Supporting Information

Thermodynamic Study of Interactions between ZnO and ZnO Binding Peptides using Isothermal Titration Calorimetry

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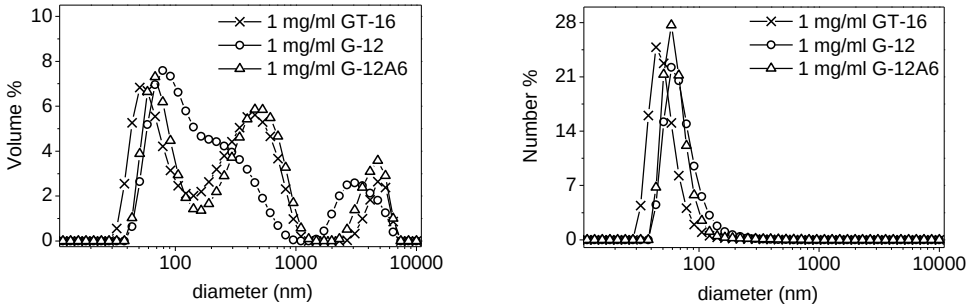
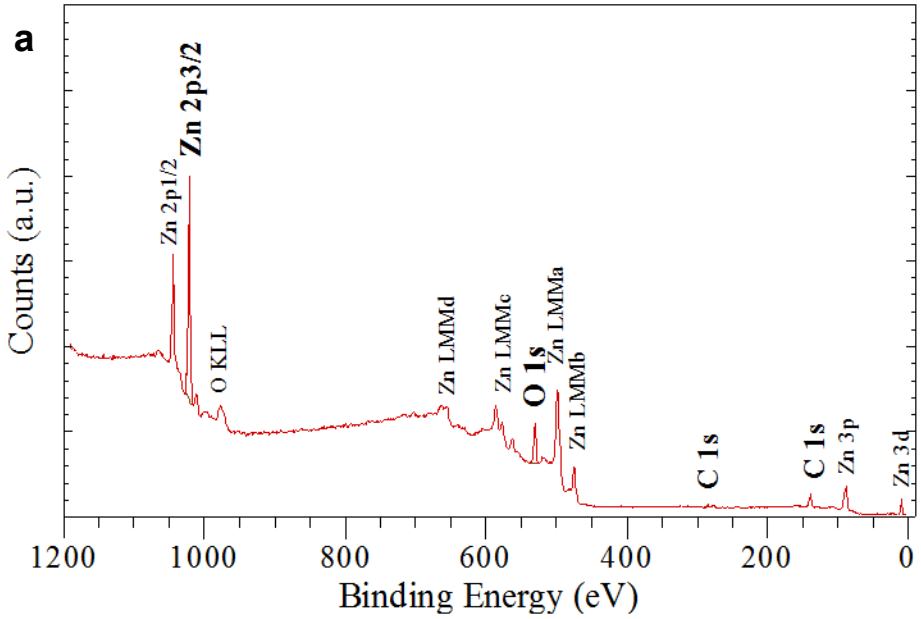
S1. Support for the choice of aqueous media for ITC experiments.

