Supporting material

The interactions of the ruthenium(II)-cymen complexes with egg white lysozyme and horse heart cytochrome c in the presence of carbonate ions

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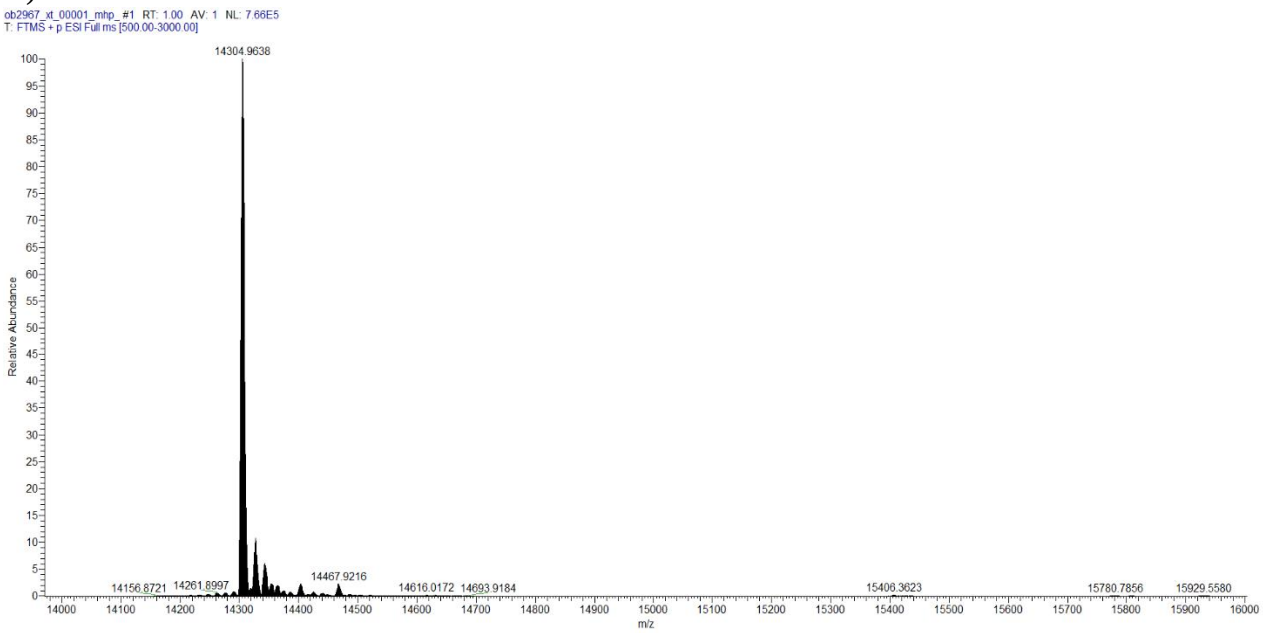
A)



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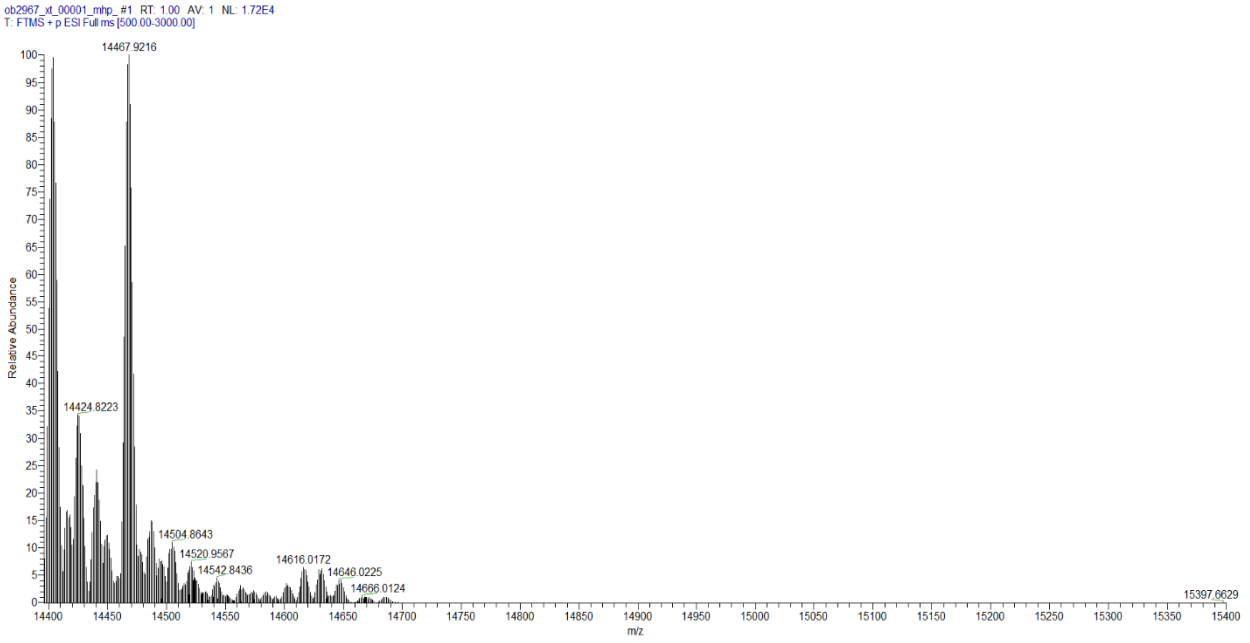
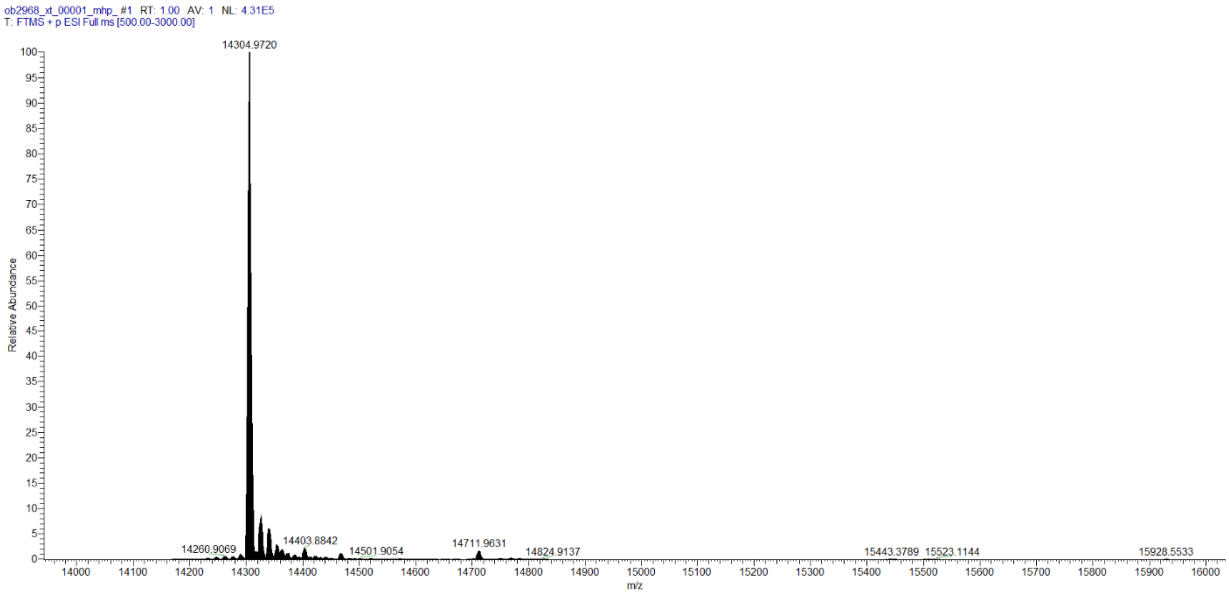


Figure S1. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 24 h in 20 mM ammonium hydrogen carbonate pH 7.4; B) Zoom out spectra in the mass region 14400-15400 Da

A)



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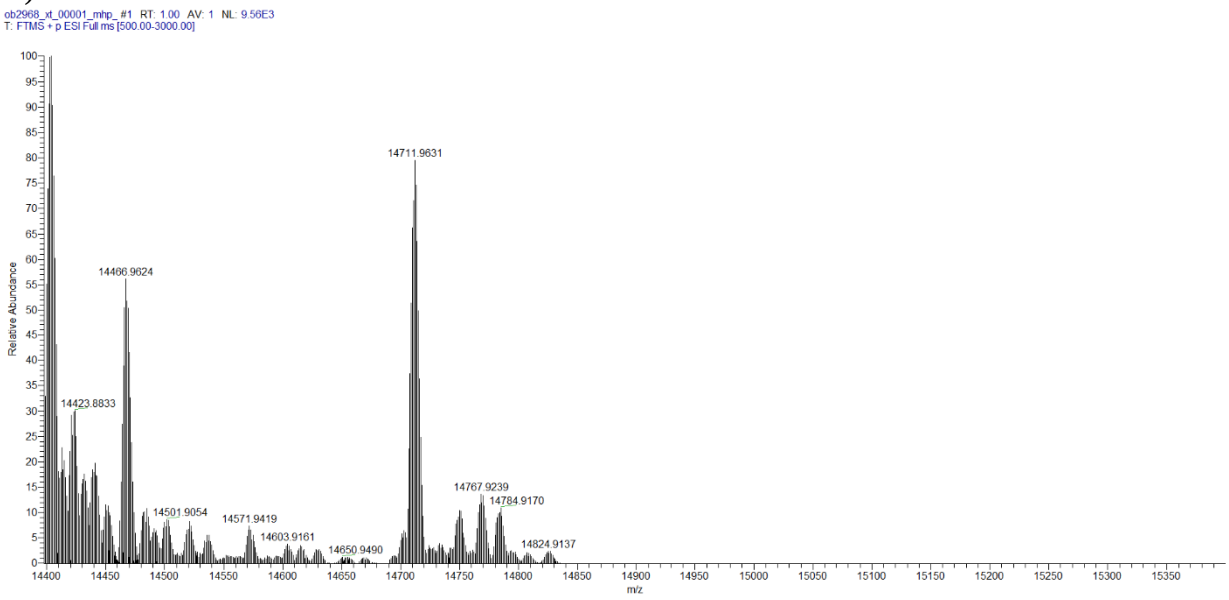
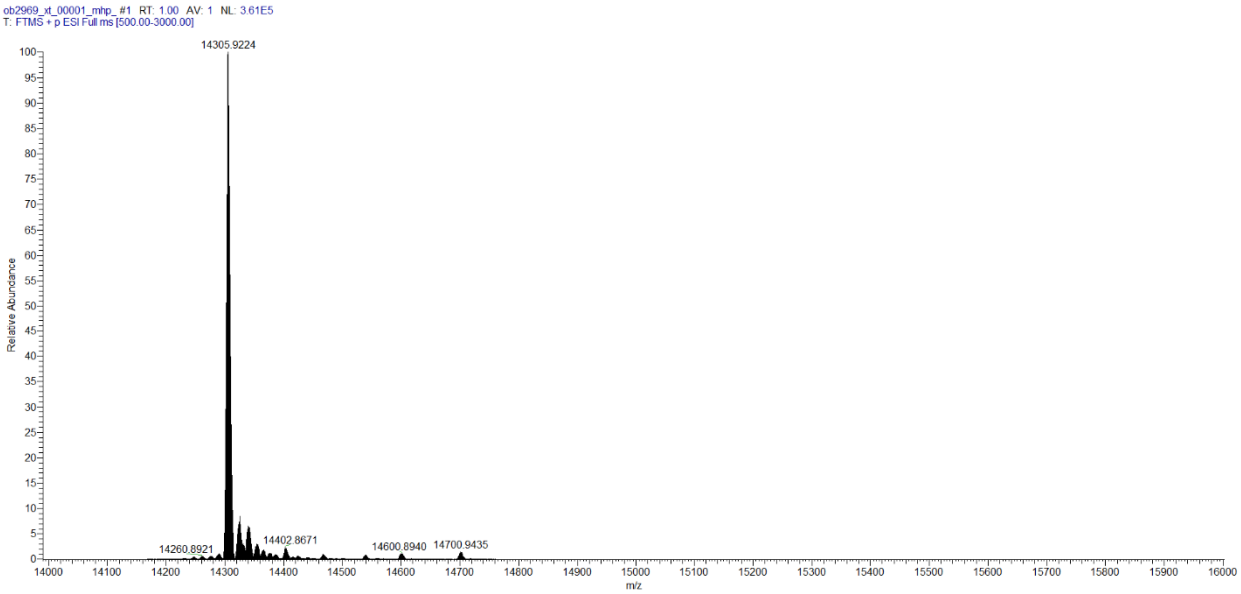


Figure S2. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 24 h with C1 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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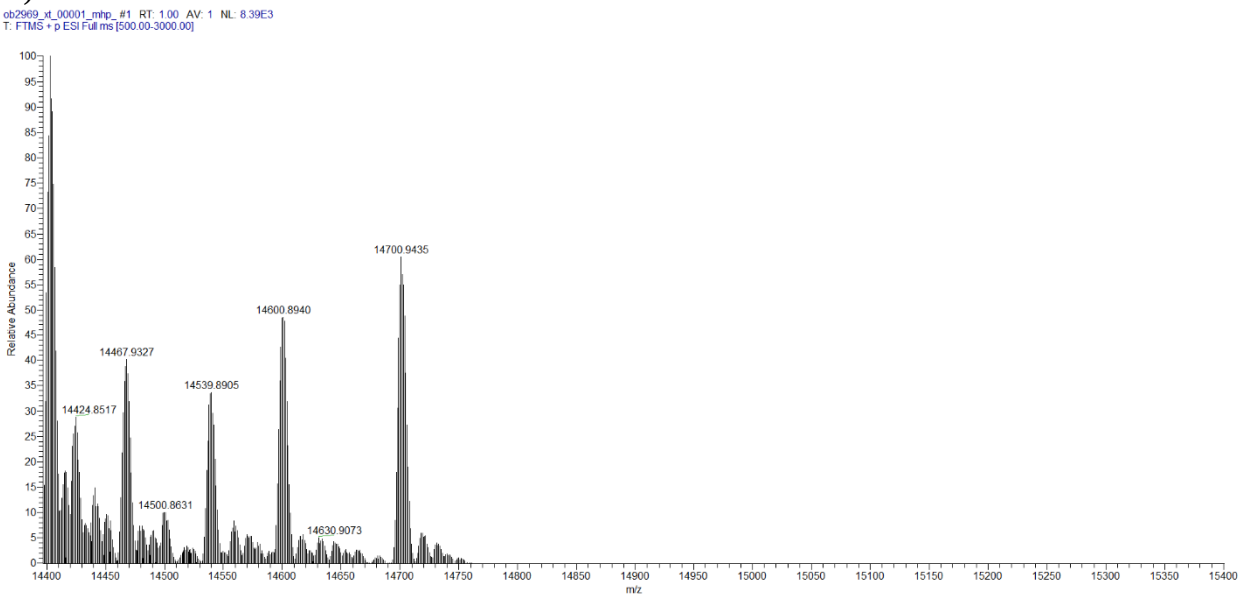
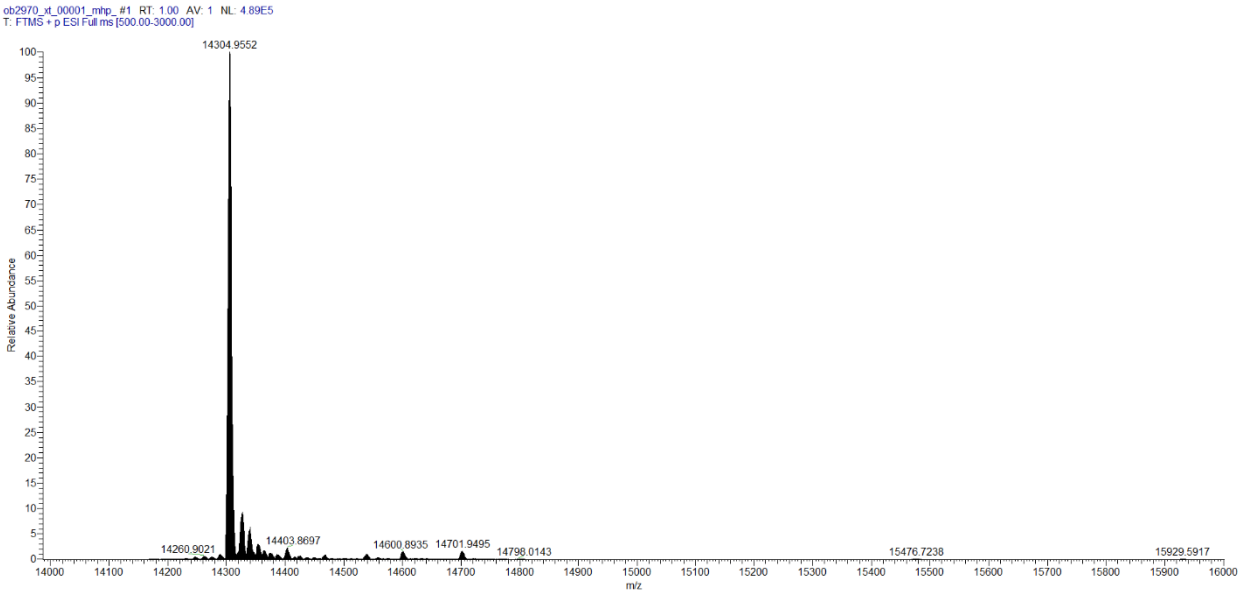


