



Nomen est omen



From antioxidant activity to complex redox modulation and intracellular signalling via the cellular thiolstat

Claus Jacob

**Bioorganic Chemistry, School of Pharmacy
Saarland University, Saarbrücken, Germany**



- Ischia is not Ischias
- Saarland is not Saharaland
- Johanna Wanka is not Willy Wonka
- Redox is not a Red Ox
- Polysulfides are not polysulfanes
- 80% Diallyldisulfide is not 100% Diallyldisulfide

Overview of today's talk

- Redox control and the cellular thiolstat
- Interferences using sulfur-containing nutraceuticals
- From nutrition to wider applications
- Intracellular diagnostics
- What's next ?



Organochalcogen Compounds in Chemistry and Biology

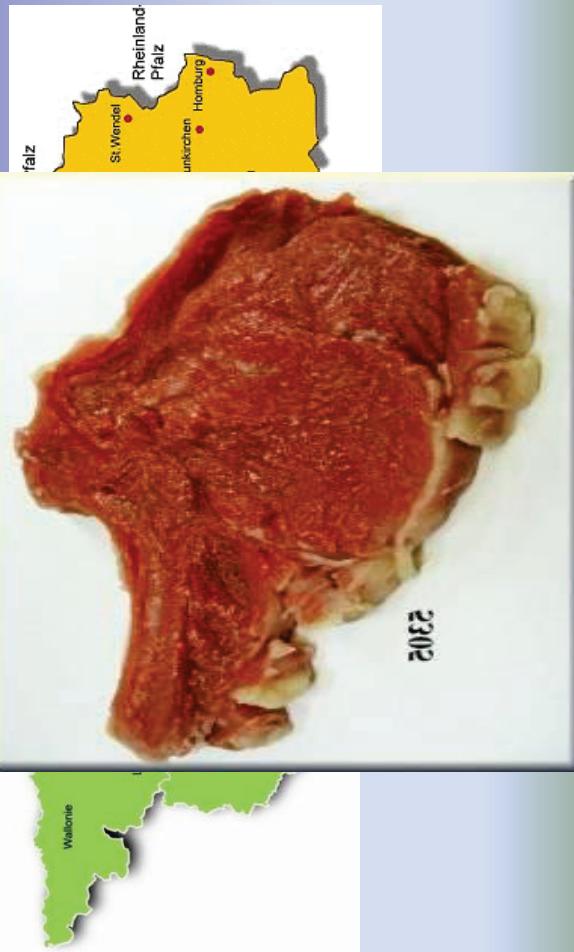
From antioxidant activity to complex redox modulation and intracellular signalling via the cellular thiolstat

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A warm welcome from the Saarland





Reactive Species and Oxidative Stress



Reactive Species

	Reactive Species	Ox. State	Occurrence
Singlet Oxygen	${}^1\text{O}_2$	0	UV-radiation, causes skin cancer
Superoxide	O_2^\bullet	-0.5	Inflammation, metabolism
Peroxide	H_2O_2	-1	Inflammation, metabolism
Hydroxyl radical	HO^\bullet	-1	Fenton reaction
Nitric oxide	$\cdot\text{NO}$	+2 (N)	NOS
Peroxynitrite	ONOO^-	-1 (O)	$\text{O}_2^\bullet + \cdot\text{NO}$

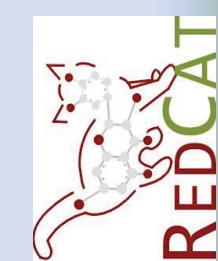
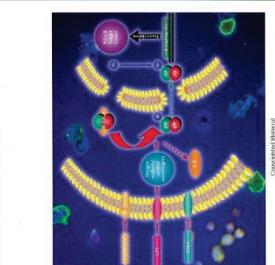
5

Redox control and the cellular

thiolstat

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Edited by Claus Jacob,
and Paul C. Winyard
**Redox Signaling and
Regulation in Biology
and Medicine**



Reactive Species and Oxidative Stress

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Sulfur in (human) Biology

- ✓ High abundance of sulfur *in vivo* (often millimolar concentrations)
- ✓ Up to 10 oxidation states of sulfur known *in vivo*
- ✓ Sulfur species may react by a variety of (redox) mechanisms
- ✓ Most cysteine redox transformations lead to biochemical consequences
- ✓ Most cysteine redox transformations are reversible
- ✓ Sensing, Signalling cascades and feedback loops are possible

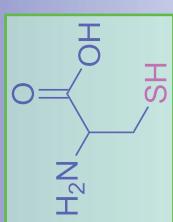
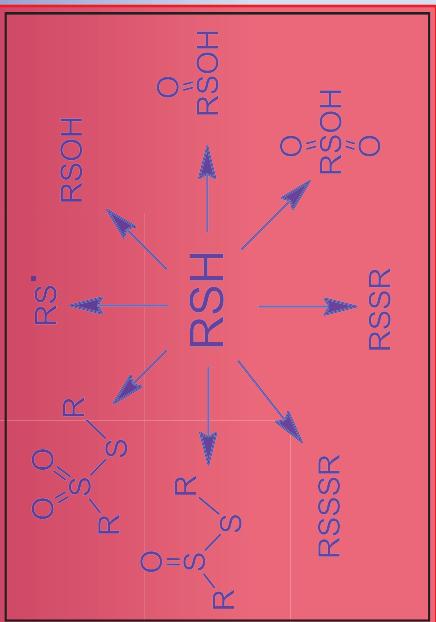


