Supplementary Material (ESI) for Molecular BioSystems

Supplement Material

Metabonomic Investigation of Human *Schistosoma mansoni* Infection

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Age related effects

The strategy of focusing on individual age groups for investigation of *S. mansoni* infection was chosen based on the fact that in the initial PCA analysis an age related trend could be observed in the scores plot already for the first two components as seen in Figure S1. Such age group related clustering has previously been described in the literature for large population studies [S6]. Therefore, selection of individual age groups will reduce age related artifacts and background variability within the data.

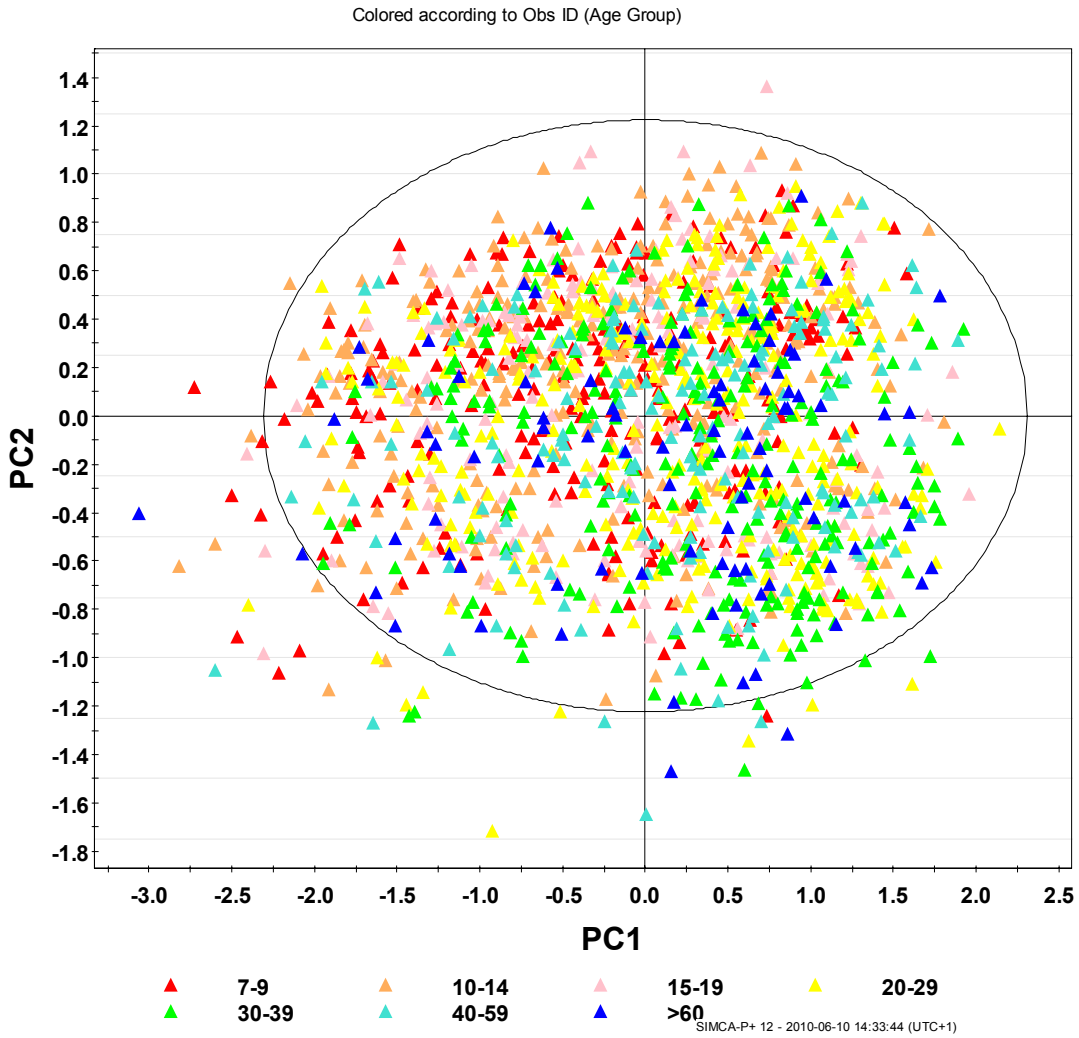


Fig. S1 Scores plot of first two components from initial PCA model using all data colored according to age groups

The age related trend was further investigated by building an OPLS model with the age group as the response variable as seen in Figure S2. In this model a clear separation between children and adults is visible with samples from adolescent individuals (age group 15-19) located in between these two clusters. No trend or clustering for the children age groups 7-9 and 10-14 years can be observed. For the individual adult age groups there is a slight trend visible, however, it is much less pronounced as for the separation between children and adults.

