Supplementary Materials

Surface-Enhanced Raman Spectroscopic Analysis of Flavoenzyme Cofactors: Guidance for Flavin-Related Bio- and Chemo- Sensors

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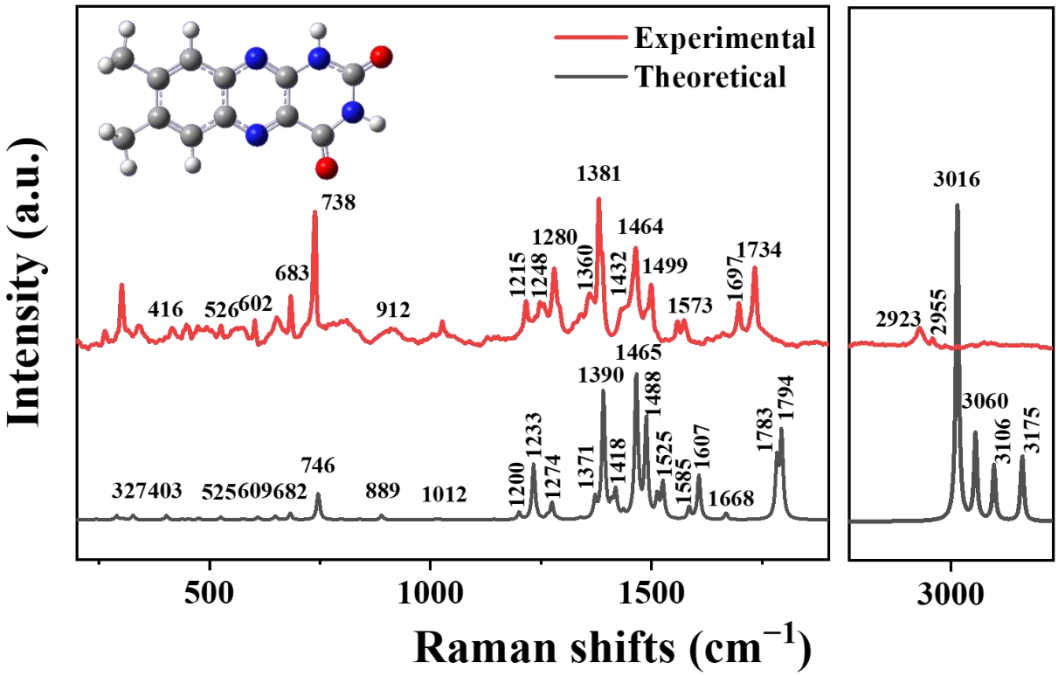

Figure S1. The comparison of the calculated Raman spetrum(black) and the experimental solid Raman spectrum(red) of lumichrome.