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The Special Relationship

- Redox processes follow a certain (molecular) mechanism
- In Biology, electron transfer is common but not necessarily always preferred
- Other mechanisms include radical reactions, atom transfer, hydride transfer and nucleophilic substitutions with simultaneous changes of oxidation states (e.g. exchange reactions)
- Exchange reactions are common with oxygen, sulfur and selenium and tellurium based molecules
- Many ROS react effectively, yet also selectively with sulfur, selenium and tellurium based molecules

Reactive Sulfur Species in the human body



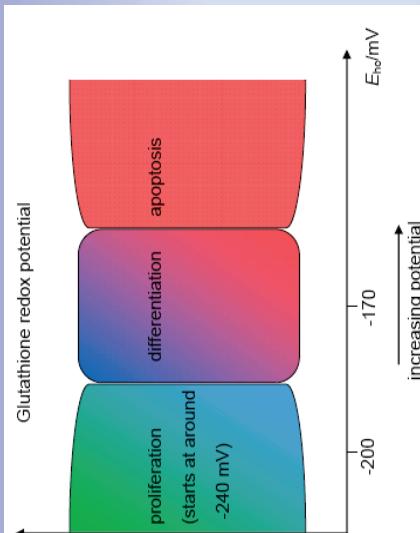
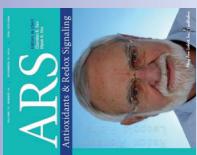
Giles et al., Free Radic. Biol. Med. 2001; Giles et al., Biochem. Biophys. Res. Commun. 2003, Chem. Biol. 2003; Jacob and Sies et al., Angew. Chem. 2003

Posttranslational modifications in peptides, proteins and enzymes

- Thiol oxidation
- S-thiolation of cysteine residues (mostly S-glutathiolation)
- Disulfide bond formation (including reversible protein-protein interactions) and - as reverse - erratic cleavage
- S-nitrosation (RSNO formation and 'NO release)
- Over-oxidation to form the cellular 'sulfenome' (sulfenic, sulfonic acids)
- "Odd" modifications (perthiols, trisulfanes)
- Metal-sulfur interactions (metal-redox interdependence)

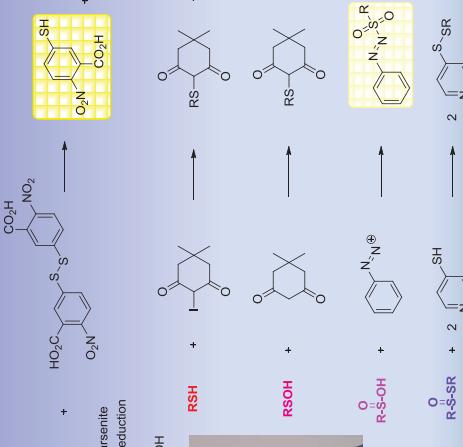
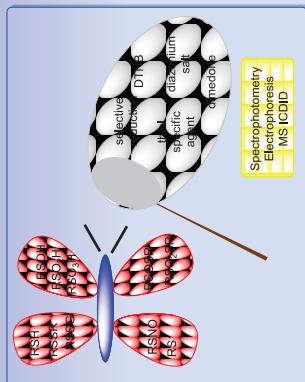


Once upon a time: Electrochemical potential, GSSG:GSH ratio and cellular responses



Schafer and Buettner Free Radical Biol. Med. 2001
Jamier et al., Chem. Eur. J. 2010

Trapping and hunting sulfur modifications



Jacob et al., ChemBioChem 2011

13 Proteins and enzymes affected by S-thiolation/dethiolation

Inactivated by protein thiolation
Pyruvate kinase
Hexokinase
Phosphofructokinase
Pyruvate dehydrogenase
Guanine nucleotide exchange factor
Thioredoxin phosphatase
Phosphoglycerate kinase
Glycogen synthase kinase 3
3-hydroxy-3-methylglutaryl-CoA reductase

Yup!

Activated by protein thiolation

Trypsin
Collagenase
Glucose-6-phosphate dehydrogenase
Fructose-1,6-biphosphatase

And many more.....

(especially cysteine-based redox proteins involved in cellular signalling)



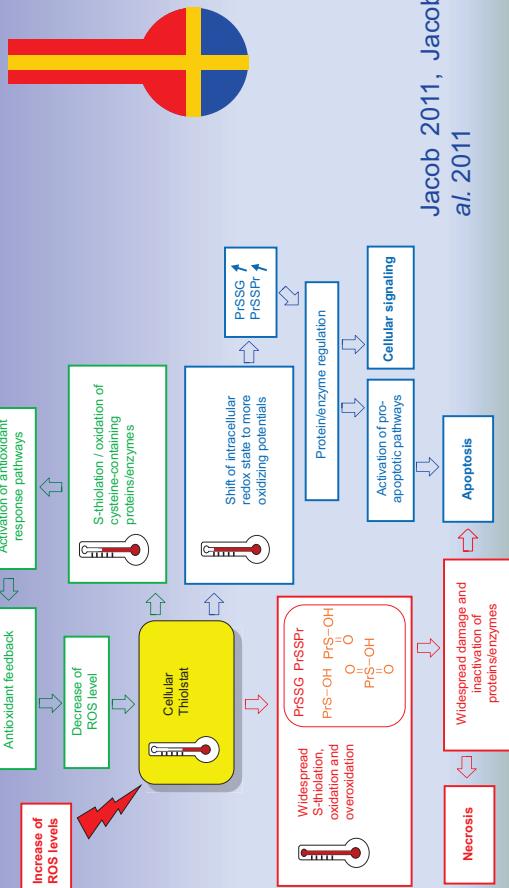
after Ghezzi et al., Redox Signaling and Regulation in Biology and Medicine, Wiley VCH 2009

