Supporting information for:

Coupled X-ray fluorescence and X-ray absorption spectroscopy for microscale imaging and identification of sulfur species within tissues and skeletons of scleractinian corals

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This supporting information contains example S-XRF maps with S-XANES points, S-XANES spectra, tables and plots of comparisons of S-XANES linear combination fits versus ME XRF map fits, images of single-energy vs. ME fitted maps, possible contamination during the polishing process

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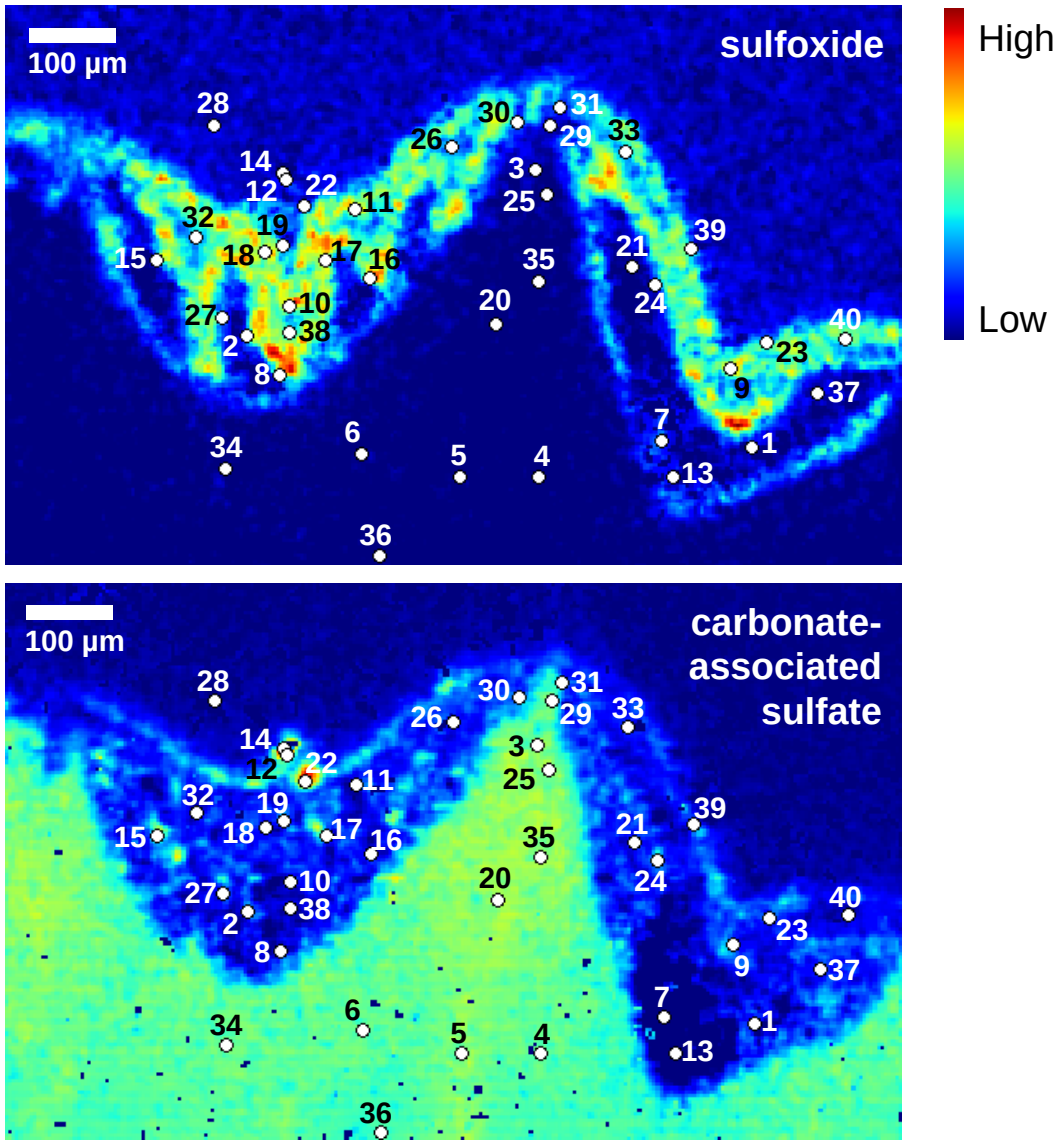

Figure S1. 40 S XANES points on *D. heliopora* sample, shown on the sulfoxide and carbonate-associated sulfate distribution heat maps.

