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Application of dyes as doping agents in MALDI-MS matrices for the signal enhancement of proteins

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Supplementary Material

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Legend

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Data S1 Statistical Data for Bovine Serum Albumin analysis.

Data S2 Statistical data for myoglobin analysis.

Data S3 Statistical data for casein analysis.

Data S4 Statistical data for insulin analysis.

Method S1 Description of spotting plan generation and statistical analysis.

The sample/spotting plan was generated using fractional factorial design (Design Expert v 9.0, Stat-Ease, Minneapolis, MN, USA). After an initial screening to identify limitations in the factor-space, the type of dye, dye concentrations, and mass range, were varied according to a face-centred central composite design (CCF). This design allows modelling of quadratic functions of the factors, using fewer experiments compared to a three-level full-factorial design. Statistical analysis was performed, such as ANOVA and t-test using the same software to study the both individual and combined effects of different factors.

Table S1 MALDI-MS data of bovine serum albumin (BSA) analysed with and without dyes.

	HCCA*					**DHB****					**SA*****				
Dye	**Dye Conc. (pM)**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc. (pM)**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc. (pM)**	**Intensity**	**S/N**	**Resolution**	**Area**
A	0.1	15341	129.9	90	17137108	100	5214	73.5	58	7789449	1	7348	169.5	90	7682927
	1	11411	101.7	81	13614231	10	4898	74.9	73	6526343	0.1	4952	133.4	99	5280801
	100	9410	106.8	74	12376452	100	4283	63.9	62	6251725	0.1	4546	109.3	83	5184041
B	10	23837	162.4	89	26941357	100	3176	42.7	36	5956328	10	5192	126.5	97	5160130
	100	16913	126.7	85	20023906	1	4128	70.8	61	5948368	0.1	4363	115.5	89	4766991
	1	12668	122.6	88	14288375	100	3021	53.5	49	5099726	100	3230	71.2	88	3661930
C	100	16437	127.1	82	19879914	10	5276	72.9	59	7705529	1	6789	154.4	66	8991997
	1	14937	121.7	83	17665216	0.1	4508	66.6	61	6613203	0.1	8370	178.8	157	8936447
	0.1	11552	130.6	99	12098975	1	4243	64.4	62	6234510	1	3675	106.1	92	3793863
D	1	16124	136.1	90	17872093	100	4712	71.4	66	6500254	10	9108	179	92	9593021
	10	9832	112.3	72	13061084	10	4017	63	58	6145665	1	5841	136.6	97	5909756
	0.1	10696	105.8	87	12272311	0.1	4205	64	72	5657242	10	4109	129.4	91	4368477
E	0.1	13006	127.7	91	14426946	1	5214	73.5	58	7789449	10	6496	138.3	78	7407662
	1	10937	111.5	86	12919051	10	5508	80.1	72	7160957	1	5958	153	41	6542150
	100	9452	111.4	80	11792833	100	3210	53.8	46	5508620	10	5786	150.5	59	5629486
Without Dye	-	2615	45.3	77	3394878	-	432	16.4	76	1037062	-	2629	83.2	94	2795285
	-	2929	47	70	3786127	-	581	18.4	64	1443673	-	2788	99.5	93	2879131
	-	3216	49.8	73	4087796	-	612	26.9	84	776639	-	1964	87.8	100	1933579

Laser Intensity (%): *50, **60, ***60

Table S2 MALDI-MS data of myoglobin analysed with and without dyes.

	HCCA*					**DHB****					**SA*****				
Dye	**Dye Conc. (pM)**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc. (pM)**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc. (pM)**	**Intensity**	**S/N**	**Resolution**	**Area**
A	1	50758	376.6	208	7070808	0.1	13629	476.7	1180	432457	0.1	20325	1037.3	1237	440640
	1	50758	376.6	208	7070808	100	14767	401.8	1296	347441	1	17801	992.7	1271	385903
	10	49481	401.6	235	6559161	10	11829	358.9	1356	275395	100	15480	913.2	1195	336568
B	10	79324	544.5	329	8722598	100	19465	530.8	1333	460513	100	22994	1187.8	1173	519320
	10	56809	494.5	328	6079946	0.1	17436	589	1331	414460	100	23551	1221.7	1257	503054
	0.1	38368	394.5	269	4569212	1	15528	491.1	1307	373268	0.1	21487	1189.1	1239	486959
C	0.1	46568	405.5	218	6401869	1	14570	459.2	1307	349786	100	35732	1499.6	1245	795324
	10	52680	419.7	267	6261088	0.1	11287	383.8	1290	272489	10	26143	1304.9	1250	581440
	0.1	46290	454.5	265	5458069	100	11595	381.8	1388	271221	0.1	20523	1164.6	1199	478987
D	10	84674	436.4	231	11019018	1	15425	448.5	1293	382366	0.1	24711	1180.9	1284	531706
	0.1	64297	386.8	233	8612477	100	13499	422.3	1249	339968	100	22680	1214.7	1211	510497
	0.1	54587	374.6	259	6755594	10	8874	435.6	1435	181950	0.1	20615	1169.5	1264	433612
E	100	69505	276.1	213	9779422	0.1	10341	317.8	1284	257574	0.1	34095	1443.7	1225	771126
	0.1	49848	425.4	260	6185621	1	10420	395.4	1355	236784	10	27080	1342.6	1203	641839
	100	42113	411.1	239	5210450	0.1	10461	356.8	1364	233011	100	19941	1128.3	1251	428944
Without Dye		32104	347.6	545	325753		11654	163.9	665	233823		20145	1014	1096	301498
		44351	423.8	516	642863		12604	162.2	886	183557		17874	972.6	1046	285911
		14485	220.7	659	58388		13797	254.3	886	204701		26273	1309.3	1174	397049

Laser Intensity (%): *40, **45, ***45

Table S3 MALDI-MS data of insulin analysed with and without dyes.

