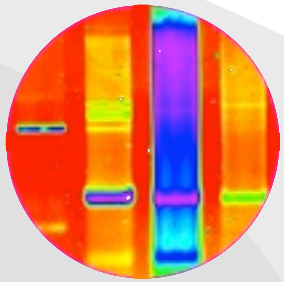
Synthetic biology *and the* limits for life



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Imagine...
Someday we will need an organism to do something for us in space. We will tweak it or make it de novo.


Imagine. . .
Fortunately there is an nearly incredible toolkit of evolved organisms and metabolic pathways on Planet Earth ready to exploit - straight and in conjunction with synthetic biology.
Rothschild, L.J. 2010. A powerful toolkit for synthetic biology: over 3.8 billion years of evolution. BioEssays 32:304–313.

Commercial Extremozymes

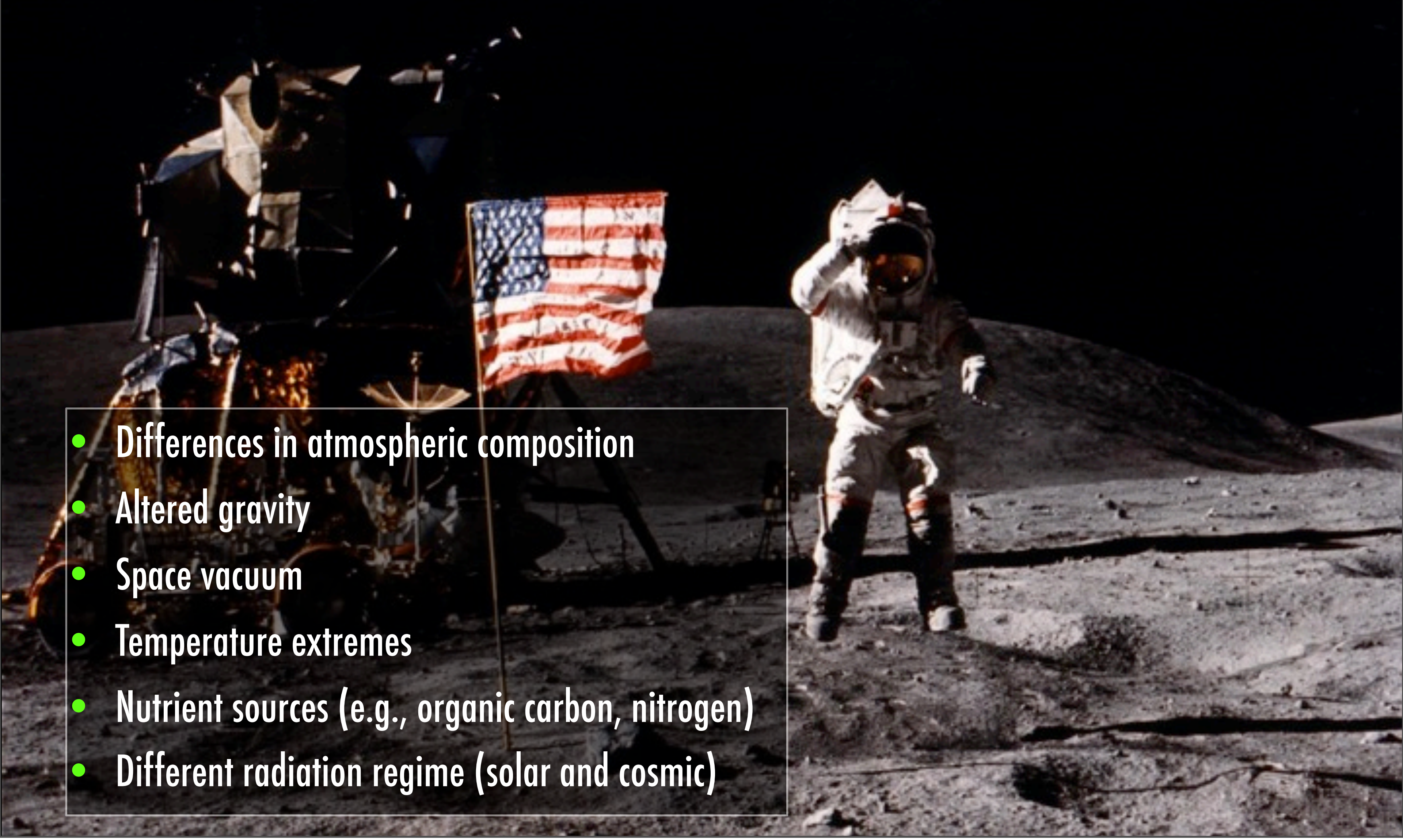
http://www.mediscover.net/Extremophiles.cfm

