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Noncovalent Interaction of Dietary Polyphenols with Common Human Plasma Proteins

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Supporting Information

ABSTRACT: Common human plasma proteins (CHPP), also called blood proteins, are proteins found in blood plasma. The molecular structure/property−affinity relationships of dietary polyphenols noncovalently binding to CHPP were investigated by comparing the binding constants obtained from the fluorescence titration method. An additional methoxy group in flavonoids increased their binding affinities for CHPP by 1.05 to 72.27 times. The hydroxylation on the 4′ position (ring B) of flavones and flavonols and the 5 position (ring A) of isoflavones weakened the binding affinities; however, the hydroxylation on other positions of flavonoids slightly enhanced or little affected the binding affinities for CHPP. The glycosylation of flavonoids weakened or slightly affected the affinities for CHPP by 1 order of magnitude. The hydrogenation of the C2═C3 double bond of flavone, 6-hydroxyflavone, 6-methoxyflavone and myricetin decreased the binding affinities about 10.02 to 17.82 times. The galloylation of catechins significantly improved the binding affinities with CHPP about 10 to 1000 times. The esterification of gallic acid increased its binding affinity. The binding affinities with CHPP were strongly influenced by the structural differences of dietary polyphenols. Polyphenols with higher affinities for purified HSA also showed stronger affinities with CHPP. The hydrophobic force played an important role in binding interaction between polyphenols and CHPP.

KEYWORDS: structure−affinity relationship, molecular property−affinity relationship, polyphenols, common human plasma proteins, protein−polyphenol noncovalent interaction

INTRODUCTION

Dietary polyphenols such as flavonoids are important nutriments in foods.[1−4] They are of great interest for complementary and alternative medicinal effects, which are basically related to the antioxidant property.[5−7] The structural differences between the various classes of polyphenols significantly affect their absorption, metabolism, and bioactivities *in vivo*.[8−10]

Common human plasma proteins (CHPP), also called blood proteins, are proteins found in blood plasma. Serum total protein in blood is about 70 g/L.[11] Briefly, CHPP consists of albumins (35−50 g/L), immunoglobulins (10−15 g/L), fibrinogens (2−4.5 g/L), and other proteins such as α_1 antitrypsin, α_1 acid glycoprotein, and α_1 fetoprotein.[11] Some of them (e.g., human serum albumin (HSA), β-lactoglobulin and γ-globulin) can bind with many kinds of endogenous and exogenous agents such as dietary polyphenols.[12−14] 60% of plasma proteins are made up of the protein albumin, which are natural vehicles for acidic and neutral bioactive molecules and food nutriments. Globulins make up 35% of plasma proteins and are used in the transport of ions, hormones and lipids assisting in immune function.[11−14]

The purified HSA (fraction V) are usually used as a modeling protein to investigate the interaction between small molecules and proteins.[15−17] However, proteins in CHPP including albumin, α-acid glycoprotein, lipoproteins and α, β, and γ globulins pay an important role in the pharmacokinetic and pharmacodynamic properties of food nutriments.[15−17] The polyphenol−protein interaction is very important for investigation the deliver of bioactive compounds *in vivo*.[18,19] Moreover, there are many metal ions, glucose and small molecules existing in blood that also affect the binding interaction between small molecules and proteins in blood. Few reports, however, have focused on the interaction between dietary polyphenols and total serum proteins. In order to investigate the binding affinities of dietary polyphenols for total plasma proteins, CHPP was purified from the blood of volunteers by centrifugation. The present work concerns the relationship between the molecular structure/properties of dietary polyphenols and their affinities for CHPP.

MATERIALS AND METHODS

Apparatus and Reagents. The fluorescence spectra were recorded on a JASCO FP-6500 fluorometer (Tokyo, Japan). Fifty-five polyphenols (Table 1) were studied. Biochanin A, genistein, apigenin, puerarin, catechin (C), epicatechin (EC), and luteolin (99.0%) were purchased from Aladin Co. Ltd. (Shanghai, China). Flavone, chrysin, and baicalein (99.5%) were obtained commercially from Wako Pure Chemical Industries (Osaka, Japan). Flavanone, 7-hydroxyflavanone, 6-hydroxyflavanone, 6-methoxyflavanone, 6-hydroxyflavone, and 6-methoxyflavone were purchased from TCI Chemical Industries (Tokyo, Japan). Other standards (Table 1) were obtained commercially from Shanghai Tauto Biotech CO.,

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Table 1. Chemical Structure of Dietary Polyphenols and Their Affinities for CHPP[a]

