This is a milieu of creativity for “thinking the unthinkable” and doing experiments about it on the Adriatic coast in Split (Croatia)

**MedILS**: Mediterranean Institute for Life Sciences([www.medils.hr](http://www.medils.hr))
Future Mediterranean Institute for Life Sciences in 2003
Split
Croatia
Scientists’ most important work: thinking!
BIOLOGY OF AGING AND AGE-RELATED DISEASES:
SIMPLE CAUSE – COMPLEX CONSEQUENCES

Designing of a new medicine in MedILS: Predictive diagnostics of predispositions to diseases and their prevention and cure at protein level

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WHAT DOES TIME TO CELLS AND ORGANISMS TO BE THE MOST EFFECTIVE CAUSE OF MORBIDITY AND MORTALITY?

WHAT IS THE CHEMISTRY OF THE BIOLOGICAL CLOCK FOR AGING AND DISEASE?

WHAT DETERMINES INDIVIDUAL PREDISPOSITIONS TO DIFFERENT AGE-RELATED DISEASES?
“All things are difficult before they are easy.”
—Thomas Fuller

(1608-1661)

All things are complicated before they become simple (through knowledge)
90% mortality by age-related diseases is associated in a similar way (5\textsuperscript{th} power of age) – THE PROJECT:

DELAYING DESTINY
Figure 3 | Pathways shared between ageing in invertebrate model organisms, mammals and human progerias. Several features of ageing, which have been
#1: ROS
ROS, PROTEIN DAMAGE & ITS IMPACT

Elimination

DAMAGE (oxidation)

Protection

- Antioxidants (protection, defence)
- Chaperones (resistance)

Proteasome (clearance)

Aggregates (garbage)

PROTEOME (Function)

to produce

TRANSCRIPTOME (Blueprints)

copied as

GENOME (Instruction)

runs

maintains and regulates

LIFE
Mutator phenotype of chaperone deletions depends on high oxidative damage to misfolded proteins, since it is reduced by Trolox (vit E) proportionally to decrease in protein carbonylation.

Surviving radiation and age *versus* acquisition of protein carbonylation

I. Radiation dose (γ or UV)

II. Age

The crossing blue/red curves:

I. Versus radiation dose: (A) *E. coli*, (B) evolved resistant *E. coli*, (C) *D. radiodurans*

(A) Nematode *C. elegans*; (B) rotifer *Adineta vaga*

(A) Primary tumor; (B) derived metastasis

II. Versus species’ life span: (A) nematode (3 weeks); (B) rat (3 years); (C) human (100 years)
Cellular functional degeneracy, aging and death by proteome oxidation: an illustration of the effect of protein carbonylation (red dots) on a cellular interactome, at three levels of oxidation (I-III).

**FUNDAMENTAL AGEING PROCESS: CORROSION OF BIOLOGICAL FUNCTION (PROTEINS)**
PERFORMANCE OF GENETICALLY IDENTICAL INDIVIDUALS (NEMATODES), OF THE SAME AGE, IN AN IDENTICAL ENVIRONMENT (running on a gel in a weak electric field) CORRELATES WITH PROTEOME CARBONYLATION
X. Manière et al., Experimental Gerontology (2014) 60C: 12.

Hard to imagine that protein oxidation is the consequence of death, since - it predicts death!
AGING AND DISEASES ARE REVERSIBLE:
Heterochronic Parabiosis or just injection of **GDF11** rejuvenate old mice by all tested criteria and reverse all tested age-related diseases and conditions (Rando, Wyss-Correy, Comboy, Rubin, etc).

GDF11 rejuvenates aged skeletal muscle and brain. (A) Heterochronic parabiosis, which couples the circulatory systems of a young and old mouse, can restore youthful properties to many aged organs. (B) Treatment with rGDF11 alone revitalizes the skeletal muscle and brain of aged mice, resulting in functional improvements in strength and odorant detection.
Accelerated ageing correlates with accelerated carbonylation

Age-related changes in the amount of oxidized proteins in cultured human dermal fibroblasts. (Aging and radiation damage proteins similarly.)

Data from: Oliver et al., JBC 262: 5488-5491 (1987)
A small fraction of human proteins are oxidable – most are “inox”! Preliminary analysis of a human Hepatocarcinoma (HCC) vs. Non-tumoral (NT) adjacent tissue, the same liver. Most oxidized (red spots) proteins are multiple isoforms with different levels of single-protein oxidation (pI change).

(2D Oxy-DIGE): green is protein, red is carbonylation

Romain Ladouce & Fernando Martin, unpublished.
**Competitive Antagonism between Folding and Oxidation**

*Krisko & Radman, PLoS Genetics 2013*

Evolved structural resistance to oxidation: protein oxidation is “punishment” for imperfect folding
The Causes of Protein Carbonylation

Control | Hydrogen peroxide | Streptomyacin

ROS

Susceptibility to ROS

Dukan et al., PNAS 2000
FIXED PROTEIN ERRORS ("SILENT" MUTATIONS/POLYMORPHISMS) ALTER PROTEIN INTRINSIC OXIDABILITY – EXEMPLE:

Human α-synuclein – single amino acid substitutions sensitize this protein to spontaneous and radiation-induced oxidation

A30P – Parkinson at 60y (red)
A53T – Parkinson at 30y (blue)
“SILENT” POLYMORPHISM AT YOUNG AGE BECOMES “LOUD” AT OLD AGE

m1, m2, m3, protein (iso)morphs
INBORN SYNDROMES

VERSUS

INBORN PREDISPOSITIONS

The nature of mutation

**Syndrome:** null mutation with immediate phenotype

*versus*

**Predisposition:** missense “silent” mutation with delayed phenotype (conditional – time/oxidation dependent – mutations)
#2: TISSUE HOMEOSTASIS

BY

“CELLULAR PARABIOSIS”

– A SORT OF TISSUE-BASED CELLULAR SOLIDARITY –
Cell fusion experiments show recessiveness (loss of function) of cellular ageing and malignant phenotypes (Henry Harris et al. in 1950-ies).