	HCCA*					**DHB****					**SA*****				
Dye	**Dye Conc.**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc.**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc.**	**Intensity**	**S/N**	**Resolution**	**Area**
A	0.1	7247.4	107.7	305	675370	100	542.93	79.1	682	24195	10	2510.09	270.2	718	110664
	100	4275.31	92	255	482033	10	400.2	44.4	614	18985	100	1882.62	223.6	725	81929
	100	4190.04	67.4	262	452627	0.1	402.79	62.6	695	17186	0.1	2739.35	326.8	680	126954
B	100	5571.08	107.7	271	589642	0.1	586.19	72.4	639	27406	1	2990.33	252.2	674	138745
	100	4098.76	79.4	240	475840	100	499.61	48.1	639	22612	1	2229.73	292.1	699	100269
	10	4733.83	75.2	272	473861	1	389.92	27.8	490	22434	100	1886.9	217.5	675	88190
C	10	3859.52	68	240	449348	100	677.2	69.8	606	33265	100	3636.24	380.3	701	164510
	0.1	4432.04	86.3	305	409214	0.1	468.72	71.1	745	18790	1	2703.2	289.6	660	129173
	10	3528.96	77.2	244	403900	10	356.9	40.3	597	17573	1	2794.01	406.1	724	124576
D	1	5523.89	78.4	226	674625	10	6803	580.4	653	68307	1	3362.15	440.7	794	135345
	0.1	4122.47	74.9	242	476341	10	562.06	102.4	773	22137	0.1	2949.04	324.2	734	127471
	10	3694.3	71.4	244	425028	100	314.77	22.7	555	15304	10	2477.16	240.2	688	111842
E	10	4021.5	72.8	238	466708	1	311.2	31.9	507	17850	0.1	2927.43	340.3	732	127126
	10	3652.49	74.9	241	420527	10	276.34	24.4	509	15336	100	2753.51	304.8	698	123491
	100	4383.48	92.7	300	411532	1	319.88	50.6	678	13911	10	2486.74	245.2	649	120376
Without Dye	-	4749	98.6	616	18016	-	50	789	214.14	8244	-	9.6	639	138.3	5365
	-	7227	151.2	444	72144	-	41.7	677	190.89	8778	-	13.5	779	149.8	5113
	-	3589	95.4	701	11380	-	27.8	692	146.67	6145	-	9.9	751	91.69	3049

Laser Intensity (%): *45, **55, ***60

Table S4 MALDI-MS data of casein analysed with and without dyes.

	HCCA*					**DHB****					**SA*****				
Dye	**Dye Conc.**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc.**	**Intensity**	**S/N**	**Resolution**	**Area**	**Dye Conc.**	**Intensity**	**S/N**	**Resolution**	**Area**
A	1	4212	72	63	1903453	1	5052	83.3	87	1983650	0.1	8769	189	131	2449170
	10	3640	56.5	63	1803637	10	5030	88.7	89	1921070	100	7770	190	126	2281790
	0.1	3043	66.8	61	1366026	1	2736	62.3	102	989079	10	8062	199	131	2246273
B	1	5298	71.6	63	2439262	1	5387	100.8	101	1943502	1	11061	237	127	3132023
	10	3505	65.5	66	1545741	1	5182	91.8	95	1879990	0.1	11350	262	136	3001613
	1	3547	64.2	66	1524057	100	4373	91.6	101	1541748	10	9717	231	137	2761127
C	10	4361	73	62	1921663	10	3955	74.4	91	1486441	10	14437	316	144	3547120
	100	3681	60.7	66	1785661	100	3620	73.2	95	1351404	0.1	10330	213	132	2915734
	0.1	3490	74.4	70	1443314	1	3438	69.5	93	1234921	1	10046	214	128	2880743
D	1	4006	66	58	1847486	100	3066	58	95	1223985	10	12229	245	134	3346870
	10	3784	64.2	58	1778733	1	2336	48.9	93	899892	10	7766	194	128	2206267
	10	3264	63.8	63	1466729	100	2141	48.6	86	811407	1	6075	131	108	2135911
E	0.1	3406	54.8	68	1552356	100	4639	80.4	89	1826347	0.1	10700	224	128	3087777
	10	3456	57.2	67	1476640	10	4453	82.3	98	1587671	100	8671	191	128	2516837
	10	2441	44.1	60	1200686	1	2632	55	91	1008935	1	8004	185	130	2286979
Without Dye	-	212	9.5	53	109044	-	105	5.9	90	70894	-	27	1.5	14	25960
	-	180	8.4	46	92591	-	205	9.3	63	98123	-	30	1.7	24	30096
	-	73	4.8	59	36378	-	365	15.9	67	163883	-	95	4.8	29	56410

Laser Intensity (%): *70, **80, ***80

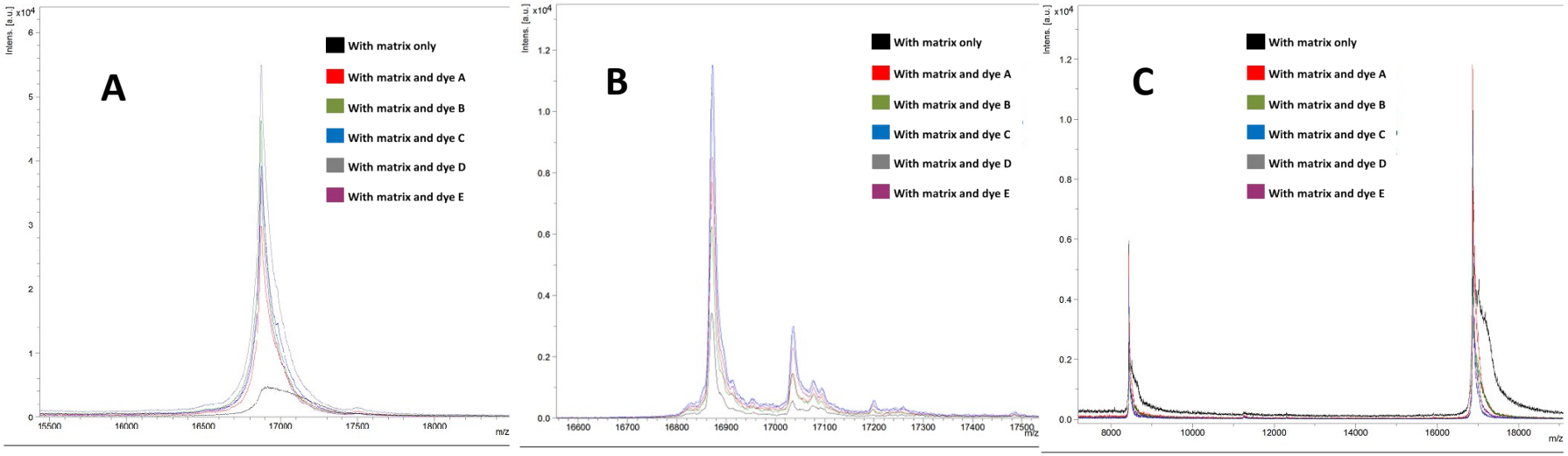

Figure S1 MALDI-MS spectra of myoglobin analysed with matrices A) HCCA, B) SA and C) DHB with dyes (A-E) and without dyes.

