SUPPORTING INFORMATION

"Rational design of novel fluorescent analogues of cholesterol: a "step-by-step" computational study"

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1. Optical properties

1.1. CASPT2 energies for probes **8** and **10**

In order to check the presence that no dark states are below the bright excited state (characterized by a HOMO → LUMO transition), we performed a CASPT2 excited state analysis based on a multireference CASSCF(8, 8)-SA11 wavefunction using the ANO-L-VDZP basis-set. These computations were performed with MOLCAS 8.0 quantum chemistry software[1]. As we can see, no dark excited states are present below the bright excited state (S_1) for both probe **8** and, more importantly, for probe **10**. As in TD-DFT computations, presented in the main article, the bright state S_1 is described by a single excitation HOMO → LUMO transition. The dominant CI-coefficient, for this HOMO → LUMO configuration, are -0.91155 and 0.74507 for probes **8** and **10**, respectively.

Table 1: CASPT2 energies for the ground state and the first 10 singlet excited state

Probe	State	CASPT2 energy [Hartree]	ΔE [eV]	ΔE [nm]	$f(S_0 \rightarrow S_n)$
8	S_0	-1124.84427339	/	/	/
	S_1	-1124.72750955	3.177	390	0.394
	S_2	-1124.69752380	3.993	311	0.000
	S_3	-1124.67543105	4.594	270	0.037
	S_4	-1124.66983915	4.747	261	0.002
	S_5	-1124.65621984	5.117	242	0.001
	S_6	-1124.63694614	5.642	220	0.006
	S_7	-1124.63500171	5.695	218	0.014
	S_8	-1124.61830540	6.149	202	0.018
	S_9	-1124.61011564	6.372	195	0.002
	S_{10}	-1124.52684766	8.638	144	0.001
10	S_0	-1124.84783247	/	/	/
	S_1	-1124.69332332	4.204	295	0.668
	S_2	-1124.68748496	4.363	284	0.001
	S_3	-1124.67038366	4.829	257	0.010
	S_4	-1124.65748752	5.180	239	0.032
	S_5	-1124.65584240	5.224	237	0.340
	S_6	-1124.62510039	6.061	205	0.012
	S_7	-1124.61558977	6.320	196	0.230
	S_8	-1124.60706099	6.552	189	0.005
	S_9	-1124.58373118	7.187	173	0.001
	S_{10}	-1124.57949729	7.302	170	0.044

2. Molecular dynamics in POPC

2.1. GAFF force field for cholesterol, probe **8** and **10**

In the following tables (Table 2, Table 3 and Table 4) we list GAFF atoms type and RESP charges used in the molecular dynamics simulation performed in this work together with their ground state geometries (obtained at B3LYP/6-31+G(d) level of theory in vacuum).

Table 2: Ground state geometry, atom names, GAFF atom-types and RESP charges and isotropic polarizabily α_{iso} for cholesterol.

