LIFE IN THE UNDERGROUND LAB

NEW DISCOVERY OPPORTUNITIES

&

Laboratorio Subterráneo de Canfranc

Carlos Peña Garay

April 27, 2022 LNGS

IN HONOR OF THE LATE PROF. L. SATTA

GINO, GIUSI AND ANTONELLA

WHY BIOLOGY UNDERGROUND?

Summary by A, Rahmeh (UPF) and I. Ruiz-Trillo (IBE):
The ubiquity of background radiation as an abiotic component of earth's surface environment may have led to the incorporation of the biophysical and biochemical effects of background radiation levels into fundamental cellular processes during the evolution of organisms living on or near surface environments.

Reduction of background radiation levels in deep underground laboratories have shown an alteration in growth kinetics in several organisms including bacteria (Smith et al. 2011, Castillo et. al 2018), unicellular eukaryotes (Satta et al. 1995), mammalian cell lines (Satta et al. 2002, liu et al. 2020), and small multicellular organisms (Morciano et al. 2019, Pirkkanen et al. 2020, Van Voorhies et al 2020).

Unicellular organisms and mammalian cell lines exhibited a decrease in growth kinetics, an increase in spontaneous mutations as well as in sensitivity to DNA damaging agents, and an up-regulation of oxidative stress related genes.

Multicellular organisms exhibited positive growth phenotypes: increased life span in Drosophila (Morciano et al. 2018), increased egg laying in C. elegans (Van Voorhies et al 2020), and increased body weight and length in lake whitefish embryos (Pirkkanen et al. 2020). In the case of Drosophila, a decrease in female and male fertility was observed which could be indicative of defective DNA repair.

ORIGINAL MOTIVATION

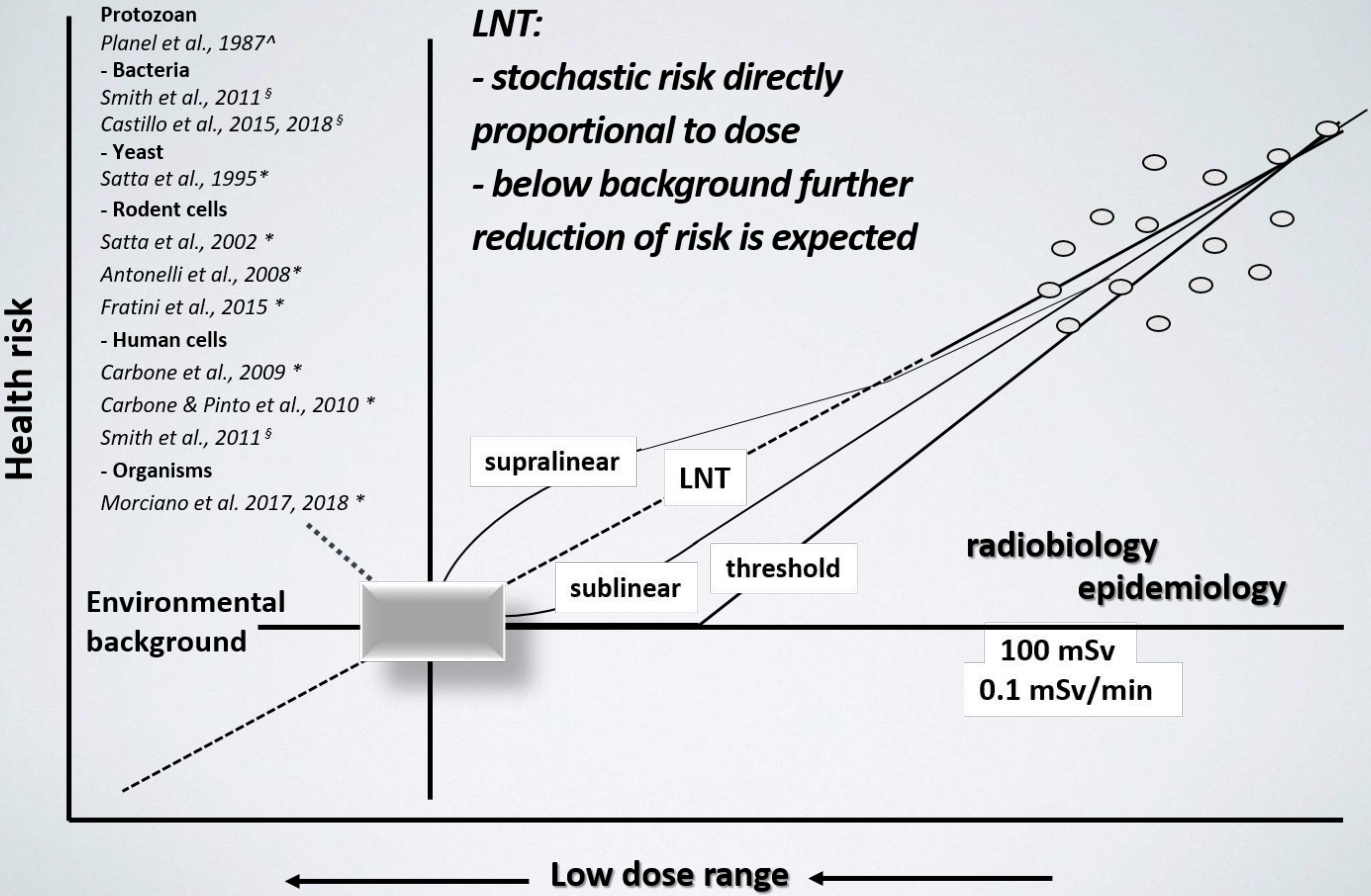

Table 2
Advancements in biological research in deep-underground laboratories.

Culture	Labs	Times	Method of shielding radiation	Value of radiation dose (LB vs. CB)	Method of research	Result
Mastigogladus I.[a]	SIMP	A few weeks	RC 2000 m	Not stated	Not stated	Died
Paramecium tetraurelia[b]	CNRS	10 days	RC 200 m + 5 cm Pb wall	0.1 vs. 1.65 mGy/y	Generation time	Cell growth decreased, inhibitory effect strengthened by adding a 5 cm lead wall.
Saccharomyces cerevisiae[c]	LNGS	120 generations	RC 1400 m	0.6 vs. 4.0 μSv/day	MMS-induced genetic damage	Higher frequency of MMS-induced recombination.
V79[d]	LNGS	6 months	RC 1400 m	Fluence ratios, cosmic rays, 10^{-6}, neutrons, 10^{-1}, radon concentration, 10^{-3}, γ-rays dose, 5×10^{-2}	Enzyme activities, apoptosis, gene expression, and mutation induction	Increased apoptotic activity, CHX-induced *c-myc* gene expression, and mutation induction at the X-linked hypoxantine-guanine phosphorybosil transferase (*hprt*) locus after UV exposure, p53 expression was unchanged.
V79[e]	LNGS	9 months	RC 1400 m	Cumulative gamma dose, 0.03 vs. 2 mGy, average radon concentration 5 vs. 120 Bq/m^3	Growth curve, apoptosis induced by CHX, antioxidant abundance, mutation induced by γ-irradiation	Growth rate unchanged at 3 months and 9 months, but an increased cell density at confluence at 9 months, CHX-induced apoptosis increased after 3 months but not after 9 months. no change in CHX-induced *p53* and *c-myc* expression at 3 months or 9 months, higher capacity to scavenge hydroperoxides but a reduced scavenging capacity towards superoxide anions, increase in both the basal *hprt* mutation frequency and sensitivity to the mutagenic effect of γ rays, downregulation of GPx activity.
V79[f]	LNGS	10 month at LB + 6 month at SB	RC 1400 m + 5-cm-thick iron wall	γ-Rays 3.6 nGy/h vs. 34 nGy/h, cosmic rays, N vs. 39 nGy/h, neutron dose rate, N vs. 2.5 nGy/h, low-LET 22.6 vs. 92.0, high-LET 0.2 vs. 2.7	Enzymatic activity, gene expression of GPx, spontaneous mutation frequency	Decreased GPx activity, increased mutation frequency after 10 months, increasing further after culture at SB for another 6 months.
TK6[g]	LNGS	6 month	RC 1400 m	^{222}Rn and daughters, 0.17 vs. 1.7 nGy/h, All rays, 3.6 vs. 300 nGy/h, cosmic rays N vs 30 nGy/h, total dose-rate 3.8 vs. 331.7 nGy/h.	Growth curve, enzymatic activity and Micronucleus assay induced by X-radiation	Cell doubling times did not vary, frequency of micronuclei caused by acute radiation exposure increased, SOD enzyme activity decreased, increase in of ROS-scavenging efficiency as measured by CAT/SOD and GPx/SOD ratios.
Drosophila melanogaster[h]	LNGS	About 9 months	RC 1400 m	Cosmic rays, N vs. 39 nGy/h, neutron dose rate, N vs. 2.5 nGy/h, γ-rays, 21.5 vs. 34 nGy/h, radon concentration, 10–70 vs.70–100 Bq m^{-3}	Life span, fertility, climbing test	Life span increased, fertility reduced, locomotion activity was not affected, positive selection of homozygotes for a semi-viable mutation in the *Drosophila* ATM-encoding gene.
Deinococcus radiodurans[i]	WIPP	75 h	RC 650 m + pre-15.24 cm World War II steel chamber	0.2 vs. 3.1 uR/h	Total cell protein, counts and optical density of the cultures	Growth inhibited, total proteins reduced.
Shewanella oneidensis, *Deinococcus radiodurans*[j]	WIPP	72 h	As above	γ-Rays, 0.16 vs. 71.3 nGy/h	Growth rate, stress-related genes	Reduction in growth of both bacterial species. Upregulation of *katB*, *recA*, *SOA0154* genes in *S. oneidensis* and upregulation of *dnaK* in *D. radiodurans*, cells were returned to background radiation levels, growth rates recovered and the stress response dissipated.
Shewanella oneidensis, *Deinococcus radiodurans*[k]	WIPP	72 h	As above	As above	As above	Retaining normal growth *for Shewanella oneidensis*, reduction in growth of *Deinococcus radiodurans*. In *Shewanella oneidensis*, 33% of the genes upregulated in the minus-radiation treatment, whereas in *Deinococcus radiodurans* only 4.5 and 6.8% up or downregulated.

