Supplementary information

Use of imaging multivariate analysis to improve biochemical and anatomical discrimination in desorption electrospray ionisation mass spectrometry imaging

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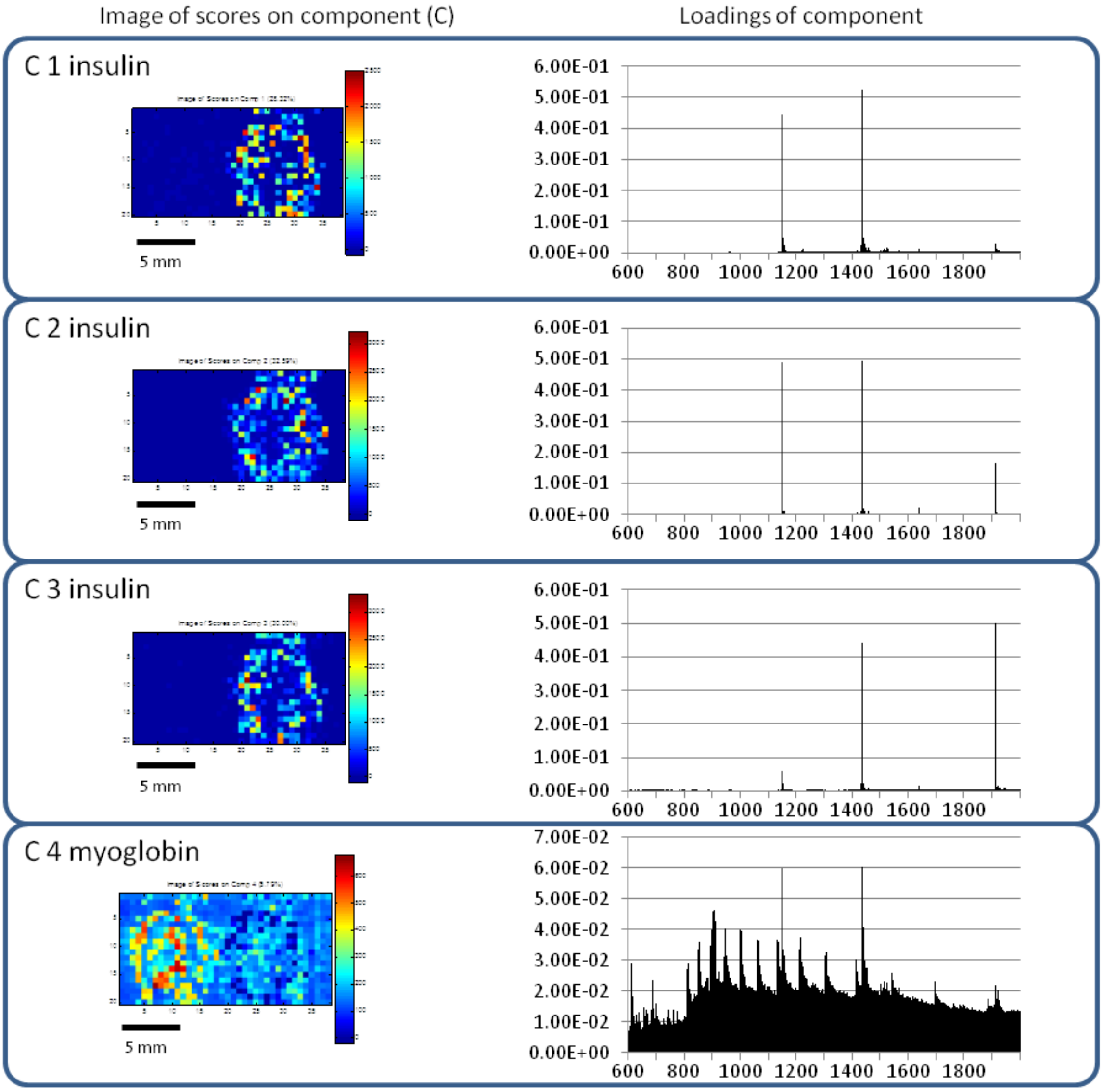
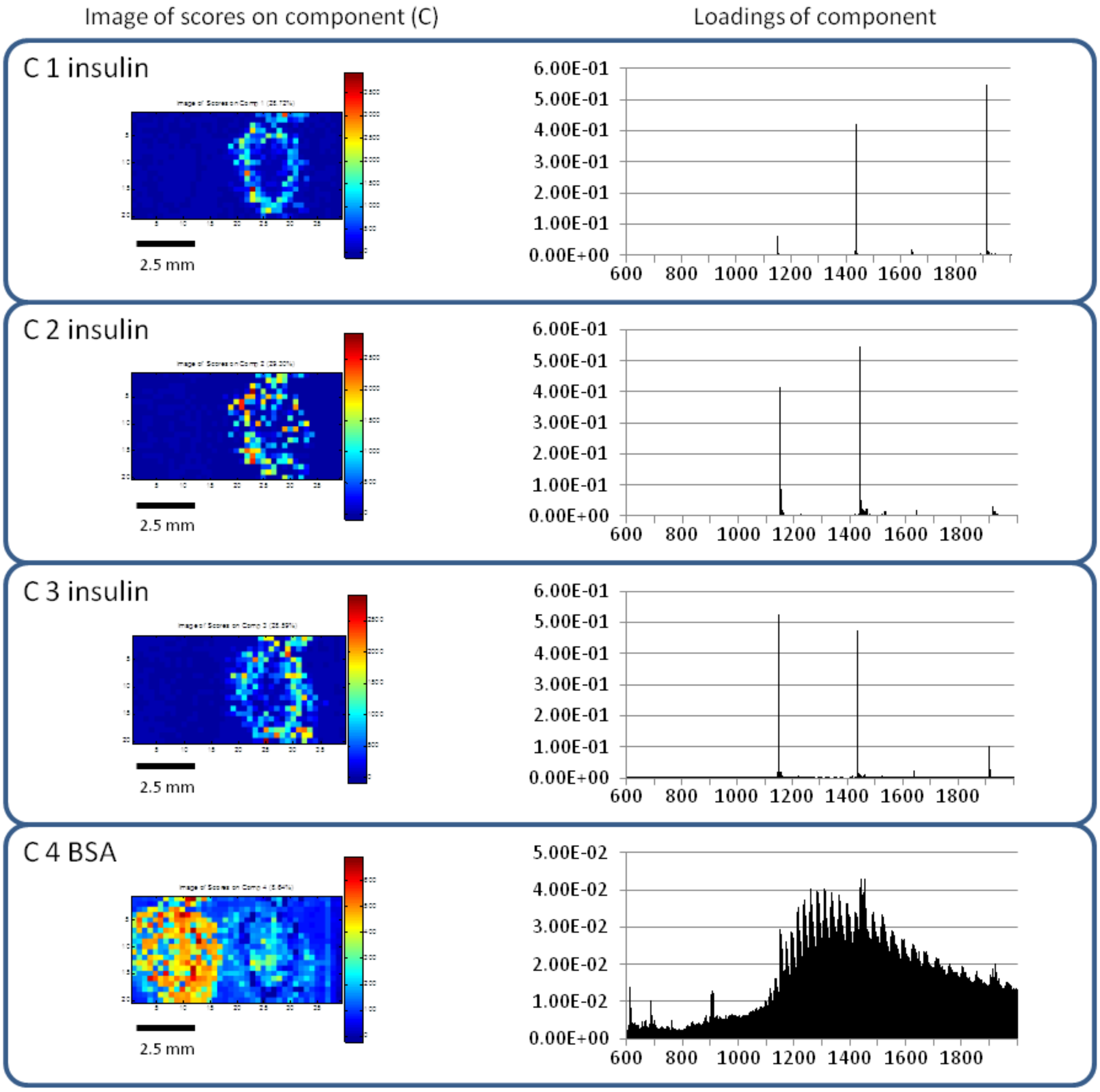
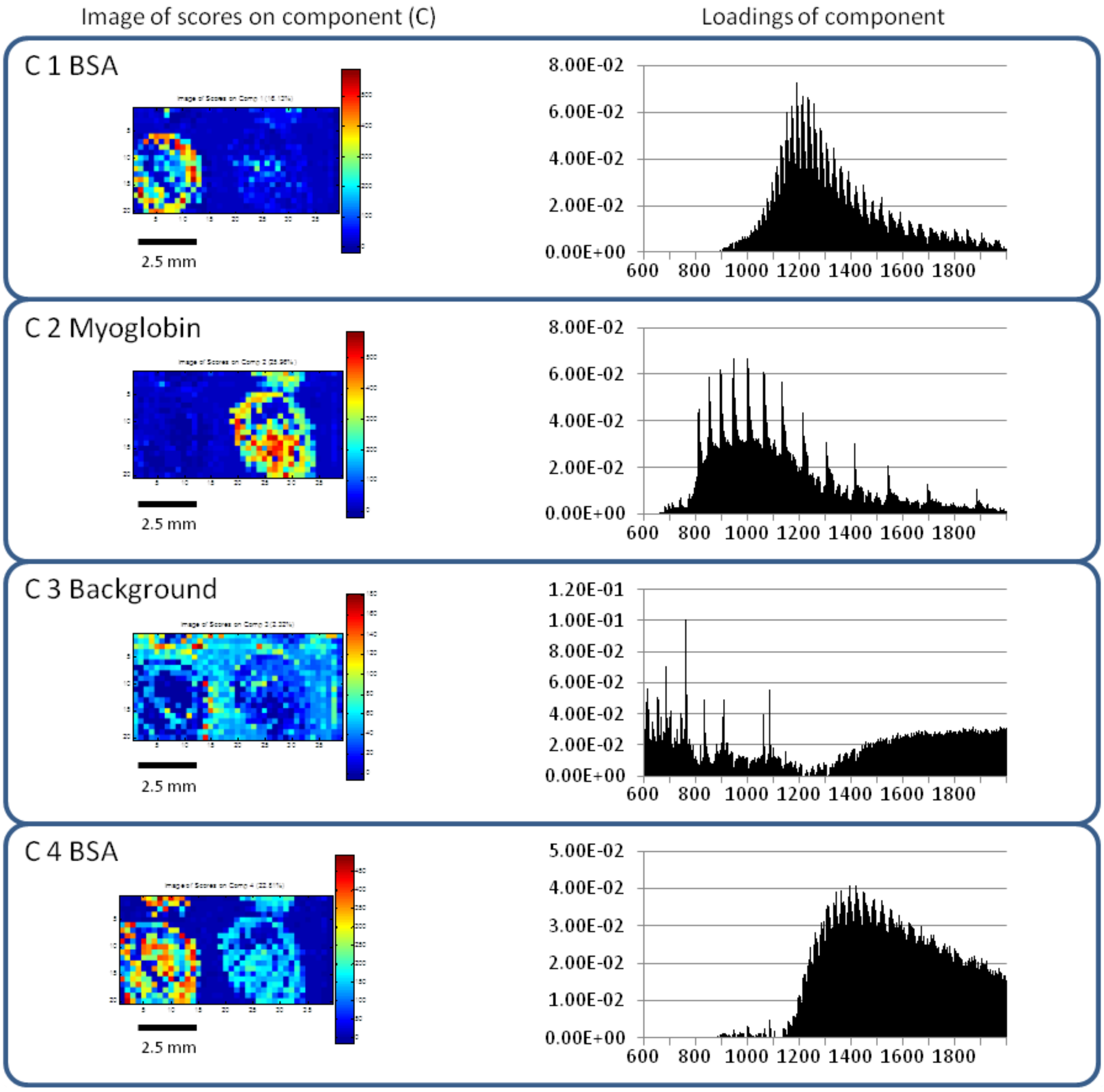


