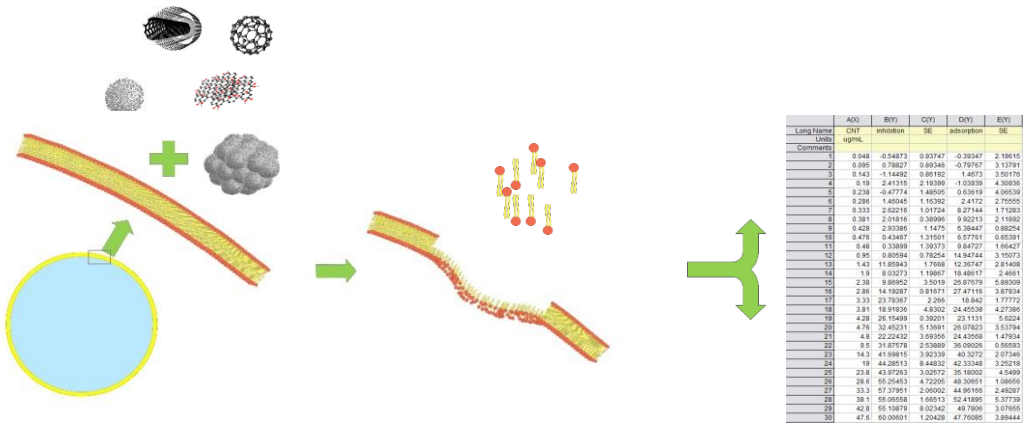
International workshop on

COMPUTATIONAL APPROACHES IN NANOSCIENCES

„COMPinNANO“

Ljubljana, 2nd and 3rd October 2015

PROCEEDINGS/ZBORNIK PREDSTAVITEV



	A(X)	B(Y)	C(Y)	D(Y)	E(Y)
Long Name	CNT	inhibition	SE	adsorption	SE
Units	ug/mL				
Comments					
1	0.048	-0.54873	0.93747	-0.39347	2.19615
2	0.095	0.78827	0.89346	-0.79767	3.13791
3	0.143	-1.14492	0.86182	1.4573	3.50176
4	0.19	2.41315	2.19399	-1.03939	4.30936
5	0.238	-0.47774	1.48505	0.63619	4.06539
6	0.286	1.46045	1.16382	2.4172	2.76555
7	0.333	2.62216	1.01724	8.27144	1.71283
8	0.381	2.01816	0.98996	9.92213	2.11892
9	0.428	2.93386	1.1475	6.38447	0.88254
10	0.476	0.43467	1.31501	6.57751	0.65391
11	0.48	0.33899	1.39373	9.84727	1.66427
12	0.95	0.80594	0.78254	14.94744	3.15073
13	1.43	11.85943	1.7668	12.36747	2.81408
14	1.9	8.03273	1.19867	18.48617	2.4661
15	2.38	9.86952	3.5019	25.87679	5.89309
16	2.86	14.19287	0.81671	27.47116	3.87934
17	3.33	23.78367	2.266	19.842	1.77772
18	3.81	18.91936	4.8302	24.45538	4.27386
19	4.28	26.15499	0.39201	23.1131	5.6224
20	4.76	32.45231	5.13691	26.07823	3.53794
21	4.8	22.22432	3.69356	24.43568	1.47934
22	9.5	31.87578	2.53889	36.09026	0.56593
23	14.3	41.59815	3.92339	40.3272	2.07346
24	19	44.28513	8.44832	42.33348	3.25218
25	23.8	43.97263	3.02572	35.18002	4.5499
26	28.6	55.25453	4.72205	48.30651	1.08656
27	33.3	57.37951	2.06002	44.96166	2.49287
28	38.1	55.06558	1.66513	52.41695	5.37739
29	42.8	56.10879	8.02342	49.7806	3.07655
30	47.6	60.00601	1.20428	47.76085	3.89444

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

Ljubljana, 2nd and 3rd October 2015

ZBORNIK PREDSTAVITEV/PRESENTATIONS

Založnik in organizator/Publisher and organizer:
Biotehniška Fakulteta, Oddelek za Biologijo, Večna pot 111, 1000 Ljubljana

Uredniki in recenzenti/Editors and reviewers: Anita Jemec (Slovenija), Damjana Drobne (Slovenija), Alok Dhawan (India), Rishi Shanker (India)

Organizacijski odbor:/Organisational committee: Anita Jemec, Barbara Drašler, Neža Rugelj, Veno Kononenko, Monika Kos, Sara Novak, Damjana Drobne

Oblikovanje/Design: Barbara Drašler and Anita Jemec

Kraj in leto izdaje/Place and date of issue: Ljubljana, 2015
Brezplačen izvod/Free copy
Dostopno/Available: http://www.bionanoteam.com

Financed by: EU FP7 project NanoValid (grant no. 263147); NanoMILE (grant no. 320451)

- CIP - Kataložni zapis o publikaciji
  Narodna in univerzitetna knjižnica, Ljubljana

  004.6:620.3(082)

  INTERNATIONAL Workshop on Computational Approaches in Nanosciences (2015 ; Ljubljana)
  Proceedings [Elektronski vir] = Zbornik predstavitev / International Workshop on Computational Approaches in Nanosciences - COMPinNANO, Ljubljana, 2nd and 3rd October 2015 ; [uredniki Anita Jemec ... et al.]. - El. knjiga. - Ljubljana : Biotehniška fakulteta, Oddelek za biologijo, 2015

  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 form 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 datebases 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.

prof. dr. Damjana Drobne and assist. prof. Anita Jemec
Ljubljana, 6.11.2015

Friday, 2nd October

16.15 – 16.30 Welcome speach & 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 (TiO_2 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:
„Chalenges 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 Sepčić**, 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 *in silico* 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; chech if resulta match
- Check the code for bugs/errors
- Try alternate aproaches/check sensitivity

Validation of the data analysis

Availability:

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

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 rights order
- Data are read at the right times

Document integrates data analysis with all the textual representations (descriptions) that everythings 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 currant 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

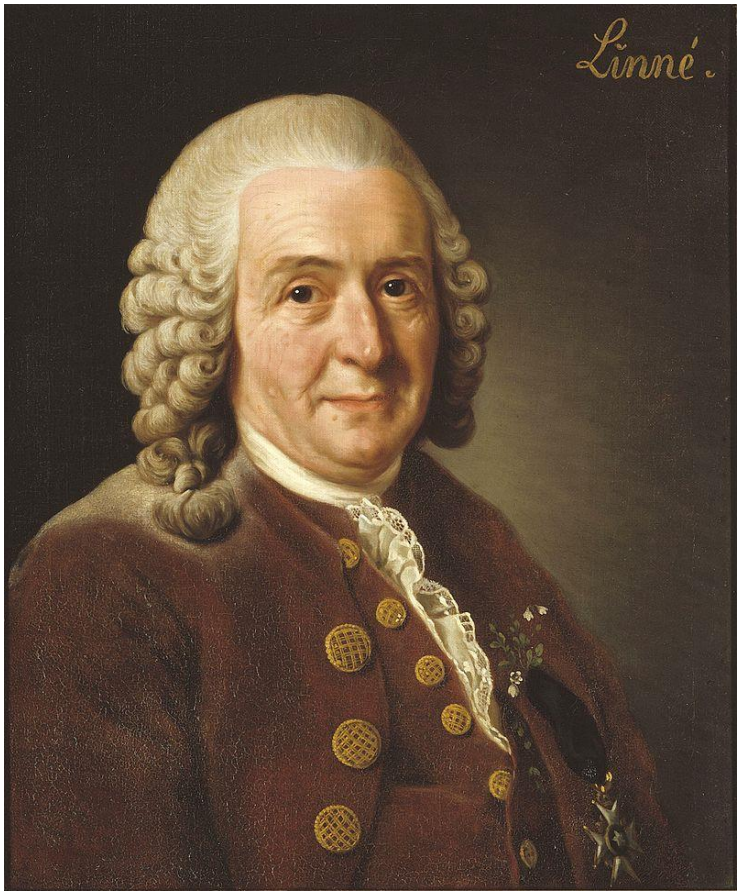
Cene Fišer
SubBio Lab
Odd. za Biologijo
Biotehniška Fakulteta
Univerza v Ljubljani

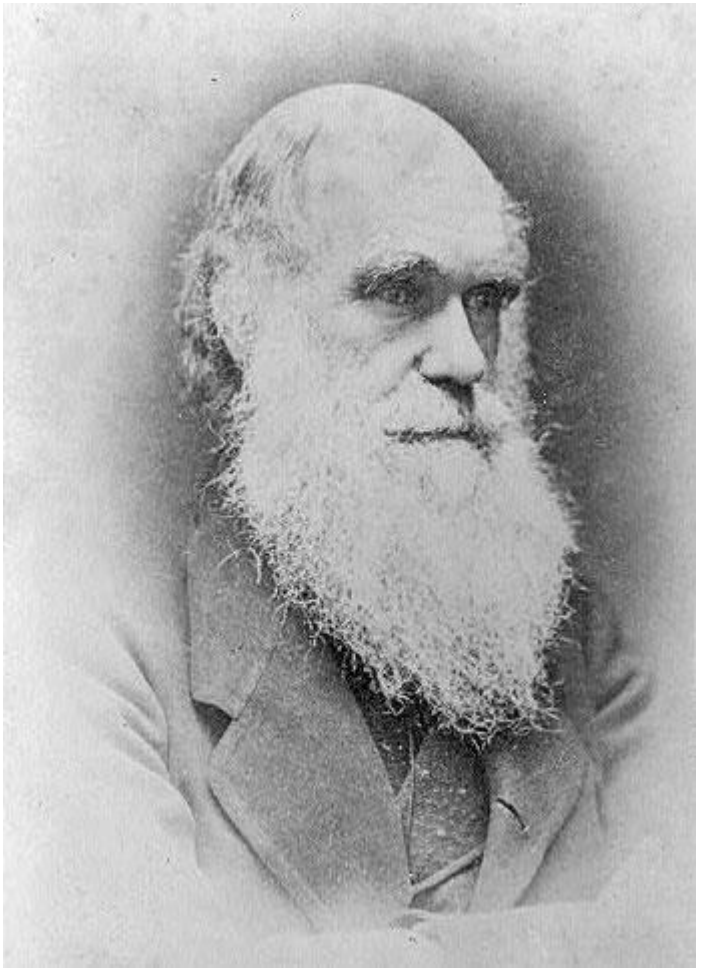
Univerza v Ljubljani

Ljubljana, 2.10.2015

taxonomy-systematics?

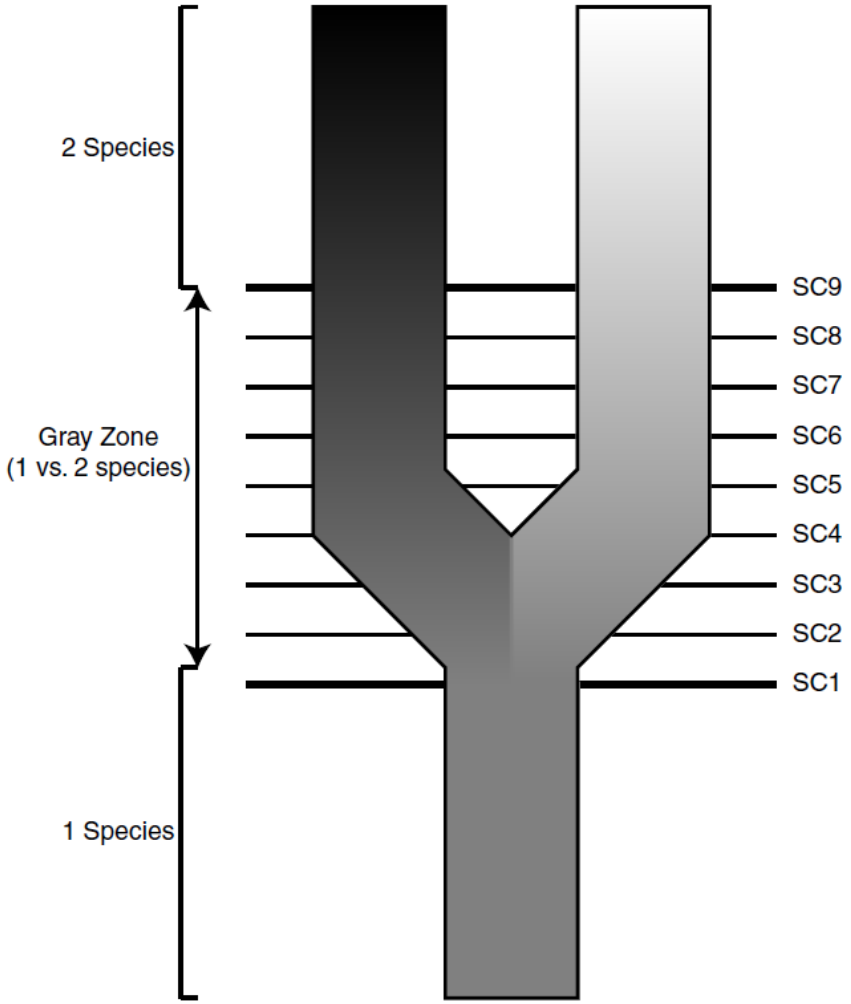
1. Taxonomy in a narrow sense: describing and naming species.
2. Taxonomy in a broad sense: species identification, research of species biology, distribution and conservation.
3. Systematics: inference of species relatedness and hierarchical categorization of species in higher taxonomic categories.





an ancient science?

Modern synthesis: grounded in evolutionary theory….



Speciation: a process of divergence

species concept
(what is species?)

↓

delimitation criteria
(how can I tell
apart?)

+

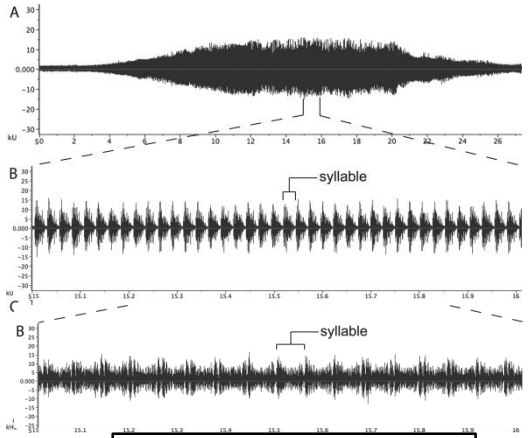
data

↓

species hypothesis

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

…..interdisciplinary science

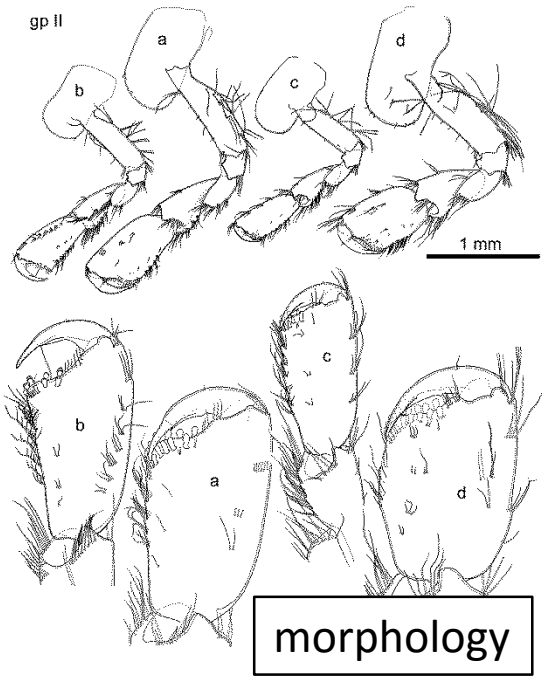


communication

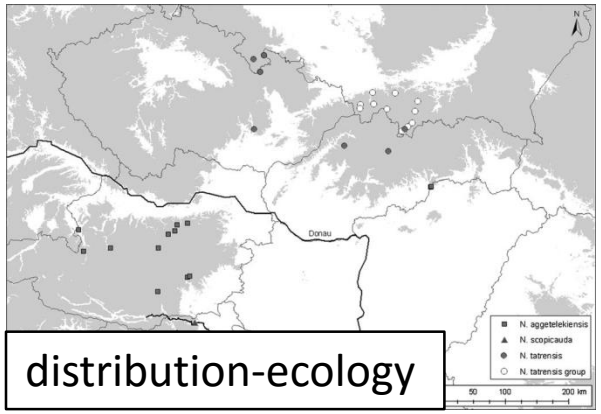
TYPE: NO LIMITS

LIMITS IN DATA DEPOSITION AND RETRIVAL

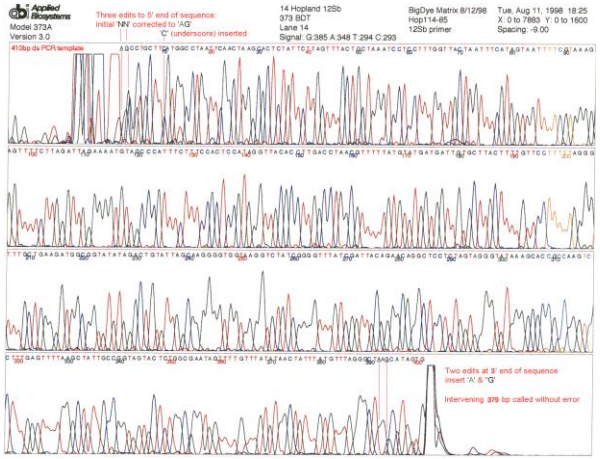
mating behavior



morphology



distribution-ecology



molecular data

Challenges for taxonomy

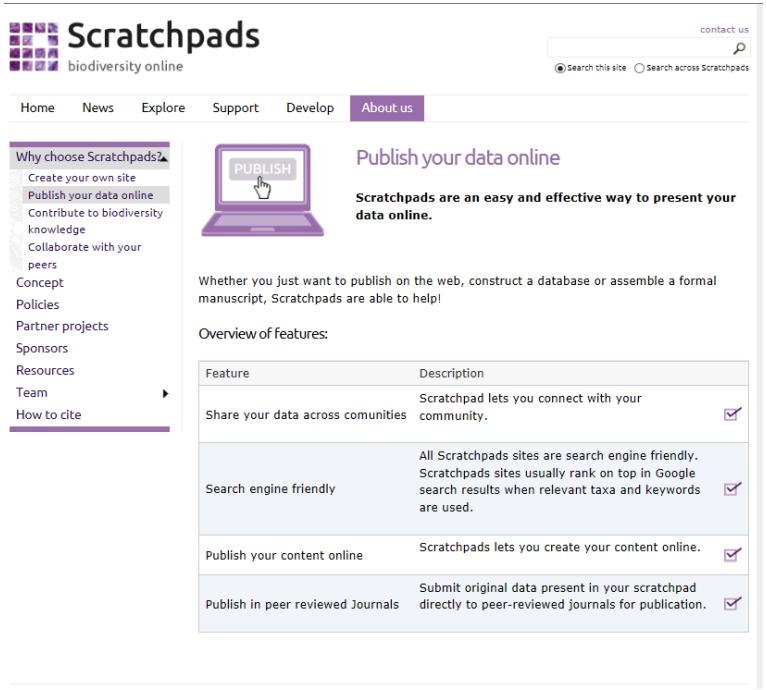
The discipline will have to reinvent itself if it is to survive and flourish.

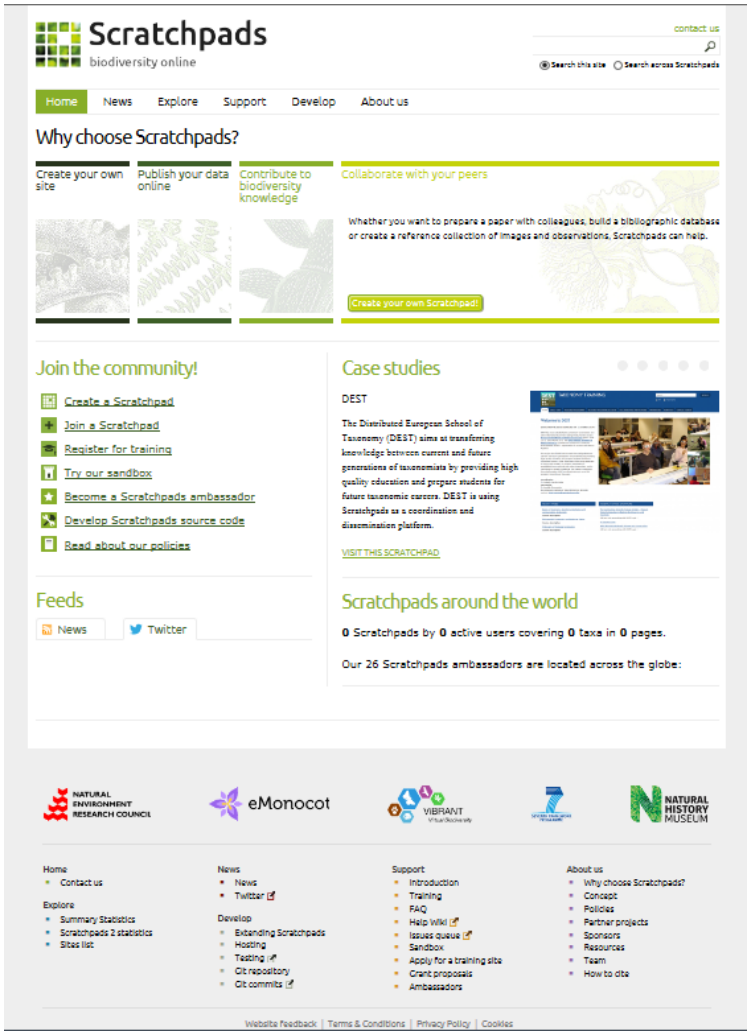
This discipline is made for the web: it is information-rich and often requires copious illustrations.

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 necesserily databases

Make your own website:
Scratchpads:
http://scratchpads.eu/





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

Analysis I : moleclar/morphological differentiation	**Analysis II : spatial circumstances of speciation**	**Analysis III : ecological niche reconstruction**
downolad sequences, quantitative or continous data	downolad spatial data	downolad spatial data
+	+	+
newly measured data	geomorphological characteristcs	Climate data (Hijmans et al. 2005, J.climat)
↓	↓	↓
analysis	analysis gene-flow barries	analysis of ecological similarity

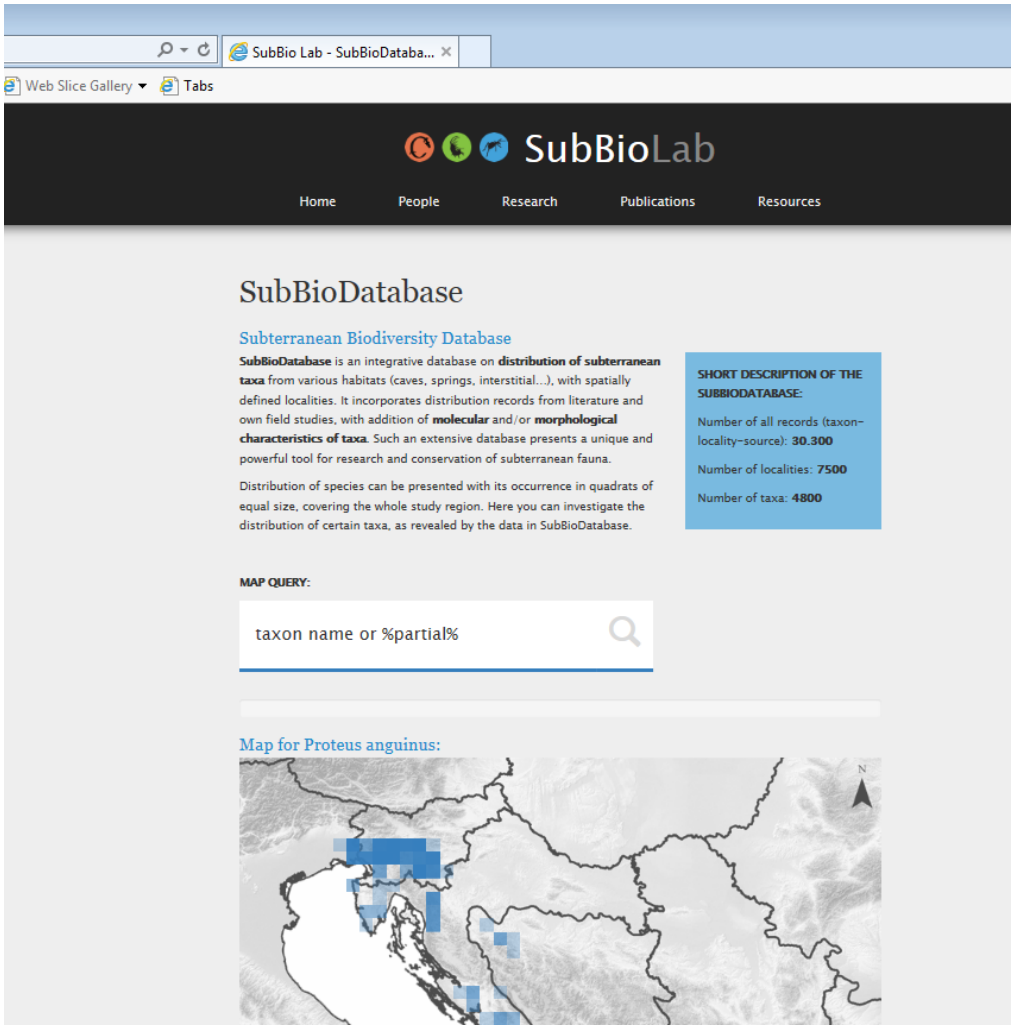
Taxon specific interactive database

SubBio Database: interactive database of subterranean fauna

Three main sources of data:
- species lists
- distributional data
- DNA sequences

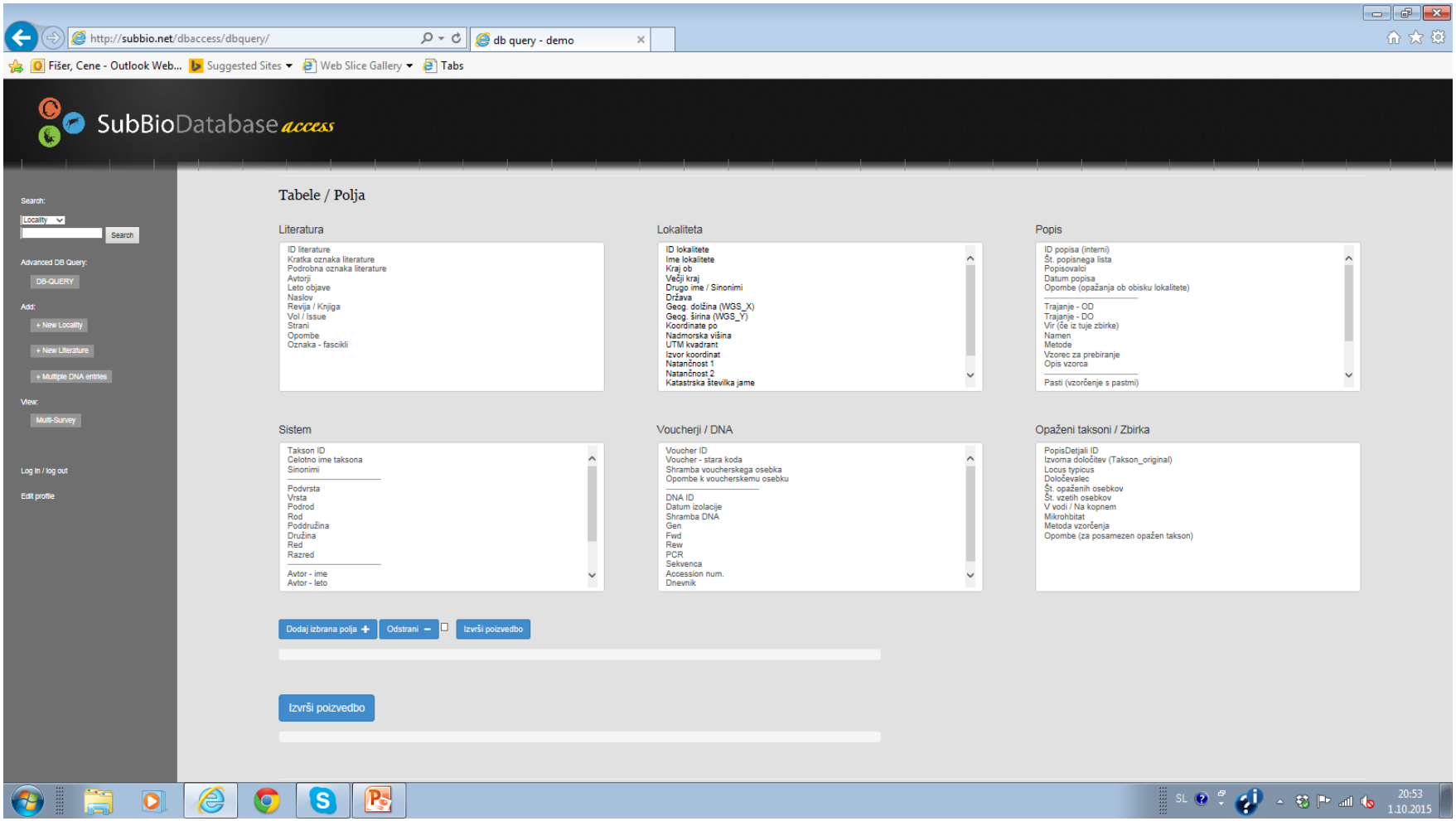
Sources of data:
- published sources
- own data

Quantity:
- number of records: 30.300
- number of localites : 7.500
- number of taxa: 4.800



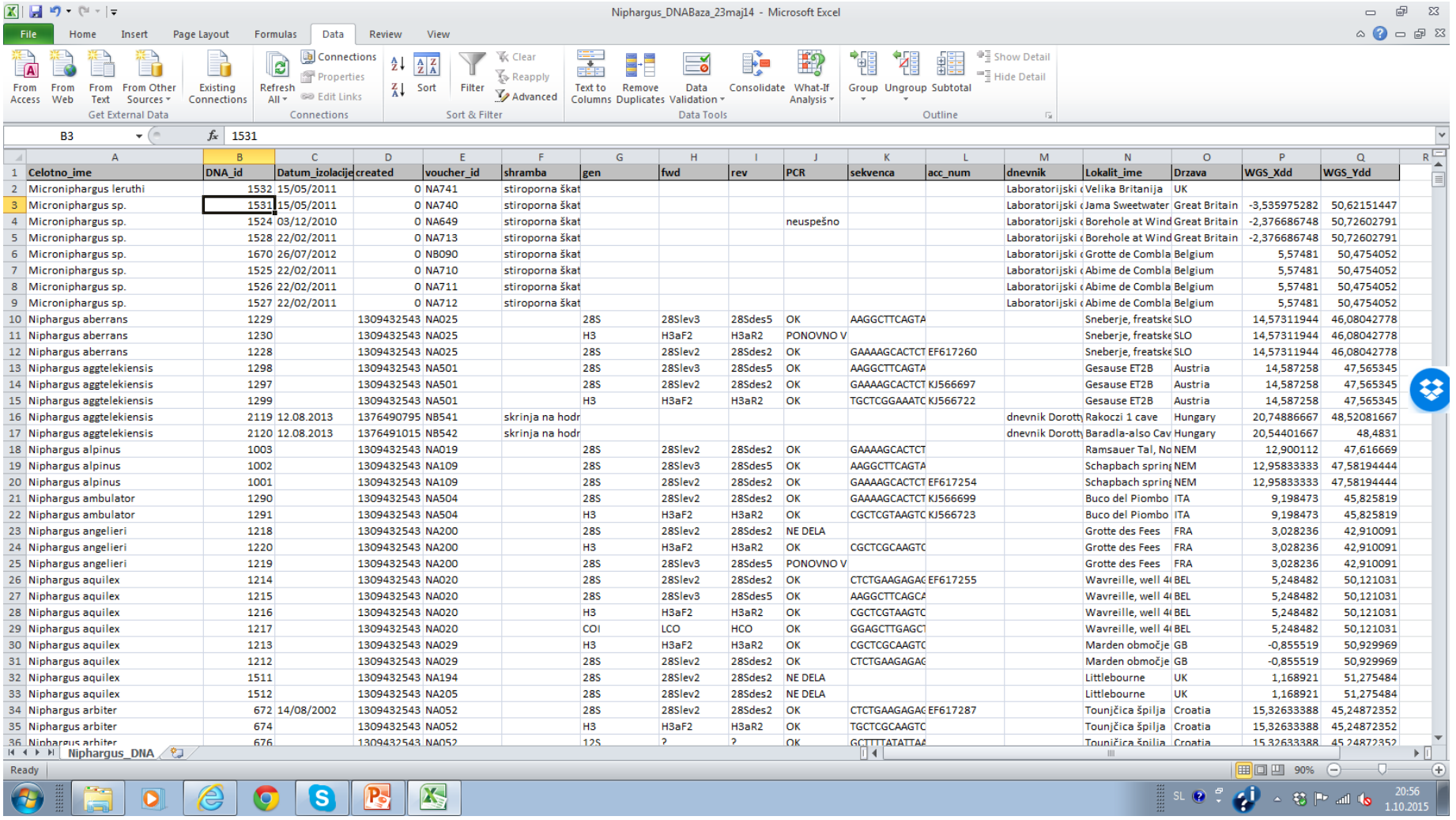
Taxon specific interactive database

SubBio Database: interactive database of subterranean fauna



Taxon specific interactive database

SubBio Database: interactive database of subterranean fauna



Niphargus_DNABaza_23maj14 - Microsoft Excel

	Celotno_ime	DNA_id	Datum_izolacije	created	voucher_id	shramba	gen	fwd	rev	PCR	sekvenca	acc_num	dnevnik	Lokalit_ime	Drzava	WGS_Xdd	WGS_Ydd
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 Combla	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	TGCTCGGAAATC	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 arbiter	672	14/08/2002	1309432543	NA052		28S	28Slev2	28Sdes2	OK	CTCTGAAGAGAC	EF617287		Tounjčica špilja	Croatia	15,32633388	45,24872352
35	Niphargus arbiter	674		1309432543	NA052		H3	H3aF2	H3aR2	OK	TGCTCGCAAGTC			Tounjčica špilja	Croatia	15,32633388	45,24872352
36	Niphargus arbiter	676		1309432543	NA052		12S	?	?	OK	GCTTTTATATTAA			Tounjčica špilja	Croatia	15,32633388	45,24872352

Niphargus_DNA

Ready

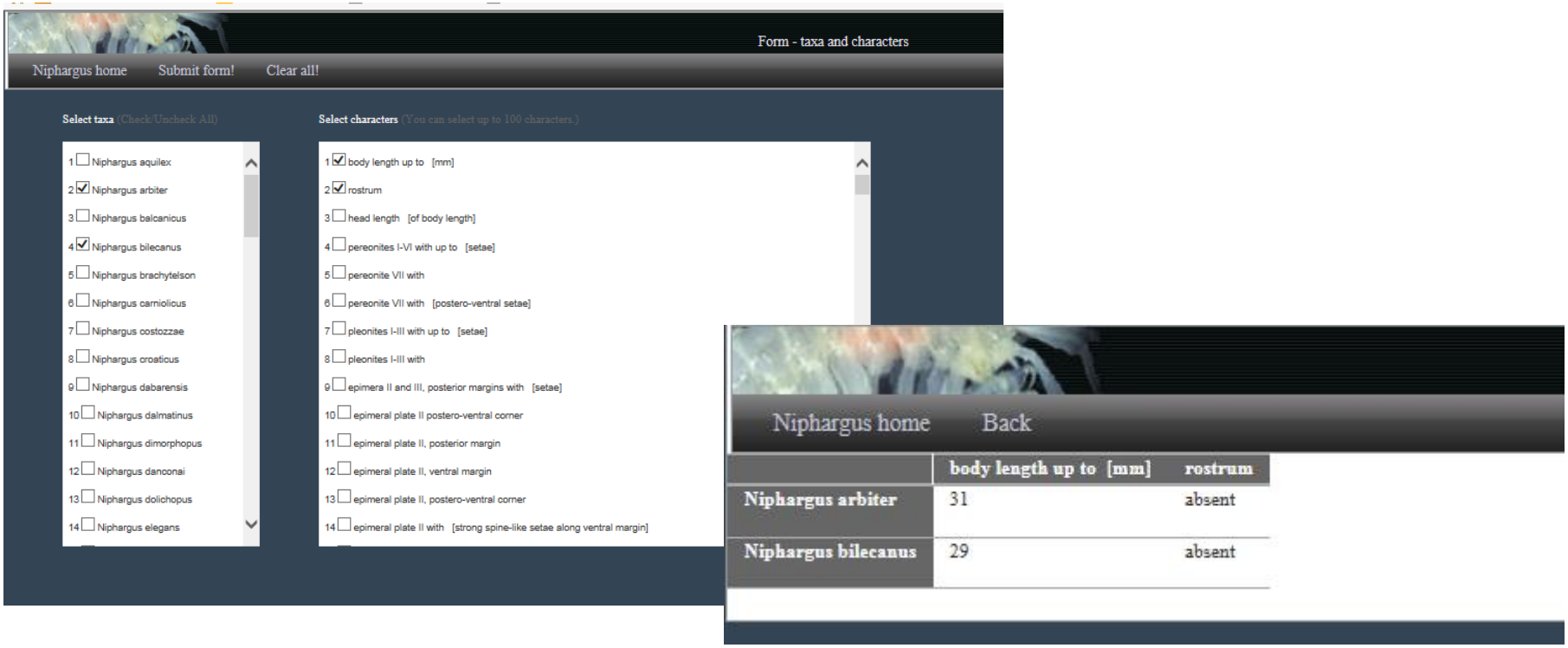
90%

SL 20:56 1.10.2015

Web-database as a collaborative tool?

Morphology: deposition of figures – deposition of measurements

Quantitative data: MYSQL



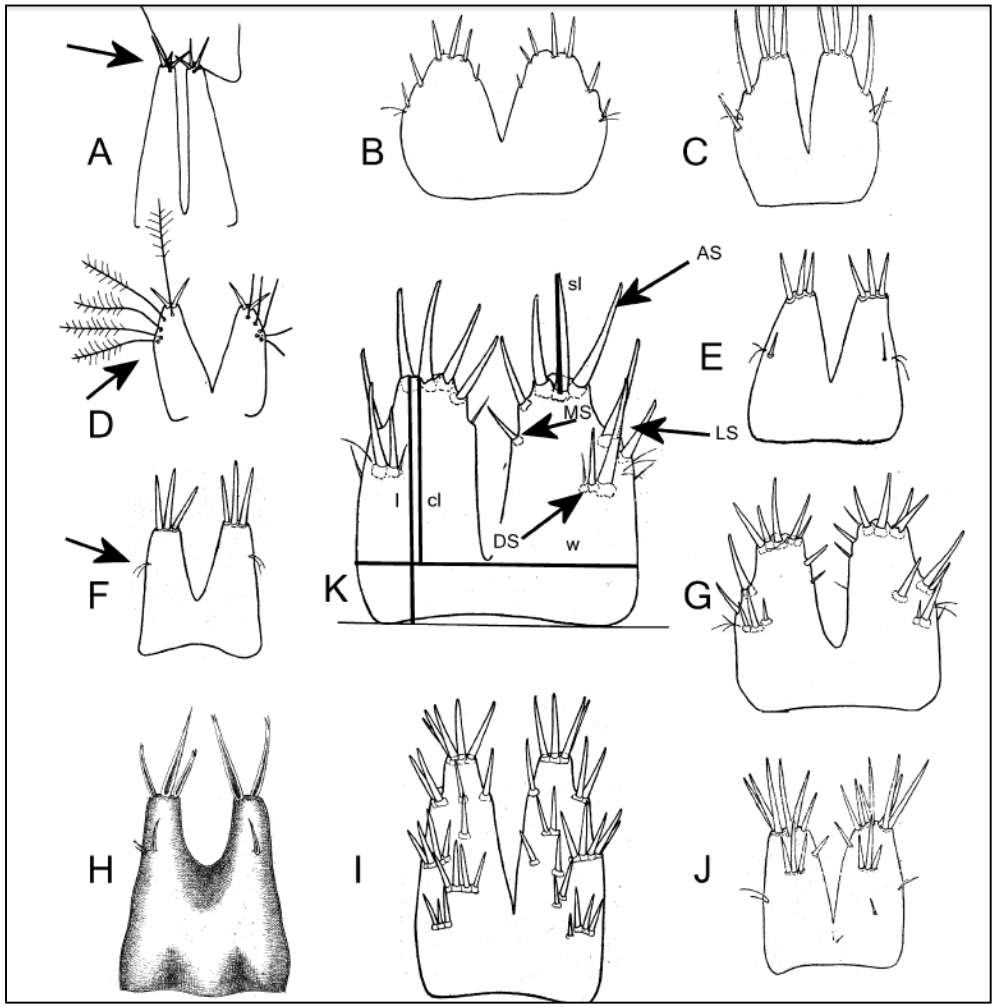
Web-database as a collaborative tool?

