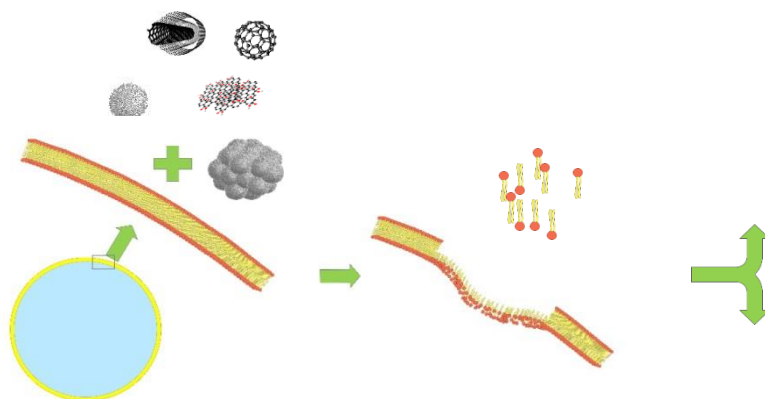




International workshop on COMPUTATIONAL APPROACHES IN NANOSCIENCES „COMPInNANO“

Ljubljana, 2nd and 3rd October 2015

PROCEEDINGS/ZBORNİK PREDSTAVITEV



	AOI	BIY	COY	DIY	EYI
Long Name	Unit	Unit	Unit	Unit	Unit
Comments	Unit	Unit	Unit	Unit	Unit
1	0.048	-0.54873	0.93747	-0.30347	2.19615
2	0.095	0.78827	0.89346	-0.79767	3.13791
3	0.143	-1.14852	0.89192	1.4577	3.50175
4	0.19	2.41315	2.19399	1.03939	4.30936
5	0.238	-0.47774	1.48505	0.63919	4.66539
6	0.285	1.48445	1.15392	2.4172	2.75555
7	0.333	2.62155	1.51524	0.27144	1.11283
8	0.381	2.91818	0.36998	0.62213	2.11882
9	0.429	2.23389	1.1475	0.38447	0.86254
10	0.476	0.43457	1.31501	0.57781	0.65301
11	0.48	0.33899	1.39373	0.84727	1.66427
12	0.95	0.80594	0.78254	1.48474	3.10573
13	1.43	11.85943	1.7668	12.30747	2.81408
14	1.9	6.03273	1.18687	18.48517	2.4661
15	2.38	9.8952	3.5019	25.87679	5.89309
16	2.86	14.10267	5.61671	27.47116	3.67934
17	3.33	23.78367	2.266	18.842	1.77772
18	3.81	18.91836	4.8302	24.45538	4.27885
19	4.29	26.15489	3.36011	23.1131	6.8224
20	4.76	32.45211	5.13681	28.07823	3.51794
21	4.8	22.24242	3.93505	24.45568	1.47834
22	5.5	31.87678	2.53889	38.09026	0.95953
23	16.3	41.88815	3.92339	40.3272	2.07346
24	19	44.29513	8.44832	42.33348	3.25218
25	23.8	43.87283	1.00772	35.18602	4.5489
26	28.8	55.25453	4.72205	48.30651	1.08656
27	33.3	57.37951	2.00502	44.95185	2.48287
28	38.1	55.95588	1.68113	52.41895	5.37739
29	42.8	55.10878	8.02342	49.7805	3.07655
30	47.6	55.05051	1.29428	47.76085	3.88444

International workshop/Mednarodni sestanek
COMPUTATIONAL APPROACHES IN NANOSCIENCES: „COMPInNANO“

Ljubljana, 2nd and 3rd October 2015

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INTERNATIONAL Workshop on Computational Approaches in Nanosciences (2015 ; Ljubljana)

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ISBN 978-961-6822-33-6 (pdf)

1. Jemec, Anita

283801856

Foreword

The production of nanomaterials has led to growing public and regulatory concern about their safety. The aim of a number of ongoing or past EU projects has been to address innovative, safe and sustainable nano-enabled products. The NanoValid project, as one of them, has developed a set of reliable reference methods and materials for their fabrication, physicochemical characterization, hazard identification and exposure assessment. However, since the experimental toxicological testing of nanomaterials is costly and time-consuming, it is necessary to develop a new approach based on knowledge, methods and tools to reach the goal of predictive nanotoxicology.

The workshop “Computational approaches in Nanosciences” hosted the participants from three large scale EU FP7 projects NanoValid, NanoMile, and Modern in order to disseminate the results on computational methods for NP hazard characterisation and exchange ideas with representatives from regulatory bodies and industry. Modellers, computational scientists, experimental (eco)toxicologists and experts from different fields of nanoscience discussed the future of *Computational Approaches in Nanosciences*.

The participants agreed that the most crucial points in the data sharing among academia, industry and regulatory bodies are (i) the organisation of data in large data sets and synchronised communication between different fields and sectors. Calibration and validation of computational models is impossible without utilizing high quality experimental data. Therefore, close collaboration between the computational chemists and experimentalists from different areas (i.e. toxicologists, specialists on characterization) would be crucial for the success.

The NanoValid project has significantly contributed toward defining the quality of nanotoxicity data and harmonization of test. In the future they have to be integrated into databases and shared. Namely, new approaches for the data gap filling are needed which have to be dynamic and consider scientific aspects and new developments in nano-sciences. The participants concluded that the future of nanosafety will rely on both, experimental data and computational methods but we have to adopt successful communication strategies.



Friday, 2nd October

16.15 – 16.30

Welcome speech & Introduction to the workshop
Prof. Dr. Damjana Drobne, Assist. Prof. Anita Jemec

TOPIC 1: ORGANISATION OF LARGE DATA SETS FOR MODELLING IN GENERAL

The topic will deal with the organisation of large data sets using different statistical approaches.

16.30 – 17.00

Assist. Prof. Ddr. David Bogataj, The Mediterranean Institute for Advanced Studies:
„Reproducible Research“

17.00 – 17.30

Assist. Prof. Dr. Cene Fišer, Biotechnical Faculty University of Ljubljana, Slovenia:
„Web-databases in systematics and taxonomy“

17.30 – 18.00

COFFEE BREAK

TOPIC 2: DATA SHARING-Academia, Industry and Regulatory bodies

The stakeholders will present their view on the nanoscience data sharing.

18.00 – 18.30

Mag. Vladimir Vrečko, Cinkarna Celje (TiO₂ producers), Slovenia:
„The importance of data interpretation and dissemination for the future of nanotechnology.“

18.30 – 19.00

Dr. Mojca Kos Durjava, National Laboratory of Health, Environment and Food
and **Mag. Karmen Krajnc**, Chemical Office of the Republic of Slovenia, Slovenia:
„Challenges of Regulation and Risk Assessment of Nanomaterials“

Saturday, 3rd October

TOPIC 3: QUANTITATIVE NANOSTRUCTURE-ACTIVITY (PROPERTY) RELATIONSHIPS (QNAR, QNPR) MODELLING

9.00 – 9.30

Prof. Dr. Marjan Vračko, National Institute of Chemistry, Slovenia:
„Chemometric analysis and QSAR modelling in NANO-toxicology“

9.30 – 10.00

Dr. Villem Aruoja, National Institute of Chemical Physics and Biophysics, Estonia:
„Toxicity of metal oxide nanoparticles to algae, bacteria and protozoa:
FP7 project MODERN“

10.00 – 10.30

COFFEE BREAK and poster session

TOPIC 4: MODELLING OF INTERACTIONS BETWEEN NANOMATERIALS AND BIOLOGICAL MODELS

Presentation of different databases aimed to collect information about Nanosafety related topics.

10.30 – 11.00

Prof. Dr. Alok Dhawan, CSIR-Indian Institute of Toxicology Research, Lucknow, India:
„Toxicity of Nanomaterials: The need for Novel Computational Tools and Approaches in Safety Assessment“

11.00 – 11.30

Dr. Lokesh Baweja, Institute of Life Sciences, Ahmedabad, Gujarat, India:
„Computational approaches to understand the interaction of nanomaterials with biomolecules“

11.30 – 12.00

Dr. Fabrice Carnal, Institut F.A. Forel, University of Geneva, Switzerland:
„Monte Carlo Modelling of Interaction Processes between Nanoparticles and Biomacromolecules of Variable Hydrophobicity“

12.00 – 12.30

Maja Sopotnik and Prof. Dr. Kristina Sepčič, Biotechnical Faculty, University of Ljubljana, Slovenia:
„Interaction of carbon-based nanomaterials with cholinesterases and serum proteins“

12.30 – 13.30

LUNCH BREAK and poster session

Saturday, 3rd October

TOPIC 5: ORGANISING DATA FOR RISK ASSESSMENT/DATA QUALITY

13.30 – 14.00	Prof. Dr. Rishi Shanker , Institute of Life Sciences, Ahmedabad, Gujarat, India: „Tracking Nano-footprints in microbial food chain: Observations, Data & Challenges“
14.00 – 14.30	Dr. Ashutosh Kumar , Institute of Life Sciences, Ahmedabad, Gujarat, India: „Importance of <i>in silico</i> approaches in understanding the interactions of nanoparticles with biological membrane“
14.30 – 15.00	Assist. Prof. Dr. Anita Jemec , Biotechnical Faculty, University of Ljubljana, Slovenia: „Quality of nanotoxicity data and the importance of harmonization“
15.00 – 16.00	COFFEE BREAK, poster session and open discussion

REPRODUCIBLE RESEARCH

ddr. David Bogataj

MEDIFAS

Reproducible research

Reproducible research is the idea that data analyses, and more generally, scientific claims, are published with their data and software code so that others may verify the findings and build upon them.

The goal of reproducible research is to tie specific instructions to data analysis and experimental data so that scholarship can be recreated, better understood and verified.

In the instructions the complex of conditions should be described precisely.

Replication

The ultimate standard for strengthening scientific evidence is replication of findings and conducting studies with independent:

- Investigators
- Data
- Analytical methods
- Instruments

Replication

- Replication is particularly important in studies that can impact broad policy or regulation decisions

What is Reproducible research?

- Scientific Question
- Research Protocol
- Nature (complex of conditions, entities and their relations)
- **1. Measured data (dataset regarding realization of an experiment – raw data)**
 - Data processing code
- **2. Analytic data (cleansed data)**
 - Analytical code
- **Computational results**
 - Presentation code (figures, tables, Numerical Summaries)
- Published article (text)

Up to now in published articles only short overview of data and code (1 and 2) is presented which is not enough.

What problem does Reproducibility solve

- Black Box Problem
- Transparency
- Data Availability
- Software/Methods Availability
- Improved Transfer of Knowledge

Reproducibility

- The premise of reproducible research is that with data/code available, researchers can check each other and the whole system is self-correcting
- Addresses the most “downstream” aspect of the research process – post-publication
- Assumes everyone plays by the same rules and wants to achieve the same goals (i.e scientific discovery)

Who Reproduces Research?

For reproducibility to be effective as means to check validity, someone needs to do something

- Re-run analysis; check if results match
- Check the code for bugs/errors
- Try alternate approaches/check sensitivity

Validation of the data analysis

Availability:

- Description of complex of conditions in which the experiment run
- Data
- Algorithms

Reasons:

- Other people can run the same algorithms on the sama data and can come to the same conclusions as the researcher

What you do not have

- Independent data
- Independent method

Impact of new technologies

- Allow us to collect data at much higher throughput
- We can get very complex high dimensional datasets almost instantaneously
- Computing power that allows us to merge databases in even bigger „megadatabases“

Minimum standards

- Experiment(observation) should be described in exactly known complex of conditions.
- If the complex of conditions is changed (environment), realization of the experiment can give different results.
- It could lead to different conclusions.

Problems

- Authors must undertake considerable effort to put data/results on the web
- Readers must download data/results individually and piece together which data go with which code section, etc
- Authors/readers must manually interact with websites
- There is no single document to integrate data analysis with textual representations; data, code, and text are not linked

Simplify the process

Put the data and the code together in the same document

- People can execute code in the right order
- Data are read at the right times

Document integrates data analysis with all the textual representations (descriptions) that everything is linked together

How do I Make My Work Reproducible

- Decide to do it (ideally from the start)
- Keep track of things, perhaps with versioning control system to track snapshots/changes
- Use statistical software whose operation can be coded (R)
- Don't save output (store raw dataset)
- Save raw data and the process that get you there
- Save data in non-proprietary formats (ASCII)

Single research report document

- To document the analysis and
- to have the code of the analysis in the same document

Documentation of total observation and research process

- Documentation preparation system – description of research process including metadata (LaTeX)
- Description of complex of conditions in which the experiment run
- Raw data - dataset
- Code – Programing Language (R) for data analysis, statistics, forecasting, optimization, sensitivity analysis and simulations.

Literate Statistical Programming with knitr (R)

- Text and code all in one place, logical order
- Data where results of observations are automatically updated that reflect external changes
- Code is live – you need to run code. When error appears it needs to be resolved.

CONCLUSION

1.- Reproducibility brings transparency (wrt code+data) and increases transfer of knowledge. Therefore complex of conditions which describe the environment for observation procedures have to be very clearly described.

2.- Important current discussion is about how to convince researchers to share data. The owners of data should have incentives to publish the dataset, metadata and code

- by the system similar as IF evaluation (citation index) of their publications and

- market system like available by ScienceDirect, Springer and others where the databases are able to buy or sell if there is not obligatory open system.

- The founder of a research should clearly determine which data should be publicly available and which are available on the data market - for sale.

Web-databases in systematics and taxonomy

Cene Fišer

SubBio Lab

Odd. za Biologijo

Biotehniška Fakulteta

Univerza v Ljubljani

Univerza v Ljubljani



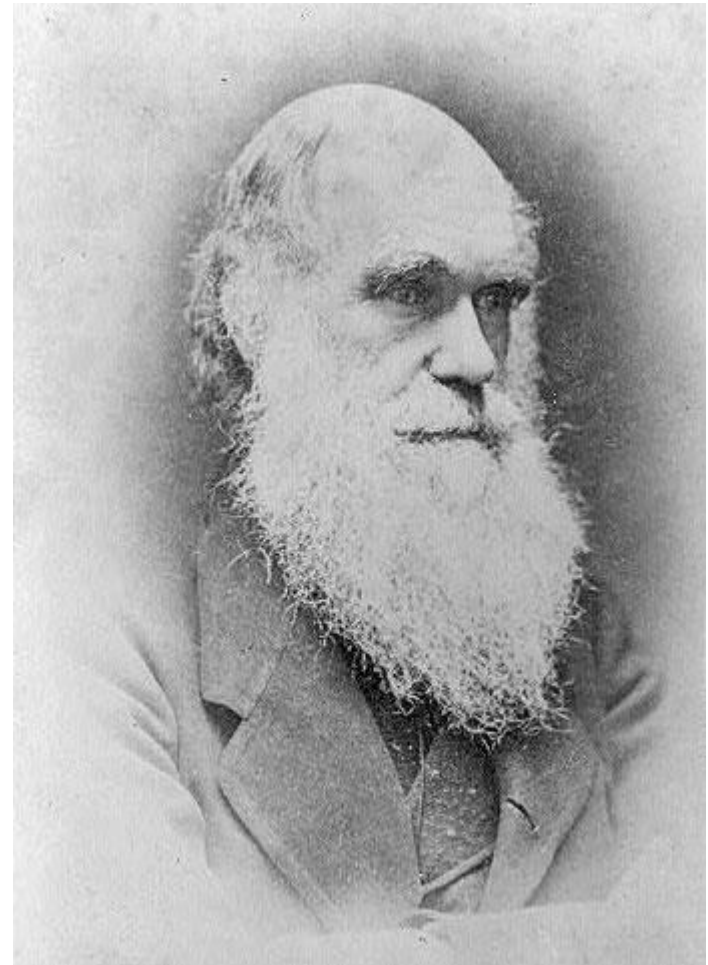
Ljubljana, 2.10.2015

taxonomy-systematics?

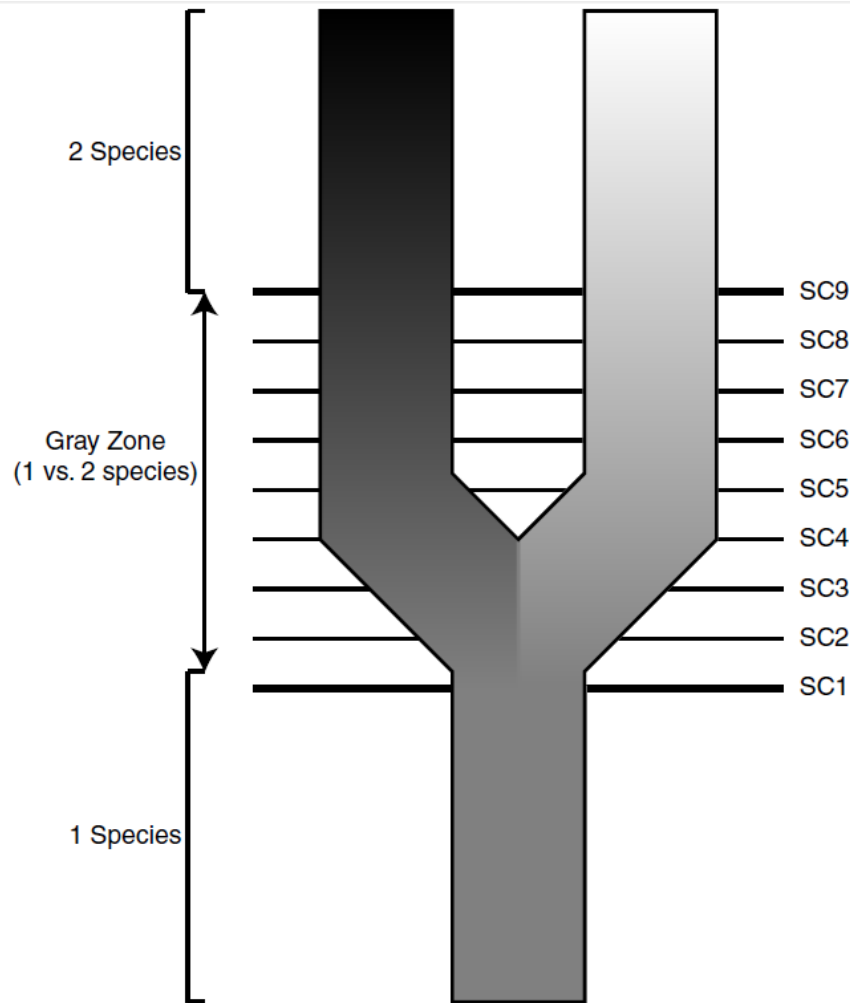
1. Taxonomy in a narrow sense: describing and naming species.
2. Taxonomy in a broad sense: species identification, research of species biology, distribution and conservation.
3. Systematics: inference of species relatedness and hierarchical categorization of species in higher taxonomic categories.



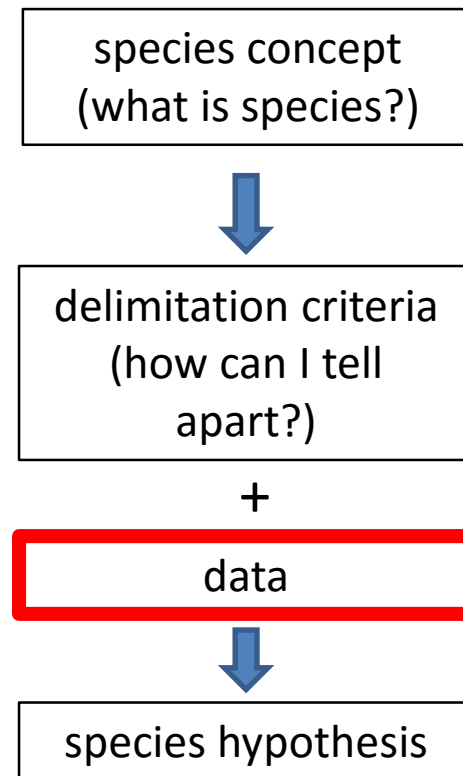
an ancient
science?



Modern synthesis: grounded in evolutionary theory....

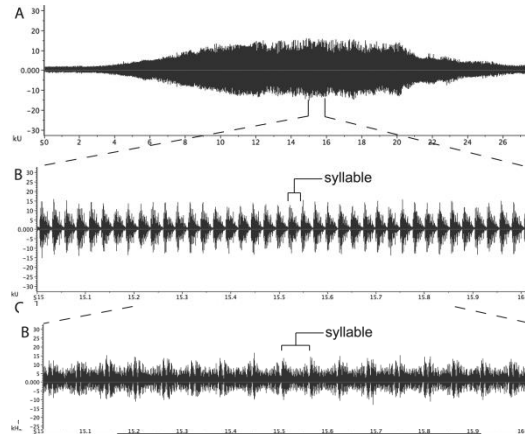


Speciation: a process of divergence



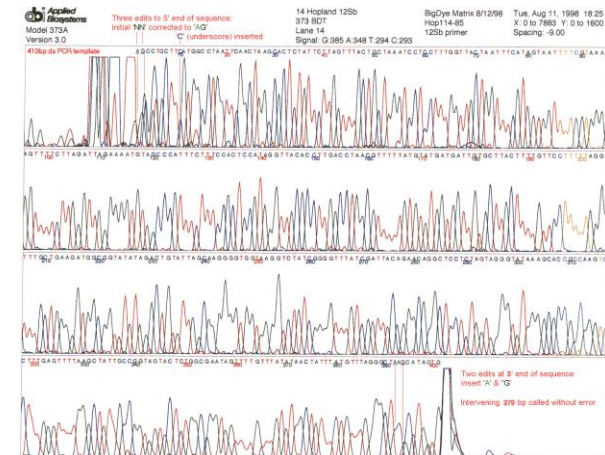
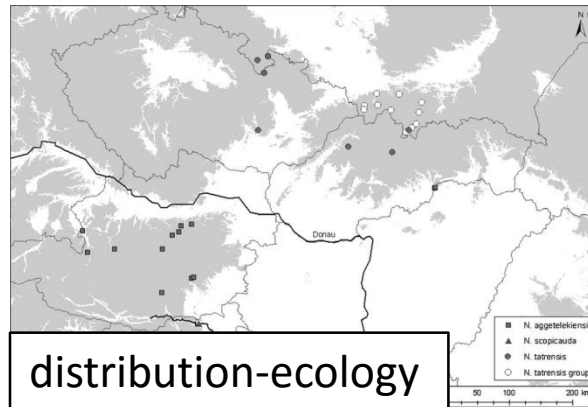
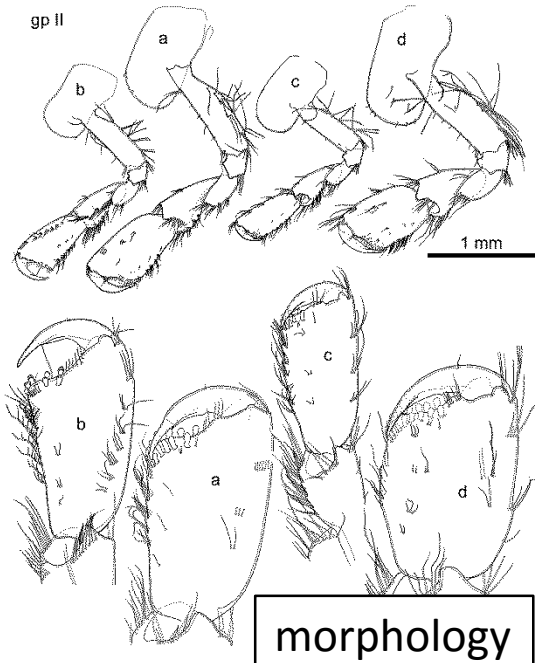
De Queiroz, Kevin (2007) 'Species Concepts and Species Delimitation', *Systematic Biology*, 56:6, 879 - 886

....interdisciplinary science



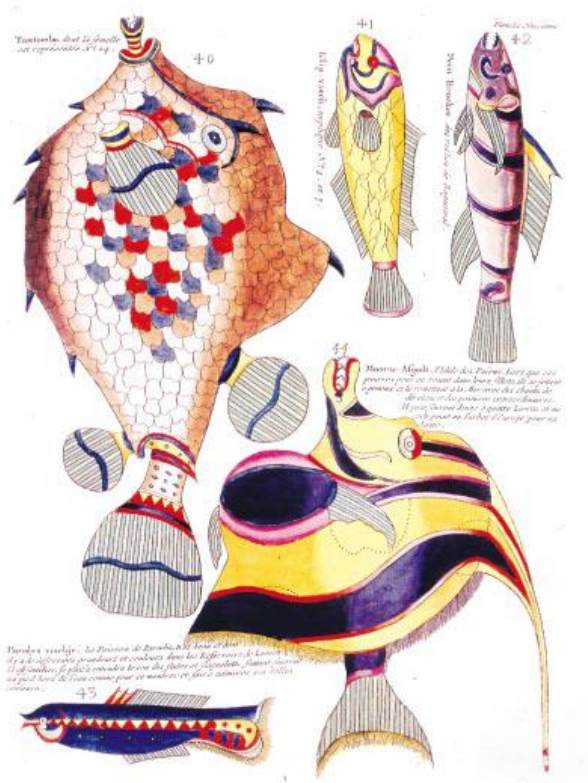
TYPE: NO LIMITS

LIMITS IN DATA
DEPOSITION AND
RETRIVAL



Challenges for taxonomy

The discipline will have to reinvent itself if it is to survive and flourish.



This discipline is made for the web: it is information-rich and often requires copious illustrations.

Godfray, H.C.J. 2002 **Challenges for taxonomy**. *Nature* 417, 17-19 (Commentary; reply to correspondence arising: “Towards taxonomy’s ‘glorious revolution’”

Taxon specialized websites: not necessarily databases

Make your own website:
Scratchpads:

<http://scratchpads.eu/>

The screenshot shows the Scratchpads biodiversity online homepage. The header includes the logo, navigation links (Home, News, Explore, Support, Develop, About us), and search options. A sidebar on the left lists various resources and a 'How to cite' link. The main content area features a 'Publish your data online' section with a 'PUBLISH' button and a description of the platform's purpose. Below this is an 'Overview of features' table.

Feature	Description
Share your data across communities	Scratchpad lets you connect with your community. ✓
Search engine friendly	All Scratchpads sites are search engine friendly. Scratchpads sites usually rank on top in Google search results when relevant taxa and keywords are used. ✓
Publish your content online	Scratchpads lets you create your content online. ✓
Publish in peer reviewed Journals	Submit original data present in your scratchpad directly to peer-reviewed journals for publication. ✓

This screenshot shows an alternative layout of the Scratchpads biodiversity online homepage. It features a 'Why choose Scratchpads?' section with four columns of benefits. Below this is a 'Join the community!' section with links to create, join, register, try, become an ambassador, develop source code, and read policies. A 'Case studies' section highlights the Distributed European School of Taxonomy (DEST). A 'Feeds' section includes links to News and Twitter. A 'Scratchpads around the world' section shows the global distribution of users and ambassadors. The footer includes logos for partner organizations and a detailed navigation menu.

Why choose Scratchpads?

- Create your own site
- Publish your data online
- Contribute to biodiversity knowledge
- Collaborate with your peers

Join the community!

- Create a Scratchpad
- Join a Scratchpad
- Register for training
- Try our sandbox
- Become a Scratchpads ambassador
- Develop Scratchpads source code
- Read about our policies

Case studies

Feeds

- News
- Twitter

Scratchpads around the world

0 Scratchpads by 0 active users covering 0 taxa in 0 pages.

Our 26 Scratchpads ambassadors are located across the globe:

...moving to level of an individual : increase in strength of analyses

**Analysis I :
moleclar/morphological
differentiation**

downolad sequences,
quantitative or
continous data

+

newly measured
data



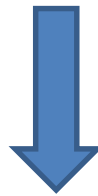
analysis

**Analysis II :
spatial circumstances of
speciation**

downolad spatial
data

+

geomorphological
characteristcs



analysis gene-flow
barries

**Analysis III :
ecological niche
reconstruction**

downolad spatial
data

+

Climate data
(Hijmans et al. 2005,
J.climat)



analysis of ecological
similarity

Taxon specific interactive database

SubBio Database: interactive database of subterranean fauna

Three main sources of data:

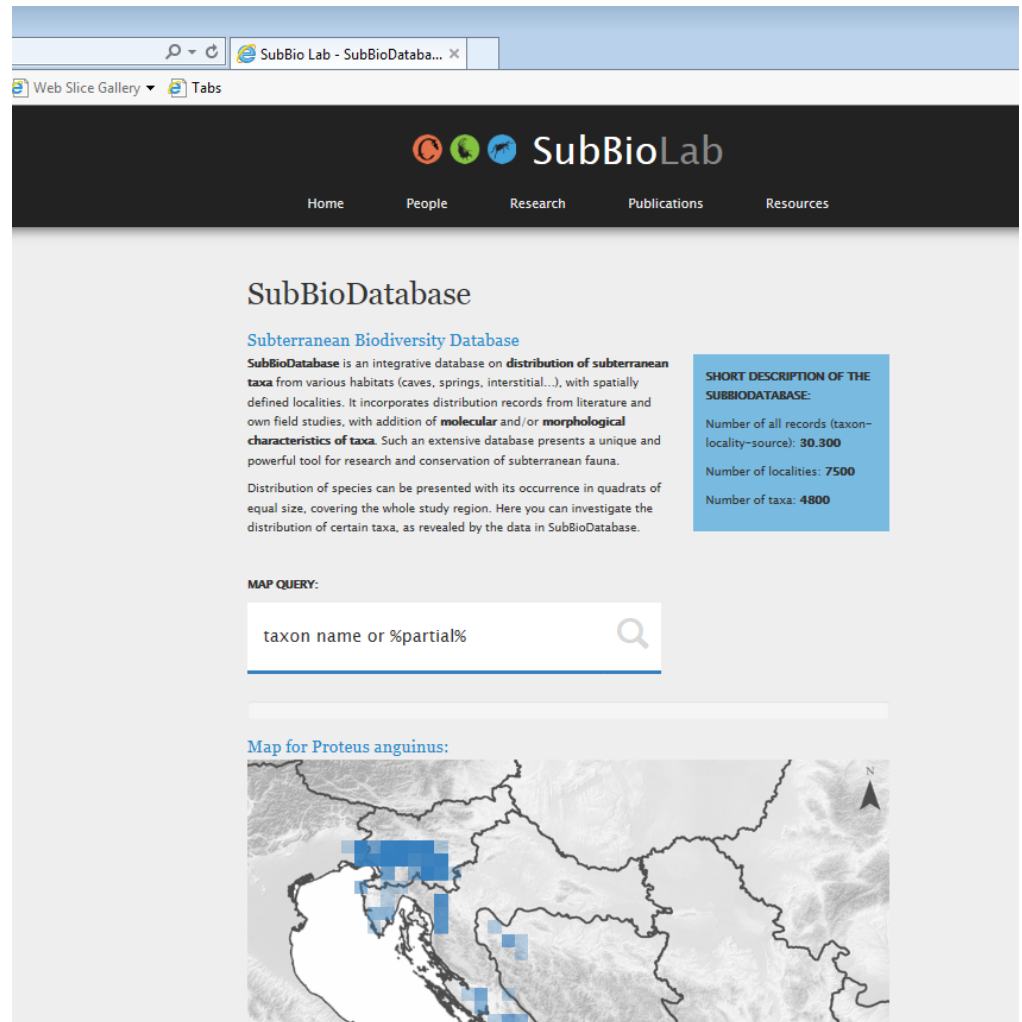
- species lists
- distributional data
- DNA sequences

Sources of data:

- published sources
- own data

Quantity:

- number of records:
30.300
- number of localities :
7.500
- number of taxa: 4.800



Taxon specific interactive database

SubBio Database: interactive database of subterranean fauna

The screenshot displays the SubBioDatabase access web interface in a browser window. The address bar shows the URL <http://subbio.net/dbaccess/dbquery/>. The page features a sidebar on the left with search and query options, and a main content area with several data entry and query sections.

Search: Locality [dropdown] Search

Advanced DB Query: DB-QUERY

Add:

- + New Locality
- + New Literature
- + Multiple DNA entries

View: Multi-Survey

Log in / log out

Edit profile

Tabele / Polja

Literatura

- ID literature
- Kratka oznaka literature
- Podrobna oznaka literature
- Avtorji
- Leto objave
- Naslov
- Revija / Knjiga
- Vol / Issue
- Strani
- Opombe
- Oznaka - fascikli

Lokaliteta

- ID lokalitete
- Ime lokalitete
- Kraj ob
- Vešči kraj
- Drugo ime / Sinonimi
- Država
- Geog. dolžina (WGS_X)
- Geog. širina (WGS_Y)
- Koordinate po
- Nadmorska višina
- UTM kvadrant
- Izvor koordinat
- Natančnost 1
- Natančnost 2
- Katastrska številka jame

Popis

- ID popisa (interni)
- Št. popisnega lista
- Popisovalci
- Datum popisa
- Opombe (opažanja ob obisku lokalitete)
- Trajanje - OD
- Trajanje - DO
- Vir (še iz tuje zbirke)
- Namen
- Metode
- Vzorec za prebiranje
- Opis vzorca
- Pasti (vzorčenje s pastmi)

Sistem

- Takson ID
- Celotno ime taksona
- Sinonimi
- Podvrsta
- Vrsta
- Podrod
- Rod
- Poddružina
- Družina
- Red
- Razred
- Avtor - ime
- Avtor - leto

Voucherji / DNA

- Voucher ID
- Voucher - stara koda
- Št. voucherskega osebk
- Opombe k voucherskemu osebk
- DNA ID
- Datum izolacije
- Št. voucherske DNA
- Gen
- Fwd
- Rew
- PCR
- Sekvenca
- Accession num.
- Dnevnik

Opaženi taksoni / Zbirka

- PopisDetalji ID
- Izvirna določitev (Takson_original)
- Locus typicus
- Določevalac
- Št. opazanih osebkov
- Št. vzeti osebkov
- V vodi / Na kopnem
- Mikrohabitat
- Metoda vzorčenja
- Opombe (za posamezen opažen takson)

Buttons:

- Dodaj izbrana polja +
- Odstrani -
- Izvrši poizvedbo
- Izvrši poizvedbo

The Windows taskbar at the bottom shows the time as 20:53 on 1.10.2015, with various system icons and open applications.

Taxon specific interactive database

SubBio Database: interactive database of subterranean fauna

Niphargus_DNABaza_23maj14 - Microsoft Excel

FileHomeInsertPage LayoutFormulasDataReviewView

From AccessFrom WebFrom TextFrom Other SourcesGet External Data

Existing Connections

Refresh All

Connections

Sort & Filter

Filter

Advanced

Text to ColumnsRemove DuplicatesData ValidationData ToolsConsolidateWhat-If Analysis

GroupUngroupSubtotal

Show DetailHide Detail

Outline

B3

fx

1531

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
		DNA_id	Datum_izolacije	created	voucher_id	shramba	gen	fwd	rev	PCR	sekvenc	acc_num	dnevnik	Lokalit_ime	Drzava	WGS_Xdd	WGS_Ydd	
1	Celotno_ime																	
2	Microniphargus leruthi	1532	15/05/2011		0 NA741	stiroporna škat									Laboratorijski	Velika Britanija	UK	
3	Microniphargus sp.	1531	15/05/2011		0 NA740	stiroporna škat									Laboratorijski	Jama Sweetwater	Great Britain	-3,535975282 50,62151447
4	Microniphargus sp.	1524	03/12/2010		0 NA649	stiroporna škat				neuspešno					Laboratorijski	Borehole at Wind	Great Britain	-2,376686748 50,72602791
5	Microniphargus sp.	1528	22/02/2011		0 NA713	stiroporna škat									Laboratorijski	Borehole at Wind	Great Britain	-2,376686748 50,72602791
6	Microniphargus sp.	1670	26/07/2012		0 NB090	stiroporna škat									Laboratorijski	Grotte de Combl	Belgium	5,57481 50,4754052
7	Microniphargus sp.	1525	22/02/2011		0 NA710	stiroporna škat									Laboratorijski	Abime de Combla	Belgium	5,57481 50,4754052
8	Microniphargus sp.	1526	22/02/2011		0 NA711	stiroporna škat									Laboratorijski	Abime de Combla	Belgium	5,57481 50,4754052
9	Microniphargus sp.	1527	22/02/2011		0 NA712	stiroporna škat									Laboratorijski	Abime de Combla	Belgium	5,57481 50,4754052
10	Niphargus aberrans	1229		1309432543	NA025		28S	28Slev3	28Sdes5	OK	AAGGCTTCAGTA				Sneberje, freatske	SLO	14,57311944 46,08042778	
11	Niphargus aberrans	1230		1309432543	NA025		H3	H3aF2	H3aR2	PONOVNO V					Sneberje, freatske	SLO	14,57311944 46,08042778	
12	Niphargus aberrans	1228		1309432543	NA025		28S	28Slev2	28Sdes2	OK	GAAAAGCACTCT	EF617260			Sneberje, freatske	SLO	14,57311944 46,08042778	
13	Niphargus aggtelekiensis	1298		1309432543	NA501		28S	28Slev3	28Sdes5	OK	AAGGCTTCAGTA				Gesause ET2B	Austria	14,587258 47,565345	
14	Niphargus aggtelekiensis	1297		1309432543	NA501		28S	28Slev2	28Sdes2	OK	GAAAAGCACTCT	KJ566697			Gesause ET2B	Austria	14,587258 47,565345	
15	Niphargus aggtelekiensis	1299		1309432543	NA501		H3	H3aF2	H3aR2	OK	TGCTCGGAATCT	KJ566722			Gesause ET2B	Austria	14,587258 47,565345	
16	Niphargus aggtelekiensis	2119	12.08.2013	1376490795	NB541	skrinja na hodr									dnevnik Dorott	Rakoczi 1 cave	Hungary	20,74886667 48,52081667
17	Niphargus aggtelekiensis	2120	12.08.2013	1376491015	NB542	skrinja na hodr									dnevnik Dorott	Baradla-also Cav	Hungary	20,54401667 48,4831
18	Niphargus alpinus	1003		1309432543	NA019		28S	28Slev2	28Sdes2	OK	GAAAAGCACTCT				Ramsauer Tal, No NEM		12,900112 47,616669	
19	Niphargus alpinus	1002		1309432543	NA109		28S	28Slev3	28Sdes5	OK	AAGGCTTCAGTA				Schapbach spring	NEM	12,95833333 47,58194444	
20	Niphargus alpinus	1001		1309432543	NA109		28S	28Slev2	28Sdes2	OK	GAAAAGCACTCT	EF617254			Schapbach spring	NEM	12,95833333 47,58194444	
21	Niphargus ambulator	1290		1309432543	NA504		28S	28Slev2	28Sdes2	OK	GAAAAGCACTCT	KJ566699			Buco del Piombo	ITA	9,198473 45,825819	
22	Niphargus ambulator	1291		1309432543	NA504		H3	H3aF2	H3aR2	OK	CGCTCGTAAGTC	KJ566723			Buco del Piombo	ITA	9,198473 45,825819	
23	Niphargus angelieri	1218		1309432543	NA200		28S	28Slev2	28Sdes2	NE DELA					Grotte des Fees	FRA	3,028236 42,910091	
24	Niphargus angelieri	1220		1309432543	NA200		H3	H3aF2	H3aR2	OK	CGCTCGCAAGTC				Grotte des Fees	FRA	3,028236 42,910091	
25	Niphargus angelieri	1219		1309432543	NA200		28S	28Slev3	28Sdes5	PONOVNO V					Grotte des Fees	FRA	3,028236 42,910091	
26	Niphargus aquilex	1214		1309432543	NA020		28S	28Slev2	28Sdes2	OK	CTCTGAAGAGAC	EF617255			Wavreille, well 4	BEL	5,248482 50,121031	
27	Niphargus aquilex	1215		1309432543	NA020		28S	28Slev3	28Sdes5	OK	AAGGCTTCAGCA				Wavreille, well 4	BEL	5,248482 50,121031	
28	Niphargus aquilex	1216		1309432543	NA020		H3	H3aF2	H3aR2	OK	CGCTCGTAAGTC				Wavreille, well 4	BEL	5,248482 50,121031	
29	Niphargus aquilex	1217		1309432543	NA020		COI	LCO	HCO	OK	GGAGCTTGAGCT				Wavreille, well 4	BEL	5,248482 50,121031	
30	Niphargus aquilex	1213		1309432543	NA029		H3	H3aF2	H3aR2	OK	CGCTCGCAAGTC				Marden območje	GB	-0,855519 50,929969	
31	Niphargus aquilex	1212		1309432543	NA029		28S	28Slev2	28Sdes2	OK	CTCTGAAGAGAC				Marden območje	GB	-0,855519 50,929969	
32	Niphargus aquilex	1511		1309432543	NA194		28S	28Slev2	28Sdes2	NE DELA					Littlebourne	UK	1,168921 51,275484	
33	Niphargus aquilex	1512		1309432543	NA205		28S	28Slev2	28Sdes2	NE DELA					Littlebourne	UK	1,168921 51,275484	
34	Niphargus arbirer	672	14/08/2002		1309432543	NA052	28S	28Slev2	28Sdes2	OK	CTCTGAAGAGAC	EF617287			Tounjčica špilja	Croatia	15,32633388 45,24872352	
35	Niphargus arbirer	674		1309432543	NA052		H3	H3aF2	H3aR2	OK	TGCTCGCAAGTC				Tounjčica špilja	Croatia	15,32633388 45,24872352	
36	Niphargus arbirer	676		1309432543	NA052		12S	?	?	OK	GCCTTTATATTAA				Tounjčica špilja	Croatia	15,32633388 45,24872352	

Ready

90%

20:56 1.10.2015

Web-database as a collaborative tool?

Morphology: deposition of figures – deposition of measurements

Quantitative data: MYSQL

Form - taxa and characters

[Niphargus home](#) [Submit form!](#) [Clear all!](#)

Select taxa (Check/Uncheck All)

- ☐ 1 Niphargus aquilex
- ☒ 2 Niphargus arbiter
- ☐ 3 Niphargus balcanicus
- ☒ 4 Niphargus bilecanus
- ☐ 5 Niphargus brachytelson
- ☐ 6 Niphargus camiolius
- ☐ 7 Niphargus costozzae
- ☐ 8 Niphargus croaticus
- ☐ 9 Niphargus dabarensis
- ☐ 10 Niphargus dalmatinus
- ☐ 11 Niphargus dimorphopus
- ☐ 12 Niphargus danconai
- ☐ 13 Niphargus dolichopus
- ☐ 14 Niphargus elegans

Select characters (You can select up to 100 characters.)

- ☒ 1 body length up to [mm]
- ☒ 2 rostrum
- ☐ 3 head length [of body length]
- ☐ 4 pereonites I-VI with up to [setae]
- ☐ 5 pereonite VII with
- ☐ 6 pereonite VII with [postero-ventral setae]
- ☐ 7 pleonites I-III with up to [setae]
- ☐ 8 pleonites I-III with
- ☐ 9 epimera II and III, posterior margins with [setae]
- ☐ 10 epimeral plate II postero-ventral corner
- ☐ 11 epimeral plate II, posterior margin
- ☐ 12 epimeral plate II, ventral margin
- ☐ 13 epimeral plate II, postero-ventral corner
- ☐ 14 epimeral plate II with [strong spine-like setae along ventral margin]

Niphargus home		
Back		
	body length up to [mm]	rostrum
Niphargus arbiter	31	absent
Niphargus bilecanus	29	absent

Web-database as a collaborative tool?

Morphology: deposition of figures – deposition of measurements

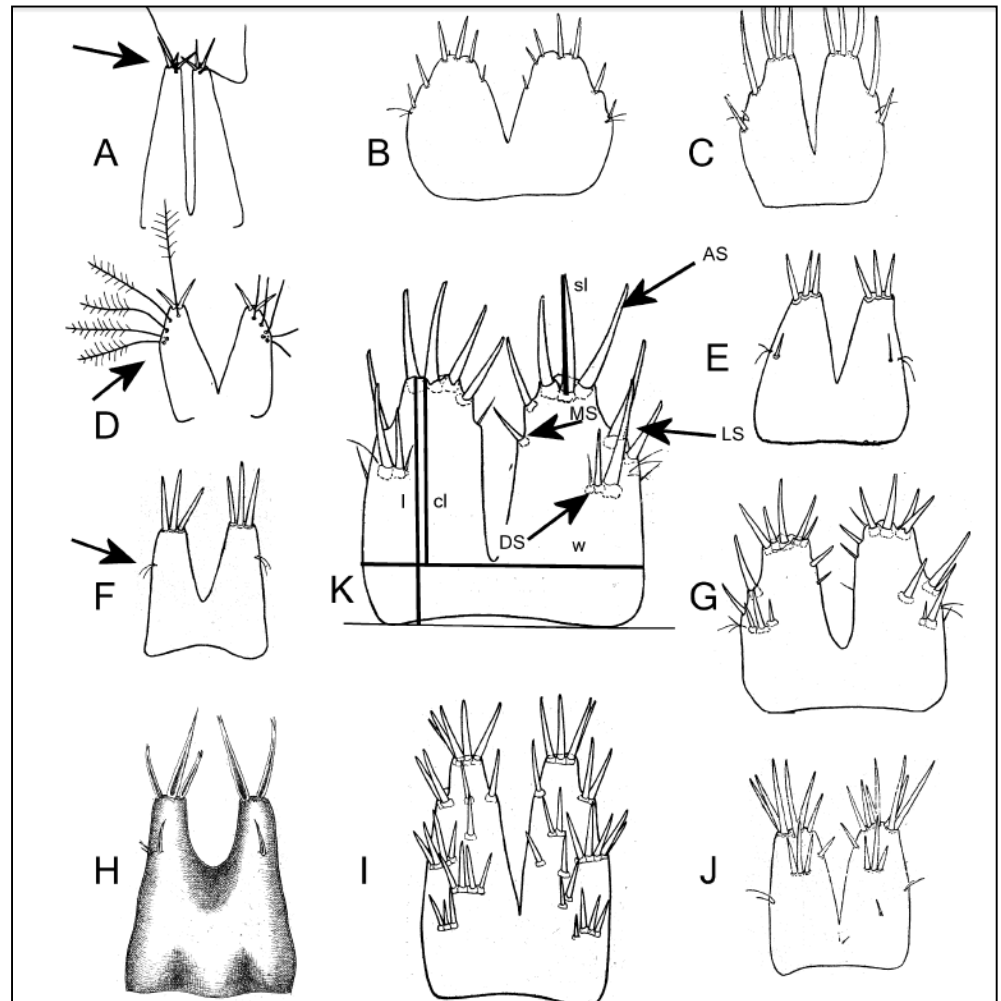
Quantitative data: MYSQL

SEVERAL IMPEDIMENT:

- taxon specific
- requires pre-defined protocols
- individual level
- unintentionally limits exploration

A CHALLENGE:

- making links with other databases (GeneBank, spatial data)



Taxon specific interactive database

Strenghts:

- precise, a possibility to correct errors
- standardized protocols
- different properties linked with voucher specimens
- eases team work

Weakness:

- nature of characters may limit extent of the database (few taxa)

Global databases – character specific

Species names on a web: a chaos

The screenshot shows the Fauna Europaea website. The header includes the logo and navigation links like 'data services', 'focal point network', 'about Fauna Europaea', 'contact', 'citation, copyright, privacy & disclaimer', and 'last update 29 August 2013 | version 2.6.2'. A left sidebar lists various database features: Name Search, Advanced Search, Taxon Tree, Distribution, Statistics, Experts, References, Taxonomic Resources, Other on-line databases, Acknowledgments, and LinkedIn. The main content area has a 'Name search' section with tabs for 'species', 'higher taxa', and 'vernacular'. It includes input fields for '(Sub) genus' and '(Sub) species', each with a dropdown menu set to 'is'. Below these are 'Search' and 'Reset' buttons. A text block explains that the database contains scientific names of all European land and freshwater animals. It also provides links for European marine species (European Register of Marine Species - ERMS), European plant species (Euro+Med PlantBase - E+M), and the integrated pan-European checklist (PESI portal). At the bottom, it states 'Fauna Europaea was supported by the European Commission under the Fifth Framework Programme...' and 'Fauna Europaea is powered by MN'. A Creative Commons BY-SA 4.0 license logo is visible.

Zoobank : Niphargus 8 taxa

Niphargus – 295 taxa

The screenshot shows the WoRMS website. The header features the 'WoRMS World Register of Marine Species' logo. A left sidebar contains a navigation menu with links: Home, About, Search taxa, Taxon tree, Literature, Distribution, Specimens, Match taxa, Editors, Statistics, Users, Webservice, Photogallery, Info downloads, Sponsors, and Activities. The main content area has a 'Search WoRMS' section with a 'Common name' dropdown set to 'contains' and a 'Scientific name' dropdown set to 'begins with'. There are input fields and a 'Search' button. Below the search section is a 'News' section with several articles, including 'MolluscaBase officially launched!' and 'New Steering Committee in place'. At the bottom, the URL 'http://www.marinespecies.org/index.php' is visible. A Creative Commons BY-SA 4.0 license logo is also present.