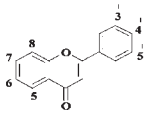
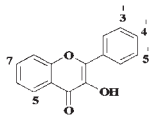
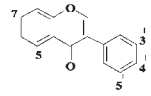
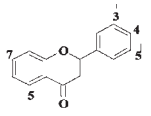
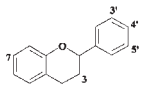
Subclass	Name	Substitutions			Affinity	
		OH	OCH_3	others	lgK_a	n
Flavones	flavone				5.111904	1.026667
	7-hydroxyflavone	7			4.976845	0.963333
	6-hydroxyflavone	6			5.357786	0.98
	6-methoxyflavone		6		5.334561	1.063333
	chrysin	5,7			5.951008	1.136667
	baicalein	5,6,7			5.849648	1.123333
	baicalin	5,6		7-β-D-glucuronide	5.320922	0.993333
	apigenin	5,7, 4'			5.288774	1.023333
	luteolin	5,7,3', 4'			5.535522	1.076667
	hispidulin	5,7, 4'	6		5.720537	1.066667
	wogonin	5,7	8		6.264464	1.203333
	tangeretin		5,6,7,8,4'		7.025346	1.283333
	nobiletin		5,6,7,8,4',5'		6.756512	1.223333
Flavonols	galangin	3,5,7			5.990456	1.123333
	kaempferide	3,5,7	4'		6.013274	1.106667
	kaempferol	3,5,7, 4'			5.171904	1.03
	kaempferitrin	5,4'		3,7-dirhamnoside	5.194862	1.023333
	quercetin	3,5,7,3', 4'			5.741354	1.103333
	quercitrin	5,7,3', 4'		3-o-β-D-glucoside	5.622433	1.04
	myricetin	3,5,7,3', 4', 5'			5.761606	1.093333
	fisetin	3,7,3', 4'			4.682642	0.92
	rutin	5,7,3', 4'		3-α-L-Rham-1,6-D-Glc	5.888382	1.193333
Isoflavones	formononetin	7	4'		4.653451	0.97
	genistein	5,7,4'			5.235177	1.05
	daidzein	7,4'			5.508845	1.083333
	daidzin	4'		7-glucoside	4.700016	0.893333
	genistin	5,4'		7-glucoside	4.29305	0.933333
	biochanin A	5,7	4'		5.608891	1.036667
	tectorigenin	5,7,4'	6		7.094332	1.293333
	puerarin	7,4'		8-C-glucoside	5.085352	1.016667
	sophoricoside	5,7		4'-O-glucoside	5.14458	1.01
Flavanone	naringenin	5,7, 4'			5.339534	1.02
	naringin	5,4'		7-neohesperidose	5.165086	1.016667
	narirutin	5,4'		7-α-L-Rham-1,6-D-Glc	5.389409	1.083333
	hesperidin	5,7, 3'	4'		5.396449	1.053333
	hesperidin-7-O-rutinose	5,3'	4'	7-α-L-Rham-1,6-D-Glc	4.166989	0.916667
	dihydromyricetin	3,5,7,3', 4', 5'			4.728982	0.956667
	flavanone				3.861855	0.856667
	7-hydroxyflavanone	7			5.168687	1.013333
	6-hydroxyflavanone	6			4.356582	0.94
	6-methoxyflavanone		6		4.18585	0.906667
	silibinin				6.068448	1.14
	alpinetin	7	5		5.363904	1.04
Flavanonol	GCG (2,3-trans)	5,7,3',4',5'		3-gallate	4.623167	0.953333
	EGCG (2,3-cis)	5,7,3',4',5'		3-gallate	3.927308	0.816667
	ECG (2,3-cis)	5,7,4',5'		3-gallate	4.284282	0.906667
	EC (2,3-cis)	3,5,7,4',5'			-	-
	EGC (2,3-cis)	3,5,7,3',4',5'			-	-
	C (2,3-trans)	3,5,7,4',5'			-	-

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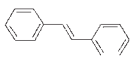
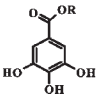
Table 1. Continued

Subclass	Name	Substitutions			Affinity	
		OH	OCH_3	others	lgK_a	n
Stilbene	resveratrol	3,5,4'			6.257709	1.163333
	polydatin	5,4'		3-glucoside	5.104716	1.033333
Gallic acid	gallic acid			R=H	3.564565	0.813333
	methyl gallate			R=methyl	5.424049	1.076667
	ethyl gallate			R=ethyl	4.679072	0.956667
	propyl gallate			R=propyl	4.84405	0.99

[a] Each polyphenol was tested with three CHPP samples from different people, and the average binding constant and the number of binding sites were obtained.

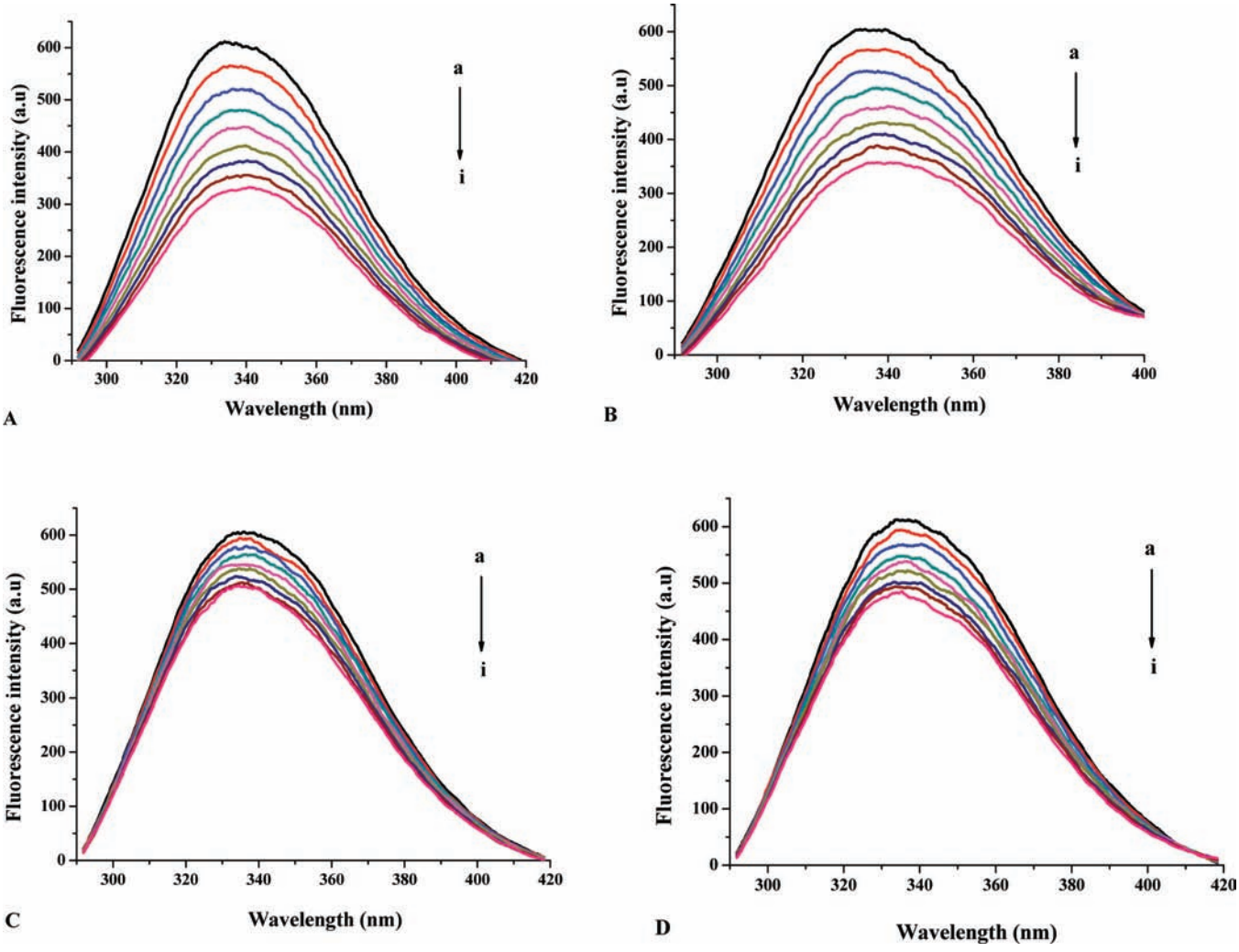


Figure 1. The quenching effects of 6-hydroxyflavone (A), 6-methoxyflavone (B), 6-hydroxyflavanone (C), and 6-methoxyflavanone (D) on CHPP fluorescence spectra at 300.15 K. λ_{ex} = 280 nm; CHPP, 1:100; a−i: 0.00, 1.00, 2.00, ..., 8.00 ($\times 10^{-6}$ mol/L) of polyphenols.