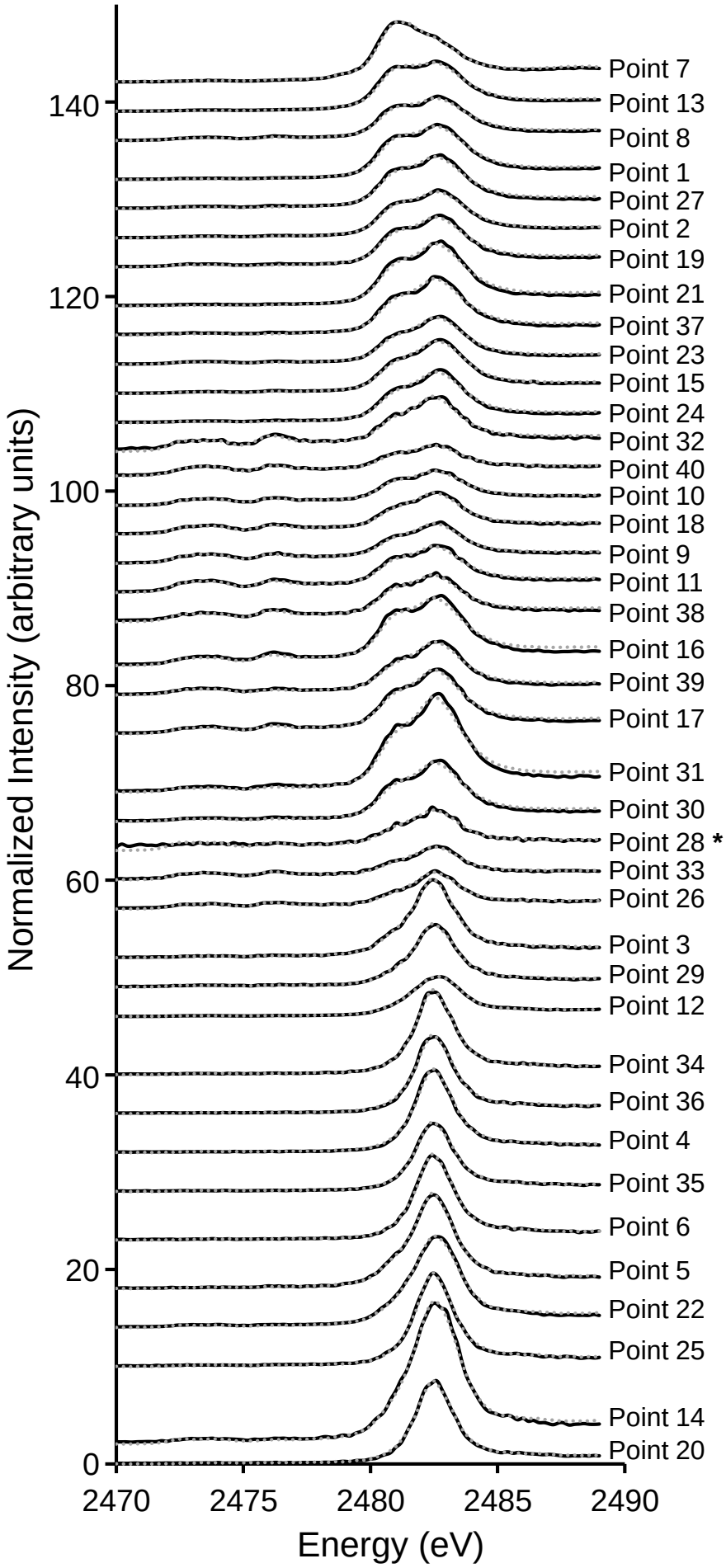

Figure S2. 40 normalized S XANES spectra collected on *D. heliopora* sample (black solid lines) with results of linear combination fitting with the seven representative S XANES species (grey dotted lines). Spectra correspond to points on Figure S1. * indicates a point on the epoxy.

