The calculation of host-guest interactions with semiempirical, and adsorption isotherms of biosorbents with consecutive docking methods

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ABSTRACT

Our Research Group has developed a sensor to detect the gene wrecking and carcinogenic mycotoxin molecules. Hence we applied functionalized gold nanoparticles and thin films to measure these analytes. During the investigations thiolated modified macrocycle molecules (mainly cyclodextrins) were applied to functionalize the gold surfaces. We found, that the gold nanoparticle based (functionalized with thiolated β-cyclodextrin, BCD) sensors, binds irreversible the Aflatoxin B1 (AfB1) and reversible the Aflatoxin B2 (AfB2) molecules. It was an interesting result and I investigated these complexes with the PM7, PM6-DH+, PM6-DH2 semiempirical quantum chemistry methods with the COSMO solvation model, implemented in MOPAC2012 software [1]. I found that the calculated standard heat of formation of the 2 : 2 AfB1 : BCD complex is slightly more negative (~20 kJ/mol), compared to the corresponding AfB2 : BCD complex.

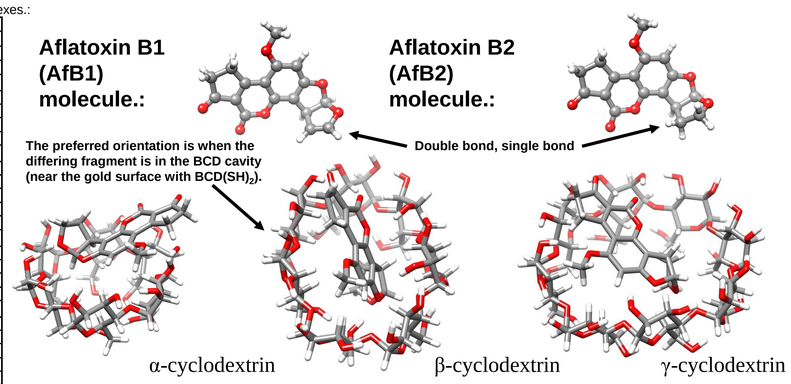
Our Research Group prepared albumin/polyelectrolyte core-shell nanoparticles for controlled drug release. During the development phase we measured the albumin/drug molecule adsorption isotherms to optimize the drug release. My former supervisor, Tamás Körtvélyesi had an idea, to dock a ligand to a receptor consecutively and use this method to calculate adsorption isotherms. Now I realized this project, and docked the ligands sequentially to the human serum albumin (HSA) and bovine serum albumin (BSA) molecules. I optimized the receptor structure with the Gromacs software, and used the AutoDock VINA docking algorithm [2]. To calculate the isotherms I used the stepwise adsorption model (like stepwise complex formation). In the future I want to deal with Monte Carlo algorithm based softwares (MMC; Mihály Mezei, MCCCS Towhee; Marcus G. Martin, BIGMAC; Prof. Berend Smit) to calculate adsorption isotherms.

The [(Aflatoxin)[BCD(SH)$_2$]] complex

A. Majzik *et al*. found from QCM and SPR adsorption measurements, that the gold nanoparticles modified, with thiolated β-cyclodextrin binds reversible the Aflatoxin B2 and irreversible the Aflatoxin B1 molecules [3]. It is a surprising result because the two toxin molecules differs in only one bond (single/double) from each other. First times I was skeptical, but I investigated these systems with AutoDock VINA docking software and the PM6-DH2, PM6-DH+, PM7 semiempirical quantum chemistry methods. In the 1:1 complexes I found that the β-cyclodextrin binds Aflatoxin the strongest (see table)!

The calculated binding enthalpies of the Aflatoxin – Cyclodextrin complexes.:

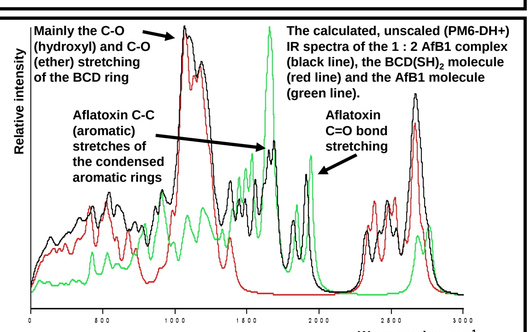
			PM6-DH2/COSMO	PM6-DH+/COSMO	PM6-DH2	PM6-DH+
		aflatoxin	ΔH^0_b / kJ/mol	ΔH^0_b / kJ/mol	ΔH^0_b ratio	ΔH^0_b ratio
Alpha-cyclodextrin	1	Aflatoxin B1	-97.12	-94.62	0.802	0.913
	2	Aflatoxin B2	-104.01	-86.41	0.832	0.676
	3	Aflatoxin G1	-105.28	-103.36	0.865	1.235
	4	Aflatoxin G2	-90.78	-104.75	0.748	0.859
	5	Aflatoxin M1	-119.89	-125.43	0.869	0.810
	6	Aflatoxin M2	-111.14	-106.29	0.823	0.866
	7	Aflatoxin Q1	-103.96	-104.57	0.917	1.017
	8	Aflatoxin P1	-100.17	-99.56	0.821	0.950
Beta-cyclodextrin	1	Aflatoxin B1	-121.11	-103.67	1	1
	2	Aflatoxin B2	-125.01	-127.83	1	1
	3	Aflatoxin G1	-121.68	-83.67	1	1
	4	Aflatoxin G2	-121.36	-121.98	1	1
	5	Aflatoxin M1	-137.94	-154.89	1	1
	6	Aflatoxin M2	-135.01	-122.76	1	1
	7	Aflatoxin Q1	-113.36	-102.82	1	1
	8	Aflatoxin P1	-121.99	-104.75	1	1
Gamma-cyclodextrin	1	Aflatoxin B1	-70.05	-60.17	0.578	0.580
	2	Aflatoxin B2	-70.93	-59.81	0.567	0.468
	3	Aflatoxin G1	-105.38	-66.60	0.866	0.796
	4	Aflatoxin G2	-106.95	-52.01	0.881	0.426
	5	Aflatoxin M1	-100.98	-104.70	0.732	0.676
	6	Aflatoxin M2	-124.71	-101.23	0.924	0.825
	7	Aflatoxin Q1	-134.57	-113.13	1.187	1.100
	8	Aflatoxin P1	-103.92	-111.28	0.852	1.062



The complexes of Aflatoxin B2 optimized with PM6-DH+/COSMO method.

The [(Aflatoxin)$_2$[BCD(SH)$_2$]] complex

At first, I prepared the 2 : 2; BCD : Aflatoxin complexes and removed one cyclodextrin from these structures and reoptimized the structure. In these complexes the Aflatoxin molecules are in reverse orientation compared to each other. One of them is deep in the CD cavity and the other is in the edge of the CD cavity. These forms C=O···H-O H-bonds with the cyclodextrin. They are in aromatic pi stacking interaction, the distance of the two planes is 3.4 Å. The calculated heat of formation of the AfB1 complexes is lower ca. 10 kJ/mol than the Afb2 complexes. The figure on the right shows the calculated IR spectra of these systems.

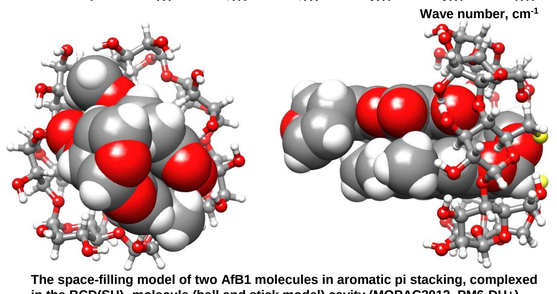


method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB1]	ΔH^0_f[(AfB1)$_2$[BCD(SH)$_2$]]	ΔH^0_b (kJ/mol)	ΔH^0_b (AfB1) – ΔH^0_b (AfB2)
PM6-DH2/COSMO	-6832.40	-875.38	-8787.64	-204.47	-7.52
PM6-DH+/COSMO	-6788.11	-875.38	-8747.37	-208.49	-12.27
PM7/COSMO	-6525.04	-784.79	-8301.75	-207.13	-10.24

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB2]	ΔH^0_f[(AfB2)$_2$[BCD(SH)$_2$]]	ΔH^0_b (kJ/mol)
PM6-DH2/COSMO	-6832.40	-1003.19	-9035.74	-196.95
PM6-DH+/COSMO	-6788.11	-1003.19	-8990.73	-196.23
PM7/COSMO	-6525.04	-906.68	-8535.28	-196.89

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB1]	ΔH^0_f[(AfB1)$_2$[BCD(SH)$_2$]]	ΔH^0_b (kJ/mol)	ΔH^0_b (AfB1) – ΔH^0_b (AfB2)
PM6-DH2	-6474.31	-784.95	-8427.57	-383.36	1.61
PM6-DH+	-6446.95	-784.95	-8433.16	-416.31	-28.68
PM7	-6250.44	-704.43	-7995.63	-336.33	-5.76

