

Microsphere packages of carotenoids: intact sea urchin eggs tracked by Raman spectroscopy tools

Photochemical & Photobiological Sciences

Electronic Supplementary Information

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Table S1 Raman intensity (counts) of $\nu_1(\text{C}=\text{C})$ band features, measured in spectra of eggs from Fig. 2a.

Egg no.	Absolute intensity	Background intensity	$\nu_1(\text{C}=\text{C})$ intensity	R ($I_{\nu_1} / I_{\text{background}}$)
Female 1				
1	1675	530	1145	2.160
2	2800	880	1920	2.181
3	5980	1855	4125	2.223
4	3910	1230	2680	2.178
5	3270	1030	2240	2.174
6	5640	1825	3815	2.090
7	4930	1685	3245	1.925
8	4325	1505	2820	1.873
9	3490	1223	2267	1.853
10	3980	1370	2610	1.905
11	4280	1328	2952	2.222
12	6575	2140	4435	2.072
13	3675	1180	2495	2.114
14	3695	1140	2555	2.241
15	4095	1255	2840	2.262
16	4480	1330	3150	2.368
17	4765	1467	3298	2.248
18	3570	1140	2430	2.131
Female 2				
19	7685	5120	2565	0.500
20	8325	4430	3895	0.879
21	9750	6950	2800	0.402
22	9750	6890	2860	0.415
23	10710	7430	3280	0.441
Female 3				
24	22890	9290	13600	1.463
25	18480	7246	11234	1.550
26	16100	6210	9890	1.592
27	15720	6060	9660	1.594
28	22645	9165	13480	1.470
29	23025	8410	14616	1.737
30	23560	10360	13200	1.274

Table S2 Raman intensity (counts) of β -carotene and echinenone $\nu_1(\text{C}=\text{C})$ modes, their ratios, and corresponding coefficients of determination (R^2) describing the accuracy of Lorentzian multi-peak fits, measured from spectra presented in Fig. 3, as indicated.

Source	β -carotene $\nu_1(\text{C}=\text{C})$ intensity (1512-1513 cm^{-1})	echinenone $\nu_1(\text{C}=\text{C})$ intensity (1521 cm^{-1})	Intensity ratio $I_{\beta\text{-carotene}} / I_{\text{echinenone}}$	Coefficient of determination (R^2)
Fig. 3a	778	520	1.496	0.997
Fig. 3b	1949	1476	1.320	0.999
Fig. 3c	783	519	1.508	0.997
Fig. 3d	457	20	22.85	0.975
Fig. 3e	Not observed	Not observed	-	0.962
Fig. 3f	Not observed	Not observed	-	0.986
Fig. 3g	61 630	75 100	0.820	0.999
Fig. 3h	22 200	32 300	0.687	0.988
Fig. 3i	24 160	39 020	0.619	0.985

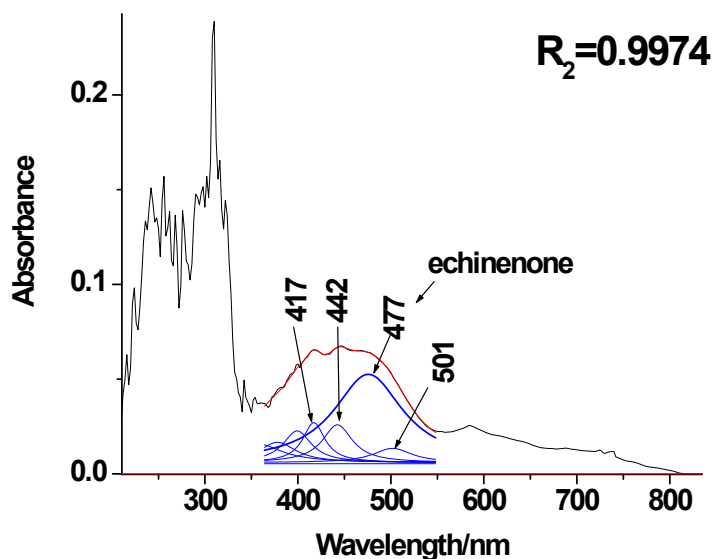


Fig. S1. UV-VIS absorbance spectrum of *P. lividus* sea urchin eggs suspended in filtered seawater. The complex shape of the 380-540 nm range has been de-convoluted using a Lorentz fit procedure and the main component at 477 nm has been assigned to echinenone. Other components at 417, 442 nm are presumably assigned to beta-carotene in specific egg fluid environment.

