Reducing Nanotoxicity With Nano-Combinatorial Chemistry Approach: The Case Of Carbon Nanotube

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Member

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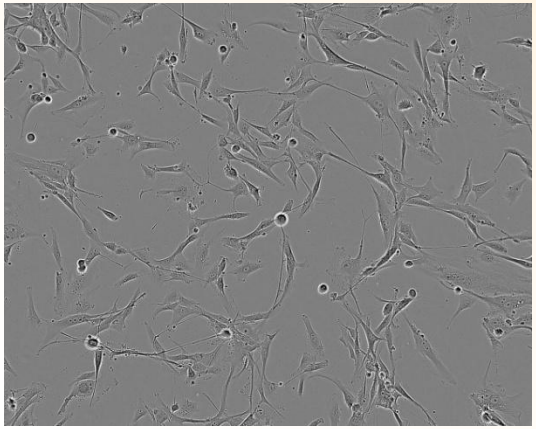
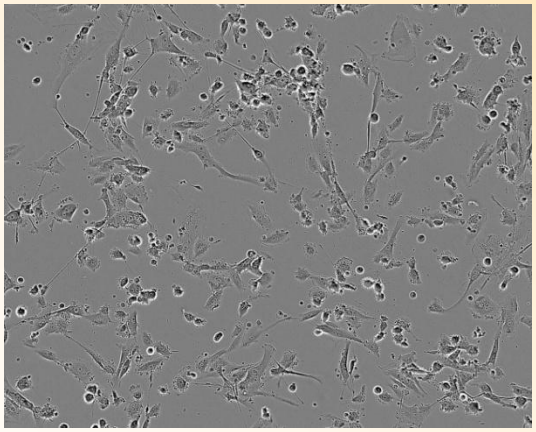
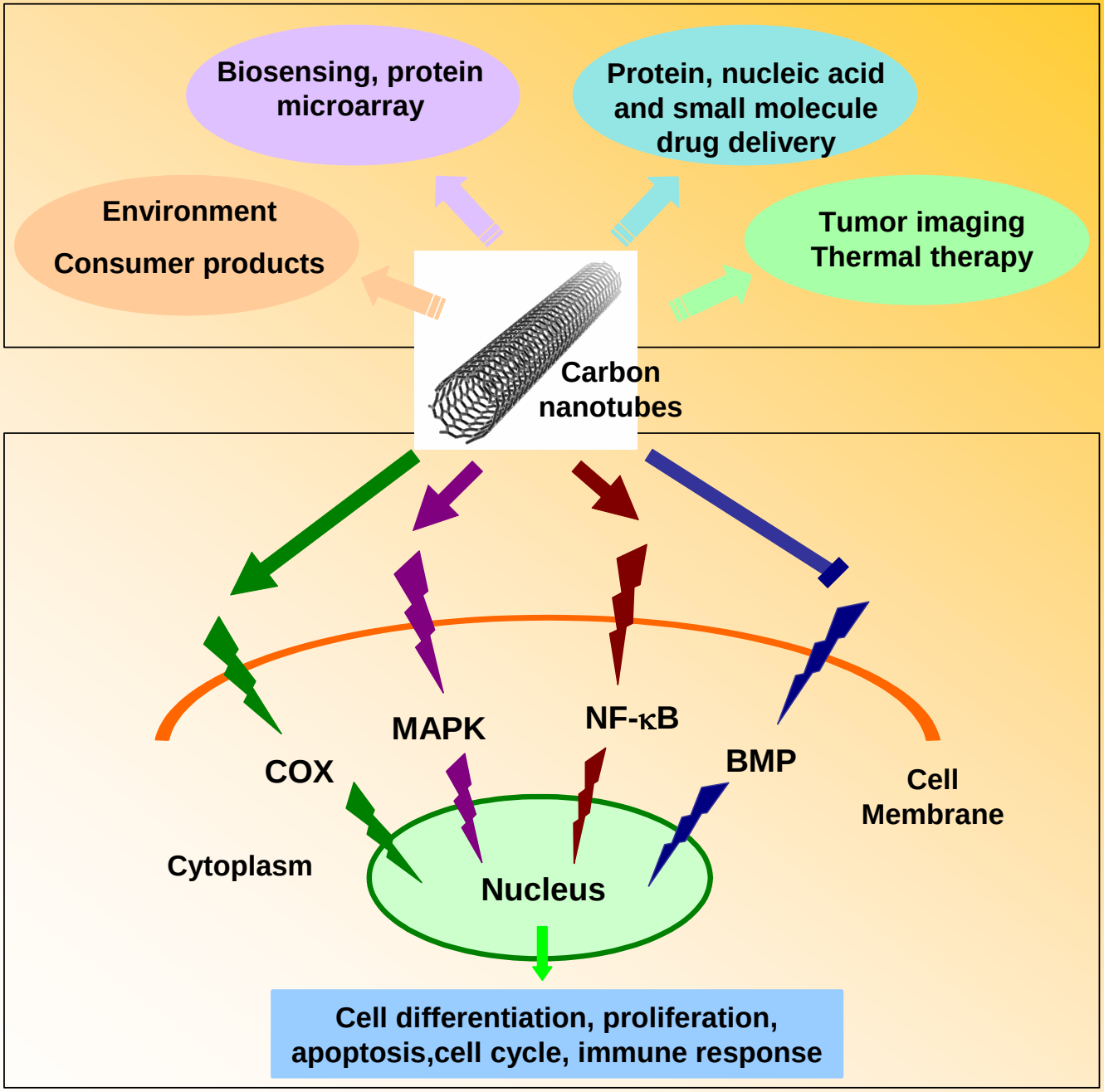
(with additional contributions from Tropsha's Group)





Carbon Nanotube Is Scaling Up!

- Hyperion (U.S.)
- Bayer (Germany) **3000 ton/year for 2012**
- Arkema (France) hundreds ton/year
- Nanocyl (Belgium) 35 ton/year now
- Pyrograf (U.S.)
- Ahwahnee (U.S.)
- Carbon- Nano-Material Technology (Korea)
- Iljin Nanotech (Korea)

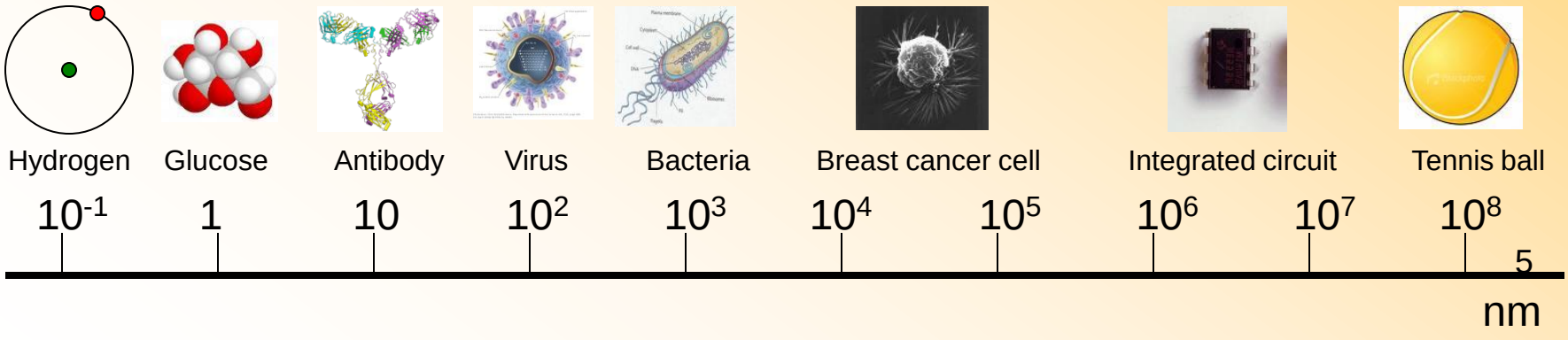

Biosensing, protein microarray
Protein, nucleic acid and small molecule drug delivery
Environment
Consumer products
Tumor imaging
Thermal therapy
Carbon nanotubes
COX
MAPK
NF-κB
BMP
Cell Membrane
Cytoplasm
Nucleus
Cell differentiation, proliferation, apoptosis,cell cycle, immune response

Public concerns on nanosafety

Protesting Eddie Bauer stain resistant "Nanopants"

Outline

- **Nanotoxicity**
- Reduce toxicity



CNT/Protein Interactions

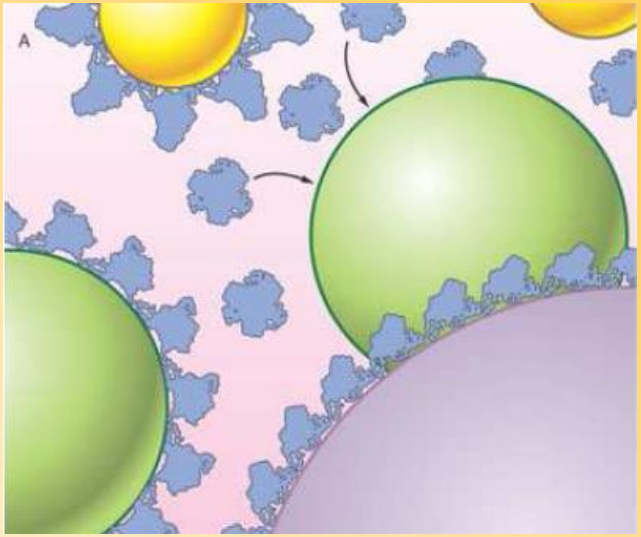
Gel electrophoresis

Mass spectrometry

Fluorescence quenching

Zeta potential

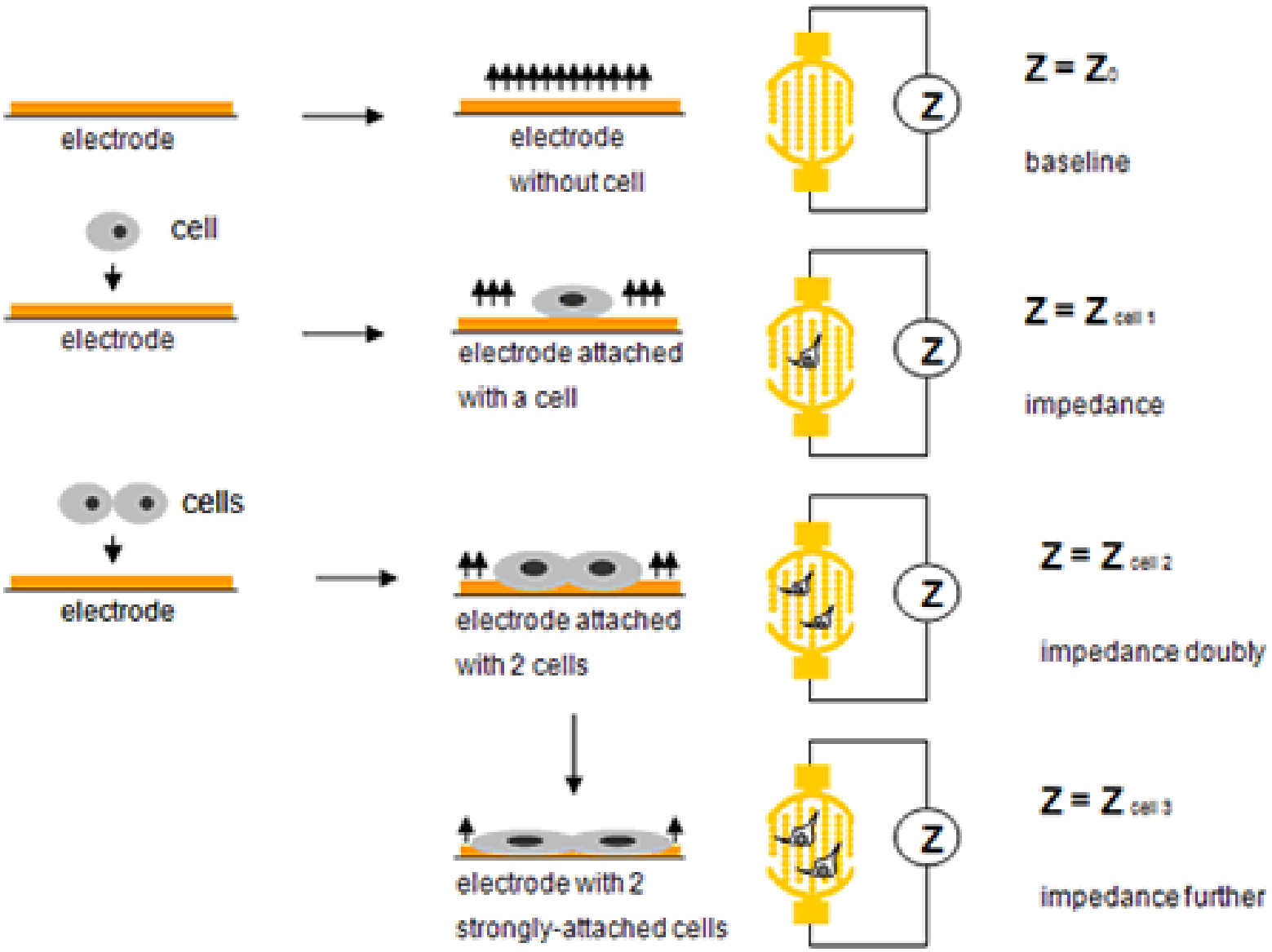
Enzyme activity



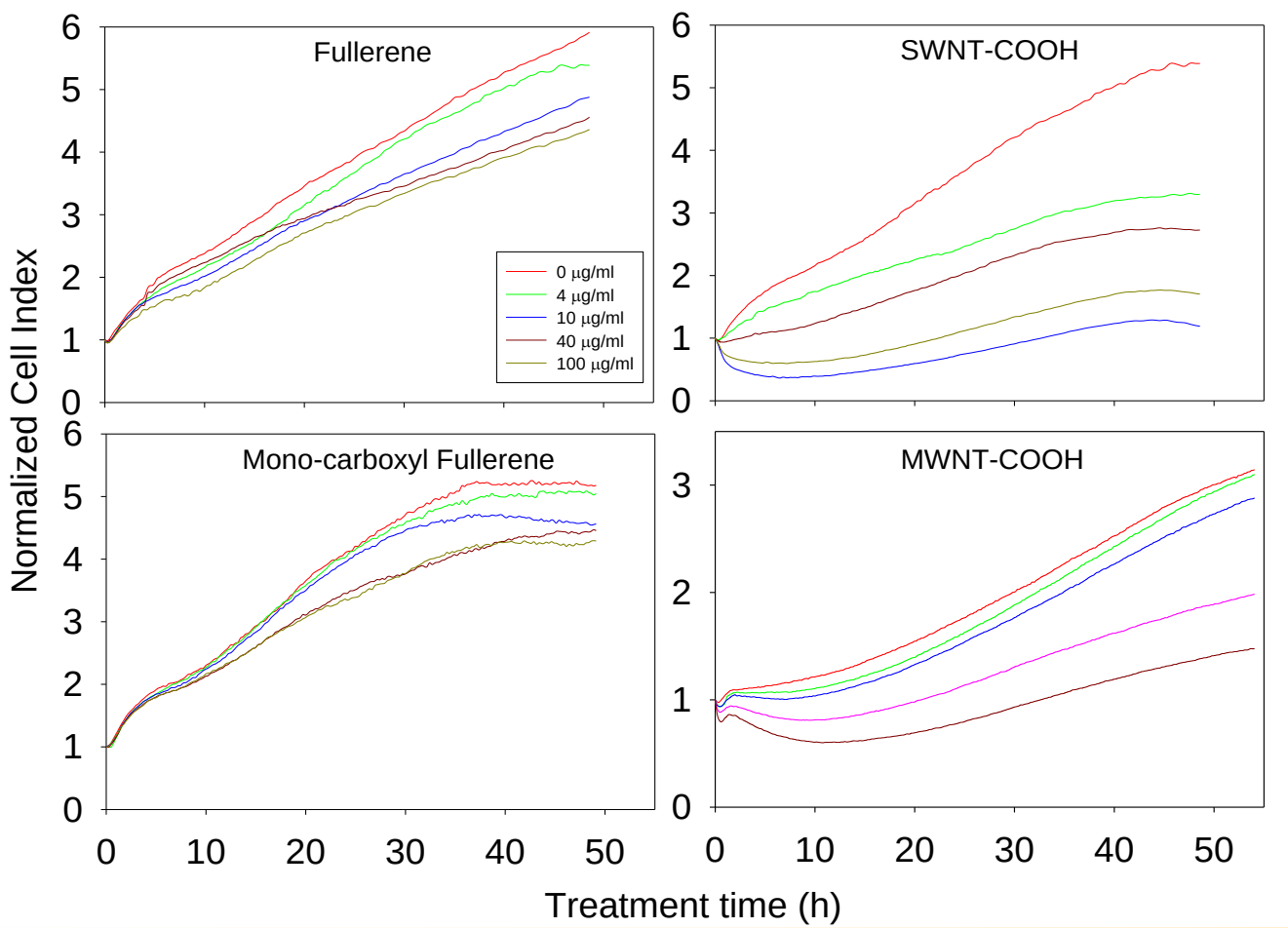
Mu, Liu, Xing, Zhou, Li, Zhang, Ji, Wang, Si, Zhang, Yan*. ***J Phys Chem C* 2008**, 112(9), 3300-3307.