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:

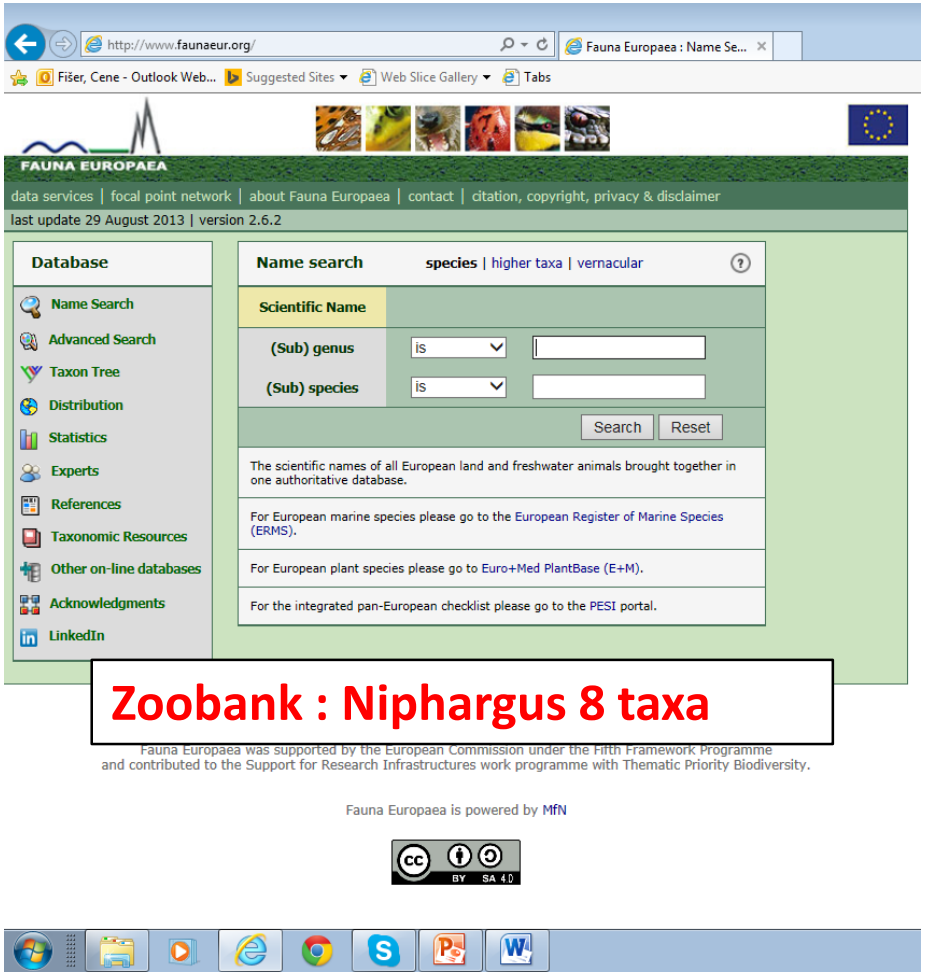
- precise, a possibility to correct errors
- standardized protocols
- different properties linked with voucher specimens
- eases team work

Weakness:

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

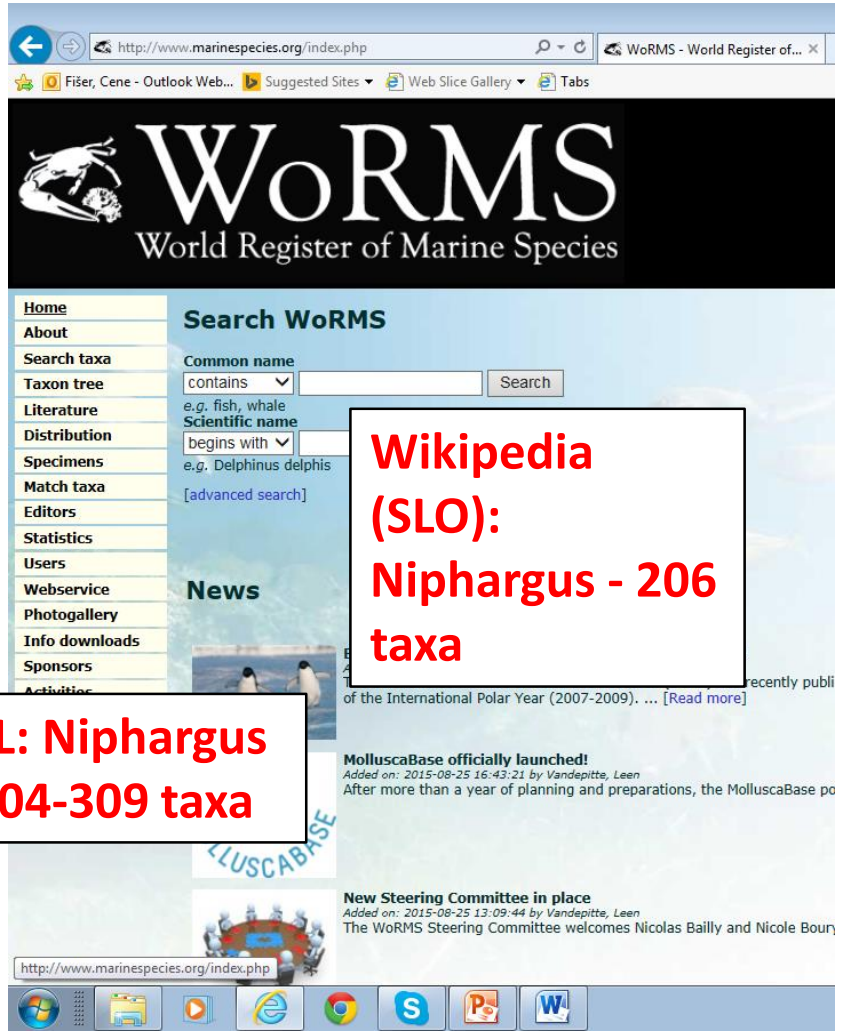
Global databases – character specific

Species names on a web: a chaos



Zoobank : Niphargus 8 taxa

Niphargus – 295 taxa



Wikipedia (SLO): Niphargus - 206 taxa

EOL: Niphargus – 204-309 taxa

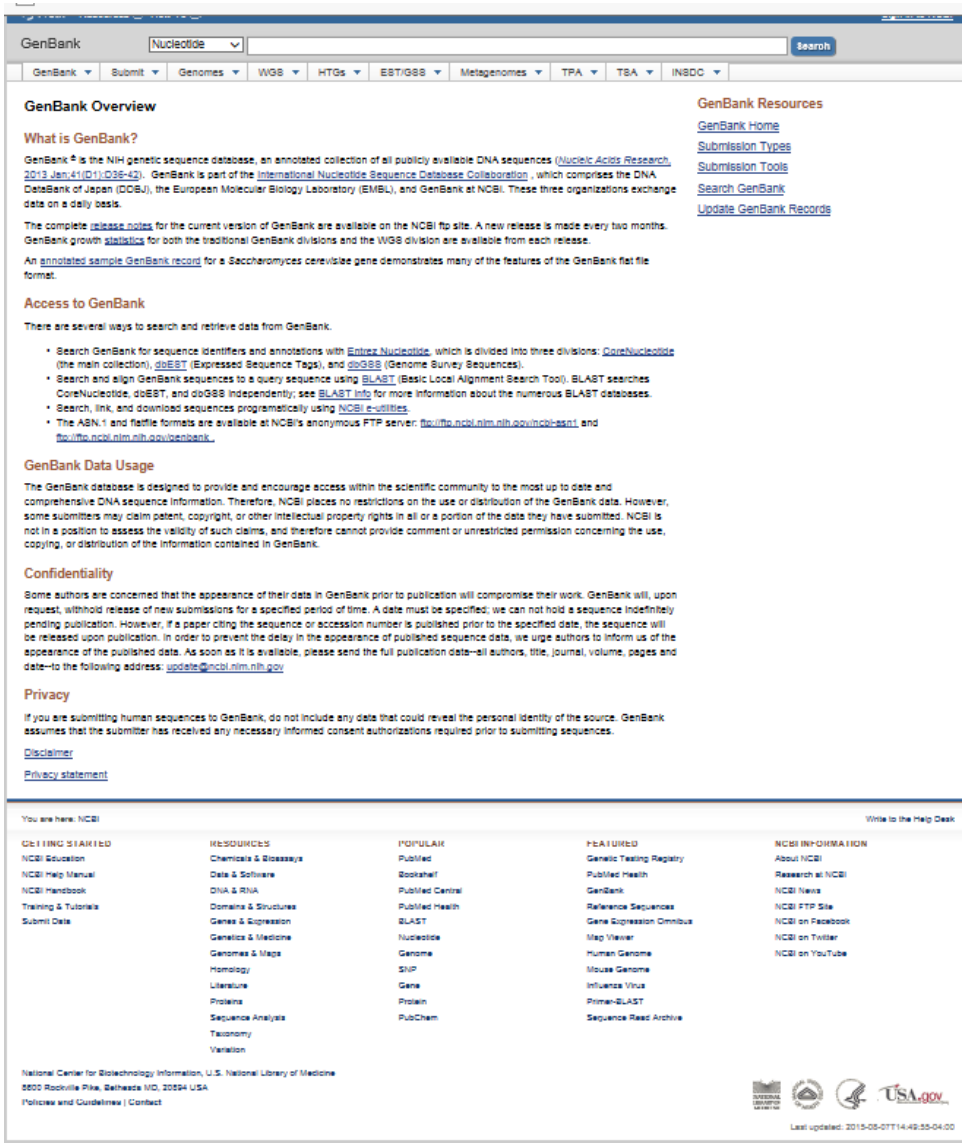
Niphargus – 261 taxa

Global databases – character specific

GeneBank: respository of sequences

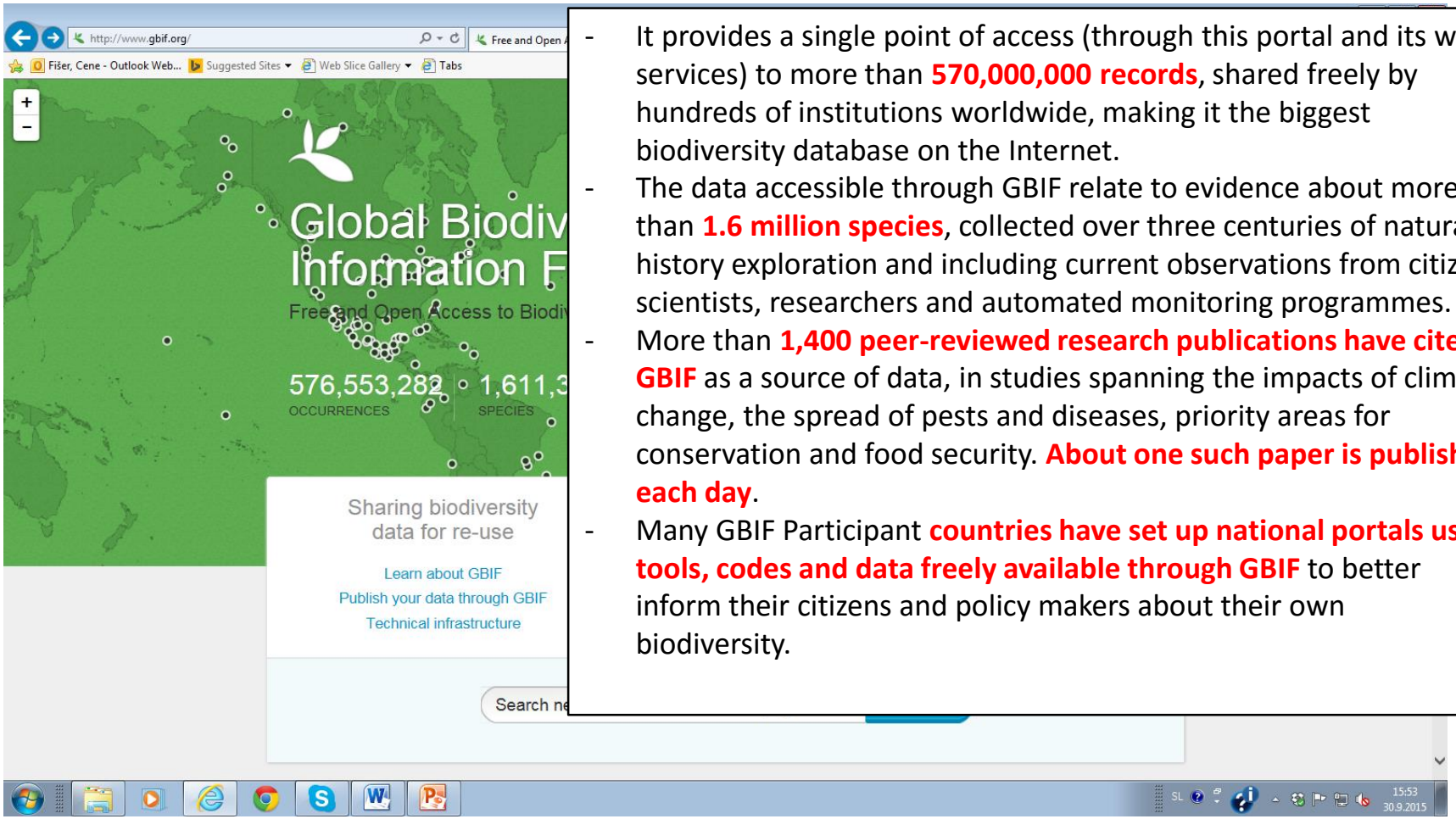
http://www.ncbi.nlm.nih.gov/genbank/

Main impediment: species id



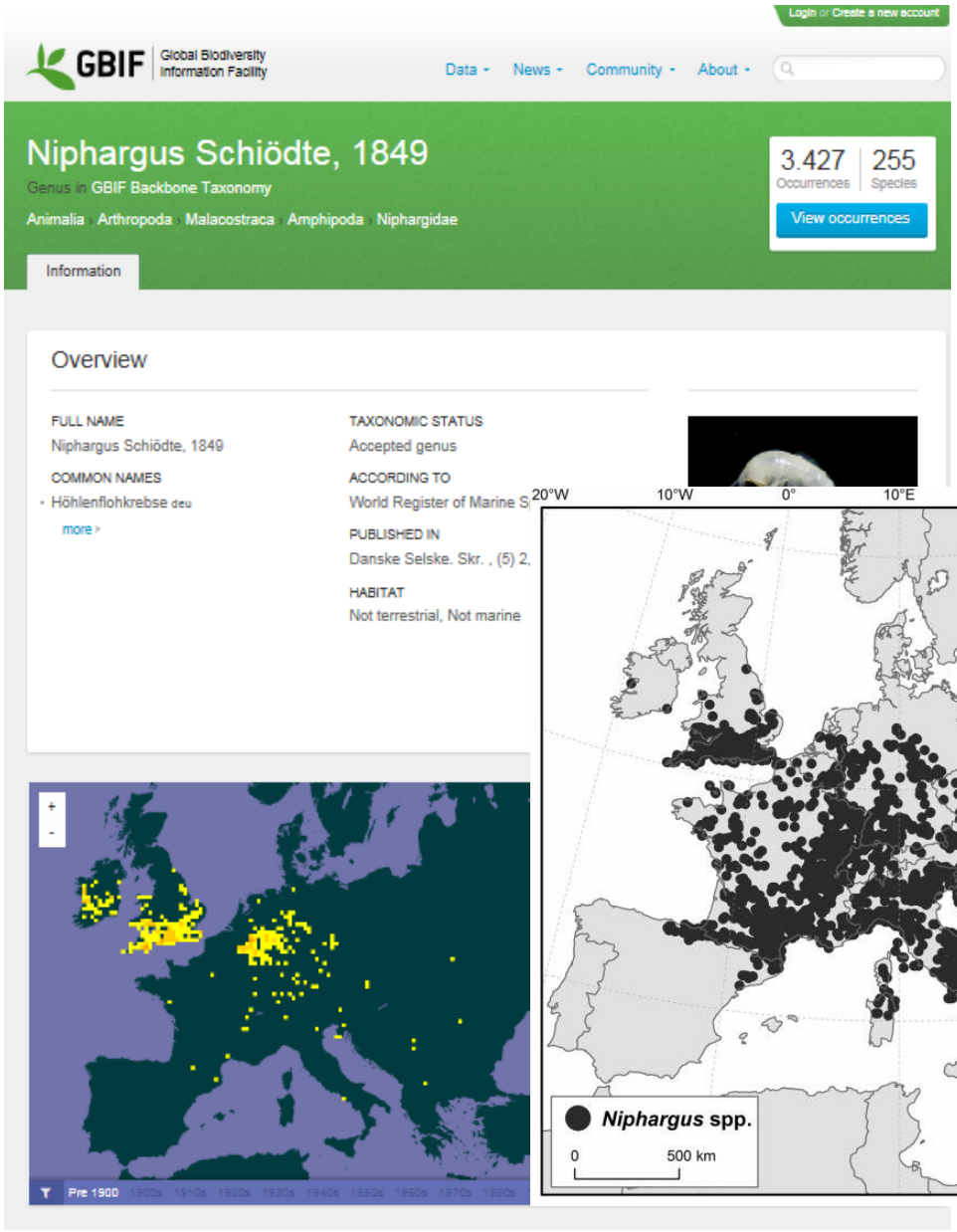
Global databases – character specific

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/



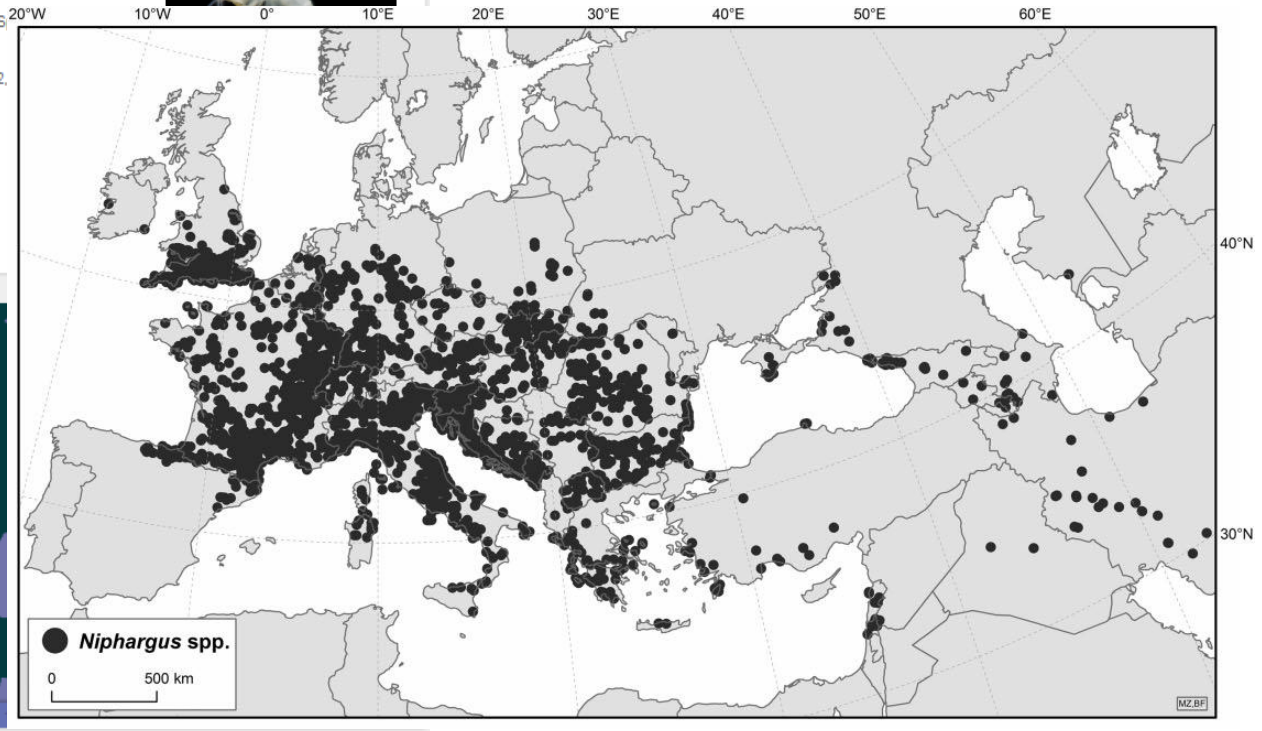
- It provides a single point of access (through this portal and its web services) to more than **570,000,000 records**, shared freely by hundreds of institutions worldwide, making it the biggest biodiversity database on the Internet.
- The data accessible through GBIF relate to evidence about more than **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
- completness 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

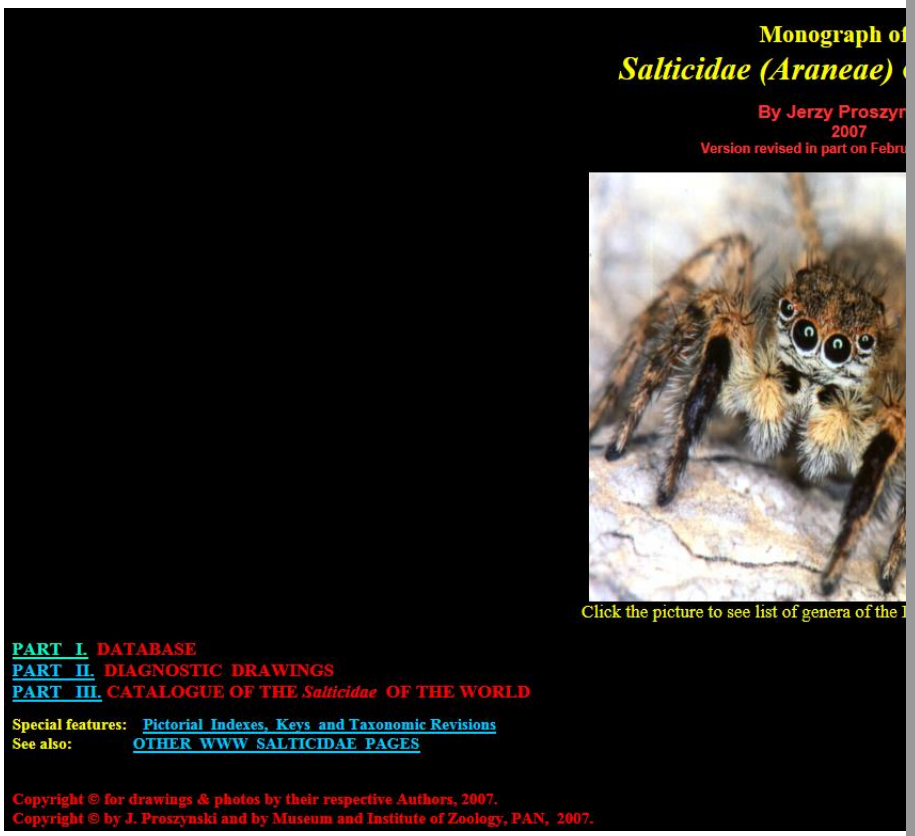
Roman Luštrik

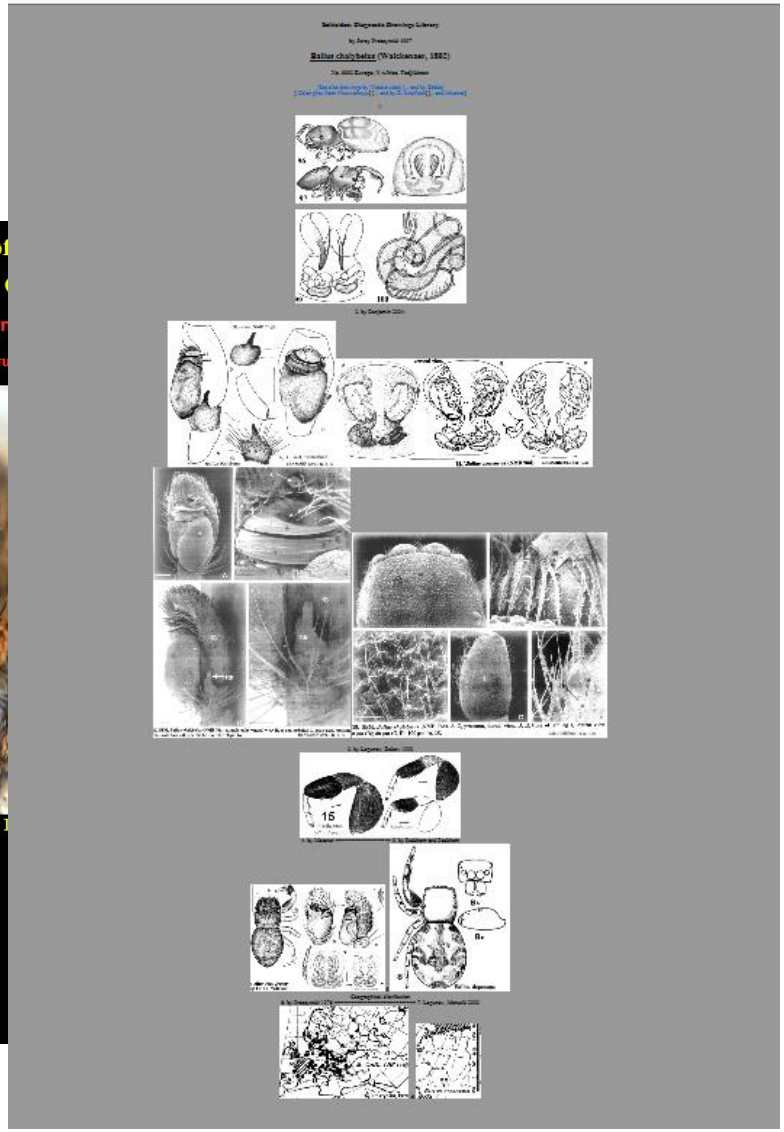
The rest of SubBio lab team

Taxon specialized websites: not necesserily databases

What is the name of this creature?

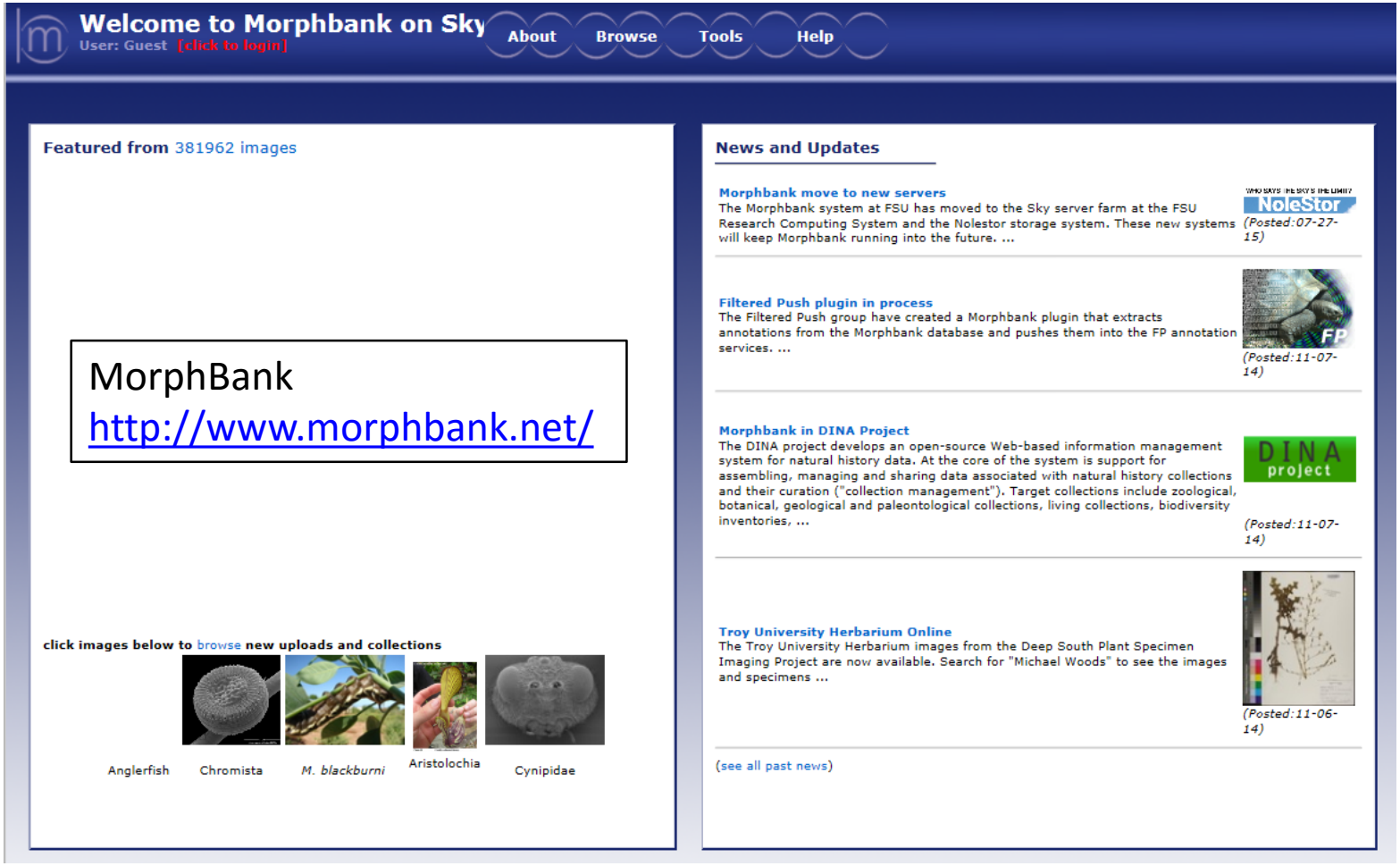
Salticid spiders
http://salticidae.org/salticid/main.htm





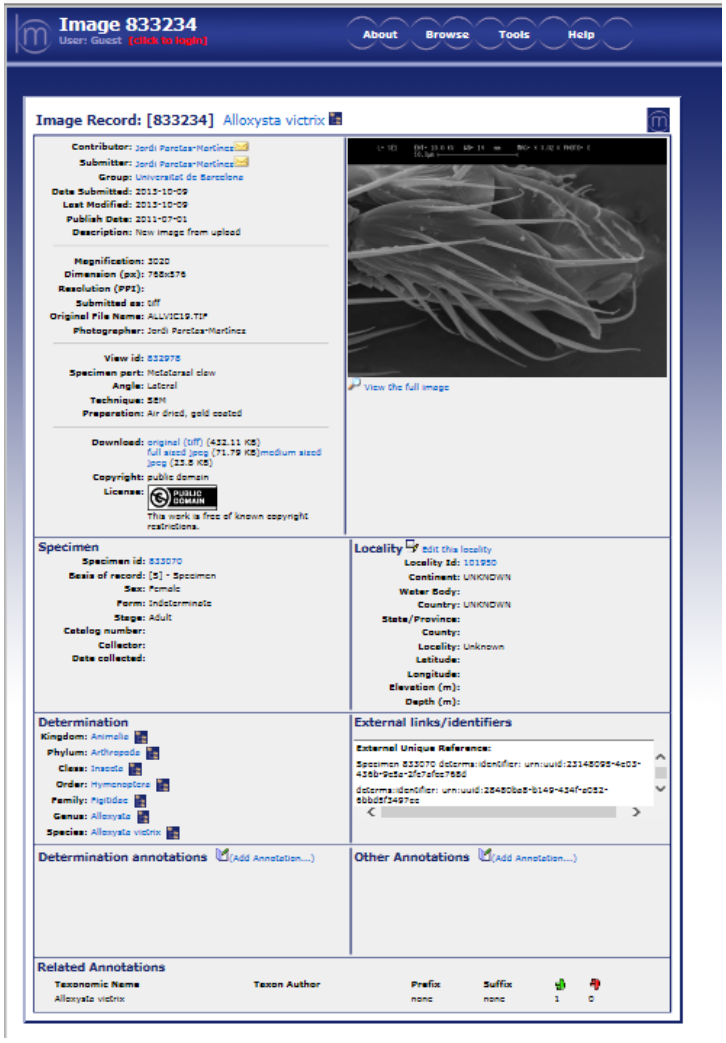
Global databases – character specific

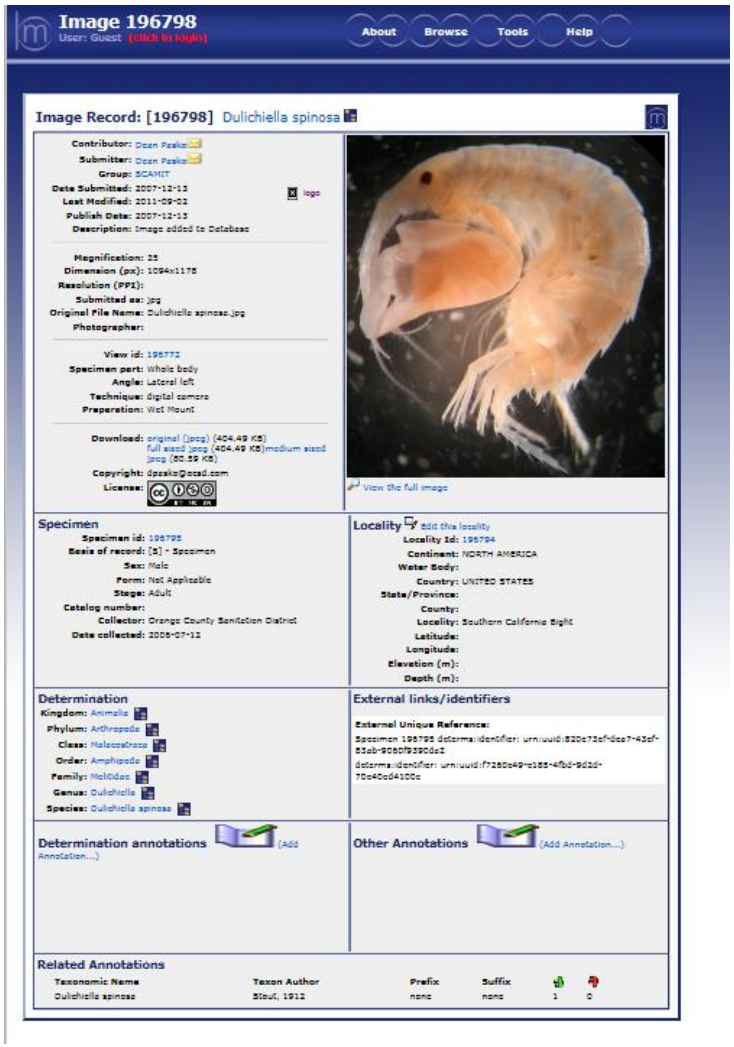
Morphology: deposition of figures – deposition of measurements



Global databases – character specific

Morphology: deposition of figures – deposition of measurements





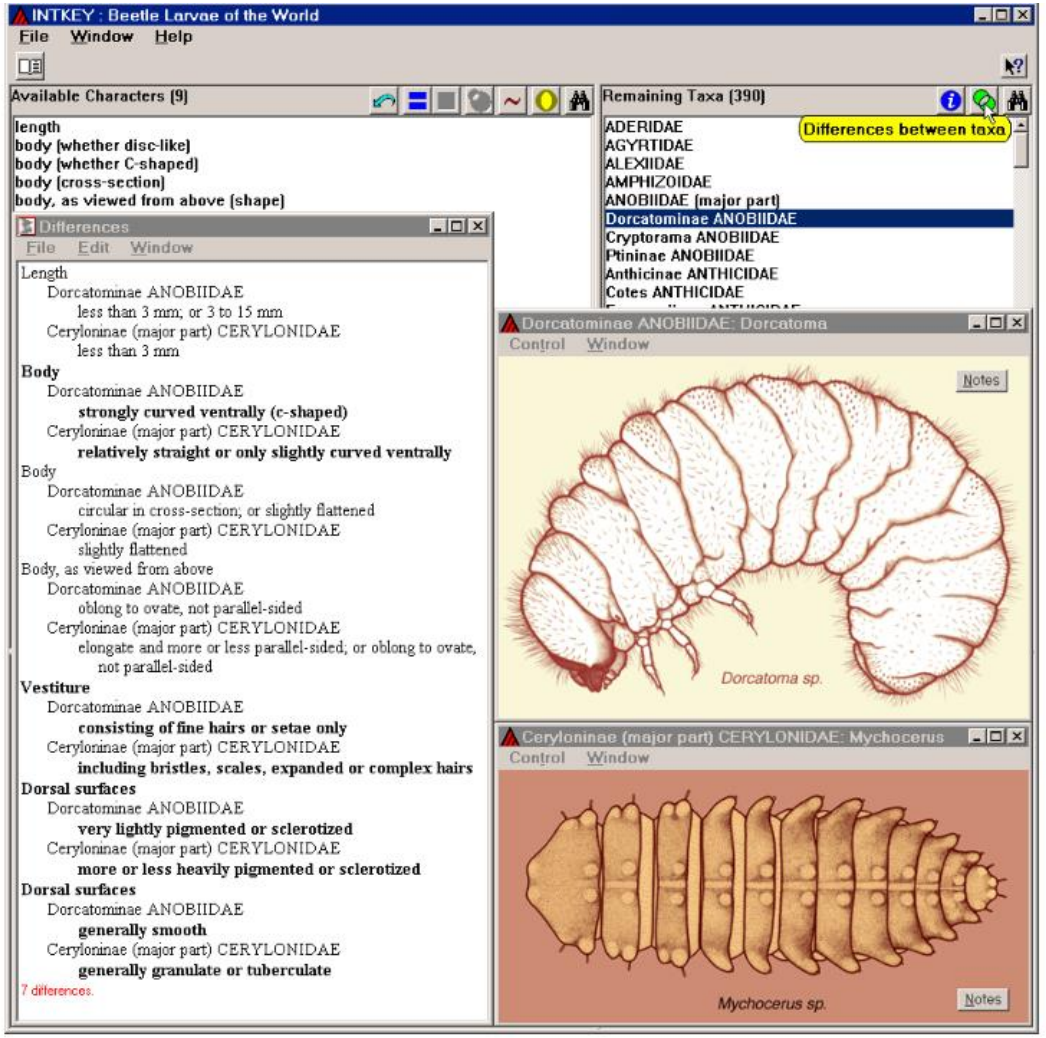
Beginnings of the databases…

Special software packages: DELTA, Lucid

Description Language for Taxonomy (DELTA)
http://delta-intkey.com/

A TOOL FOR:
- descriptions
- identifcation keys (different types)
- files for phylogenetic/phenetic analysis
- comparison of taxa

MAIN IMPEDIMENT:
limited analytic frame, species – population level



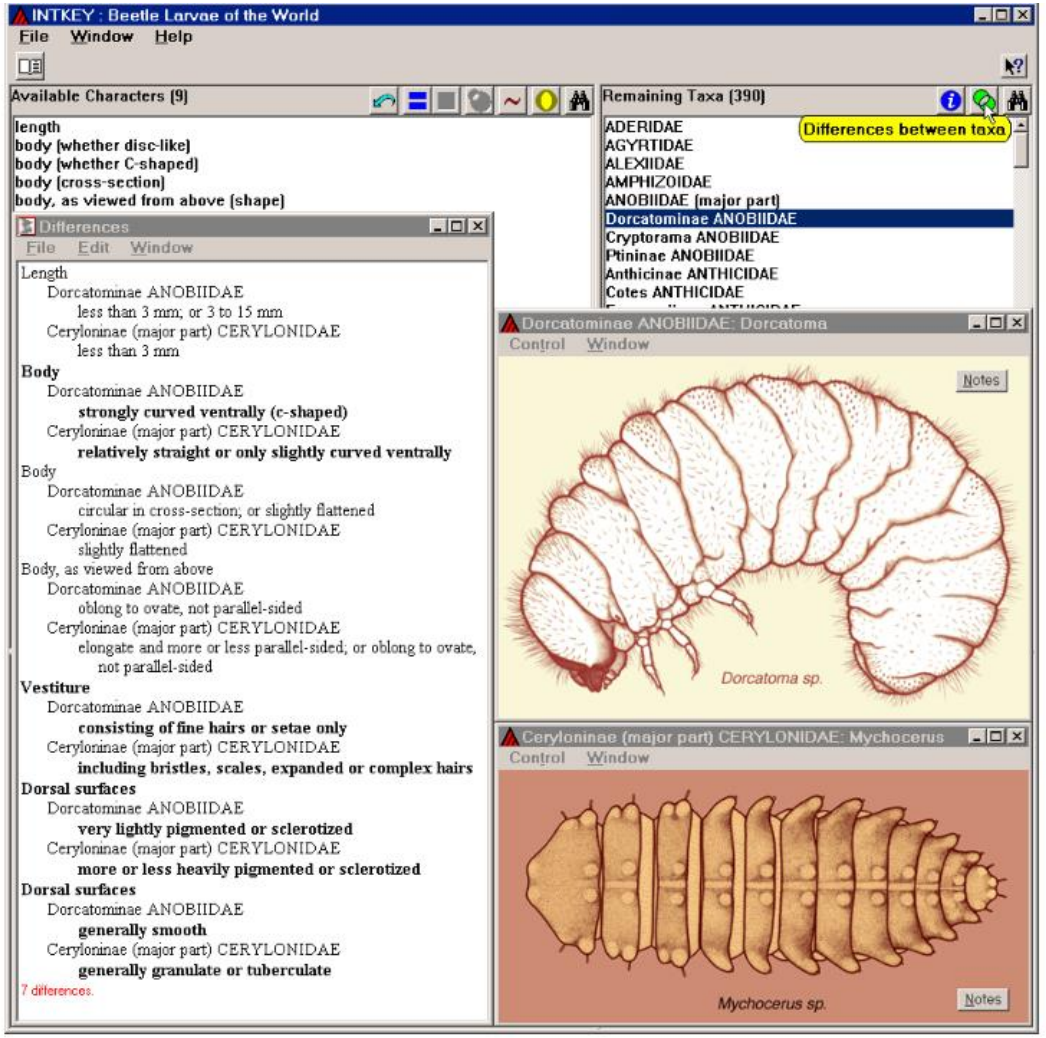
Beginnings of the databases…

Special software packages: DELTA, Lucid

Description Language for Taxonomy (DELTA)
http://delta-intkey.com/

SOURCE OF INFORMATION THAT CAN BE INCLUDED:

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



CINKARNA

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

Mag. Vladimir Vrečko

Future of nanotechnology


Hope you enjoyed and
thank you for your attention!

Public point of view

- Yet another potentialy 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 untill 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 disapointment 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 precautonary 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, normaly, some potential risks, which we can however control through joint activities of researchers, legal bodies and manufacturers.

