High-Throughput Cellular-Based Toxicity Assays for Manufactured Nanoparticles and Nanostructure-Toxicity Relationship Models

(*Task Number: 425.035)*

Subtask: "Computational Models"

PIs:

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Objectives

- **Develop QNAR models that correlate the compositional/physical/chemical/geometrical and biological descriptors of MNPs with known toxicological endpoints.**
- **Employ QNAR models for virtual screening of libraries of compound considered attachable to CNT surfaces to prioritize compounds for the experimental validation of predicted cellular toxicity and protein binding**
- **In a joint study with the experimental collaborator (Dr. Bing Yan, St. Jude) evaluate the accuracy of QNAR models by testing the selected MNPs in biological experiments.**

ESH Metrics and Impact

1. ***Obtain predictive knowledge of the physical and chemical properties of manufactured nanoparticles.***

2. ***Develop predictive computational models that correlate physical-chemical descriptors of MNPs with their toxic effects.***

Impact: Utilize the knowledge gained through above metrics for improved MNP experimental design and prioritized toxicity testing toward the manufacturing of safe nanomaterials.

Research Hypothesis

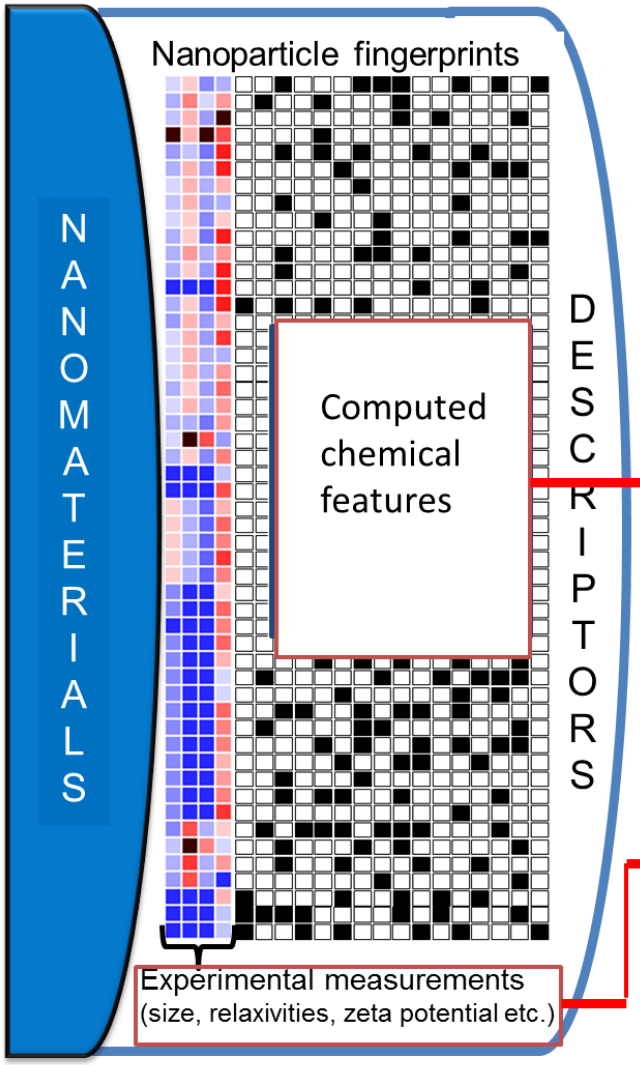
- The biological/toxicological properties of MNPs (exemplified by carbon nanotubes, CNTs) depend on the compositional/physical/chemical/ geometrical properties of the CNTs.

- Toxicological data obtained from *in-vitro* cellular-based toxicity assays will correlate reasonably with *in-vivo* results.

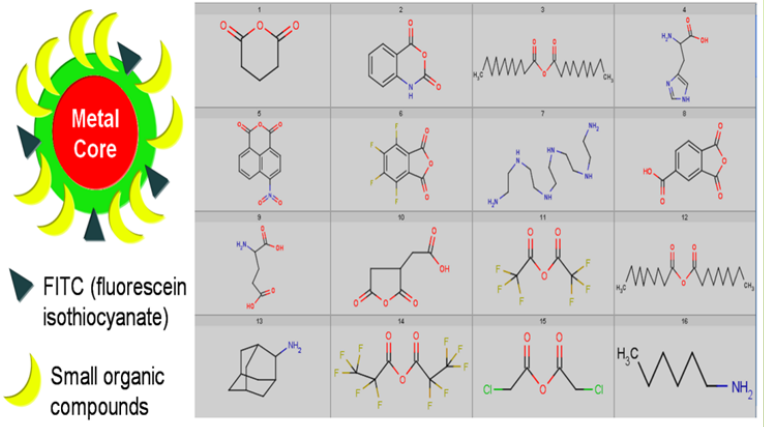
- Surface chemistry could provide sufficient information in predicting the behavior of CNTs in both in vitro and in vivo assays.

