

Supplementary Material to

SSPaQ: A subtractive segmentation approach for the exhaustive parallel quantification of the extent of protein modification at all possible sites

for JASMS

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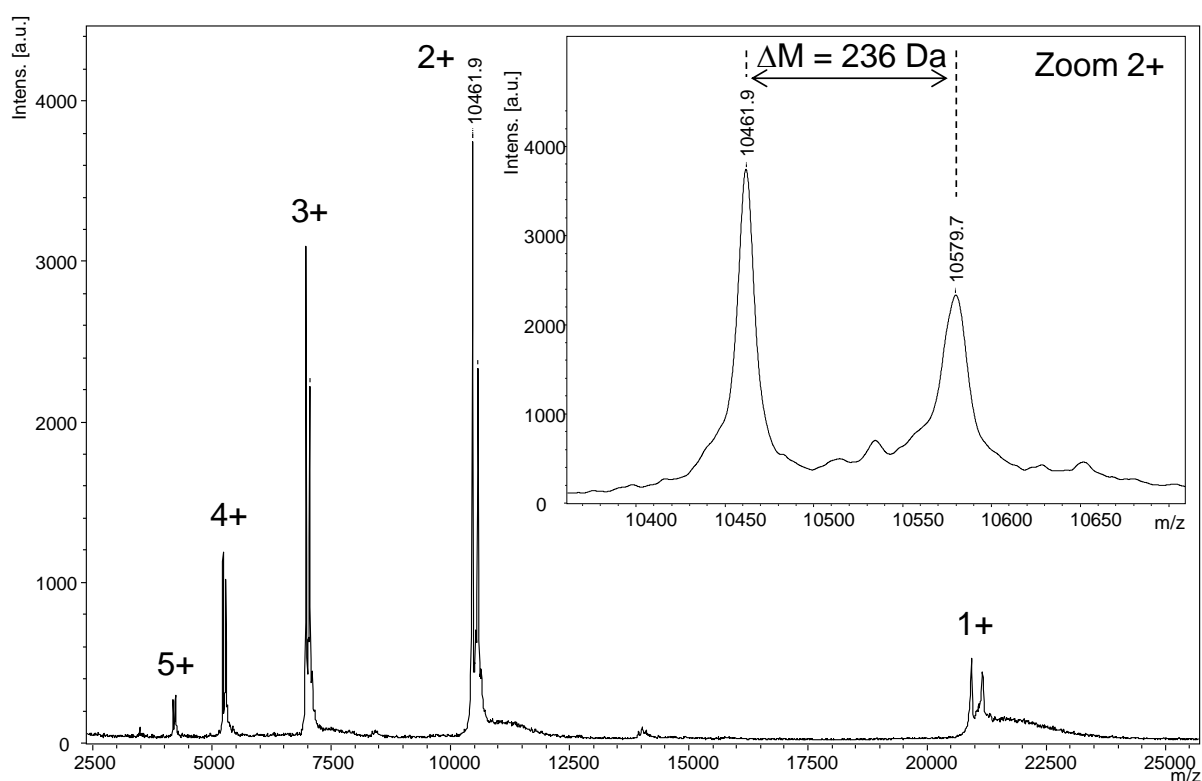
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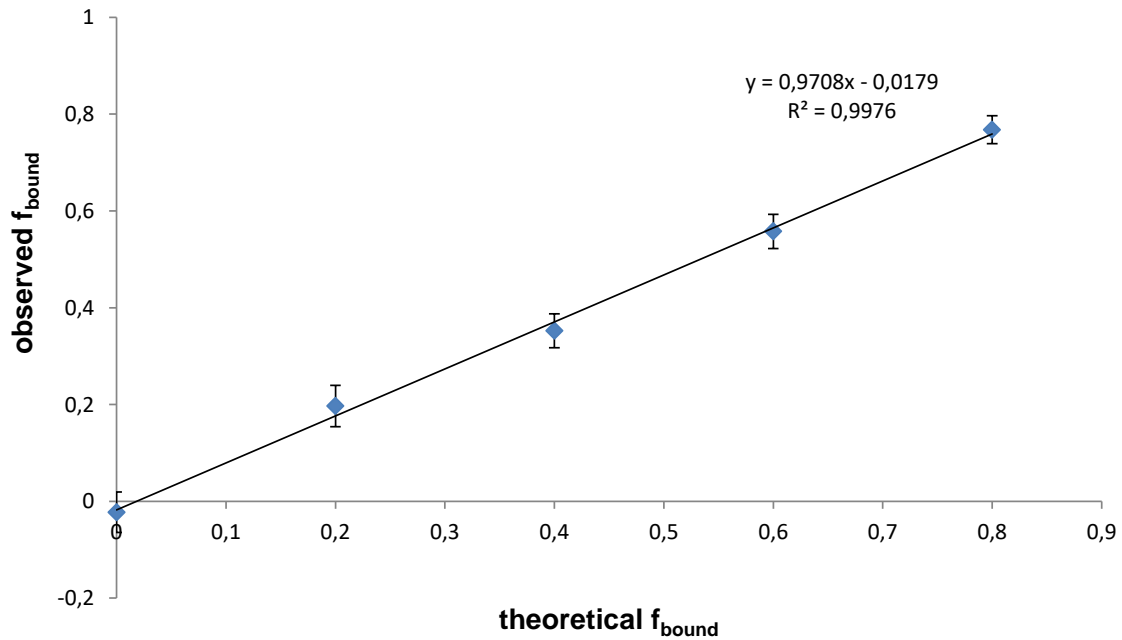
Figure S1. **Metabolic incorporation measurement on hPEBP1.** A 1:1 mixture of $^{14}\text{N}/^{15}\text{N}$ hPEBP1 protein solutions was analyzed by MALDI-TOF MS.



