Analysis of radiation damage to the tardigrade Dsup protein by small-angle X-ray scattering

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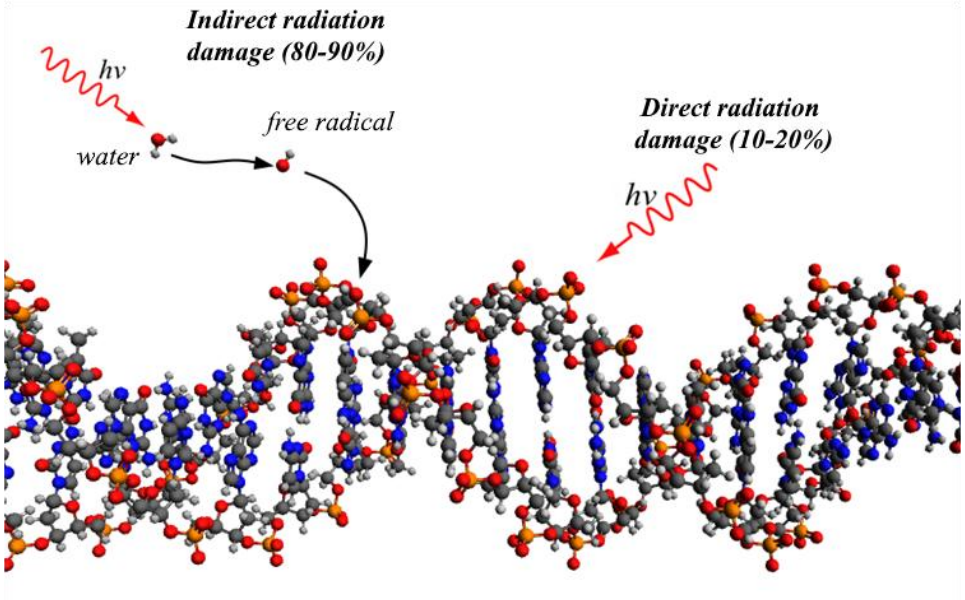
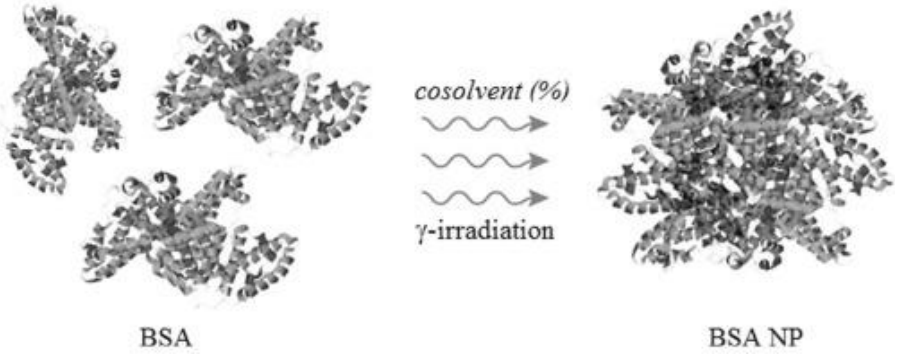
Effects of radiation on biological systems



DNA model

Reactive oxygen species generated during exposure to ionizing radiation damage and modify DNA and cause structural and conformational changes in proteins.

Among the structural changes that irradiation induces in proteins, the most common are oxidation, fragmentation, aggregation, and cross-linking.