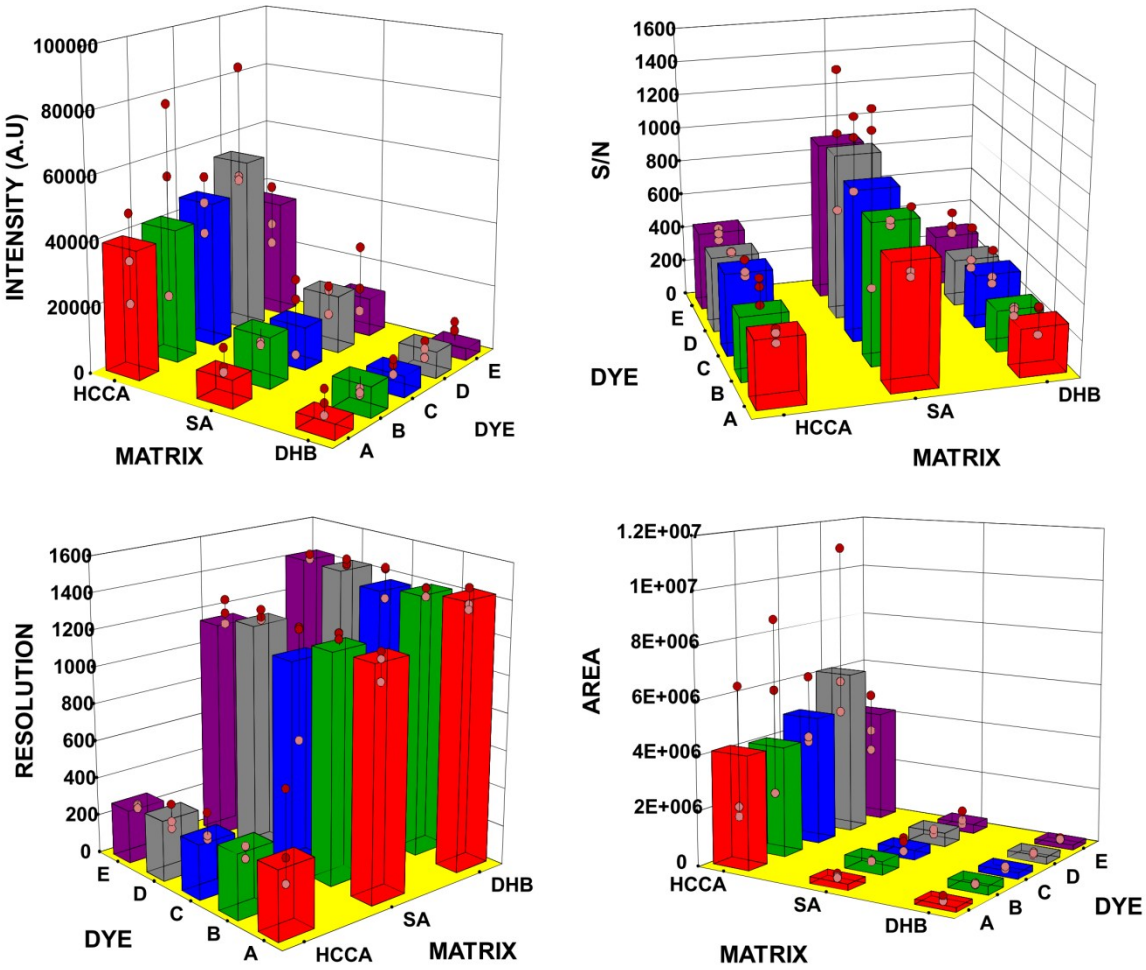

Figure S2 Graphs showing A) Intensity, B) S/N ratio, C) Resolution, and D) Area of myoglobin observed in MADLI-MS spectra vs. Matrix and Dyes used for analysis.

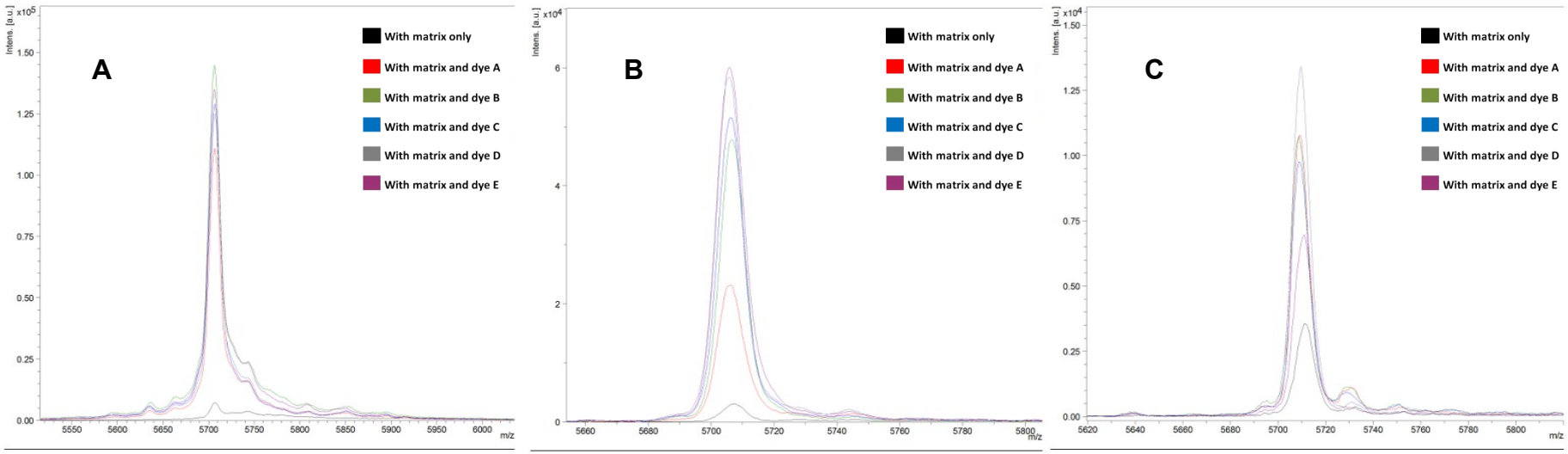

Figure S3 MALDI-MS spectra of insulin analysed with matrices A) HCCA, B) SA and C) DHB with dyes (A-E) and without dyes.

