59th meeting of the PAC
for Condensed Matter Physics

Protection against physical and chemical stresses with tardigrade proteins (TARDISS)

(Status report on the project)

DLNP JINR, Sector of Molecular Genetics of the Cell

Speaker: Mikhail Zarubin
Project Leader: Dr. Elena Kravchenko

“Protection against physical and chemical stresses with tardigrade proteins (TARDISS)” - since 2024 new title

“Study of the radioprotective properties of the Damage suppressor (Dsup) protein on the model object *D. melanogaster* and human cell culture HEK293” - 2021-2023

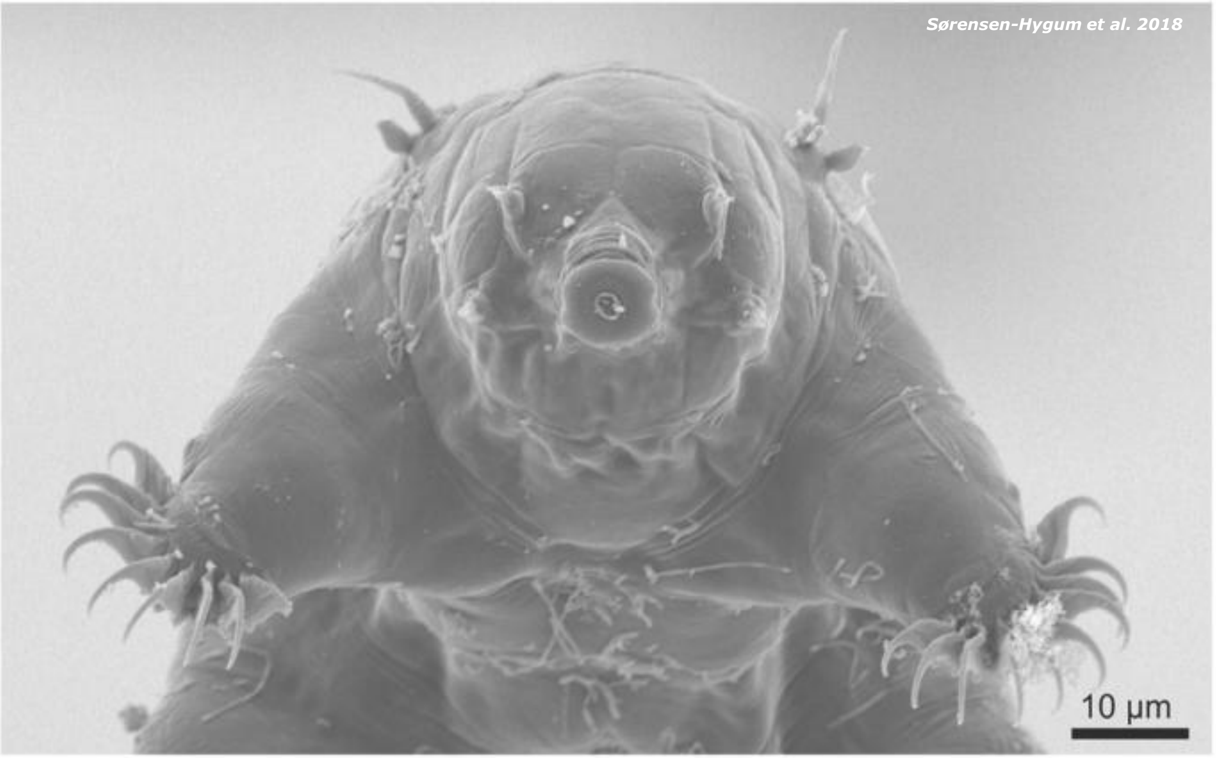
Participants:

E. Kravchenko, M. Zarubin, T. Azorskaya, T. Murugova, A. Rzyanina, K. Tarasov, A. Yakhnenko, S. Apraksina

Natural mechanisms of extreme stress- and radioresistance – important scientific topic being in focus of innovative researches linking genetics, molecular biology, cellular biology, biophysics

Studies of extremophiles have an immensive progress due to an emergence of omics-technologies in 2000s and are actively conducted in various scientific centers including BNL (USA), RIKEN (Japan), IMP (China) and recently JINR

Tardigrades (phylum Tardigrada) – Wasserbär (water bear)



Tardigrades are present in all biomes on Earth including most extreme environments

Tardigrades posses developed neural system with brain, muscle, digestive and other systems consisting of differentiated tissues

Multiple stress resistance of tardigrades

Drying (anhydrobiosis): complete desiccation (up to 10 years)

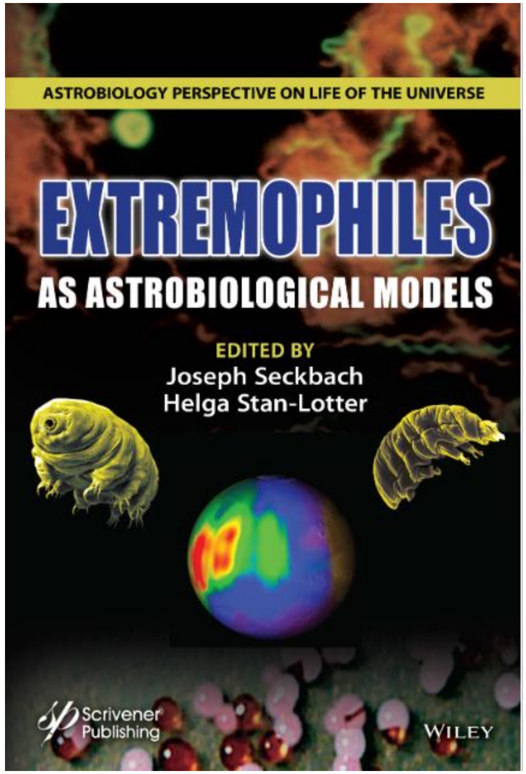
Toxic media: organic reagents, heavy metals, extreme salinity, acid and alkaline pH, anoxic conditions, oxidants

Pressure: vacuum and extreme pressure (7.5 GPa 12 hours)

Ultralow and high temperatures: −196°C to 90°C

Mechanic impact: shock upon gun shot (0.9 km/s)

Tardigrades as a model organism for spaceflight studies – impact of deep-space conditions and outer space



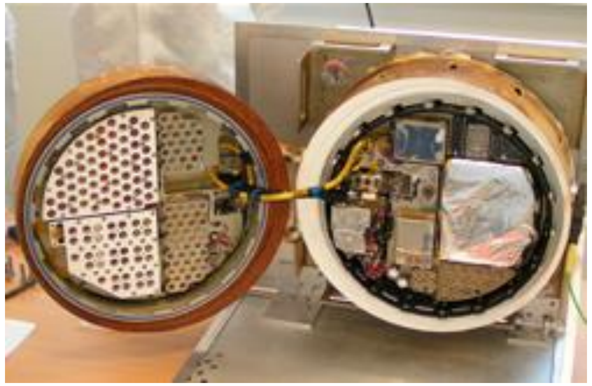
FOTON-M3 mission

TARDIS (*Jönsson et al., 2008*), RoTaRad (*Persson et al., 2011*), TARSE (*Rebecchi et al., 2011, 2009*)

TARDIS (Tardigrades in Space) - 10 day exposure to space vacuum (10^{-6} Pa), cosmic radiation (100 mGy) and harsh UV-radiation

Tardigrades managed to survive (*Jönsson et al., 2016, 2008*)

http://teamtardigrades.blogspot.com/



Current Biology CellPress

Correspondence

Tardigrades survive exposure to space in low Earth orbit

K. Ingemar Jönsson [1], Elke Rabbow [2], Ralph O. Schill [3], Mats Harms-Ringdahl [4], Petra Rettberg [2]

Prominent resistance to high and low LET radiation of species of phylum *Tardigrada*

The resistance to γ-radiation of several organisms

Organism	LD_{50}	Data source
Homo sapiens	$LD_{50/30d}$ =2.5–4.5 Gy	*Bolus et al. 2001*
Mice	$LD_{50/30d}$ = 4.5 Gy	*Bolus et al. 2001*
Goldfish	$LD_{50/30d}$ = 8 Gy	*Bolus et al. 2001*
Cockroach	$LD_{50/30d}$ = 50 Gy	*Bolus et al. 2001*
Drosophila melanogaster (Insecta)	$LD_{50/3}$ = 1238–1339 Gy	*Parashar et al. 2008*
Deinococcus radiodurans (Bacteria)	LD_{50} = 10000 Gy	*Makarova et al. 2001*
Escherichia coli (Bacteria)	LD_{50} = 600 Gy	*Krisko and Radman 2010*
Tardigrades	**LD_{50} = 1270–5000 Gy**	***Hashimoto and Kunieda 2017***

One of the most radioresistant animals (LD_{50}>5000 Gy)

Studies of tardigrade resistance to ionizing radiation

May et al., 1964
X-rays

Jönsson et al., 2005
γ-radiation

Horikawa et al., 2008, 2006
α-particles

Jönsson and Wojcik, 2017
heavy ions

Nilsson et al., 2010
protons

Outstanding radioresistance with determined LD_{50} = 4-6 kGy for various types of ionizing radiation and tardigrade species

For tardigrade embryo LD_{50} = 509 Gy of α-particels (*Horikawa et al., 2012*)

Specific protective mechanism in tardigrades?

Dsup protein (Damage suppressor) – the mechanisms of extreme radioresistance and DNA protection

In 2016 the genome sequencing of tardigrade *Ramazzottius varieornatus* was performed by *Hashimoto et al.* Bioinformatics analysis afforded to reveal the unique protein - Damage suppressor protein (Dsup)

nature COMMUNICATIONS

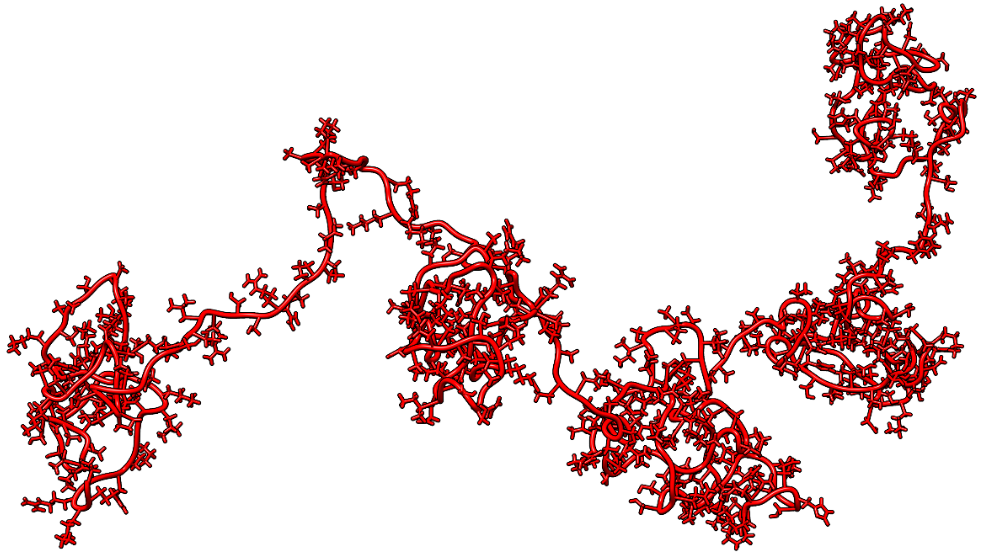
Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein

