Electronic Supplementary Material (ESI) for Soft Matter

Experimental Section

Materials

Berberine (as berberine hemisulfate, Mol wt = 384.4 g/mol) was purchased from Sigma Aldrich Inc., USA. Epigallocatechin gallate. Mol wt = 458.4 g/mol (as TEAVIGOTM tea extract) was received as a gift sample from DSM, Switzerland. Water purified by the MilliQ system was used for all the experiments.

Preparation of colloidal dispersions

Stock solutions (1μM) of berberine (pH=6.5) and EGCG (pH=6.5) respectively were prepared in MilliQ water. Colloidal dispersions were prepared by rapid mixing of stock solutions under vigorous stirring (1000 rpm) using magnetic stirrer (Model EM3300T, Labotech Inc, Germany) at various molar ratios as listed in Table 1. The final pH of the reaction mixture was around 6.5. Samples for solid state characterisation including FTIR and XRD were obtained by subjecting the colloidal dispersion to freeze drying at -85 °C using a vacuum of -0.040 mbar (Labconco Freezone 6 plus, Labconco Corporation, USA).

Analysis of particle size, surface charge and morphology

The particle size (volume weighted mean, d 4,3) and ζ-potential of dispersions were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd, UK) after appropriate dilution. All measurements were carried out at 25°C and the results reported are averages of three readings.

The shape of synthesized particles was analysed by taking TEM photographs using Technai transmission electron microscope (FEI Company, The Netherlands). The dispersion was diluted in MilliQ water and one drop of the diluted dispersion was placed on a 200-mesh carbon coated copper grid. The photographs were taken at various magnifications and 100 kV voltage. For cryo-TEM, A small volume of the sample was put on a "holey carbon" 200 mesh copper grid (Ted Pella, USA) and subjected to freezing using Controlled Environment Vitrification System (CEVS, ex Technion, Haifa). The thin film obtained was plunged and vitrified into liquid ethane and transferred under liquid nitrogen into the electron microscope using an Oxford CT3500 cryo transfer holder. The vitrified samples were investigated at low

temperature (approx. -170°C) in a Technai Sphera (FEI Company) transmission electron microscope operating at 200 kV accelerating voltage and under low dose imaging conditions. Images were recorded using a Megaview G2 wide angle camera and a Tietz 214 slow scan CCD camera.

Spectral and solid state characterisation

Fluorescence emission spectral scans were obtained using Microplate Spectrofluorometer SpectraMax M2 Microplate Reader (Molecular devices Corp., USA). The samples were excited at 421 nm and 449 nm (with a cut off filter at 530 nm), the emission spectral scan was recorded from 500 to 750nm. Berberine concentration in the samples was kept constant at (1 X 10-5 M) and the concentration of EGCG was varied to obtain samples with different Berberine: EGCG proportions.

Fourier Transform Infrared (FTIR) experiments were performed on a Bio-Rad FTS-6000 spectrometer-V 4.0 (Cambridge, MA, USA) which was equipped with a deuterated triglycine sulphate (DTGS) detector. The accessory used was a Micro-ATR equipped with a Si-crystal. For both samples and background, 64 interferograms with a resolution of 4 cm-1 were co-added and Fourier transformed. The spectral information was obtained over a frequency window from 4000 to 400 cm-1.

Diffraction lines of the samples were obtained with a Bruker AXS (Karlsruhe, Germany) D8 Discover diffractometer. The instrument was equipped with a copper anode that produces Cu Kα X-rays with an accelerating voltage 40kV and a tube current 40mA. The diffractogram was collected with a monocap collimator of 0.3 mm during 300 sec. An angular range of theta1 at 4.5° and theta2 at 10°, 25° and 40°, were use with a theta rocking of 1° and XY amplitude of 2 mm resulted in a 2θ between 4° and 55° after merging the separate recordings. Rocking and amplitude oscillation were used to obtain an average diffractogram of the sample and minimize a preferred orientation of crystals.

Molecular interactions

The thermodynamics of the binding was assessed using an isothermal titration calorimeter (VP-ITC Microcal, Northampton, MA). The solutions were degassed by using a Microcal Thermo Vac degassing unit. The reference cell was filled with degassed milliQ water. The sample cell of volume 1.4422 ml was filled with 0.1mM

EGCG and thermostated at 20 °C and 37 °C. The syringe of volume 250 µL was filled with the titrant (2mM Berberine), and the rotating speed of the syringe was set at 307 rpm. The titrant solutions were added in 10 µL aliquots (24 injections with 10 second duration each) at 180 seconds intervals. The heat released or absorbed upon each injection was measured as function of time. The heat of dilution from the blank titration of titrants into pure milliQ water was subtracted from the raw data. Data acquisition and analysis were performed with Microcal Origin software (version 7 SR4), and the single set of binding sites was applied to fit binding isotherms. Thermodynamic parameters, including the binding constant (Ka), observed binding enthalpy (ΔH), binding stoichiometry (n) and binding entropy (ΔS) were calculated by iterative curve fitting of the binding isotherms using following equation.

$$Q = \frac{\left(1+[M]_t.n.K_a + K_a.[L]_t\right) - \sqrt{\left[\left(1+[M]_t.n.K_a + K_a.[L]_t\right)^2 - 4.[M]_t.n.{K_a}^2.[L]_t\right]}}{2.K_a \big/ V.\Delta H}$$

Where Q is the cumulative heat, [M]t the total concentration of reactant in the sample cell, [L]t the total concentration of titrant added, and V the volume of the sample cell.

Gibbs free energy (ΔG) was determined from the binding constant using equation: ΔG = - RT lnKa, Where R is the gas constant (8.314 $J.mol^{-1}K^{-1}$) and T is the absolute temperature (in Kelvin) and the entropy (ΔS), from the second law of thermodynamics (ΔG = ΔH - TΔS).

