Supplementary information

Efficient protein renaturation using tunable hemifluorinated anionic surfactants as additives

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Materials:

Sodium hydride (NaH), Heptanol, Nonanol and 1,3-propane sultone, 4-Nitrophenyl acetate and dry acetonitrile were purchased from Aldrich. Perfluoroalcohols was purchased from Matrix Scientific. Carbonic anhydrase, from bovine erythrocytes was obtained from Sigma-Aldrich Co. as dialyzed and lyophilized powders and was used in the experiments without further purification. Tris base was purchased from Fisher scientific. Guanidine HCl was purchased from fluka.

Synthesis of anionic hemifluorinated surfactants and their hydrocarbon analog:

The simple one-step synthetic approach to this class of surfactants involves the reaction of 1,3-propane sultone with sodium alkoxide or perfluoroalkoxide (Scheme 1).

Scheme 1

In a typical experiment as shown above in scheme 1, alcohol ROH (1 equivalent) was deprotonated in the presence of sodium hydride (1.2 equivalents) in THF at room temperature. The reaction mixture was stirred at room temperature for 15 min., then 1,3 propane sultone (1 equivalent) was added slowly and the reaction mixture was stirred overnight. The excess sodium hydride was quenched by slow addition of methanol. The solvent was removed by rotary evaporation. Pure sodium sulfonate was obtained by recrystallization from ethanol as white solid. All other anionic surfactants having hydrocarbon or fluorous tails are prepared in 61-77% yields under similar conditions by reacting the respective alcohols with propane sultone.

Determination of Critical Micelle Concentration of anionic hemifluorinated surfactants and their hydrocarbon analog: Surface Tension method

Solution of surfactants in deionized water at various concentrations was prepared. The solution was poured into an outer tube and a capillary tube was inserted into it. For each concentration, the height of the solution in the capillary tube was measured (Anatrace tech. bulletin). The height was plotted against surfactant concentrations (mM) and the CMC obtained from the intersection of two straight lines.

Thermal denaturation – renaturation of Bovine Carbonic Anhydrase (CAB):

Thermal denaturation studies were conducted by denaturing CAB (0.25 mg/mL) at 70 °C for 6 min in 16 mM Tris-sulfate buffer, pH 7.75 in the absence or presence of the anionic fluorous surfactant, F13PS (3.1 mM, 6.1 mM), F17PS (0.5 mM, 2.1 mM) and the hydrocarbon analog C13PS (15.6mM). Renaturation was carried out by cooling and keeping the denatured sample at room temperature for 24 hrs. After the renaturation step, the fluorous

surfactant was slowly removed using dialysis (3 cycles) in the presence of 0.1 M Tris-sulfate buffer, pH 7.75 at room temperature. The dialyzed and refolded protein was then used for activity assay and far-UV circular dichroism measurements.

Chemical denaturation – renaturation of CAB:

Chemical denaturation studies were performed by denaturizing CAB (30 mg/ml) for more than 16 h in 5 M Guanidinium Chloride (GdmCl). The solution of the denatured CAB was diluted to a concentration of 0.25 mg/ml in 16 mM Tris buffer (pH 7.75) in the absence or presence of the anionic fluorous surfactant, F13PS (3.1 mM, 6.1 mM), F17PS (0.5 mM, 2.1 mM) and the hydrocarbon analog C13PS (15.6 mM). Renaturation was carried out by cooling and keeping the denatured sample at room temperature for 24 hrs. After the renaturation step, the fluorous surfactant was slowly removed using dialysis (3 cycles) in the presence of 0.1 M Tris-sulfate buffer, pH 7.75 at room temperature. The dialyzed and refolded protein was then used for activity assay and far-UV circular dichroism measurements.

Enzymatic activity assay of CAB:

All the CAB samples were diluted to 0.03 mg/mL prior to assay. Enzymatic activity of CAB was monitored using p-nitrophenyl acetate (pNPAc) as a substrate (Pocker, 1967). 63 µL 52 mM pNPAc in dry acetonitrile was added to 630 µL of diluted CAB sample to make a solution of 0.026 mg/mLCAB, 14 mM Tris-sulfate, pH 7.75 and 4.7 mM pNPAc. After 10s of mixing, the measurement of the absorbance at 400 nm over the period of 60 sec was monitored. Hydrolysis of pNPAc due to CAB results in phenolate ion (yellow color). The

increase in absorbance at 400 nm is a measure of the increase in amount of the hydrolyzed acid product as the reaction proceeds. The rate of hydrolysis of pNPAc is proportional to the amount of active protein in the solution. The refolding yield was calculated as the percentage of activity for the native protein. Buffer, with or without additives (C13PS, F13PS, F17PS), showed negligible activity compared to the activity of native protein.

