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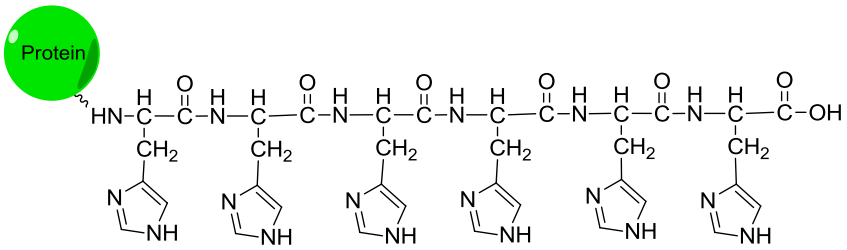
Solubilization of IMP by Noncovalent Chelation with mPEG Polymers

To conduct *in vitro* structure-function studies of Integral Membrane Proteins (IMPs), one usually employs detergents to extract the protein from the lipid membrane and for its solubilization.[1] This can be technically challenging and can lead to protein denaturation and precipitation because the amphiphile may disrupt interactions between the protein and the lipid membrane which stabilize the three-dimensional structure.[1] To overcome these obstacles, a method was developed in which hydrophilic polymers, such as monomethoxypolyethylene glycol (mPEG), are coupled to IMPs under mild conditions in place of detergents.[1]

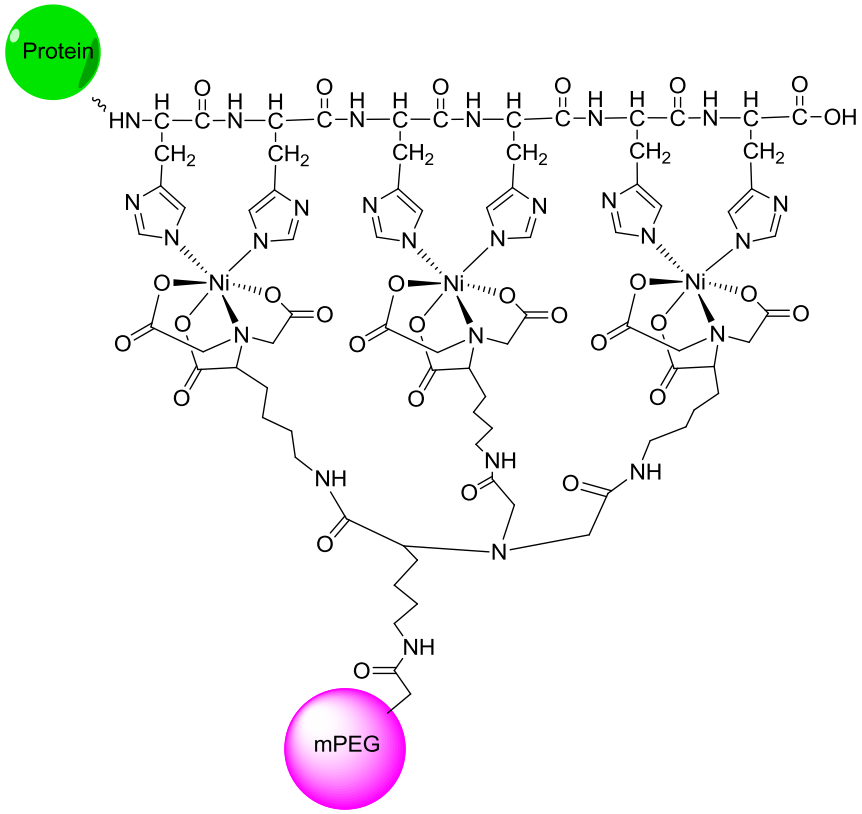
In this approach, the researchers took advantage of the ability to add a hexa-histidine tag (i.e., a series of six histidine residues) to the N-terminal end of the protein (Scheme 1)[1] through genetic recombination and with only minimal effects on protein structure and function.[1] The researchers achieved solubilization with two mPEG polymers of different length by reversible attachment to the hexa-histidine tags through nickel (Ni^{2+})-chelated NTA (nitrilotriacetic acid), see Scheme 2.[1]

This method allows for the proteins to be solubilized in low-detergent or aqueous buffer solutions.[1] The mPEG polymers were added to the recombinant IMPs.[1] After mixing, the detergent was completely removed, leaving behind the chelated proteins.[1] The solubilized protein did not change in its backbone configuration (and therefore has not been denatured).[1] The spectrum shows the activity of the chelated protein, which is similar to the spectra obtained from the native protein, which can be seen in Figure 1.[1]

(1) *Solubilization of Native Integral Membrane Proteins in Aqueous Buffer by Noncovalent Chelation with Monomethoxy Poly(ethylene glycol) (mPEG) Polymers*. Janarante, T. K.; Okach, L.; Brock, A.; Lesley, S. A.; *Bioconjugate Chemistry* **2011**, *22*, 1513-1518.