Supplementary Figure 1. Four-component MCR analysis of insulin/myoglobin two-protein side-by-side spots imaged at 250 µm resolution. DESI MS imaging data was mass-binned to 0.5 m/z unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are show. Components C1, C2 and C3 correspond to peaks observed for insulin and component C4 corresponds to peaks observed for myoglobin.



Supplementary Figure 2. Four-component MCR analysis of insulin/BSA two-protein side-by-side spots imaged at 250 µm resolution. DESI-MS imaging data was mass-binned to 0.5 *m/z* unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are shown. Components C1, C2 and C3 correspond to peaks observed for insulin and component C4 corresponds to peaks observed for BSA.



Supplementary Figure 3. Four-component MCR analysis of myglobin/BSA two-protein side-by-side spots imaged at 250 μm resolution. DESI-MS imaging data was mass-binned to 0.5 *m/z* unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are shown. Component C2 corresponds to peaks observed for myoglobin, components C1 and C4 correspond to peaks observed for BSA and component C3 corresponds to peaks in the background.

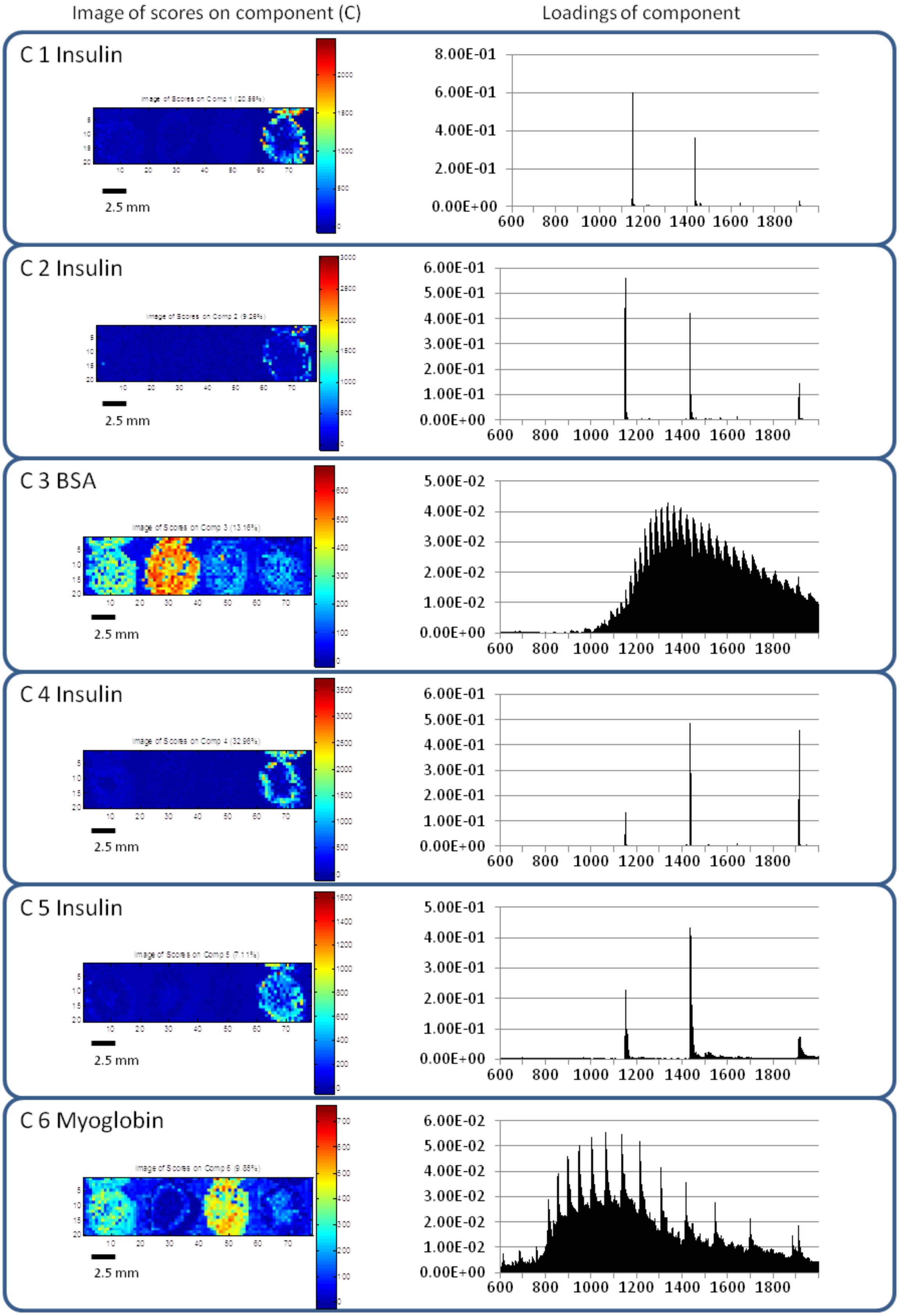
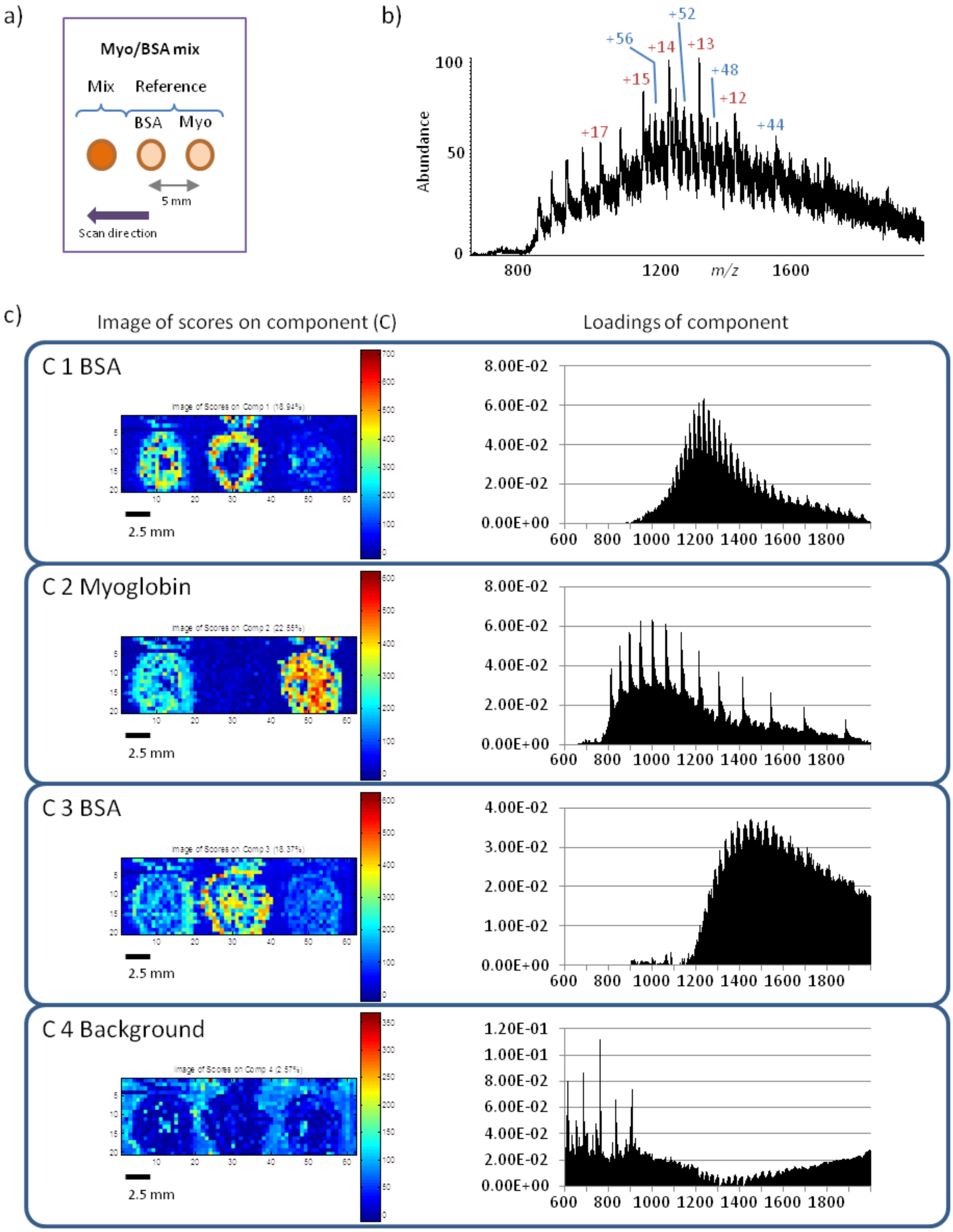
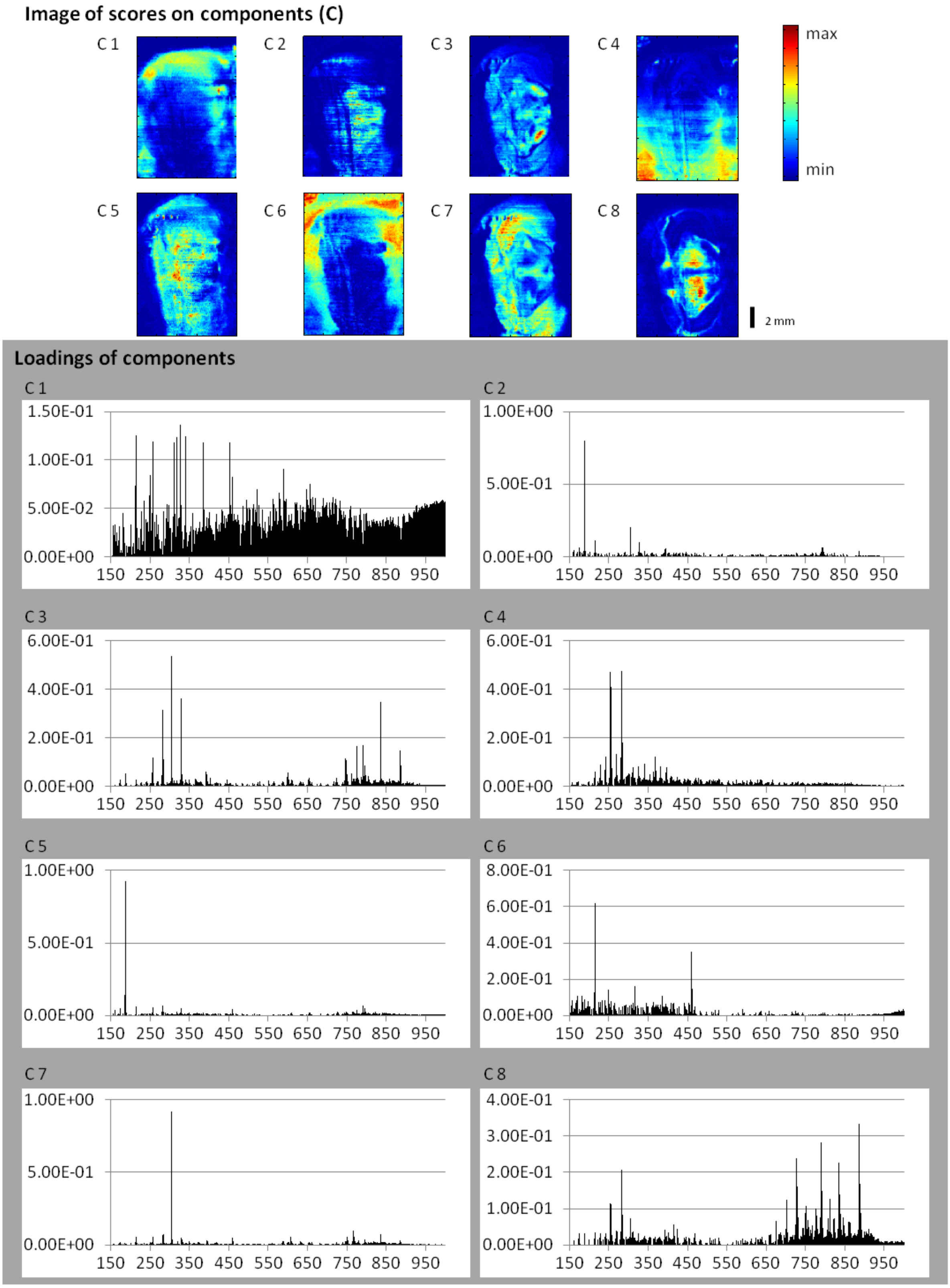