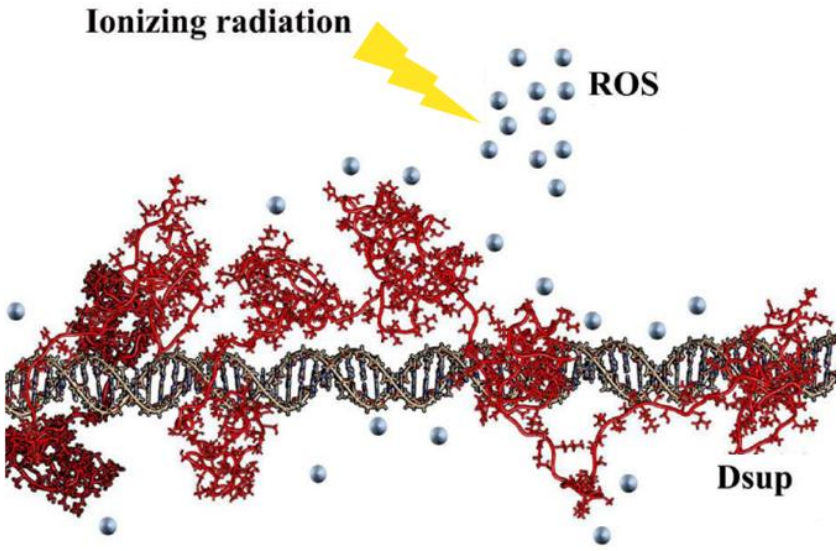


Queiroz R. G. et al. Radiation-synthesized protein-based drug carriers: Size-controlled BSA nanoparticles //International journal of biological macromolecules. – 2016. – V. 85. – P. 82-91.000

The tardigrade damage suppressor protein (Dsup)

Tardigrades are small aquatic animals, some species of which tolerate almost complete dehydration and exhibit exceptional resistance to various physical extremes in a dehydrated state.

The tardigrade damage suppressor protein (Dsup) increases radioresistance and resistance to oxidative stress in various organisms and in human cell culture.



Kravchenko E.V. "Study of the radioprotective properties of the Damage suppressor (Dsup) protein on the model object D. melanogaster and human cell culture HEK293“ (in Russian)

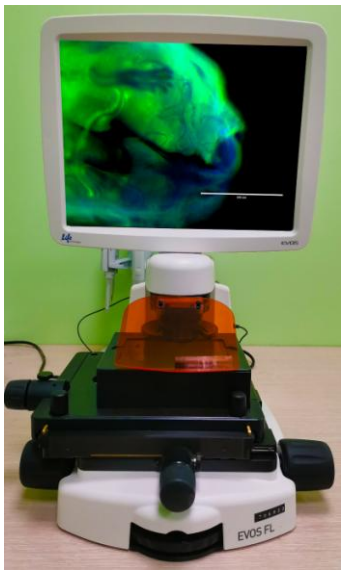
Relevance

The tardigrade Dsup protein can be used:

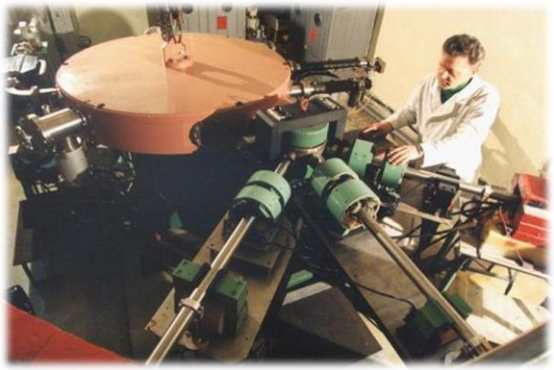
- ✓ as a DNA protector for model organisms and cell cultures;
- ✓ as a stabilizer for DNA-containing drugs;
- ✓ for manned spaceflight.

Radiation damage to the tardigrade Dsup protein

1) Protein synthesis – JINR Dzhelepov Laboratory of Nuclear Problems



2) Proteins irradiation – JINR Flerov Laboratory of Nuclear Reactions

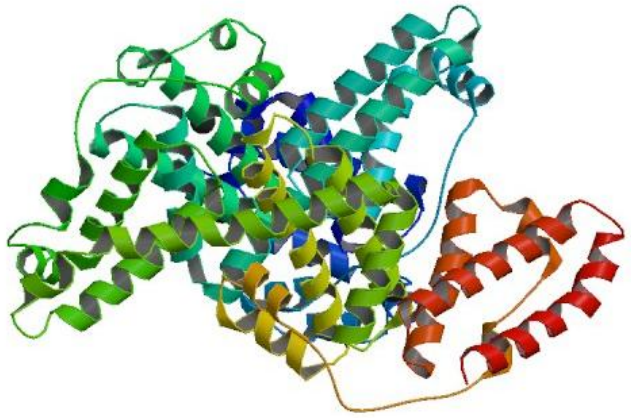


MT-25 microtron

3) Experiments – JINR Frank Laboratory of Neutron Physics and JINR Dzhelepov Laboratory of Nuclear Problems