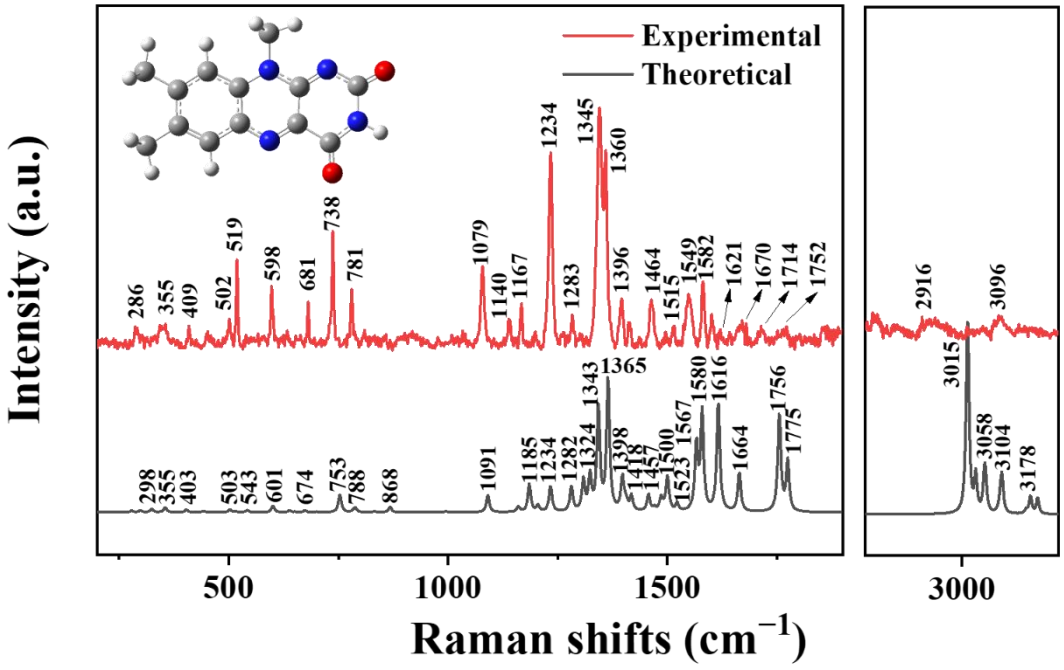

Figure S2. The comparison of the calculated Raman spetrum(black) and the experimental solid Raman spectrum(red) of lumiflavin.

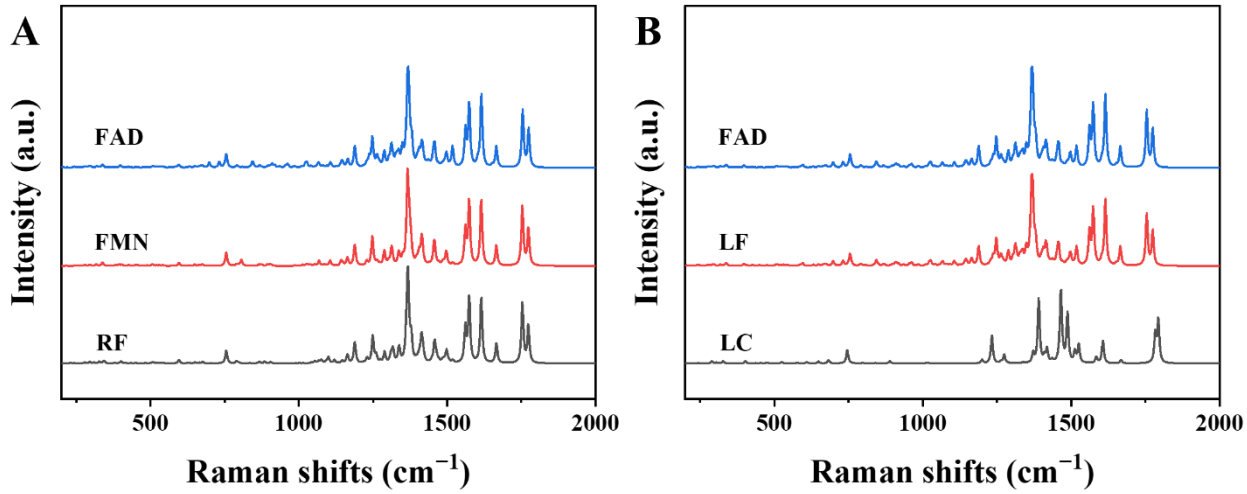

Figure S3. (**A**) The comparison of calculated Raman spectra of RF, FMN and FAD. (**B**) The comparison of calculated Raman spectra of LC, LF and FAD.