Cholesterol							
Atom number	**Name**	**X [Å]**	**Y [Å]**	**Z [Å]**	**GAFF atom-type**	**RESP Charge**	α_{iso}
1	C3	6.879	-0.082	-0.801	c3	0.378828	7.52532704
2	O1	8.294	-0.065	-0.568	oh	-0.751689	5.78273641
3	HO1	8.718	-0.682	-1.185	ho	0.423029	1.45927443
4	H31	6.675	0.260	-1.830	h1	-0.019871	1.85606657
5	C4	6.254	0.909	0.180	c3	-0.008496	8.28625582
6	H41	6.591	0.624	1.188	hc	0.072237	1.85981222
7	H42	6.651	1.913	-0.008	hc	0.072237	1.90550381
8	C5	4.738	0.922	0.100	c2	-0.410084	9.38013989
9	C6	4.079	2.079	-0.056	c2	-0.181935	9.48966611
10	H61	4.656	3.000	-0.150	ha	0.118276	2.13067117
11	C7	2.584	2.222	-0.114	c3	-0.008375	8.01595289
12	H71	2.274	3.084	0.494	hc	0.026749	2.00261433
13	H72	2.283	2.471	-1.146	hc	0.026749	1.87565226
14	C8	1.840	0.959	0.350	c3	0.055053	8.30976134
15	H81	1.896	0.925	1.448	hc	0.002266	1.64573912
16	C14	0.367	0.998	-0.079	c3	0.018813	8.42204347
17	H141	0.379	1.013	-1.184	hc	-0.016960	1.71879767
18	C15	-0.500	2.186	0.360	c3	-0.119530	7.74240985
19	H151	-0.352	2.401	1.426	hc	0.018976	1.74644610
20	H152	-0.256	3.107	-0.182	hc	0.018976	1.94576940
21	C16	-1.949	1.704	0.078	c3	-0.052708	7.57859951
22	H161	-2.593	1.868	0.952	hc	0.017247	1.83108783
23	H162	-2.399	2.269	-0.745	hc	0.017247	1.89258326
24	C17	-1.872	0.176	-0.268	c3	-0.032017	8.75505836
25	H171	-1.796	0.080	-1.364	hc	-0.037496	1.76402732
26	C13	-0.475	-0.252	0.304	c3	0.460490	8.56285985
27	C18	-0.563	-0.454	1.834	c3	-0.453169	6.99109867
28	H181	0.423	-0.511	2.302	hc	0.087135	1.90436703
29	H182	-1.107	0.360	2.325	hc	0.087135	1.72393117
30	H183	-1.089	-1.385	2.073	hc	0.087135	1.87649897
31	C12	0.209	-1.463	-0.366	c3	-0.056081	7.73584487
32	H121	-0.296	-2.401	-0.114	hc	-0.010733	1.94597956
33	H122	0.134	-1.352	-1.459	hc	-0.010733	1.74600306
34	C11	1.693	-1.581	0.040	c3	-0.112061	7.54481247
35	H111	1.741	-1.831	1.105	hc	0.018586	1.60138308
36	H112	2.132	-2.434	-0.492	hc	0.018586	1.93903499