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB2]	ΔH^0_f[(AfB2)$_2$[BCD(SH)$_2$]]	ΔH^0_b (kJ/mol)
PM6-DH2	-6474.31	-909.26	-8677.81	-384.97
PM6-DH+	-6446.95	-909.26	-8653.10	-387.63
PM7	-6250.44	-823.46	-8227.92	-330.57



The space-filling model of two AfB1 molecules in aromatic pi stacking, complexed in the BCD(SH)$_2$ molecule (ball and stick model) cavity (MOPAC2012, PM6-DH+).

The [(Aflatoxin)[BCD(SH)$_2$]$_2$] complex

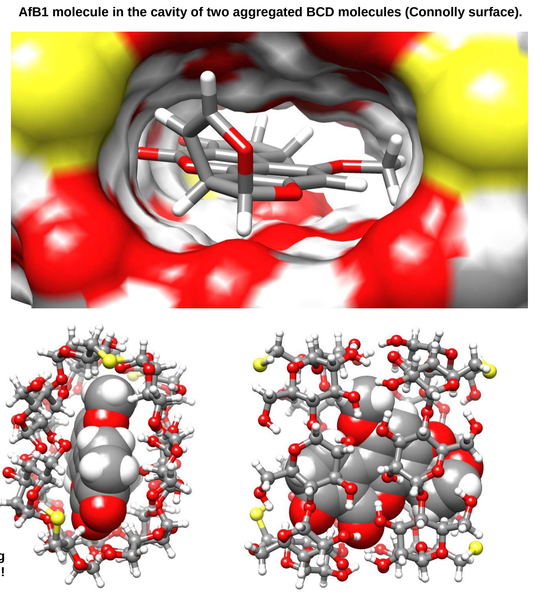
To calculate the 2 : 1; BCD : Aflatoxin complexes, I docked the toxins to the BCD molecule, then docked this complex (AutoDock VINA) to an another BCD. This procedure gives nice 2 : 1 host-guest complex structures. Then I optimized the structures with the MOPAC2012 software. The docking showed that the formation of 2 : 1 complexes has no steric hindrance. The BCD molecules bind with H-bonds to each other. The methoxy group of the Aflatoxin is in the edge of the cyclodextrin cavities, one oxo group of the Aflatoxin molecule binds to the cyclodextrin OH group with H-bond. The cyclodextrin ring is elongated in the plane of the Aflatoxin, and compressed in the perpendicular direction. The figure on the right top shows the Aflatoxin B1 in the cavity with Connolly (Solvent-excluded surface). Unfortunately the docking do not connects the CDs perfectly to each other (depends from optimization method). The calculated binding energy depends strongly from the number of the H-bonds between the two CDs.

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB1]	ΔH^0_f[(AfB1)[BCD(SH)$_2$]$_2$]	ΔH^0_b (kJ/mol)	ΔH^0_b(AfB1) – ΔH^0_b(AfB2)
PM6-DH2/COSMO	-6824.51	-874.81	-14920.66	-396.83	-2.20
PM6-DH+/COSMO	-6781.52	-874.81	-14745.95	-308.08	14.70
PM7/COSMO	-6510.36	-783.94	-14165.58	-360.92	40.69

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB2]	ΔH^0_f[(AfB2)[BCD(SH)$_2$]$_2$]	ΔH^0_b (kJ/mol)
PM6-DH2/COSMO	-6824.51	-1003.08	-15046.73	-394.63
PM6-DH+/COSMO	-6781.52	-1003.08	-14888.92	-322.79
PM7/COSMO	-6510.36	-906.28	-14328.61	-401.61

The BCD molecule deforms during the host-guest complex formation!

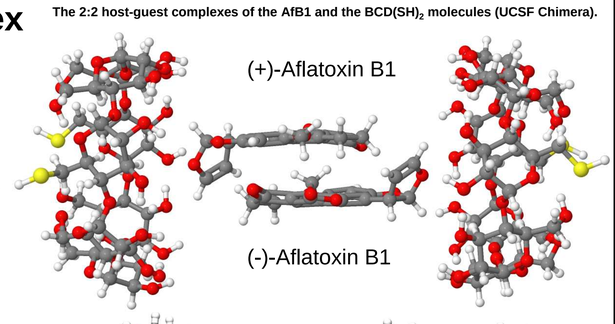
AfB1 molecule in the cavity of two aggregated BCD molecules (Connolly surface).