Ltd. (Shanghai, China). The working solutions of standards (1.0×10^{-3} mol/L) were prepared by dissolving each polyphenol with methanol. All other reagents and solvents were of analytical grade, and all aqueous solutions were prepared using newly double-distilled water.

Collection of Plasma Proteins Samples. Blood-derived serum was obtained using blood from 19 healthy adult volunteers. The blood was taken and analyzed in Shanghai Dahua Hospital. We allowed blood to clot in glass centrifuge tubes for 2−4 h to obtain serum. Blood-derived serum was clarified by centrifugation at 3000 rpm for 10 min to separate

serum from the blood cells to get CHPP. The working solution of CHPP (1:100) was prepared by directly diluting the above CHPP with double-distilled water prior to use.

Fluorescence Spectra. 3.0 mL of working solution of CHPP (1:100) was transferred to a 1.0 cm quartz cell. Then it was titrated with successive addition of 3.0 μL of polyphenol solution (1.0×10^{-3} mol/L). Titrations were performed manually by using trace syringes. In each titration, the fluorescence spectrum was collected with the working solution of CHPP (1:100). The fluorescence spectra were recorded in the wavelength range of 310−450 nm upon excitation at 280 nm when diluted CHPP was titrated with polyphenol solution. Slit widths, scan speed and excitation voltage were kept constant within each data set, and each spectrum was the average of three scans. Quartz cells (1 cm path length) were used for all measurements. The results of the time course experiments for the equilibration are not given here. Fluorescence emissions of these polyphenols within the range of 300−400 nm were not observed under the excitation wavelength of 280 nm. The polyphenols were stable during the fluorescence measurements, as shown by HPLC analyses (not given here). Each fluorescence intensity determination was repeated and found to be reproducible within experimental error. Each polyphenol was tested with three CHPP samples from different people, and the average binding constant and the number of binding sites were obtained.

Data Process. Fluorescence quenching was described by the Stern−Volmer equation:[16−19]

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F represent the fluorescence intensities of CHPP in the absence and in the presence of polyphenols, K_q is the quenching rate constant, K_{SV} is the dynamic quenching constant, τ_0 is the average lifetime, and [Q] is the concentration of polyphenols.

The binding constants were calculated according to the double-logarithm equation:[14−17]

$$\log_{10}[(F_0 - F)/F] = \log_{10}K_a + n\log_{10}[Q] \quad (2)$$

where F_0 and F represent the fluorescence intensities of CHPP in the absence and in the presence of polyphenols, K_a is the binding constant, n is the number of binding sites, and [Q] is the concentration of polyphenols. According to eq 2 the values of "$(F_0 - F)/F$" can be obtained in each "[Q]". Then, the linear regression equation between the "$\log_{10}[(F_0 - F)/F]$" values and "$\log_{10}[Q]$" values was obtained on the Origin 7.5 software. The slope factor means "n", and the intercept refers to "$\log_{10}K_a$".

RESULTS AND DISCUSSION

Quenching Effect of Polyphenols on CHPP Fluorescence. As representative examples, the fluorescence spectra of CHPP after addition of 6-hydroxyflavone (A), 6-methoxyflavone (B), 6-hydroxyflavanone (C), and 6-methoxyflavanone (D) are shown in Figure 1 (The fluorescence spectra of CHPP quenched by other polyphenols are not given here.) Except for EC, (−)-epigallocatechin (EGC), and C, all polyphenols tested can quench the fluorescence of CHPP remarkably with increasing concentration of polyphenols. There are no obvious shifts of the maximum λ_{em} of CHPP fluorescence for polyphenols tested.

The quenching ratio (F/F_0) of CHPP fluorescence with addition of 6-hydroxyflavone, 6-methoxyflavone, 6-hydroxyflavanone, and 6-methoxyflavanone are shown in Figure 2. The intensities of CHPP fluorescence decreased rapidly with the addition of 6-hydroxyflavone and 6-methoxyflavone. However, 6-hydroxyflavanone and 6-methoxyflavanone quenched CHPP fluorescence slowly. 8.0 μmol/L of 6-hydroxyflavone was found

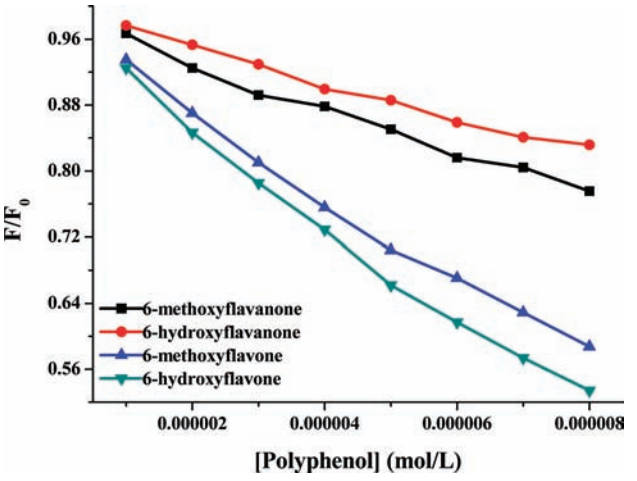


Figure 2. The quenching ratio (F/F_0) of CHPP fluorescence with addition of 6-hydroxyflavone, 6-methoxyflavone, 6-hydroxyflavanone, and 6-methoxyflavanone.