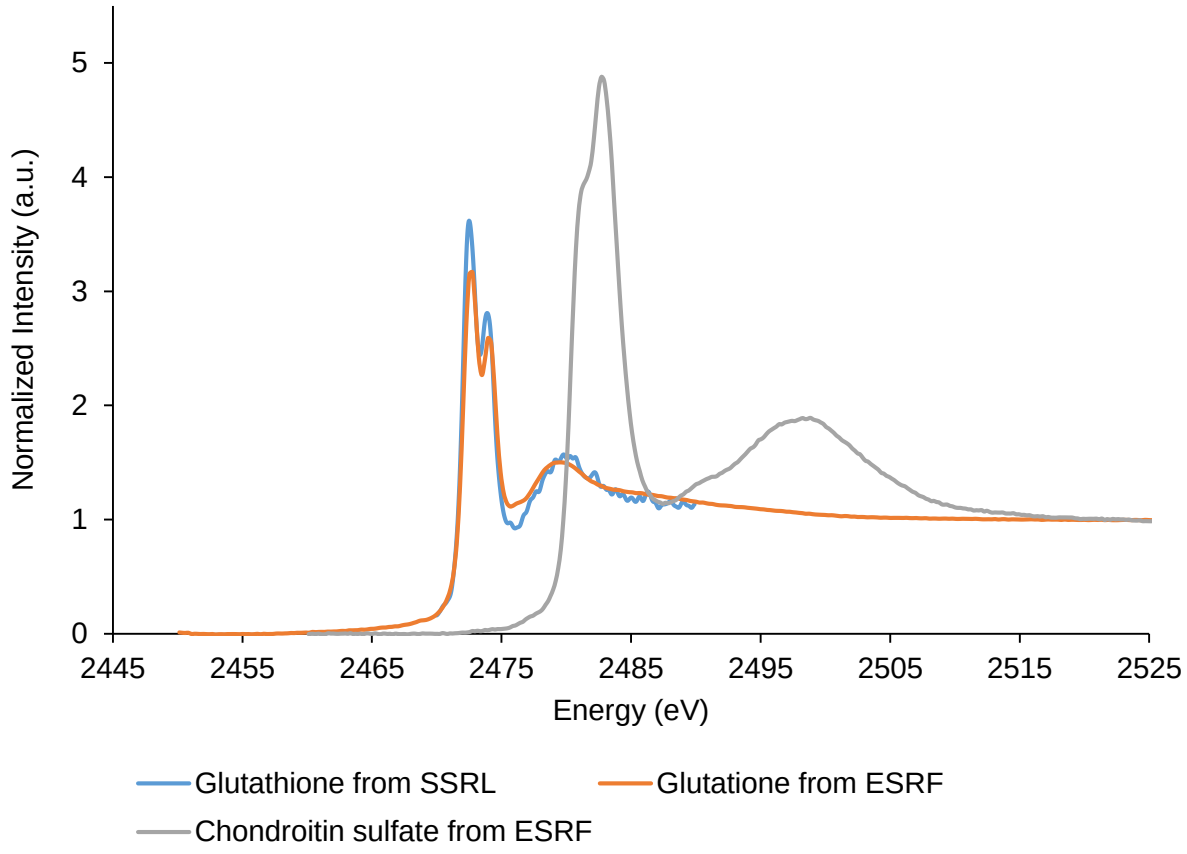

Figure S3. A comparison of reference spectra collected at two different beamlines shows that overall peak shape and positions are consistent between beamlines (SSRL and the ESRF database). In this example we show the similarities between glutathione samples measured at SSRL (blue) and from the ESRF database (orange). These glutathione compounds produce very different spectra from other S compounds, such as this chondroitin sulfate in grey from the ESRF database.

