Rapid Electrophoretic Screening for the Inhibition of Homocysteine Thiolactone-Induced Protein Oligomerization

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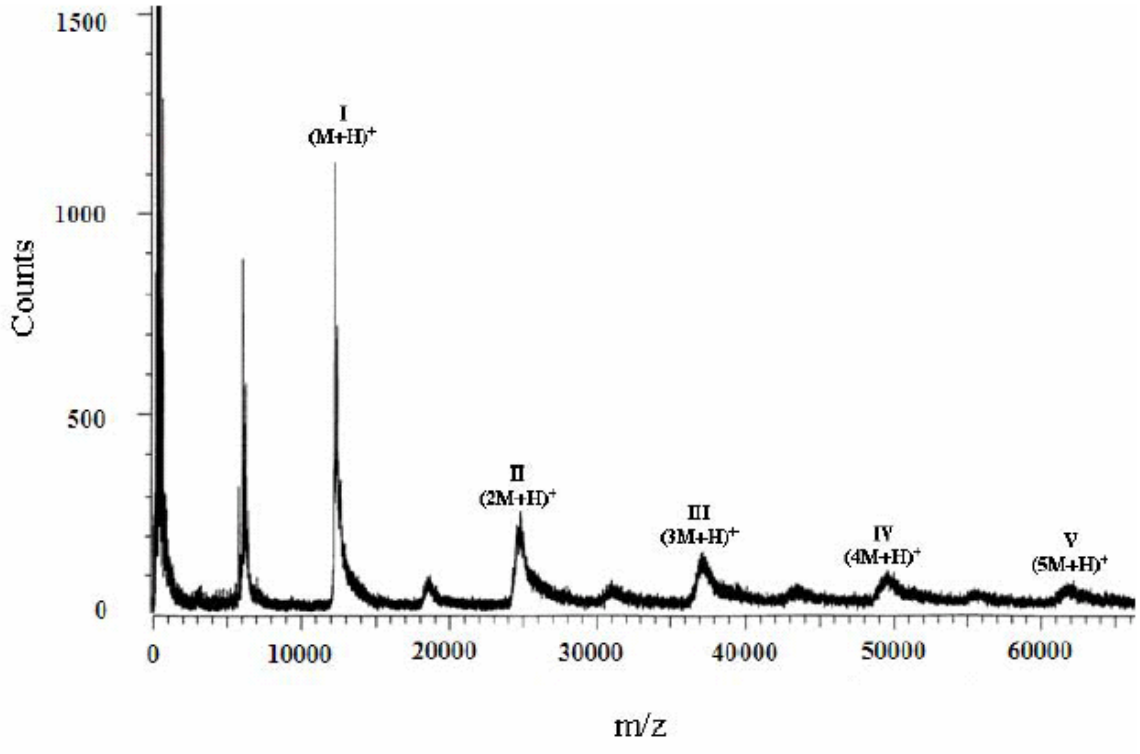


Figure S1. MALDI-TOF mass spectrum for the protein reaction mixture acquired after 24 hrs. The molecular ion $(M+H)^+$ for monomeric bovine cyt c was detected at approximately 12.3 kDal, which is consistent with the literature. The roman numerals indicate molecular ion species corresponding to monomeric (I), dimeric (II), trimeric (III), tetrameric (IV), and pentameric (V) bovine cytochrome c. The peak preceding the monomer molecular ion is due to the doubly protonated monomeric species. The minor peaks preceding the aggregate molecular ion peaks are due to various trace higher order aggregate species.

Mass spectrometry experiments were performed using a Bruker ProFLEX III™ matrix assisted laser desorption ionization time of flight- mass spectrometer (MALDI TOF-MS) in linear mode with a sinapinic acid (SA) matrix. The matrix was prepared by dissolving SA in a 2:3 acetonitrile/0.1% TFA mixture to obtain a 10 mg/mL SA solution. Protein reaction mixture samples and SA were mixed to obtain a 1:1 protein/matrix solution. The resultant solution was deposited drop-wise onto a MALDI target and allowed to dry and crystallize. Mass spectra were acquired using 100 shots with a 0 ns delay. Relevant instrumental parameters: linear mode, 100 shots, 0 ns delay, and sinapinic acid matrix.

