Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

for

Native Mass Spectrometry Analysis of Conjugated HSA and BSA Complexes with Various Flavonoids

Nicolas Alexander[t,a], Lucas McDonald[t,a], Chrys Wesdemiotis[a]*, Yi Pang[a]*

[a]*Department of Chemistry, The University of Akron, OH 44325, USA*

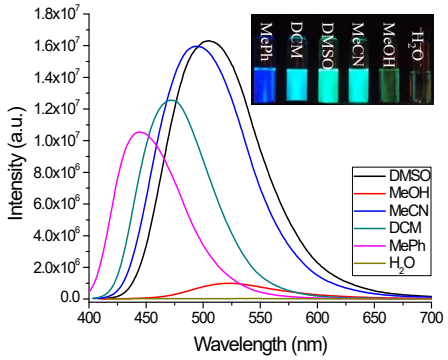

Figure S1. Emission spectra of compound **1** (20 μM) in different solvents.

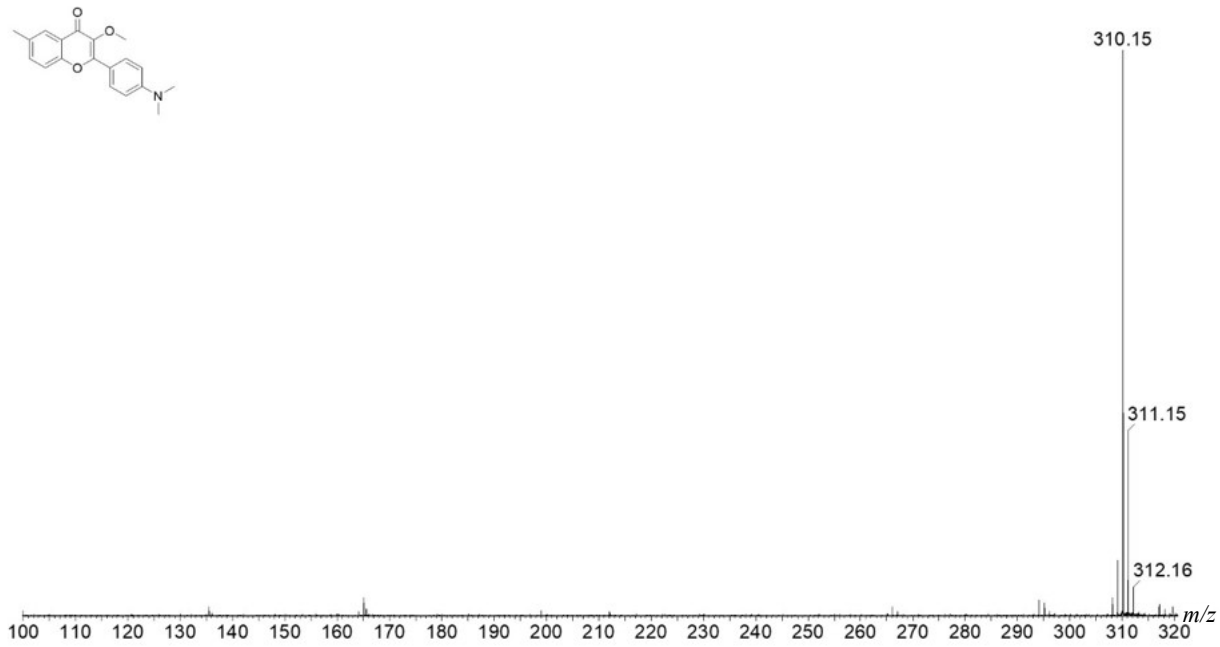

Figure S2. ESI-MS spectrum of flavone dye **1** at pH < 4. The presence of an intense $[M+H]^+$ peak at *m/z* 310 and lack of any appreciable fragmentation indicates the dye is not degraded under acidic pH conditions.

Table S1: Experimentally measured m/z values for native BSA. K^+ charges were used to obtain the calculated m/z values.

	Native BSA	
Charge State	Measured *m/z*	Measured molecular mass[a]
15	4475	66540
16	4193	66464
17	3943	66368
18	3726	66366
		Avg: 66434

[a]The average of the measured molecular mass matches well with the BSA MW (=66430).

Table S2: Experimentally measured and calculated m/z values for BSA-flavone **1** complex. Different combinations of K^+ and H^+ charges were used to obtain the calculated *m/z* values. The molecular masses are calculated by using the respective m/z values and counter ions.

BSA-Flavone **1** complex					
Charge State	Measured *m/z*	[a]MW from measured *m/z*	[b]Calculated *m/z*	[c][% Error]	Charges [M+ xK +yH]
15	4482	66758	4481	0.02	$12K^+ + 3H^+$
16	4206	66785	4203	0.07	$13K^+ + 3H^+$
17	3958	66736	3958	0.00	$14K^+ + 3H^+$
18	3743	66785	3741	0.05	$15K^+ + 3H^+$
		Avg: 66766			

[a]MW of the complex from the measured *m/z* = "measured *m/z* values" × charge – molecular mass of counter ions ($xK^+ + yH^+$).