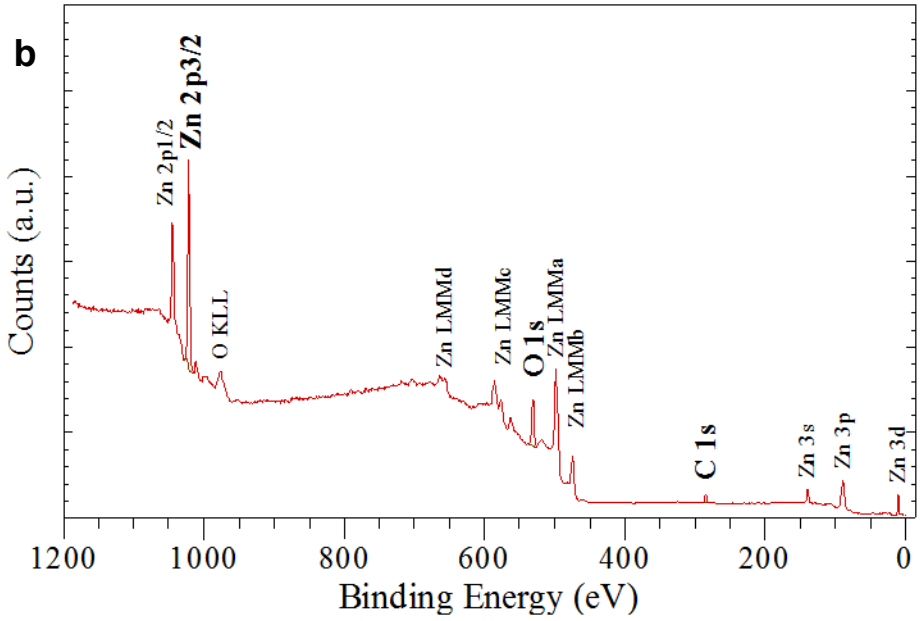
The ideal ITC experiment to probe interfacial changes would include all ionic species and solid phases present in solution during synthesis of ZnO, test their interaction with peptide simultaneously and be able to identify individual events that contribute to the global heat change measured. However, this is not always feasible, in most cases ITC experiments require a simplification of the actual event being studied to be able to interpret data and enable separation of individual events. Interactions were studied using aqueous media like in the synthesis studies but this meant that there was a decrease in the pH in the sample cell with continued titration of peptide into the sample cell. At a concentration of 3.1 mM, the pH of the peptides was pH 3.08 ± 0.35 and the pH of the water was pH 5.77 ± 0.23 without and with the ZnO particles. However the same decrease in pH occurred in the control dilution experiment (titration of peptide into water) and the interaction experiment (titration of peptide into water containing ZnO particles). At the end of the titration of the controls and interaction experiments the pH in the sample cell had been decreased to pH 3.92 ± 0.35. In ITC experiments, matching the composition of the components in the sample cell and syringe such as concentration of salts used, pH and buffers helps to minimize dilution heat changes that may plausibly override binding signals. The heat changes measured from control dilution experiments were small in comparison to heat changes of interaction with ZnO therefore there was no interference.

S2. DLS size measurements of ZnO binding peptides 1 mg/ml (GT-16 = 0.62 mM, G-12 = 0.75 mM, G-12A6 = 0.78 mM) in water, close to the concentration of peptide in the ITC cell at the end of each experiment (0.52 mM). DLS data in volume percentage distribution shows that there were aggregates of different sizes in solution but the number percentage distribution shows that the average hydrodynamic diameter of the majority of aggregates was ~60 nm in size.