**Wikipedia
(SLO):
Niphargus - 206
taxa**

**EOL: Niphargus
– 204-309 taxa**

Niphargus – 261 taxa

Global databases – character specific

GeneBank: repository of sequences

<http://www.ncbi.nlm.nih.gov/genbank/>

Main impediment: species id

The screenshot shows the GenBank website interface. At the top, there is a search bar with a dropdown menu set to 'Nucleotide' and a 'Search' button. Below the search bar is a navigation menu with links to GenBank, Submit, Genomes, WGS, HTGs, EST/GSS, Metagenomes, TPA, TSA, and INSDC. The main content area is titled 'GenBank Overview' and includes a section 'What is GenBank?' which describes the database as a collection of publicly available DNA sequences. It also mentions the 'GenBank Resources' section with links to Home, Submission Types, Submission Tools, Search GenBank, and Update GenBank Records. A 'GenBank Data Usage' section explains the database's purpose and the lack of restrictions on data use. A 'Confidentiality' section addresses concerns about data publication. A 'Privacy' section states that human sequences should not include personal data. At the bottom, there is a 'Disclaimer' and a 'Privacy statement' link. The footer contains a table with links to various resources, a 'Write to the Help Desk' link, and a 'National Center for Biotechnology Information' logo. The footer also includes the address '5800 Rockville Pike, Bethesda MD, 20894 USA' and a 'Last updated: 2015-05-07T14:49:55-04:00' timestamp.

GenBank Overview

What is GenBank?

GenBank® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences ([Nucleic Acids Research](#), 2013 Jan 41(D1):D36-D43). GenBank is part of the [International Nucleotide Sequence Database Collaboration](#), which comprises the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI. These three organizations exchange data on a daily basis.

The complete [release notes](#) for the current version of GenBank are available on the NCBI ftp site. A new release is made every two months. GenBank growth [statistics](#) for both the traditional GenBank divisions and the WGS division are available from each release.

An [annotated sample GenBank record](#) for a *Saccharomyces cerevisiae* gene demonstrates many of the features of the GenBank flat file format.

Access to GenBank

There are several ways to search and retrieve data from GenBank.

- Search GenBank for sequence identifiers and annotations with [Entrez Nucleotide](#), which is divided into three divisions: [CoreNucleotide](#) (the main collection), [dbEST](#) (Expressed Sequence Tags), and [dbGSS](#) (Genome Survey Sequences).
- Search and align GenBank sequences to a query sequence using [BLAST](#) (Basic Local Alignment Search Tool). BLAST searches CoreNucleotide, dbEST, and dbGSS independently; see [BLAST info](#) for more information about the numerous BLAST databases.
- Search, link, and download sequences programmatically using [NCBI e-utils](#).
- The ASN.1 and flatfile formats are available at NCBI's anonymous FTP server: [ftp://ftp.ncbi.nlm.nih.gov/ncbi-sql/](#) and [ftp://ftp.ncbi.nlm.nih.gov/genbank/](#).

GenBank Data Usage

The GenBank database is designed to provide and encourage access within the scientific community to the most up-to-date and comprehensive DNA sequence information. Therefore, NCBI places no restrictions on the use or distribution of the GenBank data. However, some submitters may claim patent, copyright, or other intellectual property rights in all or a portion of the data they have submitted. NCBI is not in a position to assess the validity of such claims, and therefore cannot provide comment or unrestricted permission concerning the use, copying, or distribution of the information contained in GenBank.

Confidentiality

Some authors are concerned that the appearance of their data in GenBank prior to publication will compromise their work. GenBank will, upon request, withhold release of new submissions for a specified period of time. A date must be specified; we can not hold a sequence indefinitely pending publication. However, if a paper citing the sequence or accession number is published prior to the specified date, the sequence will be released upon publication. In order to prevent the delay in the appearance of published sequence data, we urge authors to inform us of the appearance of the published data. As soon as it is available, please send the full publication date—all authors, title, journal, volume, pages and date—to the following address: [update@ncbi.nlm.nih.gov](#)

Privacy

If you are submitting human sequences to GenBank, do not include any data that could reveal the personal identity of the source. GenBank assumes that the submitter has received any necessary informed consent authorizations required prior to submitting sequences.

[Disclaimer](#)

[Privacy statement](#)

You are here: NCBI

Write to the Help Desk

GETTING STARTED	RESOURCES	POPULAR	FEATURED	NCBI INFORMATION
NCBI Education	Chemicals & Bioscience	Published	Genetic Testing Registry	About NCBI
NCBI Help Manual	Data & Software	Bookshelf	Public Health	Research at NCBI
NCBI Handbook	DNA & RNA	Published Central	GenBank	NCBI News
Training & Tutorials	Domains & Structures	Public Health	Reference Sequences	NCBI FTP Site
Submit Data	Genes & Expression	BLAST	Gene Expression Omnibus	NCBI on Facebook
	Genetics & Medicine	Nucleotide	Map Viewer	NCBI on Twitter
	Genomes & Maps	Genome	Human Genome	NCBI on YouTube
	Homology	SNP	Mouse Genome	
	Literature	Gene	Influenza Virus	
	Proteins	Protein	Primer-BLAST	
	Sequence Analysis	PubChem	Sequence Read Archive	
	Taxonomy			
	Variation			

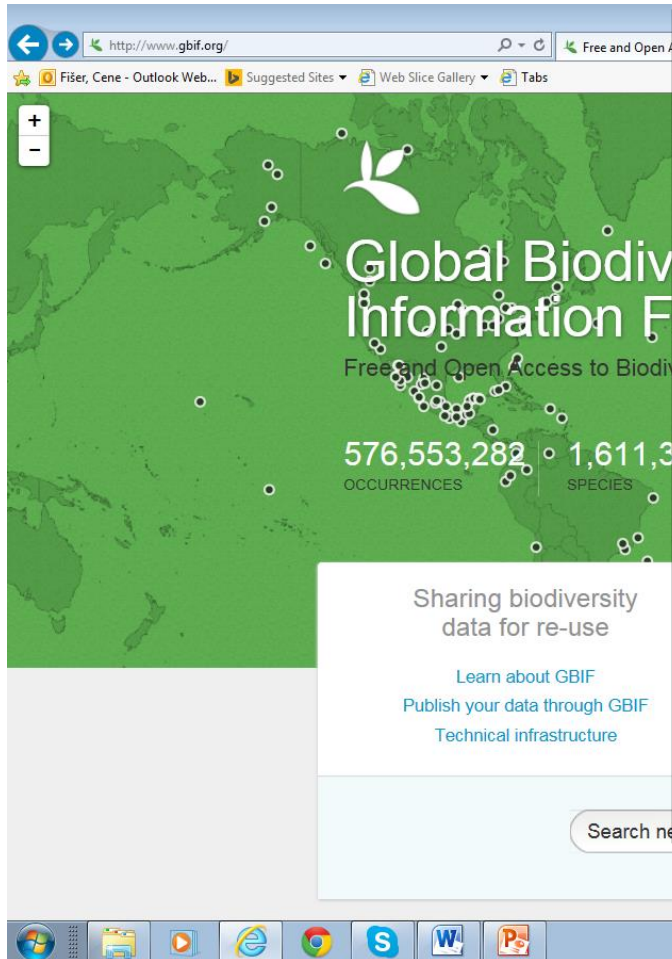
National Center for Biotechnology Information, U.S. National Library of Medicine
5800 Rockville Pike, Bethesda MD, 20894 USA
[Policies and Guidelines](#) | [Contact](#)

Last updated: 2015-05-07T14:49:55-04:00

Global databases – character specific

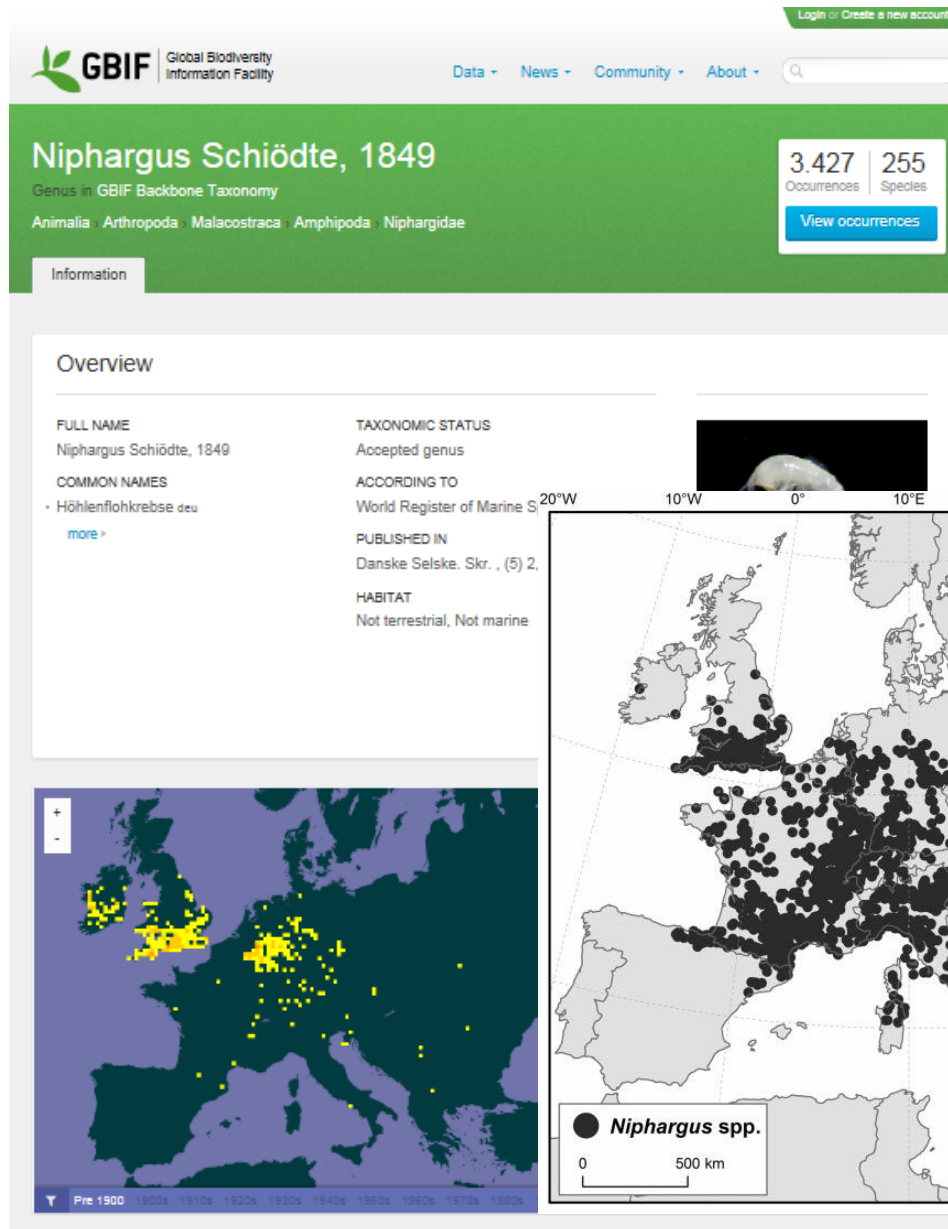
GBIF's vision: *"A world in which biodiversity information is freely and universally available for science, society and a sustainable future.,,"*

<http://www.gbif.org/>



- It provides a single point of access (through this portal and its web services) to more than **570,000,000 records**, shared freely by hundreds of institutions worldwide, making it the biggest biodiversity database on the Internet.
- The data accessible through GBIF relate to evidence about more than **1.6 million species**, collected over three centuries of natural history exploration and including current observations from citizen scientists, researchers and automated monitoring programmes.
- More than **1,400 peer-reviewed research publications have cited GBIF** as a source of data, in studies spanning the impacts of climate change, the spread of pests and diseases, priority areas for conservation and food security. **About one such paper is published each day.**
- Many GBIF Participant **countries have set up national portals using tools, codes and data freely available through GBIF** to better inform their citizens and policy makers about their own biodiversity.

Global databases – character specific



Main impediments:

- species id
- completeness of data for selected taxon
- quality of coordinates

Global databases – character specific

Strenghts:

- different taxa in one place, a promising source of information for ecology and evolutionary biology

Weakness:

- difficult or impossible to revise errors
- poor connection between databases dealing with different types of characters

Global issues

1. Quality of database-quality of data.
2. Linking the taxon oriented databases with character oriented databases: increase of accuracy and number of taxa.
3. Promotion database publishing:
 - Citation problem
 - Rewarding in funding agencies
 - Role of journals
4. Collaborative network – stimulating collaborators

Thanks...

Maja Zagmajster

Martin Turjak

Roman Luštrik

The rest of SubBio lab team

Taxon specialized websites: not necessarily databases

What is the name of this creature?

Salticid spiders

<http://salticidae.org/salticid/main.htm>

**Monograph of
*Salticidae (Araneae)***
By Jerzy Prószyński
2007
Version revised in part on February 2008

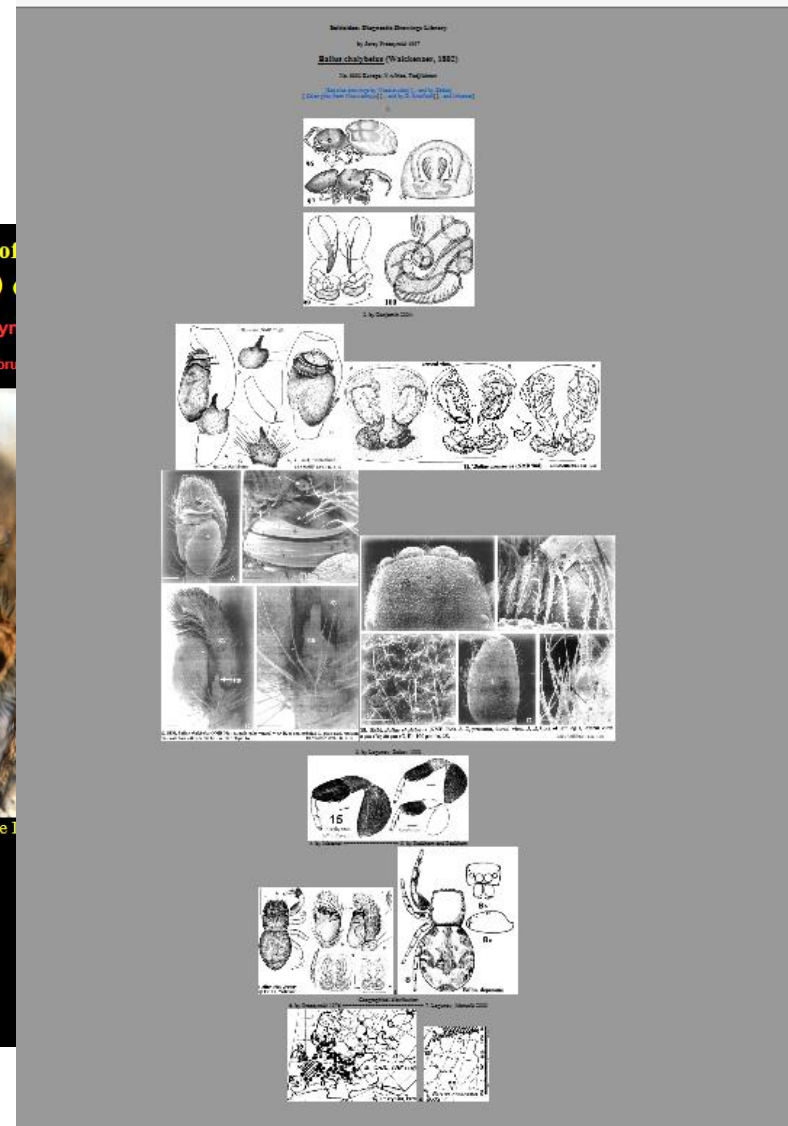


Click the picture to see list of genera of the 1

PART I. DATABASE
PART II. DIAGNOSTIC DRAWINGS
PART III. CATALOGUE OF THE *Salticidae* OF THE WORLD

Special features: [Pictorial Indexes, Keys and Taxonomic Revisions](#)
See also: [OTHER WWW SALTICIDAE PAGES](#)

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Global databases – character specific

Morphology: deposition of figures – deposition of measurements

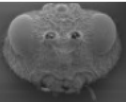
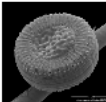
**Welcome to Morphbank on Sky**
User: Guest [\[click to login\]](#)

AboutBrowseToolsHelp

Featured from 381962 images

MorphBank
<http://www.morphbank.net/>

click images below to [browse](#) new uploads and collections



AnglerfishChromistaM. blackburniAristolochiaCynipidae

News and Updates

Morphbank move to new servers
The Morphbank system at FSU has moved to the Sky server farm at the FSU Research Computing System and the Nolestor storage system. These new systems will keep Morphbank running into the future. ...

(Posted: 07-27-15)

Filtered Push plugin in process
The Filtered Push group have created a Morphbank plugin that extracts annotations from the Morphbank database and pushes them into the FP annotation services. ...

(Posted: 11-07-14)

Morphbank in DINA Project
The DINA project develops an open-source Web-based information management system for natural history data. At the core of the system is support for assembling, managing and sharing data associated with natural history collections and their curation ("collection management"). Target collections include zoological, botanical, geological and paleontological collections, living collections, biodiversity inventories, ...

(Posted: 11-07-14)

Troy University Herbarium Online
The Troy University Herbarium images from the Deep South Plant Specimen Imaging Project are now available. Search for "Michael Woods" to see the images and specimens ...

(Posted: 11-06-14)

[\(see all past news\)](#)

Global databases – character specific

Morphology: deposition of figures – deposition of measurements

Image 833234
User: Guest (click to login)

About Browse Tools Help

Image Record: [833234] *Alloxysta victrix*

Contributor: Jordi Parotx-Martinez
Submitter: Jordi Parotx-Martinez
Group: Universitat de Barcelona
Date Submitted: 2013-10-09
Last Modified: 2013-10-09
Publish Date: 2011-07-01
Description: New image from upload

Magnification: 3000
Dimension (px): 750x576
Resolution (PPI):
Submitted as: tiff
Original File Name: ALXVIC19.TIF
Photographer: Jordi Parotx-Martinez

View id: 833275
Specimen part: Notolateral claw
Angle: Lateral
Technique: SEM
Preparation: Air dried, gold coated

Download: original (tiff) (432.11 KB)
full sized jpeg (71.70 KB) medium sized jpeg (22.5 KB)

Copyright: public domain
License: This work is free of known copyright restrictions.

Specimen
Specimen id: 833070
Basis of record: [s] - Specimen
Sex: Female
Form: Indeterminate
Stage: Adult
Catalog number:
Collector:
Date collected:

Locality
Locality id: 101950
Continent: UNKNOWN
Water Body:
Country: UNKNOWN
State/Province:
County:
Locality: Unknown
Latitude:
Longitude:
Elevation (m):
Depth (m):

Determination
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hymenoptera
Family: Pteromalidae
Genus: *Alloxysta*
Species: *Alloxysta victrix*

External links/identifiers
External Unique Reference:
Specimen 833070 dds:identifier: urn:uuid:23148009-6603-455b-9d5a-257af5c7186d
dds:identifier: urn:uuid:28480ba8-b149-434e-a032-5b6b072497ec

Determination annotations [Add Annotation...]
Other Annotations [Add Annotation...]

Related Annotations
Taxonomic Name: *Alloxysta victrix*
Taxon Author:
Prefix: none
Suffix: none
1 0

Image 196798
User: Guest (click to login)

About Browse Tools Help

Image Record: [196798] *Dulichella spinosa*

Contributor: Olan Pezko
Submitter: Olan Pezko
Group: SCANIT
Date Submitted: 2007-12-15
Last Modified: 2011-09-01
Publish Date: 2007-12-15
Description: Image added to Database

Magnification: 25
Dimension (px): 1094x1176
Resolution (PPI):
Submitted as: jpg
Original File Name: Dulichella spinosa.jpg
Photographer:

View id: 196772
Specimen part: Whole body
Angle: Lateral left
Technique: Digital camera
Preparation: Wet Mount

Download: original (jpg) (424.49 KB)
full sized jpeg (404.49 KB) medium sized jpeg (80.59 KB)
Copyright: Olan Pezko
License:

Specimen
Specimen id: 196795
Basis of record: [s] - Specimen
Sex: Male
Form: Not Applicable
Stage: Adult
Catalog number:
Collector: Orange County Sanitation District
Date collected: 2008-07-12

Locality
Locality id: 196794
Continent: NORTH AMERICA
Water Body:
Country: UNITED STATES
State/Province:
County:
Locality: Southern California Bight
Latitude:
Longitude:
Elevation (m):
Depth (m):

Determination
Kingdom: Animalia
Phylum: Arthropoda
Class: Malacostraca
Order: Amphipoda
Family: Haldidae
Genus: *Dulichella*
Species: *Dulichella spinosa*

External links/identifiers
External Unique Reference:
Specimen 196795 dds:identifier: urn:uuid:820c73ef-dea7-43ef-830b-906079390a62
dds:identifier: urn:uuid:f7260c4e-9185-4f8d-9d5d-70e40d4100e

Determination annotations [Add Annotation...]
Other Annotations [Add Annotation...]

Related Annotations
Taxonomic Name: *Dulichella spinosa*
Taxon Author: Stout, 1912
Prefix: none
Suffix: none
1 0

Beginnings of the databases...

Special software packages: DELTA, Lucid

Description Language for
Taxonomy (DELTA)

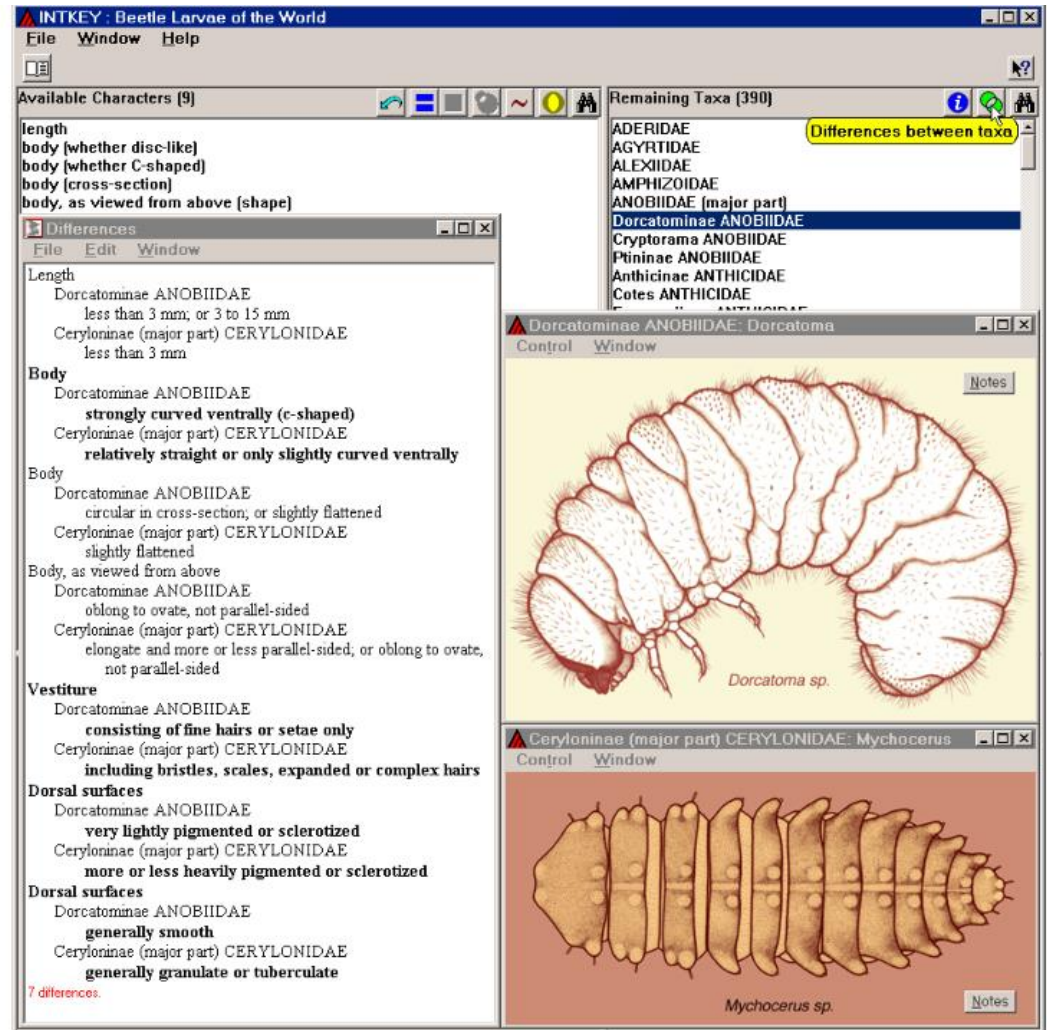
<http://delta-intkey.com/>

A TOOL FOR:

- descriptions
- identification keys (different types)
- files for phylogenetic/phenetic analysis
- comparison of taxa

MAIN IMPEDIMENT:

limited analytic frame, species – population level



Beginnings of the databases...

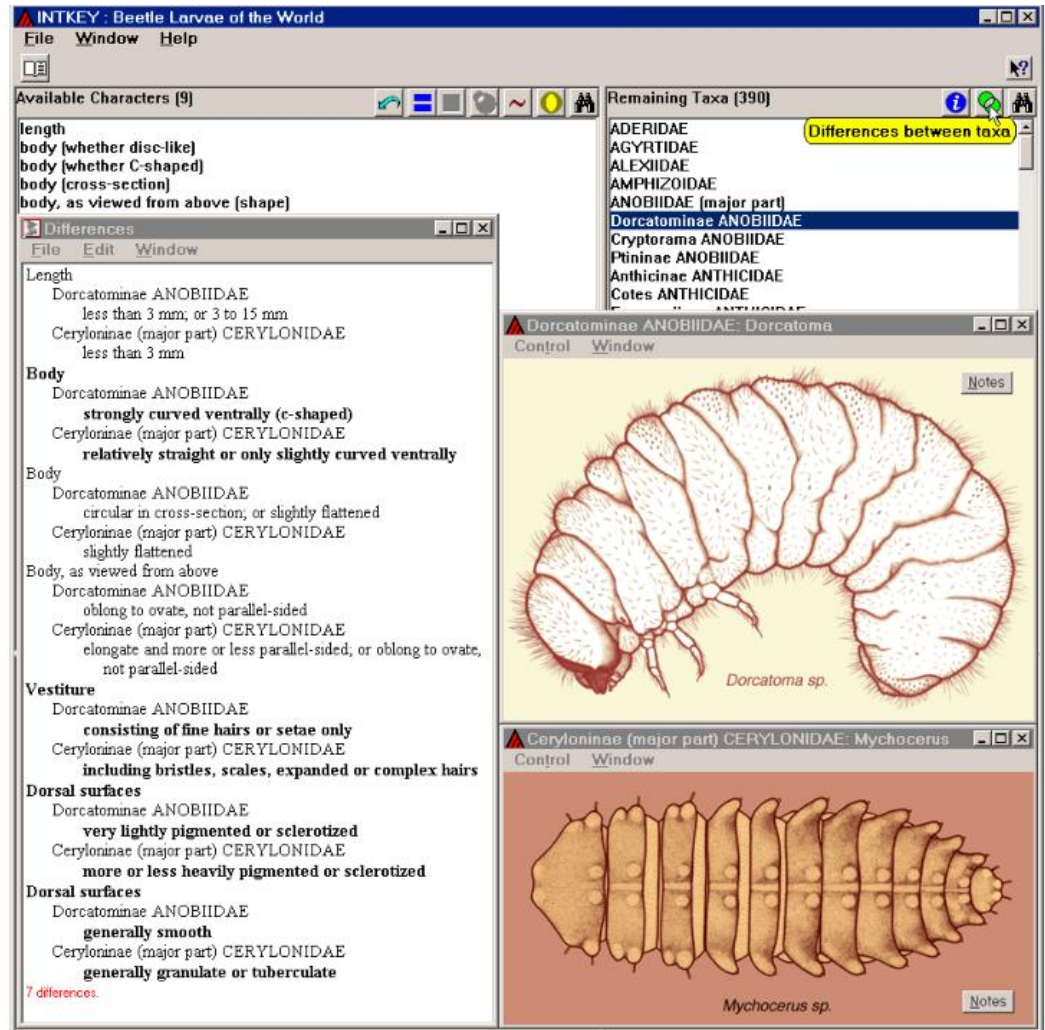
Special software packages: DELTA, Lucid

Description Language for
Taxonomy (DELTA)

<http://delta-intkey.com/>

SOURCE OF INFORMATION THAT
CAN BE INCLUDED:

- discrete and continuous morphological characters
- illustrations and photos
- sonograms
- videoclips





CINKARNA

The importance of data interpretation and dissemination for the future of nanotechnology

Mag. Vladimír Vrečko

Future of nanotechnology





**Hope you enjoyed and
thank you for your attention!**

Public point of view

- Yet another potentially dangerous technology.
- Industry pursues its own interests and can not be trusted.
- Research which is funded by private funds can not be trusted.
- Even scientists don't agree with each other, so there is obviously something wrong.
- Public media informs us of the research results that prove nanotechnology is dangerous.
- We don't want the technology until we are sure it is safe.

Scientists point of view – Material scientists

- We have discovered numerous new materials and developed their applications.
- Everyone would benefit from their use.
- We don't see, why industry does not recognise the potential and finance us extensively.
- If we could just get money, we would have immediate success on market (everyone would buy our products).
- The potential risks, costs, availability of the materials and public opinion are not our concern.
- Yet another proof that we are not understood and appropriately valued.

Scientists point of view – Risk Assessment groups

- We got financed to reveal the safety risks.
- Public expects us to prove we are independent.
- To prove that nano materials are dangerous is therefore a success and worth publishing.
- Such findings are great for PR (ensures headlines and recognition of the research group).
- If the material or technology does not manifest risky properties, it is a disappointment and not worth mentioning.
- Only if we reveal new safety risks will we have a chance for future funding of our research.

Industry point of view

- We are interested in new technologies, but we take decisions based on economy.
- We can manage the costs, however market is still underdeveloped because the risks and legal frameworks are not yet sufficiently defined.
- Public opinion is very sceptical and media are not in favour, publishing frightening stories.
- Customers and investors don't want to take premature financial risks.
- Can it happen to nanotechnology that it will follow the footsteps of GMOs?

How about that:

Scientific research title:

Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice.

Title in newspaper:

Scientists say nanoparticles cause DNA damage.

Implications for the customers:

Be aware of the products that contain nanoparticles.

Demand that all products containing nanoparticles shall be labeled (as dangerous?).

Don't buy such products as they can damage your DNA (scientist have said so).

What is needed?

- Industry needs stable and known market conditions and legal boundaries.
- It is willing to take reasonable precautionary measures, but doesn't want unpredictable environment for investments.
- Scientists should be aware of their responsibility at shaping public opinion.
- They should publish also the results in which they confirm that some nanomaterials do not pose safety risks.
- Market will grow only if all the stakeholders will feel secure.

What is needed?

- Scientist shall pursue balanced and objective approach and help regulatory bodies at designing legal frameworks.
- Scientists shall not forget that they are after all financed from the money which is mainly created by the industry.
- Scientists and industry shall join forces and through public media present nanotechnology as mature technology, which has many advantages. And, normally, some potential risks, which we can however control through joint activities of researchers, legal bodies and manufacturers.

What is needed?

We shall persuade all stakeholders that they have nothing to be afraid of.

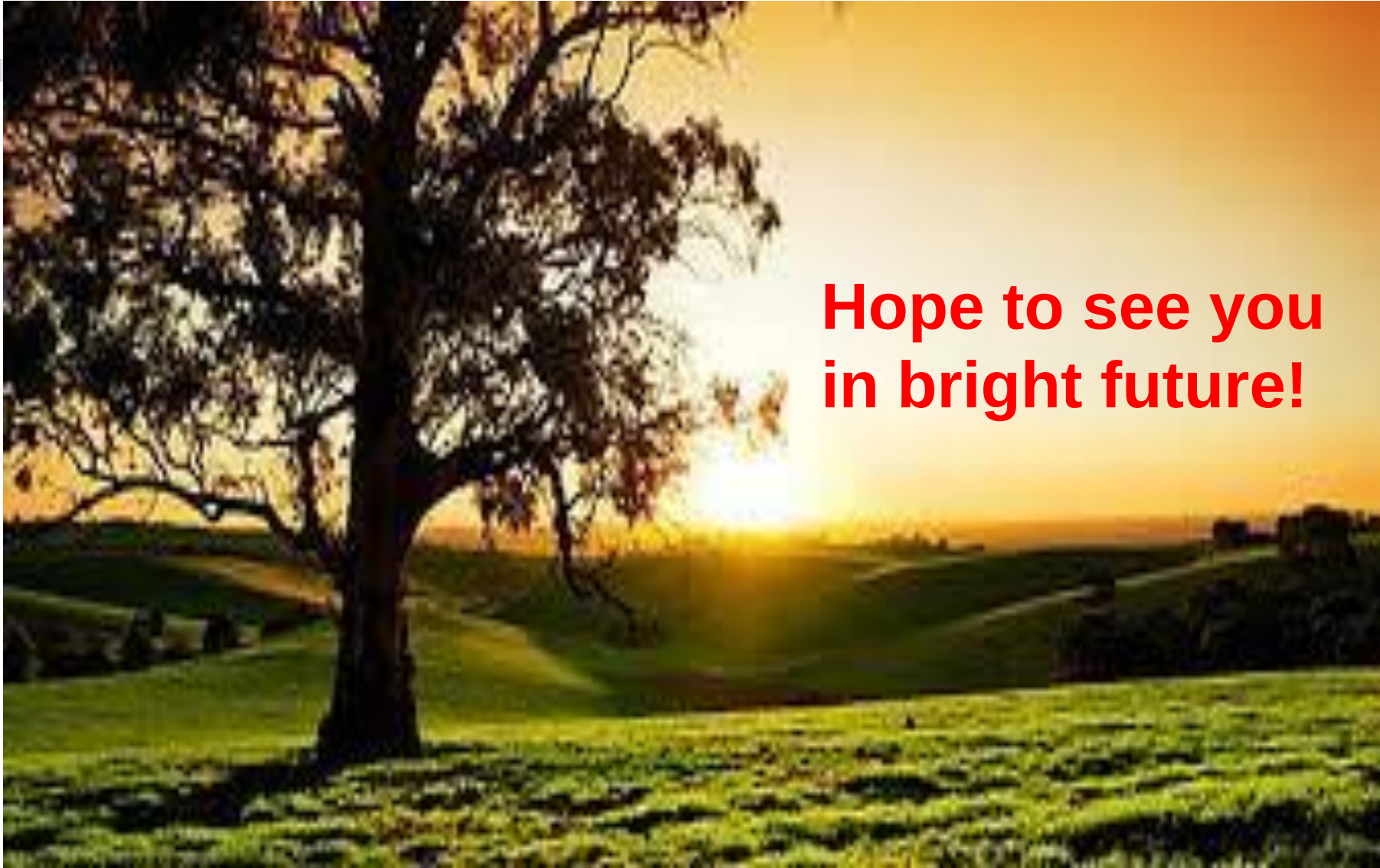
How shall we act?



So?

Or so?



A large, dark tree stands on the left side of a green field. The sun is setting in the background, creating a bright orange and yellow glow that illuminates the sky and the field. The text "Hope to see you in bright future!" is written in red on the right side of the image.

**Hope to see you
in bright future!**



**NACIONALNI LABORATORIJ ZA
ZDRAVJE, OKOLJE IN HRANO**

Prvomajska ulica 1, 2000 Maribor

Challenges of Regulation and Risk Assessment of Nanomaterials

Part 2: ECHA - NMWG

Dr. Mojca Kos Durjava

COMPInNANO, Ljubljana, 2.-3.10.2015

Risk Assessment of Nanomaterials

EC and OECD - Risk assessment of nanomaterials can be managed through existing regulatory frameworks, adapted to take into account the specific properties of manufactured nanomaterials.

REACH, CLP - EC is modifying some of the technical provisions in the REACH Annexes - amendments in 2016

Biocides – Biocidal Products Regulation (BPR), 2013: contains a definition of nanomaterial.

Risk Assessment of Nanomaterials

EC launched a comprehensive REACH Implementation Project on Nanomaterials (RIPoN) in 2009:

- RIPON1 : Substance Identity
- RIPON2 : Information Requirements
- RIPON3 : Chemical Safety Assessment

CARACAL is an expert group which advises the European Commission and ECHA on questions related to REACH and CLP.

CASG nano - Competent Authority Subgroup on Nanomaterials

- Nanomaterials in REACH
- Classification, Labelling and Packaging of Nanomaterials in REACH and CLP.

OECD - WPMN

Working Party on Manufactured Nanomaterials - WPMN

- is linked to the implementation of REACH - TG and guidance.

OECD and WPMN SG on Risk Assessment and Regulatory Mitigation SG-AP

- SG on Exposure and Exposure Mitigation Exp. Mitigation
- SG on the Environmentally Sustainable use of Nanomaterials LCA
- **SG on Testing Assessment of Manufactured Nanomaterials SG-TA:**
 - TG update
 - Assessment of data
 - In vitro work
 - Alternative methods (e.g. read-across)

ECHA - NMWG

NMWG – Nanomaterials Working Group

The aim – to discuss topical scientific and technical issues relevant to the implementation of REACH, CLP and Biocidal Product Regulation in relation to nanomaterials.

From 2013, 7th meeting in November 2015, chair Frank Le Curieux from ECHA

50 or more participants at meetings, twice a year:

- MSCA – Member States Competent Authorities
- European Commission (DG ENT and DG ENV)
- DG Joint Research Centre
- ECHA-NMWG Accredited Stakeholders Observers (ASO)
- ECHA representatives
- Invited speakers from industry, science

ECHA - NMWG

NMWG – Nanomaterials Working Group

- ECHA presentations on their work on NM
- Industry presentations of dossiers of registered NM
(10 NM – cerium oxide, calcium carbonate, zinc oxide, multi-wall nanotubes, titanium dioxide,...)
- Scientists presentations on their research in the field of NM (NanoREG, NanoValid, Marina,...)
- Other presentations

Discussion, working in groups.

ECHA - NMWG

ECHA guidance on RA of NM

Already developed by ECHA: a technical manual on how to include information on nanomaterial in a IUCLID dossier which is an integral part of every REACH registration.

REACH amendments of Annexes (VII-X) for nanomaterials



ECHA guidance on RA of NM before 2018 registration deadline

- ECHA guidance (updating)
- Practical guides/examples

Advice and expertise from the NMWG.

ECHA - NMWG

IUCLID 6

IUCLID is a software application to capture, store, maintain and exchange data on intrinsic and hazard properties of chemical substances. Report generator for Chemical Safety Report - CSR.

„Assessment entity“ concept – a feature for IUCLID 6

- Enable transparent reporting of hazard, use and exposure information for NM.

Multiple composition or multiple forms of the same substance.

Many constituents within the substance.

Variable composition of the substance.

Forming of transformation products on use.

ECHA - NMWG

Read across and grouping of NM

Colaboration of ECHA, JRC, NanoREG and RIVM.

Many nanoforms on the market:

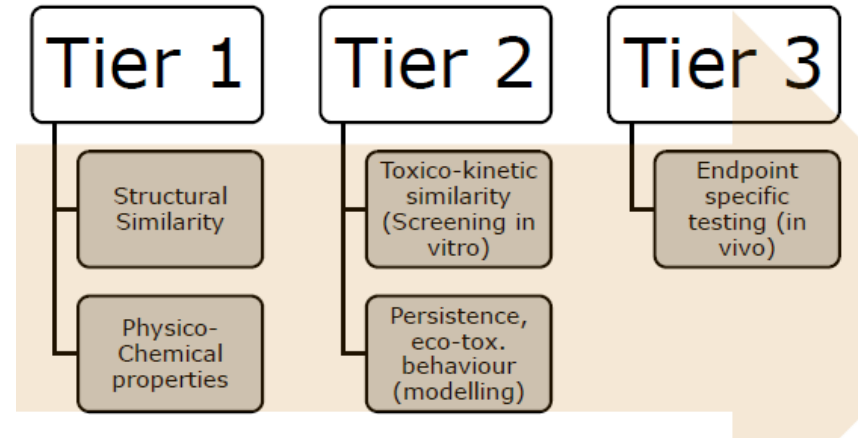
- Are studies from one form applicable to other nanoforms?
- How and when can data be used on nanoforms or beetwen non-nano and nanoforms?

Case studies presented;

Develop a decision framework;

Link to the OECD discussion;

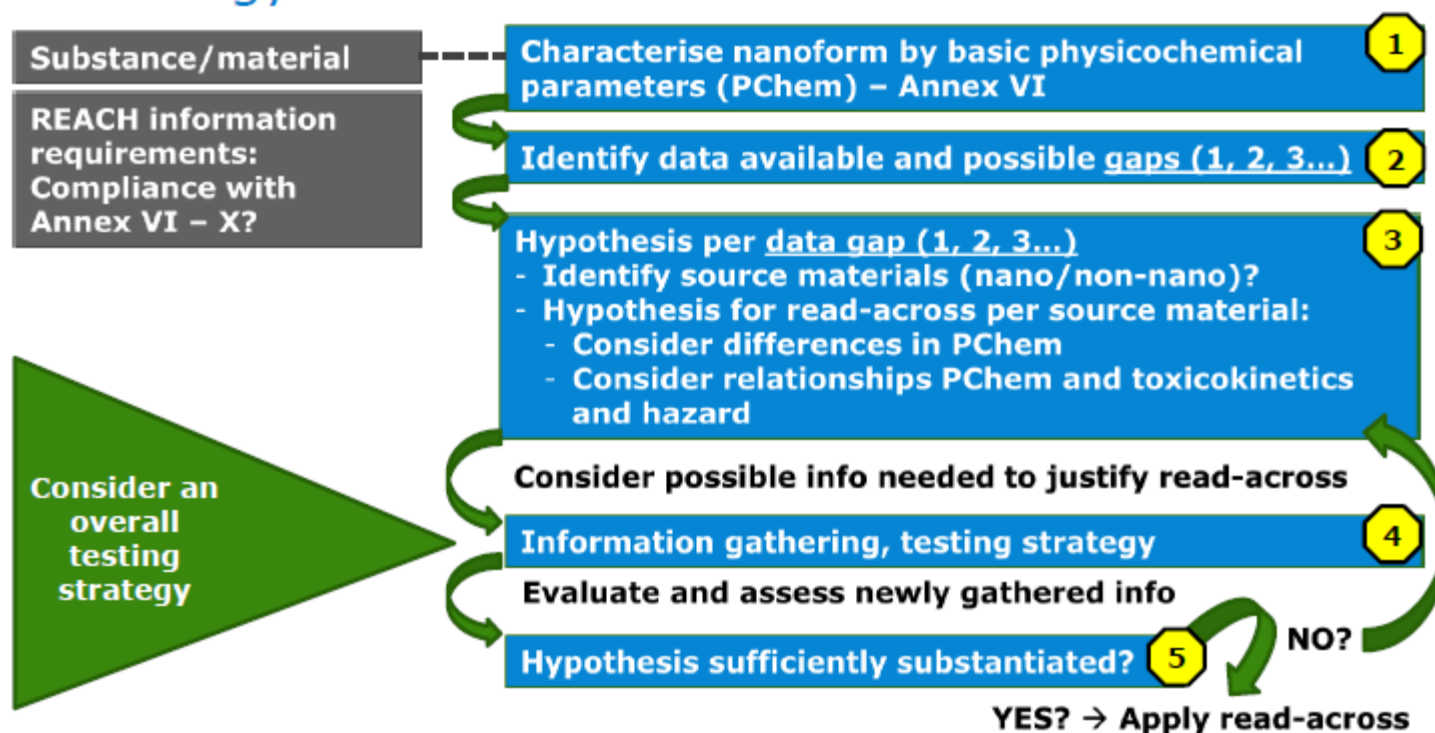
Template for reporting on read – across.



ECHA - NMWG

Read across and grouping of NM

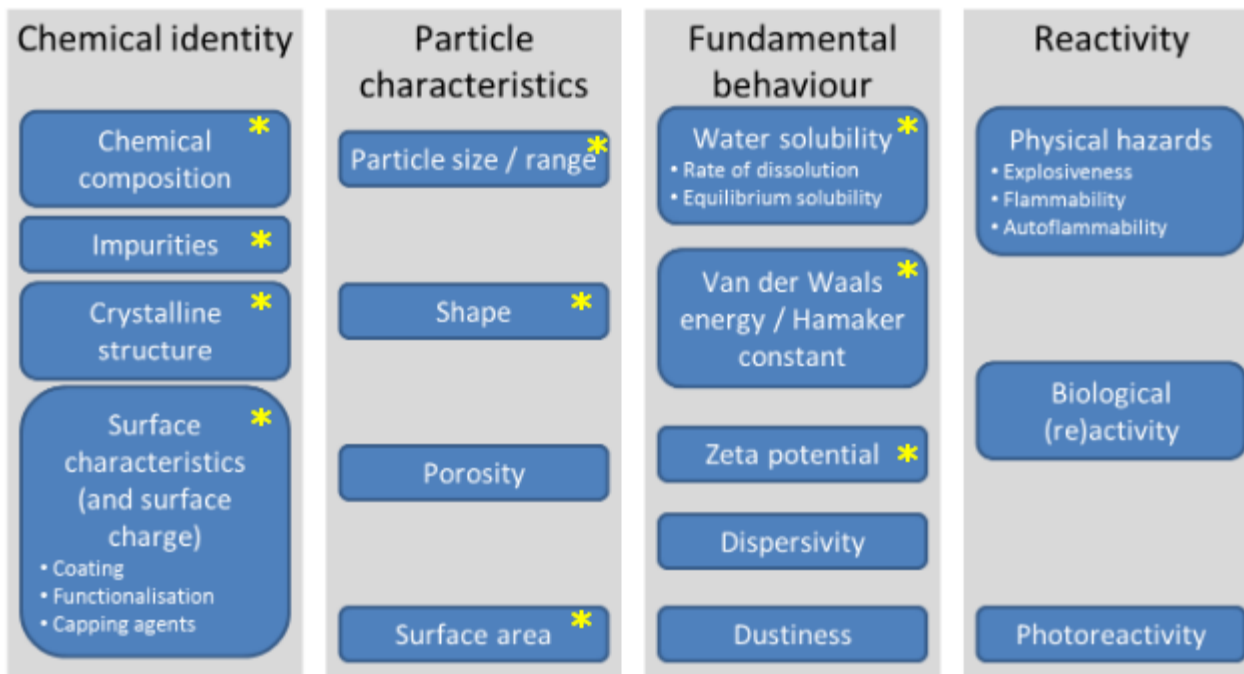
Strategy for read-across of nanomaterials



ECHA - NMWG

Read across and grouping of NM

Parameters influencing nanomaterial behaviour:



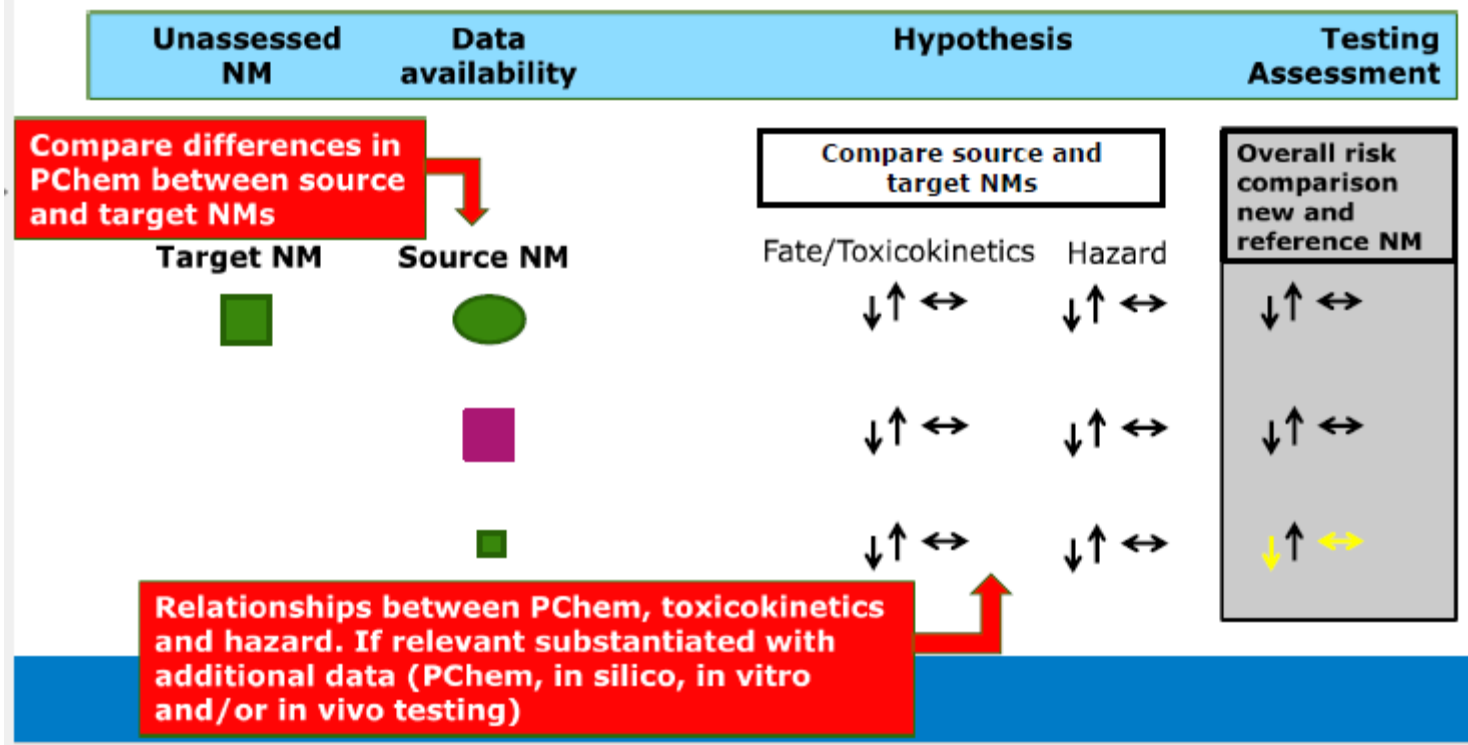
* Essential information to characterise nanomaterials

ECHA - NMWG

Read across and grouping of NM

Hypothesis on read across per data point

- Find source materials with information for same exposure route
- Assess:



ECHA - NMWG

Read across and grouping of NM

- The focus of the framework is on Pchem data as well as their relation to the different steps in the life cycle and biological pathway (exposure, kinetics, hazard).
- Identify Pchem that matter and provide strategy for systematic assessment.
- Needs to be validated in practice.
- Future implementation of growing experience and understanding on behaviour, fate, toxicokinetics and toxicity.

