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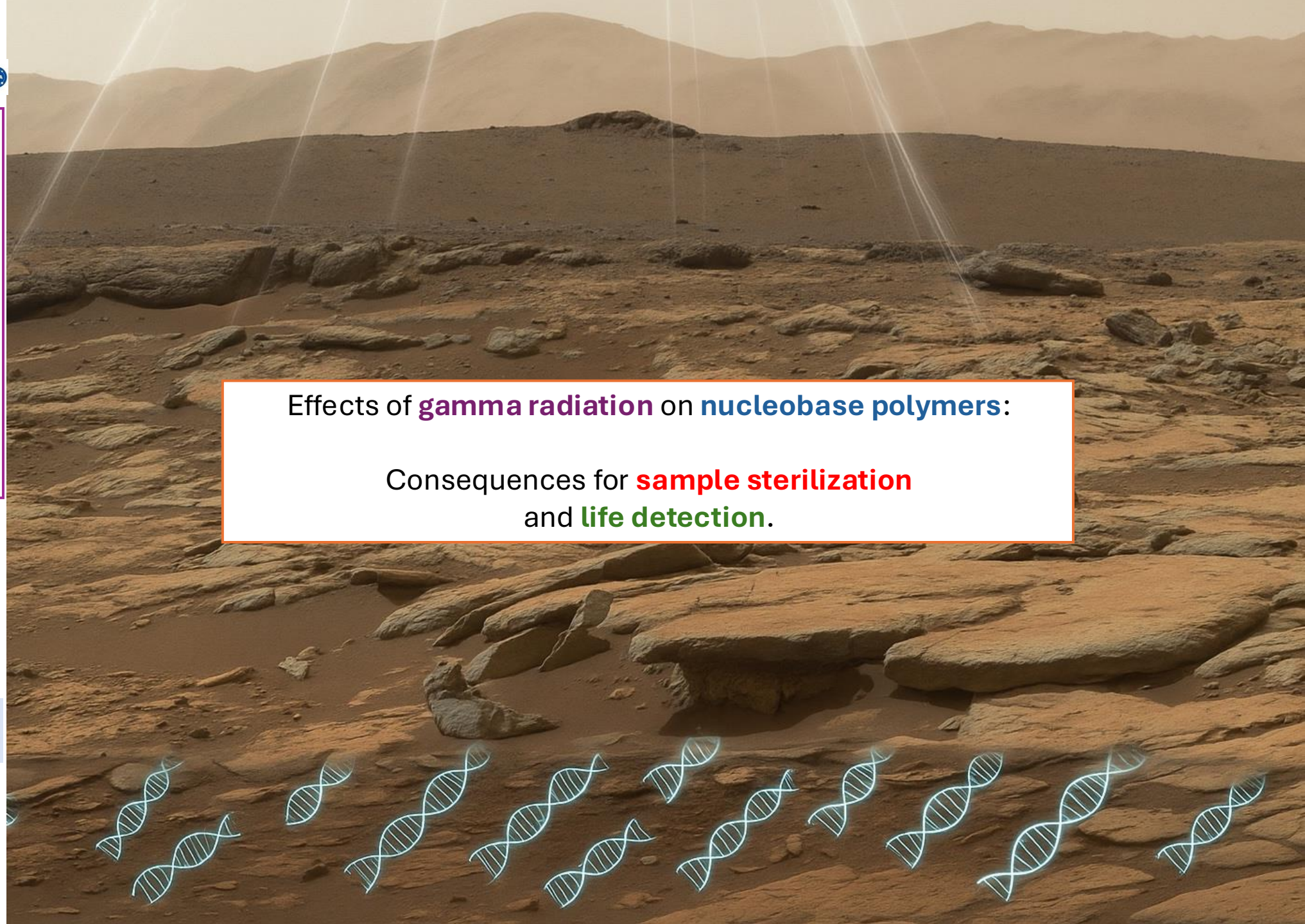
Effects of **gamma radiation** on **nucleobase polymers**:

Consequences for **sample sterilization** and **life detection**.



