About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

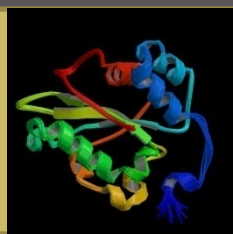
OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

EFFECTS OF ENGINEERED METAL AND METAL OXIDE NANOPARTICLES ON MODEL AQUATIC ORGANISMS: FINDING PROTEIN TARGETS BY REDOX PROTEOMICS

Prof David Sheehan
School of Biochemistry and Cell Biology
& Environmental Research Institute
University College Cork, Ireland





Where am I from?





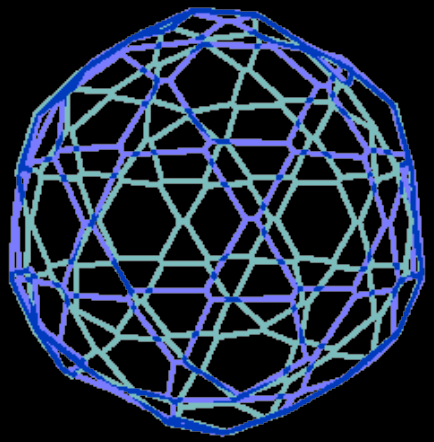


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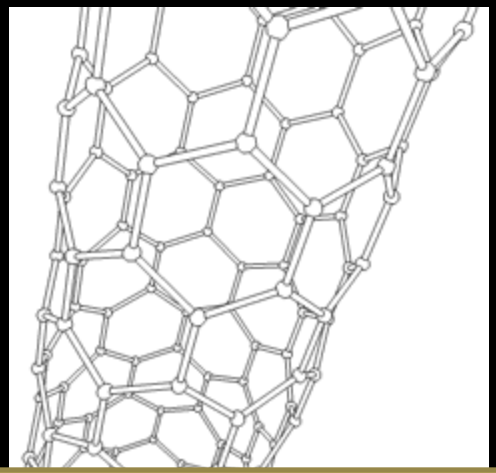
Nanoparticles

Materials in a size range, with at least one dimension between 1-100nm. (SCENHIR 2005; Moore 2006).

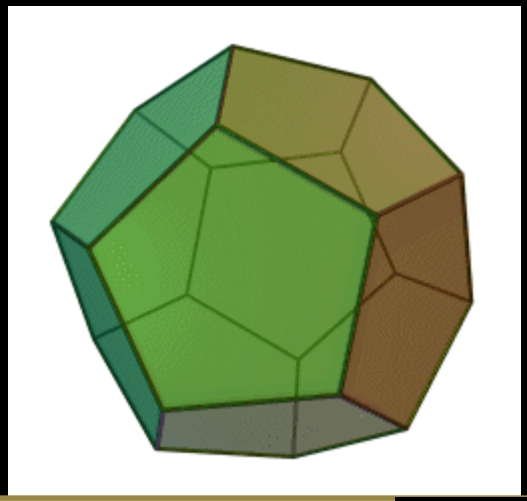
Globular

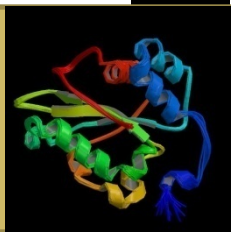


Tubular



Complex geometries





Nature

Nanoparticles Market = $1trillion by 2015 (US EPA)







+





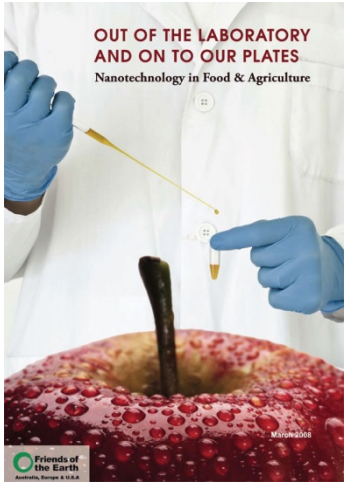




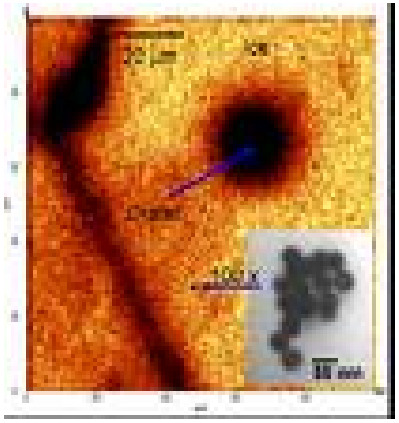




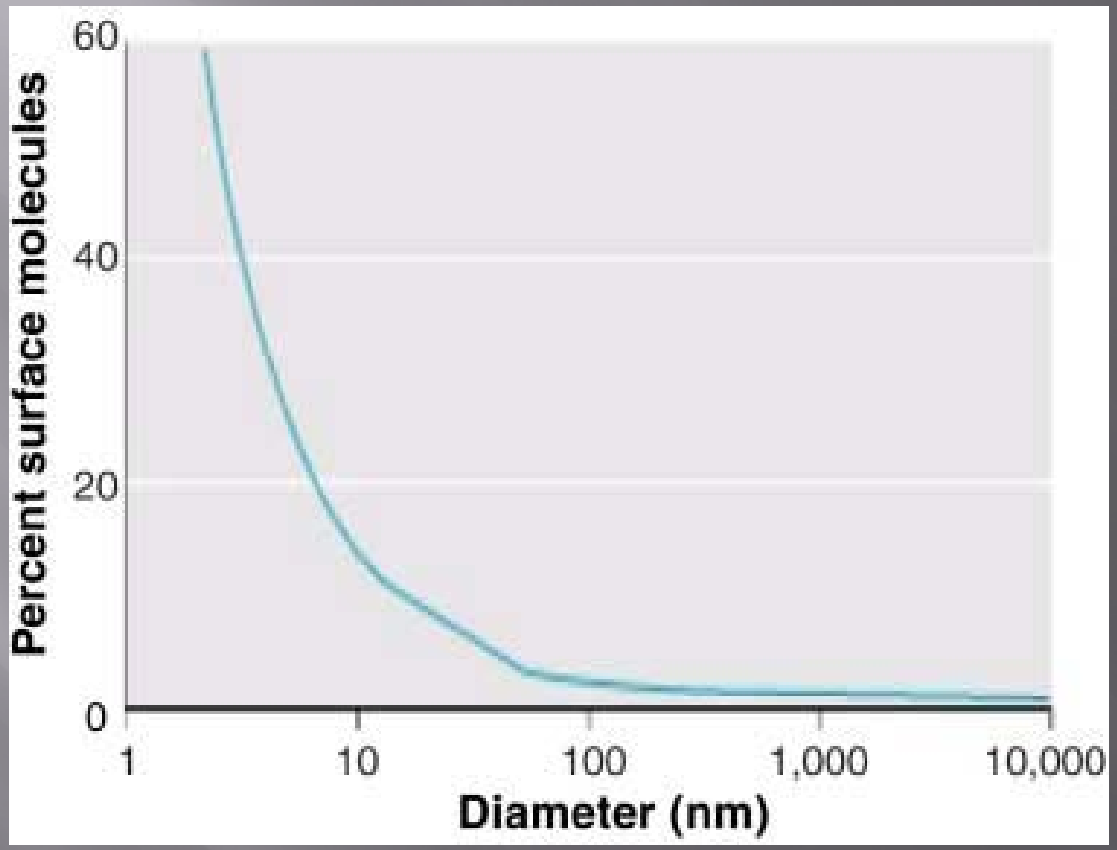






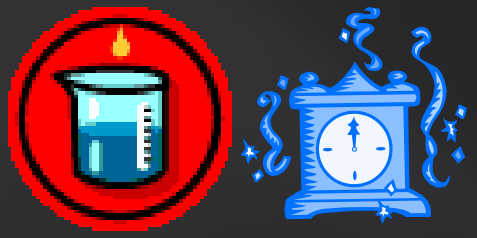


NANOMATERIALS HAVE LARGE PERCENTAGE OF MOLECULES ON THEIR SURFACE



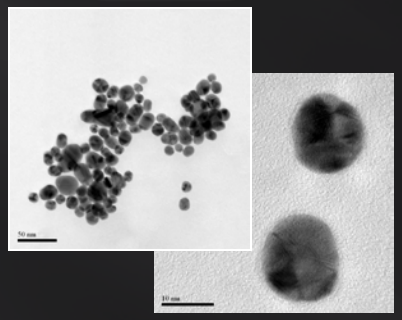
Nel et al., 2006. *Science*. **311**, 622-627

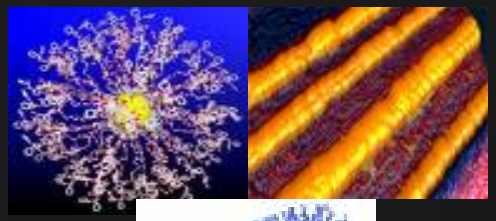
Nanoparticle Effects depend on…



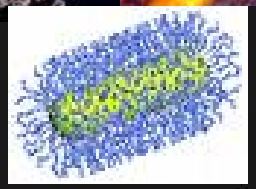
Dose and time

Size





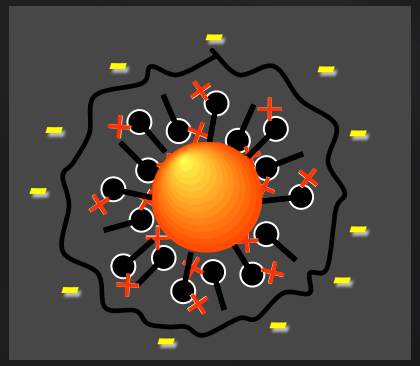
Shape





Oxidation state:

-I, 0, I, II, III, IV,V

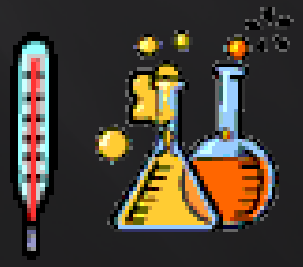


Surface charge





Synthesis method



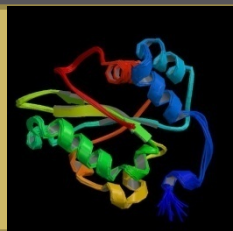
Environmental conditions (pH, temperature, chemical compounds…)

A New Toxicology?