Takuma Hashimoto[1,*], Daiki D. Horikawa[1,2,3,*], Yuki Saito[1], Hirokazu Kuwahara[1,4], Hiroko Kozuka-Hata[5], Tadasu Shin-I[6], Yohei Minakuchi[7], Kazuko Ohishi[6], Ayuko Motoyama[7], Tomoyuki Aizu[7], Atsushi Enomoto[8], Koyuki Kondo[1], Sae Tanaka[1], Yuichiro Hara[9], Shigeyuki Koshikawa[10,11], Hiroshi Sagara[5], Toru Miura[10], Shin-ichi Yokobori[12], Kiyoshi Miyagawa[8], Yutaka Suzuki[13], Takeo Kubo[1], Masaaki Oyama[5], Yuji Kohara[6], Asao Fujiyama[7,14], Kazuharu Arakawa[3], Toshiaki Katayama[15], Atsushi Toyoda[7] & Takekazu Kunieda[1]

Dsup protein is localized in nucleus

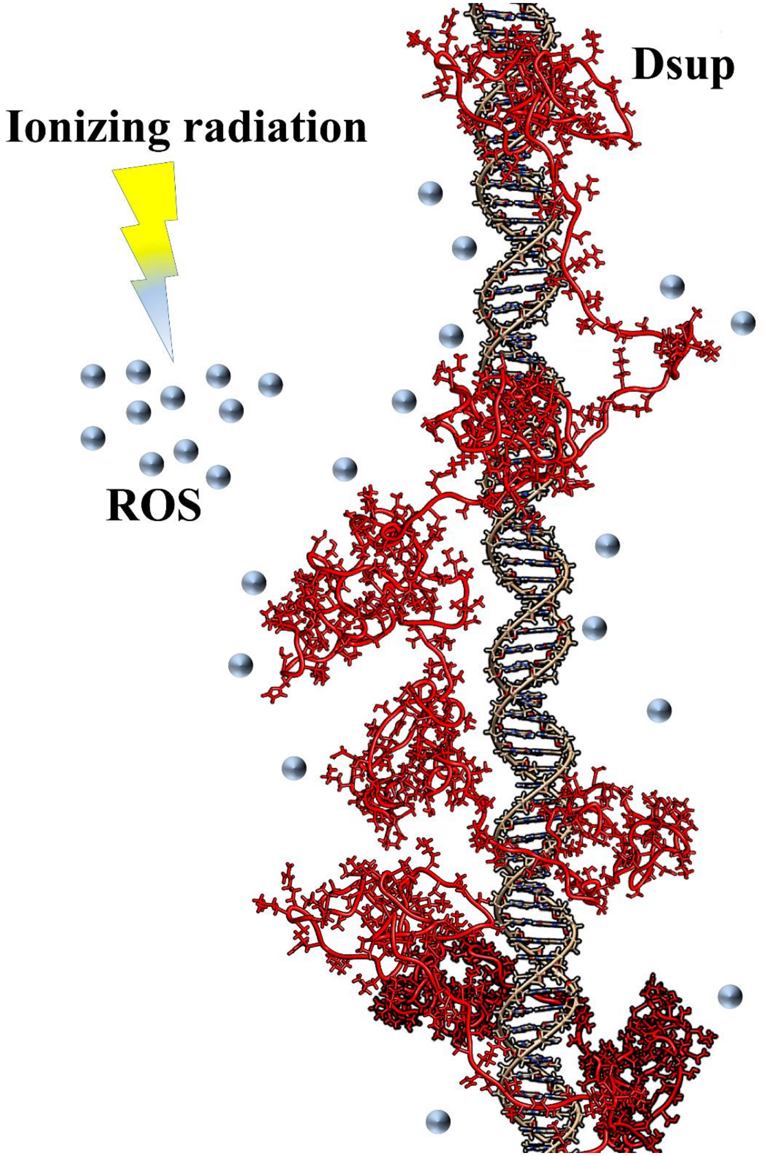
Non-specific DNA-binding

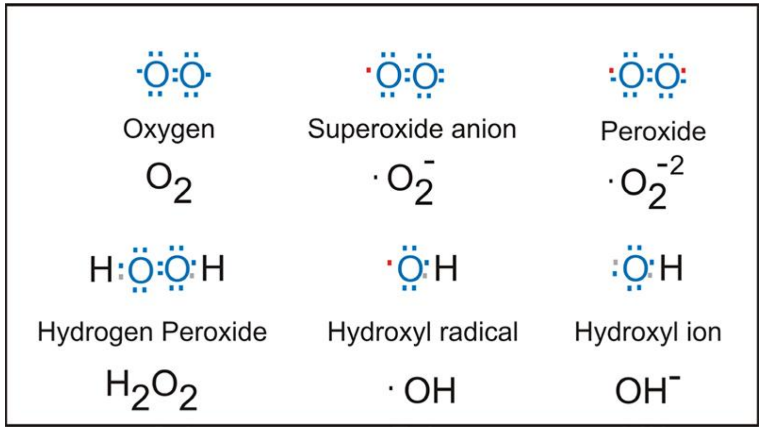
Dsup protects DNA and induces stress-resistance in human cells



Predicted model obtained with I-TASSER algorithm

Hypothetical molecular mechanisms of Dsup action in tardigrade





Reactive oxygen species (ROS)

Applications of tardigrade Dsup protein

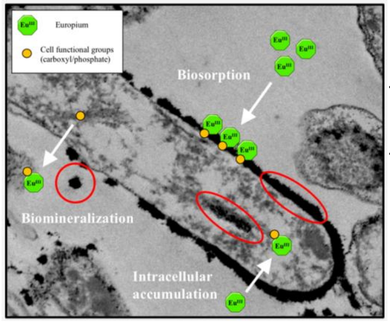
ACS Publications
Most Trusted. Most Cited. Most Read.

Natural and Designed Proteins Inspired by Extremotolerant Organisms Can Form Condensates and Attenuate Apoptosis in Human Cells

Mike T. Veling, Dan T. Nguyen, Nicole N. Thadani, Michela E. Oster, Nathan J. Rollins, Kelly P. Brock, Neville P. Bethel, Samuel Lim, David Baker, Jeffrey C. Way, Debora S. Marks, Roger L. Chang*, and Pamela A. Silver*

Cite this: *ACS Synth. Biol.* 2022, 11, 3, 1292−1302

Genetics, medicine, biotechnology, pharmacy, spaceflights: instrument for an induction of multiple-stress resistance to cellular cultures and model organisms, creation of effective producer or bioremidiating microorganisms, model organisms for non-human deep-space biological studies



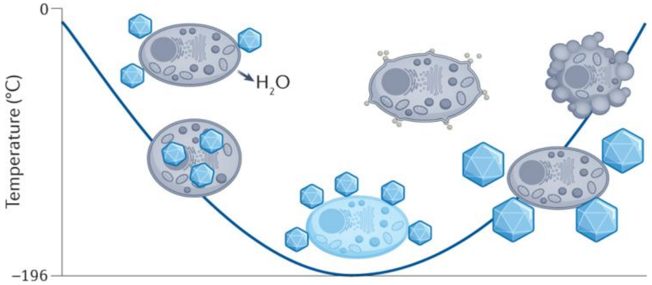
Ruiz-Fresneda et al. 2020

sor.org

nasa.gov

Medicine: protector for complicated medical procedures as radiotherapy, chemotherapy etc. and pharmaceutical support for astronauts and human microbiome

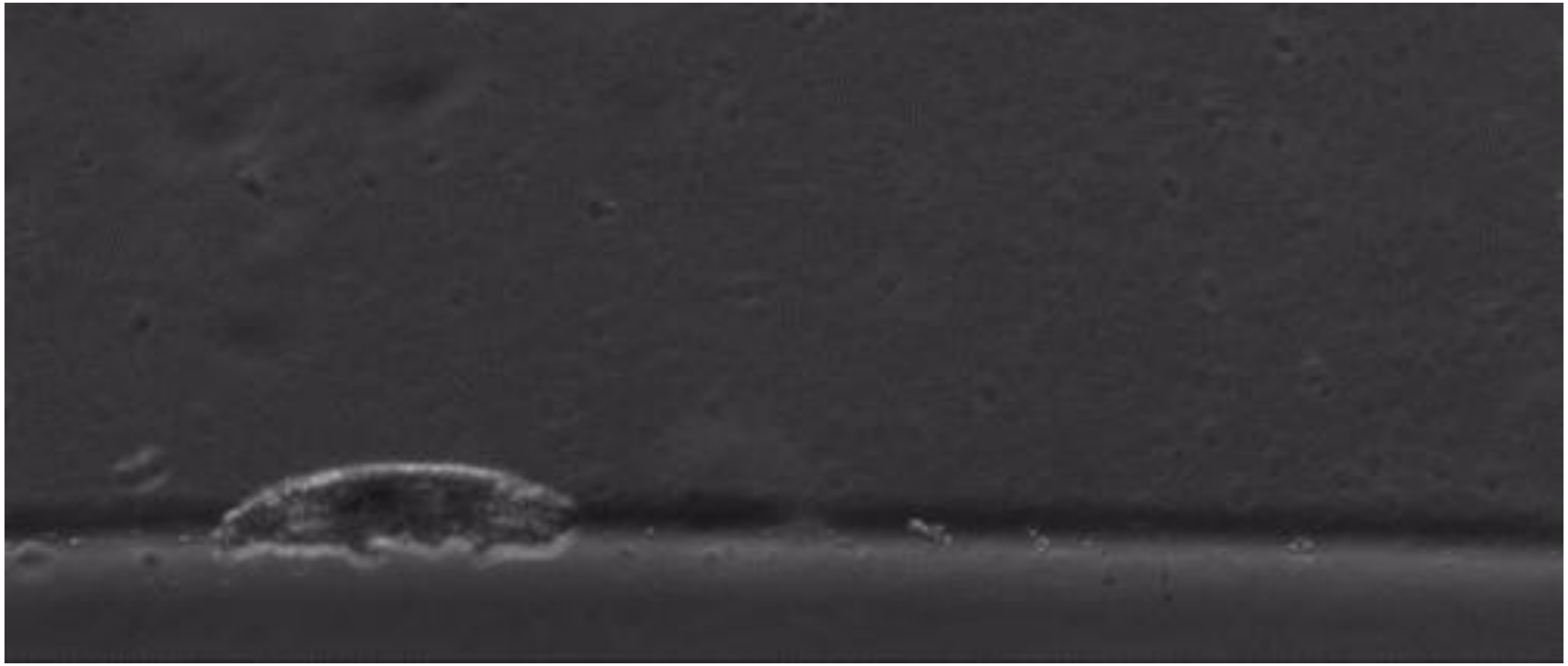
Material design, biotechnology, pharmacy: agents or stabilizers for long-term storage and cryoprotection of DNA-containing pharmaceuticals, vaccines and biological materials. Opportunities for the material design inspired by tardigrade proteins



