Supplementary information

A generalized approach for NMR studies of lipid-protein interactions based on sparse fluorination of acyl chains

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Supplementary Materials and Methods.

Lipid solution preparation

Lipids ($DHPC_7$ and 4F-$DHPC_7$) were purchased from Avanti Polar Lipids as dry powders. Solutions of the lipids in deuterated methanol or in aqueous buffer (20 mM sodium phosphate pH 7.3) were prepared by weighing the appropriate amount of dry lipid, adding the necessary volume of solvent, and vortexing until complete dissolution.

Protein sample preparation

The pSRII protein sample was prepared as described[1], except that 4F-$DHPC_7$ was used in the final NMR sample instead of $DHPC_7$. In brief, ^{15}N-labeled pSRII was expressed in *E. coli* using M9 minimal medium supplemented with $^{15}NH_4Cl$. The protein was extracted from the membrane using DDM. After His-tag affinity purification, the protein was exchanged into 4F-$DHPC_7$ or $DHPC_7$, respectively, and concentrated. Final NMR samples were prepared in 50 mM sodium phosphate buffer at pH 5.9 and supplemented with additional detergent stock solution to ~ 3% (w/v).

NMR spectroscopy

For NMR signal assignment of 4F-DHPC$_7$ micelles at 298 K, an aqueous 50 mM sample with 20 mM sodium phosphate pH 7.3, 5% ^2H_2O, was used in a 5 mm NMR tube.

2D heteronuclear ^1H,^{19}F COSY, COLOC with and without J_{HF} refocussing (12 ms $^1H \rightarrow ^{19}F$ transfer and optional 5 ms J_{HF} refocussing delay), and TOCSY-COLOC (65 ms MOCCA mixing) were recorded on a Bruker AvanceIII 600 MHz spectrometer equipped with a dual SEF probehead with z-gradients.

2D homonuclear ^1H,^1H DQF-COSY and TOCSY (65 ms DIPSI2 or MOCCA mixing) and heteronuclear ^1H,^{13}C HSQC, HSMQC (= long-range HSQC with suppression of $^1J_{CH}$ correlation and long 30 ms INEPT transfer delay), HSQC-TOCSY (65 ms DIPSI2 mixing) and TOCSY-HSQC (120 ms DIPSI2 mixing) were recorded on a Bruker AvanceIII 800 MHz spectrometer equipped with a cryogenically cooled triple resonance probehead with z-gradients.

Temperature and concentration dependent diffusion coefficients were measured on the cited 600 MHz hardware using double stimulated echo[2] (i.e. convection compensated) ^{19}F and ^1H NMR diffusion experiments with variation of the gradient strength.

The 2D ^1H,^1H TOCSY spectrum on DHPC$_7$ was measured using identical experimental conditions as for 4F-DHPC$_7$.

2D ^1H,^{15}N-TROSY and 3D ^{15}N-edited NOESY-HSQC spectra of pSRII were recorded at 308 K on a Bruker DRX 800 MHz spectrometer equipped with a cryogenically cooled triple resonance probehead with z-gradients. The sample was 0.6 mM ^{15}N-pSRII in 50 mM sodium phosphate buffer pH 5.9, 50 mM NaCl, containing 2.9% DHPC$_7$ or 3.1% 4F-DHPC$_7$. The reassignment of pSRII backbone amide resonances in 4F-DHPC$_7$ micelles (with 84% coverage) was based on proximity to corresponding signals in DHPC$_7$ micelles and pertaining NOE correlations in the 3D NOESY-HSQC spectrum.

Chemical shift perturbations in micelle embedded pSRII caused by the H/F substitution in 4F-DHPC$_7$ were calculated as CSP = $\sqrt{(_{\Delta}\nu_N^2/50 + _{\Delta}\nu_H^2/2)}$ from TROSY amide signal displacements in both ^{15}N and ^1H dimensions. Average CSP and standard deviation were computed from all reassigned backbone amide signals (n = 194).

