Task ID: 425.035

Task Title: High-Throughput Cellular-Based Toxicity Assays for Manufactured Nanoparticles and Nanostructure-Toxicity Relationships Models

Deliverable #5: Report on pre-processing experimental data and calculate MNPs chemical descriptors to prepare all data for QNTR modeling

Summary/Abstract:

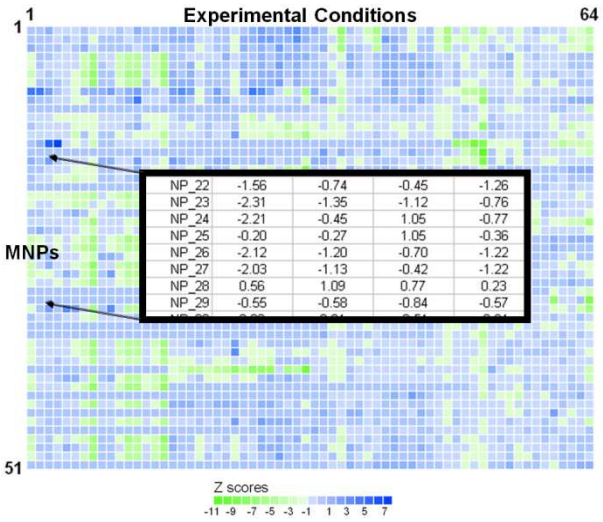
This report summarizes the result of pre-processing the experimental nanotoxicity data (three different datasets) collected from the literature (cf. Deliverable 4). In all cases, different nanostructures were shown to have distinct biological/toxicological profiles. In the first case study, experimental properties of nanoparticles were used as descriptors. In the other two case studies, conventional chemical descriptors of surface-modifying organic molecules were employed; descriptors were calculated using two different software packages, MOE and Dragon. Non-supervised hierarchical clustering was used to elucidate structure-activity/toxicity relationships for nanoparticles and identify features of nanoparticles that could serve as alerts for specific toxicity endpoint.

Technical Results and Data:

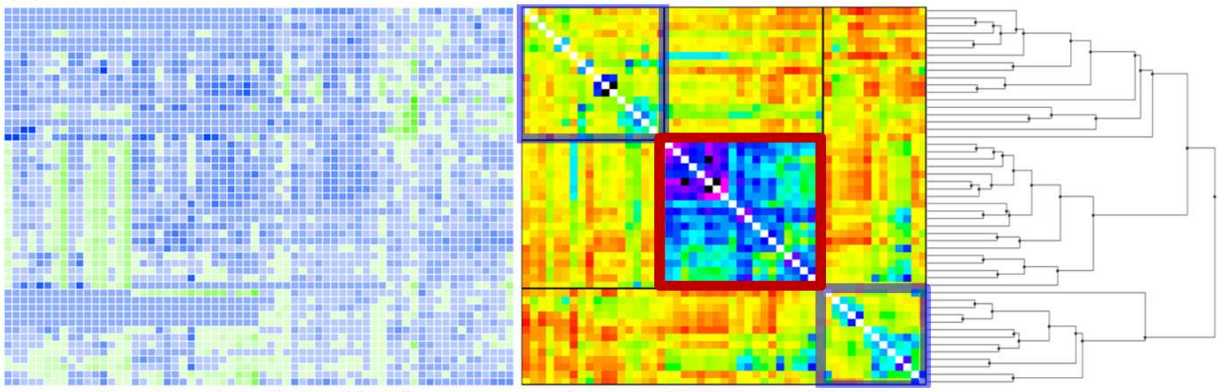
Three datasets were pre-processed and analyzed.

1) In the first case study, the data for all tested manufactured nanoparticles (MNPs) were clustered by the normalized value of activity (Figure 1A). In a separate investigation, hierarchical clustering analysis revealed three major clusters of MNPs (Figure 1B); all MNPs within a cluster have similar profiles of cellular activities. The structural analysis of MNPs found within the same activity cluster revealed that cluster members had similar core structure (Figure 1C), which suggests that there are intrinsic relationships between structure and activity of MNPs.
2) In the second study, the distributions of cellular uptake of MNPs with similar core but different surface modifying organic molecules in four different cell lines were investigated. The results indicate that only cellular uptake in pancreatic cancer cell line showed variability as a function of chemical structure of surface modifiers (Figure 2).
3) In the third case study, 84 NPs were tested for protein binding *in vitro*. Protein binding affinity profiles of NPs were found to be highly correlated in most cases except for BSA (Figure 3A). This could result from species difference since BSA is not a human protein. The result of non-supervised hierarchical clustering analysis shows that the surface molecule of NPs with similar scaffold could have similar protein binding profile (Figure 3B).

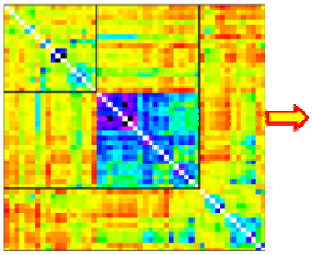
(A)



(B)



(C)



NP type	CLUSTER 1	CLUSTER 2	CLUSTER 3	Total
CLIO	7	13	3	23
PNP	7	2	10	19
MION	0	4	0	4
Qt-dot	3	0	0	3
Feridex	0	1	0	1
Ferrum Haussmann	1	0	0	1
Total	18	20	13	51

NP Core	CLUST 1	CLUST 2	CLUST 3	Total
Fe_2O_3	5	0	9	14
Fe_3O_4	9	20	4	33
Cd-Se	3	0	0	3
Fe(III)	1	0	0	1
Total	18	20	13	51

Figure 1. (A) Activity Heat Map of the biological responses induced by 51 nanoparticles tested against four cell lines in four different assays at four different concentrations. (B) Hierarchical

clustering analysis of 51 nanoparticles using their biological profiles and the ISIDA/Cluster program [on the distance matrix, blue colors = high similarity between particles, red/green/yellow colors = low/medium similarity between particles]. (C) Analysis of three clusters. A given metal core (i.e, Fe3O4) or NP category (i.e, Qt-dot), will induce similar biological effects in most cases, independent of the surface modifications.

