Supplementary data for article:

Vesic, J.; Stambolic, I.; Apostolovic, D.; Milcic, M.; Stanic-Vucinic, D.; Cirkovic Velickovic, T. Complexes of Green Tea Polyphenol, Epigalocatechin-3-Gallate, and 2S Albumins of Peanut. *Food Chemistry* **2015**, *185*, 309–317.
https://doi.org/10.1016/j.foodchem.2015.04.001

Supporting on-line material (SOM)

Complexes of green tea polyphenol, epigalocatechin-3-gallate, and 2S albumins of peanut

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2.2 Fluorescence spectroscopy

Fluorescence spectra were recorded on FluoroMax-4 spectrofluorometer (Horiba Scientific, Kyoto, Japan).

Experiments were carried out at +25°C in a 3.5 ml quartz cuvette. Preliminary Ara h 2 (50μM dissolved in 50 mM phosphate buffer pH 8) spectra were acquired at two excitation wavelengths: 280nm and 295nm, while emission was recorded in the 290 (305 for excitation at 295 nm) – 445 nm range. Ara h 6 (50μM dissolved in 50 mM phosphate buffer pH 8) spectrum was acquired at 275nm, while emission was recorded in the 285 - 500 nm range (**Figure E1**). Quenching experiments were performed with Ara h 2 solution (25 μg mL^{-1}), prepared in 20 mM phosphate buffer pH 7.2. Same phosphate buffer was used as a blank for the titration experiment. Ara h 2 solution (25 μg mL^{-1}) was titrated with 11 x 1 μL and 4 x 1,25 μL of EGCG (2.5 mg mL^{-1}) at pH 7.2. After the addition of each aliquot fluorescent spectrum was immediately recorded under the following conditions - excitation wavelength: 280 nm, 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. Spectra were further analyzed by OriginPro 8 software package (Northampton, MA, USA).

2.4 ITC measurements

ITC measurements were taken on MicroCal iTC200 (GE Lifesciences, USA). Both Ara h 2 and Ara h 6 were prepared as 30μM solution (in 20 mM phosphate, pH 7.2). Protein solution (30 μM) was placed in a 1.4 mL calorimeter sample cell and EGCG solution (370 μM) was loaded into the injection syringe. Each protein was titrated by EGCG in a sequence of twenty 10μL aliquots. In order to allow equilibration, there was a time delay of 1 min, between each injection. The contents of the sample cell were stirred throughout the experiment at 100 rpm to ensure thorough mixing. Raw data were obtained as a plot of heat (μcal/sec) against time (min) and were then integrated to obtain a plot of observed enthalpy change per mole of injectant (ΔH_{obs}, kcal mol^{-1}) against molar ratio. As a control, buffer was also titrated by 370 μM EGCG. Corrected data refer to experimental data after subtraction of the EGCG into buffer control data. ITC data were analyzed using the MicroCal ITC data analysis program.