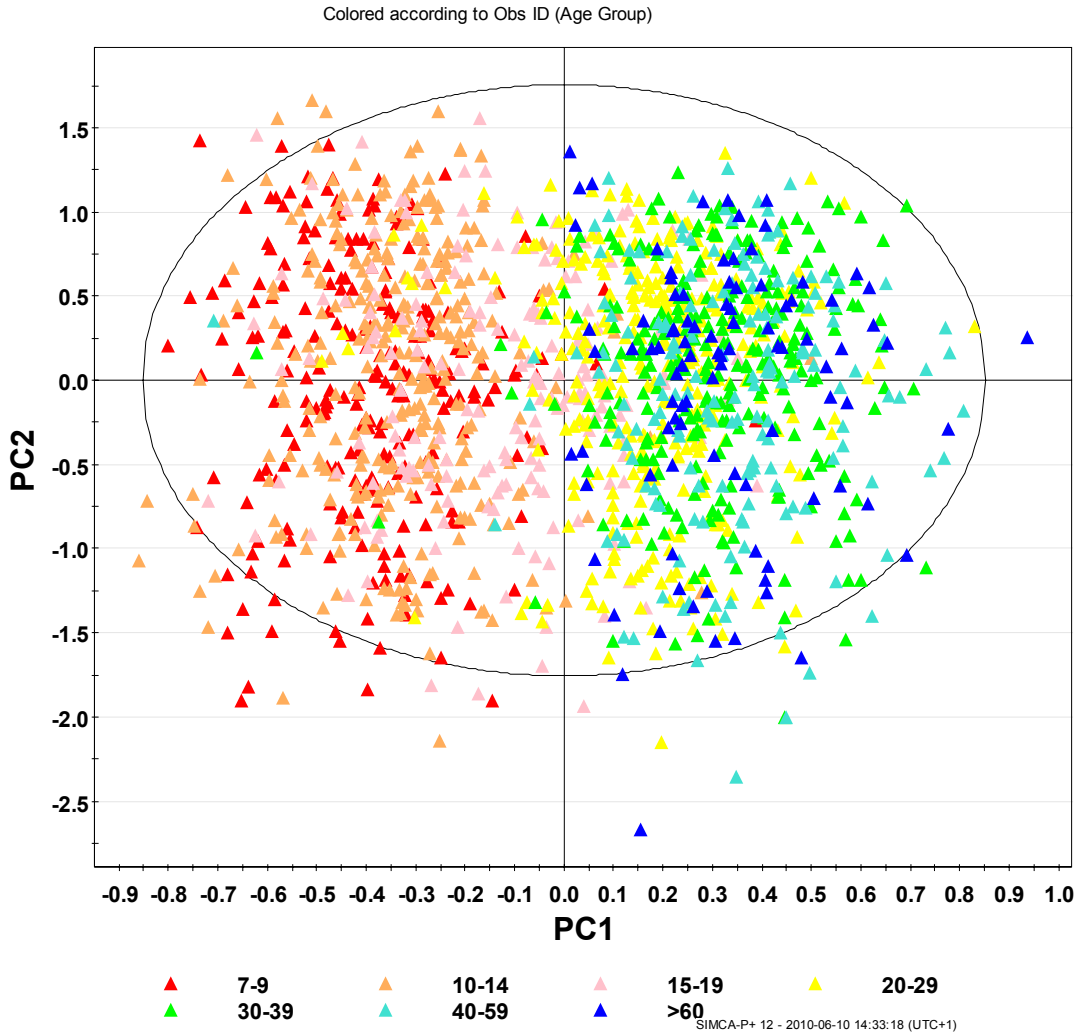


Fig. S2 Scores plot of first two orthogonal components from OPLS model build using age groups as response variable