organisms	enzyme	applications
Thermophiles	DNA polymerases	DNA amplification
	Lipases, pullulanases and proteases	Baking and brewing
	Xylanases	Paper bleaching
Halophiles	bacteriorhodopsin	Optical switches and photocurrent generators
	lipids	Liposomes for drug delivery and cosmetics
	Compatible solutes e.g. Ectoin	Protein, DNA and cell protectants
	g-Linoleic acid, b-carotene and cell extracts,e.g. Spirulina and Dunaliella	Health foods, dietary supplements, food colouring and feedstock
Psychrophiles	Alkaline phosphatase	Molecular biology
	Proteases, lipases, cellulases and amylases	detergents
	Polyunsaturated fatty acids	Food additives, dietary supplements
	Ice nucleating proteins	Artificial snow, food industry e.g. ice cream
Alkaliphiles and Acidophiles	Proteases, cellulases, lipases and pullulanases	detergents
	Elastases, keritinases	Hide de-hairing
	Cyclodextrins	Foodstuffs, chemicals and pharmaceuticals

It isn't limitless because there are constraints on evolution

category	definition	examples
Formal	Result of physical laws	Temperature, diffusion rates, gravity
Historical	Result of evolutionary history	Wing design in insects, birds and bats
Developmental	Result of developmental program	Differentiation leading to commitment, e.g., nerve cells

Physical laws are are even more important in ....
Space: a new category of extreme environment



- Differences in atmospheric composition
- Altered gravity
- Space vacuum
- Temperature extremes
- Nutrient sources (e.g., organic carbon, nitrogen)
- Different radiation regime (solar and cosmic)