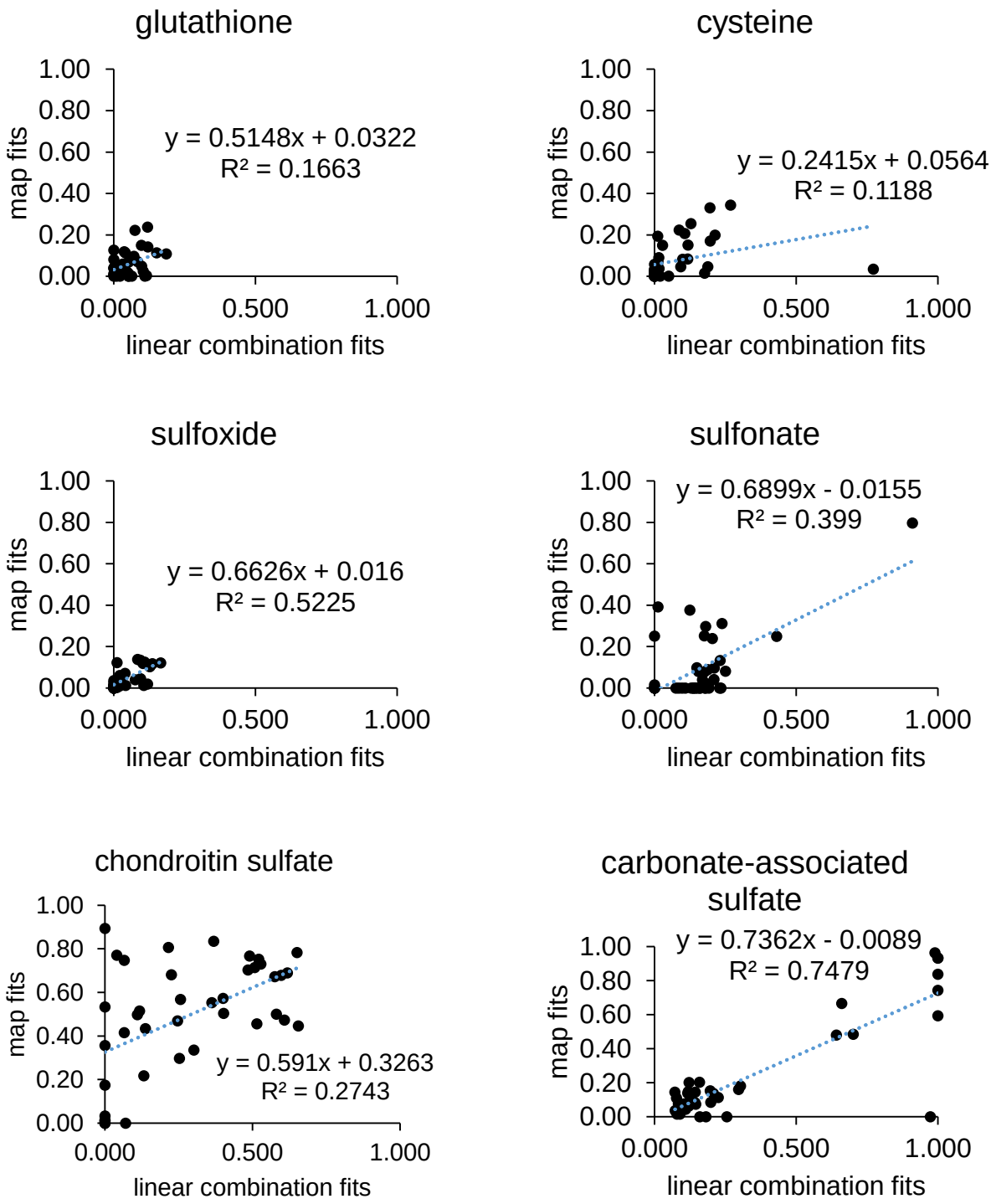

Figure S4. Validation step for ME map fittings. Fraction contributions of different S compounds calculated by linear combination fitting versus map fitting on S XANES for the *D. heliopora* sample. An ideal correlation between XANES versus map fits should yield a 1:1 relationship. Due to the very heterogeneous nature of the sample, as well as the difficulty fitting compounds present in low concentrations, these slopes are not always an ideal 1:1 fit.

Table S1. *D. heliopora* S XANES points fit with S XANES standards using linear combination fitting as well as fit with Multiple Energy (ME) maps.