- WHAT IS MEANT BY DOSE IN THIS CONTEXT?
- HOW TO CONTROL FOR LEACHING OF METALS FROM NPs?
- HOW TO MODEL PARTITIONING?
  - IN ENVIRONMENTAL COMPARTMENTS
  - IN TISSUES
  - IN CELLS
- RISK ASSESSMENT?
- IS ANOTHER ASBESTOS LURKING?
- WILL THE POLLUTER REALLY PAY?

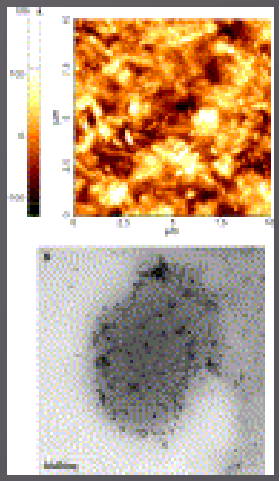


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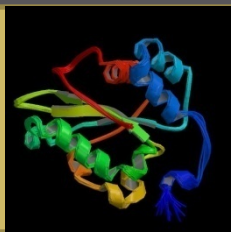


EFFECTS OF NANOPARTICLES

- THEY CAN CROSS BIOBARRIERS
- OFTEN THEY DAMAGE ORGANELLES
  - ESPECIALLY MITOCHONDRIA
  - ACCUMULATE IN LYSOSOMES?

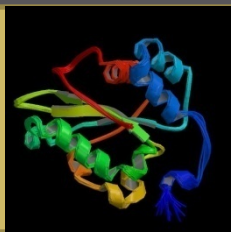


- THEY CAN BE COATED WITH PROTEINS
- THEY OFTEN TRIGGER OXIDATIVE STRESS



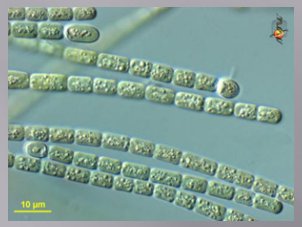
TALK SUMMARY

- NANOPARTICLES CAN BE TOXIC TO MODEL ORGANISMS IN AQUATIC TOXICOLOGY
- REDOX PROTEOMICS PROVIDES A MEANS TO DETECT AND EXPLORE THIS
- THREE EXPERIMENTS
  - *MYTILUS EDULIS*
  - *DAPHNIA MAGNA*
  - **CULTURED HUMAN ASTROCYTES**



THE OXYGEN PARADOX

OXYGEN IS ESSENTIAL FOR AEROBIC METABOLISM BUT PRODUCES TOXIC REACTIVE OXYGEN SPECIES



PHOTOSYNTHETIC CYANOBACTERIA

APPEARANCE OF MOLECULAR O_2



ORIGIN OF EARTH

ENDO-SYMBIOSIS

OZONE (O_3) LAYER

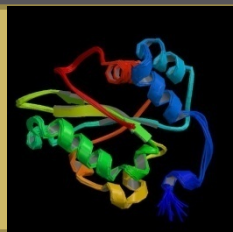
4,500 MYA **3,800 MYA** **2,500 MYA**

REDUCED ENVIRONMENT

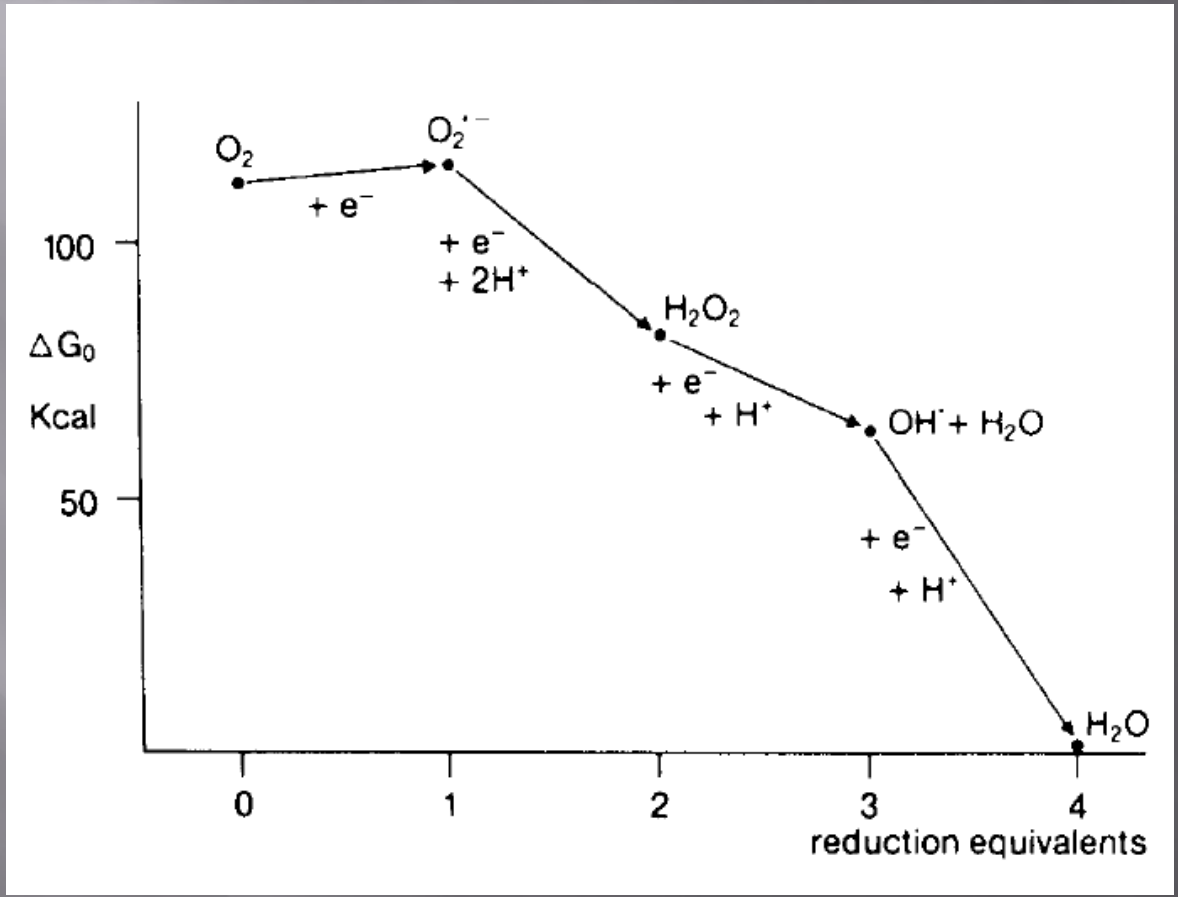
OXIDISED ENVIRONMENT

PRESENT

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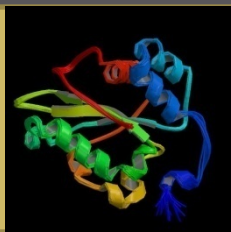
REACTIVE OXYGEN SPECIES (ROS) ARE INEVITABLY FORMED FROM O_2



ROS ARE HIGHLY REACTIVE WITH BIOMATERIALS

- IN MANY CASES, ROS REACTIONS ARE ONLY LIMITED BY RATE OF DIFFUSION – THEY REACT VERY QUICKLY
- BIOMATERIALS CAN ABSORB ROS (e.g. $\cdot$HO)
  - **DNA (6%)**
  - **RNA (7%)**
  - **PROTEINS (69%)**
  - **SMALL MOLECULES (18%)**

PROTEINS ARE THEREFORE KEY TARGETS FOR ROS



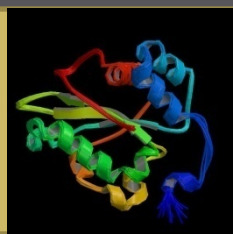
PROTEOMICS: THE STUDY OF THE PROTEOME

- **THE *PROTEOME* IS THE COMPLEMENT OF PROTEINS EXPRESSED UNDER A GIVEN SET OF CONDITIONS.**



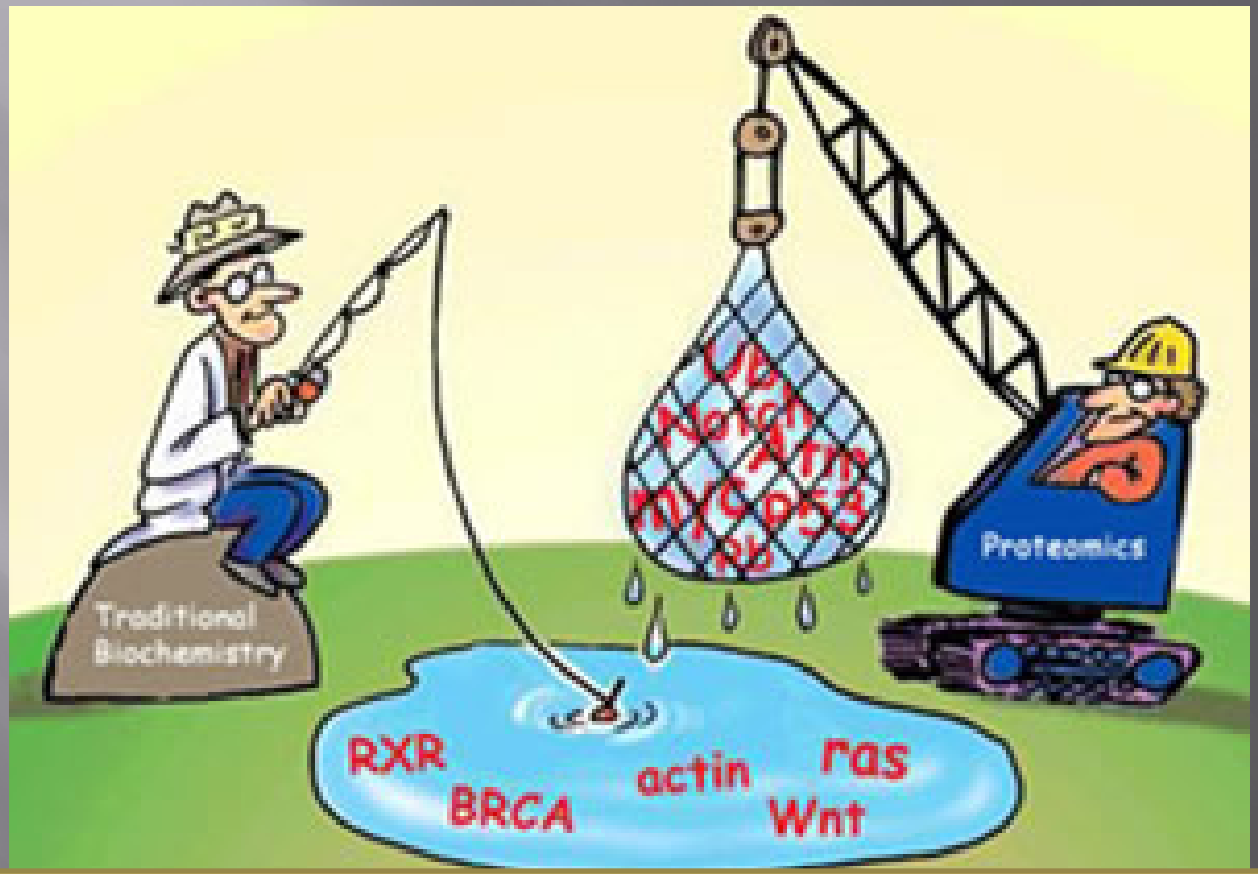
Mark Wilkins first Proposed "proteome" In Siena, 1994