(*continued on next page*)

INCREASE IN REACTIVE OXYGEN SPECIES

SUPPRESSED DNA REPARATION MECHANISMS

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TASK 5.7 - BIOLOGY IN DULs

The main objective of the Work Package is the development of i**nnovative approaches and tools** to explore life in cosmic silence and zero dose environment as well as implementation of a **scientific network**.

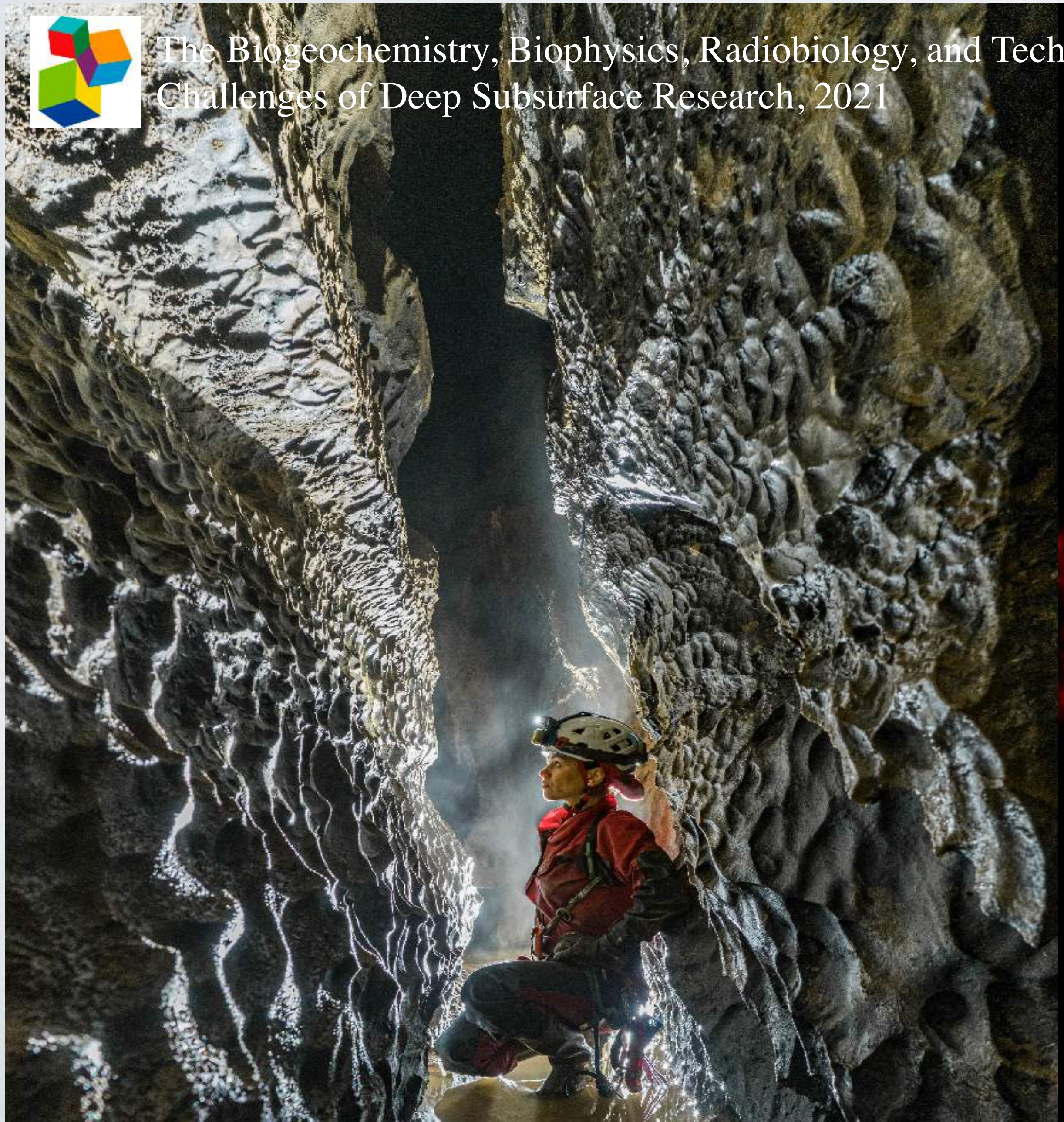
Deep Underground Laboratories represent an **extreme ultra-low radiation** environment ideal to conduct **controlled and systematic** biological studies.

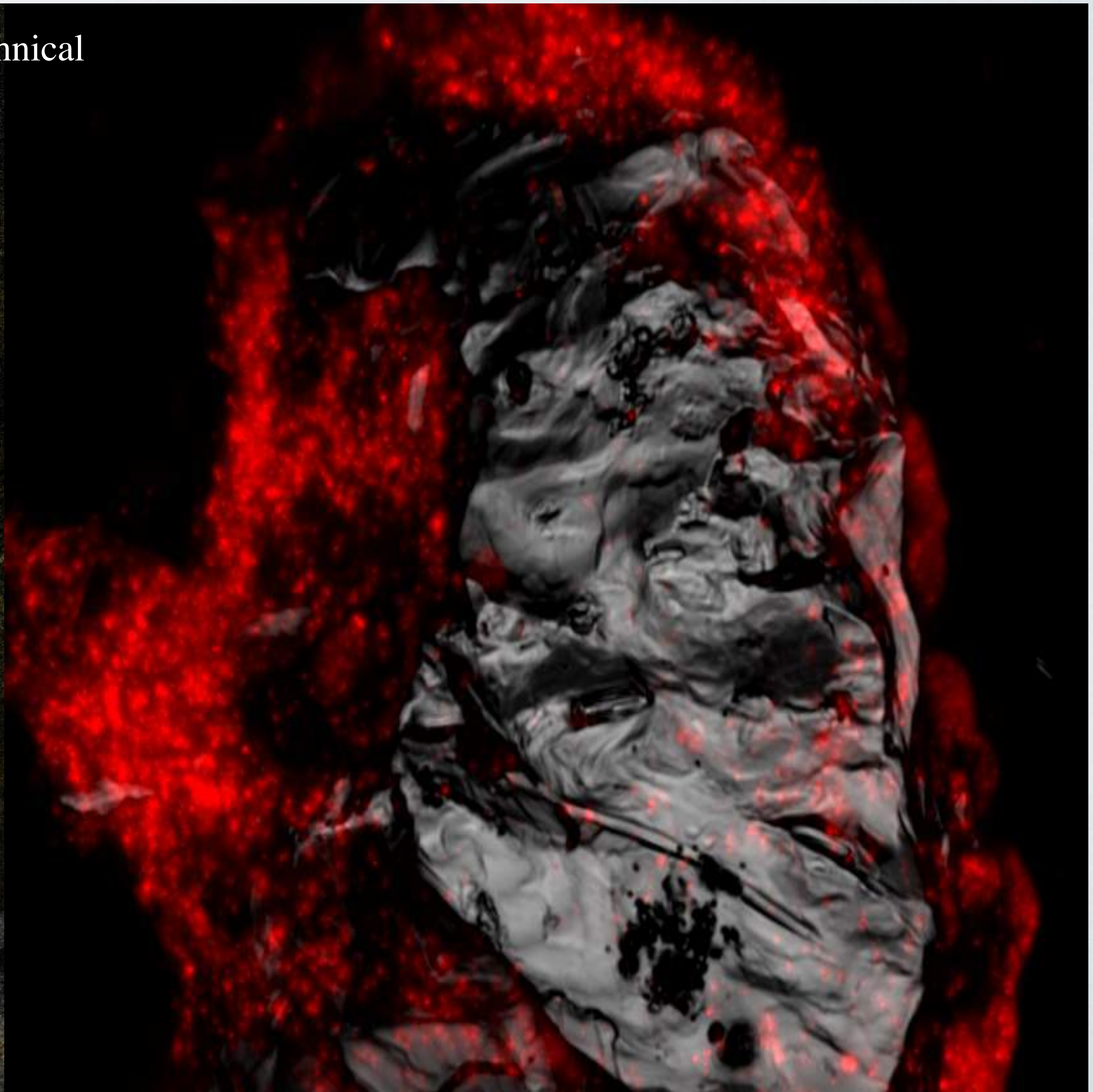
The **heterogeneity** among Laboratories offers a great opportunity for low radiation quality studies to validate the impact of cosmic silence:
- increase of ROS
- reduction of DNA repair
- "omics" investigation"
- modulation of ageing
- cancer
- adaptive response
- immune system.