Figure E1. Fluorescence spectrum of Ara h 6 upon excitation at 275 nm

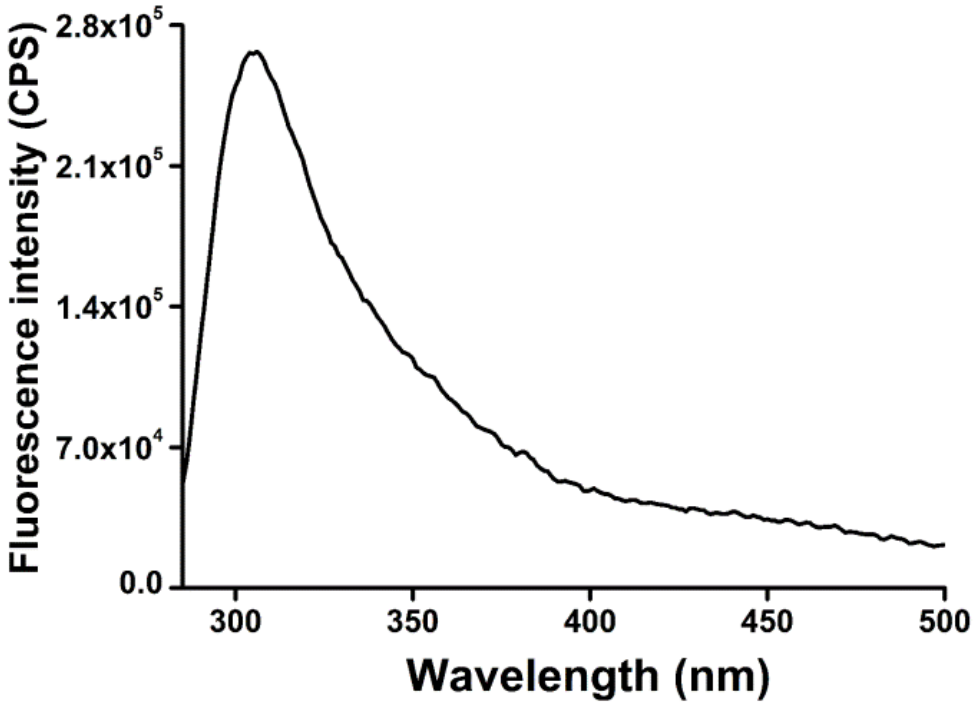

Table E1. The binding parameters (expressed as mean ± SD) describing the Ara h 2/EGCG complex determined by the fluorescence quenching method.

Equation	Binding parameter	Value
Stern-Volmer (Eq. 1)	*Ksv* (M^{-1})	(3.5 ± 0.2) x 10^4
Lehrer (Eq. 3)	*fa*	0.80 ± 0.02
	K_Q (M^{-1})	(5.5 ± 0.7) x 10^4
Double logarithm (Eq. 4)	*Ka* (M^{-1})	(1.7 ± 0.6) x 10^4
	Kd (*M*)	(8 ± 3) x 10^{-5}
	n	0.92 ± 0.04
Langmoir isotherm (Eq. 5)	*Ka* (M^{-1})	(4 ± 1) x 10^4
	Kd (*M*)	(2.6 ± 0.8) x 10^{-5}

3.4 Docking analysis and structural modeling of the complexes of EGCG and 2S albumins of peanut

Molecular docking simulations and theoretical calculations suggested several potential EGCG-binding sites in Ara h 6 protein.

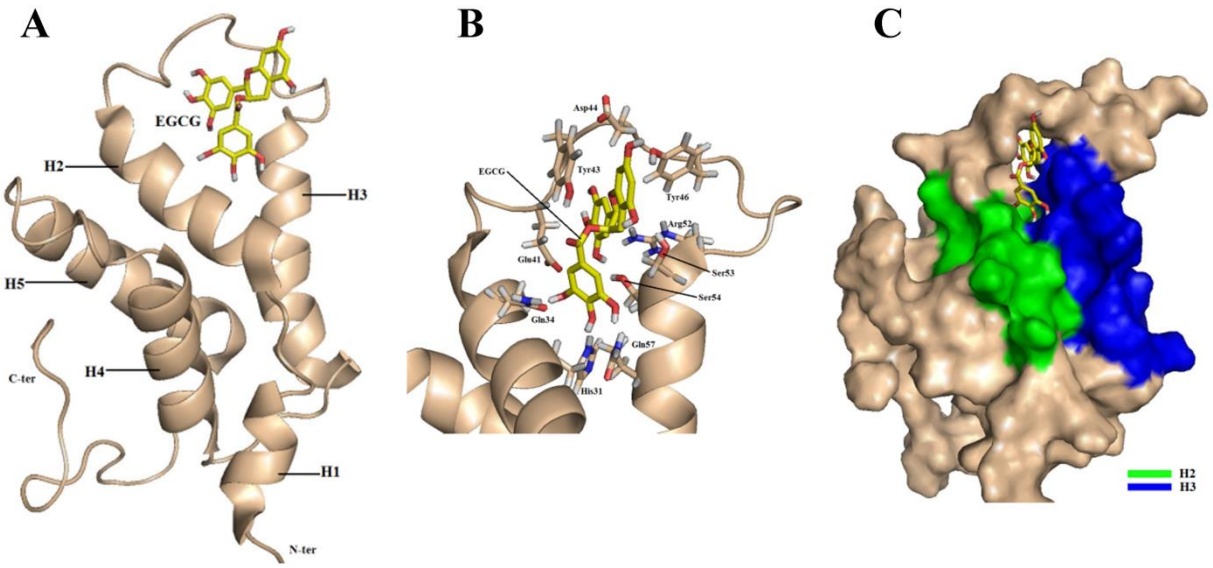

Figure E2. A. The second high-affinity site for binding EGCG to Ara h 6. **B.** Close-up view. **C**. The second high-affinity site for binding EGCG to Ara h 6 protein with H2 and H3 shown in blue and green, resp.