- **A *DYNAMIC* QUANTITY**
- ***HIGH-THROUGHPUT* METHODS WITH WIDE DYNAMIC RANGE (e.g. 2D SDS PAGE; MS).**



PROTEOMICS: DISCOVERY-LED APPROACH TO BIOMARKERS

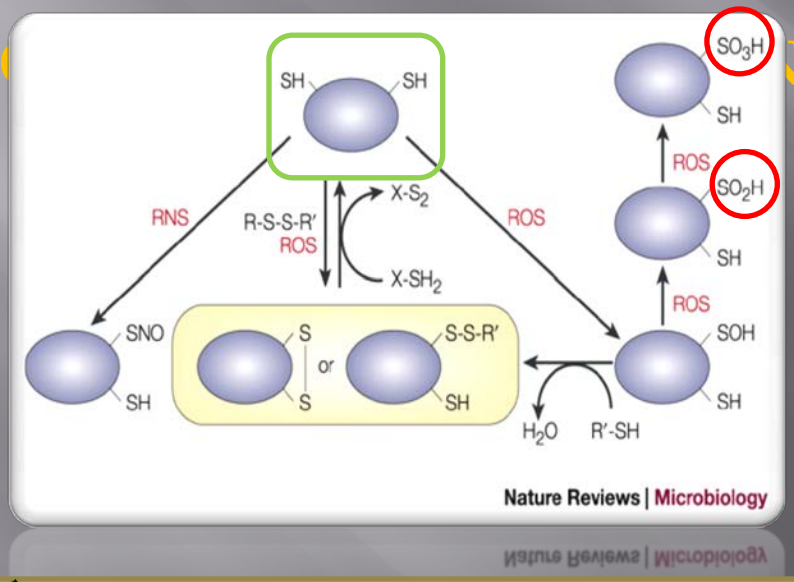
ucc
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

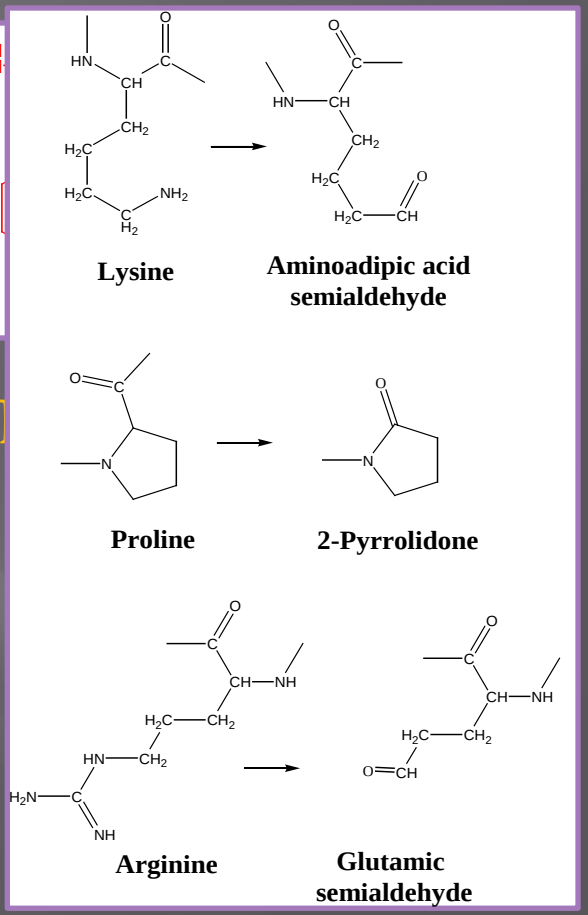


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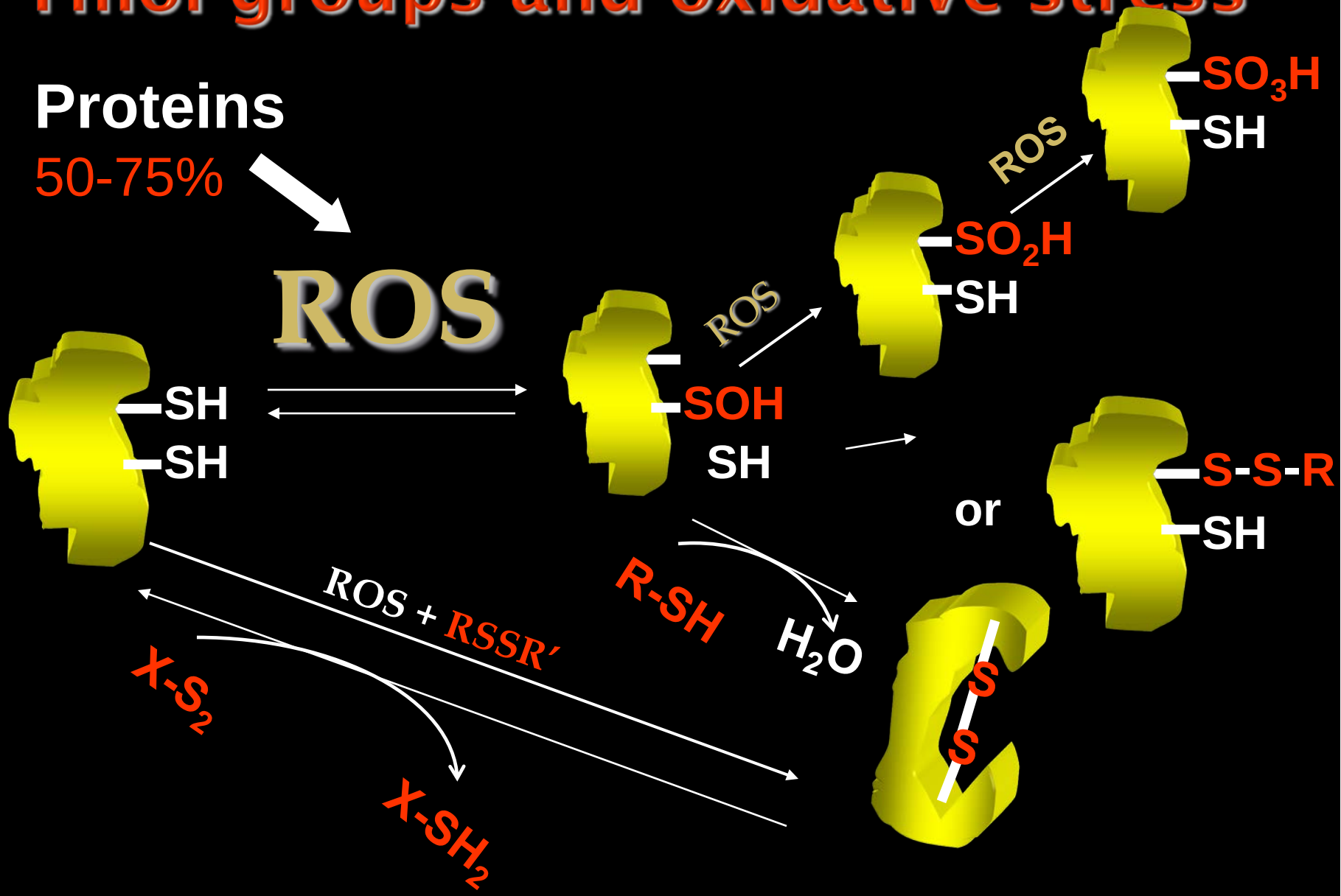
REDOX PROTEOMICS

- **PROTEINS CAN BE COVALENTLY MODIFIED BY ROS/RNS**
  - **NITROSYLATION OF TYROSIN**
  - **CARBONYLATION**
  - **NE THIO**