37	C9	2.526	-0.306	-0.231	c3	-0.036960	8.42607955
38	H91	2.542	-0.159	-1.325	hc	-0.016648	1.68257052
39	C10	4.027	-0.436	0.207	c3	0.570986	8.82640231
40	C19	4.150	-0.949	1.667	c3	-0.209529	7.19731538
41	H191	3.844	-1.997	1.752	hc	0.031785	1.84347048
42	H192	5.179	-0.882	2.032	hc	0.031785	1.92703743
43	H193	3.529	-0.353	2.345	hc	0.031785	1.74234186
44	C1	4.744	-1.431	-0.757	c3	-0.098195	7.73358052
45	H11	4.342	-2.441	-0.613	hc	0.004995	1.87933166
46	H12	4.501	-1.145	-1.791	hc	0.004995	1.72468700
47	C2	6.273	-1.473	-0.607	c3	-0.127133	7.82100959

48	H21	6.693	-2.175	-1.342	hc	0.030841	2.19436286
49	H22	6.560	-1.844	0.386	hc	0.030841	1.79025242
50	C20	-3.150	-0.592	0.158	c3	0.187074	8.78398973
51	H201	-3.283	-0.452	1.243	hc	-0.031548	1.78696205
52	C21	-3.076	-2.104	-0.116	c3	-0.298829	7.28927144
53	H211	-2.860	-2.304	-1.175	hc	0.059599	1.74095443
54	H212	-4.022	-2.598	0.129	hc	0.059599	2.11061672
55	H213	-2.301	-2.594	0.479	hc	0.059599	1.86141947
56	C22	-4.392	0.022	-0.536	c3	-0.039615	8.56967625
57	H221	-4.379	1.110	-0.401	hc	0.003047	1.69440175
58	H222	-4.312	-0.150	-1.622	hc	0.003047	1.82432582
59	C23	-5.755	-0.492	-0.043	c3	0.048794	8.37314366
60	H231	-5.875	-1.558	-0.279	hc	-0.002549	1.74572690
61	H232	-5.790	-0.411	1.053	hc	-0.002549	1.74419898
62	C24	-6.928	0.282	-0.665	c3	-0.183603	8.45937794
63	H241	-6.863	1.342	-0.372	hc	0.027806	1.80320613
64	H242	-6.816	0.264	-1.759	hc	0.027806	1.84661334
65	C25	-8.334	-0.246	-0.312	c3	0.440534	8.42696324
66	H251	-8.368	-1.312	-0.587	hc	-0.075146	1.94260581
67	C26	-8.644	-0.136	1.190	c3	-0.313515	7.48903306
68	H261	-9.655	-0.502	1.409	hc	0.058435	2.12100669
69	H262	-7.944	-0.719	1.799	hc	0.058435	1.86713877
70	H263	-8.588	0.909	1.523	hc	0.058435	1.77742806
71	C27	-9.405	0.483	-1.138	c3	-0.313515	7.77477783
72	H271	-9.219	0.380	-2.215	hc	0.058435	1.88779141
73	H272	-10.407	0.086	-0.932	hc	0.058435	2.12417547
74	H273	-9.420	1.557	-0.904	hc	0.058435	1.82240413