[b]Calculated *m/z* = ["66430 (BSA MW)" + 309 (MW for flavone **1**) + $xK^+ + yH^+$] ÷ "charge state"

By assuming a 1:1 ratio in the complex, the calculated *m/z* values are closely matching the measured *m/z* for all the charge states.

[c][% Error] is calculated by comparing the "measured *m/z*" versus the "calculated *m/z*" values.

Table S3. Experimentally measured *m/z* values for HSA.

HSA Native					
Charge State	Measured *m/z*	[a]MW from measured *m/z*	Calculated *m/z*	[b][% Error]	[M+xK$^+$ +yH$^+$]
15	4464	66451	4463	0.02	13K$^+$ + 2H$^+$
16	4185	66412	4187	0.05	14K$^+$ + 2H$^+$
17	3939	66414	3940	0.03	14K$^+$ + 3H$^+$
18	3718	66413	3722	0.11	13K$^+$ + 4H$^+$
		Avg: 66422			

[a]MW of the complex from the measured *m/z* = "measured *m/z* values" × charge – molecular mass of counter ions (xK + yH).

[b][% Error] is calculated by comparing the "measured *m/z*" versus the "calculated *m/z*" values.

Table S4. Experimentally measured and calculated *m/z* values for HSA-flavone **2** complex.

HSA-Flavone **2** complex					
Charge State	Measured *m/z*	[a]MW from measured *m/z*	Calculated *m/z*	[b][% Error]	Charges [M+ xK +yH]
15	4482	66797	4482	0.00	11K$^+$ + 4H$^+$
16	4200	66804	4200	0.00	10K$^+$ + 6H$^+$
17	3959	66792	3960	0.03	13K$^+$ + 4H$^+$
18	374*8*	66800	3748	0.00	17K$^+$ + 1H$^+$
		Avg: 66798			

[a] MW of the complex from the measured *m/z* = "measured *m/z* values" × charge – molecular mass of counter ions (xK + yH).

[b][% Error] is calculated by comparing the "measured *m/z*" versus the "calculated *m/z*" values.

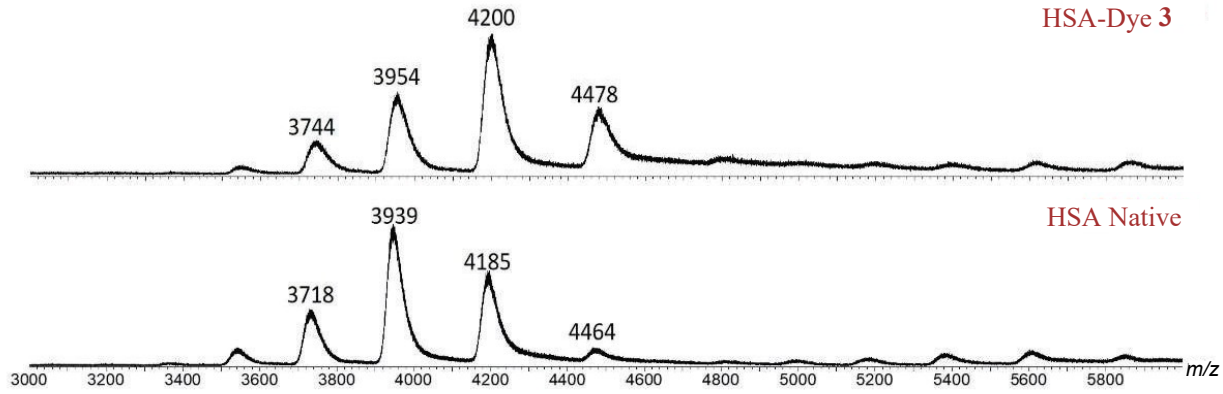

Figure S3. ESI-MS spectrum of native HSA (bottom) and HSA cmplexed to dye **3** [MW=419 Da] (top). The *m/z* values along with the predominant charge state shift in the spectra of the complex indicate a successful complexation.

Table S5: Measured vs expected *m/z* values for flavone dyes binding in 1:1 stoichiometric ratio with HSA.

HSA-Flavone **3** complex					
Charge State	Measured *m/z*	[a]MW from measured *m/z*	Calculated *m/z*	[% Error]	Charges [M+ xK +yH]
15	4478	66737	4478	0.00	$8K^+ + 7H^+$
16	4200	66804	4201	0.02	$9K^+ + 7H^+$
17	3954	66707	3954	0.00	$9K^+ + 8H^+$
18	3744	66728	3745	0.03	$14K^+ + 4H^+$
		Avg: 66744			

[a] MW of the complex from the measured m/z = "measured *m/z* values" × charge – molecular mass of counter ions (xK + yH).