Exchange and Comparison among labs
Innovative background studies in this context and protocols will be delivered.

DELIVERABLES 5.7.1&4 - BIOLOGY IN DULs

D5.7.1 Protocols to compare biological experiments in different underground labs. Development of a systematic and coordinated research model for qualitative and quantitative analysis of the underground investigations. Discussions ongoing (DULIA-bio)
D5.7.4 Rules to share services and personnel for scientists to access multiple DULs. Much more needed in Biology. Lack of expertise in UltraLow Background.

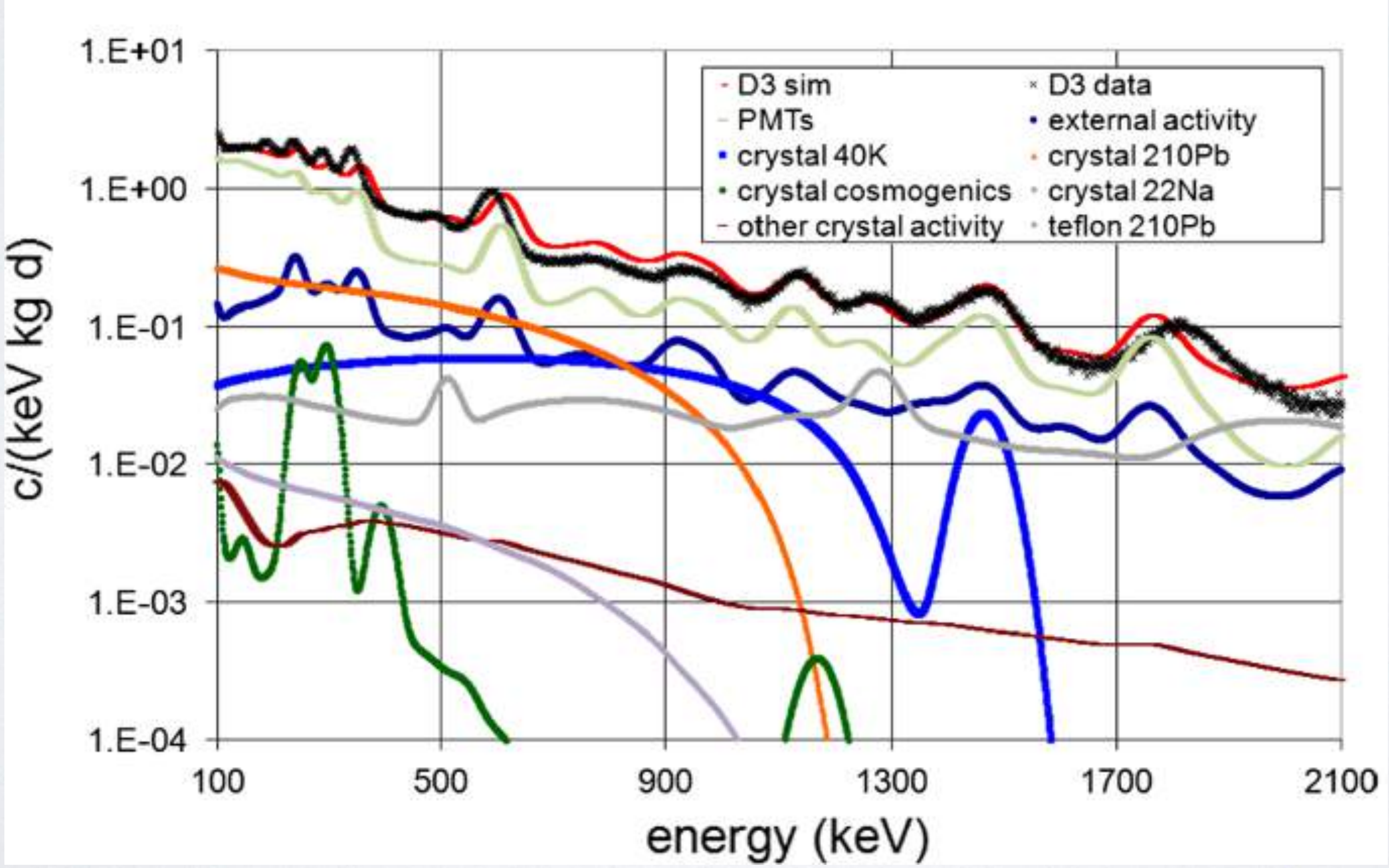




DELIVERABLES 5.7.2 - BIOLOGY IN DULs

D5.7.2 Identification and monitoring of radioactive backgrounds; development of a background model of radioactivity in the experimental site(s) and set up(s).

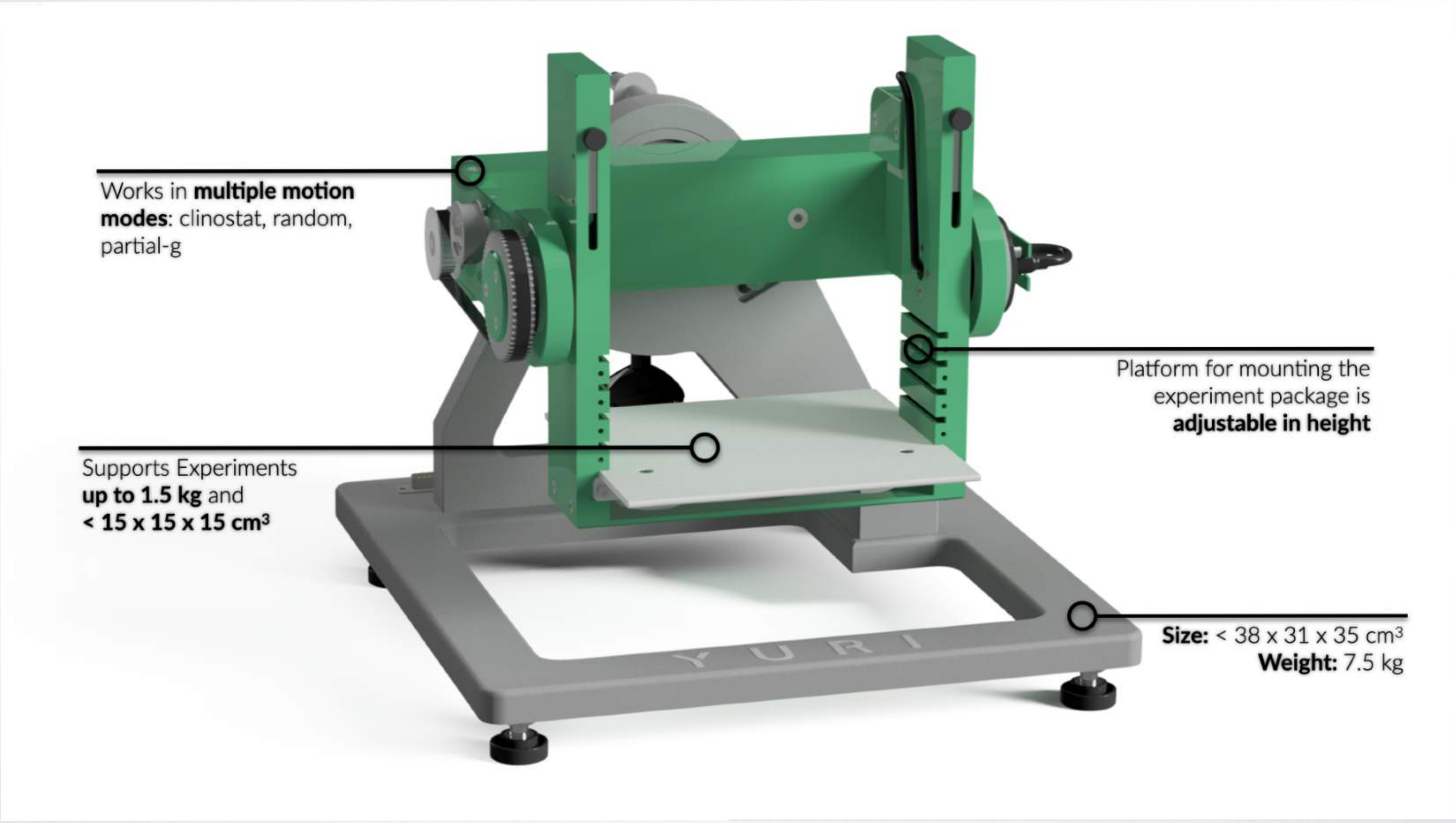
Measurements of several backgrounds have been monitored in previous experiments. But, comparison will require published background models. See, Dark Matter experiments (ANAIS, as an example).