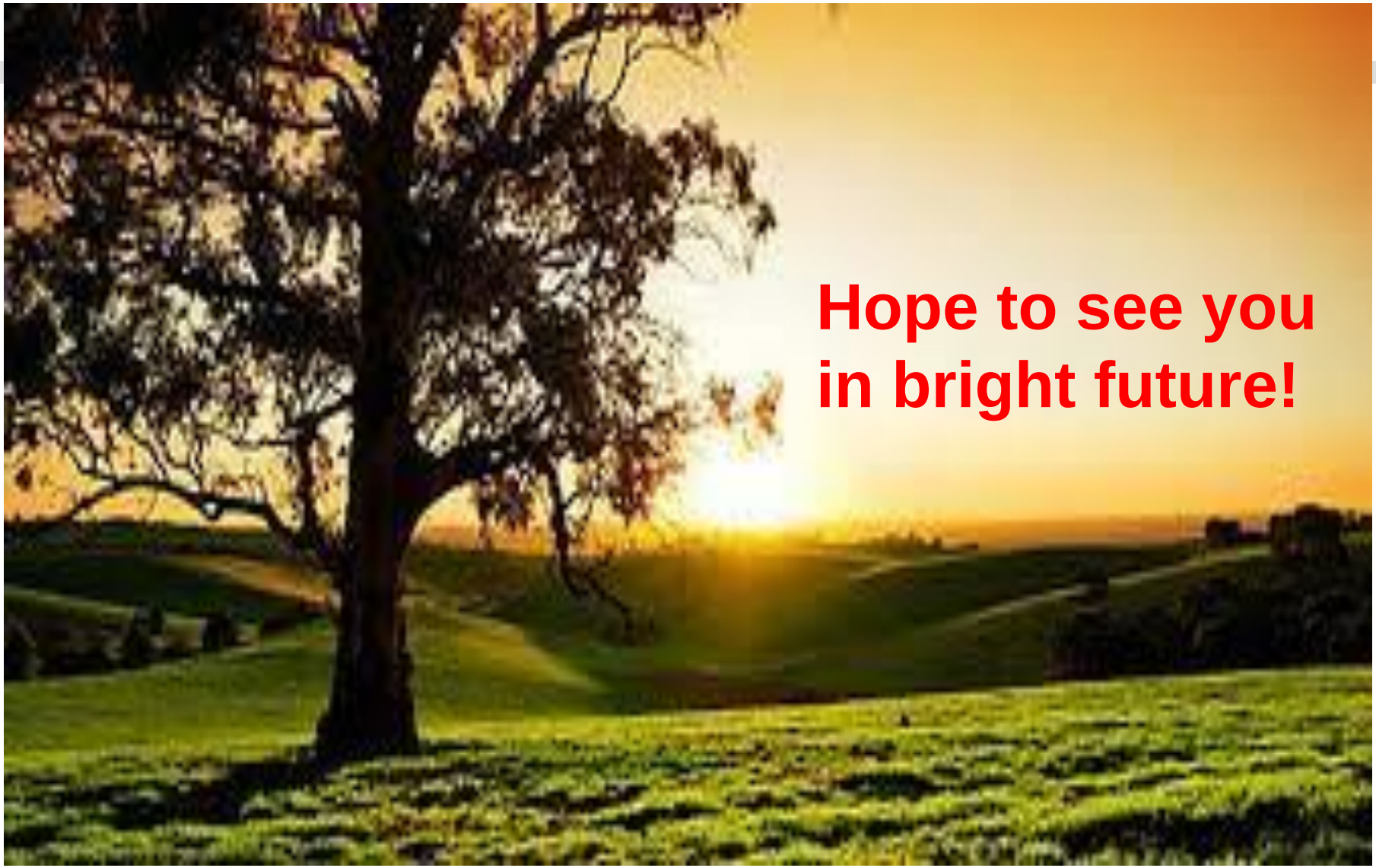
What is needed?

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

How shall we act?

So?

Or so?


Hope to see you
in bright future!

NACIONALNI LABORATORIJ ZA ZDRAVJE, OKOLJE IN HRANO
Prvomajska ulica 1, 2000 Maribor

Chalenges 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 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 tranformation products on use.

ECHA - NMWG

Read across and grouping of NM

Colaboration of ECHA, JRC, NanoREG and RIVM.

Many nanoforms on the market:

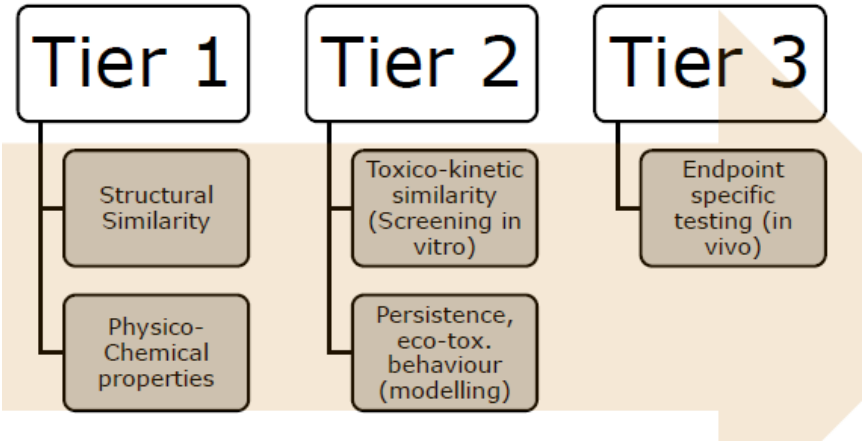
- Are studies from one form applicable to other nanoforms?
- How and when can data be used on nanoforms or beetwen non-nano and nanoforms?

Case studies presented;

Develop a decision framework;

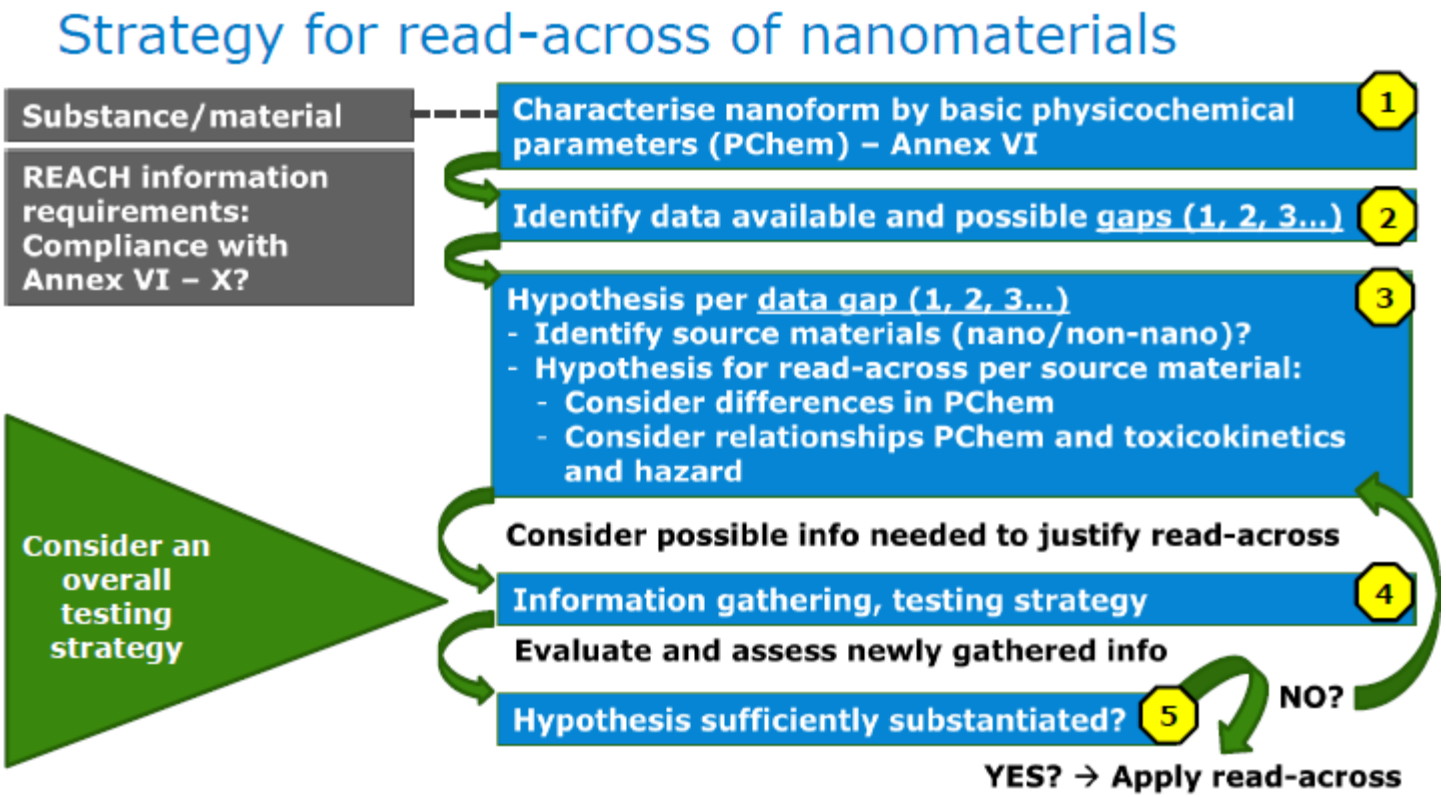
Link to the OECD discussion;

Template for reporting on read – across.



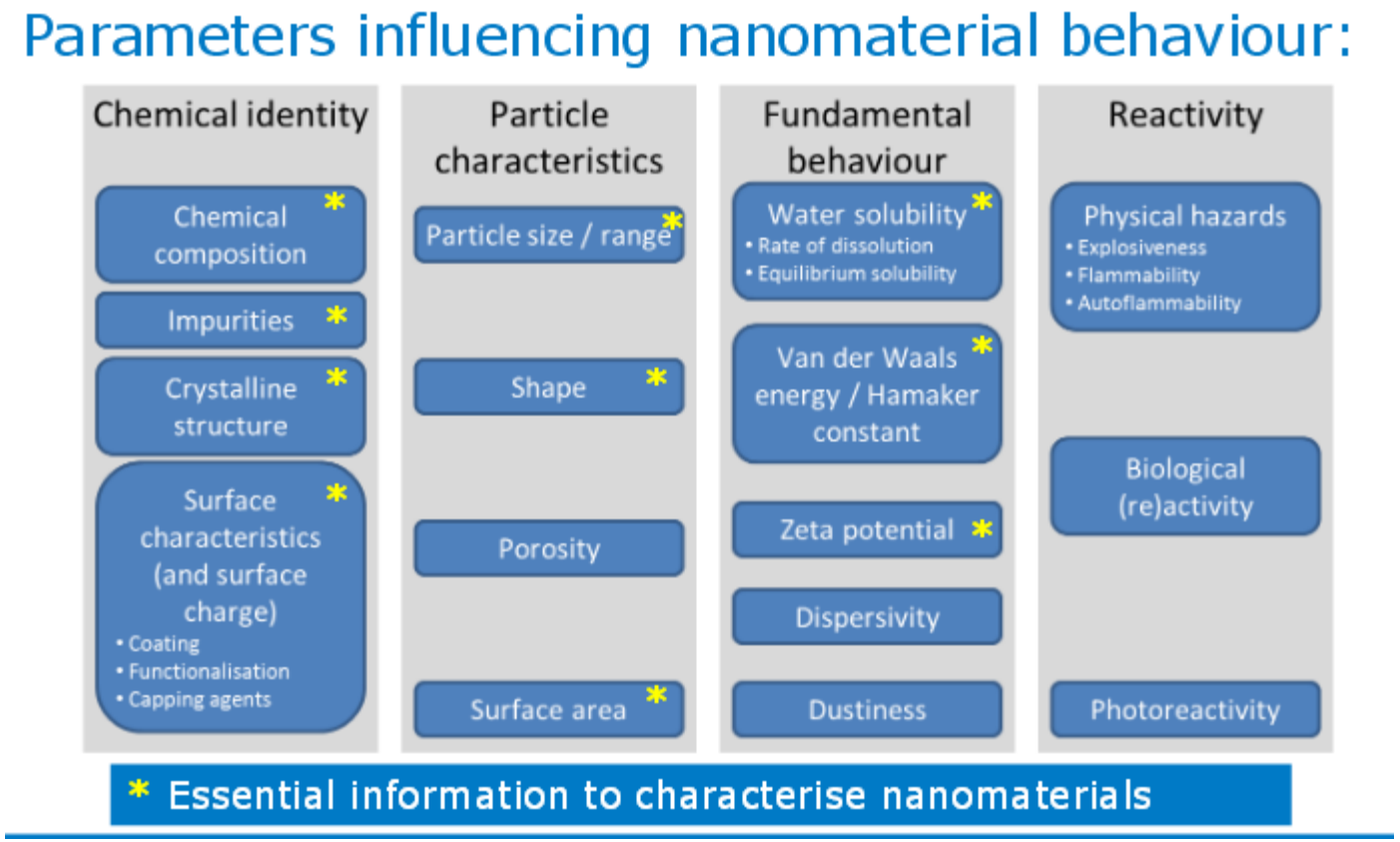
ECHA - NMWG

Read across and grouping of NM



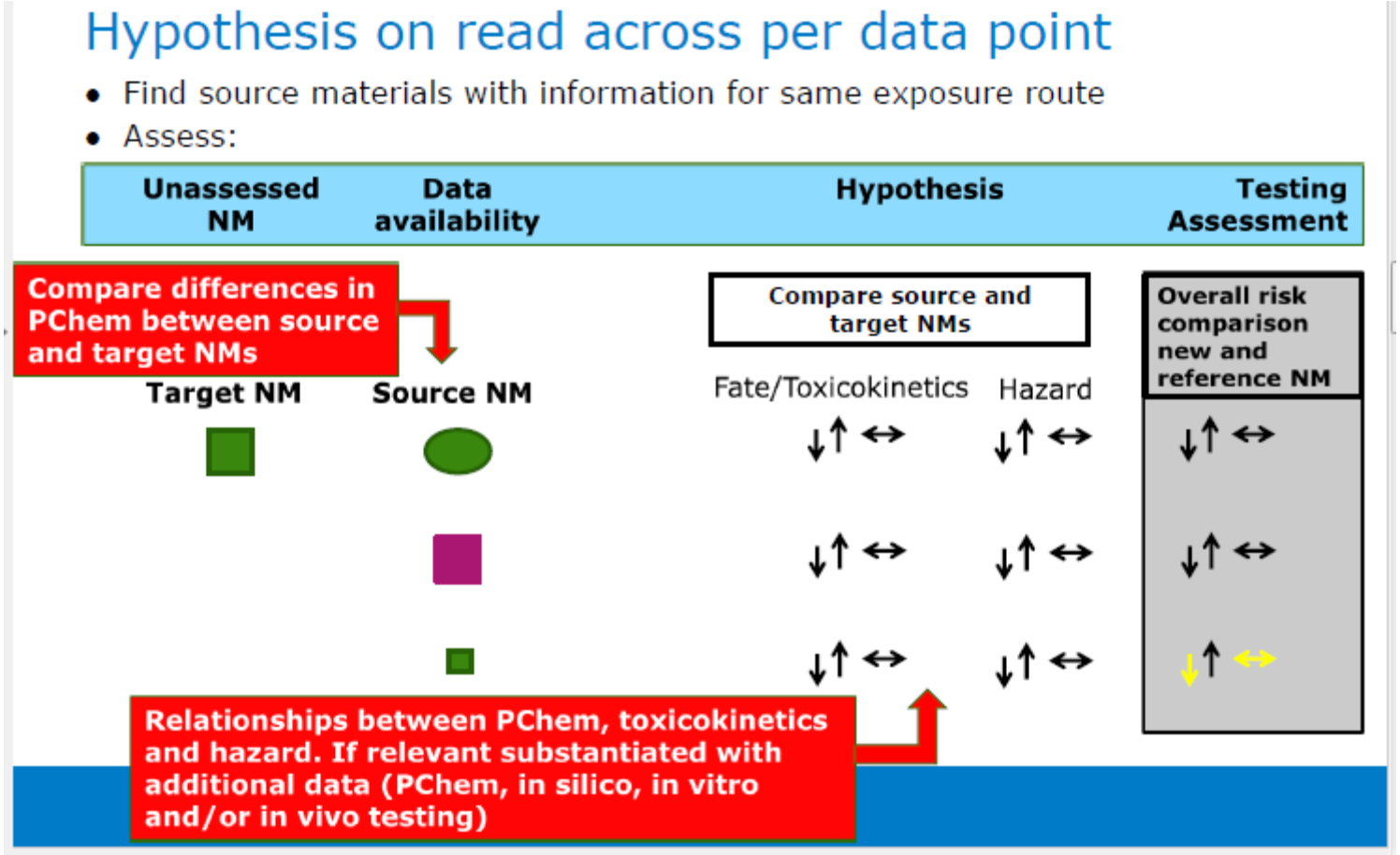
ECHA - NMWG

Read across and grouping of NM



ECHA - NMWG

Read across and grouping of NM



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
Dr. Mojca Kos Durjava
mojca.durjava@nlzoh.si
+386 24500 234

Challenges of Regulation and Risk Assesment 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 biopersistant 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/

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)gov.si)

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 projektnega 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 ocetne 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

DRUGE VSEBINE

Registracija, evalvacija,

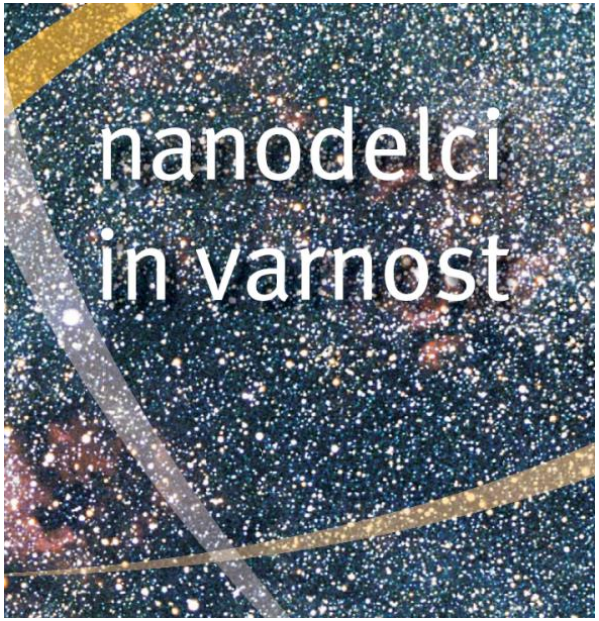
Razvrščanje, pakiranje in

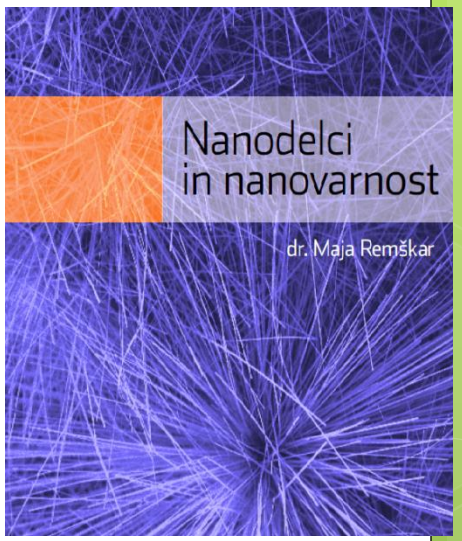
Biocidni proizvodi

A21 | Institut "Jožef Stefan"

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	MoIx
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
Inštitut "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,
Kemijski inštitut	Dominko Robert	robert.dominko(at)ki.si	LiMPO4 (M=Mn,Fe) Li2FeSiO4, Ca
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





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

Additional links:

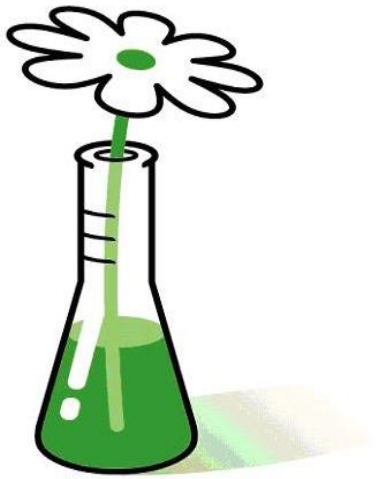
- http://ec.europa.eu/nanotechnology/index_en.html,
- http://ec.europa.eu/environment/chemicals/nanotech/index_en.htm
- http://echa.europa.eu/regulations/nanomaterials
- https://ec.europa.eu/jrc/en/scientific-tool/jrc-web-platform-nanomaterials
- https://ec.europa.eu/jrc/en/scientific-tool/nanohub
-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

Thank you for your attention

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

karmen.krajnc@gov.si

tel:01/478 6051



National
Institute of Chemistry
Slovenia

SI-1000 Ljubljana
Hajdrihova 19, POBox 660
Phone: +386 (0)1/476 02 00
Fax: +386 (0)1/476 03 00
http://www.ki.si

Chemometric analysis and QSAR modelling in NANO-toxicology

Marjan Vračko
National Institute of Chemistry,
Hajdrihova 19, 1000 Ljubljana, Slovenia,
marjan.vracko@ki.si

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

National
Institute of Chemistry
Slovenia

SI-1000 Ljubljana
Hajdrihova 19, POBox 660
Phone: +386 (0)1/476 02 00
Fax: +386 (0)1/476 03 00
http://www.ki.si

Chemometric analysis and QSAR modelling in NANO-toxicology

Outline

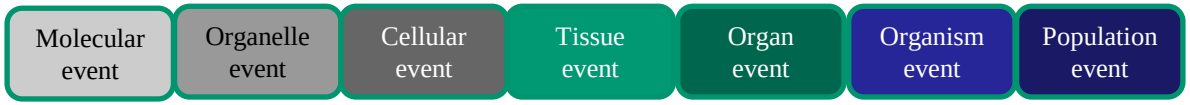
1. Introduction
2. New toxicological paradigm
3. QSAR (**Q**antitative **S**tructure-**A**ctivity **R**elationship) working sheme and strategy
4. NANO-descriptors
5. Chemometrical analysis of proteomic data

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



In chemico

In vitro

In vivo

Clinical, Epidemiological observations

IN SILICO

DOCKING, QSAR, POPULATION ANALYSIS

OECD document…
Adverse Outcome Pathway (AOP)

OECD.org | Data | Publications | More sites | News | Job vacancies

OECD
BETTER POLICIES FOR BETTER LIVES

> A to Z
Search oecd.org

OECD Home | About | Countries | Topics | > Français

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

- Testing of chemicals
- Assessment of chemicals
- Risk management of chemicals
- Chemical accident prevention, preparedness and response
- Pollutant release and transfer register
- Safety of manufactured nanomaterials
- Agricultural pesticides and biocides
- Biosafety - BioTrack

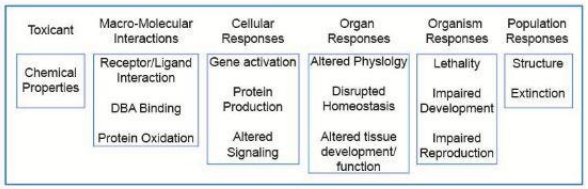
Adverse Outcome Pathways, Molecular Screening and Toxicogenomics

- 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

Example: skin sensitization (chemicals)…

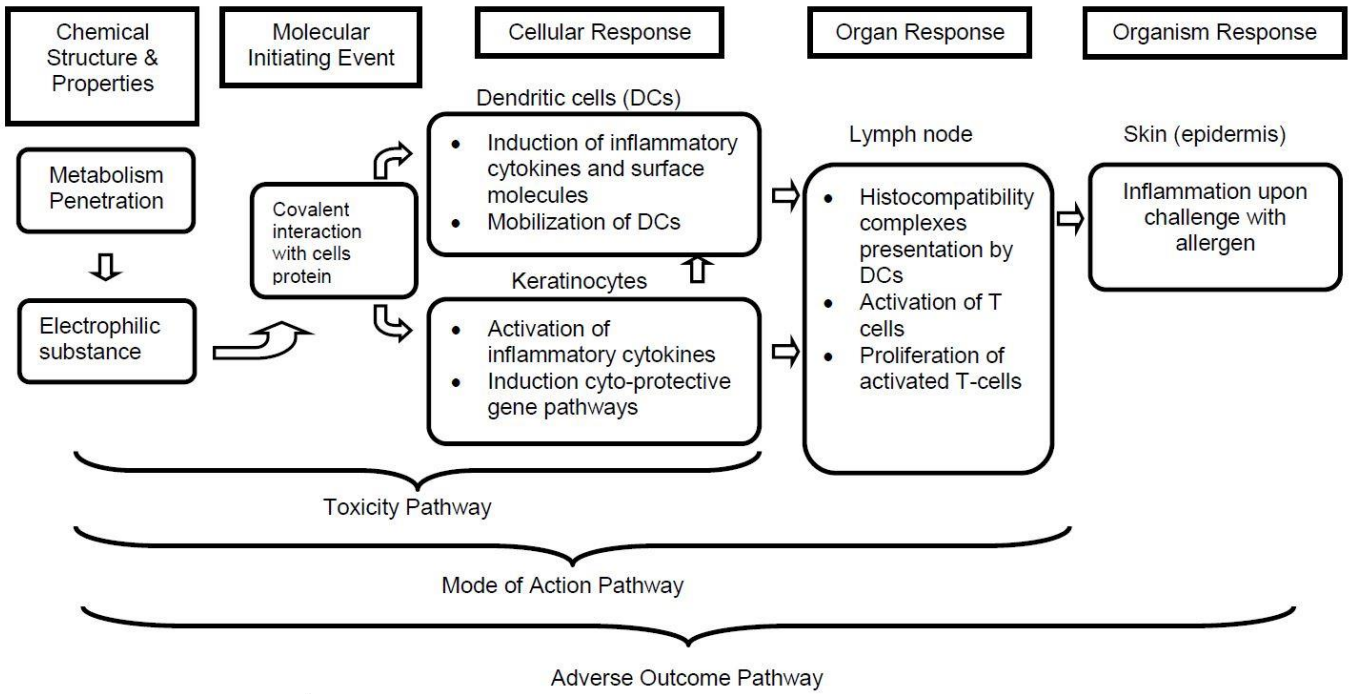
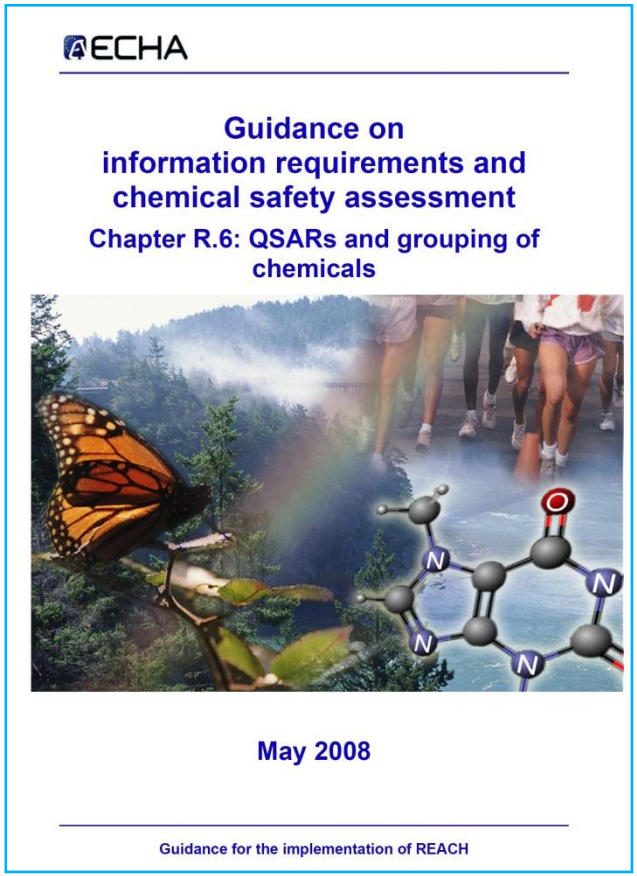


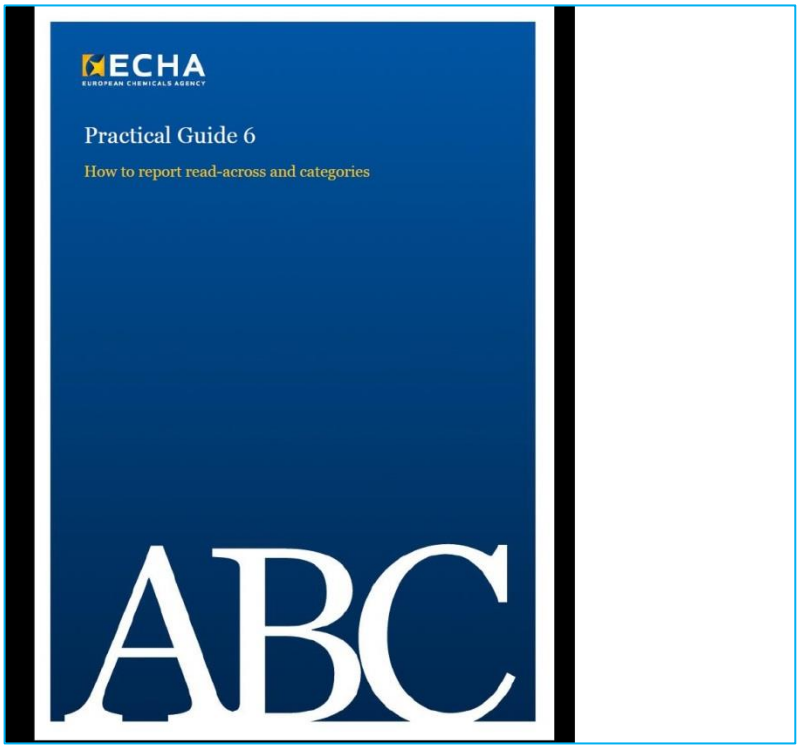
Figure 3. **Flow diagram of the pathways associated with skin sensitisation.**

In silico methods…

- *In silico* methods are important to aquire 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 accross (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}

Scheme of QSAR – strategy
How to extract the hidden knowledge from DATA SET

DATA BASE
Information about molecules
Information on toxicity and properties
Different details about experiments

Molecular structures
•Experimental (X-ray, NMR…)
•Optimized (different approximations)
Physical/chemical properties (logP,MW)

Uniformly presented toxicity or property
•dose
•Affiliation to toxicity class, mode of action, ..

Descriptors

QSAR Model is mathematical relationship between descriptors and toxicity (property)

QSAR model must be VALIDATED!

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

DATA BASE
Information about molecules
Information on toxicity and properties
Different details about experiments

Molecular structures
•Experimental (X-ray, NMR…)
•Optimized (different approximations)
Physical/chemical properties (logP,MW)

Uniformly presented toxicity or property
•dose
•Affiliation to toxicity class, mode of action, ..

Descriptors

NANO?

QSAR Model is mathematical relationship between descriptors and toxicity (property)

QSAR model must be VALIDATED!

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:

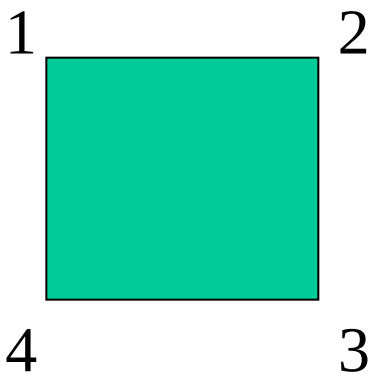
- Shape indices
- Analysis from electron microscopy pictures.

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:



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

$$W = d12 + d13 + d14 + d23 + d24 + d34$$
$$= 1 + 2 + 1 + 1 + 2 + 1 = 8$$

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

Atempts to lower the degree of degeneration:

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

Second generation topology indices

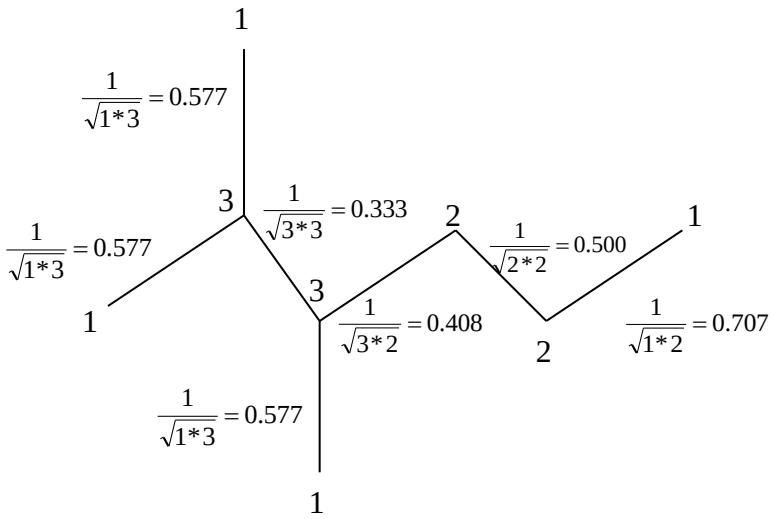
They are real numbers deduced from graphs. One of the most successful is molecular connectivity index c proposed by Randic:

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

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

Calculation of connectivity index for 3-methylheptane

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



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

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 crystaline form, in water environment, or in protein environment.

Experimental determinations:

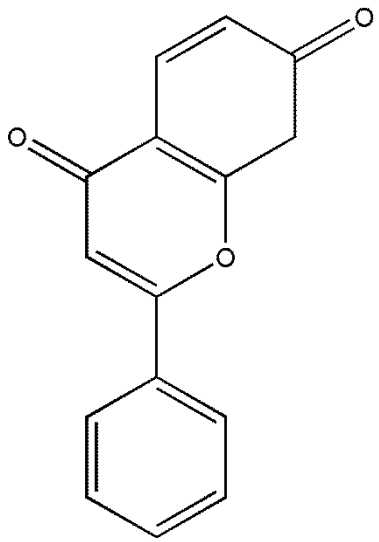
- X ray diffraction measurements
- 2D-NMR measurements – method enables determination of ligand-receptor geometry

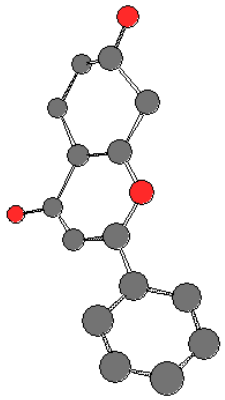
Theoretical determination:

- Quantum chemical optimization
- Molecular dynamics

<u>Result (3D structure) depends on the selected method.</u>

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



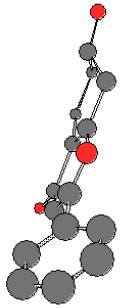


Tautomer of 7-hydroxy-2-phenyl-4-benzopyrone

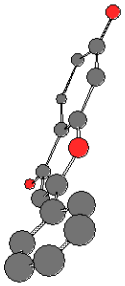
C1(=CC=CC=C1)C3=CC(C2=C(CC(C
=C2)=O)O3)=O

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

Rule based system



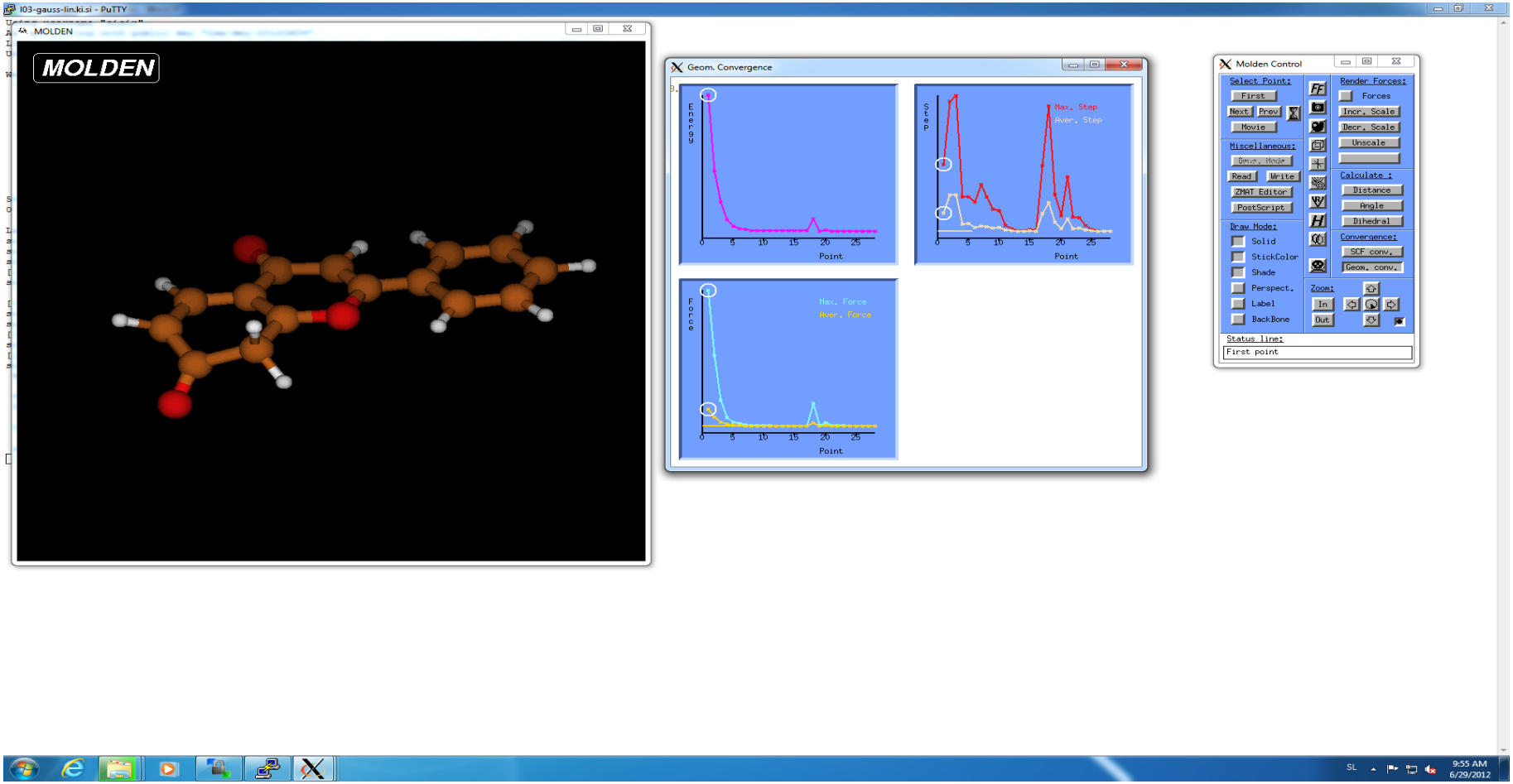
MM optimization



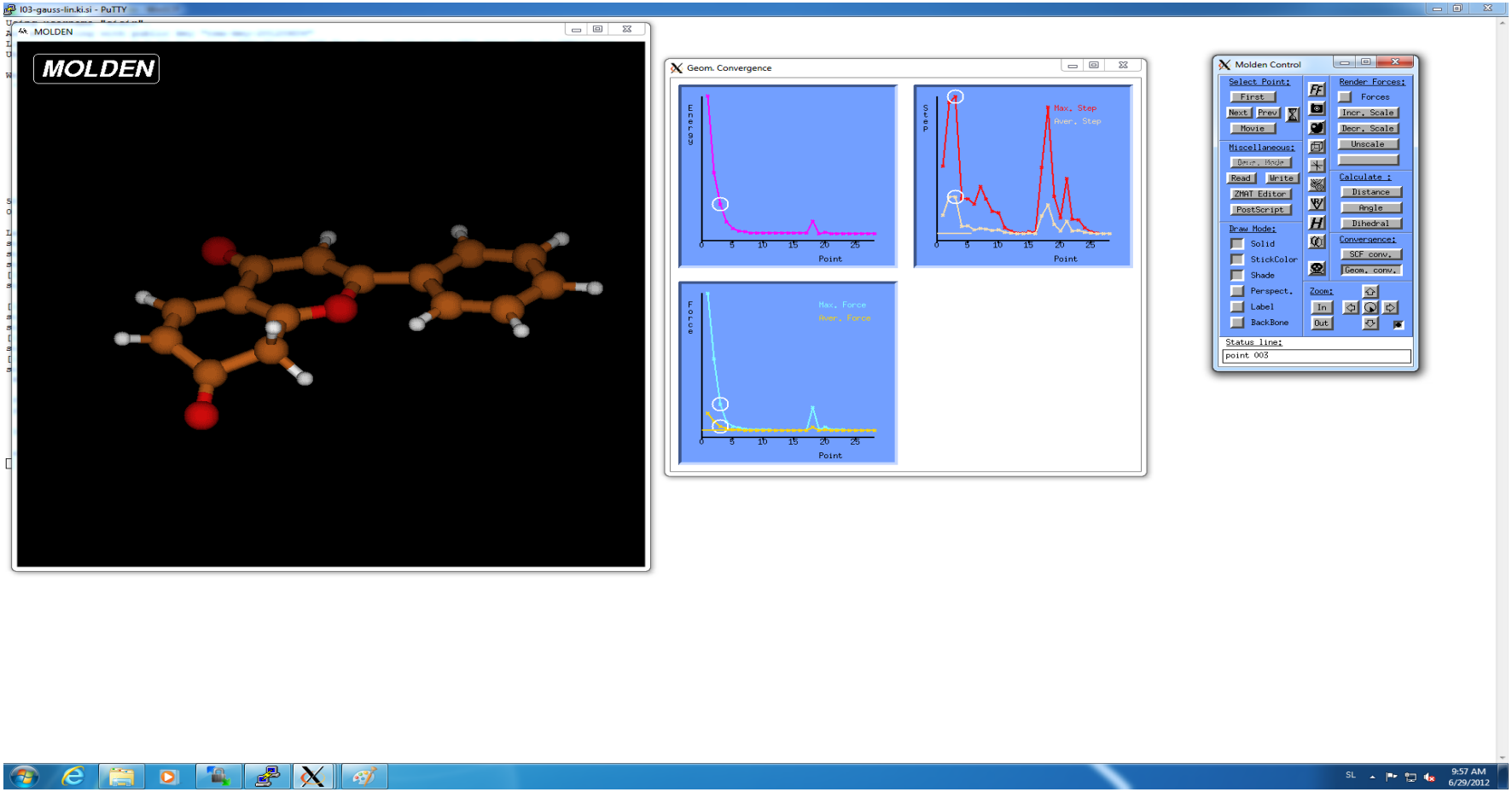
A Flavonoid derivate: 3D structure determinated with rule based generator or with QC program GAUSSIAN (HF-6-31g approximation)
Input

```
# 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
```

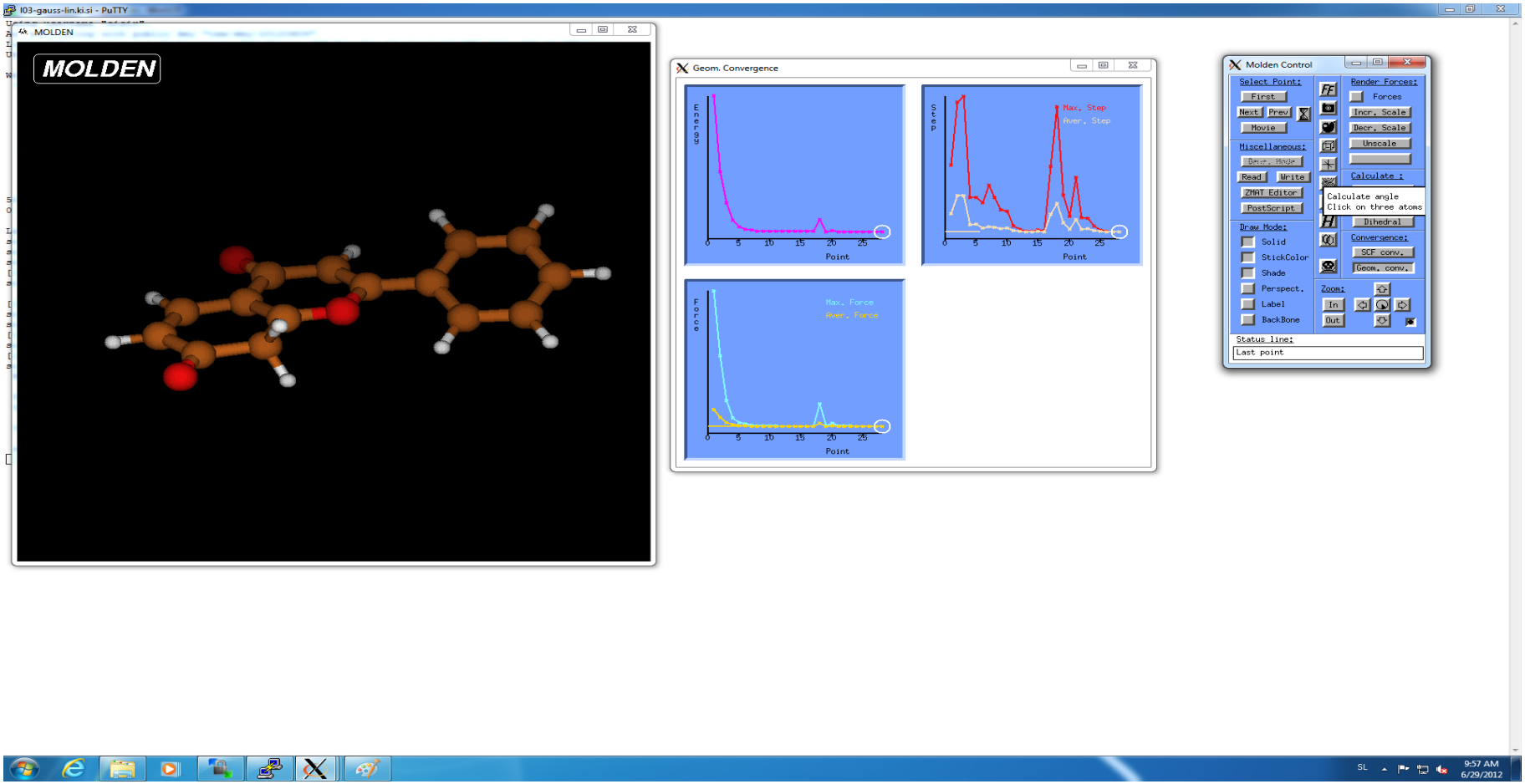
A Flavonoid derivate: 3D structure determinated with rule based generator or with QC program GAUSSIAN (HF-6-31g approximation)
Original



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 = $-E_{HOMO}$

Electron affinity = $-E_{LUMO}$

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

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_{HOMO} = -0.33343 Hartree, E_{LUMO} = 0.06510 Hartree
3. Multipole moments:

Electronic spatial extent (au): <R**2>= 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	XYY=	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	YYYX=	-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:

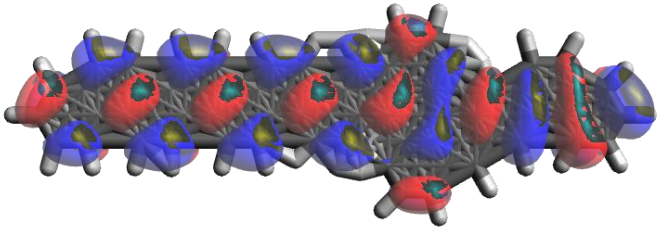
- gaps,
- proton, electron affinity,
- surface charges,
- electronic properties related to reactivity (formation of metal cations).