K.A. Murray et al. 2022

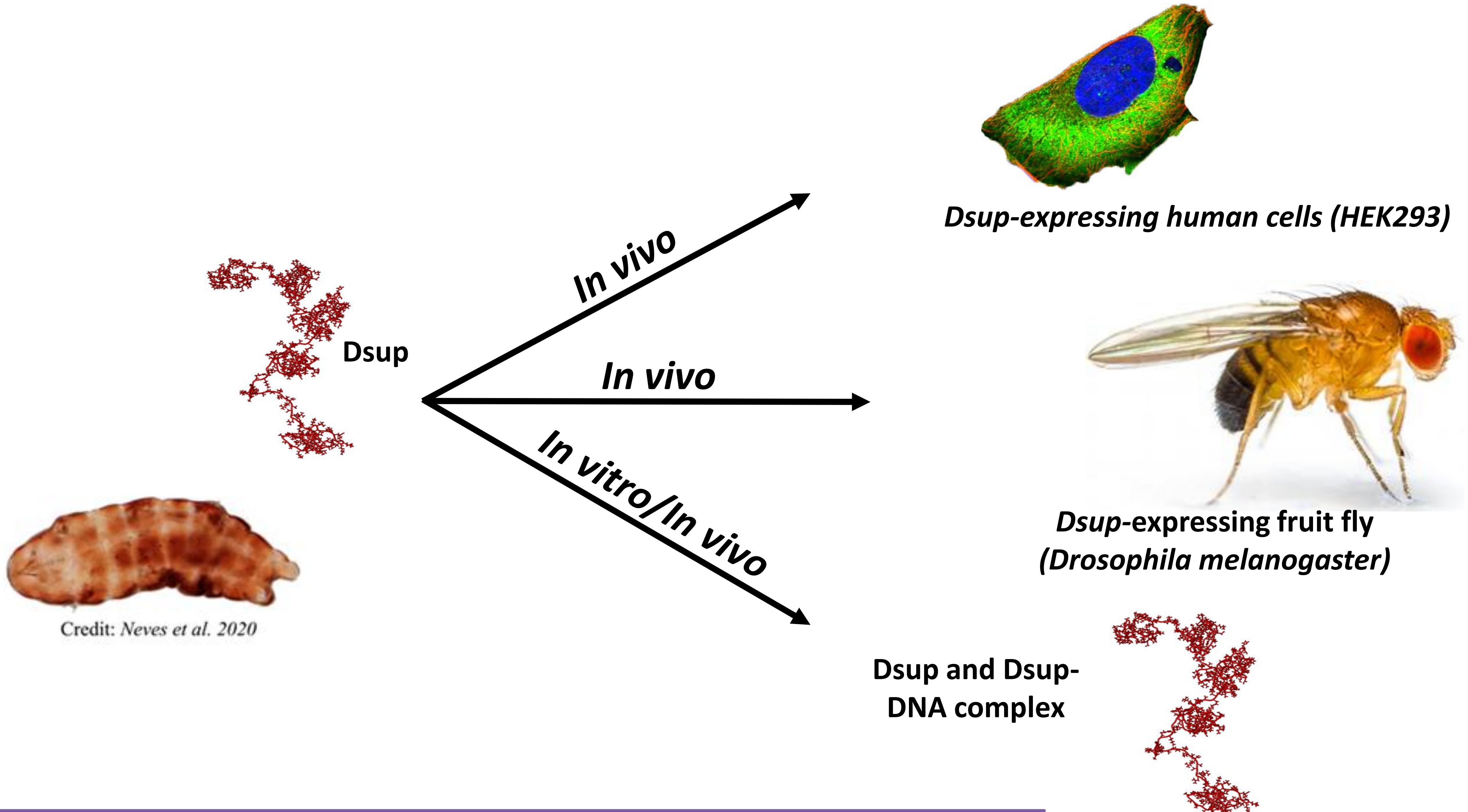
Main goals of our studies:

- **An *in vivo* and *in vitro* investigation of protective mechanism by Dsup protein**
- **The determination of applicability and optimization of Dsup protein to complex animals for an improvement of resistance to multiple stresses**
- **Applications of Dsup protein: radioprotector, biotechnological cultures, stabilizer, cryoprotectant, material design**

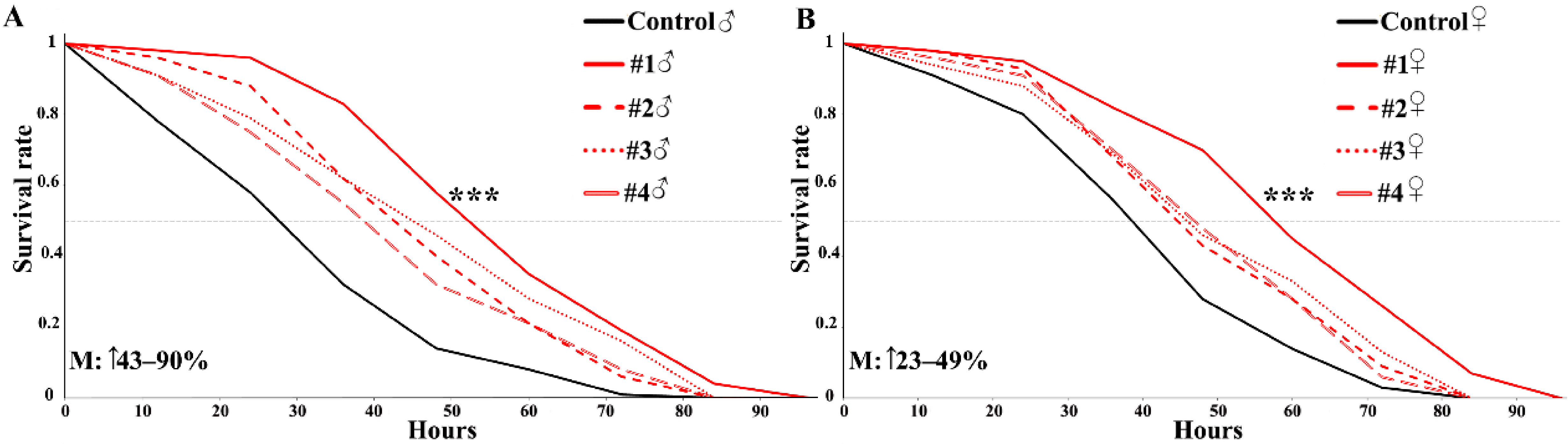


Tardigrades exhibit robust interlimb coordination across walking speeds and terrains Jasmine A. Nirody et al. PNAS 2021

SMGC DLNP studies of tardigrade DNA-protective Dsup protein



An increase of resistance to oxidative stress in *D. melanogaster*

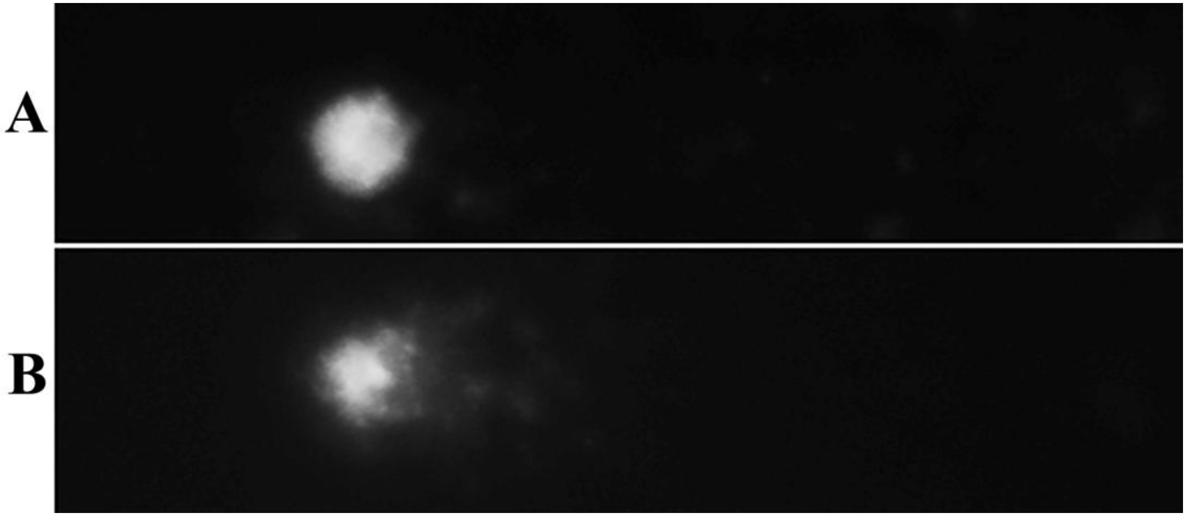


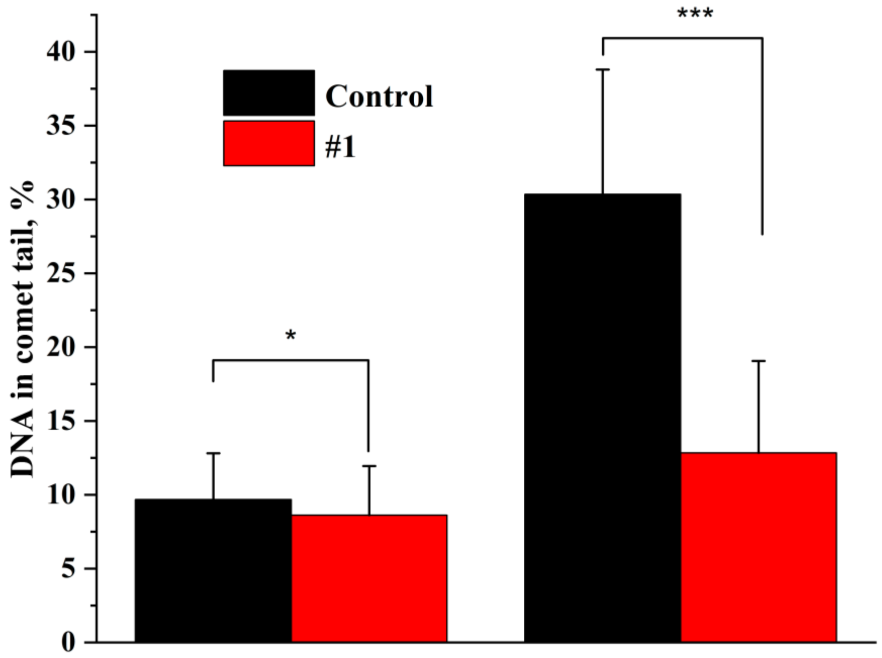
*** - p<0.001

The reduction of DNA damage by Dsup protein in *D. melanogaster*

The comet assay is a method for measuring DNA strand breaks in eukaryotic cells.

