Supplementary material for the article:

Al-Hanish, A.; Stanic-Vucinic, D.; Mihailovic, J.; Prodic, I.; Minic, S.; Stojadinovic, M.; Radibratovic, M.; Milcic, M.; Cirkovic Velickovic, T. Noncovalent Interactions of Bovine α-Lactalbumin with Green Tea Polyphenol, Epigalocatechin-3-Gallate. *Food Hydrocolloids* **2016**, *61*, 241–250. https://doi.org/10.1016/j.foodhyd.2016.05.012

Supporting material

Noncovalent interactions of bovine α-lactalbumin with green tea polyphenol, epigalocatechin-3-gallate

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Experimental part:

2.2. Fluorescence quenching analysis

Experiments were carried out at +25°C in a 3.5 ml quartz cuvette. ALA solution (25μg ml^{-1}dissolved in 50 mM sodium phosphate buffer pH 7.2) was titrated with 11 x 1 μL and 4 x 1,25 μL of EGCG (2.5 mg mL^{-1}). After the addition of each aliquot fluorescent spectrum was immediately recorded with excitation wavelength: 280 nm and emission wavelength: 290-500 nm. Between each measurement, the cell was washed three times with deionized water and a blank was made for each polyphenol concentration. The blank spectrum was automatically subtracted from the emission spectrum of the corresponding solution. All experiments were performed in triplicate and the averaged data obtained from the binding studies were used for the calculations of the binding parameters. Binding parameters were expressed as mean value of three experiments and standard deviation of measurements was shown.

2.2.1. Stern-Volmer equation

Fluorescence quenching is described by Stern-Volmer equation (Eq. 1) (Liang, Tajmir-Riahi, & Subirade, 2008):

$$\frac{F_0}{F} = 1 + K_{SV} \times [Q]$$

where $\boldsymbol{F_0}$ and $\boldsymbol{F}$ and the fluorescence intensities before and after addition of a quencher; $\boldsymbol{K_{SV}}$ is the Stern-Volmer quenching constant and ***[Q]*** is the concentration of the quencher.

The Stern-Volmer constant $\boldsymbol{K_{SV}}$ can be interpreted as the binding constant of the complex formation, assuming the observed changes in fluorescence come from the interaction between EGCG and protein (Hasni, et al., 2011).

The obtained Stern-Volmer plots were linear in all conditions, thus allowing the calculation of the fluorescence-quenching rate constant ($\boldsymbol{k_q}$) using Eq. 2 (Lakowicz, Gryczynski, Gryczynski, & Dattelbaum, 1999):

$$K_{SV} = kq \times \tau_0$$

The known value for Trp-fluorophore fluorescence lifetime in biopolymers without a quencher ($\boldsymbol{\tau_0}$) is 10 ns (Lakowicz, et al., 1999; Wang, et al., 2013).

2.2.2. Lehrer equation

The quenching constant (K_Q) and the fraction of fluorophore accessible to solvent (*fa*) can be calculated according to Lehrer equation (also called modified Stern-Volmer equation) (Eq. 3) (Keppler, Stuhldreier, Temps, & Schwarz, 2014):

$$\frac{Fo}{Fo - F} = \frac{1}{[Q] \times fa \times K_Q} + \frac{1}{fa}$$

2.2.3. Double-logarithmic equation

For the static quenching, the binding constant *Ka* and a number of binding sites can be calculated according to a double-logarithmic equation (Eq.4) (Lakowicz, et al., 1999):

$$\log \frac{F_0 - F}{F} = \log K_a + n \times \log[Q]$$

The slope of the double logarithmic plot yields the number of binding sites and the intercept provides the binding constant ($\boldsymbol{K_a}$).

2.2.4 Langmoir isotherm

Fluorescence titration data were also interpreted using Langmoir isotherm (Eq. 5) (Keppler, et al., 2014):

$$F_0 - F = \frac{(F_0 - F_\infty)\,[Q]}{K'_d + Q}$$

The non-linear regression gives the dissociation constant ***K'd*** and the maximum fluorescence difference ($\boldsymbol{F_0 - F_\infty}$).

The average values were used for calculation of binding parameters. Statistical analysis was performed using descriptive statistics tool available in Excel.

2.4. FT-IR spectroscopy measurements

Spectra were collected via the ATR method with a resolution of 4 cm^{-1} and 128 scans. The infrared spectra of α-Lactoalbumin (50 μM in 50 mM sodium phosphate buffer pH 7.2) and the α-Lactoalbumin - ligand complex (the molar ratios of EGCG to α-Lactoalbumin were 0.5:1, 1:1 and 10:1) were obtained in the featured region of 4000–400 cm^{-1}. Corresponding absorbance contribution of buffer and free ligand solution were recorded and subtracted to get the FT-IR spectra of protein and of protein−EGCG complexes, respectively. The subtraction was performed in order to obtain baseline in the region between 2000 and 1750 cm^{-1}(Dong, Huang, & Caughey, 1990).

Results

Figure S1. FTIR spectra of ALA/EGCG complexes. Curve-fitted amide I (1700-1600 cm^{-1}) regions of free ALA (A) and ALA-EGCG complexes I different molar ratio :(B) 1:0.5, (C) 1:1 and D) 1:10.