Scheme 1. Hexa-histidine tag connected to protein.



Scheme 2. Hexa-histidine tagged IMP bound to mPEG-$NTA_3(Ni^{2+})_3$.

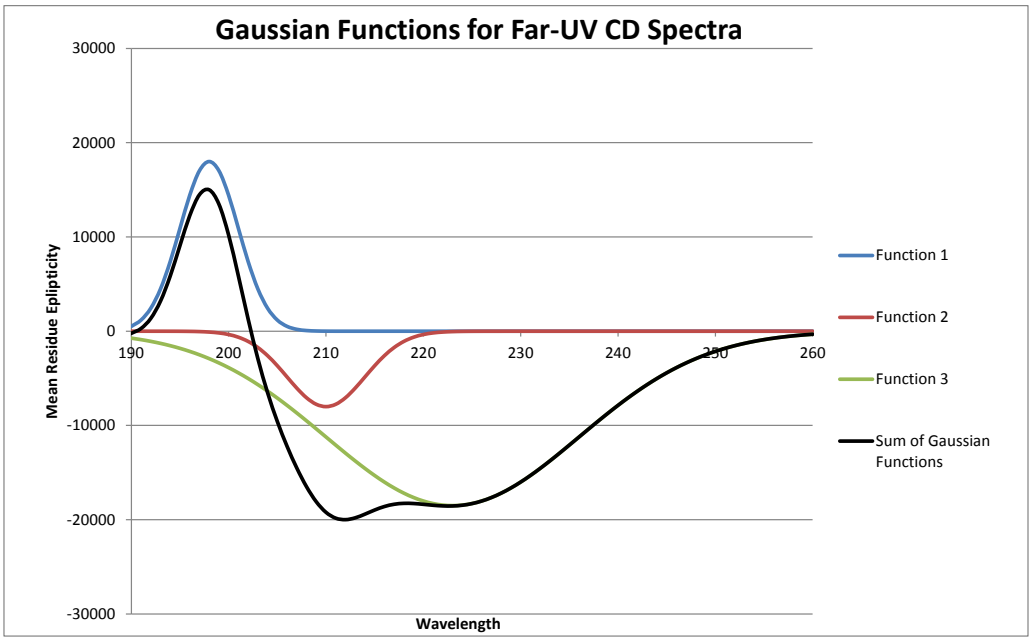


Figure 1. Simulated Far-UV circular dichroism spectrum of DGK-mPEG(10K)-$NTA_3(Ni^{2+})_3$.

Function 1	Function 2	Function 3
x = wavelength	x = wavelength	x = wavelength
height = 18000	height = -8000	height = -18500
max = 0.132	max = 0.099	max = 0.031
$a = \lambda_{max}$, 198 nm	a = 210 nm	a = 223 nm