Linear combination fits of S XANES

point	glutathione disulfide	cysteine	sulfoxide	sulfonate	chondroitin sulfate	carbonate-associated sulfate	inorganic sulfate	components sum	reduced chi squared	R factor
1	0.007 ± 0.005			0.283 ± 0.012	0.736 ± 0.016	0.106 ± 0.007		1.13	0.01635	0.0033
2	0.023 ± 0.009	0.016 ± 0.012	0.012 ± 0.004	0.184 ± 0.013	0.604 ± 0.025	0.076 ± 0.016	0.1 ± 0.04	0.98	0.00648	0.00178
3	0.023 ± 0.003		0.012 ± 0.003	0.259 ± 0.007	0.076 ± 0.009	0.719 ± 0.004		1.09	0.0049	0.00081
4						0.902 ± 0.002		0.90	0.00763	0.00136
5			0.005 ± 0.003	0.29 ± 0.006	0.082 ± 0.008	0.884 ± 0.004		1.26	0.00414	0.00048
6						0.927 ± 0.001		0.93	0.00339	0.00057
7				1.19 ± 0.007		0.117 ± 0.007		1.31	0.00788	0.00142
8	0.04 ± 0.012	0.05 ± 0.016	0.039 ± 0.006	0.202 ± 0.01	0.574 ± 0.013	0.083 ± 0.006		0.99	0.01181	0.00359
9	0.106 ± 0.01	0.234 ± 0.012	0.112 ± 0.005	0.163 ± 0.014	0.276 ± 0.027	0.128 ± 0.017	0.11 ± 0.05	1.09	0.00728	0.00238
10	0.069 ± 0.007	0.163 ± 0.009	0.086 ± 0.003	0.149 ± 0.01	0.333 ± 0.019	0.097 ± 0.012	0.04 ± 0.03	0.92	0.00362	0.00159
11	0.16 ± 0.014	0.263 ± 0.019	0.145 ± 0.007	0.207 ± 0.012	0.403 ± 0.016	0.159 ± 0.007		1.34	0.01639	0.0037
12	0.006 ± 0.003	0.005 ± 0.004	0.002 ± 0.001	0.044 ± 0.001		0.042 ± 0.003	0.719 ± 0.005	0.58	0.01033	0.00576
13	0.005 ± 0.005			0.477 ± 0.01	0.538 ± 0.014	0.089 ± 0.006		1.11	0.01211	0.00269
14	0.111 ± 0.023	0.026 ± 0.03	0.022 ± 0.011	0.232 ± 0.033	0.14 ± 0.06	0.65 ± 0.04	1.43 ± 0.11	2.13	0.06335	0.00284
15	0.031 ± 0.011	0.013 ± 0.014	0.021 ± 0.005	0.133 ± 0.015	0.54 ± 0.029	0.074 ± 0.019	0.32 ± 0.05	1.02	0.00959	0.00229
16	0.12 ± 0.031	0.16 ± 0.04	0.142 ± 0.014	0.392 ± 0.025	0.68 ± 0.03	0.201 ± 0.015		1.69	0.07397	0.00871
17	0.091 ± 0.019	0.124 ± 0.024	0.107 ± 0.009	0.252 ± 0.016	0.57 ± 0.021	0.282 ± 0.009		1.43	0.02781	0.00426
18	0.107 ± 0.01	0.206 ± 0.013	0.115 ± 0.005	0.195 ± 0.014	0.235 ± 0.027	0.133 ± 0.017	0.16 ± 0.05	1.10	0.00768	0.00243
19	0.059 ± 0.016	0.012 ± 0.021	0.012 ± 0.008	0.178 ± 0.023	0.64 ± 0.04	0.138 ± 0.029	0.02 ± 0.08	1.05	0.02037	0.00493
20						0.898 ± 0.002		0.90	0.00612	0.0011
21	0.008 ± 0.008			0.22 ± 0.018	0.8 ± 0.023	0.194 ± 0.01		1.22	0.03582	0.00587
22	0.057 ± 0.005	1.015 ± 0.066	0.009 ± 0.005	0.128 ± 0.019	0.05 ± 0.04	0.39 ± 0.024		1.32	0.02781	0.00311
23	0.046 ± 0.011	0.027 ± 0.014	0.015 ± 0.005	0.128 ± 0.016	0.472 ± 0.03	0.122 ± 0.019	0.18 ± 0.05	0.93	0.00969	0.00291
24	0.031 ± 0.006		0.007 ± 0.006	0.172 ± 0.021	0.5 ± 0.04	0.119 ± 0.027	0.22 ± 0.07	0.98	0.01812	0.00467
25	0.015 ± 0.004		0 ± 0.003	0.013 ± 0.004		1.004 ± 0.003		1.03	0.0062	0.00087
26	0.093 ± 0.008	0.092 ± 0.01	0.093 ± 0.004	0.097 ± 0.011	0.103 ± 0.021	0.2 ± 0.013	0.16 ± 0.04	0.78	0.0047	0.00249
27	0.042 ± 0.006		0.011 ± 0.006	0.226 ± 0.013	0.643 ± 0.018	0.155 ± 0.008		1.08	0.02048	0.00462
28	0.186 ± 0.021	0.107 ± 0.027	0.095 ± 0.01	0.212 ± 0.03	0.12 ± 0.06	0.14 ± 0.04	0.22 ± 0.103	1.01	0.03613	0.01287
29	0.035 ± 0.003		0.016 ± 0.003	0.073 ± 0.01	0.117 ± 0.02	0.545 ± 0.013	0.1 ± 0.03	0.85	0.00409	0.00106
30	0.062 ± 0.021	0.023 ± 0.027	0.022 ± 0.01	0.213 ± 0.018	0.615 ± 0.023	0.245 ± 0.01		1.18	0.03525	0.00664
31	0.098 ± 0.035	0.03 ± 0.05	0.049 ± 0.017	0.361 ± 0.029	0.92 ± 0.04	0.423 ± 0.017		1.88	0.09829	0.00729
32	0.222 ± 0.017	0.172 ± 0.022	0.244 ± 0.008	0.234 ± 0.025	0.33 ± 0.05	0.268 ± 0.031	0 ± 0.08	1.47	0.02384	0.00433
33	0.097 ± 0.008	0.164 ± 0.011	0.107 ± 0.004	0.122 ± 0.012	0.092 ± 0.023	0.102 ± 0.015	0.237 ± 0.04	0.84	0.0058	0.00312
34	0.008 ± 0.002			0.001 ± 0.003		0.914 ± 0.002		0.92	0.00359	0.00062
35						0.752 ± 0.001		0.75	0.00364	0.00094
36						0.85 ± 0.002		0.85	0.00771	0.00155
37	0.04 ± 0.007		0 ± 0.007	0.209 ± 0.015	0.631 ± 0.02	0.216 ± 0.009		1.10	0.02707	0.00561
38	0.14 ± 0.016	0.164 ± 0.02	0.161 ± 0.007	0.299 ± 0.013	0.325 ± 0.017	0.185 ± 0.008		1.27	0.01898	0.00448
39	0.097 ± 0.016	0.114 ± 0.021	0.047 ± 0.008	0.173 ± 0.023	0.42 ± 0.05	0.185 ± 0.029	0.17 ± 0.08	1.15	0.02079	0.00473
40	0.101 ± 0.008	0.26 ± 0.01	0.132 ± 0.004	0.133 ± 0.007	0.238 ± 0.009	0.106 ± 0.004		0.97	0.00501	0.00235