- Using physical/chemical characterization and toxicological screens for an ensemble of MNPs, it will be possible to develop and experimentally validate **predictive Quantitative Nanostructure – Activity (QNAR) models.**

Approach: MNP Types and Descriptors



ALL PARTICLES HAVE THE SAME CORE BUT DIFFERENT SURFACE MODIFIERS



Classical molecular descriptors can be computed for a molecule representing surface modifier.

ALL PARTICLES HAVE DIFFERENT CORES/ARCHITECTURES

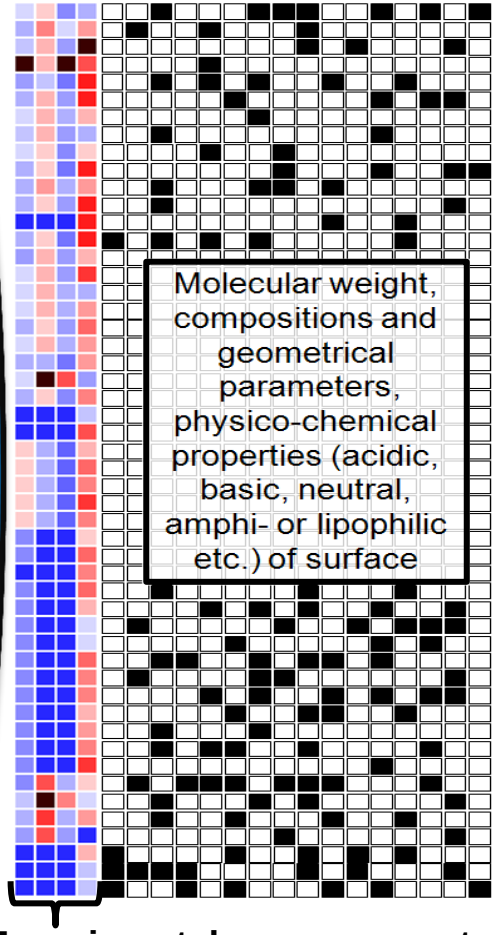
MNP	CLIO	PNP	MION	QD	Feridex IV	Ferrum Hausmann
#. particle	23	19	4	3	1	1

In the absence of defined three-dimensional structure only experimentally mesured properties could be used as descriptors

Approach: QNAR Modeling

NANOMATERIALS

Nanoparticule fingerprints



Molecular weight, compositions and geometrical parameters, physico-chemical properties (acidic, basic, neutral, amphi- or lipophilic etc.) of surface

DESCRIPTORS

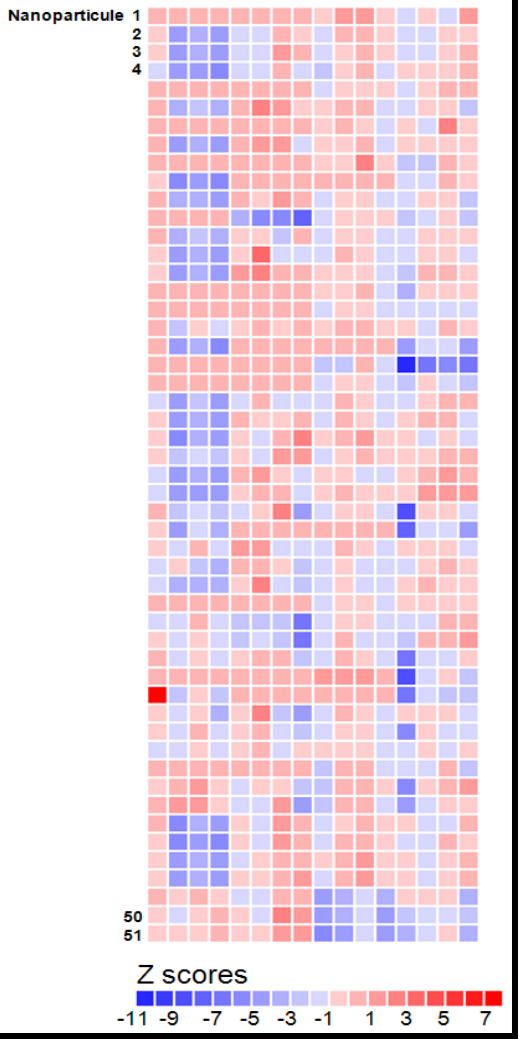
Experimental measurements
(size, relaxivities, zeta potential etc.)