DELIVERABLES 5.7.3 - BIOLOGY IN DULs

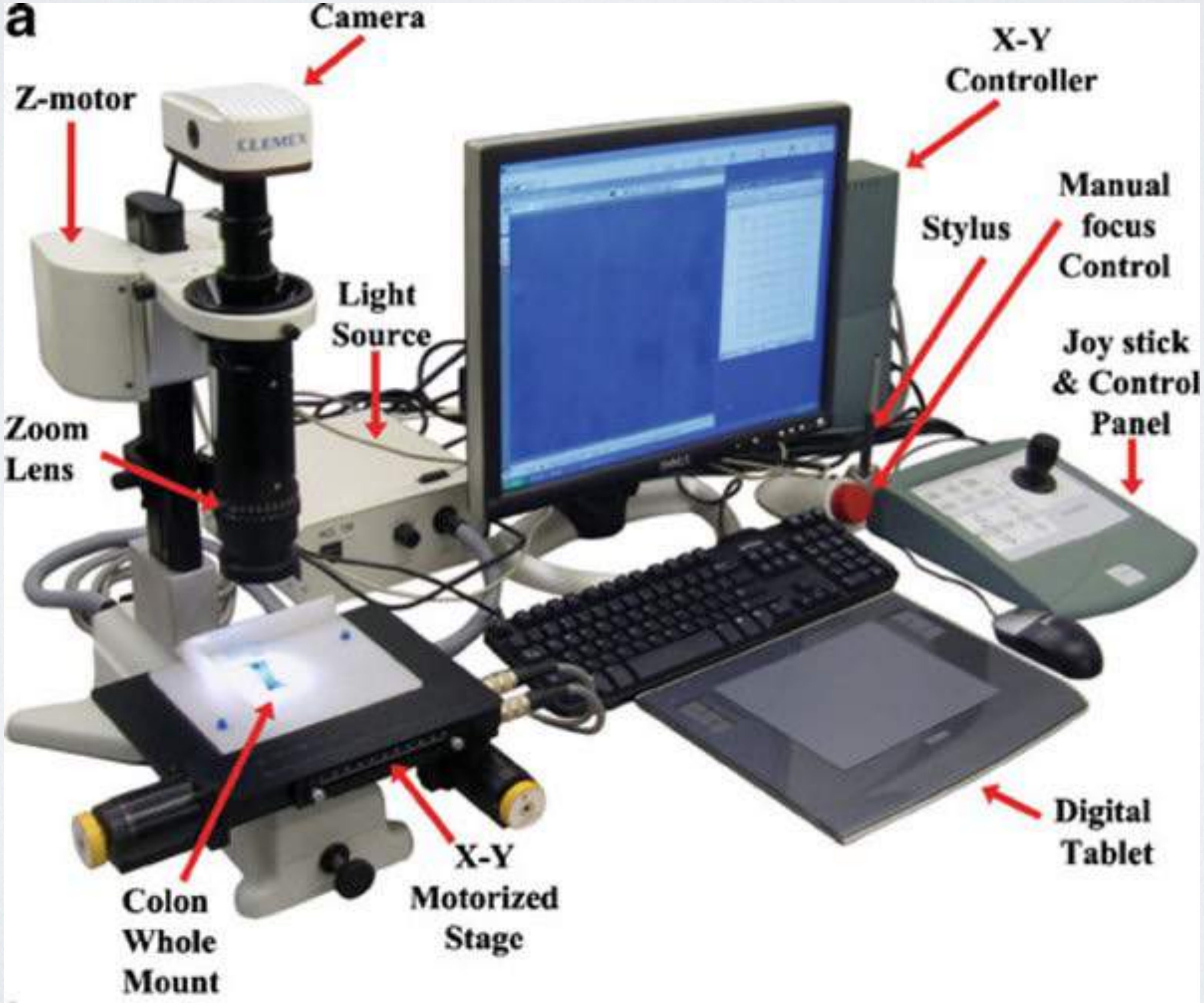
D5.7.3 Design and Implementation of microgravity investigation approach in ultra-low radiation background environment for biology experiments *and integration of biological monitoring*

Life in Space could be better mimicked by microgravity (RPM) in DUL (lack of muons)



DELIVERABLES 5.7.5 - BIOLOGY IN DULs

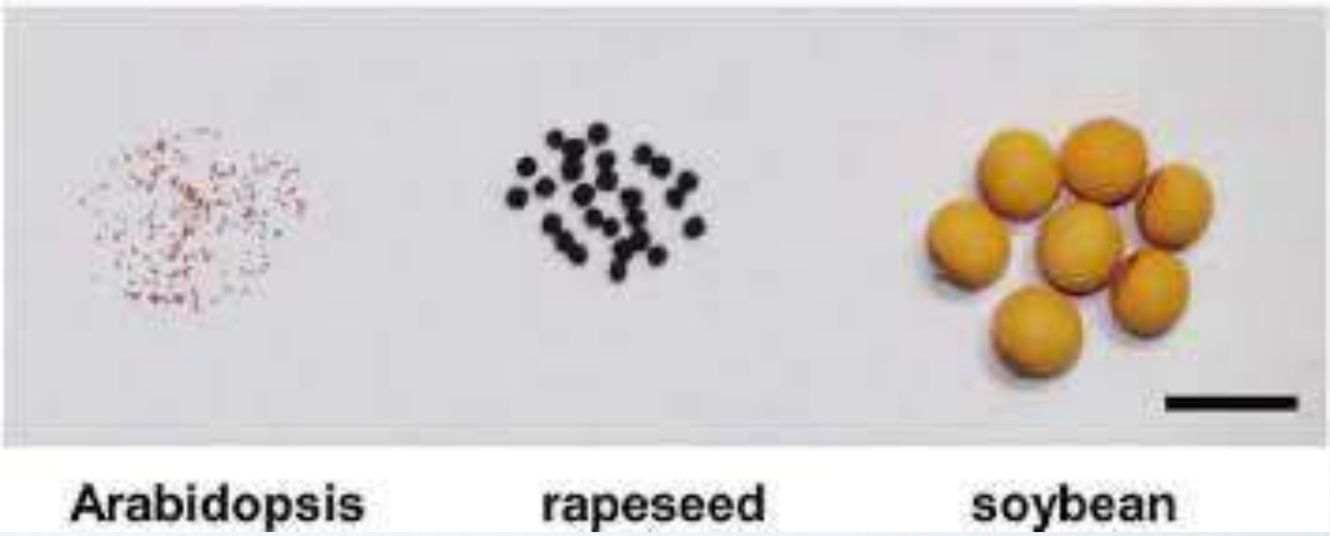
D5.7.5 Implementation of an automated workstation for morphometric tumor analysis. Genetic and molecular analysis of phenotypic traits of solid tumours in Drosophila maintained in reference and extremely-low radiation environments



DELIVERABLES 5.7.6 - BIOLOGY IN DULs

D5.7.6 Installation and testing of equipment for biological studies in specialized clean room (Zero Dose Environment). Studies of influence of radiation on storage of Arabidopsis seeds.

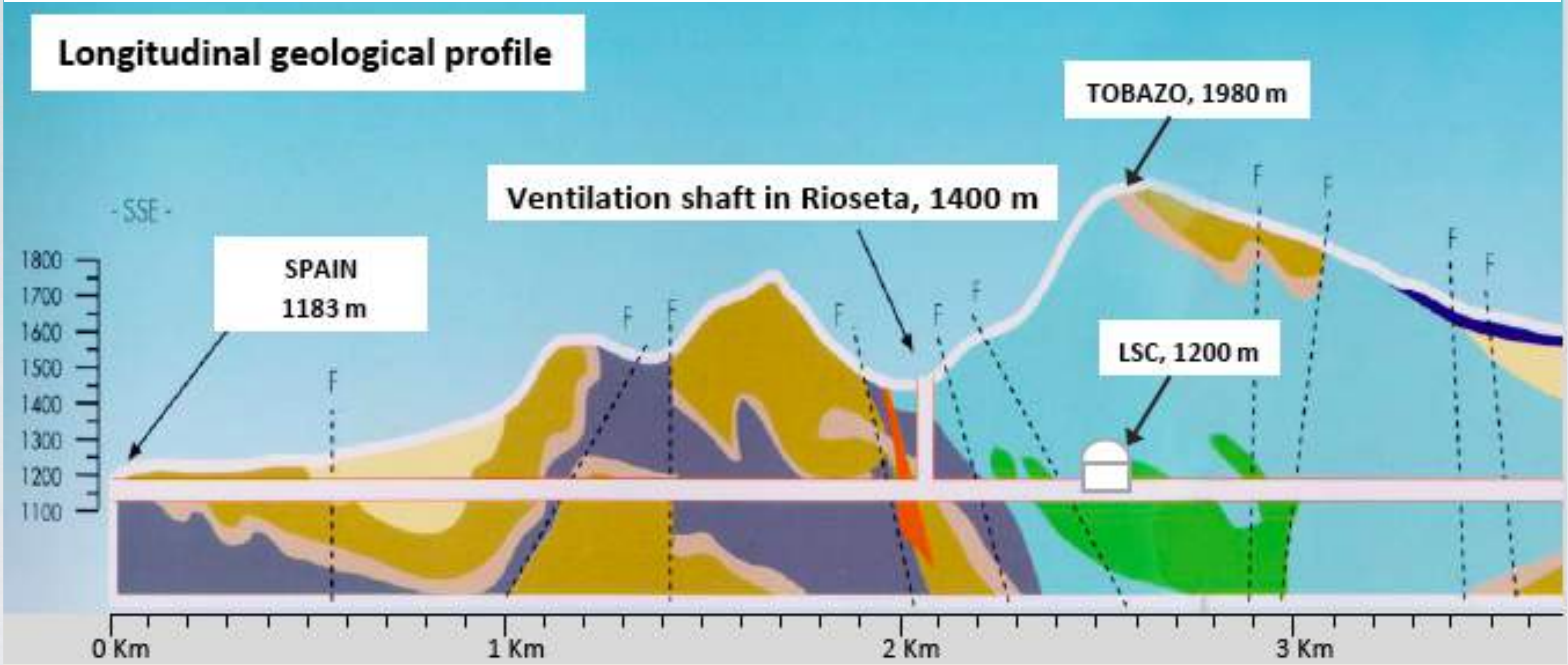
The vascular plant Arabidopsis thaliana is a central genetic model and universal reference organism in plant and crop science. Long-term studies on preservation of crops.