14 From the “Sulfur Redoxome” towards the “Cellular Thiolstat”



Modulation of protein function and enzyme activity by redox manipulation of cysteine residues enables fast, appropriate, measured and largely reversible cellular responses – in one word: effective cellular signalling and regulation.

15 Signalling and Control via the Cellular Thiolstat



Jacob 2011, Jacob et al. 2011

16 Is it possible to interfere with the cellular thiolstat?

Interferences using sulfur-containing nutraceuticals



Organic Sulfur Compounds: Plants and Fungi

Sulfur-based remedies: Haarlem oil

17

Haarlem oil provides a highly bioavailable sulfur !
Haarlem oil has demonstrated its effectiveness in preventing respiratory illness, passive and active smoking, unhealthy diets and rheumatoid arthritis. It is an energy supplement that promotes recovery after exercise, avoid aches and reduces mental fatigue. The Genuine Haarlem Oil is composed of three simple elements inside a 200mg capsule:

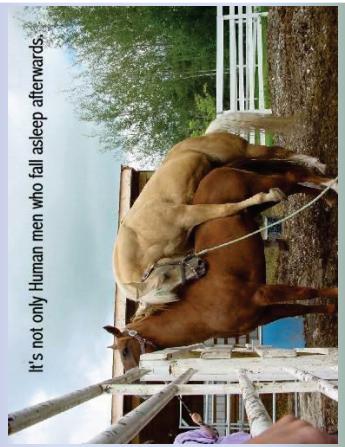
- Sulphur 16%**
- Pine turpentine 80%**
- Linseed oil 4%**

The Elderly

The demand of Sulphur is more important with its free **anti-radical actions**, as the first signs of aging are numbness of the joints (often at night or when arising in the morning).

Young people, Athletes and Intellectuals

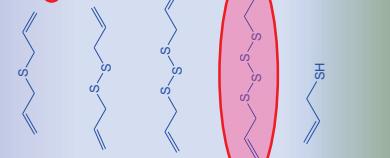
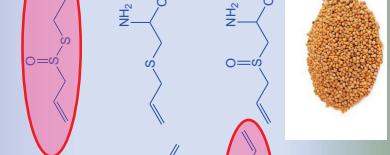
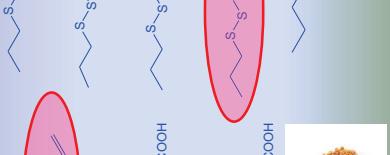
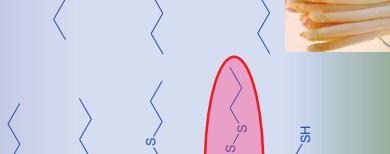
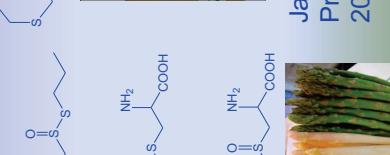
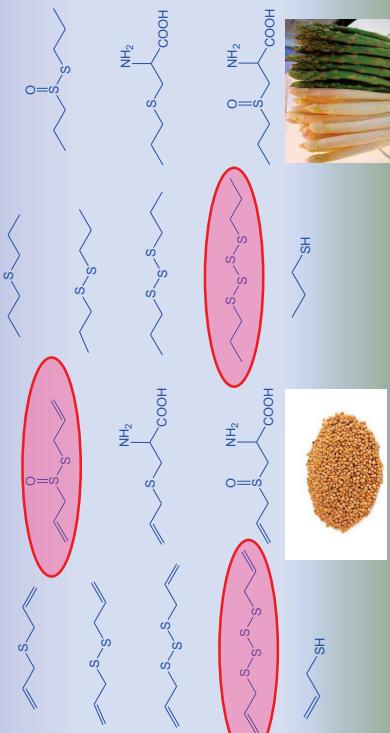
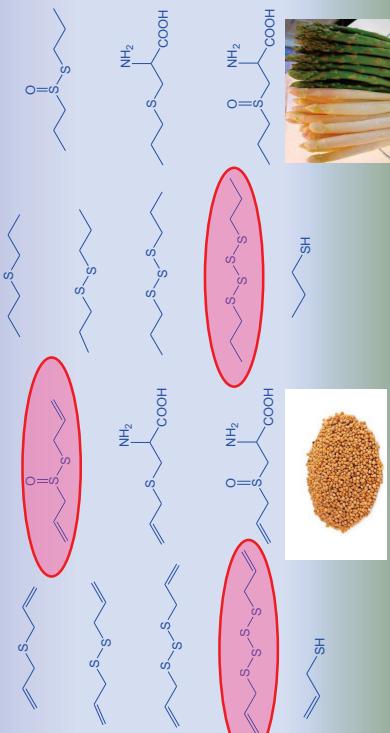
Sulphur is an important detoxification for the liver and kidneys. It plays an energetic and anti-fatigue role as it preserves the energetic potential that is reactivated during a physical and intellectual effort. It also prevents muscle aches after strenuous physical efforts.



