



Supplementary Materials

# Thermodynamic Characterization of the Interaction of Biofunctionalized Gold Nanoclusters with Serum Albumin Using Two- and Three-Dimensional Methods

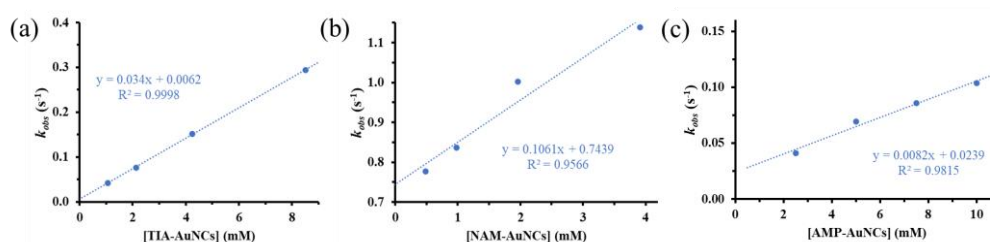
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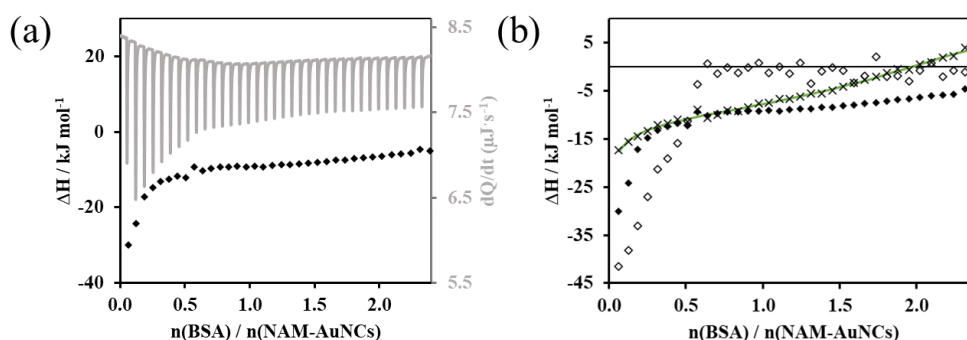
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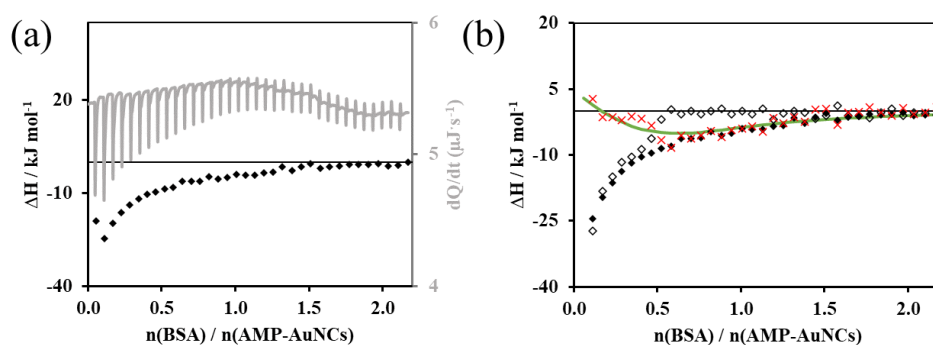
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**Figure S1.** Determination of the real rate constants ( $k_a$  and  $k_d$ ) of the binding process to BSA based on the concentration dependence of the apparent rate constant ( $k_{obs}$ ) for TIA (a), NAM (b) and AMP (c) stabilized AuNCs.



**Figure S2.** (a) Calorimetric curve (gray line) and enthalpogram (♦) recorded during the ITC examination of the BSA/NAM-AuNCs system; (b) Experimental- (♦), background- (◇) and dilution corrected- (×) enthalpogram fitted based on the model assuming two binding sites (green line).



**Figure S3.** (a) Calorimetric curve (gray line) and enthalpogram (♦) recorded during the ITC examination of the BSA/NAM-AuNCs system; (b) Experimental- (♦), background- (◇) and dilution corrected- (×) enthalpogram fitted based on the model assuming two binding sites (green line).

**Table S1.** The value and standard deviation of the thermodynamic parameters determined based on the calorimetric (ITC) analysis of the interaction between TIA-AuNCs and BSA.

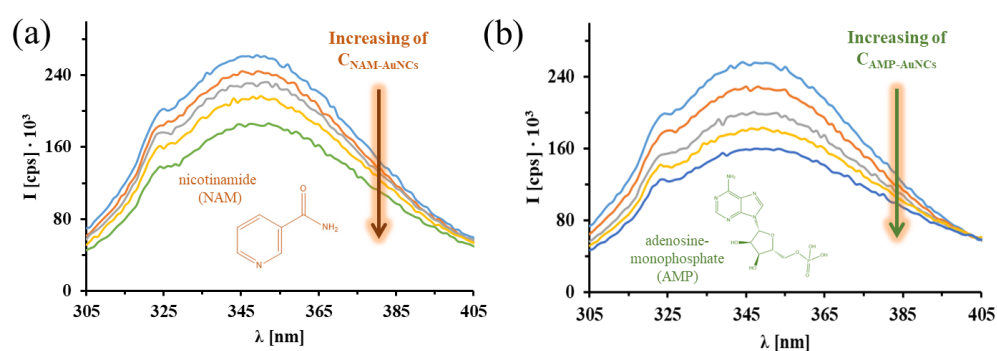
$K_{a,1}$ ( $M^{-1}$ )	$K_{a,2}$ ( $M^{-1}$ )
$4.17 \cdot 10^4 \pm 9.20 \cdot 10^3$	$1.04 \cdot 10^5 \pm 7.60 \cdot 10^4$
$\Delta G_1$ ( $kJ \cdot mol^{-1}$ )	$\Delta G_2$ ( $kJ \cdot mol^{-1}$ )
$-26.36 \pm 0.55$	$-28.62 \pm 1.81$
$\Delta H_1$ ( $kJ \cdot mol^{-1}$ )	$\Delta H_2$ ( $kJ \cdot mol^{-1}$ )
$-230 \pm 181$	$1400 \pm 3600$
$N_1$	$N_2$
$1.19 \pm 0.14$	$0.29 \pm 0.18$