Table S1

Infection classification by age group and time point (TP)

	TP	EPG > 400	EPG = 0 & CCA <1	CCA = 0 & CCA = 0.5	CCA = 1	CCA = 2	CCA = 3
Complete Cohort	A	145	29	77	40	95	143
	B	-	-	183	70	79	16
	C	-	-	201	69	53	10
	D	-	-	177	47	39	6
	E	4	147	206	65	49	10
Age group 7-15	A	55	0	6	18	36	58
	B	-	-	52	33	33	9
	C	-	-	58	29	30	9
	D	-	-	48	24	25	5
	E	2	36	56	33	25	9
Age group 20-40	A	62	19	39	15	40	56
	B	(51)[a]	(15)[a]	74	21	30	6
	C	-	-	90	24	14	0
	D	-	-	72	13	8	1
	E	1	69	90	19	16	1

[a] based on EPG of time point A

Structure elucidation of unknown metabolite M01

^{1}H detected 1D and 2D NMR spectra of urine sample 65803240905 TubeID: WELL0032#NMR#D03 were obtained using a Bruker 600 MHz AVANCE II spectrometer equipped with a 5mm TCI cryo probe and a z-gradient system. 1D proton spectra with water suppression[S1] were recorded using the first increment of a NOESY pulse sequence[S2] with presaturation (γB_1 = 50Hz) during 4 sec relaxation delay and a mixing time of 10 msec. 8 scans of 32768 points covering 6002.4 Hz were recorded at 300K and zero filled to 65536 complex points prior to Fourier transformation, an exponential window function was applied with a line-broadening factor of 1.0 Hz.

All 2D experiments were recorded at a temperature of 300K with presaturation (γB_1 = 50Hz) during a relaxation delay of 2 sec. For DQF-COSY[S3] spectra a data matrix of 256 x 2048 points covering 6009.6 x 6009.6 Hz was recorded with 8 scans for each increment. Data was zero filled to 2048 x 2048 points prior to States-TPPI type 2D Fourier transformation and a sine bell shaped window function shifted by $\pi/2$ in the F1 and $\pi/4$ in the F2 dimension was applied. Coherence order selective gradient HSQC[S4] spectra were recorded for a data matrix of 256 x 2048 points covering 24146 x 6009.6 Hz with 4 scans for each increment. Data was zero filled to 512 x 2048 points prior to echo-anti echo type 2D Fourier transformation and a sine bell shaped window function shifted by $\pi/2$ in both dimensions was applied. For HMBC[S5] spectra a data matrix of 256 x 2048 points covering 33202 x 6009.6 Hz with 16 scans for each increment. Data was zero filled to 512 x 2048 points prior to echo-anti echo type 2D Fourier transformation and a sine bell shaped window function shifted by $\pi/2$ in the F1 dimension and $\pi/6$ in the F2 dimension was applied. Magnitude mode spectra were obtained by magnitude calculation in F2. All spectra were referenced according to the internal TSP = 0.0 ppm.