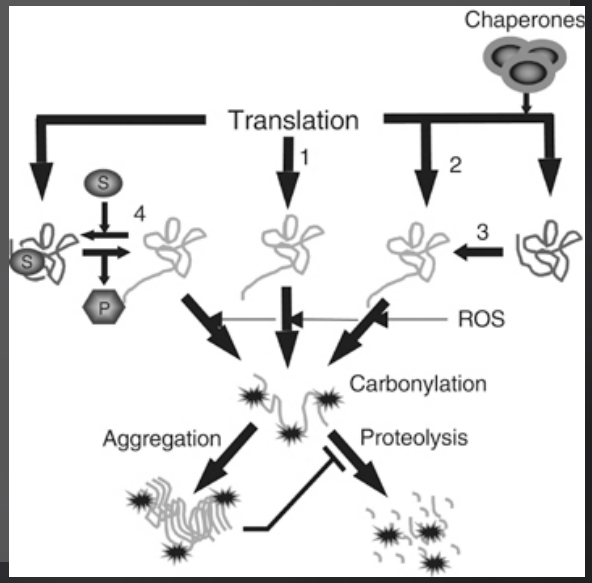


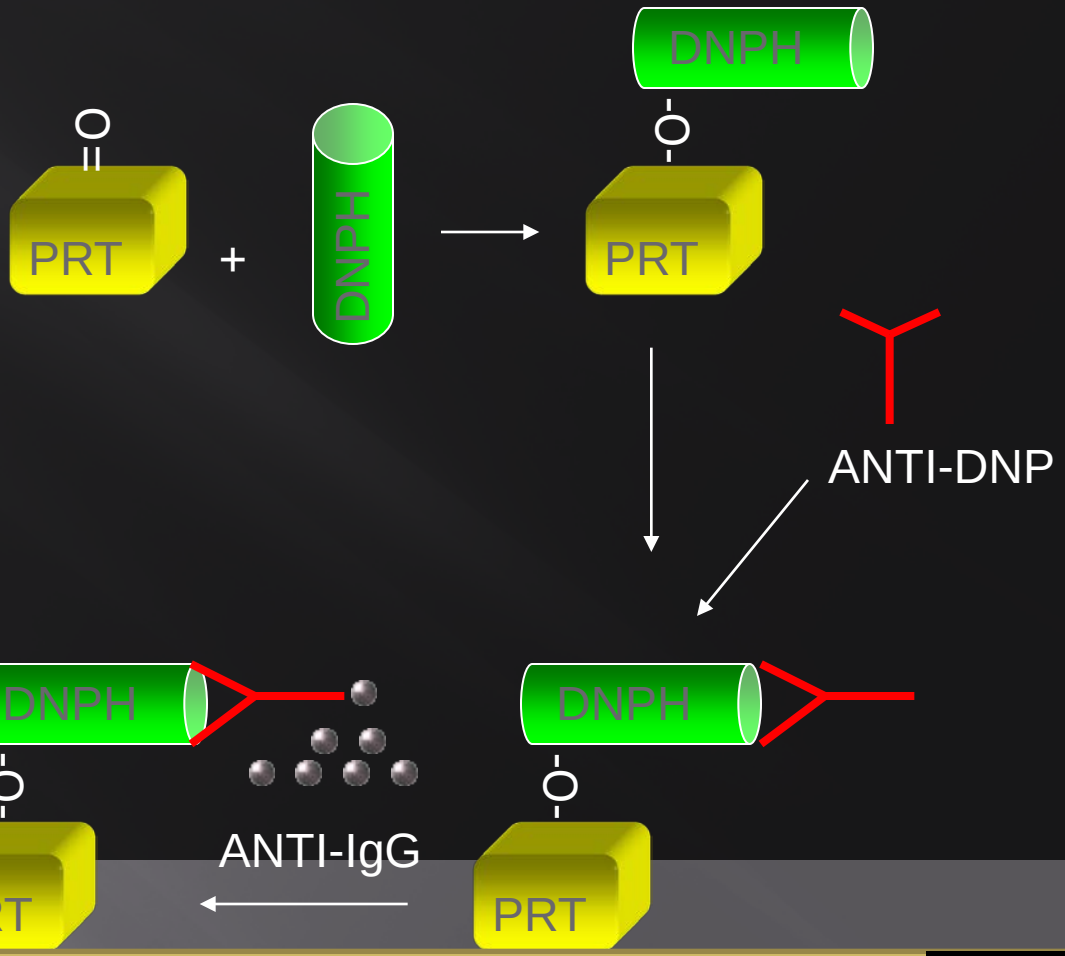


Thiol groups and oxidative stress



Carbonylation





THREE MODEL EXPERIMENTS

1. *Mytilus edulis* exposed to copper NPs

Hu et al. (2014) *Chemosphere*, **108** :289-299.

2. *Daphnia magna* exposed to copper NPs

Rainville (2014) *Analyst*, 139 :1678-1686.

3. Cultured human astrocytes exposed to polystyrene nanoparticles





NeuroNano

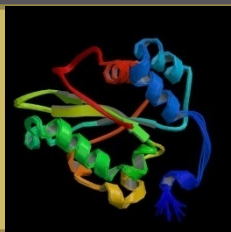
SEVENTH FRAMEWORK PROGRAMME

NeuroNano is a Small Collaborative Project funded by the European Commission 7th Framework Programme, contract number NNP4-SL-2008-214547 which commenced on February 1st 2009 for 36 months.

What is NeuroNano? "Do nanoparticles induce neurodegenerative diseases?

Understanding the origin of reactive oxidative species and protein aggregation and mis-folding phenomena in the presence of nanoparticles."

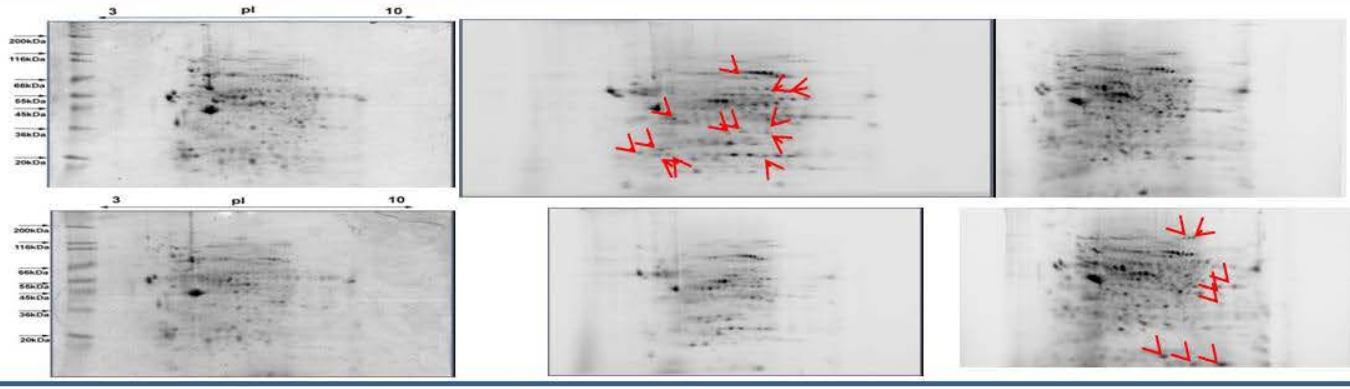
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DIGESTIVE GLAND