Figure S3. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 24 h with C2 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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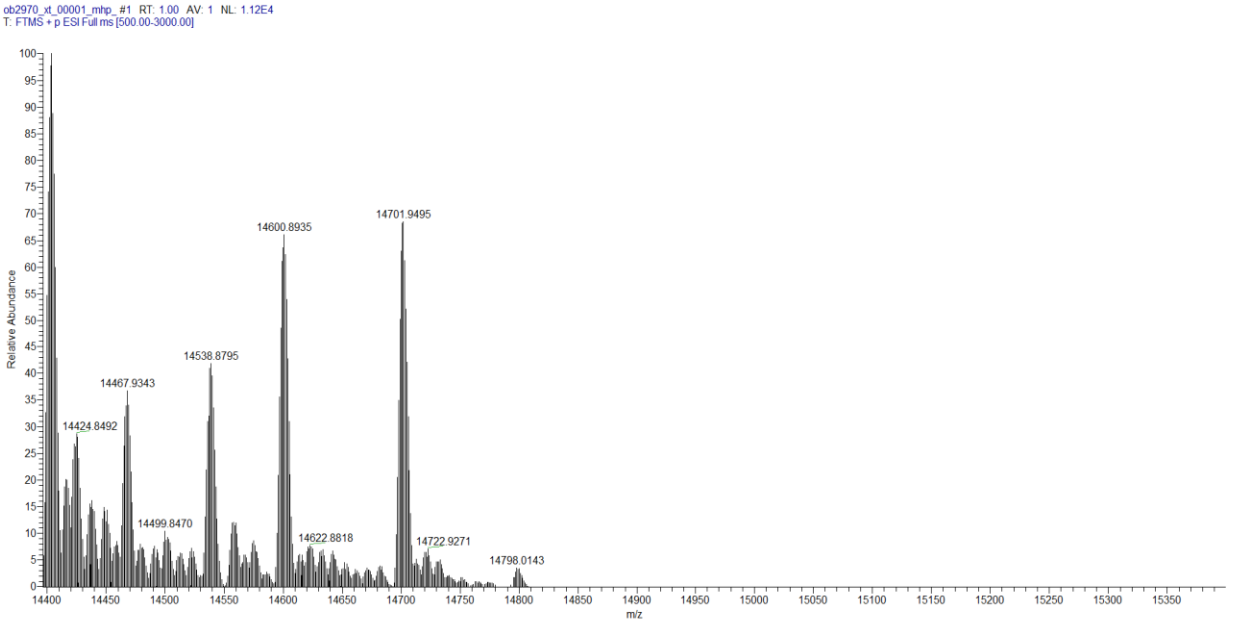
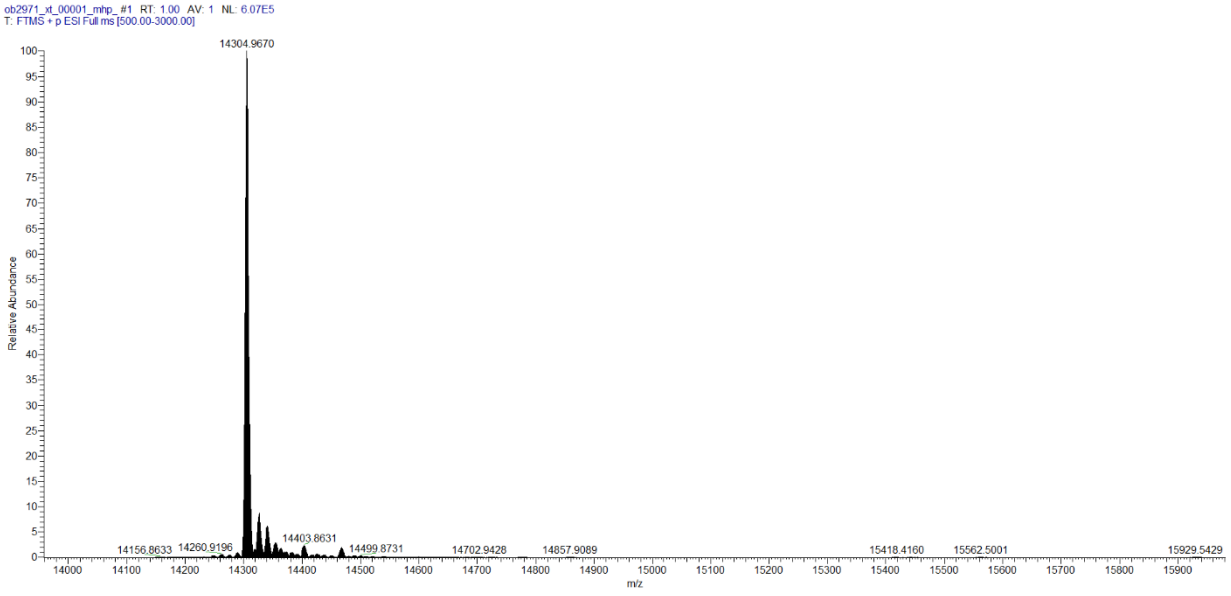


Figure S4. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 24 h with C3 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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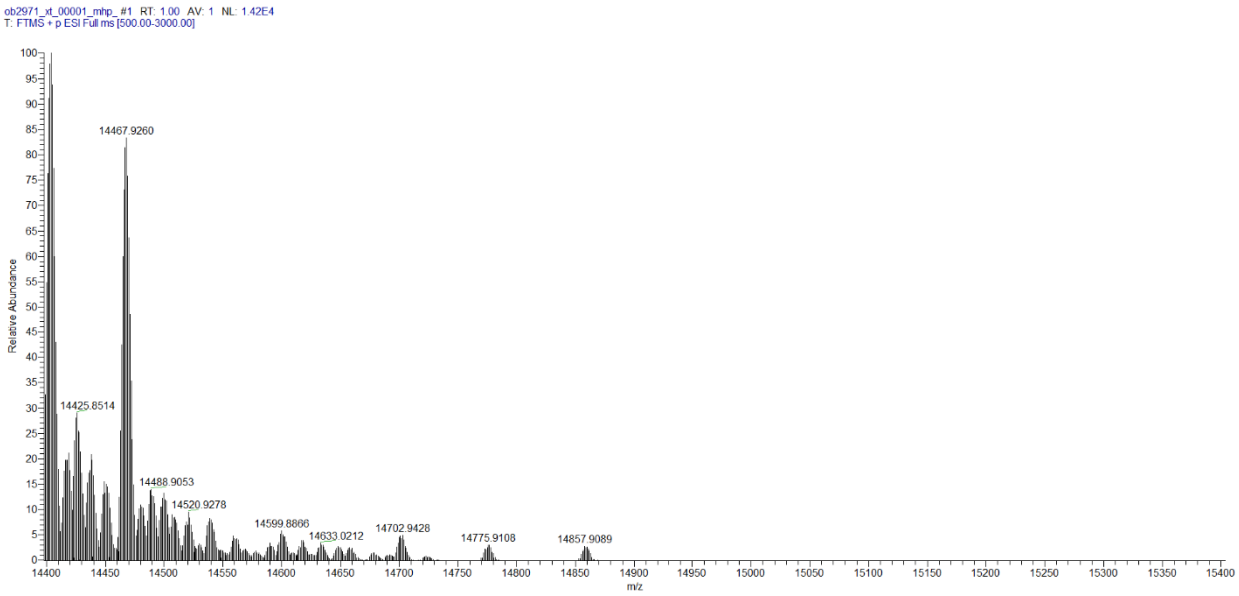
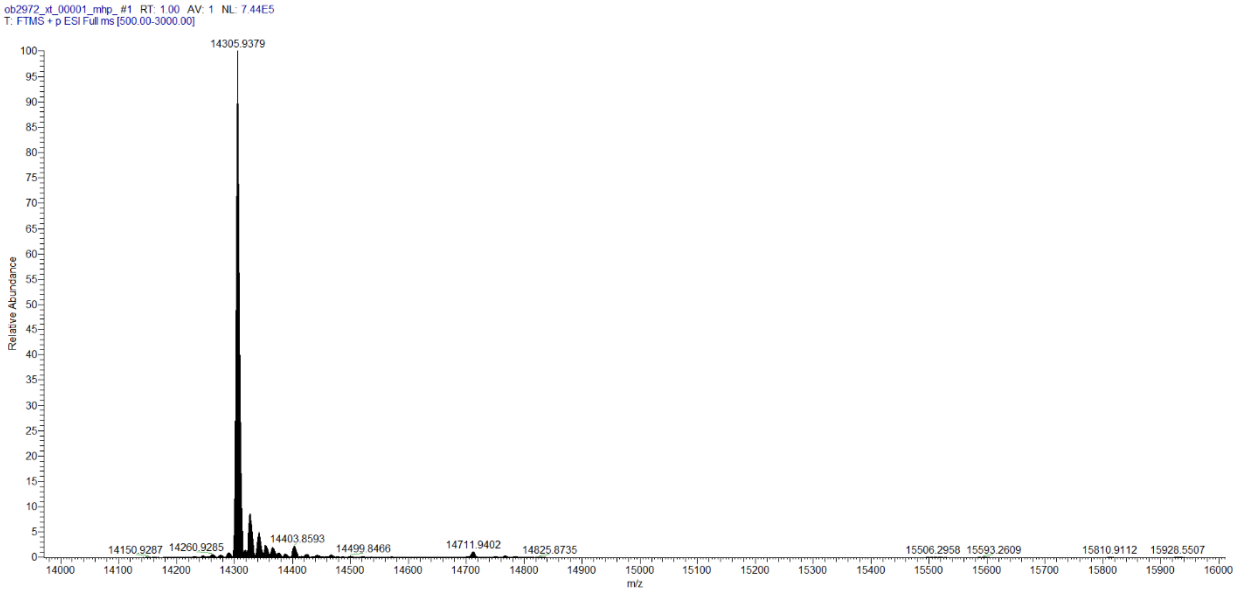


Figure S5. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 24 h with C4 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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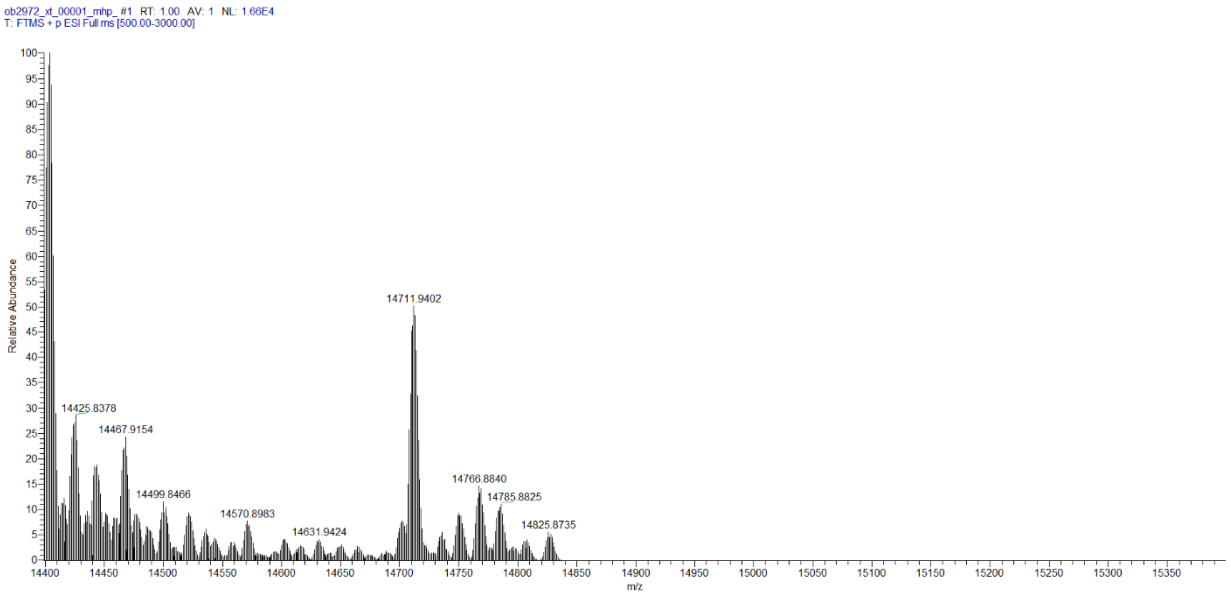
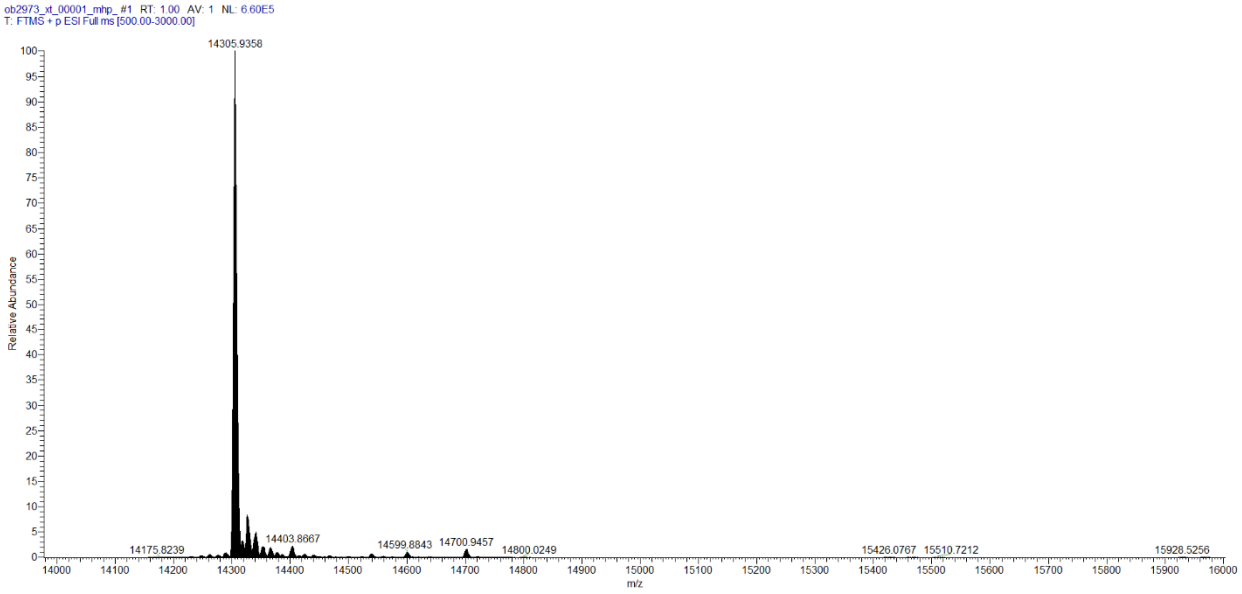


Figure S6. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 48 h with C1 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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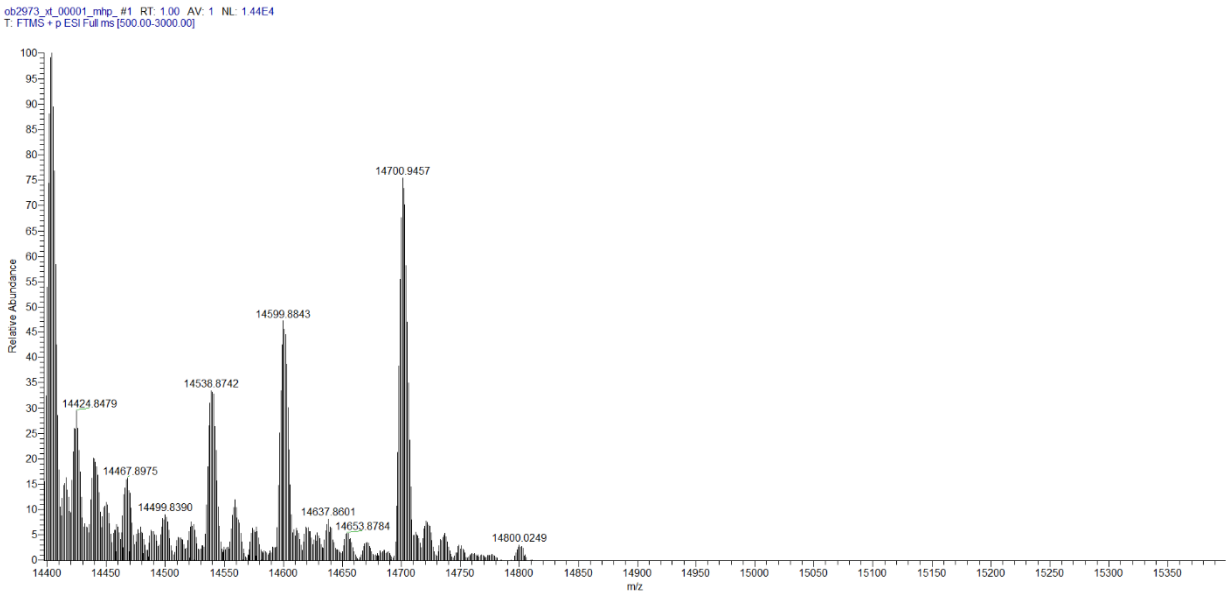
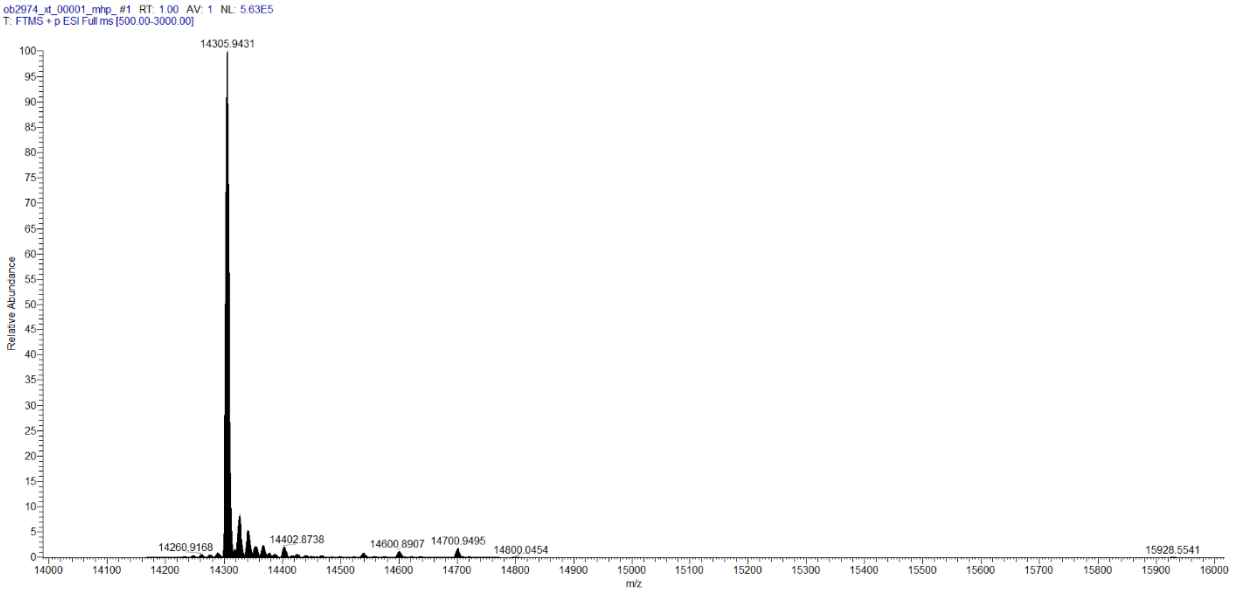