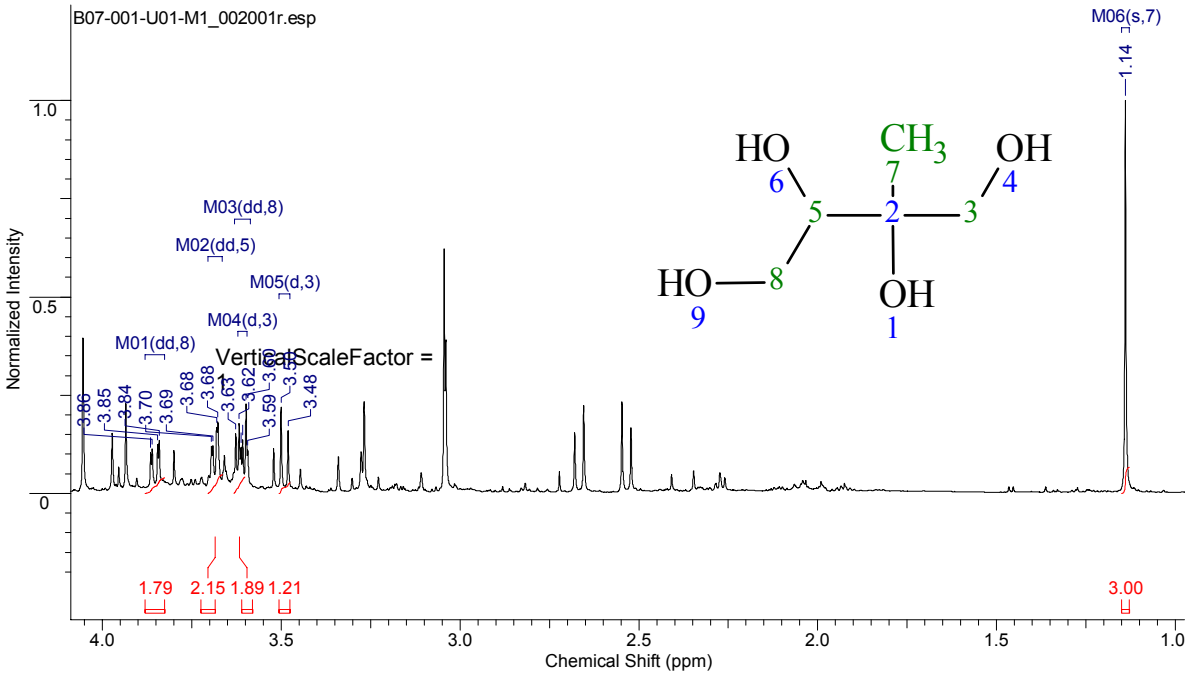
Unknown Metabolite M01 from Urine - 3-C-Methylerythritol

17/10/2007 14:04:18

Formula $C_5H_{12}O_4$	***FW***	136.1464

Acquisition Time (sec)	2.7296	***Comment***	Sample ID : 65803240905 TubeID: WELL0032#NMR#D03		
Date	16 Oct 2007 08:17:04			***Date Stamp***	16 Oct 2007 08:17:04
File Name	C:\Documents and Settings\All Users\Shared Documents\data\Biomarker\nmr\B07-001-U01-M1_002001r				
Frequency (MHz)	600.13	***Nucleus***	1H	***Number of Transients***	8
Origin	spect	***Original Points Count***	16384	***Owner***	nmrsu
Points Count	65536	***Pulse Sequence***	noesygppr1d	***Receiver Gain***	16.00
SW(cyclical) (Hz)	6002.40	***Solvent***	H2O+D2O	***Spectrum Offset (Hz)***	2868.6404
Sweep Width (Hz)	6002.31	***Temperature (degree C)***	28.700		

1H NMR (600 MHz, H2O+D2O) δ ppm 1.14 (s, 3 H) 3.49 (d, *J*=11.72 Hz, 1 H) 3.61 (dd, *J*=11.54, 8.52 Hz, 1 H) 3.61 (d, *J*=11.72 Hz, 1 H) 3.69 (dd, *J*=8.56, 2.34 Hz, 1 H) 3.85 (dd, *J*=11.63, 2.56 Hz, 1 H)



No.	Shift1 (ppm)	H's	Type	J (Hz)	Atom1	Multiplet1	(ppm)
1	1.14	3	s	-	7	M06	[1.13 .. 1.15]
2	3.49	0	d	11.72	3	M05	[3.48 .. 3.51]
3	3.61	0	d	11.72	3	M04	[3.60 .. 3.62]
4	3.61	1	dd	11.54, 8.52	8	M03	[3.59 .. 3.63]
5	3.69	1	dd	8.56, 2.34	5	M02	[3.66 .. 3.70]
6	3.85	0	dd	11.63, 2.56	8	M01	[3.83 .. 3.88]

No.	(ppm)	(Hz)	Height
1	1.14	683.8	1.0000
2	3.48	2089.2	0.1605
3	3.50	2100.9	0.2190
4	3.59	2156.8	0.1101
5	3.60	2159.6	0.2275
6	3.61	2165.2	0.1361
7	3.61	2168.2	0.1167
8	3.62	2171.3	0.1785
9	3.63	2176.8	0.1522
10	3.68	2206.8	0.1819
11	3.68	2209.0	0.1678
12	3.69	2215.2	0.1207
13	3.70	2217.7	0.1205
14	3.84	2305.4	0.1356
15	3.85	2308.0	0.1246
16	3.86	2317.0	0.1137
17	3.87	2319.6	0.1052