Comet assay for validation of DNA damage

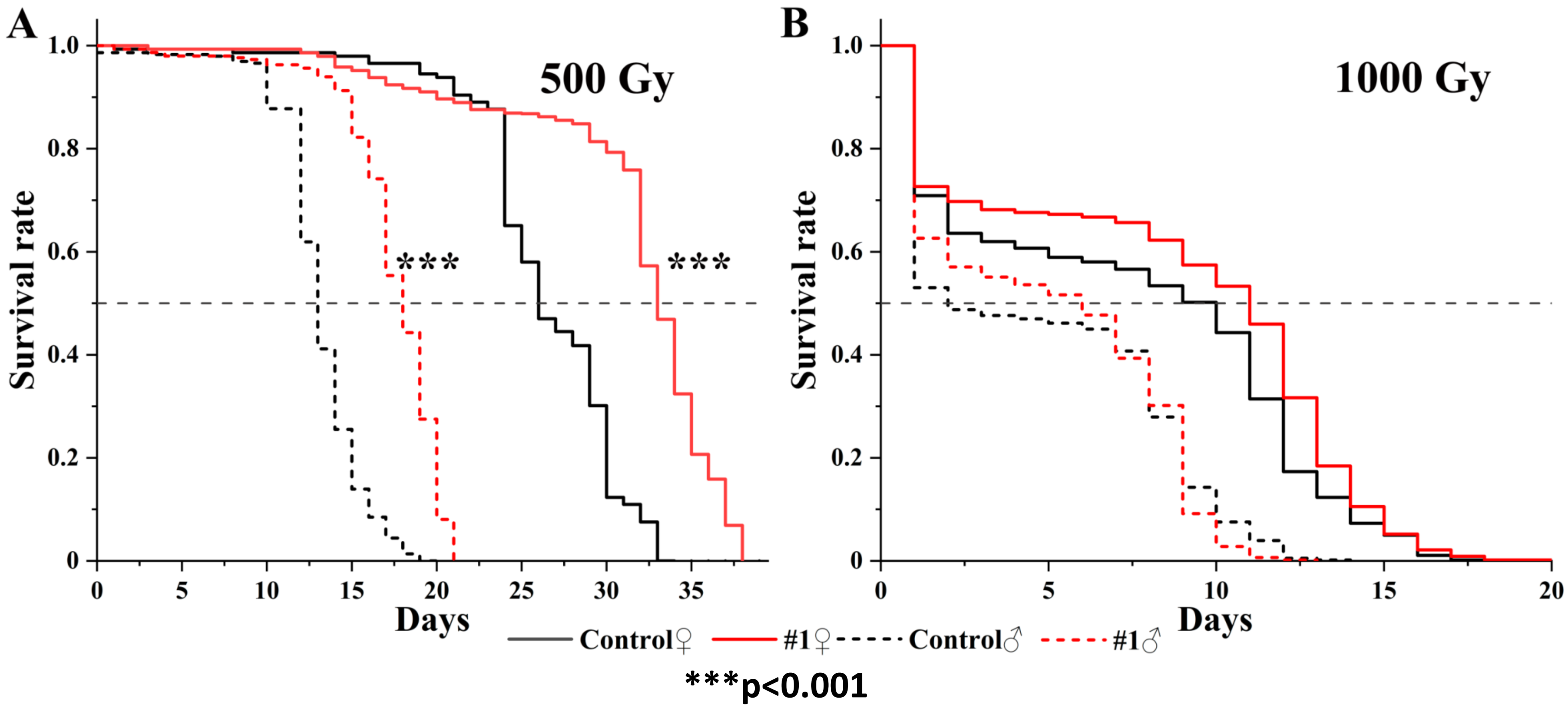




pre-treatment **oxidant treatment**

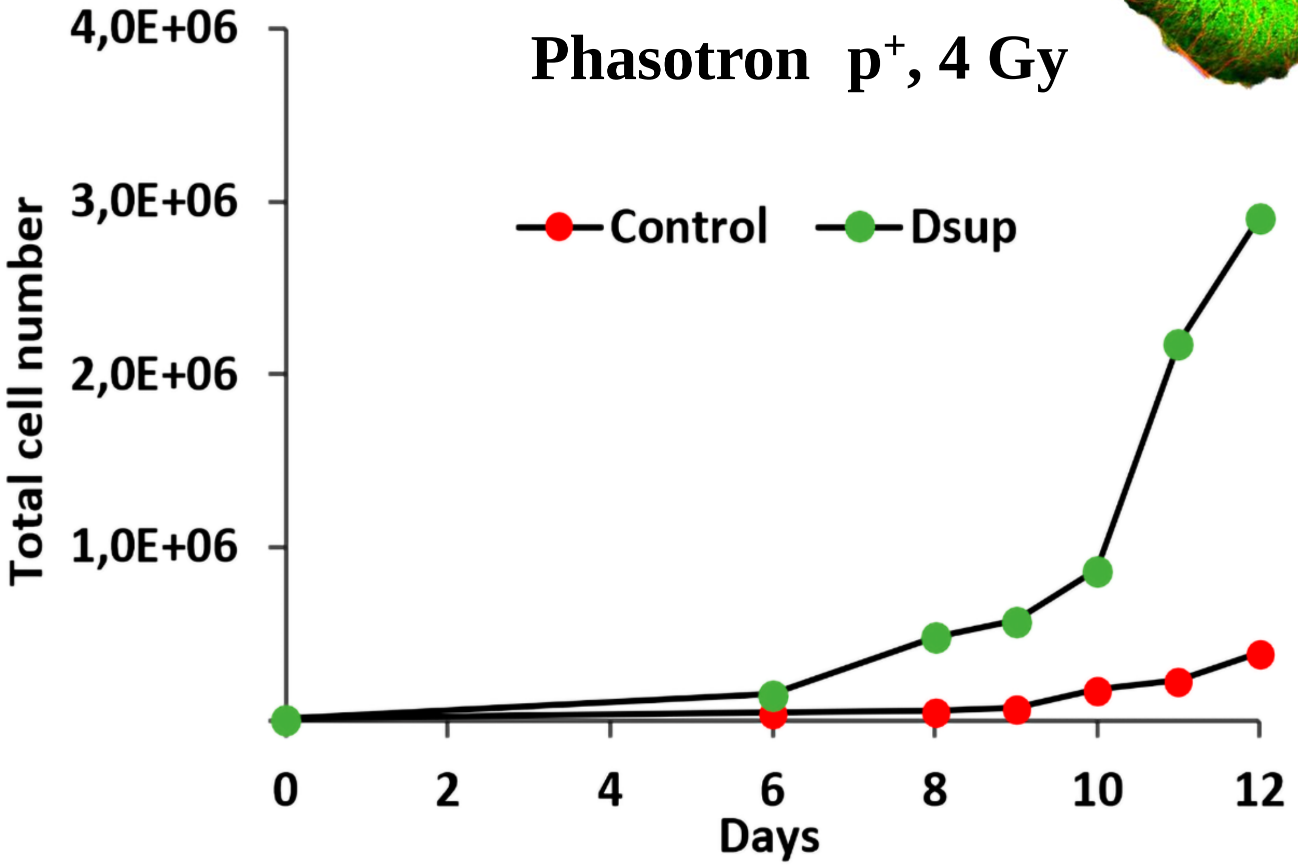
*** p<0.05; *** p<0.001**

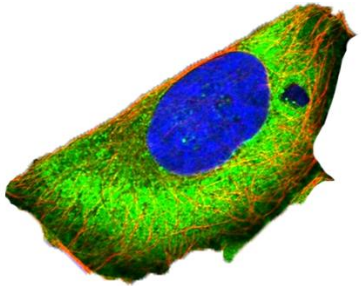
An induction of radioresistance to *D. melanogaster*



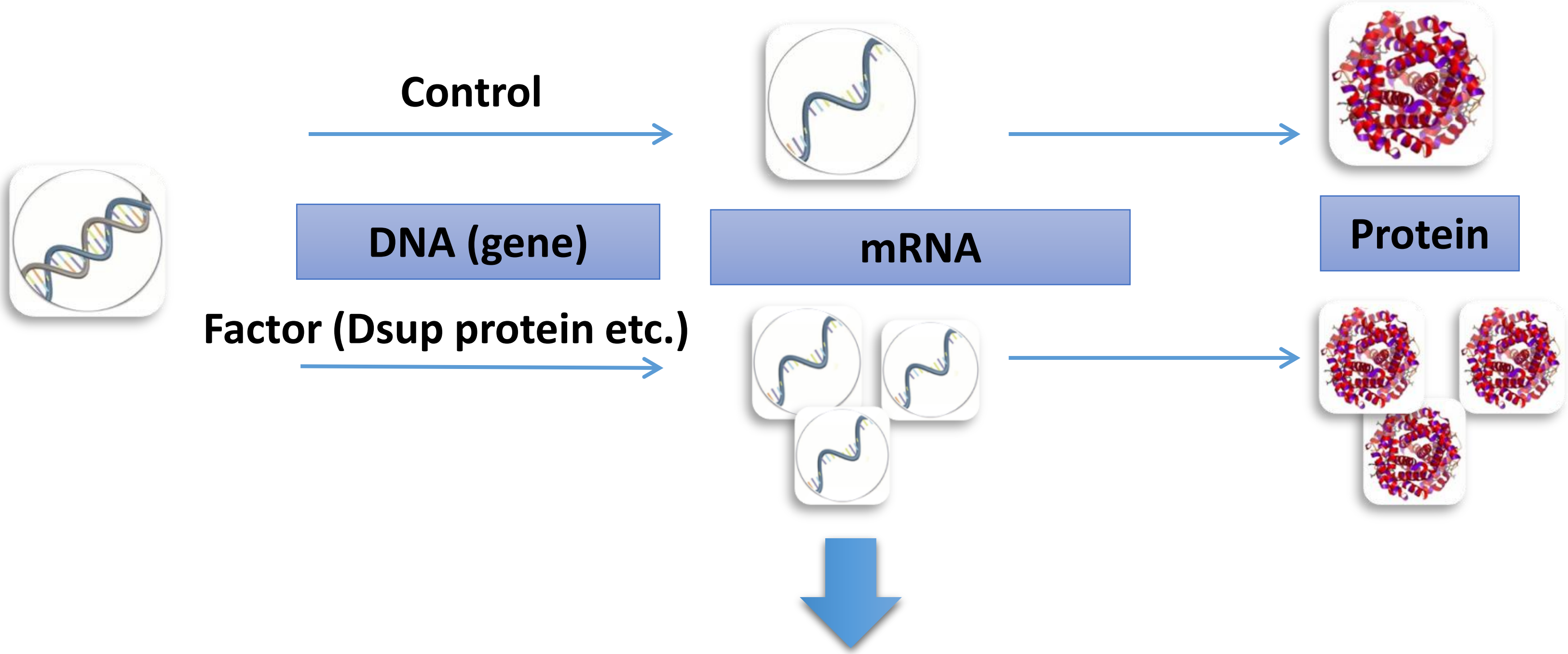
Exposure to γ-rays at microtron MT-25, FLNR JINR

Preliminary results outline: the significant improvement of radioresistance in human cellular culture (HEK293) exposed to the beam of hadrons (p^+)