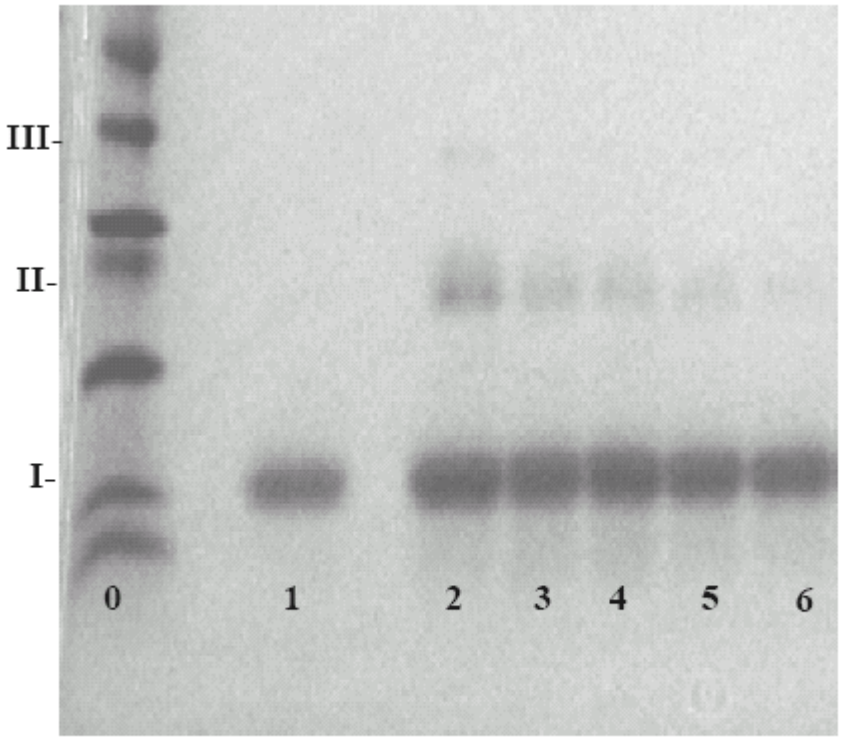
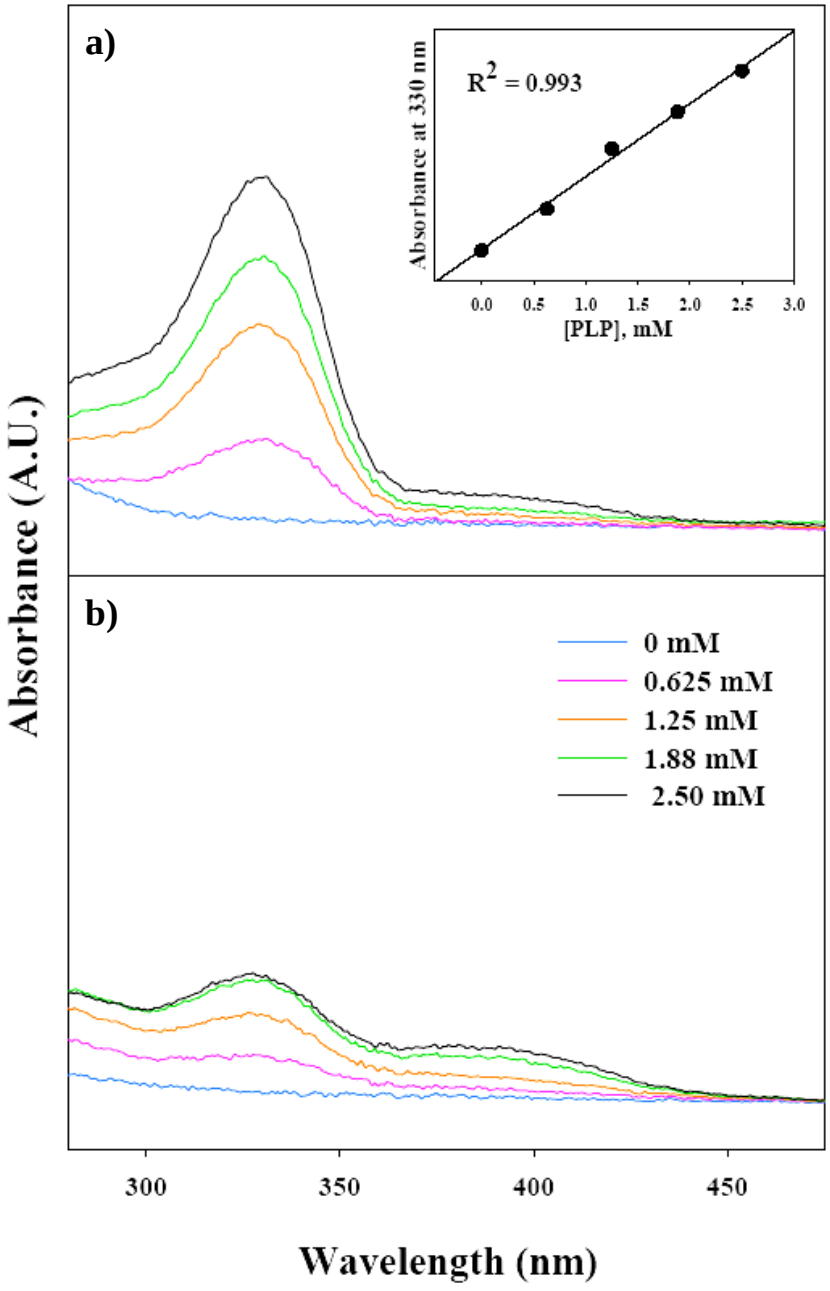


