Cucurbit[7]uril nanoencapsulation reduces the unpalatability of bitter phytochemicals

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Supporting Information:

Experimental details:

Chemicals used: Sucrose (AR, Loba), caffeine (AR, Loba), strychnine (≥98%, Sigma), thiamine hydrochloride (Sigma), potassium ferricyanide (Sigma), blue dye – brilliant blue FCF (Vidhi Speciality Food Ingredients Ltd, Mumbai), red dye – amaranth (Vidhi Speciality Food Ingredients Ltd, Mumbai). Cucurbit[7]uril (CB7) was synthesized and purified according to previous reports[17] and further characterized by ^{1}H NMR spectroscopy and mass spectrometry. The spectroscopic experiments were performed using Milli-Q water obtained from the Milli-Q grade water system from Merck (USA) with resistivity 18.2 MΩ·cm at 298 K.

Flies used: Canton S strain of *Drosophila melanogaster* (obtained from the laboratory of Dr. N.G. Prasad at IISER Mohali) was used in this study. Flies were cultured in *Drosophila* culture medium (cornmeal, agar, yeast, and sucrose mixed with an ethanolic solution of p-hydroxy methyl benzoate and propionic acid) and were reared at room temperature (20 to 25º C) under the natural light-dark cycle, at the Zoology laboratory of Shoolini University (30° 86’ N, 77° 12’ E) at Solan (Himachal Pradesh, India). Flies were starved for 24 h (with access to water, but no food) before using them in food choice assays.

Food choice assay: Twenty flies were anesthetized by cooling a vial containing flies over ice for 5-10 min and were introduced into a pair of plastic petri plates (65 mm diameter) where the lower plate had 8 droplets (each droplet – 2 µL) of one kind of solution (either 10 mM caffeine/strychnine + 5 mM sucrose or 10 mM CB7 encapsulated caffeine/strychnine + 5 mM sucrose) mixed with either red (2 mg/mL) or blue dye (1 mg/mL), and 8 droplets of another kind of solution (1 mM or 5 mM sucrose) mixed with the alternate dye (blue/red). The droplets were placed alternately (Figure 3a). The flies were allowed to revive, and then the setup was kept in a horizontal position in the dark for 2 hours at room temperature (so that flies cannot see the color of the liquid they are consuming). Then the petri plates were placed at 0°C inside a refrigerator for 30 to 40 min to kill

the flies. A binocular stereo zoom microscope (Magnus MSZ-Bi) was used to check the color of their abdomens, and thereby the kind of solution consumed by each fly was verified (red abdomen – fed on solution mixed with red dye, blue abdomen – fed on solution mixed with blue dye, purple abdomen – fed on both kinds of solutions, abdomen without any extra color – did not feed on any solution). A preference index for the solution mixed with red dye was calculated for each replicate by dividing the total number of flies with red + purple abdomens by the total number of flies with colored abdomens (red + blue + purple).[32] Likewise, a preference index for the solution mixed with blue dye was also calculated.

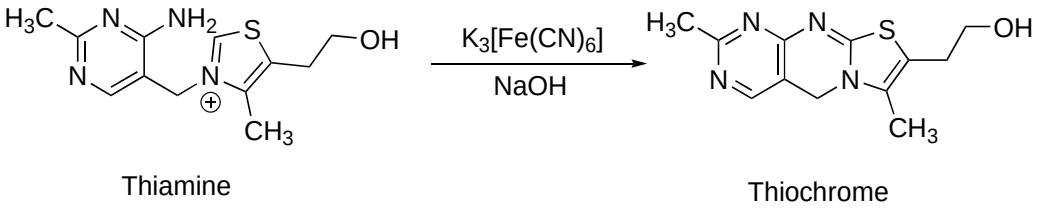

Figure S1: Synthesis of thiochrome from thiamine using potassium ferricyanide.

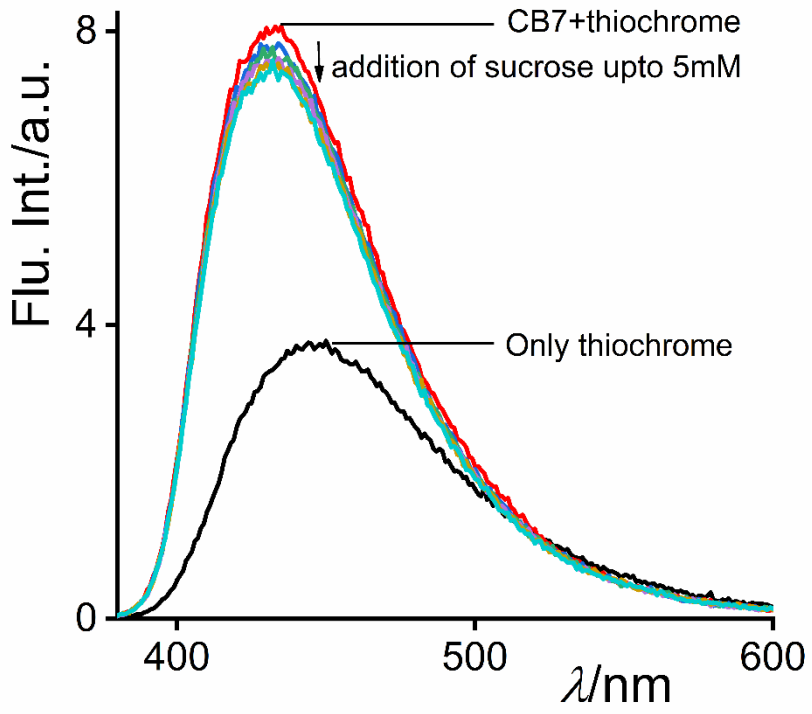

Figure S2: Plot showing fluorescence intensity of only thiochrome (5 μM), the intensity of the CB7-thiochorme complex followed by the addition of sucrose (up to 5 mM) into it.