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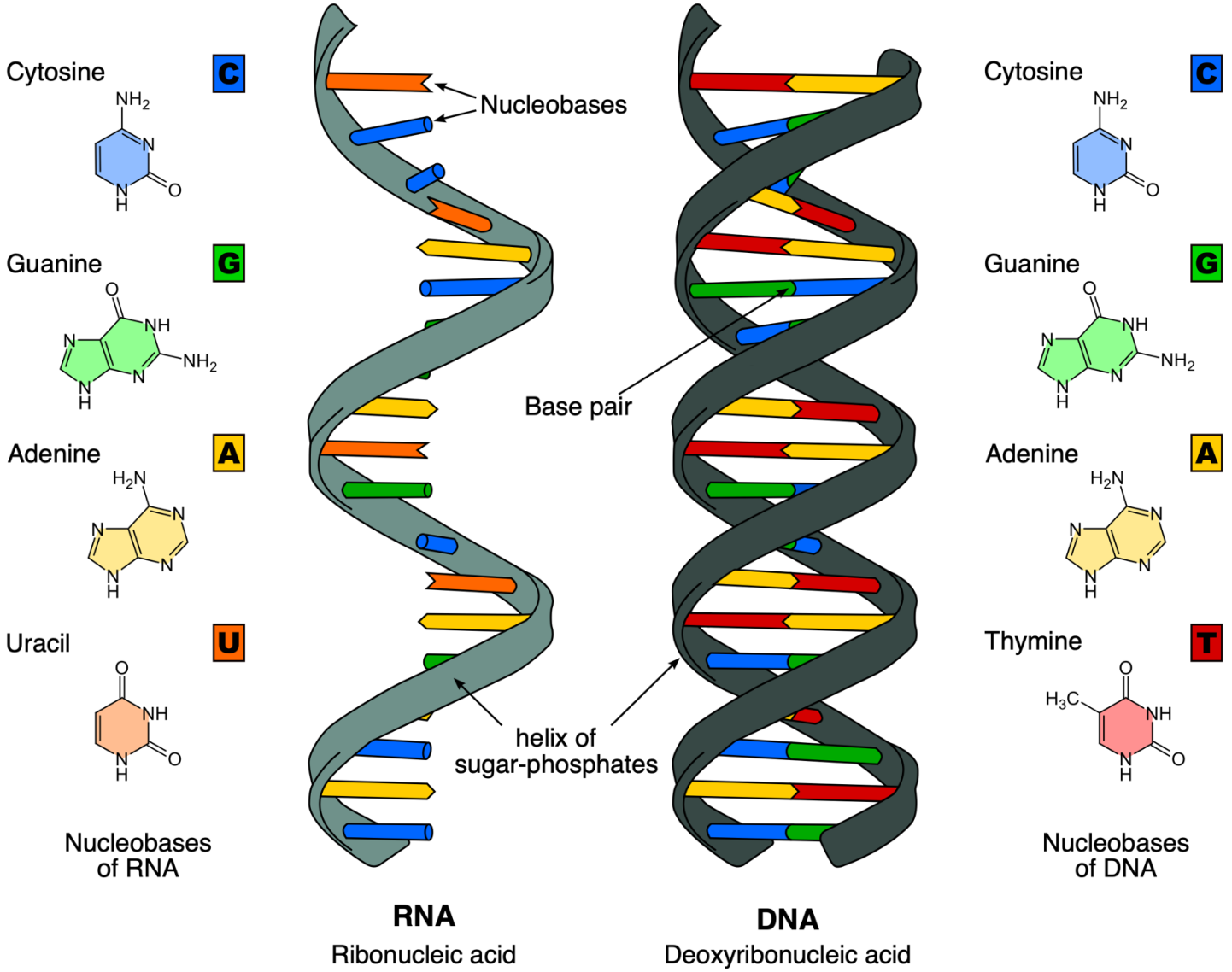
Preamble: nucleobase polymers are the essential signature of life