NPR

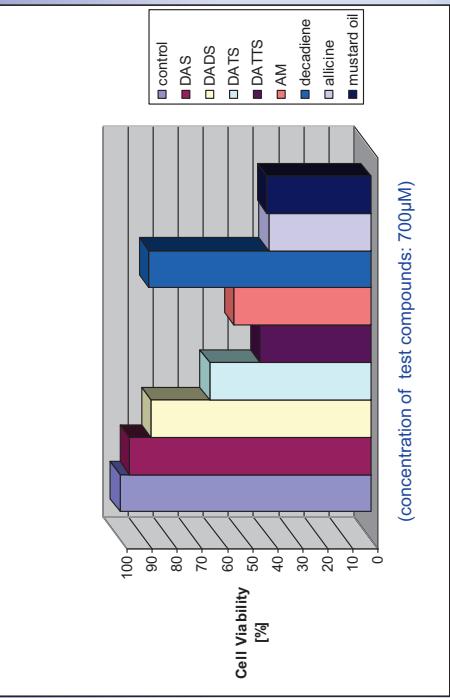


Jacob, Natural Product Reports 2006

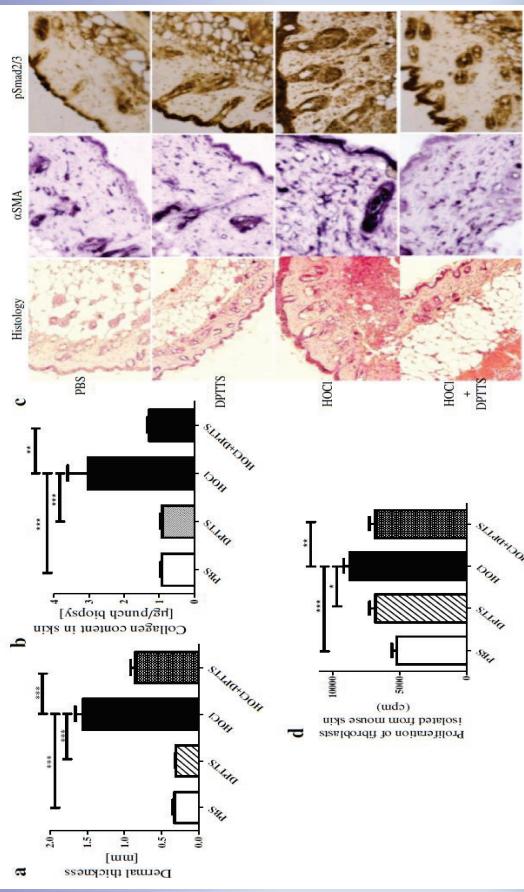


19 Diallylpolysulfanes and Caco-2 Cells: Inhibition of Proliferation

Allyl disulfide technical grade, 80%
 $\text{H}_2\text{C}=\sim\text{S}-\sim\text{CH}_2$

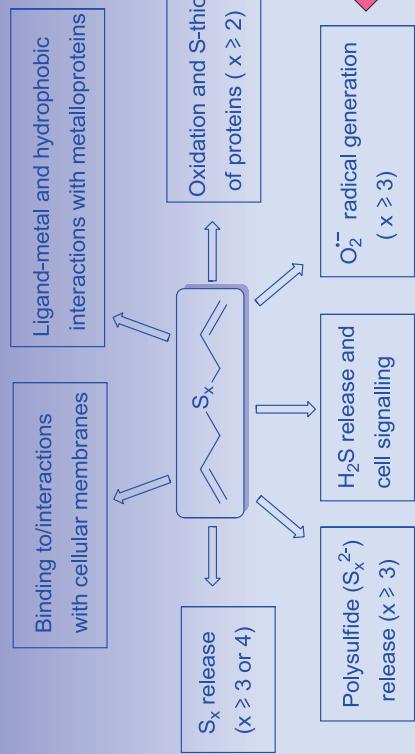


20 Dipropyltetrasulfane and Scleroderma



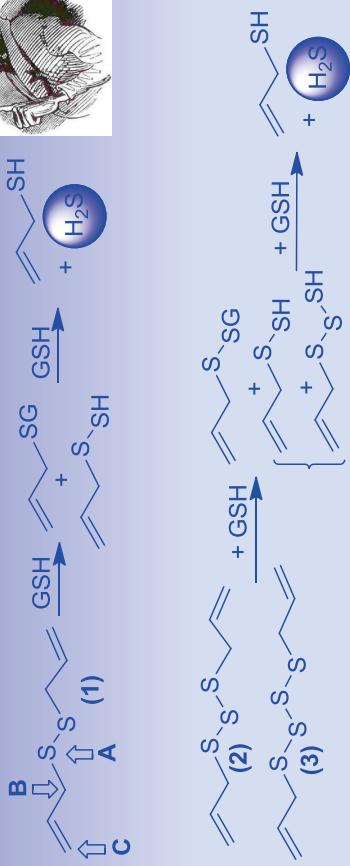
Facets of the biological chemistry of polysulfanes