Quantitative Nanostructure Activity Relationships

- **Building of models using machine learning methods (NN, SVM etc.);**

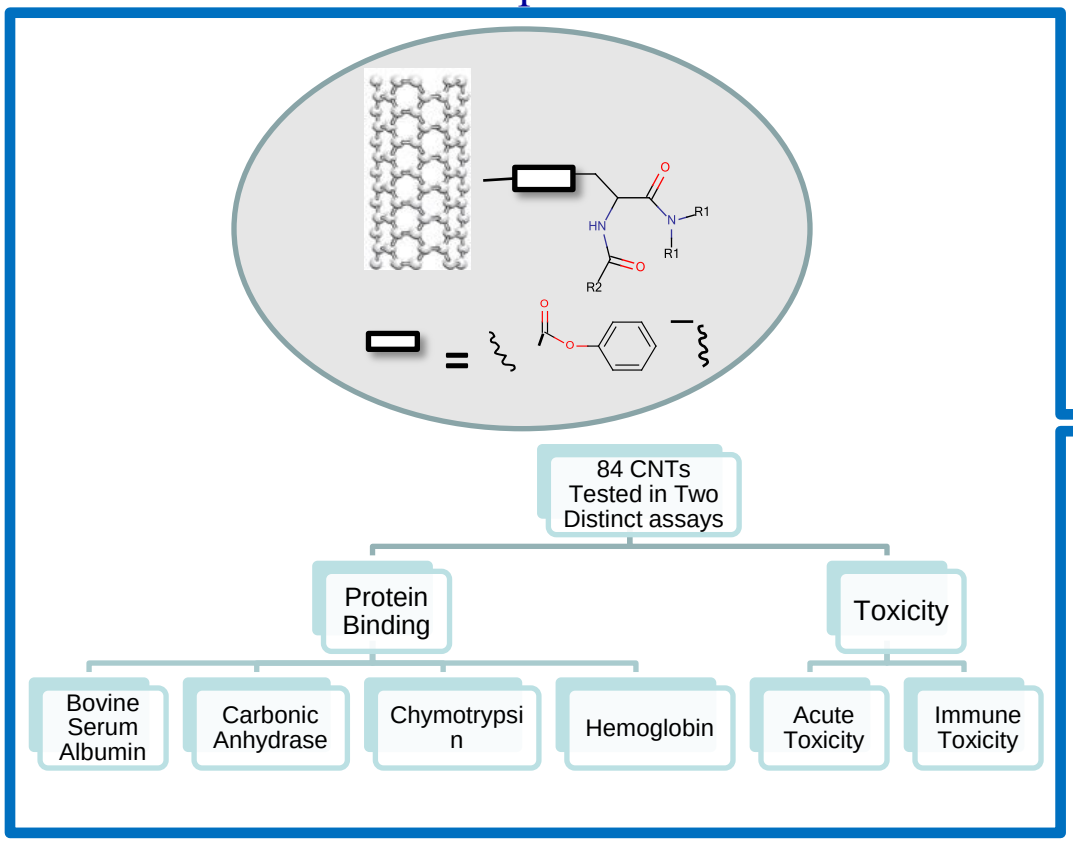
- **Validation of models according to numerous statistical procedures, and their applicability domains.**