Spectrum By Gaussian Fit Data — Submitted By Lauren Ahrens and Craig McKinney

λ_{max}	198	210	223
height	18000	-8000	-18500
width	3	4	13

	$f_1(\lambda)$		$f_2(\lambda)$		$f_3(\lambda)$		$f_4(\lambda)$	
x	Original	Normalized	Original	Normalized	Orignal	Normalized	Original	Normalized
190	0.003798662	514.1790141	3.717E-07	-0.02981323	0.001223816	-737.7702929	0.00502285	-223.62109
191	0.00874063	1183.113515	1.257E-06	-0.10085684	0.001483318	-894.2096113	0.01022521	288.803047
192	0.017996989	2436.035098	3.996E-06	-0.32052238	0.001787239	-1077.426649	0.01978822	1358.28793
193	0.033159046	4488.339758	1.193E-05	-0.95690307	0.002140727	-1290.524647	0.0353117	3196.85821
194	0.054670025	7400.021229	3.346E-05	-2.68370102	0.002549001	-1536.650506	0.05725248	5860.68702
195	0.080656908	10917.55187	8.815E-05	-7.07061046	0.003017235	-1818.922158	0.08376229	9091.55911
196	0.106482669	14413.27325	0.0002182	-17.4999289	0.003550408	-2140.34279	0.11025125	12255.4305
197	0.125794409	17027.27044	0.0005073	-40.6885538	0.004153151	-2503.70274	0.13045482	14482.8791
198	0.13298076	18000	0.001108	-88.8719723	0.004829558	-2911.470576	0.13891828	14999.6575
199	0.125794409	17027.27044	0.0022734	-182.353447	0.005582995	-3365.675616	0.13365079	13479.2414
200	0.106482669	14413.27325	0.0043821	-351.495469	0.006415896	-3867.784934	0.11728064	10193.9928
201	0.080656908	10917.55187	0.0079349	-636.47607	0.007329554	-4418.578638	0.09592138	5862.49717
202	0.054670025	7400.021229	0.0134977	-1082.68227	0.008323923	-5018.027882	0.07649169	1299.31108
203	0.033159046	4488.339758	0.0215693	-1730.12133	0.009397422	-5665.180631	0.0641258	-2906.9622
204	0.017996989	2436.035098	0.0323794	-2597.21974	0.010546774	-6358.060607	0.06092316	-6519.2452
205	0.00874063	1183.113515	0.0456623	-3662.66689	0.011766865	-7093.584971	0.06616977	-9573.1384
206	0.003798662	514.1790141	0.0604927	-4852.24528	0.013050648	-7867.506265	0.07734199	-12205.573
207	0.001477283	199.9619377	0.0752844	-6038.71682	0.014389099	-8674.38372	0.09115074	-14513.139
208	0.000514093	69.58656251	0.0880163	-7059.97522	0.015771222	-9507.5884	0.10430165	-16497.977
209	0.00016009	21.66947991	0.096667	-7753.86588	0.017184121	-10359.34567	0.11401124	-18091.542
210	4.46101E-05	6.038327302	0.0997356	-8000	0.018613133	-11220.8172	0.11839331	-19214.779
211	1.11236E-05	1.50567025	0.096667	-7753.86588	0.020042036	-12082.22337	0.11672019	-19834.584
212	2.48202E-06	0.335960444	0.0880163	-7059.97522	0.021453316	-12933.00494	0.10947213	-19992.644
213	4.95573E-07	0.067079757	0.0752844	-6038.71682	0.022828492	-13762.02165	0.09811335	-19800.671
214	8.85434E-08	0.011985051	0.0604927	-4852.24528	0.024148504	-14557.78296	0.08464127	-19410.016
215	1.41563E-08	0.001916167	0.0456623	-3662.66689	0.025394136	-15308.70498	0.07105642	-18971.37
216	2.02529E-09	0.00027414	0.0323794	-2597.21974	0.026546475	-16003.38589	0.05892588	-18600.605
217	2.59282E-10	3.50958E-05	0.0215693	-1730.12133	0.027587383	-16630.89078	0.04915671	-18361.012
218	2.9703E-11	4.02054E-06	0.0134977	-1082.68227	0.028499966	-17181.0363	0.04199771	-18263.719
219	3.04491E-12	4.12152E-07	0.0079349	-636.47607	0.029269034	-17644.66469	0.03720395	-18281.141
220	2.79314E-13	3.78074E-08	0.0043821	-351.495469	0.029881518	-18013.89696	0.03426359	-18365.392
221	2.29275E-14	3.10342E-09	0.0022734	-182.353447	0.030326838	-18282.35547	0.03260023	-18464.709
222	1.68409E-15	2.27955E-10	0.001108	-88.8719723	0.030597209	-18445.34716	0.03170517	-18534.219
223	1.10693E-16	1.49831E-11	0.0005073	-40.6885538	0.030687868	-18500	0.03119513	-18540.689
224	6.51056E-18	8.81256E-13	0.0002182	-17.4999289	0.030597209	-18445.34716	0.03081538	-18462.847
225	3.42659E-19	4.63816E-14	8.815E-05	-7.07061046	0.030326838	-18282.35547	0.03041499	-18289.426
226	1.61381E-20	2.18441E-15	3.346E-05	-2.68370102	0.029881518	-18013.89696	0.02991498	-18016.581
227	6.8012E-22	9.20596E-17	1.193E-05	-0.95690307	0.029269034	-17644.66469	0.02928096	-17645.622
228	2.56487E-23	3.47175E-18	3.996E-06	-0.32052238	0.028499966	-17181.0363	0.02850396	-17181.357
229	8.65544E-25	1.17158E-19	1.257E-06	-0.10085684	0.027587383	-16630.89078	0.02758864	-16630.992
230	2.61372E-26	3.53787E-21	3.717E-07	-0.02981323	0.026546475	-16003.38589	0.02654685	-16003.416
231	7.06273E-28	9.55997E-23	1.032E-07	-0.00827883	0.025394136	-15308.70498	0.02539424	-15308.713
232	1.70778E-29	2.31162E-24	2.692E-08	-0.00215966	0.024148504	-14557.78296	0.02414853	-14557.785
233	3.69519E-31	5.00173E-26	6.598E-09	-0.00052925	0.022828492	-13762.02165	0.0228285	-13762.022
234	7.15461E-33	9.68434E-28	1.519E-09	-0.00012184	0.021453316	-12933.00494	0.02145332	-12933.005
235	1.2396E-34	1.67789E-29	3.285E-10	-2.635E-05	0.020042036	-12082.22337	0.02004204	-12082.223
236	1.92185E-36	2.60138E-31	6.674E-11	-5.3533E-06	0.018613133	-11220.8172	0.01861313	-11220.817
237	2.66628E-38	3.60902E-33	1.274E-11	-1.0217E-06	0.017184121	-10359.34567	0.01718412	-10359.346
238	3.31005E-40	4.48042E-35	2.284E-12	-1.8318E-07	0.015771222	-9507.5884	0.01577122	-9507.5884
239	3.67714E-42	4.9773E-37	3.846E-13	-3.0852E-08	0.014389099	-8674.38372	0.0143891	-8674.3837
240	3.65536E-44	4.94781E-39	6.086E-14	-4.8815E-09	0.013050648	-7867.506265	0.01305065	-7867.5063
241	3.25158E-46	4.40127E-41	9.046E-15	-7.2558E-10	0.011766865	-7093.584971	0.01176686	-7093.585
242	2.58824E-48	3.50339E-43	1.263E-15	-1.0131E-10	0.010546774	-6358.060607	0.01054677	-6358.0606
243	1.84357E-50	2.49542E-45	1.657E-16	-1.3289E-11	0.009397422	-5665.180631	0.00939742	-5665.1806
244	1.17506E-52	1.59054E-47	2.042E-17	-1.6376E-12	0.008323923	-5018.027882	0.00832392	-5018.0279
245	6.70201E-55	9.0717E-50	2.363E-18	-1.8956E-13	0.007329554	-4418.578638	0.00732955	-4418.5786
246	3.42054E-57	4.62998E-52	2.57E-19	-2.0614E-14	0.006415896	-3867.784934	0.0064159	-3867.7849
247	1.56218E-59	2.11453E-54	2.625E-20	-2.1059E-15	0.005582995	-3365.675616	0.005583	-3365.6756
248	6.38426E-62	8.64161E-57	2.519E-21	-2.0209E-16	0.004829558	-2911.470576	0.00482956	-2911.4706
249	2.33473E-64	3.16024E-59	2.271E-22	-1.8219E-17	0.004153151	-2503.70274	0.00415315	-2503.7027
250	7.64023E-67	1.03417E-61	1.924E-23	-1.543E-18	0.003550408	-2140.34279	0.00355041	-2140.3428