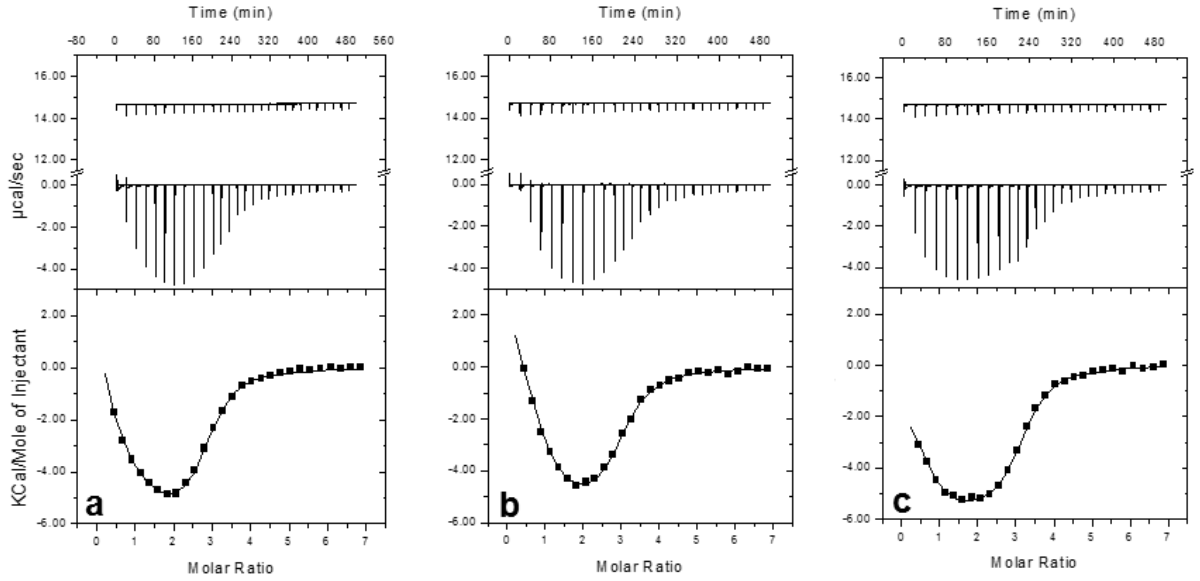


S3. XPS survey spectrum of (a) calcined ZnO rods and (b) calcined ZnO platelets used in ITC experiments.





S4. (a-c) ITC isothermal profiles (replicates) showing titrations of 3.1 mM G-12 into a suspension of 0.1 mM ZnO platelets (bulk concentration). Isotherms have been fit using two sets of independent sites model. This demonstrates the practical difficulties in modelling where inconsistencies in values can be obtained even where isothermal profiles have very similar shapes. The shapes of the isothermal profiles in (a) and (b) appear to be very similar showing reproducibility of the experiment. The profile of the third repeat (c) looks slight less similar to the first two yet the values of thermodynamic parameters obtained after fitting data using two sets of independent sites model give more similar values for (b) and (c).



Data: A110120ZnODG_NDH
Model: TwoSites
Chi^2 = 4154

N1	0.122	±0.331 Sites
K1	5.50E5	±1.11E5 M^{-1}
ΔH1	5.878E4	±1.91E5 cal/mol
ΔS1	223 cal/mol/deg	
N2	2.65	±0.319 Sites
K2	1.85E5	±4.56E4 M^{-1}
ΔH2	-7334	±635 cal/mol
ΔS2	-0.496 cal/mol/deg	

Data: A110126G12Zn_NDH
Model: TwoSites
Chi^2 = 4436

N1	0.618	±0.0755 Sites
K1	9.94E5	±2.98E5 M^{-1}
ΔH1	5819	±2.40E3 cal/mol
ΔS1	47.0 cal/mol/deg	
N2	2.31	±0.0633 Sites
K2	1.30E5	±1.10E4 M^{-1}
ΔH2	-6295	±354 cal/mol
ΔS2	2.29 cal/mol/deg	

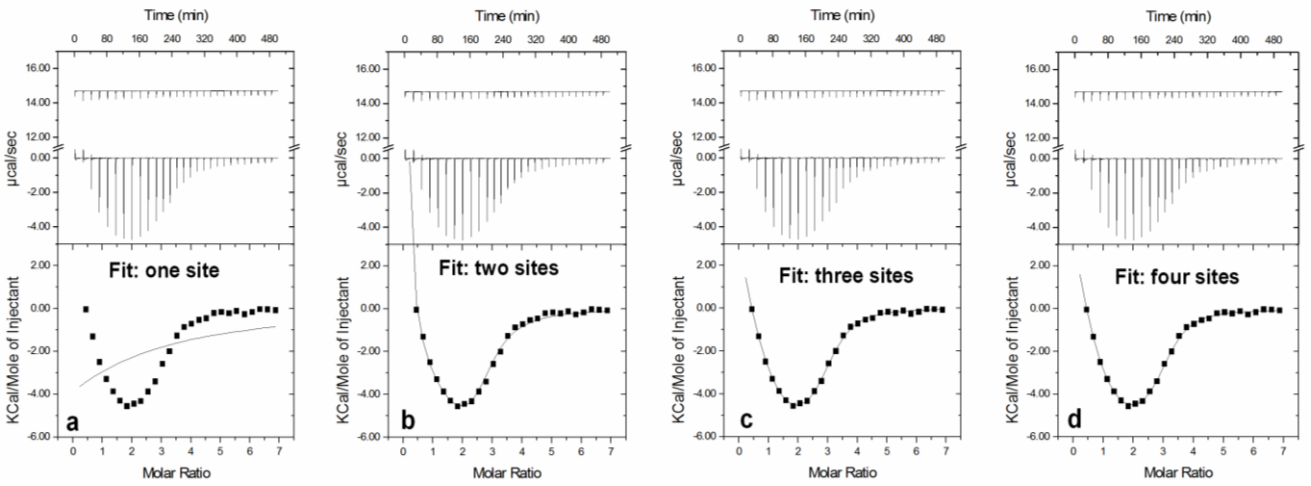
Data: A110127G12Zn_NDH
Model: TwoSites
Chi^2 = 4503