Xenocs Xeuss 3.0

Materials and methods



BSA molecule model.
Source: RCSB PDB

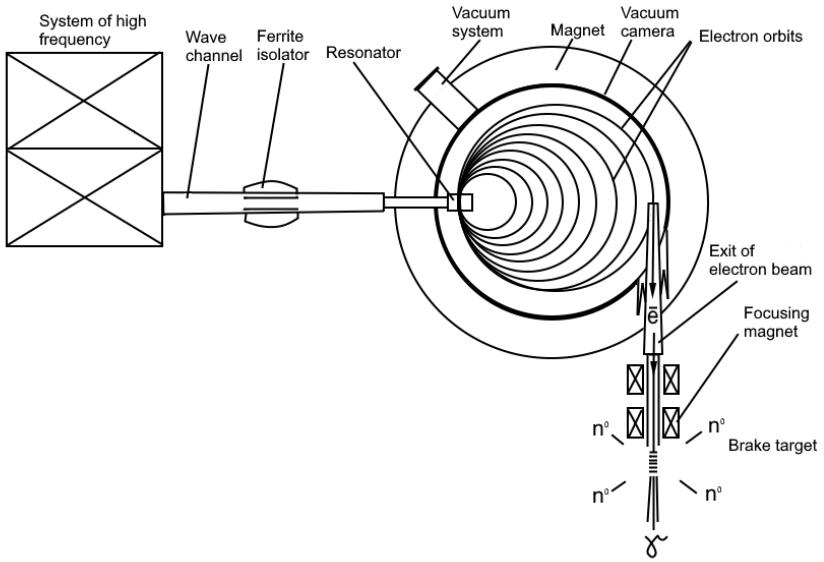
Control sample - bovine serum albumin protein (BSA):

- well-known structure;
- well-known monomer and dimer sizes;
- well-known behaviour under irradiation.

5 kGy – LD50 for tardigrades.

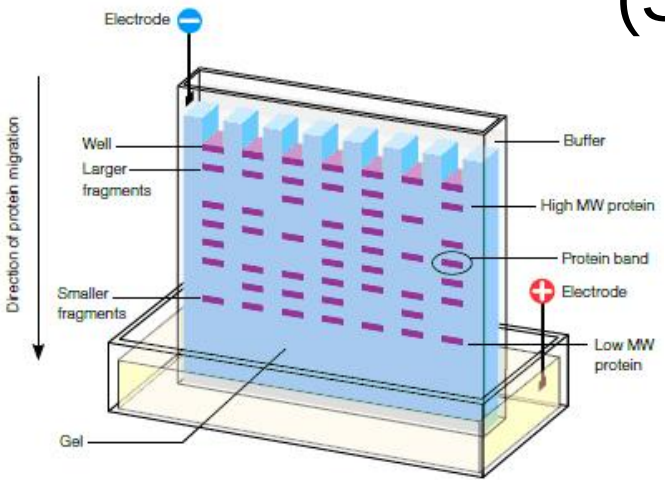
10 kGy is the LD for most known organisms (bacteria).

Irradiation of proteins – Microtron MT-25, Laboratory of Nuclear Reactions JINR.



The setup of microtron MT-25
Oprea, C. et al. Photoneutron activation analysis applied for environmental researches. 2011. Romanian Reports in Physics.

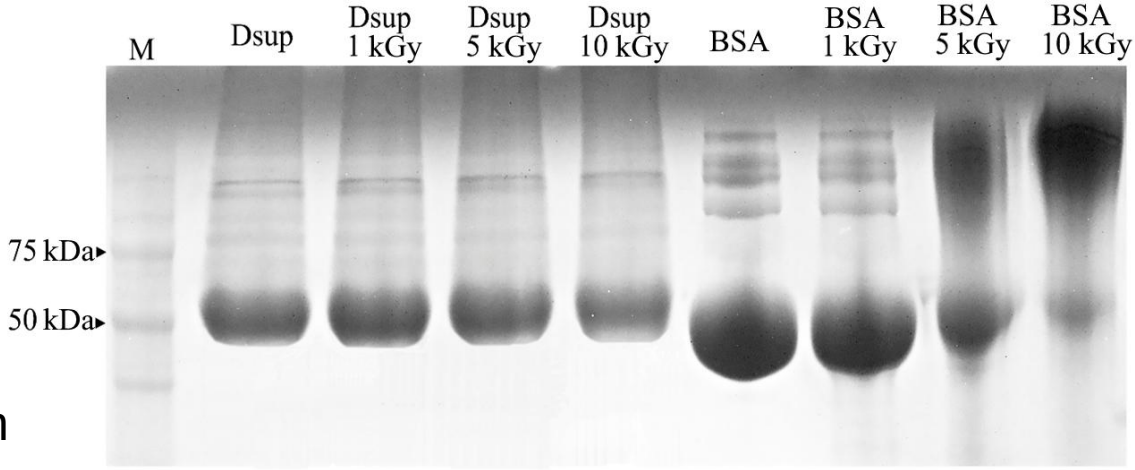
Materials and methods: sodium dodecyl sulfate - polyacrylamide gel electrophoresis of proteins (SDS-PAGE)