Table 3: Ground state geometry, atom names, GAFF atom-types and RESP charges and isotropic polarizabily α_{iso} for probe **8**.

8							
Atom number	**Name**	**X [Å]**	**Y [Å]**	**Z [Å]**	**GAFF atom-type**	**RESP Charge**	α_{iso}
1	C3	-6.821	-0.372	0.302	c3	0.379537	7.83287132
2	O1	-8.207	-0.458	-0.049	oh	-0.751834	5.97916166
3	HO1	-8.640	-1.094	0.543	ho	0.426420	1.48724839
4	H31	-6.727	-0.015	1.341	h1	-0.017557	1.85515560
5	C4	-6.191	0.653	-0.640	c3	-0.042345	8.80726285
6	H41	-6.424	0.321	-1.665	hc	0.086903	2.06119467
7	H42	-6.677	1.627	-0.513	hc	0.086903	1.98966366
8	C5	-4.696	0.787	-0.459	c2	-0.372427	10.74453013
9	C6	-4.082	1.986	-0.584	ce	-0.104696	10.85790610
10	H61	-4.682	2.877	-0.765	ha	0.120410	2.18964225
11	C7	-2.640	2.132	-0.552	ce	-0.160831	11.28198516
12	H71	-2.205	3.079	-0.865	ha	0.122079	1.79207853
13	C8	-1.838	1.128	-0.113	cf	-0.053637	13.38385678
14	C14	-0.409	1.279	0.108	cf	-0.240229	13.66594762
15	C15	0.293	2.375	0.511	ce	-0.098201	10.39188990
16	H152	-0.113	3.371	0.660	ha	0.121469	1.84788643
17	C16	1.664	1.990	0.825	ce	-0.200848	9.45199949
18	H162	2.424	2.690	1.163	ha	0.138680	2.19139163
19	C17	1.830	0.653	0.658	c2	-0.290057	11.96376277
20	C13	0.521	0.075	0.123	c3	0.611216	11.27203078
21	C18	0.761	-0.454	-1.316	c3	-0.340933	6.88150629
22	H181	-0.172	-0.761	-1.797	hc	0.066290	1.68130482
23	H182	1.214	0.327	-1.936	hc	0.066290	1.67345670
24	H183	1.436	-1.319	-1.303	hc	0.066290	1.87687509
25	C12	-0.140	-0.998	1.023	c3	-0.020602	8.76388288
26	H121	0.479	-1.906	1.053	hc	-0.005185	1.98576558
27	H122	-0.192	-0.611	2.050	hc	-0.005185	1.66070977
28	C11	-1.551	-1.359	0.526	c3	-0.086898	8.96664695
29	H111	-1.464	-1.862	-0.443	hc	-0.000889	1.54280150
30	H112	-1.985	-2.095	1.213	hc	-0.000889	1.87630289
31	C9	-2.510	-0.148	0.408	c3	0.161217	11.08238011
32	H91	-2.807	0.116	1.442	hc	-0.008512	1.70117090
33	C10	-3.852	-0.488	-0.329	c3	0.392685	11.05577120
34	C19	-3.606	-1.018	-1.769	c3	-0.131381	6.90892338
35	H191	-3.124	-2.002	-1.762	hc	0.025392	1.70777342
36	H192	-4.548	-1.124	-2.316	hc	0.025392	1.90294260
37	H193	-2.971	-0.327	-2.334	hc	0.025392	1.61651702
38	C1	-4.607	-1.563	0.506	c3	-0.048603	8.20283188
39	H11	-4.111	-2.534	0.384	hc	-0.002412	1.77831532
40	H12	-4.524	-1.302	1.571	hc	-0.002412	1.71409144
41	C2	-6.097	-1.711	0.165	c3	-0.142672	8.31944624
42	H21	-6.549	-2.458	0.834	hc	0.036590	2.21969606
43	H22	-6.238	-2.080	-0.860	hc	0.036590	1.79780880
44	C20	3.078	-0.168	0.894	c3	0.292584	10.03691504
45	H201	2.945	-1.136	0.389	hc	-0.022635	1.69452968
46	C21	3.262	-0.466	2.401	c3	-0.333432	7.97522051
47	H211	3.470	0.458	2.955	hc	0.074820	1.78140752
48	H212	4.091	-1.162	2.573	hc	0.074820	2.31056817
49	H213	2.358	-0.913	2.829	hc	0.074820	1.86735417
50	C22	4.333	0.496	0.277	c3	-0.101012	9.01100958
51	H221	4.108	0.749	-0.769	hc	0.034646	1.77569958
52	H222	4.525	1.451	0.787	hc	0.034646	1.74074811
53	C23	5.607	-0.362	0.314	c3	0.042340	8.70607620
54	H231	5.914	-0.537	1.355	hc	-0.005945	1.81779238
55	H232	5.384	-1.349	-0.115	hc	-0.005945	1.72475514
56	C24	6.773	0.291	-0.445	c3	-0.148286	8.62563957
57	H241	6.494	0.412	-1.504	hc	0.017384	1.79406452
58	H242	6.918	1.309	-0.052	hc	0.017384	1.84783087
59	C25	8.119	-0.457	-0.369	c3	0.444907	8.54011875
60	H251	8.368	-0.590	0.696	hc	-0.079539	1.96685106
61	C26	8.057	-1.850	-1.016	c3	-0.307153	7.50560426
62	H261	9.032	-2.349	-0.965	hc	0.056040	2.14834221