Control

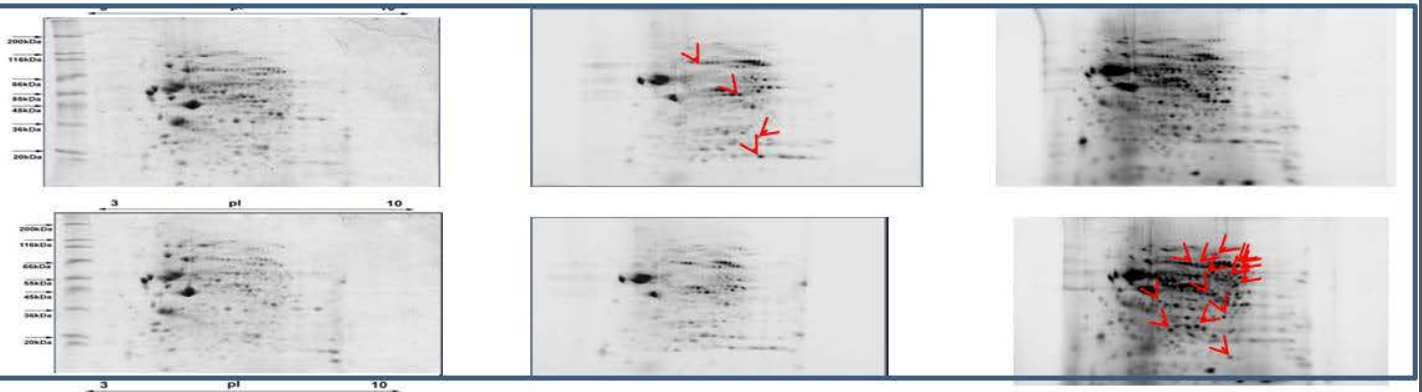
Cu NP 1PPM



GILL

Control

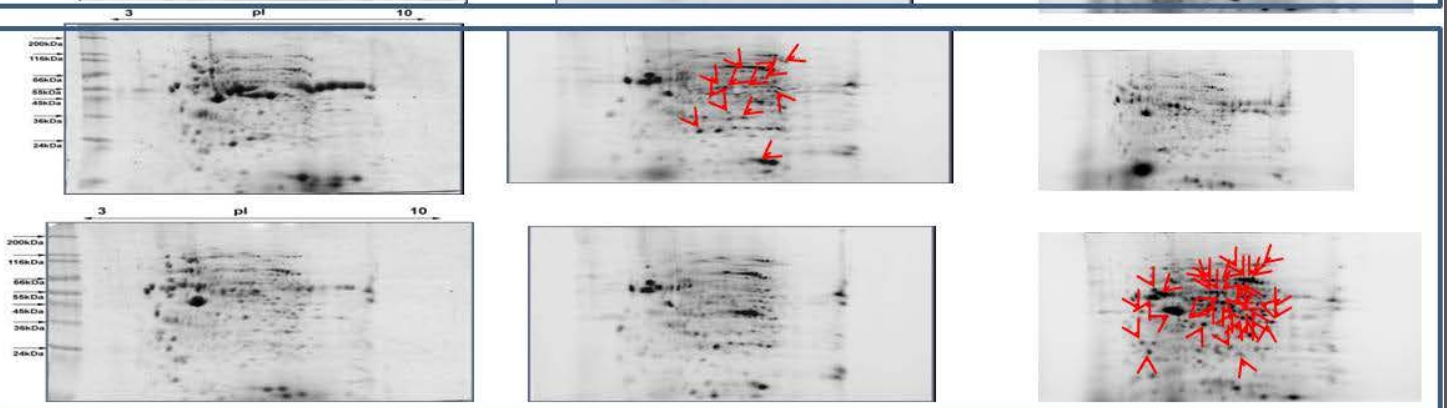
Cu NP 1PPM



MANTLE

Control

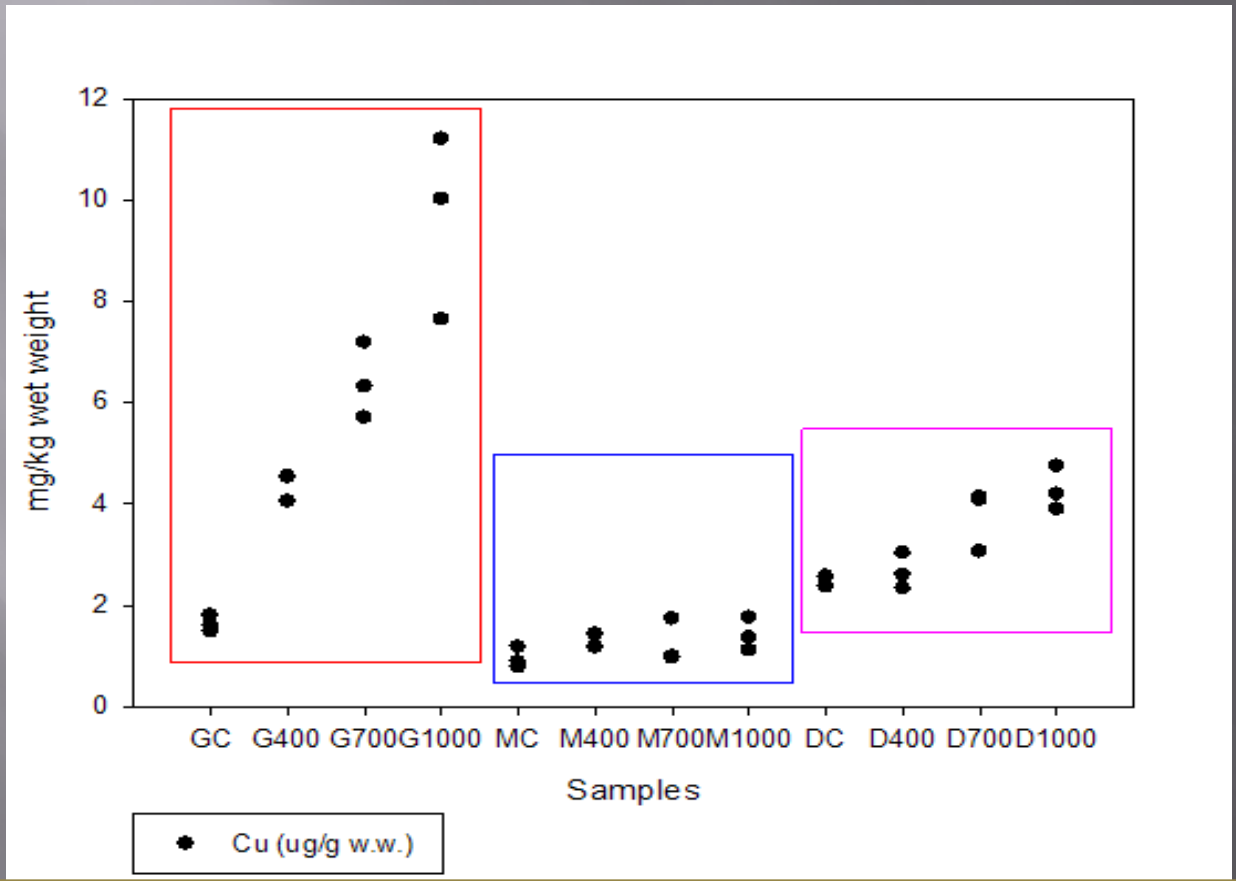
Cu NP 1PPM



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Copper accumulates mainly in gill tissue

GILL MANTLE DIGESTIVE GLAND

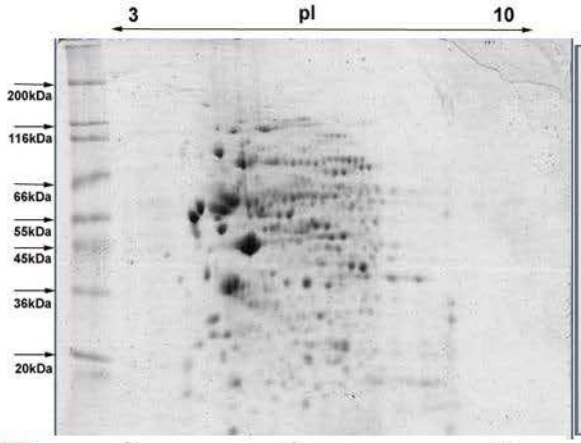


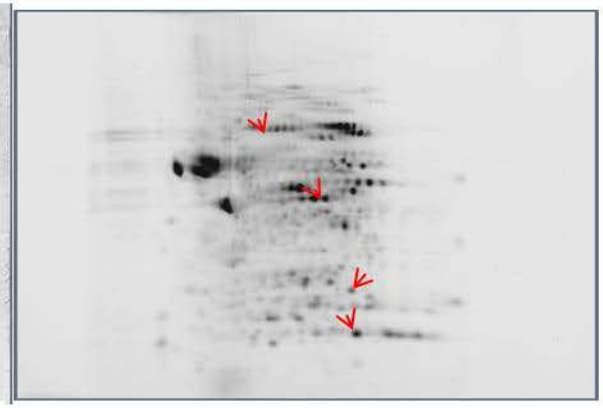
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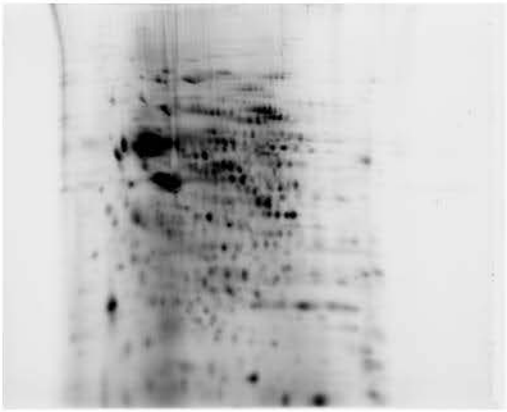
Cu NANOPARTICLES