Figure S7. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 48 h with C2 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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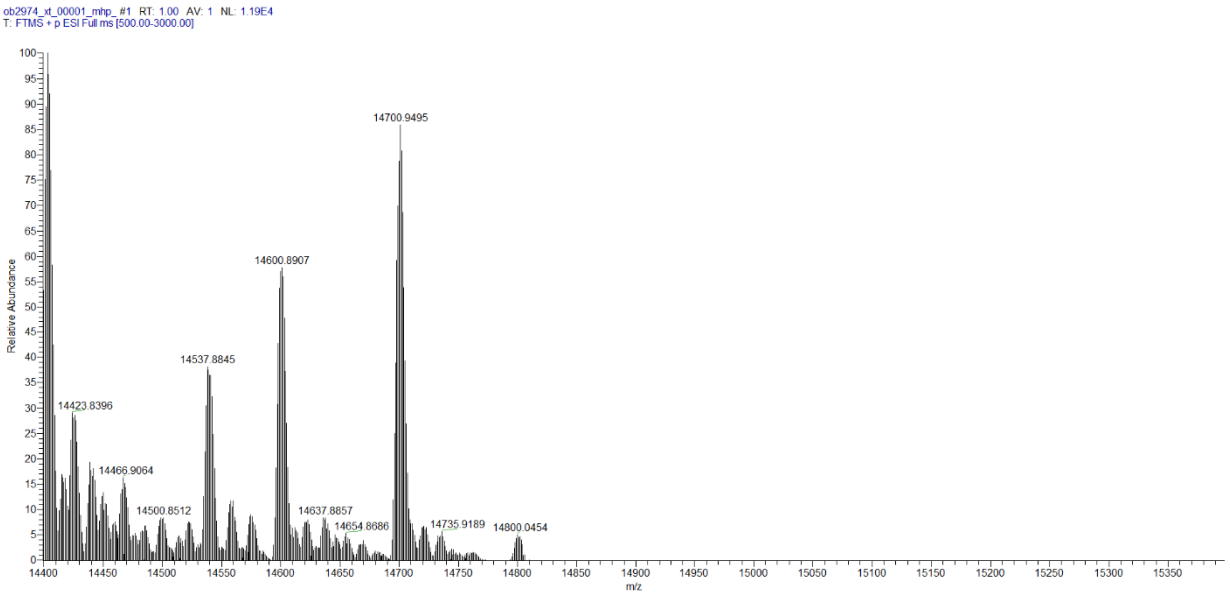
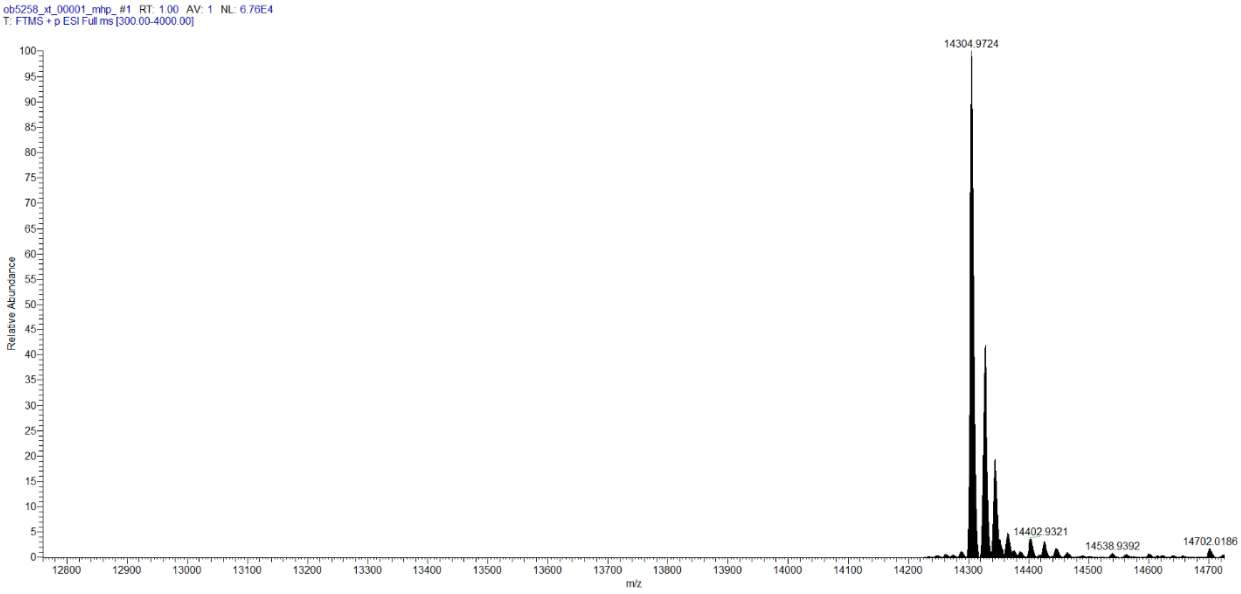


Figure S8. A) Deconvoluted ESI-MS spectra of lysozyme incubated for 48 h with C3 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-15400 Da

A)



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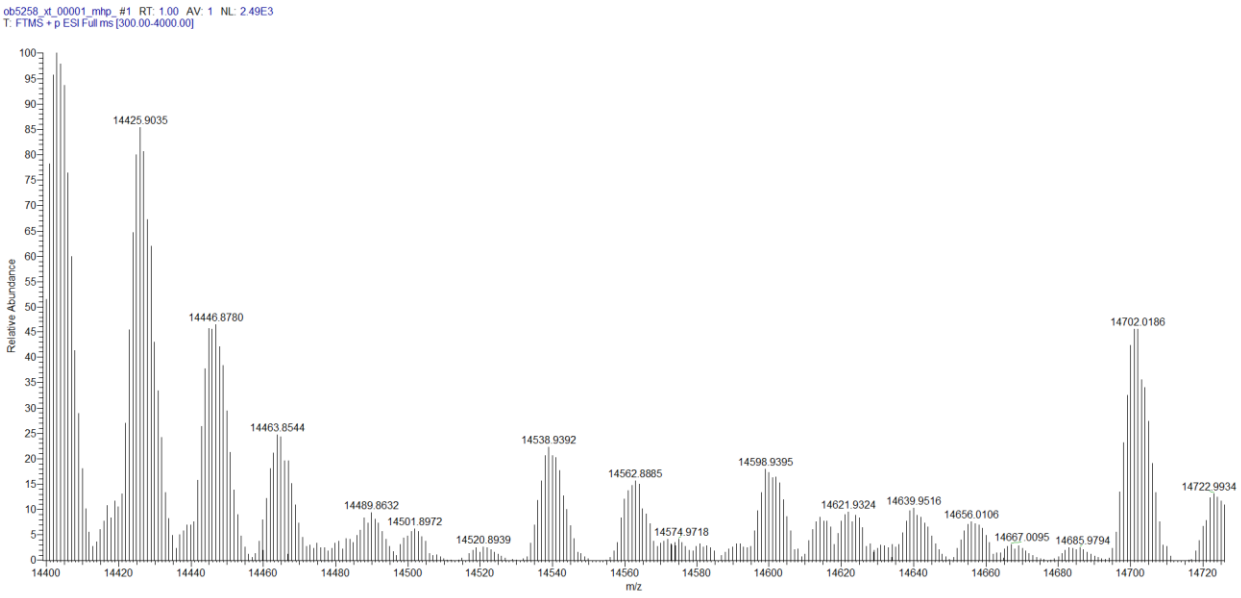
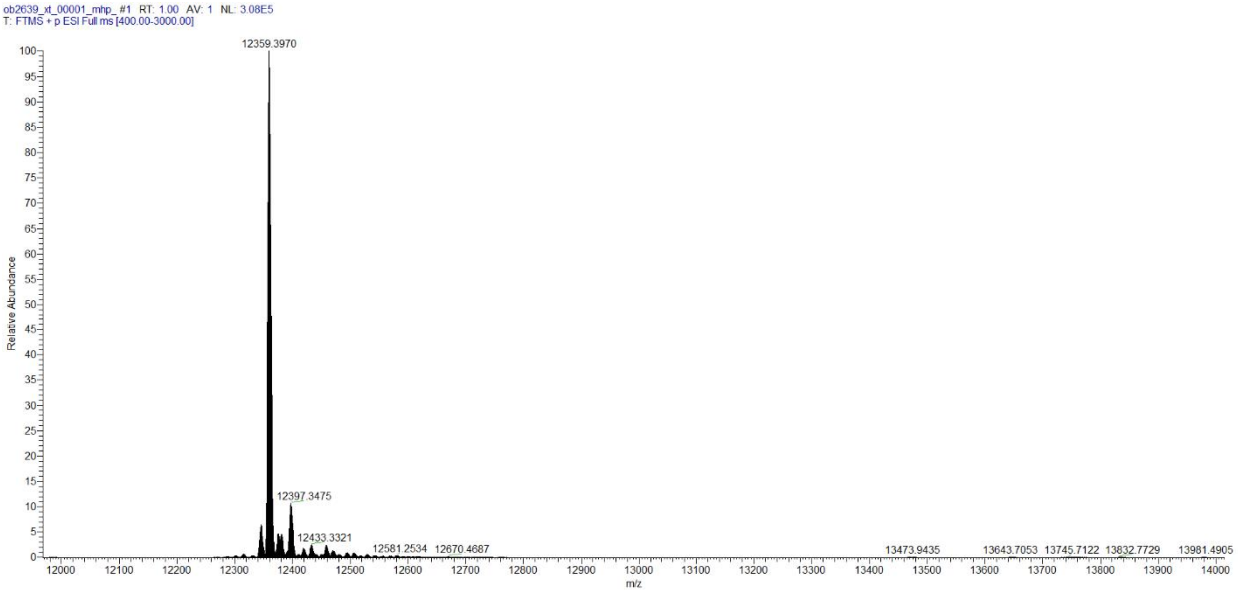


Figure S9. A) Deconvoluted ESI-MS ESI-MS spectra of lysozyme incubated for 48 h with C4 in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 14400-14800 Da

A)



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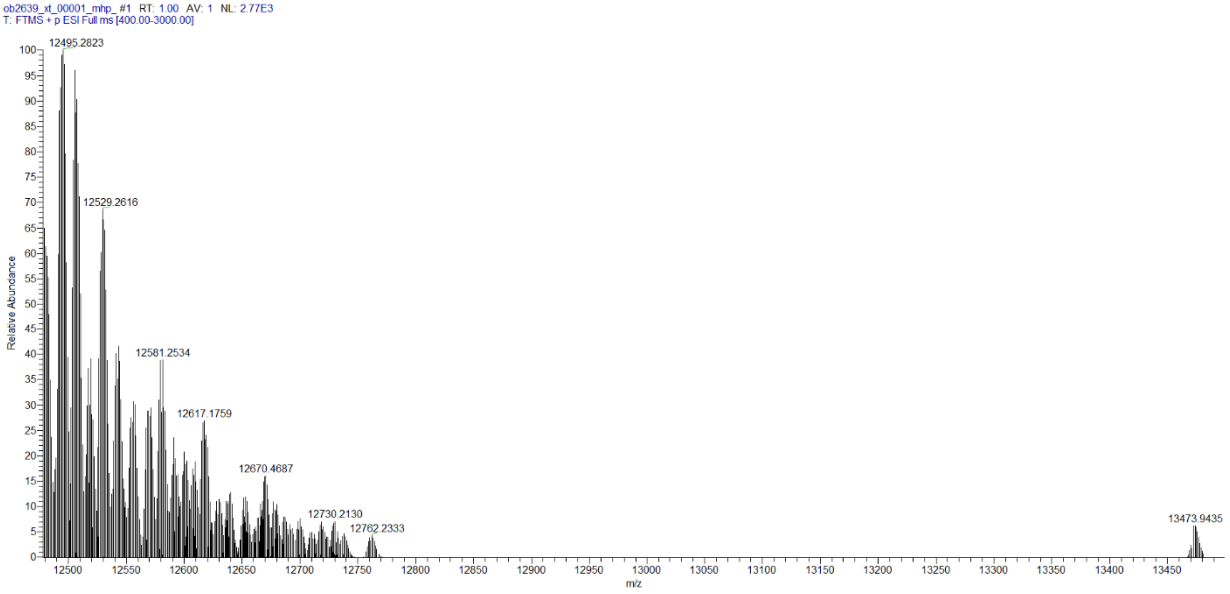
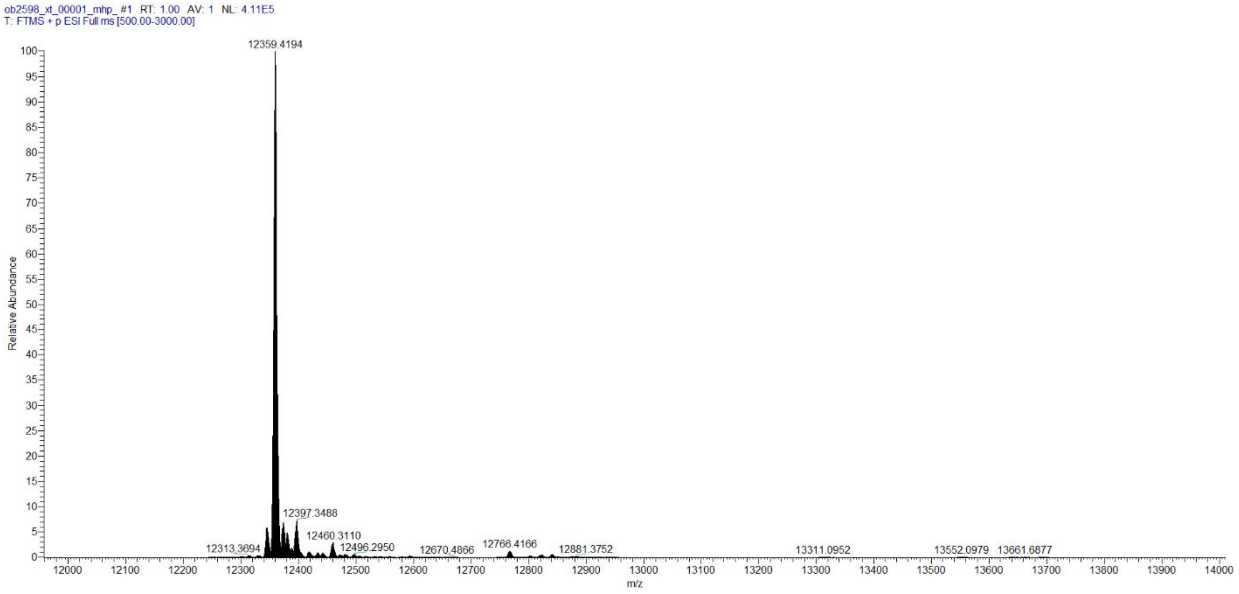


Figure S10. A) Deconvoluted ESI-MS spectra of cytochrome c incubated for 24 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



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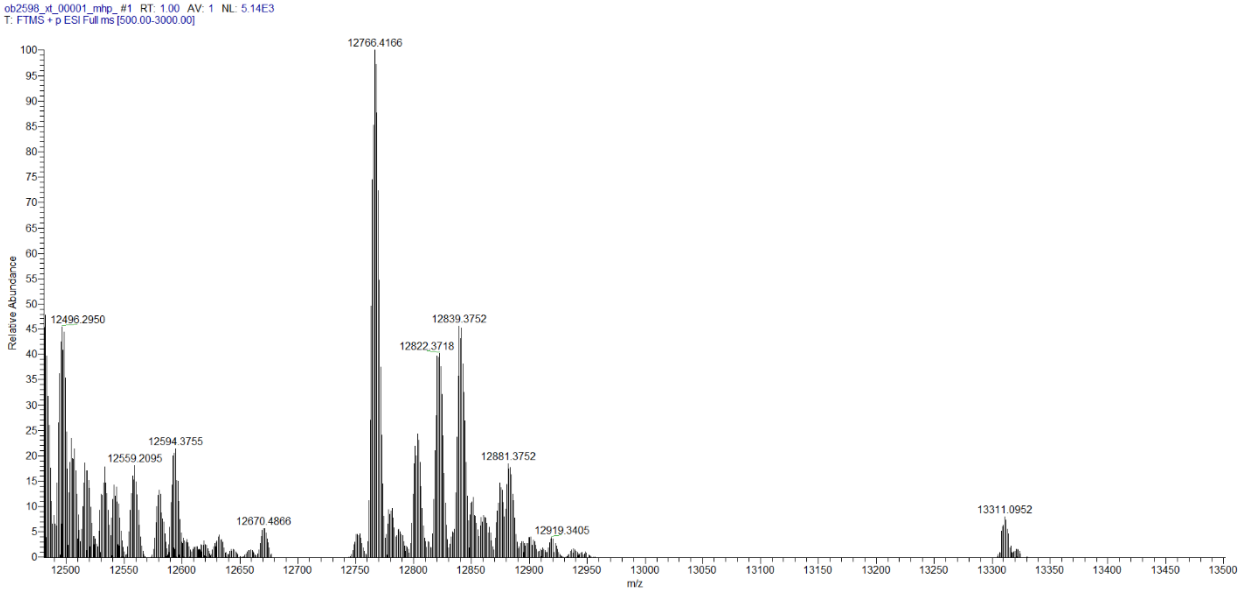
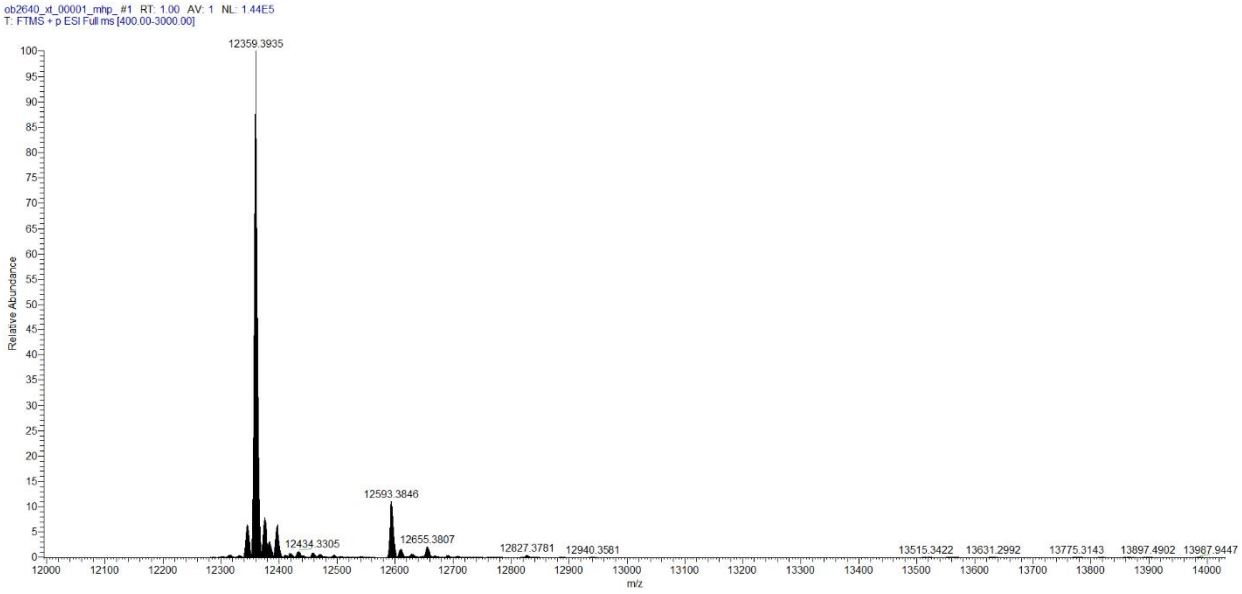


Figure S11. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C1 for 24 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



