Supplementary Material (ESI) for Chemical Communications

Squaraines as unique materials for SERRS multiplexing

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Supplementary Material

i) Materials

Squaraine dyes for SERRS were synthesised according to the methods reported previously.[8-11] **SQ2** was prepared from the aniline derivative according to the following: *N*-Methyl aniline (2.000 g, 18.7 mmol) was dissolved in *n*-butan-1-ol (100 ml), then 1-chlorohexan-6-ol (2.796 g, 22.44 mmol), potassium carbonate (7.741 g, 56.1 mmol) and potassium iodide (cat.) were added and the mixture was left to stir at reflux for 48 hrs. Upon cooling, the solvent was removed at reduced pressure, and then the residue was partitioned between diethyl ether (100 ml) and water (100 ml). The organic phase was separated and dried over sodium sulphate before filtration and removal of the solvent by evaporation. The residue was columned in 10% ethyl acetate in hexane, to afford the title compound as a colourless oil (3.01 g, 80 %). δ_H(400 MHz; $CDCl_3$) 1.3-1.5 (4H, m, 2 x CH_2), 1.5-1.7 (4H, m, 2 x CH_2), 2.94 (3H, s, CH_3), 3.33 (2H, t, *J* 7.5, CH_2), 3.64 (2H, t, *J* 6.8, CH_2), 6.70 (3H, m, 3 x ArH), 7.25 (2H, dd, *J* 8.7, 7.2, 2 x ArH).

3,4-Dihydroxy-3-cyclobutene-1,2 dione **SQ2** (0.500g, 6 mmol) and 6-(Methyl-phenyl-amino)hexan-1-ol (1.863 g, 9 mmol) were dissolved in a mixture of toluene (100 ml) and butanol (30 ml) and left to reflux overnight, with the azeotropic removal of water using Dean-Stark apparatus. The now black solution was concentrated *in vacuo*, then precipitated using a mixture of methanol and diethyl ether to afford the title compound as a blue solid (1.7 g, 58 %).
δ_H(400 MHz; DMSO) 1.2-1.6 (16H, m, 8 x CH_2), 3.16 (6H, s, 2 x CH_3), 3.50 (4H, t, *J* 7.0, 2 x CH_2), 3.67 (4H, t, *J* 6.2, 2 x CH_2), 6.95 (4H, d, *J* 9.1, 4 x ArH), 7.25 (4H, d, *J* 9.1, 4 x ArH).

ii) Sample preparation

The spectra were recorded from within standard microtitre plate wells. The samples were diluted to various concentrations using sterile water (18.2 MΩ.cm). Some Squaraine samples were dissolved in methanol prior to further dilution with water. Samples were then prepared for SERRS analysis by adding 7μL of dye or dye-labelled oligonucleotide and 10μL of 0.1 mol dm^{-3} of the aggregating agent, followed by 350 μL of silver nanoparticles. The samples were analysed within 45-60 s of the addition of the nanoparticles. Typically, single acquisitions were integrated for 1-10 s.

iii) Raman spectra acquisition

Spectra were recorded at 632.8 nm λ_{ex} (HeNe) and 785 nm (diode) using a Renishaw (Glouscestershire, UK) System 1000 and a Leica DM/LM microscope. Backscattered light was collected at 180º to the microtitre plate well using a 20x/0.4 long working distance objective and a 50x/0.75 Leica objective. Dielectric edge filters were used to reject Rayleigh scattering and a 1200 lines/mm grating was used to resolve the spectra onto a red-enhanced RenCam deep-depletion charge-coupled device. The beam was attenuated to provide ~2.5 mW at the sample. The spot size could be varied by the use of a beam expander assembly to avoid localised heating at the water surface. Other laser lines used to examine the spectra 'off-resonance' were 532 nm (frequency doubled diode), 514.5 nm (Ar^+) and 406 nm (Kr^+). In the case recording spectra from the monolayers on Klarite™ (D3 Technologies, UK) it was necessary to reduce the incident power significantly (to ~20 μW) to avoid localised sample burning. This is true for all dye reporters examined (not just squaraines) and is not a symptom of photo-bleaching. SAMs for direct SERRS intensity comparison were deposited from 1 x 10^{-5} M solutions (1% poly-L-lysine). The exact conditions for the application of these materials into SERRS systems will vary from case to case. For example **SQ2** requires the use of poly-L-lysine or a linker material to

generate strong SERRS on the Klarite™ surface, whereas **SQ6** does not. Spectra were baseline corrected using an established prodecure found in commerical available Klarite™ protocols (D3 Technologies, UK).

iv) Calculated Raman and IR intensities(I)

Below is listed the full DFT vibrational analysis for the basic anilino squaraine structure (**SQ1**). The intensities do not account for the large resonance enhancement observed for some bands. A good example of additional resonance enhancement is the symmetrical carbonyl vibration at 1740 cm^{-1}. The calculated intensity is 106 but when observed in SERRS this band is one of the strongest.