**Table S2.** The value and standard deviation of the thermodynamic parameters determined based on the calorimetric (ITC) analysis of the interaction between NAM-AuNCs and BSA.

$K_{a,1}$ ( $M^{-1}$ )	$K_{a,2}$ ( $M^{-1}$ )
$4.66 \cdot 10^5 \pm 6.90 \cdot 10^5$	$2.12 \cdot 10^6 \pm 3.80 \cdot 10^6$
$\Delta G_1$ ( $kJ \cdot mol^{-1}$ )	$\Delta G_2$ ( $kJ \cdot mol^{-1}$ )
$-32.34 \pm 3.67$	$-36.09 \pm 4.44$
$\Delta H_1$ ( $kJ \cdot mol^{-1}$ )	$\Delta H_2$ ( $kJ \cdot mol^{-1}$ )
$-5.58 \pm 8.98$	$-27.71 \pm 23.28$
$N_1$	$N_2$
$1.09 \pm 0.31$	$0.44 \pm 0.25$

**Table S3.** The value and standard deviation of the thermodynamic parameters determined based on the calorimetric (ITC) analysis of the interaction between AMP-AuNCs and BSA.

$K_{a,1}$ ( $M^{-1}$ )	$K_{a,2}$ ( $M^{-1}$ )
$1.64 \cdot 10^5 \pm 1.60 \cdot 10^5$	$1.10 \cdot 10^6 \pm 2.80 \cdot 10^6$
$\Delta G_1$ ( $kJ \cdot mol^{-1}$ )	$\Delta G_2$ ( $kJ \cdot mol^{-1}$ )
$-29.75 \pm 2.42$	$-34.47 \pm 6.31$
$\Delta H_1$ ( $kJ \cdot mol^{-1}$ )	$\Delta H_2$ ( $kJ \cdot mol^{-1}$ )
$-12.39 \pm 5.36$	$5.821 \pm 8.66$
$N_1$	$N_2$
$0.60 \pm 0.37$	$0.37 \pm 0.16$



**Figure S4.** (a) Fluorescence emission spectra of the aqueous BSA solution and the protein/cluster mixtures after addition of 0–1 mM NAM-AuNCs with constant albumin concentration ( $C_{BSA} = 5.0 \mu\text{M}$ ;  $T = 25^\circ\text{C}$ ;  $\lambda_{\text{ex}} = 280 \text{ nm}$ ) (b) Fluorescence emission spectra of the aqueous BSA solution and the protein/cluster mixtures after addition of 0–1 mM AMP-AuNCs with constant albumin concentration ( $C_{BSA} = 5.0 \mu\text{M}$ ;  $T = 25^\circ\text{C}$ ;  $\lambda_{\text{ex}} = 280 \text{ nm}$ )

**Table S4.** The value and standard deviation of the thermodynamic parameters of the examined gold nanocluster-protein interactions, evaluated from SPR, ITC and PL measurement techniques.

		$K_a (\text{M}^{-1})$	$\Delta G (\text{kJ}\cdot\text{mol}^{-1})$	$\Delta H (\text{kJ}\cdot\text{mol}^{-1})$	$N$	$\Delta S (\text{kJ}\cdot\text{mol}^{-1})$
SPR	TIA	$5468 \pm 73$	$-21.32 \pm 0.03$			
	NAM	$143 \pm 30$	$-12.29 \pm 0.53$			
	AMP	$294 \pm 90$	$-14.08 \pm 0.76$			
ITC	TIA	$4.17 \cdot 10^4 \pm 9.20 \cdot 10^3$	$-26.36 \pm 0.55$	$-230 \pm 182$	$1.19 \pm 0.14$	$-682 \pm 4$
	NAM	$4.66 \cdot 10^5 \pm 6.90 \cdot 10^5$	$-32.34 \pm 3.67$	$-5.28 \pm 8.95$	$1.19 \pm 0.14$	$91 \pm 13$
	AMP	$1.64 \cdot 10^5 \pm 1.60 \cdot 10^5$	$-34.47 \pm 2.42$	$-12.39 \pm 5.36$	$1.19 \pm 0.14$	$58 \pm 19$
PL	TIA	$1.85 \cdot 10^4 \pm 1.80 \cdot 10^3$	$-24.35 \pm 0.24$		$1.19 \pm 0.14$	
	NAM	$295 \pm 1$	$-14.09 \pm 0$		$1.19 \pm 0.14$	
	AMP	$640 \pm 3$	$-16.12 \pm 0$		$1.19 \pm 0.14$	