Supplementary data for:

Structural and functional differences in isoforms of Major Urinary Proteins: a male specific protein that preferentially binds a male pheromone

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Supplementary data A. Comparison of MALDI-ToF spectra for peak IV protein and a typical uMUP.

The proteins in chromatographic peaks II and IV were reduced and subjected to in-solution digestion with endopeptidase LysC. The peptides were analysed by MALDI-ToF MS. To aid comparison, the peak IV spectrum is vertically inverted. Peptides L1…Ln refer to the n^{th} endopeptidase LysC peptide, counted from the N-terminus.

Supplementary data B. Tandem mass spectrometry of tryptic peptides derived from peak IV uMUP.

Two peptides (doubly charged $[M+2H]^{2+}$ ions at 779.9 Th (a) and 937.5 Th (b)) derived from an in-gel digest of peak IV MUP were analysed by tandem mass spectrometry. Product ion spectra were manually interpreted to recover the sequences of the peptides from b and y ions. The symbol 'J' is used to denote leucine/isoleucine which cannot be discerned using this method.

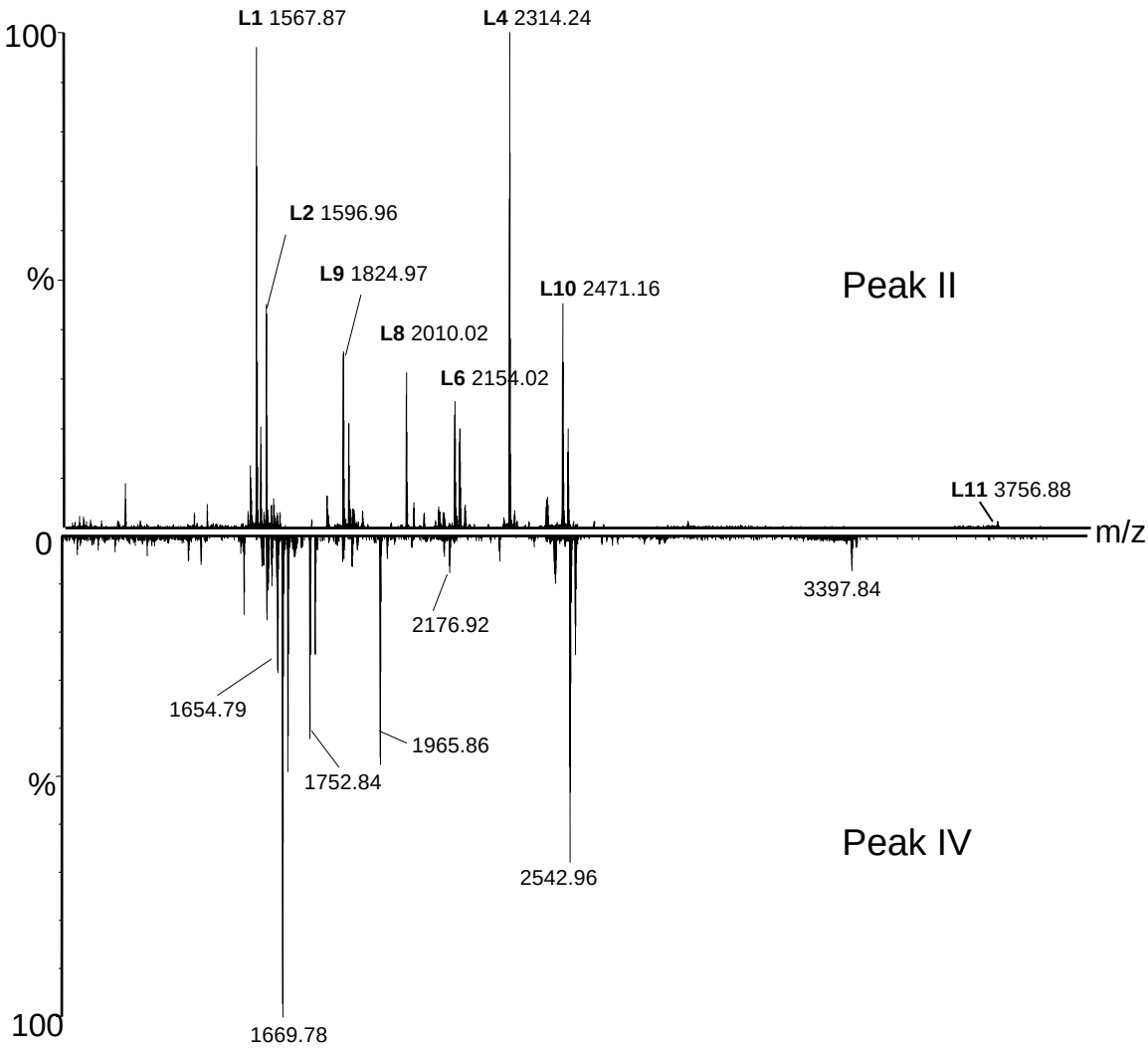
Supplementary data C. Cavity forming residues of uMUP-I in relation to peak IV MUP.

