Supplementary Information

An investigation into the altered binding mode of green tea polyphenols with human serum albumin on complexation with Copper

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Figure S1: Job's continuous variation method to determine the metal to polyphenol ratios in the complexation of Cu^{+2} with (a) EGCG and (b) ECG in methanol. Absorbance measured at 325 and 323 nm (λ_{max} of the chelates) for EGCG and ECG respectively. Job's plot shows the complexation of EGCG and Cu(II), the molar ratios involved EGCG:Cu(II) is 1:2 and that is for ECG and Cu(II) is 2:1.

Figure S2: The Stern-Volmer plots for the quenching of HSA by the (a) EGCG-Cu(II) and (b) ECG-Cu(II) complexes at different temperatures. (♦) 20, (■) 26, (▲) 32 and (—) 38 °C; $\lambda_{max} = 295 \text{ nm}$; $[HSA] = 2 \mu M$; $[EGCG/ECG-Cu(II)] = 0 \text{ to } 16 \mu M$.

Figure S3: UV-vis spectra of HSA in absence and presence (difference spectra of HSA-ligand complex minus ligand only) of (a) EGCG- Cu(II) (blue line) and (b) ECG-Cu(II) (green line) complexes in 20 mM phosphate buffer of pH 7.0. $[HSA] = [EGCG-Cu(II)] = [ECG-Cu(II)] = 25 \mu M$.

Figure S4. Regression plots for the interaction of the EGCG-Cu(II) and (b) ECG-Cu(II) complexes with HSA. (♦) 293 K; (■) 299 K; (▲) 305 K; (×) 311 K λ_{ex} : 295 nm.

Figure S5: Modified Stern-Volmer plots for the interaction of the EGCG-Cu(II) and ECG-Cu(II) complexes with HSA.

Figure S6: The Scatchard plot for the interaction of ECG with HSA (a) and the double-logarithm plots for the interaction of EGCG-Cu(II) (b) and ECG-Cu(II) (c) with HSA at different temperatures.

Figure S7: Spectral overlap between the fluorescence emission spectrum of HSA (solid line) and the absorption spectrum of ligands bound with HSA (dotted or thin line) for (a) ECG, (b) ECG-Cu(II), (c) EGCG, (d) EGCG-Cu(II) and (e) EGC at $[\text{ligand}]/[\text{protein}]=1:1$; λ_{ex} : 295 nm and λ_{em} : 347 nm.

Figure S8: Displacement of bound warfarin from HSA-warfarin complex by (a) ECG, (b) EGC, (c) EGCG, (d) ECG-Cu(II) and (b) EGCG-Cu(II). [HSA]: 2 μ M; [warfarin]: 2 μ M; [ligand]: 0 to 16 μ M; λ_{ex} : 308 nm. Arrows indicate the increase in ligand concentration.

Figure S9: Displacement of bound ibuprofen from HSA-ibuprofen complex by EGC.

[HSA] = 2 μ M; [Ibuprofen] = 1 μ M; [EGC] = 0 to 11 μ M; λ_{ex} : 265 nm. The arrow indicates the progress of titration

Figure S10: CD spectra of HSA in absence (dotted line) and presence (thick lines) of (a) ECG and (b) EGC at 1:1 and 1:2 molar ratios in 20 mM phosphate buffer of pH 7.0.

Figure S11: Curve-fitted amide I ($1700\text{-}1600\text{ cm}^{-1}$) regions of free HSA (A) and HSA-ECG (B) and HSA-EGC (C) complexes (molar ratio 1:2).

Figure S12. (a) Masking of the antioxidant property of the catechins and their copper complexes in presence of HSA. (b) The correlation between the binding affinity and the decrease in scavenging percentages for the catechins and their copper complexes.

Figure S13: Stereoview of the docked pose of (a) ECG and (b) EGC with HSA.

Table S1: Quenching parameters for the interactions of EGCG-Cu(II) and ECG-Cu(II) complexes with HSA at different temperatures

Temp (K)	EGCG-Cu(II)		ECG-Cu(II)	
	$K_{SV} (10^4 \text{ M}^{-1})$	$K_q (10^{13} \text{ M}^{-1})$	$K_{SV} (10^4 \text{ M}^{-1})$	$K_q (10^{13} \text{ M}^{-1})$
293	18.75±2.71	3.75	11.87±1.42	2.37
299	17.84±2.51	3.57	11.53±1.69	2.31
305	17.38±2.51	3.48	11.21±1.41	2.24
311	16.52±2.72	3.31	10.45±1.41	2.09

Table S2. Quenching parameters for the interactions of ECG with HSA at different temperatures

Temp (K)	ECG	
	$K_{SV} (10^4 \text{ M}^{-1})$	$K_q (10^{12} \text{ M}^{-1})$
293	1.41±0.27	2.82
303	1.36±0.32	2.72
308	1.26±0.21	2.52
313	1.23±0.19	2.46

Table S3: The effect of site markers on the binding constants of the copper complexes with HSA

	$K_b (\times 10^4) \text{ M}^{-1}$		
	No marker	Warfarin	Ibuprofen
ECG-Cu(II)	4.95±0.53	3.95±0.74	6.09±0.29
EGCG-Cu(II)	12.79±0.22	7.87±0.58	11.65±0.13

Table S4. Distances (in Å) between polar neighbors of HSA with ECG and EGC

HSA	ECG	EGC
Trp 214 Nε1	2.97 [D-3'-O]	
	2.64 [D-4'-O]	
Arg 218 Nη1	2.98 [B-3'-O]	
Arg 218 Nη2	2.12 [B-3'-O]	
Arg 222 Nη1	3.17 [B-4'-O]	
Glu 383 Oε1		2.56 [B-5'-O]
Gln 390 Oε1		2.87 [B-3'-O]
Gln 390 Nε2		3.21 [C-3-O]
Arg 410 Nε		2.04 [A-7-O]
Arg 410 Nη1		3.18 [A-7-O]
Lys 414 Nζ		2.47 [A-7-O]
Lys 436 Nζ	2.68 [A-5-O]	
Glu 492 Oε1		3.32 [A-5-O]
Glu 492 Oε2		2.98 [A-5-O]

Table S5. Changes in ASA (in Å²) of amino acid residues of HSA on complexation with ECG and EGC

Residue	ΔASA (Å ²) (HSA-ECG)	ΔASA (Å ²) (HSA-EGC)
Lys 195	54.55	
Lys 199		
Trp 214	25.53	
Arg 218	37.14	
Arg 222	16.18	
Ala 291		
Glu 292	10.50	
Asn 295		
Glu 383		29.04
Asn 386		15.59
Leu 387		18.58
Gln 390		41.30
Arg 410		27.09
Lys 414		10.06
Lys 436	21.33	
Pro 447	14.19	
Cys 448	26.62	
Asp 451	29.67	
Tyr 452	20.58	
Ser 489		25.63
Glu 492		32.44