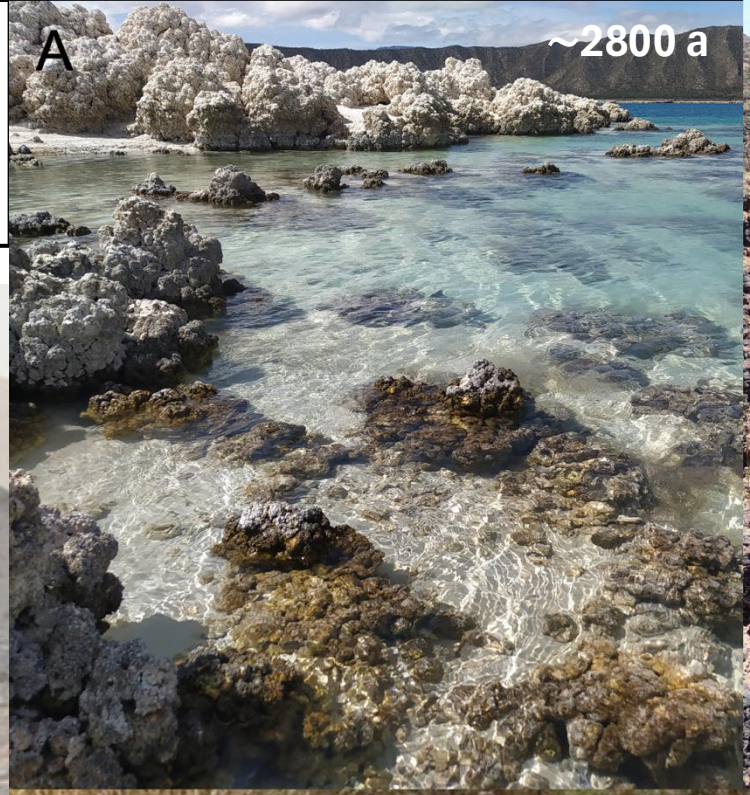


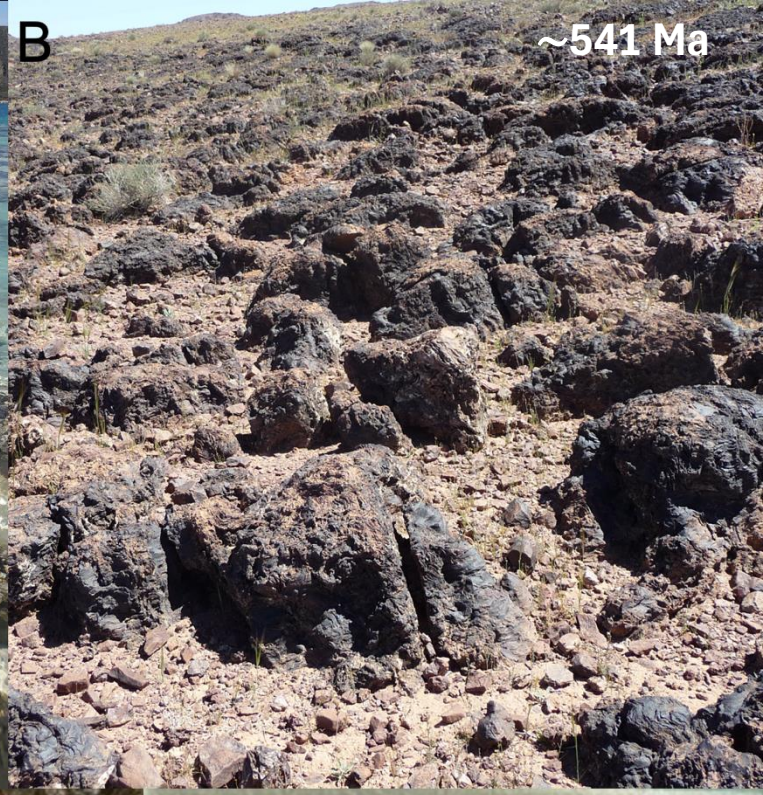
Credit: Roland1952 @ Wikipedia

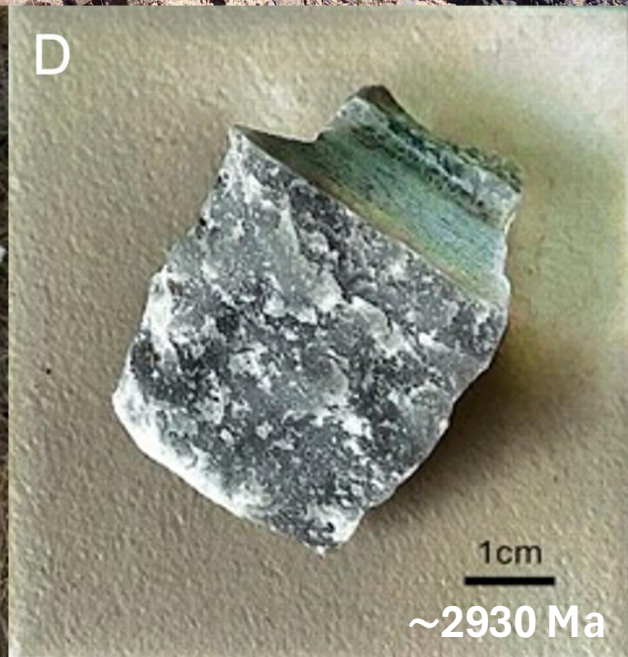
Each nucleotide consists of three components:
a phosphate group, a deoxyribose sugar molecule, and a **nucleobase**