What are the **limits** *to* **life?**

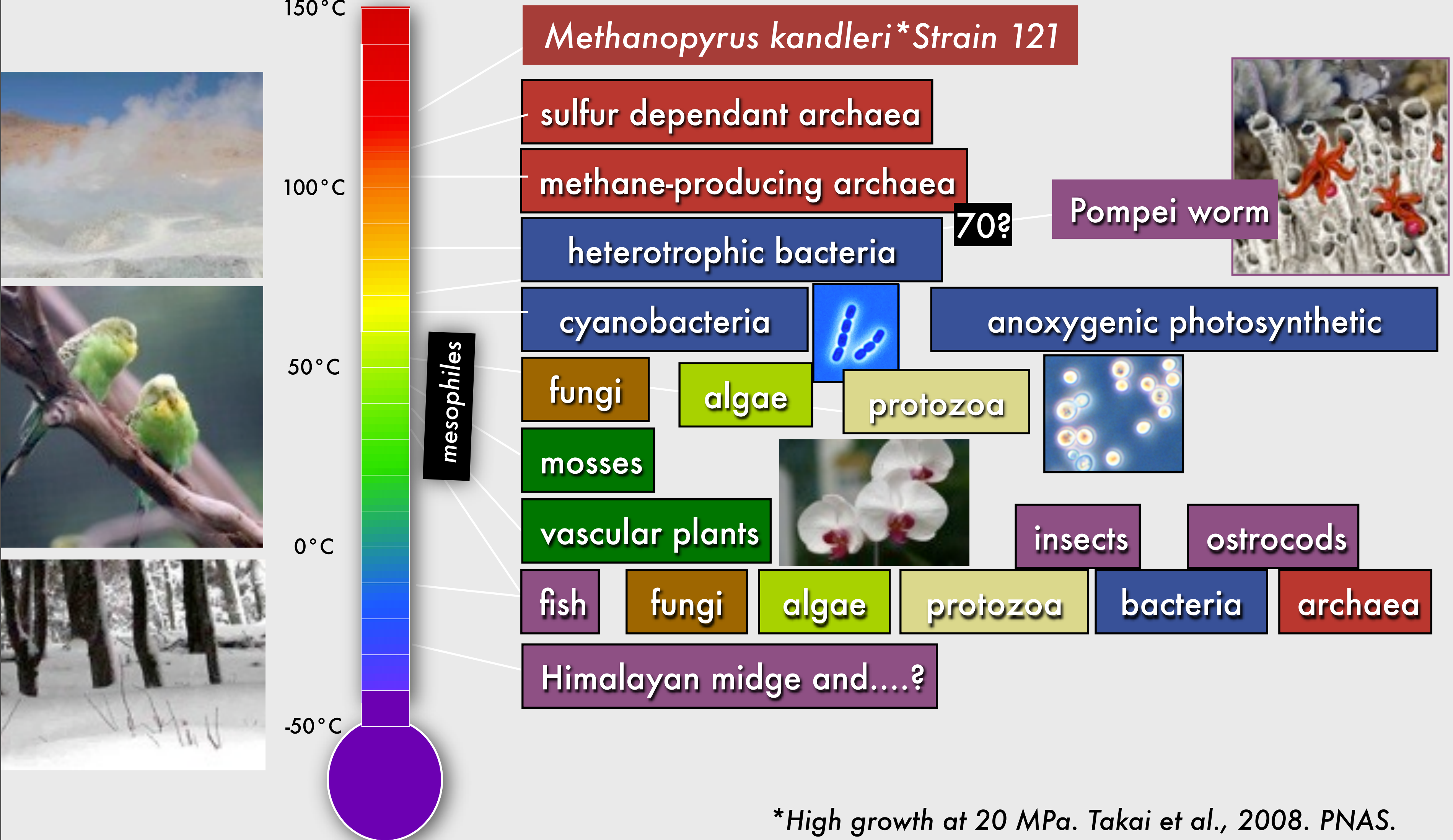
Extremophiles

give the minimum envelope for life

Categories of extremophiles

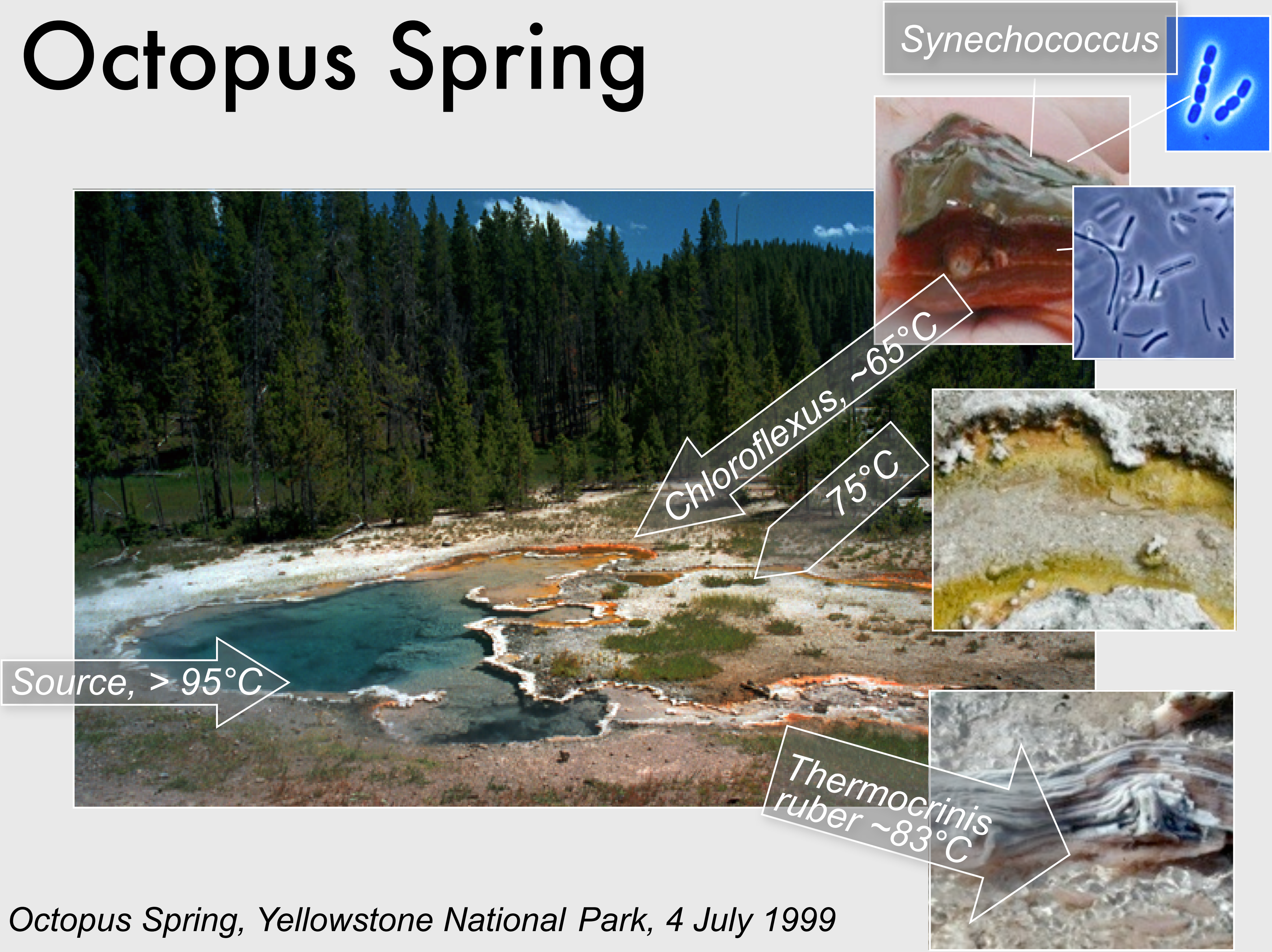
Environment	Type	Definition	Examples
Temperature	hyperthermophile thermophile mesophile psychrophile	growth >80°C growth 60-80°C growth 15-60°C growth <15°C	*Pyrolobus fumarii -113°; strain 121* *Synechococcus lividis* humans *Psychrobacter, insects*
Radiation		radiation tolerant	*D. radiodurans*
Pressure	barophile piezophile	Weight loving Pressure loving	*Shewanella* viable at 1600 MPa
Desiccation	xerophile	Cryptobiotic; anhydrobiotic	*Lyngbya, tun state of tardigrades*