WHY BIOLOGY UNDERGROUND?

A very large community, future users of Underground Labs, are interested on the influence of the lack of muons in DNA reparation and the multiple phenomena associated to it.

Two access tunnels, variable depth small spaces available



1986 - First experiments in train tunnel
2003-2006 - new lab built 1600m^2
2007-2010 - refurbishment works
Since 2010 - re-start experimental activities
Previous Directors: A. Bettini, A. Ianni

Inlet air flux: 20000 m^3/h
Radon: 50-80 Bq/m^3
Radon-free: 1mBq/m^3, 220 m^3/h
Muons: (5.3+-0.2) $\cdot 10^{-3}$ m^{-2}s^{-1}
Neutrons: 3.5 $\cdot 10^{-6}$ cm^{-2}s^{-1}

MORE INSTITUTIONS & ACTIVITIES

Delivering Connecting Blossoming

LSC IN NUMBERS

LSC is a Spanish ICTS: 10 yrs budget plan approved

People on site: 14 (2020), 19 (2021), 25 (2022)

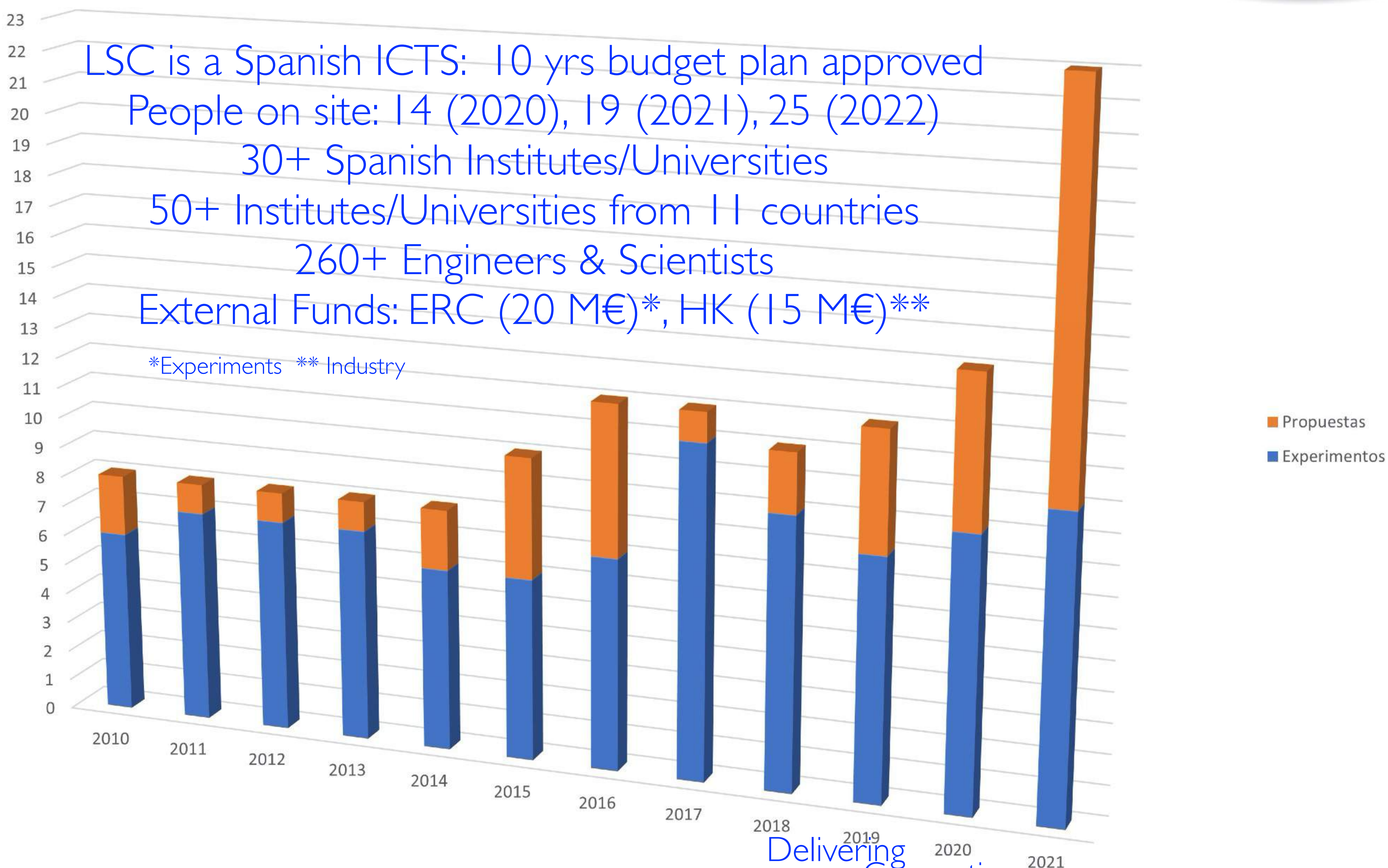
30+ Spanish Institutes/Universities

50+ Institutes/Universities from 11 countries

260+ Engineers & Scientists

External Funds: ERC (20 M€)*, HK (15 M€)**

*Experiments ** Industry



Delivering Connecting Blossoming

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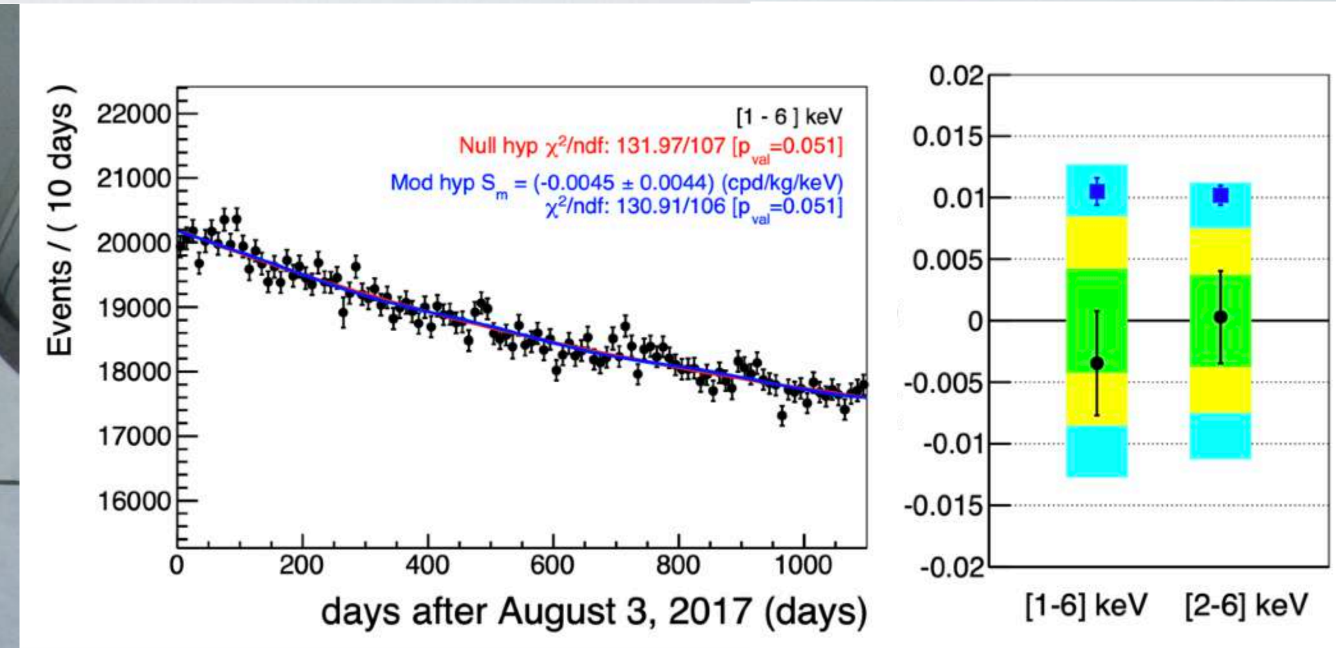
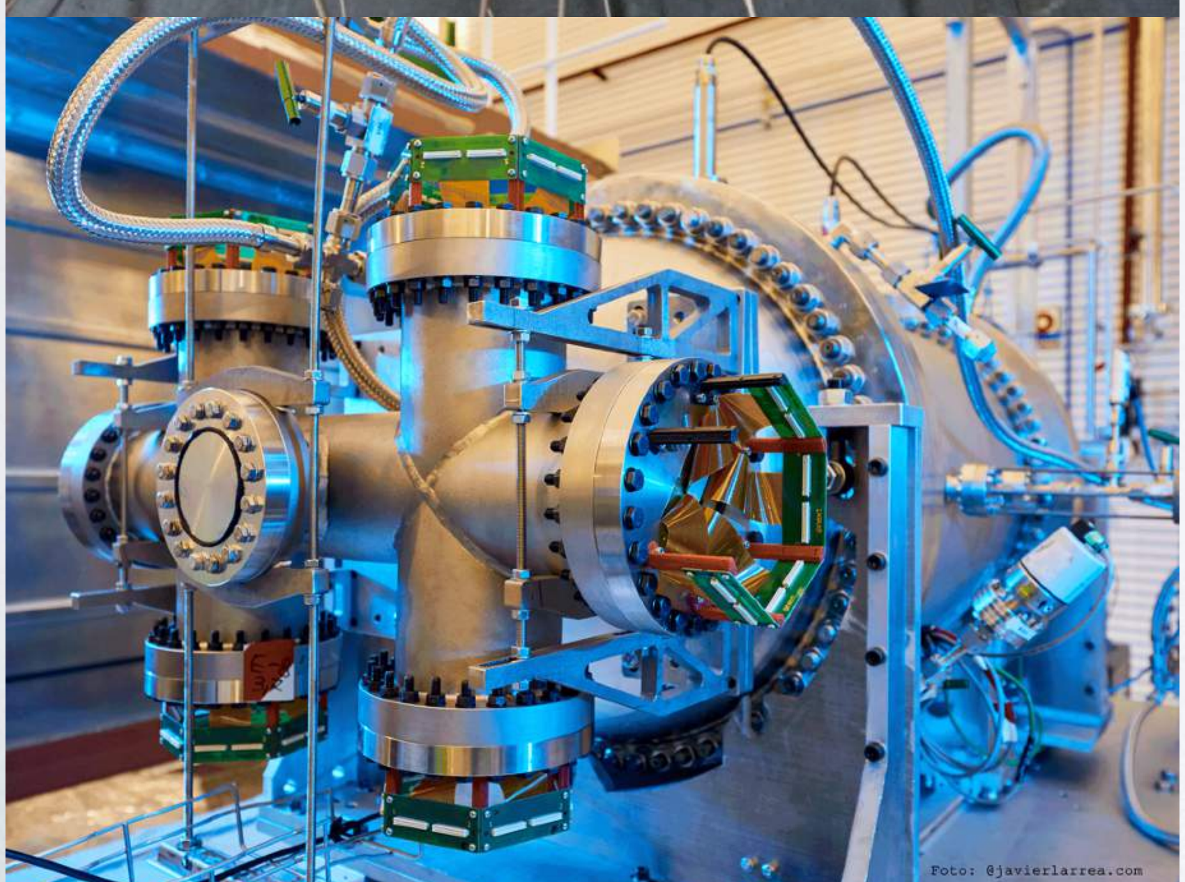
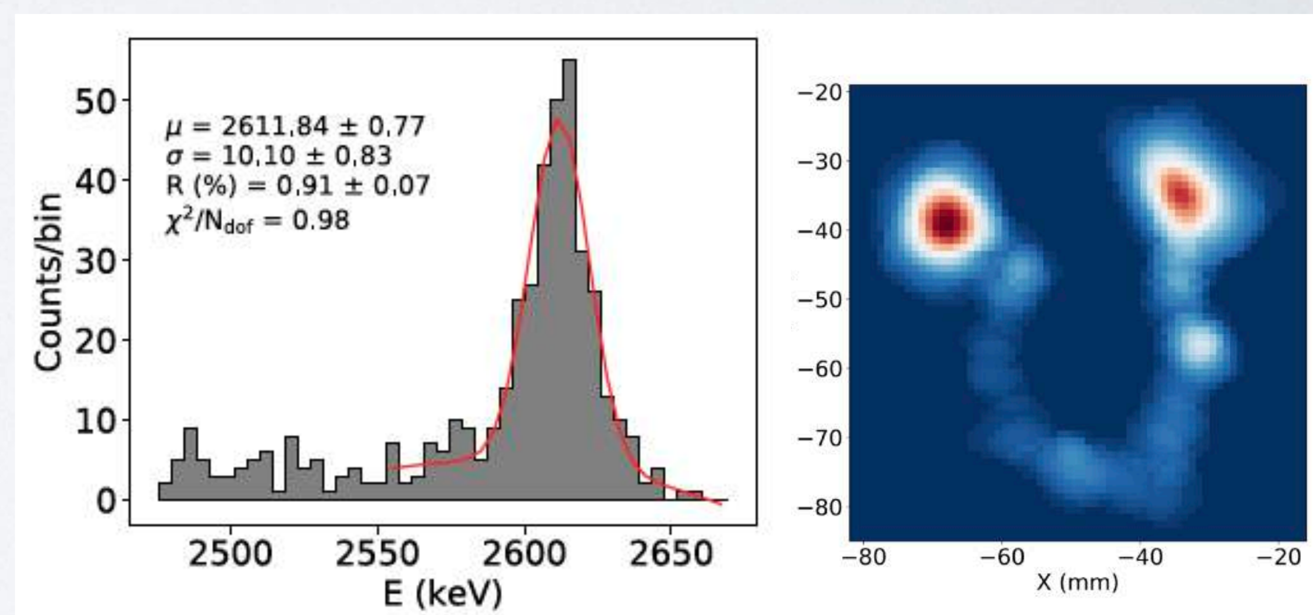
LSC MAIN SCIENCE RESULTS IN 2021