ATGCGTACTGGAACCCGATATGGCCTGCGTACTGGAATGCGTACTGGAA

Zorzano, MP., Basapathi Raghavendra, J., Carrizo, D., Cañadas, F., Reyes-Prieto, M. D'Auria, M., Martin-Torres, J . *Fragmented deoxyribonucleic acid could be extractable from Mars's surface rocks*. Commun Earth Environ 6, 838 (2025). https://doi.org/10.1038/s43247-025-02809-w

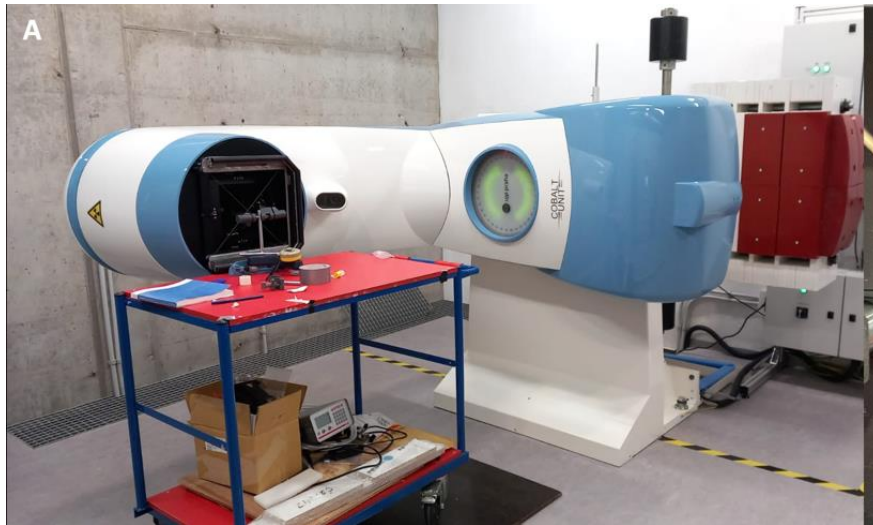






Geological exposure dose (10 MGy)

Sterilization dose (0.86 MGy)



Non-culturable microorganisms.

We analysed 0.5 g of rock per analysis in an ISO 5 cleanroom.

For illustration of sample mass: here are two examples of 0.5 g meteorites.

A note about “sterilization” in space context

- ISO 11137-1 is the international standard providing guidelines for sterilization using radionuclides Cobalt-60 and Cesium-137, the two primary isotopes that emit gamma radiation. Cobalt-60 emits gamma rays with energies of 1.173 and 1.332 MeV.

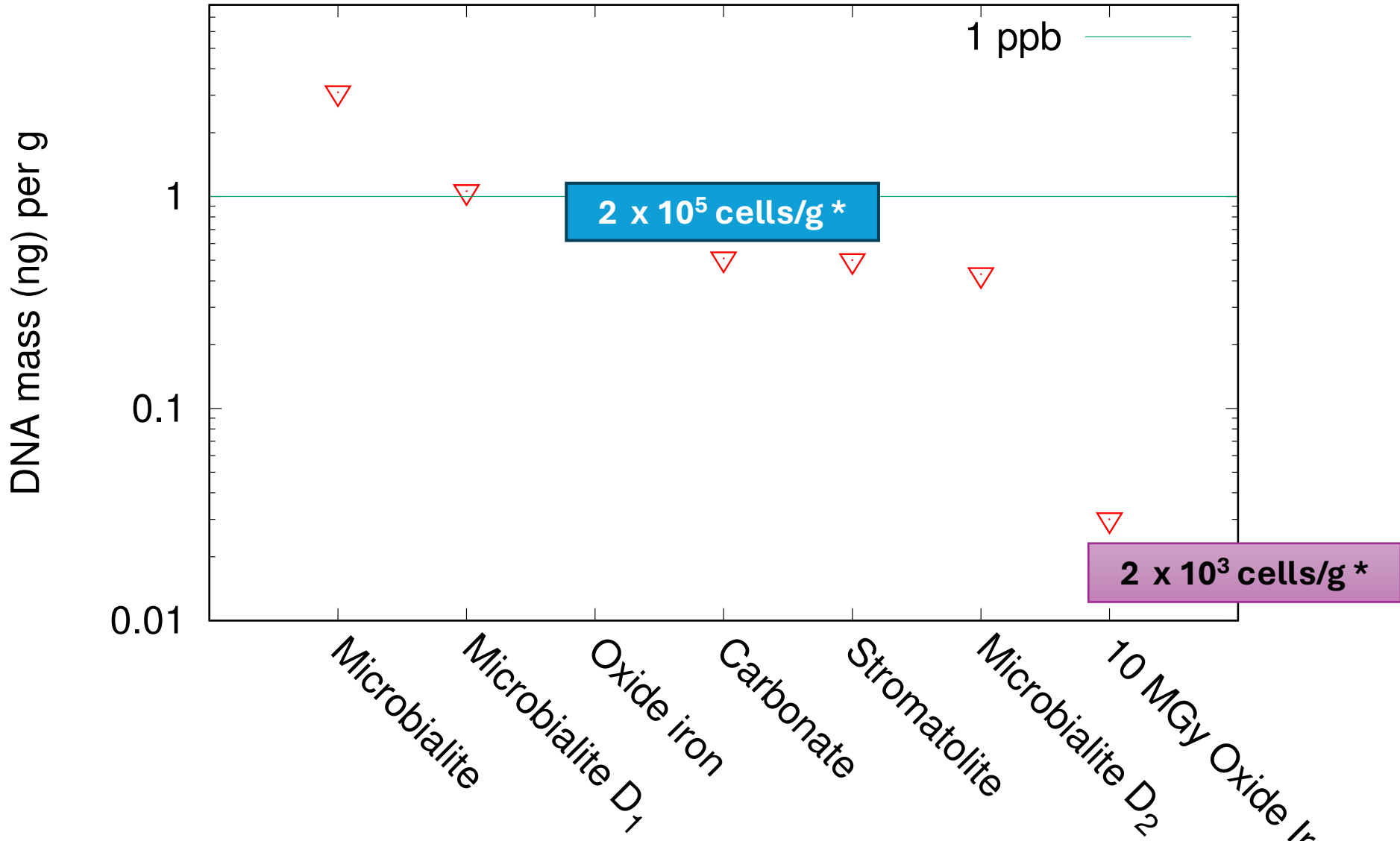
- In medical and pharmaceutical applications, gamma radiation sterilization typically employs doses ranging from 25 kGy to 50 kGy (0.025 to 0.05 MGy)* to ensure complete microbial inactivation. In space environments, doses can reach up to 100 kGy (0.1 MGy), depending on the initial bioburden level and hardware tolerance.