Salinity	halophile	Salt loving (2-5 M NaCl)	*Haloarcula, Dunaliella*
pH	alkaliophile acidophile	pH >9 Low pH loving	*Spirulina, Bacillus firmus* OF4 (10.5); 12.8? *Cyanidium, Ferroplasma*
Oxygen	anaerobe aerophile	Cannot tolerate O_2	*Methanococcus jannaschii* *Homo sapiens*
Chemical extremes		high CO_2, arsenic, mercury	*Cyanidium caldarium (pure* CO_2*)*
Vacuum		vacuum tolerant	tardigrades
Electricity			

Temperature limits for life*



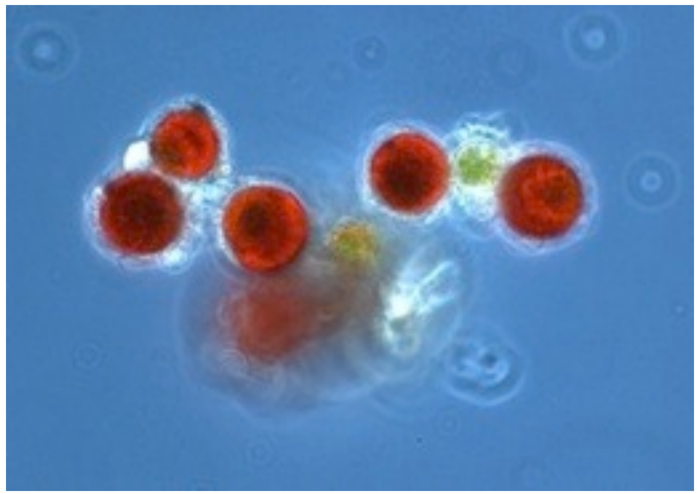
*High growth at 20 MPa. Takai et al., 2008. PNAS.
**Note many organisms, including seeds and spores, can survive at much lower and higher temperatures.