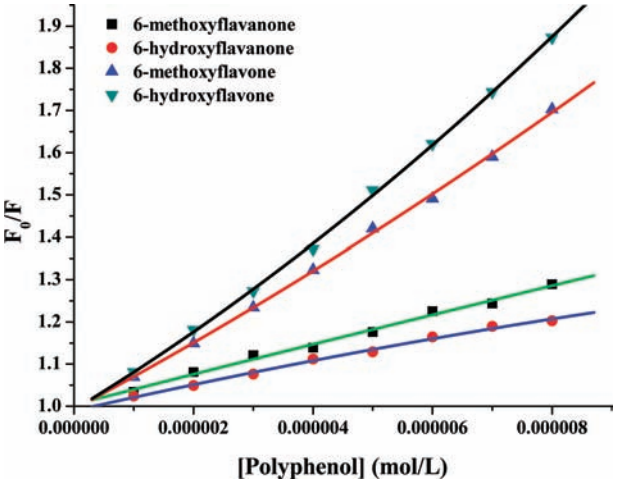


Figure 3. The Stern−Volmer plots for CHPP fluorescence quenching by polyphenols at 300.15 K.

to lead to quench CHPP fluorescence by 46.60%; however, 8.0 μmol/L of 6-hydroxyflavanone only quenched 16.83% of CHPP fluorescence. These results indicated that the quenching effect of polyphenols on CHPP fluorescence depended on the structures of polyphenols.

Figure 3 shows the Stern−Volmer plots for CHPP fluorescence quenching by 6-hydroxyflavone, 6-methoxyflavone, 6-hydroxyflavanone and 6-methoxyflavanone. As seen from Figure 3, the Stern−Volmer plots for 6-hydroxyflavanone and 6-methoxyflavanone are linear, which illustrates that 6-hydroxyflavanone and 6-methoxyflavanone quenched CHPP fluorescence mainly by the dynamic model. However, it was found that both dynamic and static quenching were involved for 6-hydroxyflavone and 6-methoxyflavone on CHPP fluorescence, which is demonstrated by the fact that the Stern−Volmer plots deviated from linearity toward the y-axis at higher concentrations.

The Binding Constants (K_a) and the Number of Binding Sites (n). Each polyphenol was tested with three CHPP samples from different people, and the average binding constant and the number of binding sites are summarized in Table 1. The values of

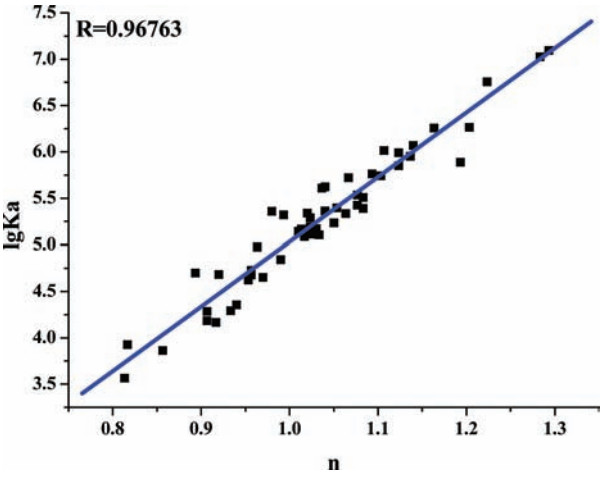


Figure 4. The relationship between the binding affinities ($\log_{10}K_a$) and the number of binding sites (n) between polyphenols and CHPP.

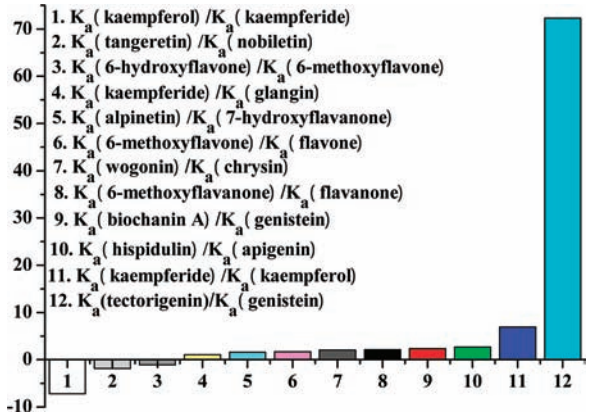


Figure 5. Effects of methoxylation and methylation of flavonoids on the affinities of the polyphenols for CHPP.

$\log_{10}K_a$ are proportional to the number of binding sites (n) (Figure 4), which indicates that the equation (eq 1) used here is suitable to study the interaction between polyphenols and CHPP.[18,19] The magnitudes of apparent binding constants for CHPP were almost in the range of 10^4-10^6 L/mol, which were much smaller than the affinities of polyphenols for BSA, bovine γ-globulin, and HSA from our previous reports (10^4-10^8 L/mol).[12,15,16]

Flavonoids. *Influence of an Additional Methoxy Group in Flavonoids.* Figure 5 shows the effect of an additional methoxy group in flavonoids on the affinities for CHPP. In general, an additional methoxy group in flavonoids increased their binding affinities for CHPP by 1.05 to 72.27 times. The affinity of tectorigenin for CHPP was found to be 72.27 times higher than that of its unmethoxylated form (genistein) (Figure 5). On the other hand, 4′-methoxylation of galangin hardly affected the affinity for CHPP. However, if a hydroxyl group in flavonoids was changed to a methoxy group (methylation), their binding affinities for CHPP were slightly affected. In our previous report, the methylation of hydroxyl group in flavonoids enhanced their binding affinities for HSA by 2−16 times.[16]

Hydroxylation. Table 2 shows the effect of hydroxylation of flavonoids on the affinities for CHPP. As seen from the data, the hydroxylation on rings A, B, and C of flavonoids slightly affected the binding affinities for CHPP. The hydroxylation on the 4′ position (ring B) of flavones and flavonols and 5 position (ring A) of isoflavones weakened the binding affinities; however, the hydroxylation on other positions of flavonoids slightly enhanced or little affected the binding affinities for CHPP. The hydroxylation on 7-hydroxyflavanone showed about 20-fold higher affinity than that of flavanone.