The second high affinity EGCG binding site is found to be situated in the cavity formed by H2 and H3 helixes and the loop connecting the two helices (**Fig. E2 A-C**). The binding site is composed of residues from several structural elements. The walls are composed from the side chains of H2 and H3 helixes and the roof of the binding site is built from the side chains of the residues from loop connecting the H2 and H3 helixes. The trihydroxybenozate ring of EGCG is stacked between the two helixes and interacts with Hd1 from His31 and Oe1 from Gln34 (the numbering from the PDB ID 1W2Q is used throughout the text). Both of these amino acids are situated at the top of helix H3. Third hydrogen bond is formed between OH group from trihydroxybenozate ring of EGCG and Ne2-Hn21 group of GLN57, which is in the H3 helix. OH groups from trihydroxyphenyl ring of EGCG form hydrogen bonds with side chains of Glu41 (from loop region) and Ser54 (from helix H3). There is also a stacking interaction between trihydroxyphenyl ring and Tyr43 from loop (Fig. E2B). Other amino acids shown in the **Fig. E2B** (Asp44, Tyr46 from loop region and Arg52 and Ser53 from helix H3) interact with dihidrobenzopyran ring of EGCG.

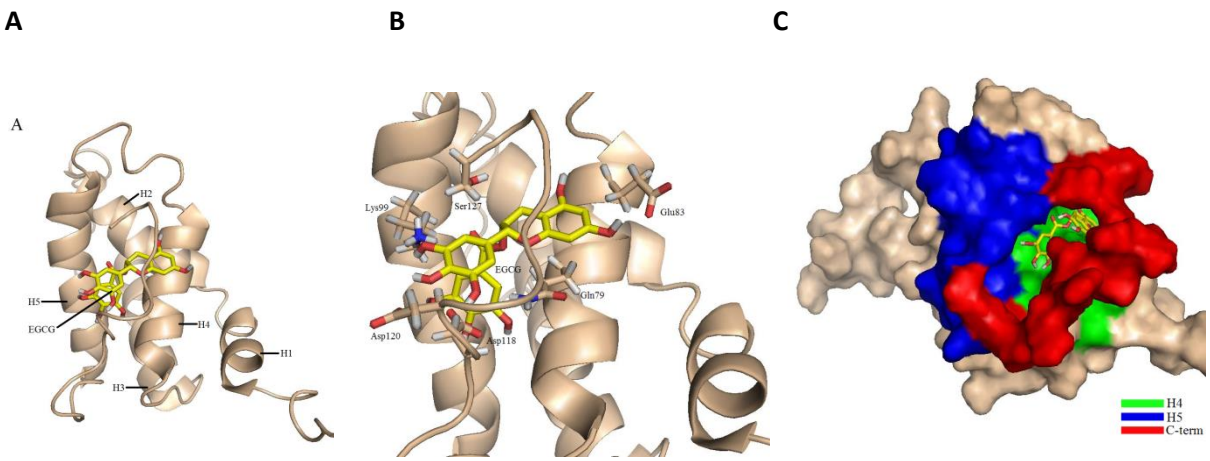

Figure E2. A. The third high-affinity site for binding EGCG to Ara h 6. **B.** Close-up view. **C**. The third high-affinity site for binding EGCG to Ara h 6 protein with H2 and H3 shown in blue and green, resp.

The third binding site is located near the C-terminal part of the protein. The hydrogen bonds are formed between trihydroxybenozate ring of EGCG and side chain of Lys99 (from H5) and Asp118 from C-terminal loop (Figure E3). The trihydroxyphenyl ring of EGCG is situated close to the C-terminal part, interacting with Asp 120 and Ser127. Amino acids from helix H4 (Gln79 and Glu83) are mainly involved in interactions with dihidrobenzopyran part of EGCG molecule. This binding site is found in only 7 out of 14 investigated structures of Ara h 6 protein. .