The ratio of amide signal intensities (maxima) in 4F-$DHPC_7$ versus $DHPC_7$ micelles was calculated for all non-overlapping TROSY signals (n = 131). For each spectrum, all signal intensities were normalized by division by the average intensity of the most intense 10 C-terminal residues that do not insert into the micelle (residues 222, 224, 228, 229, 231, 233, 234, 238 and 240). Errors were calculated by propagation using the noise level N (RMSD) in each spectrum:

$$\Delta(I_{4F\text{-}DHPC7}/I_{DHPC7}) = \sqrt{(N_{4F\text{-}DHPC7}/I_{4F\text{-}DHPC7})^2 + (N_{DHPC7}/I_{DHPC7})^2}$$

Estimation of the Critical Micelle Concentration

The critical micelle concentration (C_{CMC}) for $DHPC_7$ and 4F-$DHPC_7$ was obtained from NMR diffusion measurements at varying lipid concentrations following the procedure reported for $DHPC_6$[3]. Briefly, above the critical micelle concentration, lipid molecules rapidly exchange between free monomeric and micelle bound states; the observed diffusion coefficient is therefore a weighted average:

$$D_s = D_m \cdot (C_{total} - C_{free}) / C_{total} + D_f \cdot C_{free} / C_{total} \quad (1)$$

where D_s is the observed diffusion coefficient, D_m the diffusion coefficient of the micelle, D_f the diffusion coefficient of the free monomeric lipid; C_{total} and C_{free} are the total and free lipid concentrations, respectively. For $C_{total} > C_{CMC}$ the free monomeric lipid concentration reaches its maximum $C_{free,max} = C_{CMC}$. For $C_{total} < C_{CMC}$ no micelle formation is observed and all lipid remains in the free monomeric state: $C_{free} = C_{total}$. At low volume fractions, $\phi \leq 0.15$, the diffusion of the micelle is well approximated by

$$D_m = D_m^{\,0} \cdot (1 - 3.2 \cdot \lambda \cdot \phi) \quad (2)$$

where $D_m^{\,0}$ is the extrapolated micelle diffusion coefficient at infinite lipid dilution, λ is a factor depending on the micelle shape and here assumed to be 1 (as for non-interacting spheres), and ϕ is the micelle volume fraction defined as

$$\phi = (C_{total} - C_{free}) \cdot w/d \quad (3)$$

where w is the lipid molecular weight and d its density[4]. To calculate ϕ for 4F-DHPC$_7$ we assumed the same lipid density as for DHPC$_7$ (1.1 g/cm^3). The obstructed diffusion of the free lipid in the presence of micelles[5] is given by

$$D_f = D_f^{\,0}/(1+\phi/2) \tag{4}$$

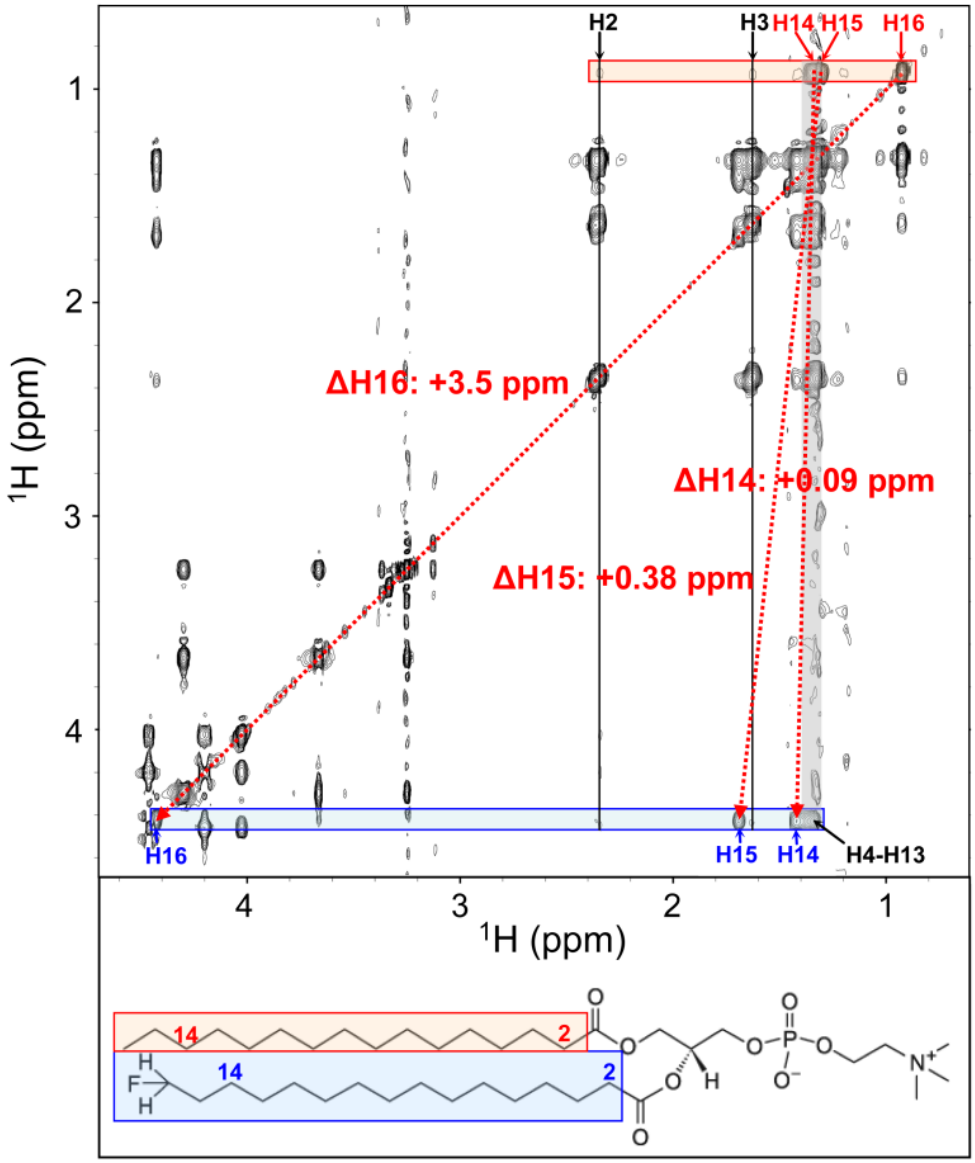
$C_{CMC} = C_{free,max}$ and the self-diffusion coefficient of the micelle at infinite lipid dilution, $D_m^{\,0}$, are extrapolated by fitting the measured concentration dependent diffusion coefficients at $C_{total} > C_{CMC}$ to equations (1) and (2), respectively. The self-diffusion coefficient of the free lipid at infinite dilution, D_f^0, was approximated as the average of several diffusion measurements at $C_{total} < C_{CMC}$.