63	H262	7.327	-2.503	-0.523	hc	0.056040	1.87954772
64	H263	7.777	-1.774	-2.076	hc	0.056040	1.77273807
65	C27	9.237	0.382	-1.008	c3	-0.307153	7.84044954
66	H271	9.328	1.364	-0.525	hc	0.056040	1.89460056
67	H272	10.209	-0.121	-0.930	hc	0.056040	2.15627136
68	H273	9.036	0.554	-2.075	hc	0.056040	1.81391535

Table 4: Ground state geometry, atom names, GAFF atom-types and RESP charges and isotropic polarizabily α_{iso} for probe **10**.

		10					
Atom number	**Name**	**X [Å]**	**Y [Å]**	**Z [Å]**	**GAFF atom-type**	**RESP Charge**	α_{iso}
1	C3	6.934	-0.131	-0.665	c3	0.462102	8.57667181
2	O1	8.221	0.196	-0.108	oh	-0.703286	6.36886752
3	HO1	8.845	-0.509	-0.347	ho	0.391200	1.71731622
4	H31	7.061	-0.283	-1.753	h1	-0.050194	2.19094507
5	C4	6.043	1.059	-0.463	c2	-0.254458	11.67352752
6	H42	6.502	2.026	-0.667	ha	0.146240	2.16403734
7	C5	4.737	0.998	-0.122	ce	-0.106399	12.17971468
8	C6	3.933	2.212	-0.036	ce	-0.181129	12.33636836
9	H61	4.460	3.164	-0.056	ha	0.138004	2.14704981
10	C7	2.581	2.193	0.067	cf	-0.214416	12.39709056
11	H71	2.046	3.134	0.172	ha	0.136980	1.82637149
12	C8	1.818	0.958	0.014	cf	0.023657	14.23670776
13	C14	0.475	0.912	0.228	ce	-0.026294	14.38557838
14	C15	-0.452	2.022	0.416	ce	-0.150062	11.07901447
15	H152	-0.167	3.031	0.697	ha	0.133945	1.82405626
16	C16	-1.708	1.633	0.123	c2	-0.317713	10.90757318
17	H162	-2.567	2.295	0.142	ha	0.160393	2.33860489
18	C17	-1.769	0.182	-0.334	c3	0.070291	10.40472434
19	H171	-1.726	0.186	-1.439	hc	0.021437	1.79648409
20	C13	-0.372	-0.366	0.176	c3	0.266833	10.57410738
21	C18	-0.501	-0.910	1.624	c3	-0.331857	7.15351214
22	H181	0.484	-1.102	2.059	hc	0.076482	1.66934731
23	H182	-1.003	-0.182	2.270	hc	0.076482	1.71084648
24	H183	-1.070	-1.845	1.657	hc	0.076482	2.02612741
25	C12	0.322	-1.390	-0.742	c3	-0.151221	9.24886275
26	H121	-0.184	-2.361	-0.705	hc	0.032766	1.94793234
27	H122	0.258	-1.039	-1.783	hc	0.032766	1.68721894
28	C11	1.802	-1.577	-0.362	c3	0.003416	9.18656977
29	H111	1.864	-2.000	0.647	hc	0.018739	1.54105062
30	H112	2.249	-2.322	-1.031	hc	0.018739	1.85092184
31	C9	2.605	-0.266	-0.450	c3	-0.101945	11.28437242
32	H91	2.800	-0.086	-1.523	hc	0.042366	1.79639515
33	C10	4.025	-0.320	0.211	c3	0.416652	11.42862728
34	C19	3.911	-0.442	1.753	c3	-0.149815	6.84847595
35	H191	3.445	-1.392	2.042	hc	0.030785	1.69613391
36	H192	4.898	-0.397	2.224	hc	0.030785	1.84016311
37	H193	3.310	0.371	2.172	hc	0.030785	1.62739712
38	C1	4.838	-1.507	-0.358	c3	-0.110359	8.88077671
39	H11	4.448	-2.452	0.040	hc	0.000629	1.74631583
40	H12	4.700	-1.546	-1.449	hc	0.000629	1.72906295
41	C2	6.339	-1.400	-0.049	c3	-0.033784	8.94577250
42	H21	6.867	-2.286	-0.428	hc	0.006495	2.27185228
43	H22	6.509	-1.366	1.034	hc	0.006495	1.70632121
44	C20	-3.068	-0.582	0.044	c3	0.155761	9.35465557
45	H201	-3.152	-0.602	1.140	hc	0.002741	1.81626404
46	C21	-3.048	-2.035	-0.467	c3	-0.318300	7.39193533
47	H211	-2.959	-2.060	-1.562	hc	0.068930	1.74719391
48	H212	-3.967	-2.565	-0.197	hc	0.068930	2.19576233
49	H213	-2.215	-2.609	-0.054	hc	0.068930	1.82397620
50	C22	-4.316	0.157	-0.501	c3	-0.042846	9.00419394
51	H221	-4.288	1.205	-0.181	hc	0.005802	1.73910424
52	H222	-4.269	0.174	-1.601	hc	0.005802	1.84508832
53	C23	-5.671	-0.422	-0.059	c3	0.071084	8.74013463
54	H231	-5.813	-1.428	-0.475	hc	-0.007237	1.77704838
55	H232	-5.669	-0.536	1.034	hc	-0.007237	1.76963068
56	C24	-6.852	0.460	-0.494	c3	-0.188363	8.72734175
57	H241	-6.765	1.449	-0.016	hc	0.026465	1.82722806
58	H242	-6.773	0.640	-1.577	hc	0.026465	1.86614238
59	C25	-8.252	-0.115	-0.197	c3	0.452439	8.60395444
60	H251	-8.307	-1.112	-0.663	hc	-0.082185	1.95936142
61	C26	-8.514	-0.281	1.308	c3	-0.301590	7.57294713
62	H261	-9.522	-0.675	1.489	hc	0.054393	2.17001359
63	H262	-7.803	-0.969	1.779	hc	0.054393	1.88913793
64	H263	-8.439	0.685	1.826	hc	0.054393	1.78802137
65	C27	-9.340	0.761	-0.839	c3	-0.301590	7.88091443
66	H271	-9.192	0.855	-1.922	hc	0.054393	1.89129709

