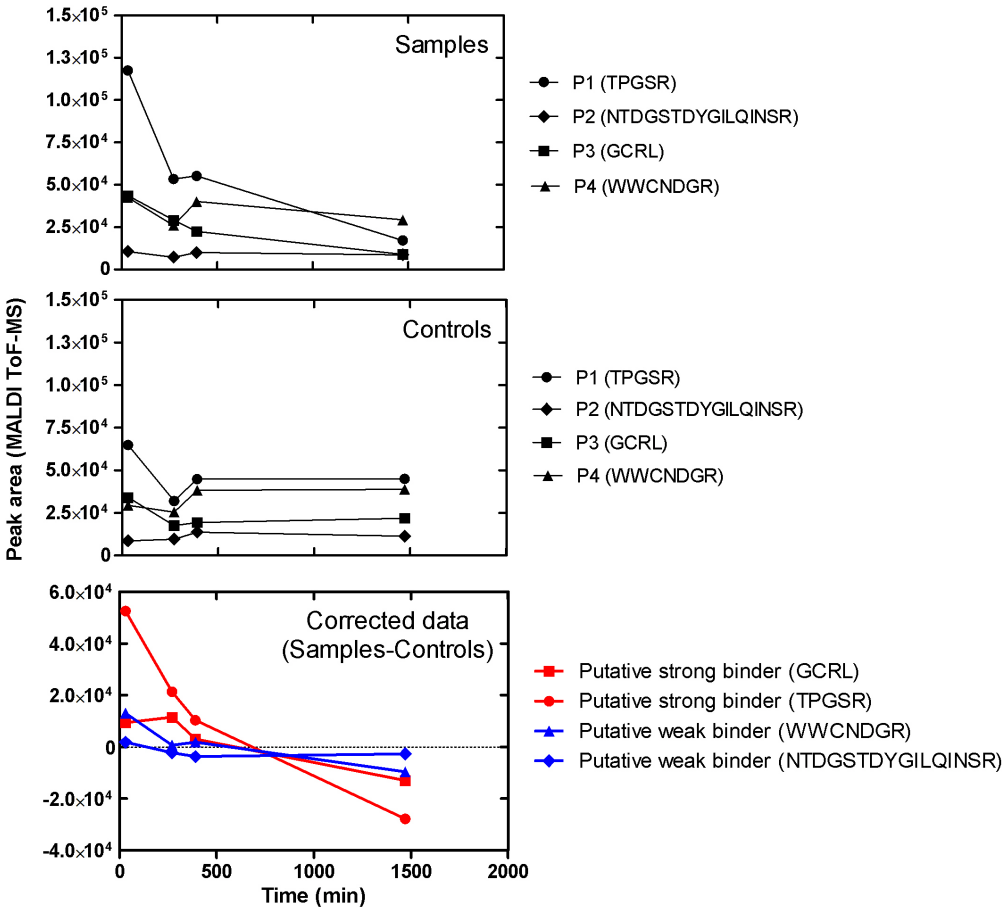


**Fig. S2:** Time evolution of peptide adsorption as measured with MALDI-ToF MS depletion technique. Sample (upper image) describes the adsorption of peptides on $SiO_2$ nano particles in Eppendorf tubes. Controls (middle) shows peptide adsorption plots on Eppendorf tubes without nano particles. The corrected data (lower image) was calculated by subtraction of controls from sample values. Although for P1 and P3 a weak adsorption onto tubes (controls) can be assumed within first 120 minutes, signals remain stable or increase slightly until 1,440 minutes.

## S3. Purity of synthetic peptides.

To assess the purity of the synthetic peptides we include exemplary MALDI ToF-MS spectra of each peptide solution, Fig. S3, in which the peaks associated to peptides used in this study are marked with black squares. The purity is acceptable in all cases except for the SBP peptide, which present also a few spurious peaks not included in our analysis. We note, however, that the intensity of the peak corresponding to the expected mass/charge ratio of the SBP peptide is high enough to permit a clear analysis of its adsorption behaviour comparably with the other sequences