Table 2. Effects of Hydroxylation of Flavonoids on the Affinities for CHPP

class	ring	position	example	effect (times)
flavone	A	7 H → OH	flavone → 7-OH flavone	little effect
		5 H → OH	7-OH flavone → chrysin	↑ 9.55
		6 H → OH	chrysin → baicalein	little effect
			flavone → 6-OH flavone	↑ 1.74
	B	4′ H → OH	chrysin → apigenin	↓ 4.57
		3′ H → OH	apigenin → luteolin	↑ 1.74
	C	3 H → OH	chrysin → galangin	little effect
			apigenin → kaempferol	little effect
			luteolin → quercetin	↑ 1.26
flavonol	A	5 H → OH	fisetin → quercetin	↑ 11.48
	B	3′ H → OH	kaempferol → quercetin	↑ 3.72
		4′ H → OH	galangin → kaempferol	↓ 6.62
		5′ H → OH	quercetin → myricetin	little effect
isoflavone	A	5 H → OH	daidzein → genistein	↓ 1.86
			daidzin → genistin	↓ 2.57
flavanone	A	6 H → OH	flavanone → 6-OH flavanone	↑ 3.09
		7 H → OH	flavanone → 7-OH flavanone	↑ 19.95

Flavonoid Isomers. In this study, we can compare two isomer groups (apigenin, galangin, baicalein, genistein and luteolin, kaempferol, fisetin). The binding constants (K_a) were determined as galangin > baicalein > apigenin > genistein and luteolin > kaempferol > fisetin. These data illustrate that flavones and flavonols show higher binding capacity with CHPP than isoflavones.

Glycosylation. The dietary flavonoids in nature occur mostly as β-glycosides. The flavonols are found mainly as the 3- and 7-*O*-glycoside, although the 4′ position may also be glycosylated in some plants (Table 1). Herein, the effect of glycosylation of dietary flavonoids on the affinities for CHPP was investigated. The sugar moieties are in 3, 7, or 4′ positions of flavonoids. In our present study (Figure 6), the glycosylation of flavonoids slightly decreased or little affected the affinities for CHPP less than 1 order of magnitude. For example, the affinity of quercetin for CHPP was similar to that of rutin and quercetin. However, recently we found that the glycosylation of flavonoids decreased the affinities for purified HSA or BSA by 1−3 orders of magnitude depending on the conjugation site and the class of sugar moiety.[16] This different influence may due to plasma proteins from common human beings having bound with some small molecules *in vivo*, which occupied the binding sites and caused competing binding.

Hydrogenation of the C2═C3 Double Bond. The C2═C3 double bond in conjugation with a 4-oxo group plays a very important role for the affinity for CHPP. As shown in the Supporting Information, it was found that the hydrogenation of the C2═C3 double bond of flavonoids decreased or little affected the binding affinities for CHPP. The hydrogenation of

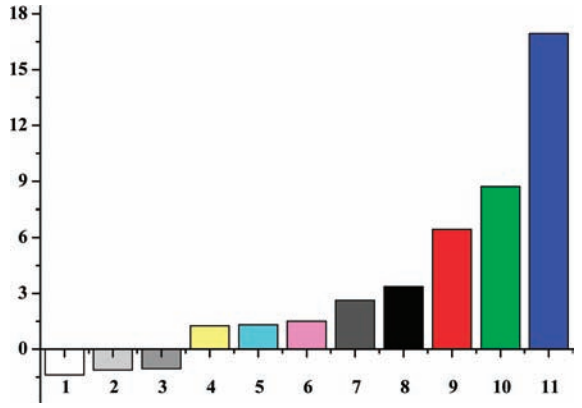


Figure 6. The glycosylation of flavonoids decreased or little affected the affinity of the polyphenols for CHPP: (1) K_a(rutin)/K_a(quercetin), (2) (narirutin)/K_a(naringenin), (3) K_a(kaempferitrin)/K_a(kaempferol), (4) K_a (genistein)/K_a(sophoricoside), (5) K_a(quercetin)/K_a(quercitrin), (6) K_a(naringenin)/K_a(naringin), (7) K_a(daidzein)/K_a(puerarin), (8) K_a-(baicalein)/K_a(baicalin), (9) K_a(daidzein)/K_a(daidzin), (10) K_a(genistein)/K_a(genistin), (11) K_a(hesperitin)/K_a(hesperitin-7-*O*-rutinose).

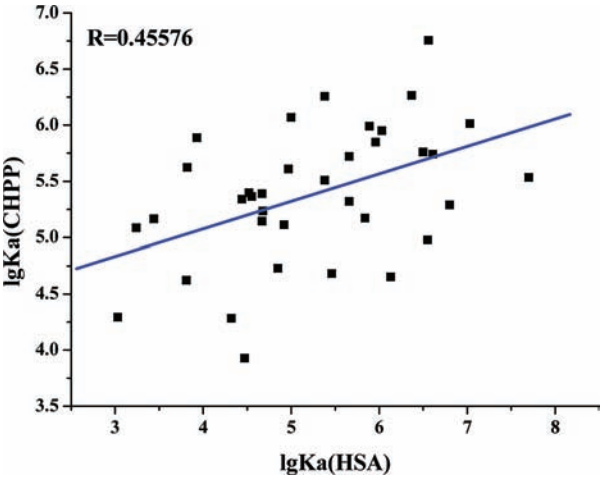


Figure 7. The relationship between the affinities of polyphenols for CHPP ($\log_{10}K_a$(CHPP)) and HSA ($\log_{10}K_a$(HSA)).

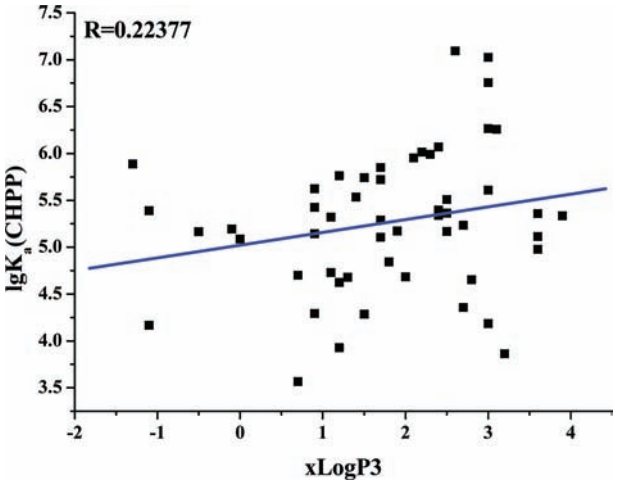


Figure 8. Relationship of apparent binding constants ($\log_{10}K_a$) with partition coefficient (XLogP3) of polyphenols. The partition coefficient values (XLogP3) were taken from PubChem Public Chemical Database.