The hydrodynamic micelle radius, r_m, was derived from $D_m^{\,0}$ by referencing to water:

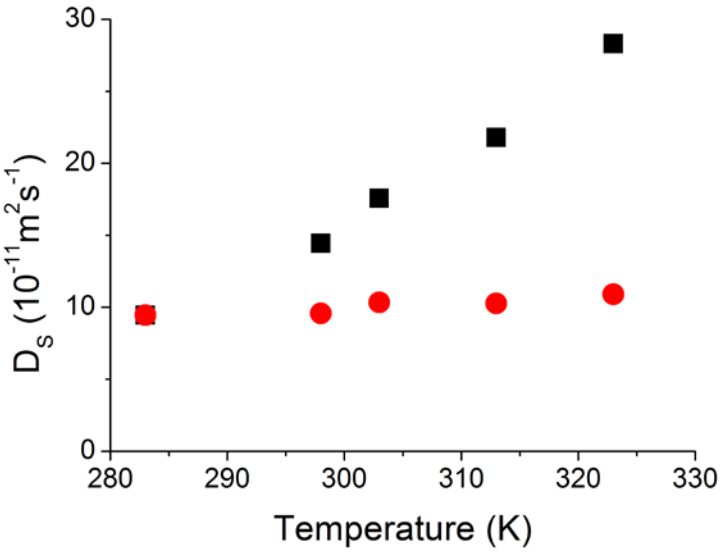
$$r_m = (D^0_{H2O}/D_m^{\,0}) \cdot r_{H2O} \tag{5}$$

where D^0_{H2O} is the self-diffusion coefficient of water at same 298 K ($2.3\cdot10^{-9}$ m^{2}s^{-1}, confirmed by NMR) and r_{H2O} its tabulated hydrodynamic radius (1.06 Å).