21



Simple H_2S release from di-, tri- and tetrasulfanes

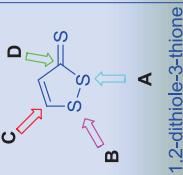
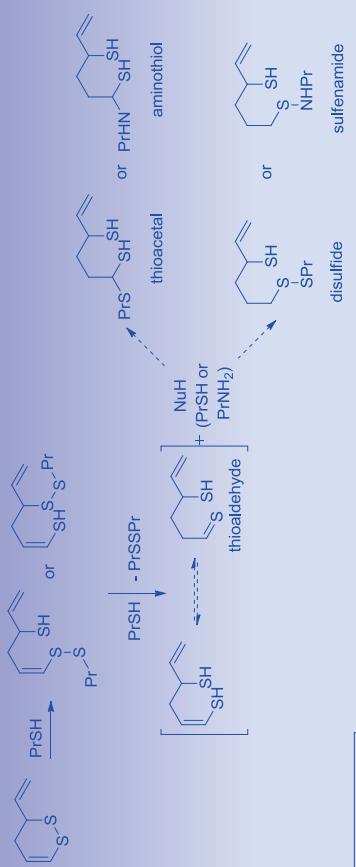
22



Benavides et al., Natl. Acad. Sci. USA 2007
Das et al., Am. J. Physiol. Heart. Circ. Physiol. 2007
Jacob et al., Planta Med. 2008

Thioaldehyde formation from α,β -unsaturated thiols

23

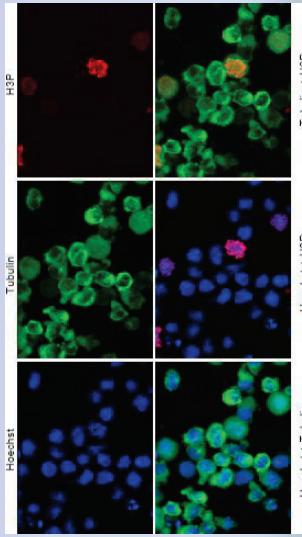


Polysulfanes trigger apoptosis in certain cells

24



Microscopic analysis of influence of the tetrasulfanes on the tubulin network in U937 cells after 4 h incubation time.



A.K. Baltases, Diploma thesis

Schematic overview of a prospective mode of action of the diallyl- and dipropyl-tetrasulfanes.

Czepukojc et al., Food and Chemical Toxicology 2013

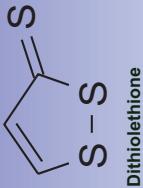


Botrytis cinerea on tomato stem.
Source: wildaboutbritain.co.uk



Botrytis cinerea on grape.
Source: acemologia.com

Botrytis assay



Growth inhibition of MDR2 strain on Dithiolethione

		Concentration		
		0.1 mM	0.2 mM	0.3 mM
Control MDR2	Dithiolethione			
		Slight inhibition	Less germination	Strong germination

Czepukojc et al., Natural Product Communications 2013

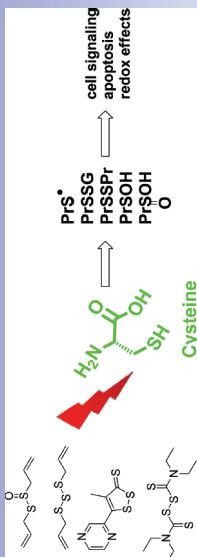
Possible applications of the chalcogen compounds

Medical Applications:

- Cancer Therapy
- Scleroderma

Agricultural Applications:

- „Green“ Pesticides and Fungicides



Reactive Sulfur Species trigger apoptosis in certain cells



Applications

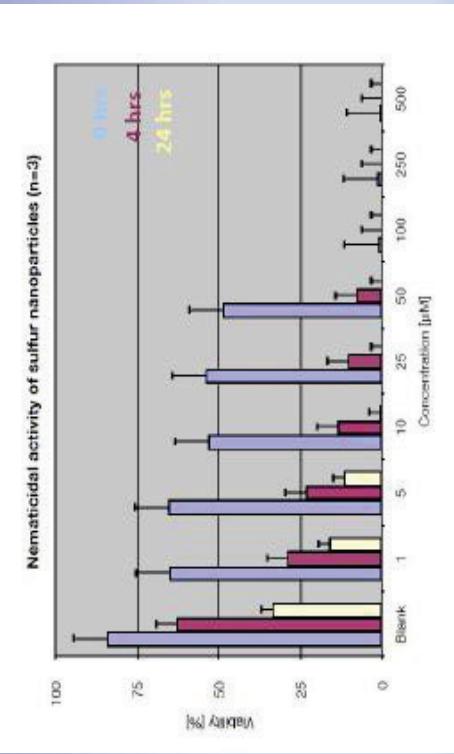
From Nutrition to Wider



The Special Role of Selenium and Tellurium

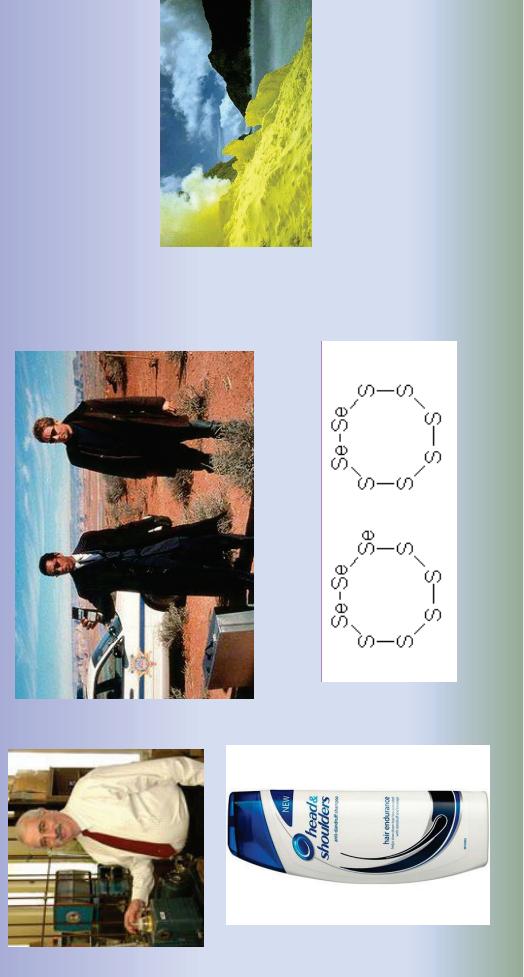


Sulfur-based remedies: Nanoparticles



Schneider et al.: J. Biomed. Nanotechnol. 2011

Chalco-gen-based remedies

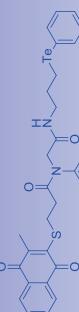


From Sulfur to Selenium and Tellurium

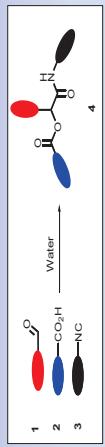


Sensor/effectors

Multicomponent Ugi-reaction:

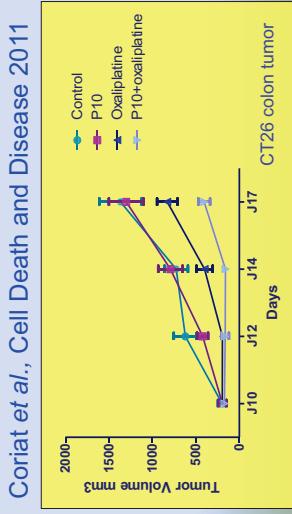
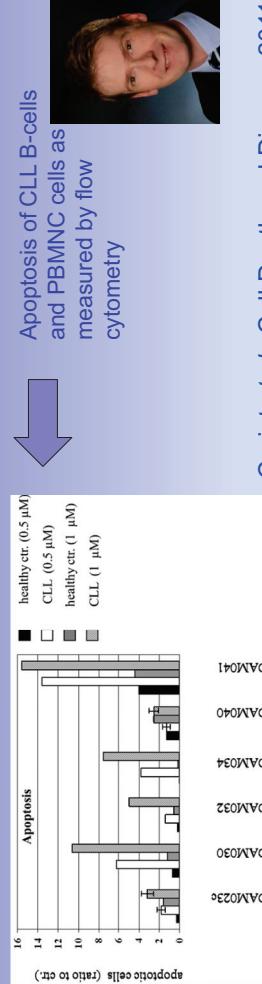


IC 5-10 M



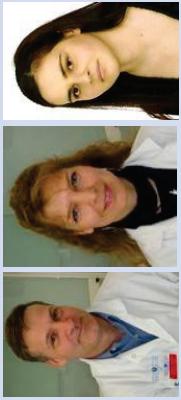
Note: There is a brand-new cluster of Se and Te chemists and biochemists emerging across Europe !!

Cytotoxicity and Apoptosis of CLL B- and PBMN³³ cells

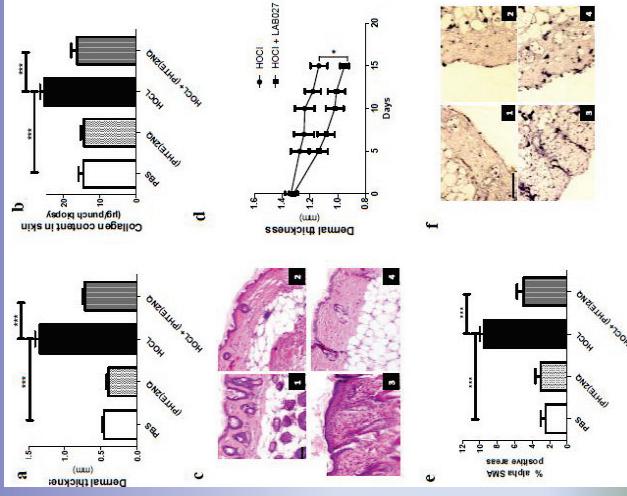


Doering et al., J. Med. Chem. 2010

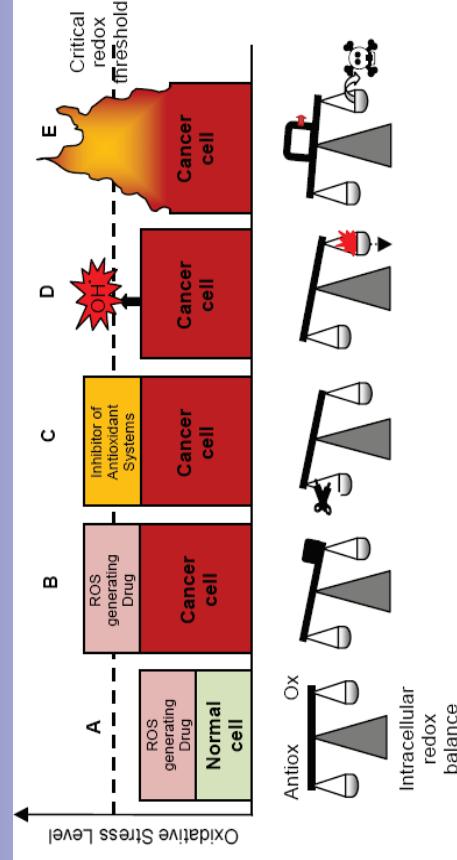
Effects of Te compounds on skin fibrosis induced by HOCl in BALB/c mice