Effect of the degree of modification on accuracy and precision.

To investigate how the error is affected by the degree of modification, accuracy and precision were measured as a function of f_{bound} , with values ranging from 0 to 0.8. For this experiment, various degrees of modification were mimicked by decreasing the quantity of nonmodified light form of hPEBP in the experiment (XP) compared to the control (CT). In this context, for a given degree of modification, all the peptides have the same expected f_{bound} value, which was used to calculate accuracy and precision. A plot of observed f_{bound} as a function of expected f_{bound} (Fig. S2) can be fitted with straight line with an R^2 close to 1.

Figure S2. **Measured f_{bound} as a function of theoretical f_{bound}** mimicked by varying nonmodified light protein concentration



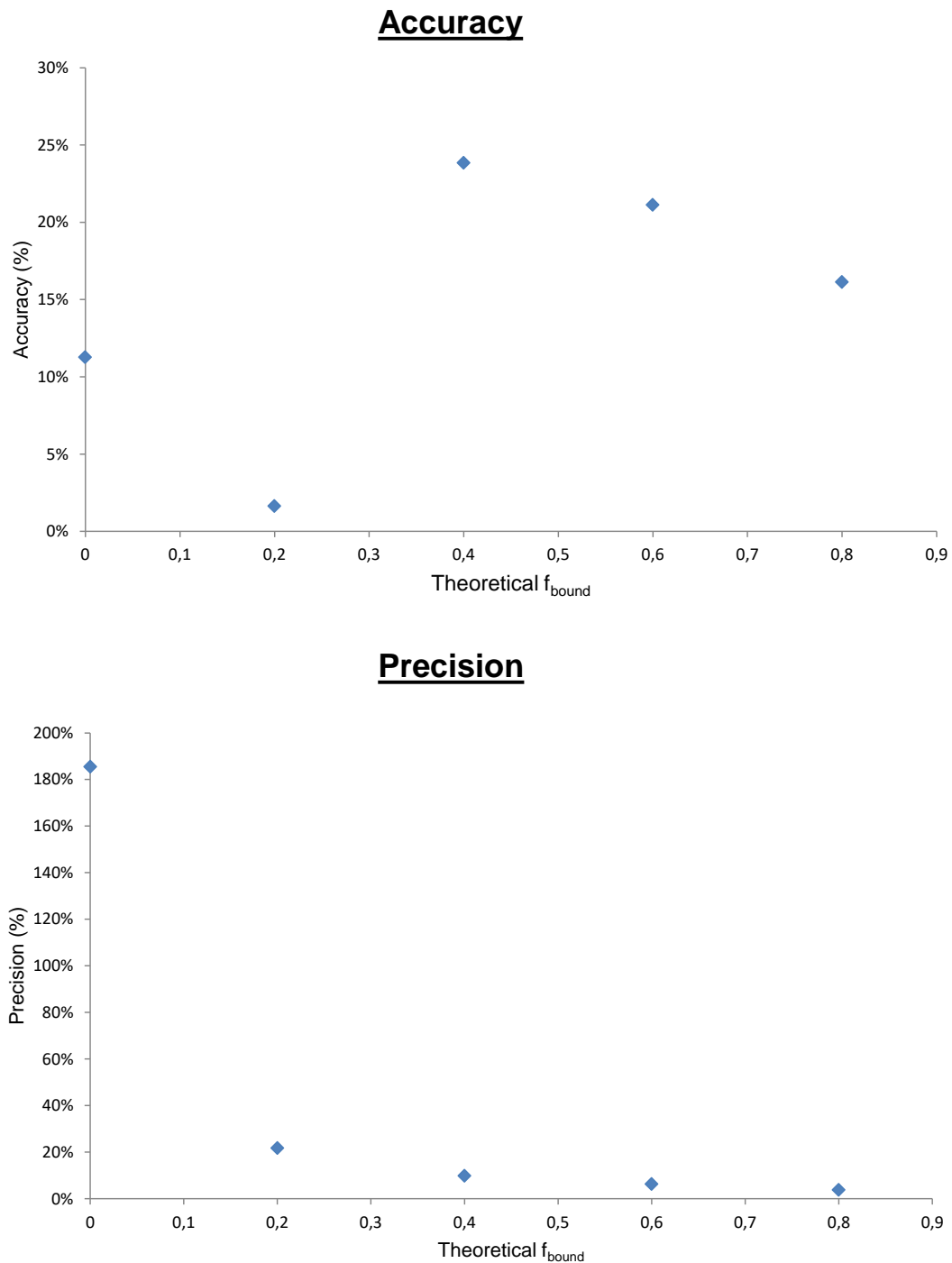
Equations 1 and 2 allow for error calculation expressed as accuracy and precision percentages:

$$Accuracy = \frac{\text{measured ratio} - \text{expected ratio}}{\text{expected ratio}} \times 100 \quad (1)$$

$$Precision = \frac{\sigma(\text{measured ratio})}{\mu(\text{measured ratio})} \times 100 \quad (2)$$

Accuracy varies from 2 to 24% in the observed range with an average of 16% (Fig. S3 upper panel) with no clear trend in the data.

Figure S3. **Accuracy and Precision of f_{bound} measurement as a function of theoretical f_{bound}** mimicked by varying nonmodified light protein concentration



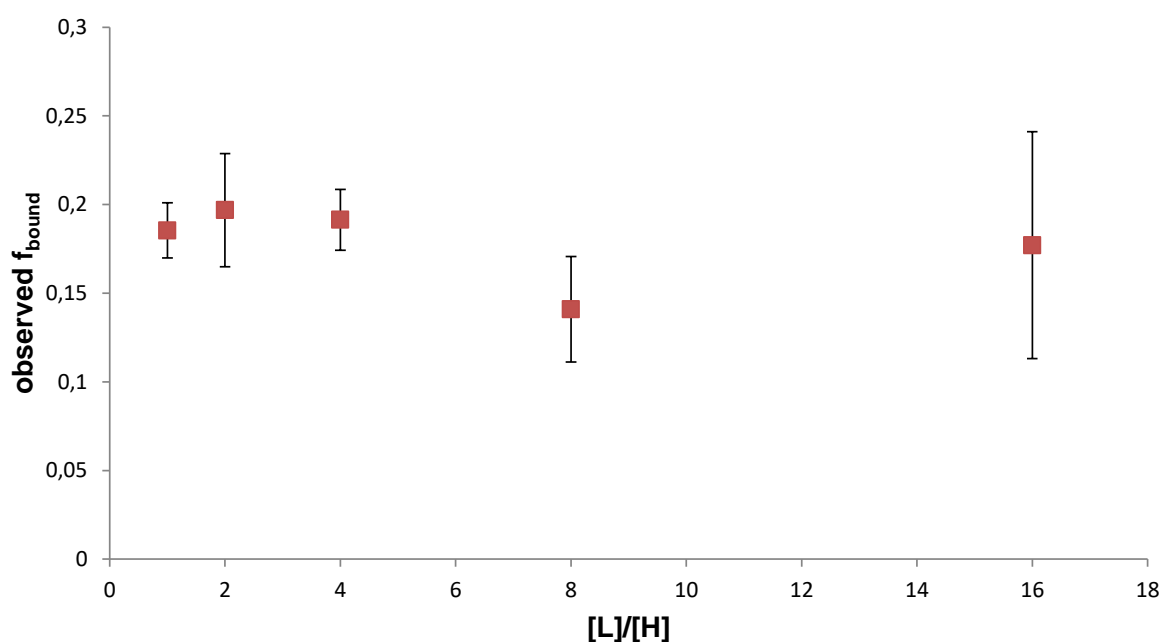
Precision tends to decrease with the degree of modification (Figure S3 bottom panel). The absolute standard deviation is quite similar for each degree of modification, ranging 0.029-0.043. The relative