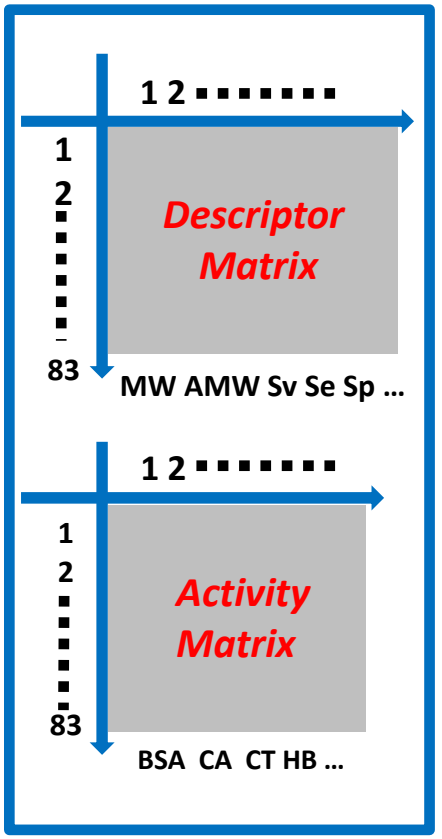
Activity Profiles



Data Retrieval and Processing

A series of 84 CNTs decorated with surface organic molecules were tested in protein binding, acute toxicity and immune toxicity assays in Dr. Bing Yan's group at St. Jude Children's Research Hospital*