Multiple energy map fits

point	glutathione disulfide	cysteine	sulfoxide	sulfonate	chondroitin sulfate	carbonate-associated sulfate	fit error
1	4.10	-2.07	0.95	6.81	65.47	6.27	394.08
2	3.14	2.87	2.65	7.03	54.28	8.73	239.90
3	-0.03	0.32	0.23	7.60	-3.73	16.26	2.12
4	0.51	-0.91	0.16	0.19	-0.21	11.59	0.00
5	1.78	-2.32	0.43	-2.99	9.10	10.62	22.85
6	0.64	-0.74	0.23	-4.26	6.09	10.15	3.42
7	0.82	-0.73	-0.84	54.97	12.08	1.07	81.50
8	8.30	-1.70	5.24	17.61	36.77	5.64	442.41
9	5.66	7.57	4.52	3.77	11.31	5.26	47.56
10	10.53	0.70	6.37	-1.19	26.32	3.60	103.59
11	5.25	6.28	4.62	2.96	12.42	5.46	13.98
12	0.98	5.26	1.03	-37.10	92.71	3.79	103.99
13	0.31	2.44	-0.34	20.89	58.87	1.26	71.37
14	-0.08	5.40	0.18	-23.25	59.14	14.33	4.49
15	3.65	1.43	4.82	-9.03	58.05	11.54	140.17
16	4.30	2.04	6.15	5.96	22.46	3.68	157.35
17	-4.44	12.95	2.33	4.55	33.27	4.93	228.34
18	3.23	3.04	0.87	-0.74	53.90	5.82	255.87
19	3.75	8.80	5.58	1.80	21.51	4.03	78.48
20	2.24	-3.10	0.45	-0.63	0.20	14.82	8.02
21	2.38	-2.61	0.27	15.30	22.97	10.48	158.00
22	2.53	3.29	0.89	-27.07	75.52	15.78	356.87
23	0.83	7.37	1.14	-0.49	35.43	4.78	245.90
24	2.75	0.05	1.41	11.73	21.26	9.36	239.02
25	1.19	0.50	-4.61	8.99	12.23	-1.17	20.71
26	3.76	2.38	0.31	5.97	3.45	-0.44	2.98
27	3.16	-1.34	1.16	5.26	36.88	7.87	210.64
28	0.57	1.08	0.24	0.22	2.70	0.44	6.63
29	0.43	1.28	0.20	-1.76	9.74	10.79	26.30
30	3.17	-0.28	1.94	-2.13	34.56	6.26	131.78
31	-0.34	3.66	1.22	-4.26	31.29	4.60	50.67
32	2.55	1.88	2.75	-5.81	15.38	-0.62	7.09
33	0.09	9.64	3.05	-4.61	14.50	1.85	21.15
34	-0.09	0.25	-0.01	0.00	0.16	11.15	3.73
35	0.14	-0.42	-0.17	6.00	-9.11	17.74	3.04
36	-0.06	0.37	-0.01	-1.63	0.36	10.30	0.96
37	3.76	-3.37	1.17	0.60	21.41	4.88	76.98
38	-0.14	6.10	2.49	-1.88	13.63	1.78	23.99
39	3.12	3.63	0.58	-9.31	37.15	-1.50	219.05
40	0.55	7.71	2.65	-0.88	10.54	0.99	17.97