Marut et al., J. Invest. Dermatol. 2012

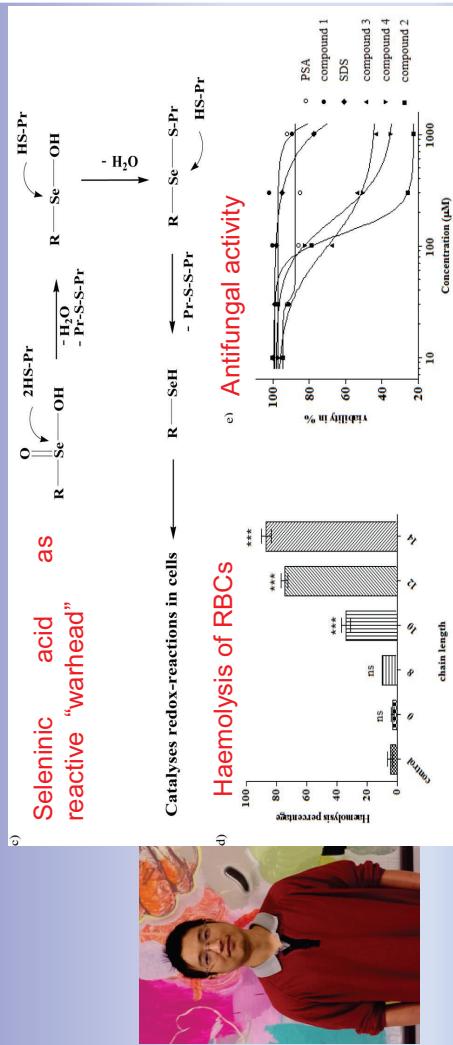


Catalysis as key to efficiency and selectivity



Jamier et al. / Chem Eur J 2010

Amphiphilic seleninic acids as 'double impact' agents



Dii et al. MedChemComm 2014

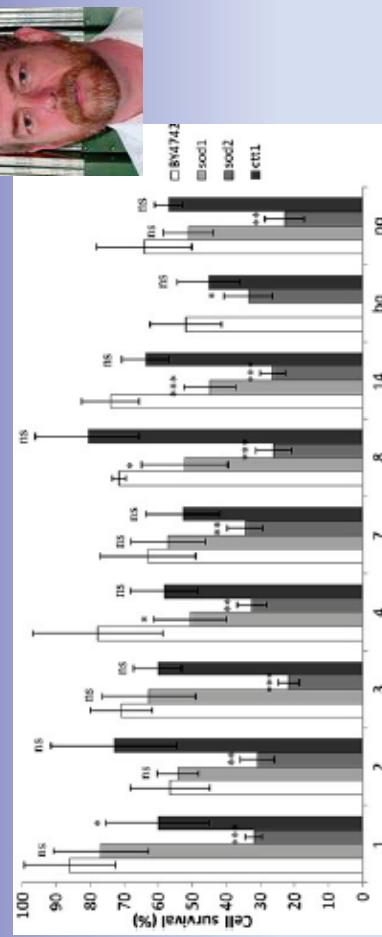
Deciphering the mode of action

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Chemogenetic analysis in yeast

39

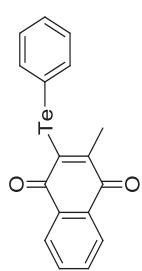


Doering *et al.*, Tetrahedron 2012
Mániková *et al.*, Molecules 2014



Impedance, cell growth curves and cluster analysis

38

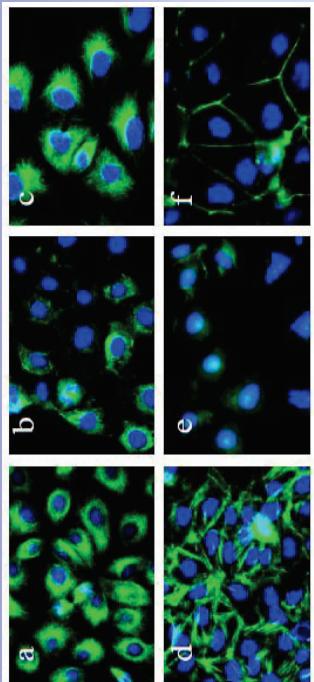


Hierarchical cluster analysis of growth data measured by impedance: Tellurium compounds cluster with tubulysin B, griseofulvin and nocodazole