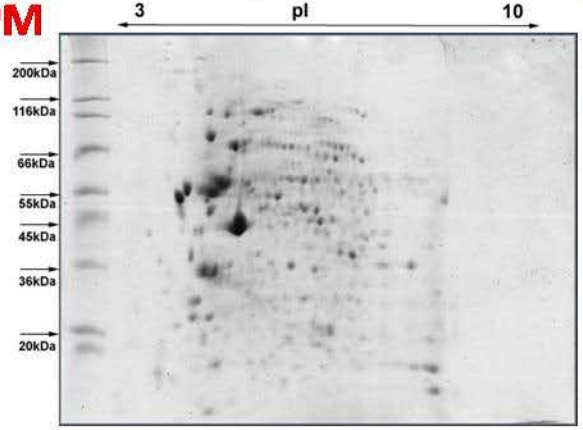
Gill, Control

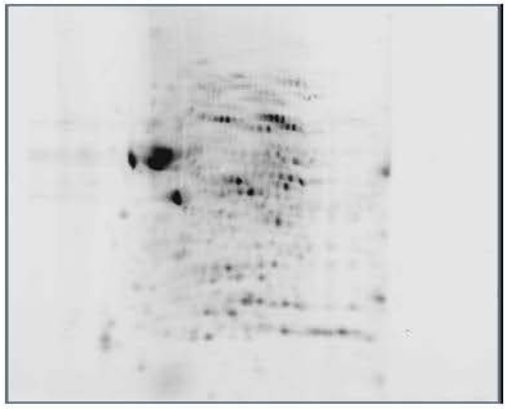


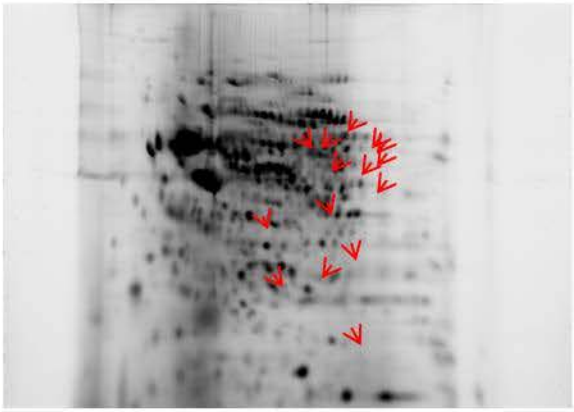




Gill, 1PPM

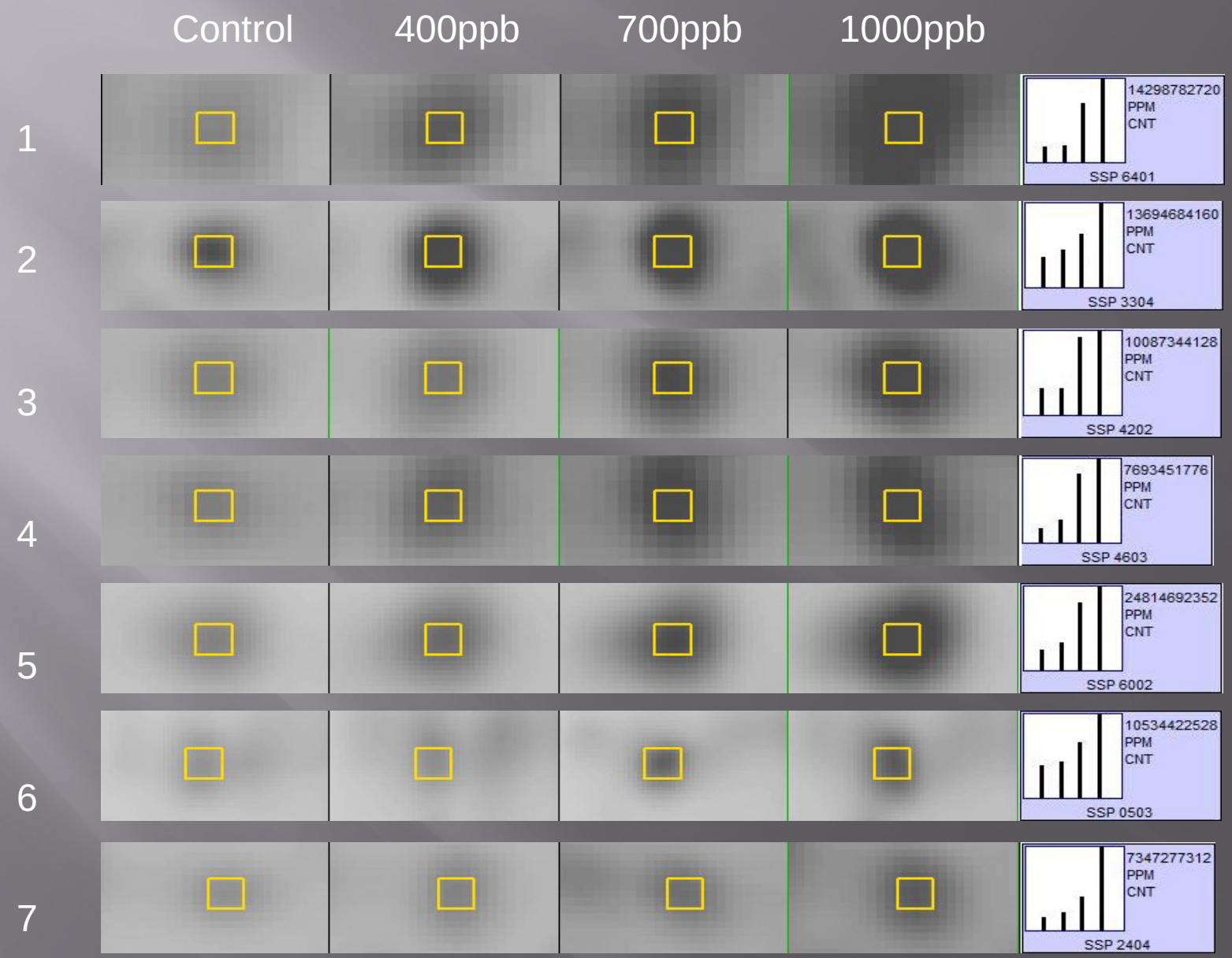




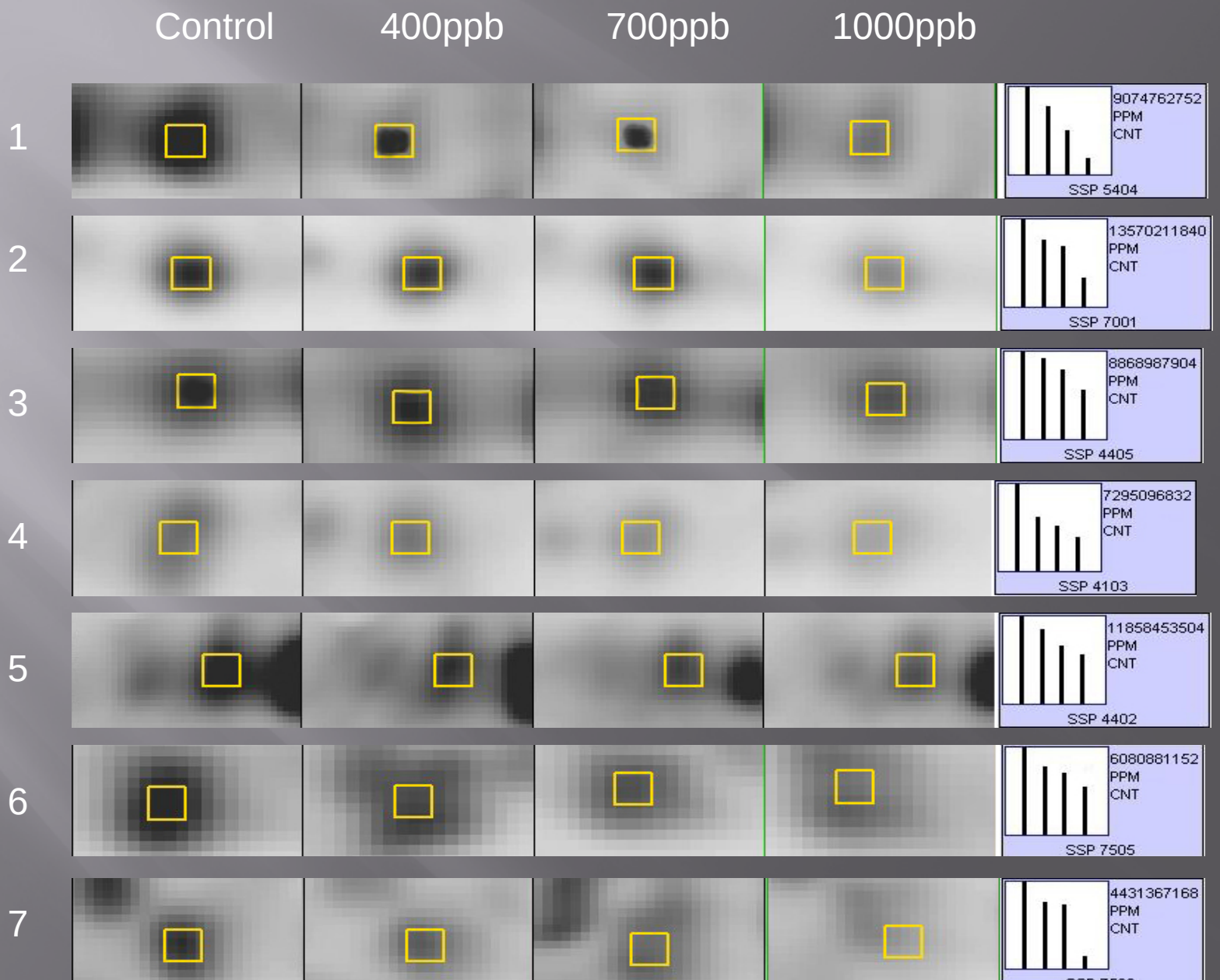


Total protein stain **IAF** **FTSC**

Copper oxide nanoparticles > carbonylation in some spots (PD Quest)



Copper oxide nanoparticles > thiol oxidation in some spots (PD Quest)