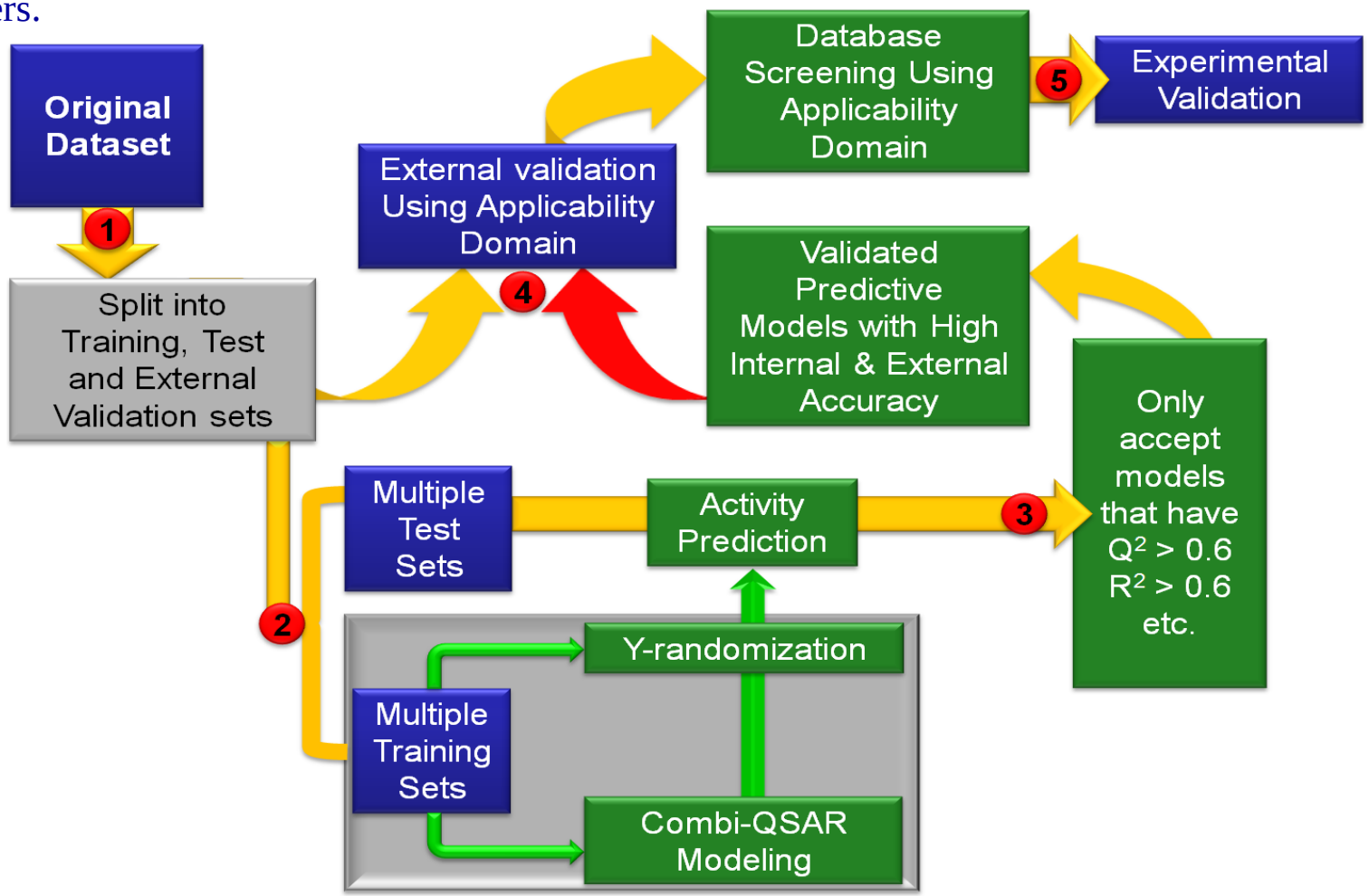




**Zhou, H., et al. A nano-combinatorial library strategy for the discovery of nanotubes with reduced protein-binding, cytotoxicity, and immune response. Nano. Lett., 2008, 8, 859-865.*

Validated QNAR Workflow

We followed a standardized, predictive QSAR workflow previously established by our group*. Models' prediction power is assessed by external cross-validation techniques as well as rigorous statistical parameters.



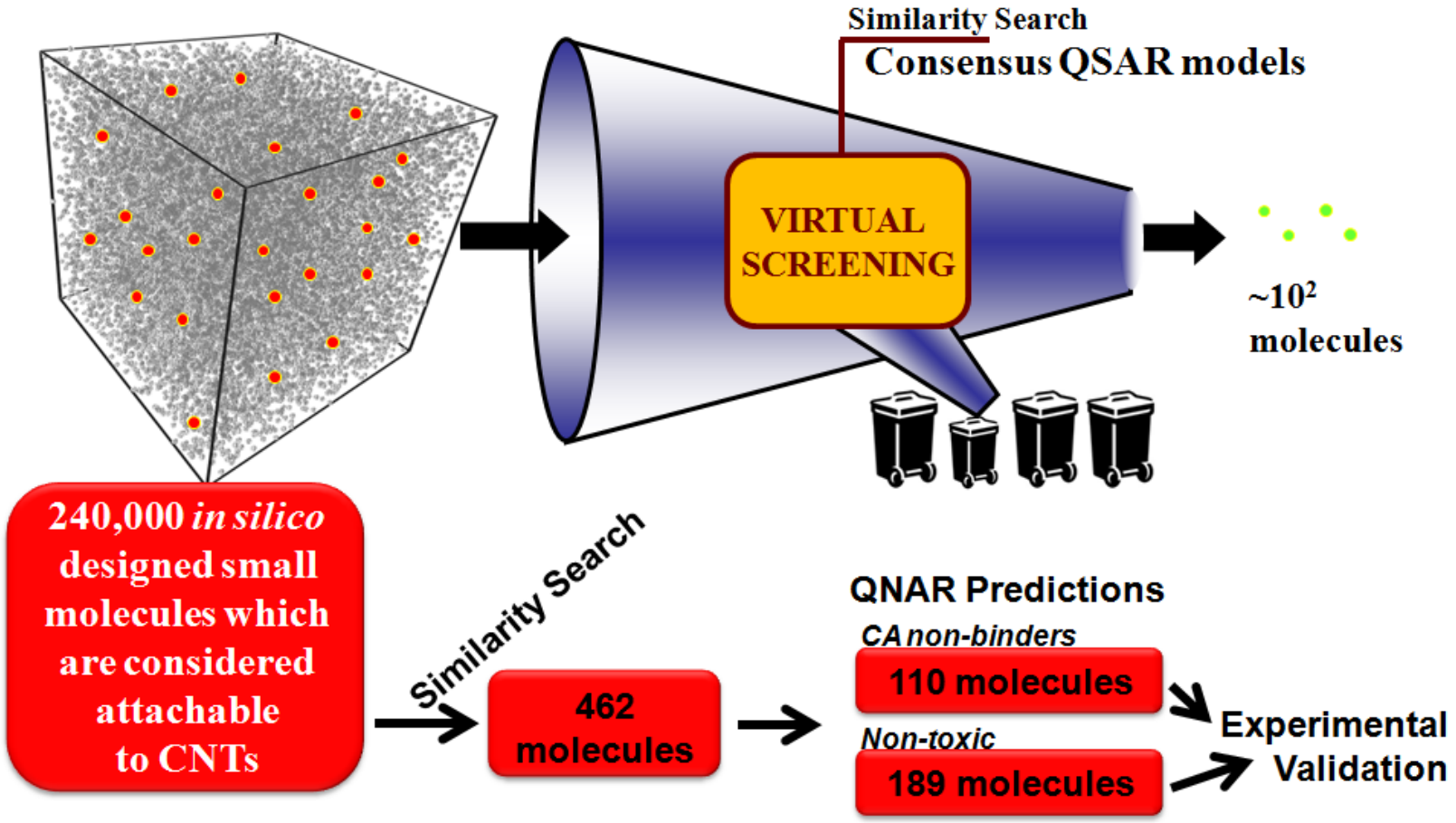
** Tropsha, A. Best Practices for QSAR Model Development, Validation, and Exploitation Mol. Inf., 2010, 29, 476 – 488).*