Problems:

- NANO particles are large, poorley 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. Jeliazkova 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..

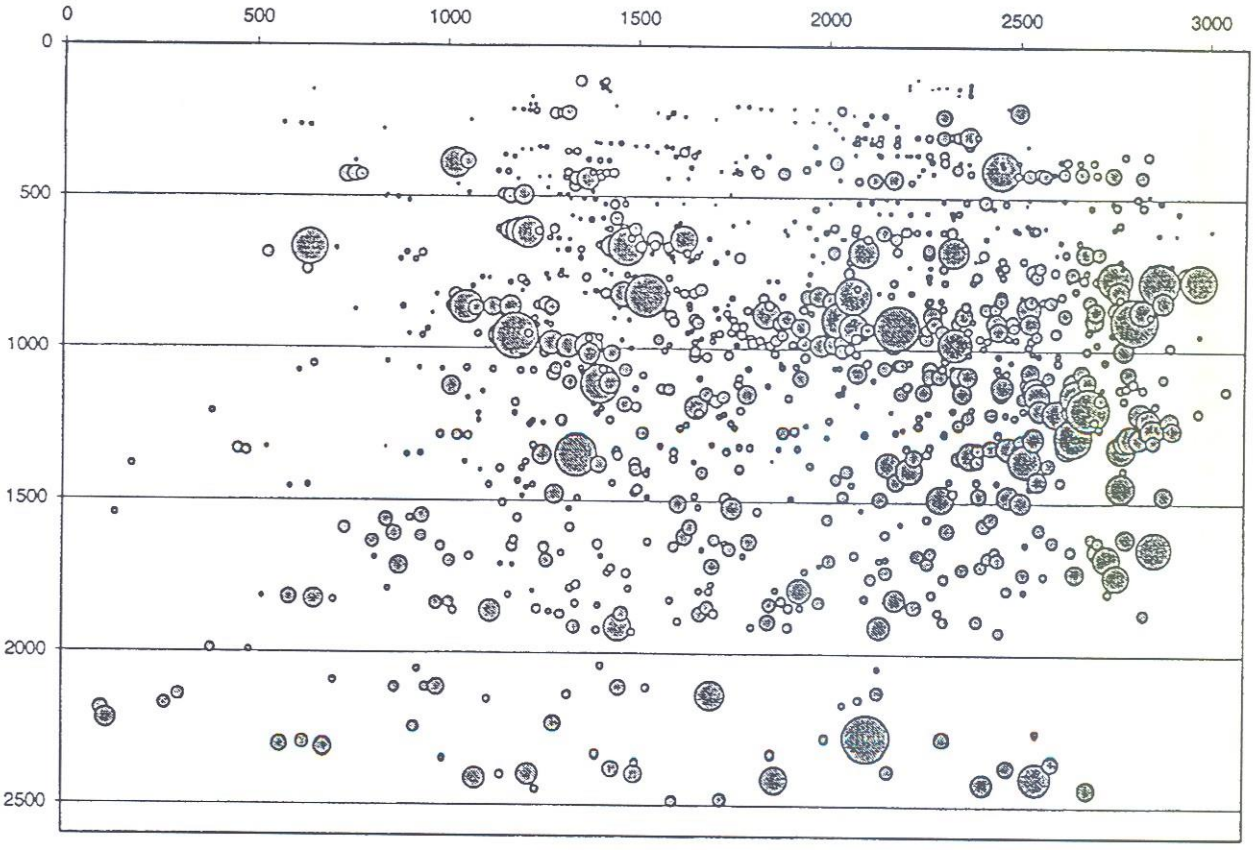
In vitro study:

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

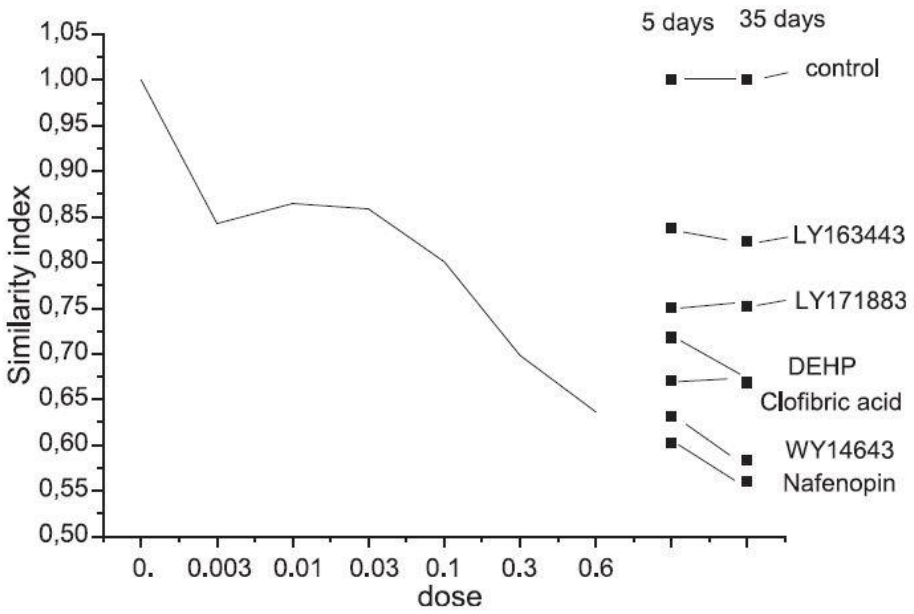


Fig. 2. Similarity index dependent on administrated dose of LY171883. On spots the right side show similarity index for 5 and 35 days exposures.

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.

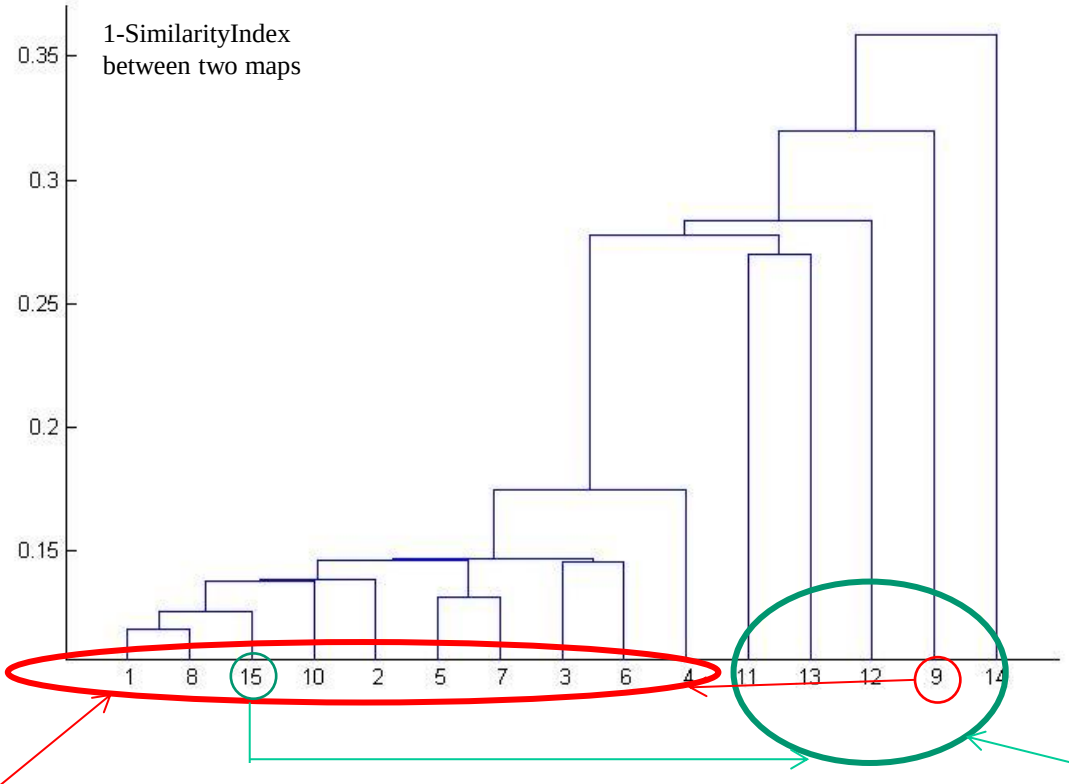
- The comparison of proteomic maps obtained from hepatocytes, which were treated 14 halocarbons.
- Six biological endpoints were determined *in vitro*: $EC50_{MIT}$, LEC_{ROS}, $EC20_{SH}$, $EC50_{LDH}$, LEC_{LP}, LEC_{CAT}
- From each map 263 spots were taken to study the similarity among maps.
- The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

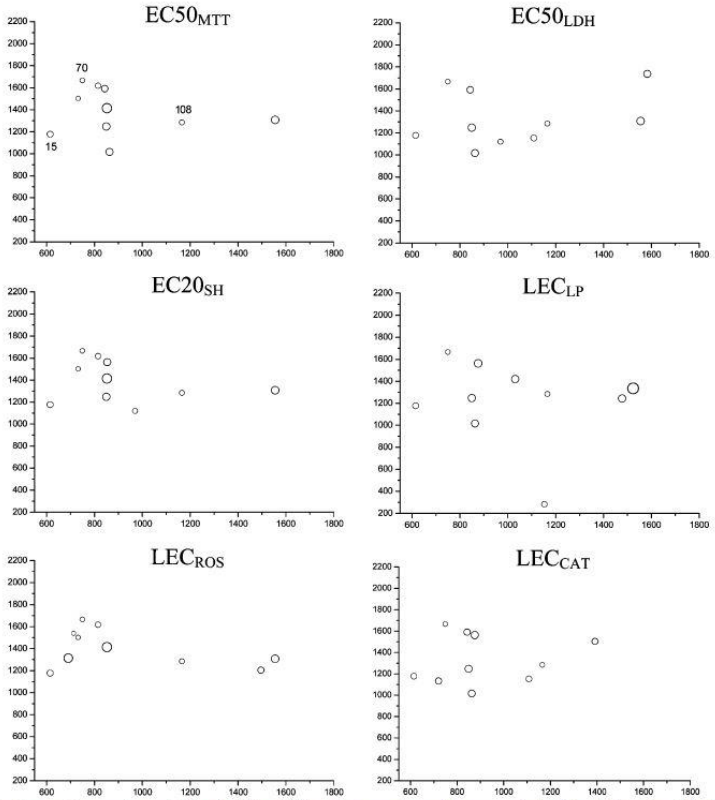


Figure 6. Ten mostly occurring spots for six different biological parameters as they appear in the 2D gel. The size of the bubbles is proportional to the logarithm of intensity. (The integrated intensity of protein spots was calculated by the image analysis software, PDQuest.) In the first picture ($EC50_{MTT}$)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

National
Institute of Chemistry
Slovenia

SI-1000 Ljubljana
Hajdrihova 19, POBox 660
Phone: +386 (0)1/476 02 00
Fax: +386 (0)1/476 03 00
http://www.ki.si

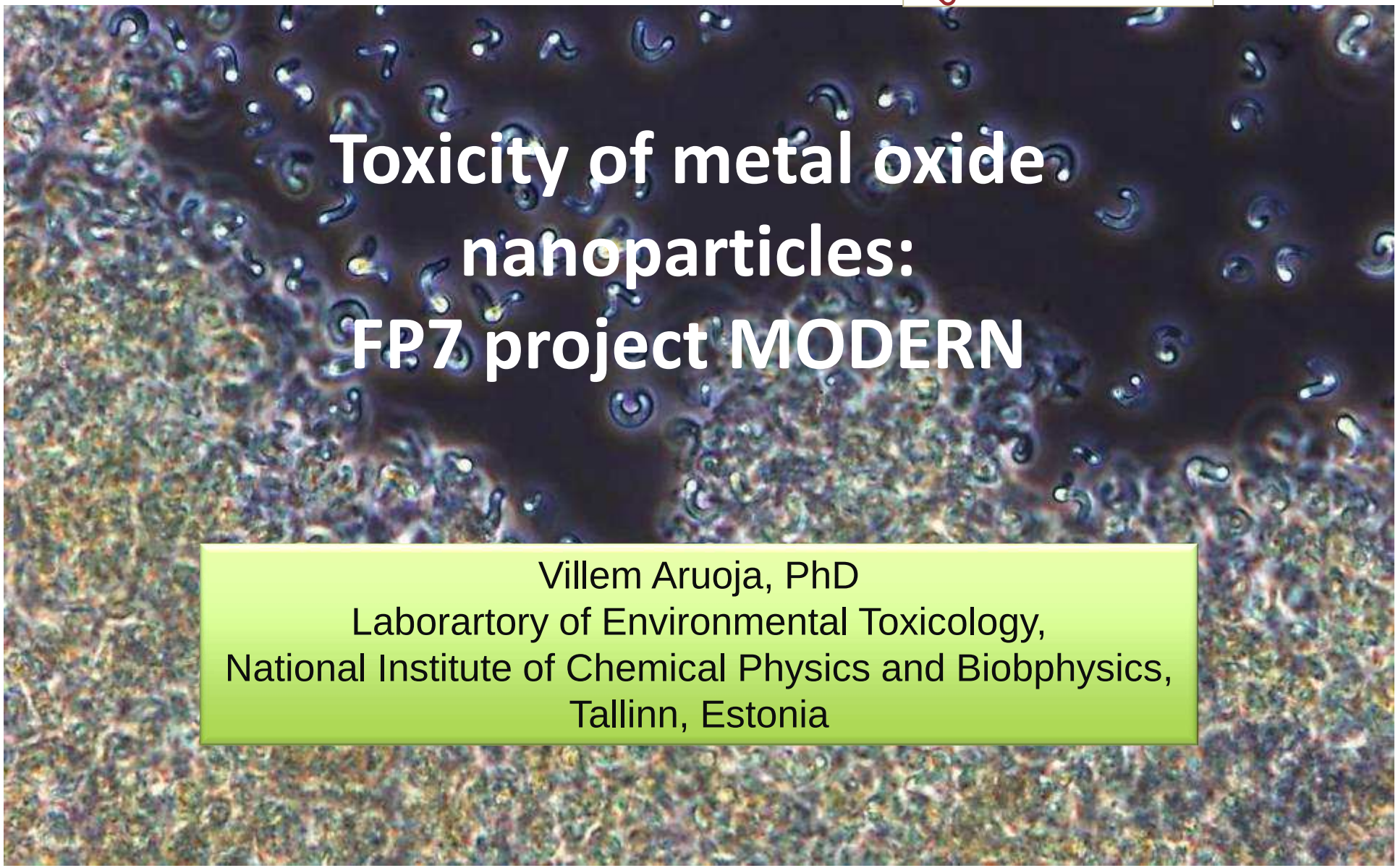
Laboratory for chemometrics

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



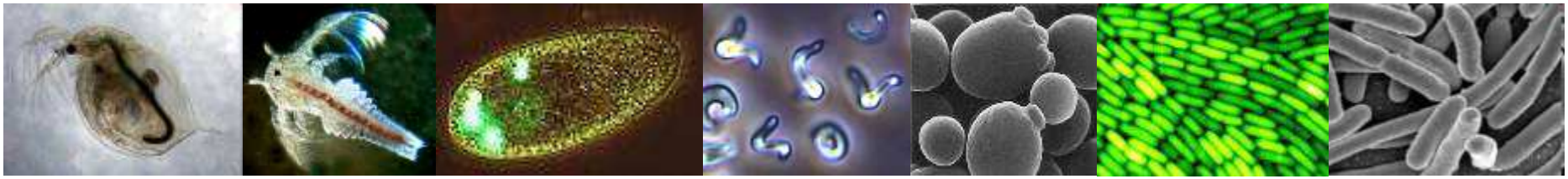
Toxicity of metal oxide nanoparticles: FP7 project MODERN

Villem Aruoja, PhD
Laborartory of Environmental Toxicology,
National Institute of Chemical Physics and Biobphysics,
Tallinn, Estonia

Our zoo

Particle ingesters	„Particle-proof“

Eukaryotes	Prokaryotes



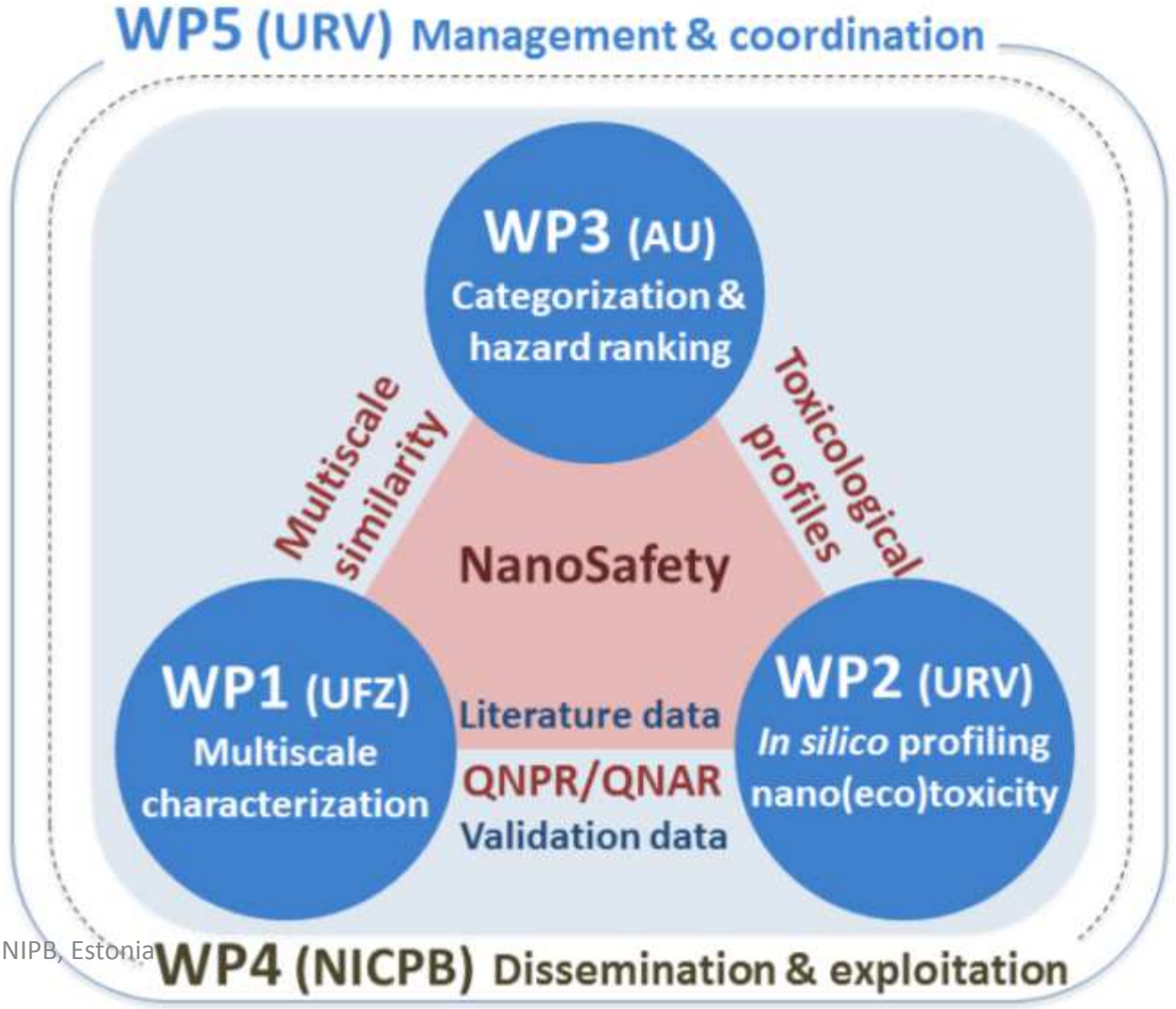
Crustacea		**Protozoa**	**Algae**	**Yeast**	**Bacteria**	
Daphnia magna	*Themnocephalus platyurus*	*Tetrahymena thermophila*	*Pseudo-kirchneriella subcapitata*	*Saccharomyces cerevisiae*	*Vibrio fischeri*	*Escherichia coli*

Consumers	Primary producers	Decomposers

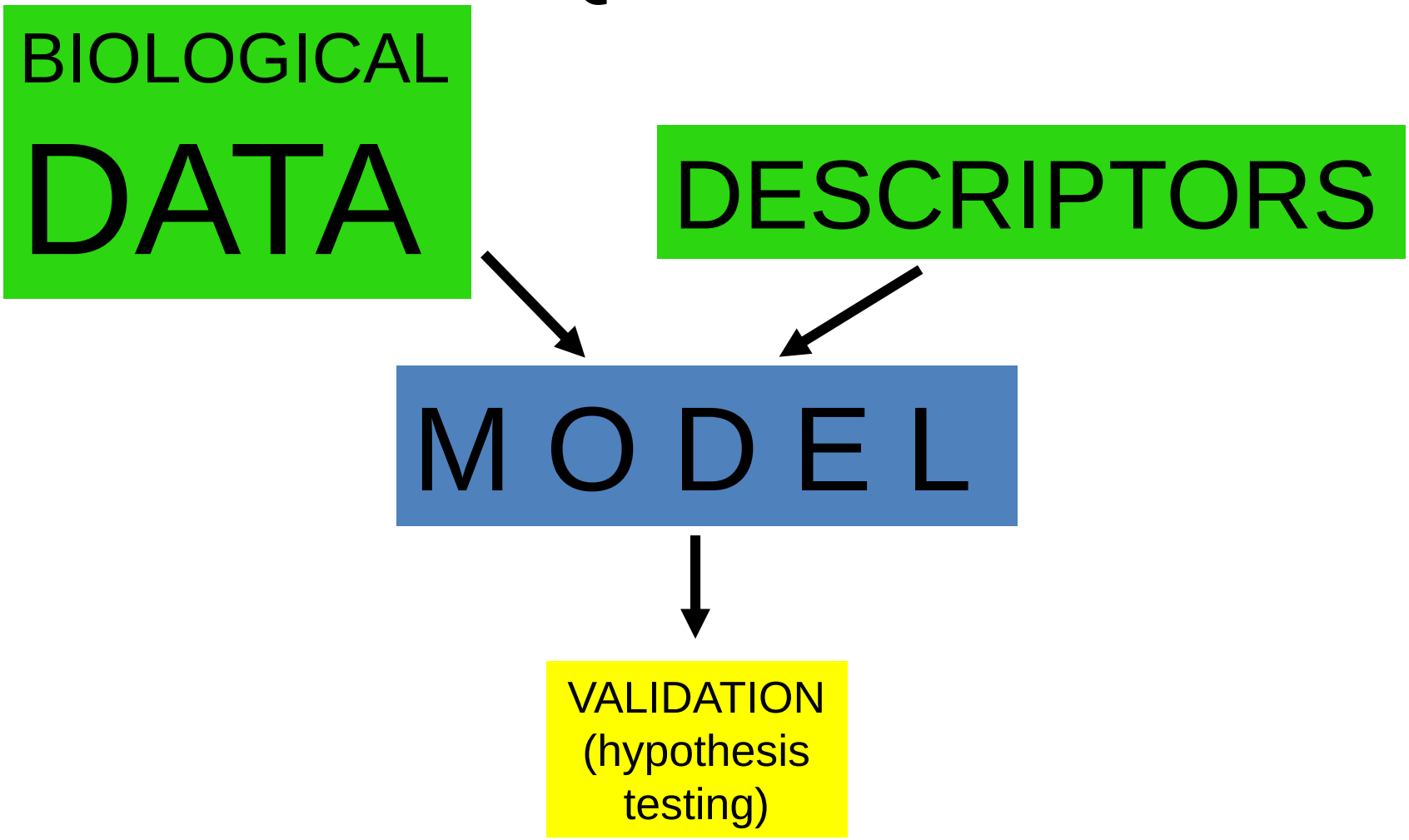
human and animal cell lines
(human alveolar epithelial cells **A549**, human epithelial colorectal cells **Caco2** , murine fibroblast cell line **Balbc/3T3**)

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)

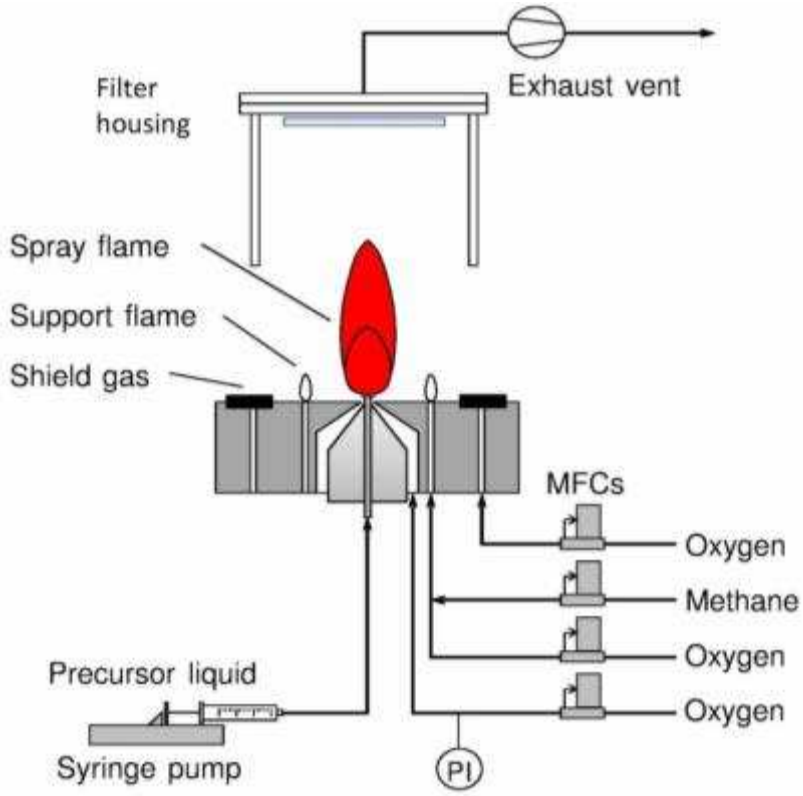
Data?

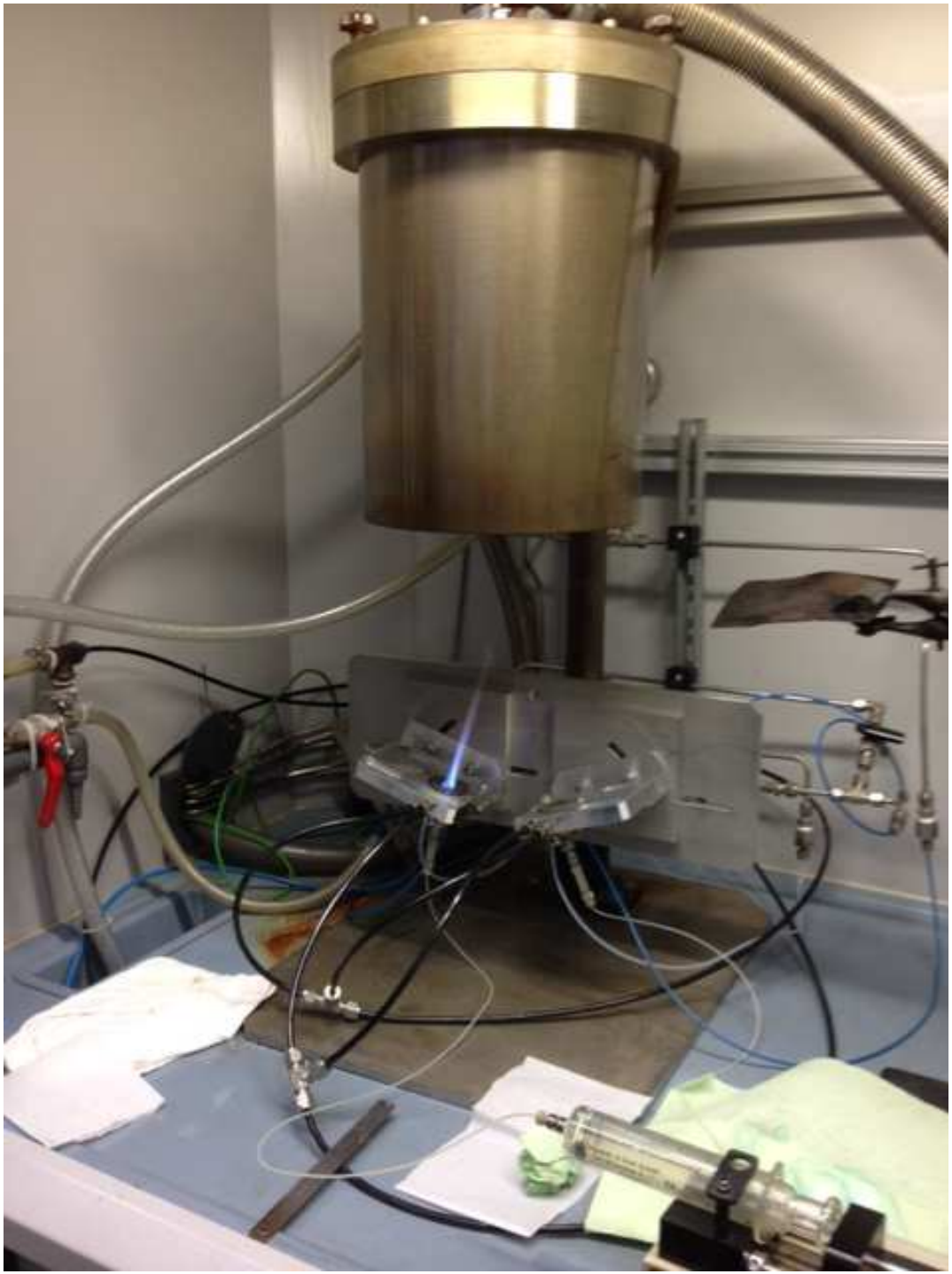
- Literature?
- Industry?

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

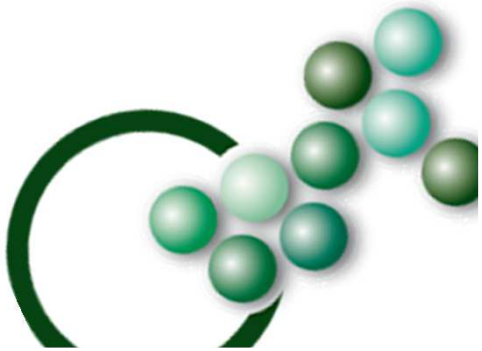


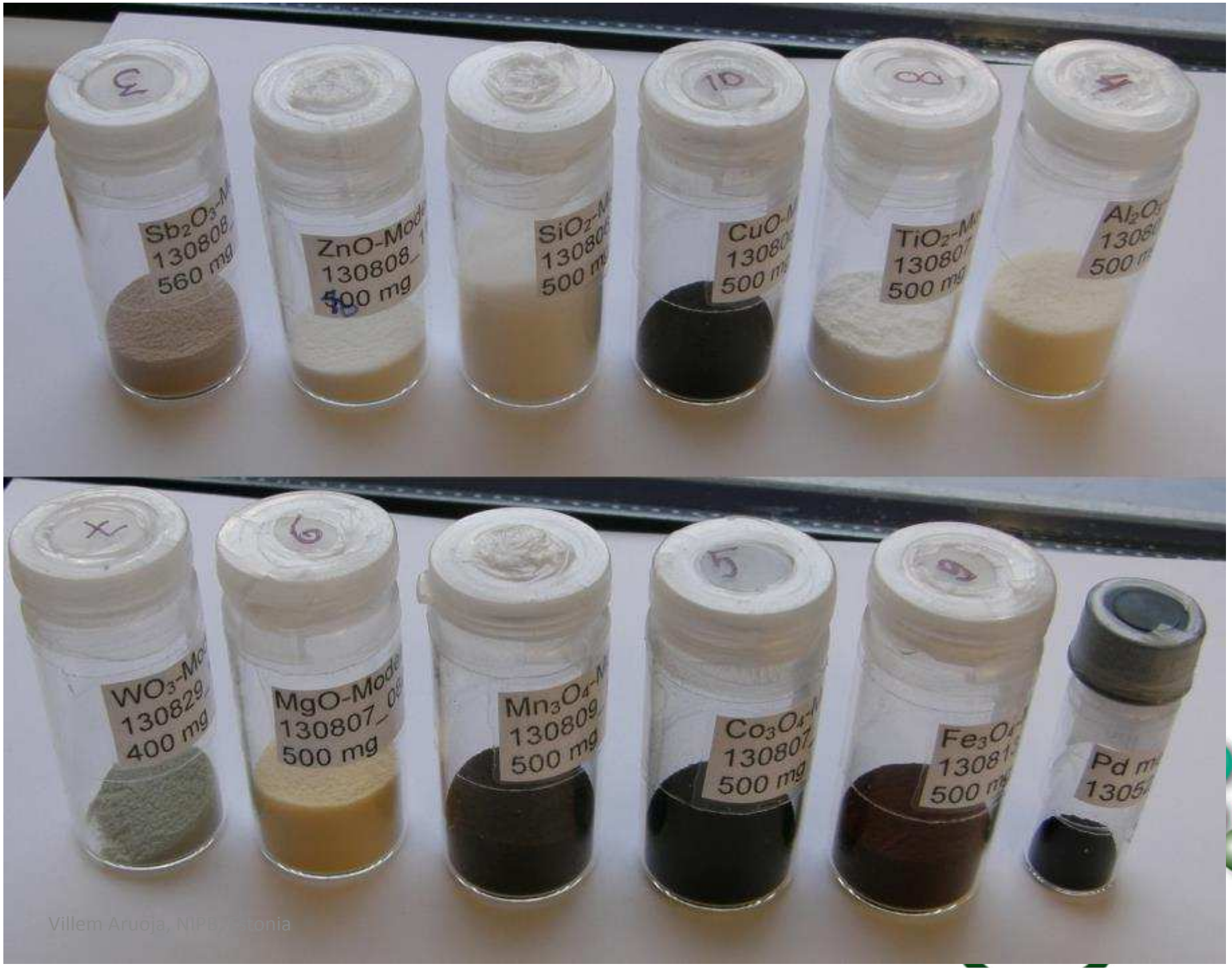


Selection of nanoparticles

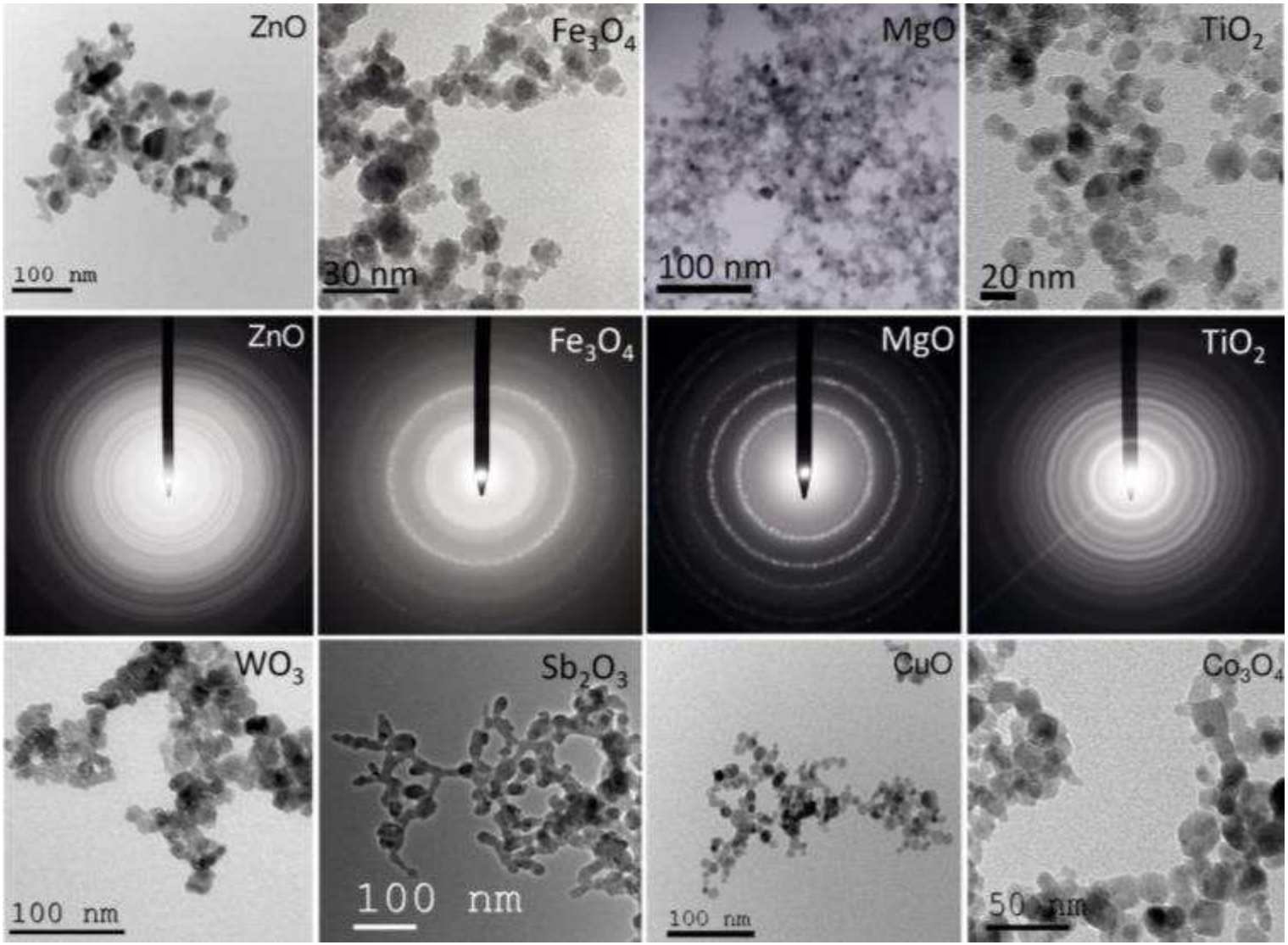
- CuO, Co_3O_4, Sb_2O_3, TiO_2, WO_3, ZnO, Mn_3O_4, Fe_3O_4, MgO, Al_2O_3, SiO_2 , Pd

  (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)



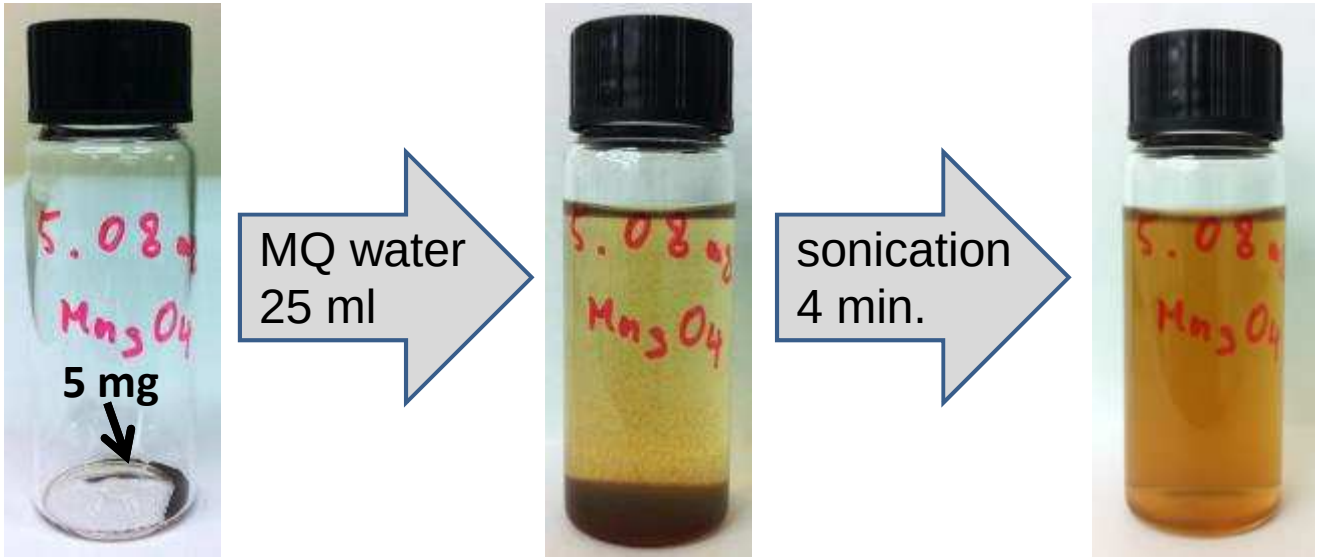

Sb2O3-M
130808_
560 mg
ZnO-Mod
130808_
500 mg
SiO2-M
130808
500 mg
CuO-M
130807
500 mg
TiO2-M
130807
500 mg
Al2O3
130808
500 m
WO3-Mo
130829_
400 mg
MgO-Mod
130807_09
500 mg
Mn3O4-M
130809_
500 mg
Co3O4-M
130807_
500 mg
Fe3O4
130813
500 mg
Pd m
1305
Villem Aruoja, NIPH, Estonia