N1	0.597	±0.0904 Sites
K1	2.15E6	±9.05E5 M^{-1}
ΔH1	-967.9	±1.20E3 cal/mol
ΔS1	25.7 cal/mol/deg	
N2	2.50	±0.0830 Sites
K2	1.60E5	±1.40E4 M^{-1}
ΔH2	-6064	±161 cal/mol
ΔS2	3.47 cal/mol/deg	

G-12 and ZnO	Dp	KA (M-1)	ΔH (Kcal mol-1)	TΔS (Kcal mol-1)	ΔG (Kcal mol-1)
Experiment a	+ve	5.50 x 105 ± 1.11 x 105	58.78 ± 191 *	66.45 *	-7.67
	- ve	1.85 x 105 ± 4.56 x 104	-7.33 ± 0.64	-0.15	-7.19
Experiment b	+ve	9.94 x 105 ± 2.98 x 105	5.82 ± 2.4	14.01	-8.19
	-ve	1.30 x 105 ± 1.10 x 104	-6.30 ± 0.35	0.68	-6.98
Experiment c	+ve	2.15 x 106 ± 9.05 x 105	-0.97± 1.2 *	7.66	-8.63
	-ve	1.60 x 105 ± 1.40 x 104	-6.06 ± 0.16	1.03	-7.10

(+ve) endothermic, (-ve) exothermic, (*) unreasonable data for the rapidly completed endothermic process

S5. (a-d) ITC isothermal profile showing titrations of 3.1 mM G-12 into a suspension of 0.1 mM ZnO platelets (bulk concentration) fit using sequential binding sites model (to consider dependent binding). This is the same isotherm from Figure S4 b which was fit using two sets of independent binding sites model. Using sequential binding sites model, fitting improved with increase in number of binding sites but this generates more thermodynamic parameters with no meaningful interpretation.



Data: A110126G12Zn_NDH
Model: Sequential Binding Sites
Chi^2/DoF = 2.032E6

K1	1.57E3 ±1.2E3 M^{-1}	
ΔH1	-2.775E4	±1.27E4 cal/mol
ΔS1	-131 cal/mol/deg	

Data: A110126G12Zn_NDH
Model: Sequential Binding Sites
Chi^2/DoF = 2.264E4

K1	1.55E3 ±9.9E2 M^{-1}	
ΔH1	2.161E5	±1.36E5 cal/mol
ΔS1	740 cal/mol/deg	
K2	9.13E5 ±6.0E5 M^{-1}	
ΔH2	-2.264E5	±1.36E5 cal/mol
ΔS2	-732 cal/mol/deg	

Data: A110126G12Zn_NDH
Model: Sequential Binding Sites
Chi^2/DoF = 4877

K1	3.08E5 ±5.8E4 M^{-1}	
ΔH1	2316	±485 cal/mol
ΔS1	32.9 cal/mol/deg	
K2	1.03E5 ±1.4E4 M^{-1}	
ΔH2	-9554	±569 cal/mol
ΔS2	-9.11 cal/mol/deg	
K3	4.51E4 ±4.6E3 M^{-1}	
ΔH3	-3573	±272 cal/mol
ΔS3	9.31 cal/mol/deg	

Data: A110126G12Zn_NDH
Model: Sequential Binding Sites
Chi^2/DoF = 4845

K1	2.80E5 ±5.6E4 M^{-1}	
ΔH1	2575	±570 cal/mol
ΔS1	33.6 cal/mol/deg	
K2	9.79E4 ±1.5E4 M^{-1}	
ΔH2	-1.004E4	±641 cal/mol
ΔS2	-10.8 cal/mol/deg	
K3	4.85E4 ±7.2E3 M^{-1}	
ΔH3	-3165	±341 cal/mol
ΔS3	10.8 cal/mol/deg	
K4	-25.0	±29 M^{-1}
ΔH4	2.170E4	±2.84E4 cal/mol