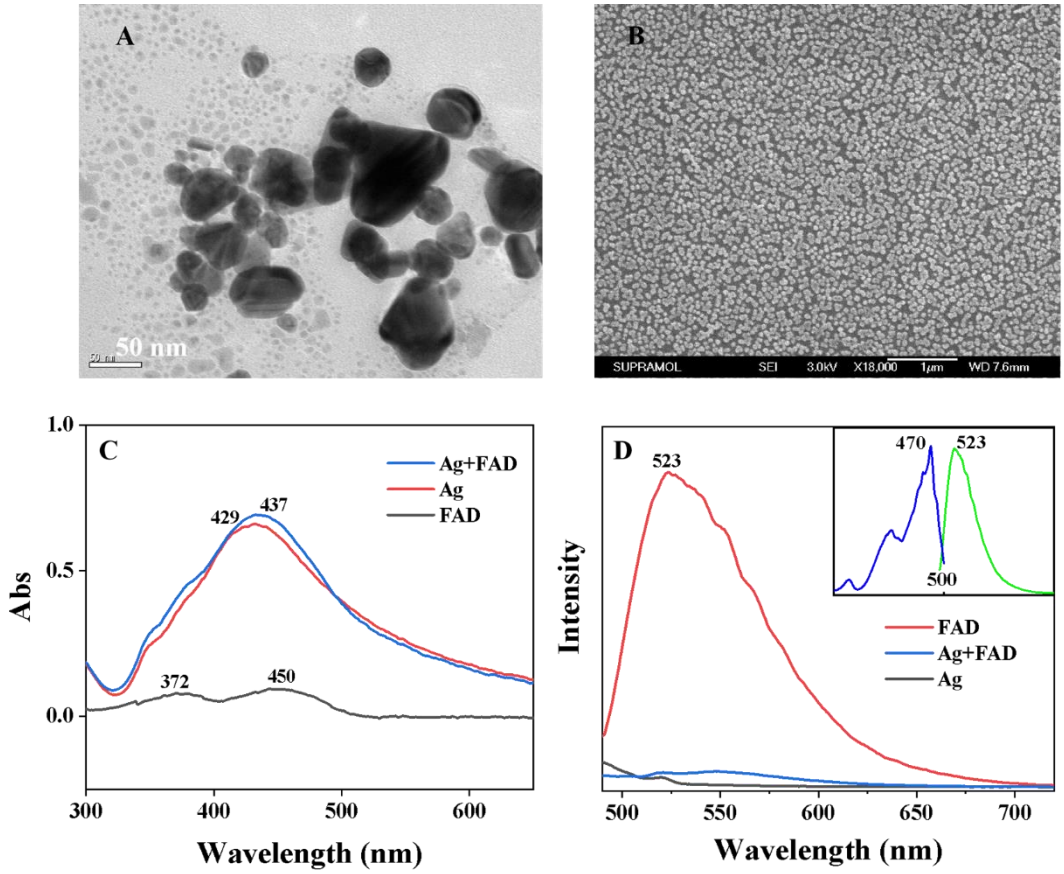

Figure S4. (**A**) TEM image of Ag nanoparticles; (**B**) SEM image of the self-assembled silver film; (**C**) UV spectra of Ag colloid, FAD and mixture of Ag colloid and FAD. The prepared Ag sol was mixed with 10^{-4} M of FAD at a volume ratio of 9:1, followed by 5-fold dilution. Next, the UV spectra were characterized and compared with the UV spectra of the diluted Ag sol and FAD mix.; (**D**) Fluorescence emission spectra of Ag colloid, FAD and mixture of Ag colloid and FAD at 470 nm excitation. The concentration of FAD for fluorescence detection was equal to that of the mixture of Ag colloid and FAD with a volume ratio of 9:1 at 10^{-5} M.

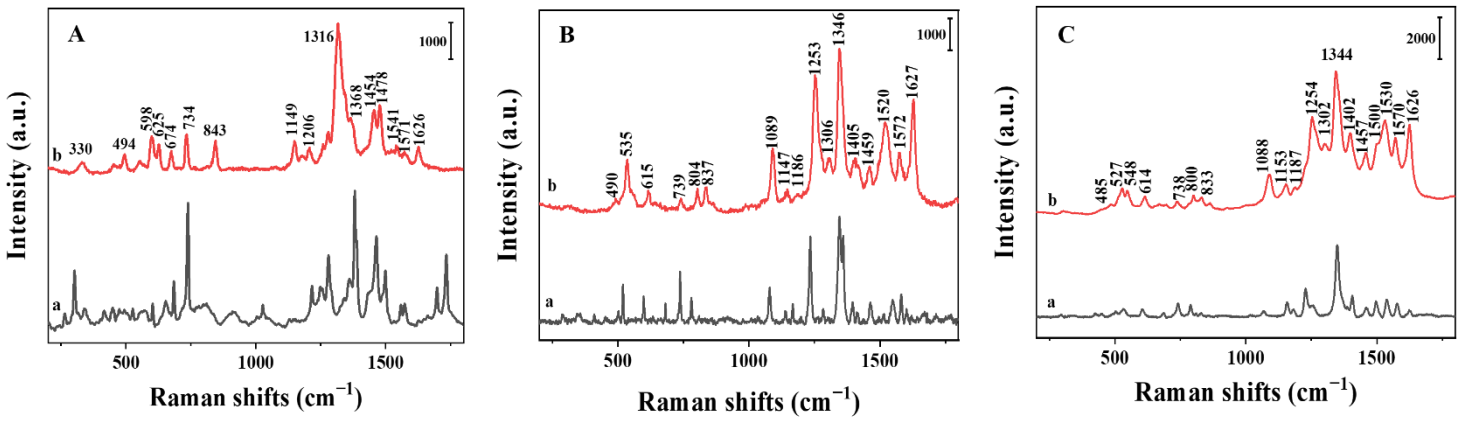

Figure S5. Normal Raman (a) and SERS (b) spectra of LC (**A**), LF (**B**) and FAD (**C**).