SDS-PAGE of proteins is a technique that separates proteins based on their electrophoretic mobility, which is determined by the size (molecular weight) of the molecule.

Schematic of electrophoretic protein separation in a polyacrylamide gel. Source: https://www.bio-rad.com

- SDS-PAGE profiles for BSA demonstrate the formation of protein aggregates, starting from a dose of 5 kGy.
- SDS-PAGE profiles of Dsup protein before and after irradiation **show its high radiation resistance** compared to BSA.



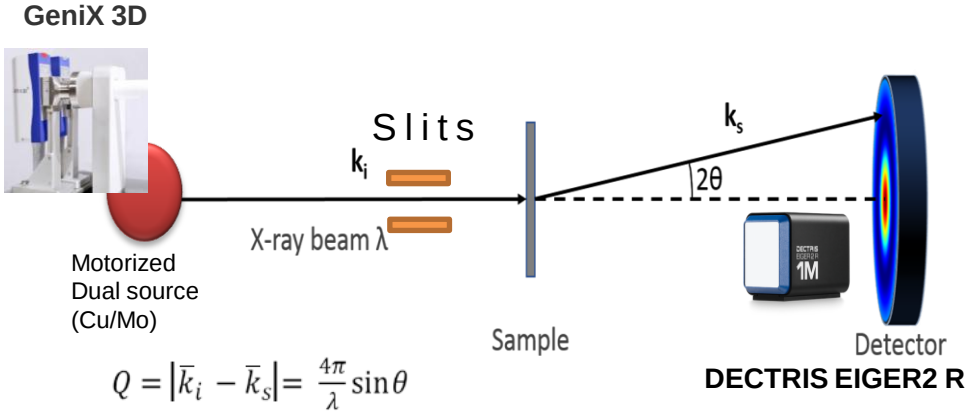
SDS-PAGE profiles for non-irradiated and irradiated at doses of 1, 5, 10 kGy proteins Dsup and BSA. M – molecular weight marker.

Materials and methods: small-angle X-ray scattering (SAXS)

Xenocs Xeuss 3.0, https://www.xenocs.com/

Small-angle X-ray scattering (SAXS) is a non-destructive method that enables structural studies of proteins in solution.

SAXS is a low-resolution method; it is a method of obtaining information about the characteristic dimensions of the object under study, the shape of the particle and the distribution of the scattering density inside it.