Supplementary Figures

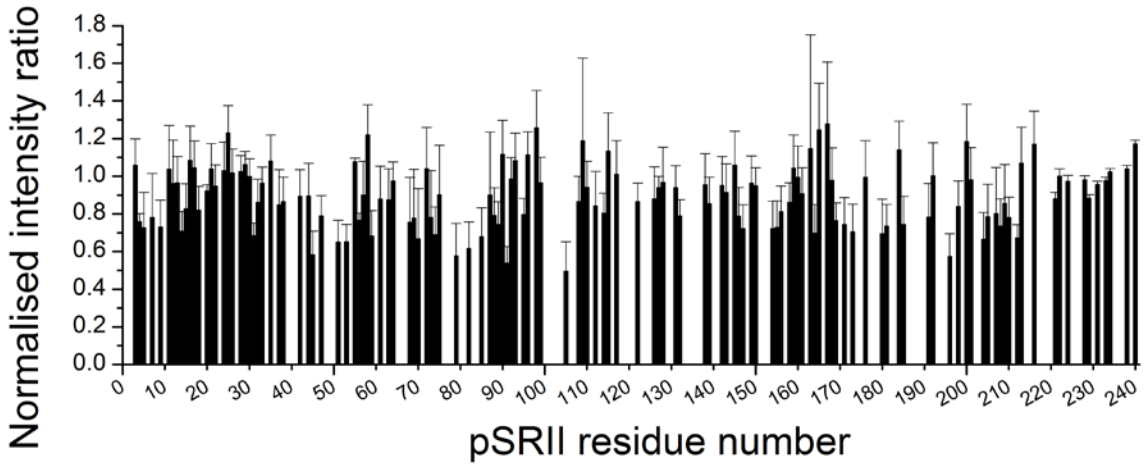


Supplementary Figure S1. Acyl chain region of the ^{1}H,^{1}H-TOCSY spectrum of 1-palmitoyl-2-(16-fluoropalmitoyl)-sn-glycero-3-phosphocholine in CD_3OD, recorded at 298 K and 600 MHz. The chemical structure of this commercially available lipid (Avanti Polar Lipids) is shown below the spectrum. Colors distinguish the unmodified (red) and terminally monofluorinated (blue) acyl chains. A comparison of corresponding ^{1}H signal frequencies in each chain yields the indicated signal shifts for H16, H15 and H14 induced by the single fluorine atom on carbon atom 16.

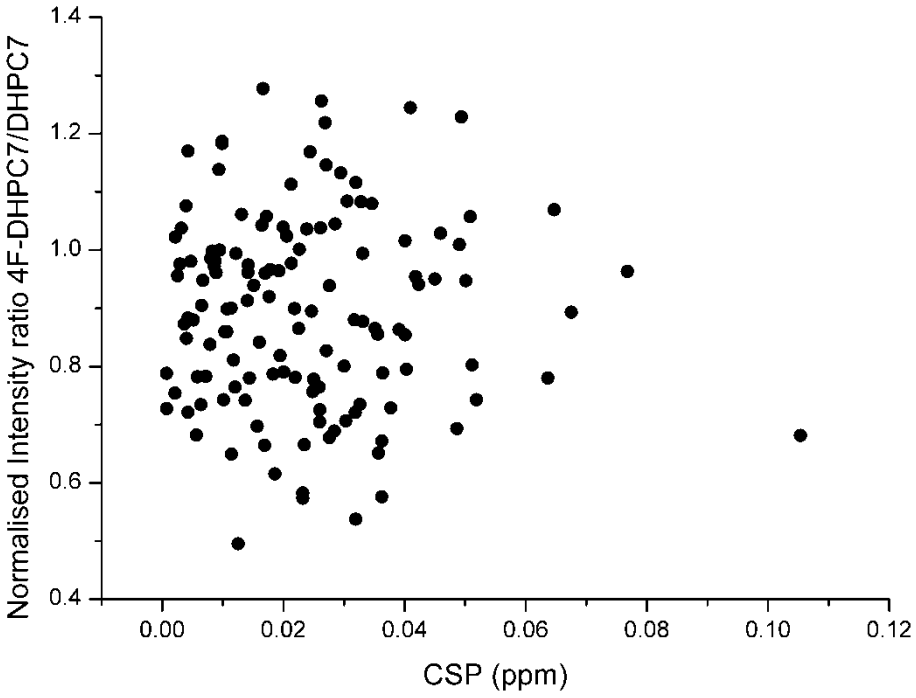


Supplementary Figure S2. Temperature dependent diffusion coefficient of 4F-DHPC$_7$ micelles. The normalised micelle diffusion coefficients (red dots), relative to water self-diffusion, were obtained by dividing the measured micelle diffusion coefficients (black squares) by $f = D_{water}[T] / D_{water}[283\ K]$. Their invariance confirms 4F-DHPC$_7$ micelle stability up to at least 323 K. The data was measured on an aqueous sample of 50 mM 4F-DHPC$_7$ in 20 mM sodium phosphate pH 7.3.

a)



b)



Supplementary Figure S3. (**a**) Normalised ratios of TROSY signal intensities in 4F-$DHPC_7$ versus $DHPC_7$ micelles, plotted against the pSRII residue number. All signal intensities of a spectrum were normalised by division by the pertaining average intensity of the most intense 10 C-terminal residues that do not insert into the micelle. (**b**) Correlation plot of normalised signal intensity ratios *vs* CSP showing *un*correlation between both parameters ($R^2 = 0.001$).