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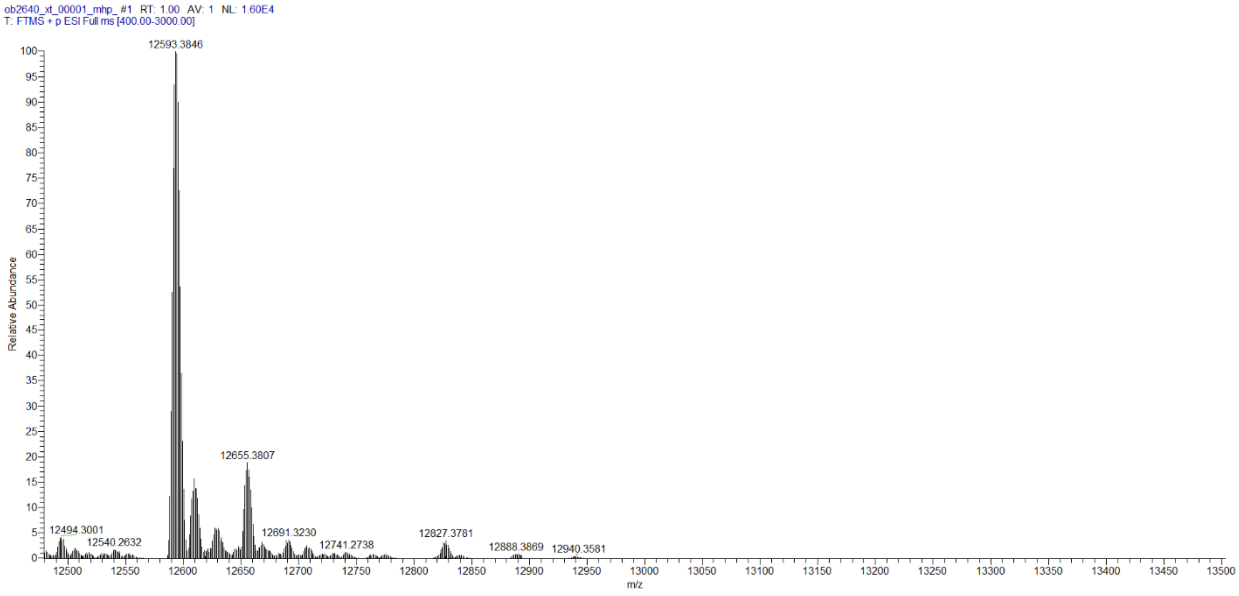
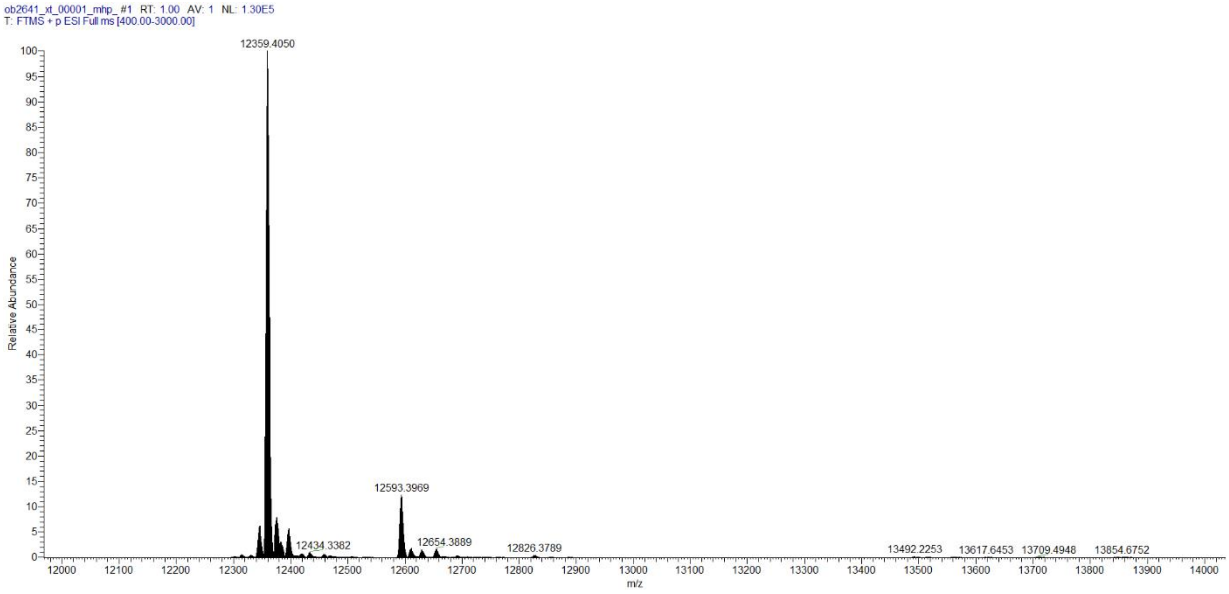


Figure S12. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C2 for 24 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



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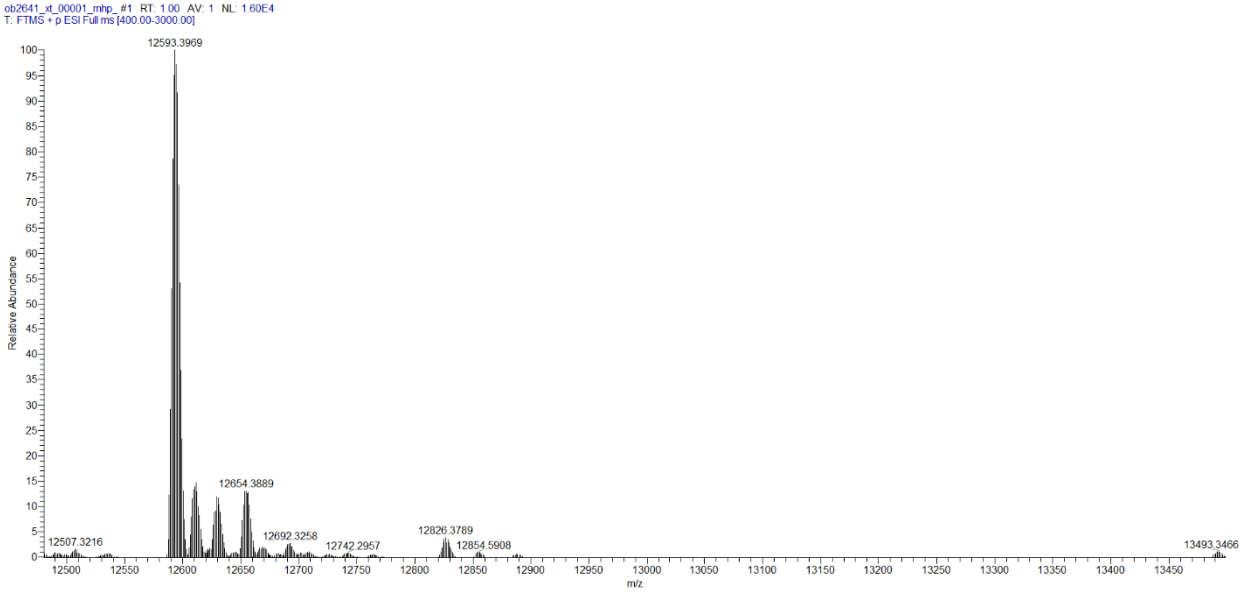
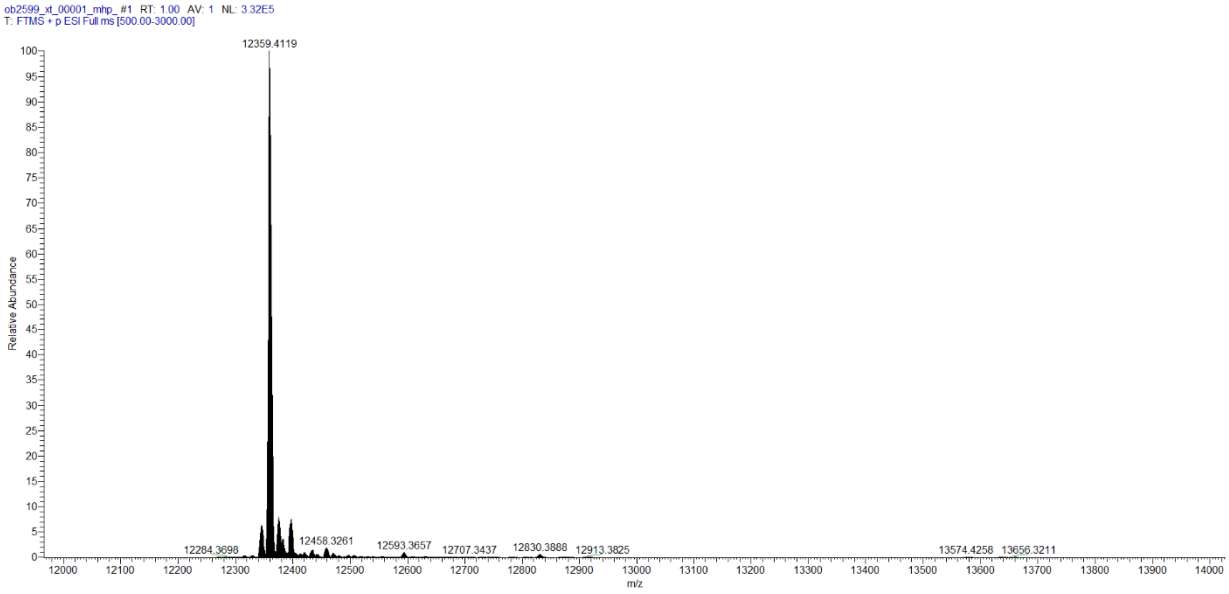


Figure S13. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C3 for 24 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



B)

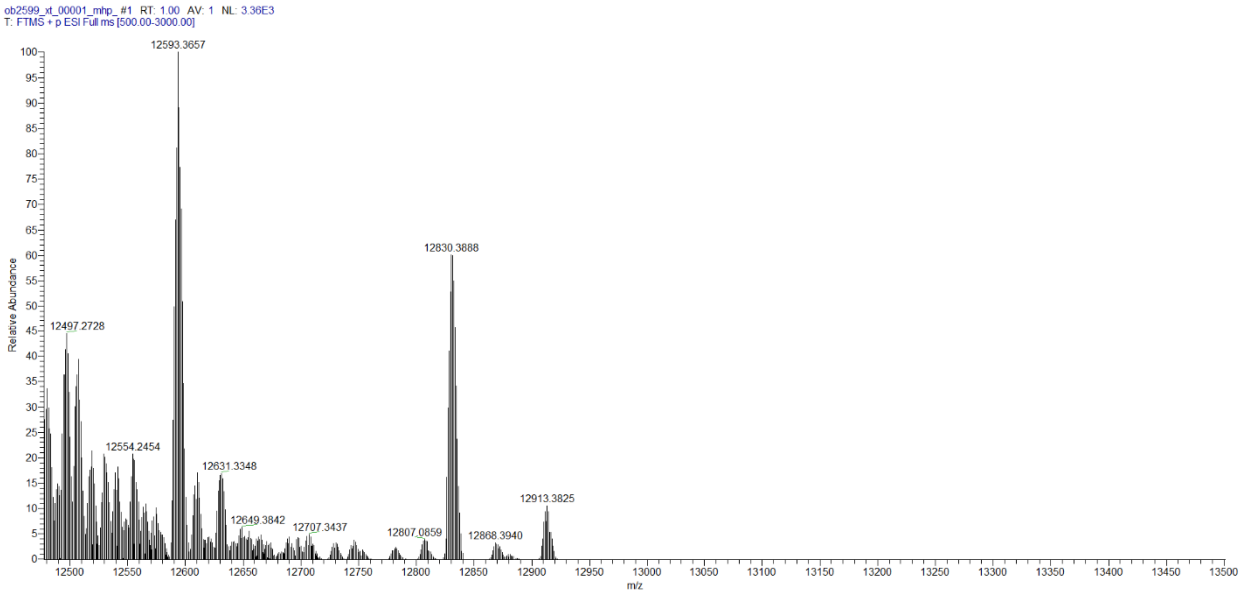
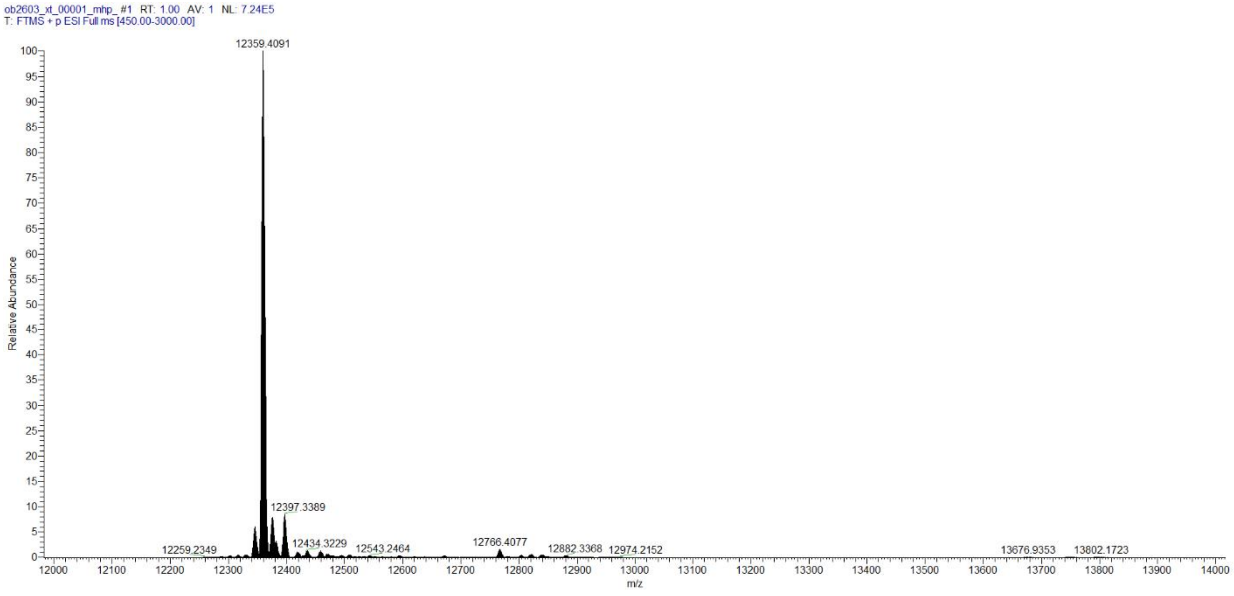


Figure S14. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C4 for 24 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



B)

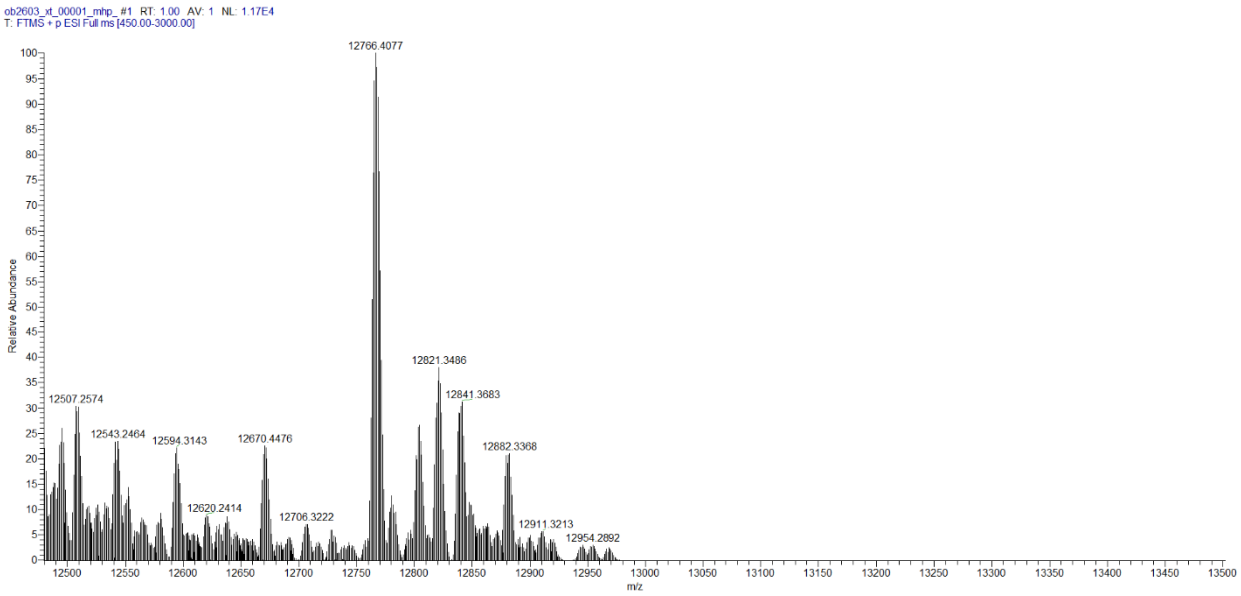
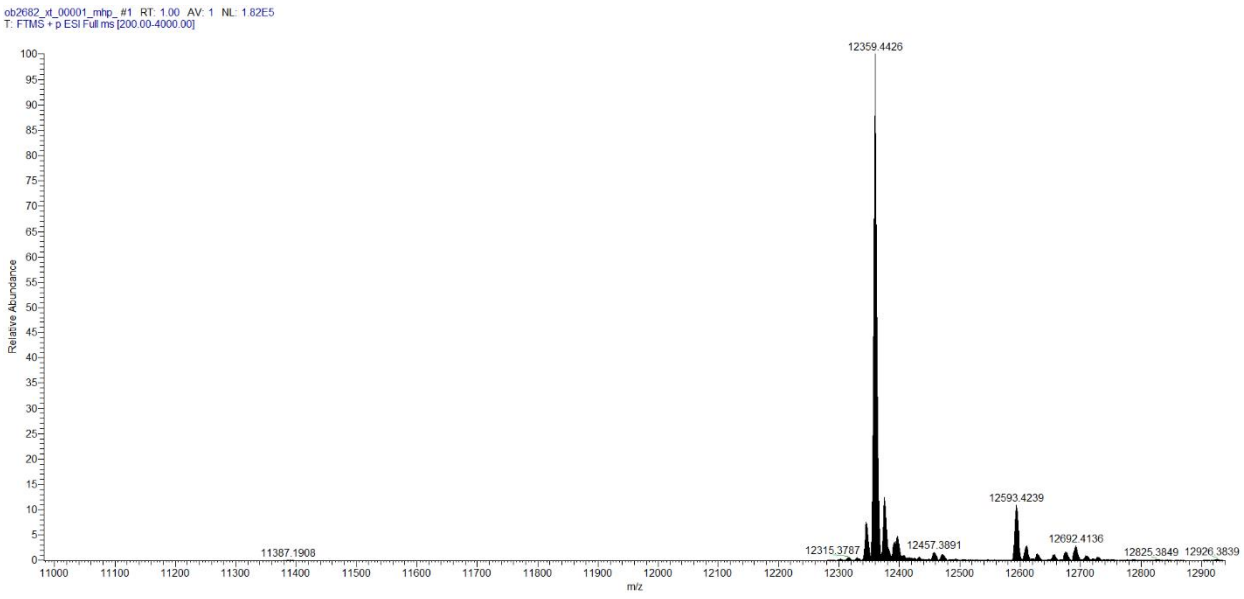