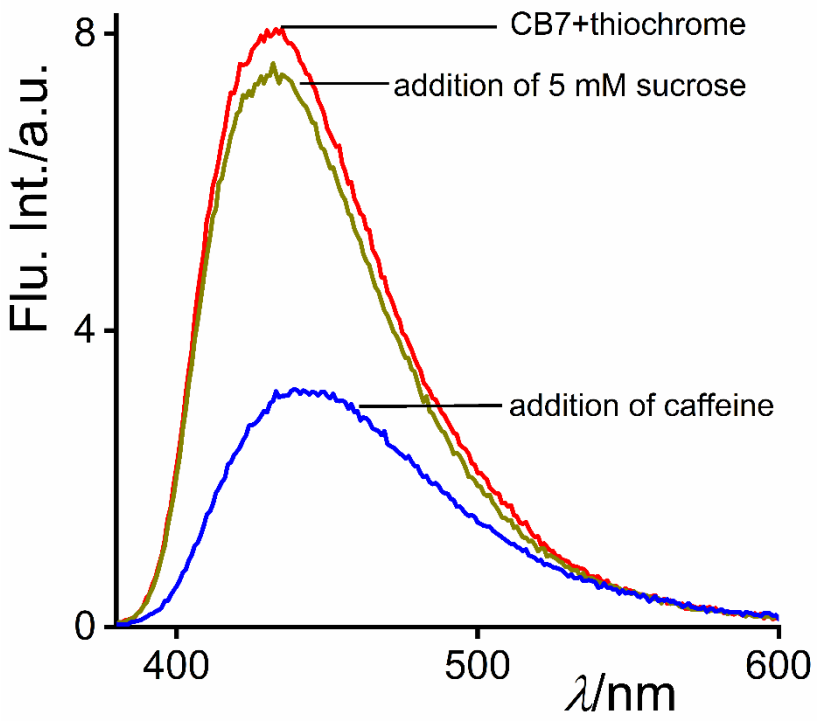

Figure S3: Fluorescence spectra of CB7-thiochorme complex (5 μM thiochrome and 0.5 mM CB7), the addition of 5 mM sucrose shows a negligible decrease in the intensity, and further addition of 0.5 mM of caffeine replaces the bound thiochrome from CB7.

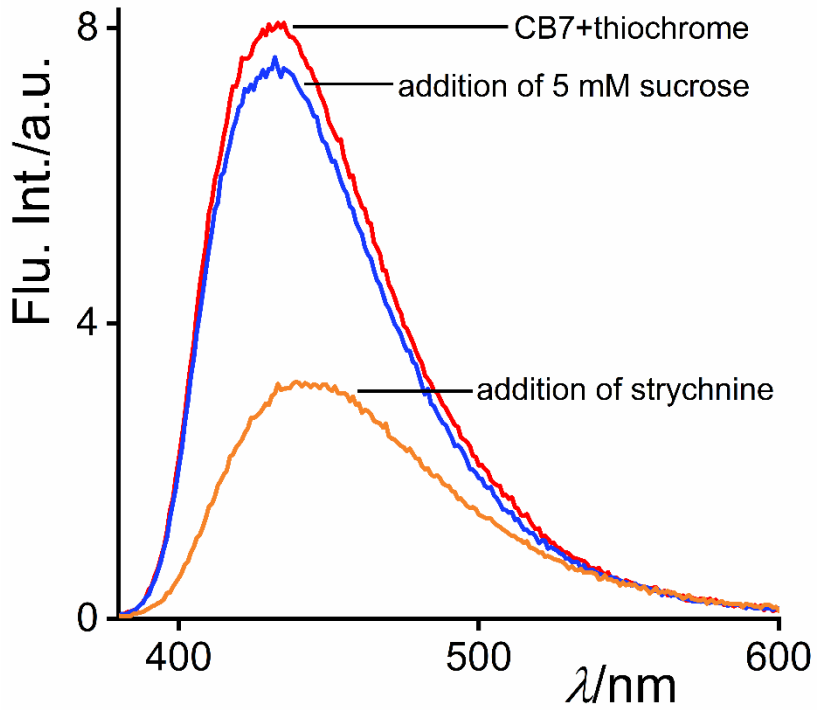

Figure S4: Fluorescence spectra of CB7-thiochorme complex (5 μM thiochrome and 0.5 mM CB7), the addition of 5 mM sucrose shows a negligible decrease in the intensity, and further addition of 0.5 mM of strychnine replaces the bound thiochrome from CB7.