The sequences of MUP-I and peak IV MUP are aligned, such that only residues that are different are identified on the peak IV MUP. Below the alignments are staves highlighting residues involved in ligand binding according to three independent studies (1:[Ref 1] 2:[Ref 2] 3:[Ref 3]). Residues highlighted in black are common to both proteins, those left unfilled are different between the two proteins.

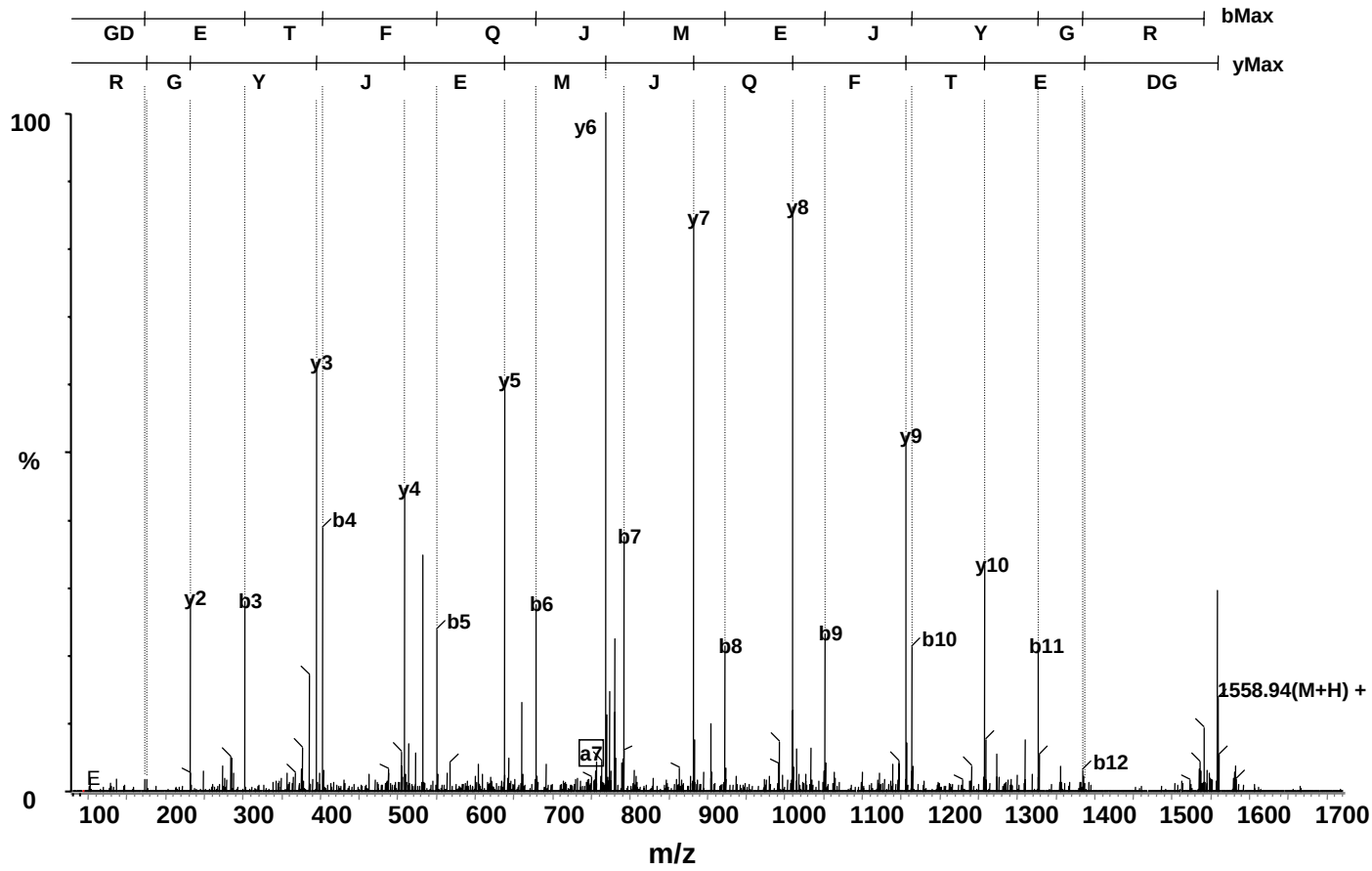
1. Lucke, C., Franzoni, L., Abbate, F., Lohr, F., Ferrari, E., Sorbi, R. T., Ruterjans, H. and Spisni, A. (1999) Solution structure of a recombinant mouse major urinary protein. Eur. J. Biochem. **266**, 1210-8