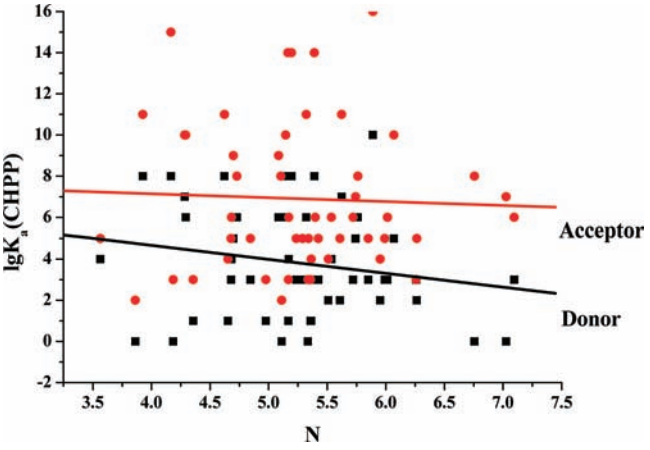


Figure 9. Relationships of the hydrogen bond acceptor/donor number of polyphenols (N) with the affinities for CHPP. The hydrogen bond acceptor/donor numbers were taken from PubChem Public Chemical Database.

the C2═C3 double bond of flavone, 6-hydroxyflavone, 6-methoxyflavone, and myricetin decreased the binding affinities about 10.02 to 17.82 times. However, the hydrogenation of the C2═C3 double bond on apigenin and 7-hydroxyflavone hardly affected the affinity for CHPP. Previously, we have investigated the effect of hydrogenation of the C2═C3 double bond in flavonoids on the affinities for purified HAS by 2−3 orders of magnitude. Planarity of the C ring in flavonoids may be important for binding interaction with proteins, as the molecules with saturated C2−C3 bonds (flavanones and certain others) permit more twisting of the B ring with reference to the C ring. A C2═C3 double bond increases the π-conjugation of the bond linking the B and C rings, which favors near-planarity of the two rings.[20] Molecules with near-planar structure more easily enter the hydrophobic pockets in proteins.

Catechins. Catechins are the major polyphenols in green tea leaves. The major catechins of green tea extract are C, EC, EGC, (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), and gallocatechin gallate (GCG).[21−24] Here, we determined the affinities between catechins and CHPP by fluorescence quenching method with double logarithm regression curve (Table 1). The binding constants ($\log_{10}K_a$) between ECG, EGCG, and GCG for CHPP were 4.28, 3.93 and 4.62, respectively. However, the binding constants of EC, EGC, and C for CHPP were very low and they hardly quenched the CHPP fluorescence. And the calculated binding constants of EC, EGC, and C for CHPP were less than 10^3 L/mol. It illustrates that the galloylation of catechins significantly improved the binding affinities with CHPP about 10 to 1000 times. The pyrogallol-type catechins showed lower affinities than catechol-type catechins. Moreover, the affinity of the catechin with 2,3-trans structure (GCG) for CHPP was shown to be higher than that of the catechin with 2,3-cis structure (EGCG).

Stilbenes. Stilbenes are phytoalexins that become activated when plants are stressed and are important polyphenols with the C6−C2−C6 structure.[25] The typical natural stilbenes are resveratrol and its 3-glucoside, polydatin.[26] Here, we determined the affinities between resveratrol/polydatin with CHPP. The

binding constants ($\log_{10} K_a$) between resveratrol and polydatin for CHPP were 6.23 and 5.10, respectively. It illustrated that the glycosylation of resveratrol slightly reduced the affinity for CHPP. The affinity of resveratrol for CHPP was about 13.48 times higher than that of polydatin.

Gallic Acid. Gallic acid is one of phenolic acids found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants. Gallic acid seems to have antifungal and antiviral properties. Gallic acid acts as an antioxidant and helps to protect our cells against oxidative damage.[27] The esters of gallic acid are termed "gallates". The typical gallates are methyl gallate, ethyl gallate, and propyl gallate (Table 1). As seen from Table 1, the esterification of gallic acid significantly improved the affinity for CHPP. The affinities of gallic acid and its esters with CHPP were determined as methyl gallate > propyl gallate > ethyl gallate > gallic acid.

Relationship between the Affinities of Polyphenols for CHPP and Purified HSA. Purified HSA and BSA are usually used as modeling proteins to investigate the interactions between small molecules and serum albumins. In our previous report,[16] the molecular property−affinity relationship of flavanoids and flavonoids for purified HSA *in vitro* was investigated. The affinities of polyphenols for purified HSA were also investigated by other groups.[28−32]

The affinities of flavonoids for purified HSA were within the range of 10^4−10^8 L/mol, which were obviously much higher than current affinities for CHPP (10^4−10^6 L/mol). In blood, there are many metal ions, such as Zn^{2+}, Cu^{2+}, Ca^{2+}, and Mg^{2+}, and small molecules, which also affect the interaction between polyphenols with plasma proteins. Cao et al. found Zn^{2+} decreased the affinity of flavonols for BSA *in vitro*.[33] The relationship between the affinities of polyphenols for CHPP and purified HSA is shown in Figure 7. As seen from these data, polyphenols with higher affinities for purified HSA also showed stronger affinities with CHPP. It illustrated that the HSA is the major transporting protein in blood for polyphenols.

The Nature of Polyphenol−CHPP Noncovalent Interaction. The noncovalent interaction between polyphenols and proteins usually is caused by four major interaction forces, namely, hydrogen bonding force, van der Waals force, hydrophobic interaction and electrostatic interaction.[34−36] The nature of polyphenol−CHPP interaction was studied by investigation of the molecular property−affinity relationship. Here the lipophilicity and hydrogen bond acceptor numbers of polyphenols were used. The lipophilicity of the compounds under study was assessed by their partition coefficient values ($XLogP_3$) according to PubChem Public Chemical Database. There is a relationship between the $XlogP_3$ values and $\log_{10}K_a$ values for polyphenols (Figure 8). The linear regression equation using the Origin 7.5 software was $\log_{10}K_a = 5.02167 + 0.13611\ XlogP_3$ ($R = 0.22377$). The affinities of polyphenols for CHPP increased with increasing partition coefficient. From this point, the hydrophobic force played an important role in binding interaction between polyphenols and CHPP.[37,38] To further investigate whether or not the hydrogen bond force plays an important role in binding polyphenols to CHPP, the relationships of the hydrogen bond acceptor/donor numbers (N, data were from reference) of polyphenols with the affinities for CHPP are shown in Figure 9. The affinities for CHPP *slightly* decreased with increasing hydrogen bond donor numbers of polyphenols *hardly* changed with hydrogen bond acceptor numbers. These results illustrate that the hydrogen bond force was not the main force to bind polyphenols to CHPP.