ECHA guidance will be developed on read across.

ECHA - NMWG

NMWG group

- until guidances for REACH and CLP are developed.

National Laboratory of Health, Environment and Food

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Challenges of Regulation and Risk Assessment of Nanomaterials

part.1

Karmen Krajnc, M.Sc.

Chemicals Office of the Republic of Slovenia

EU – strategic documents

- **Towards a European Strategy for Nanotechnology 2004**
 - **European Nanotechnology Action Plan 2005-2009**
 - Implementation Reports 2007, 2009
- **Code of conduct for nanotechnology research 2008**
- Communication from the Commission to the European Parliament, THE Council and the European Economic and Social Committee
2008 (first regulatory review)
2012 (second regulatory review)

http://ec.europa.eu/research/industrial_technologies/pdf/policy/communication-from-the-commission-second-regulatory-review-on-nanomaterials_en.pdf

Definition

(Commission Recommendation 2011/696)

- 'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for **50 %** or more of the particles in the number size distribution, one or more external dimensions is in the size range **1 nm-100 nm**.
- In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.
- By derogation, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:en:PDF>

- Consideration of possible changes to this definition

<https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/towards-review-ec-recommendation-definition-term-nanomaterial-part-3-scientific-technical>

Cosmetic regulation(1223/2009)

- definition: 'nanomaterial' means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.
- colorants, preservatives and UV-filters must be authorized (positive lists: Annexes IV, V and VI)
 - titanium dioxide (nano), zinc oxide (nano), tris-biphenyl triazine (nano) and carbon black (nano) shall be added shortly
- notifications of **safety information** for nano substances(art.16)
- **labeling: „nano“**

Biocidal regulation (528/2012)

- specific approval of nano form of biocidal substance (art. 4(4))
- labeling obligation for treated articles, art. 58(3):
the name followed by the word 'nano' in brackets
- nano-definition

REACH-nanomaterials registered

- Carbon black
- Cerium dioxide
- Calcium carbonate
- Zinc oxide
- Silver
- MWNT (multi-wall nanotubes)
- MWNT as a form of graphite
- Titanium dioxide
- Silicate(2-), hexafluoro-, disodium, reaction products with lithium magnesium sodium silicate

Substance evaluation

- **Substances selected for CoRAP** (Community rolling action plan) based on initial grounds of concern: evaluated by member states, coordinated by ECHA
- **Silicon dioxide** (synthetic amorphous silica - SAS) – the Netherlands, 2012
<http://echa.europa.eu/documents/10162/a94c8df7-81c5-4946-80ae-dfa9275897e1>
- **Silver** – the Netherlands, 2012 (ongoing)
- **Titanium dioxide** – France, 2015 (not started yet)

OECD Working Party on Nanomaterials

- TESTING PROGRAMME ON MANUFACTURED NANOMATERIAL

<http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials.htm>

- Cerium oxide
- Multi-walled carbon nanotubes (MWCNTs)
- Single-walled carbon nanotubes (SWCNTs)
- Dendrimers
- Nanoclays
- Fullerenes (C₆₀)
- Silicon dioxide
- Gold nanoparticles
- Silver nanoparticles
- Titanium dioxide, Zinc oxide (not publicly available yet)

REACH NANO legislative challenges

- Definition of „nanomaterial“
- REACH Annexes (registration purposes)
- Discussion on possible EU NANO DATABASE

Chemicals Office of the RS-nano activities

- Nanoportal
- Identified 30 companies (producers, users of nanosubstances)
 - Ag, Zn, Al, TiO₂, soot, ZnO, SiO₂, Fe.H₂SO₄, Si, Al₂O₃, graphite-C, SiC, Cu, CdS, FeCl₃, B, Fe, Cr, CuO, MoSi, MoS₂, Wox
 - Coatings/Surface modification,
 - Pigments,
 - Polymers/Composits,
 - Cosmetic,
 - Medical devices & Medicinal products,
 - Textiles,
 - Electronics.
- Awareness raising

Nanoportal

<http://www.uk.gov.si/>

www.uk.gov.si

AKTUALNO

Nanoportal



Z namenom okrepitve povezav med slovensko industrijo in številnimi priznanimi slovenskimi znanstveniki, ki se pri nas ukvarjajo s posameznimi vidiki nanomaterialov in nanovarnosti, smo v sodelovanju z slovenskimi raziskovalci, pripravili NANOPORTAL.

Portal je sestavljen iz dveh delov:

- [Splošna predstavitev področja, skupaj s koristnimi povezavami.](#)
- [Seznam več kot 100 slovenskih raziskovalcev, vključno z navedbo področja njihovega delovanja.](#)

Načrtujemo, da se bo portal sproti dopolnjeval, vse morebitne predloge za spremembe lahko posredujete na URSK, mag. Karmen Krajnc (tel: 478 6054, mail: [karmen.krajnc\(at\)qgov.si](mailto:karmen.krajnc(at)qgov.si))

DRUGE VSEBINE

[Registracija, evalvacija,](#)

[Razvrščanje, pakiranje in](#)

[Biocidni proizvodi](#)

Elektronsko sporočanje podatkov o kemikalijah



Zavezancem za sporočanje podatkov o kemikalijah na podlagi 35. člena Zakona o kemikalijah in Pravilnika o sporočanju podatkov o kemikalijah je na voljo spletna aplikacija ISK za elektronsko sporočanje podatkov. Aplikacija ISK omogoča pregled, vnos, spreminjanje in ukinjanje sporočil o kemikalijah ter pregled in vnos letnih količin o kemikalijah. Več o elektronskem sporočanju podatkov, pogojih za uporabo aplikacije, načinu registracije uporabnikov in uporabi aplikacije najdete na zavihku [e-sporočanje](#).

NOVICE

22. 9. 2015

[Objava prostega delovnega mesta projektne svetovalca Twinning projekta v Beogradu](#)

3. 9. 2015

[Javni posvet glede identifikacije snovi, ki vzbujajo veliko zaskrbljenost \(SVHC\)](#)

2. 9. 2015

[Registracija za uporabnike anhidrida očetne kisline](#)

[Več](#)

DOGODKI

27. 5. 2015

[Predstavitve s seminarja "Strokovno srečanje svetovalcev za kemikalije", 20.05.2015](#)

13. 4. 2015

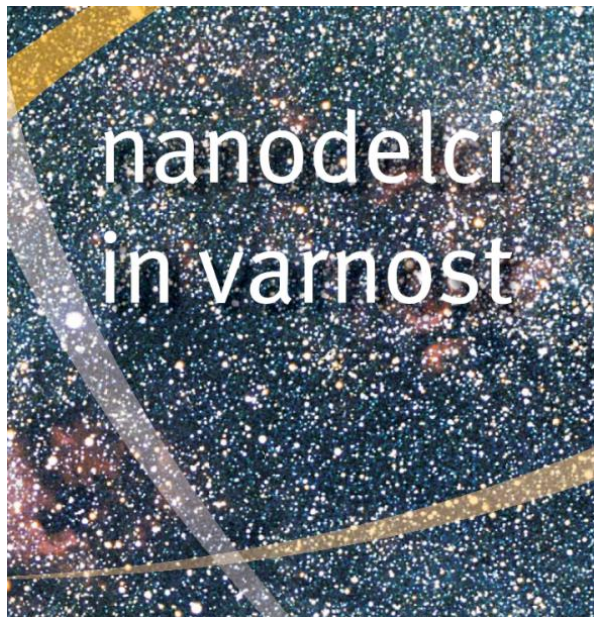
[Strokovno srečanje svetovalcev za kemikalije, 20.05.2015](#)

2. 4. 2015

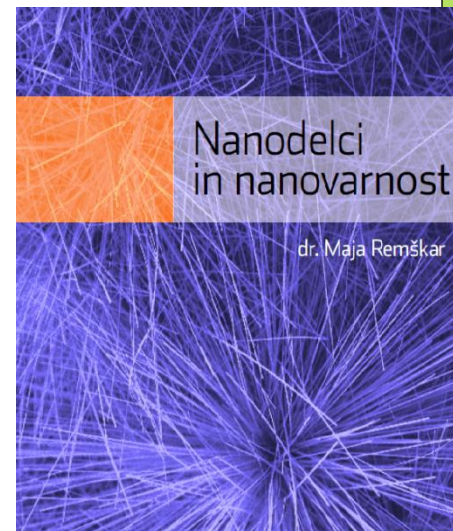
[Biocidni simpozij 2015](#)

A21		Institut "Jožef Stefan"		
A		B	C	D
Raziskovalna organizacija		Kontaktna oseba	E-mail	Nanomateriali
Cinkarna Celje		Dejan Verhovšek	dejan.verhovsek(at)cinkarna.si	TiO2
Cinkarna Celje		Nika Veronovski	nika.veronovski(at)cinkarna.si	TiO2
Helios		Znoj Bogdan	bogdan.znoj(at)helios.si	nanoclay
Industrijski razvojni center slovenske predilne industrije		Žlabravec Verica	vera.zlabravec(at)litija.com	nanodelci na tekstilu
Institut "Jožef Stefan"		Bernik Slavko	slavko.bernik(at)ijs.si	ZnO
Institut "Jožef Stefan"		Gyergyek Sašo	saso.gyergyek(at)ijs.si	magnetni nanodelci
Institut "Jožef Stefan"		Jesih Adolf	adolf.jesih(at)ijs.si	Molx
Institut "Jožef Stefan"		Kobe Spomenka	spomenka.kobe(at)ijs.si	SiC
Institut "Jožef Stefan"		Kralj Slavko	slavko.kralj(at)ijs.si	magnetni nanodelci
Institut "Jožef Stefan"		Kutnjak Zdravko	zdravko.kutnjak(at)ijs.si	MoS2
Institut "Jožef Stefan"		Lisjak Darja	darja.lisjak(at)ijs.si	feriti
Institut "Jožef Stefan"		Makovec Darko	darko.makovec(at)ijs.si	magnetni nanodelci
Institut "Jožef Stefan"		Milošev Ingrid	ingrid.milosev(at)ijs.si	nanodelci kovinskih implantov
Institut "Jožef Stefan"		Mozetič Miran	miran.mozetic(at)ijs.si	FeOx, NbOx
Institut "Jožef Stefan"		Mrzel Aleš	ales.mrzel(at)ijs.si	MoSi
Institut "Jožef Stefan"		Muševič Igor	igor.musevic(at)ijs.si	SiO2
Institut "Jožef Stefan"		Novak Saša	sasa.novak(at)ijs.si	SiC
Institut "Jožef Stefan"		Panjan Peter	peter.panjan(at)ijs.si	TiAlN/CrN
Institut "Jožef Stefan"		Pribošič Irena	irena.pribosic(at)ijs.si	KNbO3
Institut "Jožef Stefan"		Remškar Maja	maja.remskar(at)ijs.si	MoS2
Institut "Jožef Stefan"		Suvorov Danilo	danilo.suvorov(at)ijs.si	funkcionalni in bio nanodelci - ele
Institut "Jožef Stefan"		Škapin D. Srečo	sreco.skapin(at)ijs.si	poly(d,1-lactide-co-glycolide)/hy
Institut "Jožef Stefan"		Štrancar Janez	janez.strancar(at)ijs.si	TiO2
Institut "Jožef Stefan"		Vaupotič Janja	janja.vaupotic(at)ijs.si	detekcija aerosolov (5-1100 nm)
Institut "Jožef Stefan"		Vesel Alenka	alenka.vesel(at)ijs.si	Fe2O3
Institut "Jožef Stefan"		Vukomanović Marija	marija.vukomanovic(at)ijs.si	Au
Inštitut za kovinske materiale in tehnologije		Jenko Monika	monika.jenko(at)imt.si	FeOx
Kemijski inštitut		Bele Marjan	marjan.bele(at)ki.si	Carbon NP , TiO2, SiO2, LiMPO4 (M
Kemijski inštitut		Crnjak Orel Zorica	zorica.crnjak.orel(at)ki.si	Cu/ZnO, CuO/ZnO, Pd/CuO/ZnO, f
Kemijski inštitut		Dominko Robert	robert.dominko(at)ki.si	LiMPO4 (M=Mn,Fe) Li2FeSiO4, Car
Kemijski inštitut		Dražić Goran	goran.drazic(at)ki.si	TiO2
Kemijski inštitut		Gaberšček Miran	miran.gaberscek(at)ki.si	Carbon NP , TiO2, SiO2, LiMPO4 (M

Awareness raising



OGNJEMETI in druga zabavna
PIROTEHNIKA
ZASTRUPAJO OZRAČJE



- ◉ http://www.kemijskovaren.si/files/nano_knjiga.pdf

Additional links:

- http://ec.europa.eu/nanotechnology/index_en.html,
- http://ec.europa.eu/environment/chemicals/nanotech/index_en.htm
- <http://echa.europa.eu/regulations/nanomaterials>
- <https://ec.europa.eu/jrc/en/scientific-tool/jrc-web-platform-nanomaterials>
- <https://ec.europa.eu/jrc/en/scientific-tool/nanohub>
- http://ec.europa.eu/health/nanotechnology/policy/index_en.htm

Thank you for
your attention

<http://www.uk.gov.si/>

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<http://www.ki.si>

Chemometric analysis and QSAR modelling in NANO-toxicology

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Chemometric analysis and QSAR modelling in NANO-toxicology

Outline

1. Introduction
2. New toxicological paradigm
3. QSAR (**Q**uantitative **S**tructure-**A**ctivity **R**elationship) working scheme and strategy
4. NANO-descriptors
5. Chemometrical analysis of proteomic data

New paradigm in toxicology...

Adverse Outcome Pathways

Is a sequential chain of causally related events leading to the toxic effect...

Exposure:
Function of
Dose and time

Molecular
event

Organelle
event

Cellular
event

Tissue
event

Organ
event

Organism
event

Population
event

In chemico

In vitro

In vivo

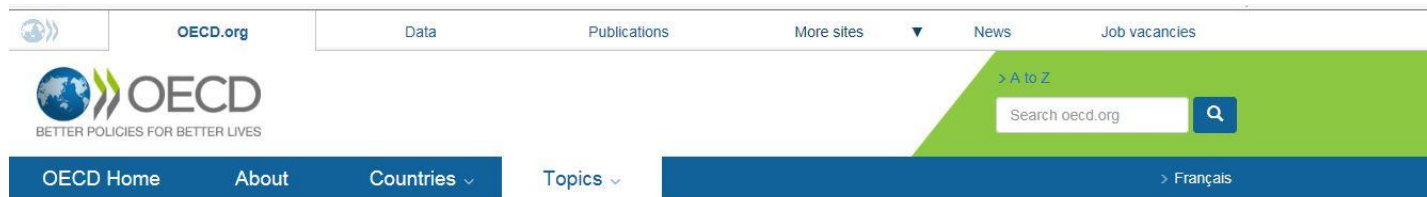
IN SILICO

*Clinical, Epidemiological
observations*

DOCKING, QSAR, POPULATION ANALYSIS

OECD document...

Adverse Outcome Pathway (AOP)



OECD Home > Chemical safety and biosafety > Testing of chemicals > Adverse Outcome Pathways, Molecular Screening and Toxicogenomics



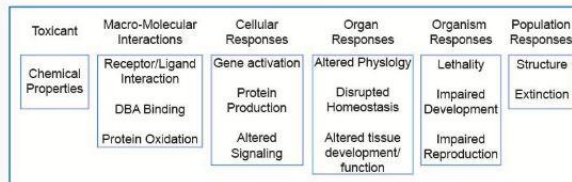
Adverse Outcome Pathways, Molecular Screening and Toxicogenomics

- [Documents](#)
- [How to make a project proposal?](#)
- [Process for the development of AOP at OECD](#)
- [List of projects on the AOP development programme workplan](#)
- [AOP wiki](#)
- [Other activities on Molecular Screening and Toxicogenomics](#)

What is an Adverse Outcome Pathway (AOP)

In 2012, the OECD launched a new programme on the development of Adverse Outcome Pathways. An Adverse Outcome Pathway (AOP) is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect (see figure). AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

Figure: schematic representation of the Adverse Outcome Pathway (AOP) illustrated with reference to a number of pathways.



The AOP development programme addresses the needs of:

- the OECD [Test Guidelines Programme](#) for the identification of new *in vitro* test methods that are candidates to become OECD Test Guidelines;
- The OECD [QSAR Project](#) for the identification of new methods/profilers for grouping chemicals, and;
- the OECD [Hazard Assessment activities](#) for the development of Integrated Approaches to Testing and Assessment (IATA), also known as Integrated Testing Strategies, for defined hazard endpoints.

The OECD co-ordinates its activities with the [WHO/IPCS work on Mode of Action](#), as the AOP concept and the Mode of Action are closely related.

Documents

[Guidance, template, format available](#)

[AOP project proposal form](#)

Workshop on COMPUTATIONAL APPROACHES IN NANOSCIENCES
„COMPInNANO“, Ljubljana, 2. - 3. October 2015

Example: skin sensitization (chemicals)...

ENV/JM/MONO(2012)10/PART1

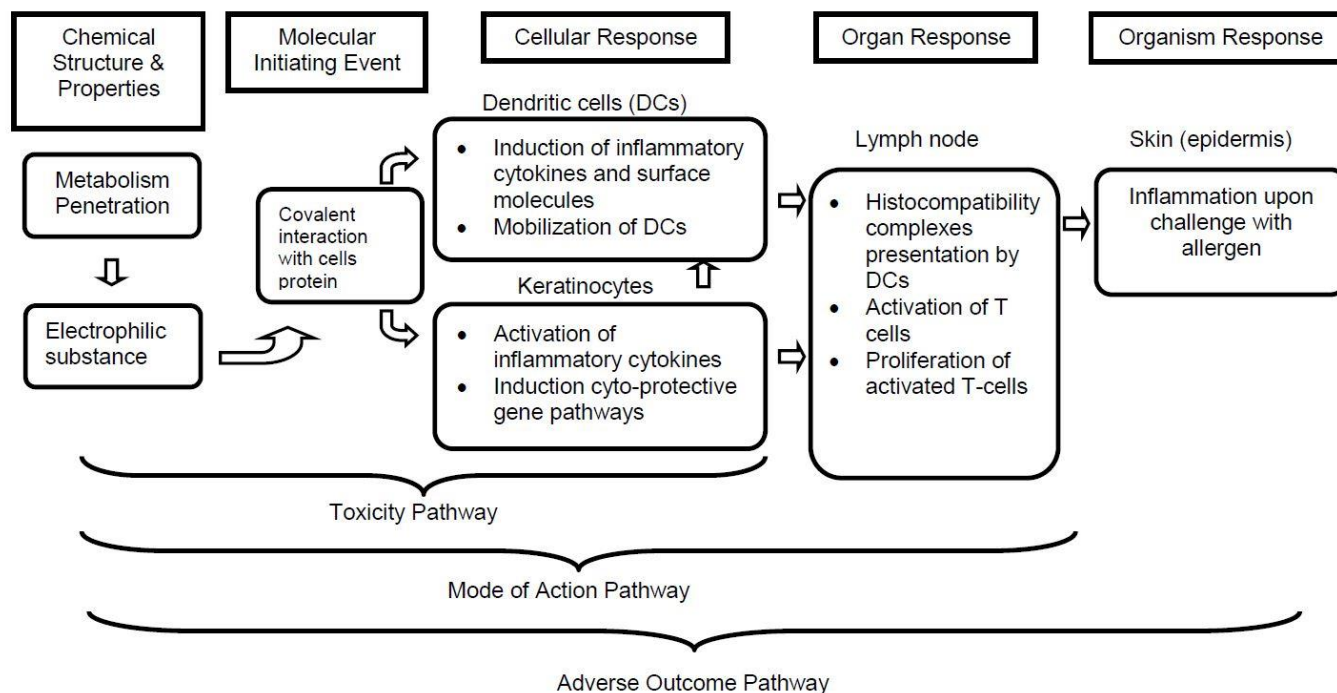
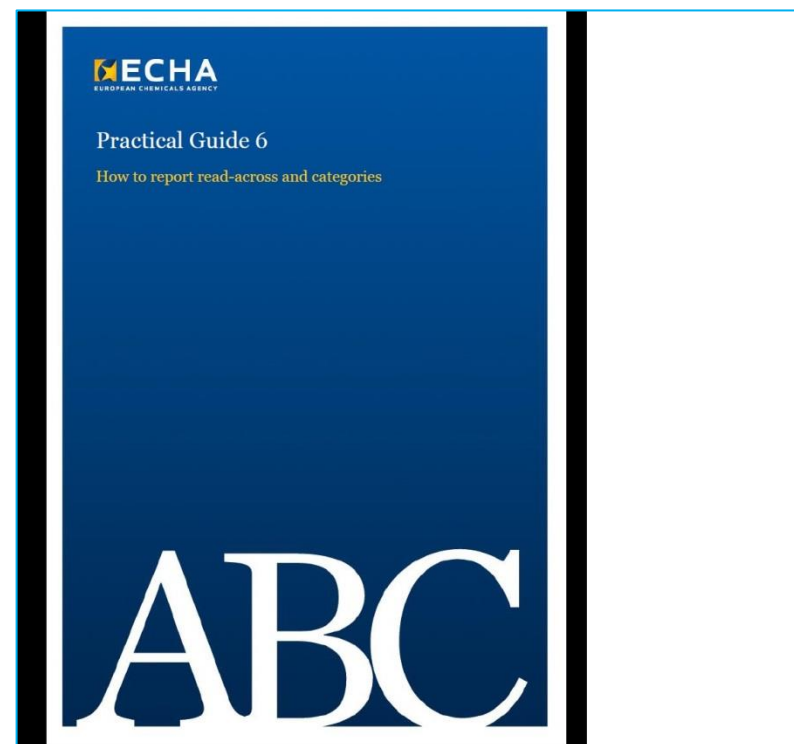
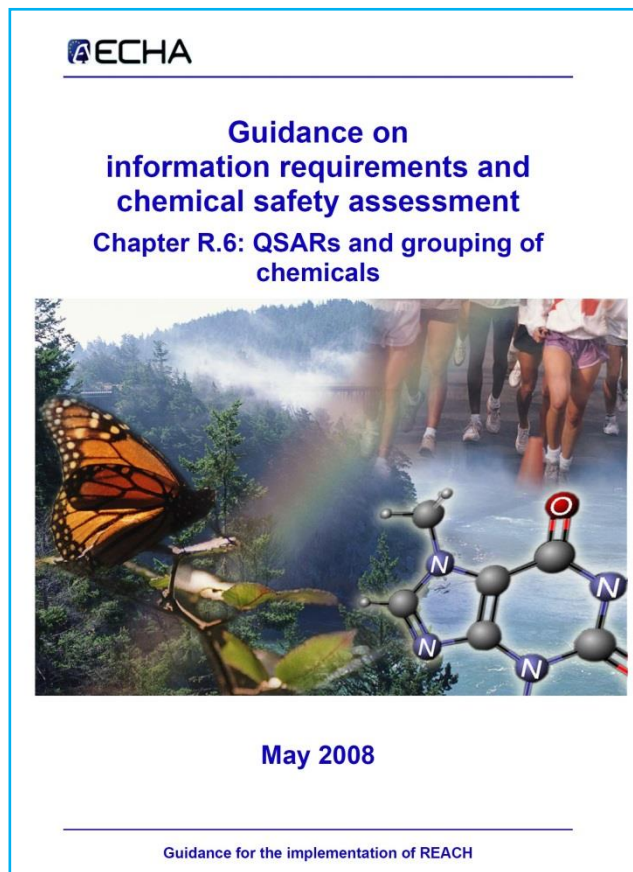


Figure 3. Flow diagram of the pathways associated with skin sensitisation.

In silico methods...

- *In silico* methods are important to acquire the data for AOP scheme...
- Under term *In silico* we understand different computational modelling approaches; molecular mechanics, dynamics, **QSAR**, modeling of ADME properties, and also **chemometrical analysis of (proteomics, genomics, metabonomics) data,...**
- **MOLECULES - NANO particles?**

QSAR, Read across (grouping) in REACH and ECHA



NANO and EU



Brussels, 3.10.2012
SWD(2012) 288 final

COMMISSION STAFF WORKING PAPER

Types and uses of nanomaterials, including safety aspects

Accompanying the

**Communication from the Commission to the European Parliament, the Council and the
European Economic and Social Committee**

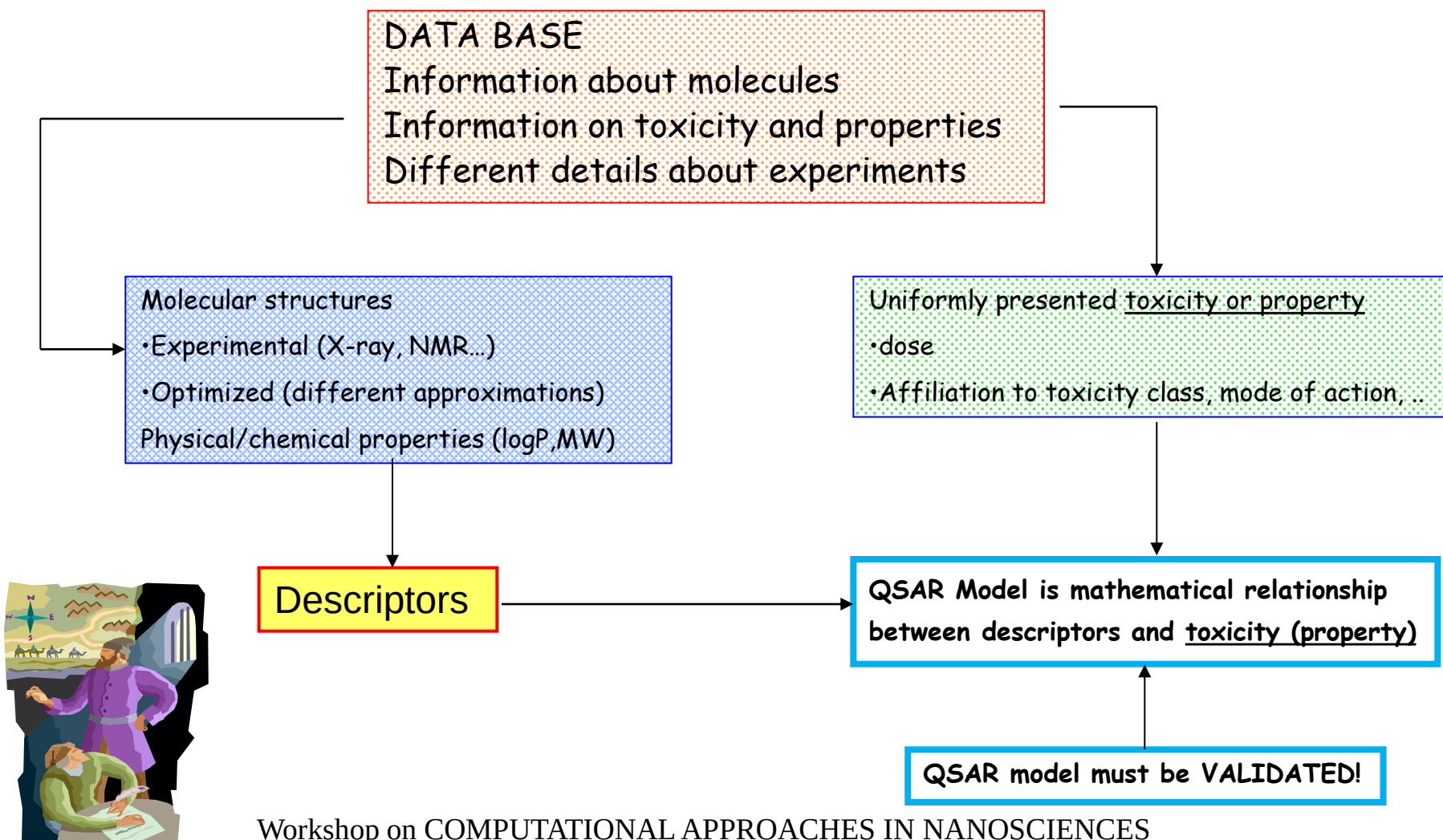
on the Second Regulatory Review on Nanomaterials

{COM(2012) 572 final}

Workshop on COMPUTATIONAL APPROACHES IN NANOSCIENCES
„COMPInNANO“, Ljubljana, 2. - 3. October 2015

Scheme of QSAR - strategy

How to extract the hidden knowledge from DATA SET



Scheme of QSAR – strategy

How to extract the hidden knowledge from DATA SET?

QSAR hypothesis: The property is a function of chemical structure!

The validated QSAR model can be used to predict the property for a '**new - hypothetical**' compound.

ONLY FOR THE PROPERTY FOR WHICH IT WAS TRAINED.



Two main application areas of QSAR models

Active substances (drug) research:

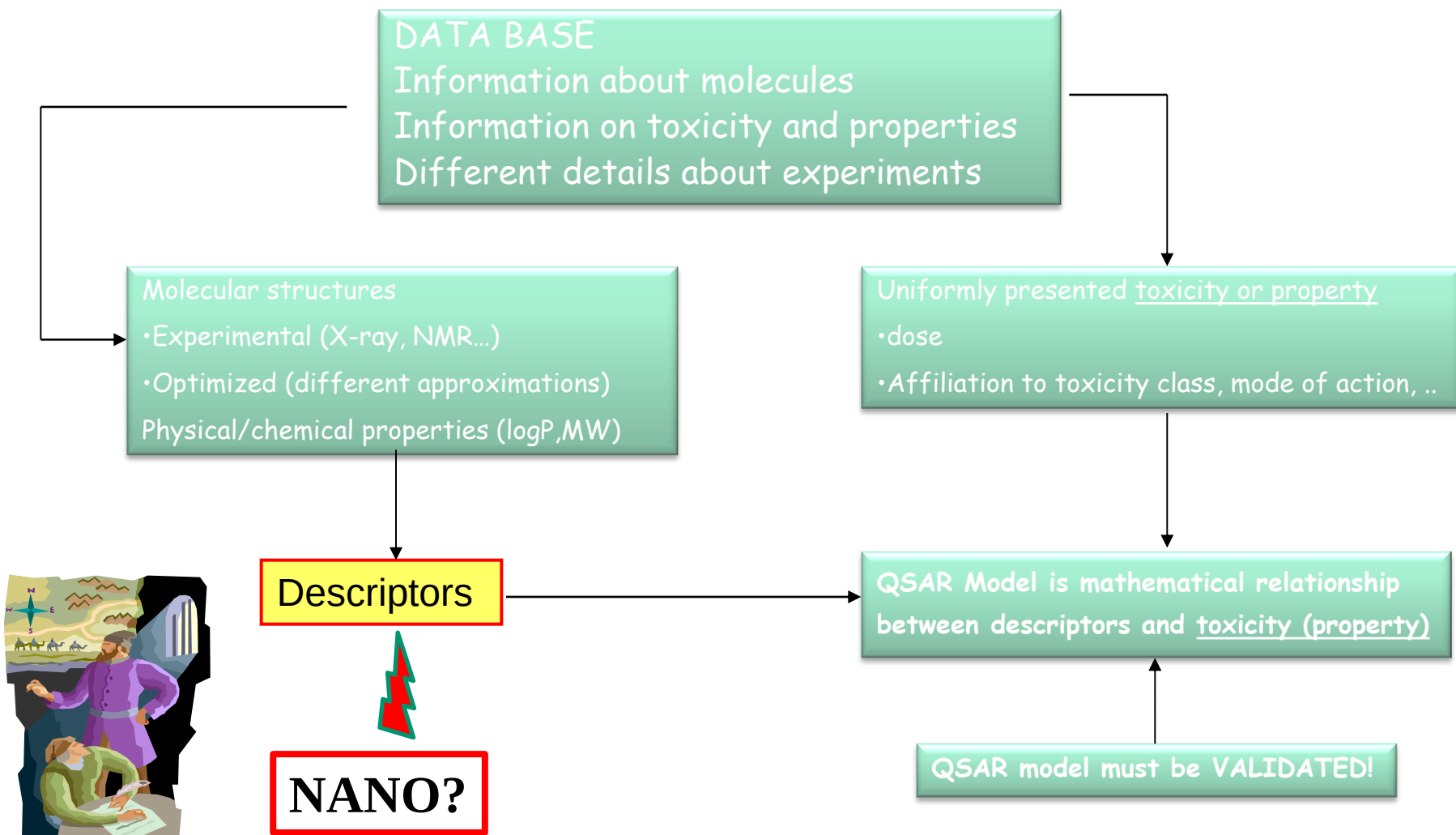
1. Searching for new lead compounds
2. Searching for better analogues

Regulatory assessment:

1. Predicting of 'missing' values for risk assessment
2. Categorisation of compounds for labeling
3. Priority setting

NANO: Scheme of QSAR - strategy

How to extract the hidden knowledge from DATA SET



Molecules – NANO particles ??

The properties of NANO particles (strongly) depend on their the size and the shape.

An accent is placed is on *descriptors*.

Gold nanoparticles are red to black.

Red glass (Wikipedia):

First produced in late Roman Empire.

The knowledge was lost and rediscovered in the 17th century by either Johann Kunckel in Potsdam or by the Florentine glassmaker Antonio Neri in Italy.

Chemist and winner of the 1925 Nobel Prize in Chemistry Richard Adolf Zsigmondy was able to understand and explain that small colloids of gold were responsible for the red colour.

Representation of a molecule - *How to apply for NANO?*

Different levels of the representation of molecules:

- 1D - Information on constituents (which atoms, or which groups of atoms)
- 2D - Structural formula gives information on atoms and bonds between atoms, but no information on metrical parameters (distances between atoms, angles between bonds)
- 3D - Coordinates of all atoms (information on all metrical properties)
- Quantum chemical descriptors are calculated from QC results - they describe the electronic properties
- Structure can be described by fragments
- Etc.



1D descriptors

Molecules (constitutional):

Information on constituents:

- Number of atoms
- Number of particular atom groups (fragments)
- ...

NANO particles:

- Size
- Shape
- Chemical constitution
- ...



2D descriptors - Topological indices

Topological indices are numbers deduced from structural formula of a molecule (2D representation).

NANO particles:

- Shape indices
- Analysis from electron microscopy pictures.

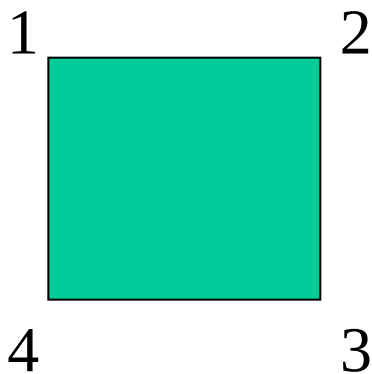
Ghorbani, Modjtaba. Computing Wiener index of C-24 fulleren.

Journal Of Computational And Theoretical Nanoscience, 2015,12, 1847-1851.

Example:

Wiener index (name proposed by H. Hosoya) is an integer number deduced from structural formula (graph).

Wiener index for cyclobutane:



<u>Molecule</u>	<u>W</u>
Methane	0
Propan	4
Cyclopropane	3
N-butane	10
Isobutane	9
Cyclobutane	8

$$\begin{aligned} W &= d_{12} + d_{13} + d_{14} + d_{23} + d_{24} + d_{34} \\ &= 1 + 2 + 1 + 1 + 2 + 1 = 8 \end{aligned}$$

Wiener index has a high degeneracy: different non-isomorphic graphs have the same W.

Attempts to lower the degree of degeneration:

- Hyper-Wiener index
- Szeged index
- Three-dimensional Wiener index
- Hosoya's index Z

Second generation topology indices

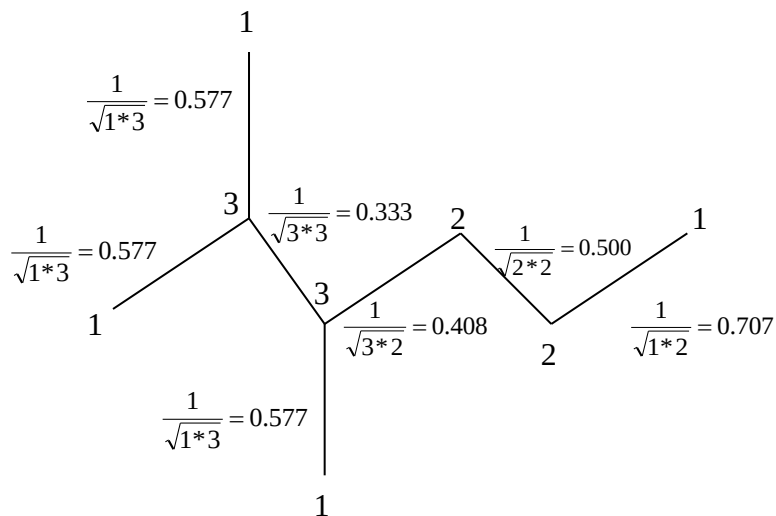
They are real numbers deduced from graphs. One of the most successful is molecular connectivity index χ proposed by Randic:

$$\chi = \sum_{ij} (v_i v_j)^{-1/2}$$

Sum runs over edges (bonds), v is the vertex degree on the endpoints.

Calculation of connectivity index for 3-methylheptane

$$\chi = \sum_{ij} (v_i v_j)^{-1/2}$$



$$\chi = 3 \cdot 0.577 + 0.333 + 0.408 + 0.500 + 0.707 = 3.679$$

3D descriptors

Three-dimensional structure of molecules is not unanimously defined.

Rigid molecules are rare, most of the molecules are flexible.

A molecule can have a different 3D structure *in vacuo*, in crystalline form, in water environment, or in protein environment.

Experimental determinations:

- X ray diffraction measurements
- 2D-NMR measurements - method enables determination of ligand-receptor geometry

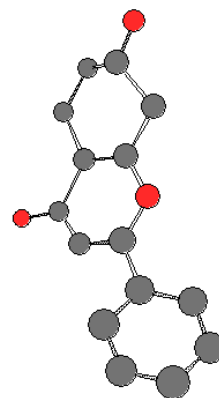
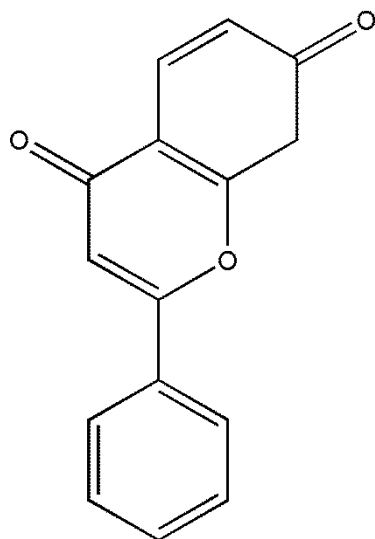
Theoretical determination:

- Quantum chemical optimization
- Molecular dynamics

Result (3D structure) depends on the selected method.



A Flavonoid derivate: 3D structure determinated with rule based generator

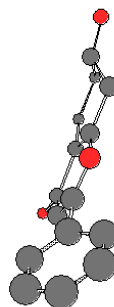


Tautomer of 7-hydroxy-2-phenyl-4-benzopyrone

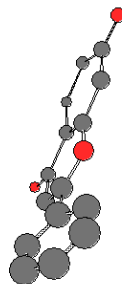
C1(=CC=CC=C1)C3=CC(C2=C(CC(C=C2)=O)O3)=O

A Flavonoid derivate: 3D structure determinated with rule based generator or with Molecular Mechanics Optimization

Rule based system



MM optimization



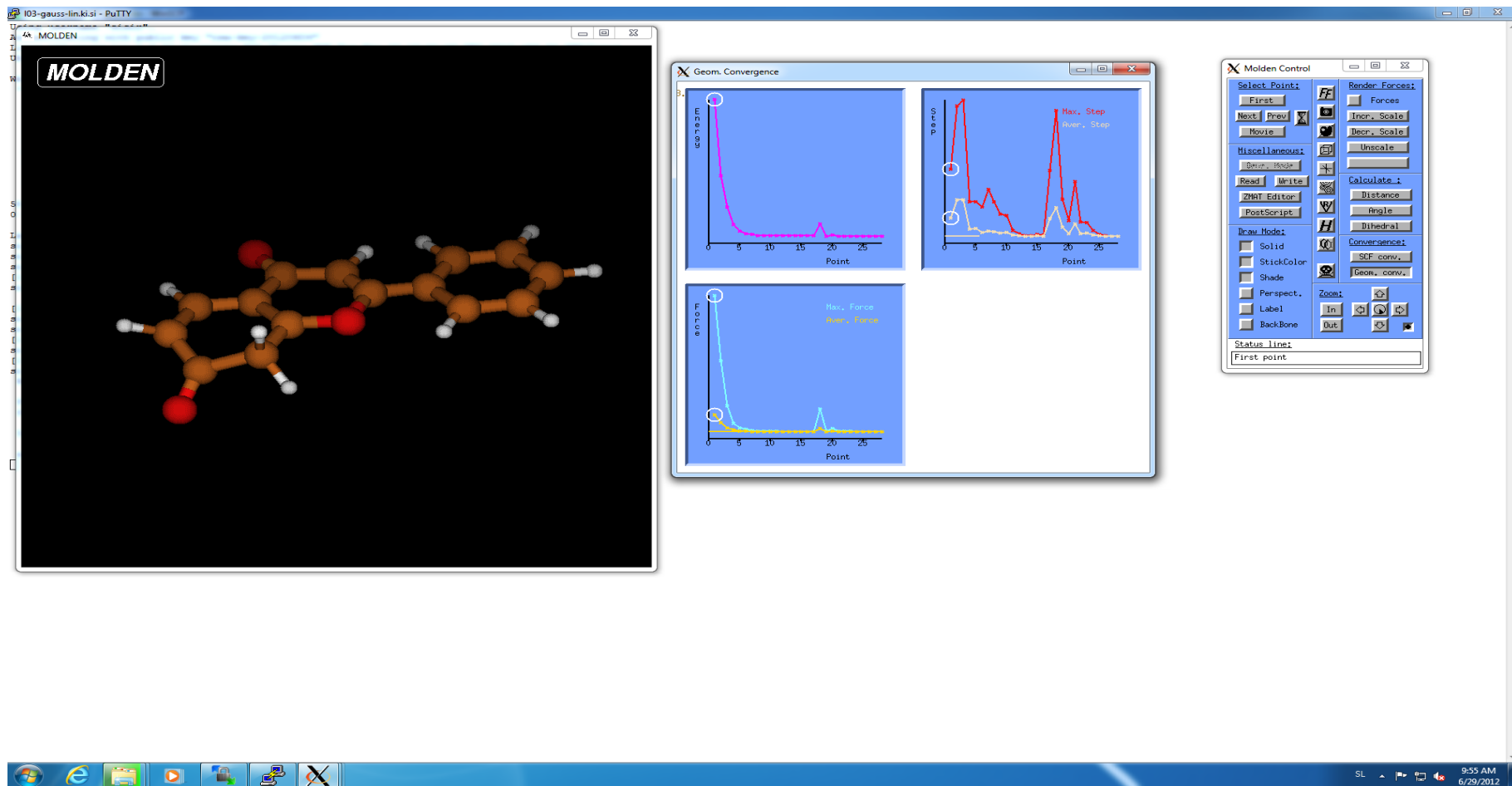
A Flavonoid derivate: 3D structure determinated with rule based generator or
with QC program GAUSSIAN (HF-6-31g approximation)
Input

```
# HF/6-31G(d) opt

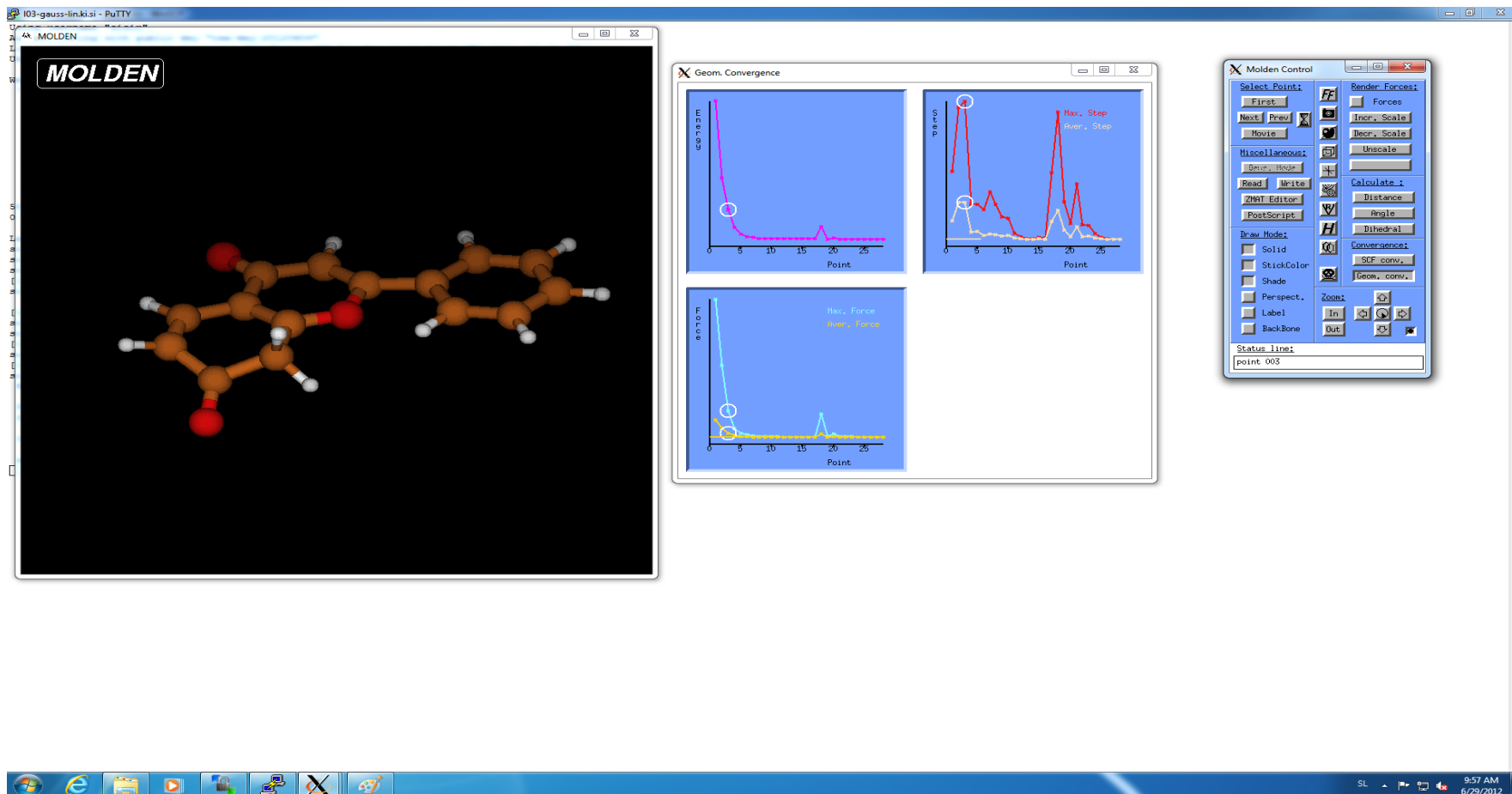
Test1

0 1
C 0 -0.809889 -2.481124 0.027570
C 0 -0.809889 -3.818124 0.027570
C 0 0.347987 -4.486624 0.027570
C 0 1.505863 -3.818124 0.027570
C 0 1.505863 -2.481124 0.027570
C 0 0.347987 -1.812624 0.027570
C 0 0.347987 -0.475624 0.027570
O 0 -0.853279 0.151261 0.027570
C 0 -0.722044 1.499890 0.027570
C 0 0.450229 2.142809 0.027570
C 0 1.574749 1.394039 0.027570
C 0 1.371375 0.058435 0.027570
C 0 -2.020291 2.245149 0.039936
C 0 -1.793136 3.659064 -0.435801
C 0 -0.663327 4.173114 0.051266
C 0 0.479580 3.479435 0.039418
O 0 2.679662 1.882333 0.027570
O 0 -2.545154 4.254249 -1.170300
H 0 -1.762517 -1.931124 0.027570
H 0 -1.762517 -4.368124 0.027570
H 0 0.347987 -5.586624 0.027570
H 0 2.458491 -4.368124 0.027570
H 0 2.458491 -1.931124 0.027570
H 0 2.266345 -0.581120 0.027570
H 0 -2.427220 2.263510 1.075717
H 0 -2.745599 1.738385 -0.635258
H 0 -0.671772 5.189531 0.471773
H 0 1.444041 4.008410 0.039418
```

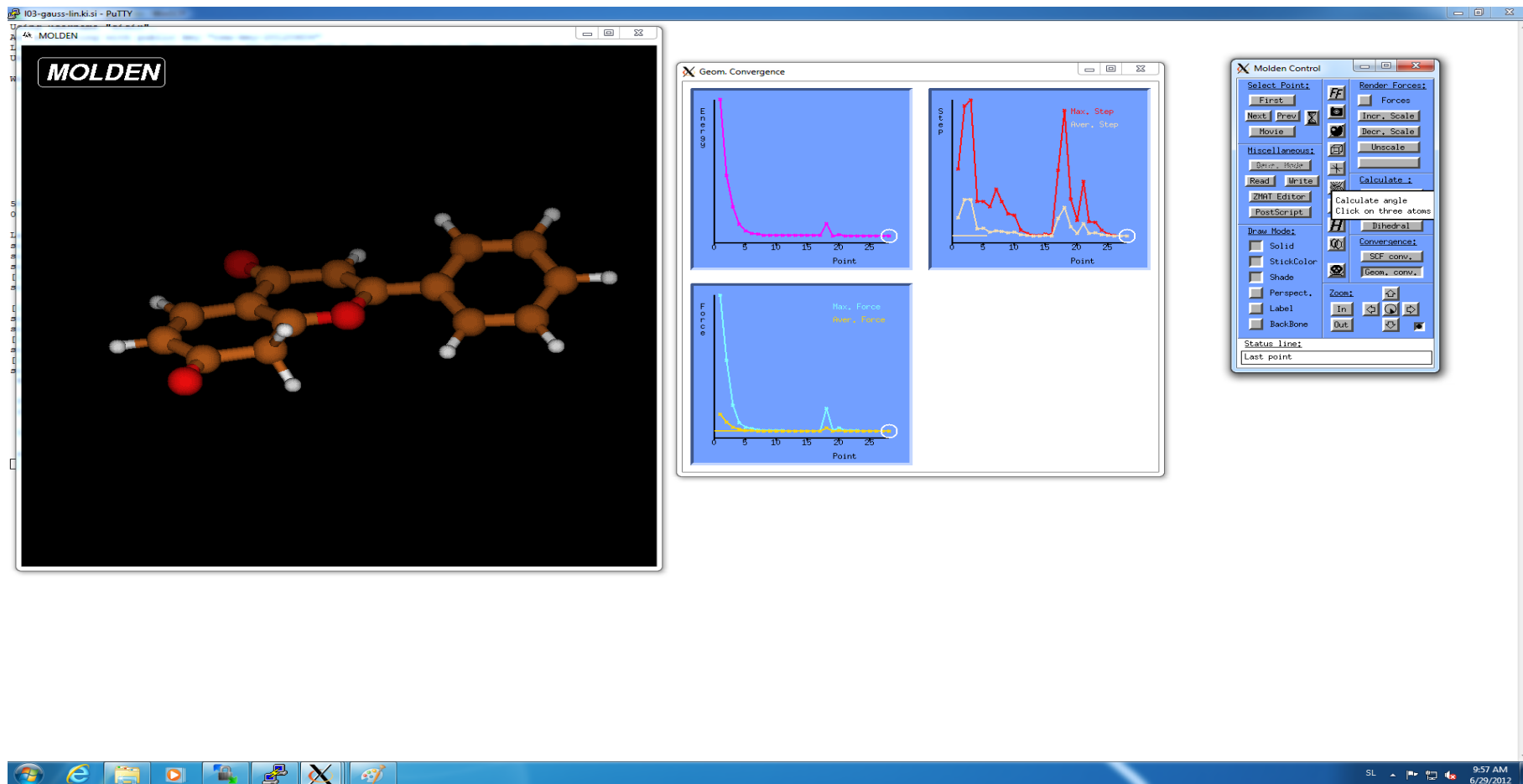
A Flavonoid derivate: 3D structure determinated with rule based generator or
with QC program GAUSSIAN (HF-6-31g approximation)
Original



A Flavonoid derivate: 3D structure determinated with rule based generator or
with QC program GAUSSIAN (HF-6-31g approximation)
Midle point



A Flavonoid derivate: 3D structure determinated with rule based generator or
with QC program GAUSSIAN (HF-6-31g approximation)
Final



Quantum chemical descriptors

HF calculations are time consuming. For our molecule from Gaussian output:

•
•

ON A TOMBSTONE, "HERE LIES LESTER MOORE,
FOUR SLUGS FROM A 44, NO LES, NO MORE".