FSP – small and crystalline NPs



Aruoja et al. Env Sci: Nano, 2015, DOI: 10.1039/C5EN00057B

Preparation of MOx NPs suspensions



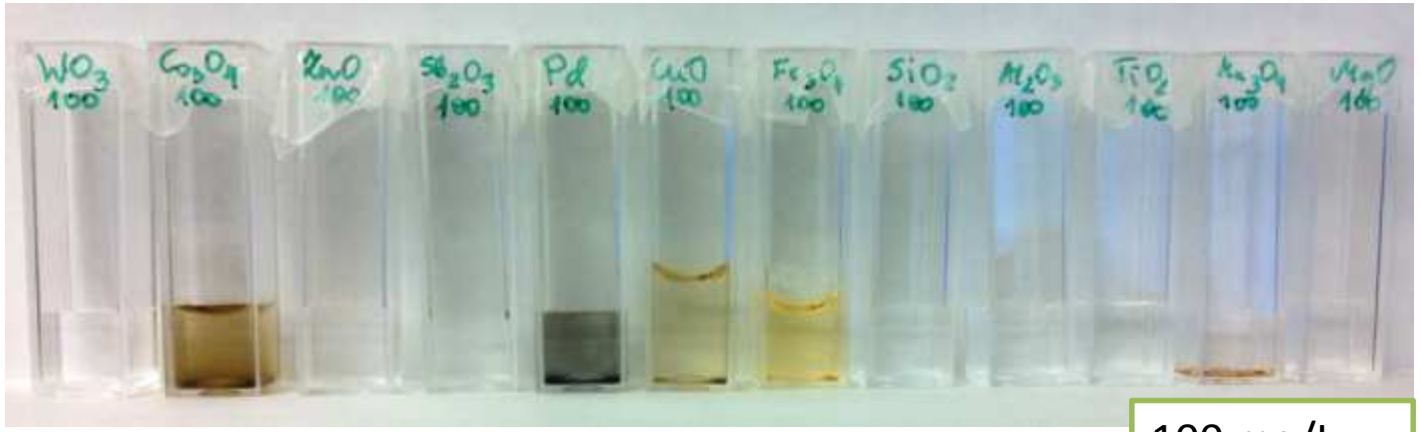
Size, hydrodynamic size

Sample	Specific surface area (SSA); m2/g	BET size (d_{BET})	DI water					Algal growth medium (pH=8.0)			
						Metal solubility				Metal solubility	
		nm	z-average hydrodynamic size, nm	ζ-potential, mV	pH	% at 10 mg/l (AAS or ICP-MS*)	% at 200 mg/l (TXRF)	z-average hydrodynamic size, nm	ζ-potential, mV	% 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_3O_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_3O_4	81	15.2	395	-14.4	7.0	11.1	4.8	920	-9.8	9.45	6.62
Fe_3O_4	120	9.7	128	22.2	5.9	<1.38	7.1	1005	-12.1	1.66	0.17
Al_2O_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	$87.9^{\dagger}$	NA
Sb_2O_3	56	20.5	125	-24.3	4.2	56.3	NA	414	-15.9	21.2	NA

Aruoja et al. Env Sci: Nano, 2015, DOI: 10.1039/C5EN00057B

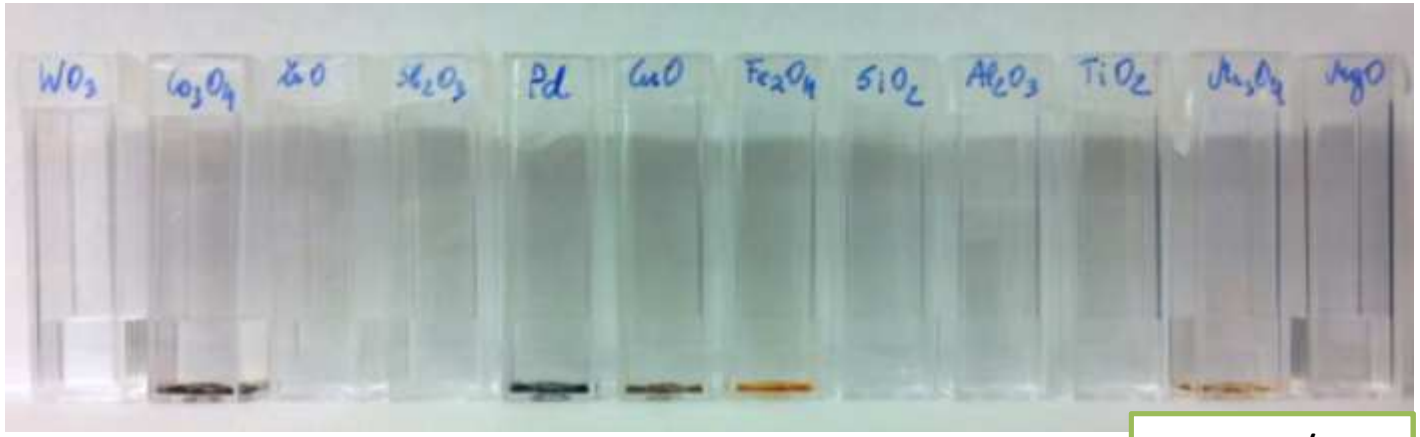
Stability of suspensions in MQ water *vs* algal test medium

In MQ water: 1 week



100 mg/L

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



100 mg/L

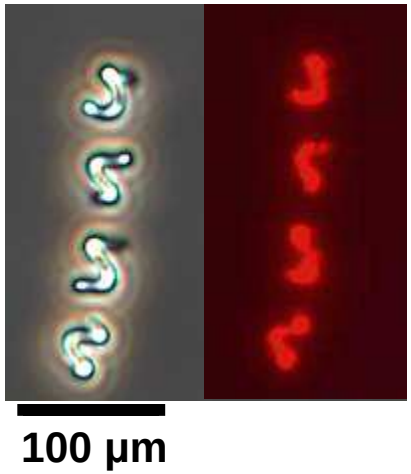
Composition of the algal test medium.

	Component	mg/L
1	NH_4Cl	15
2	$MgCl_2*6H_2O$	12
3	$CaCl_2*2H_2O$	18
4	$MgSO_4*7H_2O$	15
5	KH_2PO_4	1,6
6	$NaHCO_3$	50
7	$Na_2EDTA*2H_20$	0,1
8	$FeCl_3*6H_2O$	0,08
9	H_3BO_3	0,185
10	$MnCl_2*4H_2O$	0,415
11	$ZnCl_2$	0,003
12	$CoCl_2*6H_2O$	0,0015
13	$Na_2MoO_4*2H_2O$	0,007
14	$CuCl_2*2H_2O$	0,00001

Aruoja et al. Env Sci: Nano, 2015, DOI: 10.1039/C5EN00057B

Algal growh inhibition assay (OECD 201)

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



EC_{50}

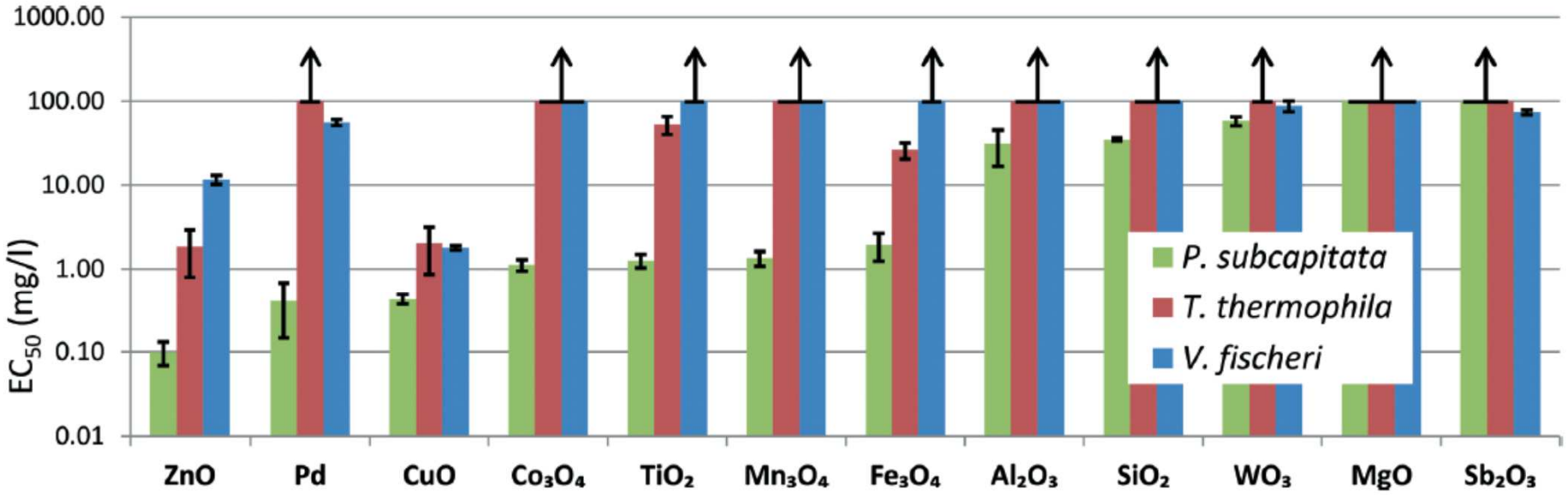
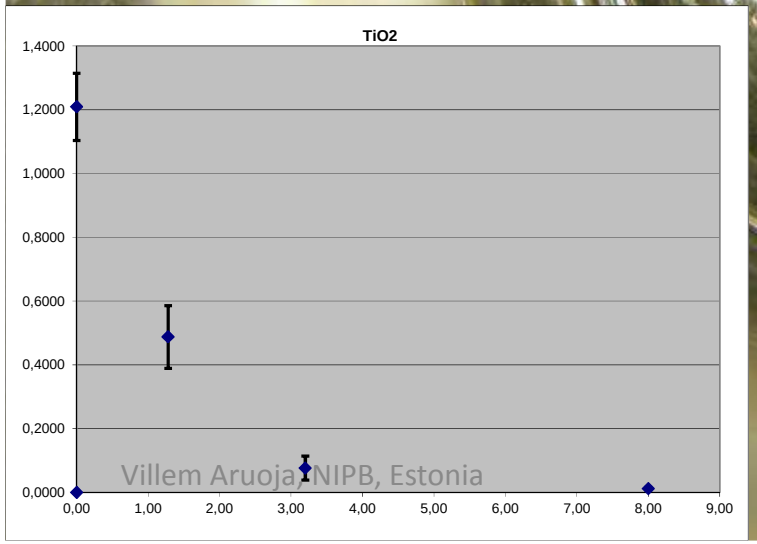


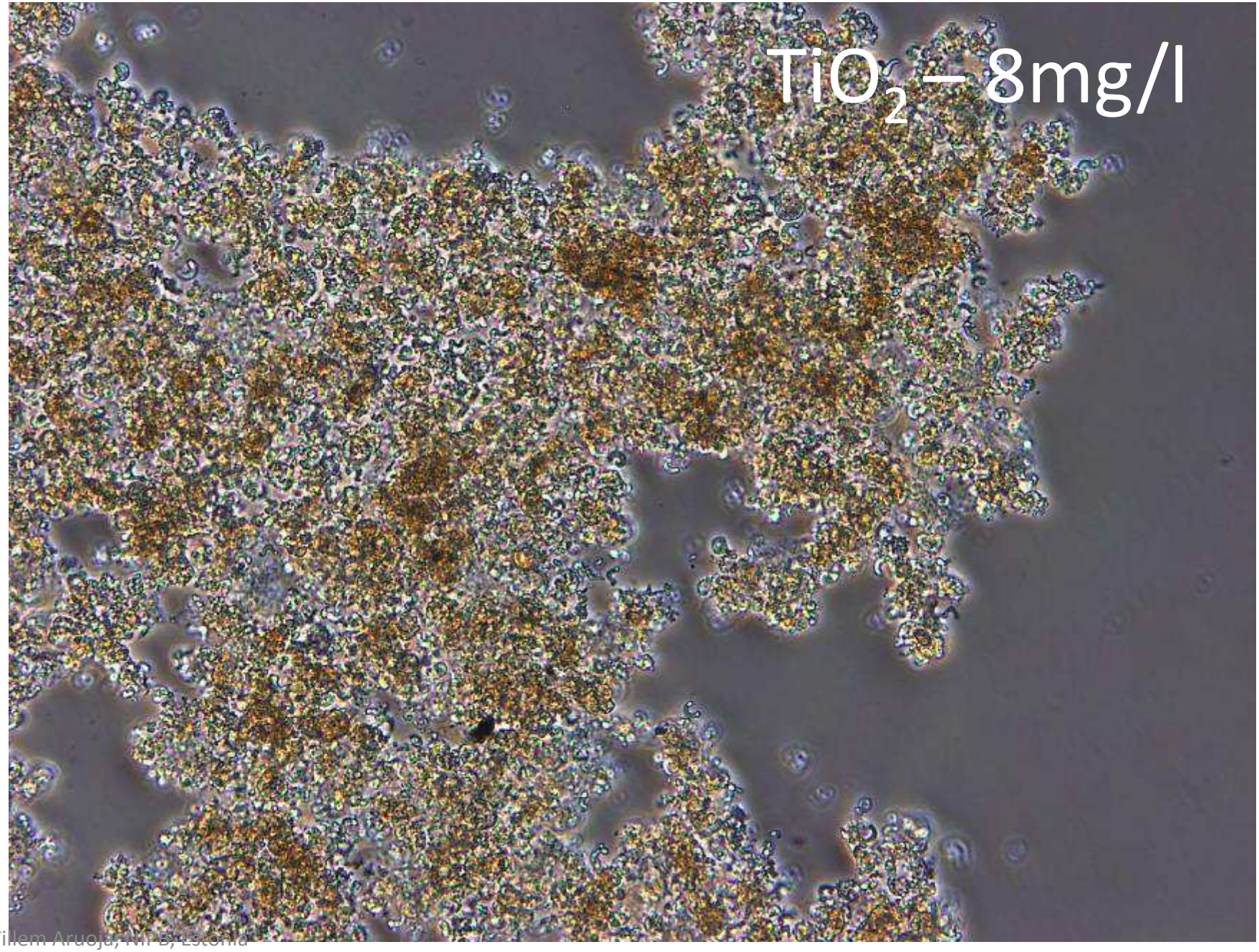
Fig. 2 Toxicity of 12 nanoparticles to alga *Pseudokirchneriella subcapitata*, protozoa *Tetrahymena thermophila* and bacterium *Vibrio fischeri*. EC_{50} values were obtained from 72 h algal growth inhibition assay, 24 h *T. thermophila* viability assay and 30 min *V. fischeri* luminescence inhibition assay (Table S3†). Arrows indicate EC_{50} values above 100 mg l^{-1}. Concentrations are nominal.

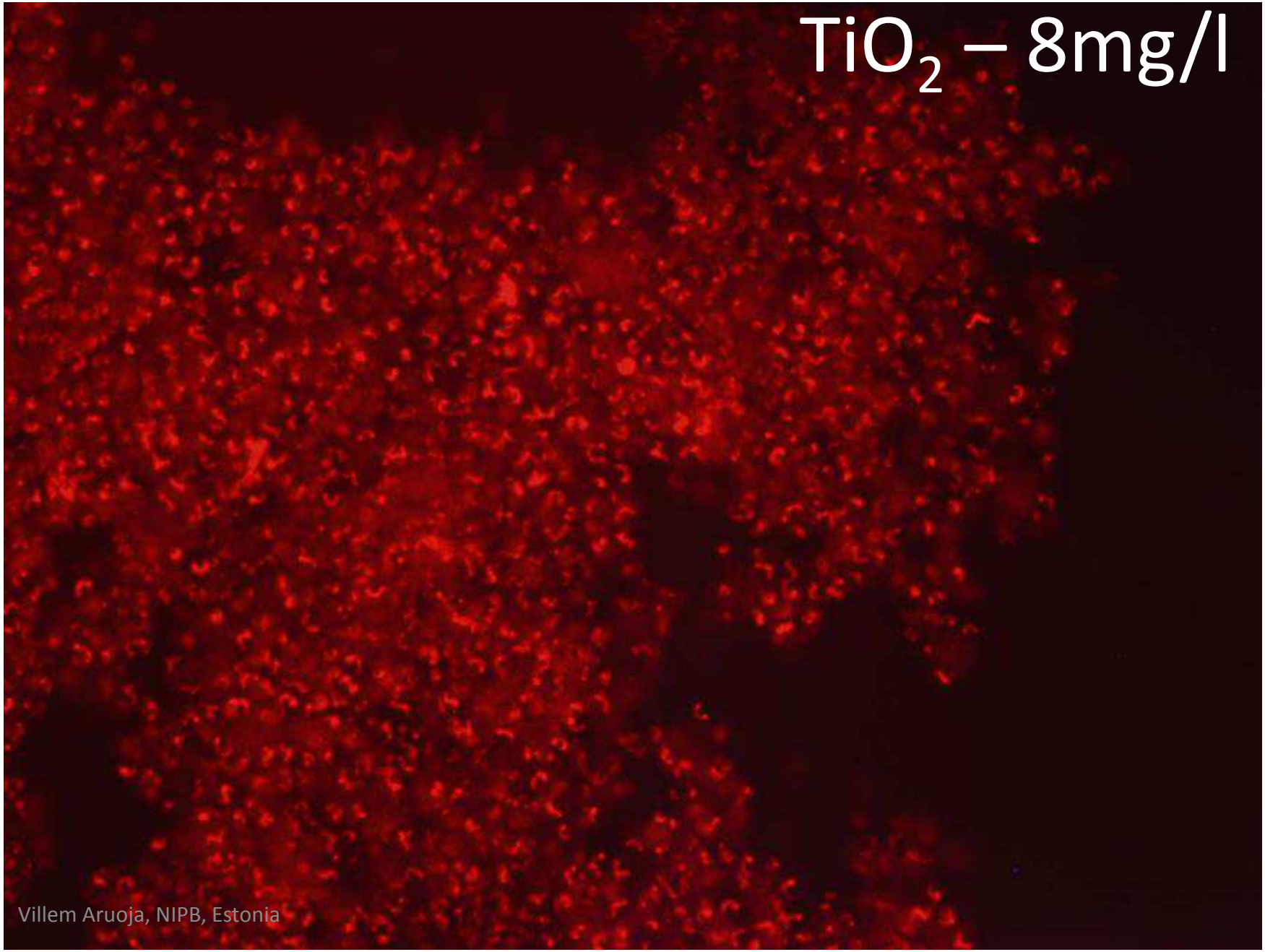
Aruoja et al. Env Sci: Nano, 2015, DOI: 10.1039/C5EN00057B

TiO_2 – 8mg/l

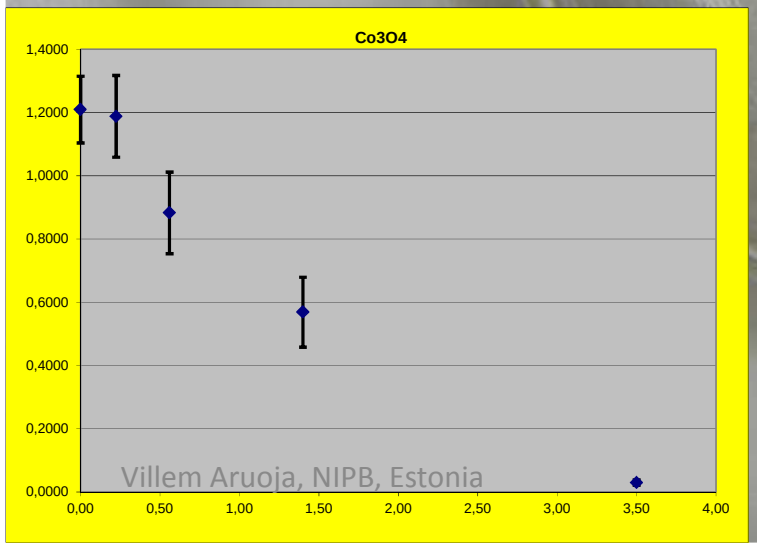


Villem Aruoja, NIPB, Estonia

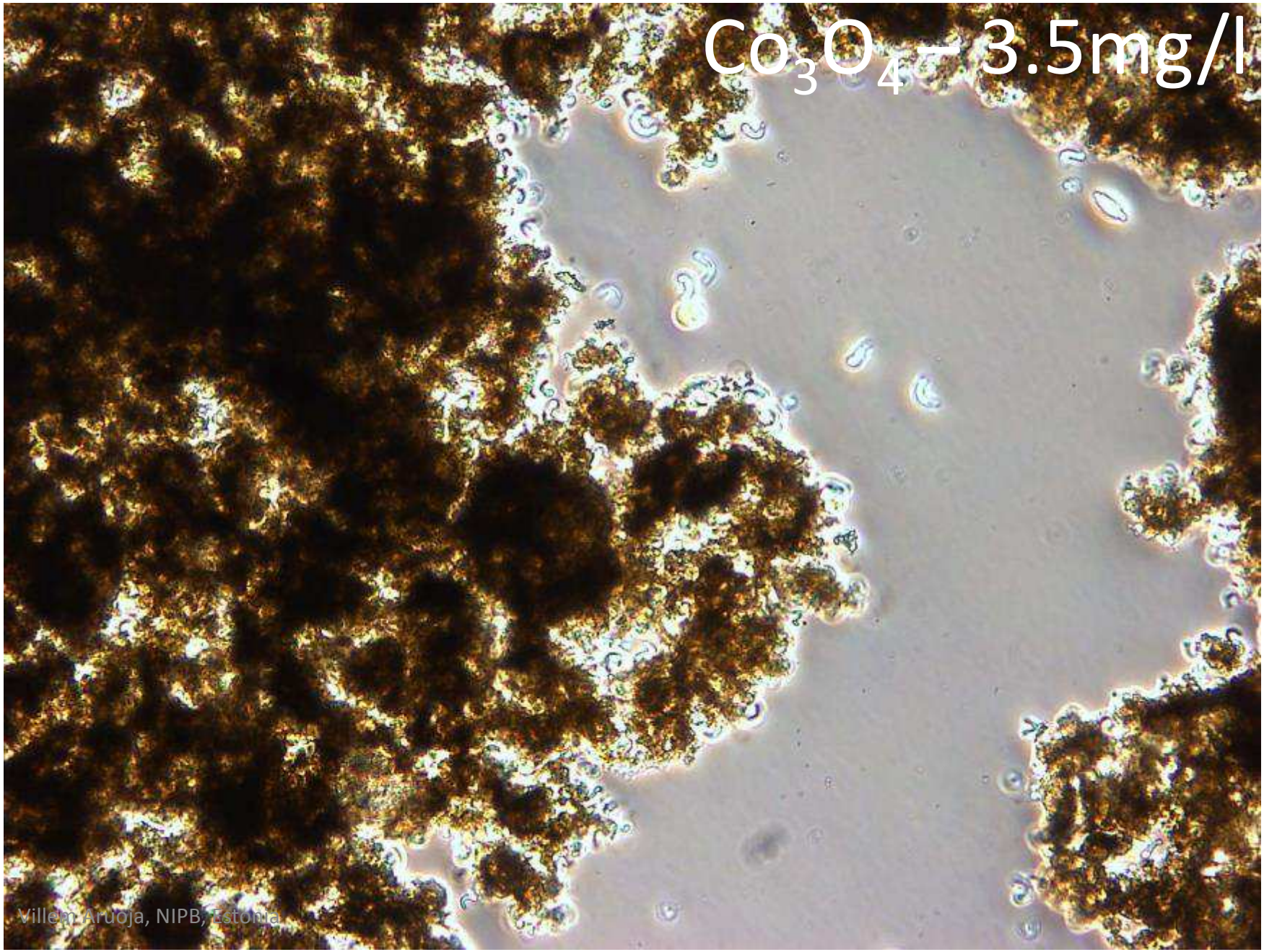

TiO_2 – 8mg/l

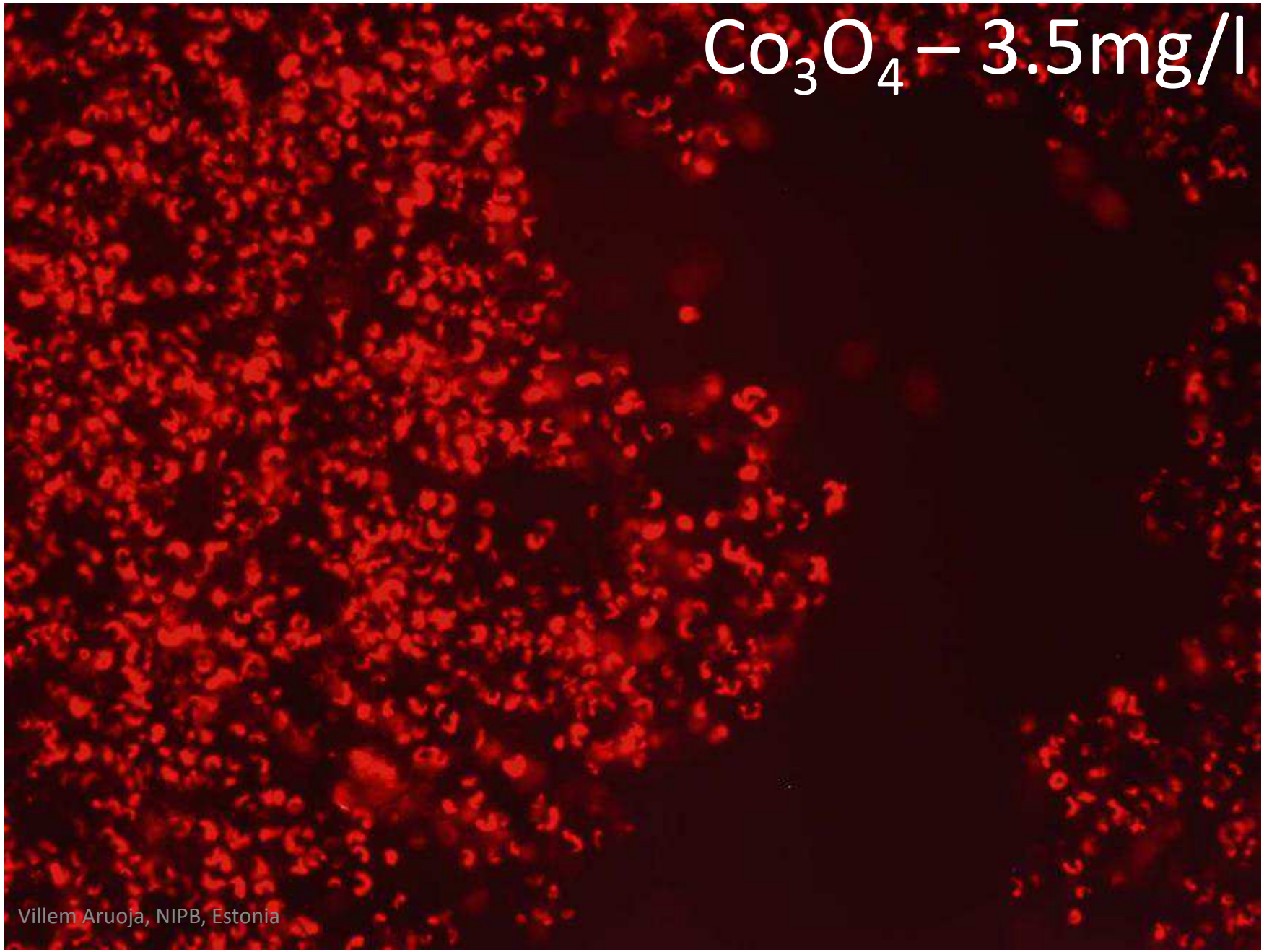

TiO_2 – 8mg/l
Villem Aruoja, NIPB, Estonia

Co_3O_4 – 3.5mg/l

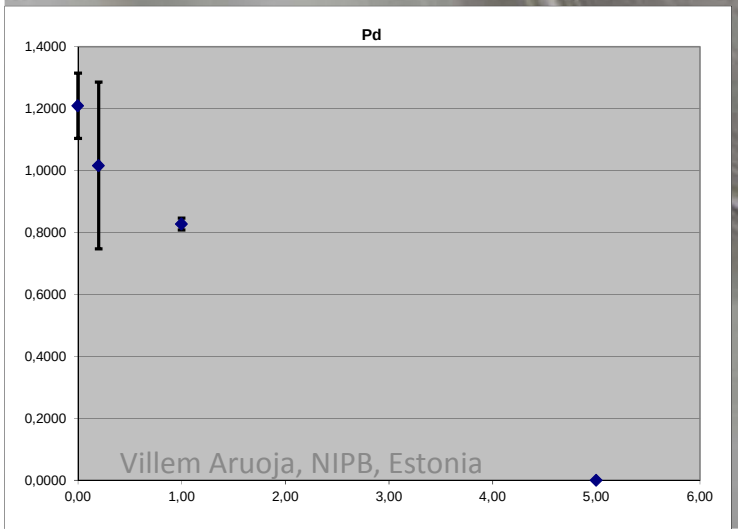


Villem Aruoja, NIPB, Estonia

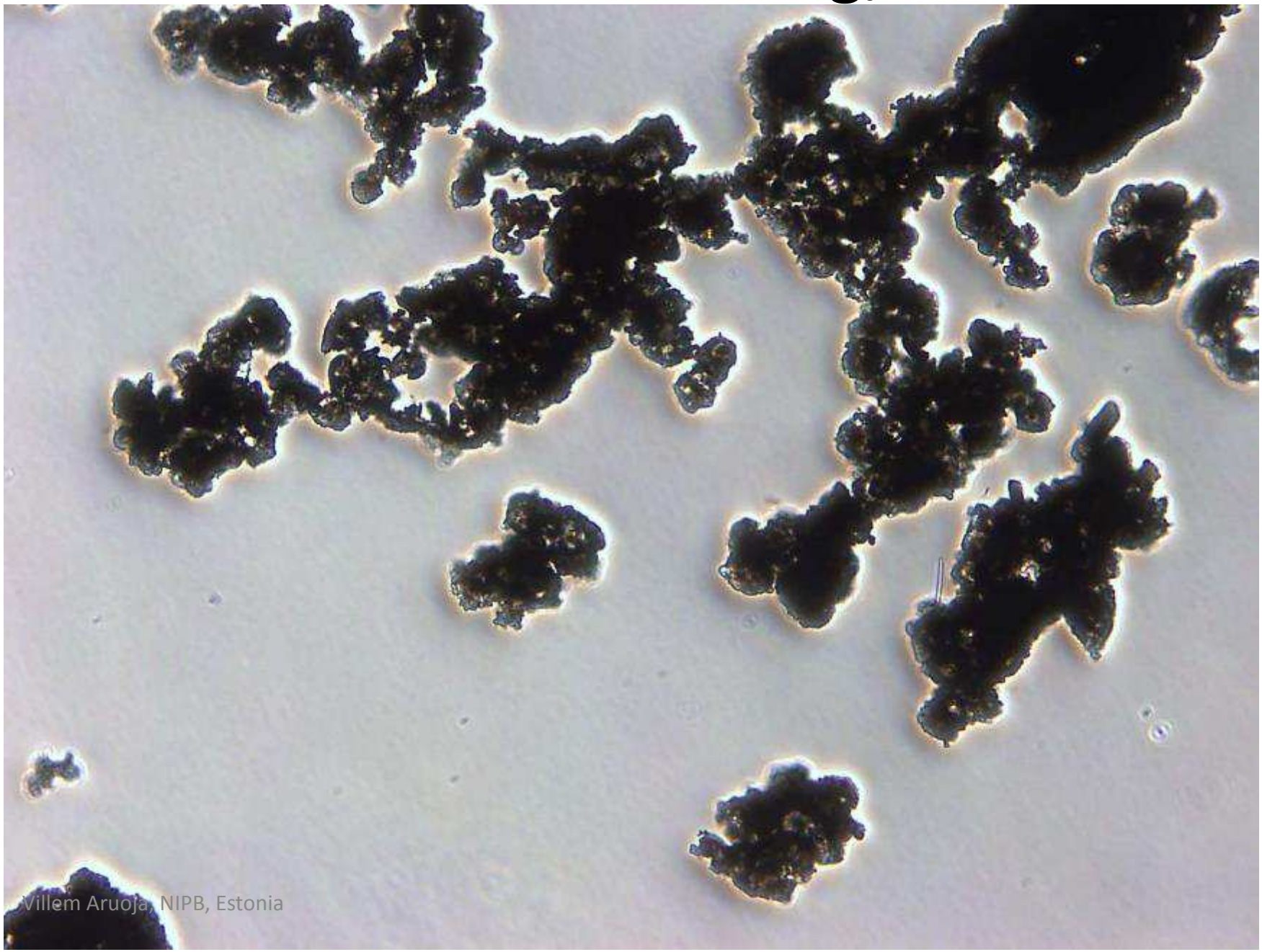

Co_3O_4 – 3.5mg/l
Villem Aruoja, NIPB, Estonia


Co_3O_4 – 3.5mg/l
Villem Aruoja, NIPB, Estonia

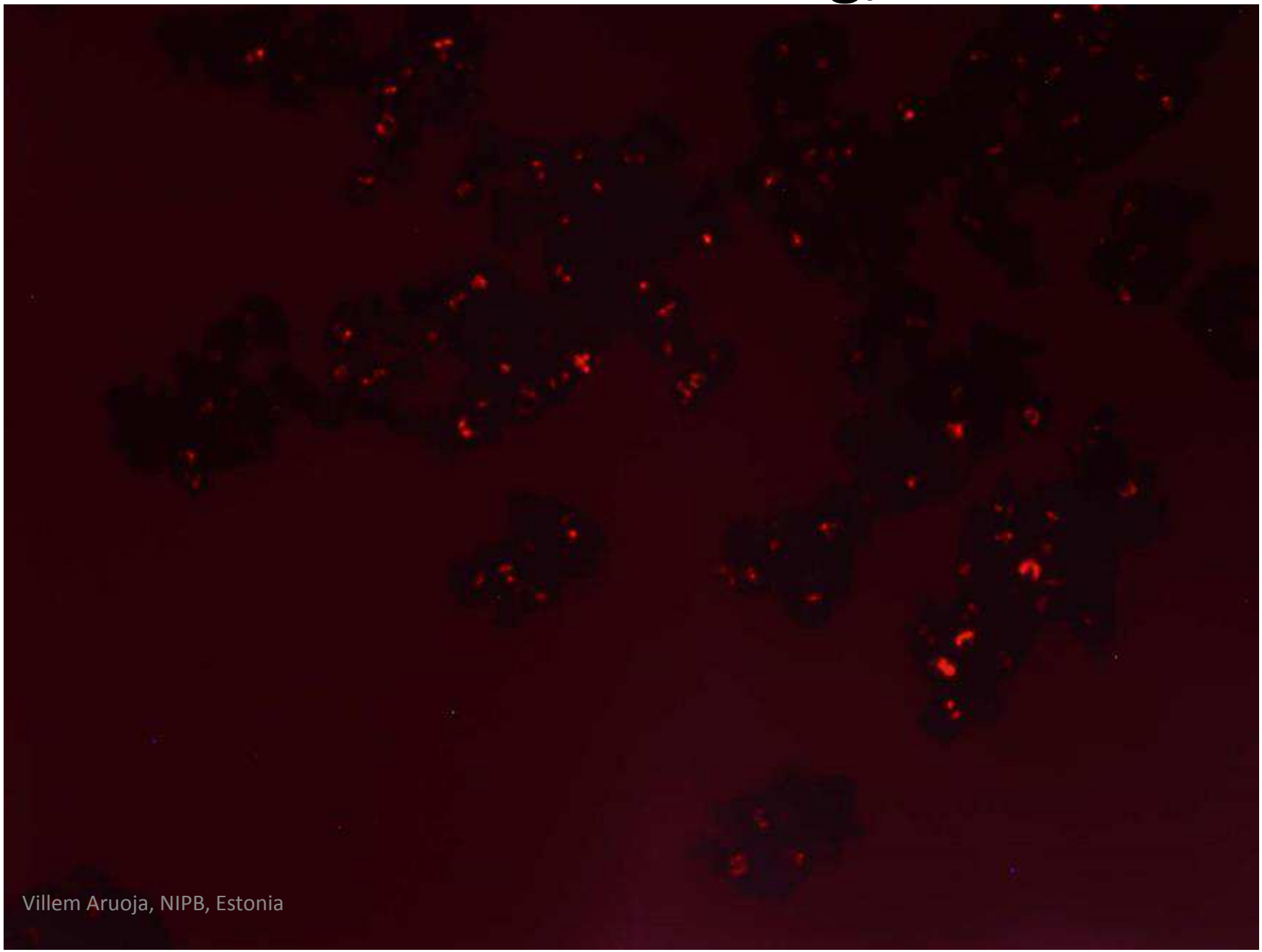
Palladium – 5mg/l



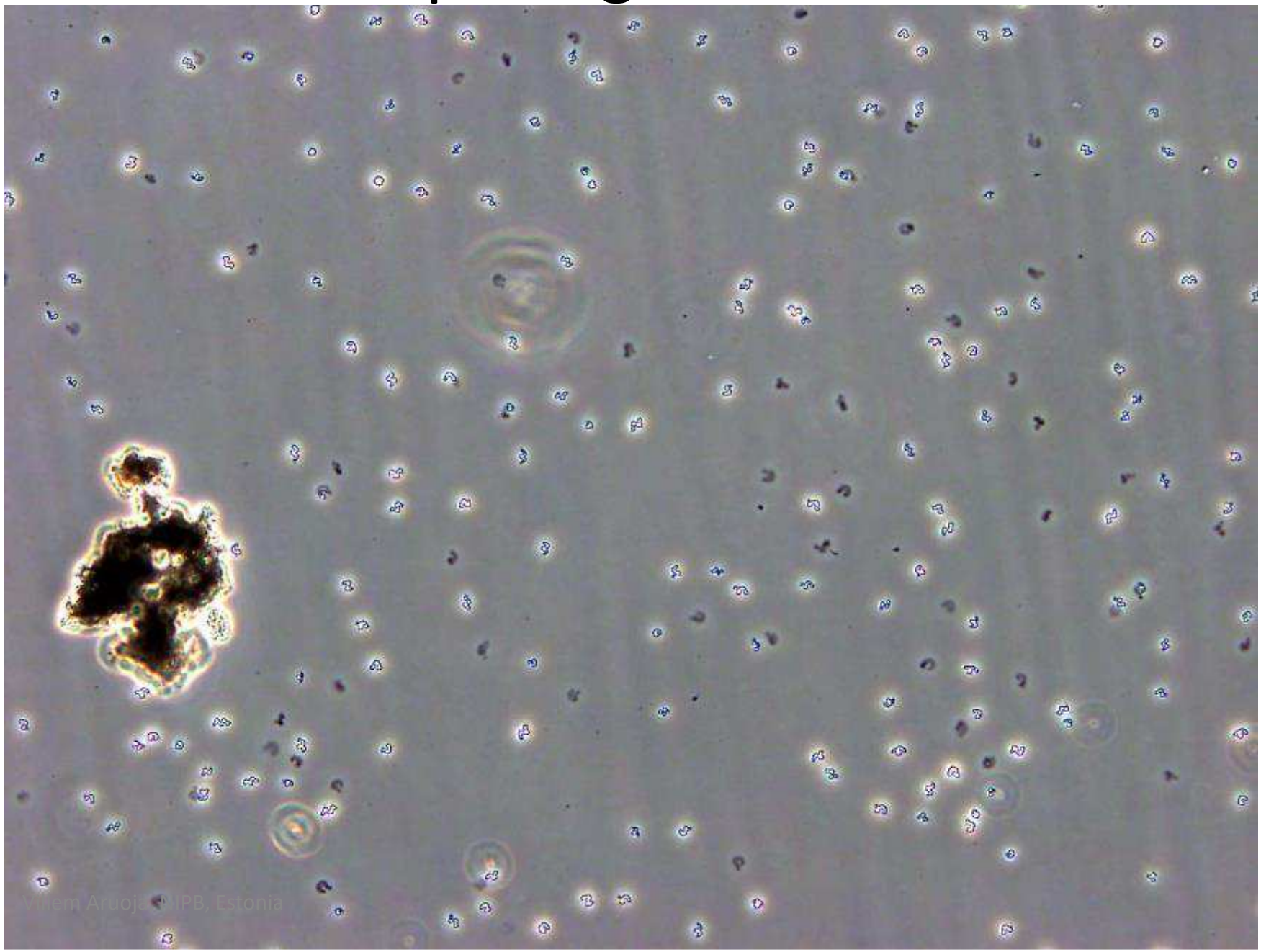
Palladium – 5mg/l



Palladium – 5mg/l



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} ~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 ~107 CFU/mL (OD_{600}=0.1).	100 µL of cells suspension and 100 µL 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 µL 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.