Image of scores on component (C)
Loadings of component
C 1 Insulin
Image of Scores on Comp 1 (20.88%)
5
10
15
20
10
20
30
40
50
60
70
2.5 mm
2000
1500
1000
500
0
8.00E-01
6.00E-01
4.00E-01
2.00E-01
0.00E+00
600
800
1000
1200
1400
1600
1800
C 2 Insulin
Image of Scores on Comp 2 (9.28%)
2.5 mm
3000
2500
2000
1500
1000
500
0
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
C 3 BSA
Image of Scores on Comp 3 (13.16%)
2.5 mm
600
500
400
300
200
100
0
5.00E-02
4.00E-02
3.00E-02
2.00E-02
1.00E-02
0.00E+00
C 4 Insulin
Image of Scores on Comp 4 (32.96%)
2.5 mm
3500
3000
2500
2000
1500
1000
500
0
6.00E-01
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
C 5 Insulin
Image of Scores on Comp 5 (7.11%)
2.5 mm
1600
1400
1200
1000
800
600
400
200
0
5.00E-01
4.00E-01
3.00E-01
2.00E-01
1.00E-01
0.00E+00
C 6 Myoglobin
Image of Scores on Comp 6 (9.85%)
2.5 mm
700
600
500
400
300
200
100
0
6.00E-02
5.00E-02
4.00E-02
3.00E-02
2.00E-02
1.00E-02
0.00E+00