Job cpu time: 0 days 1 hours 26 minutes 23.4 seconds.

File lengths (MBytes): RWF= 58 Int= 0 D2E= 0 Chk= 6 Scr= 1

Normal termination of Gaussian 09 at Thu Jun 28 17:48:11 2012.

Approximations to HF (semiempirical methods) : AM1, CNDO, MINDO, etc.

Quantum chemical descriptors

They are calculated from eigenvalues and eigenvectors of Hartree-Fock equation, or alternatively, from an approximation to it (AM1, CNDO, MINDO, etc.)

Eigenvalues are molecular orbital energies. According Koopmans' theorem:

Ionisation potential = $-E_{\text{HOMO}}$

Electron affinity = $-E_{\text{LUMO}}$

Gap = $E_{\text{LUMO}} - E_{\text{HOMO}}$

Eigenvalues are used to calculate the charge distribution and descriptors related to it (dipole moment and higher moments).

Example: CODESSA calculates about 300 quantum chemical descriptors.

Quantum chemical descriptors

Gaussian output

Our molecule:

1. Optimized geometry
2. $E_{\text{HOMO}} = -0.33343$ Hartree, $E_{\text{LUMO}} = 0.06510$ Hartree
3. **Multipole moments:**

Electronic spatial extent (au): $\langle R^2 \rangle = 5332.5111$

Charge= 0.0000 electrons

Dipole moment (field-independent basis, Debye):

X=	5.2339	Y=	-1.4411	Z=	0.0844	Tot=	5.4293
----	--------	----	---------	----	--------	------	--------

Quadrupole moment (field-independent basis, Debye-Ang):

XX=	-101.3395	YY=	-116.5999	ZZ=	-103.6957
XY=	-7.8858	XZ=	1.5863	YZ=	4.5278

Traceless Quadrupole moment (field-independent basis, Debye-Ang):

XX=	5.8722	YY=	-9.3882	ZZ=	3.5160
XY=	-7.8858	XZ=	1.5863	YZ=	4.5278

Octapole moment (field-independent basis, Debye-Ang**2):

XXX=	127.9448	YYY=	-41.1843	ZZZ=	1.2466	XXY=	66.4476
XXY=	59.6734	XXZ=	2.9705	XZZ=	-14.6642	YZZ=	-3.5759
YYZ=	-1.1079	XYZ=	6.4639				

Hexadecapole moment (field-independent basis, Debye-Ang**3):

XXXX=	-5387.5410	YYYY=	-1605.9836	ZZZZ=	-157.2575	XXXY=	-384.5756
XXXZ=	75.5886	YYXX=	-15.4537	YYYZ=	32.9132	ZZZX=	-3.2400
ZZZY=	-4.7132	XXYY=	-1293.9863	XXZZ=	-1012.2994	YYZZ=	-250.1014
XXYZ=	57.5382	YYXZ=	-5.9352	ZZXY=	21.7033		

Etc.

Quantum chemical descriptors in NANO

A promised area in NANO QSAR, possible descriptors:

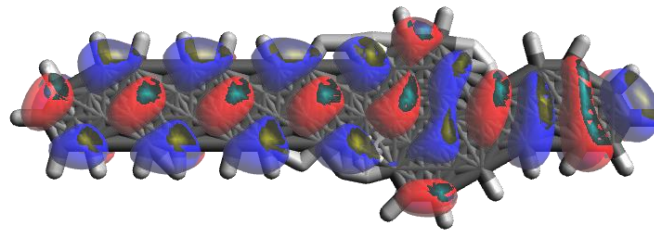
- gaps,
- proton, electron affinity,
- surface charges,
- electronic properties related to reactivity (formation of metal cations).

Problems:

- NANO particles are large, poorly defined systems. *Ab initio* calculations require large scale computer abilities.

I. Lynch et al. A strategy for grouping of nanomaterials based on key physico-chemical descriptors as a basis for safer-by-design NMs. *Nano Today*, 2014, 9, 266-270.

Electron distribution of graphene



Descriptors - résumé

- Descriptors are parameters, which represent the molecular structure in QSAR model
- Different chemical and physical parameters can be used as descriptors (logP, solubility, etc.)
- Thousands of descriptors can be calculated from chemical structures
- Dozens of programs are available (commercial and free) to calculate the descriptors (DRAGON, CODESSA, POLLY, MDL, PETRA.....)
- **We are far from clear NANO-QSAR concept....**
- **Far from NANO particle design (hypothetical NP ?)**

E. Burello, A. Worth. Predicting toxicity of nanoparticles. Nature Nanotechnology, 2011, 6, 138-139.

NANO data bases relevant for toxicity assessment

N. Jeliaskova et al. The eNanoMapper database for nanomaterial safety information. *Beilstein J. Nanotechnology*, 2015, 6, 1609-1634.

Data bases relevant for toxicity assessment:

- <http://www.nanomaterialregistry.org>
- <http://www.nanoparticlelibrary.net>
- <http://nbi.oregonstate.org>
- <http://cananolab.nci.nih.gov/caNanoLab/>
- <http://www.internano.org>
- <http://icon.rice.edu/report.cfm>
- <http://ncl.cancer.gov>
- <http://www.napira.eu>
- <http://nanopartikel.info>
- <http://nanowerk.com>
- <http://www.nanosafetycluster.eu/>

Chemometrical analysis of -omic data..

In vitro study:

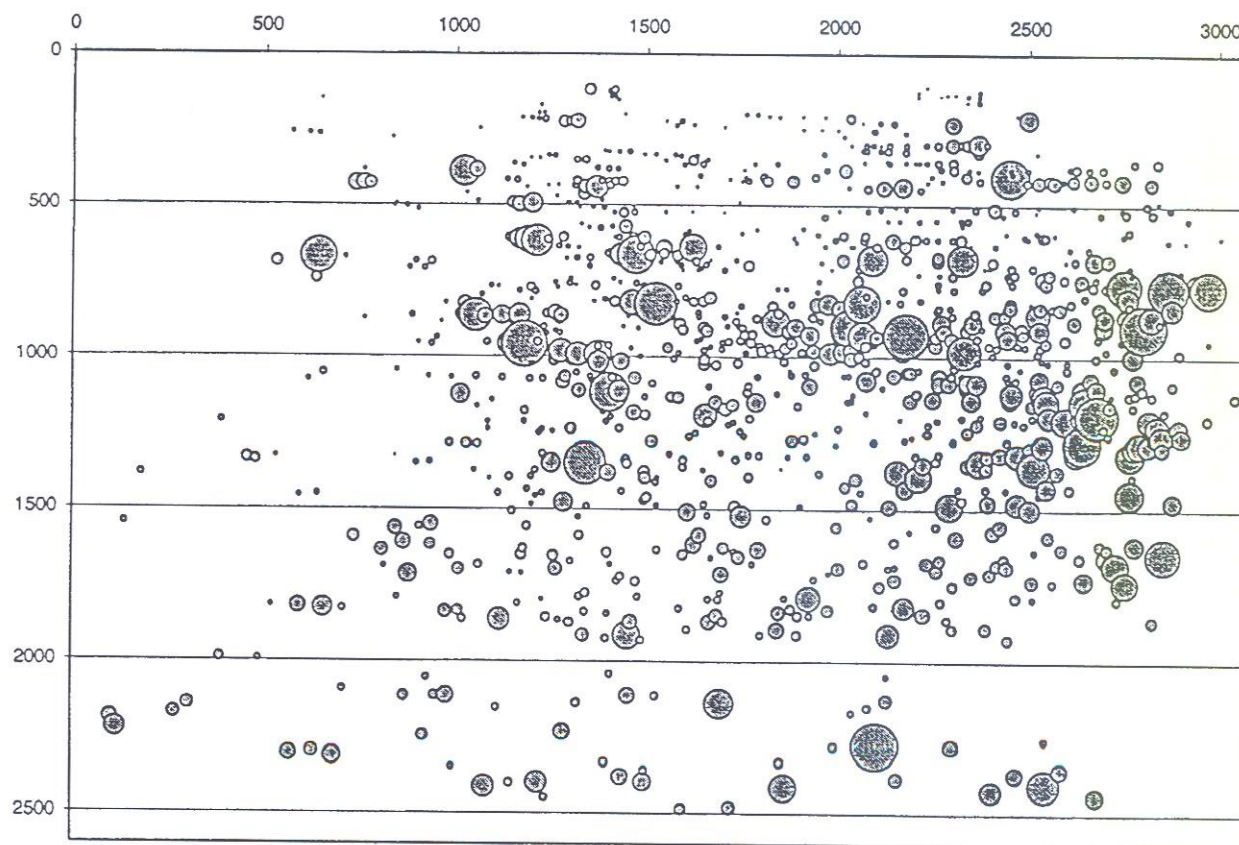
1. Cells are treated with different NANO particles...
2. The -omic status is measured (ca 3000 proteins pro measurements)
3. Statistical relevant -omics are selected?



Pilot study 1: Biodescriptors-2D proteomic maps

Study :

M. Vracko, S. C. Basak. Similarity study of proteomic maps. *Chemometrics and Intelligent Laboratory Systems* 70 (2004) 33–38



Data from:

N.L. Anderson, R. Esquer-Blasco, F. Richardson, P. Foxworthy, P. Eacho, The effects of peroxisome proliferators on protein abundances in mouse liver, *Toxicol. Appl. Pharmacol.* 135 (1996) 75– 89.

Similarity indices between control map and treated maps are reported:

The similarity index:
$$s^{a,b} = \frac{1}{N} \sum_{i=1}^N \frac{z_i^a z_i^b}{\max(z_i^a, z_i^b)^2}$$

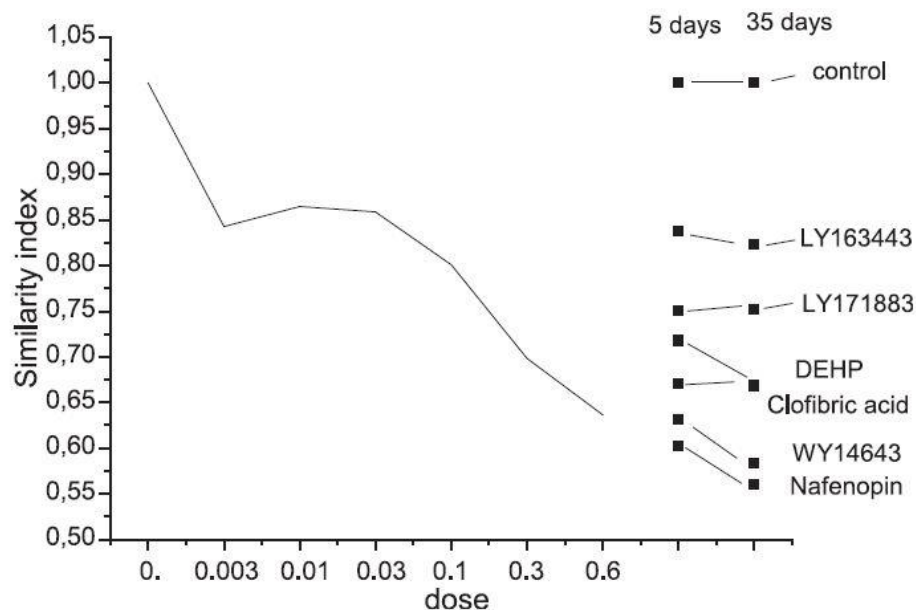


Fig. 2. Similarity index dependent on administrated dose of LY171883. On spots the right side show similarity index for 5 and 35 days exposures.

Study 2: Information on biological status of cells biodescriptors, proteomics

M. Vracko, S. C. Basak, K. Geiss, F. Witzmann. Proteomic Maps-Toxicity Relationship of Halocarbons Studied with Similarity Index and Genetic Algorithm. *J. Chem. Inf. Model.* 2006, 46, 130-136.

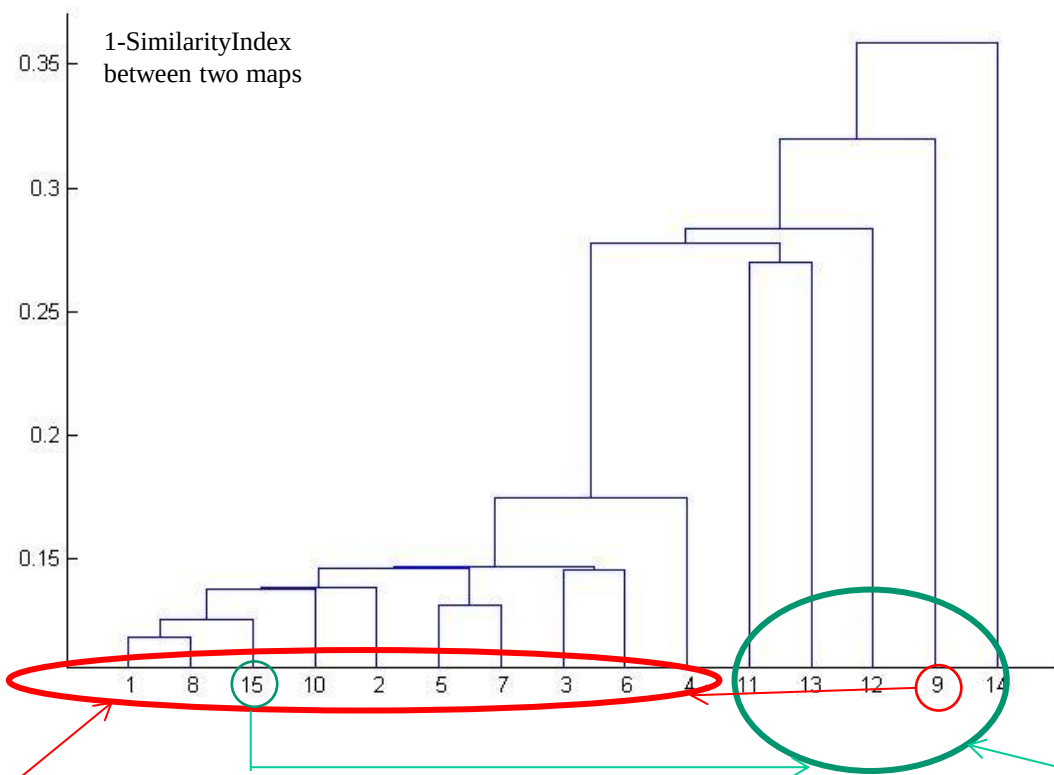
- The comparison of proteomic maps obtained from hepatocytes, which were treated 14 halocarbons.
- Six biological endpoints were determined *in vitro*: $EC50_{MIT}$, LEC_{ROS} , $EC20_{SH}$, $EC50_{LDH}$, LEC_{LP} , LEC_{CAT}
- From each map 263 spots were taken to study the similarity among maps.
- The similarity between two maps was expressed with similarity index.
- The clustering structure is graphically presented with hierarchical clustering method
- With genetic algorithm we selected proteins related to endpoints.

Information on biological status of cells biodescriptors, proteomics

The similarity index:
$$s^{a,b} = \frac{1}{N} \sum_{i=1}^N \frac{z_i^a z_i^b}{\max(z_i^a, z_i^b)^2}$$

	control	111-C2Cl3H3	112-C2Br3H3	112-C2Cl3H3	12-C2Br2H4	12-C2BrClH4	12-C2Cl2H4	C2Cl3H	C2Cl4	C2Cl4H2	CBr2H2	CBrClH2	CCl2H2	CCl3H	CHBr3
control	1.0000	0.8614	0.8529	0.7890	0.8323	0.8380	0.8540	0.8816	0.6426	0.8438	0.6850	0.6972	0.7220	0.5177	0.8748
111-C2Cl3H3	0.8614	1.0000	0.8270	0.7762	0.8128	0.8419	0.8426	0.8576	0.6685	0.8577	0.6715	0.6736	0.6914	0.5205	0.8498
112-C2Br3H3	0.8529	0.8270	1.0000	0.7812	0.7699	0.8542	0.8209	0.8413	0.6458	0.8484	0.6553	0.6751	0.6890	0.5081	0.8451
112-C2Cl3H3	0.7890	0.7762	0.7812	1.0000	0.8091	0.7777	0.8251	0.7521	0.6801	0.7607	0.6718	0.6585	0.6685	0.5404	0.7637
12-C2Br2H4	0.8323	0.8128	0.7699	0.8091	1.0000	0.7866	0.8688	0.7926	0.6483	0.7842	0.6822	0.6725	0.6737	0.5431	0.7865
12-C2BrClH4	0.8380	0.8419	0.8542	0.7777	0.7866	1.0000	0.8279	0.8409	0.6287	0.8400	0.6383	0.6489	0.6489	0.5007	0.8300
12-C2Cl2H4	0.8540	0.8426	0.8209	0.8251	0.8688	0.8279	1.0000	0.8081	0.6620	0.8208	0.6839	0.6851	0.6847	0.5269	0.8195
C2Cl3H	0.8816	0.8576	0.8413	0.7521	0.7926	0.8409	0.8081	1.0000	0.6281	0.8332	0.6618	0.6900	0.7025	0.5050	0.8746
C2Cl4	0.6426	0.6685	0.6458	0.6801	0.6483	0.6287	0.6620	0.6281	1.0000	0.6473	0.6390	0.6243	0.6187	0.5812	0.6519
C2Cl4H2	0.8438	0.8577	0.8484	0.7607	0.7842	0.8400	0.8208	0.8332	0.6473	1.0000	0.6328	0.6694	0.6837	0.5008	0.8619
CBr2H2	0.6850	0.6715	0.6553	0.6718	0.6822	0.6383	0.6839	0.6618	0.6390	0.6328	1.0000	0.6712	0.7298	0.6413	0.6657
CBrClH2	0.6972	0.6736	0.6751	0.6585	0.6725	0.6489	0.6851	0.6900	0.6243	0.6694	0.6712	1.0000	0.7162	0.5275	0.6750
CCl2H2	0.7220	0.6914	0.6890	0.6685	0.6737	0.6489	0.6847	0.7025	0.6187	0.6837	0.7298	0.7162	1.0000	0.5355	0.7084
CCl3H	0.5177	0.5205	0.5081	0.5404	0.5431	0.5007	0.5269	0.5050	0.5812	0.5008	0.6413	0.5275	0.5355	1.0000	0.5048
CHBr3	0.8748	0.8498	0.8451	0.7637	0.7865	0.8300	0.8195	0.8746	0.6519	0.8619	0.6657	0.6750	0.7084	0.5048	1.0000

Hierarchical clustering of 14 halocarbons + control



Two carbon atoms

One carbon atom

M. Vracko, S. C. Basak, K. Geiss, F. Witzmann. Proteomic Maps-Toxicity Relationship of Halocarbons Studied with Similarity Index and Genetic Algorithm. J. Chem. Inf. Model. 2006, 46, 130-136.

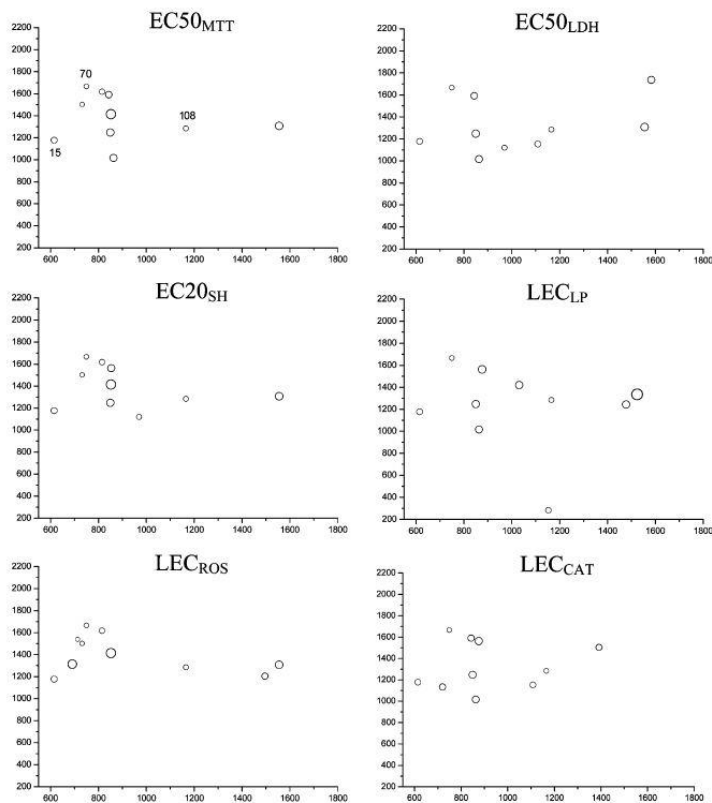


Figure 6. Ten mostly occurring spots for six different biological parameters as they appear in the 2D gel. The size of the bubbles is proportional to the logarithm of intensity. (The integrated intensity of protein spots was calculated by the image analysis software, PDQuest.) In the first picture ($EC50_{MTT}$) the spots 15, 70, and 108 are labeled.



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<http://www.ki.si>

Laboratory for chemometrics

Thank you for your attention!

Researchers

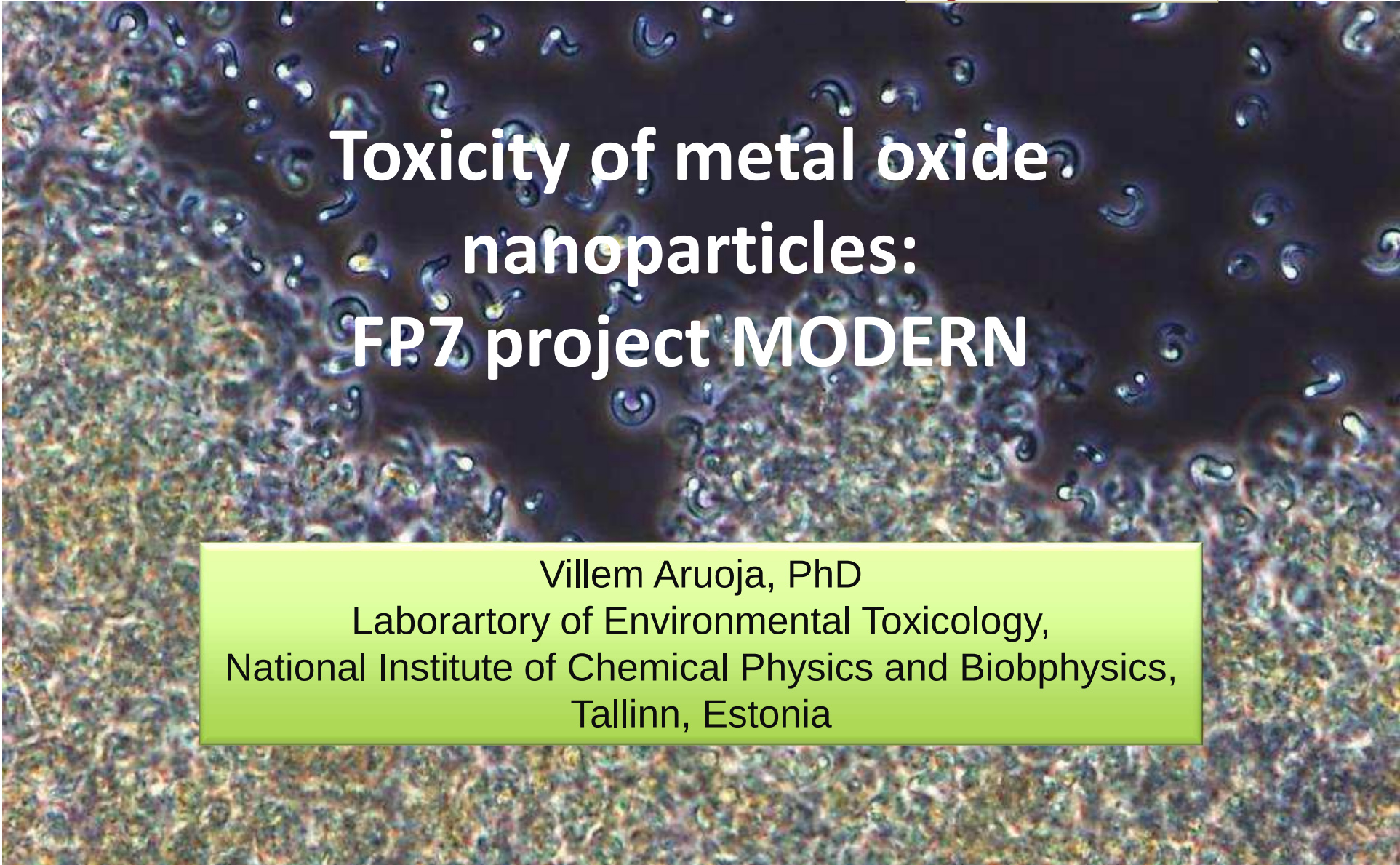
- Marjana Novič
- Marjan Tušar
- Natalja Fjodorova
- Marjan Vračko
- Špela Župerl
- Viki Drgan
- Nikola Minovski
- Katja Venko

Young researchers

- Alja Plošnik
- Jure Borišek
- Lidija Avsenik

Emeritus

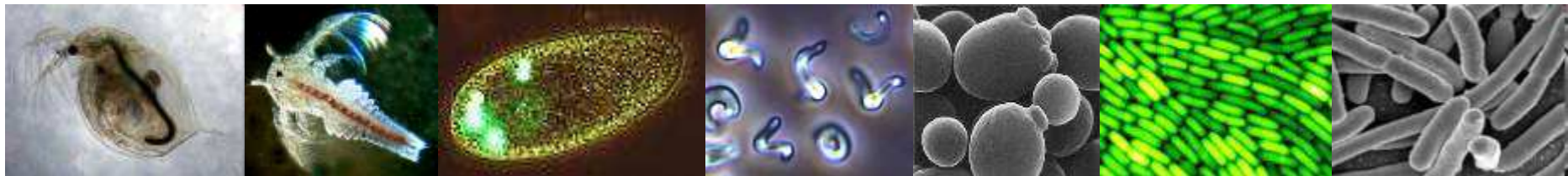
Prof. Jure Zupan
Prof. Milan Randić



Toxicity of metal oxide nanoparticles: FP7 project MODERN

Villem Aruoja, PhD
Laboratory of Environmental Toxicology,
National Institute of Chemical Physics and Biophysics,
Tallinn, Estonia

Our zoo



Crustacea

Daphnia magna

Themnocephalus platyurus

Protozoa

Tetrahymena thermophila

Algae

Pseudo-kirchneriella subcapitata

Yeast

Saccharomyces cerevisiae

Bacteria

Vibrio fischeri

Escherichia coli

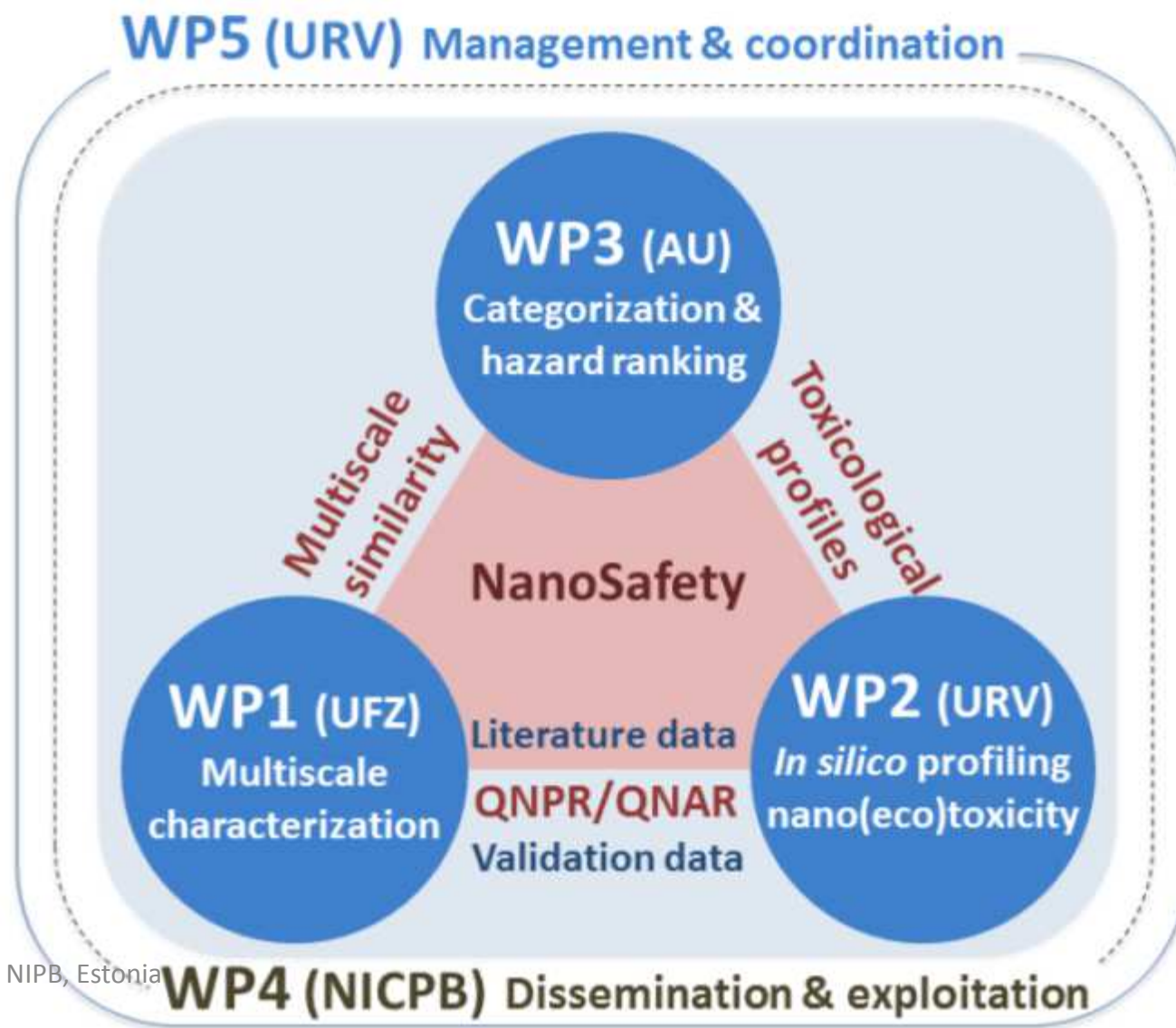


human and animal cell lines
(human alveolar epithelial cells **A549**, human epithelial colorectal cells **Caco2**, murine fibroblast cell line **Balbc/3T3**)

Villem Aruoja, NIPB, Estonia

Slide by Anne Kahru

MODeling the EnviRonmental and human health effects of Nanomaterials



QNAR

BIOLOGICAL
DATA

DESCRIPTORS

M O D E L

VALIDATION
(hypothesis
testing)

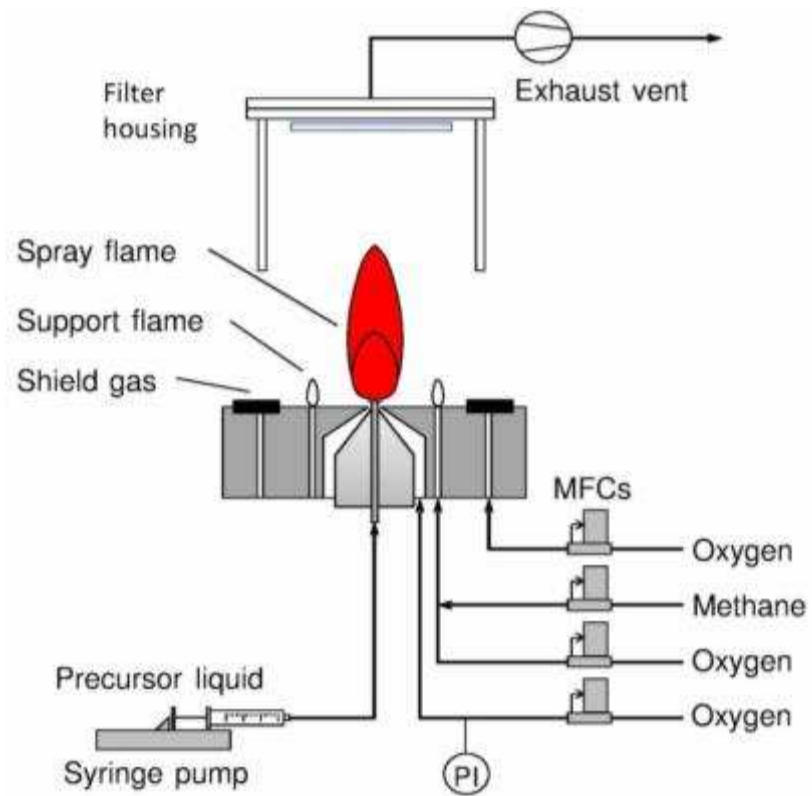
Data?

- Literature?
- Industry?

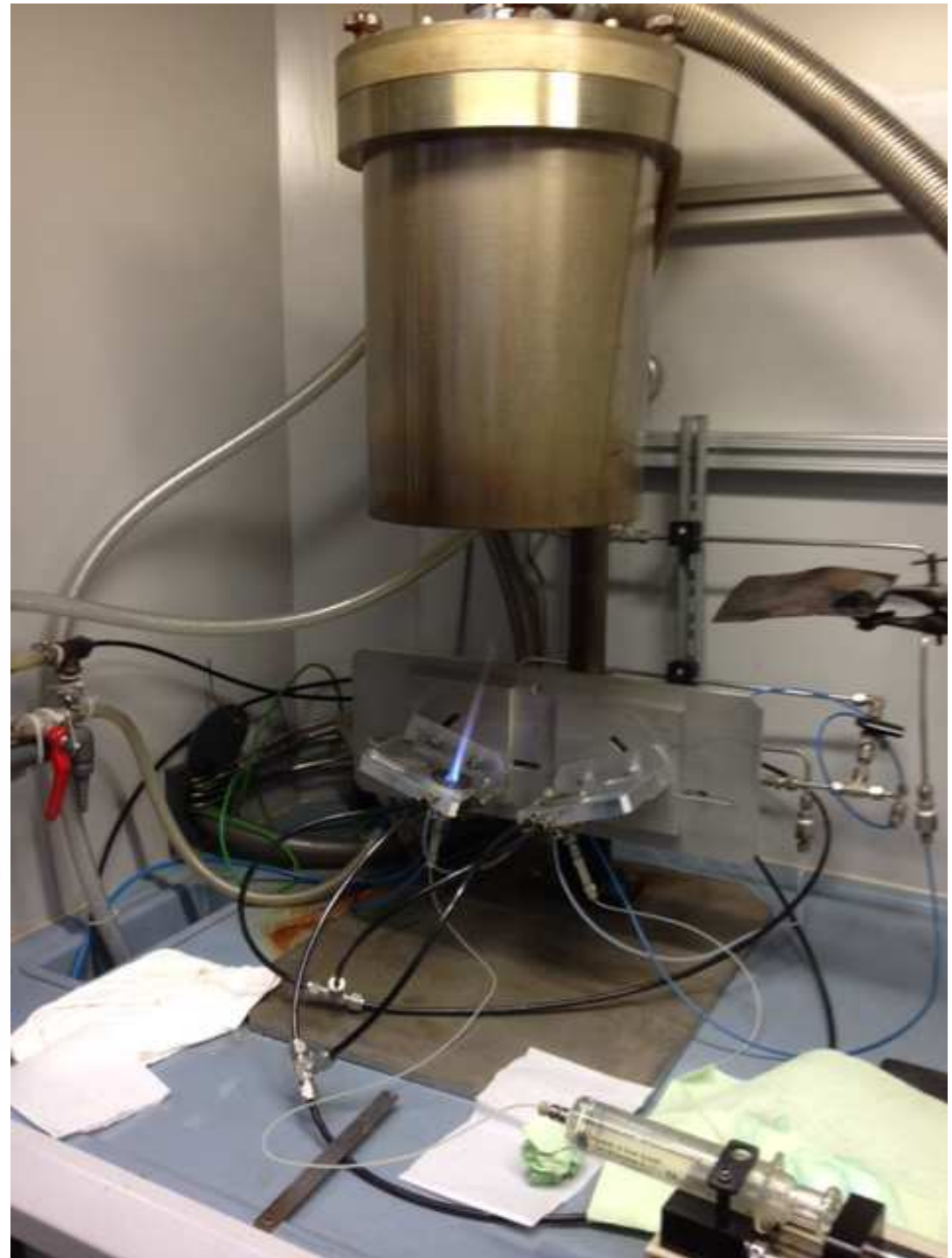
Andre Nel*: “You have 2,5 years left, start generating data now!”

- * Director of University of California's Center for Environmental Implications of Nanotechnology

Flame Spray Pyrolysis



Villem Aruoja, NIPB, Estonia



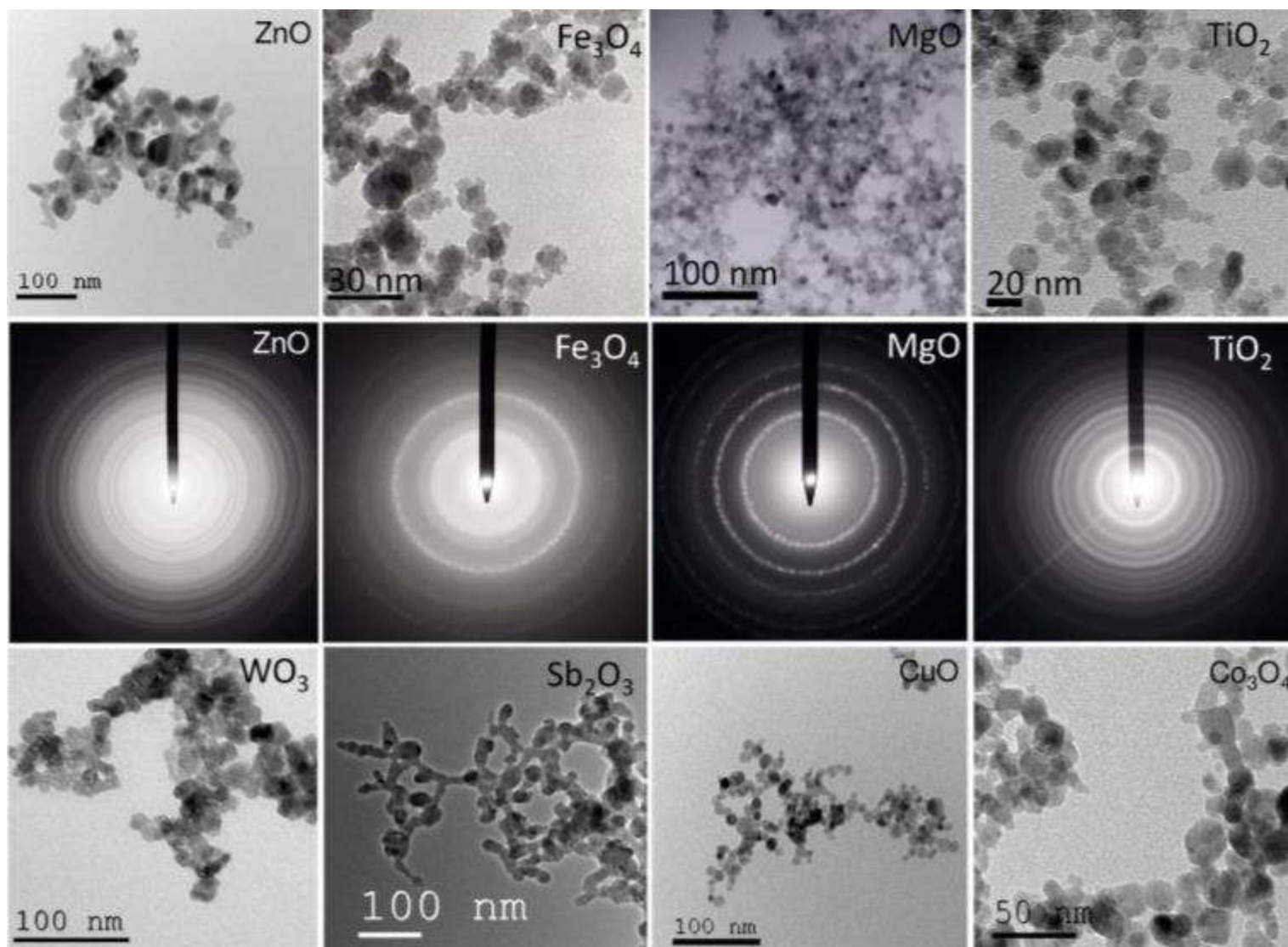
Selection of nanoparticles

- CuO, Co_3O_4 , Sb_2O_3 , TiO_2 , WO_3 , ZnO, Mn_3O_4 , Fe_3O_4 , MgO, Al_2O_3 , SiO_2 , Pd
(Initial subset in project description: 23 metal oxides: ZnO, CuO, Co_3O_4 , Fe_3O_4 , Sb_2O_3 , TiO_2 , WO_3 , Al_2O_3 , CeO_2 , Y_2O_3 , CoO, Ni_2O_3 , Cr_2O_3 , Fe_2O_3 , HfO_2 , In_2O_3 , SnO_2 , ZrO_2 , Gd_2O_3 , La_2O_3 , Mn_2O_3 , NiO, Yb_2O_3 and Ag, Au, Pt)

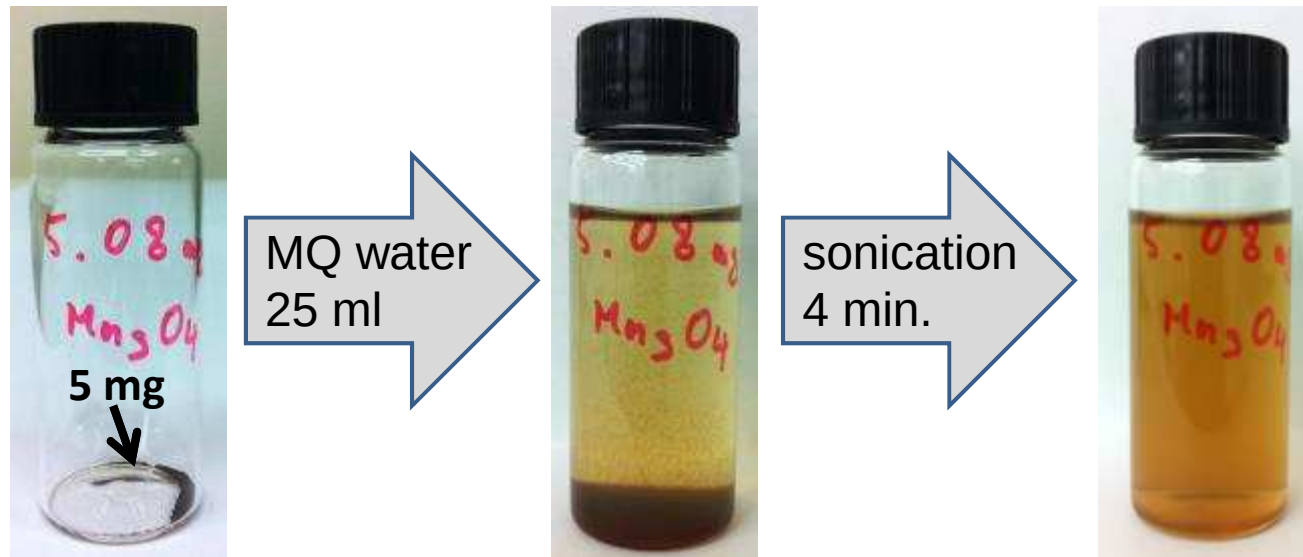




FSP – small and crystalline NPs



Preparation of MOx NPs suspensions

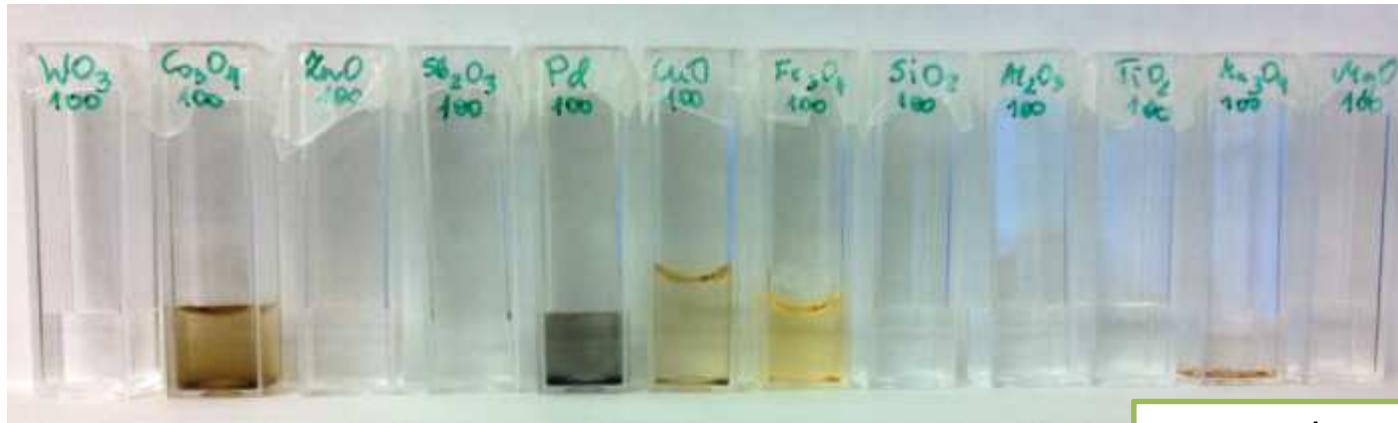


Size, hydrodynamic size

Sample	Specific surface area (SSA); m ² /g	BET size (d_{BET})	DI water				Algal growth medium (pH=8.0)				
		nm	z-average hydrodynamic size, nm	ζ -potential, mV	pH	Metal solubility		z-average hydrodynamic size, nm	ζ -potential, mV	Metal solubility	
						% at 10 mg/l (AAS or ICP-MS*)	% at 200 mg/l (TXRF)			% at 10 mg/l (AAS or ICP-MS*)	% at 100 mg/l (TXRF)
ZnO	53	20.4	171	16.4	6.6	56.1*	5.0	696	-13.1	25.7	3.18
Pd	33	15.1	127	-27.8	6.1	<0.5	NA	151	-18.6	0.24	NA
CuO	72	13.1	130	17	6.2	5.14	0.88	769	-6.2	1.16	0.26
Co ₃ O ₄	85	11.5	99	23	6.1	1.25	6.8	916	10.7	0.18	0.82
TiO ₂	123	12.2	171	-13.6	6.2	<0.83	0.10	717	-15.1	0.42	0.01
Mn ₃ O ₄	81	15.2	395	-14.4	7.0	11.1	4.8	920	-9.8	9.45	6.62
Fe ₃ O ₄	120	9.7	128	22.2	5.9	<1.38	7.1	1005	-12.1	1.66	0.17
Al ₂ O ₃	134	11.4	95	39.2	6.0	0.40*	NA	1232	8.9	0.42	NA
SiO ₂	289	7.8	148	-33.2	6.0	NA	NA	154	-19.8	NA	NA
WO ₃	79	10.6	63	-45.3	5.0	63.2*	2.3	191	-20.4	66.7	75.7
MgO	123	13.6	1964	6.9	9.6	38.1	NA	1581	6.4	87.9 [†]	NA
Sb ₂ O ₃	56	20.5	125	-24.3	4.2	56.3	NA	414	-15.9	21.2	NA

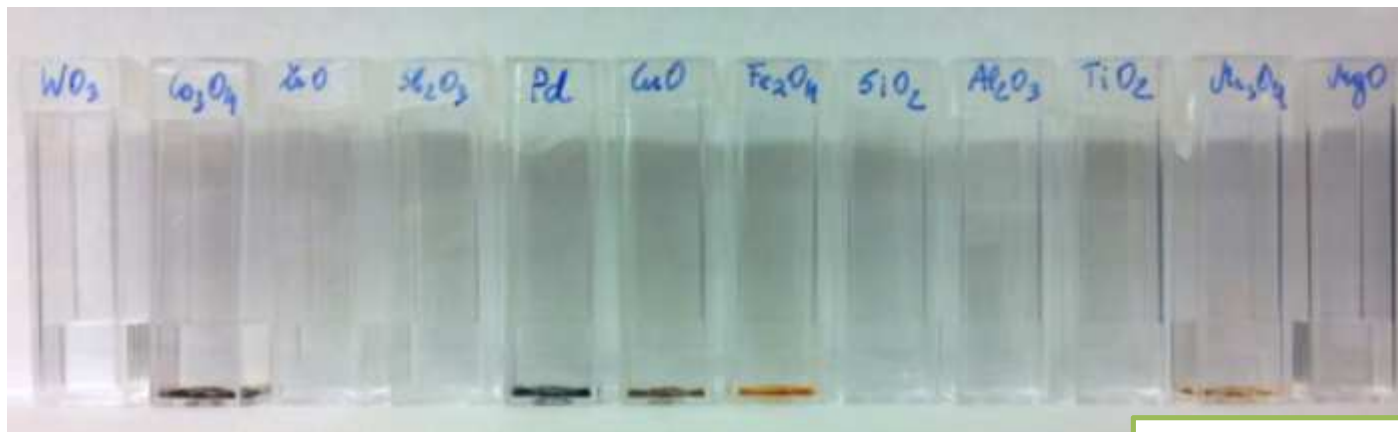
Stability of suspensions in MQ water vs algal test medium

In MQ water: 1 week



100 mg/L

In algal test medium (OECD 201): 1 day.