The [(Aflatoxin)$_2$[BCD(SH)$_2$]$_2$] complex

The 2:1 and 2:2; BCD : Aflatoxin complexes can encase one or two guest molecules while the complex links two gold nanoparticles together. (We can hypothesize that the AfB2 molecules are not able to form 2:2 complexes due to steric reasons.) This was the main idea to explain the QCM/SPR measurements. I constructed the 2:2 complex structures by „hand", and optimized with semiempirical methods. Only an Aflatoxin enantiomer pair can form the 2:2 complex. The calculated heat of formation of the AfB1 complexes is lower ca. 20 kJ/mol than the Afb2 complexes (see tables below).

The 2:2 host-guest complexes of the AfB1 and the BCD(SH)$_2$ molecules (UCSF Chimera).

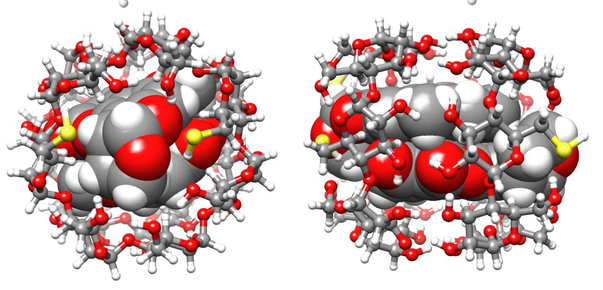


method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB1]	ΔH^0_f[(AfB1)$_2$[BCD(SH)$_2$]$_2$]	ΔH^0_b (kJ/mol)	ΔH^0_b (AfB1) – ΔH^0_b (AfB2)
PM6-DH2	-6474.31	-784.95	-15537.89	-1019.36	-16.41
PM6-DH+	-6446.95	-784.95	-15380.37	-916.58	-18.86
PM7	-6250.44	-704.43	-14695.38	-785.65	-24.52

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB2]	ΔH^0_f[(AfB2)$_2$[BCD(SH)$_2$]$_2$]	ΔH^0_b (kJ/mol)
PM6-DH2	-6474.31	-909.26	-15770.11	-1002.96
PM6-DH+	-6446.95	-909.26	-15610.13	-897.71
PM7	-6250.44	-823.46	-14908.92	-761.13

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB1]	ΔH^0_f[(AfB1)$_2$[BCD(SH)$_2$]$_2$]	ΔH^0_b (kJ/mol)	ΔH^0_b (AfB1) – ΔH^0_b (AfB2)
PM6-DH2/COSMO	-6832.40	-875.38	-15985.17	-569.59	-15.21
PM6-DH+/COSMO	-6788.11	-875.38	-15803.03	-476.04	-21.25
PM7/COSMO	-6525.04	-784.79	-15051.02	-431.36	-19.54

method	ΔH^0_f[BCD(SH)$_2$]	ΔH^0_f[AfB2]	ΔH^0_f[(AfB2)$_2$[BCD(SH)$_2$]$_2$]	ΔH^0_b (kJ/mol)
PM6-DH2/COSMO	-6832.40	-1003.19	-16225.57	-554.38
PM6-DH+/COSMO	-6788.11	-1003.19	-16037.40	-454.79
PM7/COSMO	-6525.04	-906.68	-15275.25	-411.82



Modeling adsorption/binding isotherms

My former supervisor, T. Körtvélyesi had an idea to model adsorption isotherms with molecular docking experiments. I tried it, and used the 4F5S Bovine Serum Albumin (BSA) protein X-ray structure from the RCSB Protein Data Bank. I optimized the structure with the Gromacs software using the AMBER99 force field and Gasteiger charges with explicit (TIP4P) water model. The ligand structures were prepared with the ChemAxon MarvinSketch software and a Monte Carlo conformation search was taken with the Open Babel (obconformer) software using the MMFF94 force field. The receptor structures and ligand structures were prepared with the MGL AutoDockTools software (Gasteiger charges). The total charge on the receptor was q = -17, this models neutral solution (pH = 6.5, PROPKA 3.0 and pH = 7.5, H++ online application). The docking calculations were carried out with the AutoDock VINA software, because it is fast, parallel and doesn't need the time consuming docking grid calculation. I docked the ligands sequentially to the target molecule and left the docked ligands on the target structure for the next docking step. Below, I show the equations which were used to calculate isotherms. The parameter β is the water – ligand exchange coefficient, which shows that how many waters have substituted by the ligand molecule during adsorption.