Temperature dependent dissolution

Temperature dependent dissolution was evaluated by incubating the dispersion at 37 °C (in a water bath, Model EM3300T, Labotech Inc, Germany). The samples were withdrawn periodically and release of EGCG was quantified by measuring the total anti-oxidant activity (Ferric Reducing Aanti-oxidant Power or FRAP value). The procedure followed for the assay was as follows: The working FRAP reagent was freshly prepared by mixing 2.5 ml of 20 mM $FeCl_3 \bullet 6H_2O$, 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl, and 25 ml of 300 mM acetate buffer (pH 3.6), and warmed at 37 °C prior to use. Sample (10 µl) was mixed with 300 µl of working FRAP reagent and absorbance (read at 593 nm) was measured at 0 minutes using SPECTRAmax® 190 Microplate Spectrophotometer (Molecular devices Corp., USA). The sample was incubated for 4 minutes at 37 °C followed by re-reading of the

absorbance. Ascorbic acid standards (100-1000 µmol) were processed in the same way and anti-oxidant activity was reported as FRAP value (µmol/litre). The difference in absorbance between 0 and 4 minutes was calculated for test sample (Δ At) as well as standard (Δ As) and these values were used to obtain anti-oxidant activity in terms of FRAP value (µmol/litre) as follows: FRAP value of test sample (µmol/litre) = 2 × (Δ At/ Δ As). The multiplication factor of 2 is the reported FRAP value of standard (Ascorbic acid at a concentration of 1000µM).

The particle count rate was also measured over 30 minutes of incubation at 37 °C by measuring dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd, UK) after appropriate dilution.

Figures

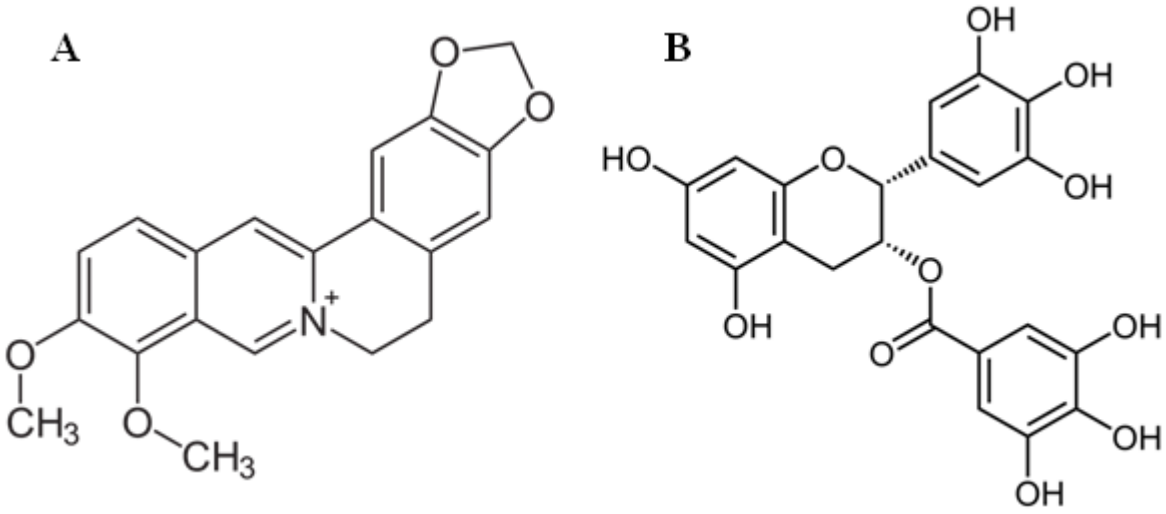

Figure S1: Molecular structures of A) Berberine base and B) Epigallocatechin galllate

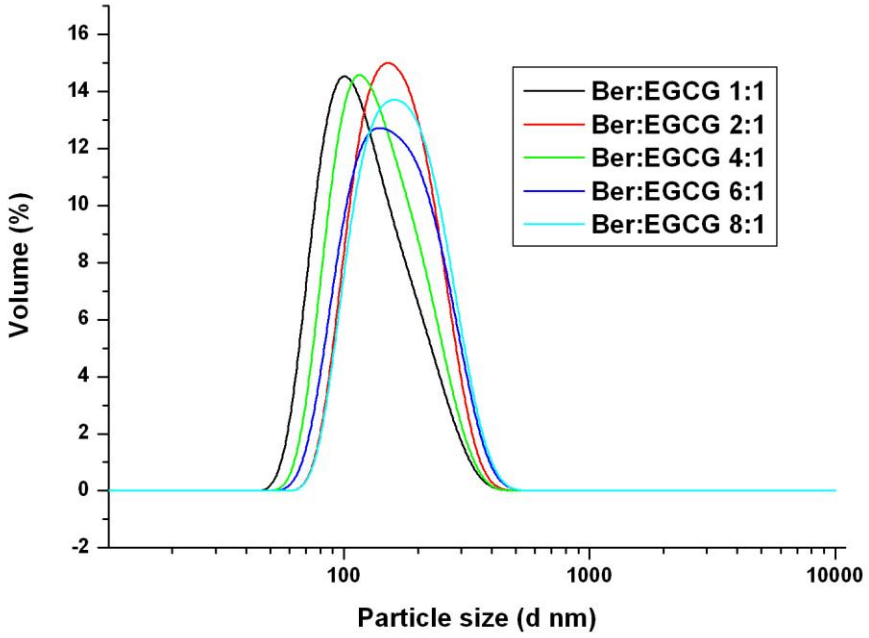

Figure S2: Particle size distribution curves for colloidal dispersions prepared at various molar ratios of Ber:EGCG.