Circular Dichroism spectroscopy:

Circlular Dichroism experiments were carried out on Jasco J-815 CD spectrometer. The data was collected and analyzed with spectra manager software, version 2 under the control of a Compaq computer. CD spectra of native and refolded CAB in the far UV region (190 – 260 nm) were recorded at 25 °C. CAB concentration was 0.25 mg/ml and C13PS, F13PS and F17PS concentration was 15.6 mM. 6.1 mM and 2.1 mM respectively. For far UV-CD spectra, quartz cuvette of 1mm path length was used. The scan rate was 50 nm $min^{-1}$and the final spectra was an average of 8 scans.

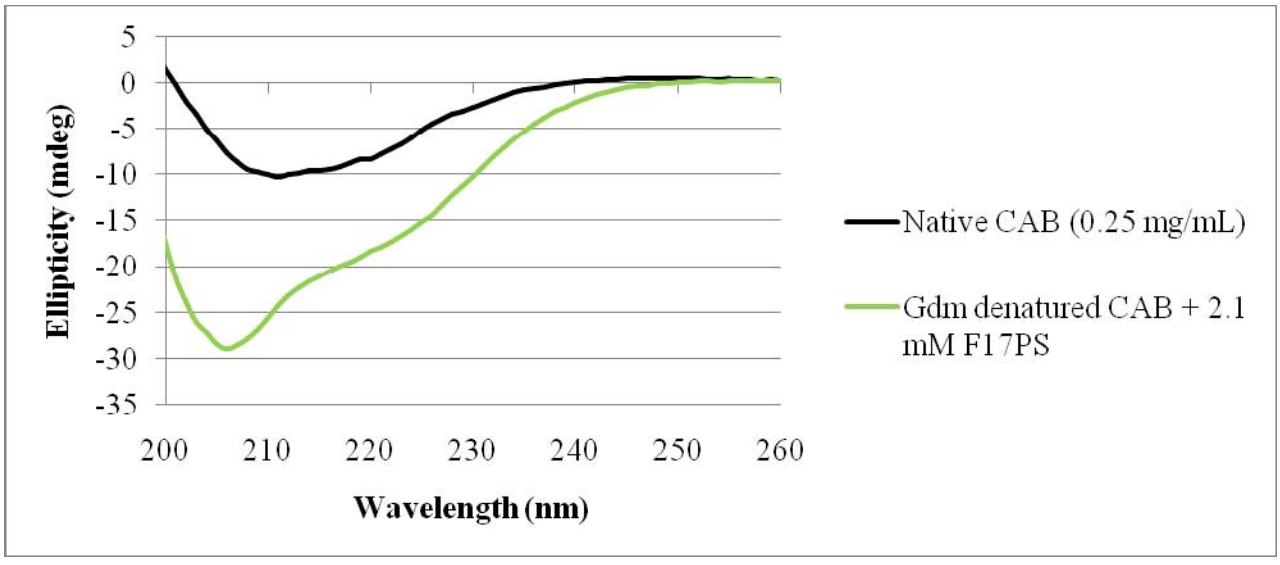

Fig. 1 Far-UV CD spectra of native CAB (0.25 mg/mL) and Gdm-denatured CAB (0.25 mg/mL) – F17PS (2.1 mM) complex.