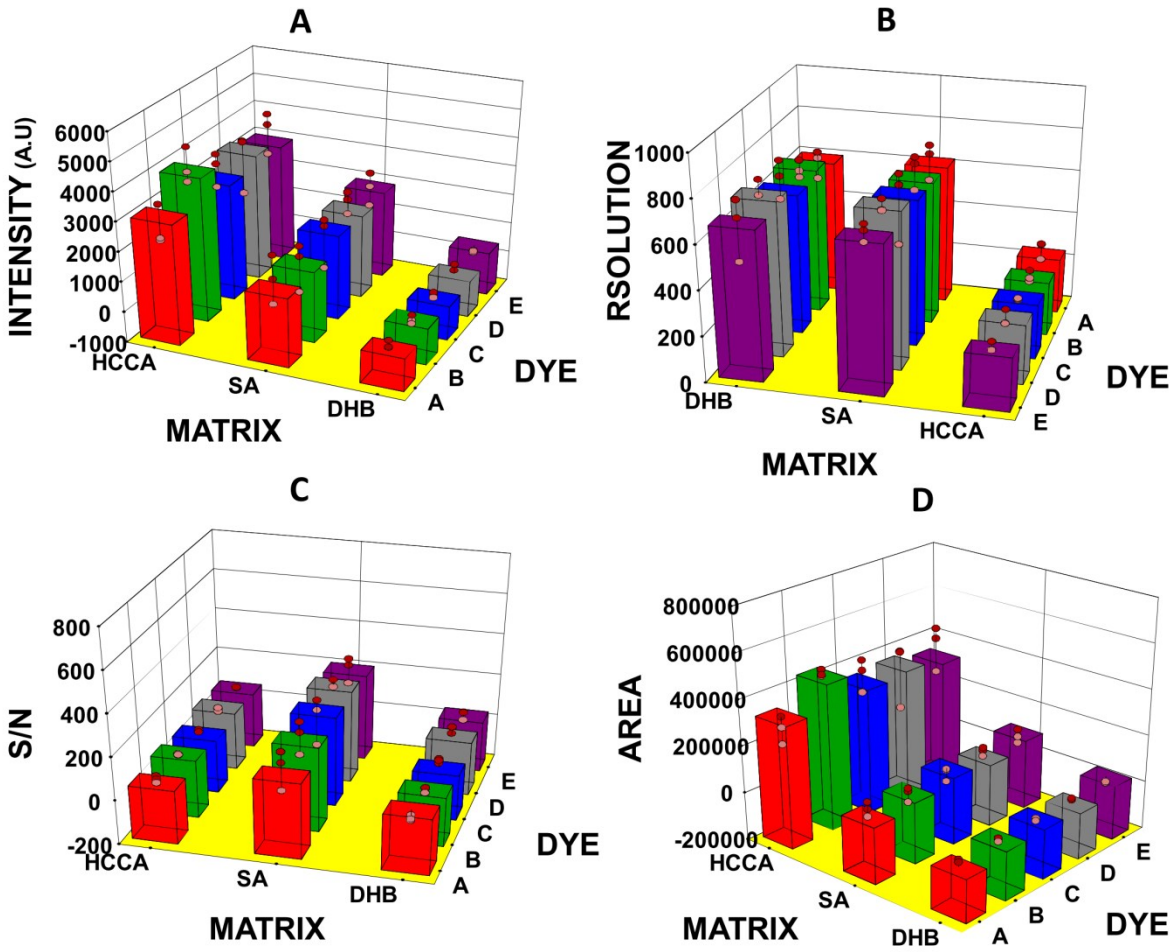

Figure S4 Graphs showing A) Intensity, B) S/N ratio, C) Resolution, and D) Area of insulin observed in MADLI-MS spectra vs. Matrix and Dyes used for analysis.

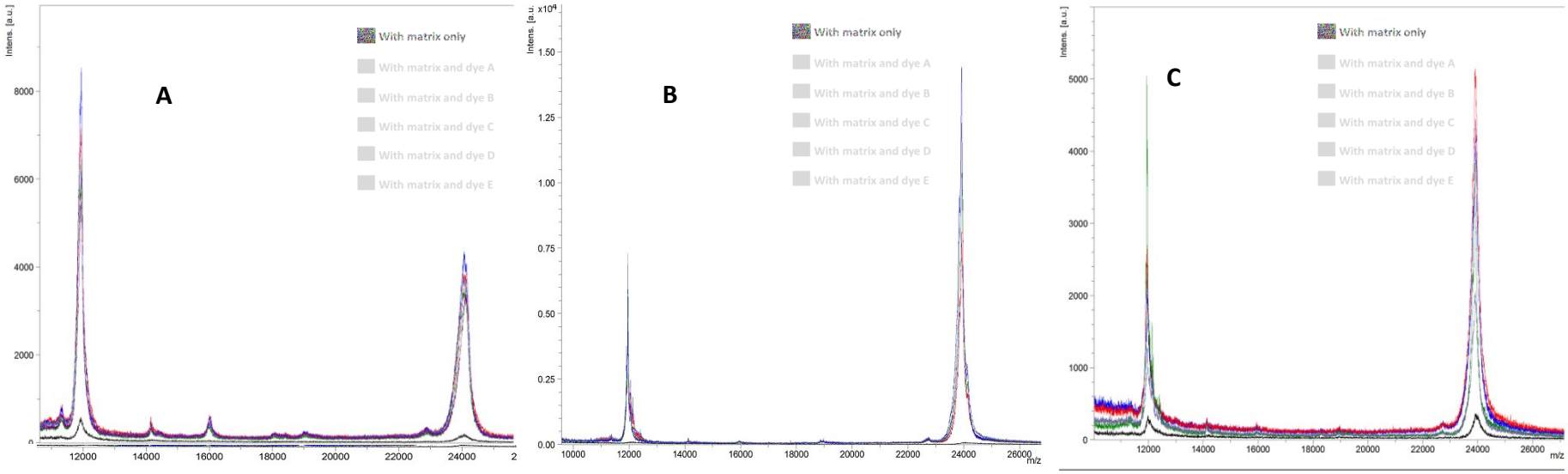

Figure S5 MALDI-MS spectra of casein analysed with matrices A) HCCA, B) SA and C) DHB with dyes (A-E) and without dyes.