^{13}C shift (ppm)	type	Atom
77.0	Cq	2
69.2	CH_2	3
77.9	CH	5
21.2	CH_3	7
64.9	CH_2	8

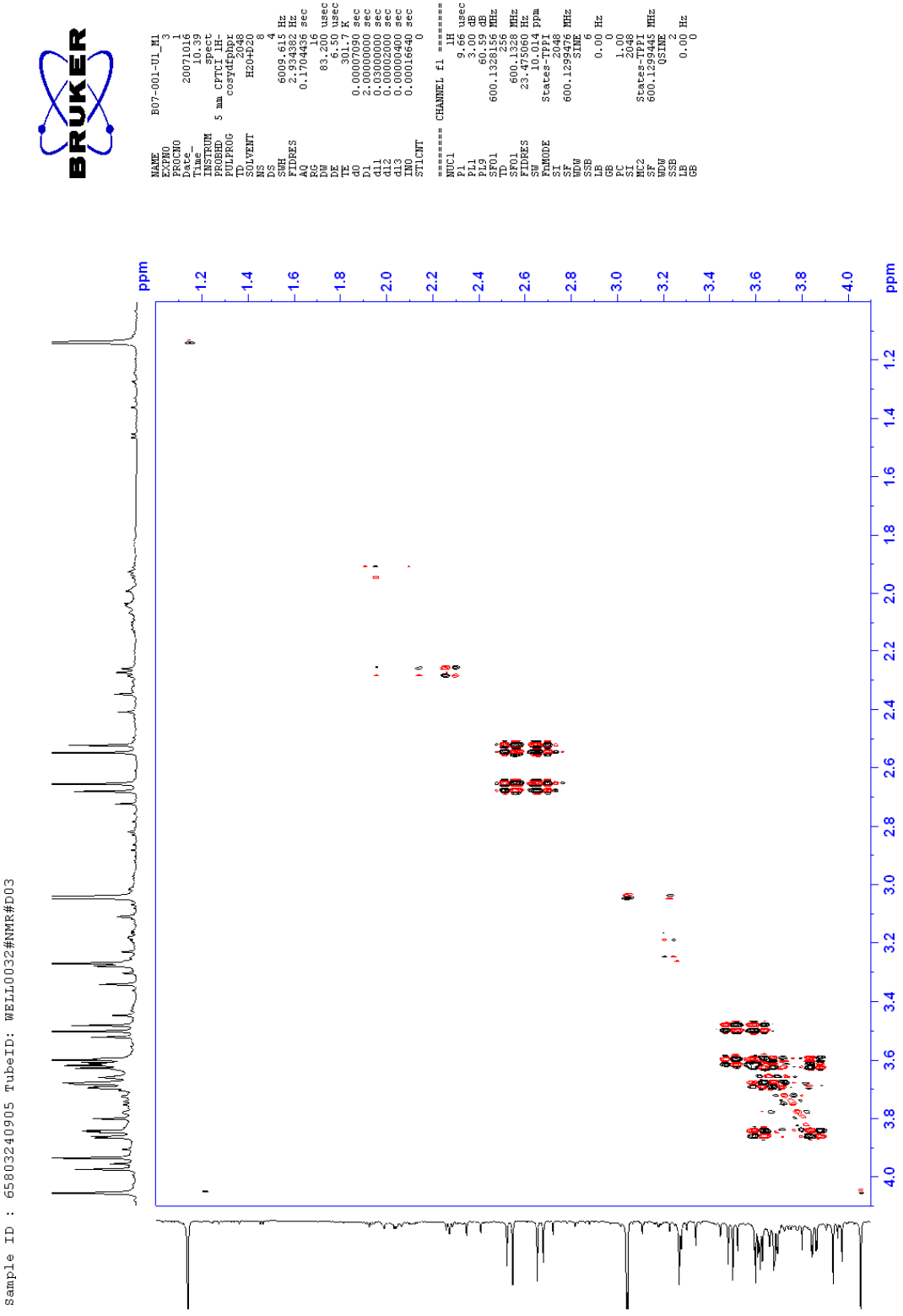


Fig. S4 Excerpt of DQF-COSY spectrum of sample 65803240905

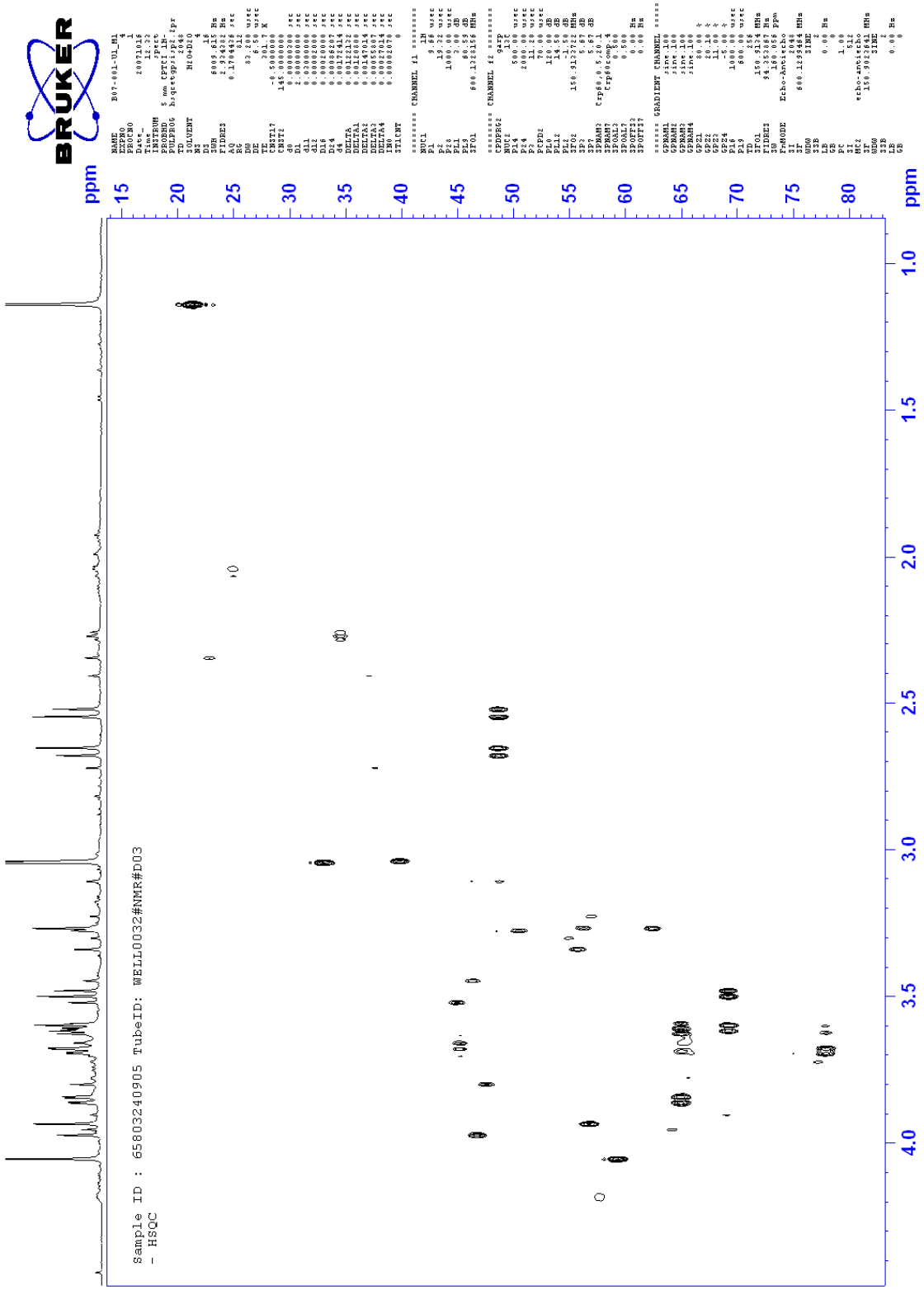