Figure S15. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C1 for 48 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



B)

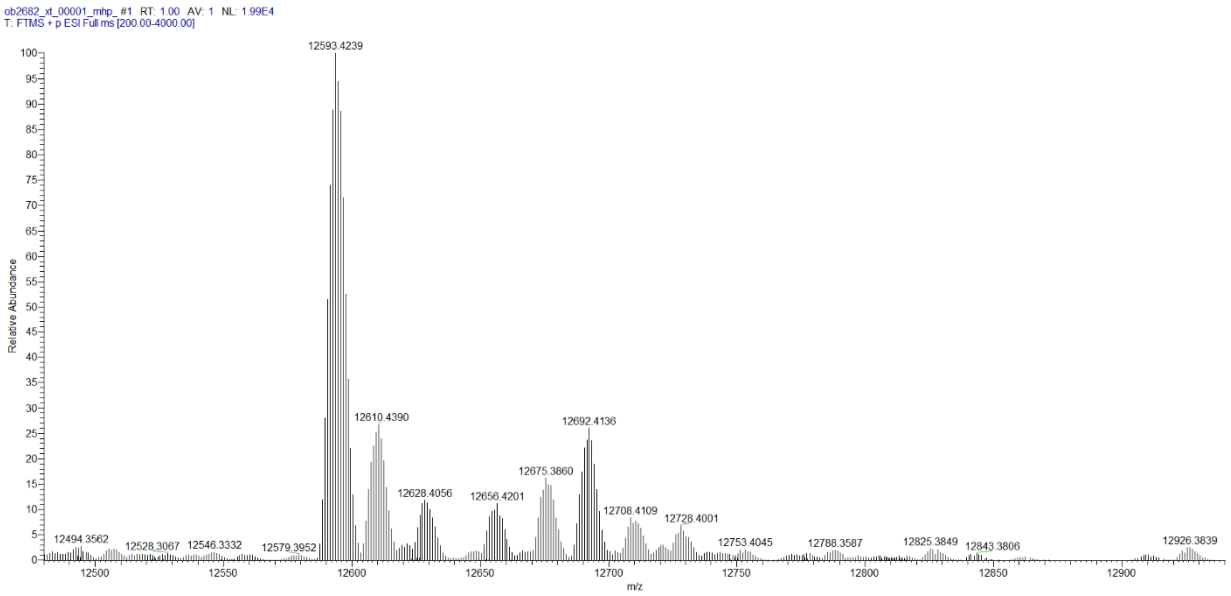
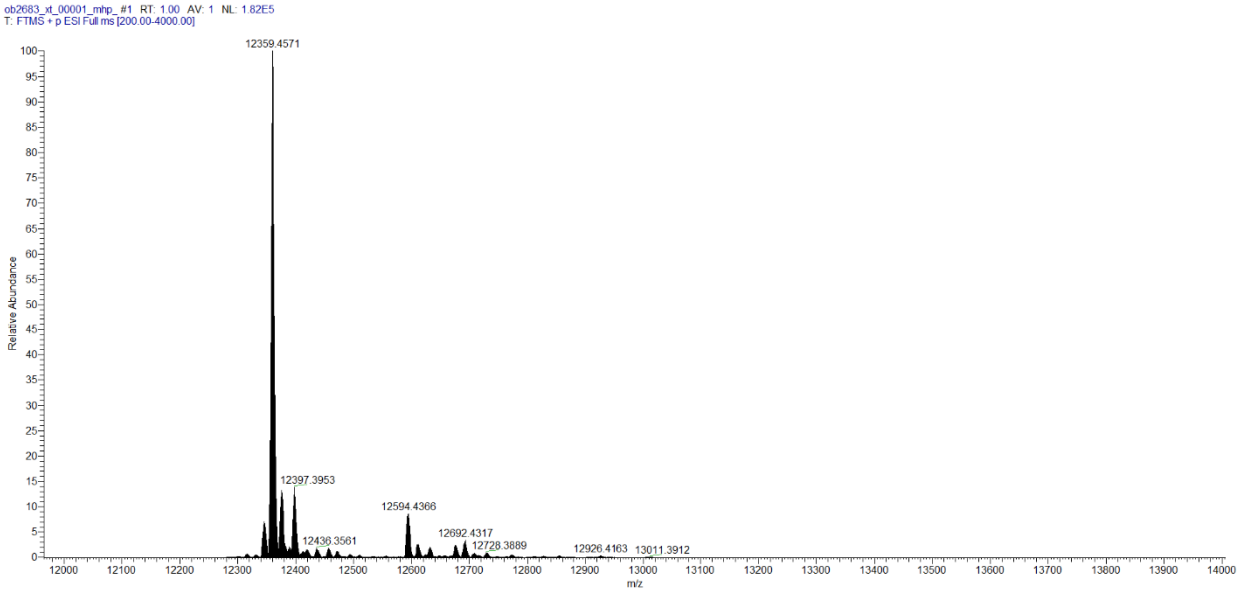


Figure S16. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C2 for 48 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13000 Da

A)



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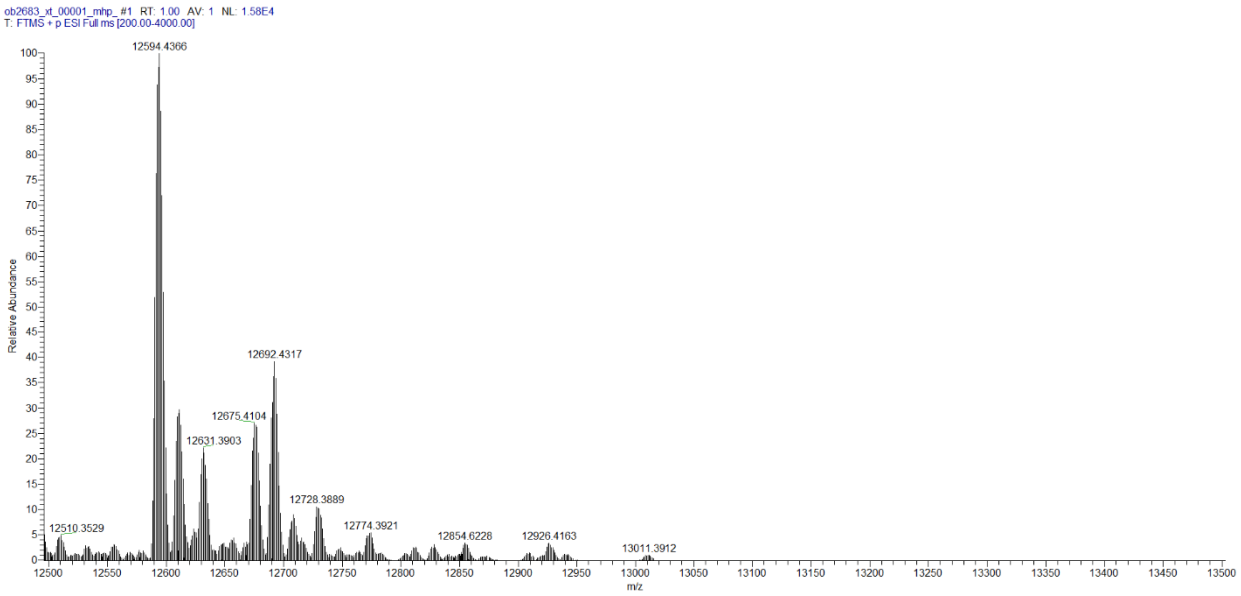
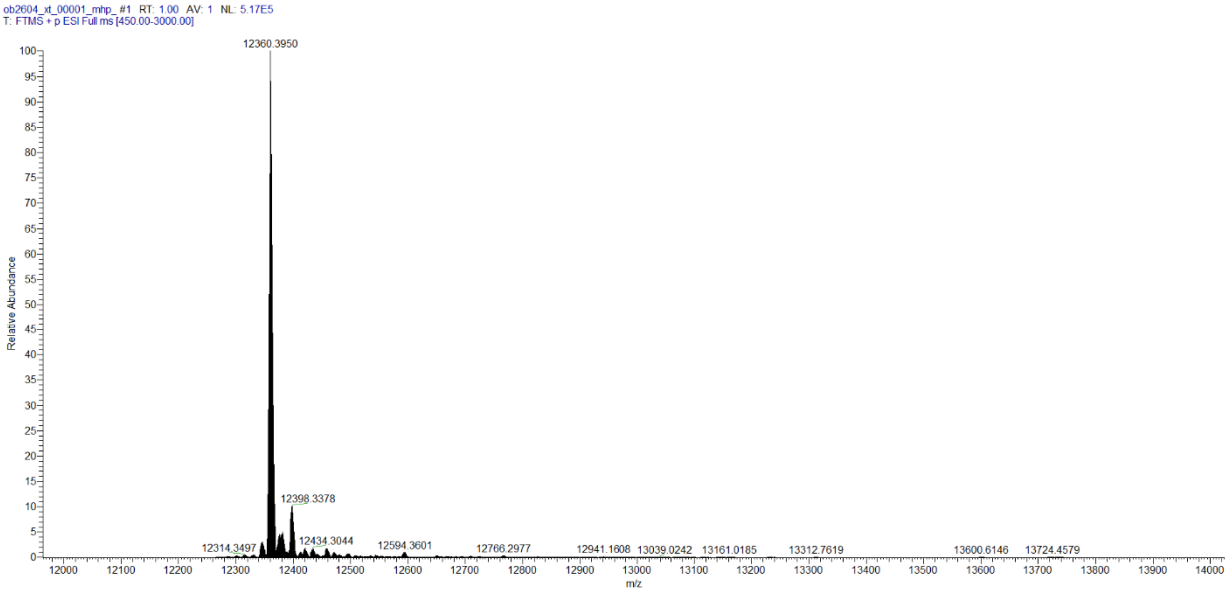


Figure S17. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C3 for 48 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13000 Da

A)



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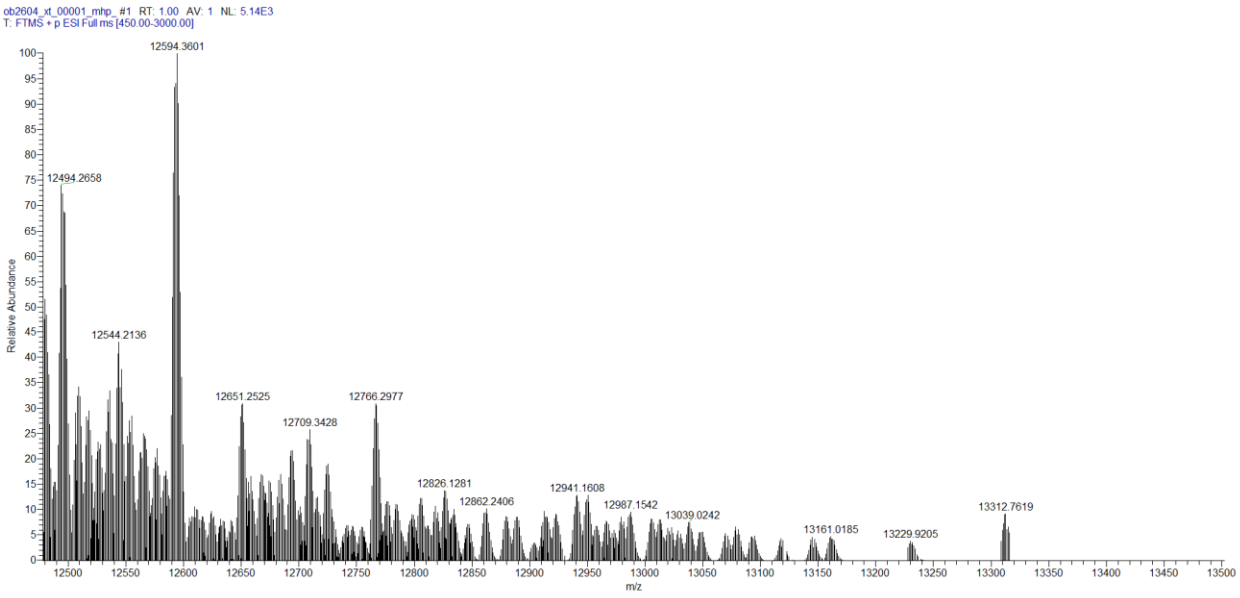


Figure S18. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C4 for 48 h in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC. B) Zoom out spectra in the mass region 12500-13000 Da

Table S1. The possible minor adducts of C1, C2, C3 and C4 with lysozyme and cytochrome c identified by deconvoluted ESI MS spectra of the after incubation of complexes with proteins in 20 mM ammonium hydrogen carbonate pH 7.4 at 37 ºC for 24 h and 48 h.

Sample	Exp. mass of adduct	Exp. mass increase	Modification	Theorethical mass of adduct	Delta mass	Relative intensity
C1						
Ly 24 h	14304.9720	0	-	14304.9720	0	100
	14732.9344	427.9624	[RuCym(CO_3)], [Ru($OH)_2$]	14736.8794	-3.9450	0.09
	14744.9339	439.9619	[RuCym($OH)_2$], [Ru($OH)_4$] $^{2-}$	14744.9056	0.0283	0.07
	14767.9239	462.9519	[RuCym(CO_3)], [Ru($OH)_4]^{2-}$	14770.8849	-2.9610	0.3
	14784.9170	479.9450	[RuCym(CO_3)], [Ru($OH)_5]^{3-}$	14787.8876	-2.9706	0.24
	14806.9128	501.9408	[RuCym(CO_3)(OH)]$^{1-}$, [Ru($OH)_5]^{3-}$	14804.8903	2.0225	0.05
	14826.9275	521.9555	[RuCym(CO_3)], [Ru(CO_3)($OH)_4]^{4-}$	14830.8696	-3.9421	0.05
Ly 48 h	14305.9379	0	-	14305.9379	0	100
	14718.9429	413.005	[RuCym(CO3)], [Ru(OH)]	14720.8425	-1.8910	0.12
	14735.9180	429.9801	[RuCym(CO_3)], [Ru($OH)_2$]	14737.8453	-1.9273	0.21
	14751.8626	445.9247	[RuCym($OH)_2$], [Ru($OH)_3]^{2-}$	14754.8480	-2.9854	0.2
	14766.8840	460.9461	[RuCym(CO_3)], [Ru(CO3)]	14763.8245	3.0595	0.33
	14785.8825	479.9446	[RuCym(CO_3)], [Ru($OH)_5]^{3-}$	14788.8535	-2.9710	0.25
	14827.9097	521.9718	[RuCym(CO_3)], [Ru(CO_3)($OH)_4]^{4-}$	14831.8355	-3.9258	0.12
Cyt 24 h	12359.4194	0	-	12359.4194	0	100
	12594.3755	234.9561	[RuCym]$^{2+}$	12595.4322	-1.0567	0.27
	12781.3928	421.9734	[RuCym($OH)_2$], [Ru($OH)_3]^-$	12782.3503	-0.9575	0.12
	12801.3404	441.9210	[RuCym($OH)_2$], [Ru($OH)_4]^{2-}$	12799.3530	1.9874	0.27
	12822.3718	462.9524	[RuCym(CO_3)], [Ru($OH)_4]^{2-}$	12825.3323	-2.9605	0.5
	12841.3790	481.9596	[RuCym(CO_3)], [Ru($OH)_5]^{3-}$	12842.3350	-0.9560	0.57
	12883.3590	523.9396	[RuCym(CO_3)], [Ru(CO_3)($OH)_4]^{4-}$	12885.3170	-1.9580	0.22
Cyt 48 h	12359.4091	0	-	12359.4091	0	100
	12594.3143	234.9052	[RuCym]$^{2+}$	12595.4219	-1.1076	0.36
	12780.3803	420.9712	[RuCym($OH)_2$], [Ru($OH)_3$]-	12782.3400	-1.9597	0.21
	12801.3380	441.9289	[RuCym($OH)_2$], [Ru($OH)_4]^{2-}$	12799.3427	1.9953	0.33
	12821.3486	461.9395	[RuCym(CO_3)], [Ru($OH)_4]^{2-}$	12825.3220	-3.9734	0.61
	12841.3683	481.9592	[RuCym(CO_3)], [Ru($OH)_5]^{3-}$	12842.3247	-0.9564	0.5
	12881.3440	521.9349	[RuCym(CO_3)], [Ru(CO_3)($OH)_4]^{4-}$	12885.3067	-3.9627	0.34
C2						
Ly 24 h	14305.9224	0	-	14305.9224	0	100
	14558.8702	252.9478	[RuCym(OH)]$^+$	14558.9379	-0.0678	0.19
Ly 48 h	14305.9358	0	-	14305.9358	0	100
	14558.8717	252.9359	[RuCym(OH)]$^+$	14558.9513	-0.0796	0.26
	14720.9295	414.9937	[RuCym(CO3)], [Ru(OH)]	14720.8404	0.0891	0.17
	14736.916	430.9802	[RuCym(CO_3)], [Ru($OH)_2$]	14737.8432	-0.9272	0.12
	14802.0275	496.0917	[RuCymL]	14803.1327	-1.1052	0.06
	12359.3935	0	-	12359.3935	0	100