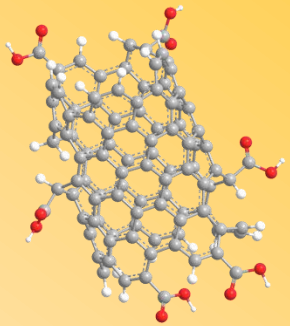
Zhang, Xing, Li, Mu, Zhou, Yan*. ***Nano Lett.* 2009**, 9(6), 2280-2284.

Real-Time Cell Electronic Sensing



Inhibition of Cell Proliferation by Carbon Nanotubes

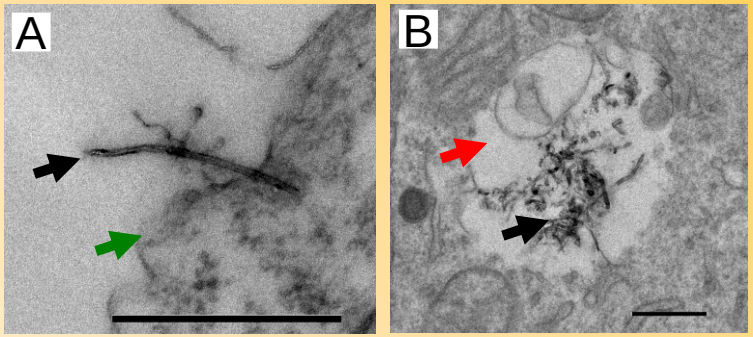


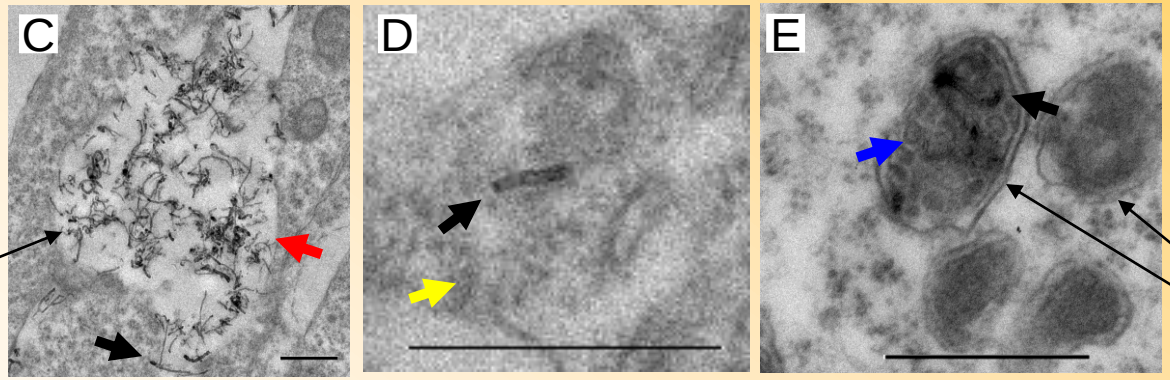


HEK293

Cellular Localization

MWCNT-COOH, 1h incubation

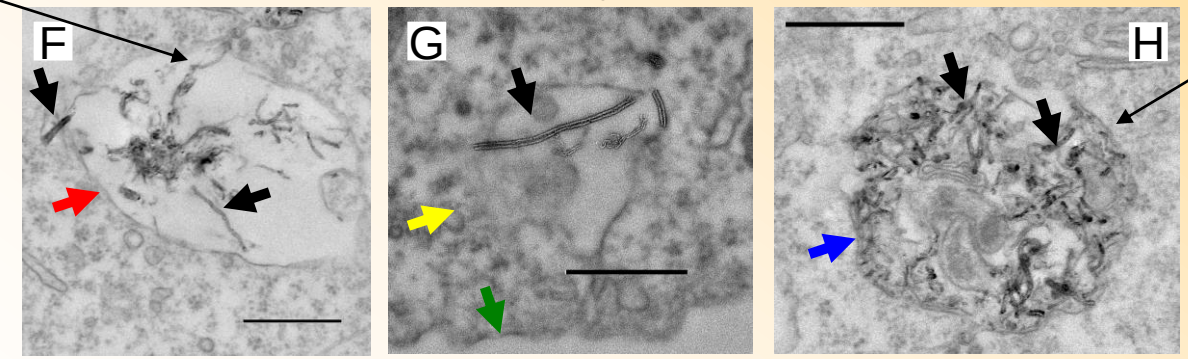




endosome

lysosome

MWCNT-COOH, 48h incubation



Plasma membrane

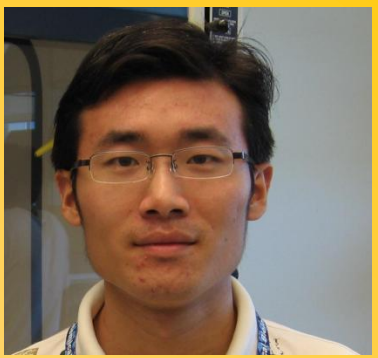
Endosome

MWCNTs

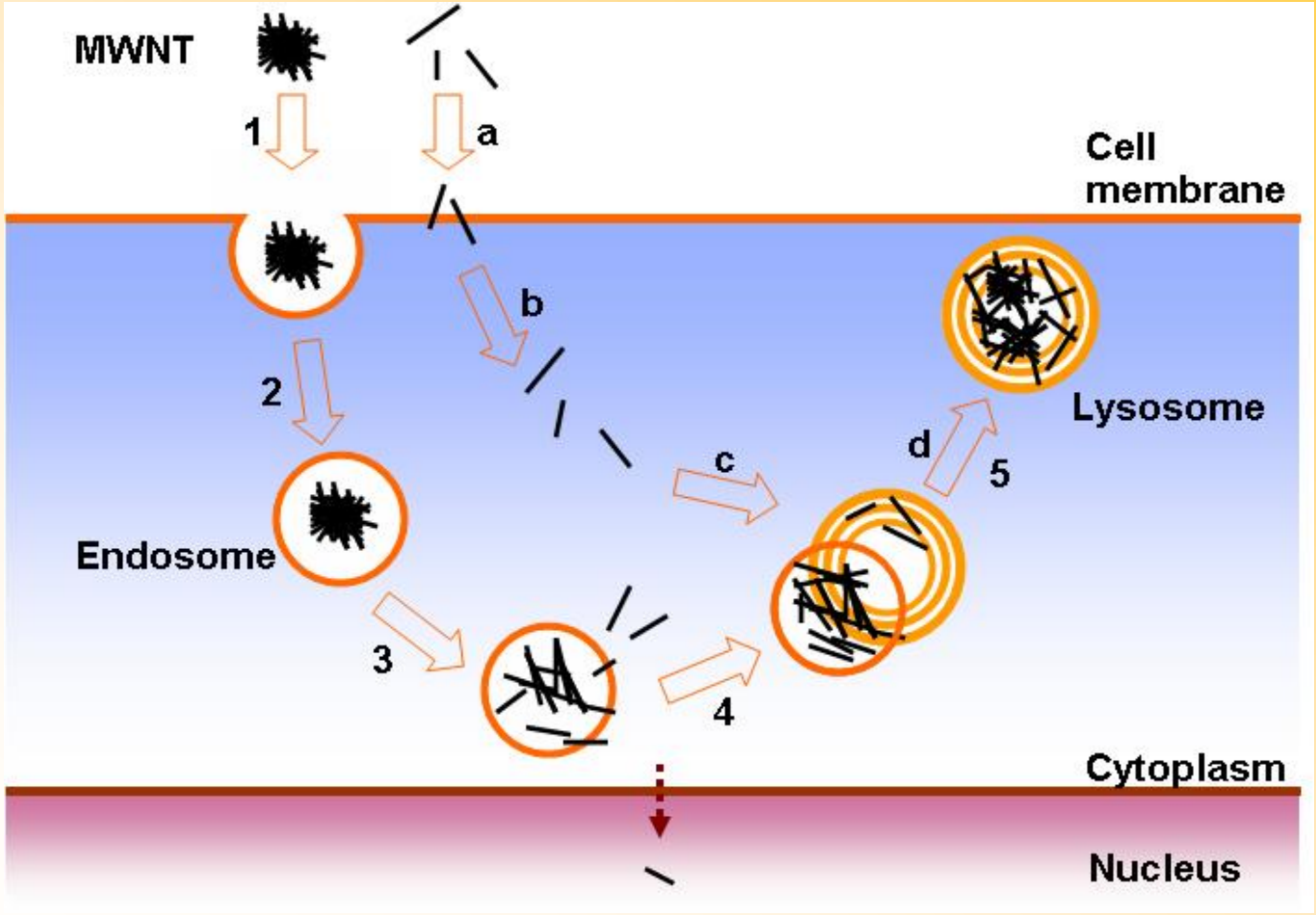
Cytoplasm

Lysosome

A Model for MWNT Cell Uptake



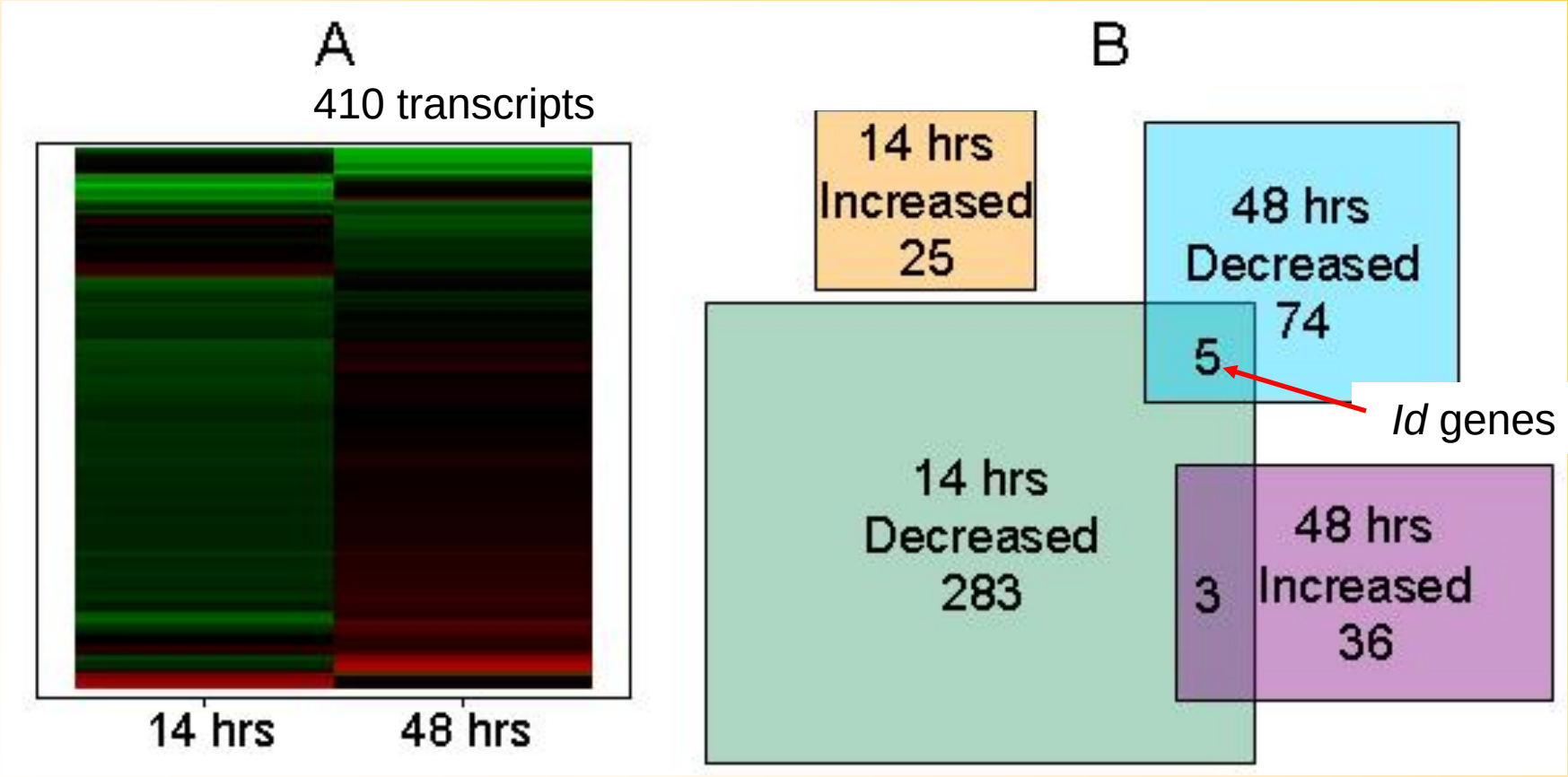
Qingxin Mu



Mu, Broughton, Yan*, ***Nano Lett.*** 2009, 9(12), 4370-4375.