standard deviation however starts very high with 185% for zero modification, then decreases rapidly down to 4% as the degree of modification increases.

Effect of the amount of added internal standard on accuracy and precision.

To investigate how accuracy and precision are affected by the amount of added internal standard, several [L]/[H] protein ratios from 1/1 to 1/16 were prepared. A f_{bound} of 0.2 was chosen for this experiment, mimicked by varying the amount of light protein in the experiment compared to the control as above, and observed f_{bound} plotted as a function of the [L]/[H] ratio (Figure S4).

Figure S4. **Effect of [L]/[H] protein ratios on f_{bound} measurement**



Our results show that the accuracy is not clearly correlated with the amount of internal standard added (Figure S5 upper panel) and that the precision tends to increase as the amount of internal standard decrease (Figure S5 lower panel). For accuracy, the relative error ranges 2-30% with an average accuracy of 11%. For precision, the relative standard deviation increases from 8% to 36% as the amount of internal standard decreases (i.e. the [L]/[H] ratio increases).

Figure S5. Accuracy and Precision of f_{bound} measurement as a function of $[L]/[H]$ protein ratios.

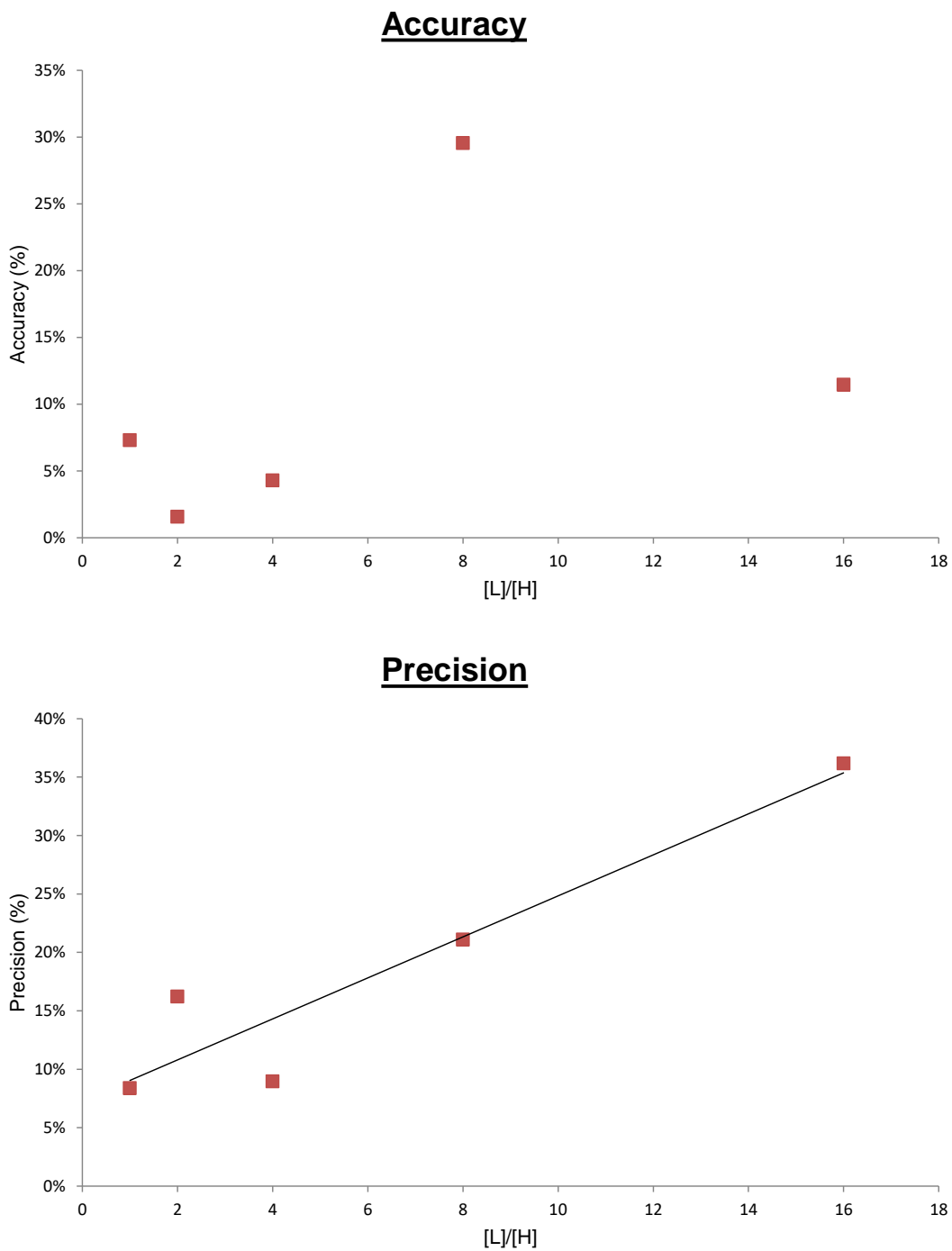


Figure S6. **Peptides f_{bound} for individual proteinases.** Graphical representation of parallel quantification of peptide modifications upon reaction of locostatin with hPEBP1, $t = 5$ h. Grey bars correspond to ± 2 standard deviations in the CT sample.