Supplementary Table S1. NMR signal assignment for 4F-DHPC$_7$ micelles (50 mM, 20 mM phosphate buffer pH 7.3, 298 K)

	Carbon position	Chemical shifts		
		^{1}H δ (ppm) [d]	^{13}C δ (ppm) [d]	^{19}F δ (ppm) [e]
Acyl chain 1	1 [c]	-	176.97; 177.00 177.01; 177.04	
	2	2.48 (x2)	32.3	-
	3 [a]	1.87^{syn} ($^{3}J_{HF}$ = 6-11 Hz) 1.88^{anti} ($^{3}J_{HF}$ = 36 Hz)	32.4	-
	4 [c]	4.53	96.5	-181.807; -181.801 -181.770; -181.742
	5 [a]	1.60^{syn} ($^{3}J_{HF}$ = 9-14 Hz) 1.51^{anti} ($^{3}J_{HF}$ = 32 Hz)	39.2	-
	6	1.36; 1.44	20.5	-
	7	0.90 (×3)	15.9	-
Acyl chain 2	1 [c]	-	176.75; 176.76 176.79; 176.80	-
	2	2.53 (×2)	32.5	-
	3 [a]	1.90^{syn} ($^{3}J_{HF}$ = 11-20 Hz) 1.92^{anti} ($^{3}J_{HF}$ = 34-41 Hz)	32.4	-
	4 [c]	4.56	96.6	-181.694; -181.689 -181.525 (×2)
	5 [a]	1.61^{syn} ($^{3}J_{HF}$ = 13 Hz) 1.54^{anti} ($^{3}J_{HF}$ = 32.5 Hz)	39.3	-
	6	1.37; 1.44	20.5	-
	7	0.91 (×3)	15.9	-
sn2-Glycerol	1' [b]	4.41^{syn} ($^{3}J_{HH}$ = 3 Hz) 4.27^{anti} ($^{3}J_{HH}$ = 15 Hz)	65.5	-
	2'	5.29	73.4	-
	3' [b]	4.02^{syn} ($^{3}J_{HH}$ < 5 Hz) 4.04^{anti} ($^{3}J_{HH}$ > 10 Hz)	66.3	-
Choline	1''	4.30 (×2)	62.0	-
	2''	3.66 (×2)	68.6	-
	Trimethyl	3.22 (×9)	56.5 (×3)	-

(a) *syn/anti* conformations with respect to F
(b) *syn/anti* conformations with respect to *sn2*-glycerol H2'
(c) Up to 4 distinct signals for ^{13}CO and ^{19}F due to resolved (R,S), (R,R), (S,R), and (S,S) diastereomers from non-stereospecific fluorination at both alkyl C4
(d) direct referencing against internal DSS
(e) indirect referencing against ^{1}H using IUPAC ratio Ξ_F %= 94.094011[6].

Supplementary Table S2. NMR backbone amide signal assignment[a] for pSRII in $DHPC_7$ or 4F-$DHPC_7$ micelles (in 50 mM sodium phosphate buffer pH 5.9, 308 K), and resulting fluorine induced chemical shift perturbations, $CSP = \sqrt{({}_{\Delta}\nu_N{}^2/50 + {}_{\Delta}\nu_H{}^2/2)}$.