Transcriptome analysis of *Drosophila melanogaster*

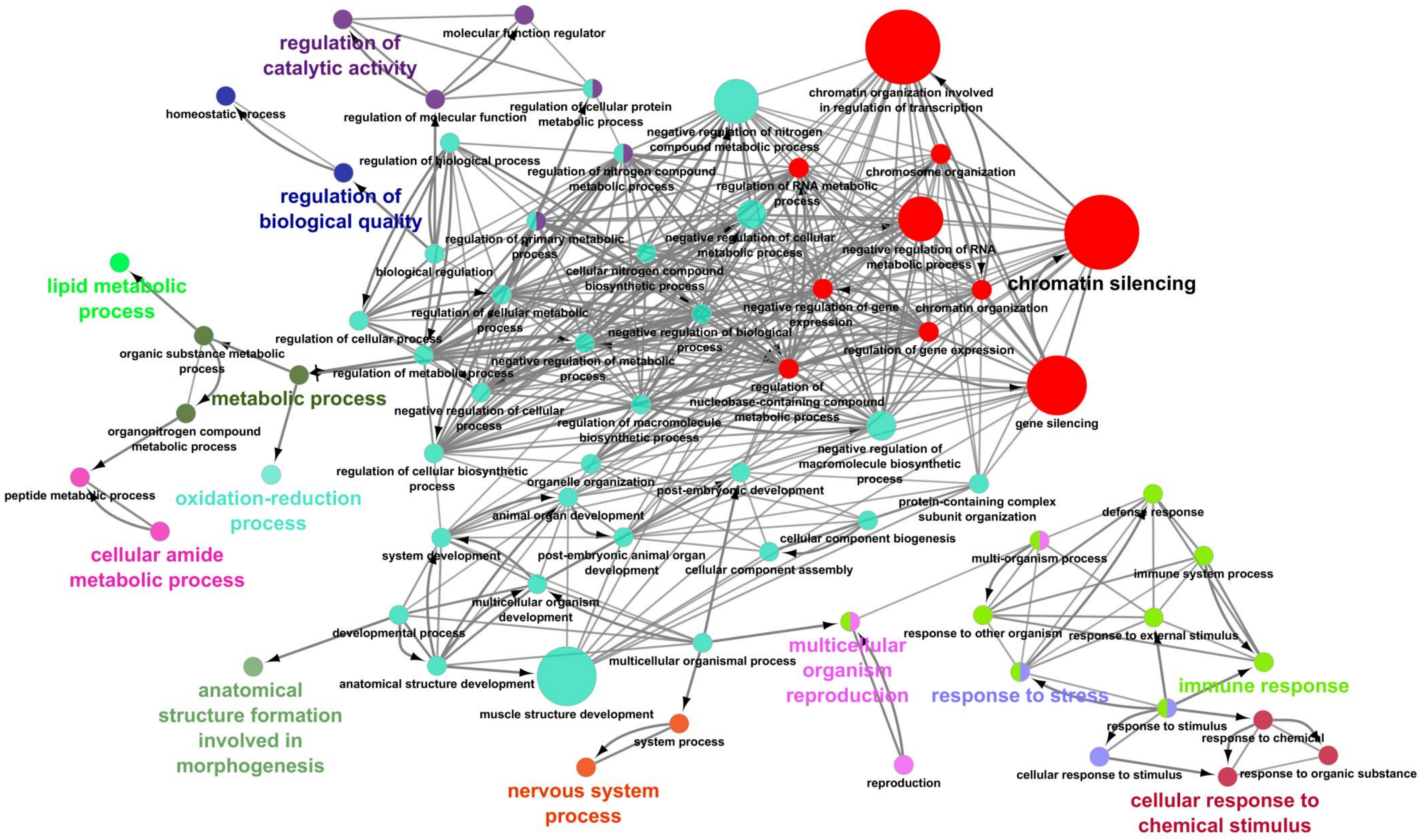


The information coded in gene (DNA) is transcribed in RNA. Transcriptome – is total RNA of the organism, which is an indicator of many systems state.

Transcriptomics: comparative analysis of expression of all genes between control and experimental groups

Transciptome analysis of *Dsup*-expressing and control lines of *D. melanogaster*

Dsup protein – is the unspecific transcriptional repressor, which suppresses an activity of ~750 genes (p<0.01) related to regulation of transcription, chromatin organization, functioning of neural system etc.



Biological network of processes impacted by Dsup protein

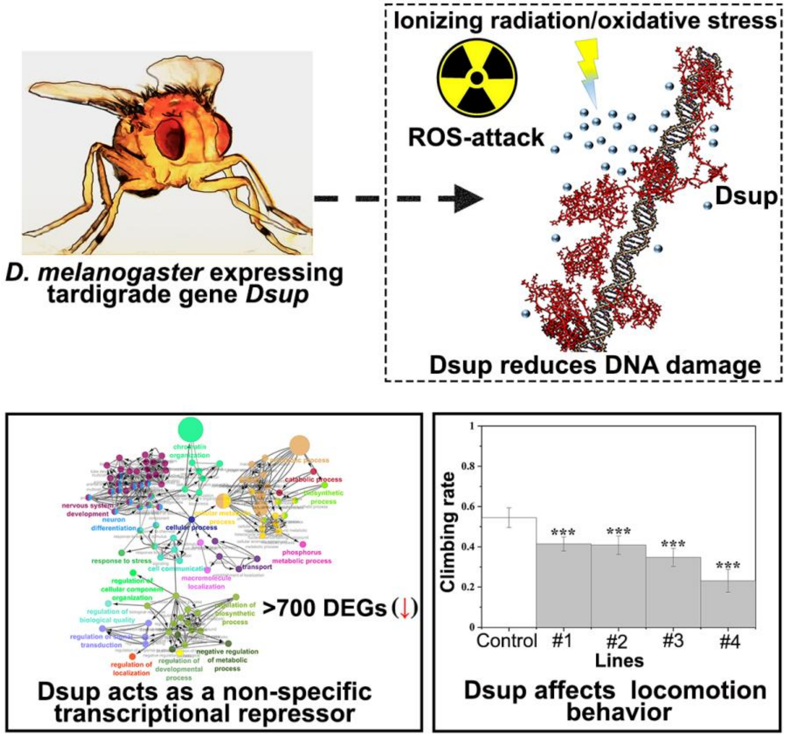
iScience

ARTICLE | ONLINE NOW, 106998

The tardigrade Dsup protein enhances radioresistance in *D.melanogaster* and acts as an unspecific repressor of transcription

Mikhail Zarubin • Talyana Azorskaya • Olga Kuldoshina • Sergey Alekseev • Semen Mitrofanov • Elena Kravchenko [3] •

Open Access • Published: May 29, 2023 • DOI: https://doi.org/10.1016/j.isci.2023.106998



iScience (Q1, IF 6.1), Cell Press

Molecular mechanism of radioprotection: an insight into structural properties of Dsup and Dsup-DNA complex

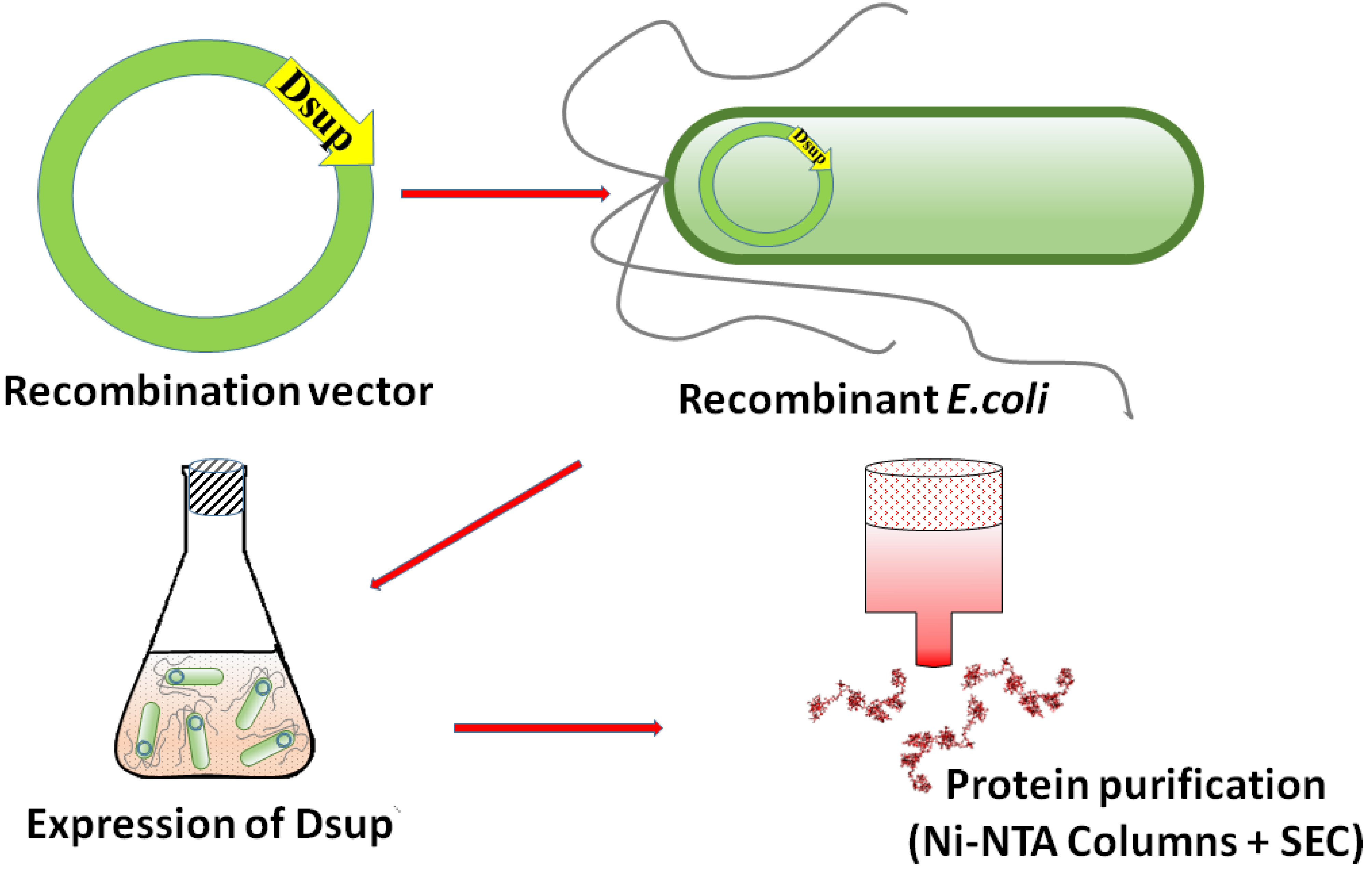
Collaboration with Frank Laboratory of Neutron Physics (+ Moscow Institute of Physics and Technology)

Structural properties of Dsup protein as a candidate significantly disordered protein