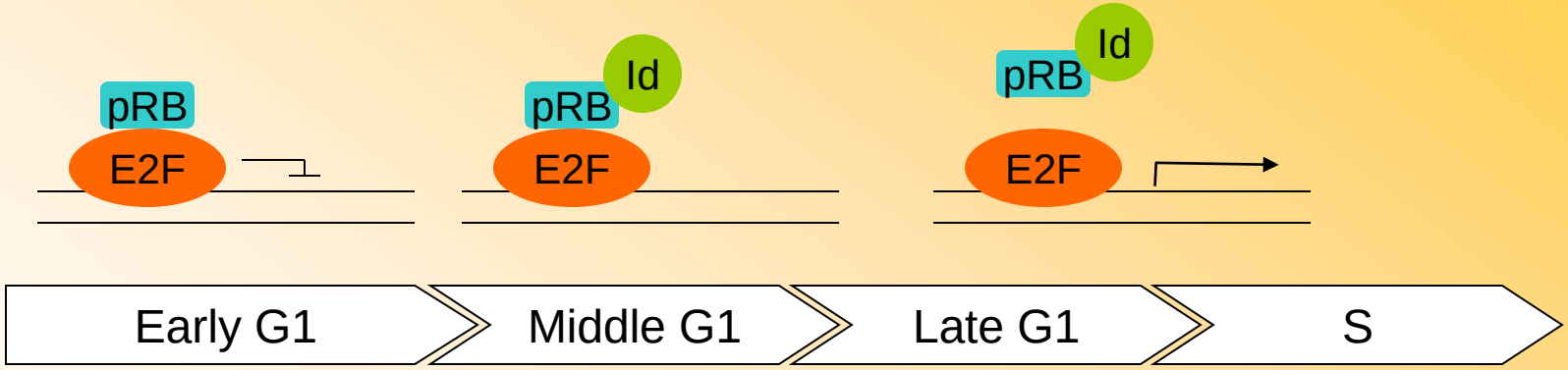
SWNT-COOH Affects Globe Gene Expression

Using Affemetrix U133v2 human genome chip (54,600 probesets)



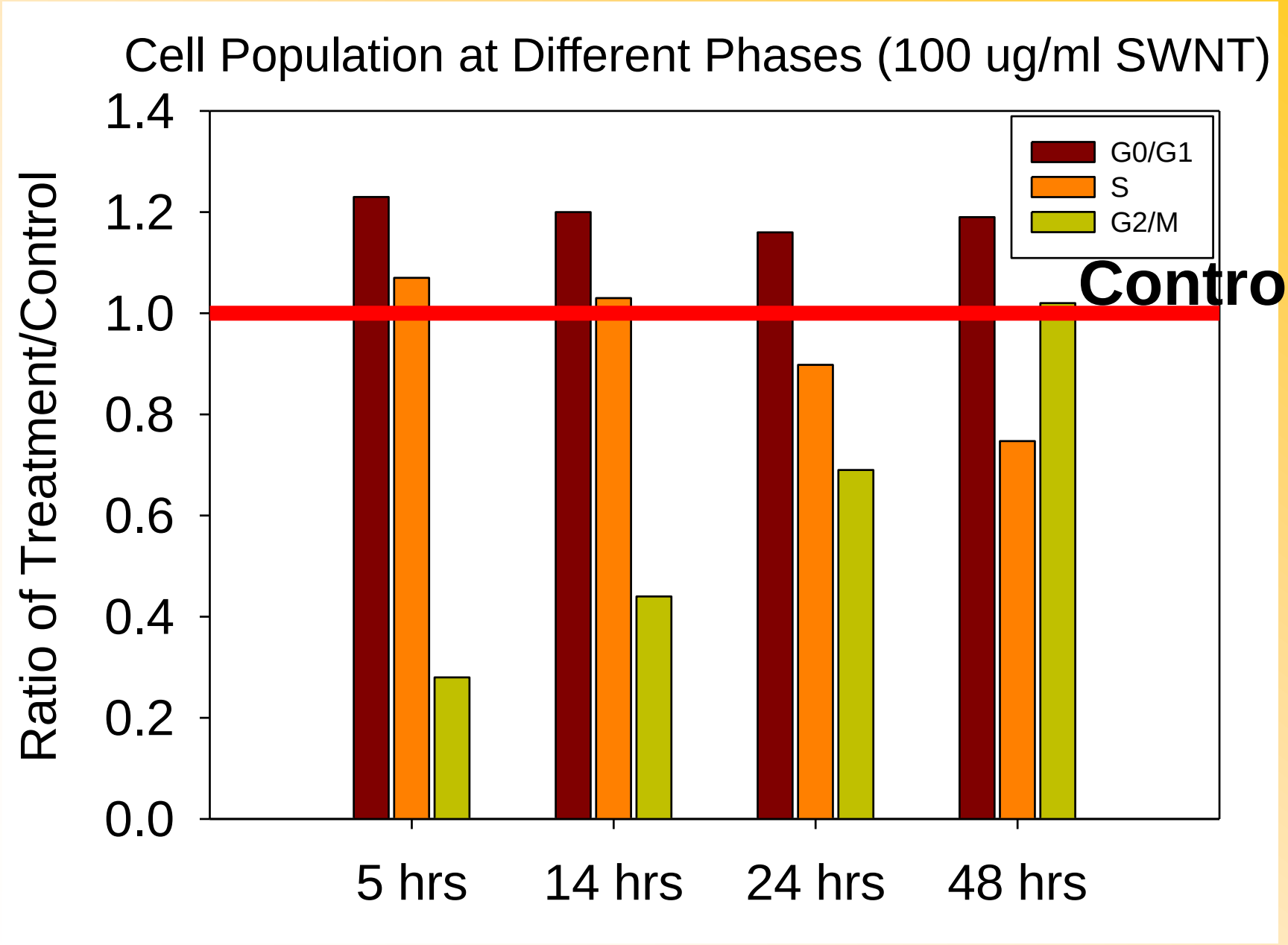
HEK293 cells, 100 μg/mL

ID Proteins Regulate Cell Cycle



Zoe Zebedee, Eiji Hara. Oncogene. (2001) 20, 8317-8325

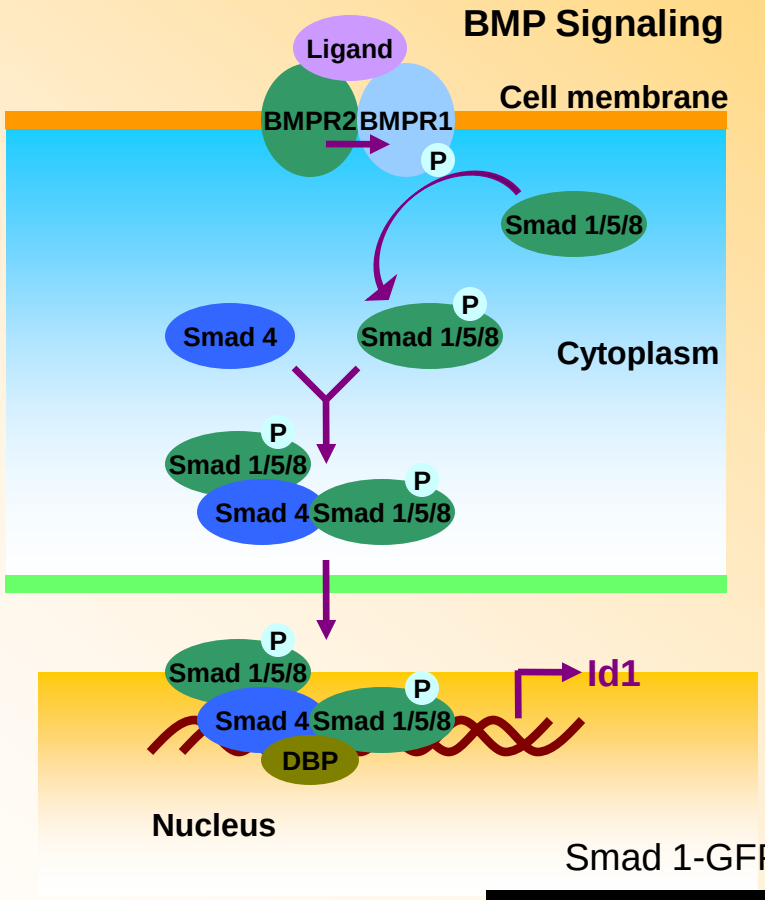
Cell Cycle Analysis

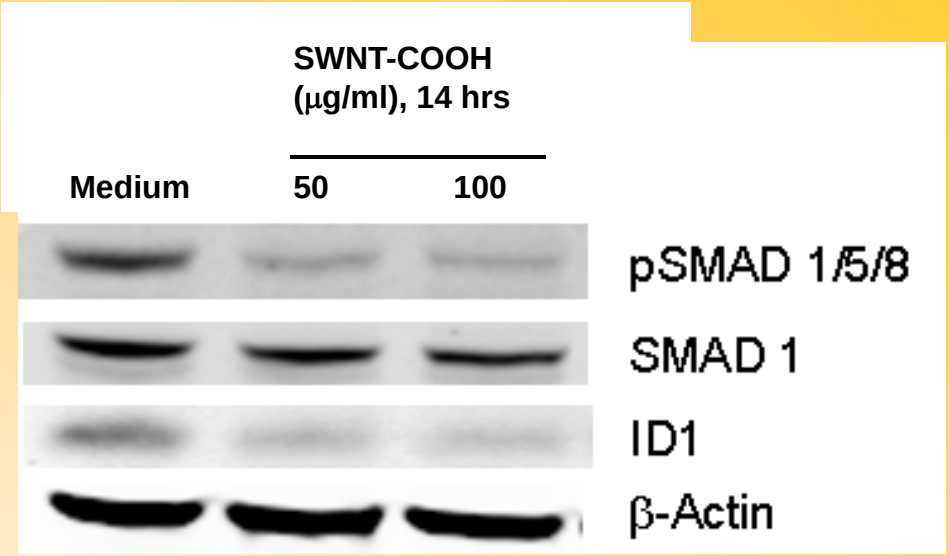


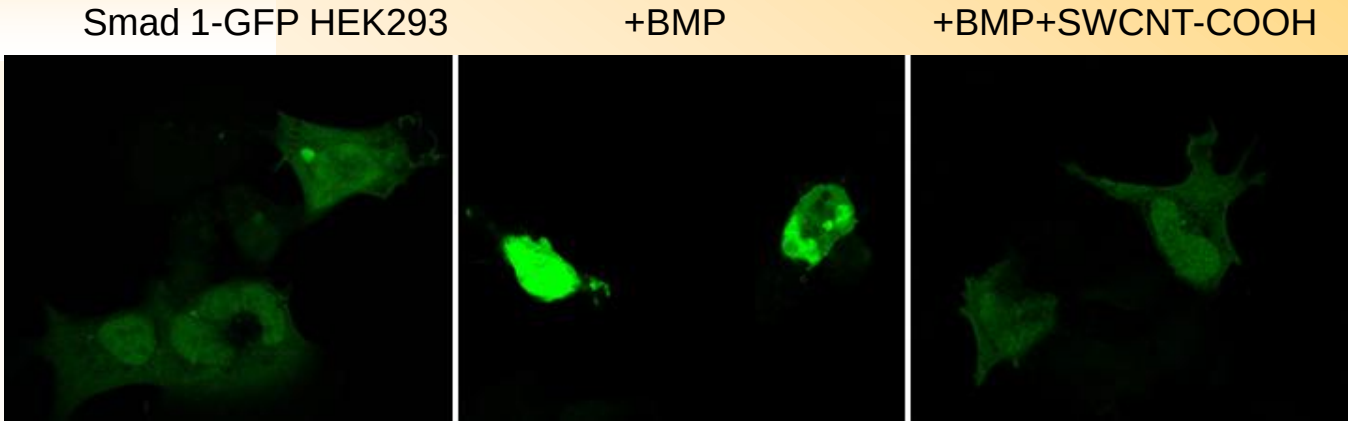
Signaling Pathways Significantly Effected

14 hours treatment		48 hours treatment	
Pathway name	$p < 0.05$	Pathway name	$p < 0.05$
Starch and sucrose metabolism	6.80E-05	Adherens junction	0.000332
Tight junction	0.004099	TGF-beta/BMP signaling pathway	0.000528
Long-term depression	0.016265	Tight junction	0.001647
Adherens junction	0.016989	Glycan structures - biosynthesis 2	0.028914
Taste transduction	0.019684	Glycosphingolipid biosynthesis - lactoseries	0.041922
Notch signaling pathway	0.020863		
Amyotrophic lateral sclerosis (ALS)	0.024071		
TGF-beta/BMP signaling pathway	0.02534		
Gap junction	0.02726		