Residue number	pSRII in $DHPC_7$		pSRII in 4F-$DHPC_7$		CSP
	^1H_N	^{15}N	^1H_N	^{15}N	
1					
2	8.01588	119.32981	7.99318	119.5099	0.0301047
3	8.55974	111.4296	8.53073	111.31877	0.02581581
4	8.04694	119.25723			
5	8.39337	107.3323	8.36107	107.22444	0.0274649
6	7.55352	116.61072	7.53853	116.51818	0.01684111
7	7.3973	119.09193	7.35508	119.07815	0.02991759
8					
9	8.68366	119.02623	8.62265	118.93335	0.04509594
10	8.25907	119.31372	8.24084	119.20489	0.020076
11	8.02032	104.3809	7.99187	104.24052	0.02826362
12	8.43554	122.36931	8.40273	122.35102	0.02334392
13	8.92287	115.62043	8.89045	115.69415	0.02518374
14	8.74791	106.81066	8.7213	106.62178	0.03267352
15	8.40481	116.8758	8.39221	116.802	0.01372257
16	8.89729	124.25857	8.89408	124.48201	0.0316806
17	8.54916	118.02895	8.57158	118.16151	0.0245514
18	7.86354	103.34783	7.86401	103.23583	0.01584268
19	8.28832	116.04685	8.27888	116.06923	0.00738743
20	8.11554	117.85406	8.15865	118.0935	0.04556168
21	8.05954	120.38993	8.0675	120.53351	0.02107095
22	8.63501	117.30173	8.65554	117.61681	0.04686415
23	8.46044	120.35039	8.41276	120.64453	0.05354492
24	8.69955	117.48323	8.72582	117.74758	0.04174536
25	8.68079	120.0202	8.71062	120.30789	0.04582821
26	7.68615	102.95429	7.7336	103.00484	0.03430535
27	7.22432	119.84483	7.2608	120.00241	0.03408848
28	7.85211	117.09571	7.82191	117.01368	0.02430223
29	7.20398	122.34813	7.18351	122.3024	0.01585355
30	8.81876	108.7873	8.82107	108.75842	0.00439877
31	8.7196	114.60407	8.71807	114.63453	0.00444147
32	9.04361	110.13482	9.03089	110.18272	0.01125999
33	8.05983	118.13235			
34	8.24631	120.26926	8.22804	120.19747	0.01643084
35	8.46442	114.90384	8.41932	114.75167	0.03847232
36	7.61675	118.01599	7.60481	117.90097	0.01832686
37	7.2429	115.9634	7.2419	115.97078	0.00126067
38	8.69631	116.98724	8.63923	116.89844	0.04227023
39	7.62576	115.38754			
40	7.3937	118.08532	7.36266	118.04847	0.0225588
41					
42	8.46507	105.72566	8.37481	105.46527	0.07368509
43					
44	7.89779	108.81712	7.9236	108.74353	0.02100923

45	8.95068	113.95794	8.95628	114.13679	0.0256013
46	8.10789	119.66091	8.10414	119.70947	0.00736157
47	6.74219	119.94194	6.73649	119.95588	0.00448681
48	8.53649	119.15053	8.52179	119.41303	0.03855088
49	7.5492	114.66705	7.55136	114.80548	0.01963645
50	8.44727	121.52553	8.41845	121.52473	0.02037913
51	8.84655	114.47724	8.85486	114.49153	0.00621387
52	8.18479	120.72057	8.20918	120.89787	0.03043258
53	8.31105	112.88612	8.35247	112.92952	0.02992456
54	7.54672	117.67539			
55	8.3825	123.912			
56	7.33852	114.94415	7.34729	115.15839	0.03092623
57	8.08367	106.33909	8.0906	106.27934	0.00976799
58	7.67748	121.97358	7.7053	122.12862	0.02945716
59	8.8655	111.27755	8.72474	110.9256	0.11128371
60	8.49494	121.77906	8.43832	121.52748	0.05356083
61	9.08529	126.18424	9.12099	126.28568	0.02903526
62					
63	8.48562	125.43025	8.47239	125.44827	0.00969592
64	8.88415	127.50292	8.87191	127.42127	0.01443064
65	8.50339	119.8926	8.49475	120.01207	0.01796626
66	8.65735	120.50941	8.66173	120.48985	0.0041526
67	8.05142	117.16085	8.05261	117.17314	0.00193104
68	9.25437	127.93552	9.23881	127.90263	0.01194537
69	8.06043	126.24615	8.05542	126.25965	0.00402431
70	8.63721	122.84449	8.60459	122.79066	0.02428962
71					
72	6.4211	115.74822	6.39236	115.67549	0.02277689
73	6.20311	111.62483	6.2124	111.68281	0.01050646
74	7.08283	116.8649	7.07848	117.08338	0.03105047
75	7.66143	116.36234	7.66802	116.37285	0.00489114
76	7.99571	114.29506			
77	8.34717	119.22127	8.36507	119.36869	0.02438971
78	7.5526	114.91784			
79	9.8484	106.5049	9.87013	106.72718	0.03498949
80	8.22388	116.80171	8.2508	116.82963	0.01944052
81					
82	6.77071	116.96086	6.78144	116.84684	0.01782071
83	7.82871	121.73435			
84	8.17519	117.39151			
85	9.03615	121.49344	9.06894	121.50161	0.0232148
86	8.60188	120.63854			
87	8.51155	115.6272	8.52993	115.72329	0.01880367
88	9.40461	107.25347	9.42274	107.34229	0.01794793
89	8.94276	125.08738	8.9303	125.026	0.01236834
90	7.71275	118.92325	7.73377	119.03812	0.02201869
91	8.14711	115.91991	8.18402	115.92204	0.02610105
92	7.6109	104.73063	7.61439	104.74794	0.00347603
93	7.30342	117.23057	7.3266	117.38162	0.02692542
94	8.43056	119.88327			