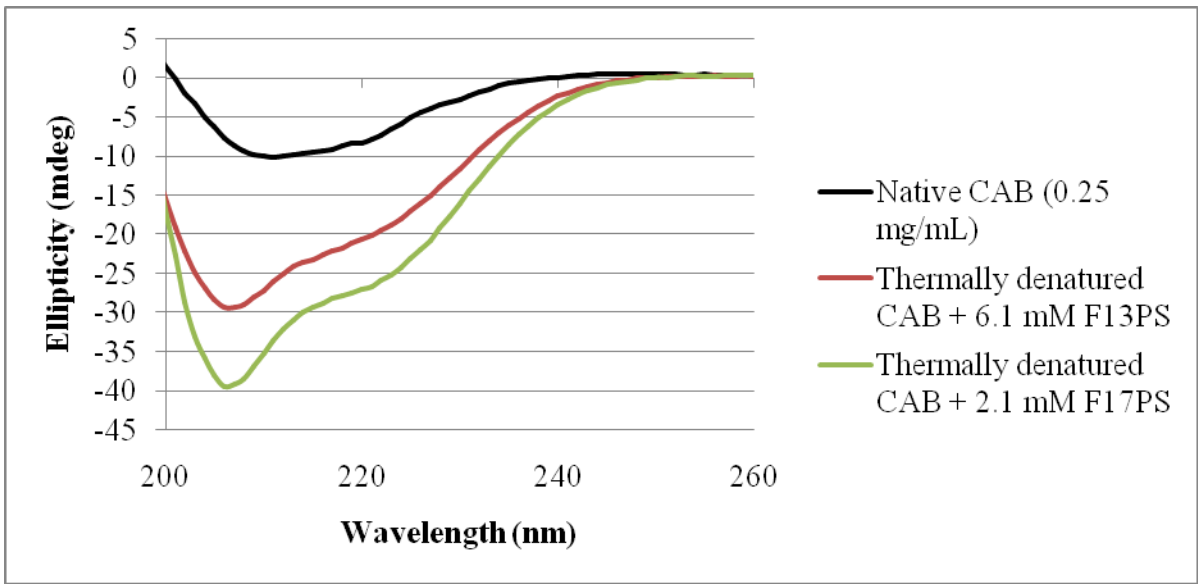

Fig. 2 Far-UV CD spectra of native CAB (0.25 mg/mL), thermally-denatured CAB (0.25 mg/mL)–F13PS (6.1 mM) and thermally-denatured CAB (0.25 mg/mL)–F17PS (2.1 mM) complex.

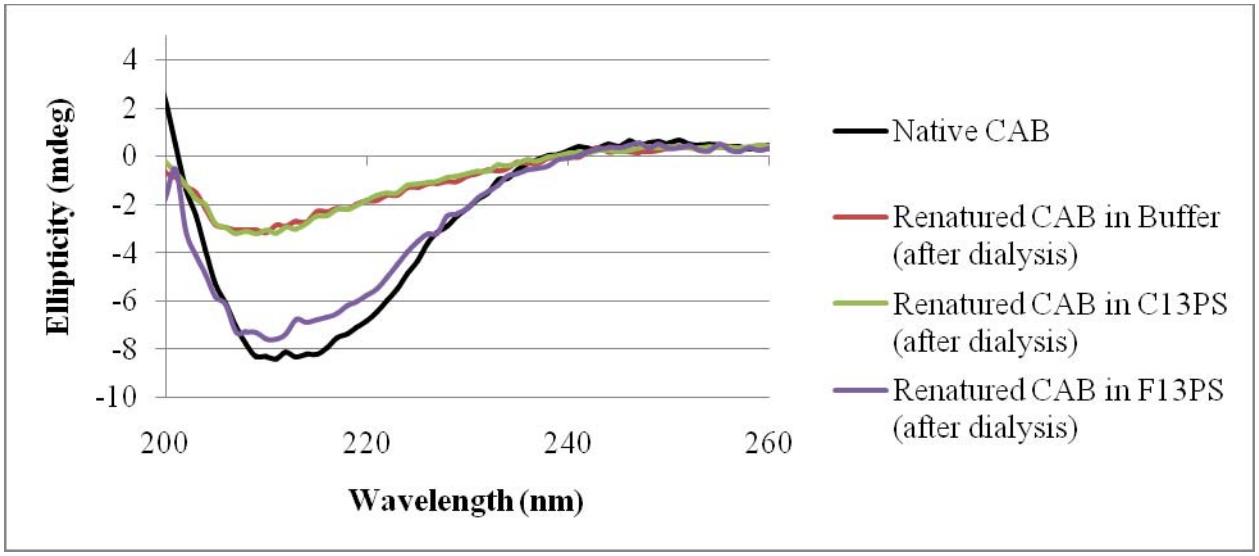

Fig. 3 Far-UV CD spectra of native CAB (0.25 mg/mL), thermally- denatured, renatured and dialyzed CAB (0.25 mg/mL) in the presence of Tris buffer (16 mM), C13PS (15.6 mM) and F13PS (6.1 mM).