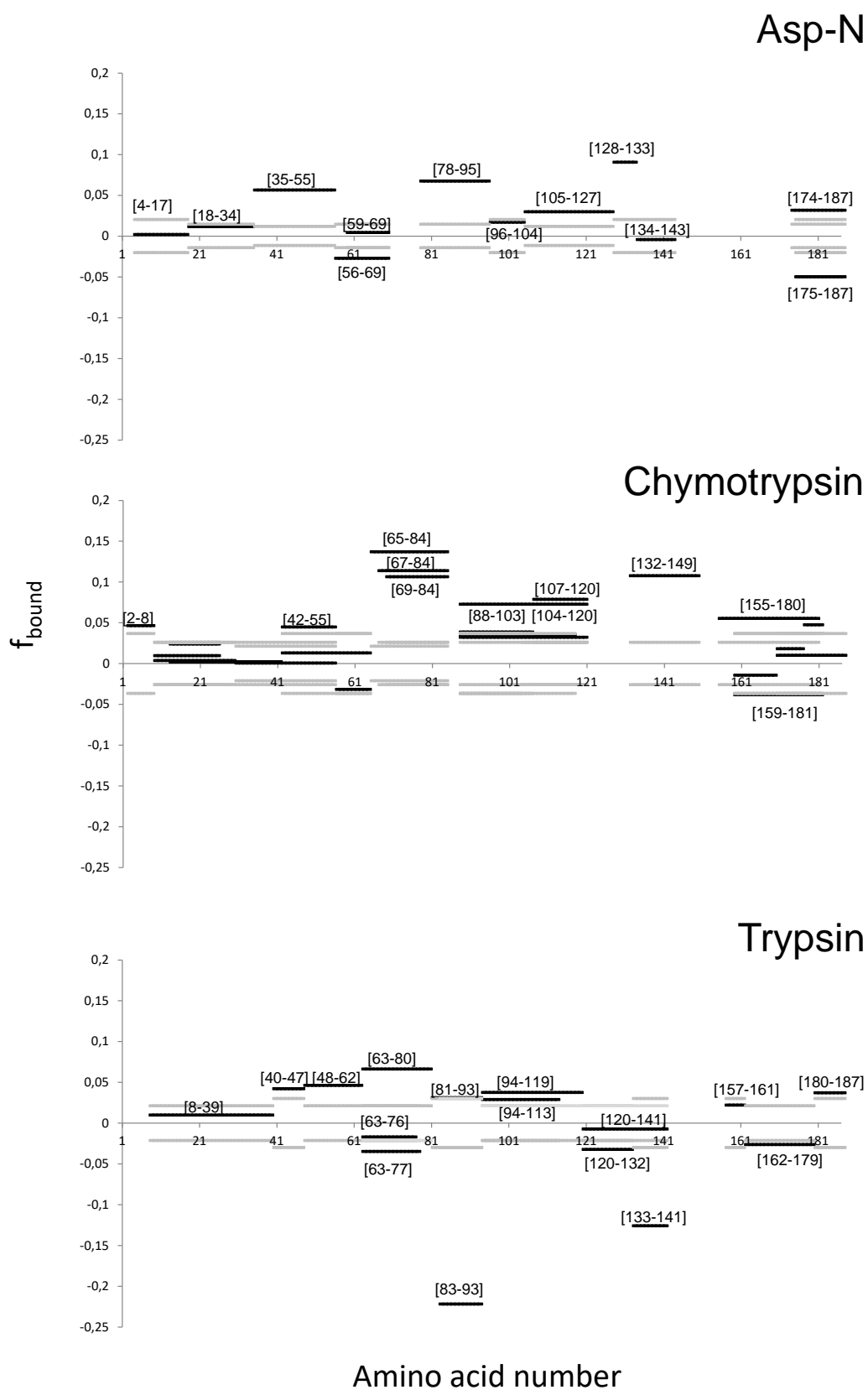


Figure S7. **Combined results for the three proteinases.** Graphical representation of parallel sites quantification upon