95	8.62226	114.02511	8.60822	114.28491	0.03805892
96	8.28037	122.34454	8.28816	122.23103	0.01697152
97	8.30101	118.46605	8.26097	118.45983	0.02832622
98	8.58227	117.25222	8.52995	117.30313	0.03768989
99	8.2335	104.20493	8.14449	103.8446	0.08098237
100	7.81024	122.56608	7.6754	122.17006	0.11057825
101	7.71847	115.05241	7.57541	115.10945	0.10147982
102	8.58137	119.63832	8.55317	119.20655	0.06423493
103	8.01069	121.70673			
104	8.2733	119.96174	8.23498	119.91435	0.02791285
105	8.09853	114.31413	8.08409	114.23396	0.01525783
106	8.11667	113.5861	8.10461	113.56422	0.00907174
107	8.23116	120.35207	8.22768	120.43641	0.01217867
108	7.62875	117.11521	7.64293	116.99862	0.01929769
109	7.43818	111.67095	7.435	111.5983	0.01051745
110	8.88497	117.84441	8.90863	118.0947	0.03915098
111	8.57873	120.3958	8.61707	120.47812	0.0295044
112	8.16424	101.86954	8.16273	101.78568	0.01190756
113	8.03214	121.10481	8.02697	121.22367	0.01720228
114	8.77497	118.3442	8.81731	118.63214	0.05054233
115	8.58443	100.39578	8.61863	100.46808	0.02625578
116	7.8591	120.5329	7.86913	120.57688	0.0094332
117	7.16693	111.83552	7.20363	112.1133	0.04708163
118	7.2585	120.3779	7.2842	120.65664	0.04340697
119					
120	7.91715	104.76173			
121	8.6428	119.3443	8.59501	119.24994	0.03633206
122	10.34065	121.65746	10.28001	121.69361	0.04318265
123	8.13082	119.62641			
124	8.07638	118.05627			
125	7.88217	121.36752	7.81268	121.54089	0.05491423
126	7.04021	117.52939	6.99825	117.37251	0.03704791
127	7.94146	118.05103	7.94251	118.08116	0.00432523
128	8.4536	104.73119	8.43818	104.86243	0.02152596
129	7.91512	119.61841			
130	7.94244	104.99138	7.92825	104.81572	0.02679192
131	8.59911	123.25191	8.6188	123.40942	0.02626854
132	7.95524	116.04786	7.99015	116.18101	0.03104726
133	7.83447	120.0384	7.84079	120.1286	0.01351636
134	8.40986	118.27922	8.41297	118.33915	0.00875603
135	8.07296	119.28564			
136	7.86326	104.87212	7.87472	104.83355	0.00976825
137	8.42874	123.6168	8.43824	123.74253	0.01900752
138	8.6227	118.12883	8.64886	118.38454	0.04061927
139	8.46483	118.5343	8.51129	118.65534	0.03704429
140	8.18857	119.60594	8.23118	119.60155	0.03013622
141	8.36112	116.27533			
142	8.54061	114.6798	8.59306	114.79659	0.04059925
143	8.1584	110.74703	8.16397	110.80495	0.00908884
144					