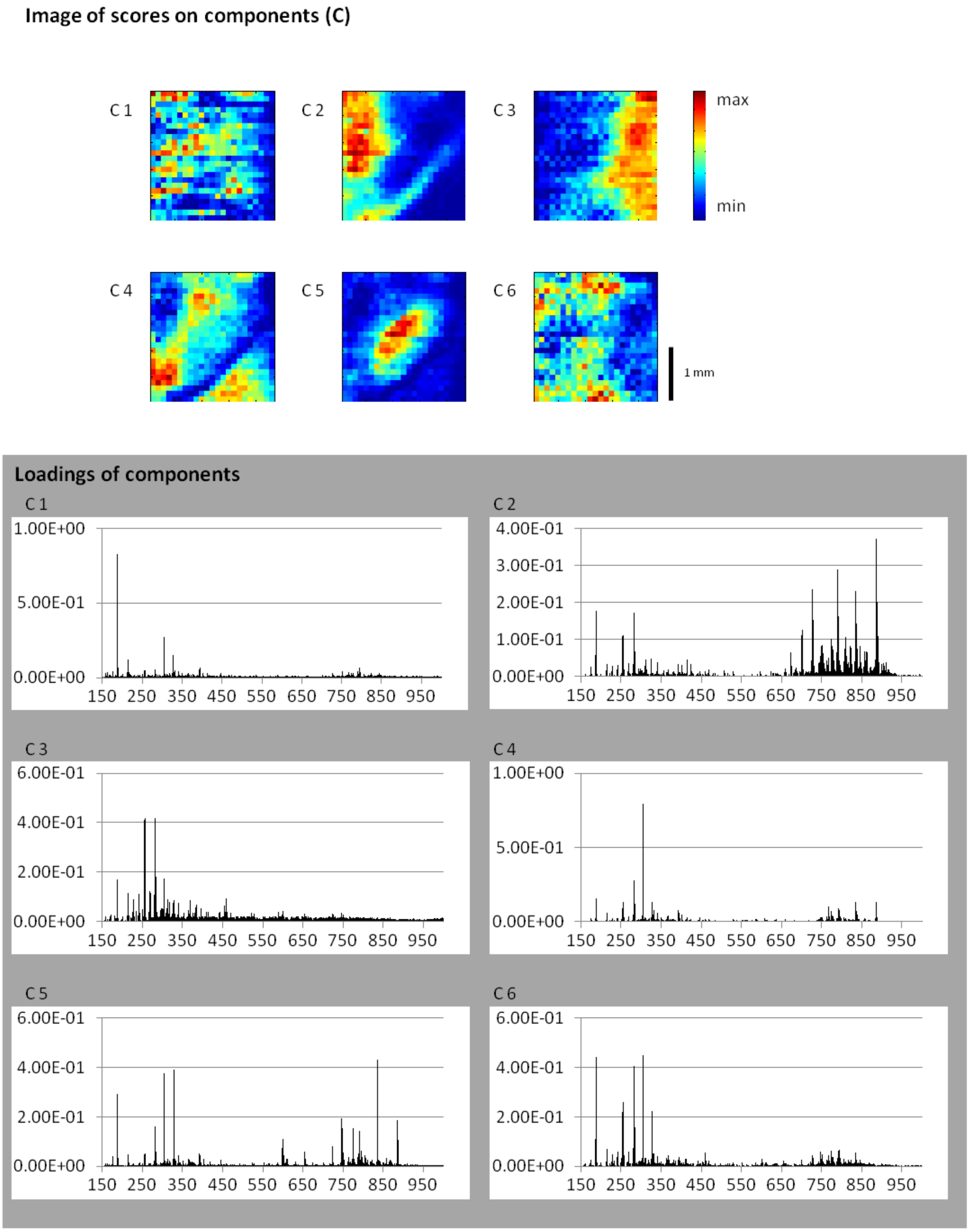
Supplementary Figure 4. Six-component MCR analysis of insulin/myoglobin/BSA protein mixture spots imaged at 250 µm resolution. DESI-MS imaging data was mass-binned to 0.5 *m/z* unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are shown. Components C1, C2, C4 and C5 correspond to peaks observed for insulin, component C6 corresponds to peaks observed for myglobin and component C3 corresponds to peaks observed for BSA.