Small-angle X-ray scattering experiment

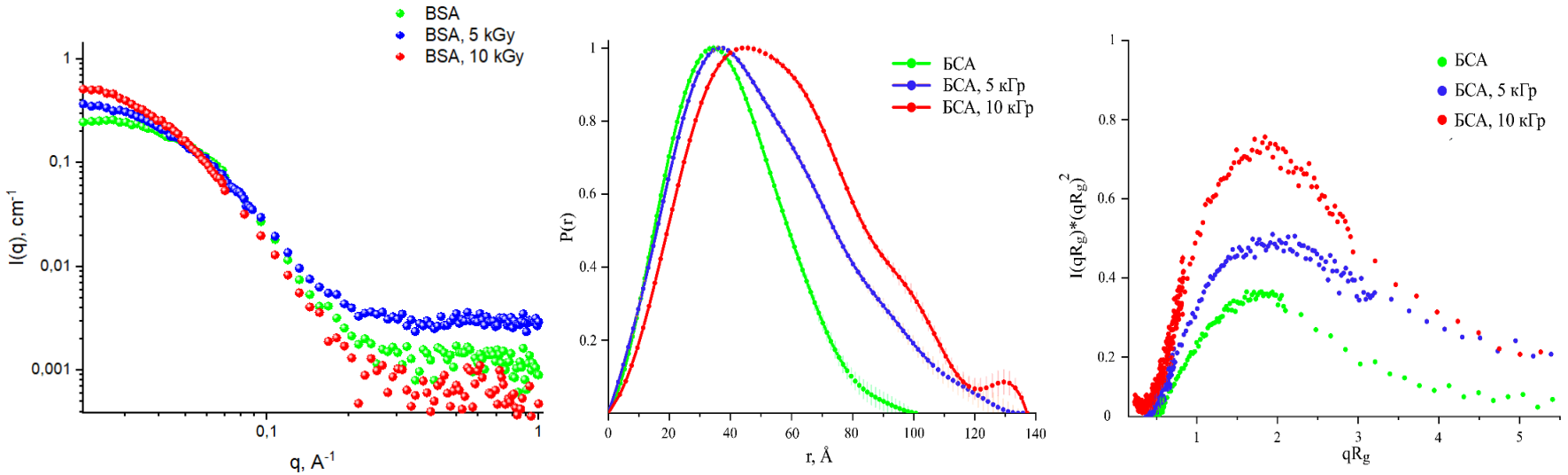
Experimental data processing

Scattering intensity :

$$I(q) = 4\pi \int_0^{D_{max}} p(r) \frac{\sin(qr)}{qr} dr$$

Pair distance distribution function:

$$P(r) = \frac{r^2}{2\pi^2} \int_0^{\infty} q^2 I(q) \frac{\sin(qr)}{qr} \mathrm{d}q$$

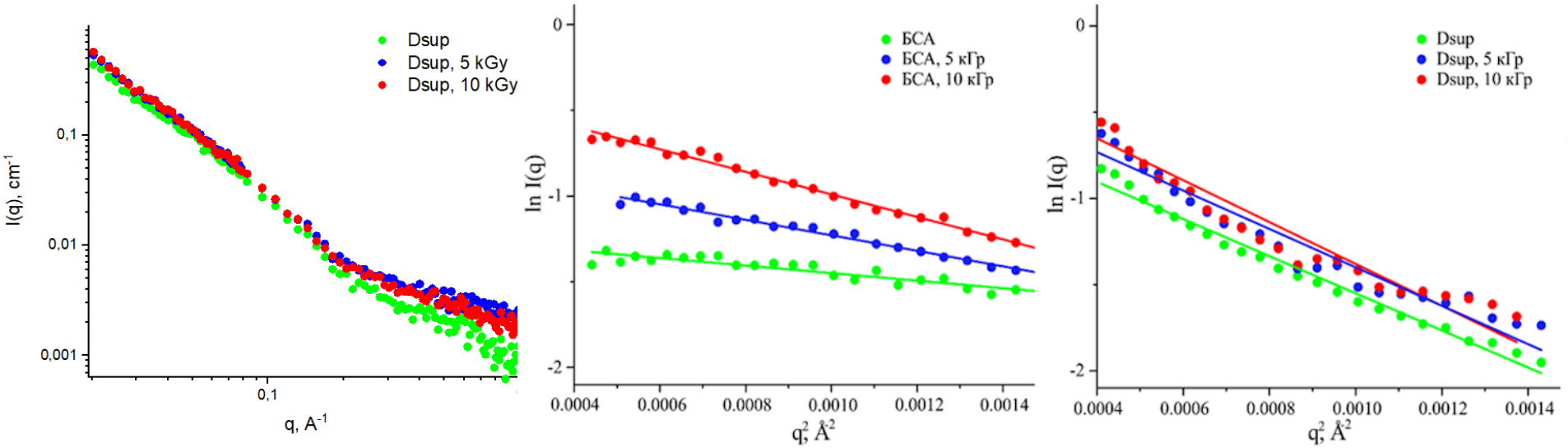


SAXS data, pair distance distribution function, and a dimensionless Kratky plot for BSA irradiated with various doses of γ-radiation and in the absence of irradiation.

The graphs show the process of larger aggregates formation.