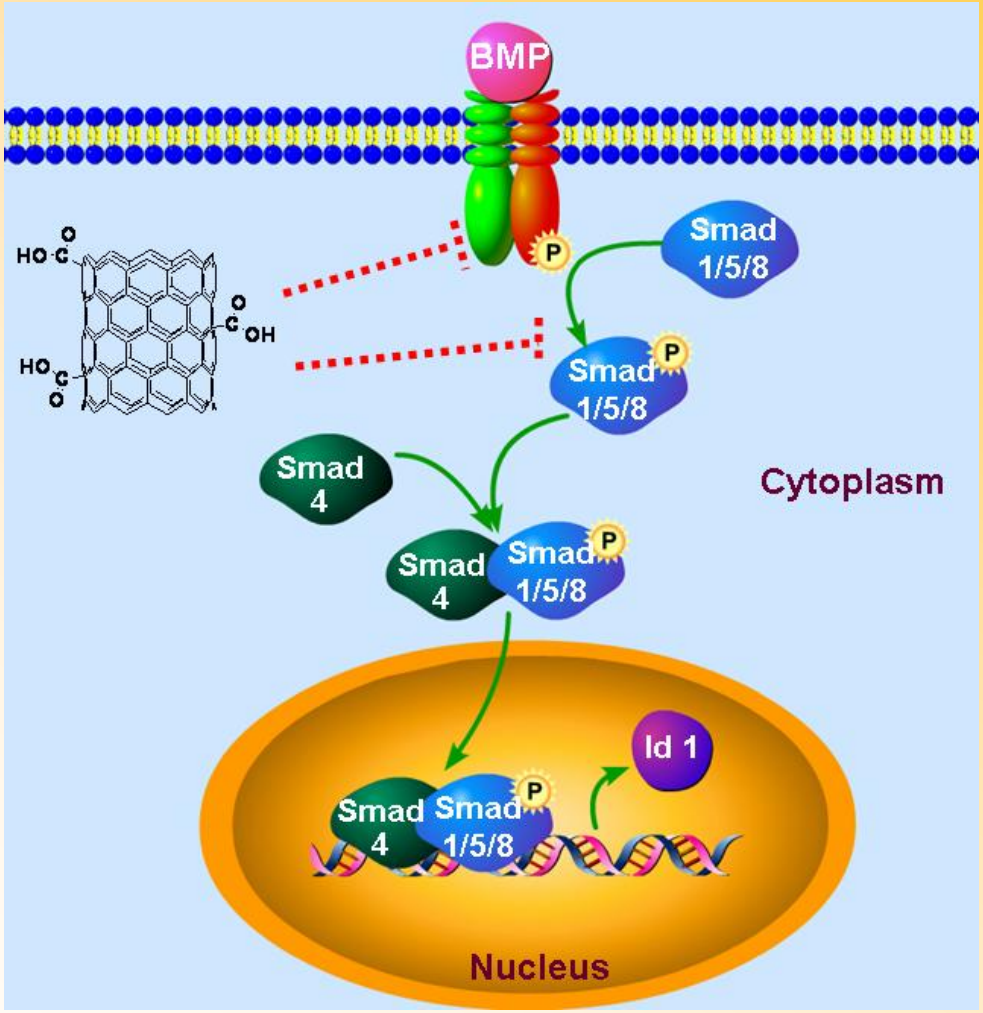
Possible Mechanisms

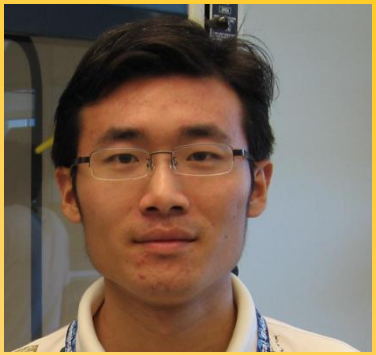






Suppression of BMP Signaling by SWCNT-COOH



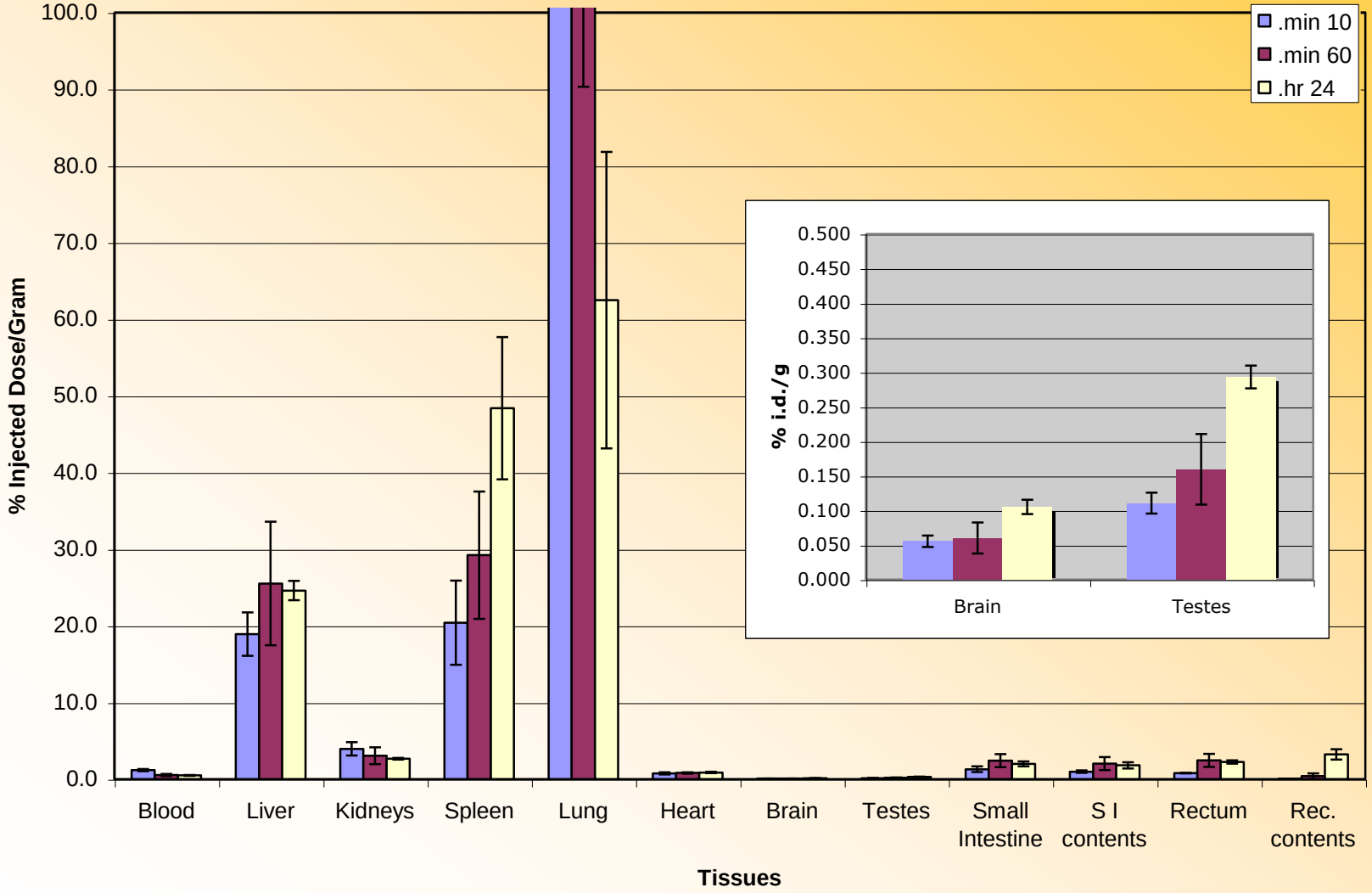


Qingxin Mu

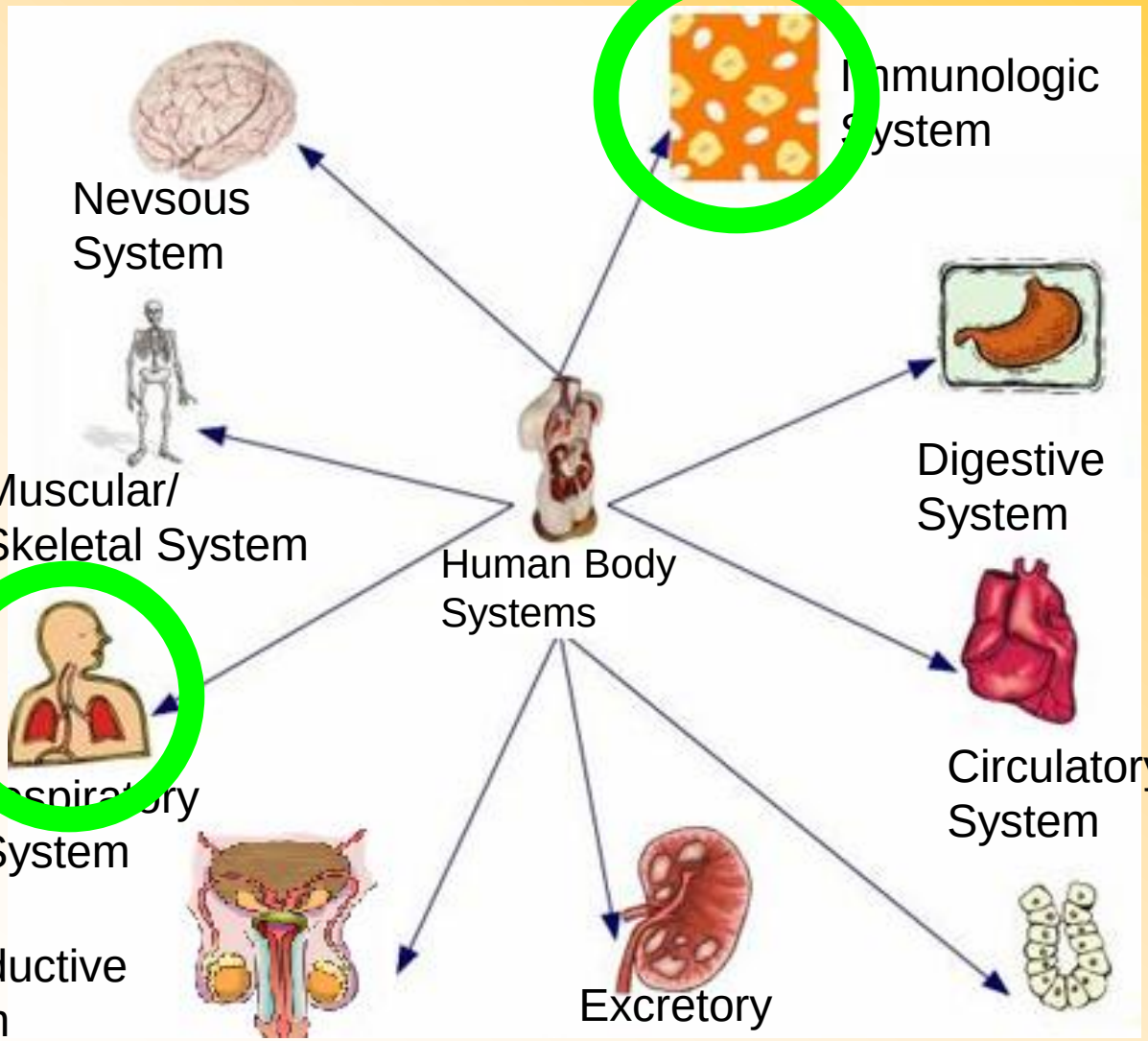
Mu, Du, Chen, Zhang, Yan*. ***ACS Nano*** 2009, 3, 1139-1144.

Distribution of Carbon Nanotubes in Mice

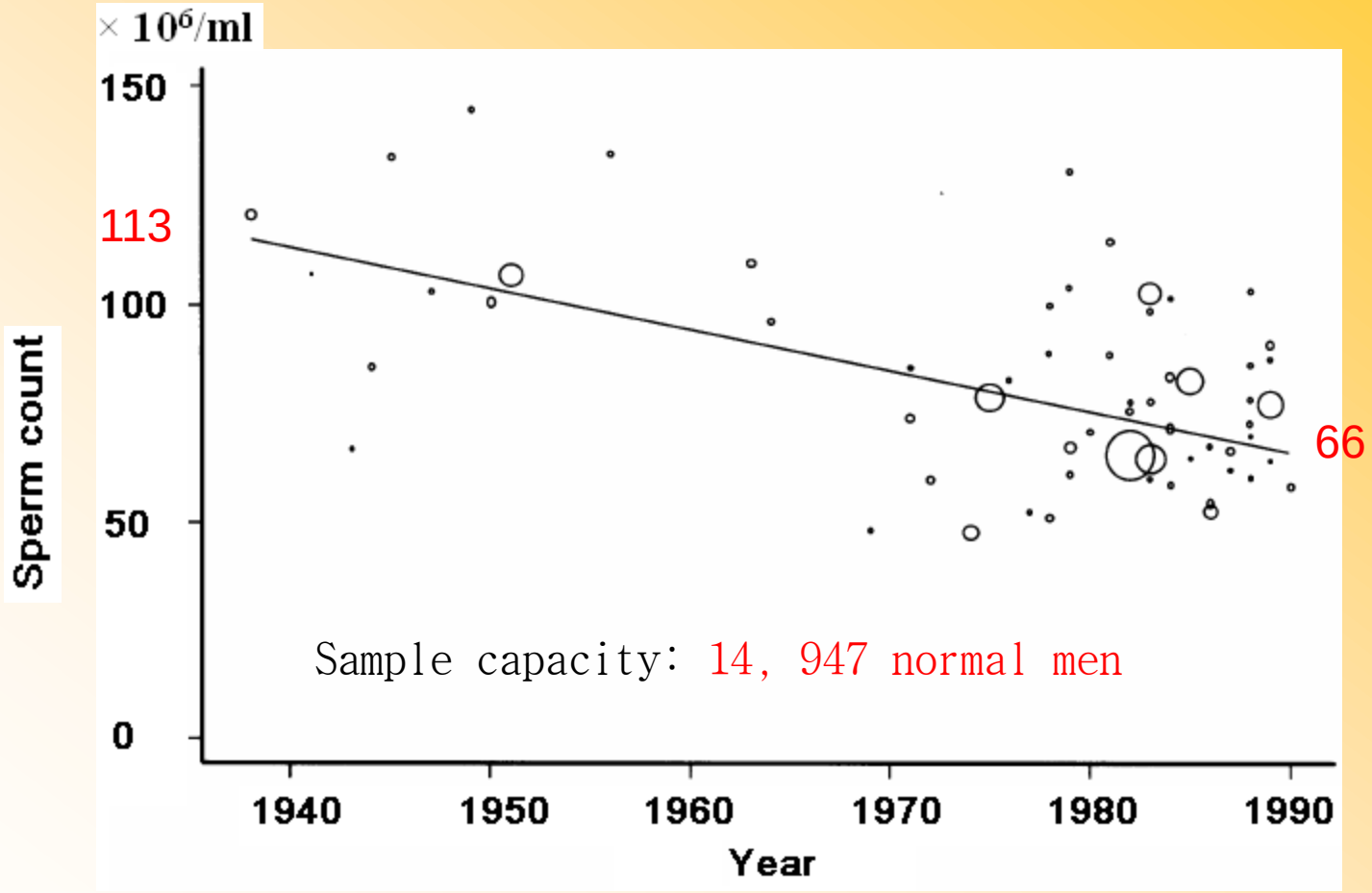
Scott Snyder



Lack reproductive nanotoxicity research

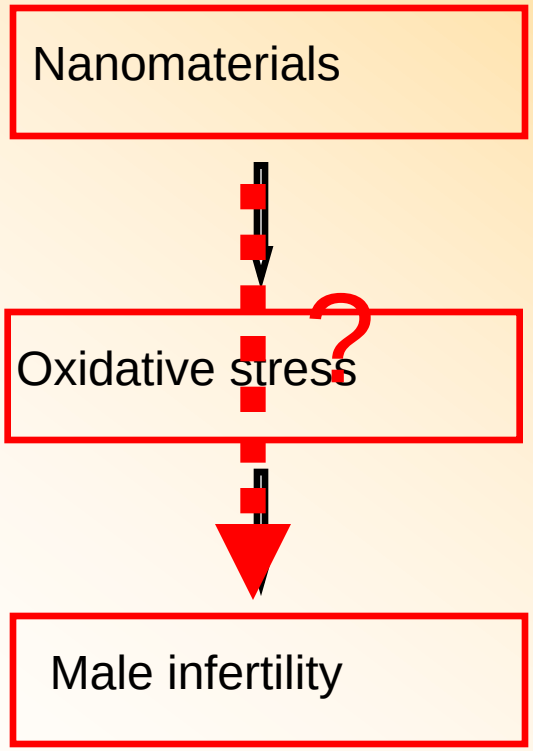


Sperm count keeps decreasing

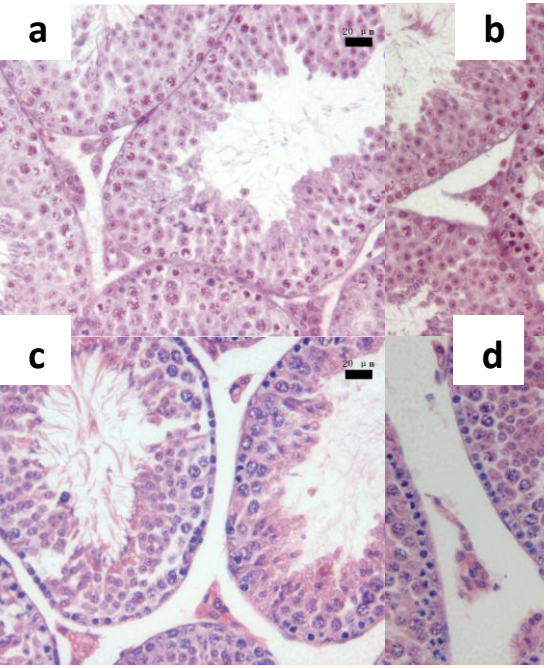


Carlsen E, Giwercman A, Keiding N, Skakkebæk NE. Evidence for decreasing quality of semen during past 50 years. Br Med J 305:609–613 (1992).