Schneider *et al.*, MedChemComm 2012

Cellular staining techniques

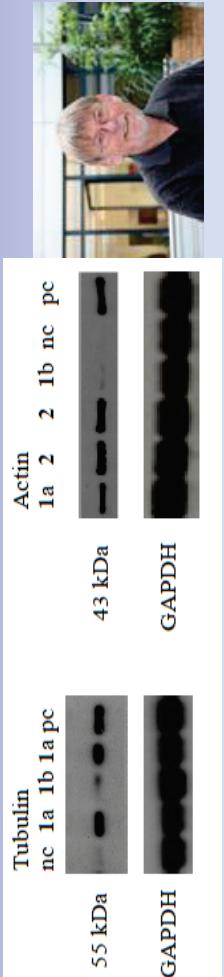
40



Fluorescent microscopy of microtubuli (upper row) and microfilament structures (lower row) of PtK2 cells after incubation with Te compounds for 18 h (a and d as negative controls)

Schneider *et al.*, MedChemComm 2012

DARTS with Western Blot analysis



Drug Affinity Responsive Target Stability Analysis of compound-protein interactions using KB-3-1 cell extracts and Pronase

Schneider et al., MedChemComm 2012

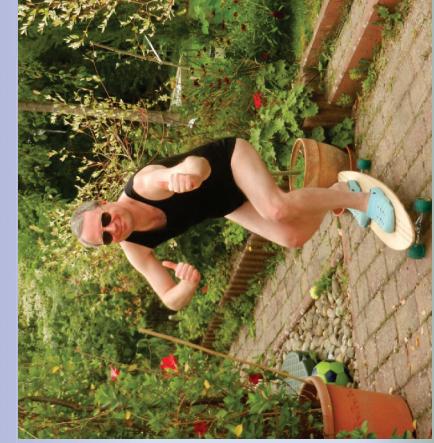
Possible applications in the context of inflammatory disease(s)

ASK FOR THE COINS AT THE CAFE!
ԱԵՍԱՌՈՂԻ ՀԱՍՈՒ ԿՐՈՒՔ ՍՈԲՈՐԱԾ
ДЛЯ ПРИОБРЕТЕНИЯ МОНЕТ ОБРАЩАЙТЕСЬ В КАФЕ

Warning! Persons having serious known acute disease, heart problem, people receiving medical treatment, people with heart pacemakers, serious blood circulation problem in the legs, high fever, abnormal spinal problems, respiration apparatus problem should not use the product.

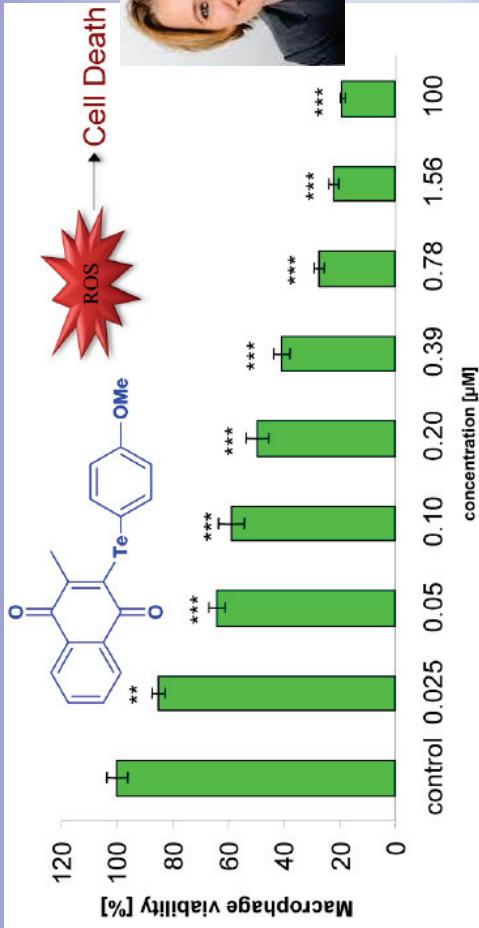
Линейка

What's next ?



A photograph showing a person's lower legs and feet. They are wearing blue flip-flops and light-colored pants. They are standing on a paved surface made of large, rectangular stones. To their left is a garden bed with various plants, including red flowers and green leafy vegetables. A wooden bucket is resting on the ground near the plants.

High efficiency of nanomolar concentrations of Te compounds against macrophages



Doering et al., Tetrahedron 2012

Exotic Adventures

45



The Team: "Academiacs International"

46



Dr. Torsten Burkholz
Dr. Ethiene Estevam
Dr. Uma M. Viswanathan
Nassifatou Koko Tittikpina
Lisa Faulstich
Adel Al-Marby
Jawad Nasim

Aman Bhasin
Nashrawan Lababedi
Marina Hakenesch
Sandra Hübgen
Javeria Iftikhar
Nour Jamal Aldabbas
Sharoon Griffin



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Money for nothin' and chicks for free

M. Montenath (UdS)
I. Bernhardt (UdS)
A. Kiemer (UdS)
C.-M. Lehr (UdS)
F. Sasse (Braunschweig)
A. Slusarenko (Aachen)
M. Herling (Cologne)
Karl-Herbert Schäfer (Zweibrücken)
Ludger Wessjohann (Halle)
F. Batteux (Fr)
M. Diederich (Lux/Korea)
G. Kirsch / D. Bagrel (Fr)
N. Latruffe / M. Cherkaoui Malki
M. Chovanc
P. Arsenjans



"NutriOx" 2014 in Metz

48



Workshop NutriOx 2014
Nutrition and Ageing
1 – 3 October 2014
Metz Technopôle
France



<http://www.ijb.univ-lorraine.fr/sip.php?article176&lang=fr>