Octopus Spring



Octopus Spring, Yellowstone National Park, 4 July 1999

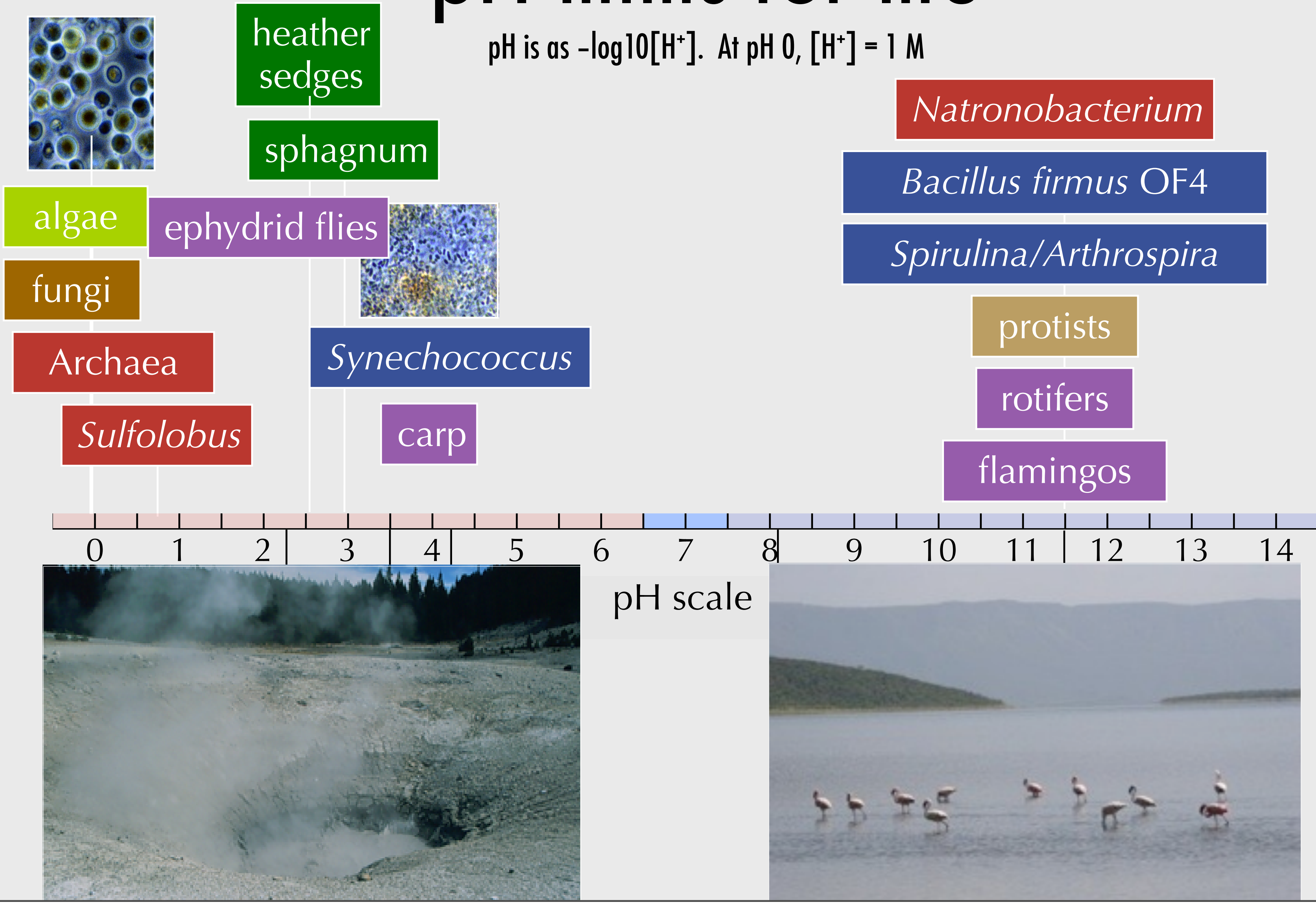
Snow algae (watermelon snow)