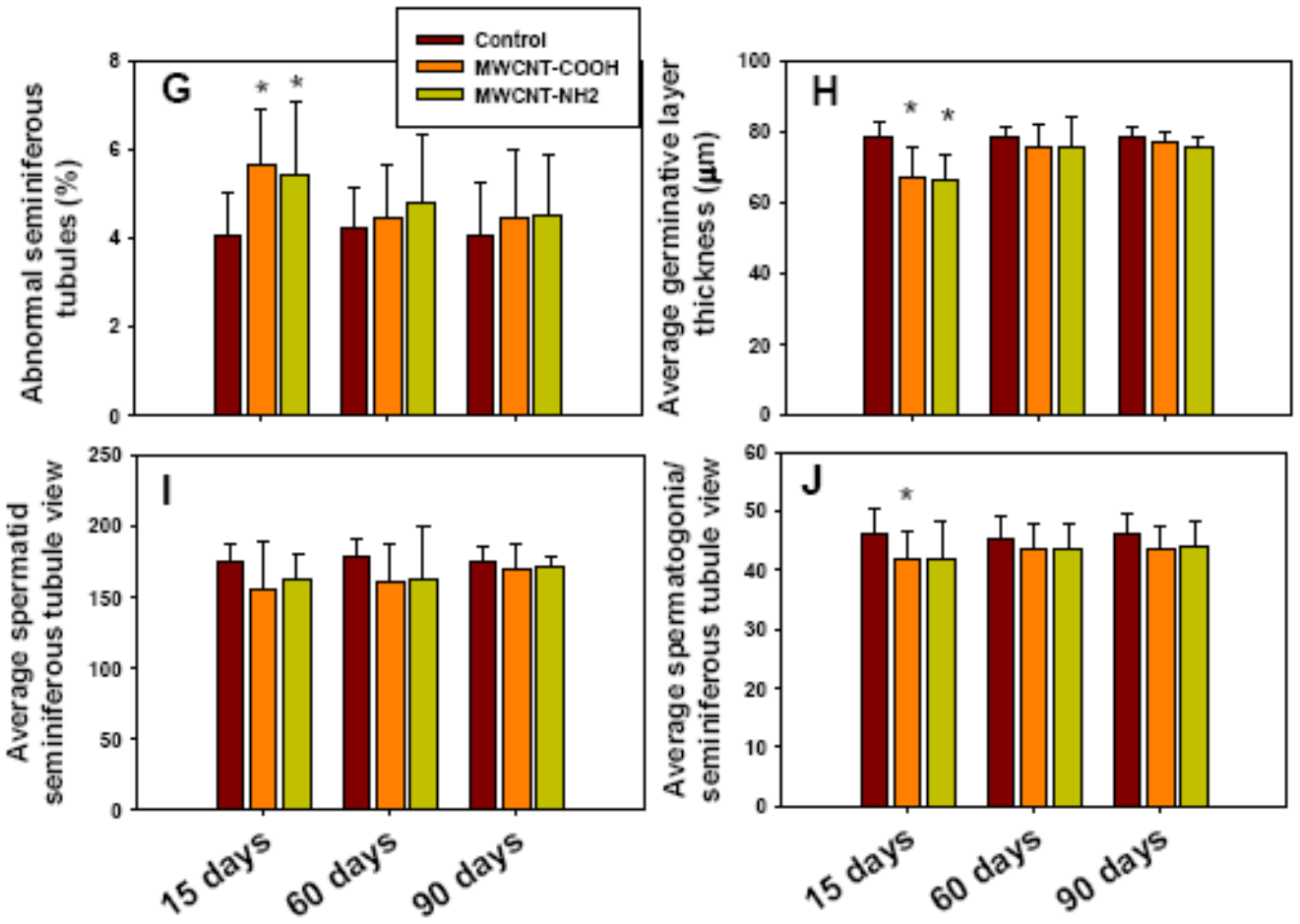
Oxidative stress, nanomaterials and male infertility



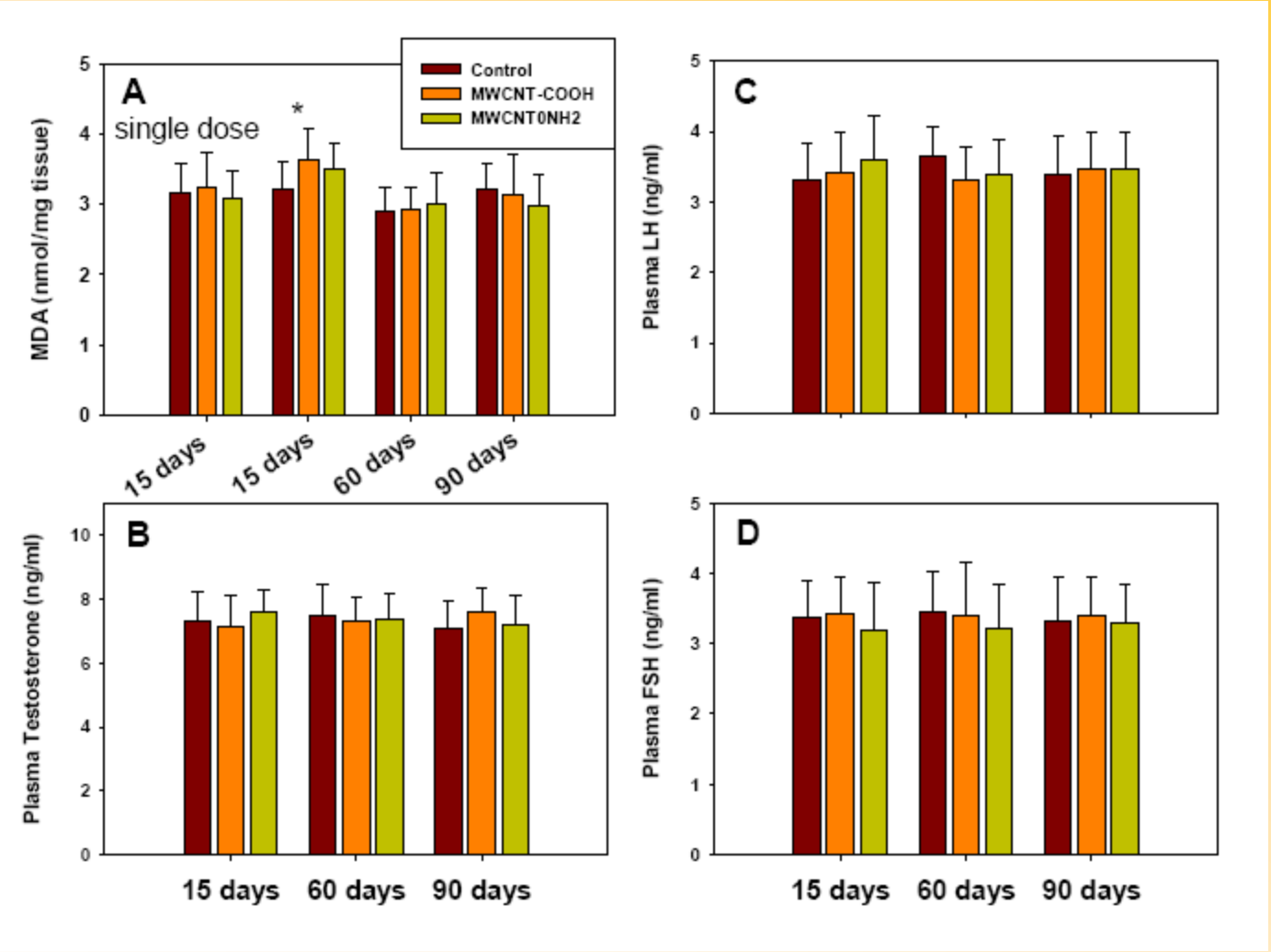
Carbon Nanotubes Cause Reversible Damage to Mouse Testis



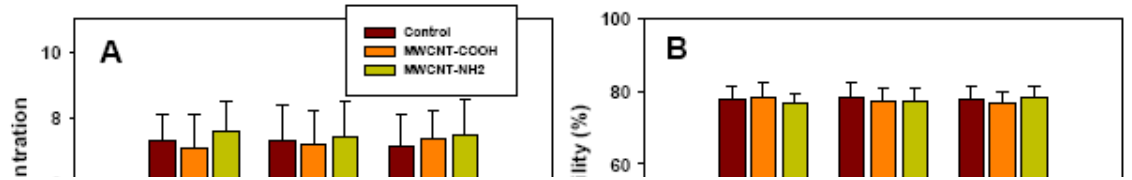
A: Control
B: 15days
C: 60days
D: 90days



Oxidative Stress and Sex Hormone Levels



Sperm Health are Unaffected



Acrosome integrity

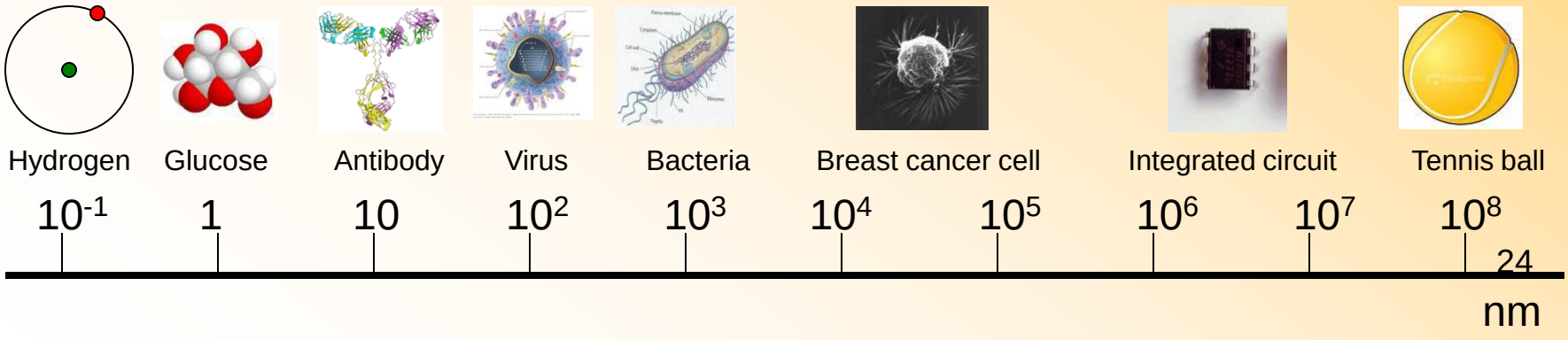
Male Fertility Mice 15- and 60-Day after Exposure (Exposure Scheme 2)

	Copulation Index(%)[a]	Fertility Index(%)[b]	Gestation Index (%)[c]	Average no. of live pups/pregnant female	Viability index PND4[d]
Control	100.0	91.6	100	7.4	95.6%
MWCNT-COOH-15 days	100.0	91.7	100	7.8	97.7%
MWCNT-NH2-15 days	100.0	100.0	100	7.5	100.0%
MWCNT-COOH-60 days	100.0	83.3	100	7.2	95.8%
MWCNT-NH2-60 days	100.0	91.7	100	6.8	97.3%

*Bai,Zhang,Zhang,Mu,Zhang,Butch,Snyder,Yan**, ***Nature Nanotechnology***, 2010, Advanced online publication, August 7.