67	H272	-10.340	0.341	-0.672	hc	0.054393	2.16941131
68	H273	-9.331	1.774	-0.413	hc	0.054393	1.82702304

2.2. Atom numbering for POPC

In Figure 1 we presents the atom numbering convention used for the analysis of the order parameter for the lipid 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC).

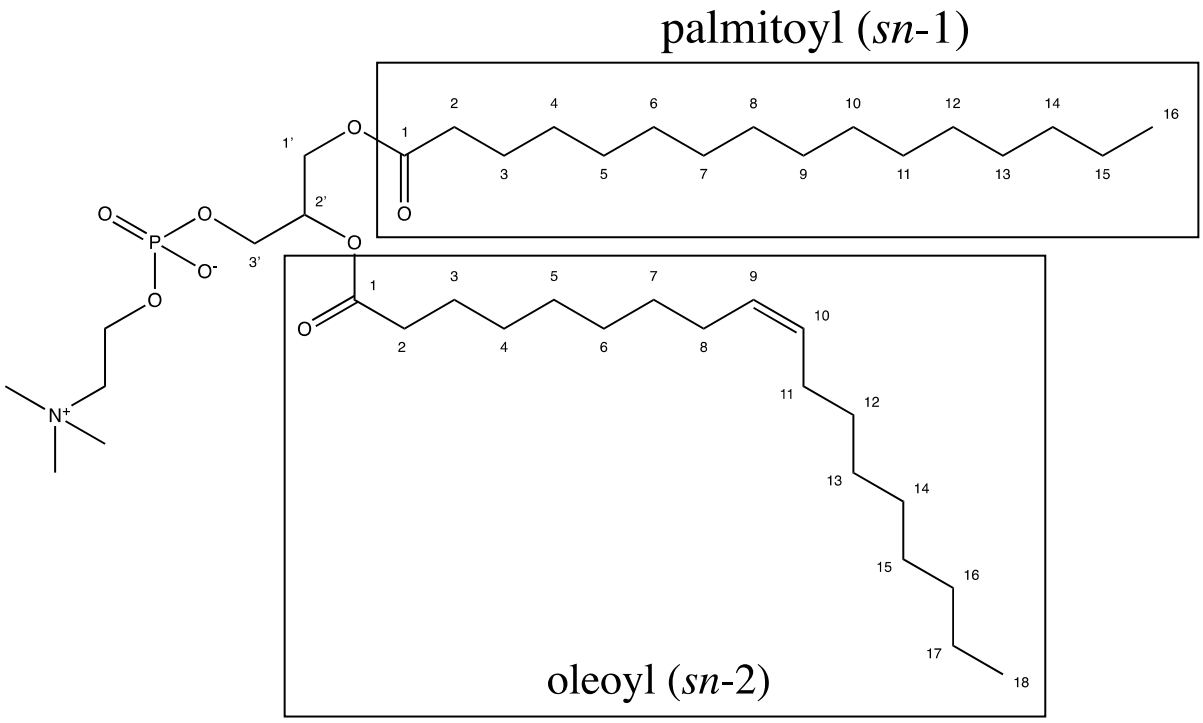

Figure 1: Chemical structure of of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) with the atom numbering of the sn-1, sn-2 used in this study. Atoms labelled with 1', 2' and 3' are those used to extract the rotational autocorrelation function.