Fig. S5 Excerpt of HSQC spectrum of sample 65803240905

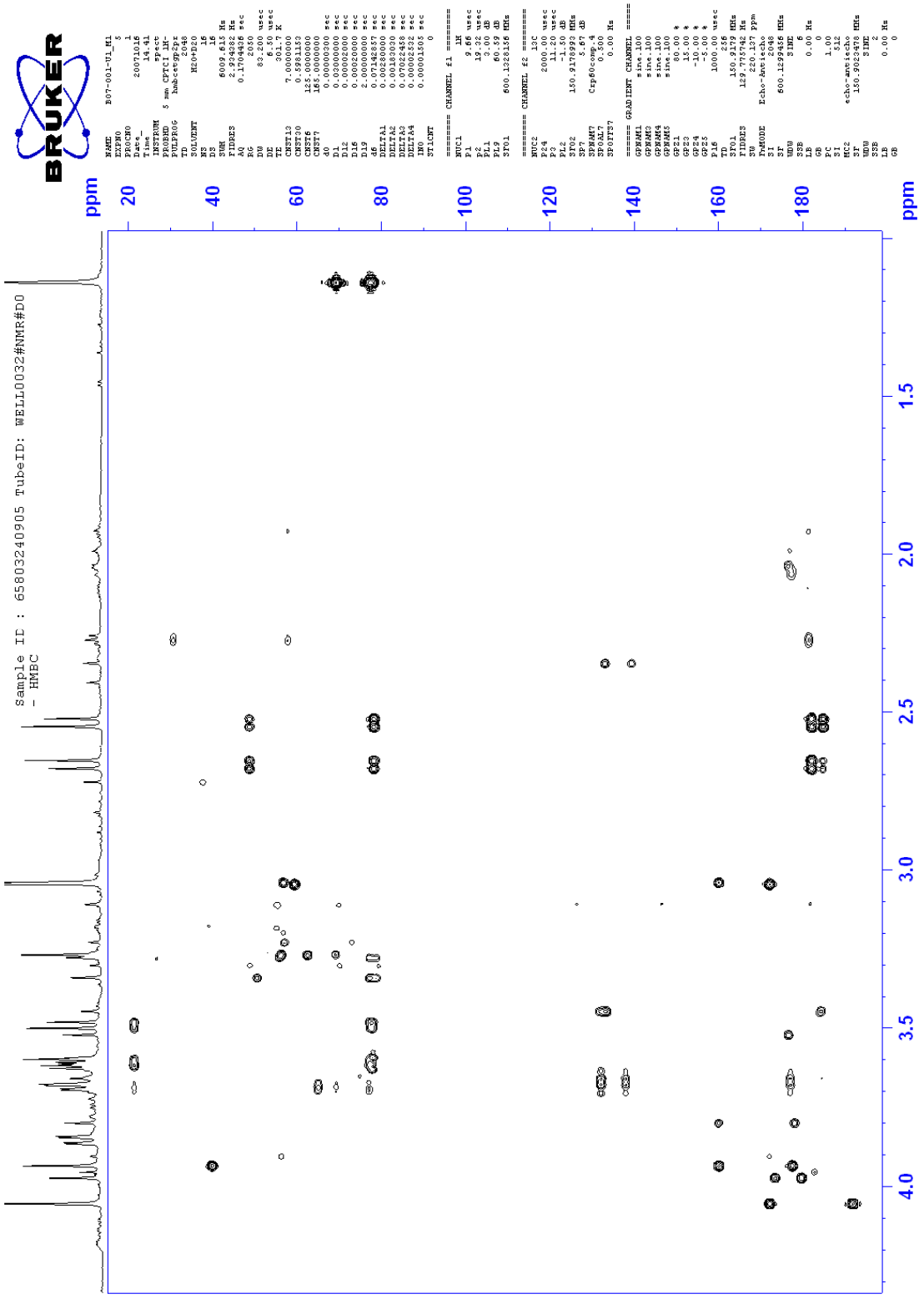


Fig. S6 Excerpt of HMBC spectrum of sample 65803240905

Treatment effect

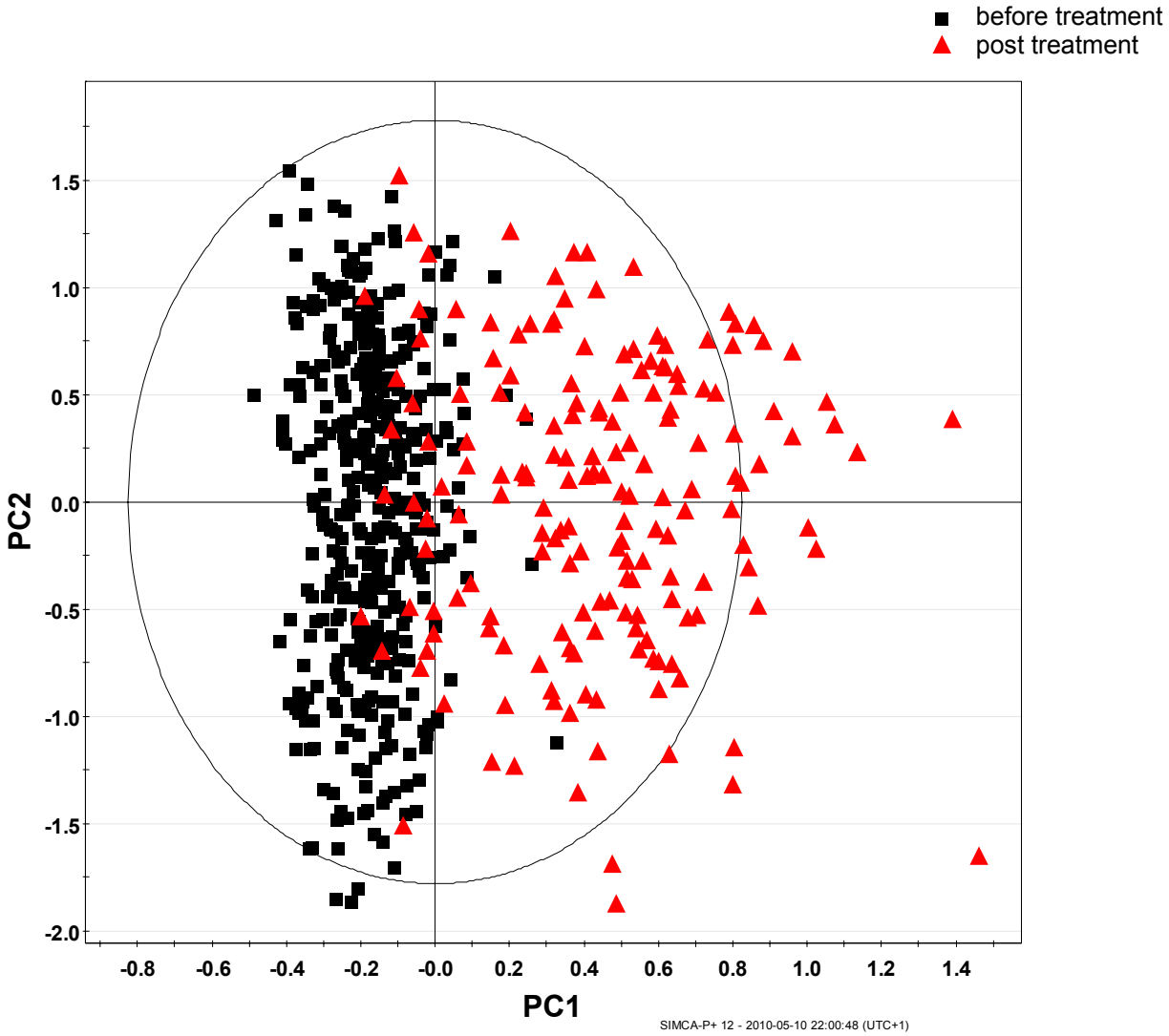


Fig. S7 Scores plot of first and orthogonal component from OPLS-DA two class model built using 602 NMR spectra of urine with treatment status as response variable illustrating Praziquantel treatment effect. Data points are colored according to before treatment (■) and 24h post treatment (▲) time points including initial and 2 weeks follow-up visits

References

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