Outline

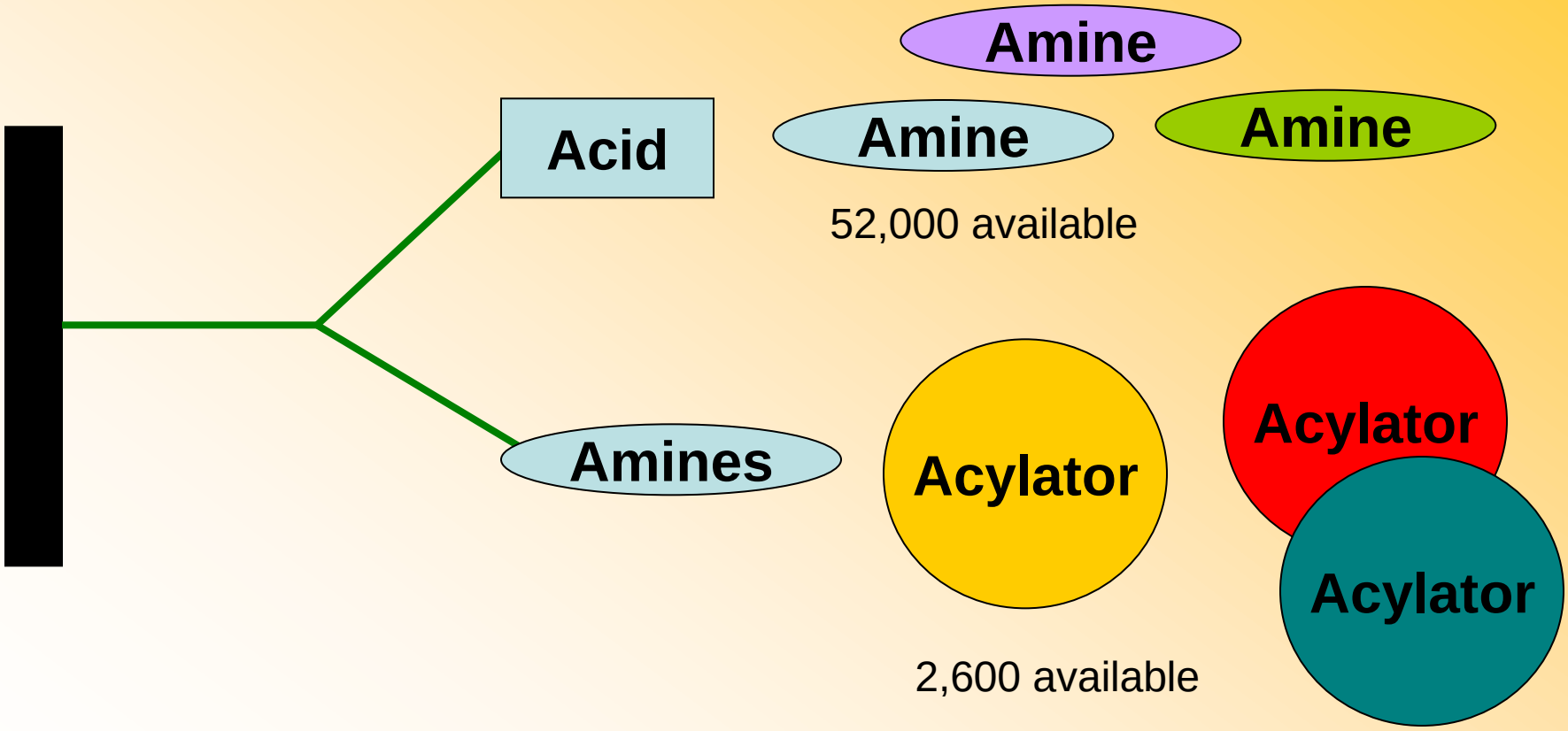
- Nanotoxicity
- **Reduce toxicity**



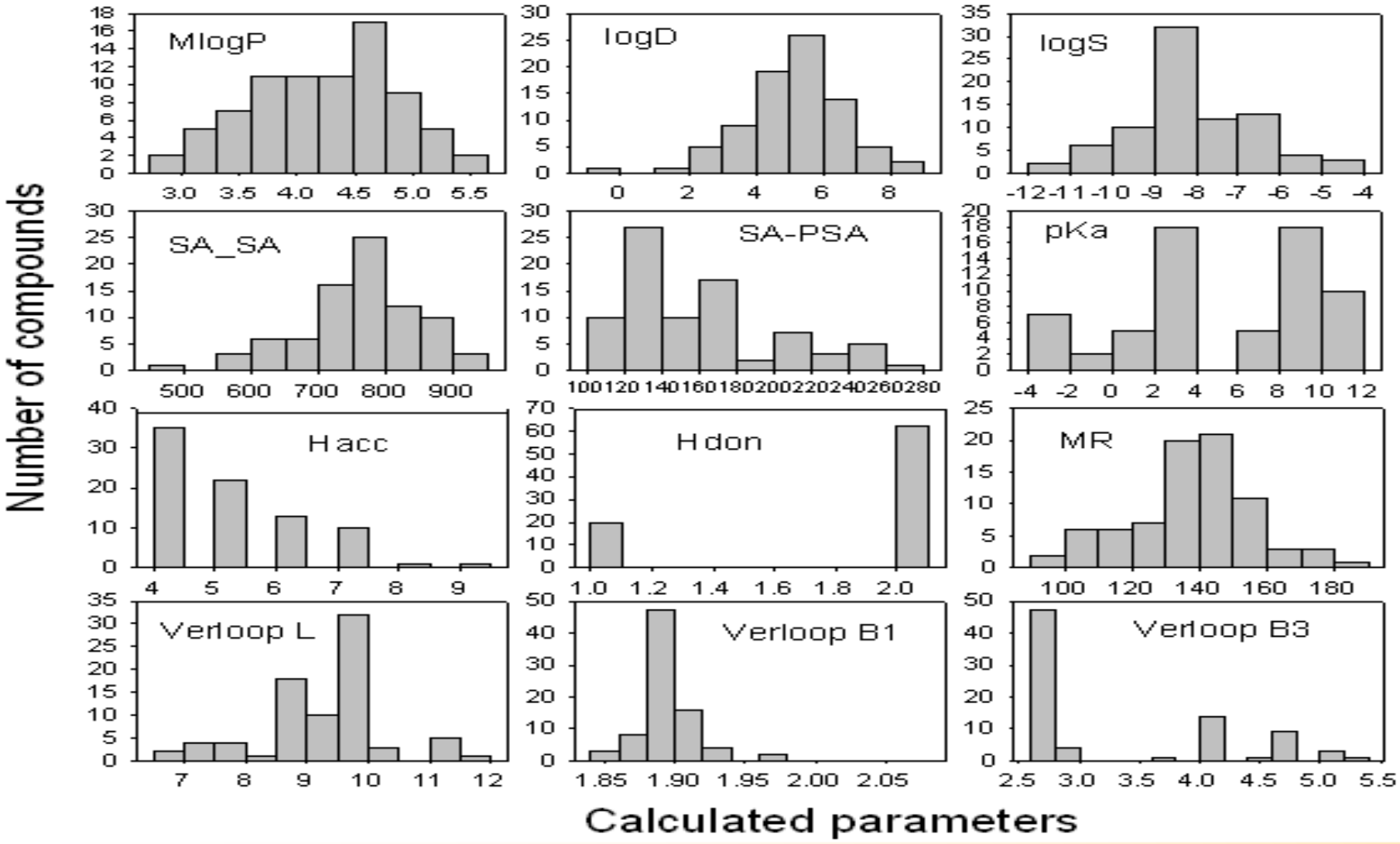
Rationales

- Nanoparticles have extremely high surface area. By modifying their surface chemistry, we may modulate their biological interactions.
- Compared with the linear method, nano-combinatorial library method (mapping chemical space) may be a much more effective approach

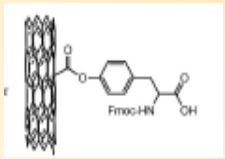
CNT Library Design



Selection of Building Blocks by Computation



Diverse Carbon Nanotube Library

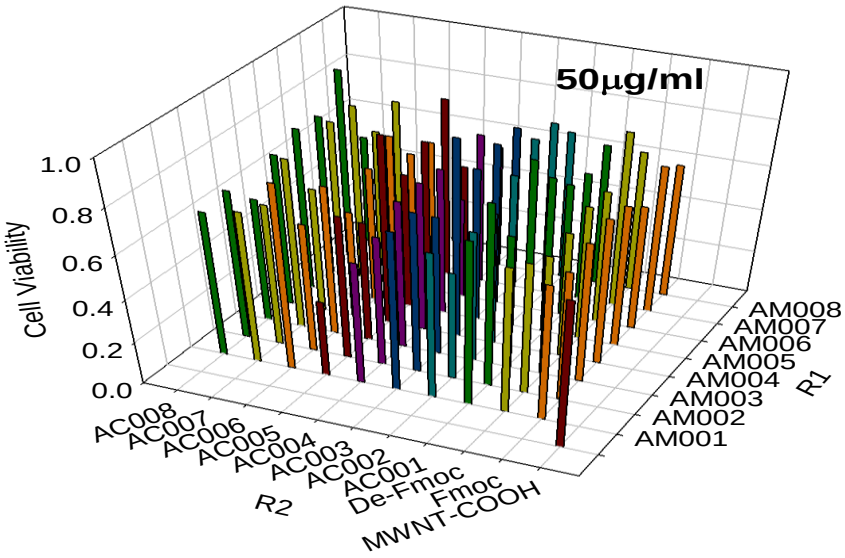


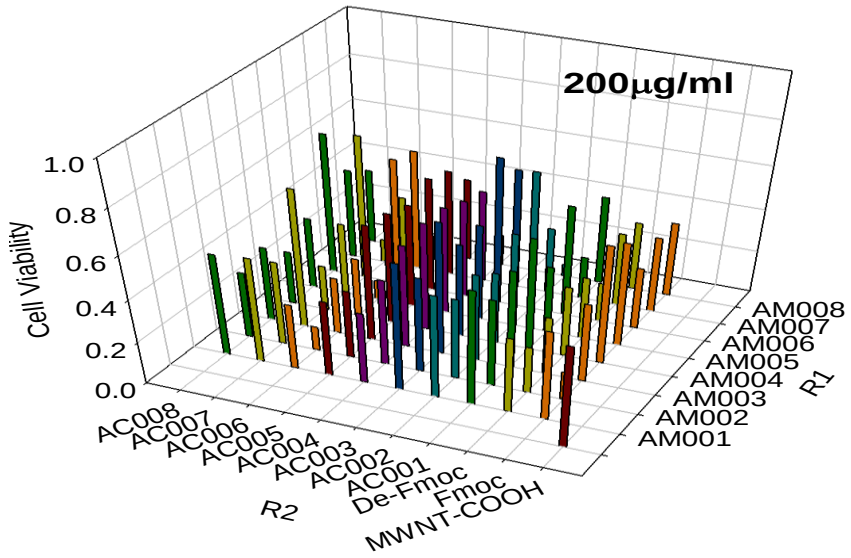
R1

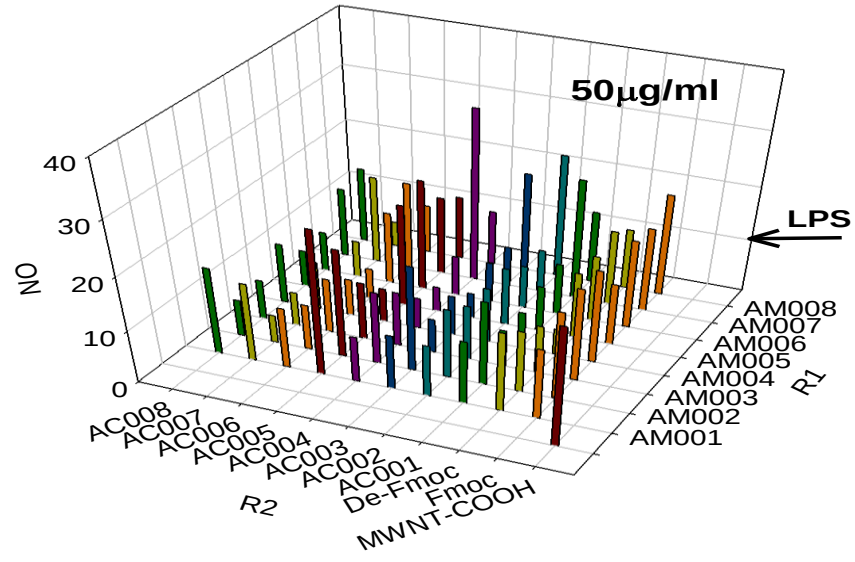
R2

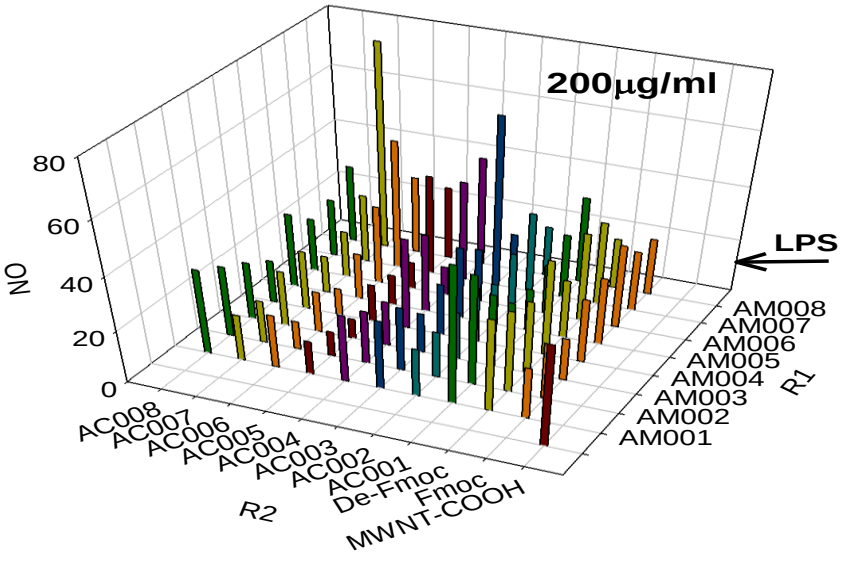
	AM001	AM002	AM003	AM004	AM005	AM006	AM007
Fmoc	5	6	7	8	9	10	11
De-Fmoc	13	14	15	16	17	18	19
AC001	21	29	37	45	53	61	69
AC002	22	30	38	46	54	62	70
AC003	23	31	39	47	55	63	71
AC004	24	32	**40**	**48**	56	**64**	72
AC005	**25**	**33**	**41**	**49**	**57**	**65**	**73**
AC006	26	**34**	**42**	**50**	58	66	74
AC007	27	35	43	51	59	67	75
AC008	**28**	**36**	**44**	**52**	**60**	**68**	76

Reduce Cytotoxicity and Immune Responses

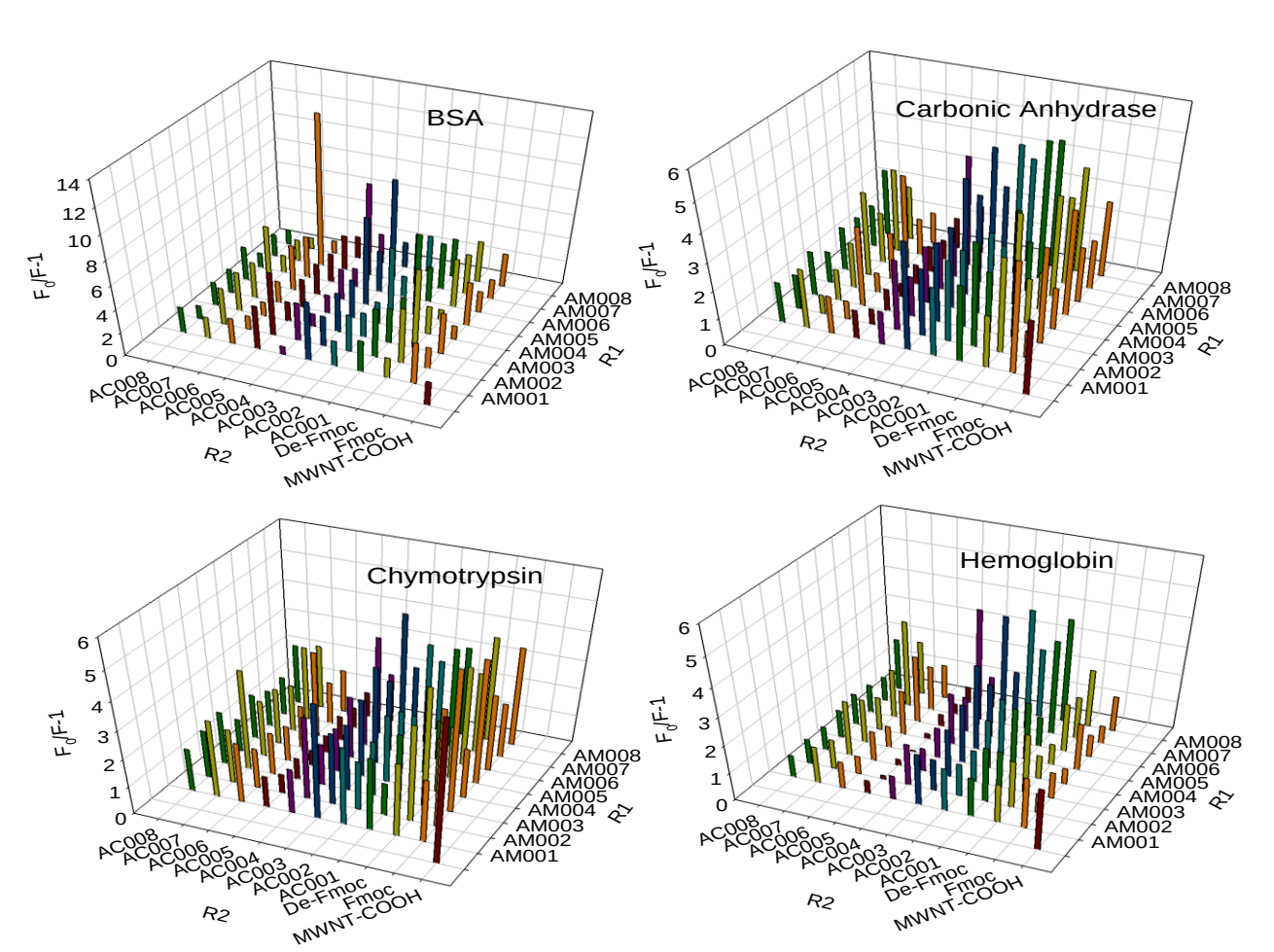


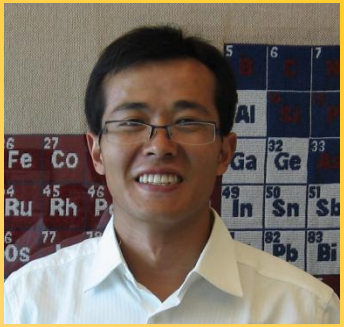






Surface Ligand Modulates Protein Binding





Dr. Hongyu Zhou

Zhou, Mu, Gao, Liu, Xing, Gao, Zhang, Qu, Chen, Liu, Zhang, Yan.* ***Nano Lett*** *8(3), 859-865, 2008*.