$$(1)\quad BSA + nL \rightleftharpoons BSA(L)_n \qquad E^L_n = \sum_{i=1}^{n} E^L_i$$

$$(2)\quad BSA + mH_2O \rightleftharpoons BSA(H_2O)_m \qquad E^{H_2O}_m = \sum_{j=1}^{m} E^{H_2O}_j; \quad n = \frac{m}{\beta}$$

$$(1)-(2)\quad BSA(H_2O)_{n\beta} + nL \rightleftharpoons BSA(L)_n + n\beta H_2O$$

$$(3)\quad \Delta E_n = \sum_{i=1}^{n} E^L_i - \beta \int_{n=1}^{m/\beta} E^{H_2O}(n)\,dn \qquad (4)\quad K_n = \prod_{i=1}^{n} K_i = \exp\left(\frac{-\Delta E_n}{RT}\right)$$

$$BSA + L \rightleftharpoons BSA(L) \qquad K_1 = \frac{[HSA(L)]}{[HSA][L]}$$

$$BSA(L) + L \rightleftharpoons BSA(L)_2 \qquad K_2 = \frac{[HSA(L)_2]}{[HSA(L)][L]}$$

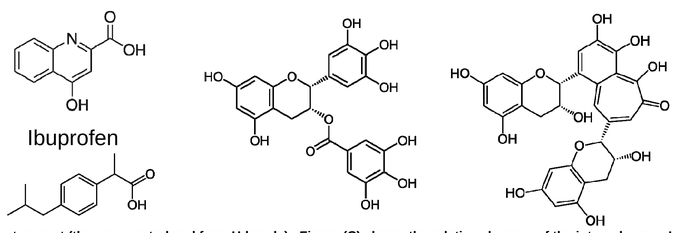
$$BSA(L)_2 + L \rightleftharpoons BSA(L)_3 \qquad K_3 = \frac{[HSA(L)_3]}{[HSA(L)_2][L]}$$

$$(5)\quad \frac{\sum c_{adsorbed\ L}}{\sum c_{BSA}} = \frac{[BSA]\left(K_1[L] + 2K_1K_2[L]^2 + 3K_1K_2K_3[L]^3 + \ldots\right)}{[BSA]\left(1 + K_1[L] + K_1K_2[L]^2 + K_1K_2K_3[L]^3 + \ldots\right)}$$

The used ligands and the raw data of docking calculations

I docked 250 ligand molecules and 1000 H_2O molecules to the BSA receptor structure. In the configuration file the AutoDock VINA parameters were.: cpu = 4, random seed = 1987, exhaustiveness = 100, num_modes = 1, energy_range = 3. The docked water molecules were SPC models. Below the figures shows the docking energies (**A**), the integrated docking energies (**B**) and the integrated, normalized docking energies (**C**). The KYNA and Ibuprofen had q = -1 charge.

Kynurenic acid (KYNA), Epigallocatechin gallate (EGCG), Theaflavin



Ibuprofen

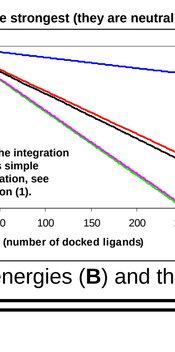
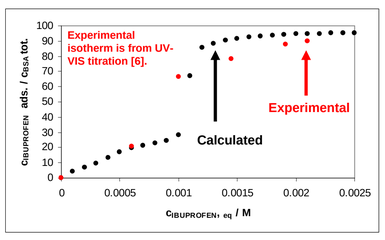


Figure (A) and figure (B) shows that the EGCG and the Theaflavin molecules binds the strongest (they are neutral and form H-bonds). Figure (C) shows the relative changes of the integral curves!

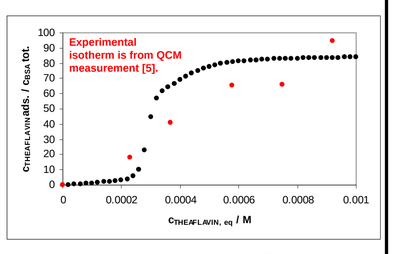
The docking energies (**A**), integrated docking energies (**B**) and the normalized integrated docking energies (**C**).