Results

Guinier equation: $$I(q) = I(0)\exp\left(-\frac{q^2 R_g^2}{3}\right)$$



SAXS data for the tardigrade Dsup protein before and after irradiation with γ-rays at doses of 5 and 10 kGy. Guinier plot for solutions of BSA and Dsup before and after irradiation with γ-rays at doses of 5 and 10 kGy.

The Guinier plot for Dsup shows the absence of changes in the spatial structure of this protein when irradiated in solution with γ-rays at doses of 5 and 10 kGy.

Conclusions

The data obtained using SAXS demonstrates:

- ✓ absence of radiation-induced aggregation and changes in the spatial structure of the Dsup protein when irradiated with γ-rays at doses of 5 and 10 kGy;
- ✓ high radiation resistance of the disordered Dsup protein compared to the globular BSA protein.

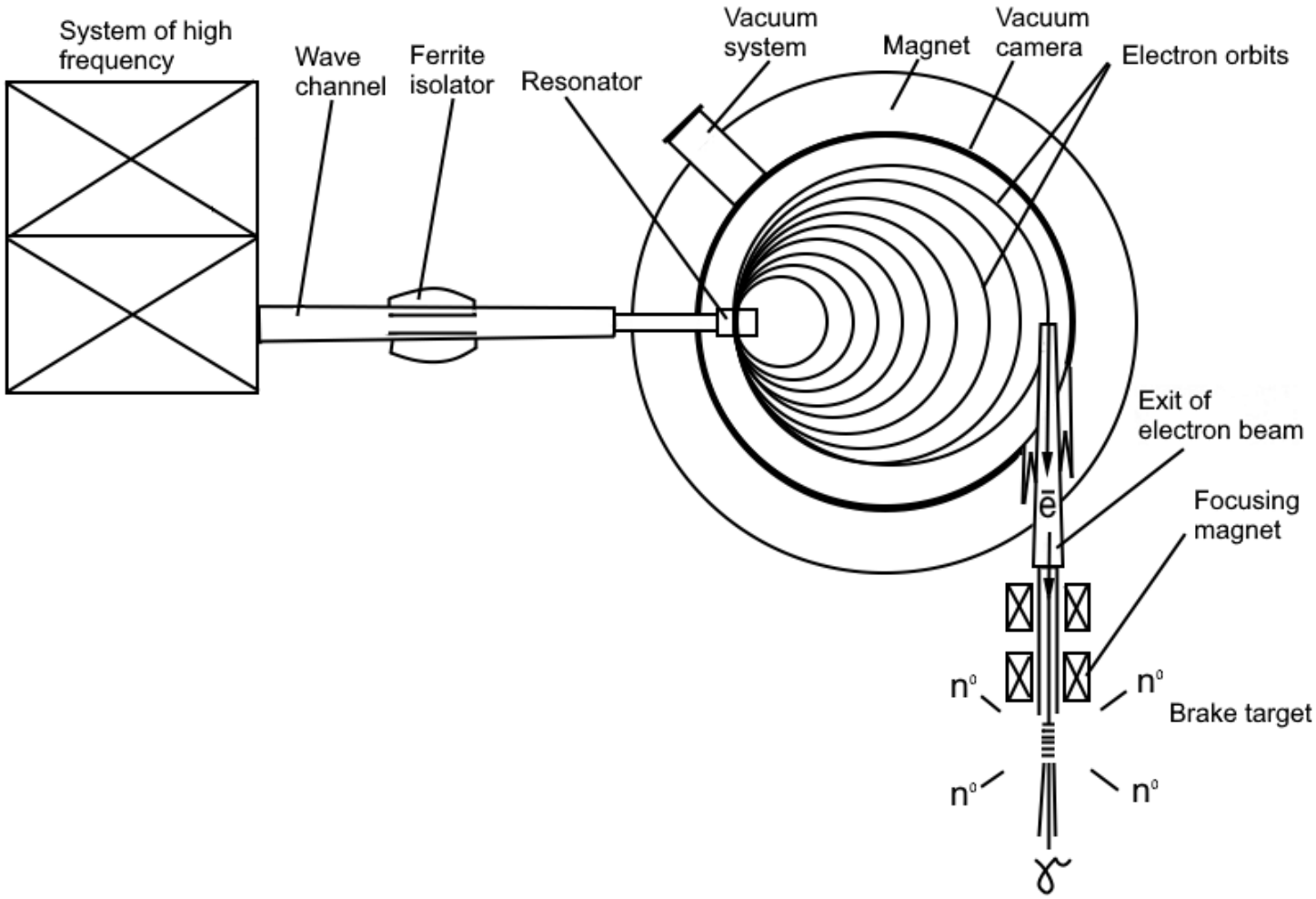
Zarubin M.P., Nizamieva A.F., Gorshkova Yu.E., Kravchenko E.V., Alekseev S.I., Mitrofanov S.V. (2023). Resistance to γ-irradiation of the radioprotective tardigrades protein Dsup (damage suppressor). Moscow University Physics Bulletin (accepted)