100 mg/L

Composition of the algal test medium.

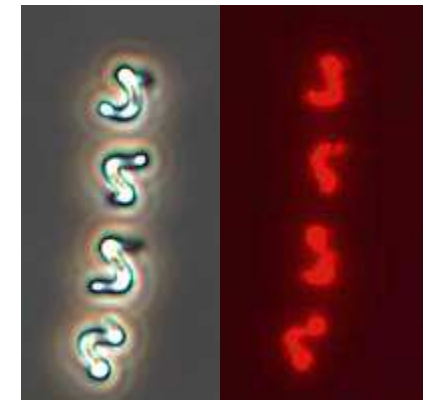
	Component	mg/L
1	NH ₄ Cl	15
2	MgCl ₂ *6H ₂ O	12
3	CaCl ₂ *2H ₂ O	18
4	MgSO ₄ *7H ₂ O	15
5	KH ₂ PO ₄	1,6
6	NaHCO ₃	50
7	Na ₂ EDTA*2H ₂ O	0,1
8	FeCl ₃ *6H ₂ O	0,08
9	H ₃ BO ₃	0,185
10	MnCl ₂ *4H ₂ O	0,415
11	ZnCl ₂	0,003
12	CoCl ₂ *6H ₂ O	0,0015
13	Na ₂ MoO ₄ *2H ₂ O	0,007
14	CuCl ₂ *2H ₂ O	0,00001

Algal growth inhibition assay (OECD 201)

- Primary producers
- Sensitive to toxicants
- Very sensitive to heavy metals



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100 μm

EC₅₀

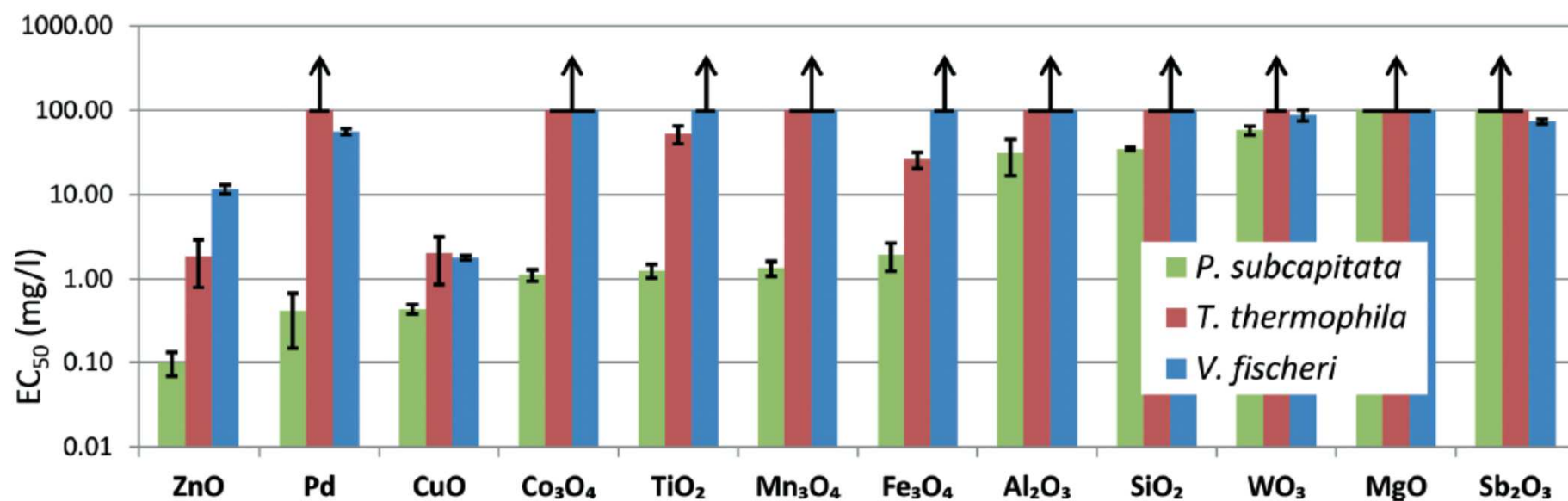
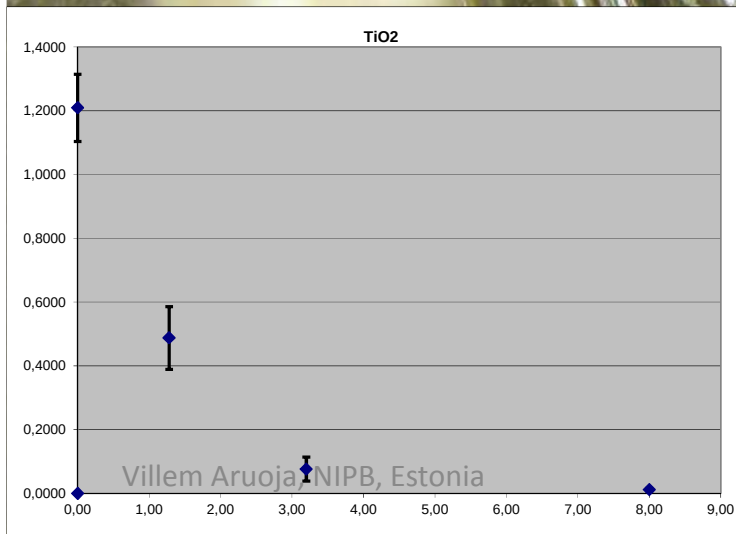
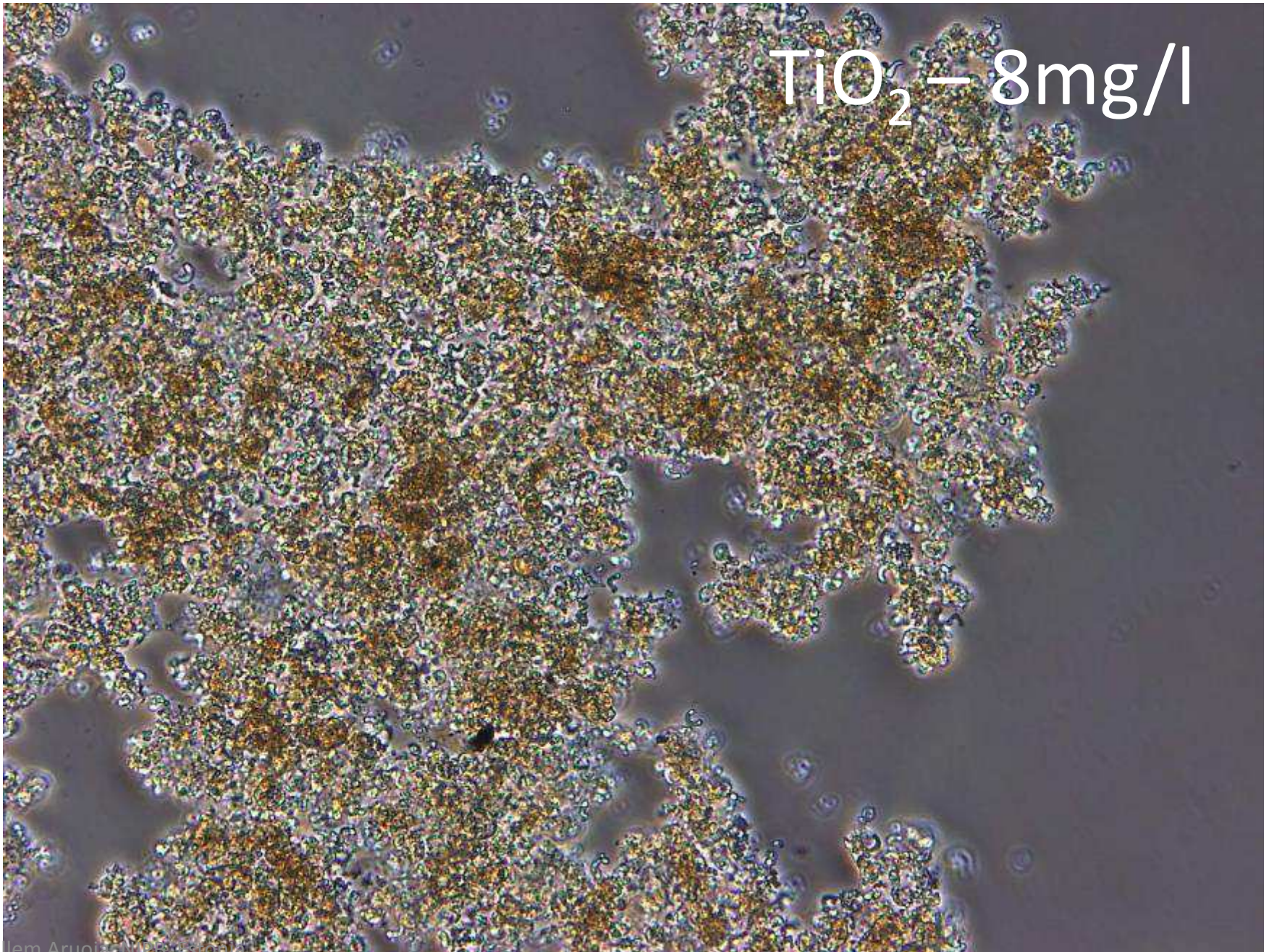


Fig. 2 Toxicity of 12 nanoparticles to alga *Pseudokirchneriella subcapitata*, protozoa *Tetrahymena thermophila* and bacterium *Vibrio fischeri*. EC₅₀ values were obtained from 72 h algal growth inhibition assay, 24 h *T. thermophila* viability assay and 30 min *V. fischeri* luminescence inhibition assay (Table S3†). Arrows indicate EC₅₀ values above 100 mg l⁻¹. Concentrations are nominal.

TiO_2 – 8mg/l

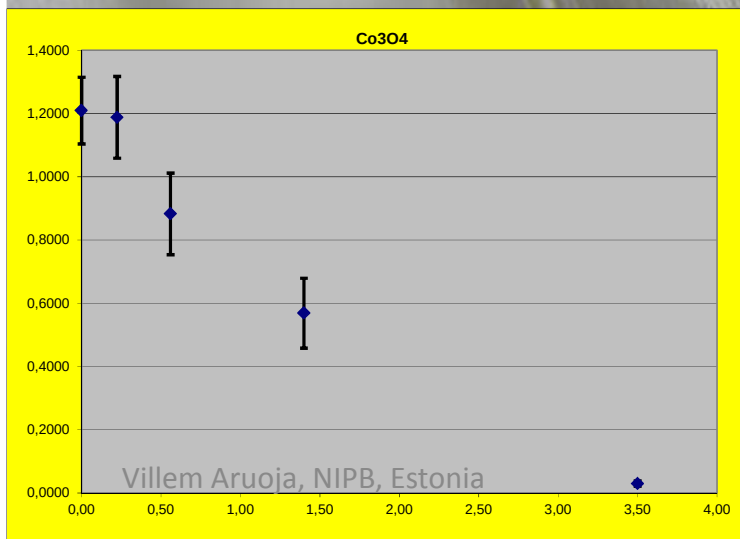


TiO_2 – 8mg/l

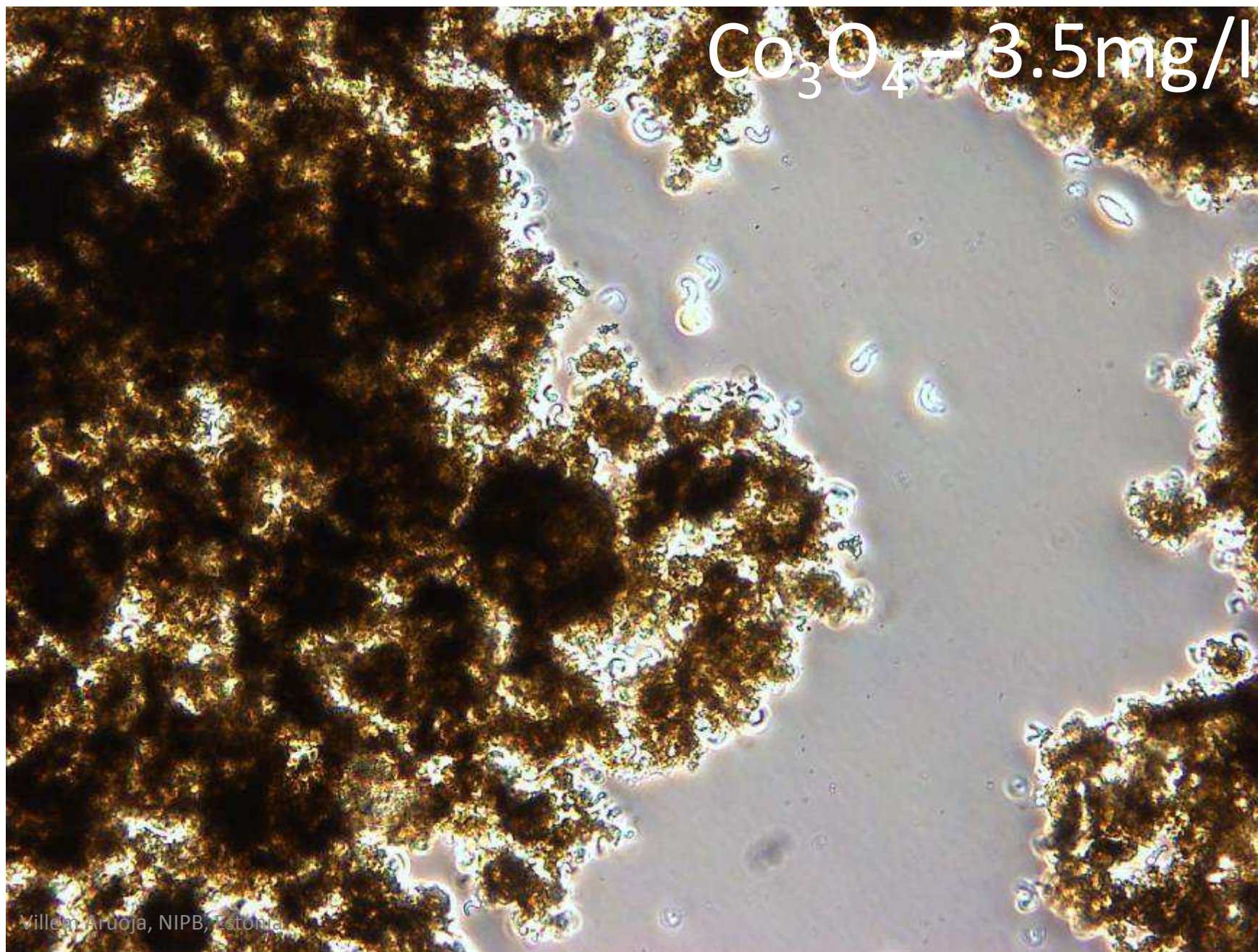


TiO_2 – 8mg/l

Co_3O_4 – 3.5mg/l

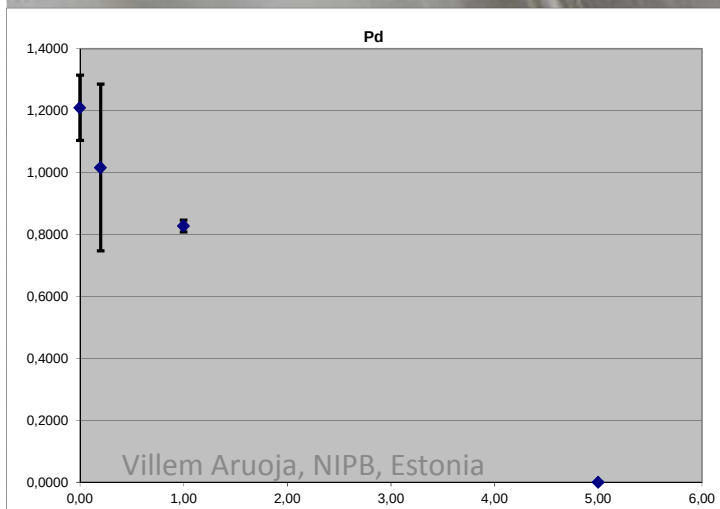


Co_3O_4 – 3.5mg/l

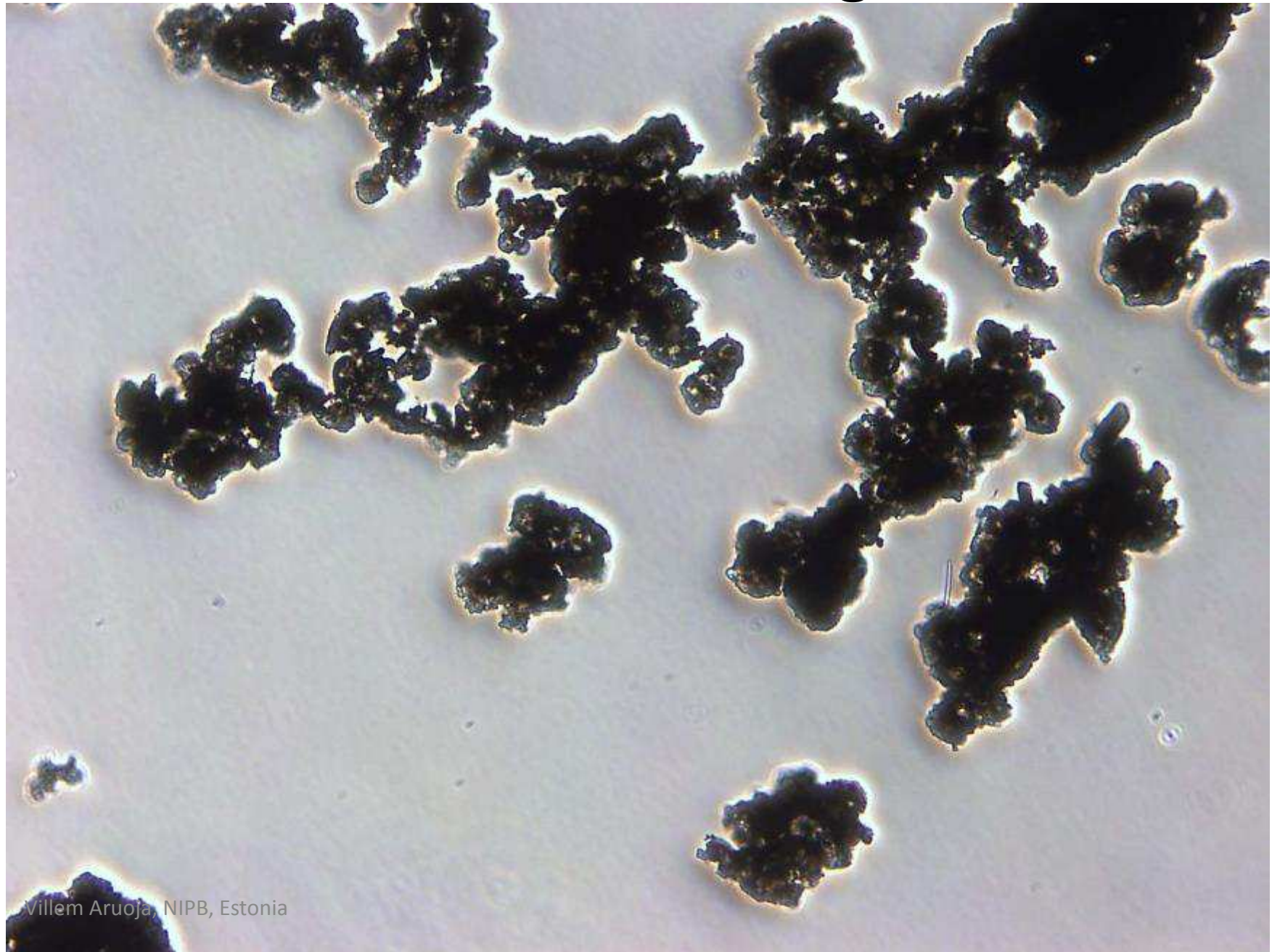


Co_3O_4 – 3.5mg/l

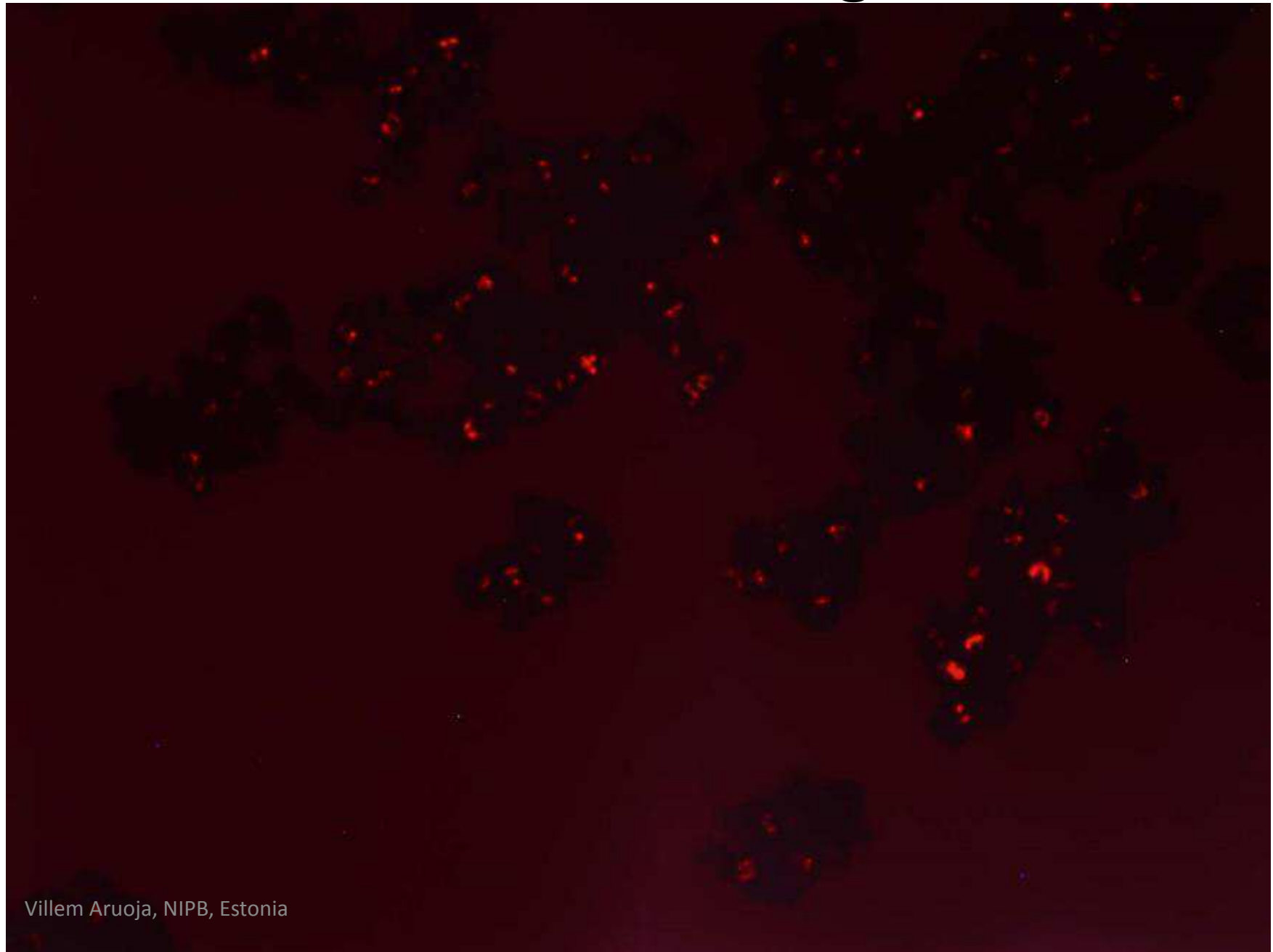
Palladium – 5mg/l



Palladium – 5mg/l

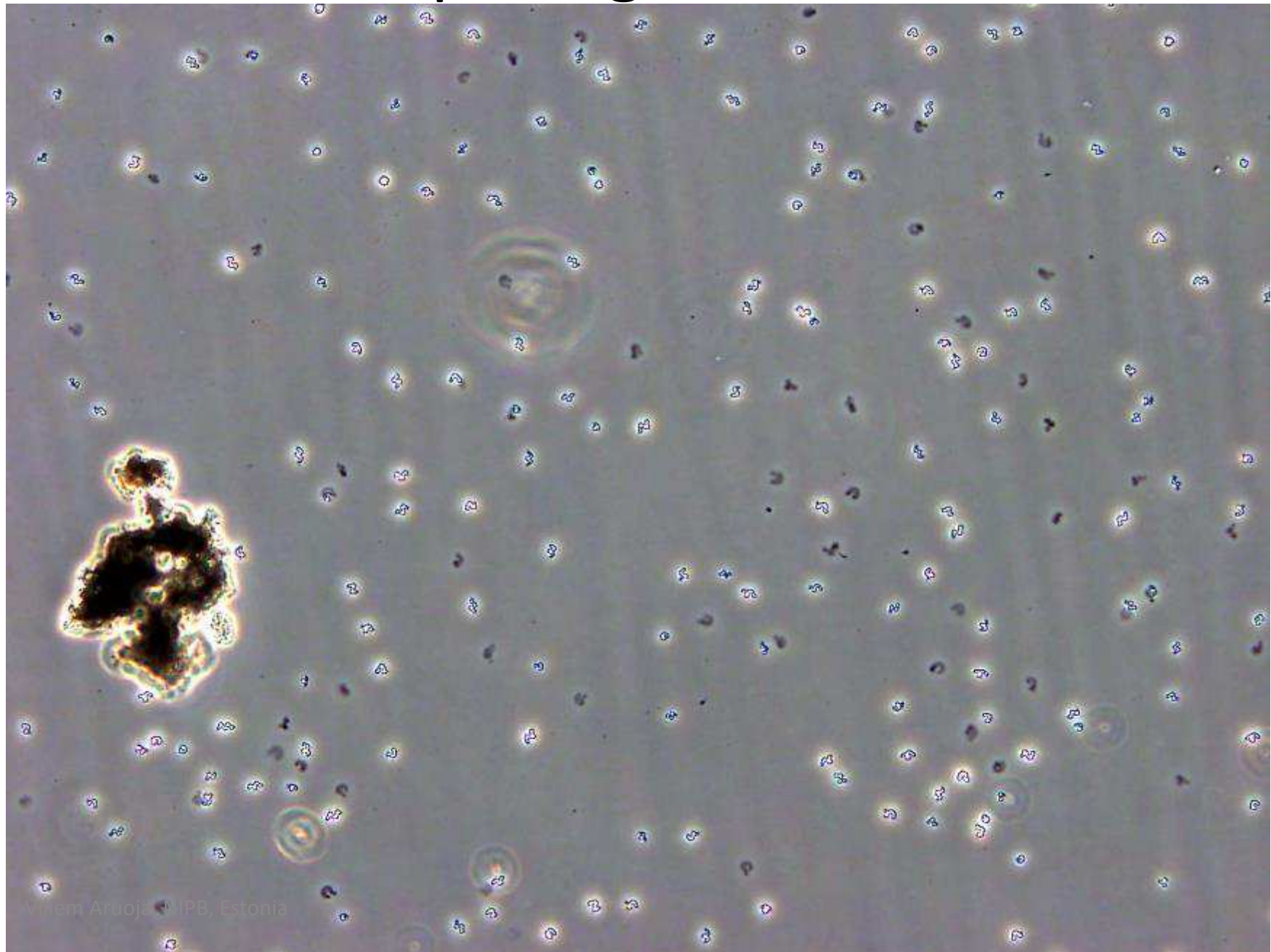


Palladium – 5mg/l



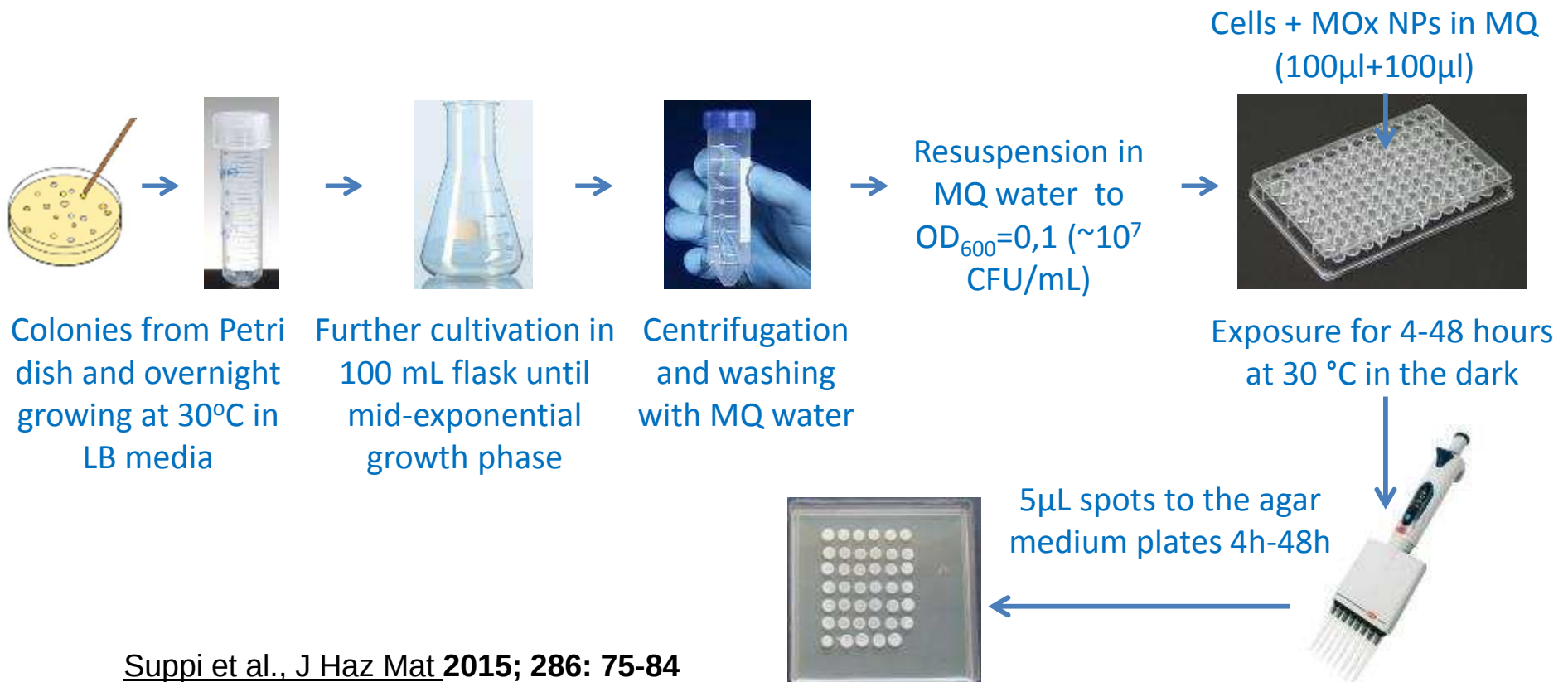
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Palladium – passage to clean medium



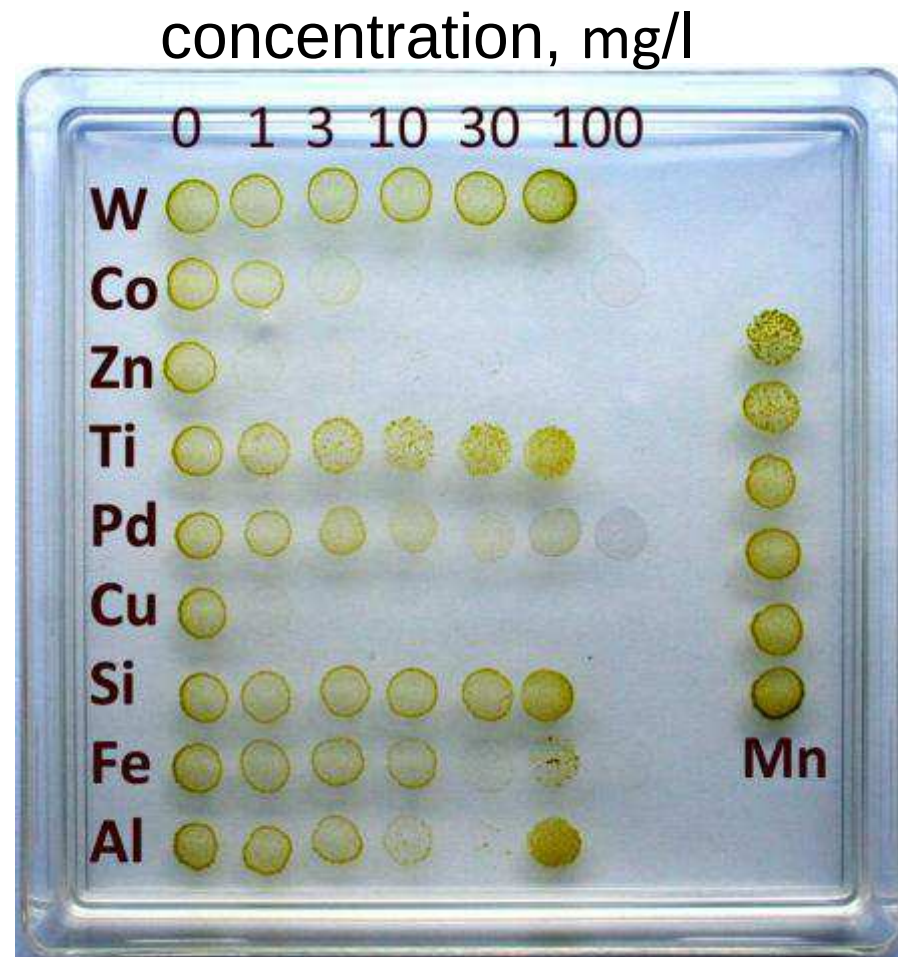
'Spot'-test

Inoculation	Bacterial growth to log phase	Washing with MQ water	Exposure to MOx NPs	4-48h spot
Bacterial colonies from Petri dish are transferred to 3ml liquid LB growth media and grown overnight in 14 mL round-bottom polypropylene culture tube at 30 °C on a shaker (200 rpm).	Dilution (1:50) of the overnight culture in 20 mL of fresh medium (in 100 mL culture flask) and further cultivation at 30 °C on a shaker at 200 rpm for 4-4.5 hours until bacteria reach mid-exponential growth phase ($OD_{600nm} \sim 0.6$).	The cells are harvested by centrifugation at 7000 rpm for 7 min at 20 °C in 50 mL centrifuge tubes. After that the cells were washed twice with MQ water and resuspended in MQ water to a density of $\sim 10^7$ CFU/mL ($OD_{600}=0.1$).	100 μ L of cells suspension and 100 μ L of MOx NPs suspension in MQ water were pipetted into the 96-well microplates. MQ water without test chemicals was inoculated with test strains in parallel and served as a control culture (not treated). The microplates were incubated for 4 hours or 24 hours at 30 °C in the dark, without shaking.	After 4 h or 24 h of exposure, 5 μ L of the cell culture from each well (treated or not treated) was pipetted ("spotted") onto the LB agar medium plates and incubated at 30 °C for 24 h. The growth of bacteria (formation of colonies) was evaluated visually on LB agar.

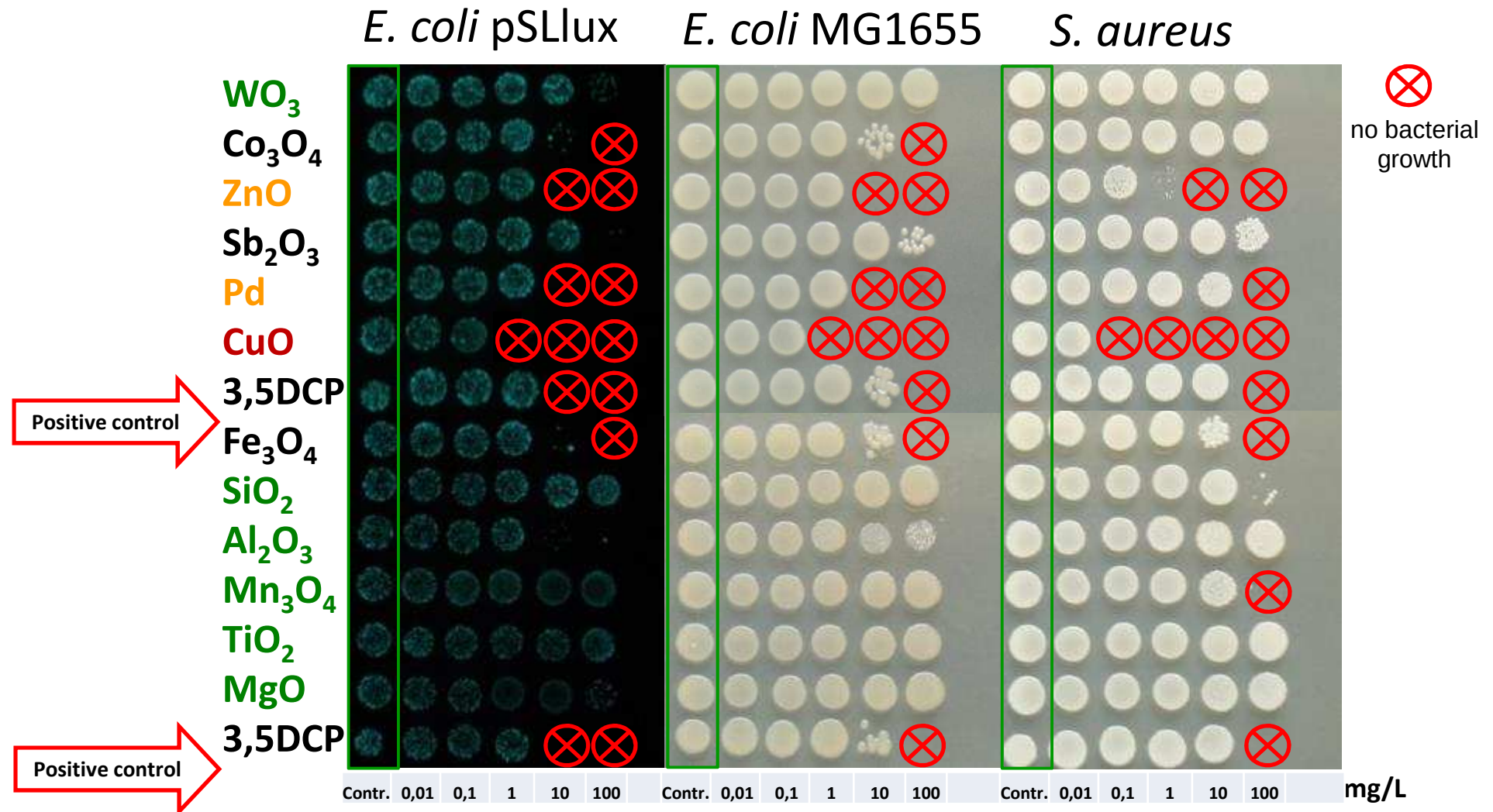


„Spot test“

Algal cells after
24h incubation
with NPs in
deionized water



Results from 24-h 'spot'-test with bacteria

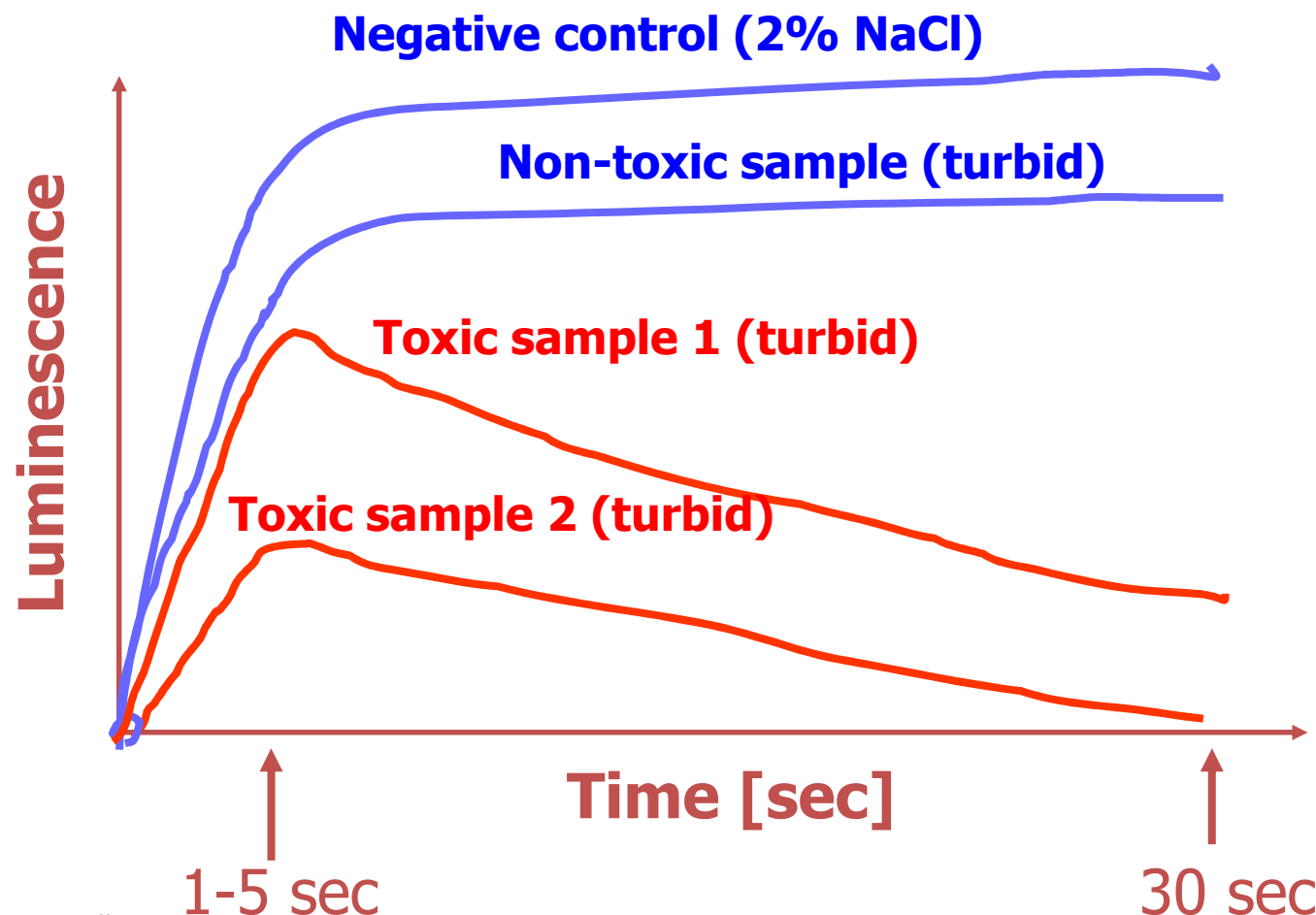


→ Co₃O₄, CuO, ZnO, Fe₃O₄ and Mn₃O₄ inhibited bacterial colony forming ability in the 'spot'-test at concentrations ≤ 100 mg/L.

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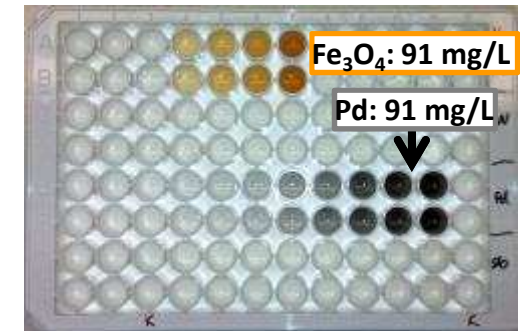
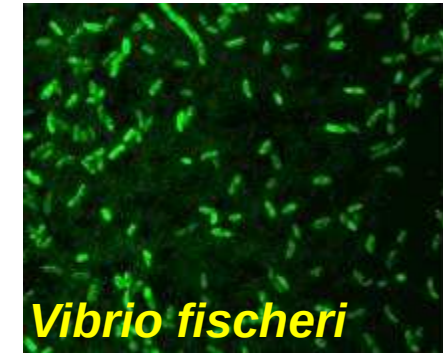
Environmental Science: Nano, 2015, DOI: 10.1039/C5EN00057B

Kinetic bioluminescence inhibition test with *Vibrio fischeri* (ISO 21338:2010)

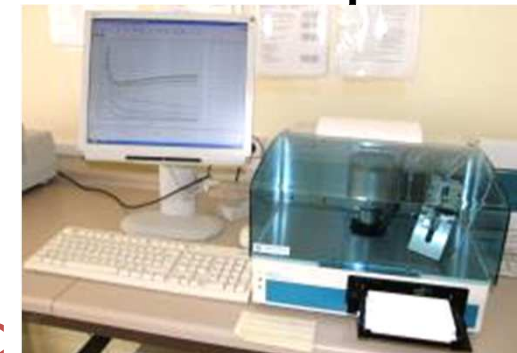


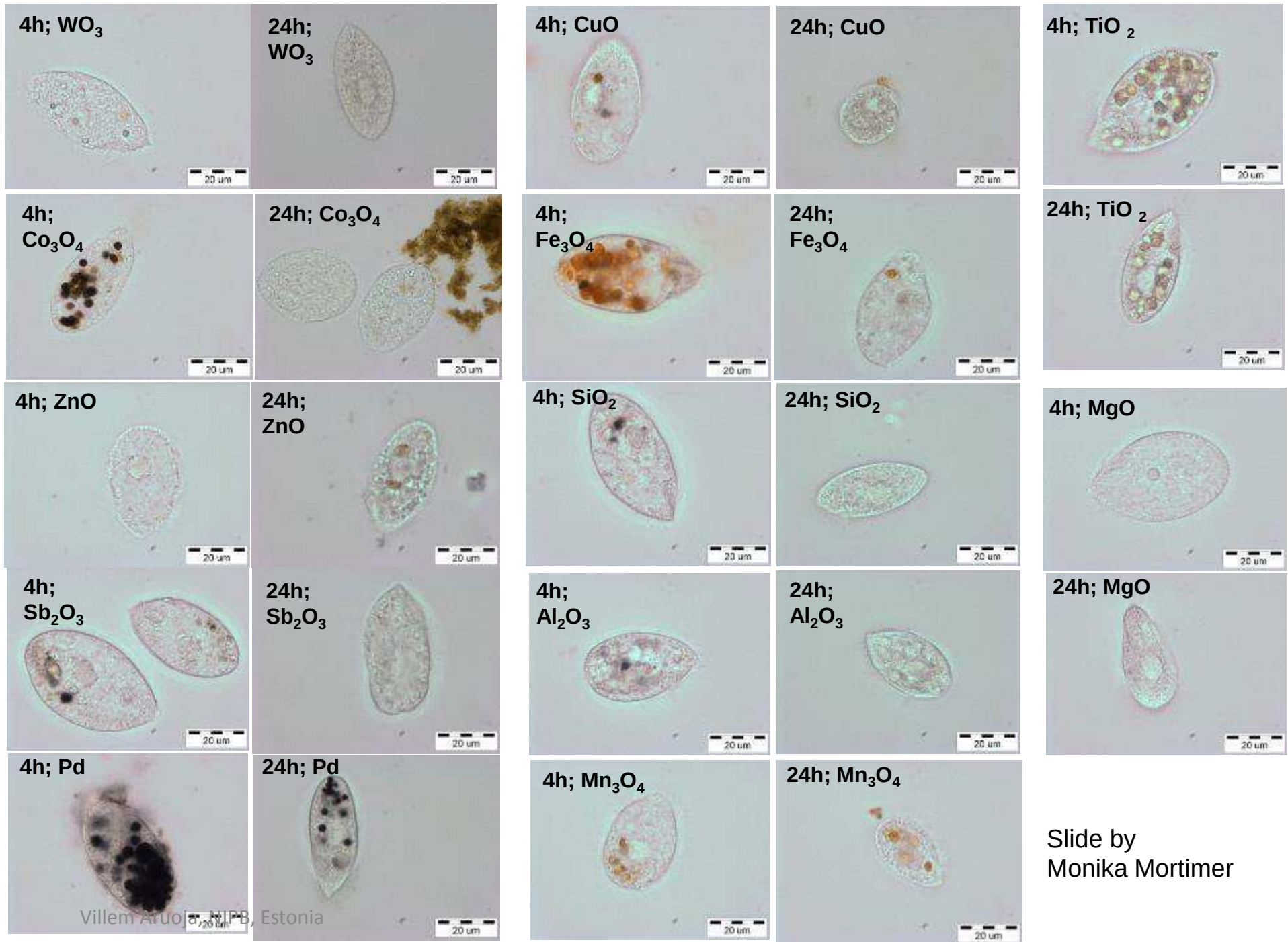
Villem Aruoja, NIPB, Estonia

Slide by Anne Kahru



96-well microplate





Slide by
Monika Mortimer

Table 1. Toxicity of metal oxide and Pd nanoparticles to protozoa (*Tetrahymena thermophila*) and bacteria (*Vibrio fischeri*, *Escherichia coli*, *Staphylococcus aureus*). The presented toxicity values are based on nominal initial exposure concentrations used in testing.