Summary of QNAR models for CA binding and acute toxicity*

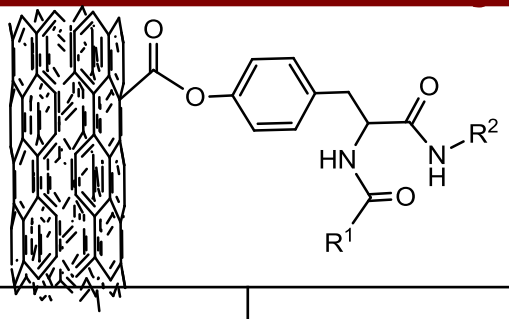
		kNN-Dragon	SVM-Dragon	RF-Dragon	kNN-MOE	SVM-MOE	RF-MOE
CA binding	**Sens.**	0.77	0.70	0.74	0.70	0.67	0.67
	Spec.	0.73	0.68	0.73	0.68	0.58	0.68
	Accr.	**0.75**	0.69	**0.73**	0.69	0.63	0.67
Acute toxicity	Sens.	0.71	0.79	0.79	0.68	0.63	0.63
	Spec.	0.69	0.74	0.69	0.63	0.63	0.63
	Accr.	**0.70**	**0.77**	**0.74**	0.66	0.63	0.63

**The reported values are averaged over 5-fold external validation*

Virtual Screening of External Library

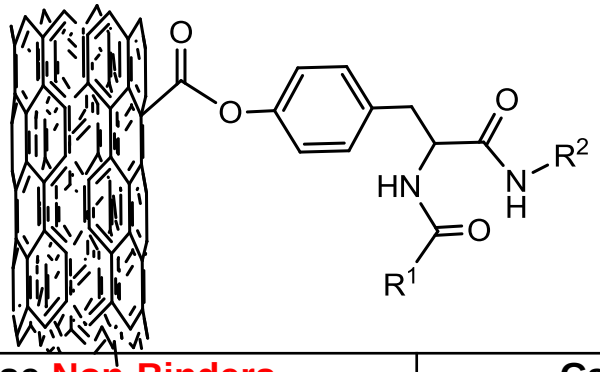


Selected Hits for the Cytotoxicity Assay



Non-Toxic			Toxic		
Id	**R1**	**R2**	**Id**	**R1**	**R2**
II-1	Phenethyl	Cyclopentyl	**II-11**	4-Methylphenyl	4-Nitro-3-(trifluoromethyl)phenyl
II-2	Benzyl	Cycloheptyl	**II-12**	4-Fluorophenyl	4-Nitro-3-(trifluoromethyl)phenyl
II-3	Benzyl	Cyclopentyl	**II-13**	Phenyl	4-Nitro-3-(trifluoromethyl)phenyl
II-4	Phenethyl	Cyclohexyl	**II-14**	4-Trifluoromethylphenyl	3-Hydroxyphenyl
II-5	4-Chlorophenyl	Benzyl	**II-15**	4-Trifluoromethylphenyl	4-Fluorophenyl
II-6	4-Methylbenzyl	Phenyl	**II-16**	4-Trifluoromethylphenyl	4-Hydroxyphenyl
II-7	Cyclopentylmethyl	Phenyl	**II-17**	4-Trifluoromethylphenyl	1-Phenylethyl
II-8	Benzyl	4-Methylcyclohexyl	**II-18**	4-Fluorophenyl	3-Trifluoromethyl
II-9	Benzyl	Butyl	**II-19**	4-Trifluoromethylphenyl	3-Ethynylphenyl
II-10	4-Chlorophenyl	Cycloheptyl	**II-20**	4-Trifluoromethylphenyl	3-Methoxyphenyl