Fig. S2. Representative Raman images of *Paracentrotus lividus* mature eggs carotenoid signal distribution in confocal mode, generated according to carotenoids ν_1 band (a) and ν_2 band intensity (b) under 532 nm excitation, using 100x objective; (c) a representative Raman spectrum used for constructing the images programs: Origin 6.1 (OriginLab), Powerpoint

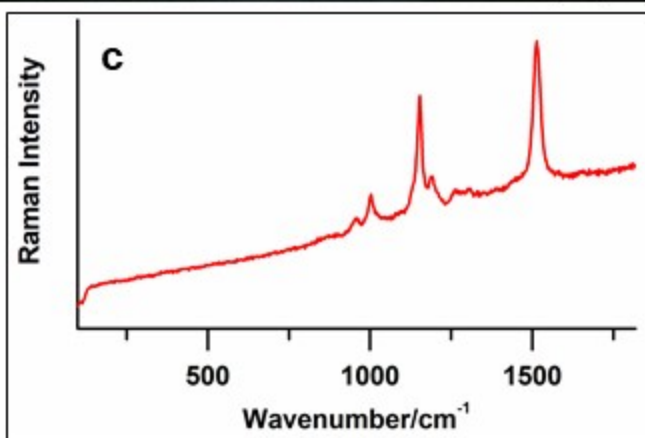
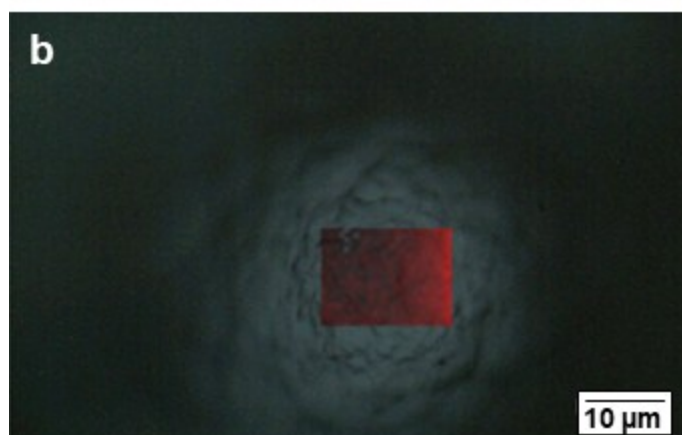
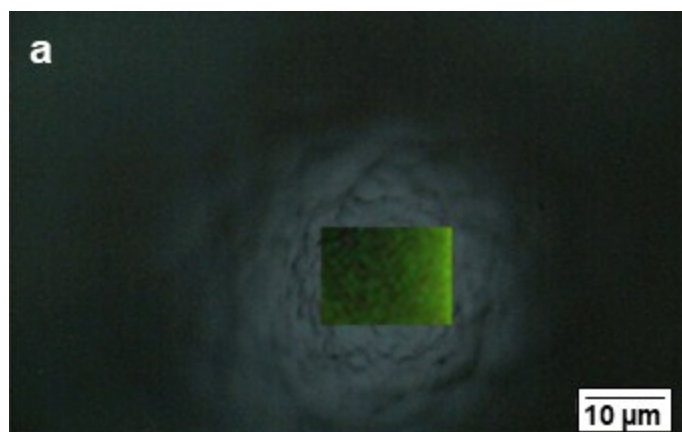


Fig. S3. Representative Raman images of *Paracentrotus lividus* mature eggs protein signal distribution in confocal mode, generated according to amide I band under 785 nm excitation. (a) protein signals are detected on the four eggs at the edge of imaging area (20x objective); (b) slight variations in amide I band intensity between image pixels indicates that the distribution of proteins is not uniform (100x objective); (c) a representative Raman spectrum used for constructing the images **programs: Origin 6.1 (OriginLab), Powerpoint**