Colonies from Petri dish and overnight growing at 30°C in LB media

Further cultivation in 100 mL flask until mid-exponential growth phase

Centrifugation and washing with MQ water

Resuspension in MQ water to OD_{600}=0,1 (~10^7 CFU/mL)

Cells + MOx NPs in MQ (100µl+100µl)

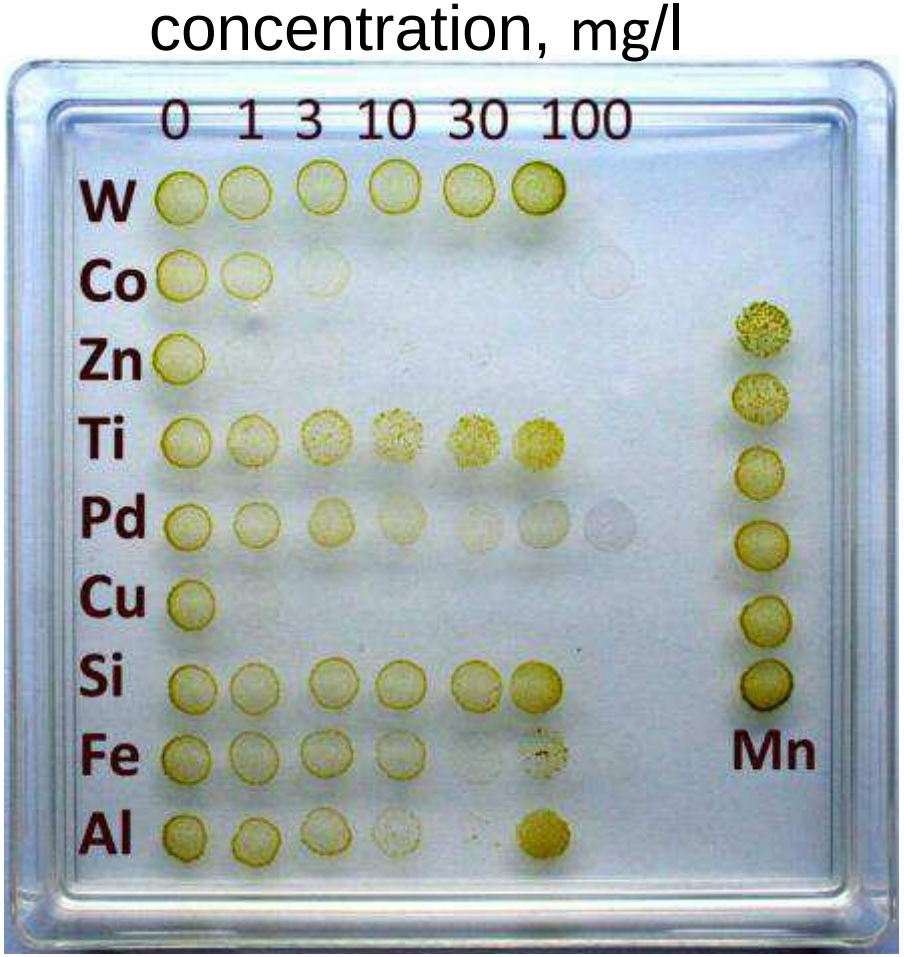
Exposure for 4-48 hours at 30 °C in the dark

5µL spots to the agar medium plates 4h-48h

Suppi et al., J Haz Mat **2015; 286: 75-84**

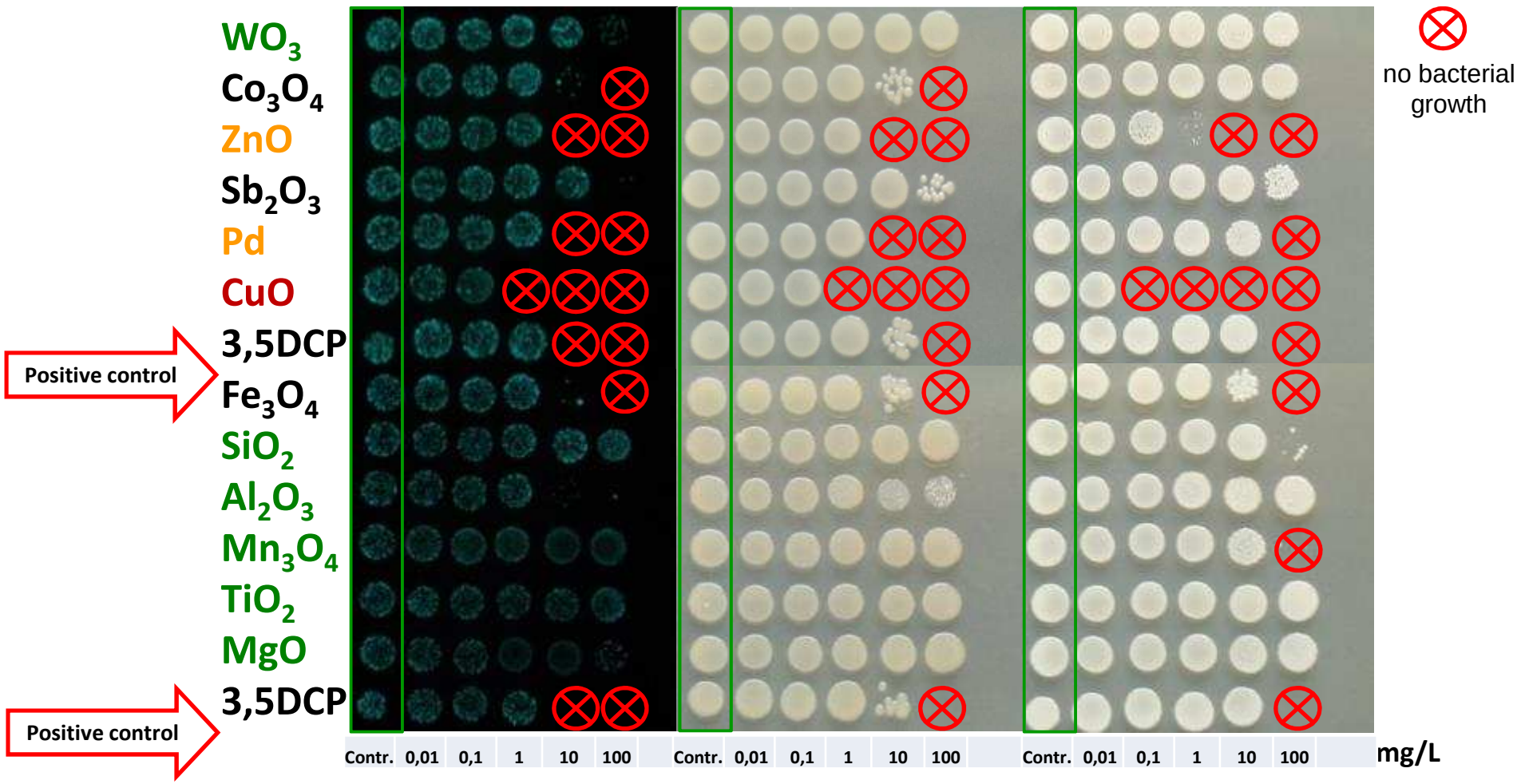
„Spot test“

Algal cells after 24h incubation with NPs in deionized water



Suppi et al., J Haz Mat **2015; 286: 75-84**

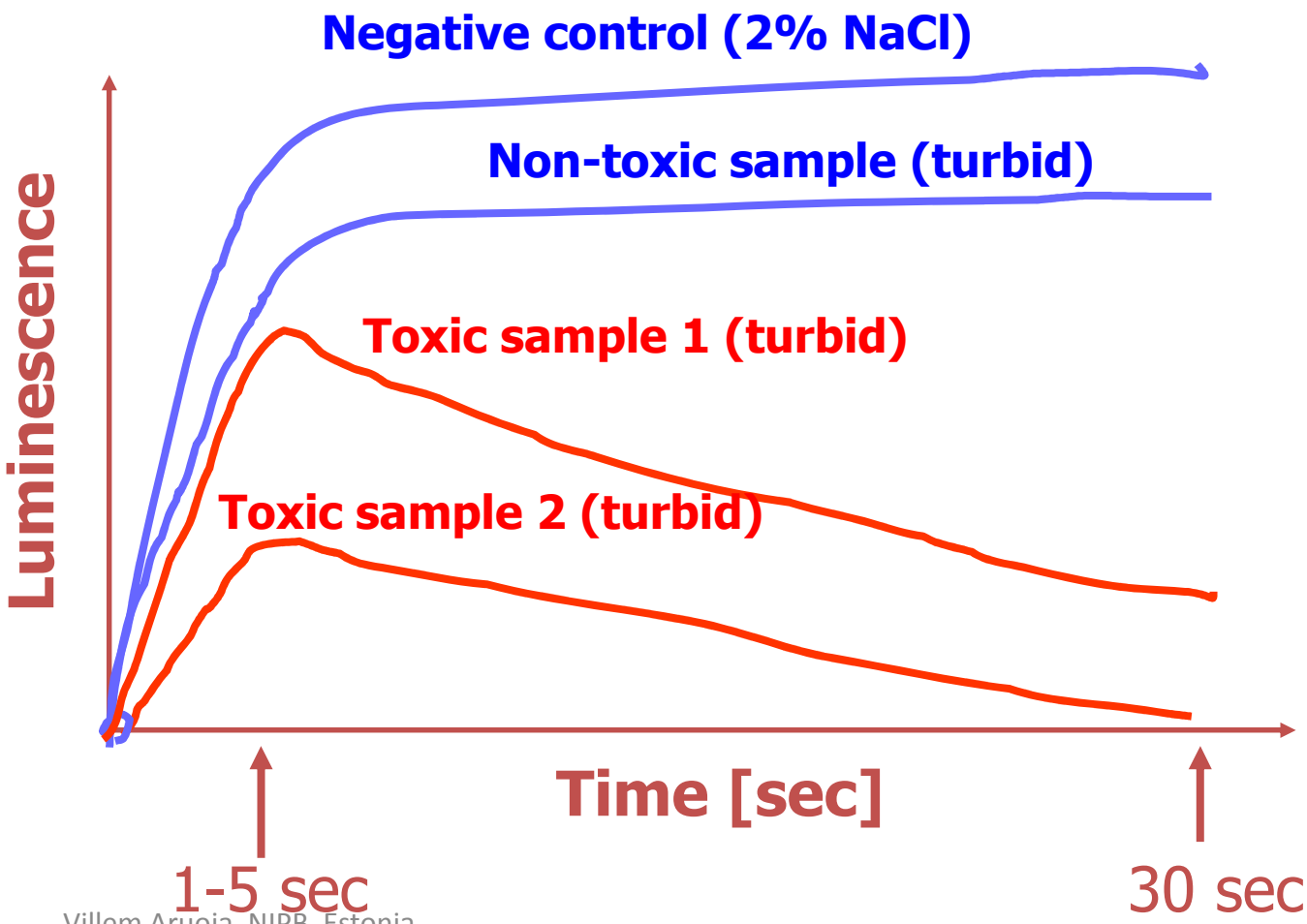
Results from 24-h 'spot'-test with bacteria

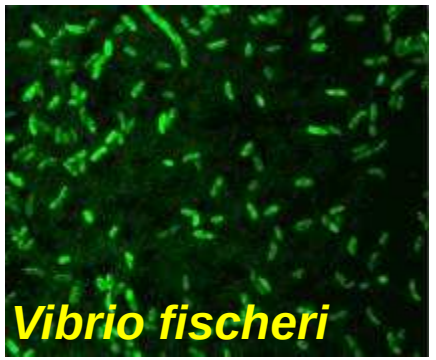


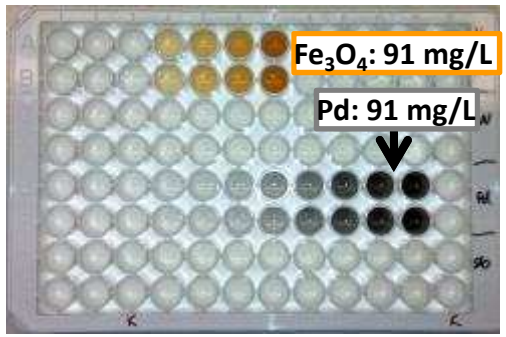
→ **Co_3O_4, CuO, ZnO, Fe_3O_4 and Mn_3O_4** inhibited bacterial colony forming ability in the 'spot'-test at concentrations ≤ 100 mg/L.

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

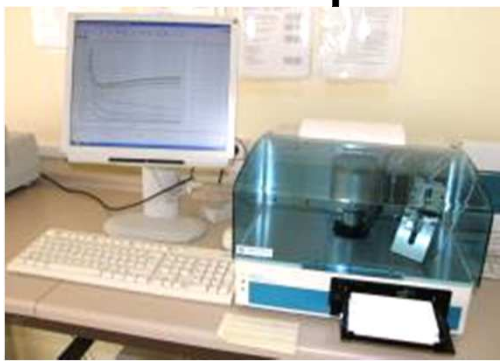
Kinetic bioluminescence inhibition test with *Vibrio fischeri* (ISO 21338:2010)







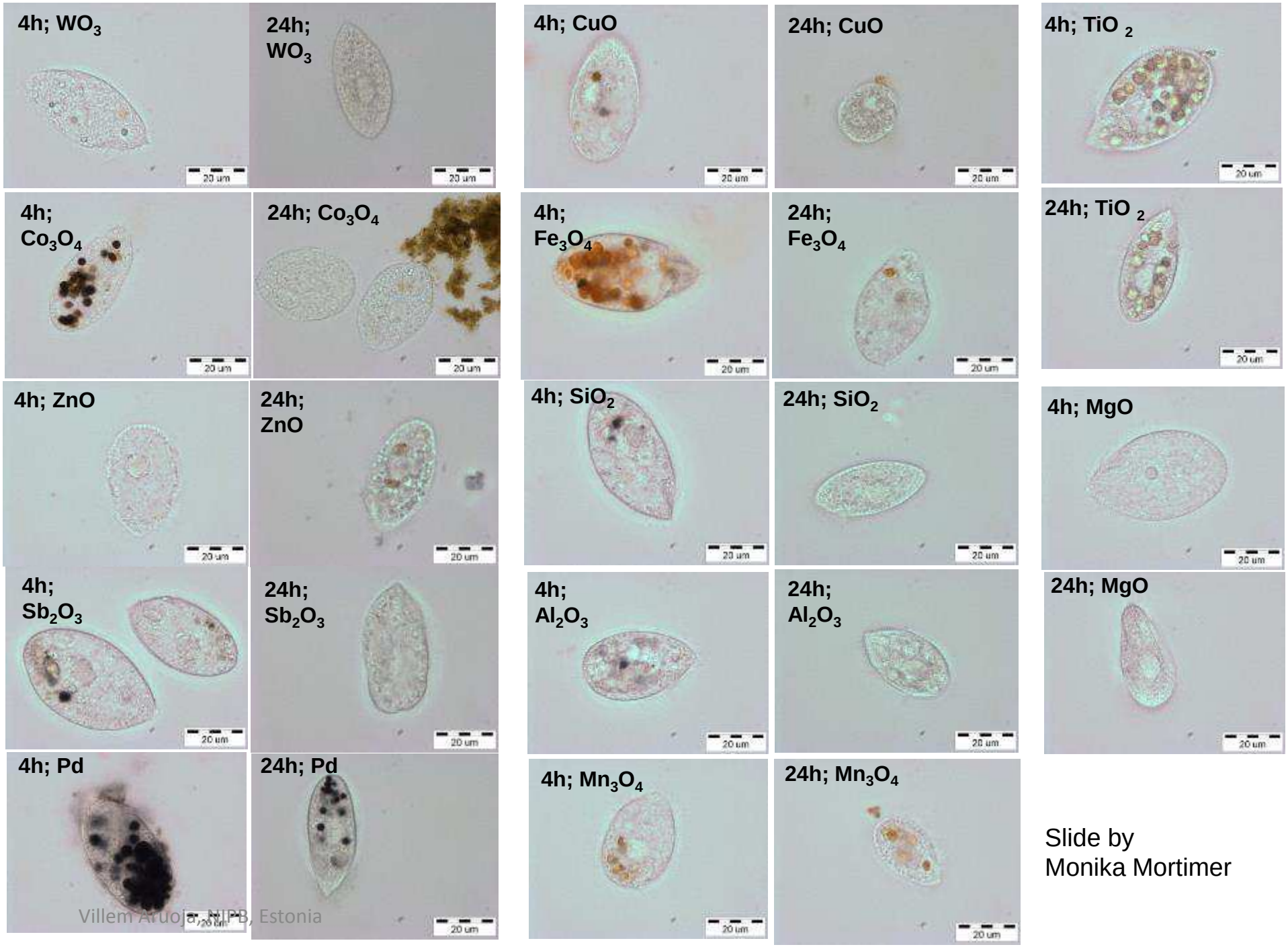
96-well microplate



luminometer

Villem Aruoja, NIPB, Estonia

Slide by Anne Kahru



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] *T. termophila*	30-min EC50[1] *V. fischeri* (gram –)	24-h MBC[2] *E. coli* pSLux (gram –)	24-h MBC[2] *E. coli* MG1655 (gram –)	24-h MBC[2] *S. aureus* (gram +)
	mg/L (compound)				
CuO	2.0	1.8	1	1	0.1
ZnO	1.8	11.6	10	10	10
Fe_3O_4	20.1	>100	100	100	100
Mn_3O_4	22.0	>100	>100	>100	100
Al_2O_3	61.3	>100	100	>100	>100
TiO_2	69.1	>100	>100	>100	>100
Pd	>100	56.3	10	10	100
Co_3O_4	>100	>100	100	100	>100
Sb_2O_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 ( ).

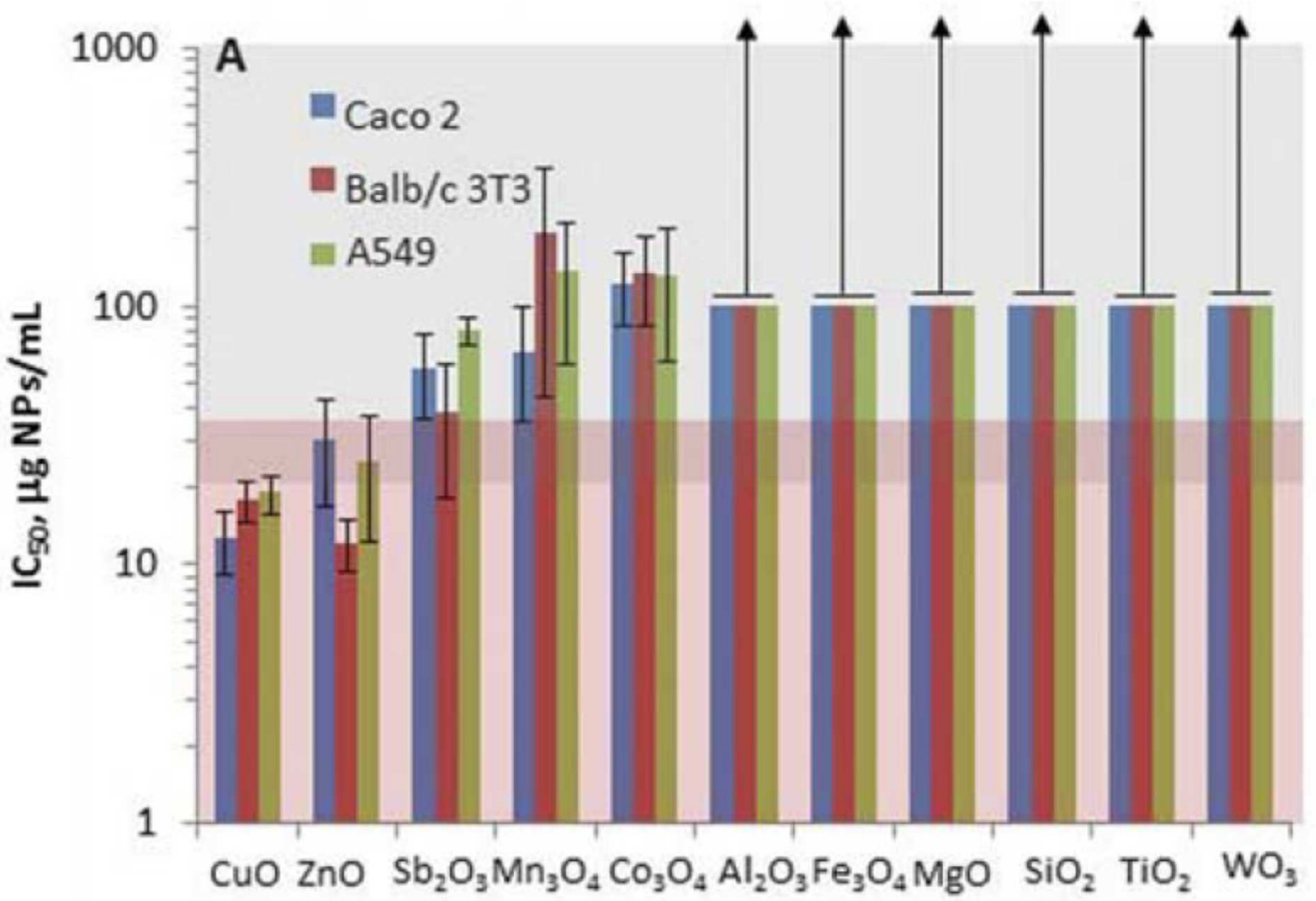
Aruoja et al. Env Sci: Nano, 2015, DOI: 10.1039/C5EN00057B

Mechanism: solubility, ROS

Nano-particle[¥]	Solubility (%)[§]	ROS (HPF)[$]	ROS (DCF)[+]	Trophic level: Primary producer; Cell type: Eukaryote; Internalizes nanoparticles: No?; Algal 72 h growth inhibition OECD 201	Consumer; Eukaryote; Yes; Protozoan 24 h viability	Degrader; Prokaryote; No?; Bacterium 30 min. lumi-nescence 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	
Co_3O_4	1.25	+	+	1 …2	>100	>100	ROS	
TiO_2	<0.83	+++	+	1 …2	10 ... 50	>100	ROS	 Acute aquatic hazard?
Mn_3O_4	11.1	-	+++	1 …2	>100	>100	ROS	
Fe_3O_4	<1.38	+*	-	1 …2	10 ... 50	>100	ROS	
Al_2O_3	0.40	+	-	10 ... 50	>100	>100		
SiO_2	NA	-	-	10 ... 50	>100	>100		
WO_3	63.2	-	-	10 ... 50	>100	50...100		
MgO	38.1	-	-	>100	>100	>100		
Sb_2O_3	56.3	+	-	>100	>100	50...100		

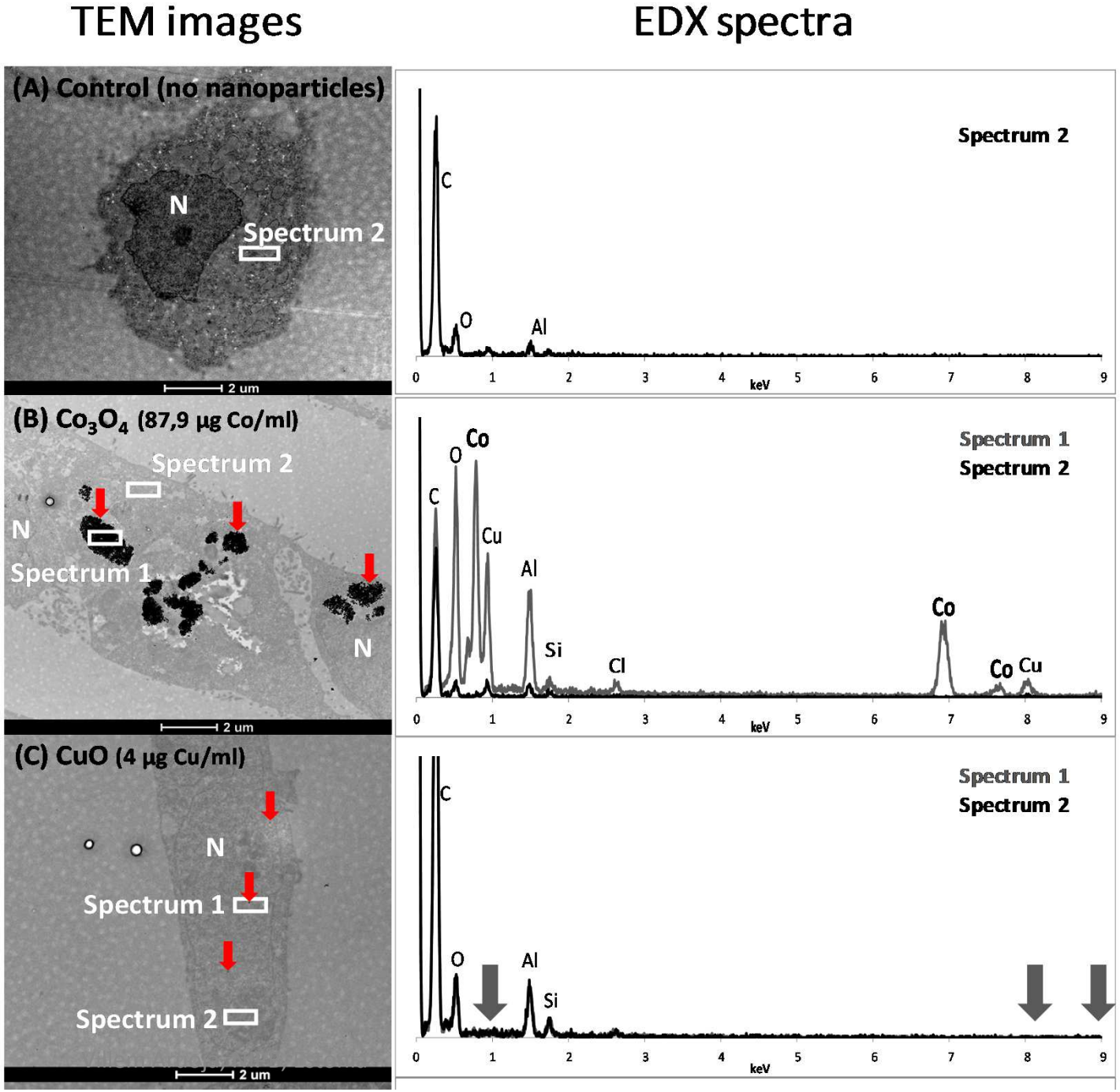
Aruoja et al. Env Sci: Nano, 2015, DOI: 10.1039/C5EN00057B

Cell cultures, EC_{50}



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

Endo-cytosis



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

Send Orders for Print-Reprints and e-prints to reprints@benthamscience.ae

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

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 Vija[1], Aleksandr Käkinen[1], Mariliis Sihtmäe[1], Suman Pokhrel[5], Lutz Mädler[5], Margit Heinlaan[1], Vambola Kisand[4], Ruth Shimmo[3] and Anne Kahru[1]

Environmental Science Nano

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

CrossMark click for updates

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]

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

Table of Contents

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

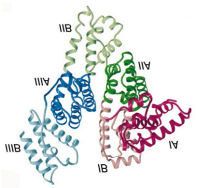
Introduction

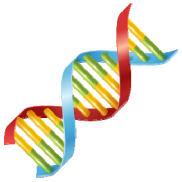
I. Introduction

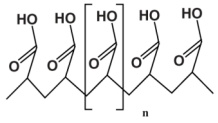
Polyelectrolytes

Charged polymers

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





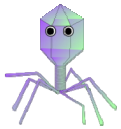


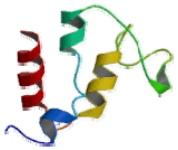
Nanoparticles

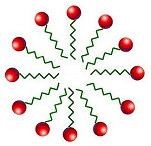
Objects constituted by tens or hundreds of atoms which have structure sizes comprised between 1 to 100 nm in at least one dimension

- Spherical, tubes, needle-like, etc.

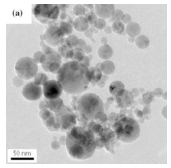
Organic nanoparticles

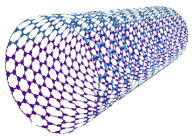






Inorganic nanoparticles





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

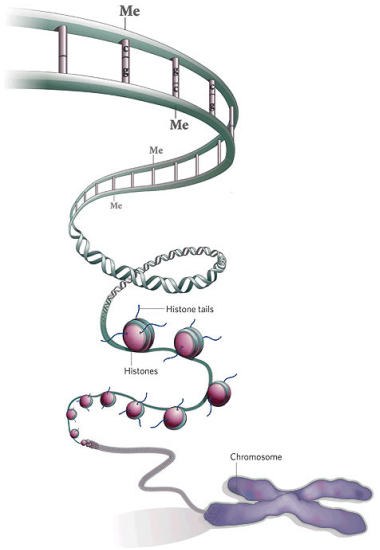
Biomedical

Contrast improvement in Magnetic Resonance Imaging

Cancer therapy by accumulation of active drug

Environment

Wastewater treatment

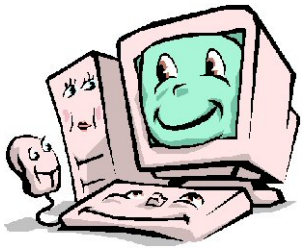


I. Introduction

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

II

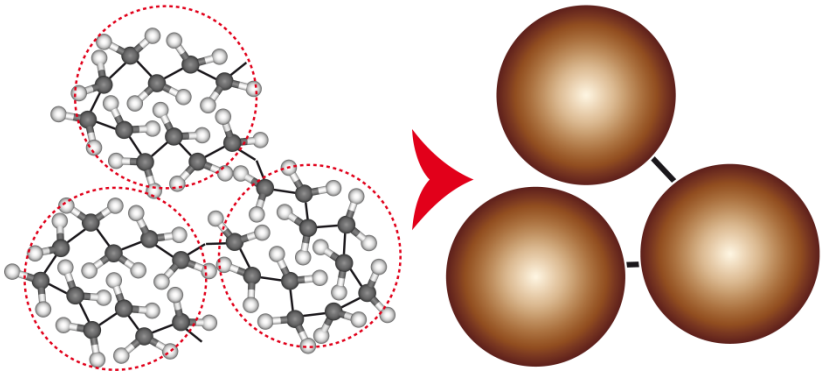
Simulation method and model

II. Simulation method and model

Polyelectrolytes

- Several thousands of atoms !
- CPU consuming

Coarse-grained models



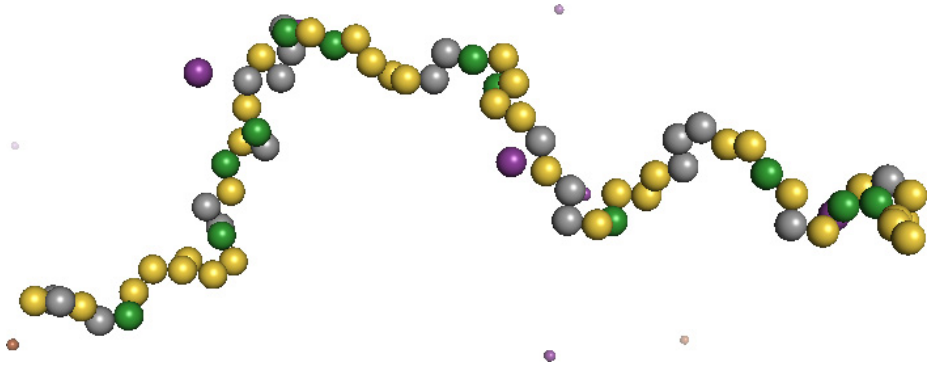
Atomistic details (bond length, vibrations, etc.) are omited

⇩

Group of atoms → effective monomer

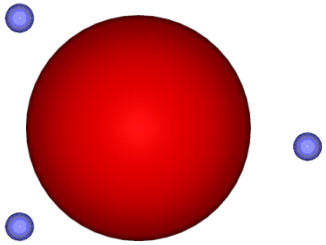
II. Simulation method and model

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

$$U_{el}\left(r_{ij}\right)=\begin{cases}\infty, & r_{ij} < R_i + R_j \\ \dfrac{z_i z_j e^2}{4\pi\varepsilon_r\varepsilon_0}\dfrac{1}{r_{ij}}, & r_{ij} \geq R_i + R_j\end{cases}$$

e	Elementary charge	r_{ij}	Distance between two particles
ε_0	Permittivity of the vacuum	R	Particle radius
ε_r	Water dielectric constant	z	Particle charge

2) Lennard-Jones

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

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

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

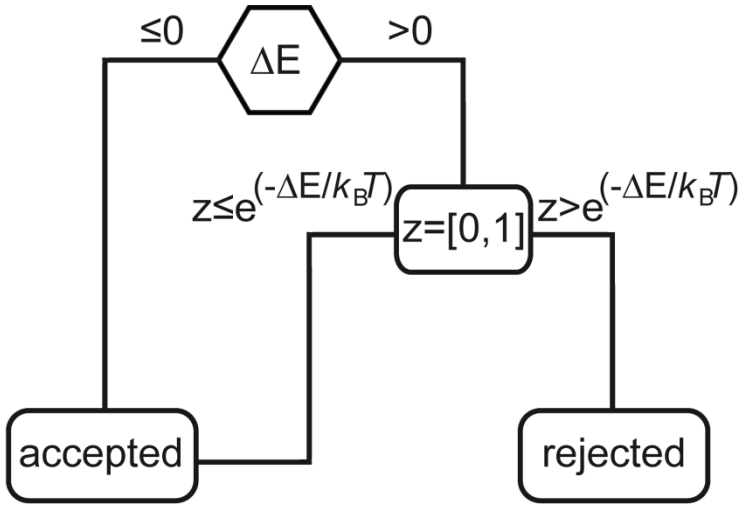
Minimum energy investigation

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

⇩

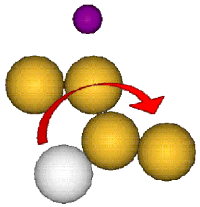
Recording of observables (macroscopic properties) → **average values**

II. Simulation method and model

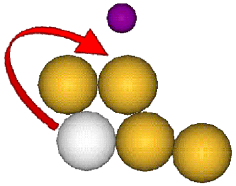
Elementary movements

Biomacromolecules

Internal movements

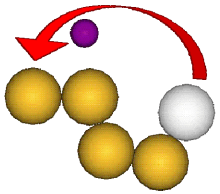


End-bond

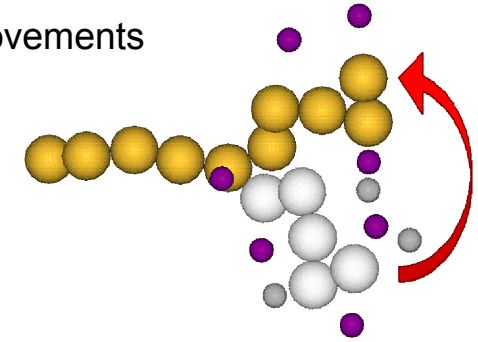


Kink-jump

Global movements

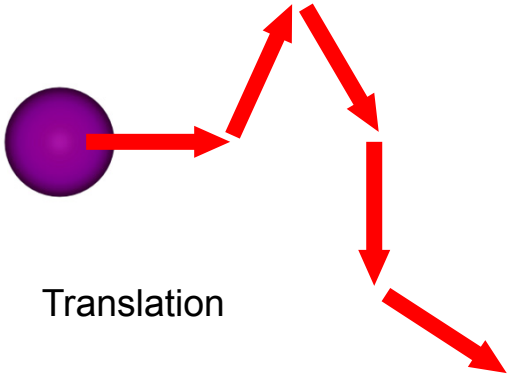


Reptation



'Partially clothed' Pivot

Counterions



Translation

II. Simulation method and model

pH dependency of biomacromolecules

⇩

Protonation/deprotonation cycle

10'000 MC steps

Chain relaxation

Elementary movements

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

Electrostatic, excluded volume and hydrophobic potential

Protonation/deprotonation process

N/4 monomers

$$\Delta E = \Delta E_{tot} \pm k_B T \ln(10) \times (\text{pH-p}K_0)$$

Chemical potential

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

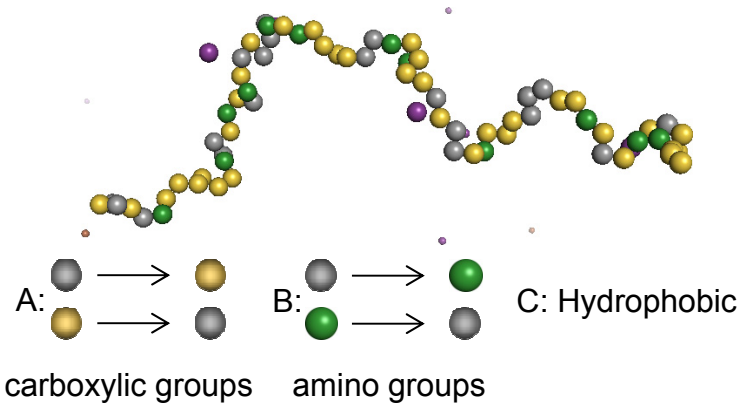
C.E. Reed and W.F. Reed, *Journal of Chemical Physics*, vol. 96, 1992, p. 1609

II. Simulation method and model

2 models

Simplified protein structure

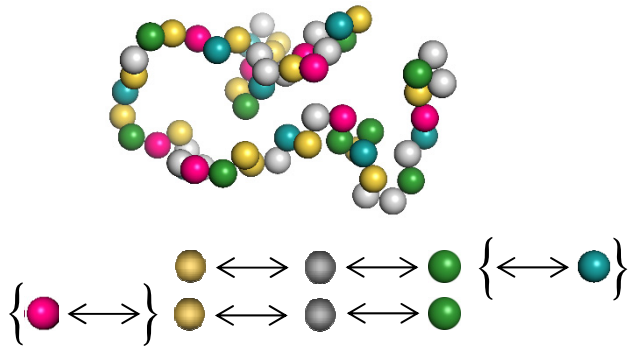
0 Neutral monomers
-1 Negatively charged monomers
1 Positively charged monomers



- 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

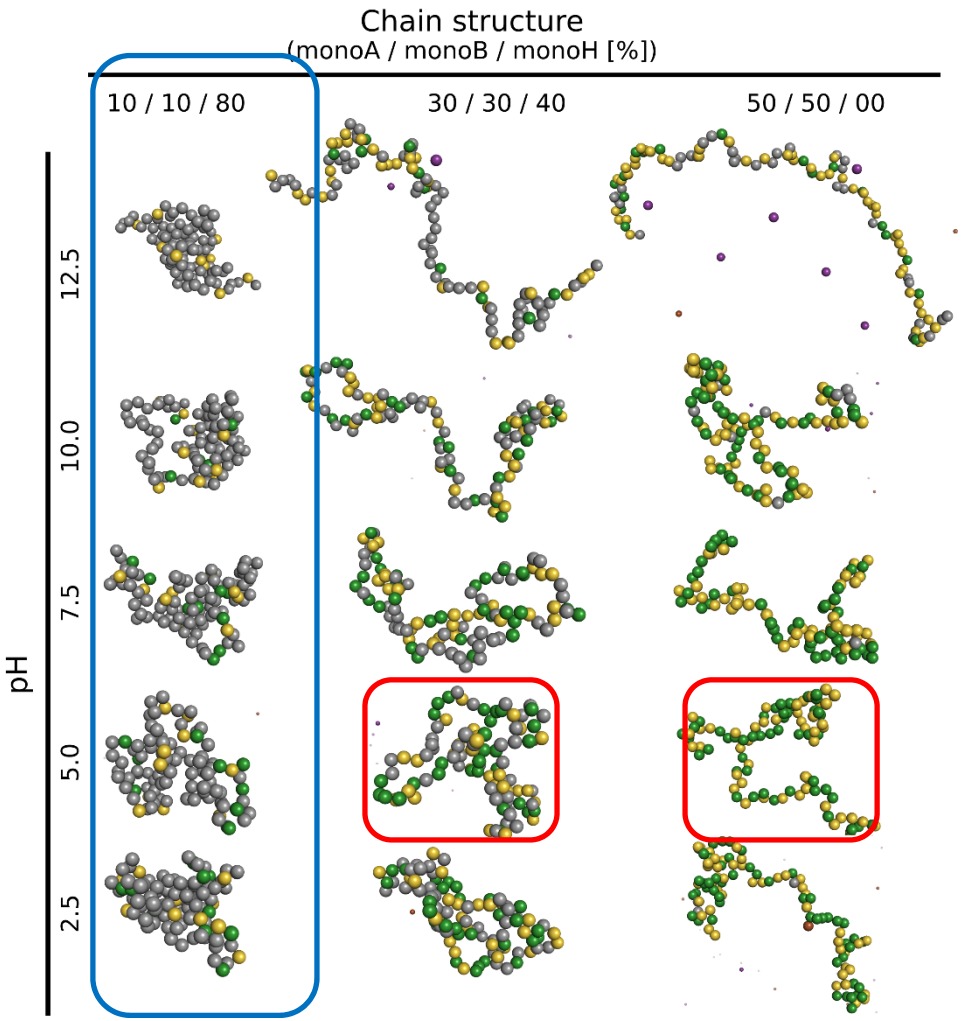
III

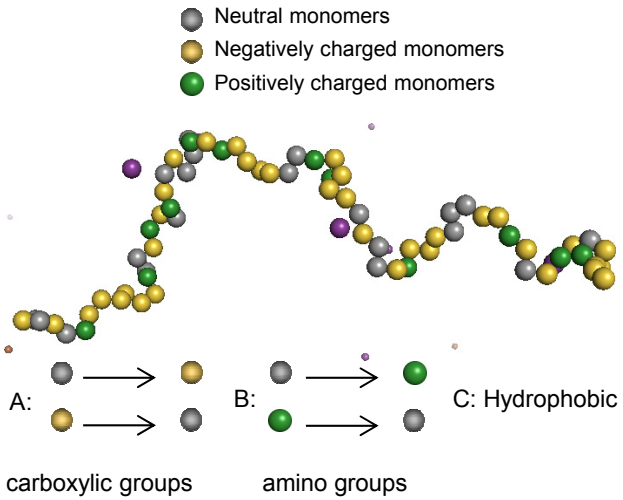
Results

Simplified protein structure

- pH
- Chain hydrophobicity
- Presence of nanoparticle

III. Simplified protein structure





highly hydrophobic chains

⇩

folded conformation within the whole pH range

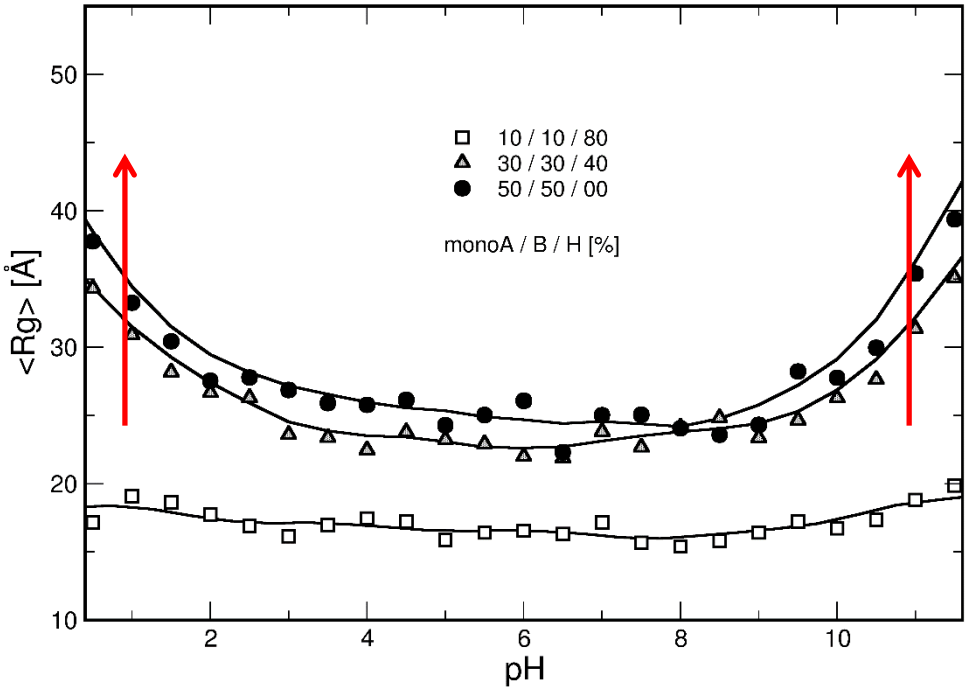
hydrophobic/intermediate chains

⇩

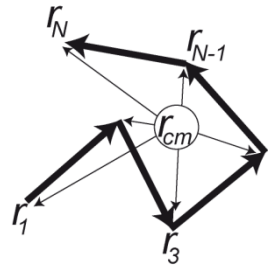
more folded conformations when the global chain charge is neutral