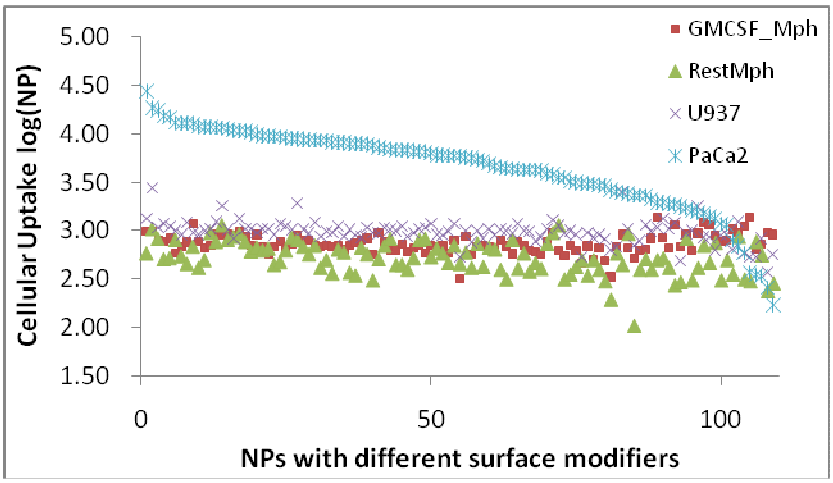
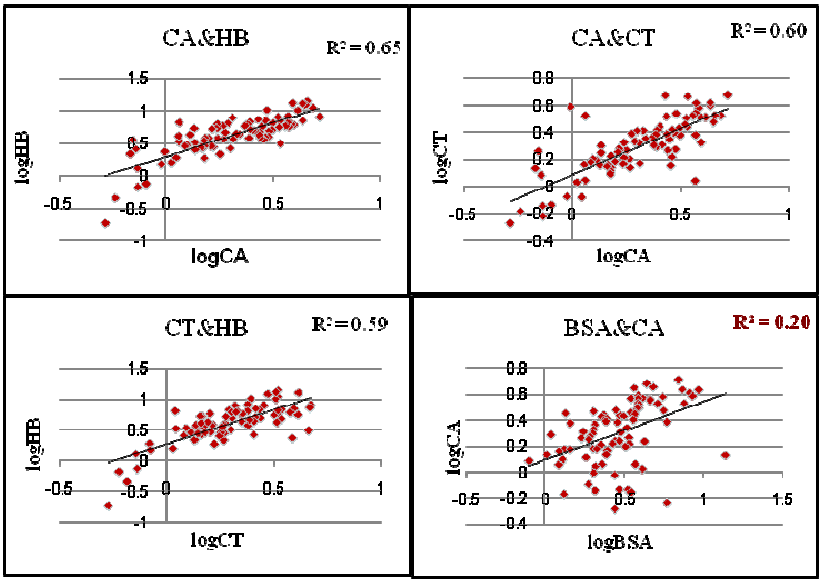


Figure 2. Cellular uptake data in four different cell lines for 109 nanoparticles with the same core but different surface modifiers.

(A)



(B)

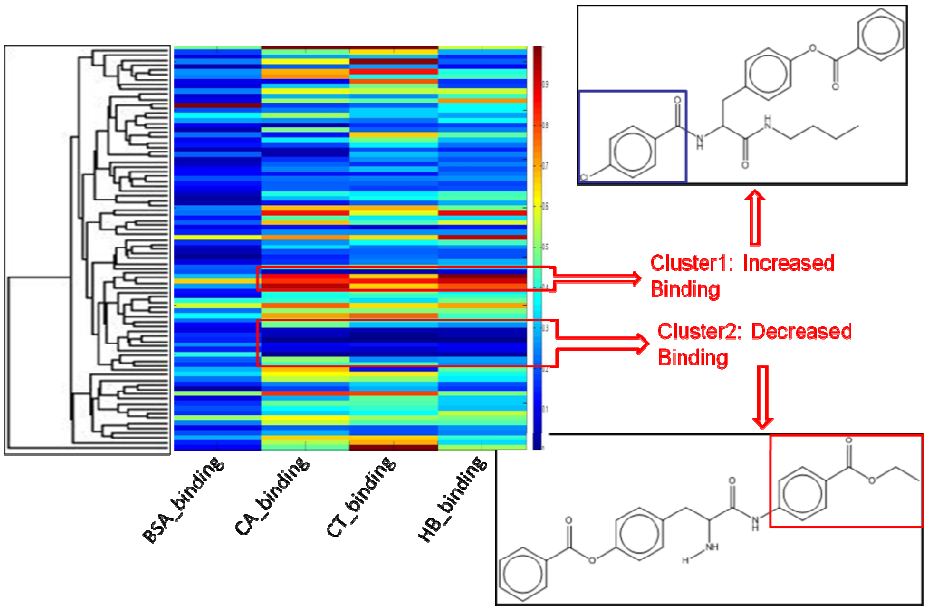


Figure 3. Pre-processing a dataset including 84 NPs. (A) Pairwise correlation between protein binding affinities of NPs in different assays. (B) Non-supervised hierarchical clustering analysis based on chemical descriptors results in clusters with similar protein binding profiles.