Supplementary Figure 5. Four-component MCR analysis of myoglobin/BSA protein mixture spots imaged at 250 µm resolution. a) setup for DESI imaging of myoglobin (Myo) and BSA spots. b) mass spectrum of protein peaks summed across the mixture spot. Numbers on each spectrum indicate the charge state of the protein for its corresponding spike on the multiply charged peaks, with red for myoglobin and blue for BSA. c) DESI-MS imaging data was mass-binned to 0.5 *m/z* unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are show. Component C2 corresponds to peaks observed for myoglobin, components C1 and C3 correspond to peaks observed for BSA and component C4 corresponds to peaks in the background.



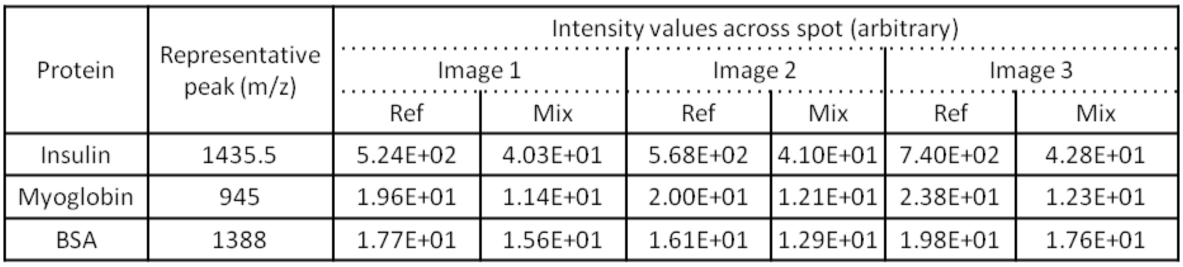
Supplementary Figure 6. Eight-component MCR analysis of rat brain coronal section imaged at 100 µm resolution. DESI-MS imaging data was mass binned to 1 *m/z* unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are show. Images for components C2, C3, C5, C7 and C8 correspond to areas that show distinct structures within the brain. Components C1, C4 and C6 show images of peaks that correspond to background, such as areas that are outside of the brain section.



Supplementary Figure 7. Six-component MCR analysis of the *substantia nigra* region of the rat brain coronal section imaged at 100 µm resolution. DESI-MS imaging data was mass-binned to 0.5 *m/z* unit and analysed with MCR with no data pre-treatment. The image of the scores and loadings plot for each component are show. The image of component C5 shows a concentration of signals that map to the *substantia nigra* region. Images for components C1, C2, C3, C4 and C6 correspond to other features within the brain as well as general background.