2. Timm, D. E., Baker, L. J., Mueller, H., Zidek, L. and Novotny, M. V. (2001) Structural basis of pheromone binding to mouse major urinary protein (MUP-I). Protein Sci. **10**, 997-1004.

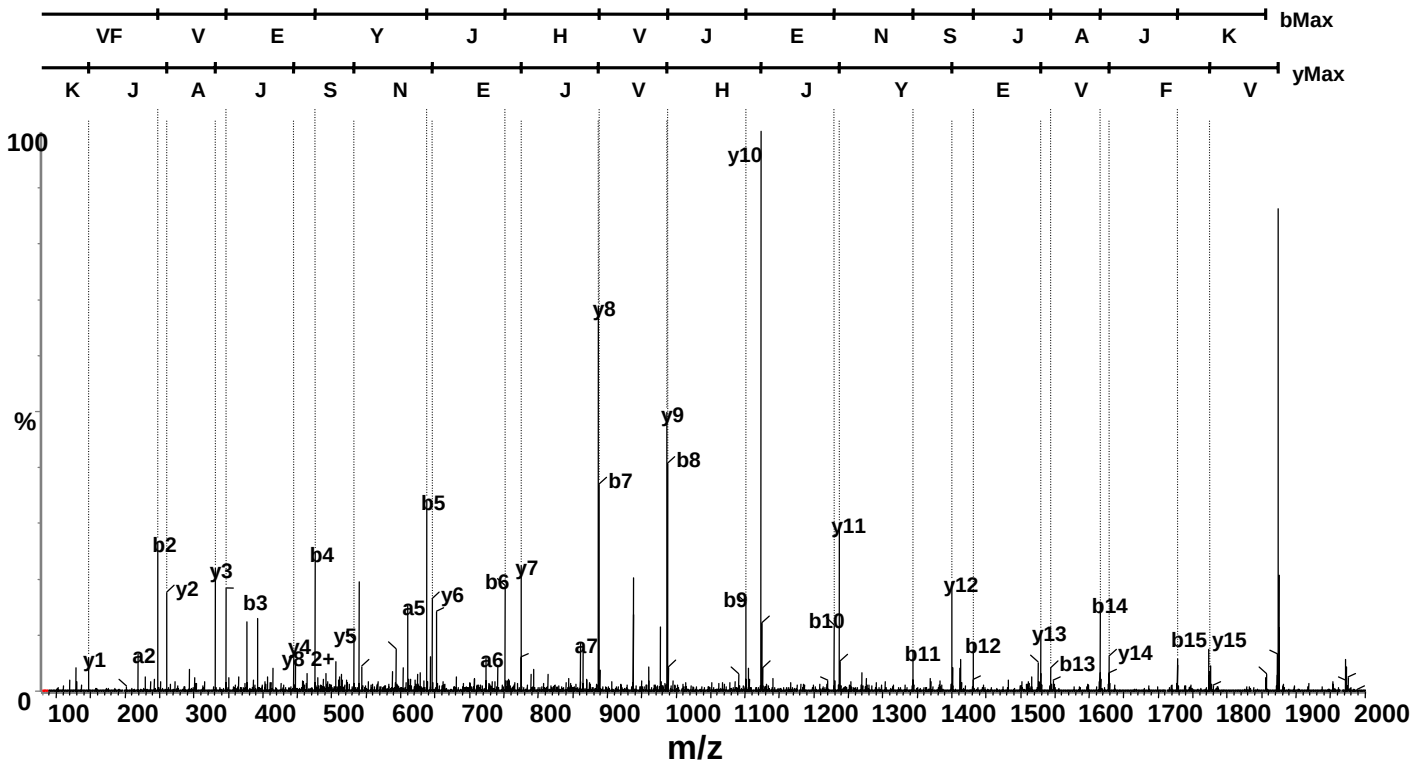
3. Bingham, R. J., Findlay, J. B., Hsieh, S. Y., Kalverda, A. P., Kjellberg, A., Perazzolo, C., Phillips, S. E., Seshadri, K., Trinh, C. H., Turnbull, W. B., Bodenhausen, G. and Homans, S. W. (2004) Thermodynamics of binding of 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-isobutylpyrazine to the major urinary protein. J. Am. Chem. Soc. **126**, 1675-81