Thank you for your attention!

Experimental data processing

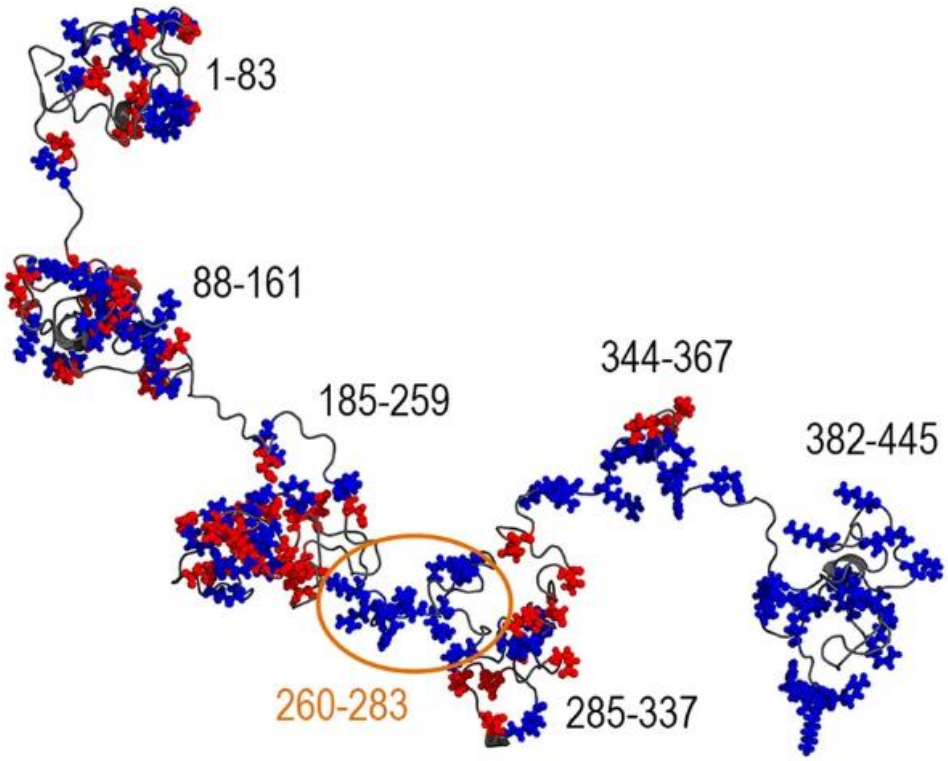
Table 1. Molecular characteristics of BSA calculated from SAXS data. R_g – radius of gyration, I(0) – intensity at q = 0, D_{max} – maximum transverse size, V_p – rock volume, MM – molecular weight

Образец	R_g, Å	$I(0)$	D_{max}, Å	V_p, Å^3	MM , кДа		MM (UniProt), кДа
					Q_p	V_c	
БСА	29,6±0,7	0,32±0,01	100,7	92450	66,95	65,7	69,3
БСА, 5 кГр	36,0±1,0	0,45±0,01	135,8	121520	75,30	74,00	
БСА, 10 кГр	42±1,6	0,69±0,02	137,2	251000	182,15	170,85	
БСА, мономер	28		92,0	92000			
БСА, димер	41		138,0	200000			138,6



MT-25 microtron setup.
Oprea, C. et al. Photoneutron activation analysis applied for environmental researches. 2011. Romanian Reports in Physics.

Белок тихоходок Dsup (damage suppressor)



Структурная 3D-модель белка Dsup, предсказанная методом I-TASSER. Заряженные аминокислоты представлены в виде шариков (положительно заряженные обозначены синим цветом, а отрицательно заряженные - красным).