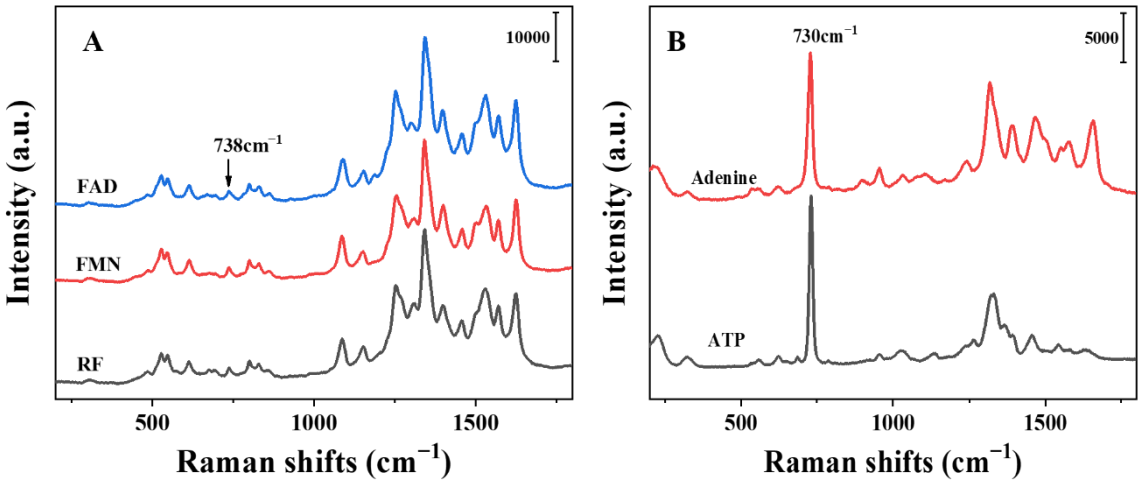

Figure S6. SERS spectra of 10^{-5} M RF, FMN, FAD, adenine and ATP on Ag film at 532 nm laser.

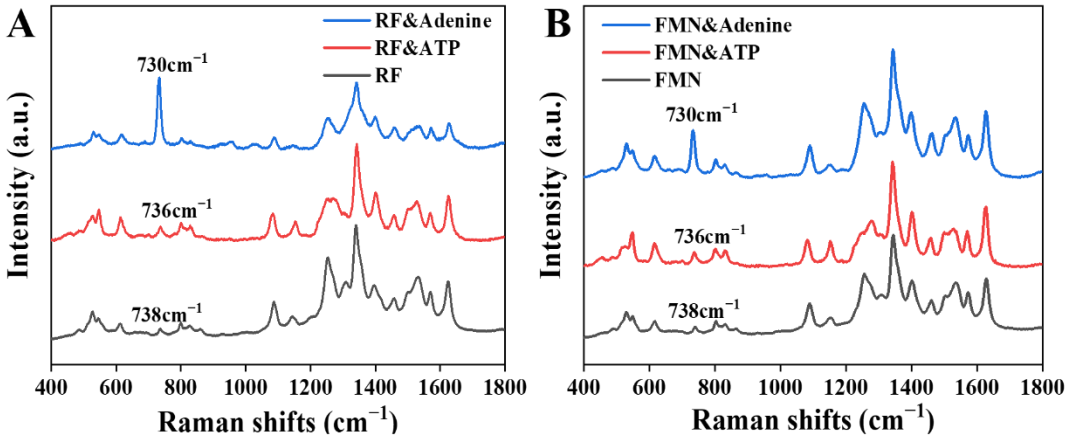

Figure S7. 532 nm laser Raman spectra of RF(**A**) and FMN (**B**), mixed with the same concentration of adenine or ATP.