145	8.69903	113.48235	8.74736	113.70008	0.04600023
146	6.96744	114.14154	6.95399	114.27191	0.02074555
147	8.08674	123.53086	8.11057	123.52156	0.0169016
148	7.97875	114.55231	7.97338	114.53726	0.00435299
149	7.88823	122.63892	7.89462	122.65235	0.00490136
150	7.7721	109.20257	7.77318	109.22447	0.00318989
151	7.42213	117.25484	7.42222	117.27958	0.00349934
152	7.43463	118.01357	7.43795	118.03745	0.00411294
153	7.94788	112.60695	7.96166	112.65517	0.01189317
154	8.90408	116.18264	8.89754	116.15473	0.0060799
155	9.01441	109.83095	9.00734	109.83847	0.00511111
156	7.47172	123.08527	7.44752	123.14456	0.01905587
157	8.54116	120.31088	8.5325	120.28896	0.00686349
158	8.2225	111.24309	8.20357	111.2214	0.0137325
159	7.58593	122.21734	7.58758	122.3304	0.01603161
160	8.98497	117.72955	8.96832	117.68733	0.01320083
161	8.51122	117.08651	8.49398	117.01096	0.01621002
162	7.60909	118.13443			
163	8.10839	116.70579			
164	9.47243	123.86079	9.45373	123.77883	0.01758391
165	8.58179	118.5436	8.5349	118.30778	0.0470272
166					
167	7.73421	113.33741	7.70873	113.29942	0.01880106
168	8.74715	116.15078	8.7108	116.17808	0.02599167
169	7.37199	111.54291	7.35051	111.61266	0.01811067
170	7.79072	112.62521	7.80827	112.75874	0.0225966
171	9.77459	118.42625	9.78094	118.50825	0.01243548
172	7.61792	115.30934	7.60204	115.30112	0.01128887
173	7.71489	112.82692	7.73366	112.92449	0.01914561
174	7.43744	117.77571	7.36047	117.71782	0.05503831
175					
176	7.41558	114.94565	7.41489	115.16254	0.03067676
177	7.74635	119.13758			
178	8.05538	122.29167	8.05576	122.53666	0.03464786
179	8.03183	112.86281			
180	8.29218	112.48562	8.3548	112.55314	0.04529692
181	7.94987	109.72956	7.9335	109.72145	0.01163202
182					
183					
184	7.04132	104.90199	7.0342	104.84106	0.0099798
185	8.97975	119.11605	9.03346	119.31105	0.04693487
186	7.91372	119.49049	7.91067	119.52727	0.00563086
187					
188	7.90985	114.76922	7.93075	114.71086	0.01692698
189	7.77851	112.62622			
190					
191	7.71327	108.1491			
192	7.14345	120.77378	7.10364	120.78382	0.02818571
193					
194	8.04494	114.1569			

195	8.27053	120.32993	8.27076	120.45952	0.01832752
196	8.41167	113.73246	8.43952	113.78622	0.02110957
197	7.91129	117.19647	7.88619	117.1741	0.01802813
198	8.60109	121.31258	8.59146	121.26573	0.00950089
199	7.40074	117.58167			
200	8.46436	117.02269			
201	9.59216	120.15028	9.57379	120.12727	0.01339095
202	8.37482	118.27339	8.37698	118.25793	0.00266703
203	8.2755	118.96341	8.24853	118.99818	0.0196944
204	9.27087	109.6865	9.26168	109.59566	0.01439674
205	7.551	113.09499	7.55181	113.0837	0.00169627
206			7.71396	120.19254	
207	8.99755	109.09834	9.01845	109.25732	0.02690535
208	7.36058	120.35045	7.39725	120.35197	0.0259305
209	7.96069	102.98621	7.96806	103.24535	0.03701661
210	9.07354	120.72301	9.14386	120.94896	0.059106
211	8.3745	120.42333			
212	7.67748	121.28091	7.69999	121.51864	0.0371976
213	8.54931	116.22209	8.62468	116.39413	0.05858561
214	8.29441	119.90042	8.27015	119.79231	0.02297889
215	7.77865	120.08745	7.79424	120.23783	0.02395427
216	8.12488	120.11994	8.12723	120.31434	0.02754248
217					
218	7.87286	113.7233	7.86026	113.72123	0.00891435
219	7.95101	121.16706	7.97089	121.27528	0.02078073
220	7.95192	117.70546	7.95737	117.63996	0.01003276
221	7.74502	121.32749	7.73421	121.28313	0.00988859
222	8.00884	117.71287	8.00687	117.66857	0.00641796
223	8.20278	117.13562	8.17933	117.1436	0.01662001
224	8.28246	108.67407	8.26655	108.63396	0.01259922
225	8.36561	120.1377	8.35115	120.14182	0.01024135
226	8.34101	115.95158	8.33427	115.94823	0.00478939
227	8.30838	123.59404	8.29899	123.56776	0.00760913
228	8.16158	123.29263	8.15279	123.25671	0.00802726
229	8.27868	107.6001	8.27321	107.58666	0.00430965
230	7.89059	117.90049	7.88592	117.90217	0.00331072
231	8.45566	123.043	8.45177	123.05744	0.00342583
232	8.05731	113.06021	8.05392	113.06936	0.00272406
233	8.36764	122.07413	8.36532	122.08192	0.00197608
234	8.05118	116.09285	8.04734	116.09403	0.00272041
235					
236	8.34644	123.69866	8.3442	123.7155	0.00286016
237	7.97622	118.2583	7.9743	118.26295	0.00150853
238	8.26521	126.13966	8.26234	126.15503	0.00297375
239	8.16089	118.60048	8.15807	118.60298	0.00202514
240	8.11537	121.60123	8.10731	121.5831	0.00624946
241	8.2031	118.63339	8.20214	118.72119	0.01243534

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