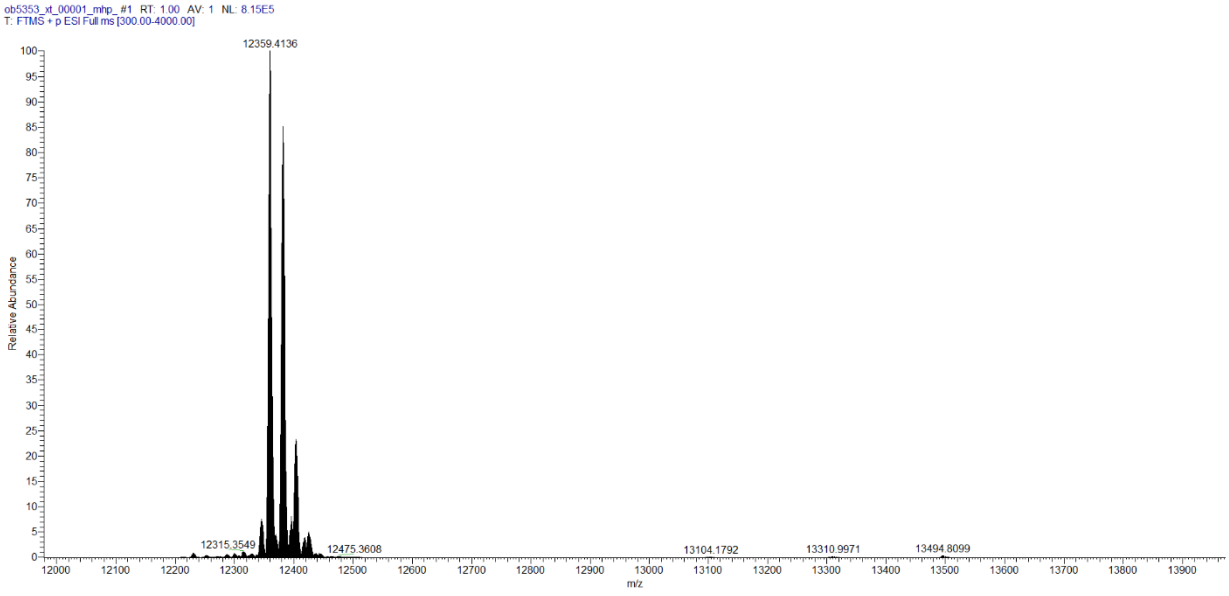
Cyt 24 h	12609.3870	249.9935	[RuCym(OH)]$^{+}$	12612.4090	-3.0220	1.74
	12627.3428	267.9493	[RuCym(OH)$_2$]	12629.4118	-2.0690	0.66
	12655.3807	295.9872	[RuCym(CO$_3$)]	12655.3911	-0.0104	2.08
	12670.3794	310.9859	[RuCym(CO3)(OH)]-	12672.3938	-2.0144	0.24
	12689.3245	329.931	[RuCym(CO3)], [Ru(OH)4]2-	12691.1980	-1.8734	0.39
	12827.3781	467.9846	[RuCym(CO$_3$)], [Ru(CO$_3$)(OH)$_4$]$^{4-}$	12825.3064	2.0717	0.39
Cyt 48 h	12359.4426	0	-	12359.4426	0	100
	12610.439	250.9964	[RuCym(OH)]$^{+}$	12612.4581	-2.0191	2.92
	12628.4056	268.963	[RuCym(OH)$_2$]	12629.4609	-1.0553	1.29
	12656.4201	296.9775	[RuCym(CO$_3$)]	12655.4402	0.9710	2.22
	12675.3860	315.9434	[RuCym(CO$_3$)(OH)]$^{-}$	12672.4429	2.9431	1.77
	12692.4136	332.971	[RuCym]$^{2+}$, Ru^{2+}	12697.3598	-4.9462	2.85
	12708.4109	348.9683	[RuCym(CO3)], [Ru(OH)$_5$]$^{3-}$	12708.2498	0.1612	0.91
	12728.4001	368.9575	[RuCym]$^{2+}$, [Ru(OH)$_2$]	12731.3652	-2.9651	0.75
	12825.3849	465.9423	[RuCym(OH)$_2$], [Ru(CO$_3$)(OH)]$^{-}$	12825.3555	0.0294	0.24
	12925.4000	565.9574	[RuCym(CO$_3$)], [RuCym(OH)$_2$]	12925.4584	-0.0584	0.27
C3						
Ly 24 h	14304.9978	0	-	14304.9978	0	100
	14557.8989	252.9437	[RuCym(OH)]$^{+}$	14557.9707	-0.0718	0.28
	14800.0130	495.0578	[RuCymL]	14802.1521	-2.1391	0.08
Ly 48 h	14305.9431	0	-	14305.9431	0	100
	14557.8586	251.9155	[RuCym(OH)]$^{1+}$	14558.9586	-1.1000	0.25
	14719.9400	413.9969	[RuCym(CO3)], [Ru(OH)]	14720.8477	-0.9077	0.14
	14735.9189	429.9758	[RuCym(CO$_3$)], [Ru(OH)$_2$]	14737.8505	-1.9316	0.12
	14800.0454	494.1023	[RuCymL]	14803.1400	-3.0946	0.11
Cyt 24 h	12359.4050	0	-	12359.4050	0	100
	12611.4030	251.998	[RuCym(OH)]$^{1+}$	12612.4205	-1.0175	1.82
	12629.3584	269.9534	[RuCym(OH)$_2$]	12629.4233	-0.0649	1.46
	12654.3889	294.9839	[RuCym(CO$_3$)]	12655.4026	-1.0137	1.6
	12670.3660	310.9610	[RuCym(CO3)(OH)]-	12672.4053	-2.0393	0.25
	12692.3258	332.9208	[RuCym(CO3)], [Ru(OH)4]2-	12691.2094	1.1164	0.34
	12826.3789	466.9739	[RuCym(CO$_3$)], [Ru (OH)$_4$]$^{2-}$	12825.3179	1.0610	0.48
	12854.5908	495.1858	[RuCymL]	12856.6019	-2.0111	0.12
Cyt 48 h	12359.4571	0	-	12359.4571	0	100
	12611.4476	251.9904	[RuCym(OH)]$^{+}$	12612.4726	-1.0250	2.57
	12631.3903	271.9331	[RuCym(OH)$_2$]	12629.4754	1.9149	1.92
	12675.4104	315.9532	[RuCym(CO$_3$)(OH)]$^{-}$	12672.4574	2.9530	1.87
	12692.4317	332.9745	[RuCym]$^{2+}$, Ru^{2+}	12697.3743	-4.9426	3.39
	12708.4164	348.9592	[Ru(CO$_3$)], [Ru(OH)$_5$]$^{3-}$	12714.3770	-5.9606	0.78
	12728.3889	368.9317	[RuCym]$^{2+}$, [Ru(OH)$_2$]	12731.3797	-2.9908	0.95
	12774.3921	414.9349	[RuCym]$^{2+}$, [Ru(CO$_3$)OH)]$^{-}$	12774.3617	0.0304	0.47
	12854.6228	495.1656	[RuCymL]	12856.6540	-2.031	0.29
	12925.4059	565.9487	[RuCym(CO$_3$)], [RuCym(OH)$_2$]	12925.4729	-0.0670	0.29

C4						
Ly 24 h	14304.9670	0	-	14304.967	0	100
Ly 48 h	14304.9724	0	-	14304.9724	0	100
Cyt 24 h	12359.4119	0	-	12359.4119	0	100
	12610.3974	250.9855	$[RuCym(OH)]^+$	12612.4274	-2.0300	0.17
	12630.3343	270.9224	$[RuCym(OH)_2]$	12629.4302	0.9041	0.17
	12655.3483	295.9364	$[RuCym(CO_3)]$	12655.4095	-0.0611	0.06
	12911.3622	551.9503	$[RuCym(CO_3)]$, $[RuCym(OH)]$	12908.4250	2.9372	0.1
Cyt 48 h	12359.3950	0	-	12359.3950	0	100
	12651.2525	290.8575	$[Ru(OH)_2]$, $[Ru(OH)_3]^-$	12649.2174	2.0351	0.31
	12667.4161	307.0211	$[Ru(OH)_3]^-$, $[Ru(OH)_3]^-$	12666.2201	1.1960	0.17
	12693.2686	332.8736	$[Ru(CO_3)]$, $[Ru(OH)_4]^{2-}$	12692.1994	1.0691	0.21
	12709.3428	348.9478	$[Ru(CO_3)]$, $[Ru(OH)_5]^{3-}$	12709.2022	0.1407	0.26
	12766.2977	405.9027	$[RuCym(OH)]^+$, $[Ru(OH)_3]^-$	12766.3231	-0.0254	0.31

Table S2. The roughly estimated extent of protein modification (%) by tested ruthenium complexes, based on relative intensity of unmodified and modified proteins in each deconvoluted ESI MS spectra

	Ly 24h	Ly 48h	Cyt 24h	Cyt 48h
C1	2.5	2.3	3.2	4.1
C2	3.6	3.9	14.2	19.5
C3	4.2	4.3	15.5	17.5
C4	0.6	3.1	2.1	2.2

A)



B)

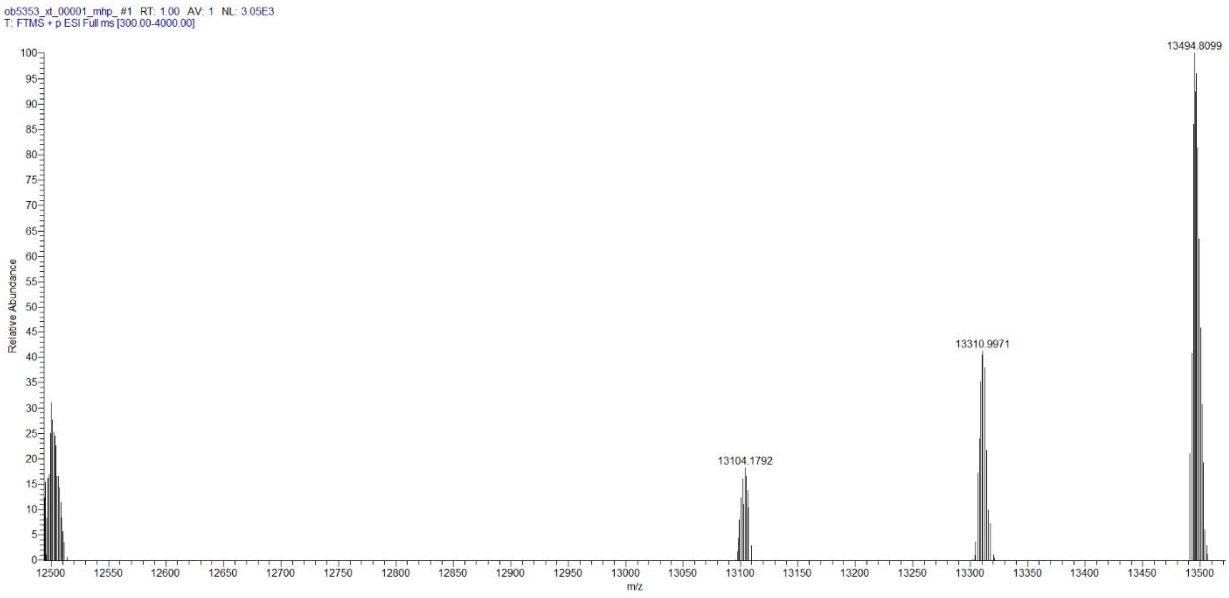
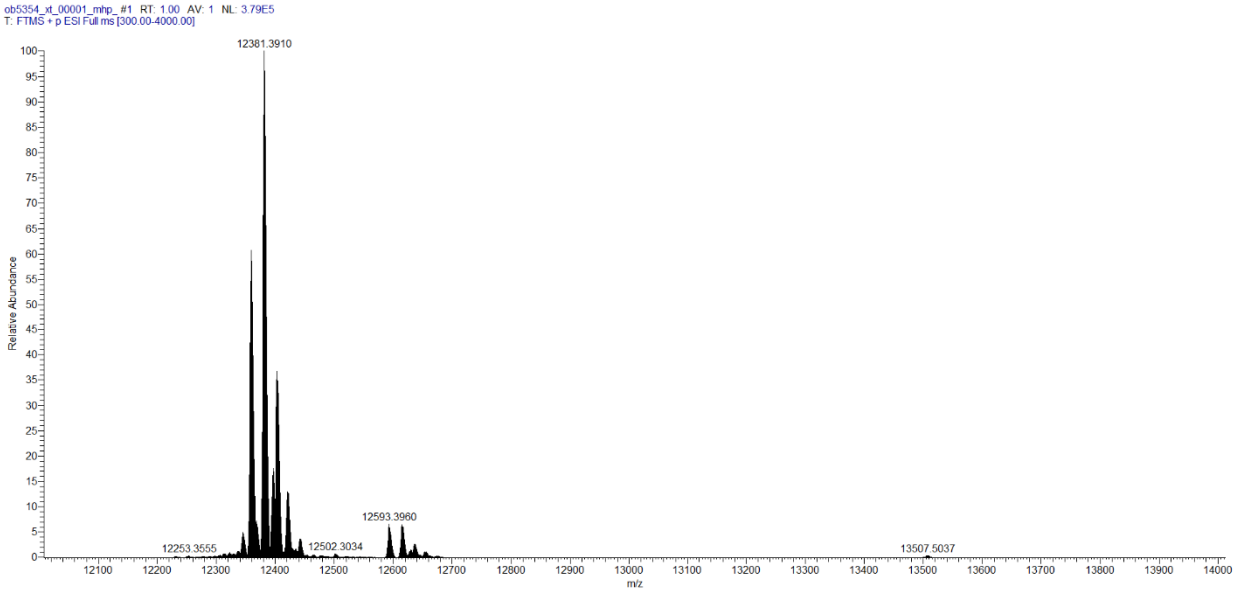


Figure S19. A) Deconvoluted ESI-MS spectra of cytochrome c incubated for 48 h in water at 37 ºC. B) Zoom out spectra in the mass region 12500-13500 Da

A)



B)

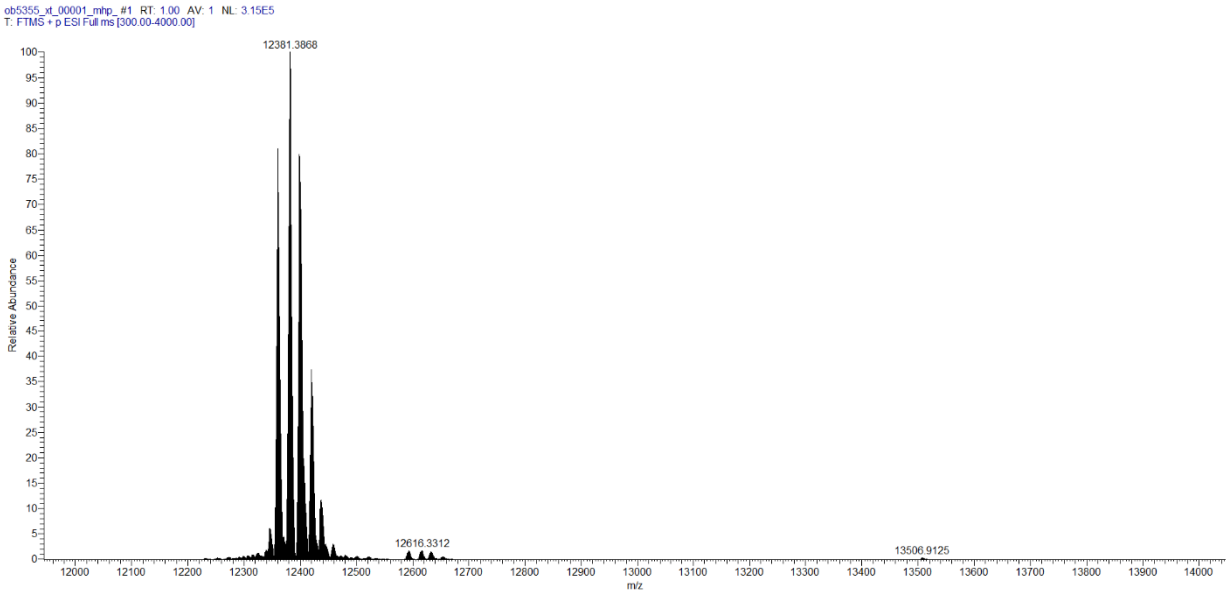
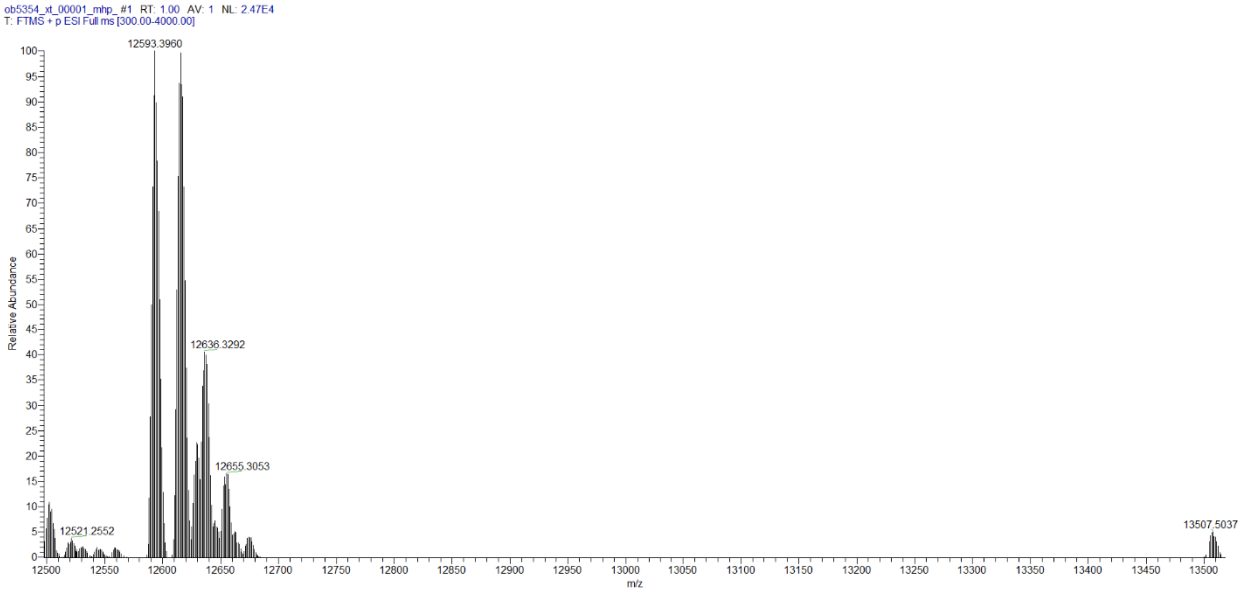


Figure S20. A) Deconvoluted ESI-MS spectra of cytochrome c incubated with C3 (A) and C4 (B) for 48 h in water at 37 ºC.

A)



B)

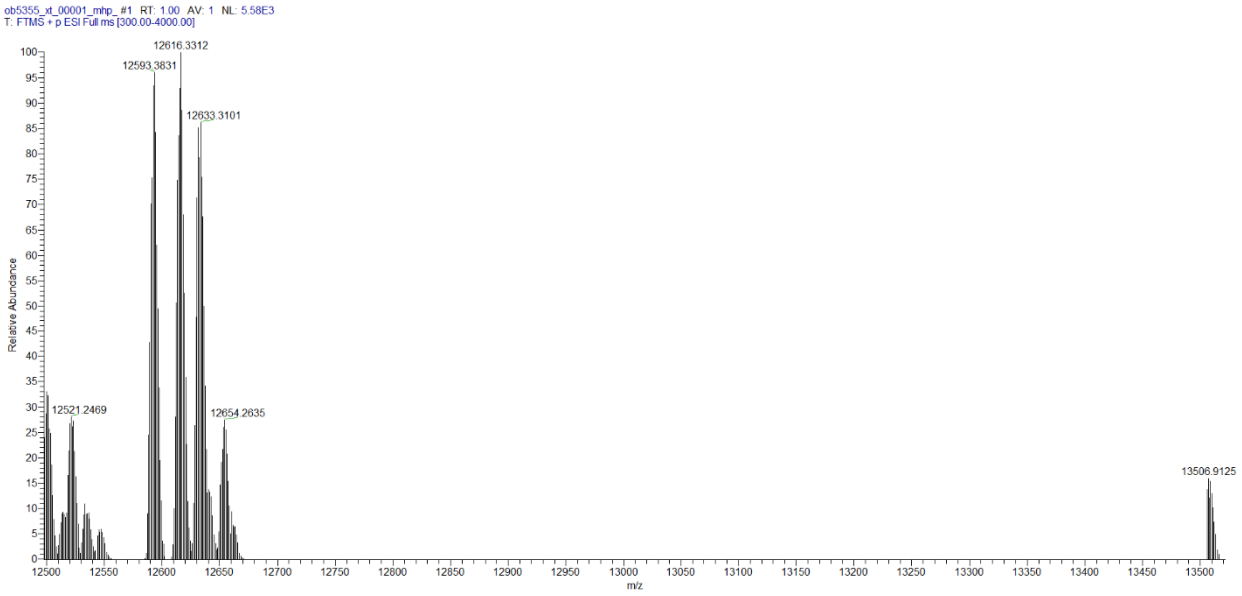


Figure S21. Zoom out of deconvoluted ESI-MS spectra in the mass region 12500-13500 Da of cytochrome c incubated with C3(A) and C4 (B) for 48 h in water at 37 ºC.