ANAIS: 3 years data published

5 years data in 2022 (on DAMA/LIBRA claim)





NEXT-NEW phase completed

Great Energy res. & e-reconstruction!

LSC STRATEGIC PLAN: 2022-2031

Host one fundamental physics frontier experiment: NEXT-ton scale
Technology able at the LSC (size, depth,…)
1-ton Enriched Xenon in ^{136}Xe, gas purification system, SiPM detectors electronics, ultra-pure copper and steel.

Hub of the Spanish Community contributing to International Projects
Spanish Contribution to the Construction of HyperKamiokande
Small/Medium size experiments: CROSS, DAMIC, DArT, NaI, … that contribute to bigger experiments in other labs. Coordinate with EU DUL.

Explore. Influence of (lack of) cosmic rays on:

- Superconducting Devices
- Biology Underground

And improve/innovate on low background services to match requirements of larger and cleaner detectors

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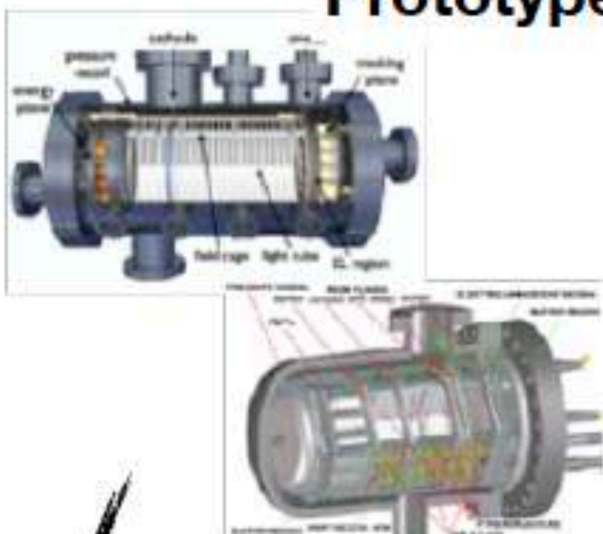
Gas phase detectors: NEXT

Prototypes



NEXT-100 (100 kg)

NEW (~10kg)

NEXT-HD/BOLD (~1000 kg)

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SPANISH CONTRIBUTION TO THE HK CONSTRUCTION

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Hyper-Kamiokande

- ~2027 onwards
- 260 kton (188 kton FV)