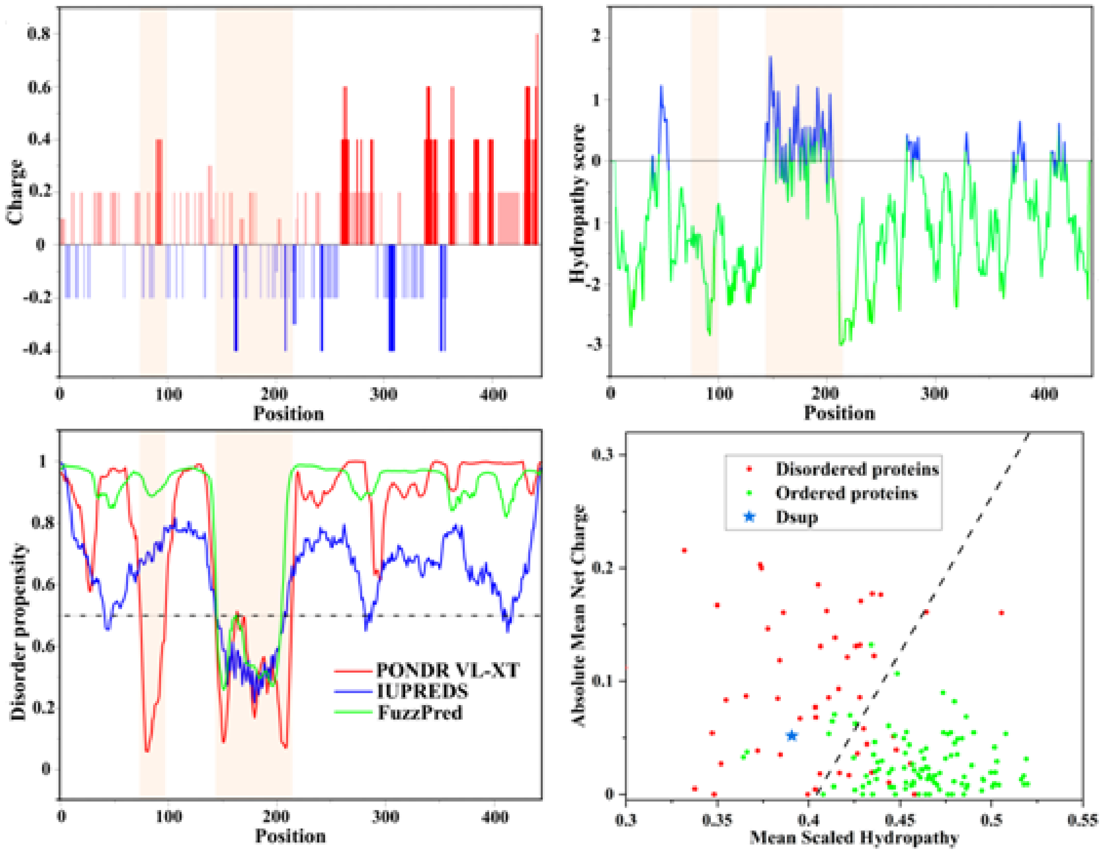
Effects of physicochemical conditions on Dsup molecule

Assessment of Dsup-DNA complex properties to validate mechanism of radioprotection

Production of Dsup protein



Computational analysis



Dsup – is predicted to be an intrinsically disordered protein (IDP) (Alphafold 2.0, PONDR etc.)

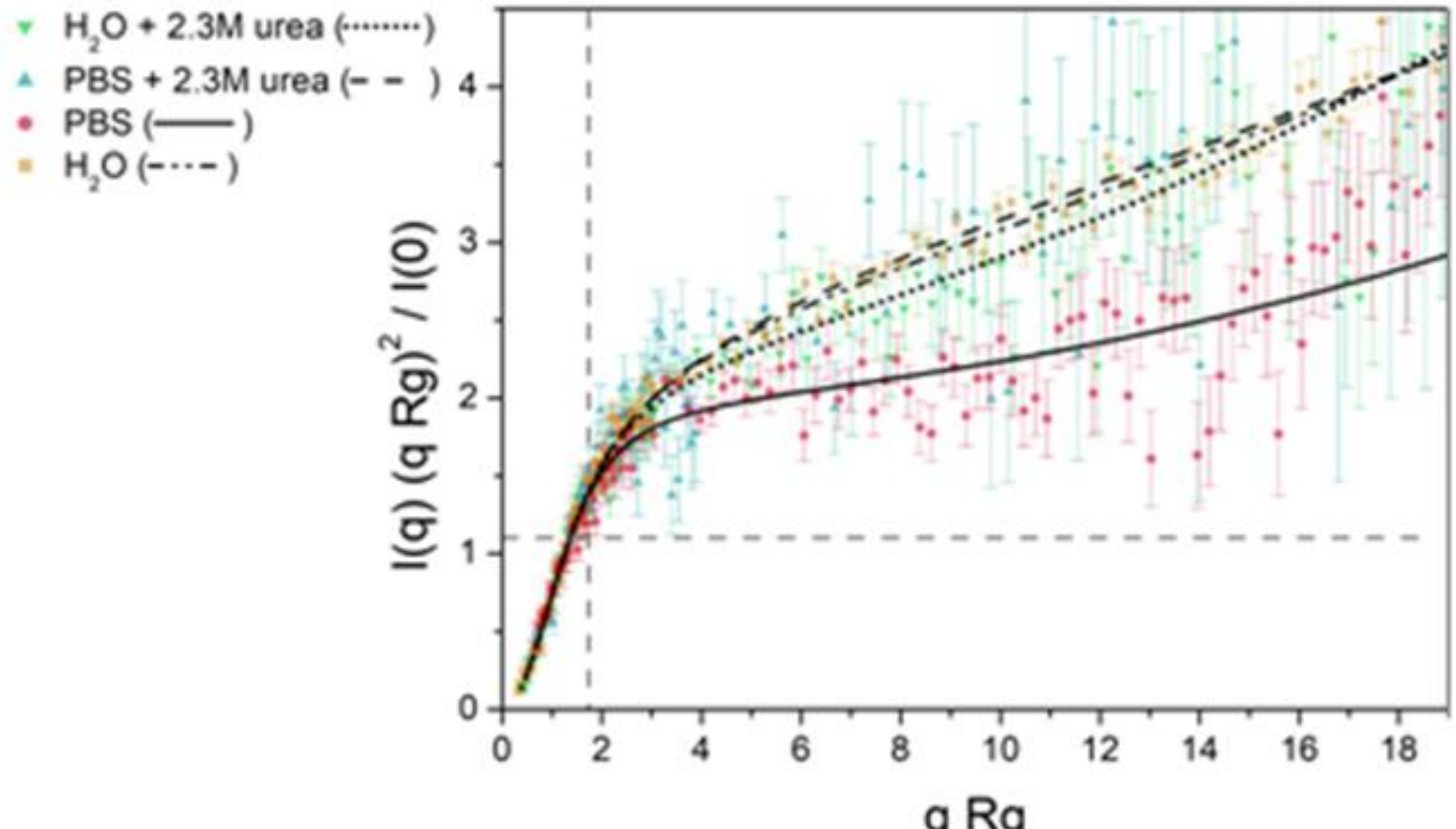
Polyampholyte with total positive net charge (24 at pH 7) and pI 10.56

Dsup has a significant charge mixing

Dsup is hydrophilic protein

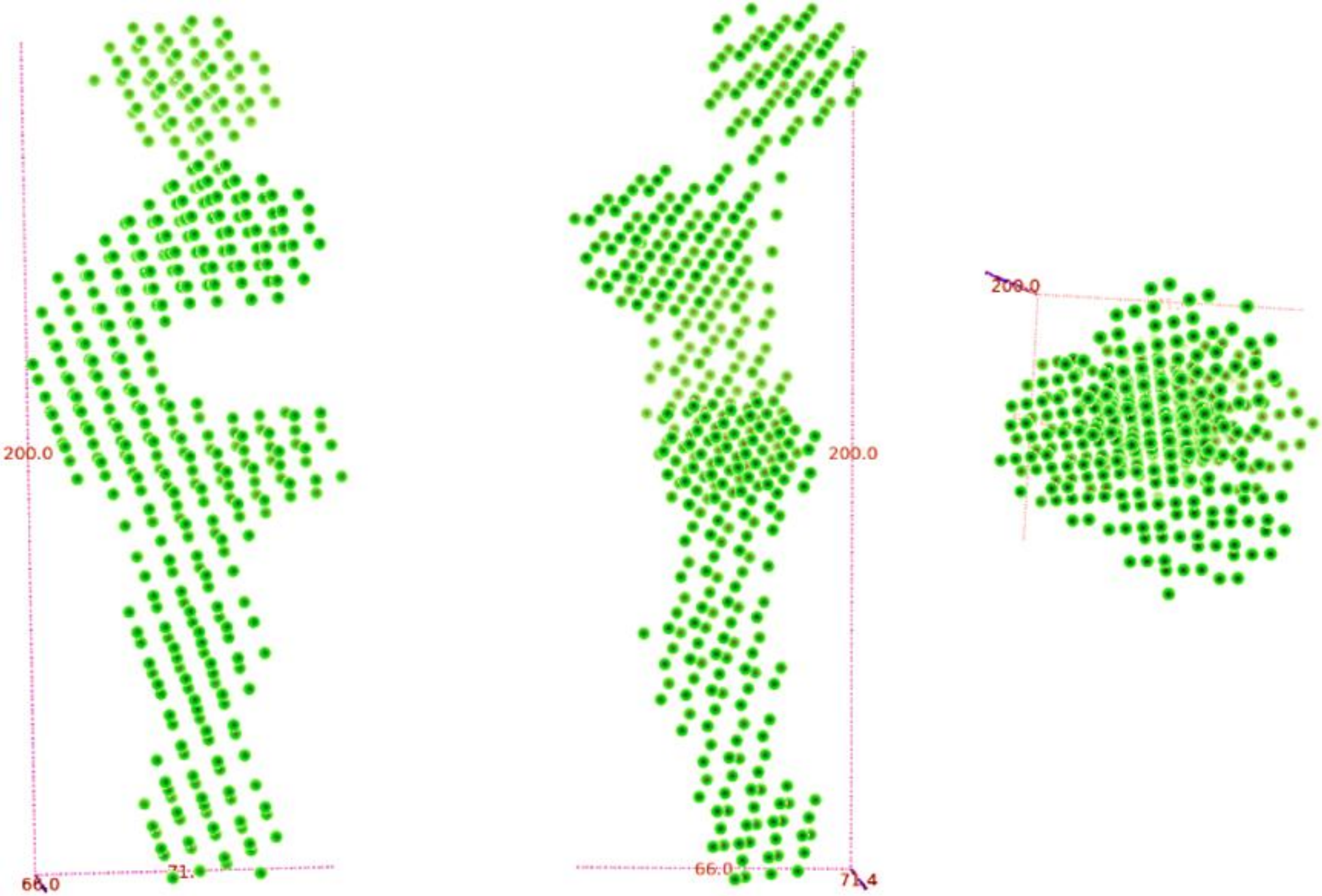
SAXS study of Dsup protein and Dsup-DNA complex