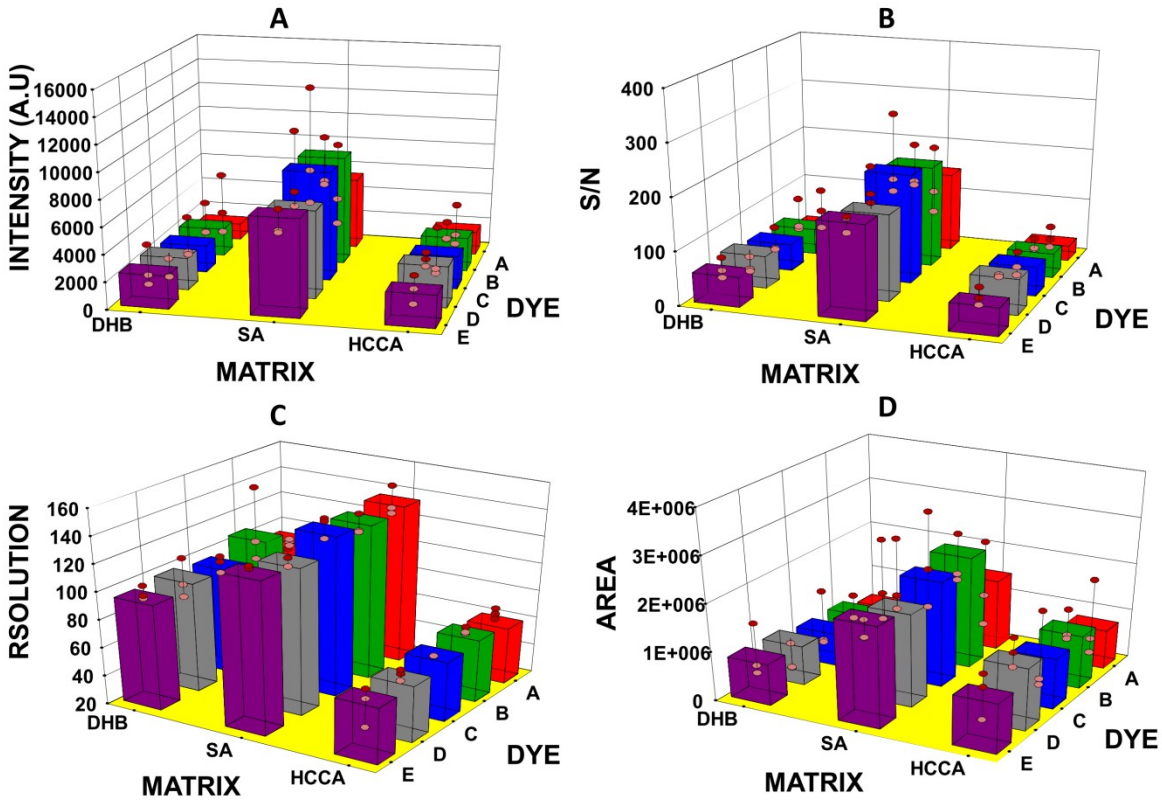

Figure S6 Graphs showing A) Intensity, B) S/N ratio, C) Resolution, and D) Area of casein observed in MADLI-MS spectra vs. Matrix and Dyes used for analysis.

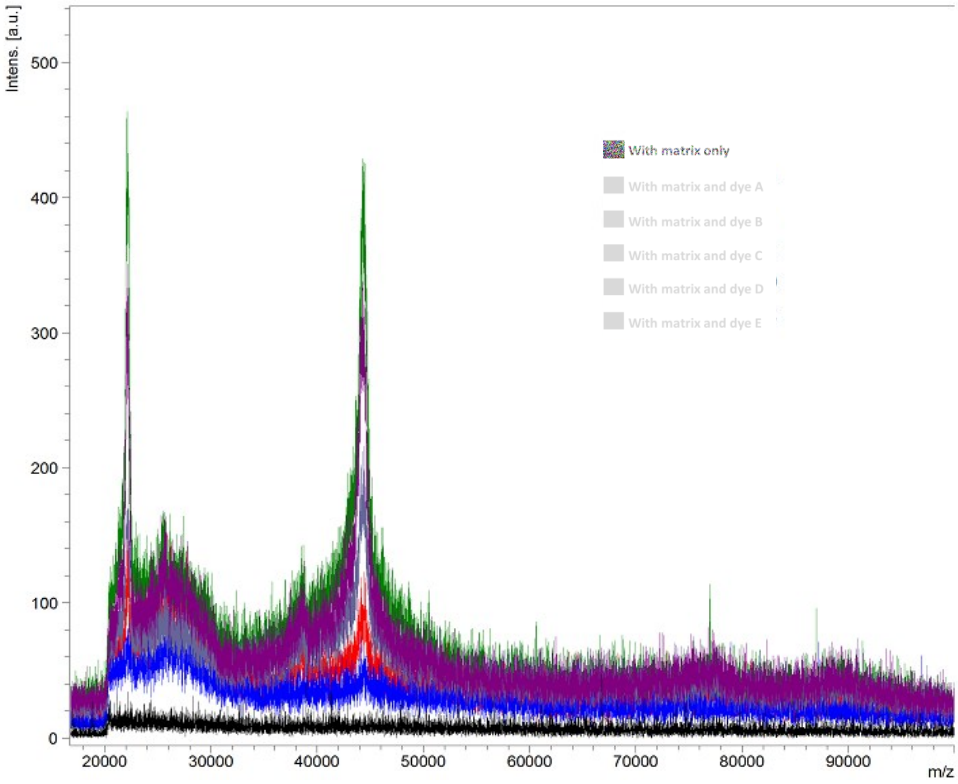

Figure S7 MALDI-MS spectra of egg white sample analysed with HCCA with and without dyes (A-E) for the possible detection of proteins.