X 8.4

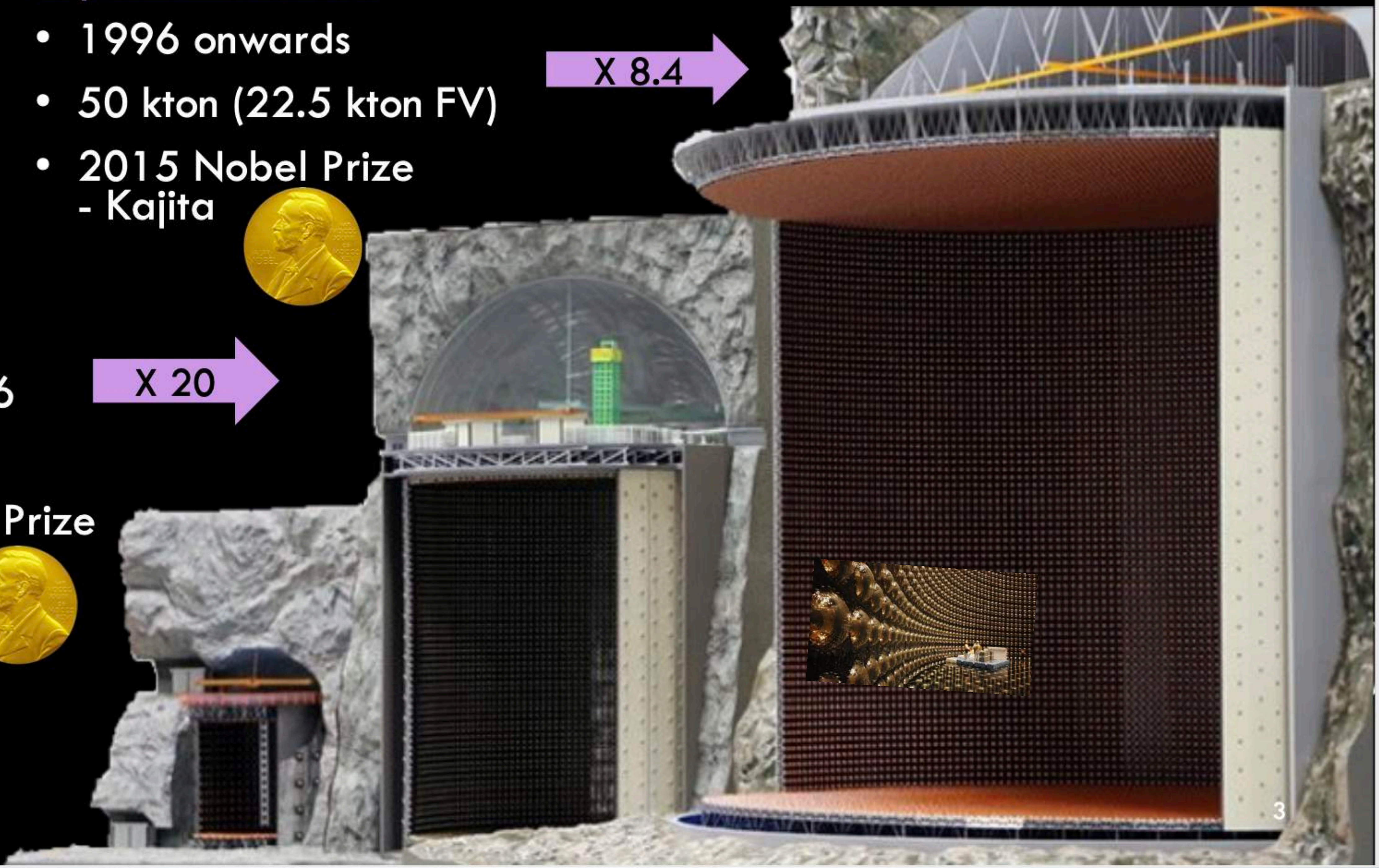
Super-Kamiokande

- 1996 onwards
- 50 kton (22.5 kton FV)
- 2015 Nobel Prize
  - Kajita

Kamiokande

- 1983 – 1996
- 3 kton
- 2002 Nobel Prize
  - Koshiba

X 20



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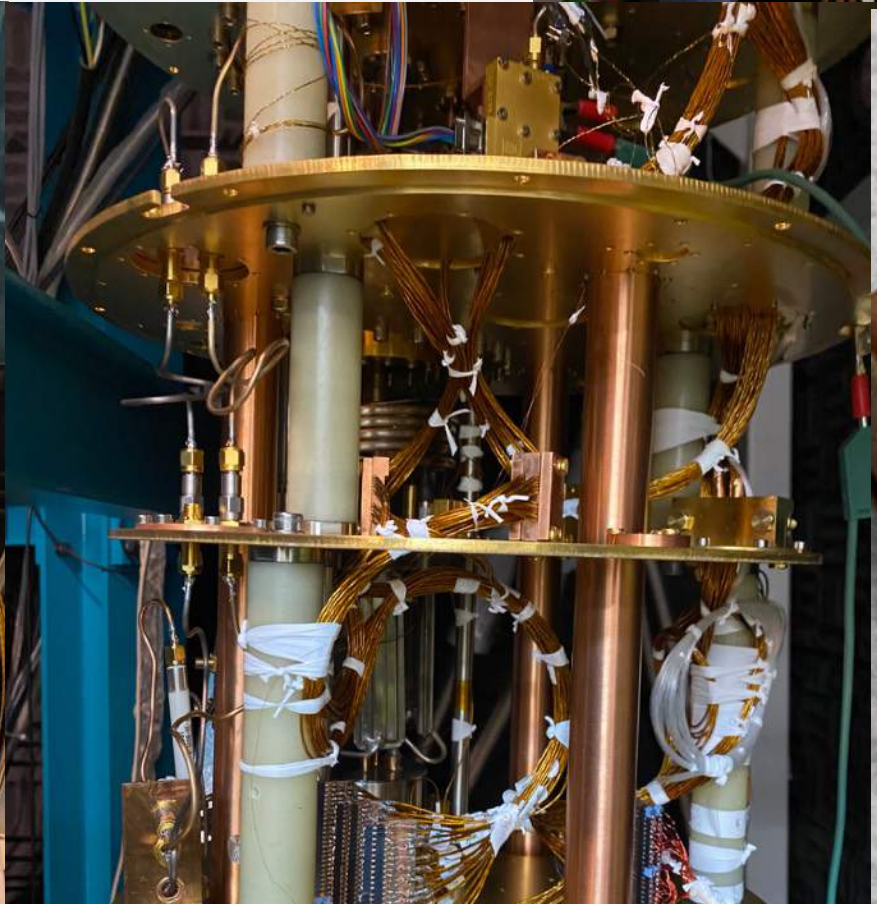
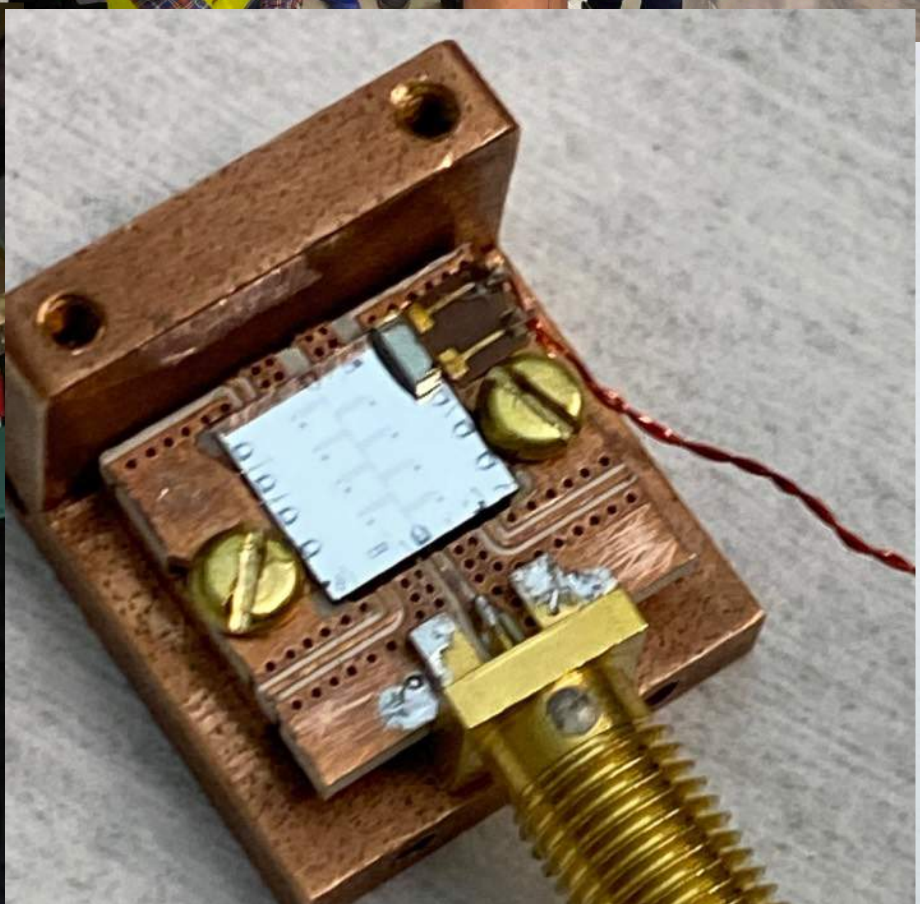
NEW LINE : SUPERCONDUCTING DEVICES