Table S3. The main adducts of C3 and C4 with Cyt identified by deconvoluted ESI MS spectra of the after incubation of complexes with proteins in water at 37 ºC for 24 h and 48 h. The relative

peak intensity of adduct is in comparison to nonderivatized Cyt in each individual mass spectrum, which is 100.

	Exp. mass of adduct	Exp. mass increase	Adduct	Theor. mass of adduct	Delta mass	Rel. intensity of adduct
C3	12359.4319	0	-	12359.4319	0	60.7
	12381.3910	21.9591	Na^+	12381.4139	-0.0228	100
	12397.3645	37.9326	K^+	12397.3878	-0.0233	16.88
	12403.3633	43.9314	$2Na^+$	12403.3958	-0.0325	36.77
	12420.3266	60.8947	Na^+, K^+	12419.3697	0.9569	12.95
	12442.3152	82.8833	$2Na^+$, K^+	12441.3517	0.9635	3.66
	12593.3960	233.9641	$[RuCym]^{2+}$	12595.4447	-2.0487	6.52
	12616.3483	256.9164	$[RuCym]^{2+}$ Na^+	12617.4266	-1.0783	6.09
	12633.3258	273.8939	$[RuCym]^{2+}$ K^+	12633.4006	-0.0748	1.45
	12636.3292	276.8973	$[RuCym]^{2+}$ $2Na^+$	12639.4086	-3.0794	2.65
	12655.3053	295.8734	$[RuCym]^{2+}$ Na^+, K^+	12655.3825	-0.0773	1.08
	12674.2974	314.8655	$[RuCym]^{2+}$ $2Na^+$, K^+	12677.3645	-3.0671	0.26
C4	12360.4256	0	-	12360.4256	0	80.99
	12381.3868	20.9612	Na^+	12382.4076	-1.0207	100
	12398.3510	37.9254	K^+	12398.3815	-0.0305	79.94
	12420.3233	59.8977	Na^+ K^+	12420.3634	-0.04018	37.33
	12436.2816	75.856	$2K^+$	12436.3374	-0.0558	11.56
	12593.3831	232.9575	$[RuCym]^{2+}$	12596.4384	-3.0553	1.7
	12616.3312	255.9056	$[RuCym]^{2+}$ Na^+	12618.4204	-2.0891	1.77
	12633.3101	272.8845	$[RuCym]^{2+}$ K^+	12634.3943	-1.0841	1.53
	12654.2635	293.8379	$[RuCym]^{2+}$ Na^+, K^+	12656.3762	-2.1127	0.49

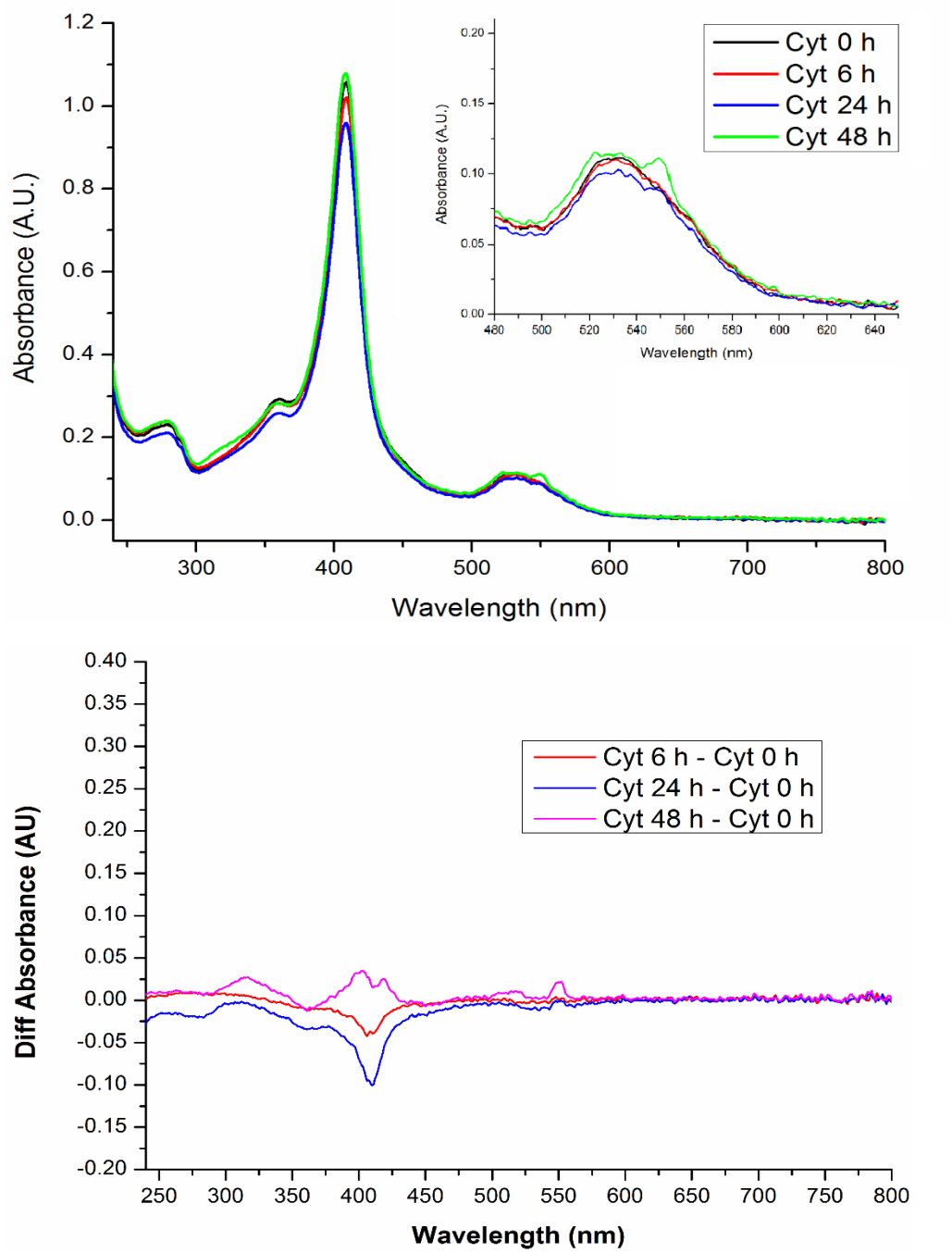


Figure S22. A) UV VIS spectra of Cyt during incubation in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C. B) Difference UV VIS spectra between Cyt incubated for 6 h, 24 h and 48 h, and Cyt incubated for 0 h.

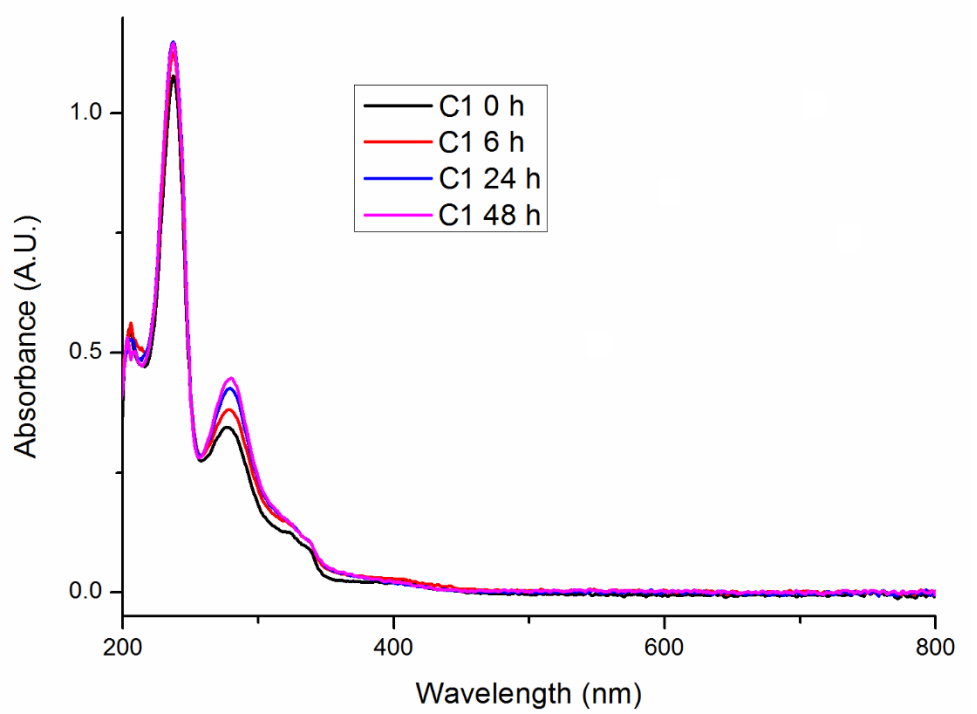


Figure S23. UV VIS spectra of C1 during incubation in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C.

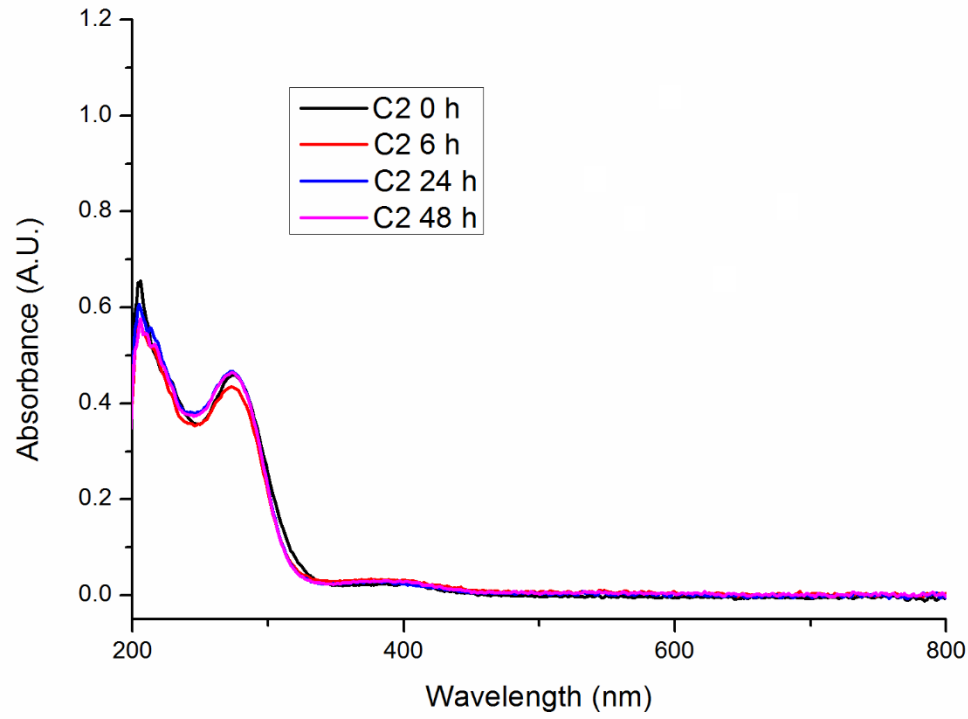


Figure S24. UV VIS spectra of C2 during incubation in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C.

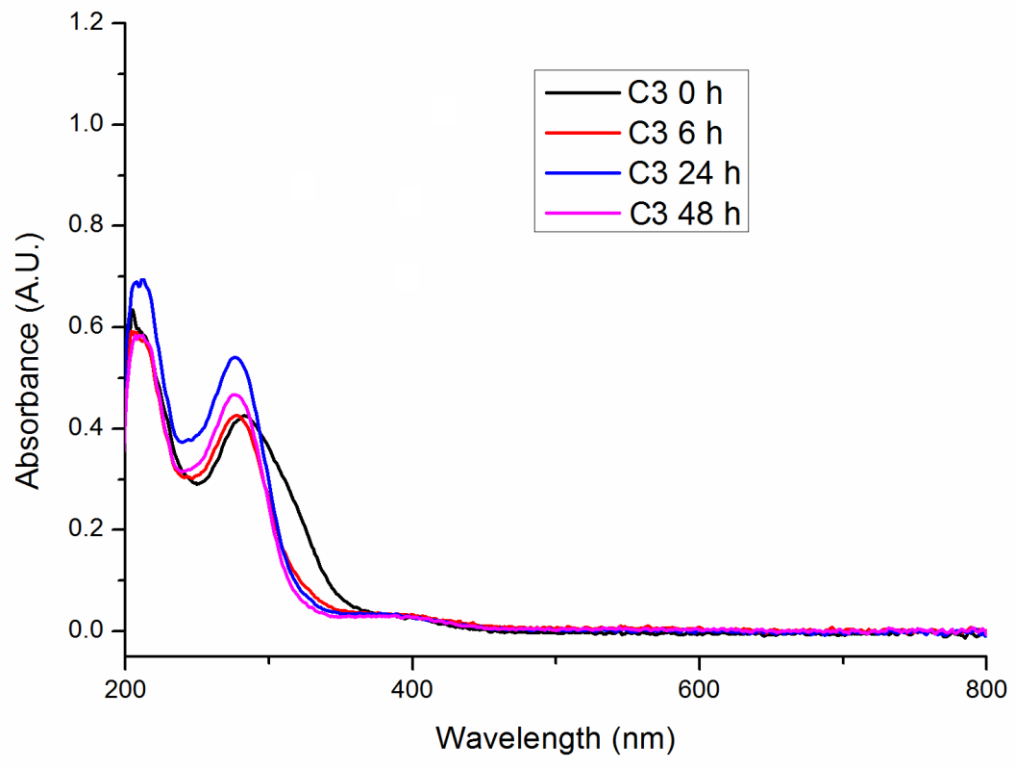


Figure S25. UV VIS spectra of C3 during of incubation in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C.

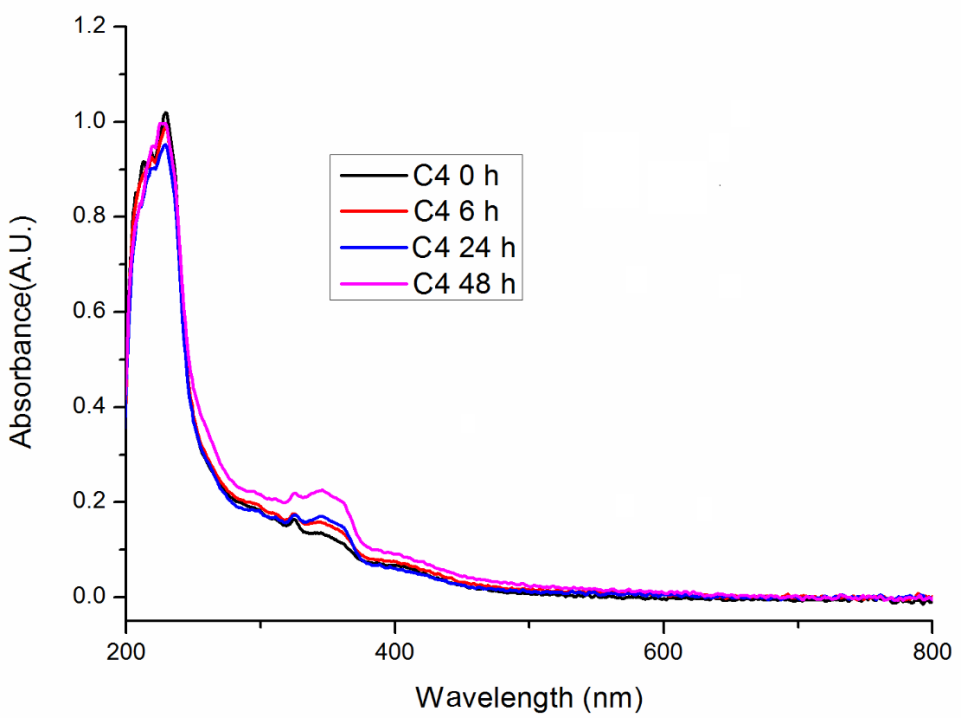
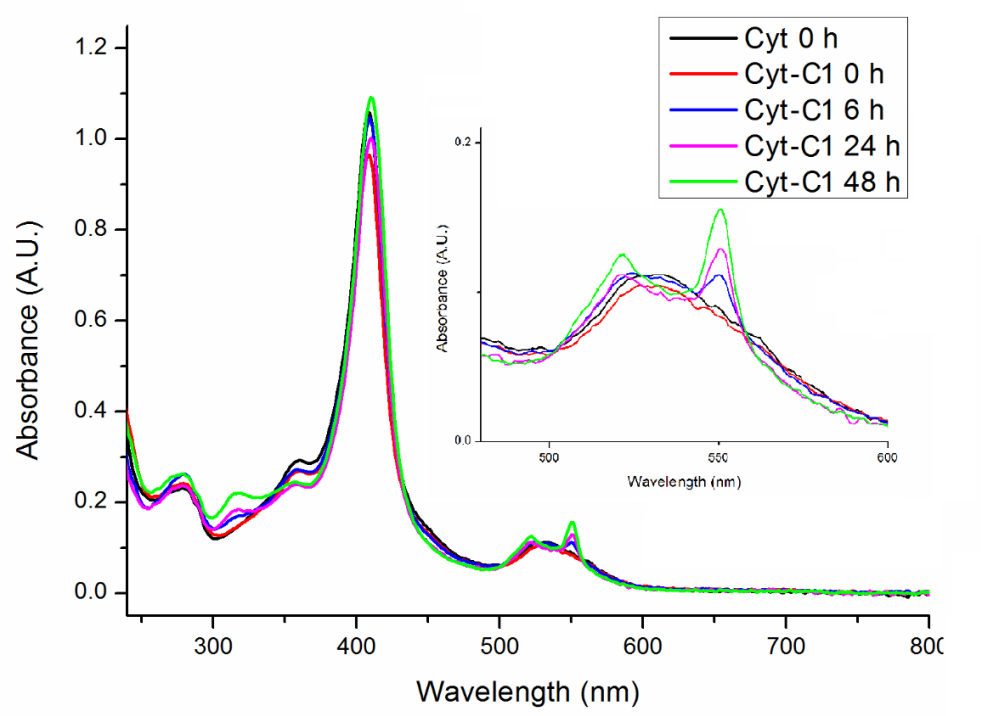


Figure S26. UV VIS spectra of C4 during incubation in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C.