reaction of locostatin with hPEBP1, $t = 5$ h.

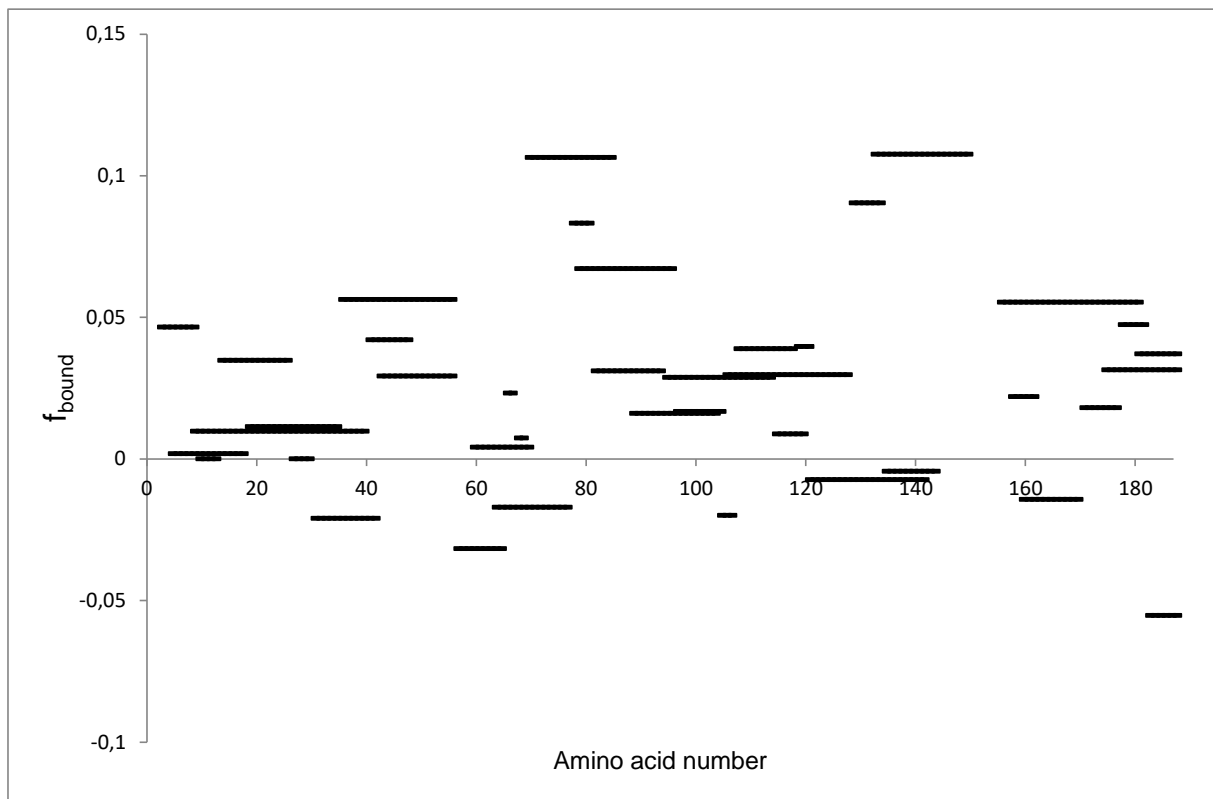


Figure S8. Local Minima for f_{bound} derived from the dataset in Figure S7.