Kratky plot



SAXS Xeuss 3.0, FLNP JINR

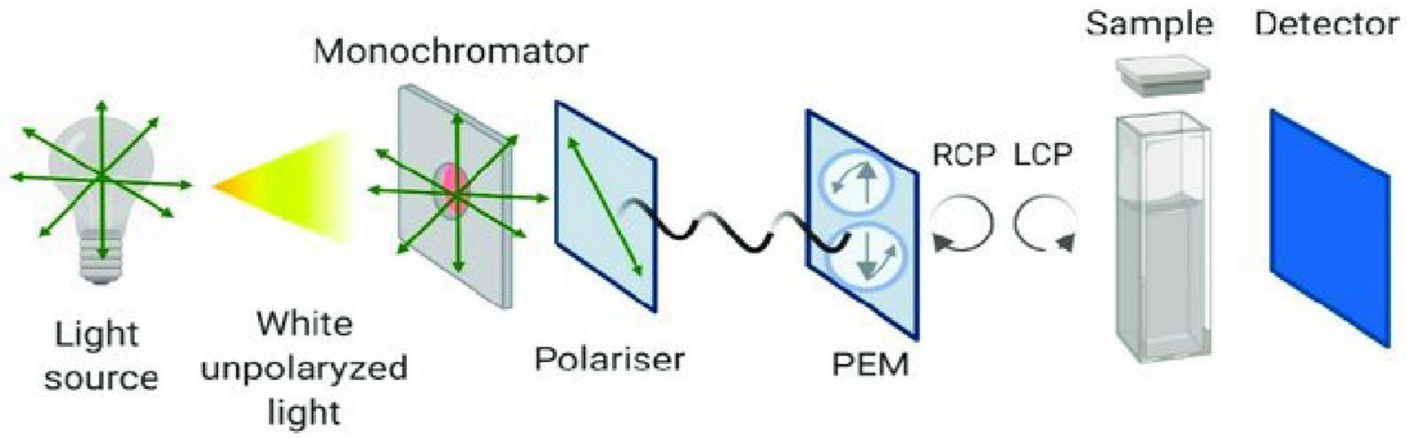
Low-resolution model of Dsup protein



SAXS-based *ab initio* model of Dsup protein (DAMMIF package)

Secondary structure of Dsup: Circular dichroism spectroscopy (CD)

Collaboration with MIPT



M.F. Pignataro *et al.* 2020

Jasco J-1100 CD Spectrometer, MIPT

Secondary structure content in Dsup - 5-7% α-, 30-31% β-, 60-62% disordered regions

Dsup is an intrinsically disordered protein

Transitions of secondary structure due to physicochemical environment are revealed

Dsup-DNA is fuzzy complex

New results on properties of Dsup-DNA complex

Demonstration of Dsup-DNA complex formation

(agarose gel electrophoresis, SAXS, MST)

Dsup binds to DNA and RNA

(agarose gel electrophoresis)

Dsup-DNA - fuzzy and highly dynamic complex

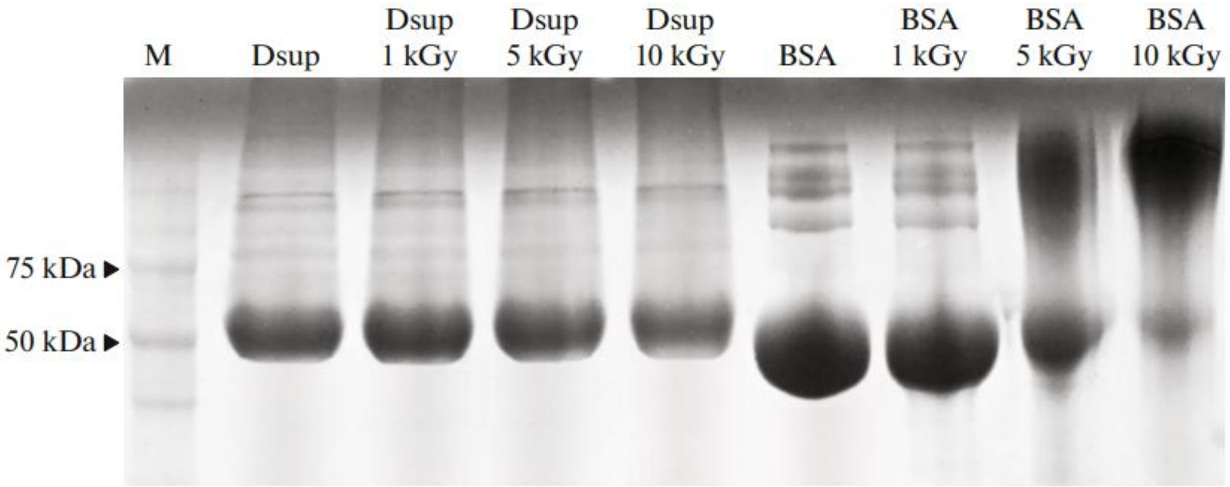
(CD-spectroscopy)

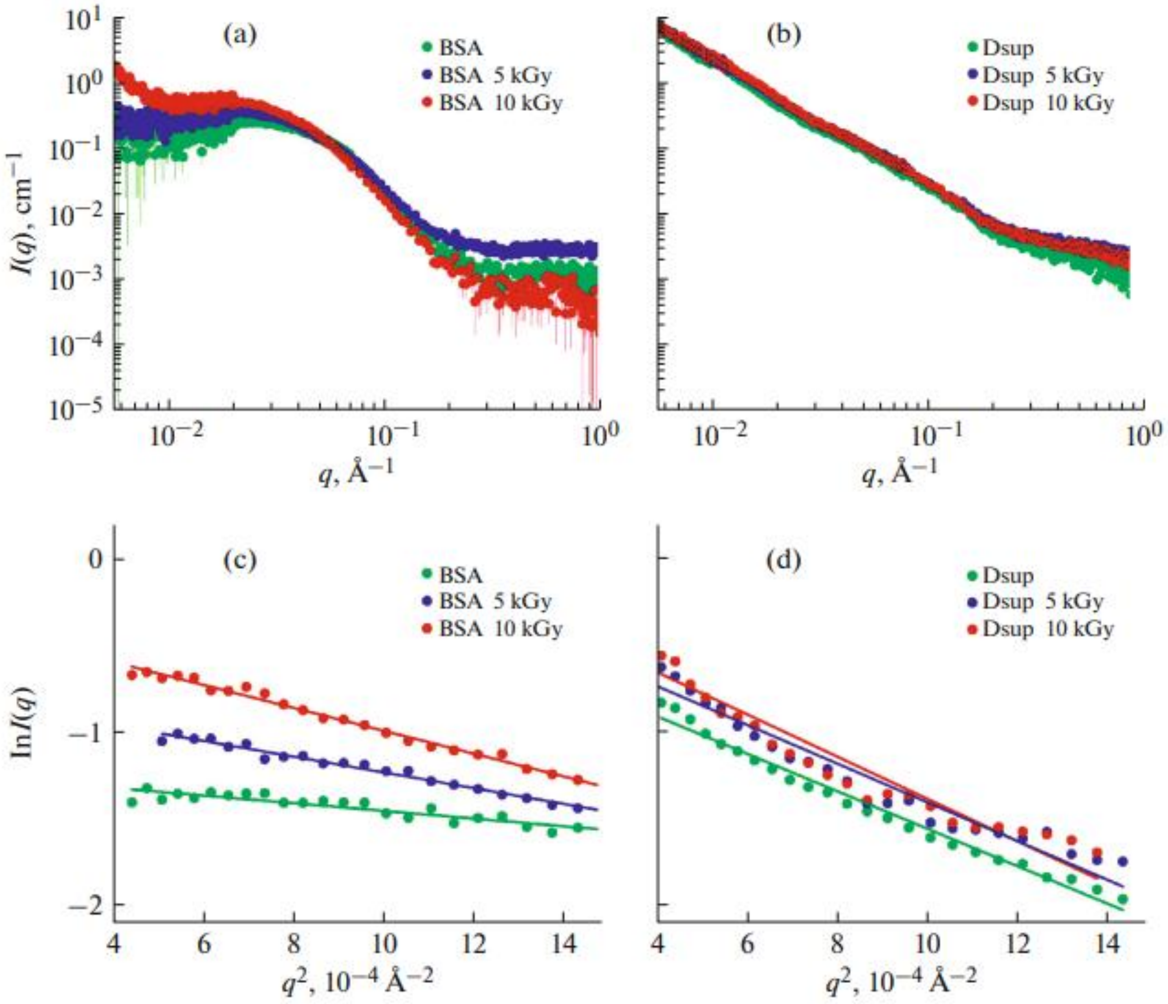
First data on dissociation constants (K_d) for Dsup and DNA interaction

(MST)

Applied research: validation of Dsup's stability properties

Collaboration with Frank Laboratory of Neutron Physics





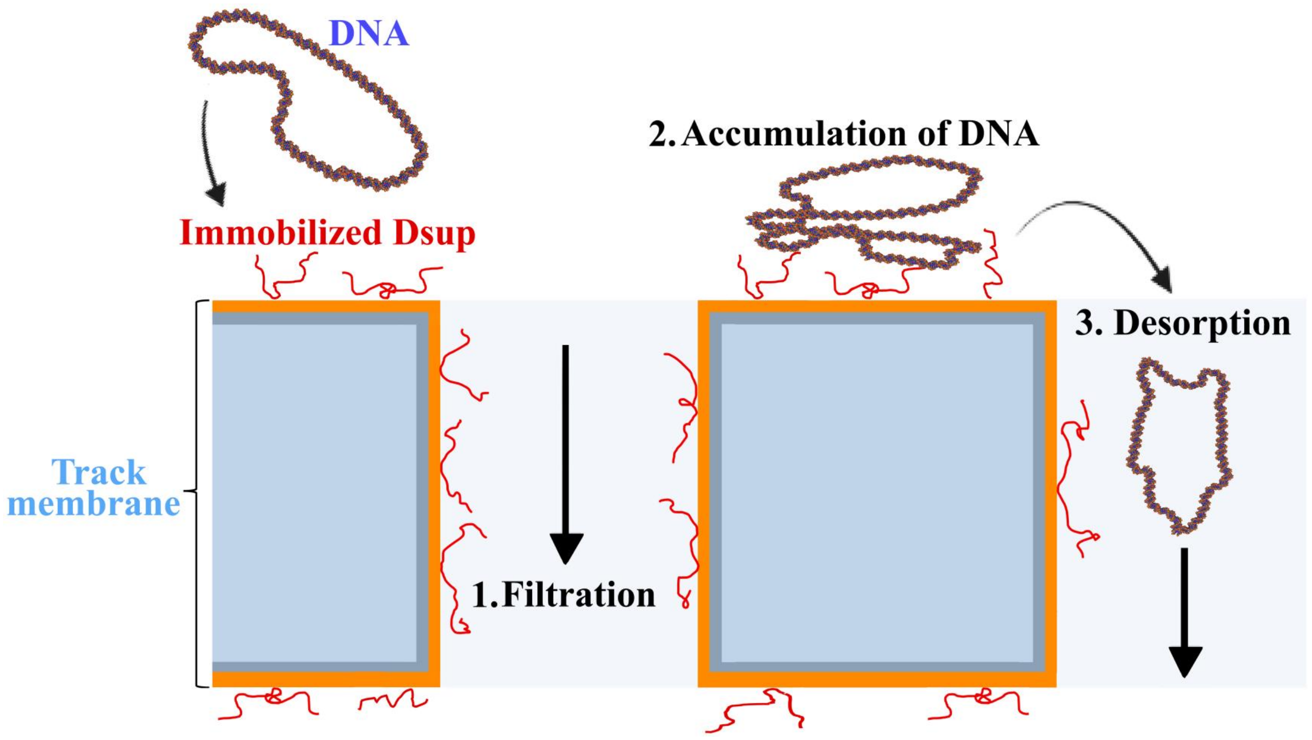
Dsup is resistant to high doses of ionizing radiation

https://doi.org/10.3103/S0027134924700024

Material design with proteins of extremophiles:
Track membranes functionalized with Dsup protein for cell-free DNA isolation