Selected Hits for CA Binding Assay



Carbonic Anhydrase Non-Binders			Carbonic Anhydrase Binders		
Id	**R1**	**R2**	**Id**	**R1**	**R2**
II-21	4-Chlorophenyl	3-Methylphenyl	**II-9**	Benzyl	Butyl
II-22	4-Chlorophenyl	3-Ethylphenyl	**II-31**	Benzyl	Cyclohexyl
II-23	4-Chlorophenyl	4-Methylphenyl	**II-32**	Benzyl	Benzyl
II-24	4-Chlorophenyl	2-Methylbutyl	**II-33**	1-Butenyl	Cycloheptyl
II-25	4-Chlorophenyl	2-Methylphenyl	**II-34**	Butyl	Cycloheptyl
II-26	4-Chlorophenyl	Cyclohexylmethyl	**II-35**	Phenyl	Cyclohexyl
II-27	4-Chlorophenyl	Pentyl	**II-36**	Butyl	Cyclohexyl
II-28	4-Chlorophenyl	1-Phenylethyl	**II-37**	Cyclohexyl	Cyclohexyl
II-29	4-Chlorophenyl	3-Methylbenzyl	**II-38**	Pentyl	Cyclohexyl
II-30	4-Chlorophenyl	4-Methylcyclohexyl	**II-39**	Cyclohexyl	Benzyl

Summary of experimental validation results for cytotoxicity of selected hits.*

CNT ID	II-1	II-2	II-3	II-4	II-5	II-6	II-7	II-8	II-9	II-10
Average cell viability (%)	48	51	51	46	48	55	58	62	58	49
Standard Deviation (%)	5	3	3	3	2	10	6	7	3	6
Class	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
Predicted Class	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***0***
CNT ID	**II-11**	**II-12**	**II-13**	**II-14**	**II-15**	**II-16**	**II-17**	**II-18**	**II-19**	**II-20**
Average cell viability (%)	29	39	36	39	42	31	41	39	45	40
STDEV (%)	9	8	7	5	8	11	5	9	11	10
Class	***1***	***1***	***1***	***1***	0	***1***	0	***1***	0	0
Predicted Class	***1***	***1***	***1***	***1***	1	***1***	1	***1***	1	1

Cell viability of THP-1 cells treated with 200 μg/mL. Threshold of 40% was applied to classify CNTs as non-toxic or toxic. CNTs are labeled as "0" (non-toxic) if their cell viability is greater than 40% and "1" (toxic) if their cell viability is less than 40%. The prediction statistics is: **sensitivity=100% (10/10), specificity=60% (6/10), accuracy=80% (16/20).**

*Experimental data is generated by Dr. Bing Yan and colleagues (St. Jude).

Summary of experimental validation* results for selected CA binders and non-binders.

CNT ID	II-21	II-22	II-23	II-24	II-25	II-26	II-27	II-28	II-29	II-30
Average protein binding (F0/F1)	1.68	1.66	1.92	1.72	1.83	2.60	1.74	2.01	1.60	2.65
Standard Deviation	0.05	0.06	0.02	0.03	0.02	0.02	0.02	0.01	0.06	0.02
Class	***0***	***0***	***0***	***0***	***0***	1	***0***	1	***0***	1
Predicted Class	***0***	***0***	***0***	***0***	***0***	0	***0***	0	***0***	0
CNT ID	**II-9**	**II-31**	**II-32**	**II-33**	**II-34**	**II-35**	**II-36**	**II-37**	**II-38**	**II-39**
Average protein binding (F0/F1)	4.29	2.78	2.48	2.51	2.59	3.69	2.37	2.77	3.41	2.90
STDEV	0.03	0.05	0.08	0.01	0.04	0.00	0.02	0.06	0.05	0.11
Class	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***
Predicted Class	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***	***1***

CA (50 μg/mL) was excited at 280 nm and emission spectra (300-400 nm) were recorded. Spectra were measured before and immediately after addition of MWCNT (15 μg/mL). Ratio of F_0 (initial) to F (modified) at 340 nm was used to evaluate protein binding affinity; the threshold was set at 2.00 (F0/F1) in the modeling process, and CNTs are labeled as "0" (non-binder) if their CA bindings are smaller than 2.00 and "1" (binder) if their CA bindings are greater than 2.00. Statistics: **sensitivity 77% (10/13), specificity 100% (7/7) and prediction accuracy is 85% (17/20).**

*Experimental data is generated by Dr. Bing Yan and colleagues (St. Jude).