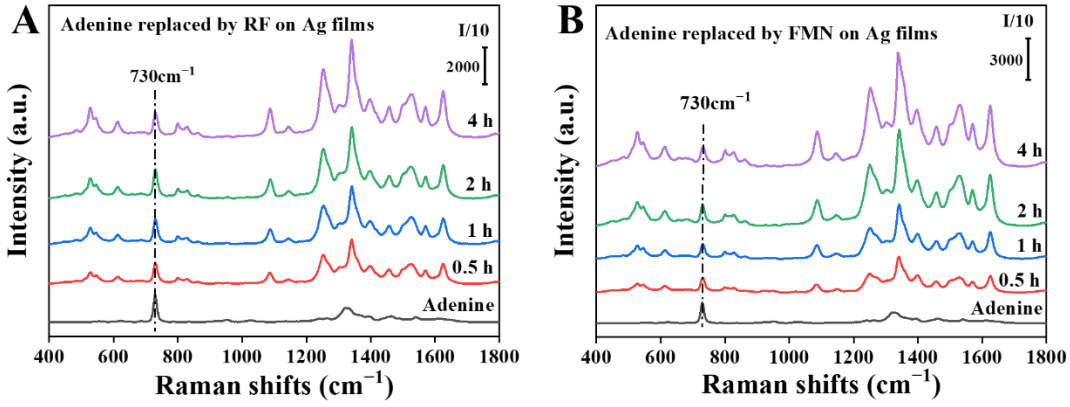

Figure S8. 532 nm laser Raman spectra of adenine replaced by RF (**A**) and FMN (**B**) on Ag films, respectively.

Table S1. Band assignments of Raman and SERS spectra from DFT calculation and experimental results of LC.

Calculation (cm^{-1})	Raman (cm^{-1})	SERS (cm^{-1})	Assignments
327	339	330	C_6-C_7-C_{Me}, C_9-C_8-C_{Me} bending
403			N_3-C_2=O, N_3-C_4=O bending
525	526	494	C_{4a}-C_4-N_3, C_{10a}-N_1-C_2 bending
607	602	598	Ring I, II bending
649	652	625	N_3-C_2=O, N_3-C_4=O, Ring II bending
682	683	674	C_{9a}-C_{4a}-N_5, C_{5a}-C_{9a}-N_{10}, Ring III bending
746	738	734	C_7-C_8, C_{5a}-C_{9a} stretching, Ring II breath
889		843	C_6-C_{5a}-C_5, Ring III bending
1012	1026	1149	N_1-C_2, C_2-N_3 stretching
1200			C_8-C_9-H, C_7-C_6-H bending, C_7-C_{Me}, C_8-C_{Me} stretching
1233	1215	1206	N_3-C_4, C_{5a}-C_6, C_{9a}-N_{10} stretching
1264	1248		C_{5a}-N_5, C_{5a}-C_6, C9-C_{9a}, C_{9a}-N_{10} stretching
1274	1280		C_7-C_6-H, C_8-C_9-H, C_{5a}-N_5-C_{4a} bending
1339	1339		C-N stretching, C_2-N_1-H bending
1371	1360		C_{4a}-C_{10a}, C_{5a}-C_{9a}, C_7-C_8 stretching
1390	1381	1316	C_{5a}-C_6, N_1-C_2, C_2-N_3 stretching
1418			C_2-N_3-H bending
1436	1432		C_2-N_3-H, C_2-N_1-H bending, N_1-C_{10a}, N_{10}-C_{10a}, C_5-C_{4a} stretching
1465	1464	1454	C_6-C_7, N_1-C_{10a} stretching
1488, 1512, 1525	1499		H-C-H(Me) bending
1585	1558	1541	C_6-C_7, C_8-C_9, C_{5a}-C_{9a}, C_{4a}-N_5 stretching
1607	1573	1571	C_6-C_7, N_{10}-C_{10a} stretching
1668			C_6-C_7, C_8-C_9 stretching
1783, 1794	1734	1626	C_4=O, C_2=O stretching
3016, 3060	2923, 2955		C-H stretching
3585	3390		N-H stretching

Table S2. Band assignments of Raman and SERS spectra from DFT calculation and experimental results of LF.