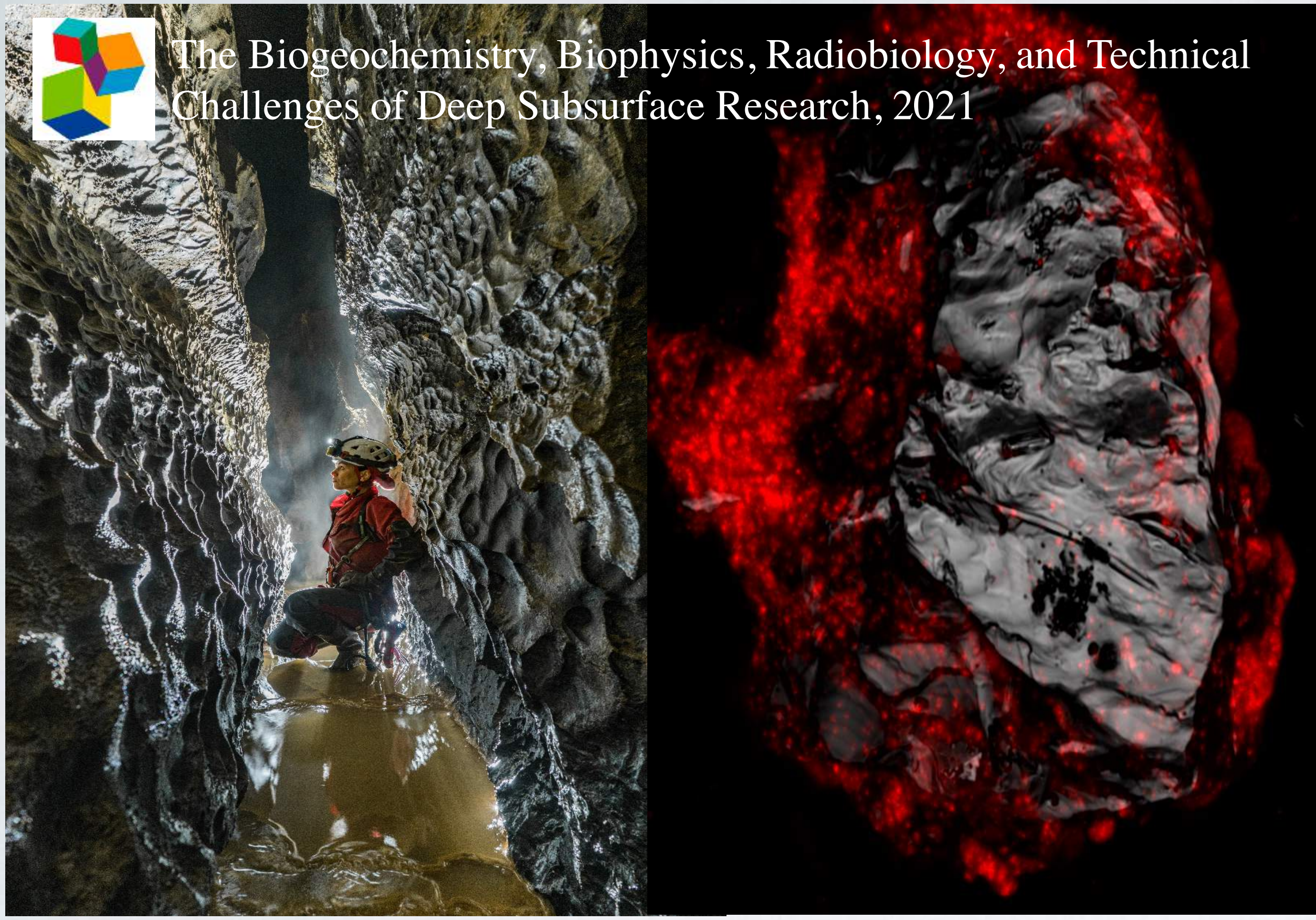
CROSS (2018-) and LSC (2022-) dilution refrigerators:

- ARQ/MIRQ- Mitigating Radiation in Qubits
IFAE, LSC, UZ and CROSS teams.
Ongoing experiments with flux qubits underground to demonstrate the impact of cosmic muons on coherence time.
- CADEX: Axion haloscope based on microwave filters, antenas and KIDs at 10 mK.
- Test new particle physics detectors
- Materials characterization





NEW LINE: BIOLOGY UNDERGROUND



New biolab underground. Explore suppression of DNA reparation mechanisms.
Expressions of Interest approved:

- Life in Heavy Water and its Energy Source [CBMSO] - Microorganisms in D_2O
- Darwinian evolution (De Luria-Delbruck) [I2SysBio] - E. Coli
- DNA Damage repair [UPF] - E. coli
- Multicellular Structure Formation [IBE] - S. Arctica, C. Owczarzaki
- Interaction between host and pathogens [I2SysBio] - C. Elegans, O. Nodavirus

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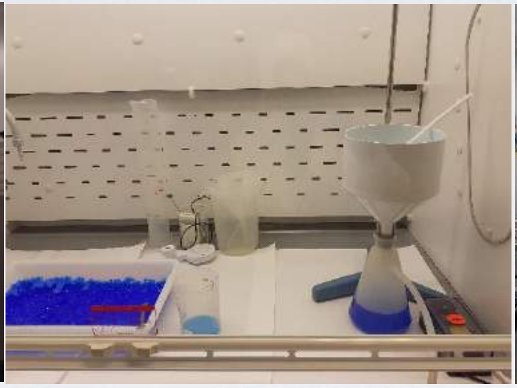
IMPROVE SERVICES UNDERGROUND

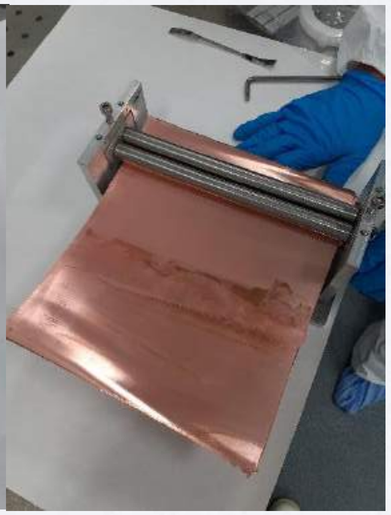
EF-Cu PIECE – DAMIC Collaboration

Setup installed in the LSC Clean Room (Class 10.000, underground). Preparation done in the Clean Room: copper sulphate recristallization (purification), electrolyte, OFHC copper bars (anodes) cleaning…



Also, new ICPMS in clean room (Sep'22) to improve sensitivity to sub-ppt in U/Th

1st STEP: Electroforming, EF-Cu hole cylinder preparation (0.3-0.5 mm tickness, 82mm diameter, 205mm height)	**2nd STEP**: cut, open and flatten the EF-Cu cylinder	**3rd STEP**: plastic support setup	**4th STEP**: Electroforming, EF-Cu plane (in progress)

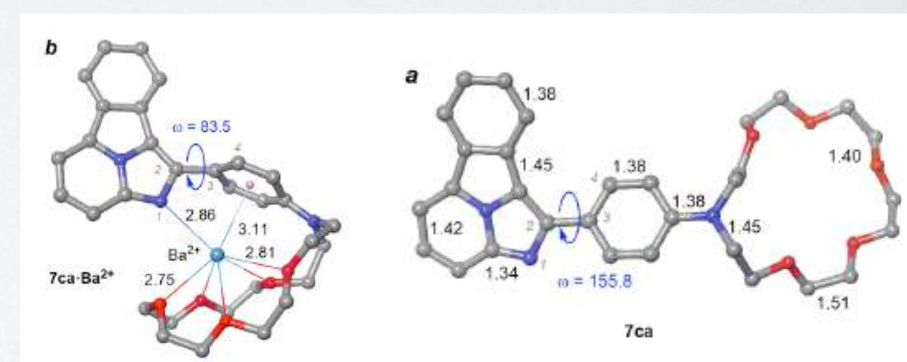


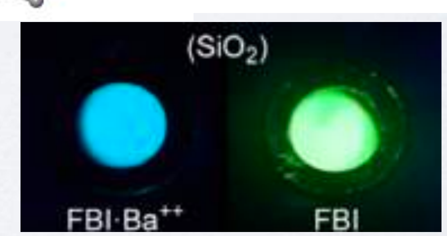
Produce large amounts of EFCu for LNGS additive manufacturing service

EXPLORE NEW TECHNIQUES
RadIum TAgging (RITA) BASED ON SMFI

New detection method of Ra with $<10^3$ atoms sensitivity
Goal: Screening of UltraLow background materials
Makes use of <u>single molecule fluorescence imaging</u> techniques
Molecule with unchelated/chelated fluorescence bicolor

Ra^{++} beam preparation from sample droplets **Mass Spectrometry** → Deceleration **ion trapping** → Capture by molecules on gold plate and SMFI induce by strong laser **Ion capture and counting**





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THANK YOU

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