2.3. POPC rotational correlation time

The estimation of POPC rotational correlation time was based on a previous study (see Supporting Information of ref. 2). The autocorrelation function $C_f(t)$ is defined by[3]:

$$C_f(t) = \langle f(\xi) f(\xi + t) \rangle_{\xi},$$

where the average is over the time origin ξ. The integral over the time define the correlation time τ_f :

$$\tau_f = \int_0^\infty C_f(t)dt$$

.

We performed the fitting of the rotational correlation function $C_f(t)$ using a double exponential function defined by:

$$y(t) = a_1 e^{-(t/\tau_1)} + a_2 e^{-(t/\tau_2)}$$

.

The mean correlation time is the integral of the fitting function y from time origin 0 to infinity (last frame), this equals to the sum of correlation time τ_1 and τ_2 each one multiplied by their prefactors a_1 and a_2:

$$\tau_f = \int_0^\infty a_1 e^{-(t/\tau_1)} + a_2 e^{-(t/\tau_2)} dt$$

.

In Figure 2 we presents the rotational correlation function $C_f(t)$ for each simulated sterol (cholesterol, probe **8** and probe **10**, continuous lines) together with their double exponential fitting function.

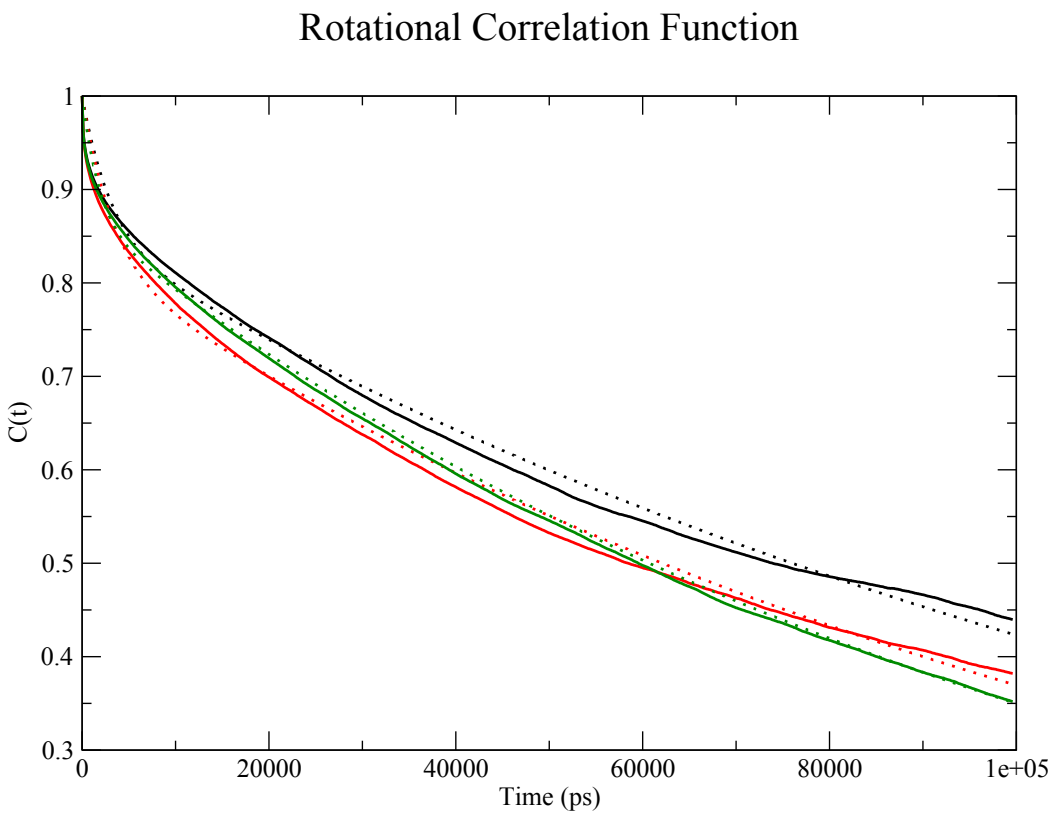

Figure 2: Rotational correlation function (straight lines) and fitting (dotted lines) for: cholesterol (black), probe 8 (red) and probe (10).

3. Bibliography

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