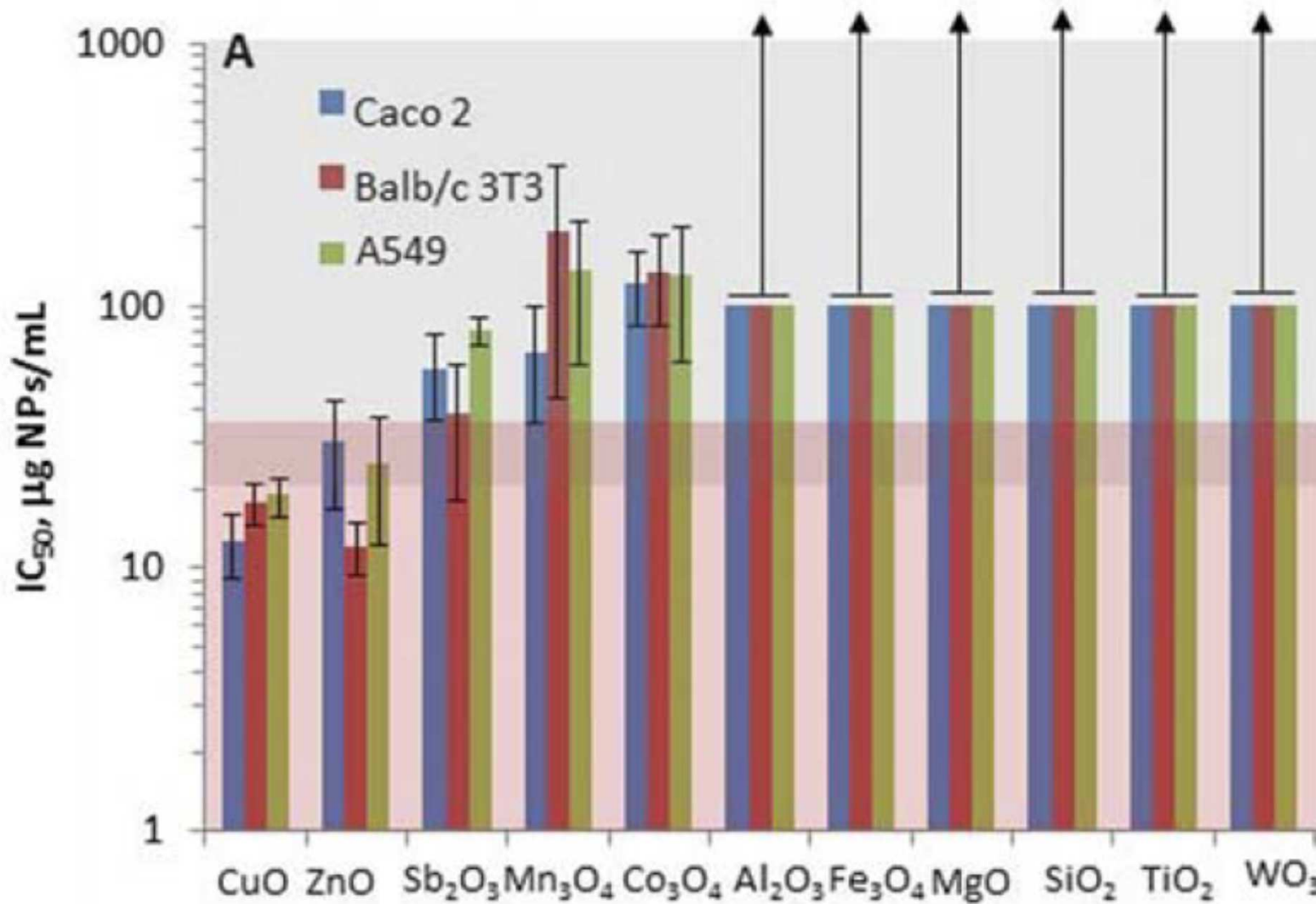
NPs	24-h EC50 ¹ <i>T. thermophila</i>	30-min EC50 ¹ <i>V. fischeri</i> (gram –)	24-h MBC ² <i>E. coli</i> pSLux (gram –)	24-h MBC ² <i>E. coli</i> MG1655 (gram –)	24-h MBC ² <i>S. aureus</i> (gram +)
	mg/L (compound)				
CuO	2.0	1.8	1	1	0.1
ZnO	1.8	11.6	10	10	10
Fe ₃ O ₄	20.1	>100	100	100	100
Mn ₃ O ₄	22.0	>100	>100	>100	100
Al ₂ O ₃	61.3	>100	100	>100	>100
TiO ₂	69.1	>100	>100	>100	>100
Pd	>100	56.3	10	10	100
Co ₃ O ₄	>100	>100	100	100	>100
Sb ₂ O ₃	>100	74.5	>100	>100	>100
WO ₃	>100	92.8	>100	>100	>100
SiO ₂	>100	>100	>100	>100	>100
MgO	>100	>100	>100	>100	>100

¹ **EC50** - half effective concentration; ² **MBC** - minimum bactericidal concentration. The lowest tested concentration that completely inhibited the visible growth of bacteria on the agarized test medium at 30°C in the dark after 24-h of incubation to MOx NPs. **Colour code:** ≤0.1 mg/L (■); >0.1–10 mg/L (■); 10–50 mg/L (■); 50–100 mg/L (■); =100 (■) mg/L; >100 mg/L (■).

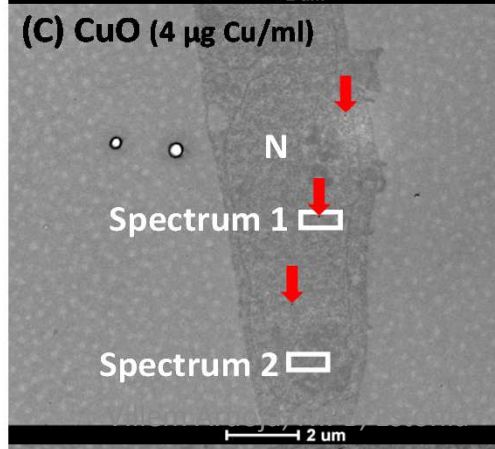
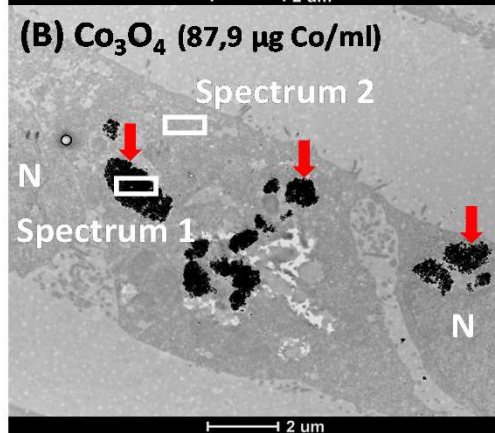
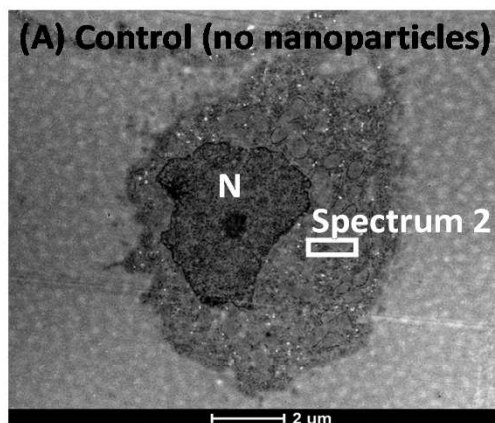
Mechanism: solubility, ROS

Trophic level				Primary producer	Consumer	Degrader		
Cell type				Eukaryote	Eukaryote	Prokaryote		
Internalizes nanoparticles				No?	Yes	No?		
Nano-particle [¥]	Solubility (%) [§]	ROS (HPF) ^{\$}	ROS (DCF) ⁺	Algal 72 h growth inhibition OECD 201	Protozoan 24 h viability	Bacterium 30 min. luminescence ISO 21338	Mechanism of toxicity	Classification [†]
ZnO	56.1	-	-	< 1.0	1 ...2	10 ... 50	Zn ions	 Acute aquatic hazard
Pd	<0.5	+	++	< 1.0	>100	10 ... 50	ROS	
CuO	5.14	+++*	-	< 1.0	1 ...2	1 ...2	Cu ions & ROS	
Co₃O₄	1.25	+	+	1 ...2	>100	>100	ROS	 Acute aquatic hazard?
TiO₂	<0.83	+++	+	1 ...2	10 ... 50	>100	ROS	
Mn₃O₄	11.1	-	+++	1 ...2	>100	>100	ROS	
Fe₃O₄	<1.38	+*	-	1 ...2	10 ... 50	>100	ROS	
Al₂O₃	0.40	+	-	10 ... 50	>100	>100		
SiO₂	NA	-	-	10 ... 50	>100	>100		
WO₃	63.2	-	-	10 ... 50	>100	50...100		
MgO	38.1	-	-	>100	>100	>100		
Sb₂O₃	56.3	+	-	>100	>100	50...100		

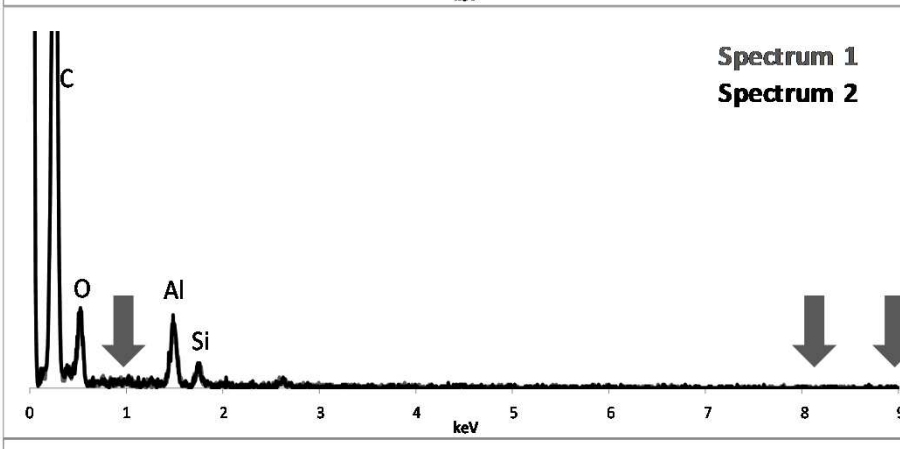
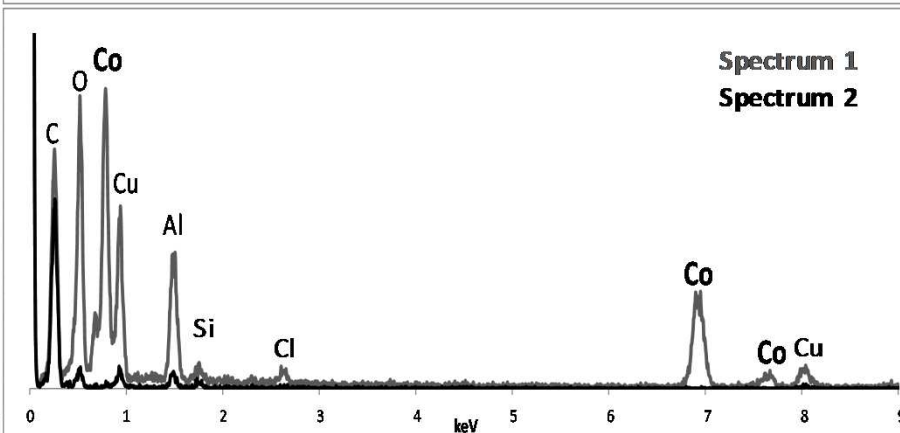
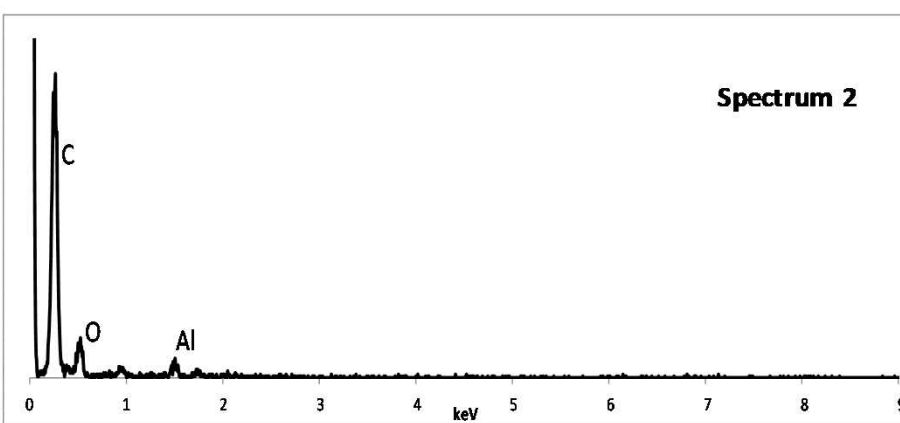
Cell cultures, EC₅₀



TEM images



EDX spectra



Endo- cytosis

Ivask et al. Current Topics in Medicinal Chemistry [2015, 15(18):1914-1929]



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Current Topics in Medicinal Chemistry, 2015, 15, 1-16

1

Toxicity of 11 Metal Oxide Nanoparticles to Three Mammalian Cell Types *In vitro*

Angela Ivask^{1,2,*}, Tiina Titma^{1,3}, Meeri Visnapuu^{1,4}, Heiki Vija¹, Aleksandr Käkinen¹, Mariliis Sihtmäe¹, Suman Pokhrel⁵, Lutz Mädler⁵, Margit Heinlaan¹, Vambola Kisand⁴, Ruth Shimmo³ and Anne Kahru¹



Environmental
Science
Nano



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Cite this: DOI: 10.1039/c5en00057b

Toxicity of 12 metal-based nanoparticles to algae, bacteria and protozoa†

Villem Aruoja,^{*a} Suman Pokhrel,^b Mariliis Sihtmäe,^a Monika Mortimer,^a and Anne Kahru^a



Villem Aruoja, NIPB, Estonia

Anne Kahru, EUROTOX 2015, Porto, Sept
13-16

34

Monte Carlo Modelling of Interaction Processes between Nanoparticles and Biomacromolecules of Variable Hydrophobicity

Fabrice Carnal

October 3rd, 2015



CompinNano, Ljubljana



**UNIVERSITÉ
DE GENÈVE**
FACULTÉ DES SCIENCES

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I. Introduction

II. Simulation method and model

III. Results

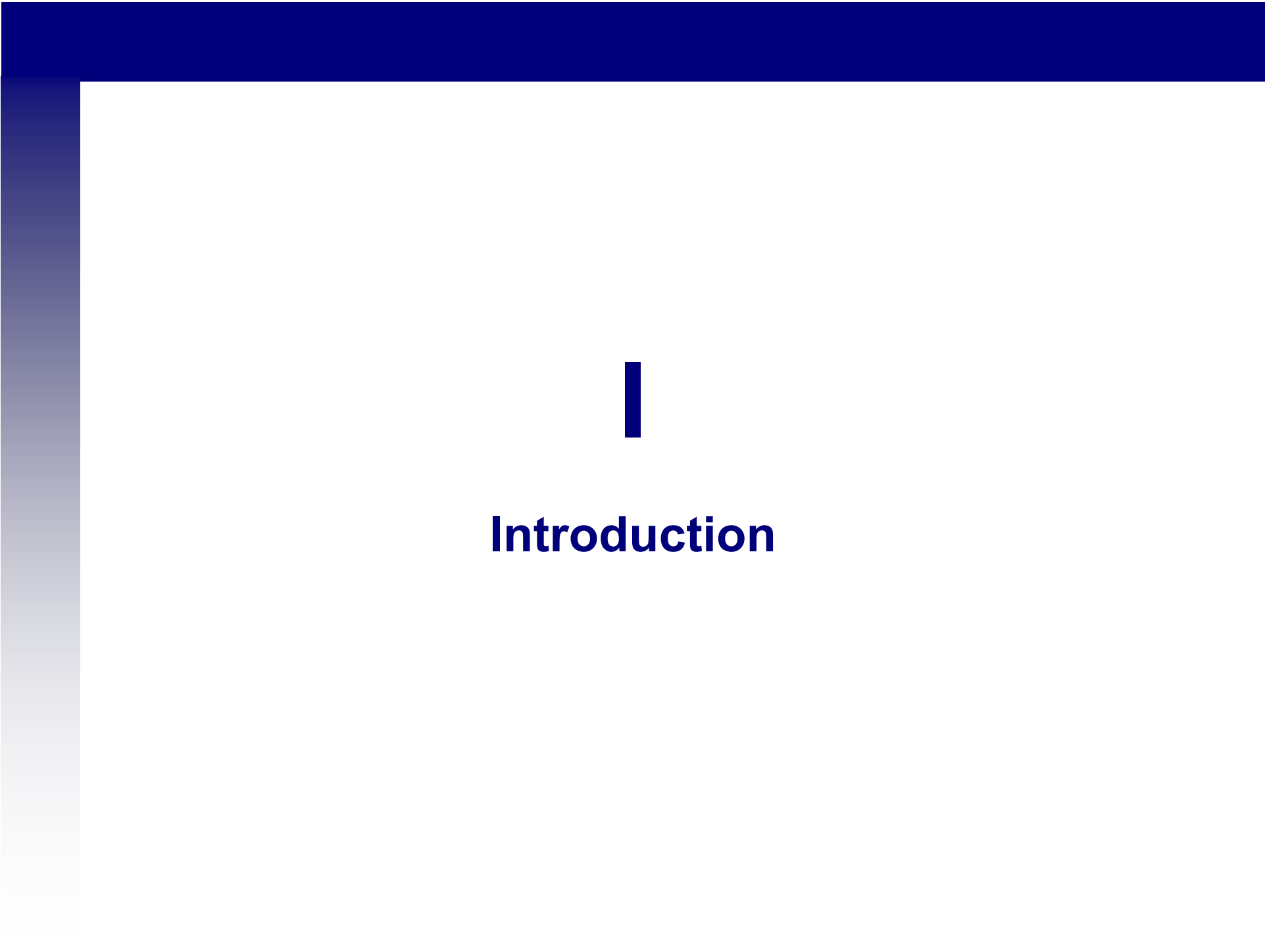
Simplified protein structure

- pH
- Chain hydrophobicity
- Presence of nanoparticle

BSA protein

- Parametrization
- pH
- Nanoparticle surface charge density

IV. Conclusions and perspectives



I

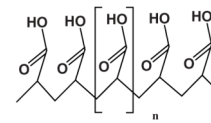
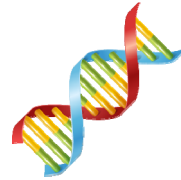
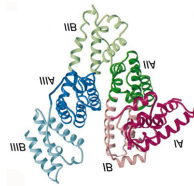
Introduction

I. Introduction

Polyelectrolytes

Charged polymers

- Functional groups are dissociated to charged monomers and counterions (weak polyelectrolyte charge varies in function of pH)

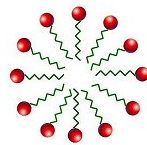
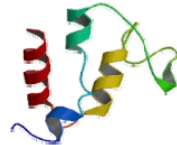
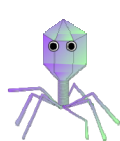


Nanoparticles

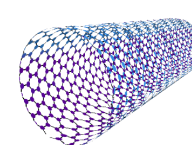
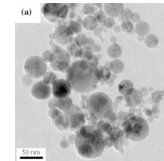
Objects constituted by tens or hundreds of atoms which have structure sizes comprised between 1 to 100 nm in at least one dimension

- Spherical, tubes, needle-like, etc.

Organic nanoparticles



Inorganic nanoparticles



High specific surface → High reactivity

I. Introduction

Electrostatic interactions in polyelectrolyte systems



Self-assembled complexes between polyelectrolytes and nanoparticles, dendrimers, flat surfaces, biomacromolecules, charged polymers

Biology

DNA packaging in eukaryote cells

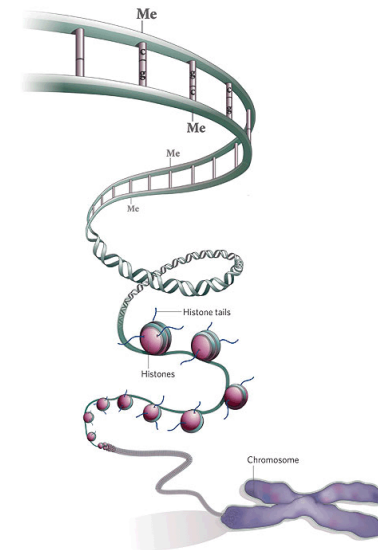
Biomedical

Contrast improvement in Magnetic Resonance Imaging

Cancer therapy by accumulation of active drug

Environment

Wastewater treatment

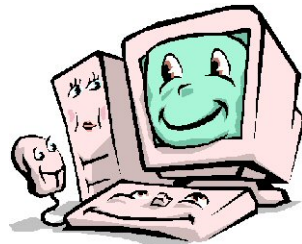


I. Introduction

Goal

How the chain hydrophobicity is playing a role on the conformational properties of biomacromolecules and on the formation of complexes with nanoparticles.

R_m	β
2	0
3	0.3
4	1
5	3



Systematic investigations



Numerical simulations



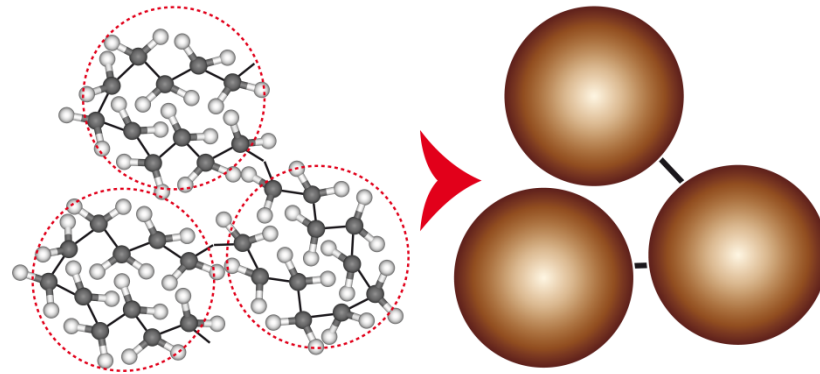
Simulation method and model

II. Simulation method and model

Polyelectrolytes

- Several thousands of atoms !
- CPU consuming

**Coarse-grained
models**



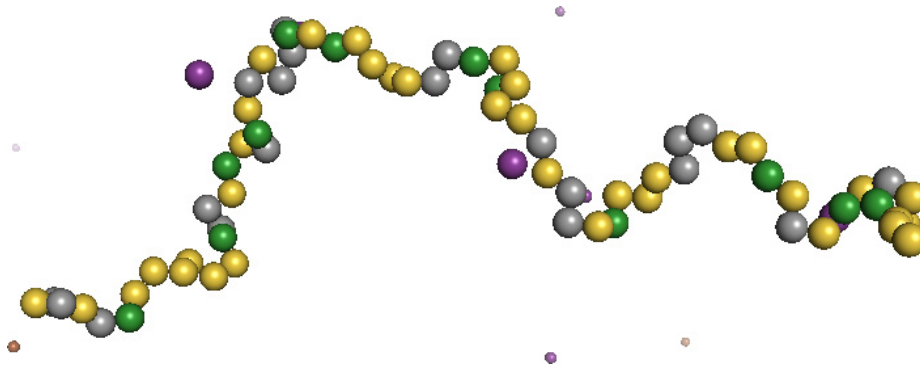
Atomistic details (bond length, vibrations, etc.) are omitted



Group of atoms → effective monomer

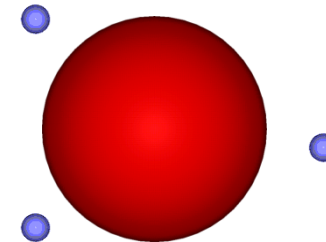
II. Simulation method and model

Biomacromolecules



- Neutral monomers
- Negatively charged monomers
- Positively charged monomers

Nanoparticles



- Nanoparticle counterions
- Chain counterions

- Each object is represented by a hard sphere
- The solvent (water) treated implicitly as a dielectric medium
- Monomer charge varies depending on the solution pH

II. Simulation method and model

Potentials

1) Electrostatic

Each charged objects interact via a full Coulomb electrostatic potential and excluded volume potential

$$U_{el}(r_{ij}) = \begin{cases} \infty, & r_{ij} < R_i + R_j \\ \frac{z_i z_j e^2}{4\pi\epsilon_r \epsilon_0} \frac{1}{r_{ij}}, & r_{ij} \geq R_i + R_j \end{cases}$$

e Elementary charge

ϵ_0 Permittivity of the vacuum

ϵ_r Water dielectric constant

r_{ij} Distance between two particles

R Particle radius

z Particle charge

2) Lennard-Jones

Lennard-Jones potential is used to take into account hydrophobic interactions between monomers

$$U_{vdW}(r_{ij}) = vdW \left[\left(\frac{r_0}{r_{ij}} \right)^{12} - 2 \left(\frac{r_0}{r_{ij}} \right)^6 \right]$$

r_0 Usually $R_i + R_j$

vdW Minimum depth of the potential curve located at a distance r_0

II. Simulation method and model

Minimum energy investigation

Monte Carlo Metropolis

- Random conformation

→ System energy calculation $E_{initial} = \sum_{ij} U_{ij}$

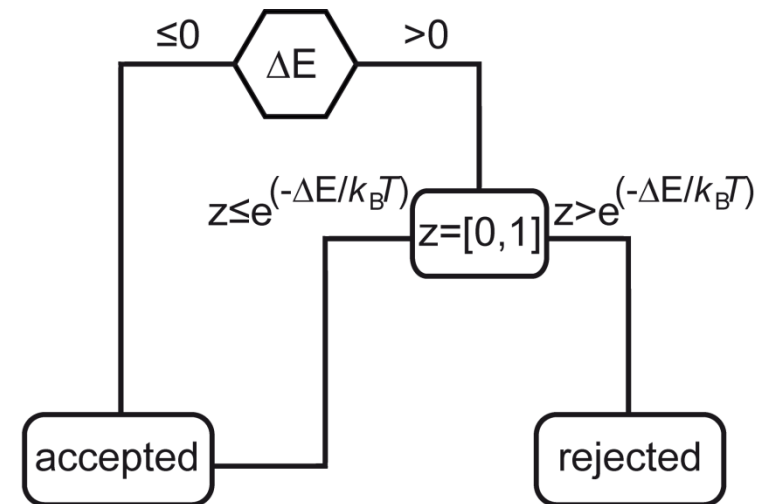
- Elementary Movements

→ System energy calculation E_{final}

- Energy difference

$$\Delta E = E_{final} - E_{initial}$$

- Metropolis test



Several thousands successive trials to achieve equilibrium state



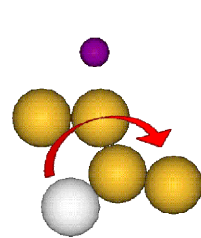
Recording of observables (macroscopic properties) → **average values**

II. Simulation method and model

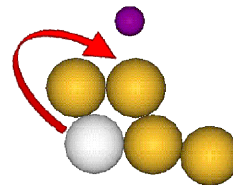
Elementary movements

Biomacromolecules

Internal movements

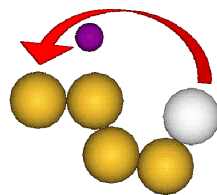


End-bond

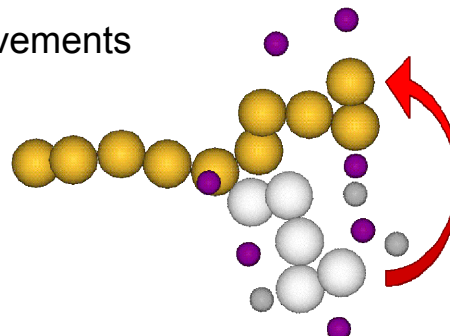


Kink-jump

Global movements

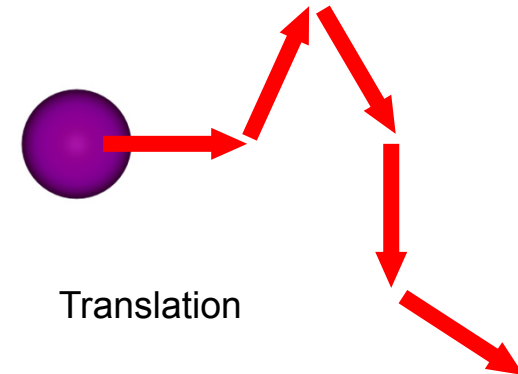


Reptation



'Partially clothed' Pivot

Counterions



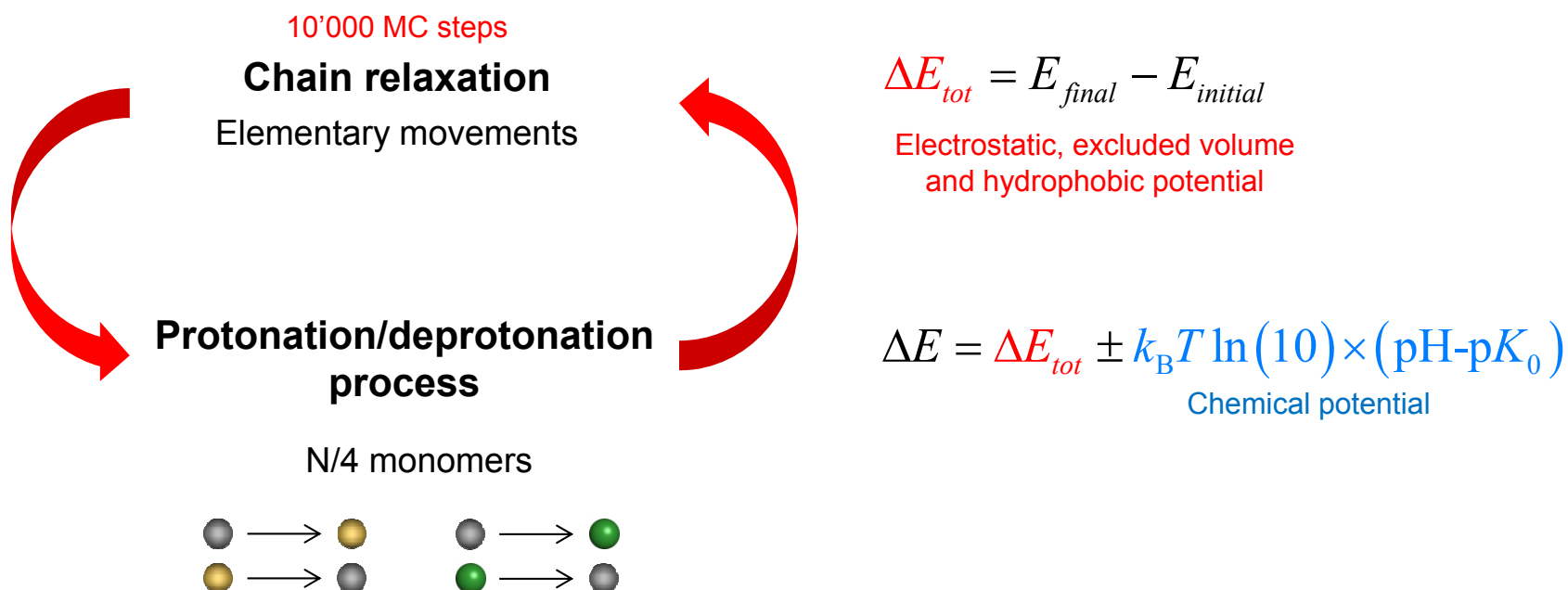
Translation

II. Simulation method and model

pH dependency of biomacromolecules



Protonation/deprotonation cycle



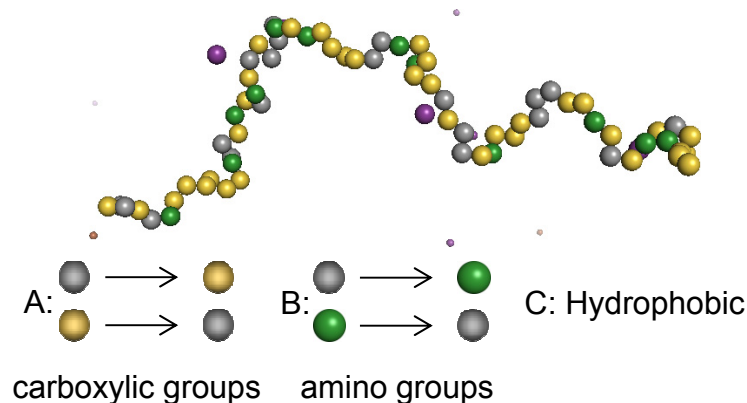
Several million MC steps to obtain a reasonable sampling of low energy conformations

II. Simulation method and model

2 models

Simplified protein structure

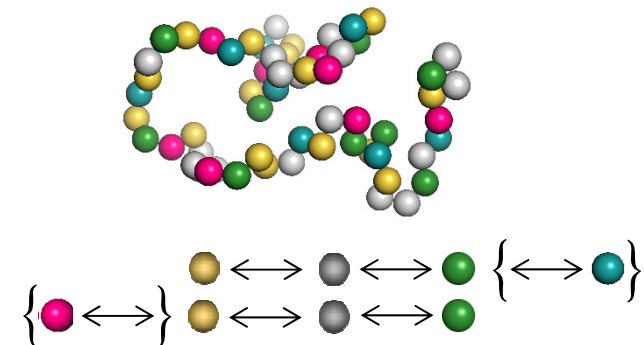
- 0 Neutral monomers
- 1 Negatively charged monomers
- 1 Positively charged monomers



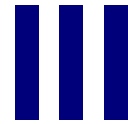
- 100 monomers
- sequence of monomers randomly determined in the beginning of the simulation
- $pK_A = 2.17$ / $pK_B = 9.53$

BSA protein

- 0 Neutral monomers
- 2 -1 Negatively charged monomers
- 2 1 Positively charged monomers



- 583 amino acids
- sequence of amino acids known (x-ray structure)
- each amino acid can be neutral, positively and negatively charged
- pK_A , pK_B and pK_C (if present) different for each amino acid

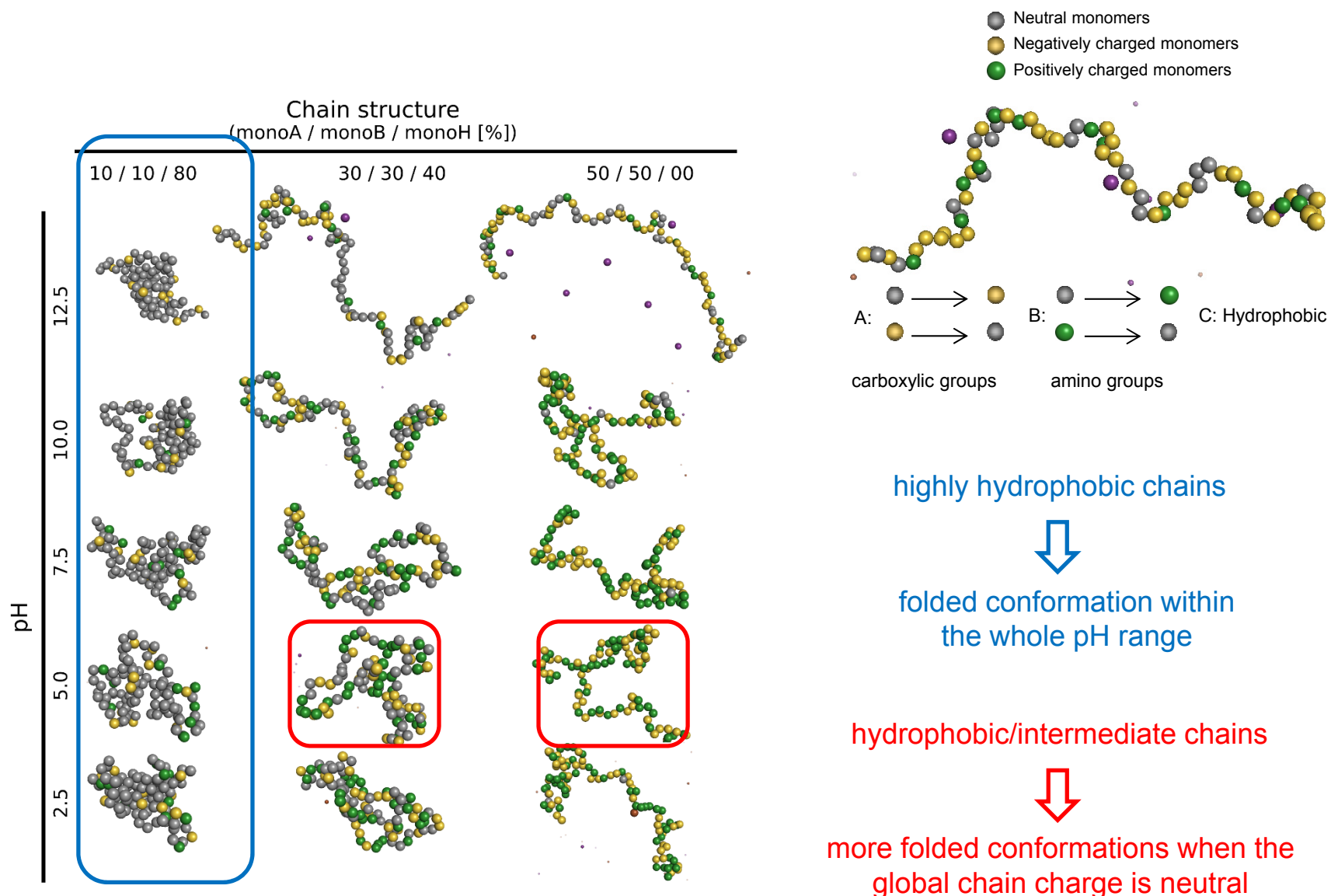


Results

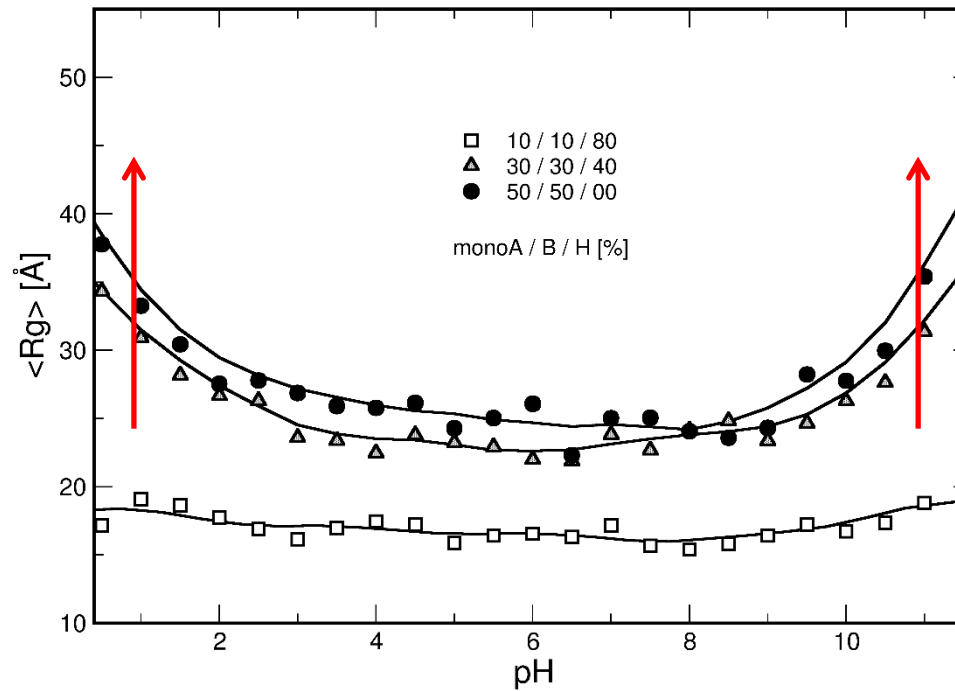
Simplified protein structure

- pH
- Chain hydrophobicity
- Presence of nanoparticle

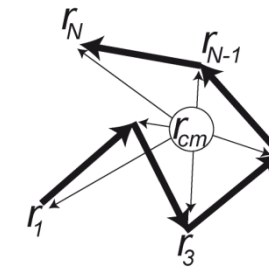
III. Simplified protein structure



III. Simplified protein structure



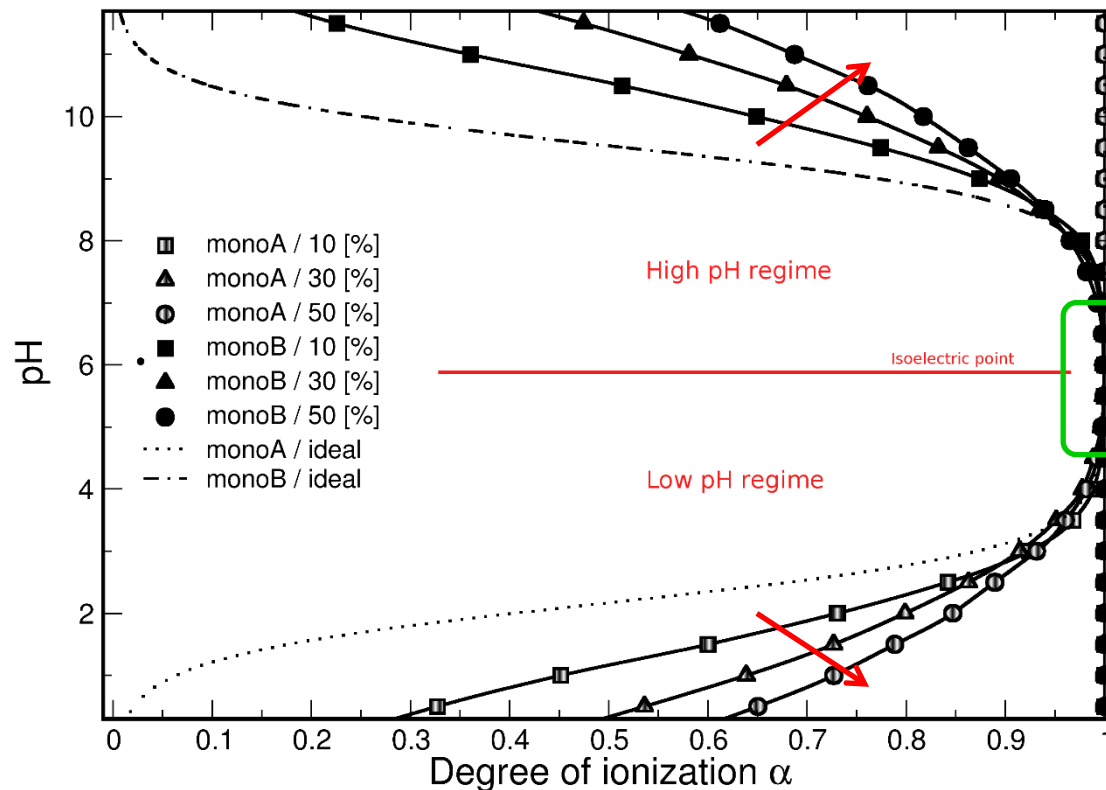
Square radius of gyration



$$R_g^2 = \frac{1}{N} \sum_{i=1}^N (r_i - r_{cm})^2$$

- Extended conformations at low and high pH in the case of hydrophilic and intermediate chains
- Strong repulsive monomer-monomer electrostatic interactions favorise less compact conformations

III. Simplified protein structure

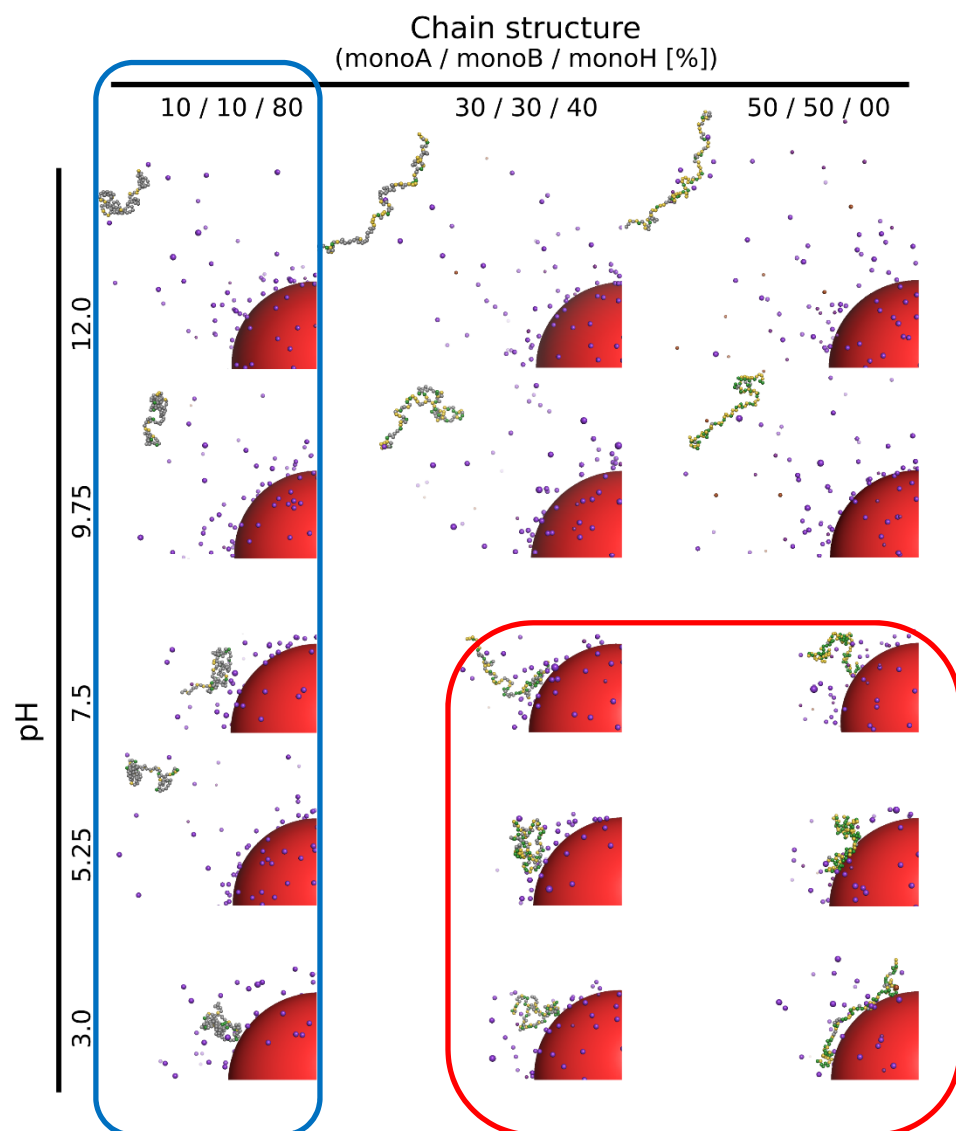


$pK_0^A = 2.17$ (carboxylic groups)