Supplementary Table 1

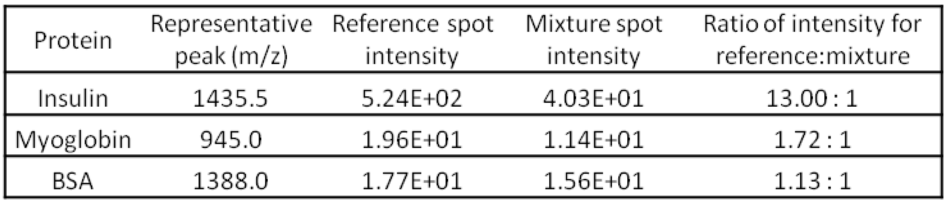
Intensity values of protein peaks across a protein spot from three replicates of images for the insulin/myoglobin/BSA protein mix. The representative peak is the most representative *m/z* value for one of the multiply charged peaks for each protein. The intensity values for each of the proteins for both the reference (Ref) and mixture (Mix) spots are similar across all of the three images produced.



Protein	Representative peak (m/z)	Intensity values across spot (arbitrary)					
		Image 1		Image 2		Image 3	
		Ref	Mix	Ref	Mix	Ref	Mix
Insulin	1435.5	5.24E+02	4.03E+01	5.68E+02	4.10E+01	7.40E+02	4.28E+01
Myoglobin	945	1.96E+01	1.14E+01	2.00E+01	1.21E+01	2.38E+01	1.23E+01
BSA	1388	1.77E+01	1.56E+01	1.61E+01	1.29E+01	1.98E+01	1.76E+01

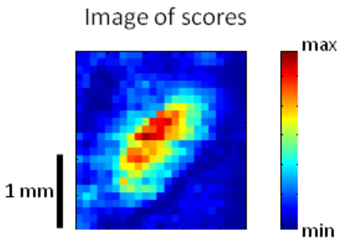
Supplementary Table 2

Intensity ratios for protein peaks comparing reference spot to mixture spot summed from the original mass spectrum of the image for the insulin/myoglobin/BSA protein mix. The representative peak refers to the most representative *m/z* value for one of the spikes of the multiply charged peaks of each protein. The reference:mixture spot ratio for insulin is an order of magnitude greater in the reference, while the same ratios for myoglobin and BSA are much more similar between the reference and mixture spots.

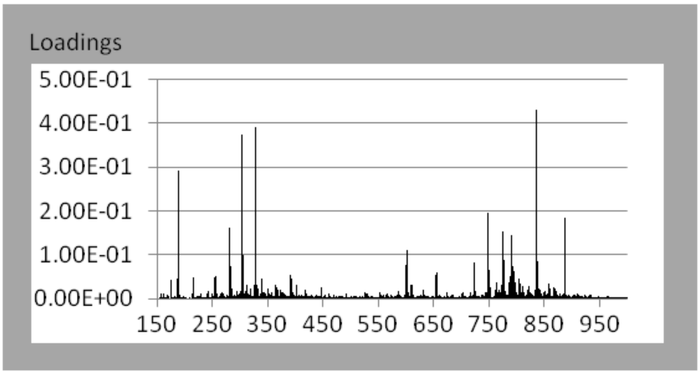


Protein	Representative peak (m/z)	Reference spot intensity	Mixture spot intensity	Ratio of intensity for reference:mixture
Insulin	1435.5	5.24E+02	4.03E+01	13.00 : 1
Myoglobin	945.0	1.96E+01	1.14E+01	1.72 : 1
BSA	1388.0	1.77E+01	1.56E+01	1.13 : 1

a) **Component 5**



b)



c)

m/z	Loadings score on Comp. 5	Rank
834	4.30E-01	1
327	3.93E-01	2
303	3.76E-01	3
187	2.93E-01	4
835	2.21E-01	5
746	1.96E-01	6
885	1.85E-01	7
281	1.63E-01	8
747	1.55E-01	9
774	1.53E-01	10
790	1.44E-01	11
600	1.10E-01	12
886	1.06E-01	13
328	1.03E-01	14
304	9.98E-02	15
775	8.77E-02	16
836	8.43E-02	17
722	8.11E-02	18
598	7.57E-02	19
281.5	7.41E-02	20
791	7.40E-02	21
748	6.51E-02	22
794	6.32E-02	23
654	5.86E-02	24
283	5.79E-02	25
303.5	5.51E-02	26
750	5.49E-02	27
652	5.45E-02	28
391	5.40E-02	29
788	5.19E-02	30
255.5	5.09E-02	31
214	4.73E-02	32
253.5	4.73E-02	33
255	4.71E-02	34
393	4.66E-02	35
186	4.61E-02	36
339	4.57E-02	37
601	4.50E-02	38
804	4.47E-02	39
282	4.28E-02	40

Supplementary Table 3

The top mass peaks with the highest loadings score from component 5 of the *substantia nigra* MCR analysis in supplementary figure 7. a) The original image of scores for component 5. b) loadings plot for component 5. c) The top 40 mass peaks ranked by loadings score for component 5. Note the data was mass-binned at 0.5 *m/z* and some peaks that differ in 0.5 *m/z* units may refer to the same metabolite.