Figure S2. Bovine cytochrome *c* and hcy thiolactone (10 mg/mL and 2.5 mM, respectively) were reacted with pyridoxal-5-phosphate (PLP) for 24 h at room temperature. The resultant samples were denatured by heating at 90°C for 5 min in the presence of SDS and separated using SDS-PAGE on 4–20% gels at approximately pH 9. A representative gel is shown above. Unmodified cytochrome *c* migrated as a single band (lane 1). Lanes 2-6 show 1:0, 1:0.25, 1:0.5, 1:0.75, and 1:1 hcy thiolactone:PLP, respectively. Cytochrome *c* monomers, dimers, and trimers are denoted as I, II, and II, respectively as determined by comparison with protein mass standards (lane 0). Note the decrease in aggregate band intensities as the concentration of PLP increases, eventually resulting in the disappearance of the trimeric species as well as an appreciable reduction in the dimeric species.



Figures S3. Spectrophotometric detection of in situ pyridoxal tetrahydrothiazine formation after 12 h in a) control reaction mixtures containing 2.5 mM Hcy thiolactone and PLP in the indicated concentrations (inset: pyridoxal tetrahydrothiazine production monitored at 330 nm), and b) protein reaction mixtures containing 10 mg/mL bovine cyt c, 2.5 mM Hcy thiolactone, and PLP in the indicated concentrations after 12 h. All samples were diluted 10-fold with pH 7.4, 100 mM sodium phosphate, 0.2 mM EDTA buffer prior to analysis. UV-Vis spectra acquired in triplicate at 25°C in a 1 cm quartz cell with a Shimadzu model UV-3101PC spectrophotometer.

column 1								
monomer	**1**	**2**	**3**	**4**	**5**	**avg**	**stdev**	**% stdev**
RT (min)	1.9184	1.9250	1.9230	1.9093	1.9184	1.9188	0.0060	0.31
alpha	213.6	217.5	235.1	221.2	215.3	220.5	8.6	3.90
sigma	0.02060	0.02000	0.02149	0.02069	0.02069	0.02079	0.00040	1.91
w1/2	0.04840	0.04816	0.05049	0.04862	0.04863	0.04886	0.00093	1.91
N	8.70.E+05	8.85.E+05	8.04.E+05	8.54.E+05	8.62.E+05	8.55.E+05	2.77.E+04	3.24
dimer								
RT (min)	1.7489	1.7523	1.7530	1.7383	1.7580	1.7500	0.0073	0.42
alpha	63.89	62.52	61.10	68.43	62.89	63.76	2.80	4.38
sigma	0.02310	0.02277	0.02200	0.02433	0.02300	0.02314	0.00072	3.12
w1/2	0.05427	0.05351	0.05266	0.05717	0.05425	0.05437	0.00169	3.12
N	5.75E+05	5.94E+05	6.13E+05	5.12E+05	5.82E+05	5.75E+05	3.41E+04	5.93
trimer								
RT (min)	1.6250	1.6330	1.6360	1.6430	1.6430	1.6360	0.0074	0.45
alpha	12.4	14.1	13.0	17.5	14.0	14.2	2.0	13.98
sigma	0.01666	0.01800	0.01800	0.02000	0.01700	0.01784	0.00129	7.22
w1/2	0.03914	0.04222	0.04115	0.04693	0.04014	0.04192	0.00303	7.22
N	9.55E+05	8.29E+05	8.76E+05	6.79E+05	9.28E+05	8.53E+05	1.09E+05	12.77

Table S1. Representative data resulting from five consecutive runs. Electropherograms were exported into a spreadsheet program and fit to Gaussian peaks. The outputs of the best fit analysis are migration time (MT), peak area (alpha), standard deviation of the Gaussian peak (sigma). Sigma corresponds to the electrophoretic peak width. The full-width at half height (w1/2) and number of theoretical plates (N) were calculated using equations 1 and 2 in the text.