Frequency/cm^{-1}	**Symmetry**	**I (IR)**	**I (Raman)**	**Assignment**
3	B_{1u}	1	0	
16	B_{2g}	0	21	
21	B_{1u}	7	0	
27	B_{2u}	4	0	
47	A_u	0	0	
53	B_{2g}	0	1	
63	B_{3g}	0	0	
75	B_{1u}	0	0	
81	A_u	0	0	
84	B_{1g}	0	6	
90	B_{3g}	0	2	
159	B_{1u}	10	0	
159	B_{2g}	0	2	
166	A_g	0	151	C-C bonds connecting Ph and C4
172	A_u	0	0	
172	B_{3g}	0	0	
202	B_{1g}	0	13	
209	B_{2u}	0	0	
224	B_{1u}	0	0	
235	B_{3u}	0	0	
274	B_{2g}	0	0	
304	B_{1g}	0	5	
325	B_{1u}	6	0	
346	B_{2u}	0	0	
379	B_{3u}	27	0	
412	B_{3g}	0	0	
414	A_u	0	0	
426	A_g	0	20	
470	B_{1g}	0	0	
474	B_{2u}	7	0	
475	B_{2g}	0	8	

506	B_{3u}	74	0	
509	B_{1u}	33	0	
564	B_{2g}	0	0	
576	A_g	0	23	
615	B_{1g}	0	12	
617	B_{2u}	1	0	
636	B_{3g}	0	0	
649	A_g	0	59	
675	B_{3u}	3	0	
719	B_{1u}	1	0	
737	B_{2g}	0	3	
746	A_g	0	135	Ph C-C and C-N combinations
772	B_{1u}	23	0	
778	A_u	0	0	
779	B_{3g}	0	1	
783	B_{3u}	97	0	
792	B_{1G}	0	6	
804	B_{2G}	0	0	
807	B_{1u}	87	0	
929	A_g	0	383	C-N combinations
930	B_{3u}	108	0	C-N combinations
945	B_{1u}	5	0	
945	B_{2g}	0	0	
949	B_{3g}	0	0	
949	A_u	0	0	
978	A_g	50	0	
981	B_{3u}	1	0	
1027	A_g	0	118	Ph C-C combinations
1043	B_{2u}	32	0	
1043	B_{1g}	0	1	
1049	B_{1g}	0	135	C4 C-C combinations
1051	B_{2u}	12	0	
1093	B_{3g}	0	2	
1093	A_u	0	0	
1094	B_{2g}	0	0	
1094	B_{1u}	0	0	
1104	B_{3u}	6	0	
1118	B_{1g}	0	5	
1121	B_{2u}	12	0	
1150	B_{3u}	99	0	
1150	A_g	0	252	Me C-H and C-N combinations

1173	B_{3u}	825	0	Ph C-H combinations
1178	A_g	0	295	Ph C-H combinations
1228	B_{1g}	0	183	C-N combinations
1228	B_{2u}	45	0	
1294	B_{1g}	0	10	
1295	B_{2u}	38	0	
1339	B_{1g}	0	4	
1340	B_{2u}	0	0	
1347	B_{3u}	1033	0	Ph C-C and C-N combinations
1350	A_g	0	295	Ph and C4 C-C combinations
1386	B_{3u}	2495	0	Ph and C4 C-C combinations
1394	B_{1g}	0	52	
1394	B_{2u}	1	0	
1424	A_g	0	134	Me C-H and Ph C-H combinations
1432	B_{3g}	0	14	
1432	A_u	0	0	
1438	B_{2u}	2	0	
1438	B_{1g}	0	214	Me C-H and Ph C-H combinations
1440	B_{3u}	47	0	
1441	B_{1u}	36	0	
1441	B_{2g}	0	93	
1456	A_g	0	147	Me C-H combinations
1461	B_{1g}	0	6	
1461	B_{2u}	8	0	
1475	B_{3u}	515	0	Me C-H combinations
1485	A_g	0	4	
1506	B_{1g}	0	3	
1508	B_{2u}	8	0	
1518	B_{3u}	63	0	
1545	A_g	0	6470	Ph C-C and C4 combinations
1592	B_{3u}	1849	0	Ph C-C combinations
1603	A_g	0	2833	Ph C-C combinations
1638	B_{2u}	798	0	C-O combination
1740	A_g	0	106	Symmetrical C-O combination*
2926	B_{1g}	0	986	Me C-H combinations
2926	B_{2u}	150	0	
2932	B_{3u}	537	0	
2933	A_g	0	2678	Me C-H combinations
2975	B_{3g}	0	326	Me C-H combinations
2975	A_u	0	0	
2975	B_{2g}	0	696	Me C-H combinations

2975	B_{1u}	114	0	Me C-H combinations
3059	B_{1g}	0	35	
3059	B_{2u}	3	0	
3069	B_{3u}	79	0	
3069	A_g	0	738	Me C-H combinations
3105	B_{3u}	1	0	
3105	A_g	0	172	Ph C-H combinations
3105	B_{1g}	0	33	
3105	B_{2u}	2	0	
3127	B_{1g}	0	251	Ph C-H combinations
3127	B_{2u}	15	0	
3128	B_{3u}	67	0	
3128	A_g	0	715	Ph C-H combinations