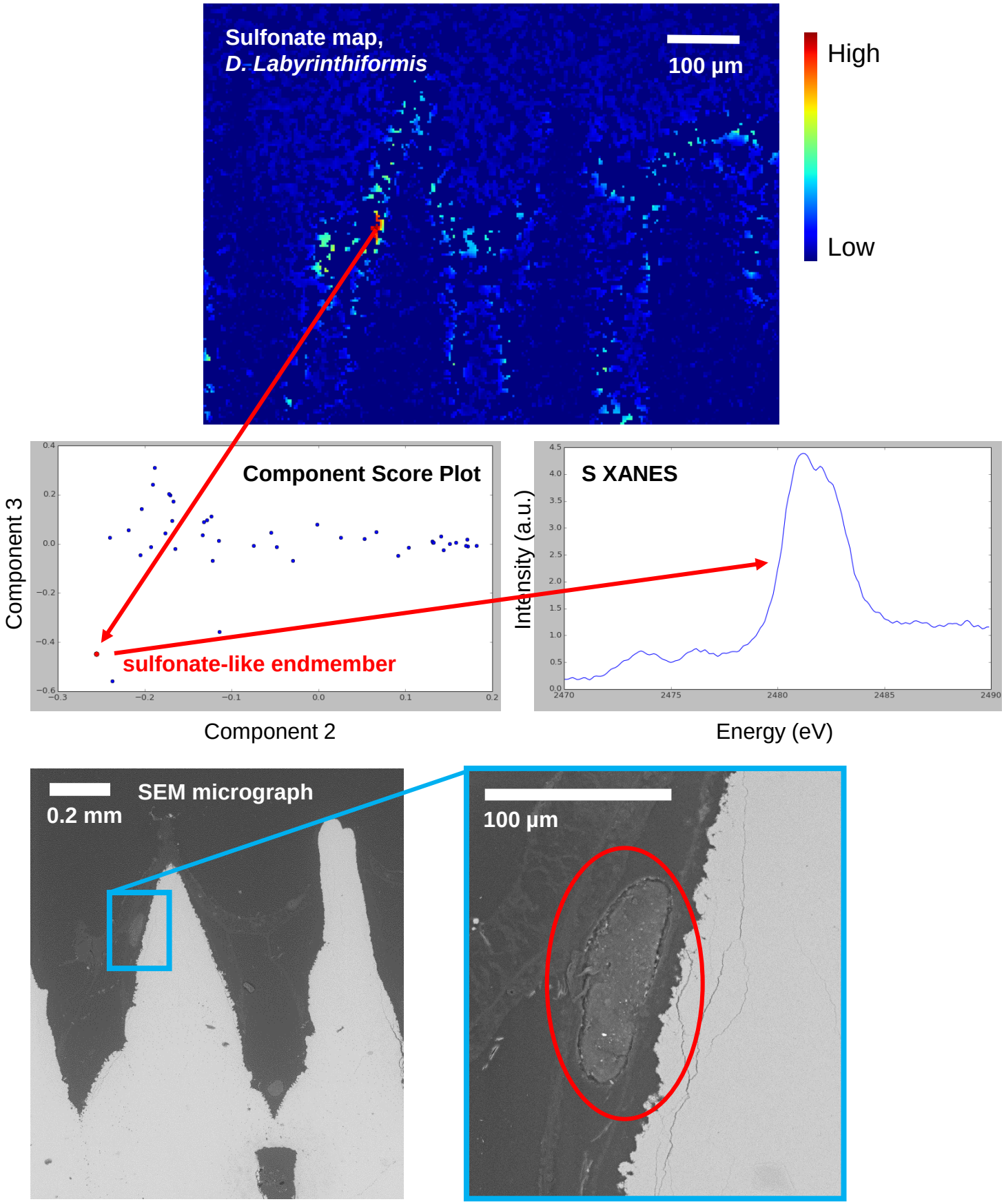

Figure S5. Potential contamination from the polishing process as shown by SEM micrographs of a filled bubble region correlated with a concentrated distribution of a sulfonate-like species that is also an end-member S XANES spectrum in our PCA component 3 vs. component 2 score plot.