A)



B)

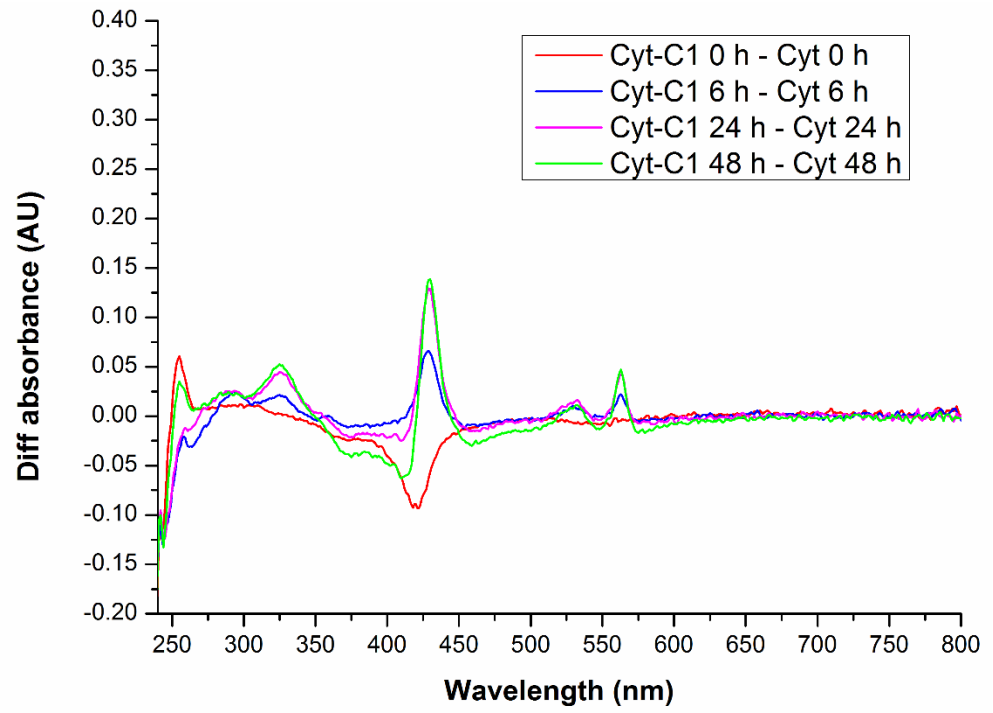
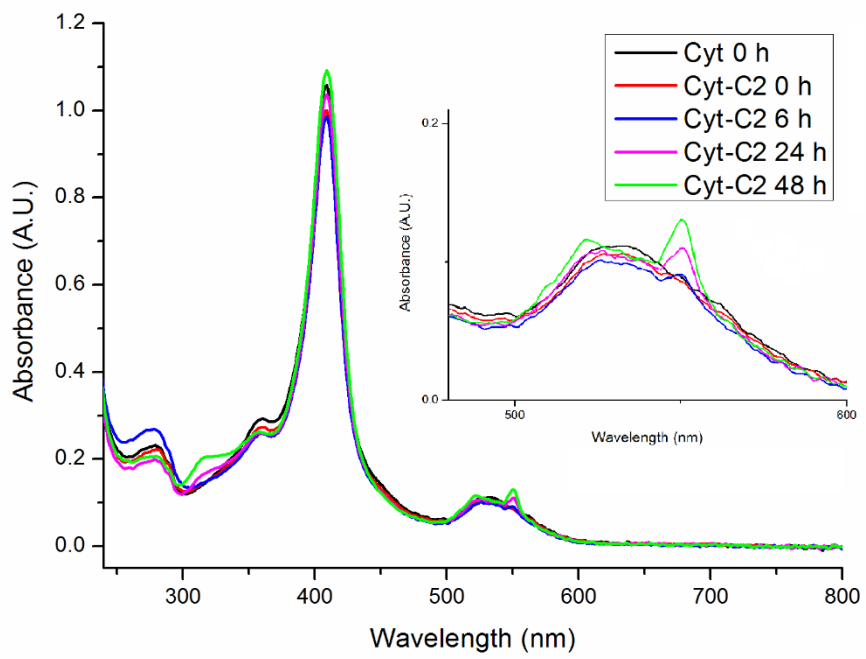


Figure S27. A) UV VIS spectra of Cyt incubated with C1 in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C. B) Difference UV VIS spectra between Cyt incubated with C1 and Cyt alone.

A)



B)

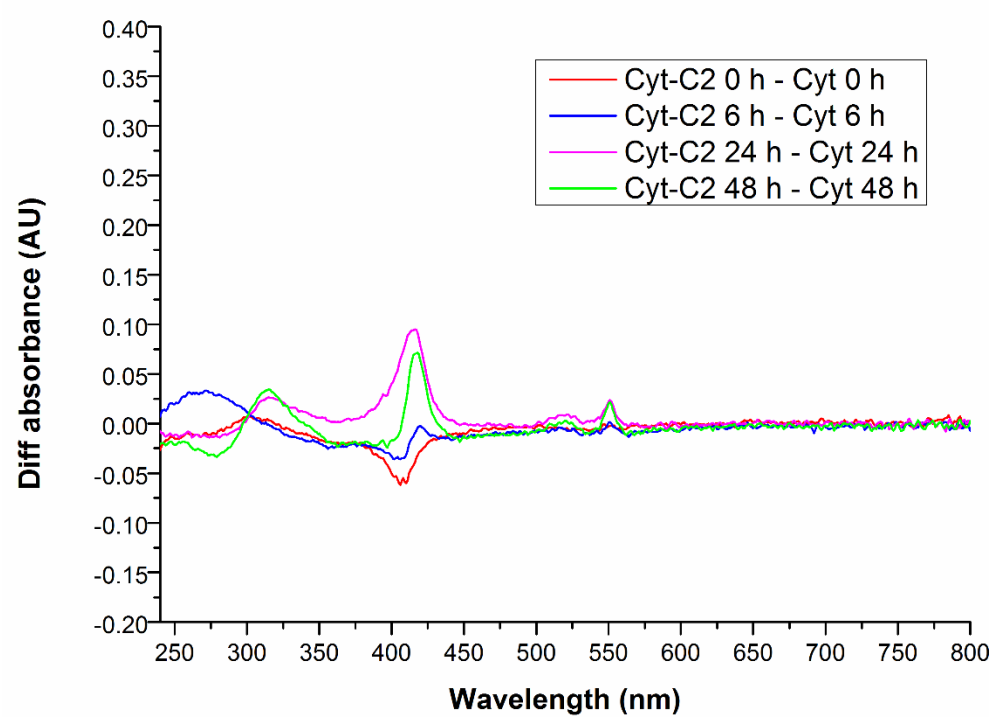
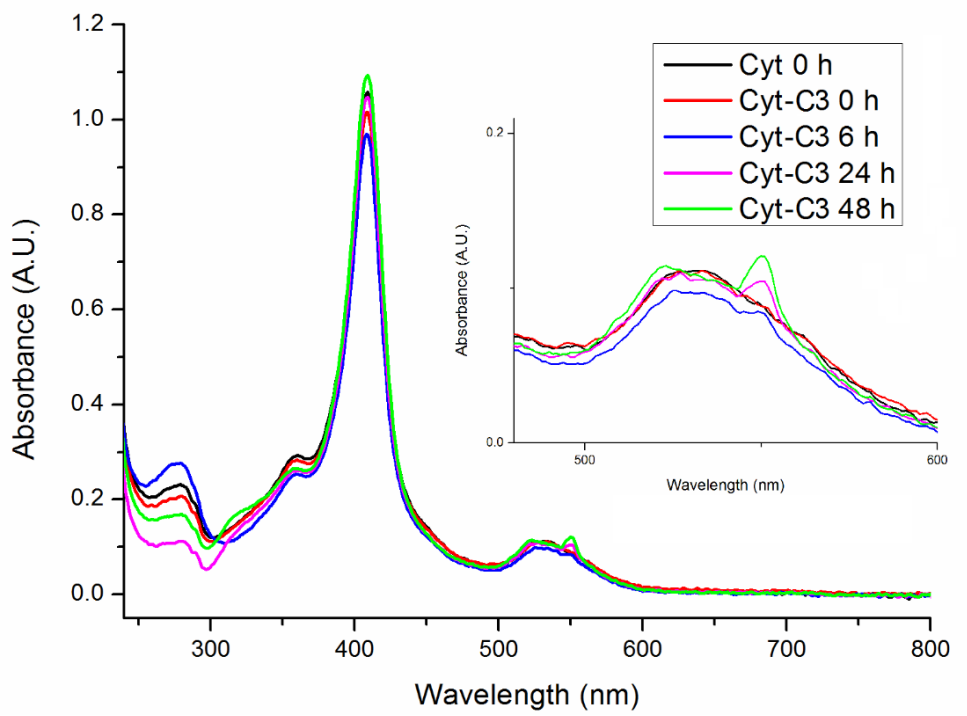


Figure S28. A) UV VIS spectra of Cyt incubated with C2 in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C. B) Difference UV VIS spectra between Cyt incubated with C2 and Cyt alone.

A)



B)

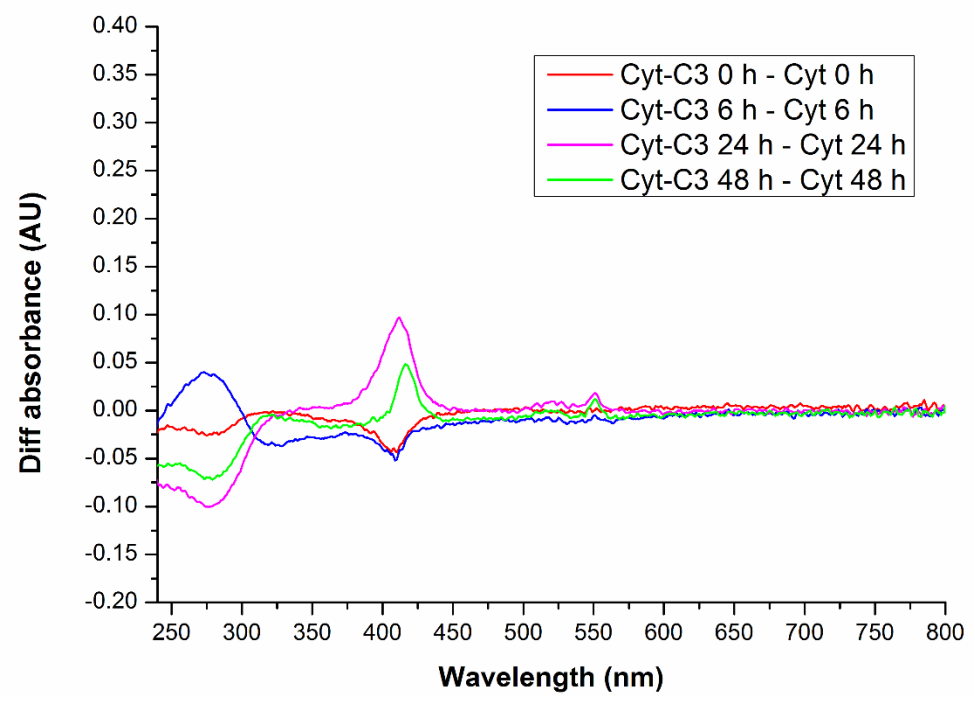
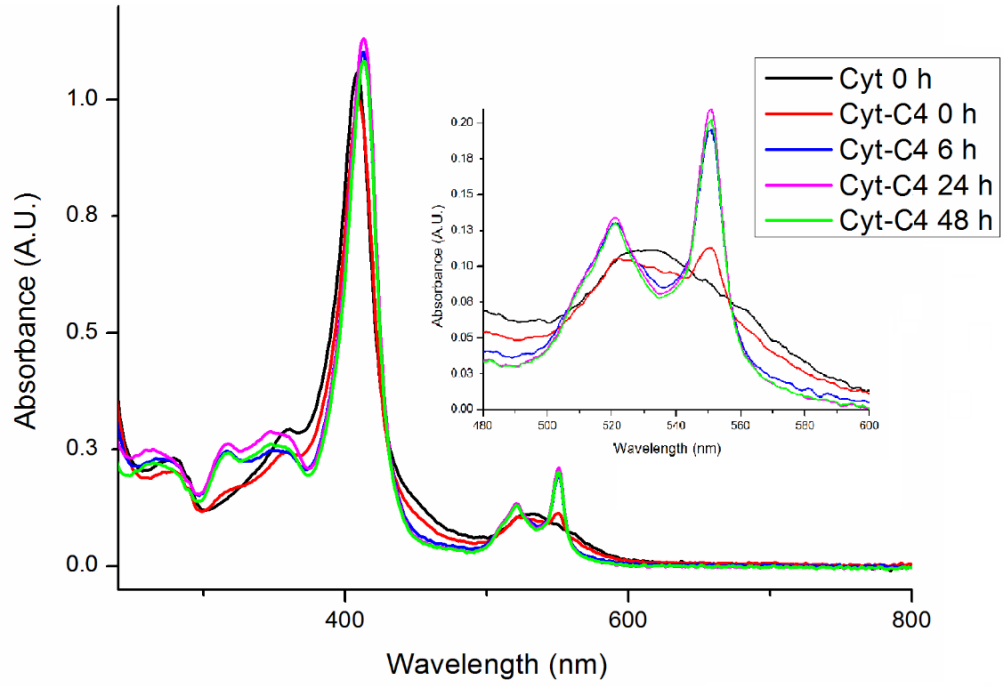


Figure S29. A) UV VIS spectra of Cyt incubated with C3 in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C. B) Difference UV VIS spectra between Cyt incubated with C3 and Cyt alone.

A)



B)

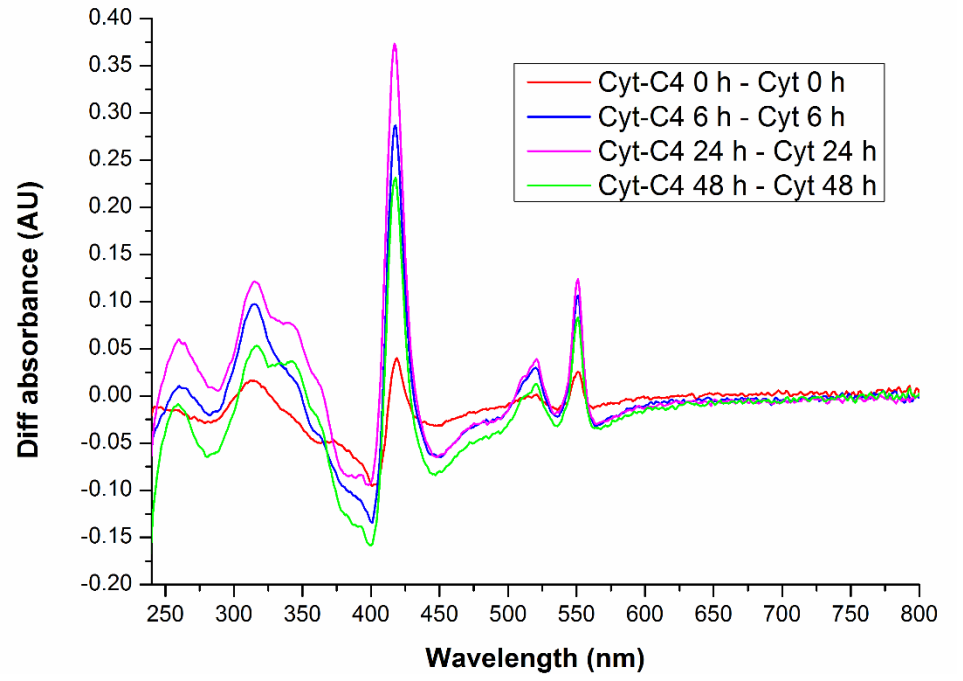


Figure S30. A) UV VIS spectra of Cyt incubated with C4 in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C. B) Difference UV VIS spectra between Cyt incubated with C4 and Cyt alone.

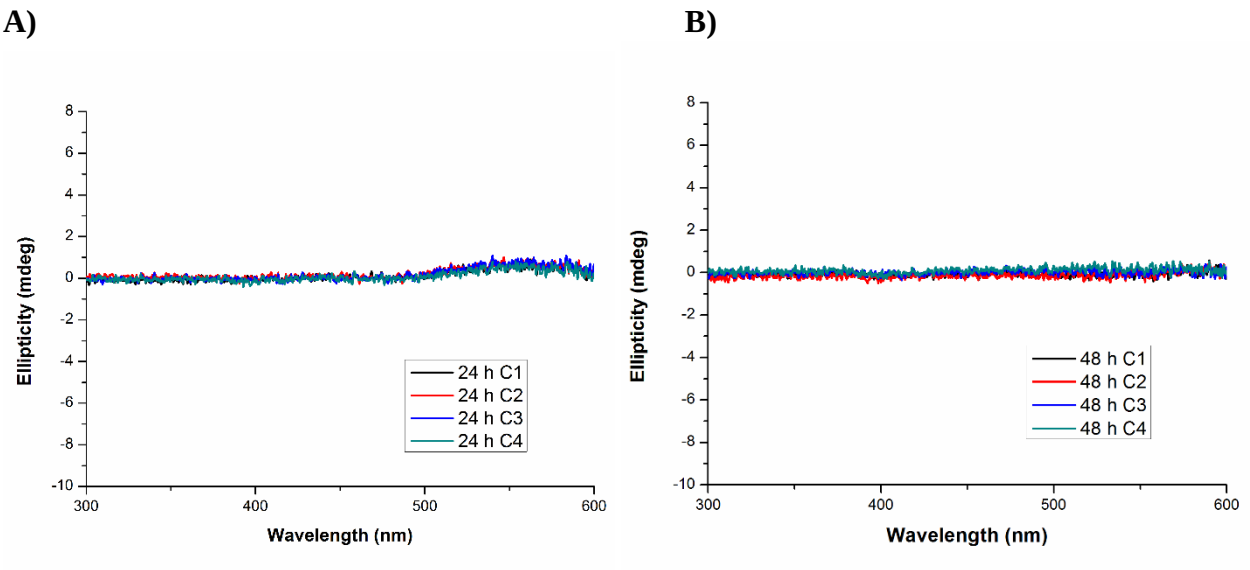


Figure S31. CD spectra of C1, C2, C3 and C4 after incubation for 24 h (A) and 48 h (B) in 20mM ammonium hydrogen carbonate pH 7.4 at 37 °C.