- Considering that certain terrestrial Arctic organisms have shown resistance to radiation doses up to 0.1 MGy under Martian-like temperatures**, the sterilization dose in our study has been increased to 0.86 MGy to ensure effectiveness.

*R. Gradini, et al. *A summary on cutting edge advancements in sterilization and cleaning technologies in medical, food, and drug industries, and its applicability to spacecraft hardware*. Life Sciences in Space Research, Volume 23, 2019.

** Vladimir S. Cheptsov, et al. *Microbial activity in Martian analog soils after ionizing radiation: implications for the preservation of subsurface life on Mars*. AIMS Microbiology, 2018, 4(3): 541-562

Good analogue for Martian-like surface-environmental cell densities



DNA mass measured with Qubit fluorometer

*Assuming a DNA_mass= 0.005 pg per bacteria/archaea cell (commonly used average in environmental samples)

ATGC

Length of chains (#)

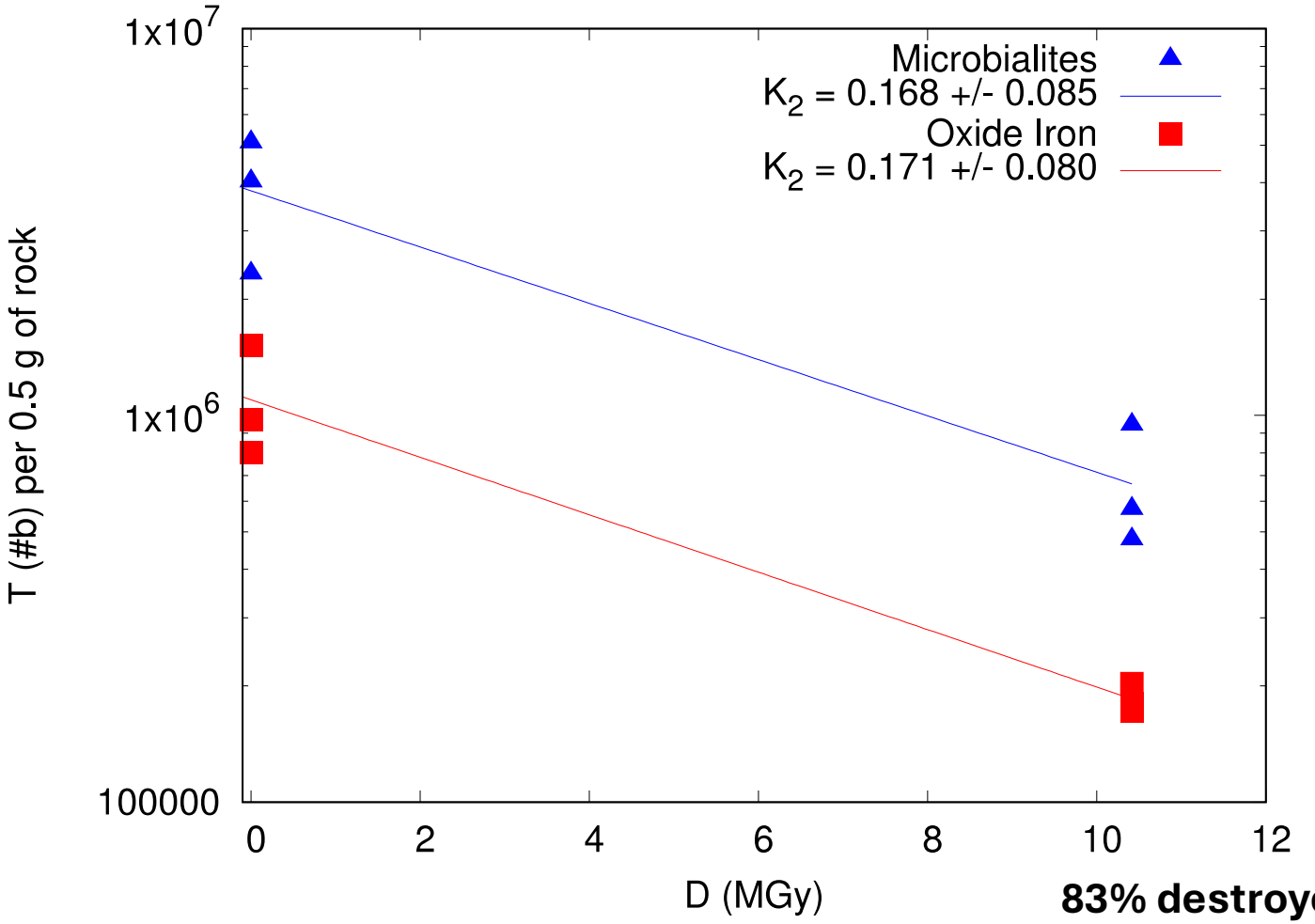
sterilization dose: 37% survive

3 replicates (0.5 g)	T ±σ_T	L ± σ_L	GC ±σ_GC	Q± σ_Q	K (1/MGy)	TOC (ppm) ±σ_TOC
Blank	153,239 ±122,063	63 ±10.47	41.33 ±5.13	3.13 ±0.23	-	-