Part II. Molecular Modeling

Dataset Overview

- *Structures of MWNT*

  NPs bearing the same core (carbon nanotube) but with different surface modifiers: 1 pristine NP, 3 intermediates and 80 modified NPs were tested

- *Endpoints*

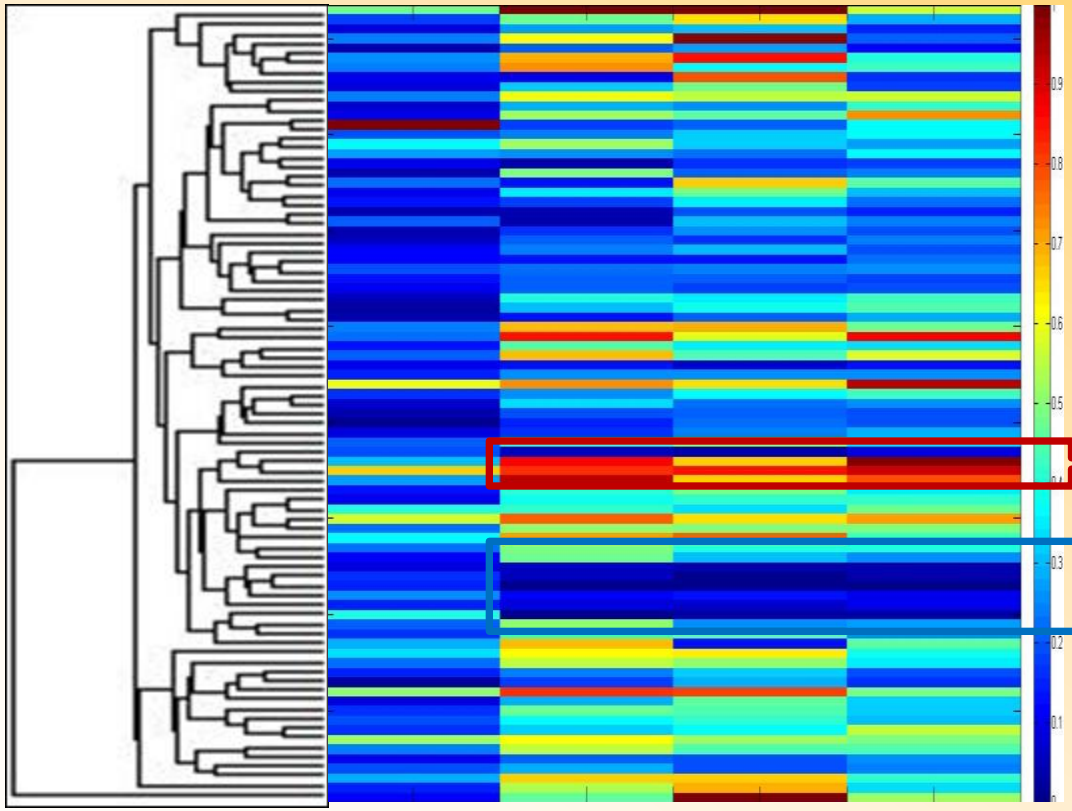
1)Protein Bindings (F_0/F_1, tested in 15 and 7.5 ug/mL)

2)Acute toxicity (survival percentage, tested in 200 and 50 ug/mL)

3)Immune Toxicity (secretion of NO, tested in 200 and 50 ug/mL)

SAR study: Non-Supervised Hierarchical Clustering Analysis

83 compounds represented by Dragon descriptors

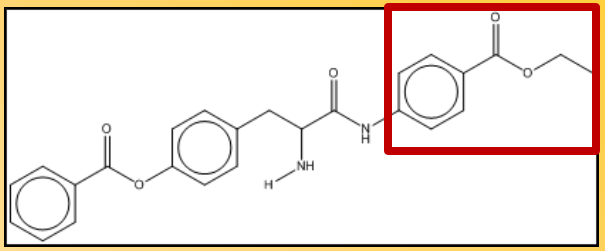


BSA_binding

CA_binding

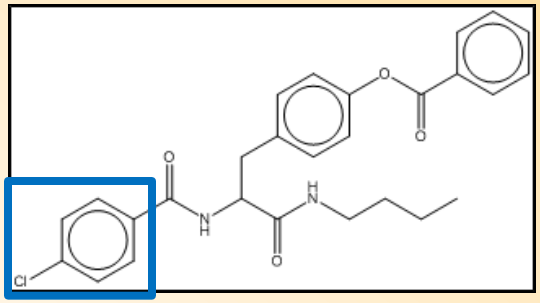
CT_binding

HB_binding

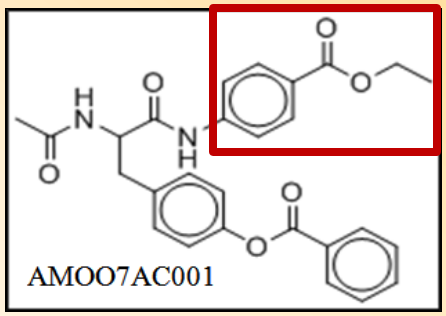


Cluster 1: strong protein binders

Cluster 2: weak protein binders

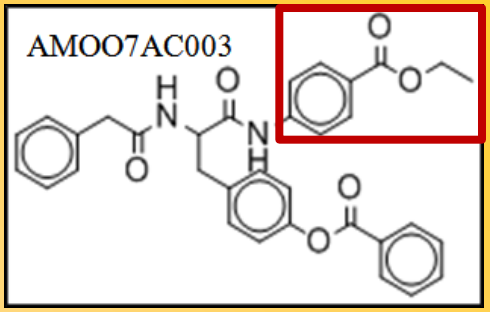


Cluster1

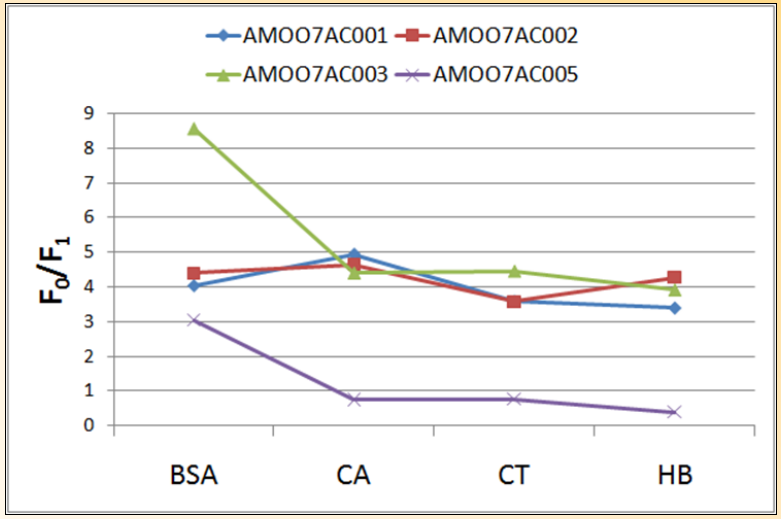


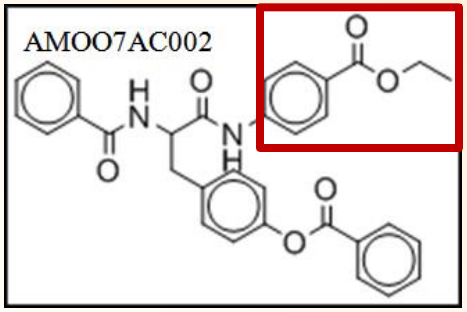
BSA Binding = 4.03
CA Binding = 4.93
CT Binding = 3.59
HB Binding = 3.39

Similar surface modifiers have similar biological/toxicological effects

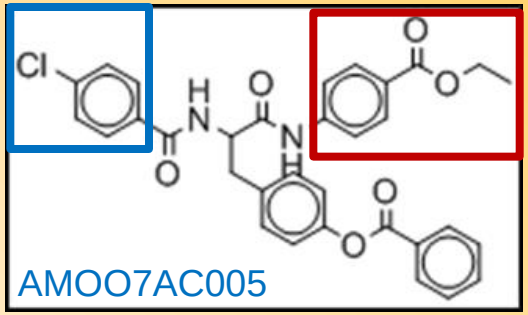


BSA Binding = 8.56
CA Binding = 4.41
CT Binding = 4.46
HB Binding = 3.92





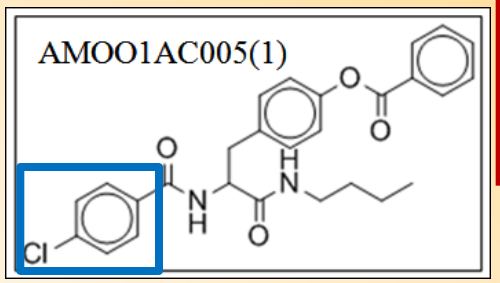
BSA Binding = 4.40
CA Binding = 4.65
CT Binding = 3.58
HB Binding = 4.28



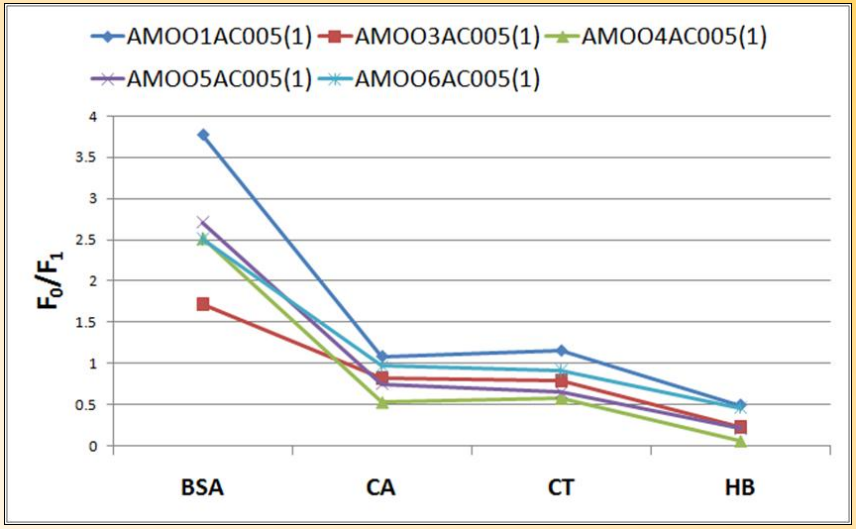
BSA Binding = 3.05
CA Binding = 0.75
CT Binding = 0.77
HB Binding = 0.40

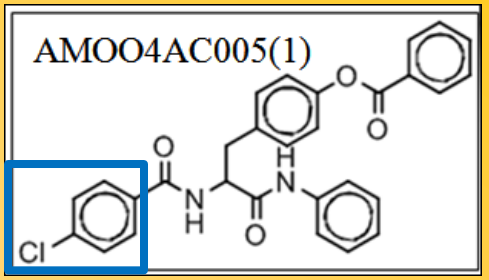
Cluster2

Similar surface modifiers have similar biological/toxicological effects

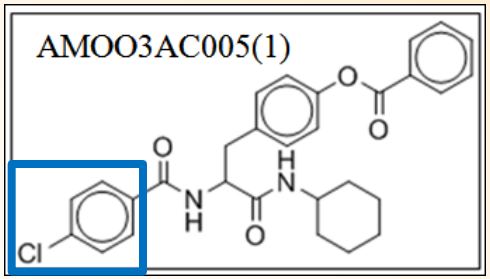


BSA Binding = 3.77
CA Binding = 1.08
CT Binding = 1.15
HB Binding = 0.49

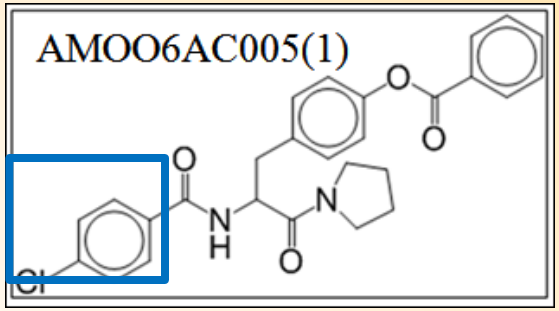




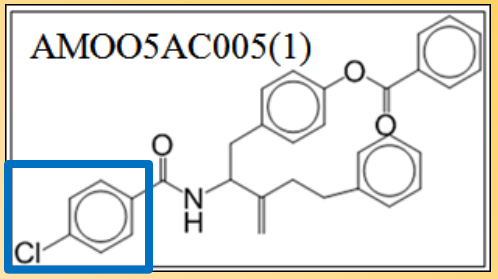
BSA Binding = 2.52
CA Binding = 0.53
CT Binding = 0.58
HB Binding = 0.06



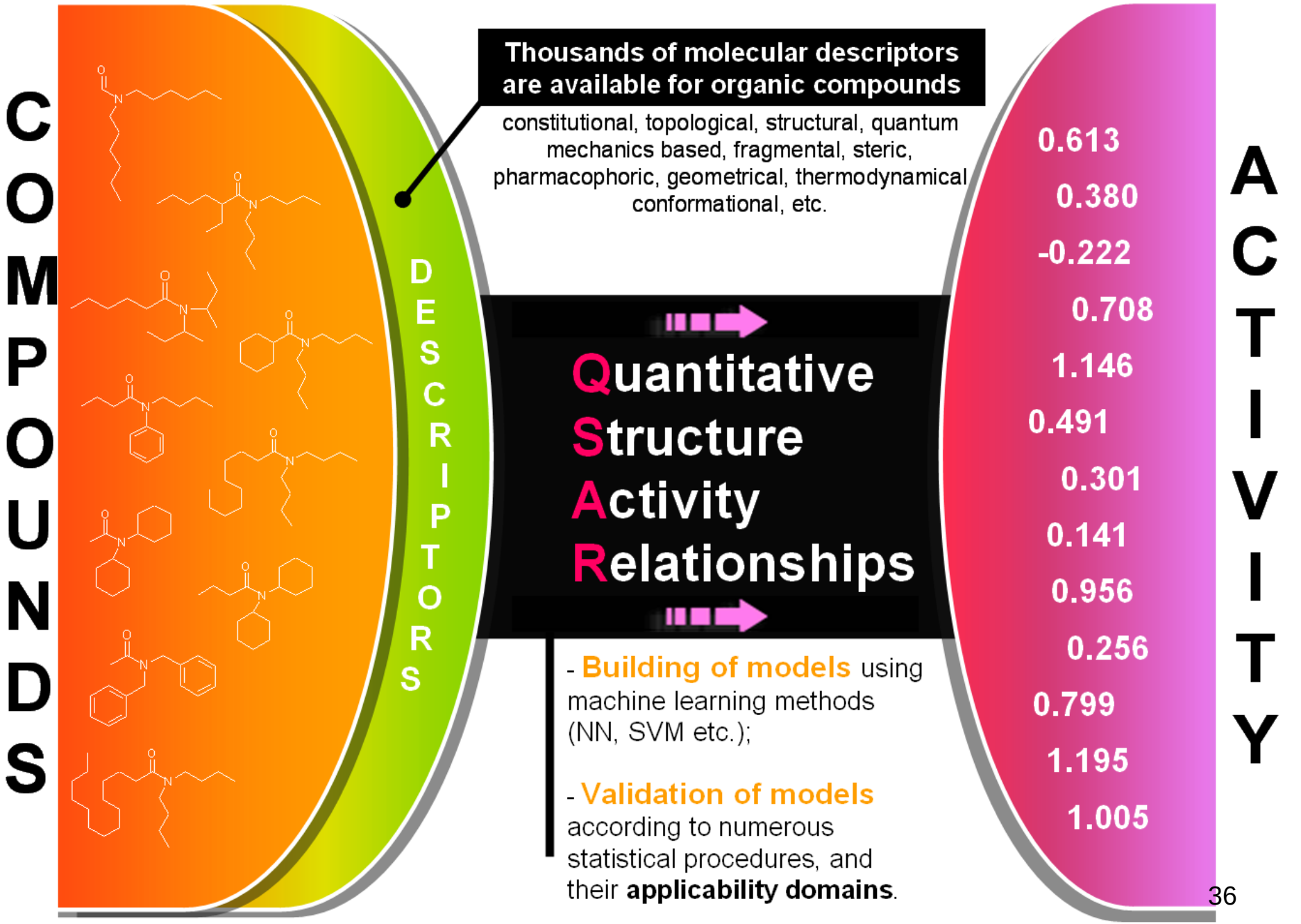
BSA Binding = 1.72
CA Binding = 0.82
CT Binding = 0.79
HB Binding = 0.23