Michael Stocker 1960-ies: No need to fuse cells – just direct cell contact suppresses malignancy!
Pre-malignant (« M ») and malignant (M) cell phenotypes...

...are suppressed by TnT. Hyaluronan protects TnT and other intercellular traffic structures.

Entire organs behave functionally like a single mega-cell with billions of nuclei.

Inflammation (tumour promotor) breaks down inter-cellular connections leading to expression of recessive (pre)malignant (« M » and M) phenotypes.

A mutant malignant (M) cellular mono-clone isolates its inside cells from the suppressing normal (N) cells and the malignant growth is ON.
Tunneling nanotubes allow intercellular traffic of RNA, proteins and entire organelles.
LIKE IN DEVELOPMENT OF B/W PHOTOGRAPHY, INFLAMMATION REVEALS HIDDEN/ LATENT PHENOTYPES OF FUNCTIONAL CELL DEFECTS (GENETIC OR EPIGENETIC) BY DESTROYING CELLULAR PARABIOSIS STRUCTURES VIA EXCRETED METALLOPROTEINASES (MMP)
Longevity versus Body Mass

? Difference in biology of a cell residing within small versus large animal ?
Specific Metabolic rate vs. Body mass

The graph shows the relationship between mass-specific metabolic rate (ml O2/g x h) and mass (kg) for various species. The x-axis represents mass in kilograms, and the y-axis represents the mass-specific metabolic rate in ml O2/g x h. Various species are labeled on the graph, including Shrew, Harvest mouse, Kangaroo mouse, Cactus mouse, Flying squirrel, Rat, Cat, Dog, Human, Horse, and Elephant. The graph demonstrates a trend where species with smaller masses have higher metabolic rates per unit mass compared to larger species.
Decreasing mutation rate with body size and longevity: result of decrease in ROS and lasting cellular parabiosis?
EMERGENCE OF MORBID PHENOTYPES AT THE LEVEL OF THE PROTEOME
and points of preventative and/or curative intervention

1. Structure correctors, site protectors
2. Chemical or physical chaperones - structure stabilizers
3. Mito-targeted anti-ROS
4. UVA / UVB screen, skin hydration
5. Antioxidants

ROS
OxPhos (mitochondria)
Environment (Chemicals, UVA, UVB, radiation)

Proteostasis Network:
- Folding Chaperones
- Misfolded Proteins
- Intrinsic Instability

Protein Synthesis
- Mutations (Polymorphism)

Genome
Phenotype (Cellular Functions)

Proteolysis (26S proteasome)
Proteolysis (20S proteasome) (Lysosomes)

: Oxidative damage
1 5: Points of Protective or Corrective intervention
THANKS FOR LISTENING
WHAT IS SO DIFFERENT IN OUR APPROACH?

We want to redesign the medicine. Since vaccination and antibiotics, our medicine does not prevent or cure diseases – no drug treatment of deadly diseases increases the duration of life, because they treat consequences not the cause!

Our approach: treat the cause (like vaccination and antibiotics)

(1) Predictive diagnostic of predispositions to diseases, i.e., identification of the health-related « weak link »

(2) Reinforcement of the « weak link » by designed molecules – the same for both prevention and therapy

(3) Cure of chronic incurable diseases by reversion of disease back to health (aging and diseases are reversible – parabiosis)

This is human biological reinforcement - not transhumanism (which is human reinforcement by the use of sophisticated prostheses)
AGGREGATION OF OXIDIZED PROTEINS IN YEAST FOLLOWS THE GOMPETZ LAW OF AGEING AND MORTALITY

Anita Krisko & MR, submitted

Figure 5. Protein aggregate appearance in budding yeast follows the Gompertz law of mortality. (A) The replicative lifespan is presented as the number of buds produced by
EMERGENCE OF PHENOTYPES IN DISEASE AND AGEING

Latency period for expression of acquired recessive mutations in solitary quiescent (stem) cells may depend on protein oxidation (e.g., in carcinogenesis).

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**Legend:**
- O: Wild type (WT)
- m: Mutated (M)
- +: Promoter activation

**Diagram:**
- "WT"
- "WT/M"
- "M"
Radiation shatters *D. radiodurans'* DNA too...

... but “superbugs” quickly repair it
90% mortality is associated with aging in a similar way (5th power of age)
PROTEINS ARE NOT EQUALLY SUSCEPTIBLE TO OXIDATION

Senescent human cells accumulate oxidized proteins but most of our proteins are ‘inox’ (B. Friguet et al).