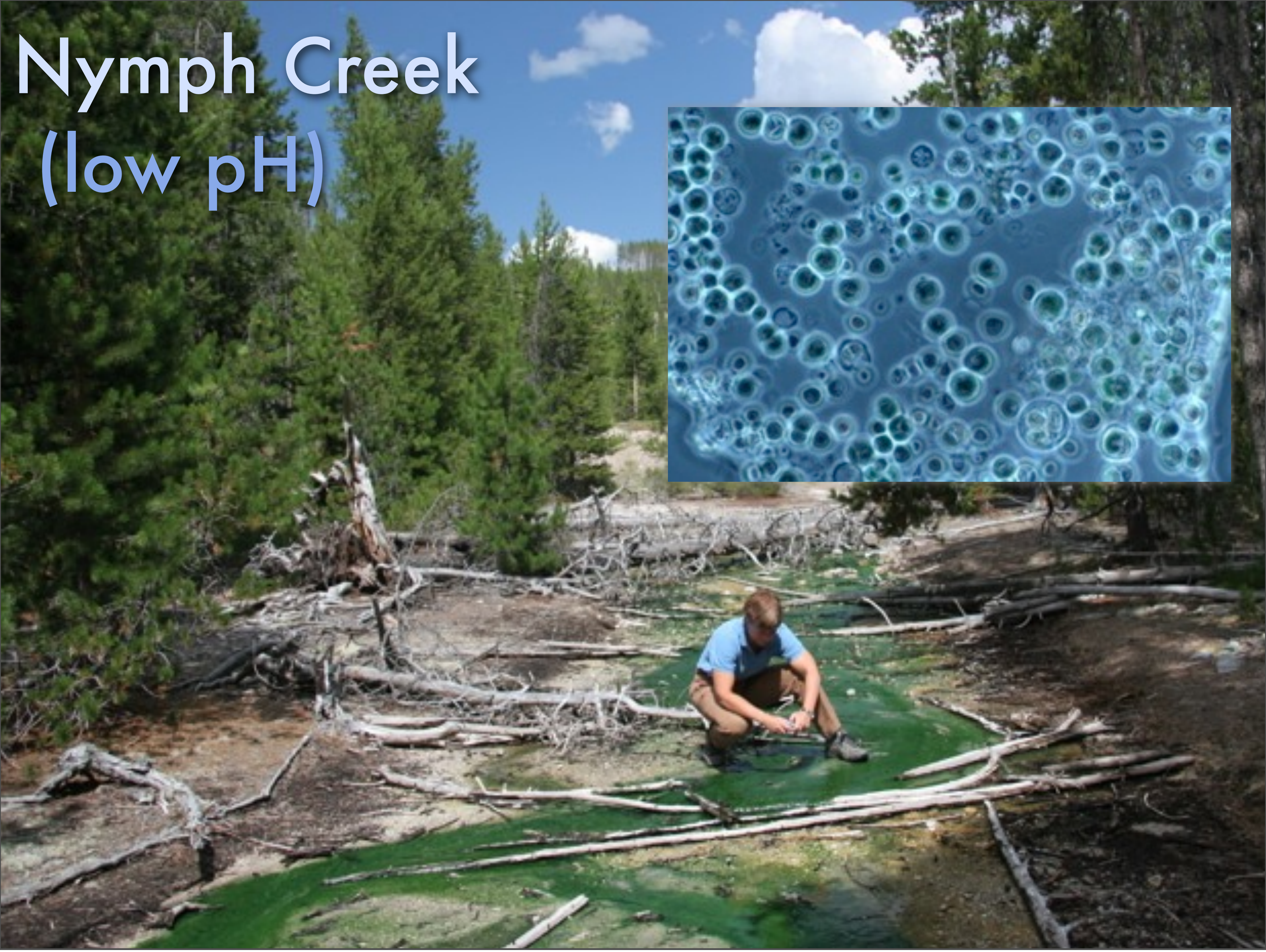
- Lassen Volcanic National Park, King's Creek, July 2005

pH limits for life

pH is as $-\log10[H^+]$. At pH 0, $[H^+] = 1$ M



Nymph Creek
(low pH)