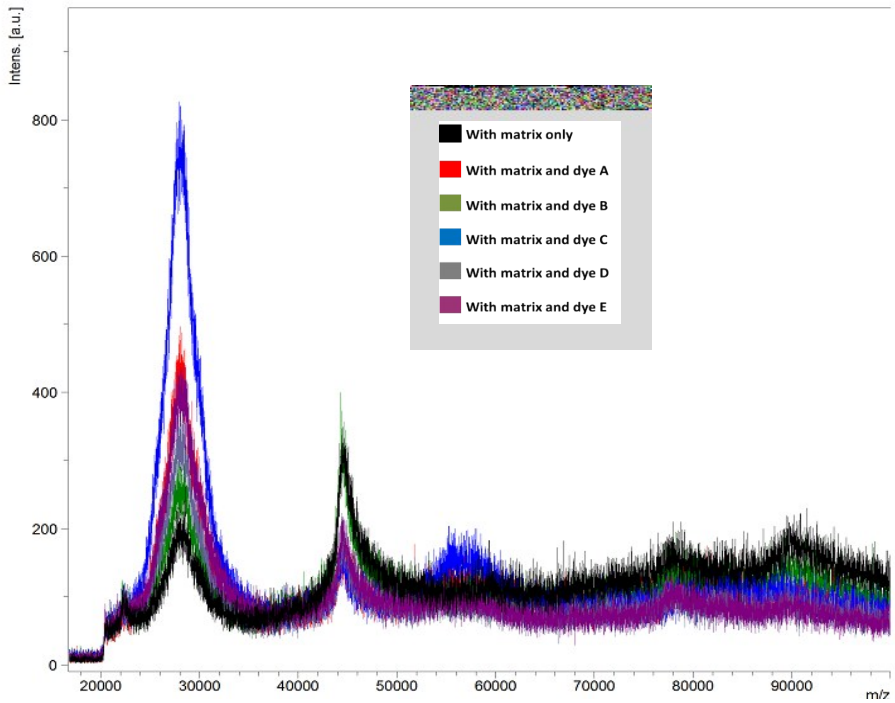

Figure S8 MALDI-MS spectra of egg white sample analysed with DHB with and without dyes (A-E) for the possible detection of proteins.

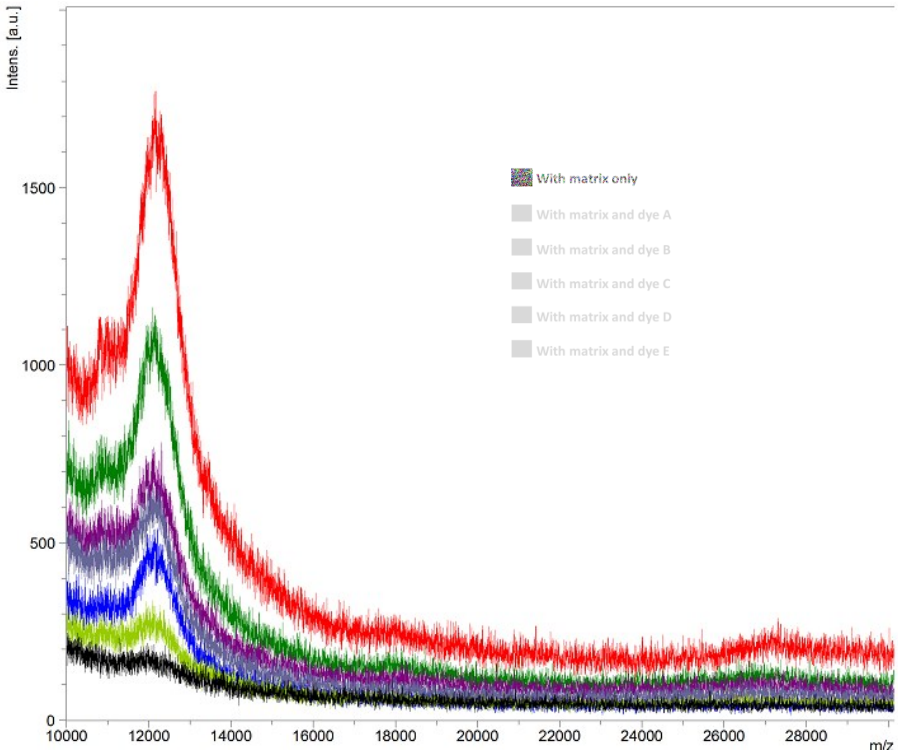

Figure S9 MALDI-MS spectra of honey sample analysed with HCCA with and without dyes (A-E) for the possible detection of polydispersed carbohydrates/ proteins.

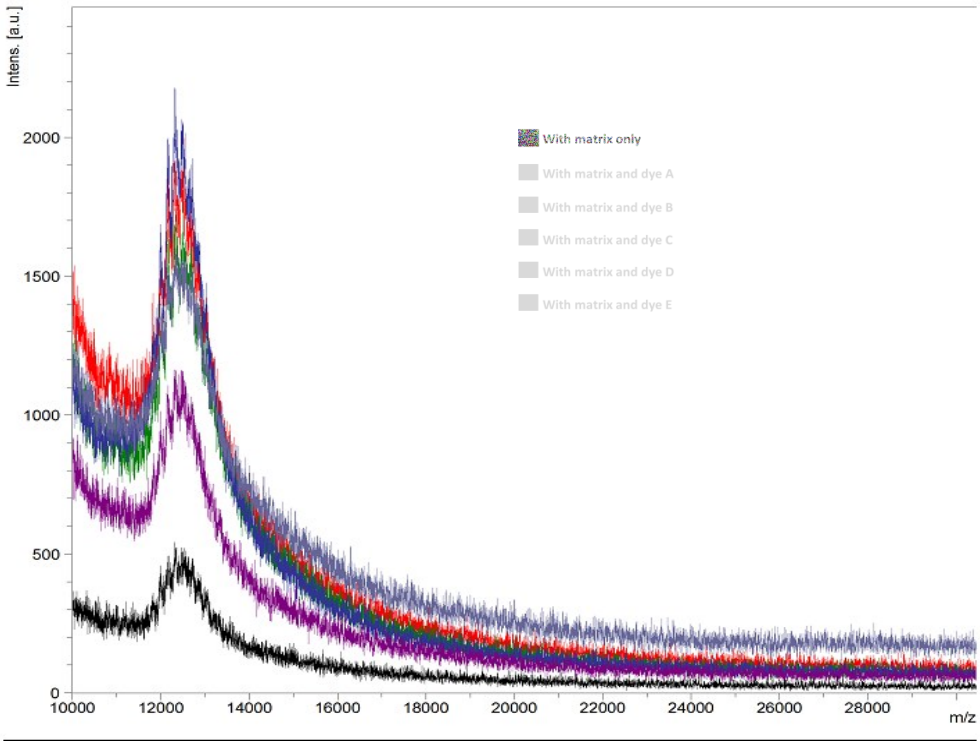

Figure S10 MALDI-MS spectra of honey sample analysed with DHB with and without dyes (A-E) for the possible detection of polydispersed carbohydrates/ proteins.