$pK_0^B = 9.53$ (amino groups)

- Charging process of carboxylic/amino groups promoted by charged amino/carboxylic groups
- Attractive electrostatic interactions weaker for hydrophobic chains resulting in a less efficient charging process

III. Simplified protein structure



$R_{NP} = 100\text{\AA}$
 $\sigma_{NP} = -39.9\text{ mC/m}^2$

highly hydrophobic chains



no elongated conformation
no formation of complexes

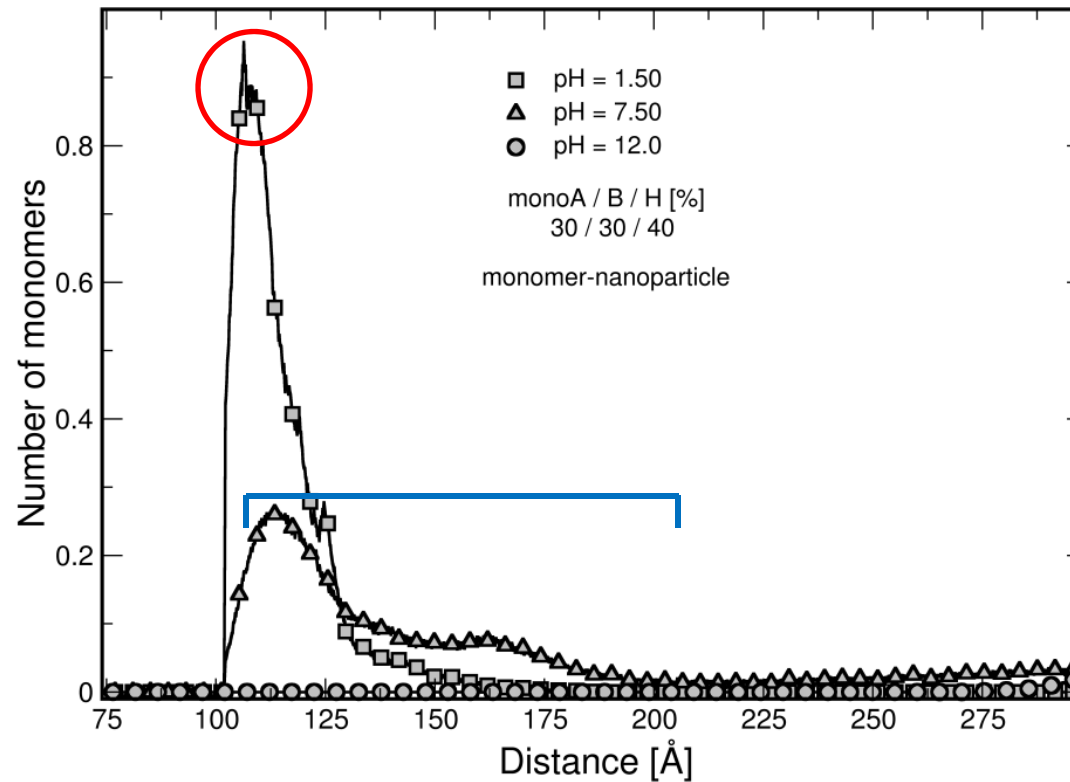
intermediate and hydrophilic chains



stronger electrostatic interactions with
the NP → adsorption at the surface

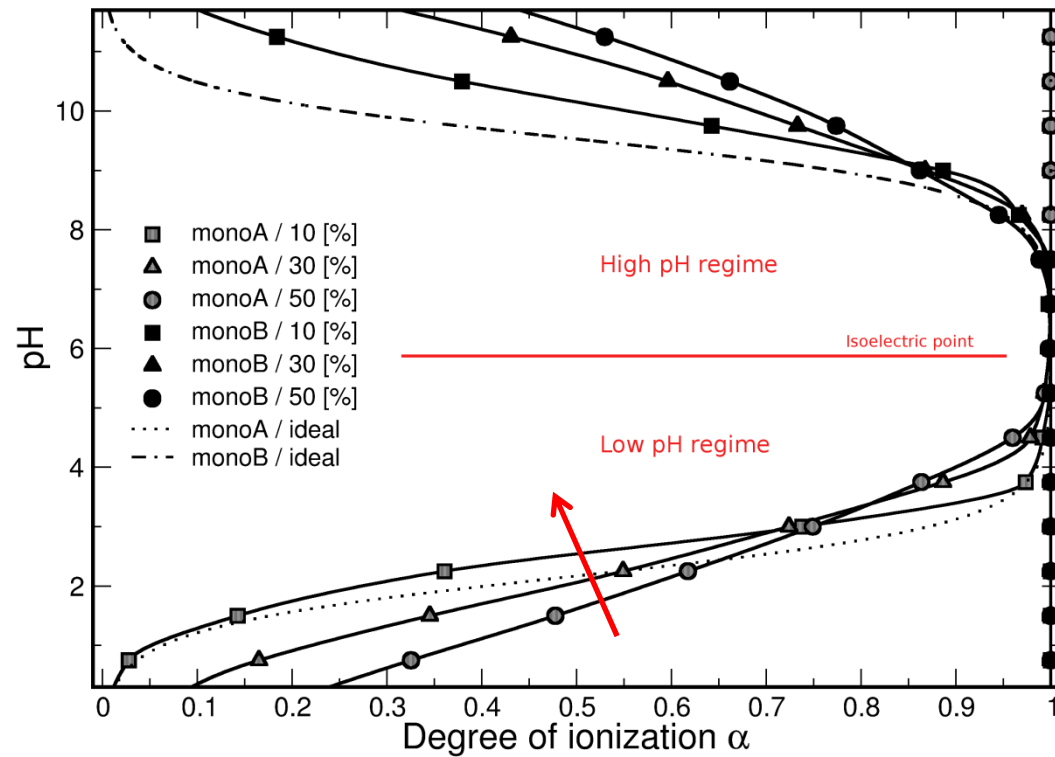
both amino and carboxylic groups
charged at physiological pH resulting in
partial chain adsorption

III. Simplified protein structure

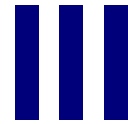


- Strong adsorption at low pH → **high peak**
- At physiological pH, local adsorption resulting in a **larger monomer distribution**

III. Simplified protein structure



- Lost of symmetry at low pH → **intersection** with the ideal curve
- Modification of the apparent pKa hence **promoting** the protonation of carboxylic groups

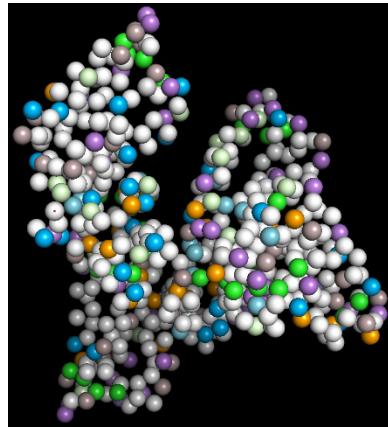


Results

BSA protein

- Parametrization
- pH
- Nanoparticle surface charge density

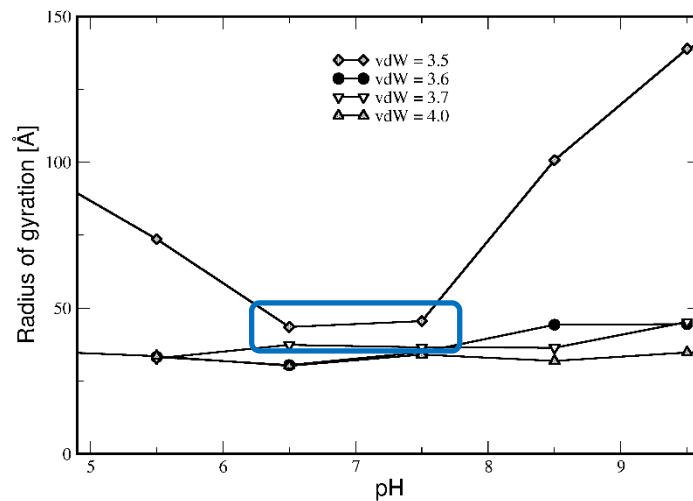
III. BSA protein



BSA x-ray structure

Parameters

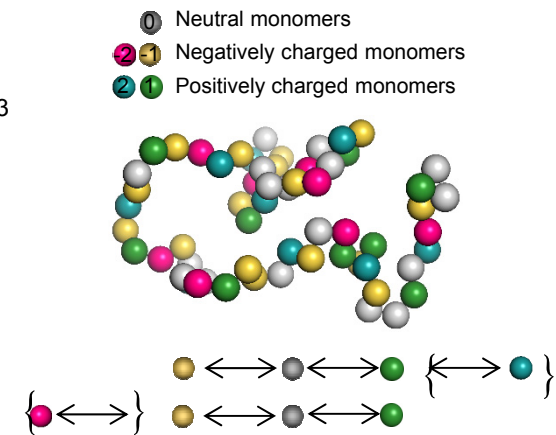
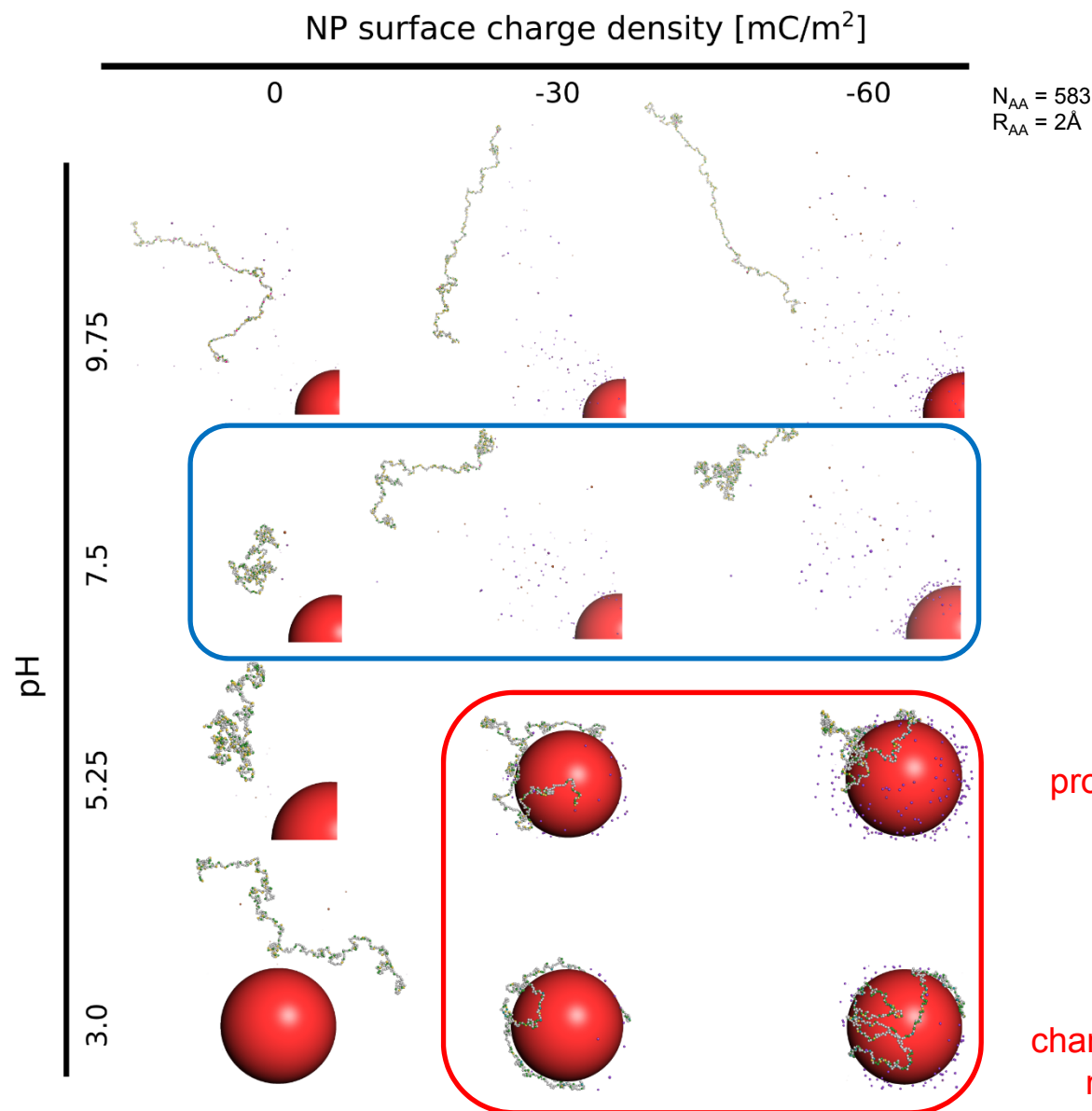
- length and contour size of the protein
- amino acid distribution
- pK_a , pK_b and pK_c values



$$U_{\text{vdW}}(r_{ij}) = \text{vdW} \left[\left(\frac{r_0}{r_{ij}} \right)^{12} - 2 \left(\frac{r_0}{r_{ij}} \right)^6 \right]$$

- hydrophobic interactions between amino acids not too strong to observe conformational changes (denaturation)
→ $\text{vdW} = 3.5 K_B T$
- hydrophobic amino acids: Ala, Met, Leu, Val, Ile, Phe

III. BSA protein



physiological pH



more folded conformations
and no adsorption with only
electrostatic interactions

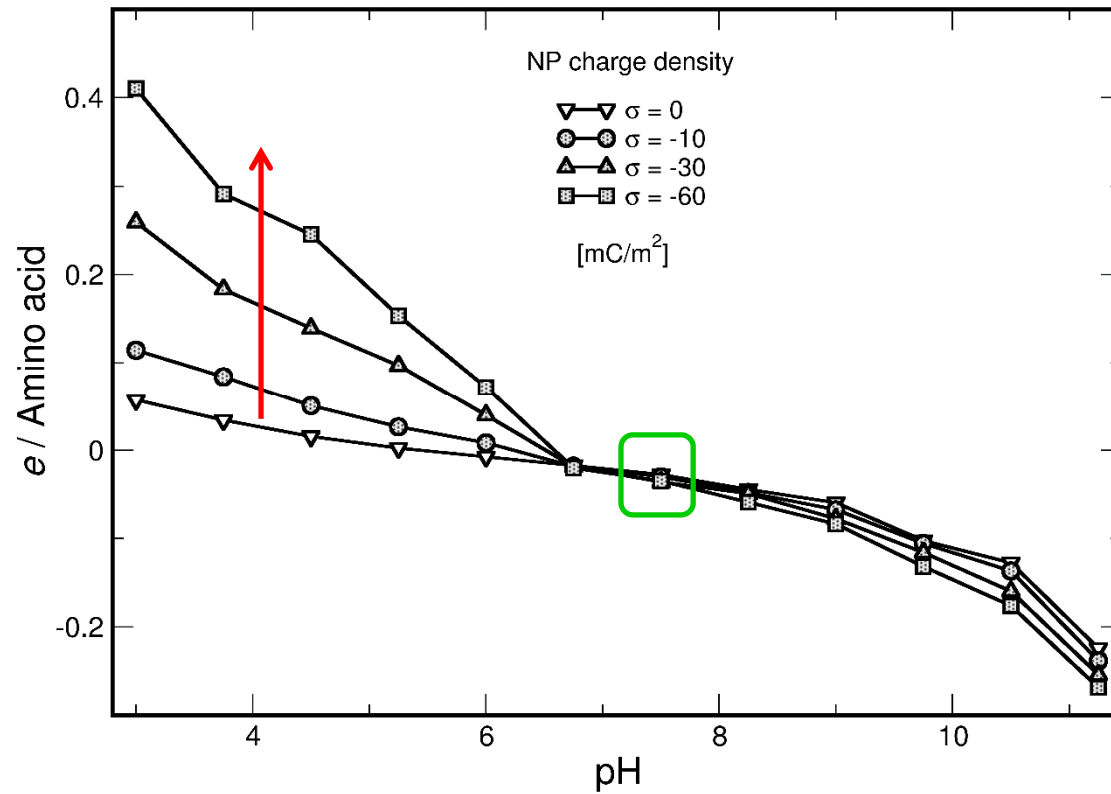
protein positively charged at low pH



adsorption at the surface of the
nanoparticle

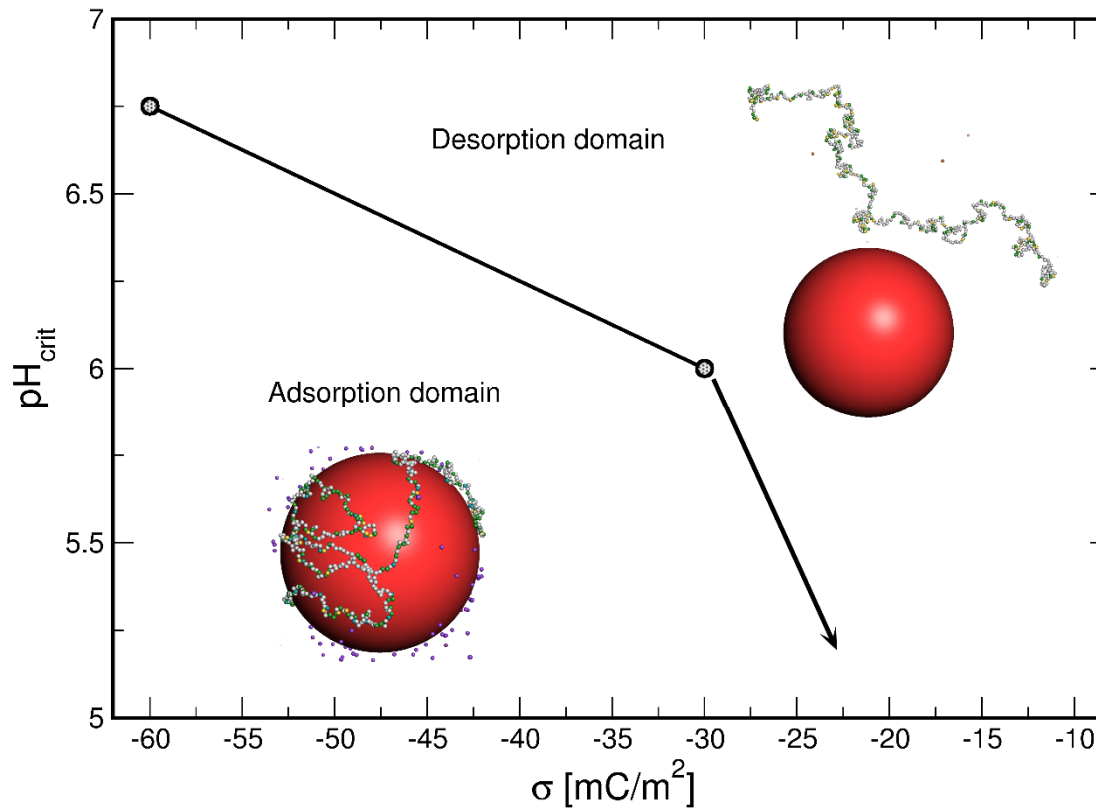
change of the protein conformation and
modification of the charge of the
nanoparticle (reactivity)

III. BSA protein



- Global charge of the protein is slightly negative at physiological pH
- Presence of the nanoparticle significantly influences the charging process of the protein

III. BSA protein



$$R_{NP} + R_{AA} \leq Ads_L \leq R_{NP} + 3R_{AA}$$

Adsorption criterion

One amino acid situated in the adsorption layer (Ads_L) more than 50% of Monte Carlo steps

- Adsorption domain increases with the nanoparticle surface charge density

Prediction of the reactivity of nanoparticles!

IV

Conclusions and perspectives

IV. Conclusions et perspectives

- Structure of strong hydrophobic chains not dependent on pH
→ Denaturation and reactivity limited
- The nanoparticle modifies the acid/base properties of chains, and thus the charging process

BSA protein

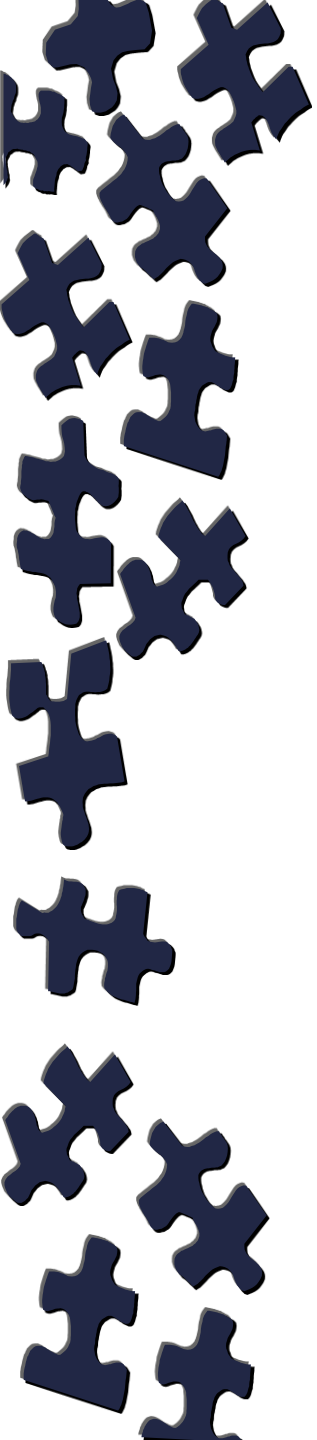
- BSA conformations strongly modified at extrem pH and with the adsorption at the nanoparticle surface
→ Denaturation
- No BSA adsorption at physiological pH considering only electrostatic interactions
→ Importance of hydrophobic and structural interactions between the nanoparticle and BSA

Work in progress

- Improvement of the **interactions** between the protein and nanoparticle
- Parametrization and **validation** of the model based on experimental data provided by the University of Ljubljana

Thank you

for your attention !



Computational Approaches in Nanosciences Workshop

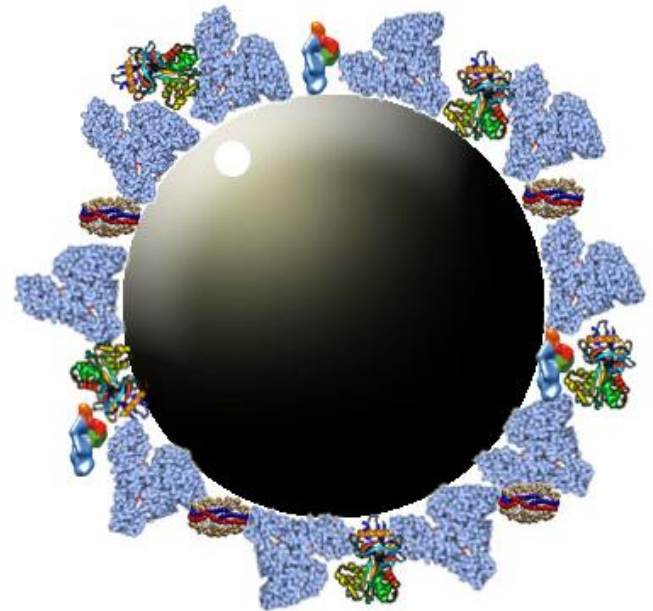
Interaction of carbon-based nanomaterials with cholinesterases and serum proteins

Maja Sopotnik

October 3rd, 2015

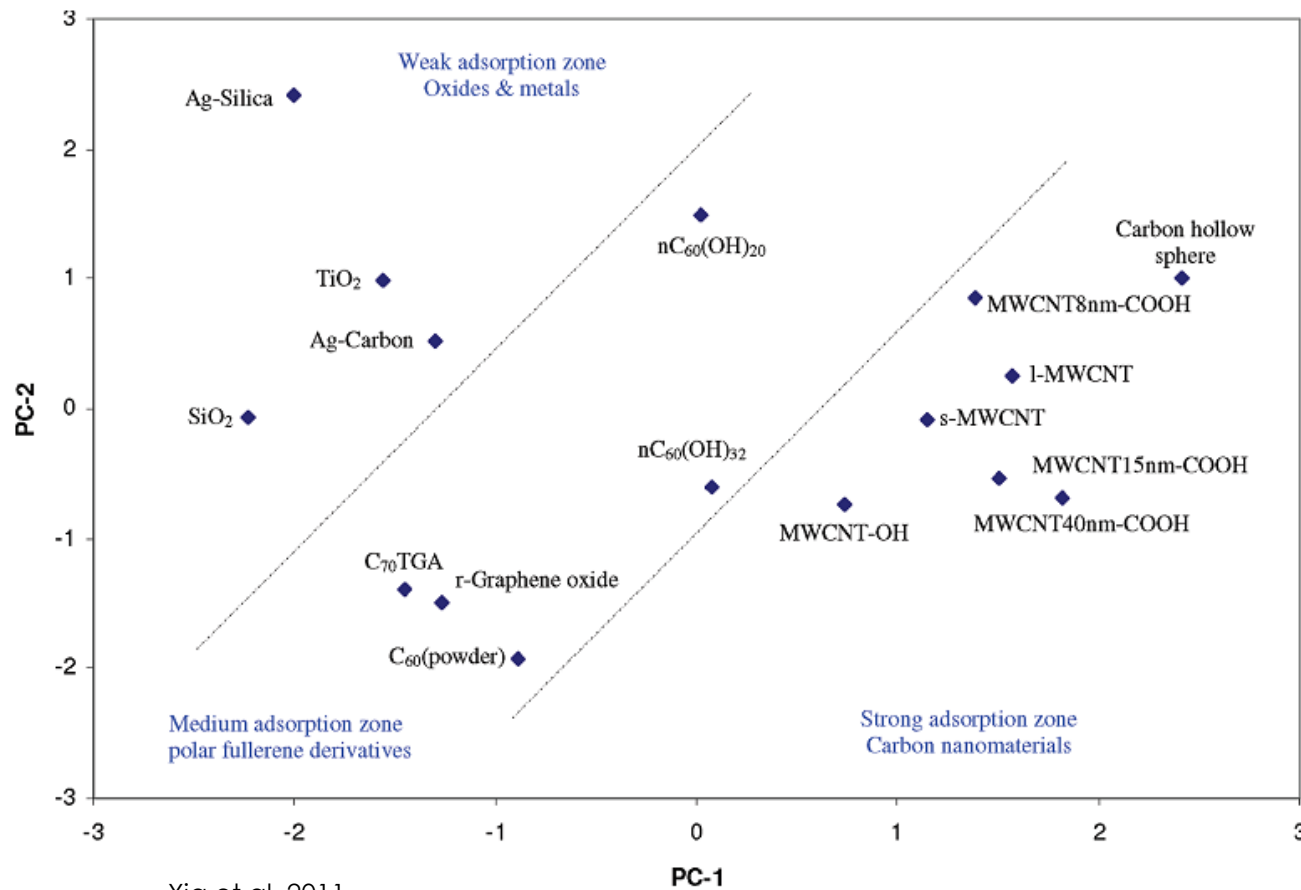
NM in biological media

- › Adsorption of biological molecules on the surface of the NM
- › Protein corona
 - depends on the properties of the NM and the proteins
- › Change in NM characteristics and behaviour
- › The need for biological characterisation of NM



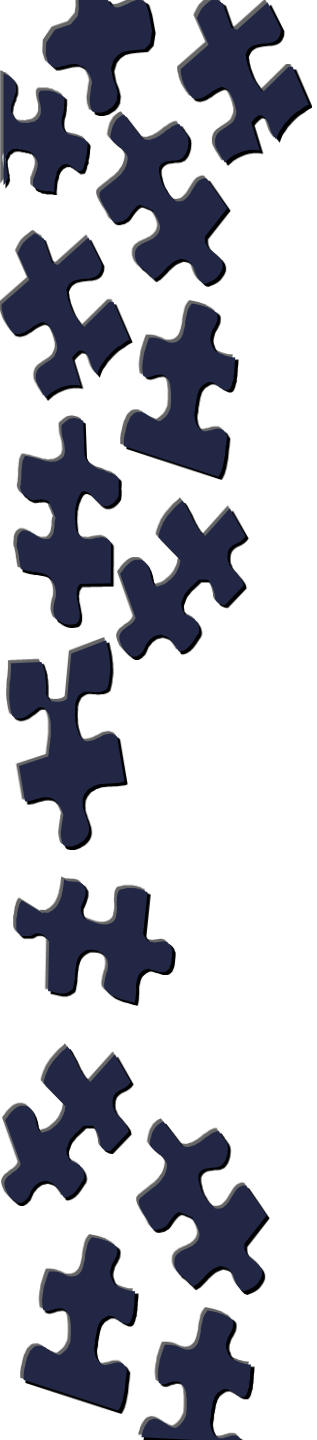
Carbon NM are highly sorptive

- › Surface adsorption index, 5 nanodescriptors



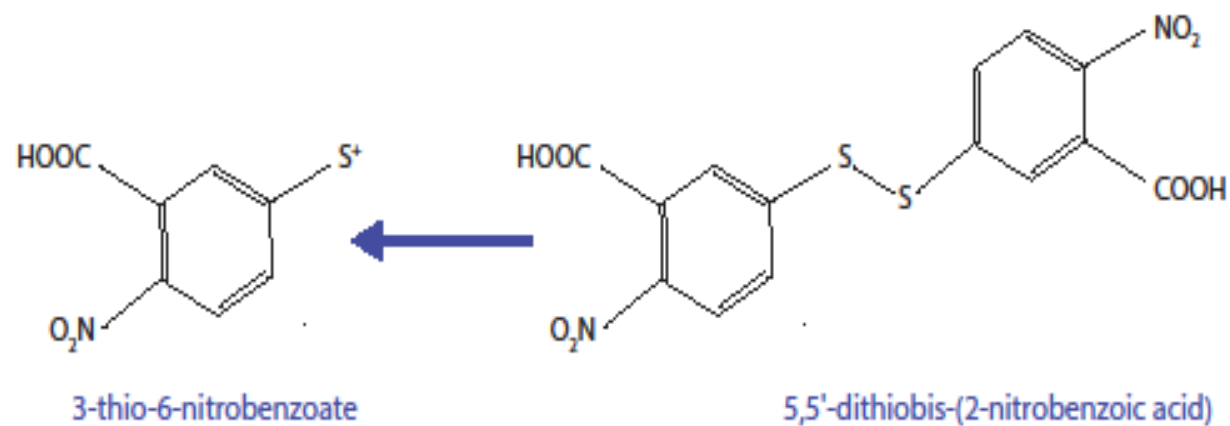
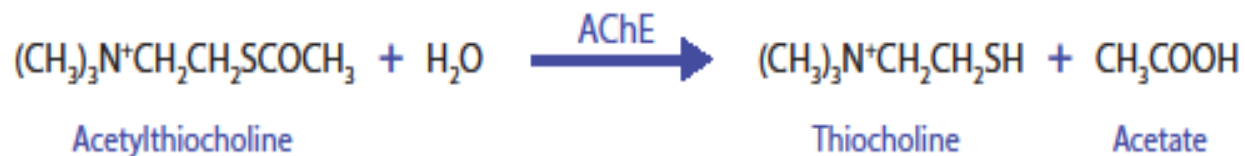
Xia et al. 2011





Cholinesterases

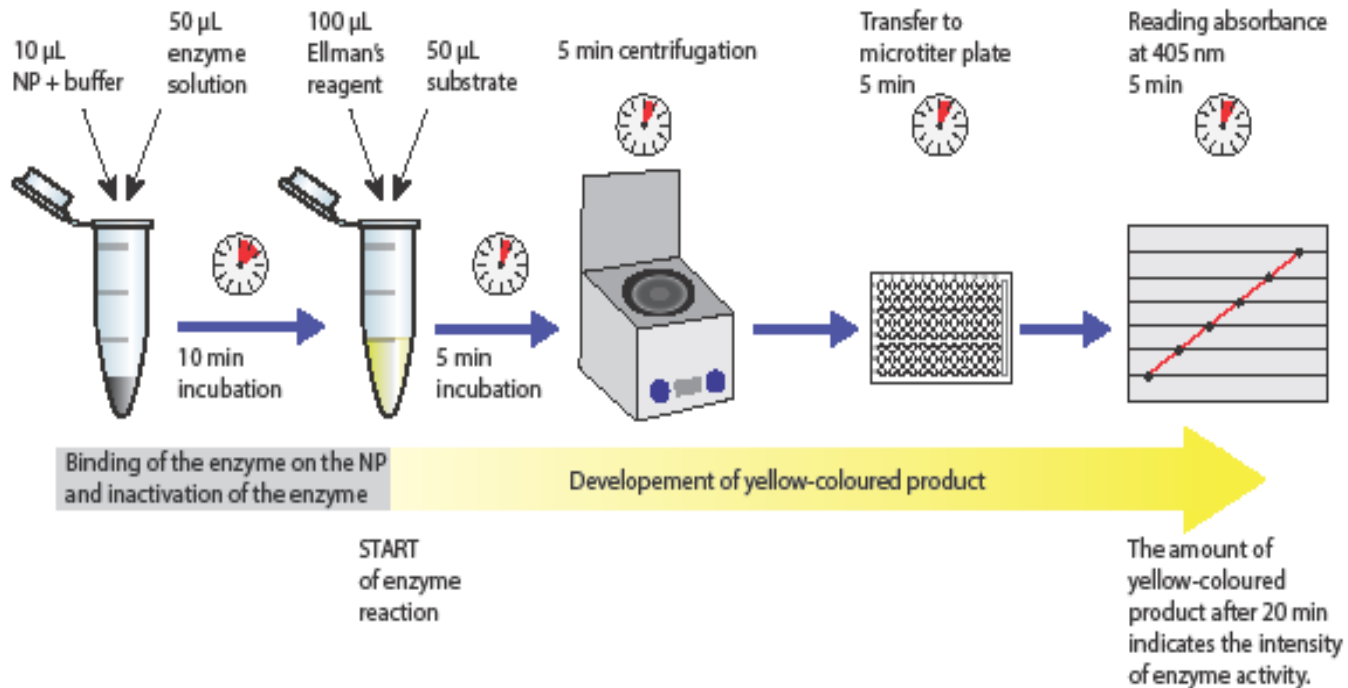
- › Acetylcholinesterase
 - Neurotransmitter acetylcholine degradation
- › Butyrylcholinesterase
 - Backup system in the blood
- › Convenient optical method of measuring the amount of the reaction product – Ellman's method



Jemec et al., in press

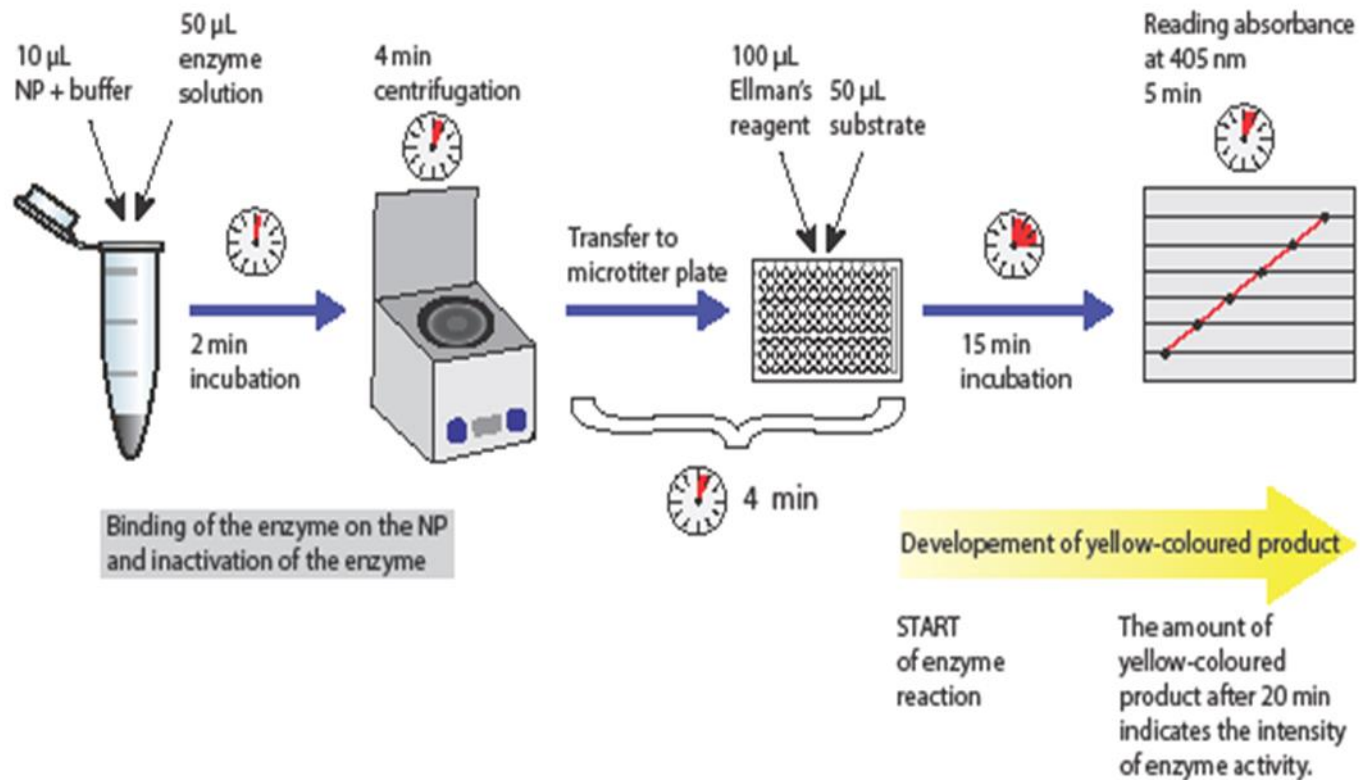


Measuring the inhibition of enzyme activity by the NM



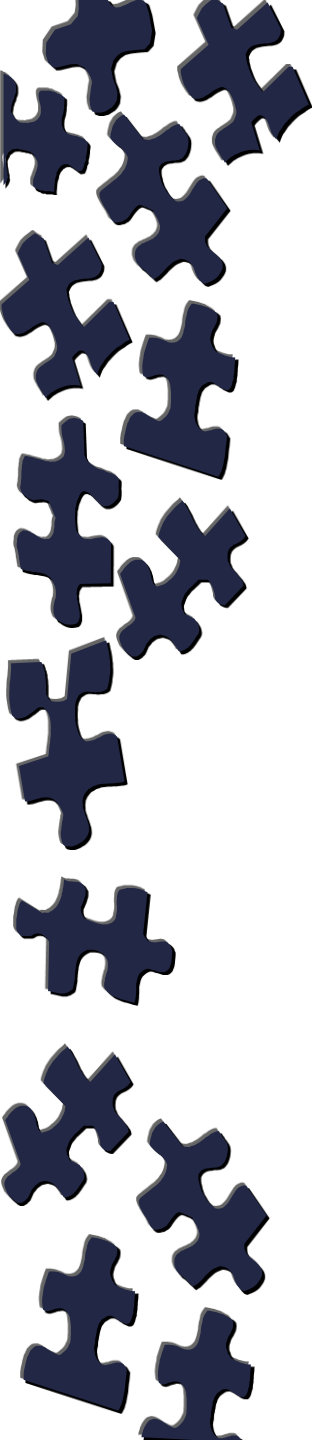
Jemec et al., in press

Measuring the adsorption of the enzyme on the NM



Jemec et al., in press

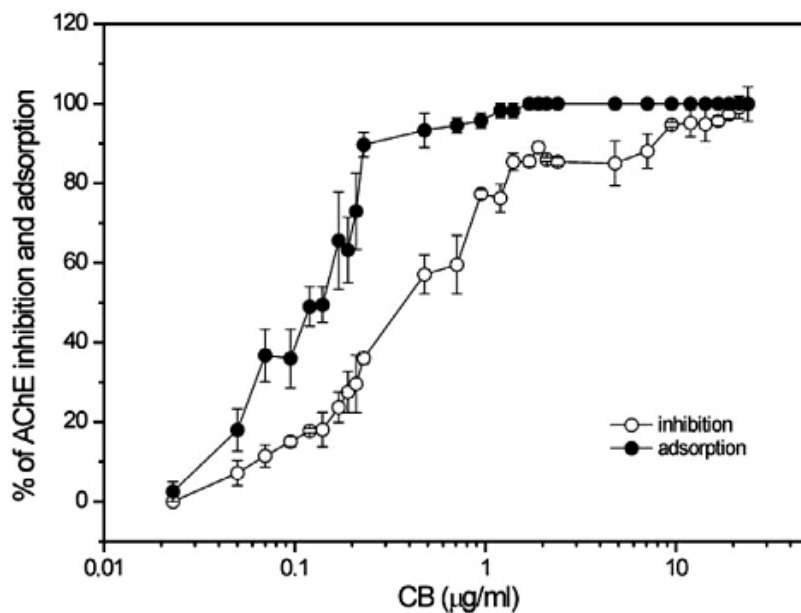




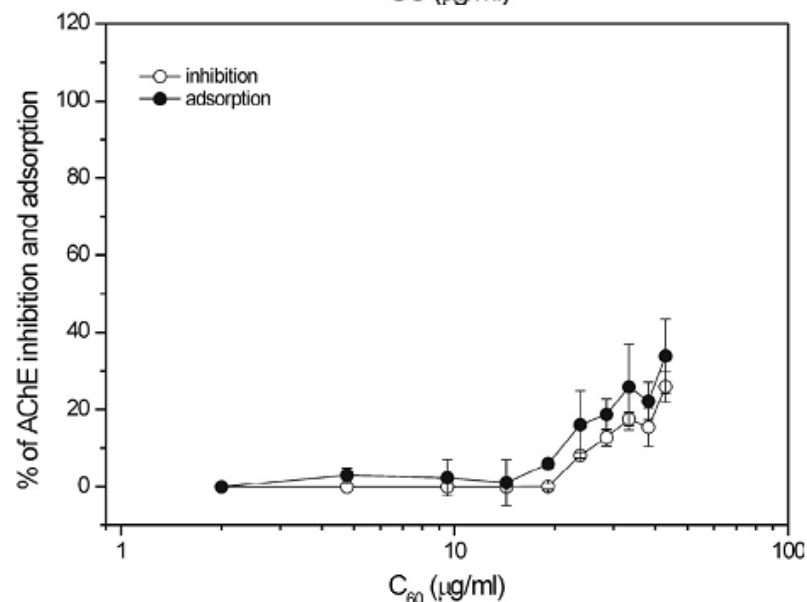
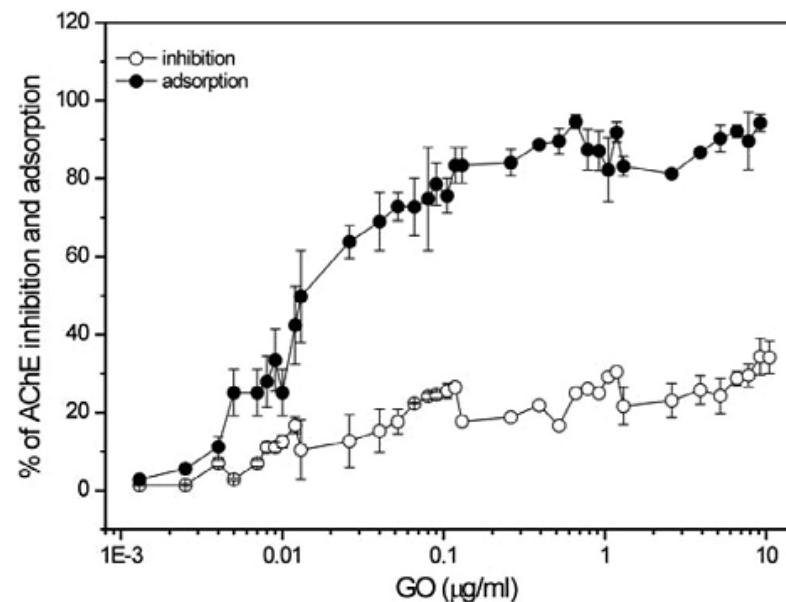
Comparison of different carbon NM

- › Graphene oxide
- › Carbon black
- › C₆₀ – fullerenes
- › Multi-walled carbon nanotubes

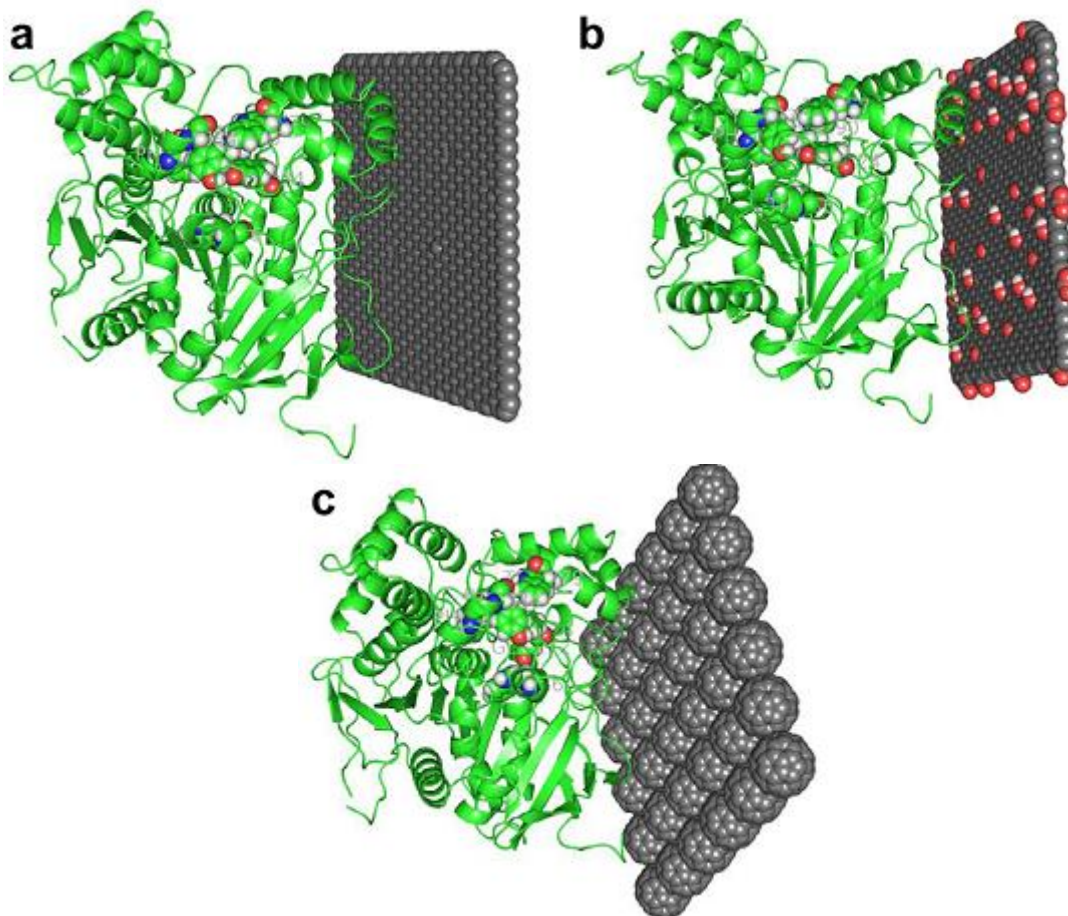
Drosophila melanogaster acetylcholinesterase