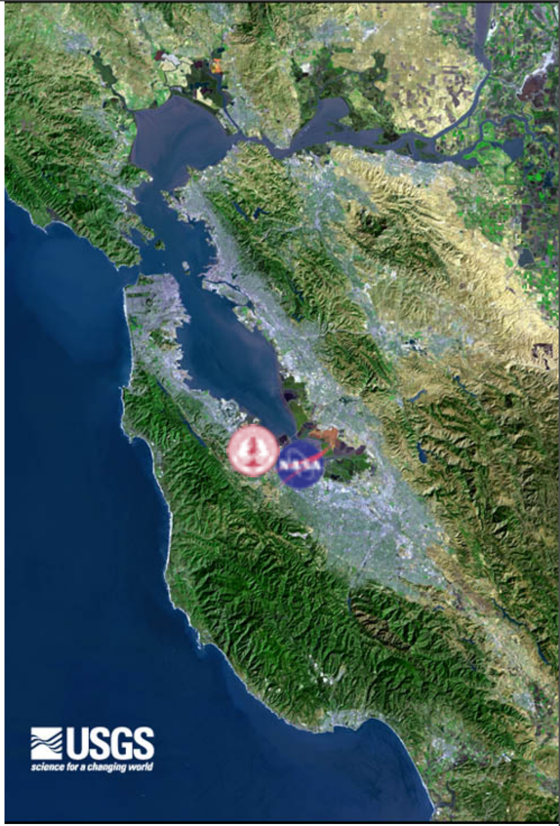


Rift Valley, Kenya (high pH)

photos, Jan. 2007

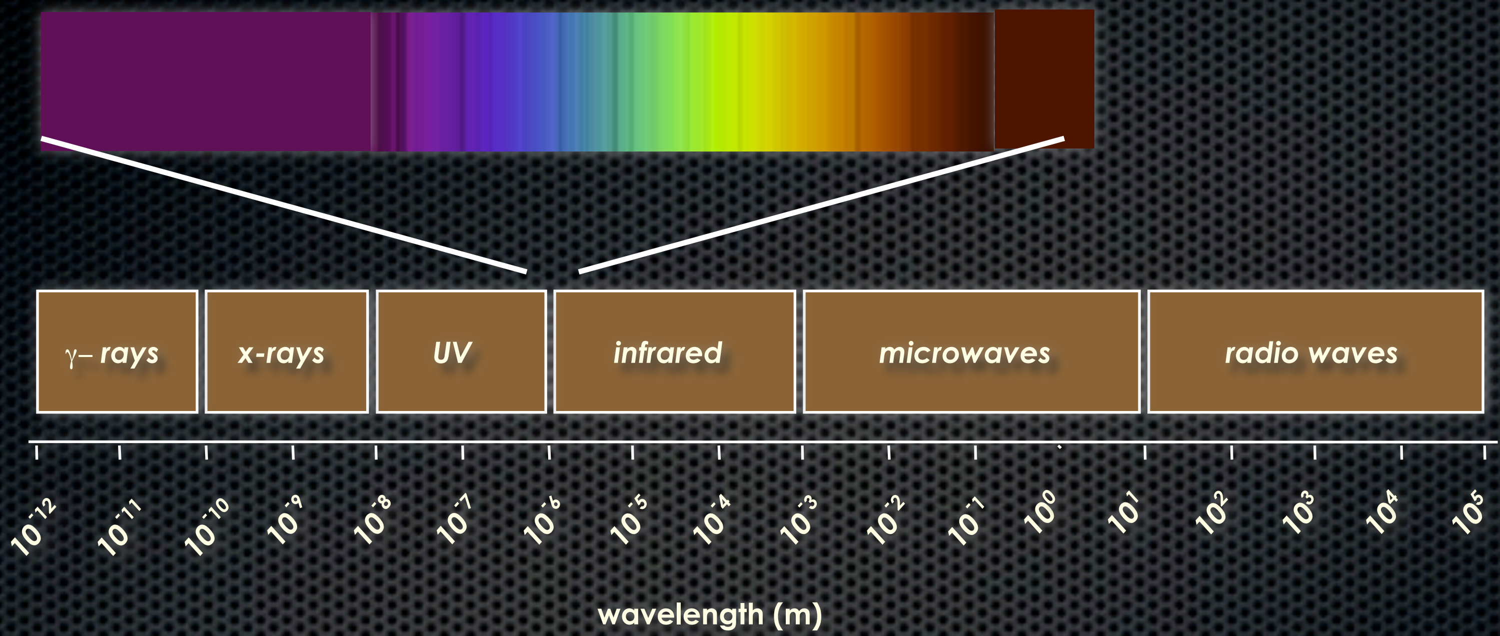
Salinity

- Halophiles: 2-5 M salt
- Include Archaea and a eukaryote.
- Dunaliella salina is used in biotech industry. Produces glycerol and β-carotene.
- Bacterial halophiles were flown in space.