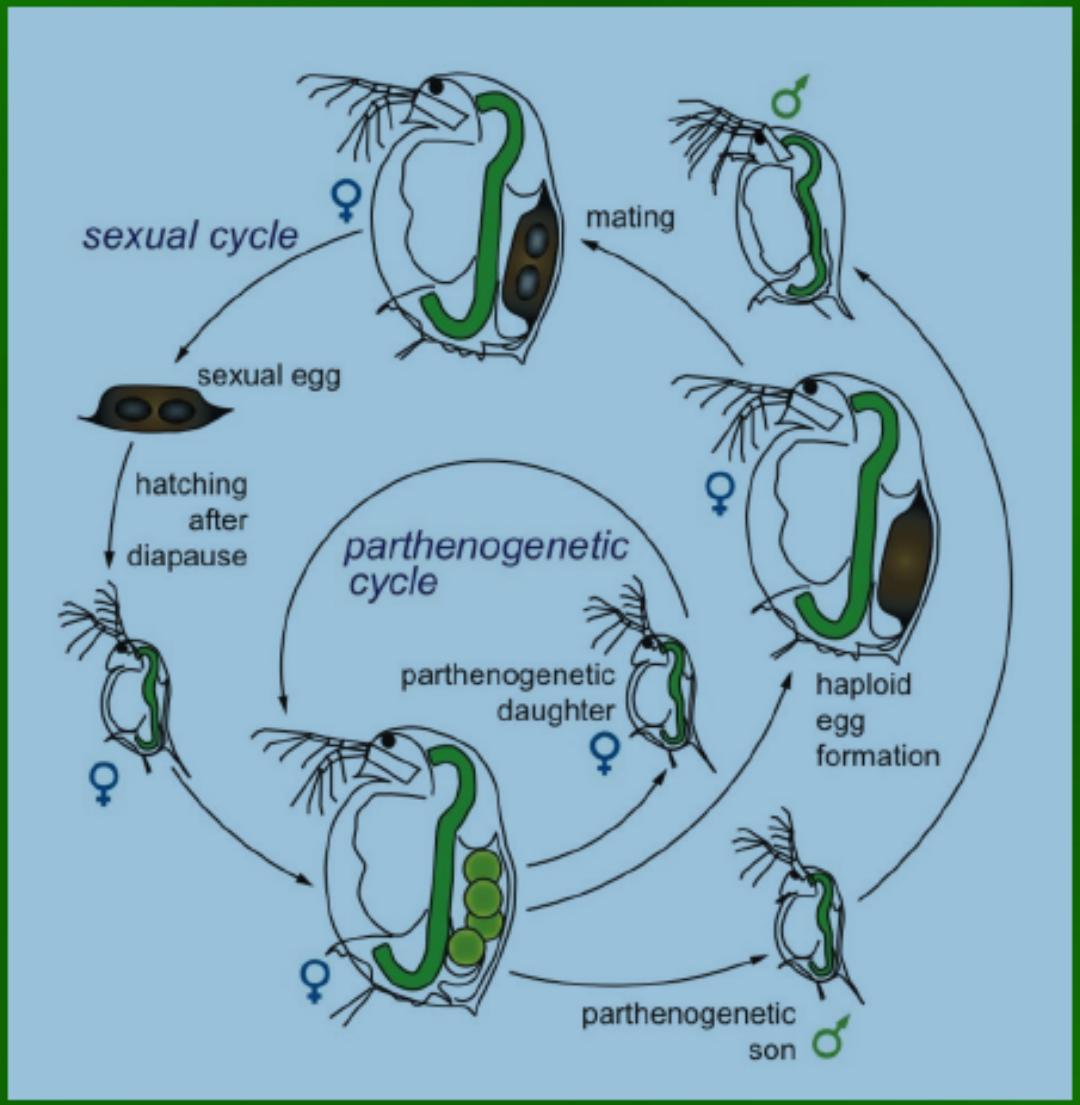


Proteomic approach to oxidative stress in *Daphnia magna*



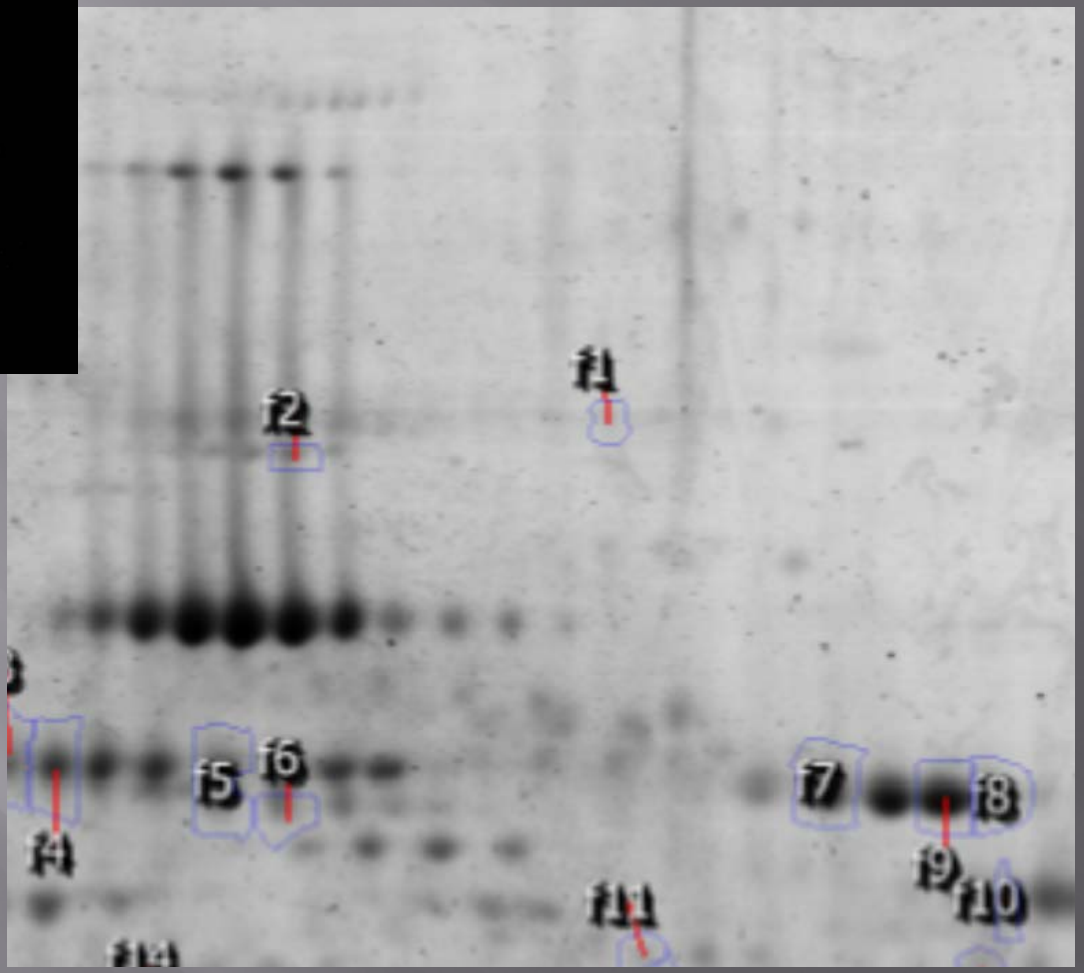





sexual cycle
mating
sexual egg
hatching after diapause
parthenogenetic cycle
parthenogenetic daughter
haploid egg formation
parthenogenetic son



Daphnia magna exposed to CuO NPs



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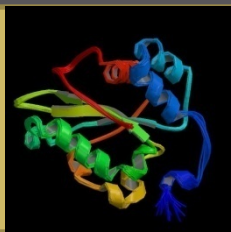


Table 2: Identified proteins using LC-MS/MS

Feature	Protein	Organism	Accession number	MW (kDa) (Measured / Predicted)	Protein Score[a] / Protein C.I. (%)	Total Ion Score / Total Ion C.I. (%)	Sequence coverage (%)	Number of peptides[b]
1	hypothetical protein DAPPUDRAFT_318420	*Daphnia pulex*	gi|321469729	98/171	362/100	289/100	17	3
3	hypothetical protein DAPPUDRAFT_213992	*Daphnia pulex*	gi|321465380	68/228	153/100	153/100	10	2
4	hypothetical protein DAPPUDRAFT_213992	*Daphnia pulex*	gi|321465380	68/228	162/100	144/100	11	2
5	hypothetical protein DAPPUDRAFT_213992	*Daphnia pulex*	gi|321465380	68/228	205/100	162/100	16	2
7	hemoglobin	*Daphnia magna*	gi|2105139	66/38	319/100	301/100	19	2
10	hypothetical protein DAPPUDRAFT_313764	*Daphnia pulex*	gi|321474716	16/170	283/100	283/100	12	
15	vitellogenin fused with superoxide dismutase	*Daphnia magna*	gi|39979307	40/225	559/100	485/100	14	2
18	hypothetical protein DAPPUDRAFT_326737	*Daphnia pulex*	gi|321460806	19/29	447/100	381/100	52	4

[a] The protein score probability limit (where $p < 0.05$) is 86.
[b] Number of MS/MS patterns assigned to peptides with confidence interval above 95 %.



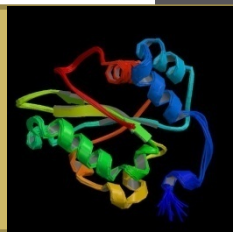


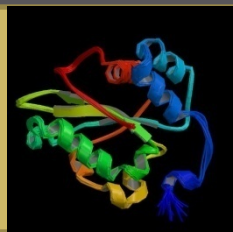
Table 3: Blast and interproscan search of hypothetical proteins

Feature	Accession Number	Best hit[a] (protein (*species*), E-Value, Identity %)	Families and Domains[b]	Go functions[b]
1	gi|321469729	Vitellogenin-like protein (*Lepeophtheirus salmonis*) E: 3e-106, Identity: 24%	von Willebrand factor, type D Domain; Vitellinogen, superhelical; Vitellinogen, beta-sheet N-terminal	Lipid transport activity; Lipid transport
3,4,5	gi|321465380	Vitellogenin fused with superoxide dismutase (*Daphnia magna*) E: 0.0, Identity: 52%	Superoxide dismutase (Cu/Zn)/chaperones; von Willebrand factor, type D Domain; Vitellinogen, open beta-sheet; Vitellinogen, beta-sheet N-terminal	Superoxide metabolic process; Metal ion Binding; Oxidation-reduction Process; Lipid transport activity; Lipid transport
10	gi|321474716	Vitellogenin-like protein (*Lepeophtheirus salmonis*) E: 3e-106, Identity: 24%	von Willebrand factor, type D Domain; Vitellinogen, superhelical; Vitellinogen, beta-sheet N-terminal	Lipid transport activity; Lipid transport
18	gi|321460806	14-3-3 protein epsilon (*Schistocerca gregaria*) E: 2e-170, Identity: 91%	14-3-3 protein	protein domain specific binding

[a] Best hit results are obtained using a blastp search (Altschul et al., 1997).

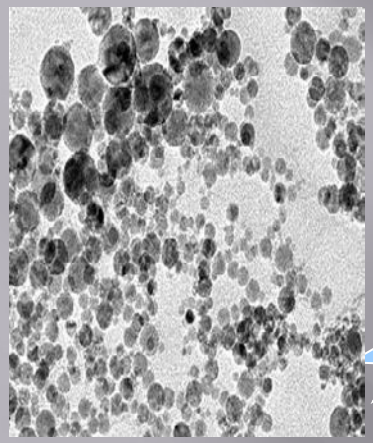
[b] Families, domains and GO functions were obtained using Interproscan (Zdobnov and Apweiler, 2001).





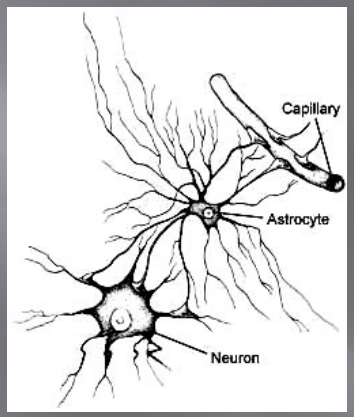
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SEVENTH FRAMEWORK PROGRAMME



Nanoparticles

Astrocytes cells



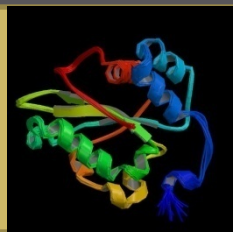
Oxidative stress?
Anti-oxidants?
NPs accumulation?

Mytilus Edulis



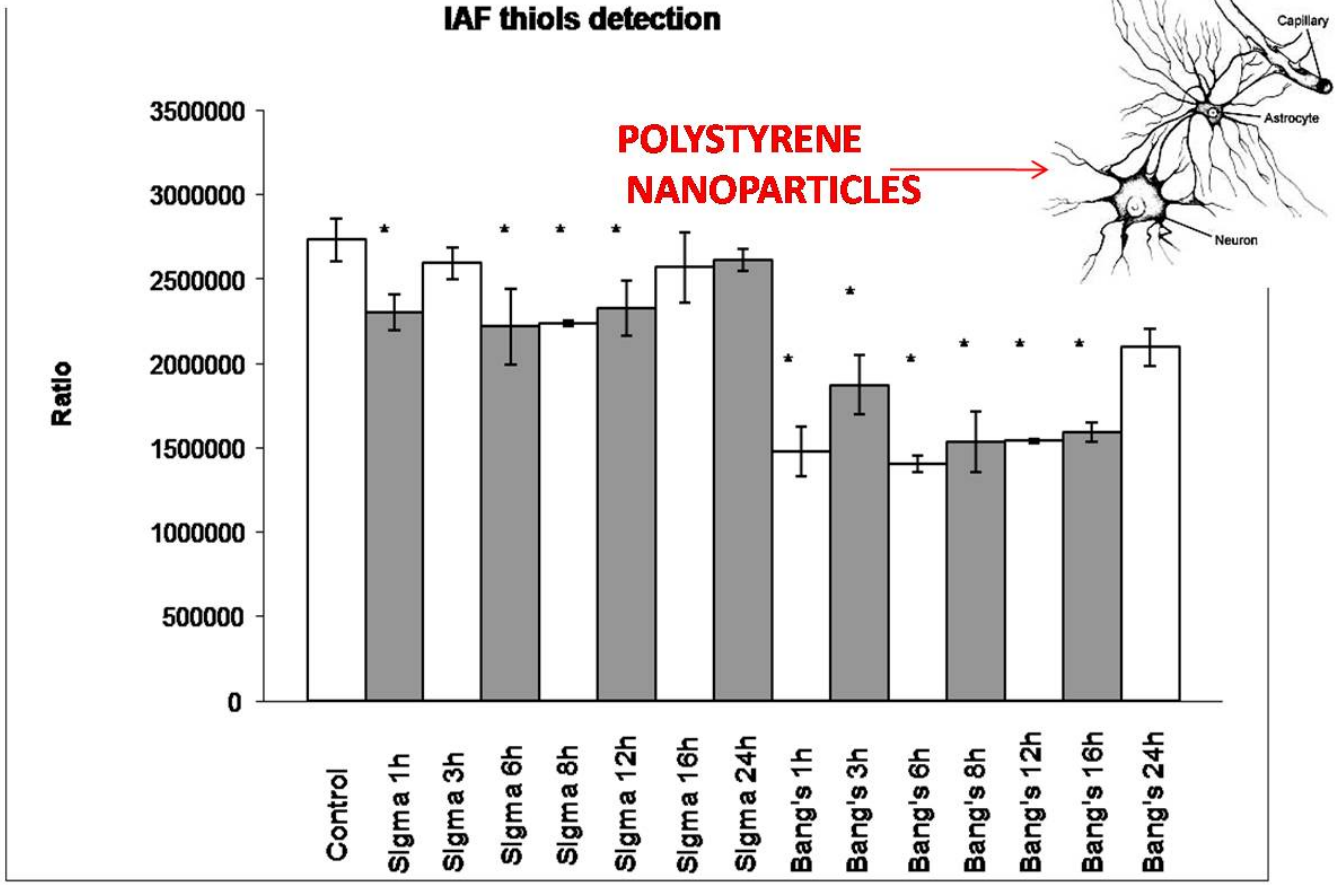
Other animal samples
(Collaboration labs)