F. Carnal, A. Clavier and S. Stoll, *Environmental Science: Nano*, vol. 2, 2015, p. 327

III. Simplified protein structure



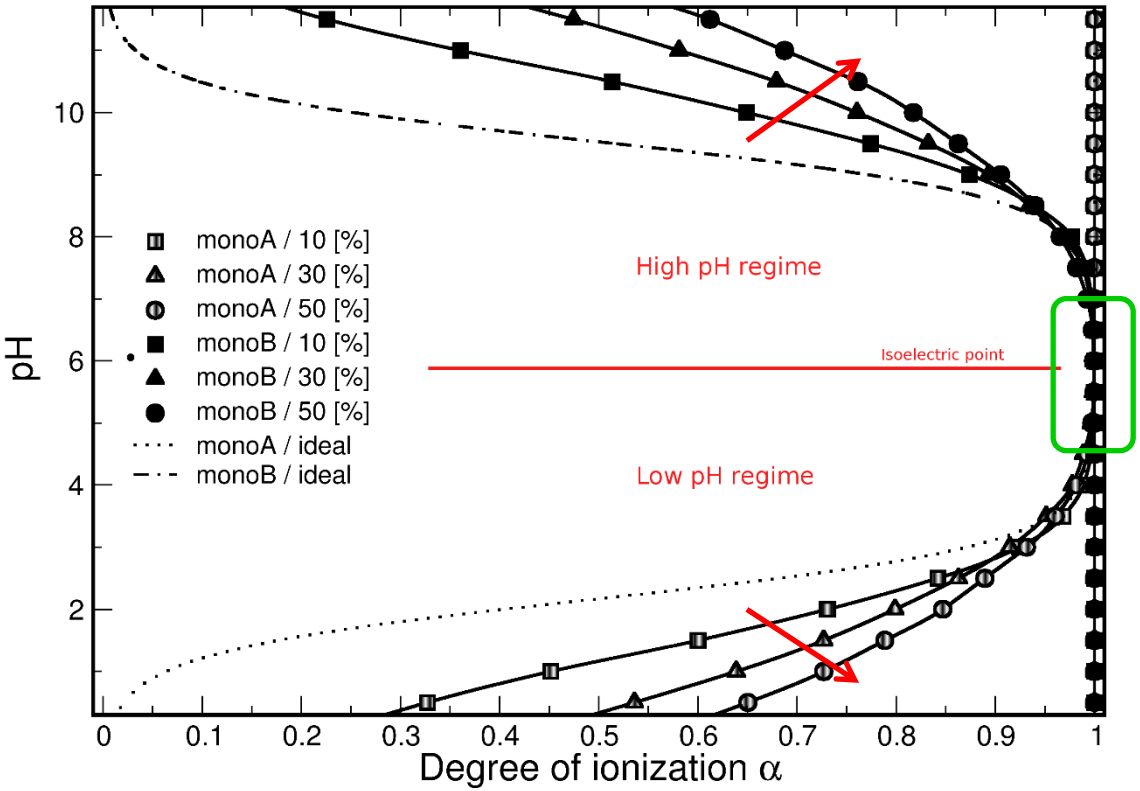
Square radius of gyration



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

- 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



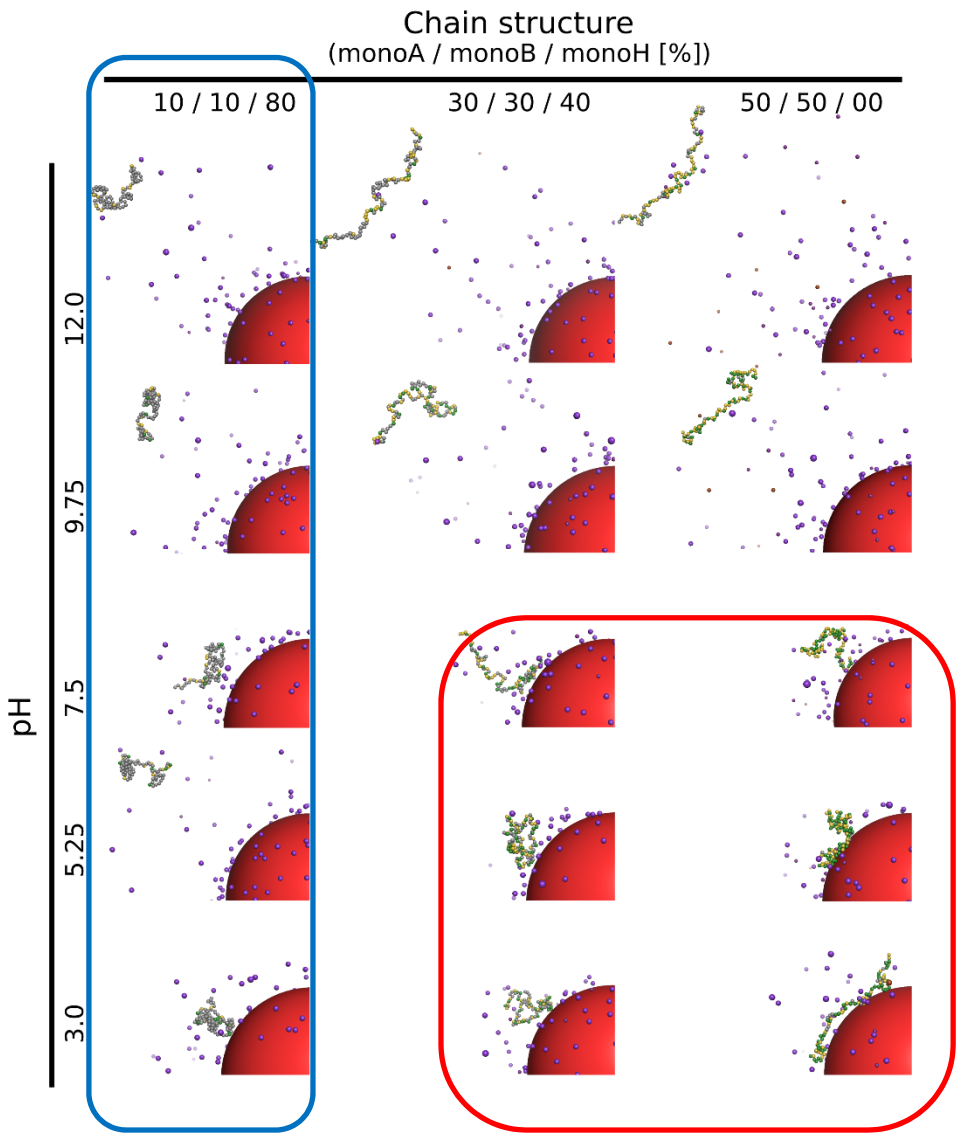
$pK_0^A = 2.17$ (carboxylic groups)

$pK_0^B = 9.53$ (amino groups)

Chain globally neutral!

- 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} = 100Å
σ_{NP} = -39.9 mC/m^2

highly hydrophobic chains

⇩

no elongated conformation
no formation of complexes

intermediate and hydrophilic chains

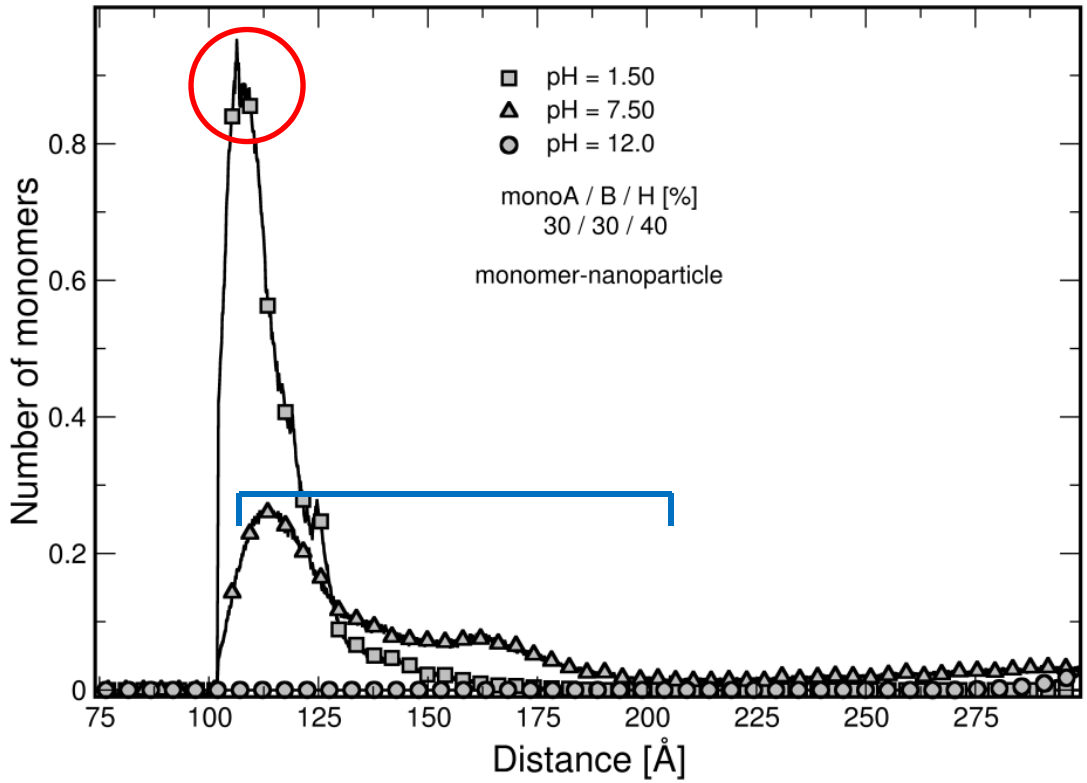
⇩

stronger electrostatic interactions with the NP → adsorption at the surface

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

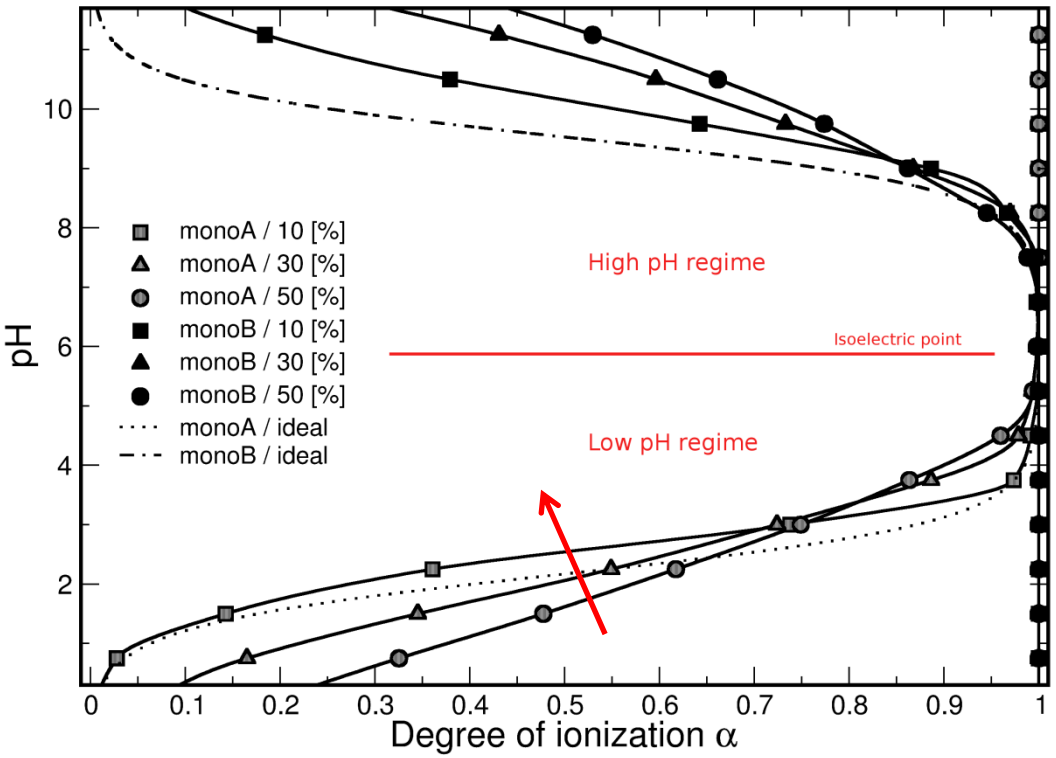
F. Carnal, A. Clavier and S. Stoll, *Environmental Science: Nano*, vol. 2, 2015, p. 327

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

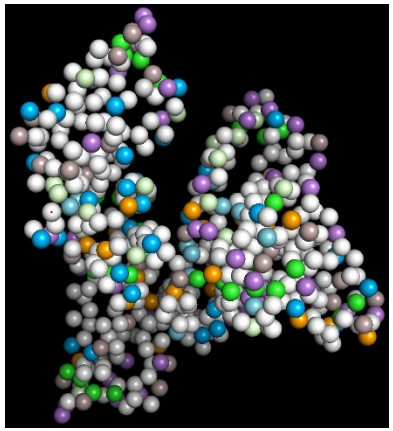
III

Results

BSA protein

- Parametrization
- pH
- Nanoparticle surface charge density

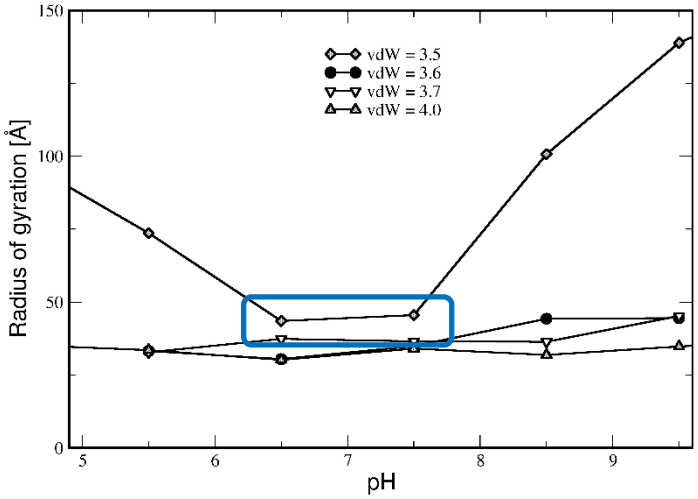
III. BSA protein



BSA x-ray structure

Parameters

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



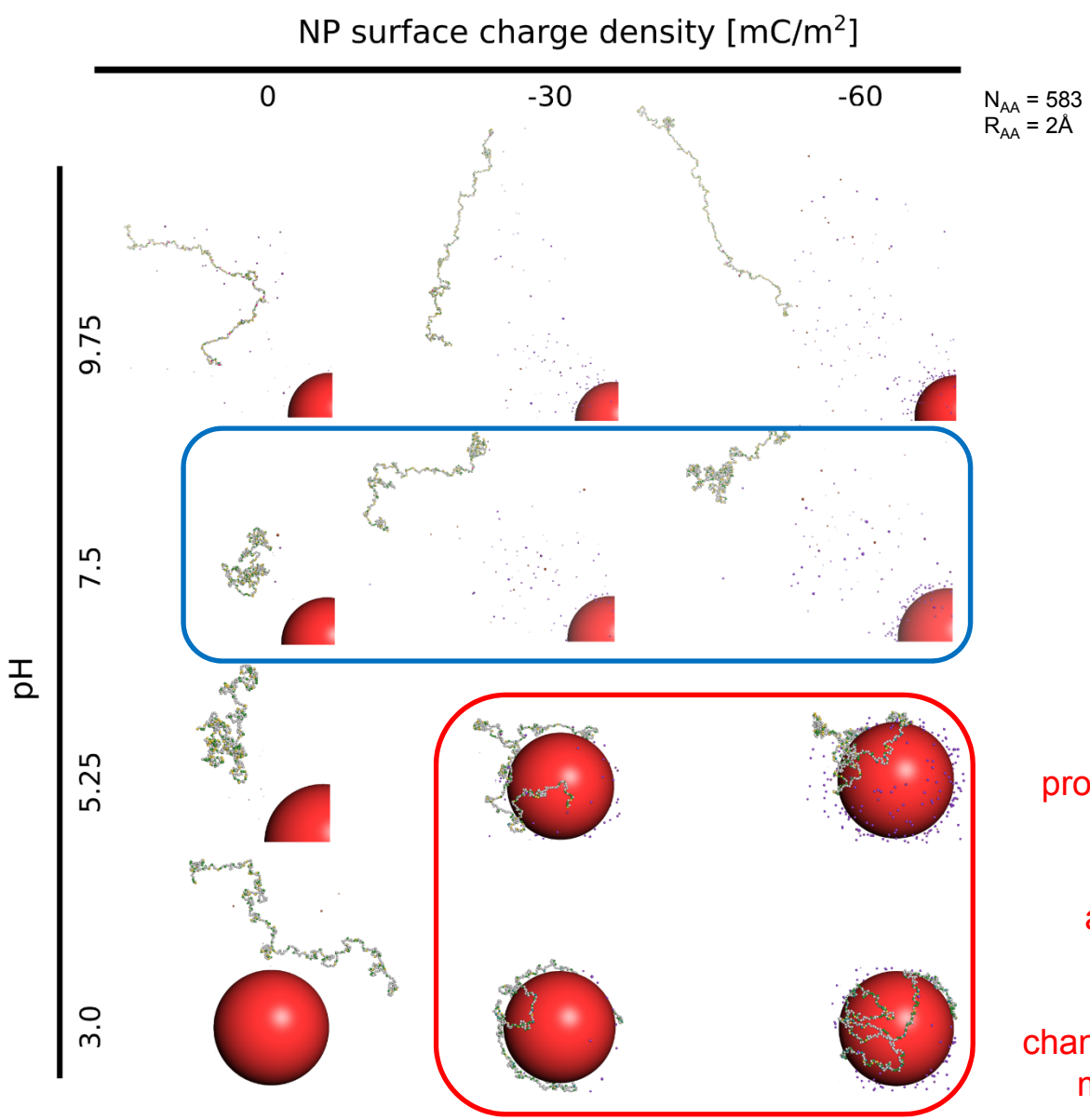
$$U_{\mathrm{vdW}}(r_{ij}) = vdW\left[\left(\frac{r_0}{r_{ij}}\right)^{12} - 2\left(\frac{r_0}{r_{ij}}\right)^{6}\right]$$

- hydrophobic interactions between amino acids not too strong to observe conformational changes (denaturation)

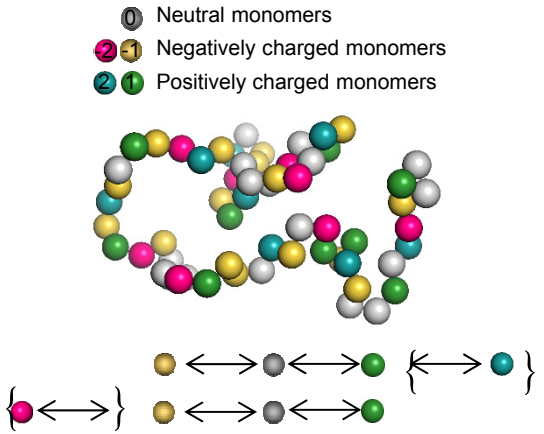
  → vdW = **3.5** $K_B T$

- hydrophobic amino acids: Ala, Met, Leu, Val, Ile, Phe

III. BSA protein



N_{AA} = 583
R_{AA} = 2Å



physiological pH

⇩

more folded conformations and no adsorption with only electrostatic interactions

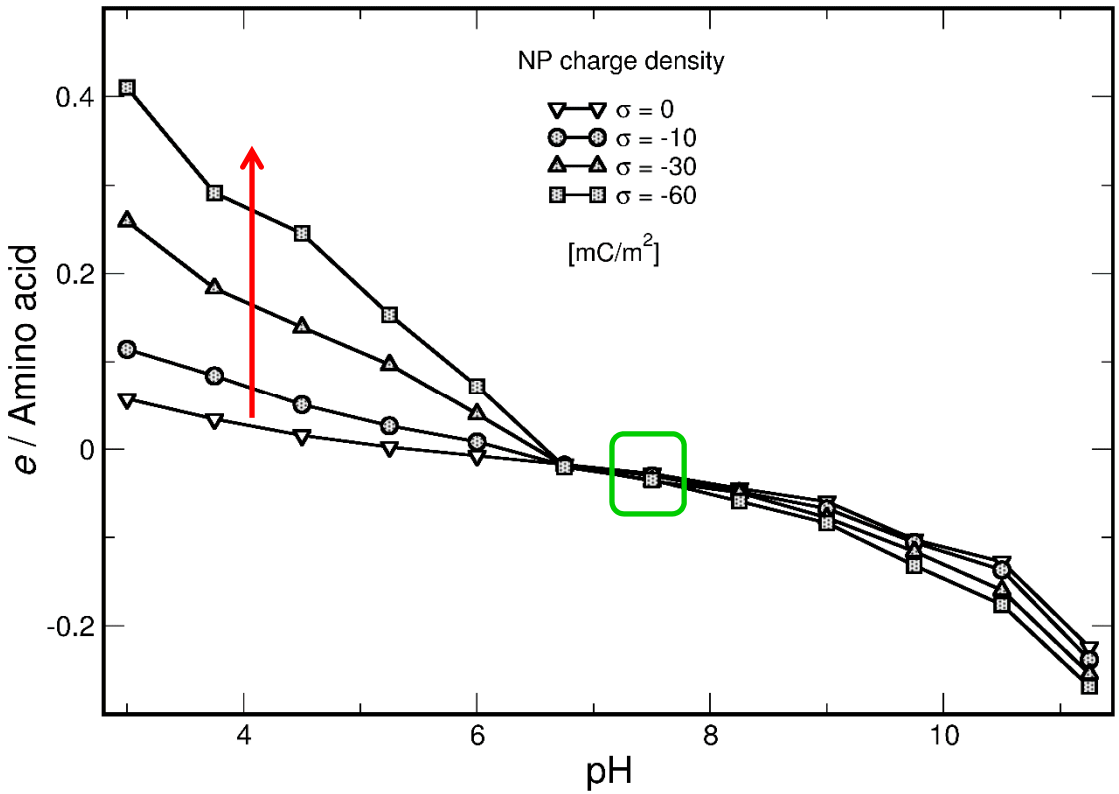
protein positively charged at low pH

⇩

adsorption at the surface of the nanoparticle

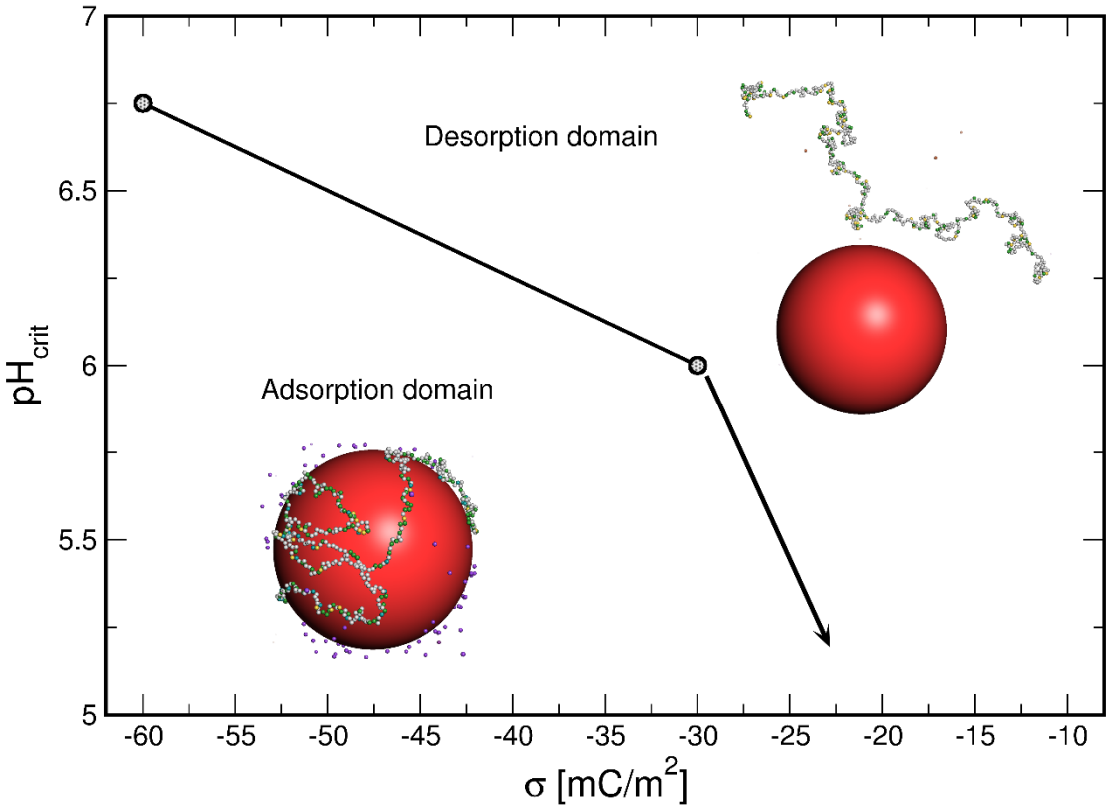
change of the protein conformation and modification of the charge of the nanoparticle (reactivity)

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

for your attention !

Computational Approaches in Nanosciences Workshop

Interaction of carbon-based nanomaterials with cholinesterases and serum proteins

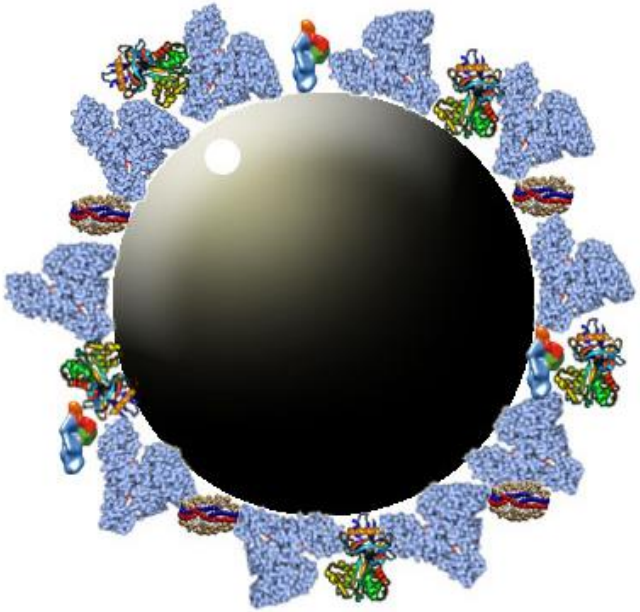
Maja Sopotnik

October 3rd, 2015

Bionanoteam
Nanobiology & Nanotoxicology Research

NM in biological media

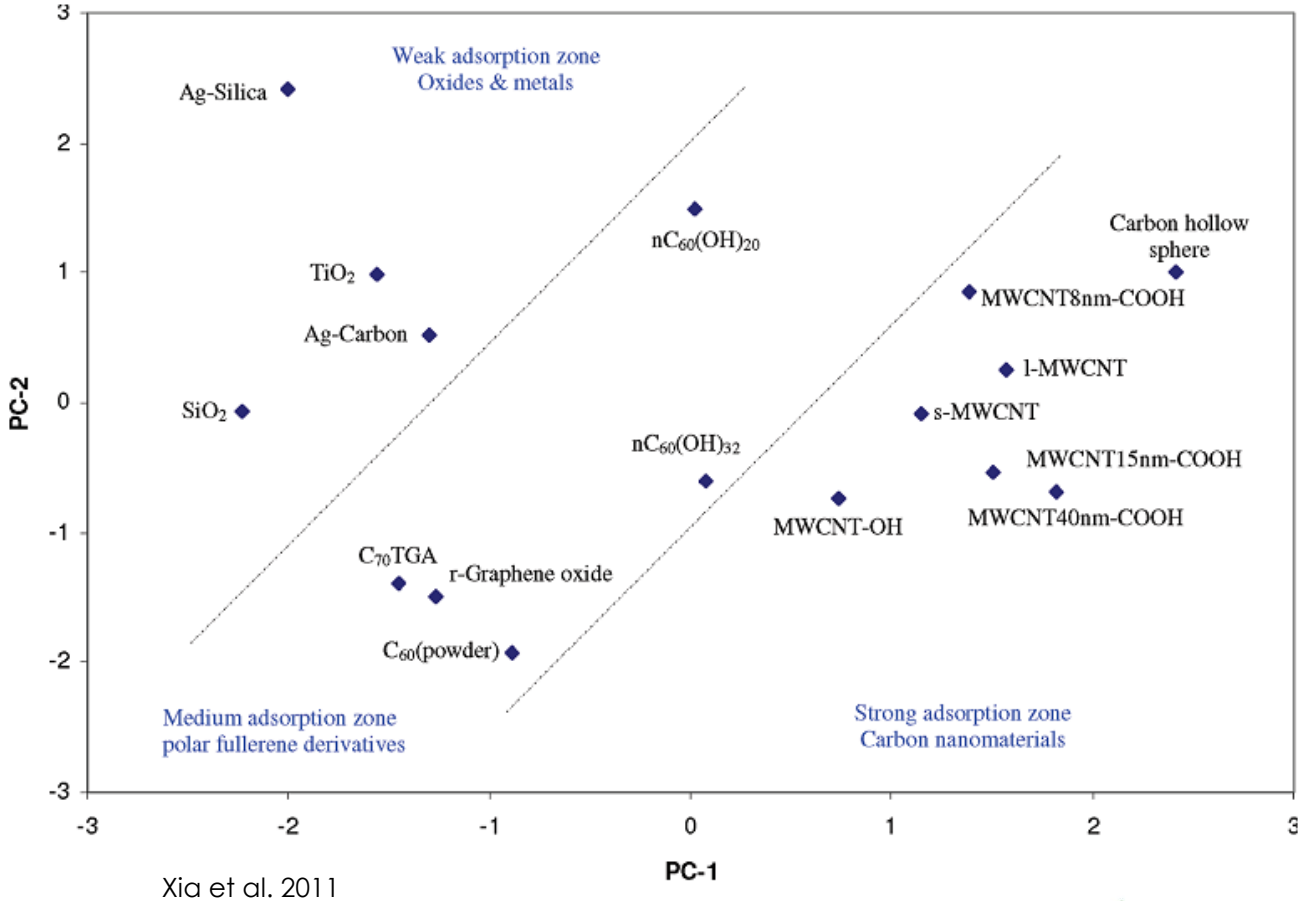
- Adsorption of biological molecules on the surface of the NM
- Protein corona
  - depends on the properites of the NM and the proteins
- Change in NM characteristics and behaviour
- The need for biological characterisation of NM



Bionanoteam
Nanobiology & Nanotoxicology Research

Carbon NM are highly sorptive

- Surface adsorption index, 5 nanodescriptors



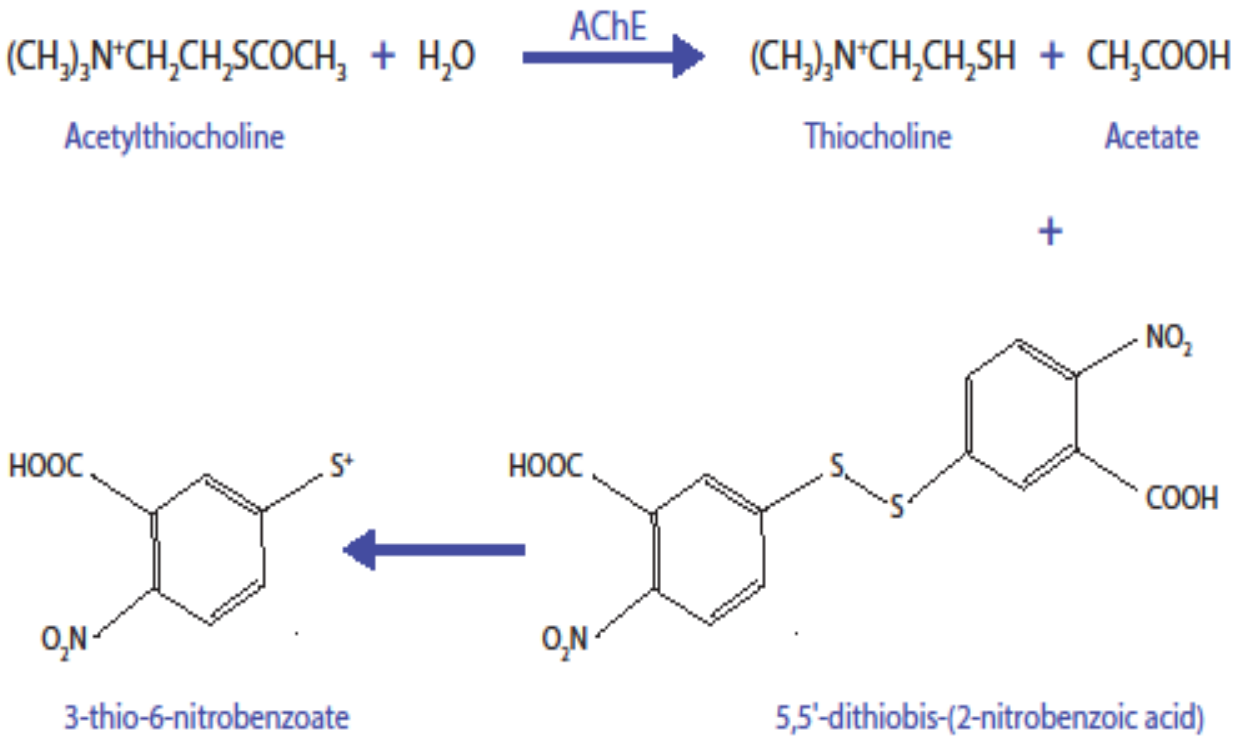
Xia et al. 2011

Bionanoteam
Nanobiology & Nanotoxicology Research

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

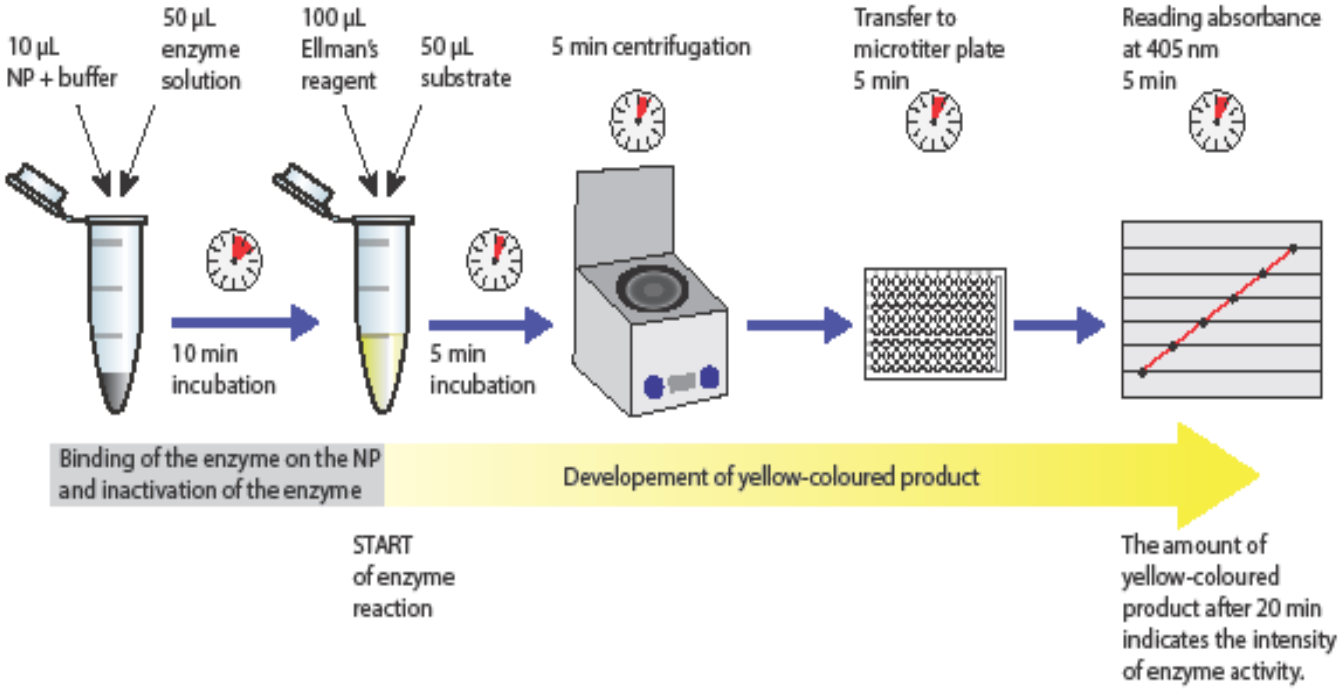
Bionanoteam
Nanobiology & Nanotoxicology Research



Jemec et al., in press

Bionanoteam
Nanobiology & Nanotoxicology Research

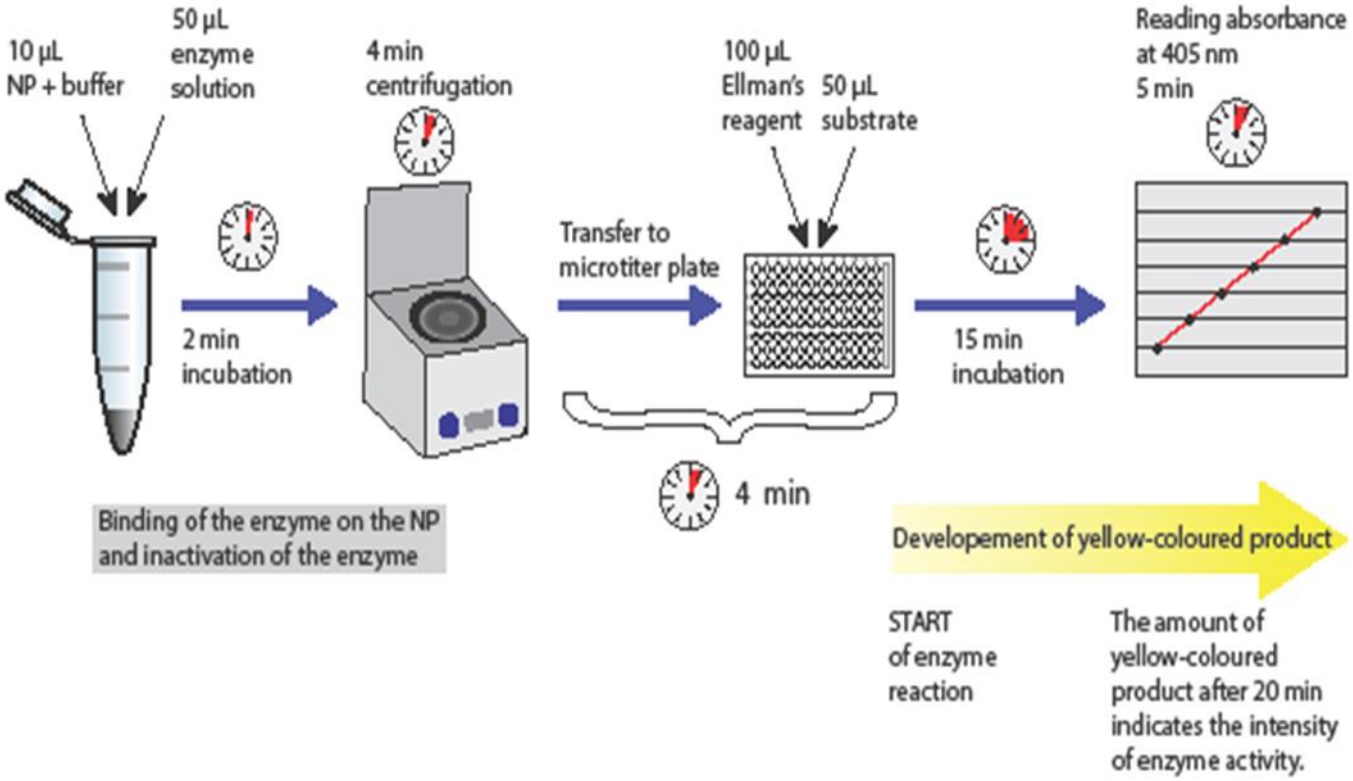
Measuring the inhibition of enzyme activity by the NM



Jemec et al., in press

Bionanoteam
Nanobiology & Nanotoxicology Research

Measuring the adsorption of the enzyme on the NM



Jemec et al., in press

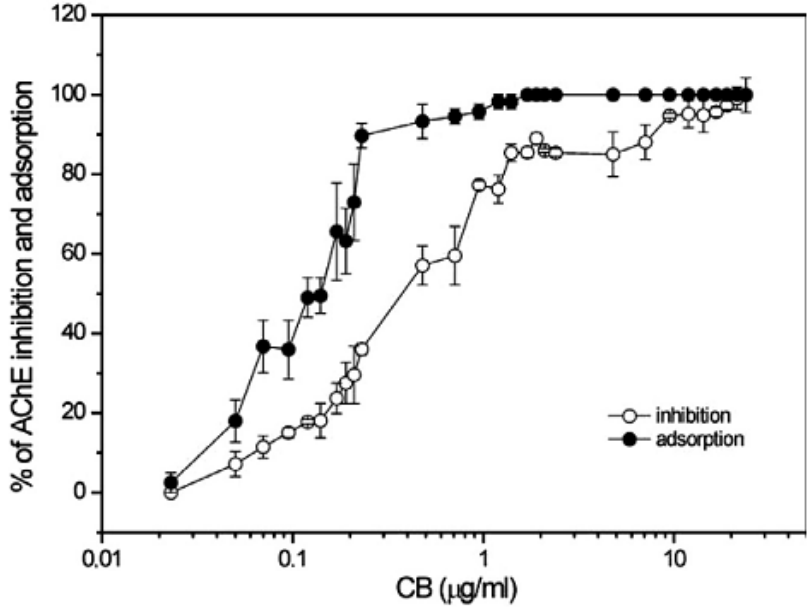
Bionanoteam
Nanobiology & Nanotoxicology Research