Microbialite (~2800 a)	3,813,449 ±1,392,360	659.23 ±362.37	59 ±1.73	8.5 ±0.85	-	63,262.30 ±9,230.3
Microbialites (~2800 a), **0.86 MGy**	1,403,205 ±835,166	169.53 ±41.75	48.33 ±4.73	4.97 ±0.67	1.161 +/- 0.585	18,870.40 ±3,951.4
Microbialites (~2800 a), 10 MGy	665,510 ±247,034	189.33 ±64.77	44.33 ±3.79	3.9 ±0.75	0.168 +/- 0.085	42,841.36 ±17,660.5
Stromatolites (~541 Ma)	748,005 ±500,367	216 ±65.76	45.67 ±2.52	4.33 ±0.71	-	448.79 ±213.7
Stromatolites (~541 Ma), 10 MGy	917,383 ±427,995	210.5 ±43.59	49.67 ±2.52	3.87 ±0.72	-	182.75 ±16.4
Oxide iron (~2930 Ma)	1,097,511 ±370,325	199.33 ±76.46	44.33 ±1.15	3.63 ±0,21	-	8500
Oxide iron (~2930 Ma), 10 MGy	184,893 ±15,993	150.37 ±40.06	44 ±5.00	3.57 ±0.25	0.171 +/- 0.080	-
Carbonate (~2930 Ma)	760,257 ±475,632	140.03 ±54.24	44 ±1.00	3.57 ±0.60	-	619.68 ±304

Nanopore sequencing of **0.01-20 ng´s of DNA** extracted from rocks, and **no amplification**

In both the microbialite and oxide iron formation samples, exposure to D_2 = 10.45 MGy resulted in a radiolytic constant of K_{bases}= 0.17 MGy^{-1}.

For lower "sterilization" dose D_1 = 0.86 MGy, we obtain for the microbialite samples, radiolytic constant K_{bases} = 1.161 +/- 0.585 MGy^{-1}.