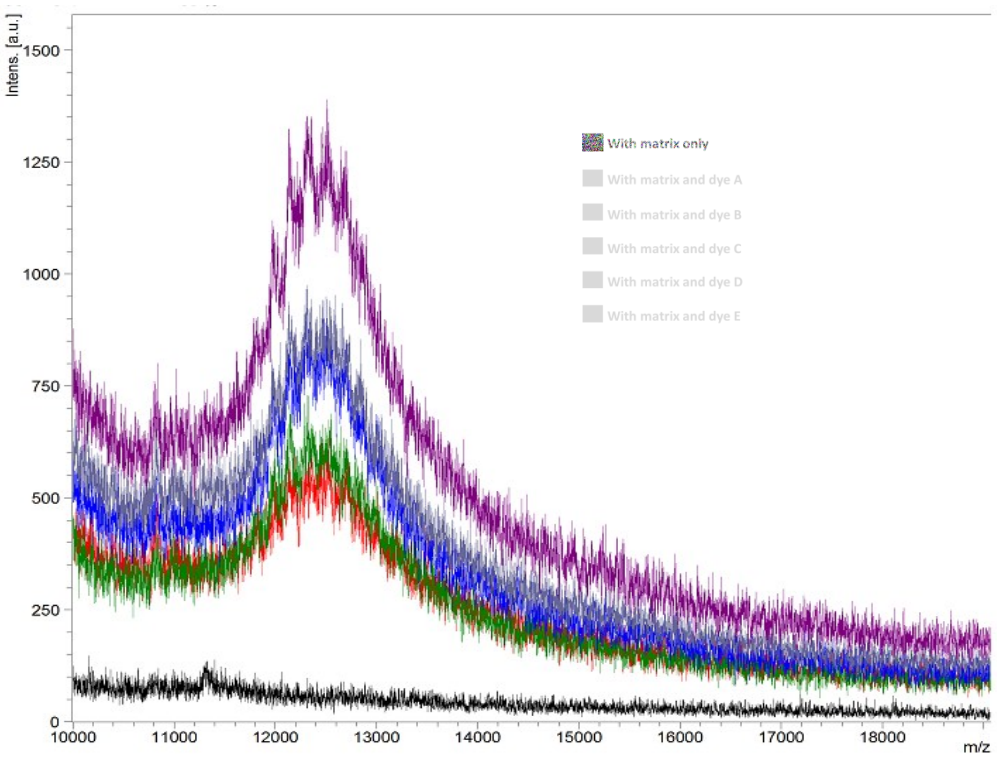

Figure S11 MALDI-MS spectra of honey sample analysed with SA with and without dyes (A-E) for the possible detection of polydispersed carbohydrates/ proteins.

Data S1. Statistical Data for Bovine Serum Albumin analysis.

Response 1 INTENSITY

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.01	6.54	0.0310	Significant
a-MATRIX	*2*	*6.01*	*6.54*	*0.0310*	
Subplot	33	137.01	0.72	0.8590	not significant
B-DYE	*4*	*137.01*	*1.06*	*0.3806*	
C-DYE CONC	*3*	*137.01*	*0.61*	*0.6102*	
aB	*8*	*137.01*	*0.82*	*0.5862*	
aC	*6*	*137.01*	*0.44*	*0.8476*	
BC	*12*	*137.01*	*0.72*	*0.7344*	

Response 2 S/N

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.01	13.47	0.0060	significant
a-MATRIX	*2*	*6.01*	*13.47*	*0.0060*	
Subplot	33	137.03	1.09	0.3523	not significant
B-DYE	*4*	*137.03*	*1.38*	*0.2429*	
C-DYE CONC	*3*	*137.03*	*1.15*	*0.3299*	
aB	*8*	*137.03*	*1.69*	*0.1053*	
aC	*6*	*137.03*	*0.95*	*0.4621*	
BC	*12*	*137.03*	*0.64*	*0.8087*	

Response 4 AREA

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.01	4.72	0.0587	not significant

a-MATRIX	*2*	*6.01*	*4.72*	*0.0587*	
Subplot	33	137.02	0.77	0.8133	not significant
B-DYE	*4*	*137.02*	*1.68*	*0.1591*	
C-DYE CONC	*3*	*137.02*	*0.60*	*0.6139*	
aB	*8*	*137.02*	*0.89*	*0.5234*	
aC	*6*	*137.02*	*0.44*	*0.8482*	
BC	*12*	*137.02*	*0.57*	*0.8604*	

Response 3 RSOLUTION

Fixed Effects

Source	**Term df**	**Error df**	**F Value**	**p-value Prob > F**	
Whole-plot	2	6.02	50.10	0.0002	significant
a-MATRIX	*2*	*6.02*	*50.10*	*0.0002*	
Subplot	33	137.04	1.65	0.0243	significant
B-DYE	*4*	*137.05*	*0.67*	*0.6117*	
C-DYE CONC	*3*	*137.05*	*2.15*	*0.0965*	
aB	*8*	*137.05*	*0.92*	*0.5042*	
aC	*6*	*137.05*	*2.31*	*0.0371*	
BC	*12*	*137.04*	*1.95*	*0.0336*	

Data S2. Statistical data for myoglobin analysis.