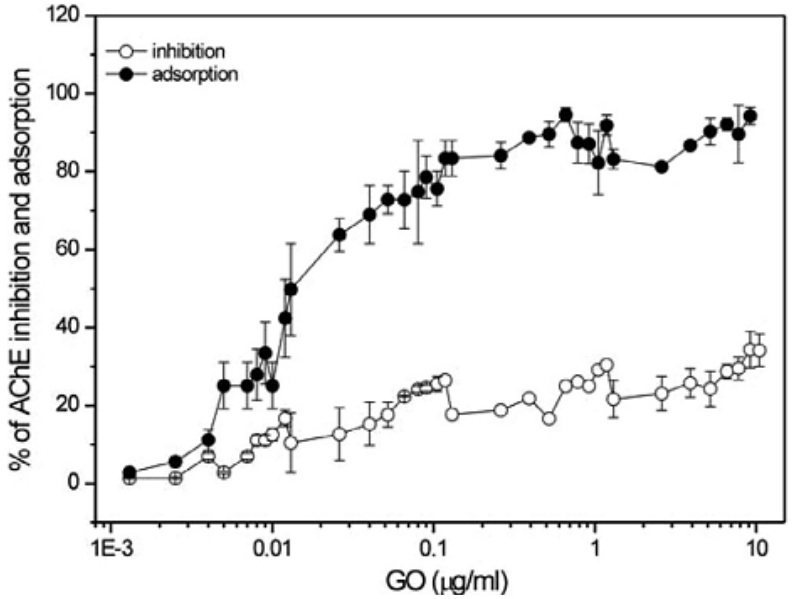
Comparison of different carbon NM

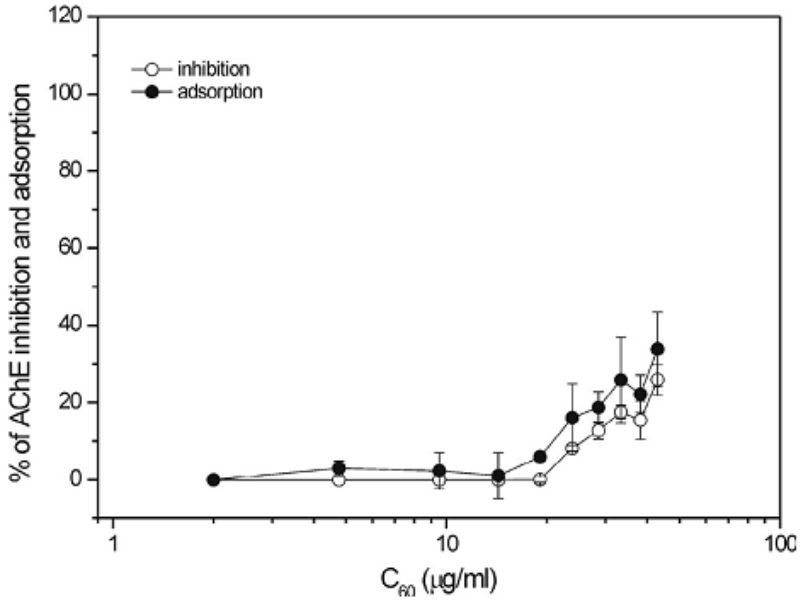
- Graphene oxide
- Carbon black
- C_{60} – fullerenes
- Multi-walled carbon nanotubes

Bionanoteam
Nanobiology & Nanotoxicology Research

Drosophila melanogaster acetylcholinesterase



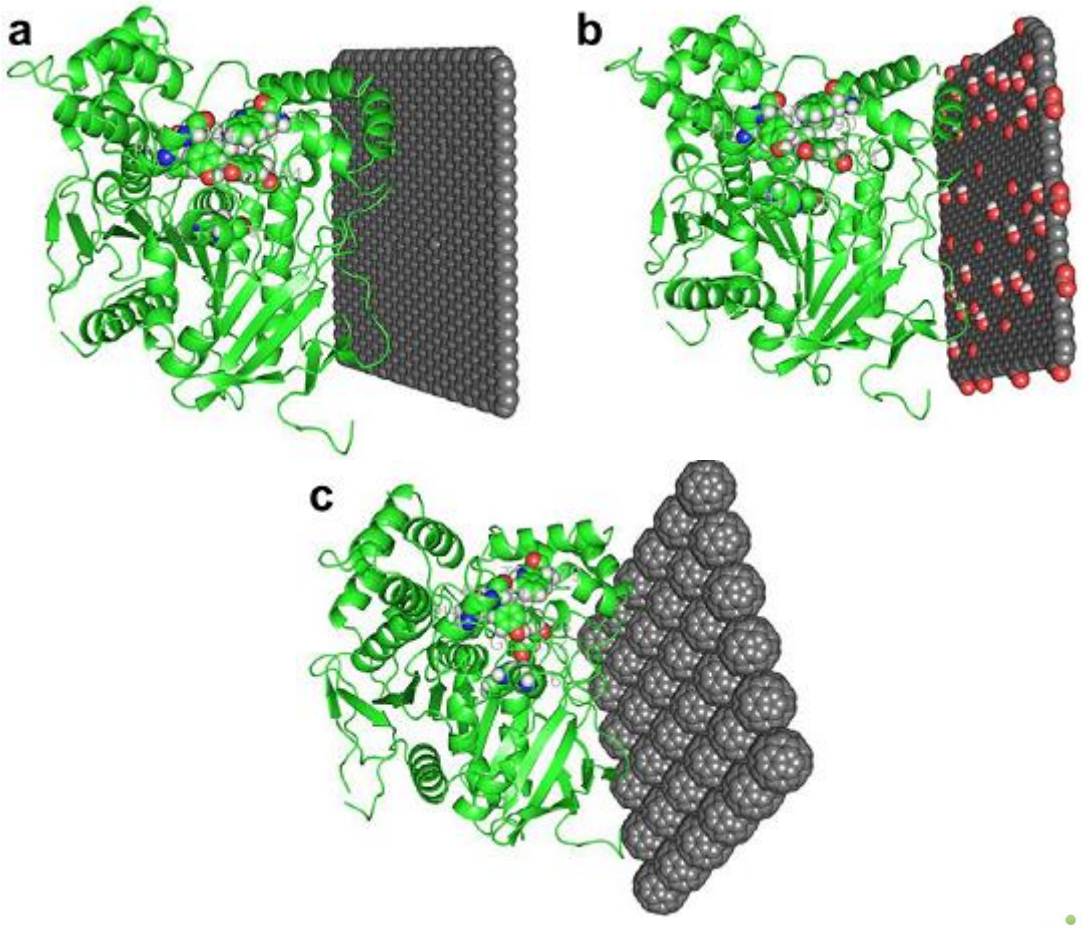




Mesarič et al. 2013

Bionanoteam
Nanobiology & Nanotoxicology Research

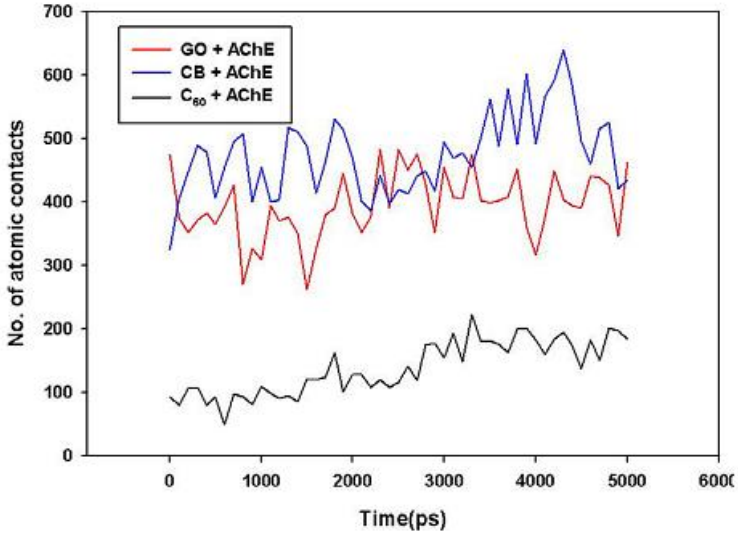
Molecular dynamics simulations



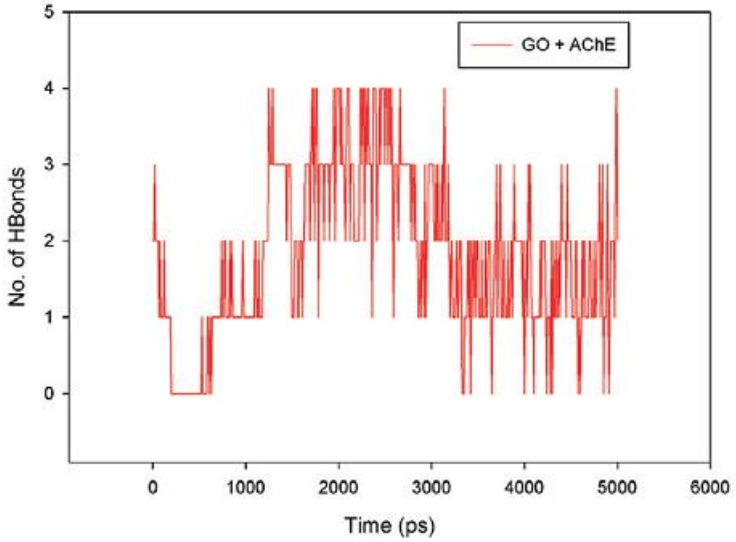
Mesarič et al. 2013

Bionanoteam
Nanobiology & Nanotoxicology Research

Molecular dynamics simulations



Atomic contacts



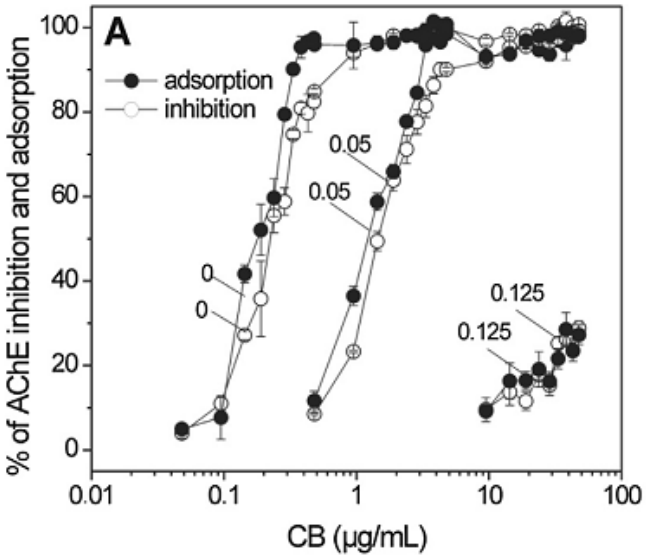
Hydrogen bonds

Mesarič et al. 2013

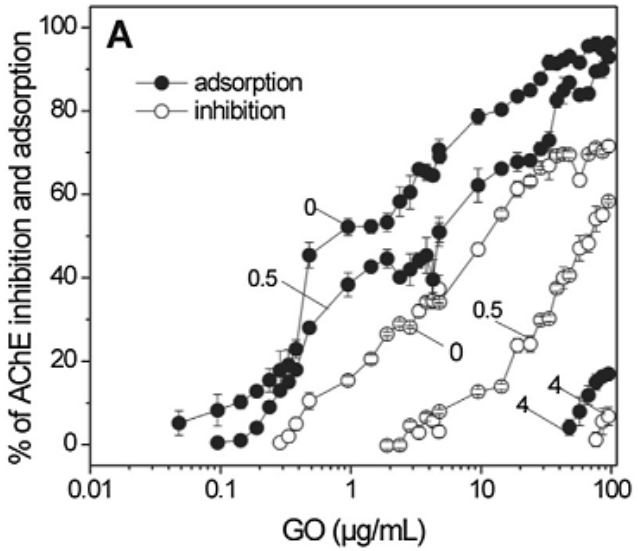
Bionanoteam
Nanobiology & Nanotoxicology Research

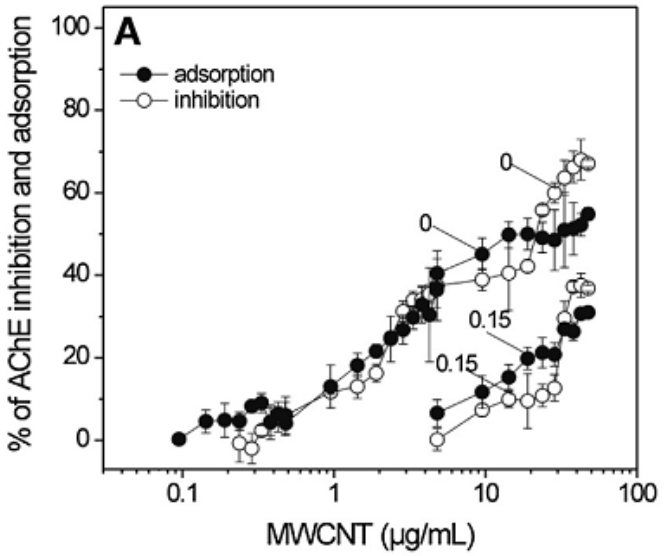
Electrophorus electricus acetylcholinesterase

- Pre-coating with BSA



Sopotnik et al. 2015

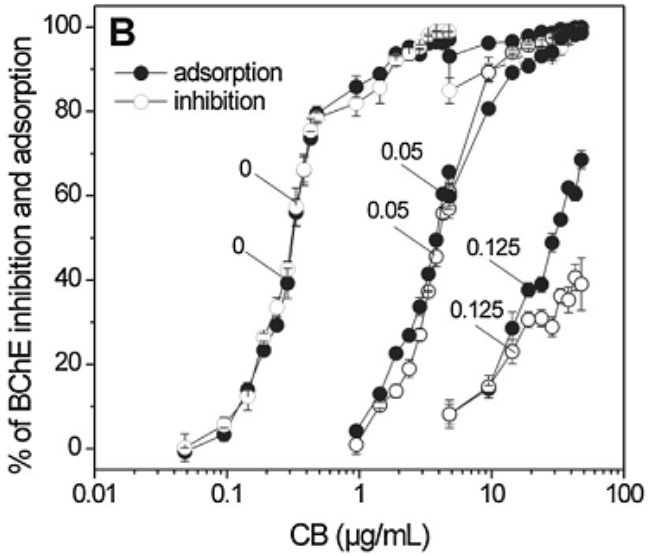


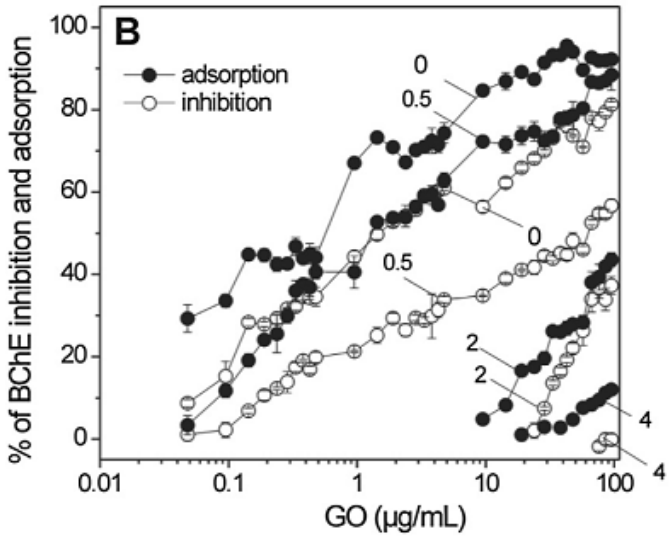


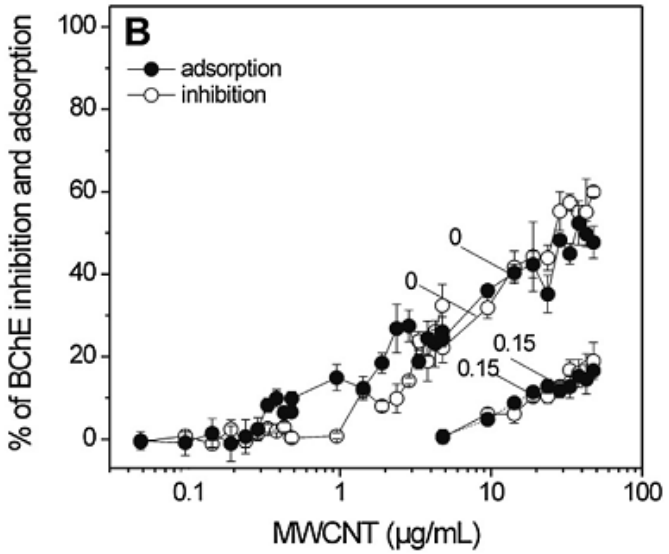
Bionanoteam
Nanobiology & Nanotoxicology Research

Equine serum butyrylcholinesterase

- Pre-coating with BSA



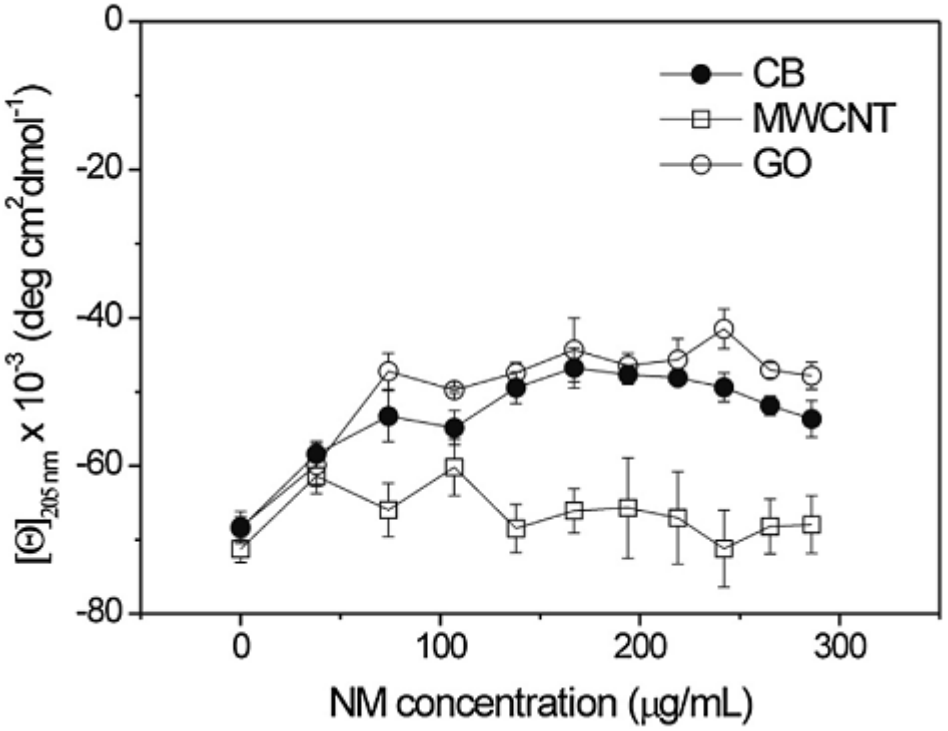




Sopotnik et al. 2015

Bionanoteam
Nanobiology & Nanotoxicology Research

CD-measurements

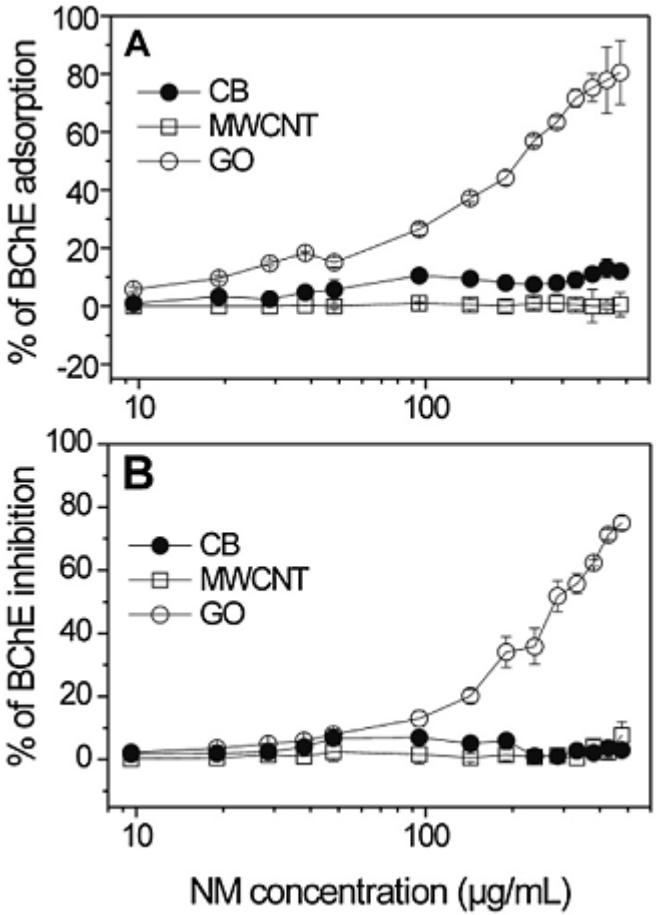


Sopotnik et al. 2015

Bionanoteam
Nanobiology & Nanotoxicology Research

Human serum

- 0,4 % human serum
- Intrinsic BChE

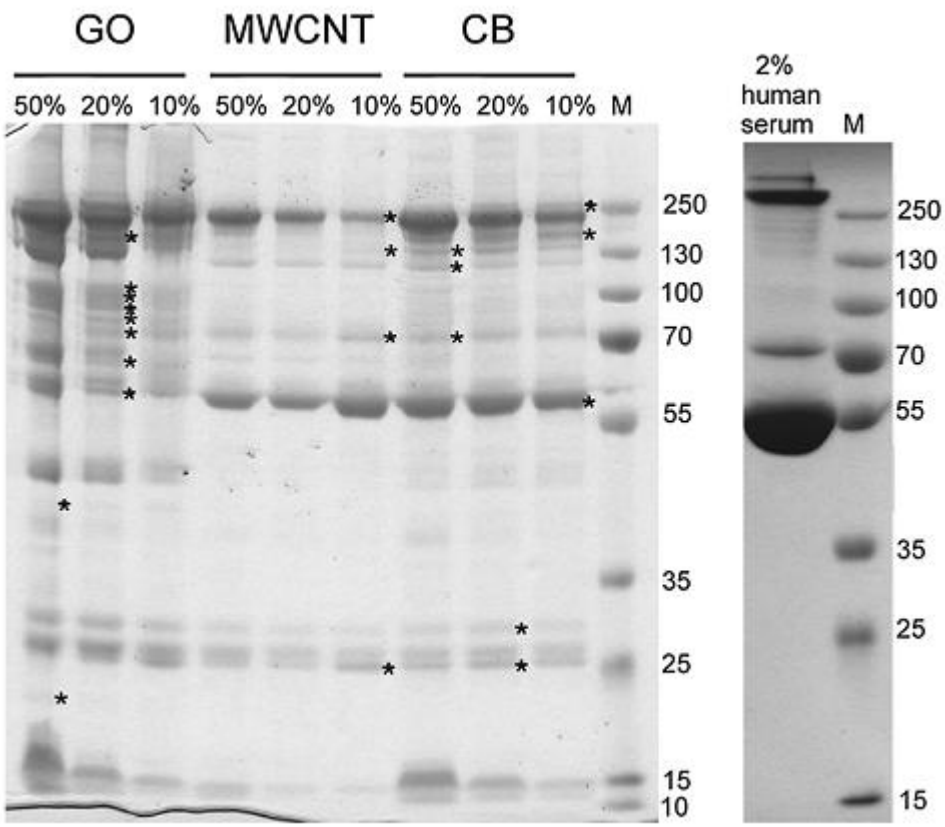


Sopotnik et al. 2015

Bionanoteam
Nanobiology & Nanotoxicology Research

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

Bionanoteam
Nanobiology & Nanotoxicology Research

Conclusions

- AChE inhibition: CB>GO>MWCNT>C_{60}
- 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

Bionanoteam
Nanobiology & Nanotoxicology Research

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., Sepčić, K. & d. Drobne. Biological characterization of nanomaterials. In press.
- Mesarič, T., Baweja L., Drašler, B., Drobne, D., Makovec, D., Dušak, P., Dhawan, A. & K. Sepčić, 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., Sepčić, K. & D. Drobne, 2015. Comparative study of serum protein binding to three different carbon-based nanomaterials. Carbon 95: 560-572.

Bionanoteam
Nanobiology & Nanotoxicology Research

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

This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No 263147

What is quality of nanotoxicity data and why we need quality data?

Angewandte Reviews
Nanotoxicology
H. F. Krug
DOI: 10.1002/anie.201403367

Nanosafety Research—Are We on the Right Track?

*Harald F. Krug**

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

Angewandte International Edition Chemie

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…„

Multiplicity of variables- an example for in vitro testing

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 a basis.

1. MinChar initiative, providing minimal material characterization recommendations for nano-toxicology studies (http://characterizationmatters.org/parameters/), see Annex 1
2. DaNa critera 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!

NANOVALID CRITERIA FOR quality data

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 µg/ml, µg/cm2 ; N (particle)/cell or pg/cell)
- Controls (positive and negative controls)
- Interferences with test system
- Appropriate methods / endpoints
- Use of reference material

NANOVALID CRITERIA quality of nanotoxicity data

General aspects

Appropiate data evaluation / statistics

Standardisation criteria (SOPs used, OECD guidelines, decison 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?

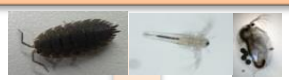
NANOVALID harmonisation efforts

STEPS in Establishing reference methods

WP 3
Development of preliminary SOPs
Pre-validation of SOPs

WP 5
Priority list of methods for NMs exposure assessment
CRITERIA for selection of reference methods set
- selection of three candidate reference methods

freshwater Daphnia magna, marine Artemia salina and terrestrial isopods Porcellio scaber



WP 5
-validation of three candidate reference methods

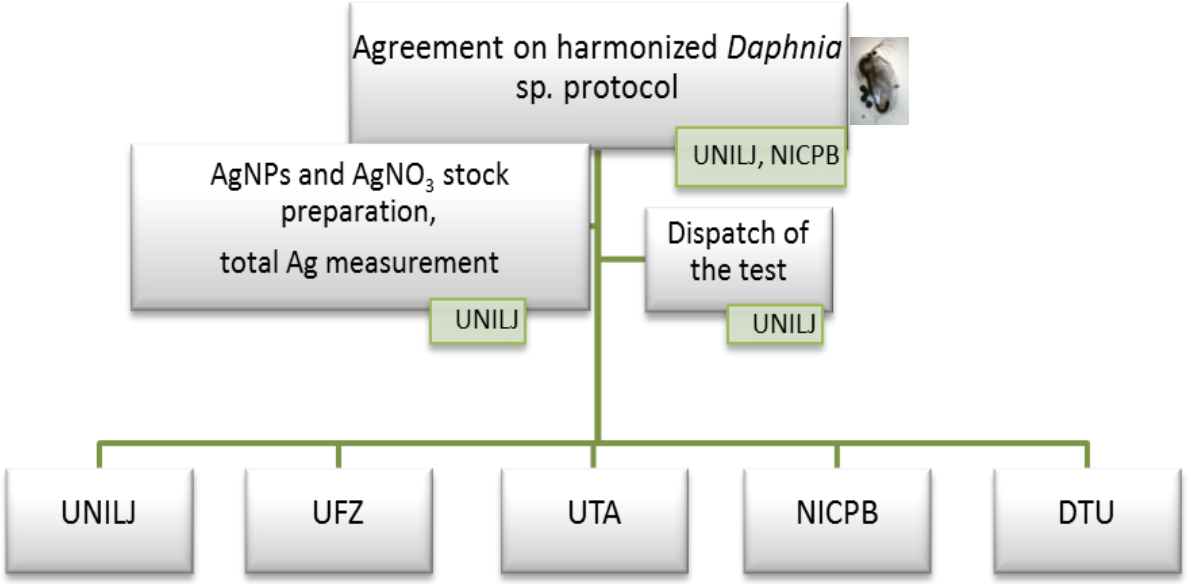
CRITERIA for reference method:

- 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



University of Ljubljana (UNILJ, Biotechnical Faculty, Slovenia) (reference laboratory), National Institute of Chemical Physics and Biophysics (NICPB, Estonia), The Helmholtz Centre for Environmental Research (UFZ, Germany) Technical University of Denmark (DTU, Denmark)

RESULTS: *Daphnia magna*

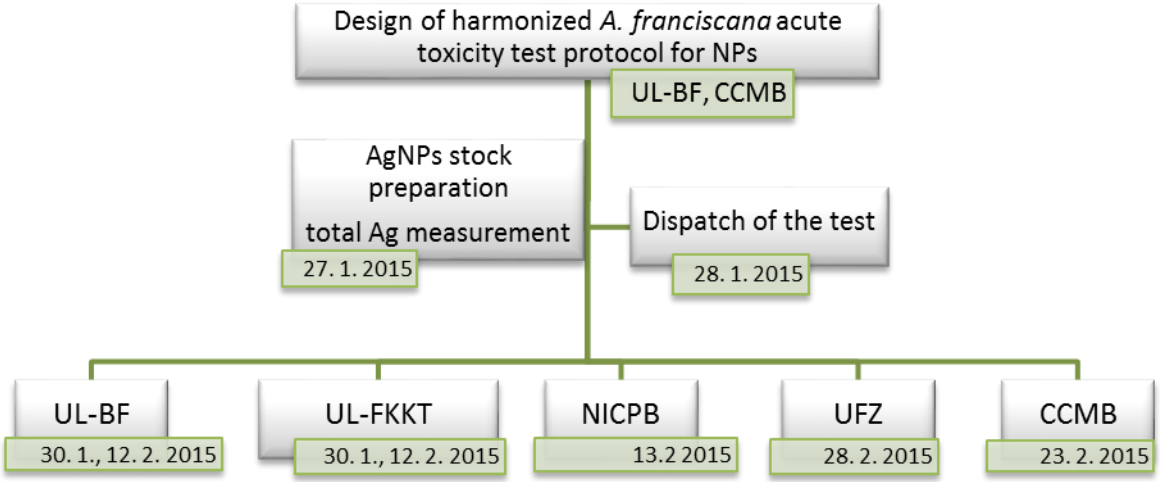
Exactly after SOP developed by NICPB (OECD 2004: No. 202)

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

hatching of the cysts

- *cysts:* *A. franciscana*
- *storage:* room T, darkness, anoxic conditions
- *hatching vessel: (volume, material, how many cysts per vol)*
- *medium*: SSW
- *time*: 24 h
- *temperature*: 25° C
- aeration: < 200 bubbles/min
- *hydratation*: No
- *bleaching*: No

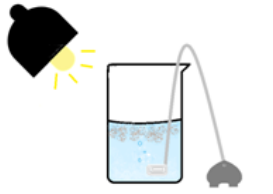
start of the exposure

- *age of nauplii*: 24 h
- *test medium*: SSW
- [1]*illumination*: 16h :8h-light:dark
- *temperature*: 25° C,
- [2]*incubation*: on the desk in temperature controlled room
- *volume*: 24-well (2 ml per well)
- *feeding*: No

reference chemical $K_2Cr_2O_7$

endpoint evaluation

- immobility
- OPTIONALLY: swimming alteration, absorption of NMs on body surface, development



24 h of hatching

Instar I nauplii

+

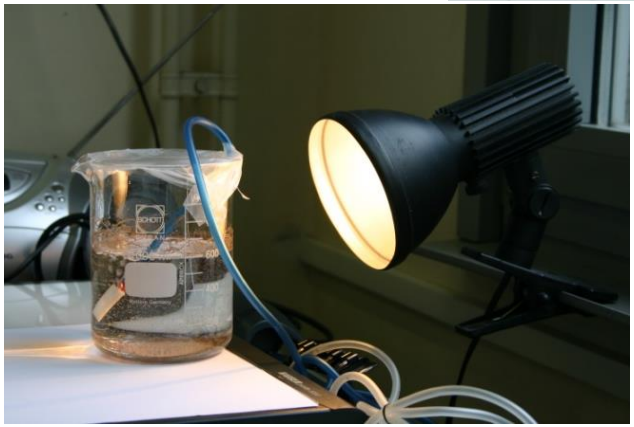
+ toxicant

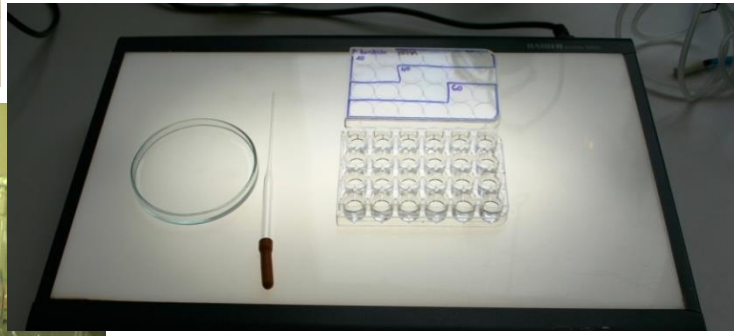
48 h of exposure

Counting the viable (mobile) nauplii

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

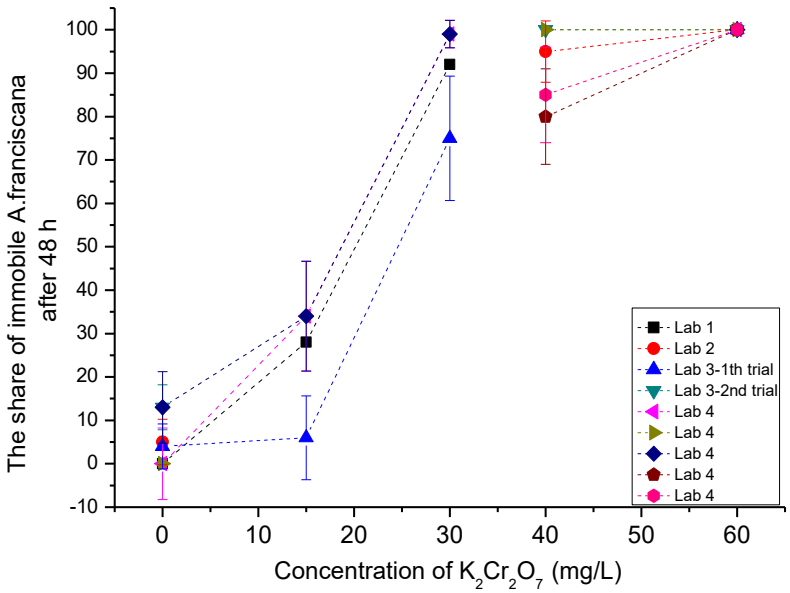




RESULTS: POTASIUM DICHROMATE

Data of the intercalibration study on **$K_2Cr_2O_7$.**

a.)



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

CONCLUSIONS:

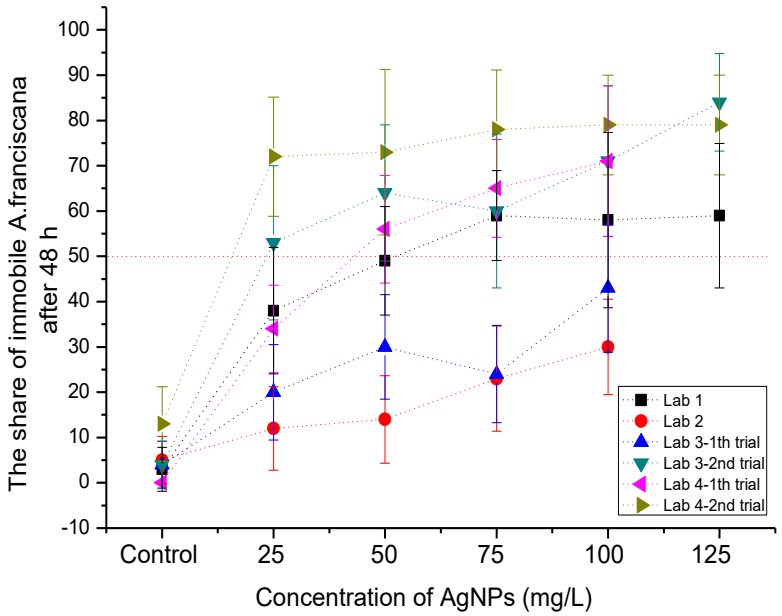
- 1 partner invalid results-high control mortality (inappropriate cysts storage, prolonged hatching)
- LOW Intra-laboratory variation (UNILJ) (relative coefficient of variation): 9.6 %.
- LOW Inter-laboratory variation: 17%

GOOD REPEATABILITY WITH CHROMIUM

RESULTS: SILVER NANOPARTICLES

Data of the intercalibration study on **AgNPs**

b.)



Each data point represents mean ± 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 VARIABILTY

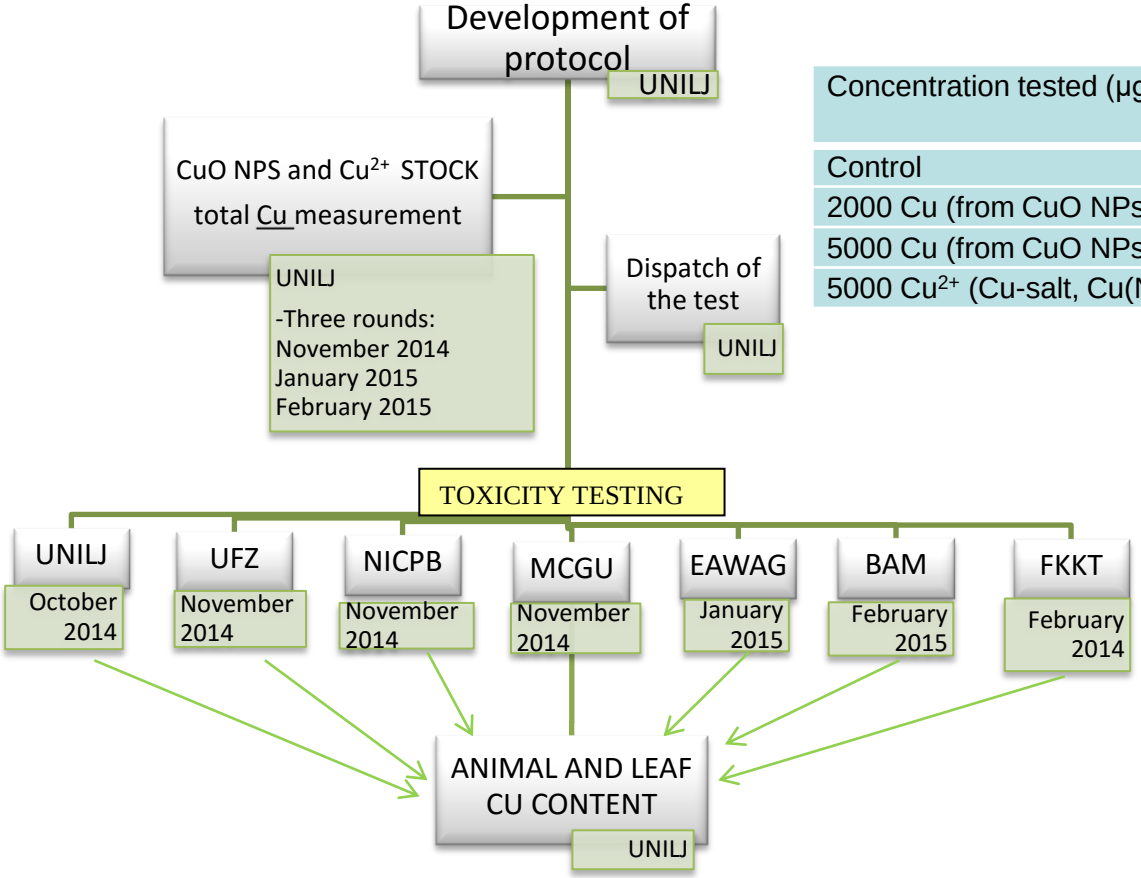
- hatching conditions
- test plate incubation
- illumination regime
- Special AgNPs properties

-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, *A. franciscana*	Commonly undefined, mostly *Artemia sp.* or *A. salina*
	Origin of Artemia cysts	° Producer, which supplies *A. franciscana*, 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 ussulally 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

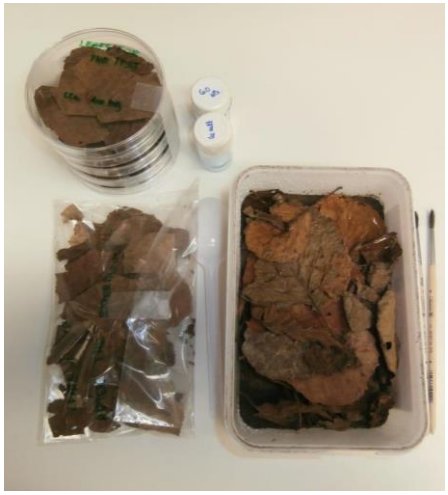
isopods Porcellio scaber

Organization of inter-laboratory comparison study

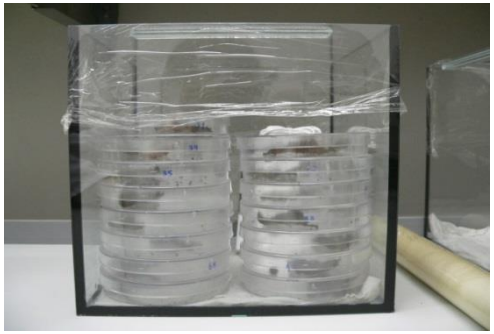


Concentration tested (µg/g leaf)	No. of animals
Control	10-12
2000 Cu (from CuO NPs)	10-12
5000 Cu (from CuO NPs)	10-12
5000 Cu^{2+} (Cu-salt, $Cu(NO_3)_2 \cdot 3H_2O$)	10-12

RESULTS: *Porcellio scaber*



Detailed instructions, video/audio material



Animal Cu content- Bioaccumulation
Leaf Cu content

Conclusions

- 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 if 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:

DaNa 2.0

Information about nanomaterials and their safety assessment

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

ACKNOWLEDGEMENTS

To All NanoValid partners involved in round robin.
Monika Kos for Artemia assay validation
All Bionanoteam for support in analytic and toxicity testing

EU FP7 project NanoValid (Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials; grant no. 263147)