K_{purine}= 1.11 MGy^{-1} after exposure to a dose of 0.072 MGy, (added in 25–50ppm to a 5 cm long kaolinite –mudstone-)

$K_{lipids, alkanes, hopanes, steranes}$ = 0.3 - 2 MGy^{-1}

$K_{isovaline}$ (SiO_2 in LN_2) = 0.45 +/- 0.01 MGy^{-1}.

$K_{glycine}$ (SiO_2 in LN_2) = 0.36 +/- 0.04 MGy^{-1}

$K_{glutamic\ acid}$ =0.172 MGy^{-1}

The variation in K values across different organic molecules, materials and doses suggests that the mineral matrix and the specific molecular structure of the organics significantly influence the radiation-induced reactivity.

83% destroyed after D_2 *(136 million years)*

CGCATTGAGAACTAAGCTTGTGGGCTTGCTAACTAATAGAGCT
GAATCCTGAAACCTAGTGCTTCAGGGCAGCTGCCCTACACAA
ACGACATAACTATCTTCTGGTTCTCCGCTCCCGGCGAGACCTTTC
TATAACTTTCCTGCTCCGGTATCTTGAATCGCTGCCAATAGACCTCG
TCGATAGAGCCTTAAATCTATTTAAGAGAGCGAAGCGCGCCCATCA
CCTCACTTGGGTCGGGACGGGCATCCATAGCCATAAGAGTATCAC
GCCCGCACGAGAAACGCTTACCAAGAAATCATTCGGTATTAAGGTT
GAAAGAGGATGATTTGACAACTAGCGTGAACGTTATATTGTACTGGT
GACCCATCGACGGGCCCTCCCCGGCGCATTTGCTCGAGTTGTAC
CTTGGCACGATGCTAGTTCGGTAAGGTAGCCTTATTCCCCGCAGA
ACTCCTACAAGCCCCCCCAAGAAACTGGCCGTAGTATATAGAGACG
GGTGCAGAGTACAGTAGTTCTCAGTTACTGATATGTACGCACGGTAA
TAAATTAGCCATTTGGATTATCGAGGTATAAGTCATCGAATCTCGGGT
TAGCACGCTATGCGGACCTGAACTCACACCACCGTAAAAGCGAG
CACTAGAACTCCGTCTCGGCCATGCAAGCGGTGCATTAACTATAGA
ACGCCCACGTGCAACCGGTAGAATCCGCAGAATAAGGTATTGACC
TTTTGTCATCCGCAGACCAAGCGTAGTTAATCTAGGTATGAAGATCG
ATATTCCTGAACGCCTAGCCGATCCACTGCATTACACATCACTTGC
ACGGGCGAACAAGGAAAAGTTAAGCAGTAGGAGGTGGGCTTCTC
AACGCGATTTGGCGGCGGTGACGGGGGATGACCACCCCAGGTA
TCCCTAGTTCGTGCTATATAGGGGGGCACTATGAGCGGTTTAACCG
CGCCTTTACGTTCCAACGGATTAGCAATGGGTGCCGCGACCT

_____
_____TATCTTC_
_____
_____
_____
_____AAAGA
CGATGATTTGACAACTAGCGTGAACGTTATATTGTA_____C
CATCGACGGGCCCTCCCCGGCGCATT_____
_____
_____
_____
_____GCACGCTATGCGGACCTGAACTCAC___
_____CCGTCTCGGCCATGCAAGCGGT
GCATTAACTATAGAACGCCCA_____
_____
_____
_____GAAAAGTTAAGCAGTAGGAGGTGGGCT_____
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_____

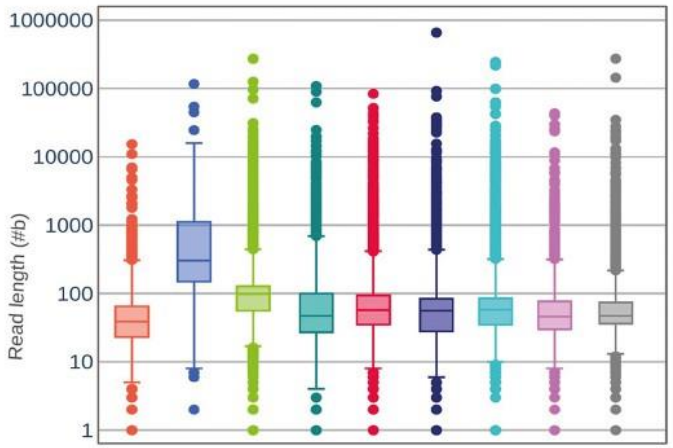
83% destroyed after D_2 ***(136 million years)***

What can we do with the fragments that survive?