Mesarič et al. 2013



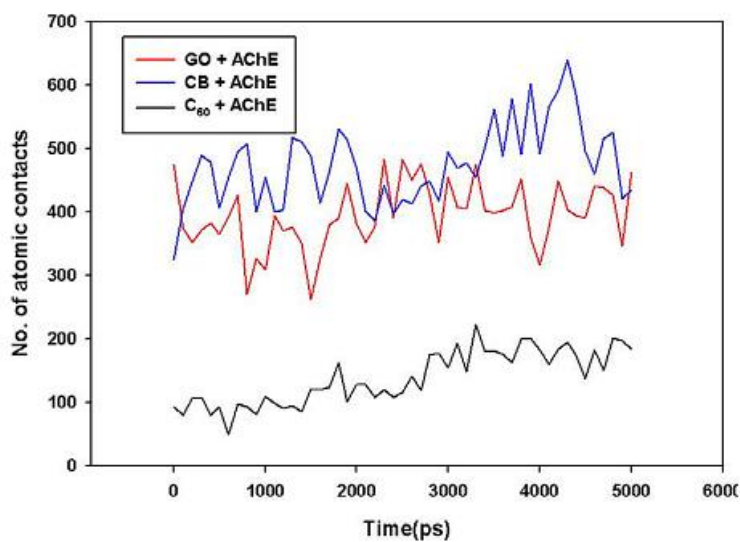
Molecular dynamics simulations



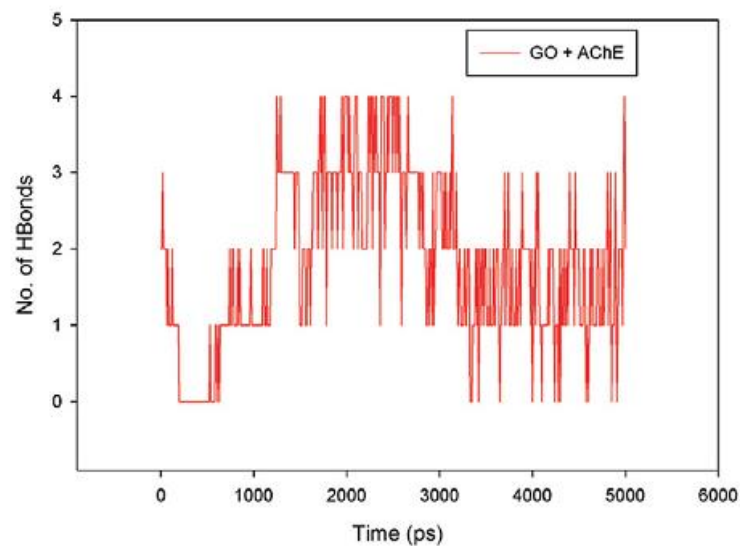
Mesarič et al. 2013



Molecular dynamics simulations



Atomic contacts



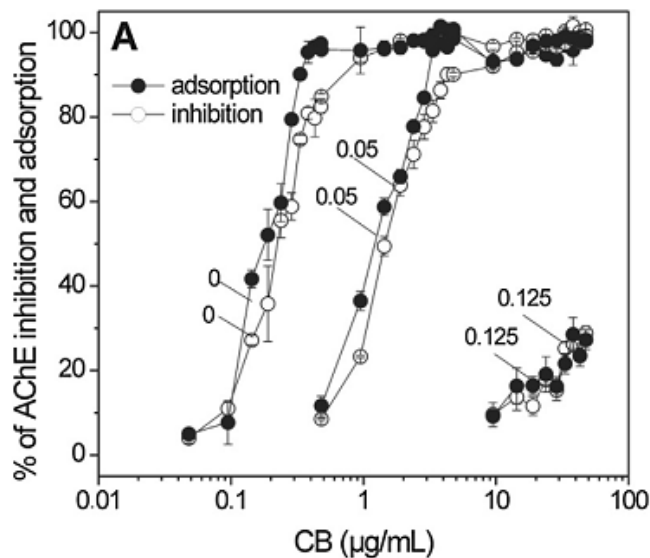
Hydrogen bonds

Mesarič et al. 2013

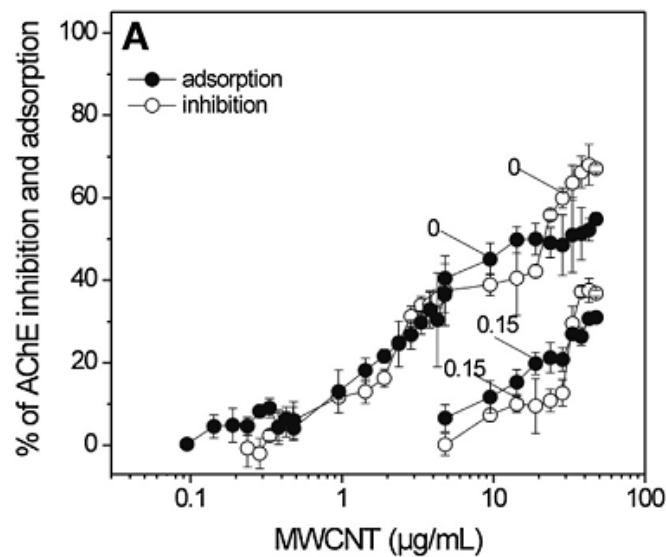
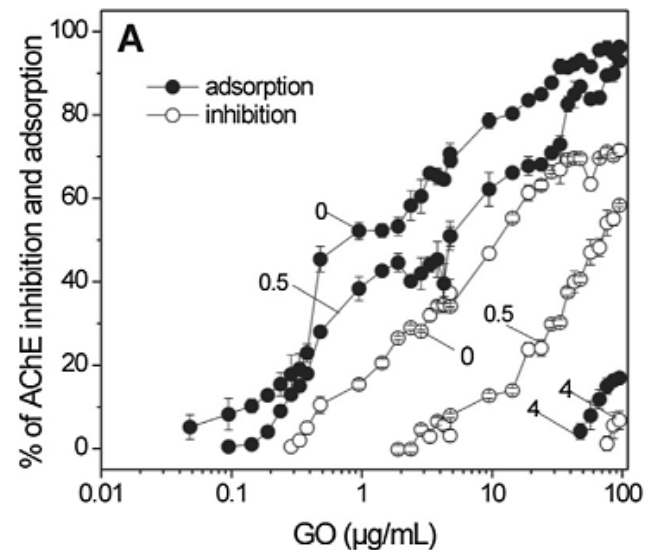


Electrophorus electricus acetylcholinesterase

› Pre-coating with BSA

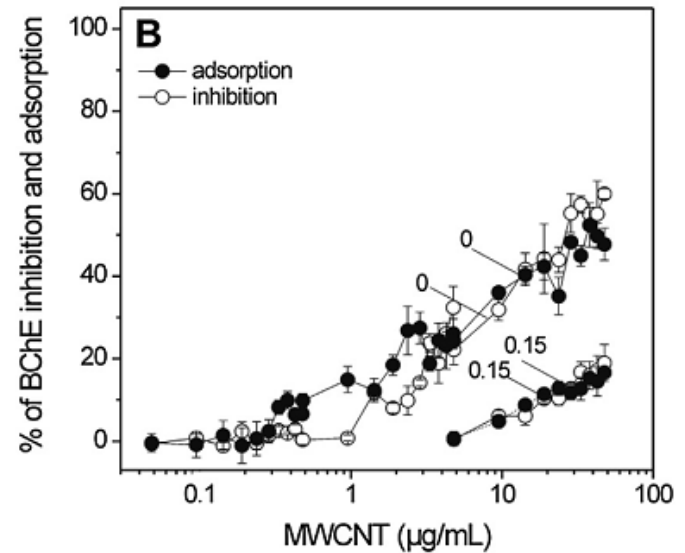
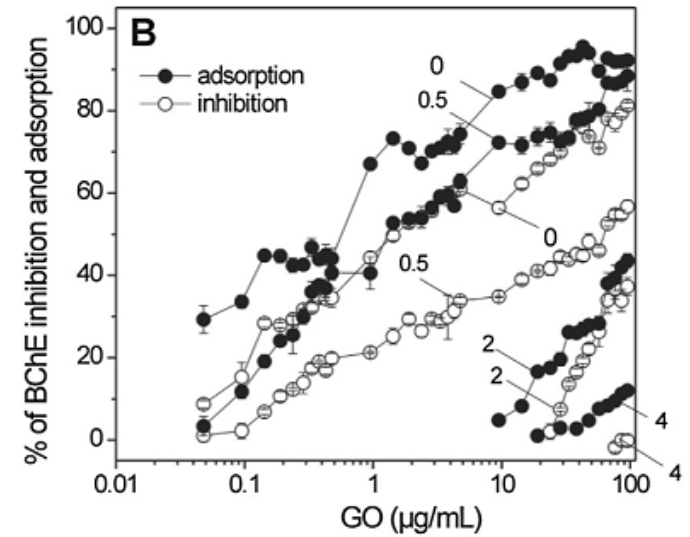
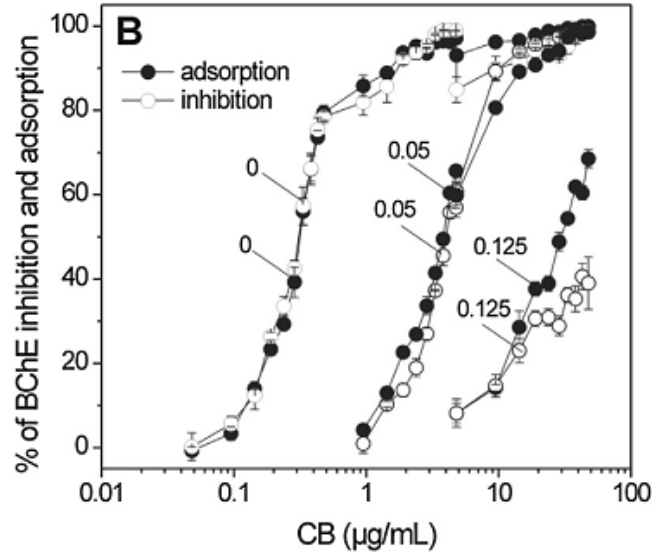


Sopotnik et al. 2015



Equine serum butyrylcholinesterase

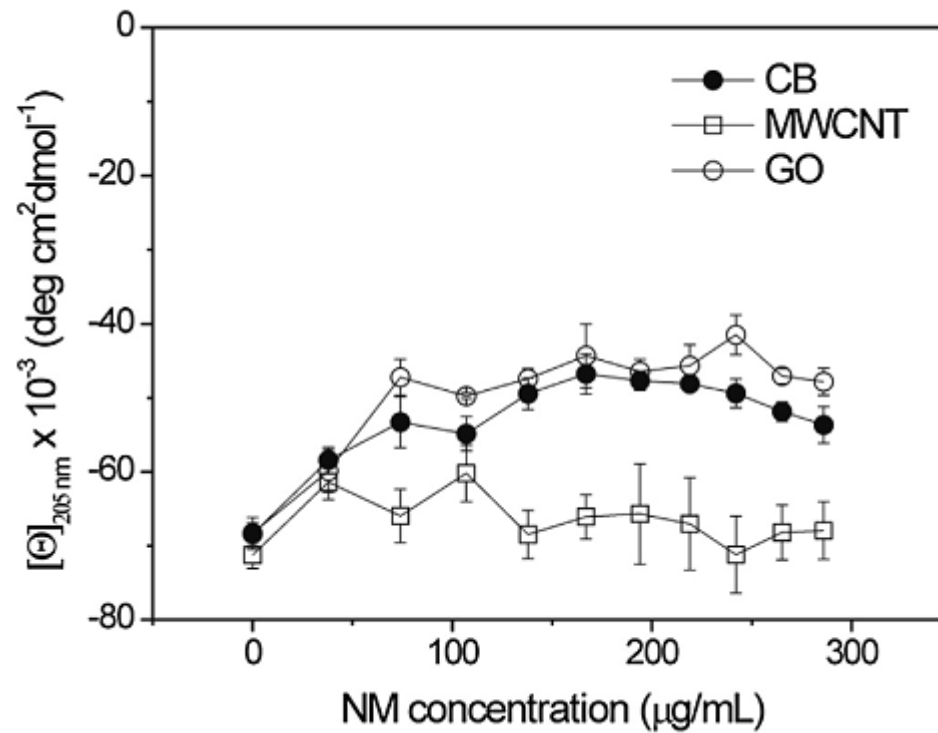
› Pre-coating with BSA



Sopotnik et al. 2015



CD-measurements

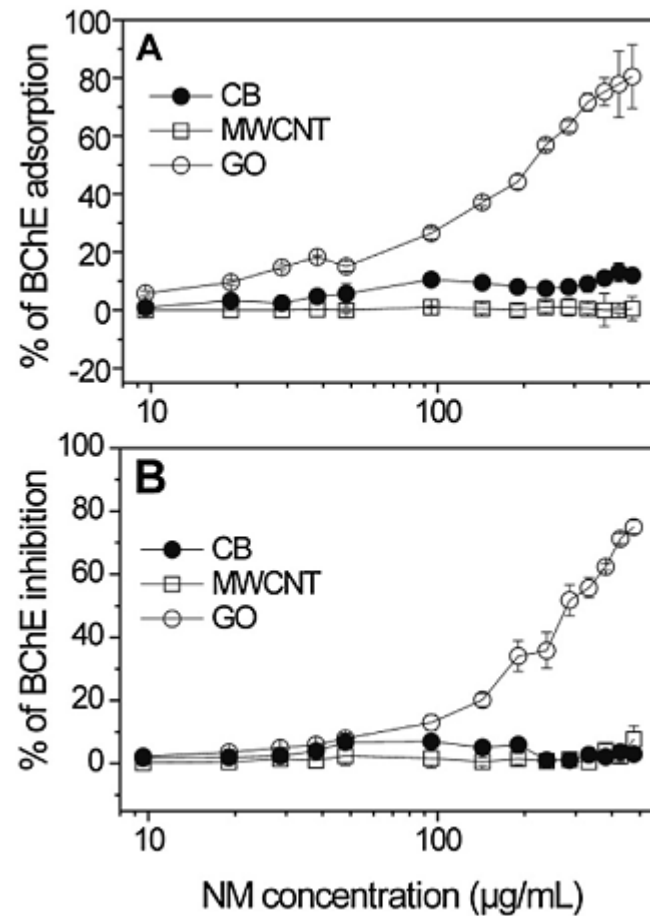


Sopotnik et al. 2015



Human serum

- > 0,4 % human serum
- > Intrinsic BChE

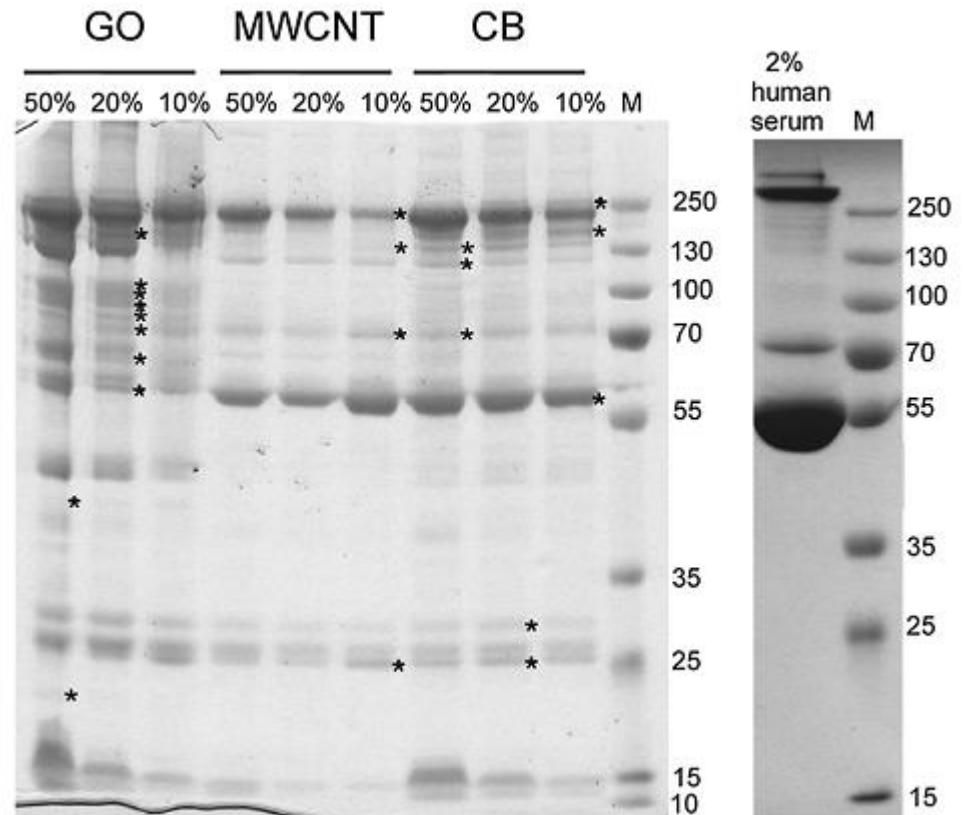


Sopotnik et al. 2015



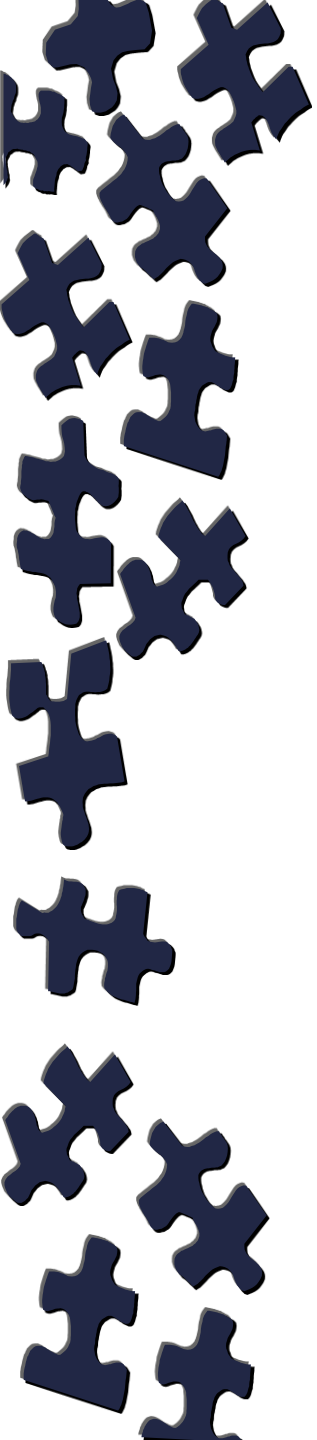
Human serum

- > Transport proteins
 - Serum albumin
 - Seroransferrin
 - Apolipoprotein A-I
 - Apolipoprotein E
- > Immune system
 - Complement C3
 - Complement C4-A
 - Copmlement C1q
 - Immunoglobulins



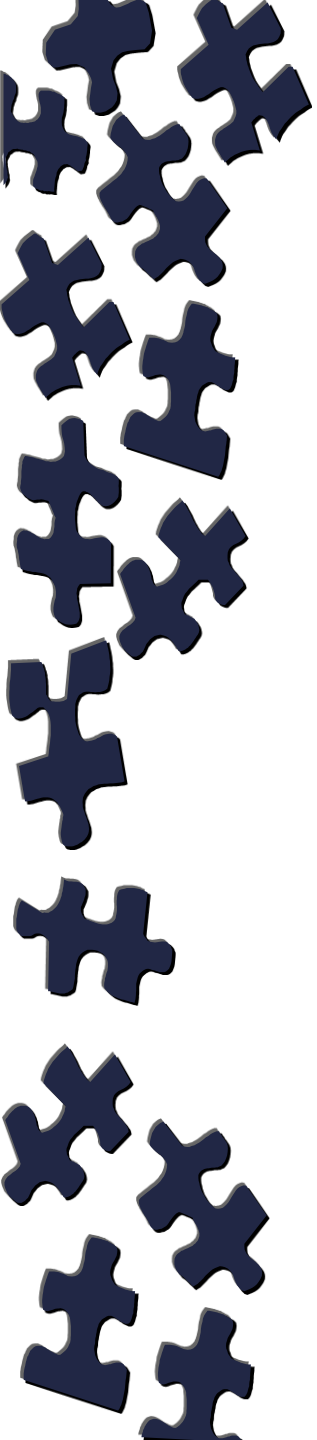
Sopotnik et al. 2015





Conclusions

- › AChE inhibition: CB>GO>MWCNT>C₆₀
- › GO : AChE adsorption >> AChE inhibition
- › Binding affinity of NMs for serum albumin was similar in pure albumin solution and in the whole serum
- › CB and MWCNT have a strong affinity for serum albumin
- › GO has a weaker affinity for serum albumin but stronger for other serum proteins, including BChE; is less specific
- › Carbon NM corona is enriched with complement factors and apolipoproteins



Literature:

- › Xia, X. R., Monteiro-Riviere, N A., Mathur, S., Song, X., Xiao, L., Oldenberg, S. J., Fadeel, B. & J. E. Riviere, 2011. Mapping the Surface Adsorption Forces of Nanomaterials in Biological Systems. *ACS Nano* 5(11): 9074-9081.
- › Jemec, A., Mesarič, T., Sopotnik, M., Sepčič, K. & d. Drobne. Biological characterization of nanomaterials. In press.
- › Mesarič, T., Baweja L., Drašler, B., Drobne, D., Makovec, D., Dušak, P., Dhawan, A. & K. Sepčič, 2013. Effects of surface curvature and surface characteristics of carbon-based nanomaterials on the adsorption and activity of acetylcholinesterase. *Carbon* 62: 222-232.
- › Sopotnik, M., Leonardi, A., Križaj, I., Dušak, P., Makovec, D., Mesarič, T., Poklar Ulrih, N., Junkar, I., Sepčič, K. & D. Drobne, 2015. Comparative study of serum protein binding to three different carbon-based nanomaterials. *Carbon* 95: 560-572.

Quality of nanotoxicity data and the importance of harmonization

Dr. Anita Jemec

University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

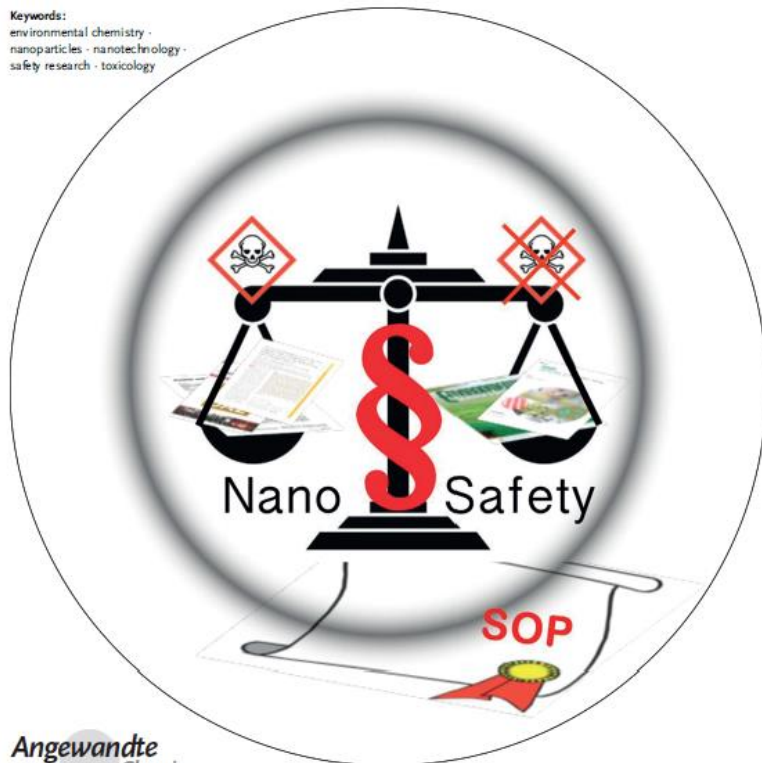
Univerza
v Ljubljani
Biotehniška
fakulteta



Nanosafety Research—Are We on the Right Track?

Harald F. Krug*

Keywords:
environmental chemistry ·
nanoparticles · nanotechnology ·
safety research · toxicology



Krug et al., 2014.

Screen the nanotoxicity literature of the last 10-15 years
>10 000 publications.

Conclusion:

„Most of these studies, do not offer any kind of clear statement on the safety of nanomaterials. On the contrary, most of them are either self-contradictory or arrive at completely erroneous conclusions...“

Table 1: Variables in the in vitro toxicity tests during the investigation of ENMs (modified according to Ref. [35]).

Variables associated with the nano-material	Variables associated with the tox assay	Variables associated with the biological model
sample purification for the removal of biologically relevant trace elements	selection of the correct test system regarding the biological end points	selection of the biological system
sample characterization of the raw material: composition and purity size shape agglomeration status etc.	different test systems for the same biological end point	cell lines: selection criteria identification age and storage number of passages etc.
sample characterization regarding biological impurities: endotoxins etc.	controls: adapted negative controls adapted positive controls comparison to reference materials	primary cells/organ systems: donor dependency donor variability culture conditions
dispersion in biological media under relevant conditions: temperature humidity gas concentrations (O ₂ , CO ₂) salinity etc.	testing of possible interferences of the ENM with the biological test system binding of indicator molecules light absorption or fluorescence of the materials etc.	culture conditions during the experiments: temperature humidity gas concentrations (O ₂ , CO ₂) salinity etc.
sample characterization in biological media: size and shape agglomeration status protein corona etc.	measurement uncertainty not considered: round robins calibration with standards or reference material	biological parameter: cell density volume of the medium serum content of the medium compatibility of the solvent or dispersion medium

CRITERIA FOR quality of nanotoxicity data

NanoValid aimed to develop criteria for good quality nanotoxicity data to be used for RA and LCA

-

In order to provide a list of criteria three web-based initiatives, one authority document, one list developed with NanoValid, and two scientific papers (Stefaniak et al., 2013, using various sources, Mills et al., 2014) were used as a basis.

1. MinChar initiative, providing minimal material characterization recommendations for nanotoxicology studies (<http://characterizationmatters.org/parameters/>), see Annex 1
2. DaNa criteria checklist, providing a list to evaluate nanotoxicity studies regarding their scientific value (<http://www.nanopartikel.info/cms/lang/en/Wissensbasis/kriterienkatalog>), (Kühnel et al., 2014))
3. The Nanomaterial Registry's Minimal Information About Nanomaterials (MIAN), <https://www.nanomaterialregistry.org/about/MinimalInformationStandards.aspx> (Mills et al., 2014), see Annex 4
4. Standard information required for Nanomaterials manufactured or imported (REACH) "Nanomaterials and REACH – Background paper on the position of the German competent authorities", (UBA, 2013)
5. List of issues and parameters to be specified for ENPs used in toxicological tests under NanoValid, see Annex 3
6. List of relevant nanomaterials properties provided by Stefaniak et al. (2013, see Table 2, page 1329), see Annex 5

UNIFICATION OF CRITERIA NEEDED!

Physical and chemical properties

- Name of substance (or CAS-No)
- Aggregation / Agglomeration State
- Shape
- Particle Size / Size Distribution (including type of dispersion medium and additives)
- form of delivery (powder, suspension)
- Composition (including chemical composition, elements, element distribution and crystal structure)
- Purity (including levels of impurities)
- Surface Chemistry (including functionalization, reactivity, hydrophobicity)
- Solubility
- Surface Area
- Porosity
- Density
- Defect density
- Surface Charge
- Stability
- Conductivity
- Magnetic properties
- Surface Reactivity
- Consideration of surface modification: exact characterization

Sample preparation

- Adequate characterization of sample: Dissolution for soluble (ion releasing) nanomaterials
- Suitable preparation of the sample: Detailed description of the dispersing procedure
- Determine size in processed sample, do not rely on producer information
- Aggregation / Agglomeration in respective media
- Dispersibility
- Consider age / storage periods of NM powders / suspensions for subsequent testing

Toxicity testing

- Determination of exposure concentration (real vs. nominal)
- Stability—how do material properties change with time, storage, handling, preparation, delivery (aging)
- Behaviour in exposure media over test duration solubility, and the rate of material release through dissolution
- Aggregation / Agglomeration in respective media
- Dose metrics used (mass, surface-area and number concentration in $\mu\text{g/ml}$, $\mu\text{g/cm}^2$; N (particle)/cell or pg/cell)
- Controls (positive and negative controls)
- Interferences with test system
- Appropriate methods / endpoints
- Use of reference material

General aspects

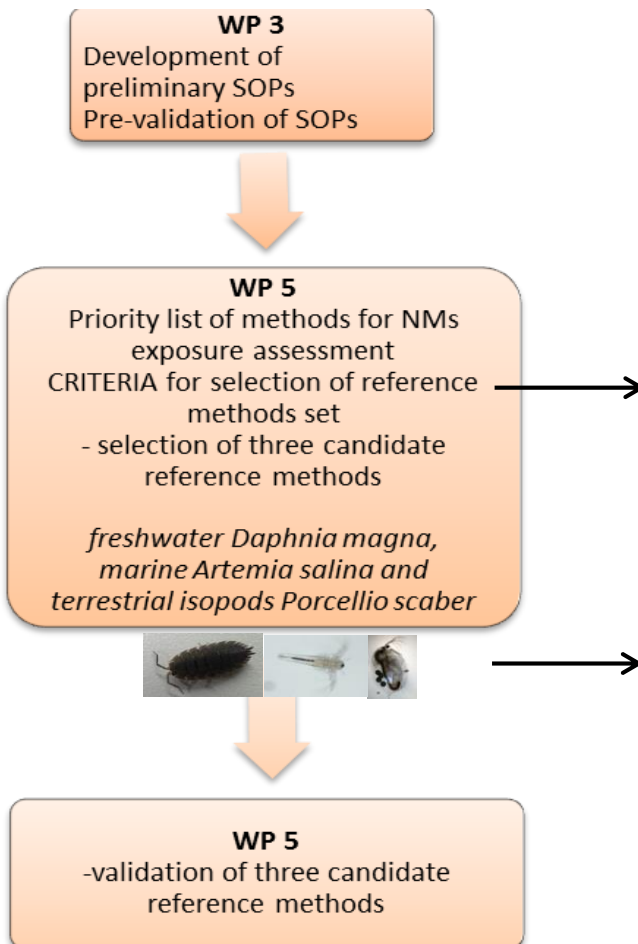
Appropriate data evaluation / statistics

Standardisation criteria (SOPs used, OECD guidelines, decision trees)

Further questions regarding the scientific validity of the data:

- 1) Are raw data provided by the data source?
- 2) Were proper controls used and reported?
- 3) Was the instrument within calibration?
- 4) How many replicates were performed?
- 5) Was the measurement protocol reported?
- 6) Was there a citation to the protocol?
- 7) Were there modifications made to the cited protocol?
- 8) Are there specifications regarding the age of the NM?

STEPS in Establishing reference methods



CRITERIA for reference method:

- sensitive to demonstrate exposure to NPs
- the signal to noise ratio have to be high enough
- the exposure should be linked to effects,
- the test should exhibit good reproducibility, ease of performance, and robustness.

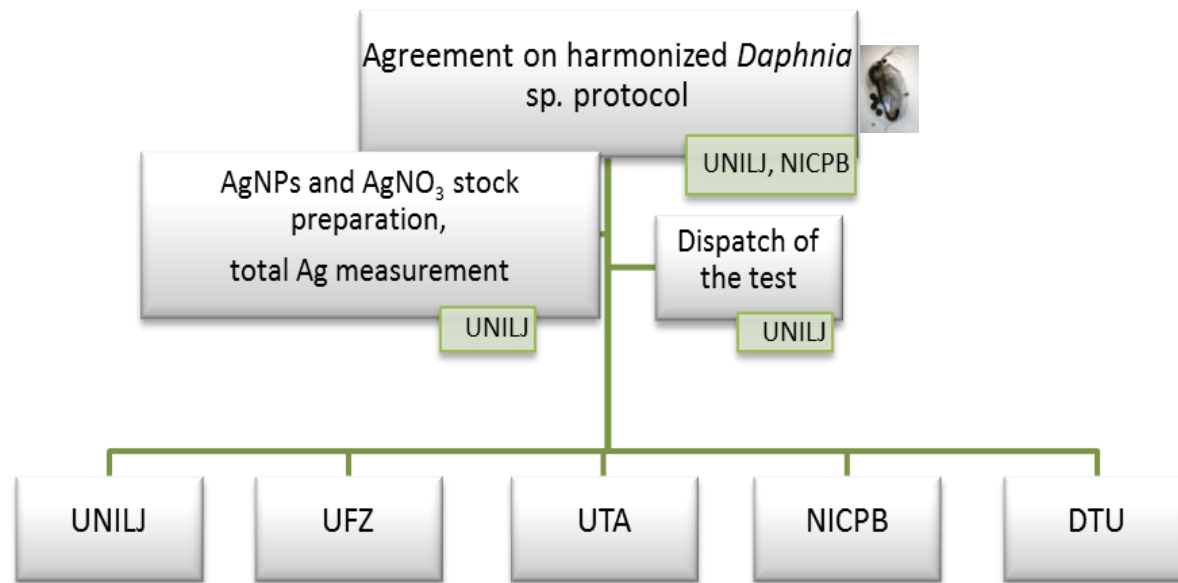
CHOICE:

Daphnia magna
Artemia franciscana
Porcellio scaber

waterflea Daphnia magna



Organization of inter-laboratory comparison study





RESULTS: *Daphnia magna*

Exactly after SOP developed by NICPB (OECD 2004: No. 202)

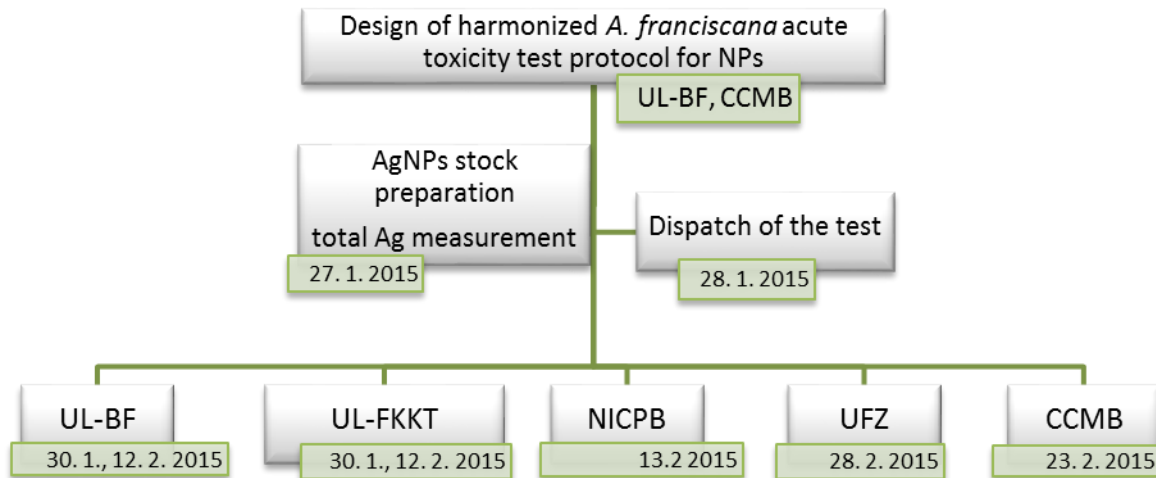
PARTNER	48h EC50, AgNPs µg/L	48h EC50, Ag ⁺ µg/L
1: NICPB reference laboratory	2.50	1.00
2: UNILJ	5.37	3.69
3: UFZ	2.39	1.28
4: DTU	4.081	3.21
5: UTA	18.85	2.02

- Good inter-laboratory repeatability

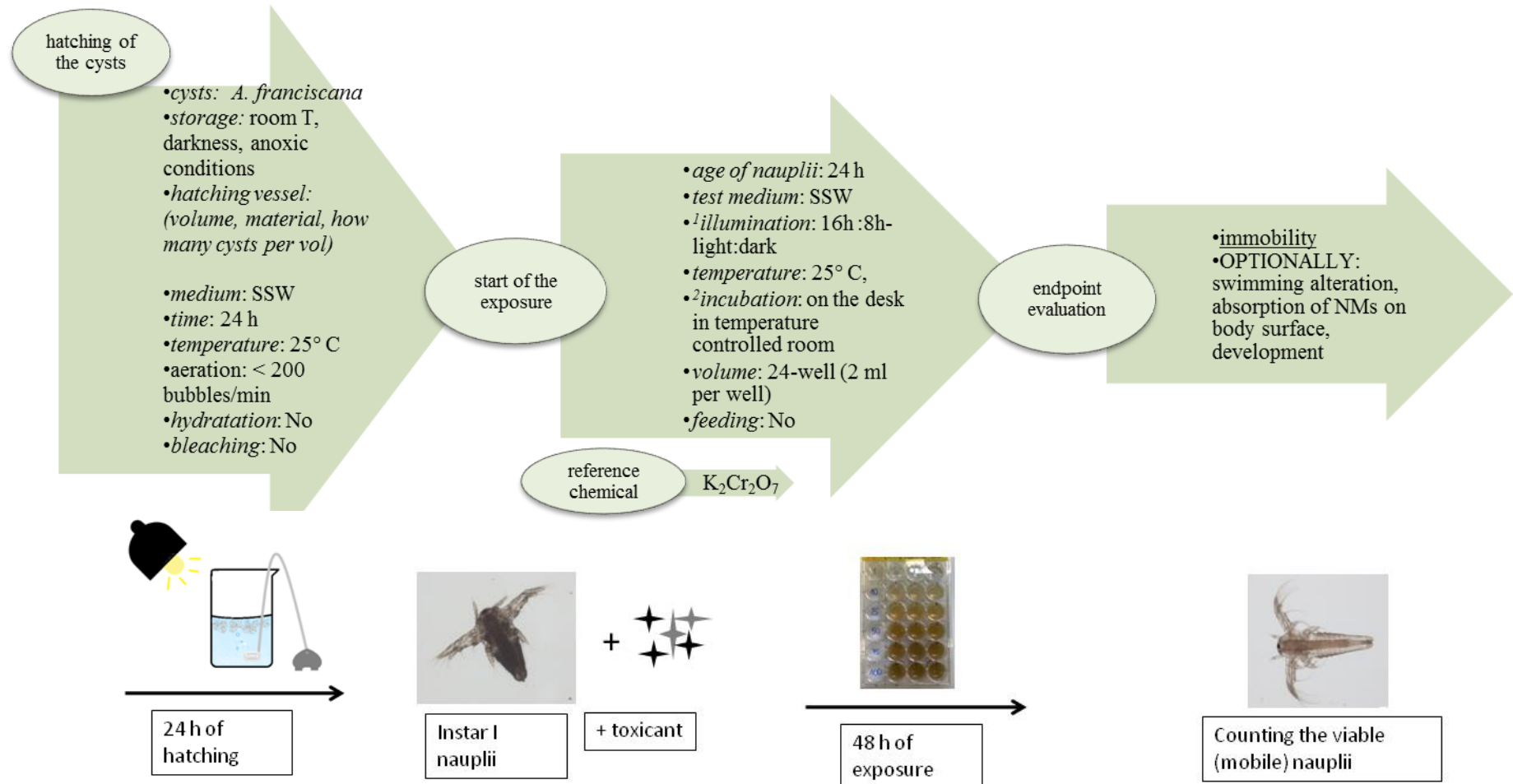
brineshrimp Artemia franciscana



Organization of inter-laboratory comparison study



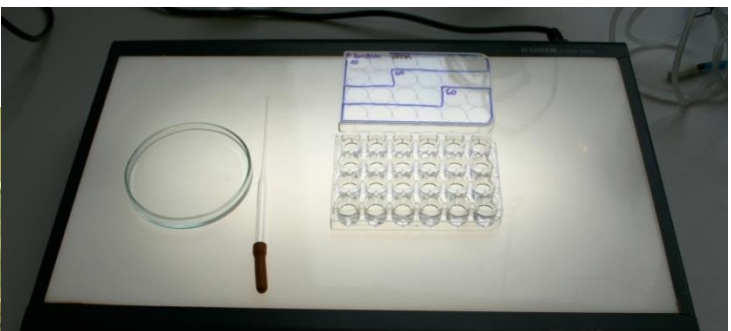
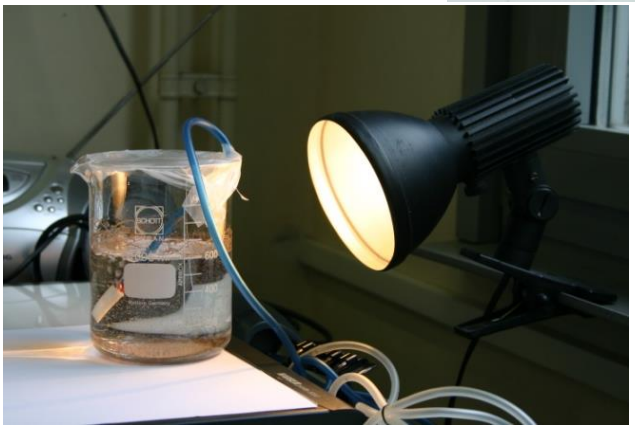
First the harmonised protocol was developed- literature review and experimentation





Experimental set-up.

Negative control: ASW medium
Test compounds AgNPs (mg/L):
25
50
75
100
125
Positive control; $K_2Cr_2O_7$ (mg/L)
15
30
60

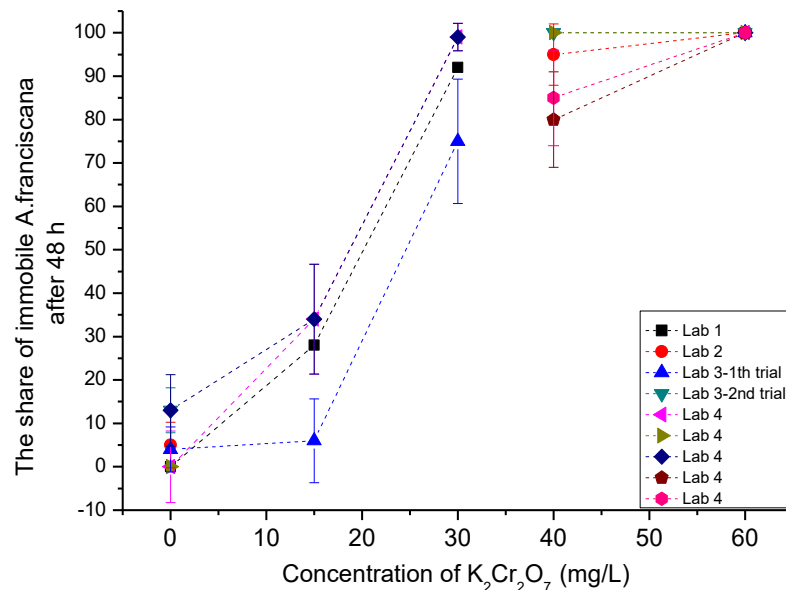




RESULTS: POTASIAM DICHROMATE

Data of the intercalibration study on $K_2Cr_2O_7$.

a.)



CONCLUSIONS:

- 1 partner invalid results-high control mortality (inappropriate cysts storage, prolonged hatching)
- LOW Intra-laboratory variation (UNILJ) (relative coefficient of variation): 9.6 %.
- LOW Inter-laboratory variation: 17%

GOOD REPEATABILITY WITH CHROMIUM

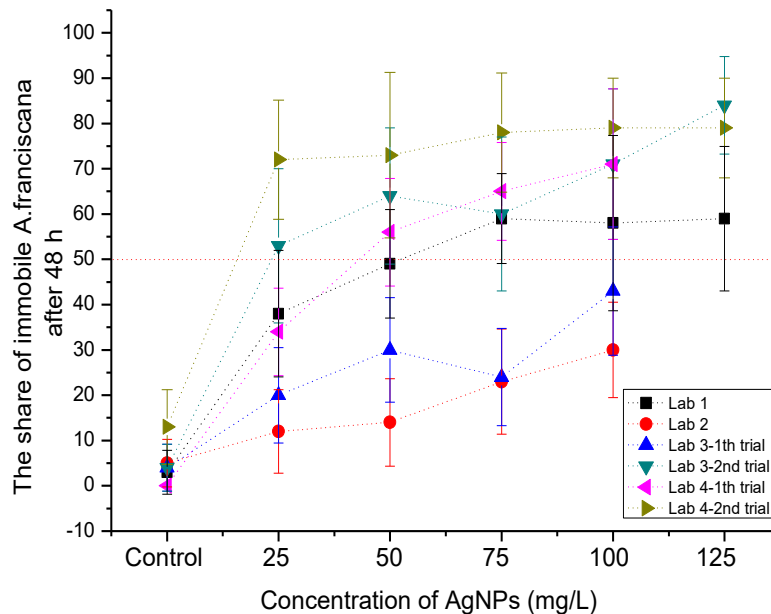
Each data point represents mean $\pm$ SD
(10 repetitions were done for each concentration).



RESULTS: SILVER NANOPARTICLES

Data of the intercalibration study on **AgNPs**

b.)



Each data point represents mean $\pm$ SD
(10 repetitions were done for each concentration).

CONCLUSIONS:

- 1 partner invalid results-high control mortality (inappropriate cysts storage, prolonged hatching)
- High Inter-laboratory variation (relative coefficient of variation): **37 %**
48h EC50 value was 36,48 mg/L

NOT REPEATABLE.
SOURCES OF VARIABILITY?

IDENTIFICATION OF SOURCES OF VARIABILITY

- hatching conditions
- test plate incubation
- illumination regime
- Special AgNPs properties

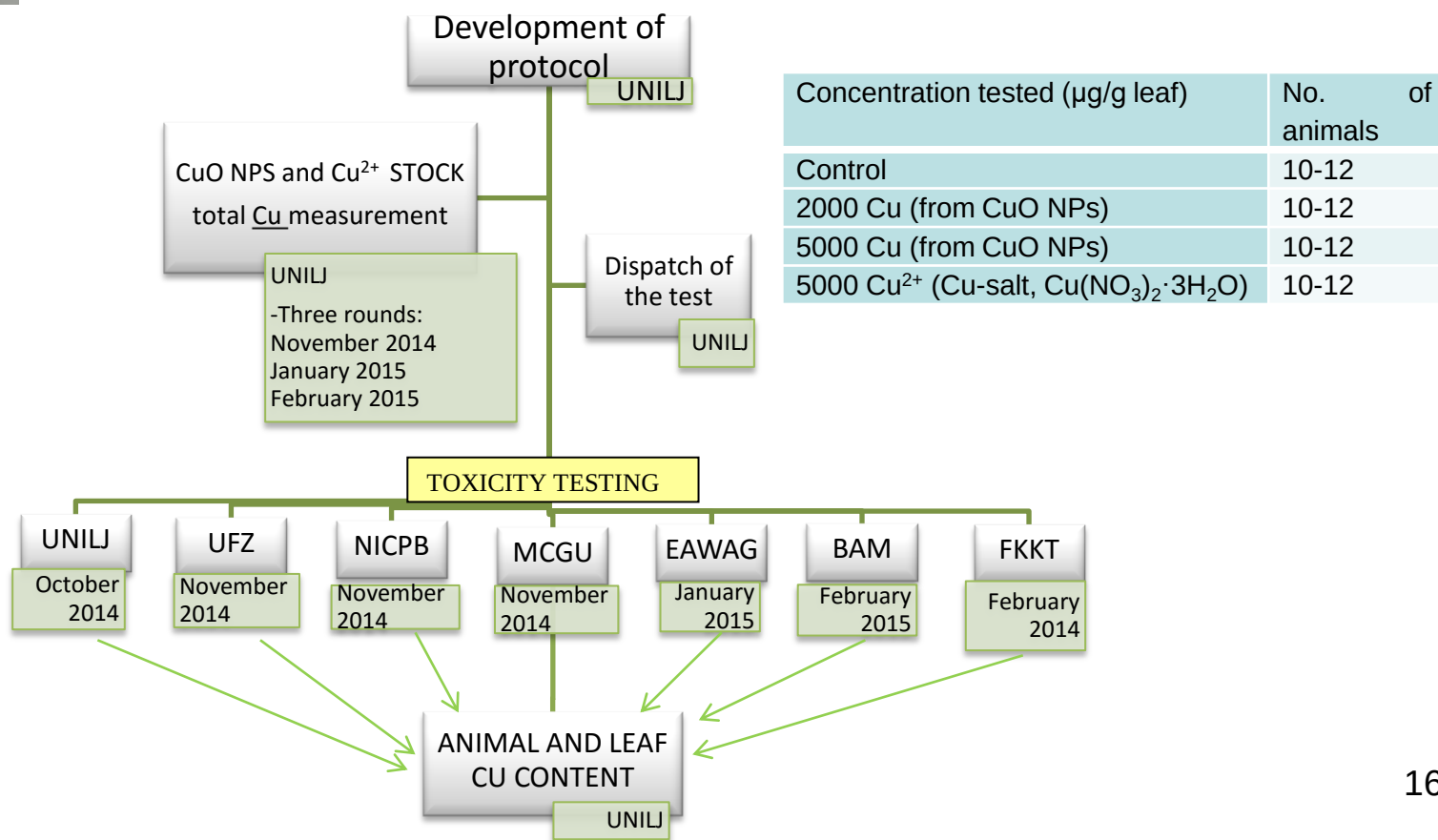
-light dependent Ag^+ speciation

	Source of variability	Actions taken to diminish the variability	Previous practises in nanotoxicity assays
HATCHING PHASE	Species of Artemia	Defined species, <i>A. franciscana</i>	Commonly undefined, mostly <i>Artemia sp.</i> or <i>A. salina</i>
	Origin of Artemia cysts	◦ Producer, which supplies <i>A. franciscana</i>, Great Salt Lake, USA ◦ Further suggestion: use of certified cysts	Very variable sources, commonly undefined
	Hatching medium	Defined composition, artificial salt water (SSW)*	Undefined composition: sea water or Instant Ocean®, Red Sea Salt®, Synthetica Sea salts®, Tropic Marin® – a synthetic sea salt mix
	Illumination	Continuous light	Continuous light, or not reported
	Temperature	Defined temperature: 25 °C	25-30 °C, often not reported
	Duration	Defined duration: 24 h	24 h or 48 h
	Aeration	Defined rate: < 200 bubbles/min, partners reported that hatching was unsuccessful at very high rates	Not defined
	Hydratation/bleaching	Not applied, hatching was successful without this step	Various practises, but usually not applied
TOXICITY TEST	Test medium	Defined composition, synthetic salt water (SSW)*	Undefined composition: sea water or Instant Ocean®, Red Sea Salt®, Synthetica Sea salts®, Tropic Marin® – a synthetic sea salt mix
	Age of cysts	Additional experiments done, 24 h old nauplii (stage I) ensure sufficient survival of controls during 48 h exposure	Various practises: 24 h, 30 h, 48 h, not reported
	Illumination	Additional experiments done, illumination is extremely important in nanotoxicity studies, we suggest	Often not reported, Various practises: dark, 16 h/8 h light/dark regime

isopods Porcellio scaber



Organization of inter-laboratory comparison study



RESULTS: *Porcellio scaber*



Detailed instructions, video/audio material



Animal Cu content- Bioaccumulation
Leaf Cu content



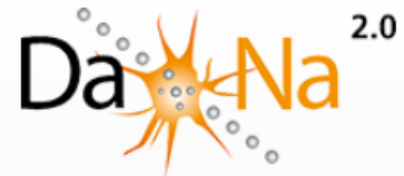
- Internationally recognized toxicity test assay the “Feeding assay with *Daphnia magna*” fulfils the criteria for the reference nanomaterial exposure and effect method.
- The assay with *Artemia franciscana* has a number of advantages as a test organism and fulfils a number of criteria as a reference method. The reproducibility of the assay with the reference chemical $K_2Cr_2O_7$ was good, but this was not the case with AgNPs. We attribute this to specific properties of these NPs.
- The “Feeding assay with isopod *Porcellio scaber*” has proved to be a reliable and reproducible assay and we therefore suggest it as reference method for terrestrial nanomaterial exposure and effect. We therefore suggest further steps to standardise the protocol.

harmonisation

quality

Data
sharing

GOOD PRACTISE EXAMPLE:



Information about nanomaterials
and their safety assessment

<http://www.nanoobjects.info/en/>

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