Conclusions

- Endpoint-specific QNAR models of protein binding and cellular toxicity have been developed for a series of functional carbon nanotubes. The calculated statistics showed that the average predictive accuracy for 5-fold external validation could be as high as 75%.
- The statistically significant QNAR models have been employed for virtual screening of a library of 240K organic compounds considered to be attachable to CNT surface. The CA binding models showed experimentally confirmed predictive accuracy of 85%. Similarly, cellular toxicity models showed experimentally confirmed predictive accuracy of 80%.
- To the best of our knowledge, this study is the first case of using *in silico* approaches to design biologically active Carbon NanoTubes with the reduced toxicity and protein binding
- The results of this study suggest that QNAR models could be used as reliable means to aid in rational design of surface-modified MNP with the desired biological properties

Industrial Interactions and Technology Transfer

- **Results of these studies have been presented at several national and international conferences and webinars attended by industry and academia including:**
  - **European Commission COST workshop on QNTR, Maastricht, Netherlands in April 2012**
  - **Nanoinformatics Conference, Arlington, DC, Dec 2011**
  - **SRC Metrology Webinar Series (January 2012)**

- **The UNC group joined the Nanoinformatics Working Group (http://www.internano.org/nanoinformatics/index.php/Main:Community_Portal) maintained by the National Nanomanufacturing Network.**

- **We have transferred all modeling techniques and models developed in our studies to the publicly available ChemBench web portal (*chembench.mml.unc.edu*). ChemBench enables the unrestricted use of our models to prioritize MNPs for the experimental validation (similar to the joint study with Dr. Bing Yan) as well as the development of new QNAR models using user-supplied data.**

Future Plans

Next Year Plans

- We plan to extend our collaboration with Dr. Bing Yan (St. Jude) to enable additional QNAR model building and experimental validation using new data from Dr. Yan.
- We have established a new collaboration with scientists at the Research Triangle Institute (Kim Guzan and colleagues) and Pacific Northwest Laboratory (Dr. Nathan Baker and colleagues) who are establishing ontology and databases related to biological activity of MNPs. We will be building new QNAR models using data collected in these nano-depositories
- In collaboration with Prof. Stefano Curtarolo (Duke University), we will be developing new computational descriptors of nanomaterials.

Long-Term Plans

- Obtain predictive knowledge of physical, geometrical, and chemical properties of MNPs that affect human cells and utilize this knowledge for improved MNP experimental design and prioritized toxicity testing.

Publications, Presentations, and Recognitions/Awards

- B. Yang et al. Chemical Basis of Nanoparticle-Biological Systems Interactions. *Chemical Reviews*, **2012**, submitted.
- Manocha, S., Tropsha, A., Mumper, R. "Effect of Carbon Coating on the Physico-Chemical Properties and Toxicity of Copper and Nickel Nanoparticles. *ACS Nano*, **2011**, submitted.
- Fourches D, Pu D, Tropsha A. Exploring quantitative nanostructure-activity relationships (QNAR) modeling as a tool for predicting biological effects of manufactured nanoparticles. *Comb Chem High Throughput Screen*. **2011**, 14(3):217-25
- Alexander Tropsha, Denis Fourches. Quantitative Nanostructure-Activity Relationships (QNAR) modeling: Applications to Rational Design of Nanomaterials with the Desired Bioactivity Profile. SRC Metrology Webinar Series, Jan. 20, **2012**.
- Alexander Tropsha, Denis Fourches. Quantitative Nanostructure-Activity Relationships (QNAR) modeling: Applications to Rational Design of Nanomaterials with the Desired Bioactivity Profile Nanoinformatics Conference, Arlington, DC, Dec. 5-7, **2011**.
- Alexander Tropsha, Quantitative Nanostructure-Activity Relationships (QNAR) modeling: Applications to Rational Design of Nanomaterials with the Desired Bioactivity Profile. Univ. of Strasbourg, Strasbourg, France, Nov. 11, **2011**
- Alexander Tropsha. Quantitative Nanostructure-Activity Relationships (QNAR) models as tools for predicting biological effects of manufactured nanoparticles. European Commission COST workshop on QNTR, Maastricht, Netherlands, April 3-5 **2011**