Relating thermodynamics to the dominant contributions determining the toxicity of nanomaterials

nanomedicine EUROPEAN TECHNOLOGY PLATFORM

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Objectives

➢The complex phenomena occurring at the bio/nano interface are evaluated by assessing the effect of the nanoparticles (NPs) on the protein stability and the thermodynamics of binding

➢The thermodynamic data were correlated with the toxicity results assessed by label- and interference-free impedance-based flow cytometry.

The control of the **Energetic Driving Forces:** important step in understanding the stability and possibilities of nanomaterials' interaction with biologic systems

The protein interaction with nanoparticles is a key issue for biotechnology and biomedical applications

Providing insight into adsorption-induced changes in the protein structure and stability, as well as into mechanism of binding interaction

Experimental measurements

Selection of materials

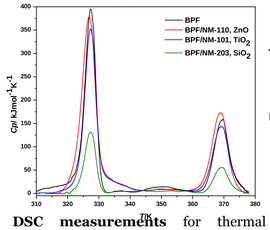
- Representative nanomaterials from the Joint Research Center (JRC) Repository used in the OECD WPMN Testing Programme):
  TiO_2 (NM-101) anatase, primary particle size 5-6 nm;
  ZnO (NM-110) uncoated, primary particle size 86 nm;
  SiO_2 (NM-203) synthetic amorphous silica, thermal, primary particle size 58 nm.
- Protein: BPF (Bovine plasma fibrinogen, type I-S)
- Water Milli Q (Direct-Q 3UV System, Millipore, 18.2 MΩ cm)

Equipment

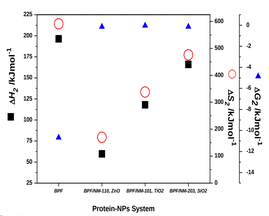
- NanoDSC calorimeter, TA Instruments
- Isothermal titration calorimeter ITC_{200}, MicroCal Inc.
- Impedance-based flow cytometer Ampha Z30, Amphasys AG, Switzerland
- Zetasizer Nano ZSP, Malvern Instr. Ltd, UK
- Transmission electron microscopy (TEM), JEM-2100, JOEL, Japan

Results

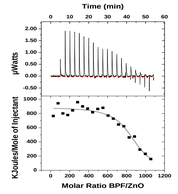
Thermodynamic data



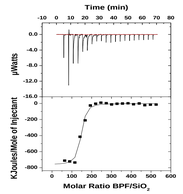
DSC measurements for thermal denaturation of BPF with NPs in water.



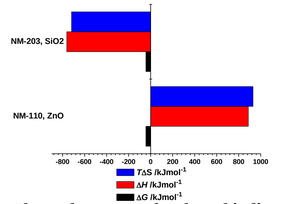
Thermodynamic parameters denaturation of BPF with NPs in water.



ITC measurements for the interaction of BPF (1.75x10^{-5} M) with ZnO NPs (2.92x10^{-9} M) in water.



ITC measurements for the interaction of BPF (1x10^{-4} M) with SiO_2 NPs (3.35x10^{-8} M) in water.



The changes of the binding thermodynamic parameters for the interaction of BPF with ZnO and SiO_2 NMs

❖ The decrease of ΔH: a less stable conformation of the protein molecule adsorbed onto the surface;

❖ The decrease of ΔS: ordering of the solvent molecules;

$\Delta S>0$: rearrangements within the protein molecule is the main reason for the adsorption of *BPF*.

❖ The change in ΔG towards less negative values indicates that *BPF* adopts a more flexible conformation on NPs surface.

❖ The unfavorable $\Delta H(>0)$ and highly favorable $\Delta S(>0)$ suggest that *BPF binding on ZnO NMs is an entirely entropy – controlled process*, usually dominated by hydrophobic forces.

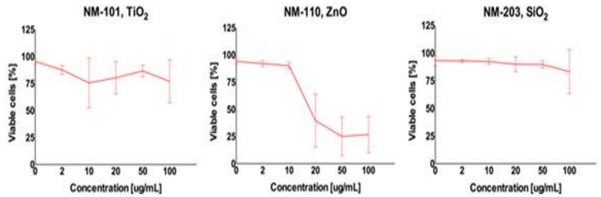
❖ High favorable $\Delta H(<0)$ and unfavorable $\Delta S(<0)$ indicate *a predominantly enthalpic binding process driven by* electrostatic forces for *BPF-SiO_2* system.

❖ ΔG are negative, showing the spontaneity of BPF binding to NPs surface.

Due to the *enthalpy/entropy compensation, the binding energies (ΔG)* values of the studied systems are near each other

✓ The decreasing structural stability of the protein associated with the affinity for the NMs followed the trend: (NM-110, ZnO)> (NM-101, TiO_2)> (NM-203, SiO_2)

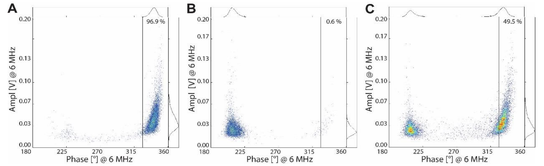
Toxicity data



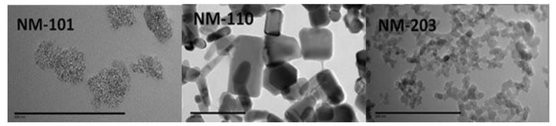
Viability of U937 cells exposed to 5 concentrations of NM-101, NM-110 and NM-203.

Table 1: Physicochemical properties of NMs

Material code	Structure (XRD)	Chemical composition [wt%] (EDS)	Primary particle size ± SD or size range [nm] (TEM)	BET [m2/g]	z-Average [nm] and PDI (DLS; 0.05% BSA-water)	Zeta potential ± SD in PBS [mV] and isoelectric point (ISP) [pH]
NM-101 TiO_2	Anatase	Ti 58.79, O (calculated) 40.35; traces (ppm) of Si, P, Al, S	5.5 ± 0.7	234 - 316	z-Average: 449 PDI: 0.315	Zeta pot: -33 ± 9 ISP: 6
NM-110 ZnO	Hexagonal zincite structure	Zn 80.90, O 19.01; traces (ppm) of Si, Al, Ca, Ni, Pb, Co (ICP-OES)	Population 1= 20 - 50 Population 2= 50 - 350	12 -13	z-Average: 233 PDI: 0.110	Zeta pot: -47 ± 9 ISP: N/A
NM-203 SiO_2	Synthetic amorphous silica	Si 46.32, O 53.21; traces (ppm) of Al, S	13 ± 6	204	z-Average: 145 PDI: 0.203	Zeta pot: -46 ISP: < 2



Gating for IFC measurements. (**A**) The population of viable cells was determined from the negative control and the gate was applied to all samples within one experiment. (**B**) Necrotic cells, and (**C**) 50:50 mixture of viable and necrotic cells. The cell number within the gates is expressed in percentage of the total cell count.



TEM images of NM-101, NM-110, and NM-203

Conclusions

✓**The thermodynamic data have been evaluated to get insight into adsorption-induced changes in the protein structure and stability, as well as into mechanism of binding interaction;**

✓**The thermodynamic results agree with the toxicity ranking assessed by label- and interference-free impedance-based flow cytometry.**

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