251	2.23729E-69	3.02835E-64	1.53E-24	-1.2276E-19	0.003017235	-1818.922158	0.00301723	-1818.9222
252	5.8625E-72	7.93536E-67	1.144E-25	-9.175E-21	0.002549001	-1536.650506	0.002549	-1536.6505
253	1.37464E-74	1.86068E-69	8.031E-27	-6.4419E-22	0.002140727	-1290.524647	0.00214073	-1290.5246
254	2.88429E-77	3.90412E-72	5.297E-28	-4.2489E-23	0.001787239	-1077.426649	0.00178724	-1077.4266
255	5.41545E-80	7.33025E-75	3.282E-29	-2.6326E-24	0.001483318	-894.2096113	0.00148332	-894.20961
256	9.09862E-83	1.23157E-77	1.91E-30	-1.5324E-25	0.001223816	-737.7702929	0.00122382	-737.77029
257	1.36792E-85	1.85159E-80	1.045E-31	-8.3791E-27	0.001003756	-605.1084317	0.00100376	-605.10843
258	1.84032E-88	2.49101E-83	5.366E-33	-4.3041E-28	0.000818409	-493.3730867	0.00081841	-493.37309
259	2.21548E-91	2.99883E-86	2.589E-34	-2.077E-29	0.00066335	-399.8967883	0.00066335	-399.89679
260	2.38665E-94	3.23053E-89	1.174E-35	-9.4153E-31	0.000534497	-322.2185858	0.0005345	-322.21859
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