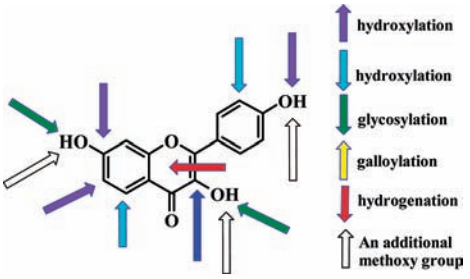


Figure 10. The structural elements that influence the affinities of polyphenols for CHPP. The up arrows represent increasing the binding affinity; the down arrows represent decreasing the binding affinity.

Concluding Remarks. As shown in Figure 10, some of the structural elements that influence the affinities of polyphenols for CHPP are the following: (1) An additional methoxy group in flavonoids increased their binding affinities for CHPP by 1.05 to 72.27 times; methylation of flavonoids hardly affected the affinities. (2) Hydroxylation on 4′ position (ring B) of flavones and flavonols and 5 position (ring A) of isoflavones weakened the affinities; however, the hydroxylation on other positions of flavonoids slightly enhanced or little affected the binding affinities. (3) Glycosylation of flavonoids weakened or little affected the affinities by 1 order of magnitude. (4) Hydrogenation of the C2═C3 double bond of flavone, 6-hydroxyflavone, 6-methoxyflavone, and myricetin decreased the binding affinities about 10.02 to 17.82 times. (5) Galloylation of catechins significantly improved the binding affinities with CHPP about 10 to 1000 times. (6) Esterification of gallic acid increased its binding affinity.

■ ASSOCIATED CONTENT

Supporting Information. Figure depicting the ffects of hydrogenation of the C2═C3 double bond on flavonoids on the affinities for CHPP. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Cohen, S. D.; Kennedy, J. A. Plant metabolism and the environment: Implications for managing phenolics. *Crit. Rev. Food Sci.* **2010**, *50*, 620–543.

(2) Hagl, S.; Deusser, H.; Soyalan, B.; Janzowski, C.; Will, F.; Dietrich, H.; Albert, F. W.; Rohner, S.; Richling, E. Colonic availability of polyphenols and D-(−)-quinic acid after apple smoothie consumption. *Mol. Nutr. Food Res.* **2011**, *55*, 368–377.

(3) Côté, J.; Caillet, S.; Doyon, G.; Sylvain, J. F.; Lacroix, M. Analyzing cranberry bioactive compounds. *Crit. Rev. Food Sci.* **2010**, *50*, 872–888.

(4) Johnson, J.; de Mejia, E. G. Dietary factors and pancreatic cancer: The role of food bioactive compounds. *Mol. Nutr. Food Res.* **2011**, *55*, 58–73.

(5) Woodward, G. M.; Needs, P. W.; Kay, C. D. Anthocyanin-derived phenolic acids form glucuronides following simulated gastrointestinal digestion and microsomal glucuronidation. *Mol. Nutr. Food Res.* **2011**, *55*, 378–386.

(6) Côté, J.; Caillet, S.; Doyon, G.; Sylvain, J. F.; Lacroix, M. Bioactive compounds in cranberries and their biological properties. *Crit. Rev. Food Sci.* **2010**, *50*, 666–679.

(7) Gonzalez, R.; Ballester, I.; Lopez-Posadas, R.; Suarez, M. D.; Zarzuelo, A.; Martinez-Augustin, O.; De Medina, F. Effects of flavonoids and other polyphenols on inflammation. *Crit. Rev. Food Sci.* **2011**, *51*, 331–362.

(8) Gosslau, A.; Li, S. M.; Ho, C. T.; Chen, K. Y.; Rawson, N. E. The importance of natural product characterization in studies of their anti-inflammatory activity. *Mol. Nutr. Food Res.* **2011**, *55*, 74–82.

(9) Blade, C.; Arola, L.; Salvado, M. J. Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms. *Mol. Nutr. Food Res.* **2010**, *54*, 37–59.

(10) Manzano, S.; Williamson, G. Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Mol. Nutr. Food Res.* **2010**, *54*, 1773–1780.

(11) Schaller, J.; Gerber, S.; Kaempfer, U.; Lejon, S.; Trachsel, C. *Human Blood Plasma Proteins: Structure and Function*; Wiley: New York, 2008.

(12) Xiao, J. B.; Kai, G. Y.; Yang, F.; Liu, C. X.; Xu, X. C.; Yamamoto, K. Molecular structure-affinity relationship of natural polyphenols for bovine γ-globulin. *Mol. Nutr. Food Res.* **2011**, *55*, S86–S92.

(13) Bae, M. J.; Ishii, T.; Minoda, K.; Kawada, Y.; Ichikawa, T.; Mori, T. Albumin stabilizes (−)-epigallocatechin gallate in human serum: Binding capacity and antioxidant property. *Mol. Nutr. Food Res.* **2009**, *53*, 709–715.

(14) Xiao, J. B.; Cao, H.; Chen, T. T.; Yang, F.; Liu, C. X.; Xu, X. C. Molecular property-binding affinity relationship of flavonoids for common rat plasma proteins *in vitro*. *Biochimie* **2011**, *93*, 134–140.

(15) Xiao, J. B.; Cao, H.; Wang, Y. F.; Yamamoto, K.; Wei, X. L. Structure-affinity relationship of flavones on binding to serum albumins: Effect of hydroxyl groups on ring A. *Mol. Nutr. Food Res.* **2010**, *54*, S253–S260.

(16) Xiao, J. B.; Chen, T. T.; Cao, H.; Chen, L.; Yang, F. Molecular property-affinity relationship of flavanoids and flavonoids for human serum albumin *in vitro*. *Mol. Nutr. Food Res.* **2011**, *55*, 310–317.