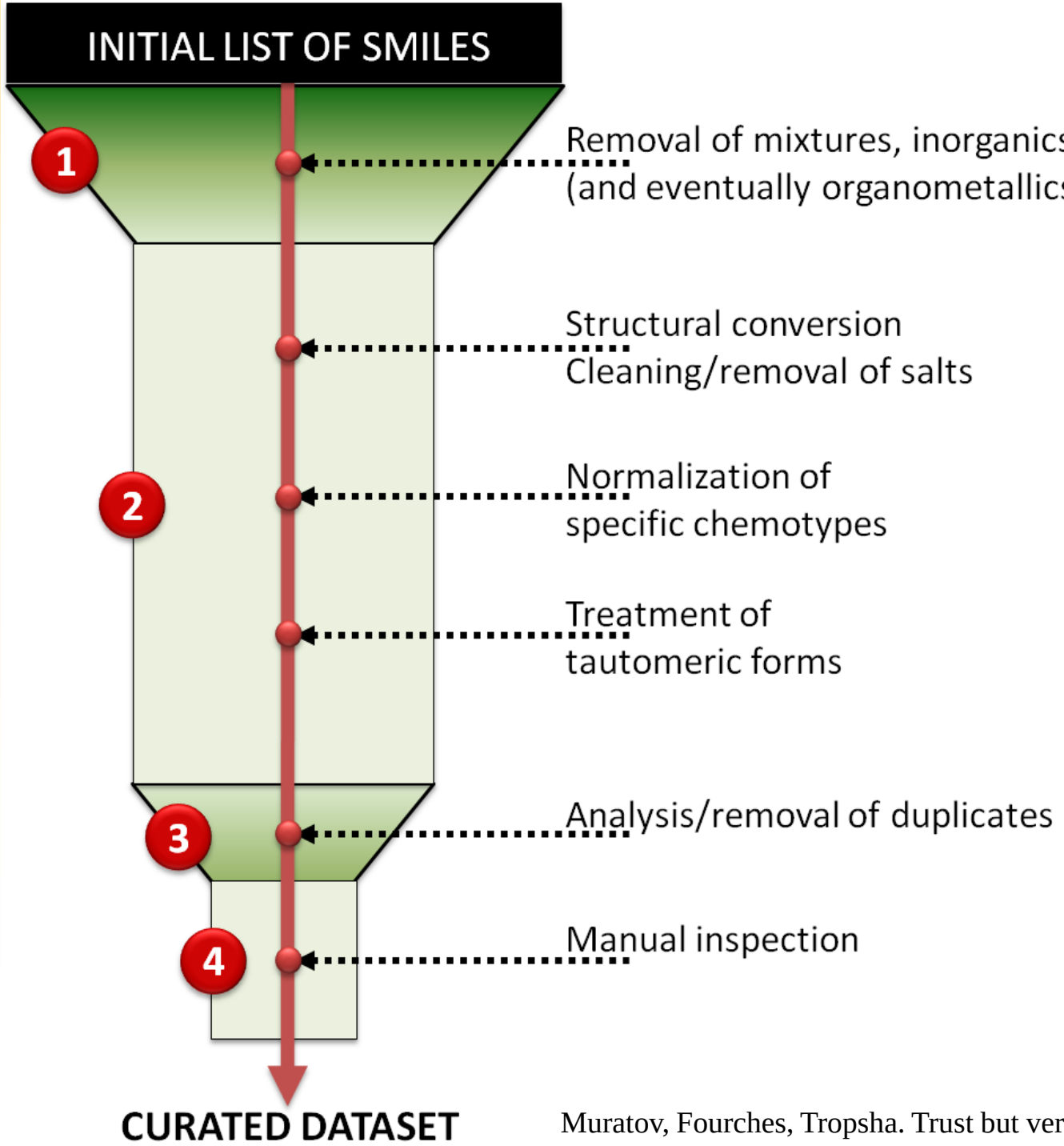


BSA Binding = 2.51
CA Binding = 0.97
CT Binding = 0.91
HB Binding = 0.46



BSA Binding = 2.71
CA Binding = 0.75
CT Binding = 0.65
HB Binding = 0.21


COMPOUNDS
DESCRIPTORS
Thousands of molecular descriptors are available for organic compounds
constitutional, topological, structural, quantum mechanics based, fragmental, steric, pharmacophoric, geometrical, thermodynamical conformational, etc.
Quantitative Structure Activity Relationships
- Building of models using machine learning methods (NN, SVM etc.);
- Validation of models according to numerous statistical procedures, and their applicability domains.
0.613
0.380
-0.222
0.708
1.146
0.491
0.301
0.141
0.956
0.256
0.799
1.195
1.005
ACTIVITY



SOFTWARE

ChemAxon - Standardizer
OpenEye - Filter

ChemAxon - Standardizer
OpenBabel
Molecular Networks - CHECK,TAUTOMER

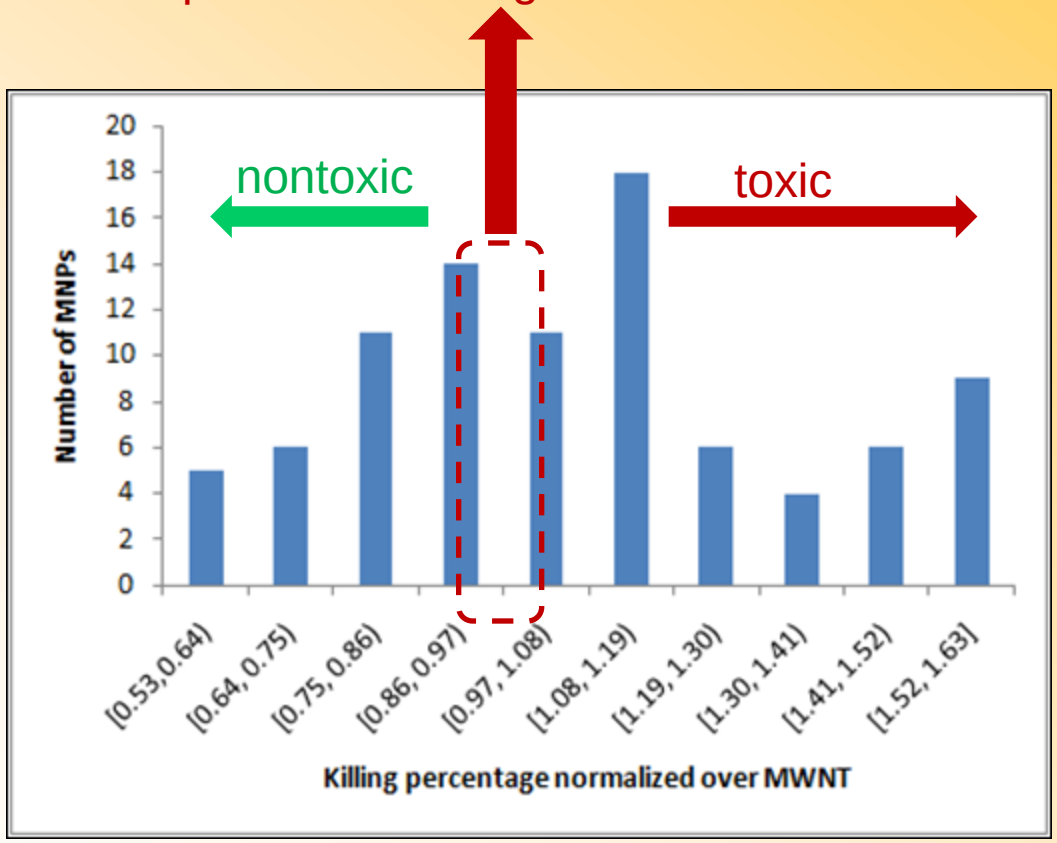
ISIDA - Duplicates
HiT QSAR
CCG - MOE

ISIDA - EdiSDF
Hyleos - ChemFileBrowser
OpenBabel
ChemAxon - MarwinView

Muratov, Fourches, Tropsha. Trust but verify. JCIM, 2010, 29, 476 – 488.

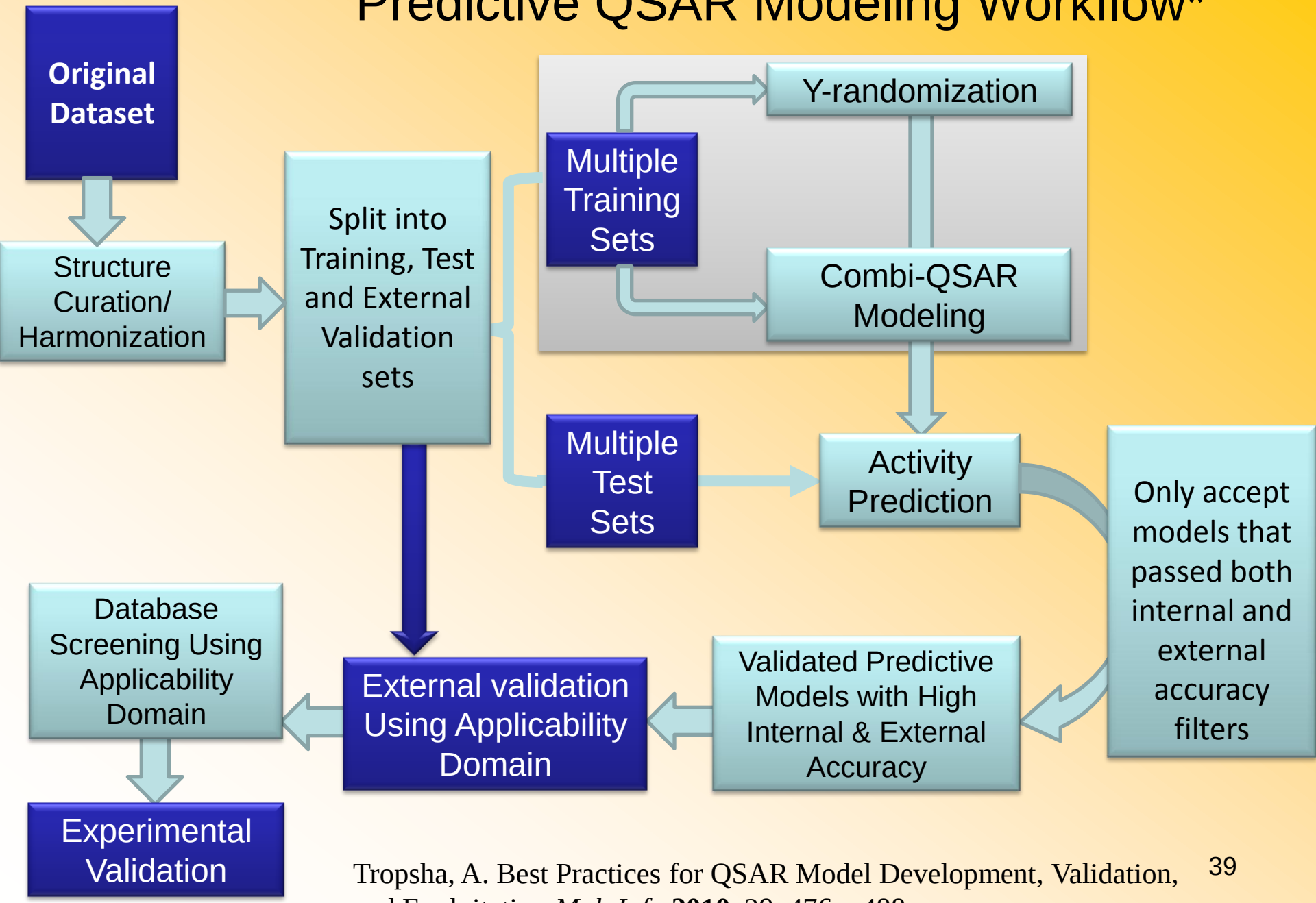
Data Pre-treatment

Carbon nanotubes with killing percentage between 0.95 and 1.05 were removed prior to modeling



38 toxic and 35 non-toxic nanoparticles were used to build models

Predictive QSAR Modeling Workflow*



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

Combinatorial QSAR Models

5-Fold External Cross-Validation

		kNN-Dragon	SVM-Dragon	RF-Dragon	kNN-MOE	SVM-MOE	RF-MOE
Fold0	Sens.(%)	88	88	88	88	88	88
	Spec.(%)	50	67	50	50	50	50
	CCR(%)	69	78	69	69	69	69
Fold1	Sens.(%)	80	80	100	80	80	80
	Spec.(%)	56	67	56	44	44	56
	CCR(%)	68	74	78	62	62	68
Fold2	Sens.(%)	71	71	71	57	57	57
	Spec.(%)	71	71	86	71	57	71
	CCR(%)	71	71	79	64	57	64
Fold3	Sens.(%)	78	89	100	78	67	60
	Spec.(%)	80	80	80	80	80	67
	CCR(%)	79	85	90	79	74	64
Fold4	Sens.(%)	44	67	44	44	33	33
	Spec.(%)	88	88	88	75	75	75
	CCR(%)	66	78	66	60	54	54
Cumulative	Sens.(%)	71	79	79	68	63	63
	Spec.(%)	69	74	69	63	63	63
	CCR(%)	**70**	**77**	**74**	66	63	63

Sens.: Sensitivity; Spec.: Specificity; CCR: Correct Classification Rate.

Summary

❖Carbon nanotubes are bio-active entities. They can potentially activate cellular signaling pathways and cause in vivo toxicity.

❖ Surface nano-combinatorial chemistry modifications on NPs can effectively modulate its bio-activities to evade interactions with proteins or cells.

❖QSAR modeling has been used to rationalize data. Models (with external predictive accuracy as high as 77%) will be used to guide the design of functional and non-toxic carbon nanotubes.