Mínguez-Toral M. et al. A computational structural study on the DNA-protecting role of the tardigrade-unique Dsup protein //Scientific reports. – 2020. – Т. 10. – №. 1. – С. 13424.

Устойчивость различных организмов к γ-радиации

Организм	LD_{50} или другие доступные данные	Автор
Человек разумный	$LD_{50/30d}$ = 2.5-4.5 Gy	Bolus (2001)
Мышь	$LD_{50/30d}$ = 4.5 Gy	Bolus (2001)
Золотая рыбка	$LD_{50/30d}$ = 8 Gy	Bolus (2001)
Таракан	$LD_{50/30d}$ = 50Gy	Bolus (2001)
Дрозофила фруктовая	$LD_{50/30d}$ = 1238-1339 Gy	Parashar et al. (2008)
Deinococcus radiodurans (Bacteria)	LD_{50} = 10 000 Gy	Makarova et al. (2001)
Коловратки	Не влияет на выживаемость до 1120 Gy	Gladyshev and Meselson (2008)
Тихоходки	LD_{50} = 1270-5000 Gy	Hashimoto and Kunieda (2017)

- Kravchenko E.V. "Study of the radioprotective properties of the Damage suppressor (Dsup) protein on the model object D. melanogaster and human cell culture HEK293“ (in Russian)

Proteins used

Recombinant Dsup protein was produced in E. coli BL21(DE3) cells using the pCold-I-Dsup plasmid (a gift from Takekazu Kunieda (Addgene plasmid #90021)). Protein purification was performed using a Ni-NTA Fast Start Kit (QIAGEN) according to the manufacturer's instructions. The resulting Dsup protein solution was concentrated to 5 mg/ml and the elution buffer was replaced with standard phosphate buffered saline PBS, pH-7.4 (Gibco) using Amicon centrifugal concentrators (Merck). Protein concentration was determined using Qubit 4 Fluorometer (Thermo Fisher Scientific) and Qubit Protein Broad Range (BR) Assay Kit (Thermo Fisher Scientific). Bovine serum albumin (BSA) (Sigma-Aldrich) was used as a solution in standard phosphate buffered saline (PBS) at a concentration of 5 mg/ml.

Irradiation of protein solutions

Protein solutions with a concentration of 5 mg/ml were irradiated with γ-quanta at the Microtron MT-25 in the Laboratory of Nuclear Reactions of the Joint Institute for Nuclear Research in doses of 1, 5, 10 kGy with an intensity of 1 Gy s^{-1}. Dosimetric monitoring was carried out using an SNC600c ionization chamber (Sun Nuclear Corporation).

Polyacrylamide gel electrophoresis of proteins under denaturing conditions

Protein electrophoresis in polyacrylamide gel under denaturing conditions was carried out according to the Laemmli method [18] in a Mini-PROTEAN Tetra electrophoresis chamber (Bio-Rad) using 12% Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad) and electrophoresis buffer, containing Tris base 25mM, glycine 192mM, SDS 0.1% w/v. Precision Plus Protein™ Dual Color Standards (Bio-Rad) was used as a molecular weight marker.

Small-angle X-ray scattering (SAXS) experiments

The measurements were carried out on a setup in point geometry using a GeniX 3D microfocus X-ray source with a wavelength λ = 1.5419 Å in a 30 W/30 μm mode. The installation is equipped with an Eiger2 R1 M (Dectris) detector with an area of 77.1 mm × 79.7 mm. Sample measurements were carried out in borosilicate capillaries with an internal diameter of 1.5 mm (Hilgenberg), which were in a vacuum at room temperature. Data acquisition time was 60 min for each sample-to-detector distance (SD). The choice of three distances SD 400, 1800 and 4500 mm in the experiment made it possible to obtain the dependence of the X-ray scattering intensity I(q) in the range of transmitted pulses $q = (4\pi/\lambda)\sin(\theta)$, where 2θ is the scattering angle, from $5 \cdot 10^{-3}$ to 0.65 $Å^{-1}$. AgBeh (silver behenate) and GC (glassy carbon) standards were used to calibrate the SD distance and absolute intensity, respectively. Primary data processing was carried out in the Xenocs XSACT program version 2.4.