(17) Xiao, J. B.; Kai, G. Y. A review of dietary polyphenol-plasma protein interactions: Characterization, influence on the bioactivity, and structure-affinity relationship. *Crit. Rev. Food Sci. Nutr.* **2011**, DOI: 10.1080/10408398.2010.499017.

(18) Berezhkovskiy, L. M. On the calculation of the concentration dependence of drug binding to plasma proteins with multiple binding sites of different affinities: Determination of the possible variation of the unbound drug fraction and calculation of the number of binding sites of the protein. *J. Pharm. Sci.* **2007**, *96*, 249–257.

(19) Xiao, J. B.; Chen, X. Q.; Jiang, X. Y.; Hilczer, M.; Tachiya, M. Probing the interaction of trans-resveratrol with bovine serum albumin: a fluorescence quenching study with Tachiya model. *J. Fluoresc.* **2008**, *18*, 671–678.

(20) Edenharder, R.; von Petersdorff, I.; Rauscher, R. Antimutagenic effects of flavonoids, chalcones and structurally related compounds on the activity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and other heterocyclic amine mutagens from cooked food. *Mutat. Res.* **1993**, *287*, 261–274.

(21) Stoupi, S.; Williamson, G.; Drynan, J. W.; Barron, D.; Clifford, M. N. A comparison of the in vitro biotransformation of (−)-epicatechin and procyanidin B2 by human faecal microbiota. *Mol. Nutr. Food Res.* **2010**, *54*, 747–759.

(22) Qin, B. L.; Polansky, M. M.; Harry, D.; Anderson, R. A. Green tea polyphenols improve cardiac muscle mRNA and protein levels of signal pathways related to insulin and lipid metabolism and inflammation in insulin-resistant rats. *Mol. Nutr. Food Res.* **2010**, *54*, S14–S23.

(23) Stalmach, A.; Mullen, W.; Steiling, H.; Williamson, G.; Lean, M. E. J.; Crozier, A. Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Mol. Nutr. Food Res.* **2010**, *54*, 323–334.

(24) Ostertag, L. M.; O'Kennedy, N.; Kroon, P. A.; Duthie, G. G.; de Roos, B. Impact of dietary polyphenols on human platelet function − A critical review of controlled dietary intervention studies. *Mol. Nutr. Food Res.* **2010**, *54*, 60–81.

(25) Wang, T. T. Y.; Schoene, N. W.; Kim, Y. S.; Mizuno, C. S.; Rimando, A. M. Differential effects of resveratrol and its naturally occurring methylether analogs on cell cycle and apoptosis in human androgen-responsive LNCaP cancer cells. *Mol. Nutr. Food Res.* **2010**, *54*, 335–344.

(26) Cottart, C. H.; Nivet-Antoine, V.; Laguillier-Morizot, C.; Beaudeux, J. L. Resveratrol bioavailability and toxicity in humans. *Mol. Nutr. Food Res.* **2010**, *54*, 7–16.

(27) Chuang, C. Y.; Liu, H. C.; Wu, L. C.; Chen, C. Y.; Chang, J. H. T.; Hsu, S. L. Gallic acid induces apoptosis of lung fibroblasts via a reactive oxygen species-dependent ataxia telangiectasia mutated-p53 activation pathway. *J. Agric. Food Chem.* **2010**, *58*, 2943–2951.

(28) Tang, J. H.; Lian, N.; He, X. H.; Zhang, G. H. Investigation of the interaction between sophoricoside and human serum albumin by optical spectroscopy and molecular modeling methods. *J. Mol. Struct.* **2008**, *889*, 408–414.

(29) Knjazeva, T.; Kaljurand, M. Capillary electrophoresis frontal analysis for the study of flavonoid interactions with human serum albumin. *Anal. Bioanal. Chem.* **2010**, *397*, 2211–2219.

(30) Xie, M. X.; Xu, X. Y.; Wang, Y. D. Interaction between hesperetin and human serum albumin revealed by spectroscopic methods. *Biochim. Biophys. Acta* **2005**, *1724*, 215–24.

(31) Wu, Q. H.; Zhou, X.; Zang, X. H.; Wang, C.; Zhang, Z. H.; Wang, Z. Spectroscopic study on the interaction between resveratrol and human serum albumin. *Guangpuxue Yu Guangpu Fenxi* **2009**, *29*, 226–30.

(32) Zhang, Y. H.; Dong, L. J.; Li, J. Z.; Chen, X. G. Studies on the interaction of gallic acid with human serum albumin in membrane mimetic environments. *Talanta* **2008**, *76*, 246–253.

(33) Cao, S. H.; Jiang, X. Y.; Chen, J. W. Effect of Zinc (II) on the interactions of bovine serum albumin with flavonols bearing different number of hydroxyl substituent on B-ring. *J. Inorg. Biochem.* **2010**, *104*, 146–152.

(34) Xiao, J. B.; Chen, L. S.; Yang, F.; Liu, C. X.; Bai, Y. L. Green, yellow and red emitting CdTe QDs decreased the affinities of apigenin and luteolin for human serum albumin *in vitro*. *J. Hazard. Mater.* **2010**, *182*, 696–703.

(35) Xiao, J. B.; Huo, J. L.; Yang, F.; Chen, X. Q. Non-covalent interaction of dietary polyphenols with bovine hemoglobin in vitro: Molecular structure/property-affinity relationship aspects. *J. Agric. Food Chem.* **2011**, *59*, 8484–8490.

(36) Xiao, J. B.; Wang, F. J.; Liu, J.; Wang, L. T.; Kai, G. Y.; Yu, X. B. Effect of ZnO#ZnS QDs heterojunctures on the stilbenes-plasma proteins interactions. *Mol. BioSyst.* **2011**, *7*, 2452–2458.

(37) Xiao, J. B.; Kai, G. Y.; Ni, X. L.; Yang, F.; Chen, X. Q. Interaction of natural polyphenols with human α-amylase in vitro: Molecular property-affinity relationship aspect. *Mol. BioSyst.* **2011**, *7*, 1883–1890.

(38) Yang, F.; Zhao, Y. R.; Kai, G. Y.; Xiao, J. B. Interaction of dietary flavonoids with gamma-globulin: Molecular property-binding affinity relationship aspect. *Food Funct.* **2011**, *2*, 137–141.