Proteomics Research Group, School of Biochemistry & Cell Biology, UCC



NeuroNano

Cultured Human Astrocytes

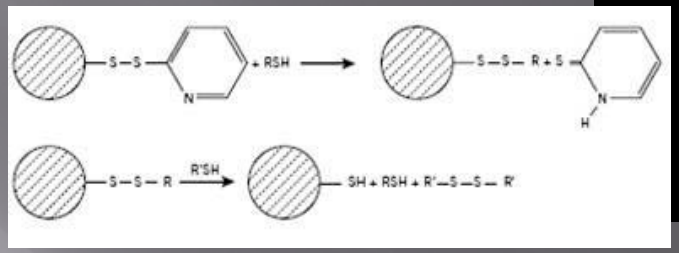


P<0.05

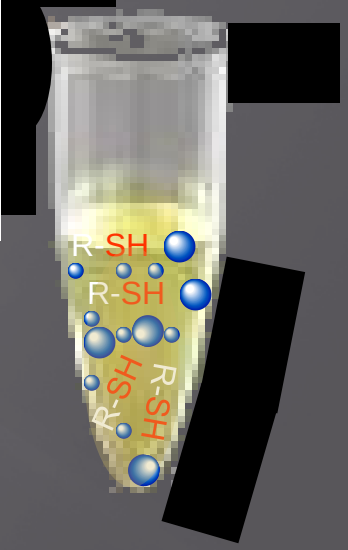
Selection of proteins with thiol groups



+



Activated thiol sepharose

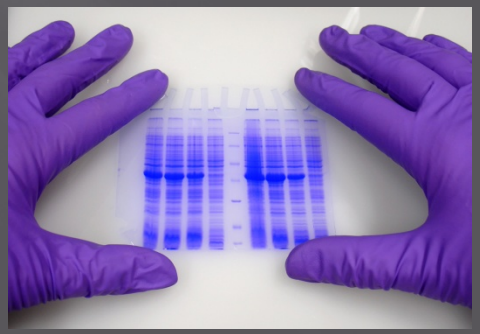


DTT @





Thiol- proteins



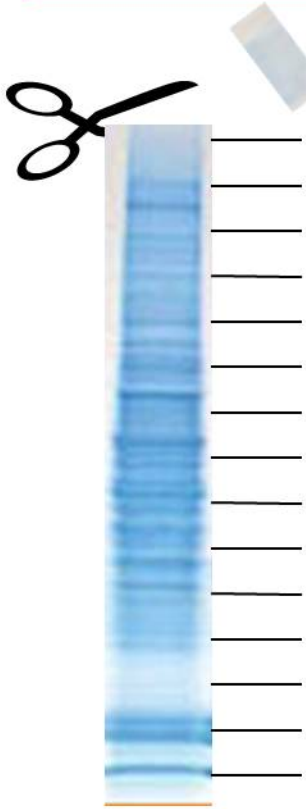
Hu et al. (2010) *Analytical Biochemistry*, **398** :245-253.

NeuroNano

CULTURED HUMAN ASTROCYTES – POLYSTYRENE NPs

Thiol fraction recovered and analysed by mass spectrometry

1) Eluted proteins are separated by 1D SDS-PAGE



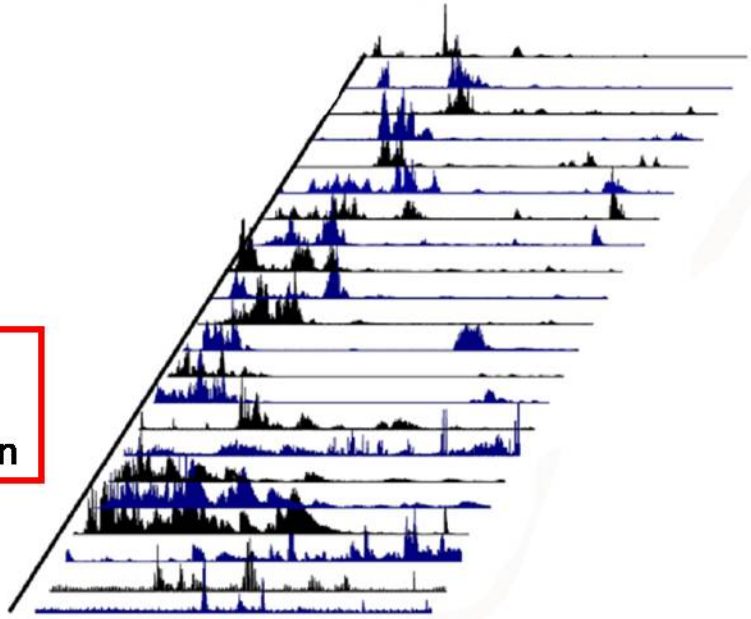
2) Gel lanes are sliced



3) In-Gel trypsin digestion, peptide extraction & purification



4) Reverse Phase HPLC – MS/MS of each gel slice



5) Protein Identification (e.g., Sequest)

~5000 peptides

~1400 unique peptides

~200 proteins/lane

NeuroNano

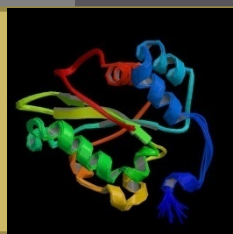
PROTEIN IDENTIFICATION

12 and 24h exposure, NH_2-PS on astrocytes

- Identified 780 proteins in the thiol fraction
- Spectral counts give semi-quantitative information on protein concentration
- All proteins containing thiol groups, no background!

			Spectral count			Relative SpC (to total SpC)*100		
Acc number	PROTEIN	Mw	Control	12h	24h	Control	12h	24h
Q15149	Plectin	531465.9	144	69	134	3.88	3.48	4.73
P08670	Vimentin	53619.2	101	25	91	2.72	1.26	3.21
P10809	60 kDa heat shock protein, mitochondrial	61016.5	57	11	36	1.54	0.55	1.27
P02545	Lamin	74094.8	53	15	30	1.43	0.76	1.06
P12111	Collagen alpha-3(VI) chain	343450.3	51	35	42	1.37	1.77	1.48
P21333	Filamin-A	280561.4	50	31	33	1.35	1.56	1.16
P07355	Annexin A2	38579.8	46	18	37	1.24	0.91	1.31
P02768	Serum albumin	69321.6	42	9	30	1.13	0.45	1.06
Q13813	Spectrin alpha chain	284362.5	41	18	26	1.11	0.91	0.92
P50454	Serpin	46411.3	37	13	28	1.00	0.66	0.99
P38646	Stress-70 protein, mitochondrial 2	73634.8	35	19	21	0.94	0.96	0.74
P06733	Alpha-enolase	47139.4	34	15	35	0.92	0.76	1.23
P60709	Actin, cytoplasmic	41709.7	32	8	21	0.86	0.40	0.74
P35579	Myosin-9	226390.6	30	20	22	0.81	1.01	0.78
P14618	Pyruvate kinase isozymes	57900.2	29	13	16	0.78	0.66	0.56
Q01082	Spectrin beta chain	274437.2	27	1	5	0.73	0.05	0.18
P62736	Actin	41981.8	27	4	22	0.73	0.20	0.78

Etc.



NeuroNano

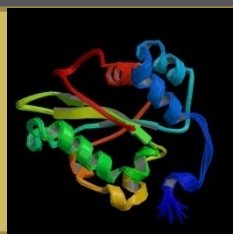
Several proteins, among the most oxidised, connected to cytoskeleton and cell structure

Selecting the proteins with higher fold change in respect to untreated

Cell movement and uptake

		0h	12h	24h
Q01082	Spectrin beta chain,	1.00	0.07	0.24
O75369	Filamin	1.00	0.94	0.65
P04083	Annexin	1.00	1.19	0.48
P37802	Transgelin	1.00	0.94	0.13
Q14204	Cytoplasmic dynein 1	1.00	0.19	0.26
P68363	Tubulin alpha	1.00	0.37	0.65
P12109	Collagen alpha	1.00	0.83	0.29
P02786	Transferrin receptor protein 1	1.00	1.17	0.49
P51149	Rab7a	1.00	0.80	0.00
P60660	Myosin light polypeptide	1.00	0.62	0.22
O00159	Myosin Ic	1.00	0.00	0.22
P61586	Transforming protein RhoA	1.00	0.31	0.00
P19105	Myosin regulatory light chain	1.00	0.75	0.26

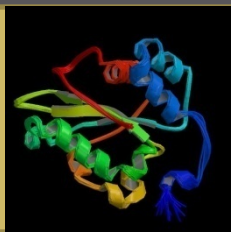
Also: proteins associated to mitochondria and their function, Ribosomal proteins ...



CONCLUSIONS

- NANOPARTICLES CAN ENTER ORGANISMS AND CROSS BIOBARRIERS
- THEY FREQUENTLY TRIGGER OXIDATIVE STRESS
- OFTEN, CYTOSKELETAL OR STRESS-RESPONSE PROTEINS ARE TARGETS
- GEL-BASED AND GEL-FREE PROTEOMICS OFFER ROUTES TO TARGET IDENTIFICATION

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www.ludesi.com)

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