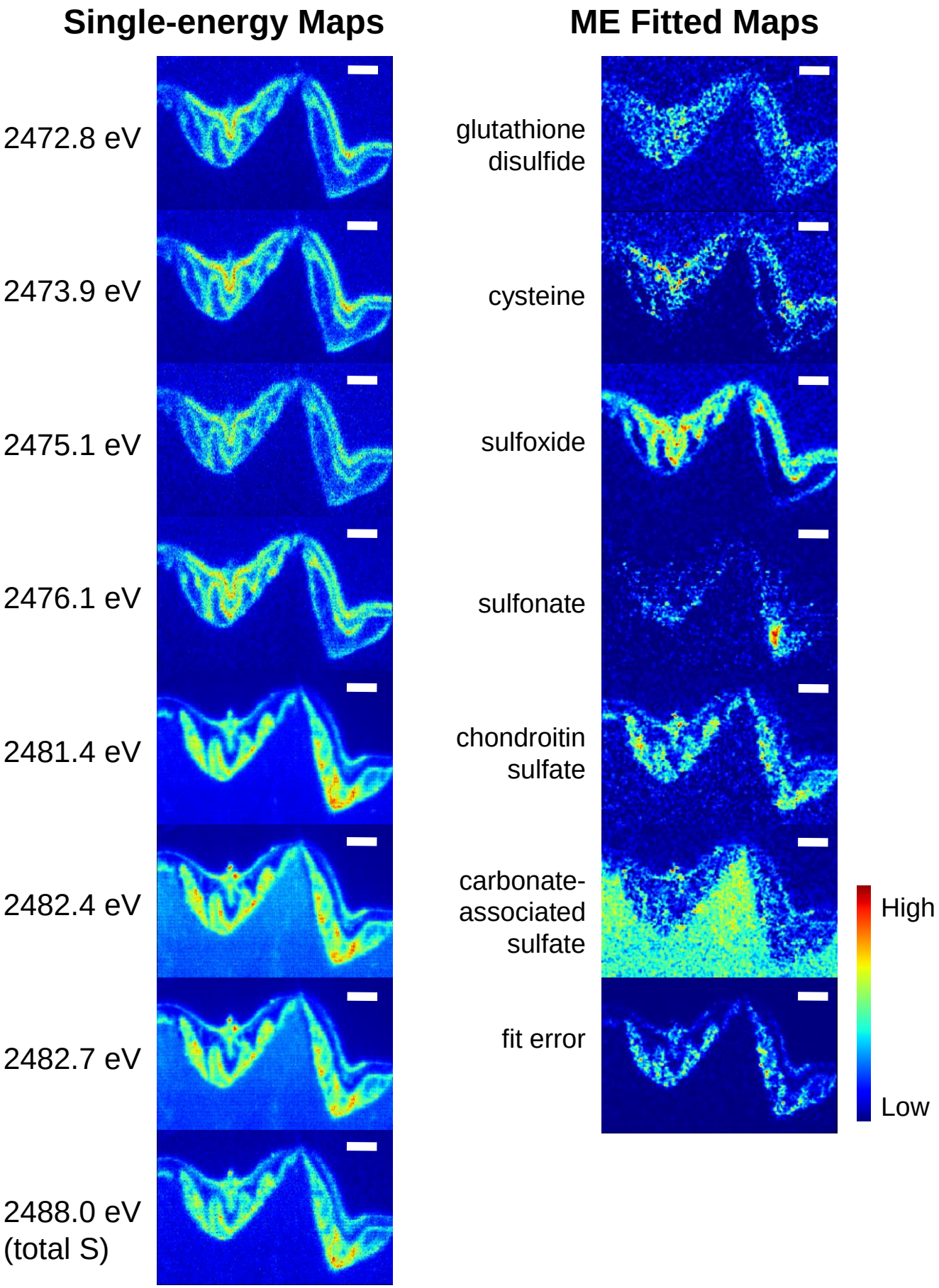

Figure S6. Comparison of single-energy maps (left) versus stacked multiple energy (ME) maps fitted to S XANES of standard S compounds (right) shows how ME fitted maps present more accurate distributions of individual S bonding environments in *D. heliopora*. Scale bars are 100 µm.