Collaboration with the Centre of Applied Physics FLNR JINR

Development of the technique and biomaterials for accumulation, concentrating and purification of cell-free nucleic acid molecules (DNA/RNA) from various biological fluids using the Dsup protein



BIOTECHNOLOGY PROGRESS

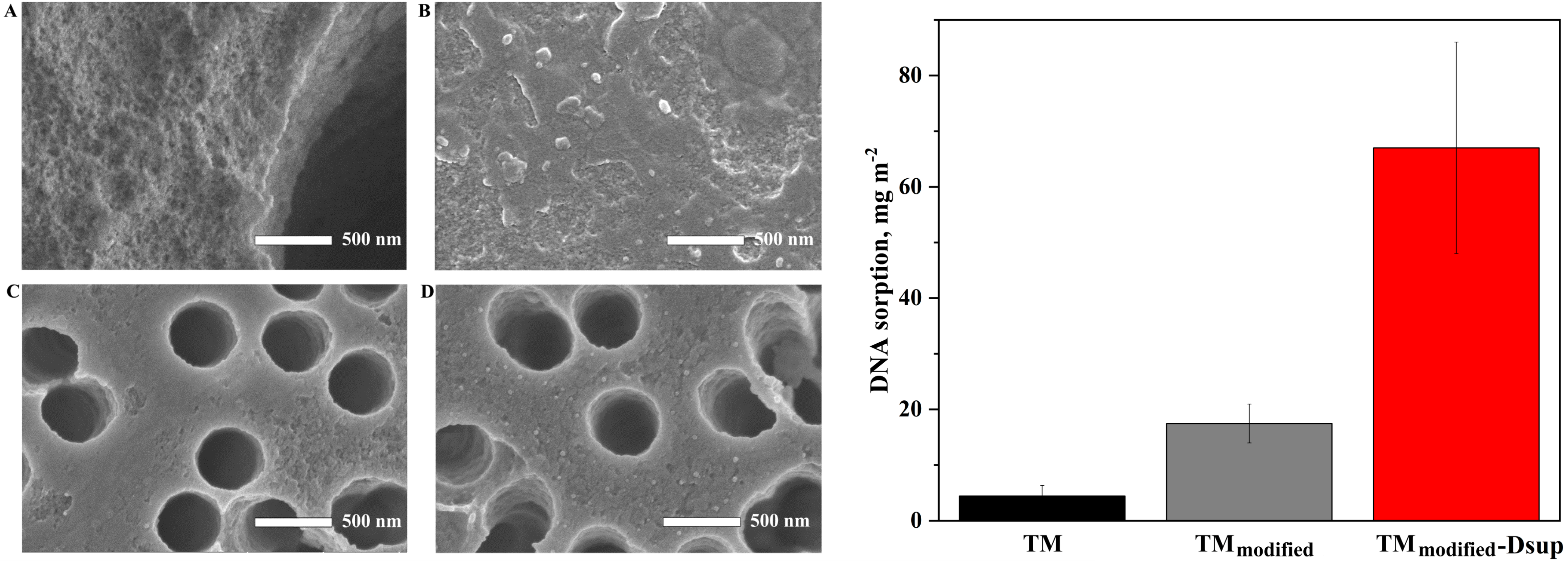
Society for Biological Engineering
An AIChE Technological Community

AIChE
The Global Home of Chemical Engineers

Developing tardigrade-inspired material: Track membranes functionalized with Dsup protein for cell-free DNA isolation

Mikhail Zarubin, Evgeny Andreev, Elena Kravchenko, Uliana Pinaeva, Alexander Nechaev, Pavel Apel

First published: 03 May 2024 | https://doi.org/10.1002/btpr.3478



Plan of experiments in 2024-2028: radioprotective mechanism

1. Evaluation of *in vivo* effects of the Dsup protein on chromatin compaction and transcriptome: combination of transcriptomic analysis (Affymetrix) with chromatin accessibility assay at a genome-wide level (ATAC-seq)

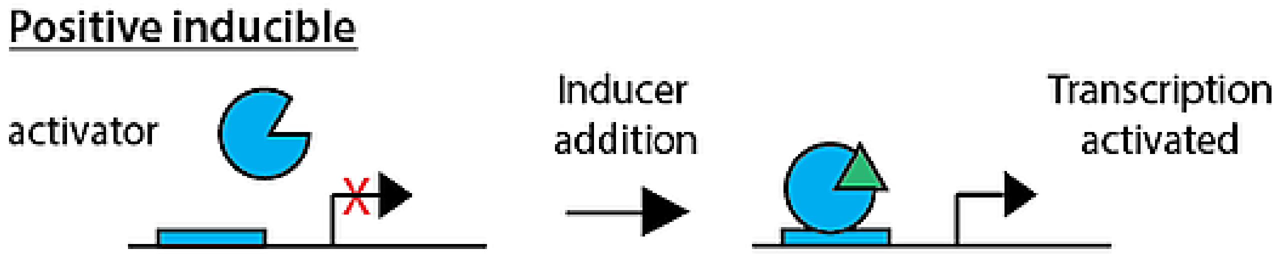
2. *In vitro* study of Dsup-DNA complex: structure (Cryo-EM) and binding properties

3. Focus on the fundamental biological role of DNA-binding intrinsically disordered proteins as crucial actors in genome regulation and architecture

Plan of experiments in 2024-2028: biotechnologies

4. Development of gene construct for stress-inducible gene expression pattern: creation of multicellular organisms or biotechnological cultures with enhanced stress-resistance requires improvement of *Dsup* gene sequence in order to optimize it's expression

Safer Dsup production: Dsup will be produced in response to a certain external stimulus as metal ions, oxidative stress, heat shock etc. and after the stimulus is removed, the inducible *Dsup* gene quickly returns to its original, inactive state



Plan of experiments in 2024-2028: applied technologies

5. Use of DNA/RNA-protective Dsup and human exosomes to create a system for increasing resistance to chemotherapy and radiotherapy of healthy cells and tissues during the treatment of oncological diseases with antitumor drugs and ionizing radiation

6. Dsup as a stabilizing substance: gels, stabilizers, cryoprotectants and radioprotectors containing Dsup or tardigrade proteins for preservation, transportation and storage of DNA/RNA-containing pharmaceuticals, vaccines and biological samples

Future experiments: natural mechanisms of extreme radioresistance

Collaboration with Frank Laboratory of Neutron Physics

Study of extremophile microorganisms from high radiation area nearby active zone(several meters) of the IBR-2 reactor (channel #3) – started on June 2024

Automatic box

BioSampling is performed with automatic box programmed for temporal opening/closing and containing nutrition media

Groups involved in TARDISS project

Dzhelepov Laboratory of Nuclear Problems, Joint Institute for Nuclear Research

Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research

Flerov Laboratory of Nuclear Reactions, Joint Institute for Nuclear Research

Research Center for Molecular Mechanisms of Aging and Age-related Diseases, Moscow Institute of Physics and Technology

School of Photonics and Quantum technologies. Digital medicine, Moscow State University

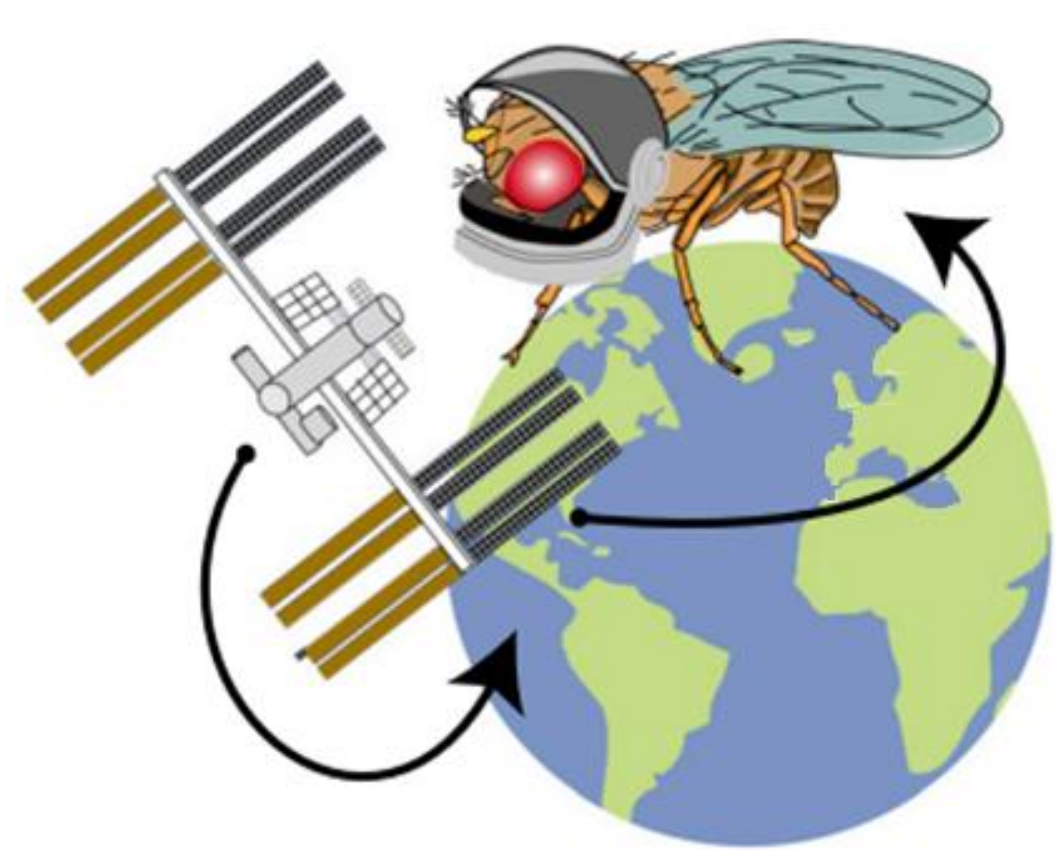
Recent publications

1. The tardigrade Dsup protein enhances radioresistance in *D. melanogaster* and acts as an unspecific repressor of transcription. (2023). *iScience.* **Q1**
2. Structural study of the intrinsically disordered tardigrade damage suppressor protein (Dsup) and its complex with DNA. (Under review). *Scientific Reports.* **Q1**
3. Developing tardigrade-inspired material: Track membranes functionalized with Dsup protein for cell-free DNA isolation. (2024). *Biotechnology Progress.* **Q2**
4. Radioprotective protein of tardigrades Dsup (damage suppressor) is resistant to high doses of ionizing radiation. (2024). *Moscow University Physics Bulletin*.
5. Unique Radioprotective Damage Suppressor Protein (Dsup): Comparative Sequence Analysis. (2022). *Physics of Particles and Nuclei Letters.*

Results are presented at international Russian and European conferences:

FEBS-2021 (Slovenia), EMBO|EMBL Symposium 2021 (Germany), EBSA-2021 (Austria),
FEBS-2022 (Portugal), BGRS-2022 (Russia),
Earth – Orbit – Deep space 2023 (Russia), EDRC-2023 (France),
PAUL Symposium 2024 (South Africa)

Theses: Master Degree (2024), PhD (planned in 2024), PhD (planned in 2025)



S. Walls et al. 2020

Thank you for your attention!