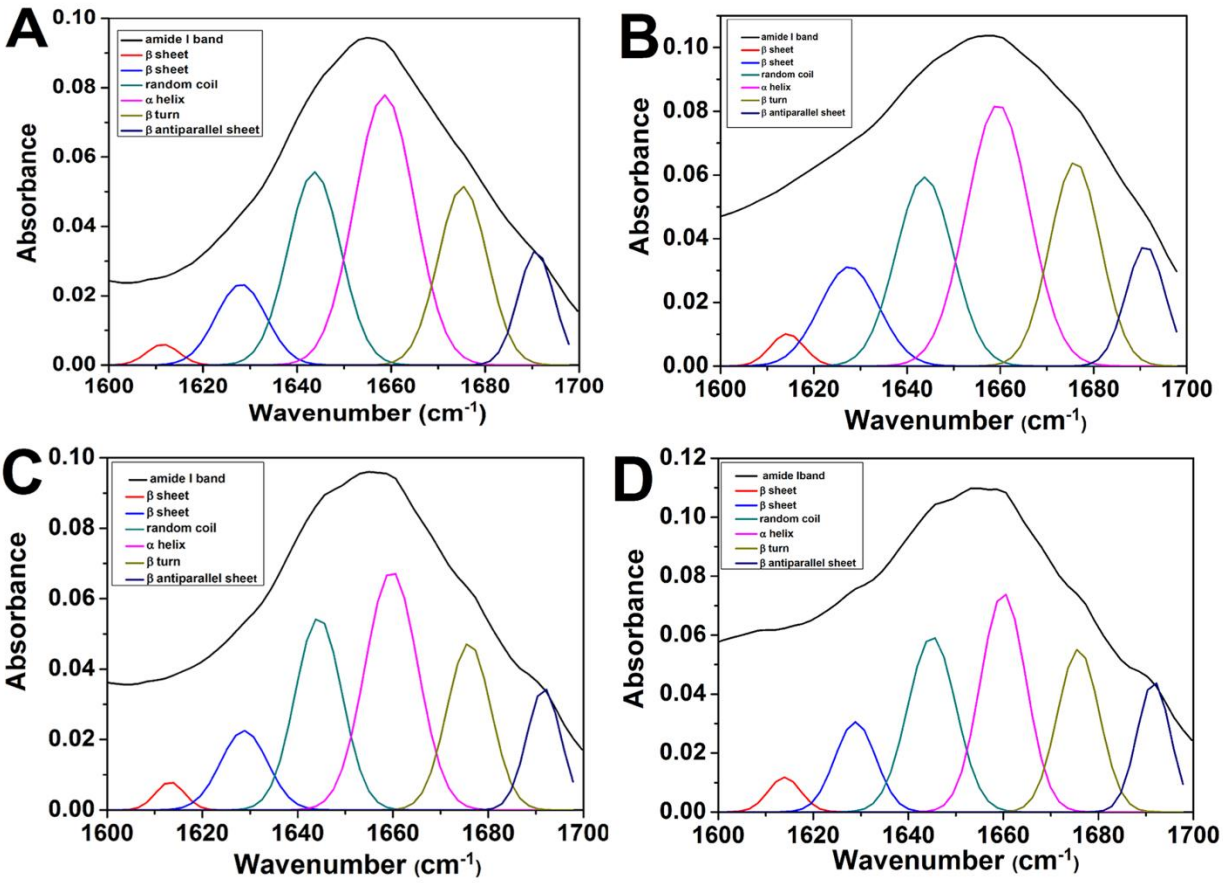
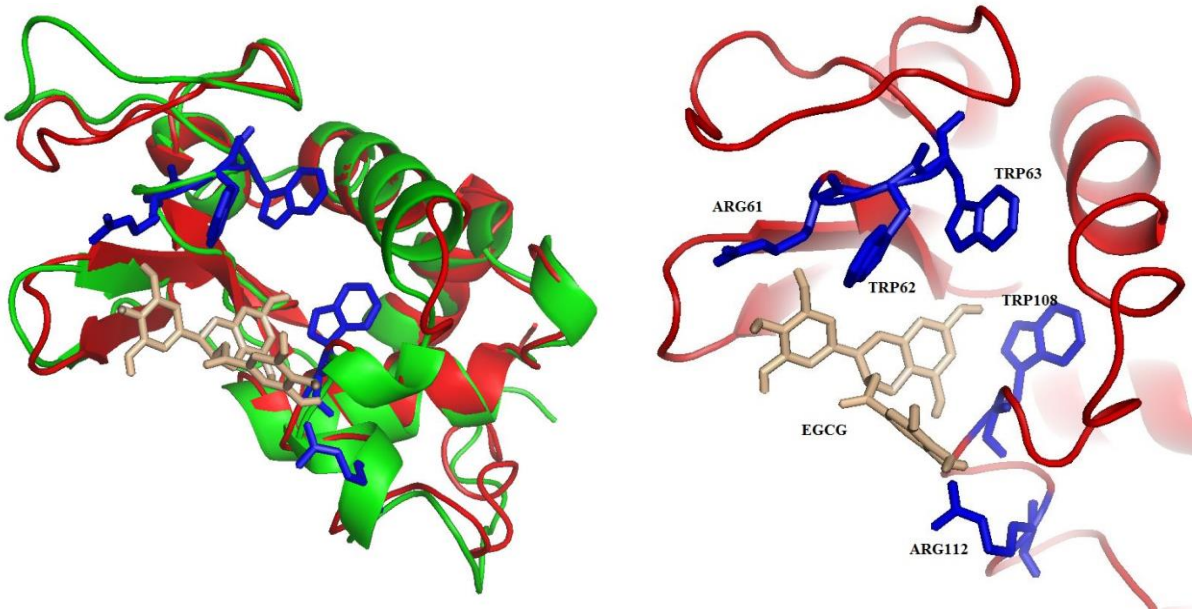


Figure S2. A) Aligned structures of native ALA (PDB 1F6S, green) and lysosyme (6LYZ, red) with EGCG (beige) molecule docked. Tyrosine and tryptophane residues of lysosyme interacting with EGCG (Ghosh, Sahoo, & Dasgupta, 2008) are in blue. B) Structure of lysosyme (6LYZ, red) only with EGCG docked to ALA.

A) B)



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