Calculation (cm^{-1})	Raman (cm^{-1})	SERS (cm^{-1})	Assignments
279			C_9-C_8-C_{8Me}, C_9-C_{9a}-N_{10}, C_4-C_{4a}-N_5 bending
298			C_6-C_7-C_{7Me}, N_5-C_{5a}-C_{9a} bending
325	286		C_8-C_9-C_{9a}, C_{5a}-C_6-C_7, C_{9a}-N_{10}-C_{11Me}, C_2-N_1-C_{10a} bending
355	355		C_{10a}-N_{10}-C_{11Me}, C_6-C_{5a}-C_{9a} bending
403	409		C_6-C_7-C_{7Me}, N_3-C_4-O, N_3-C_2-N_1, C_{9a}-N_{10}-N_{10a} bending
503	502	490	C_{5a}-C_{9a}-C_9, C_8-C_9-C_{9a}, C_{9a}-C_{5a}-C_6 bending
516	519		C_{9a}-N_{10}-C_{10a}, C_4-N_3-C_2 bending
543		535	C_{9a}-N_{10}-C_{10a}, C_9-C_8-C_{8Me} bending
601	598		C_{9a}-N_{10}, C_{5a}-C_6 stretching, C_4-N_3-C_2 bending
638			C_6-C_7-C_{7Me}, C_{9a}-C_{5a}-N_5, C_{9a}-N_{10}-C_{11Me} bending, Ring I breath
673	681	615	N_1-C_2-O, N_3-C_4-O, C_{9a}-C_{5a}-N_5 bending
753	738	736	Ring II, III breath
788	781	804	C_{4a}-C_{10a}-N_1, C_{4a}-N_5-C_{5a} bending, C_7-C_{7Me}, C_8-C_{8Me} stretching
833		837	C_4-N_3-C_2, C_{9a}-N_{10}-C_{10a}, C_2-N_1-C_{10a}, C_{9a}-C_{5a}-N_5 bending
868			Ring III breath, C_2-N_1 stretching
905			C_{7Me}-C_7-C_6-H torsion
995			N_{10}-C_{11ME} stretching
1034			C_{11Me}-H bending
1091	1079	1089	C_{5a}-N_5, C_{9a}-N_{10}, C_{5a}-C_6, C_{10a}-N_{10}, C_4-C_{4a} stretching
1160	1140	1147	C_7-C_{7Me}, C_8-C_{8Me}, N_{10}-C_{11Me} stretching
1185	1167	1186	C_9-C_{9a}, C_{5a}-N_5 stretching
1234	1234	1253	C_{5a}-N_5, C_7-C_{7Me}, C_8-C_{8Me} stretching
1282	1283		C_9-C_{9a}, C_{5a}-N_5, C_{5a}-C_6-C_7 stretching
1309		1306	C_{5a}-N_5, C_9-C_{9a} stretching, C_{9a}-C_9-H, C_{4a}-N_5-C_{5a} bending
1324			C_{9a}-N_{10}, C_{5a}-C_6 stretching
1343	1345	1346	C_{10a}-N_{10}, C_9-C_{9a} stretching
1365	1360		C_4-N_3, C_6-C_{5a}-C_{9a}, C_{10a}-N_1 stretching
1398	1386	1405	C_6-C_7, C_{10a}-N_{10}-C_{9a}, C_4-N_3 stretching
1418			$C_{7,8Me}$-H, C_4-N_3-H bending
1457	1464	1459	C_{5a}-C_6-C_7, C_{10a}-N_1 stretching
1500	1496		C_{Me}-H bending, C_7-C_8-C_{8Me}-H torsion
1523	1515		C_8-C_9-H, C_7-C_6-H, C_{Me}-H bending
1567	1549	1520	C_{4a}-N_5, C_{10a}-N_1, C_9-C_{9a}-C_{5a} stretching
1580	1582	1572	C_{5a}-C_{9a}, C_{10a}-N_1, C_7-C_8 stretching
1616	1621	1627	C_{10a}-N_1, C_{4a}-N_5, C5a-C6, C8-C9 stretching
1664	1670		C_6-C_7, C_9-C_{9a}, C_{5a}-N_5-C_{4a} stretching
1756, 17775	1714, 1752		C_2=O, C_4=O stretching
3000-3100	2916		C_{Me}-H stretching
3178, 3195	3096		$C_{benzene}$-H stretching