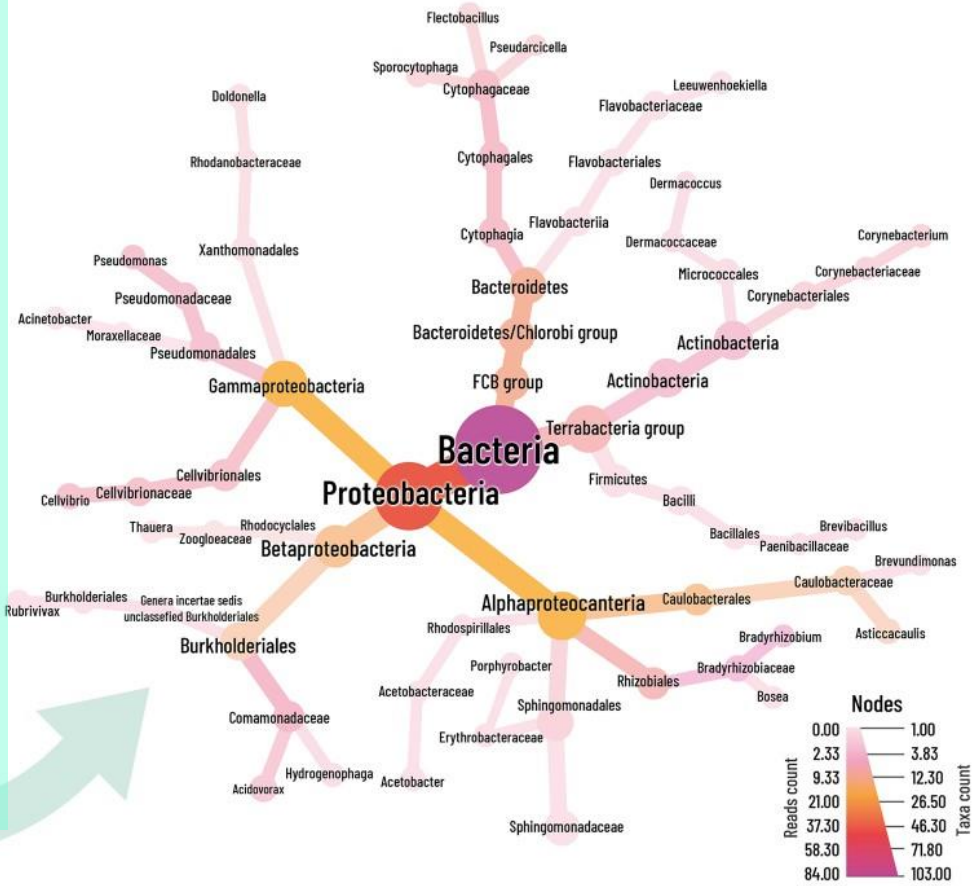
Characterize their length (L)

Non-cultured, DNA-based technologies:

Nanopore sequencing for bioburden quantification, metagenomics analysis and life detection can work effectively even after radiation exposure



ATGCGTACTGGAACCCGATATGGCCTGCGTACTGGAATGCGTACTGGAA



A few ng of DNA mass allows to investigate biodiversity, quantify and track contamination on rock samples, cleanrooms, reagents, space hardware, human habitats, etc.

We can determine if there are microorganisms of concern, quantify bioburden, with statistics and assess contamination risks.

PP implications

- Ambient, operators and reagents add unavoidable contamination (even in a ISO 5 clean room environment with cleanroom garment).
- **Secuencing and total nucleobases counts can be used to quantify bioburden/contamination.**
- **Metagenomics can be used to characterize contamination (even without amplification).**
- After exposure to 0.86 MGy (sterilization-like radiation dose) 37% of nucleobases in rocks can be extracted and sequenced. **We can sterilize returned samples for PP purposes and still identify DNA-like polymers.**
- **We can find and characterise DNA-like polymers in rocks exposed to more than 130 million years of space radiation.**
- Radiation induces DNA break but also naturally degraded DNA exhibits short reads. Probably other sterilizing methods will also fragment DNA.
- For further details: Zorzano, MP., Basapathi Raghavendra, J., Carrizo, D., Cañadas, F., Reyes-Prieto, M. D'Auria, M., Martin-Torres, J . *Fragmented deoxyribonucleic acid could be extractable from Mars's surface rocks*. Commun Earth Environ 6, 838 (2025). https://doi.org/10.1038/s43247-025-02809-w