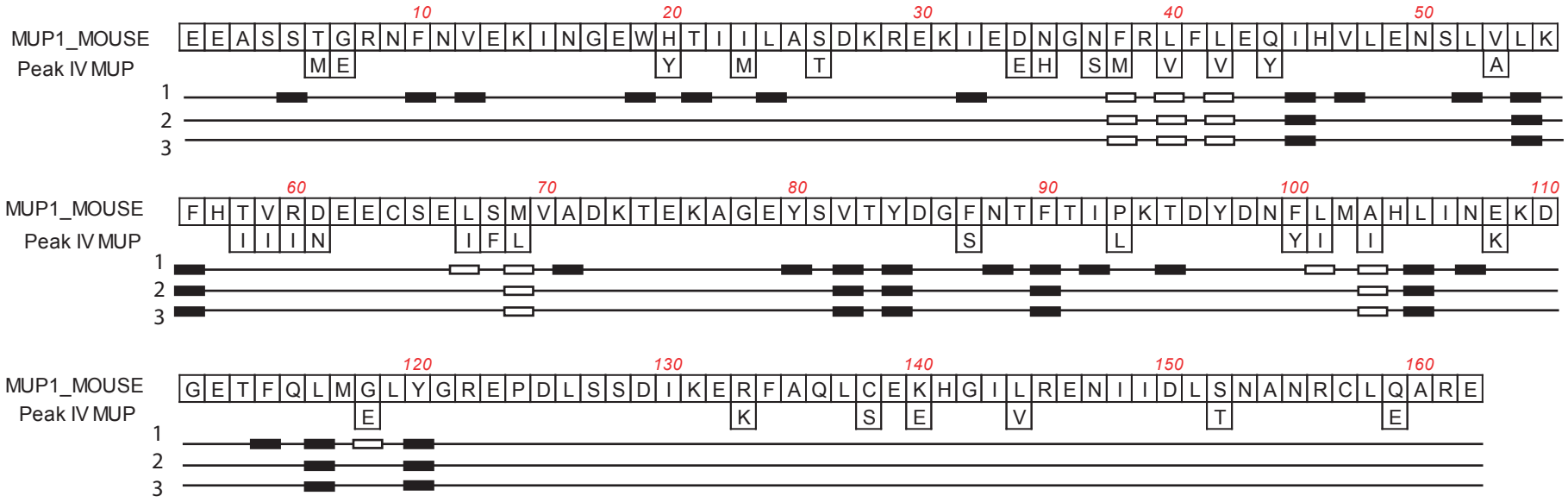

100
%
0
%
100
m/z
L1 1567.87
L4 2314.24
L2 1596.96
L9 1824.97
L10 2471.16
L8 2010.02
L6 2154.02
L11 3756.88
Peak II
3397.84
2176.92
1654.79
1965.86
1752.84
2542.96
Peak IV
1669.78

a) $[M+2H]^{2+}$ = 779.9Th



b) $[M+2H]^{2+}$ = 937.5Th




MUP1_MOUSE
Peak IV MUP
10
20
30
40
50
E E A S S T G R N F N V E K I N G E W H T I I L A S D K R E K I E D N G N F R L F L E Q I H V L E N S L V L K
M E Y M T E H S M V V Y A
1
2
3
MUP1_MOUSE
Peak IV MUP
60
70
80
90
100
110
F H T V R D E E C S E L S M V A D K T E K A G E Y S V T Y D G F N T F T I P K T D Y D N F L M A H L I N E K D
I I I N I F L S L Y I I K
1
2
3
MUP1_MOUSE
Peak IV MUP
120
130
140
150
160
G E T F Q L M G L Y G R E P D L S S D I K E R F A Q L C E K H G I L R E N I I D L S N A N R C L Q A R E
E K S E V T E
1
2
3