Response 1 INTENSITY

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.01	117.62	< 0.0001	Significant
a-MATRIX	*2*	*6.01*	*117.62*	*< 0.0001*	
Subplot	33	137.06	1.23	0.2092	not significant
B-DYE	*4*	*137.08*	*1.54*	*0.1945*	
C-DYE CONC	*3*	*137.08*	*0.87*	*0.4599*	
aB	*8*	*137.07*	*1.28*	*0.2601*	
aC	*6*	*137.08*	*2.00*	*0.0691*	
BC	*12*	*137.07*	*0.78*	*0.6731*	

Response 2 S/N

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.02	64.06	< 0.0001	significant
a-MATRIX	*2*	*6.02*	*64.06*	*< 0.0001*	
Subplot	33	137.04	0.66	0.9201	not significant
B-DYE	*4*	*137.05*	*1.04*	*0.3909*	
C-DYE CONC	*3*	*137.05*	*0.30*	*0.8228*	
aB	*8*	*137.05*	*0.95*	*0.4775*	
aC	*6*	*137.05*	*0.52*	*0.7961*	
BC	*12*	*137.04*	*0.49*	*0.9160*	

Response 3 RSOLUTION

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	5.99	1419.34	< 0.0001	significant
a-MATRIX	*2*	*5.99*	*1419.34*	*< 0.0001*	
Subplot	33	137.04	0.89	0.6415	not significant

B-DYE	*4*	*137.04*	*1.45*	*0.2208*
C-DYE CONC	*3*	*137.05*	*0.90*	*0.4433*
aB	*8*	*137.04*	*0.90*	*0.5173*
aC	*6*	*137.04*	*0.86*	*0.5276*
BC	*12*	*137.04*	*0.70*	*0.7522*

Response 4 AREA

Fixed Effects

Source	**Term df**	**Error df**	**F Value**	**p-value Prob > F**	
Whole-plot	2	6.06	375.45	< 0.0001	Significant
a-MATRIX	*2*	*6.06*	*375.45*	*< 0.0001*	
Subplot	33	137.11	1.28	0.1635	not significant
B-DYE	*4*	*137.12*	*1.27*	*0.2857*	
C-DYE CONC	*3*	*137.12*	*1.35*	*0.2623*	
aB	*8*	*137.12*	*1.29*	*0.2557*	
aC	*6*	*137.12*	*1.81*	*0.1015*	
BC	*12*	*137.12*	*0.97*	*0.4834*	

Data S3. Statistical data for casein analysis.

Response 1 INTENSITY

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.00	31.63	0.0007	significant
a-MATRIX	*2*	*6.00*	*31.63*	*0.0007*	
Subplot	33	135.11	1.53	0.0477	significant
B-DYE	*4*	*135.18*	*2.52*	*0.0444*	
C-DYE CONC	*3*	*135.17*	*3.62*	*0.0148*	
aB	*8*	*135.17*	*0.64*	*0.7392*	
aC	*6*	*135.16*	*1.29*	*0.2648*	
BC	*12*	*135.09*	*1.34*	*0.2058*	

Response 2 S/N

REML Analysis for selected model

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.04	74.33	< 0.0001	significant
a-MATRIX	*2*	*6.04*	*74.33*	*< 0.0001*	
Subplot	33	135.14	1.86	0.0073	significant
B-DYE	*4*	*135.20*	*2.94*	*0.0229*	
C-DYE CONC	*3*	*135.19*	*3.65*	*0.0143*	
aB	*8*	*135.19*	*1.55*	*0.1454*	
aC	*6*	*135.18*	*1.81*	*0.1011*	
BC	*12*	*135.12*	*1.21*	*0.2814*	

Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Response 3 RSOLUTION

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.20	744.17	< 0.0001	Significant
a-MATRIX	*2*	*6.20*	*744.17*	*< 0.0001*	
Subplot	33	135.42	1.25	0.1845	not significant
B-DYE	*4*	*135.58*	*1.32*	*0.2669*	
C-DYE CONC	*3*	*135.55*	*1.34*	*0.2645*	
aB	*8*	*135.56*	*1.79*	*0.0844*	
aC	*6*	*135.54*	*0.82*	*0.5592*	
BC	*12*	*135.40*	*1.08*	*0.3858*	

Response 4 AREA

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	5.93	17.49	0.0033	significant
a-MATRIX	*2*	*5.93*	*17.49*	*0.0033*	
Subplot	33	135.06	1.44	0.0762	not significant
B-DYE	*4*	*135.14*	*2.18*	*0.0741*	
C-DYE CONC	*3*	*135.13*	*3.29*	*0.0227*	
aB	*8*	*135.13*	*0.50*	*0.8514*	
aC	*6*	*135.12*	*1.21*	*0.3058*	
BC	*12*	*135.04*	*1.43*	*0.1613*	

Data S4. Statistical data for insulin analysis.

Response 1 Intensity

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.31	748.08	< 0.0001	significant
a-MATRIX	*2*	*6.31*	*748.08*	*< 0.0001*	
Subplot	33	136.71	0.97	0.5169	not significant
B-DYE	*4*	*136.83*	*1.07*	*0.3735*	
C-DYE CONC	*3*	*136.83*	*0.029*	*0.9932*	
aB	*8*	*136.82*	*2.05*	*0.0455*	
aC	*6*	*136.83*	*0.69*	*0.6543*	
BC	*12*	*136.80*	*0.58*	*0.8560*	

Response 2 S/N

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	5.63	192.17	< 0.0001	significant
a-MATRIX	*2*	*5.63*	*192.17*	*< 0.0001*	
Subplot	33	135.81	1.54	0.0454	significant
B-DYE	*4*	*135.86*	*0.49*	*0.7448*	
C-DYE CONC	*3*	*135.86*	*1.75*	*0.1605*	
aB	*8*	*135.85*	*1.61*	*0.1287*	
aC	*6*	*135.85*	*0.97*	*0.4481*	
BC	*12*	*135.84*	*2.04*	*0.0250*	

Response 3 RSOLUTION

Fixed Effects

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	5.88	517.07	< 0.0001	significant
a-MATRIX	*2*	*5.88*	*517.07*	*< 0.0001*	
Subplot	33	136.03	1.01	0.4684	not significant
B-DYE	*4*	*136.06*	*1.93*	*0.1081*	
C-DYE CONC	*3*	*136.06*	*0.34*	*0.7981*	
aB	*8*	*136.06*	*0.54*	*0.8272*	
aC	*6*	*136.06*	*2.23*	*0.0438*	
BC	*12*	*136.05*	*0.59*	*0.8487*	

Response 4 AREA

These rows were ignored for this analysis.

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REML Analysis for selected model

Source	Term df	Error df	F Value	p-value Prob > F	
Whole-plot	2	6.21	644.66	< 0.0001	significant
a-MATRIX	*2*	*6.21*	*644.66*	*< 0.0001*	
Subplot	33	136.46	1.23	0.2082	not significant
B-DYE	*4*	*136.53*	*1.66*	*0.1639*	
C-DYE CONC	*3*	*136.53*	*0.60*	*0.6129*	
aB	*8*	*136.52*	*2.11*	*0.0392*	
aC	*6*	*136.53*	*1.08*	*0.3791*	
BC	*12*	*136.51*	*0.70*	*0.7455*	