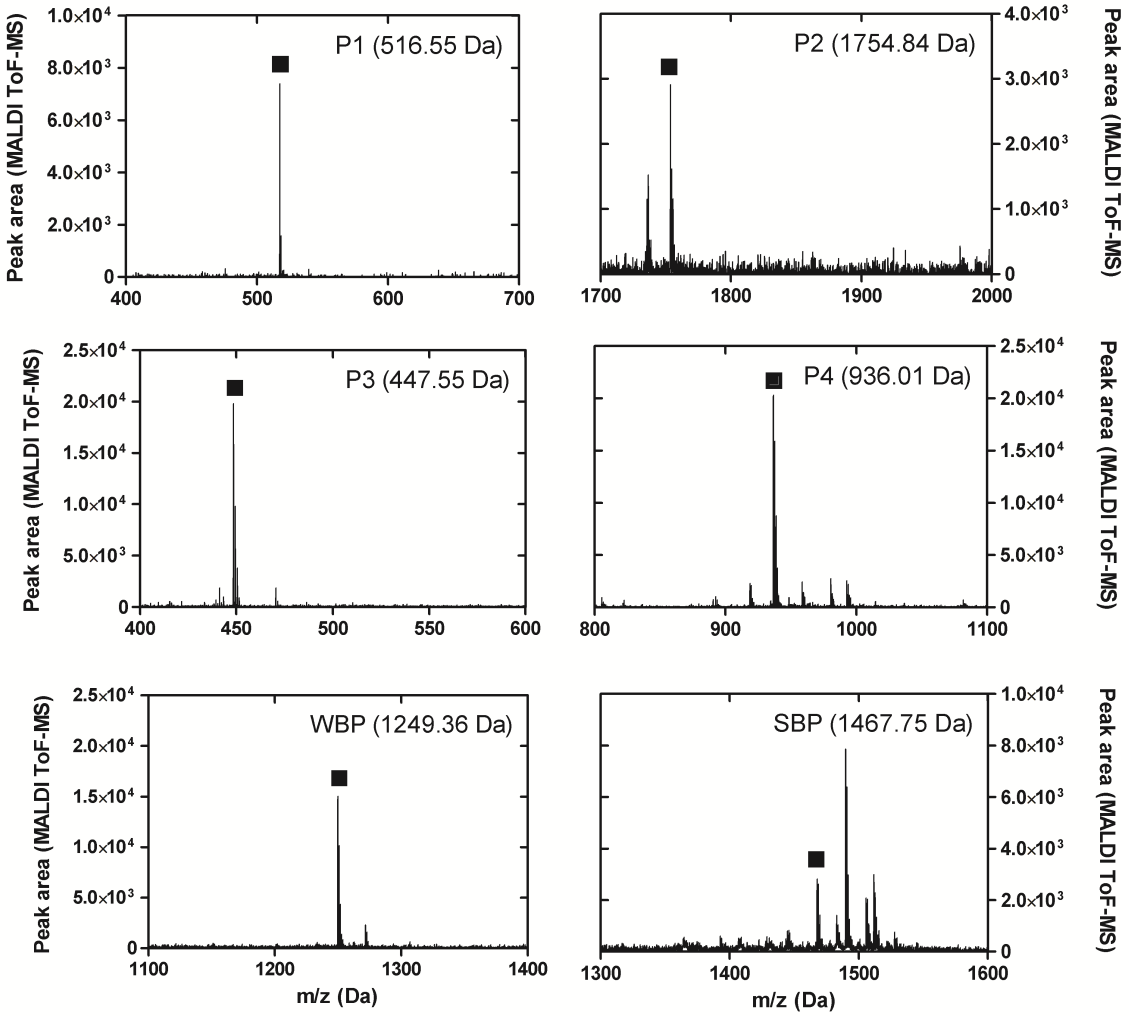


**Fig. S3:** MALDI ToF-MS of the solution of synthetic peptides P1, P2, P3, P4, WBP, SBP used in our study, as a mean of assessing the purity of the peptides after SPP synthesis. Black squares indicate the specific peptide peaks. MALDI ToF-MS is a common technique to identify peptide purity after synthesis procedure and was therefore used in this context. Given peptide masses are without modifications (e.g. alkylation). As it can be seen, peaks of interest can be separated easily from potential foreign peaks (if existing).

## S4. Oxidation of tryptophane residues.

We have monitored the MALDI peaks associated to the peptide P4 (containing 2 tryptophan residues) assuming (i) no oxidation (936.01 Da); (ii) single oxidation (+16 Da w.r.t. the non-oxidized case); (iii) twofold oxidation (+32 Da); (iv) threefold oxidation (+48 Da); and (v) fourfold oxidation (+64 Da). As reported in Figure S4, the peak areas associated with any of the oxidized states are much smaller than the one associated with the non-oxidized state. Also during the temporal evolution of the peak area when the peptide is incubated with $SiO_2$ nanoparticles, no appreciable increase of the peak areas of the oxidised states is observable. We thus conclude that possible oxidation does not interfere with the adsorption properties of P4.

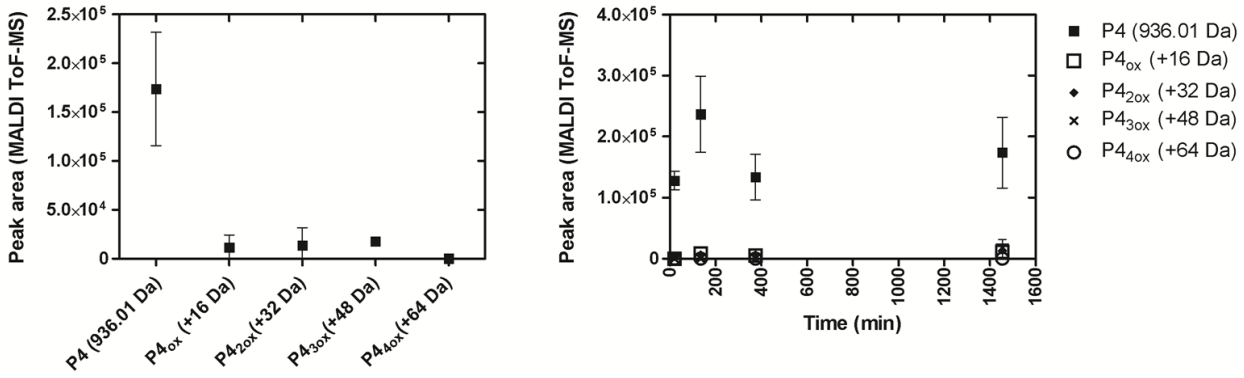


**Fig. S4:** MALDI peak areas associated with the non-oxidized or with oxidized states of tryptophan in peptide P4 (WWCNDGR). The occurrence of peaks corresponding to oxidized states after 24 hours incubation is negligible (left). The abundance of oxidation peaks did not change significantly with time during incubation of the peptide with $SiO_2$ nanoparticles (right).