The calculated adsorption/binding isotherms

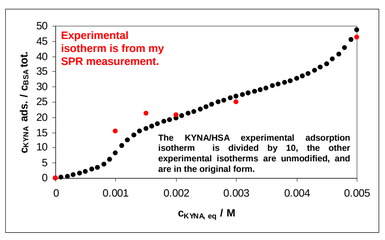
I calculated the adsorption isotherms using the equation (5). The EGCG and the Theaflavin contain lots of OH groups, so these molecules are more hydrophilic than the KYNA or the Ibuprofen. These molecules bind to the hydrophilic sites of the BSA (with H-bonds). They substitute lots of waters from this sites so their β numbers are large (2.50 and 3.55). The KYNA and the Ibuprofen have a relatively large hydrophobic region, so they bind to the hydrophobic binding sites where there aren't a lot of water molecules. The β coefficient of these molecules is small (0.85 and 1.85). The other explanation is that the vdW volume of the EGCG and Theaflavin is larger than the KYNA or Ibuprofen molecules. The shape of the calculated isotherms depends strongly from the β coefficient. I plotted the calculated (black) and the experimental (red) adsorption isotherms in the figures on the right [4,5,6].



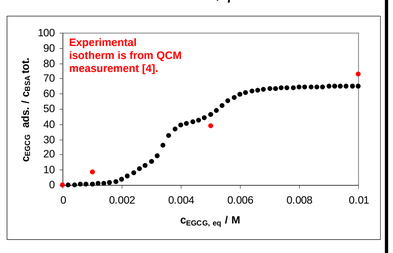
Ibuprofen, β = 0.85



Theaflavin, β = 2.50



KYNA, β = 1.85

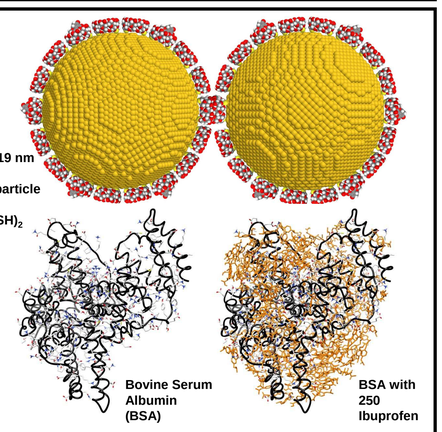


EGCG, β = 3.55

CONCLUSION

● I have calculated the β-cyclodextrin – Aflatoxin 1 : 1, 1 : 2, 2 : 1 and 2 : 2 host-guest complexes by semiempirical quantum chemistry methods. I saw that the calculated standard binding enthalpy of Aflatoxin B1 complexes is smaller about 10 kJ/mol (1 : 2) and 20 kJ/mol (2 : 2) than the corresponding Aflatoxin B2 complexes. The effect of gold surface can strengthen the complex formation [7]. The 2 : 1 and 2 : 2 complexes can bind two gold nanoparticles together and they are promising in nano sensor applications.

● In the docking experiments I calculated four adsorption/binding isotherms for the Bovine Serum Albumin molecule with consecutive docking method. I compared the calculated and the experimental adsorption/binding isotherms and found good agreements as in the adsorbed amounts as in the shapes of the isotherms. In the future I will tighten the water binding sites, using Boltzmann factor to average more docked conformers, increase the accuracy parameter to 1000, using the flexible residues option for the receptor and using PROPKA 3.0 protonation states. I am interested on the Monte Carlo based methods to calculate adsorption/binding isotherms.



[1] MOPAC2012, James J. P. Stewart, Stewart Computational Chemistry, Version 13.113L web: HTTP://OpenMOPAC.net
[2] O. Trott, A. J. Olson, *J. Comput. Chem.*, 31, 455-461, 2010
[3] A. Majzik, D. Sebők, E. Csapó, T. Bartók, I. Dékány, Biosensors prepared for detectation of aflatoxins in corn matrix, P006, SIWAN5, Szeged, Hungary, 2012
[4] X. Wang, C-T. Ho, Q. Huang, *J. Agric. Food Chem.*, 55, 4987-4992, 2007
[5] M. Chitpan, Monitoring of the binding processes of black tea polyphenols to bovine serum albumin surface using quartz crystal microbalance with dissipation, dissertation, Graduate School-New Brunswick Rutgers, The State University of New Jersey, New Brunswick, New Jersey, USA, 2009
[6] N. Varga, G. Bohus, M. Benkő, I. Dékány, Core-shell nanohybrid particles for controlled release of ibuprofen, P21, COST Action CM1101, Szeged, Hungary, 2013
[7] G. Filippini, F. Goujon, C. Bonal, P. Malfreyt, *J. Phys. Chem. C*, 116, 22350-22358, 2012

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