Desiccation

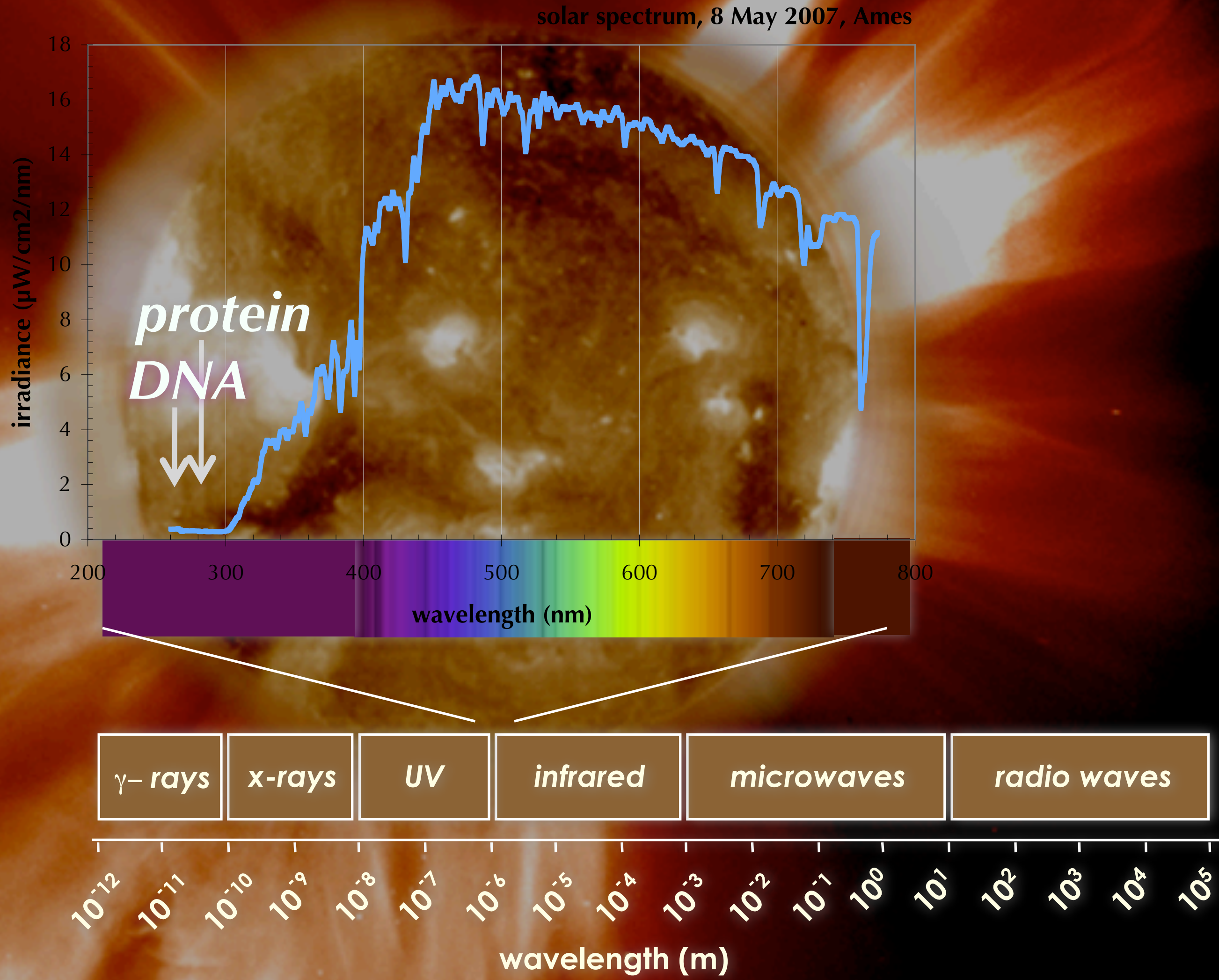
- Can be correlated with salinity tolerance.
- Cell growth at normal temperatures usually requires water potential, aw (defined as pH2O [liquid solution] / pH2O [pure liquid water], where p is the vapor pressure of the respective liquid) of >0.9 for most bacteria and >0.86 for most fungi.
- Lowest value known for growth of a bacterium at normal temperatures is aw = 0.76 for Halobacterium.
- Possibly a few organisms, e.g. lichens in the Negev Desert, can survive on water vapor rather than liquid water.
- Don't repair cell damage during desiccation, so must be good at repair upon rehydration.

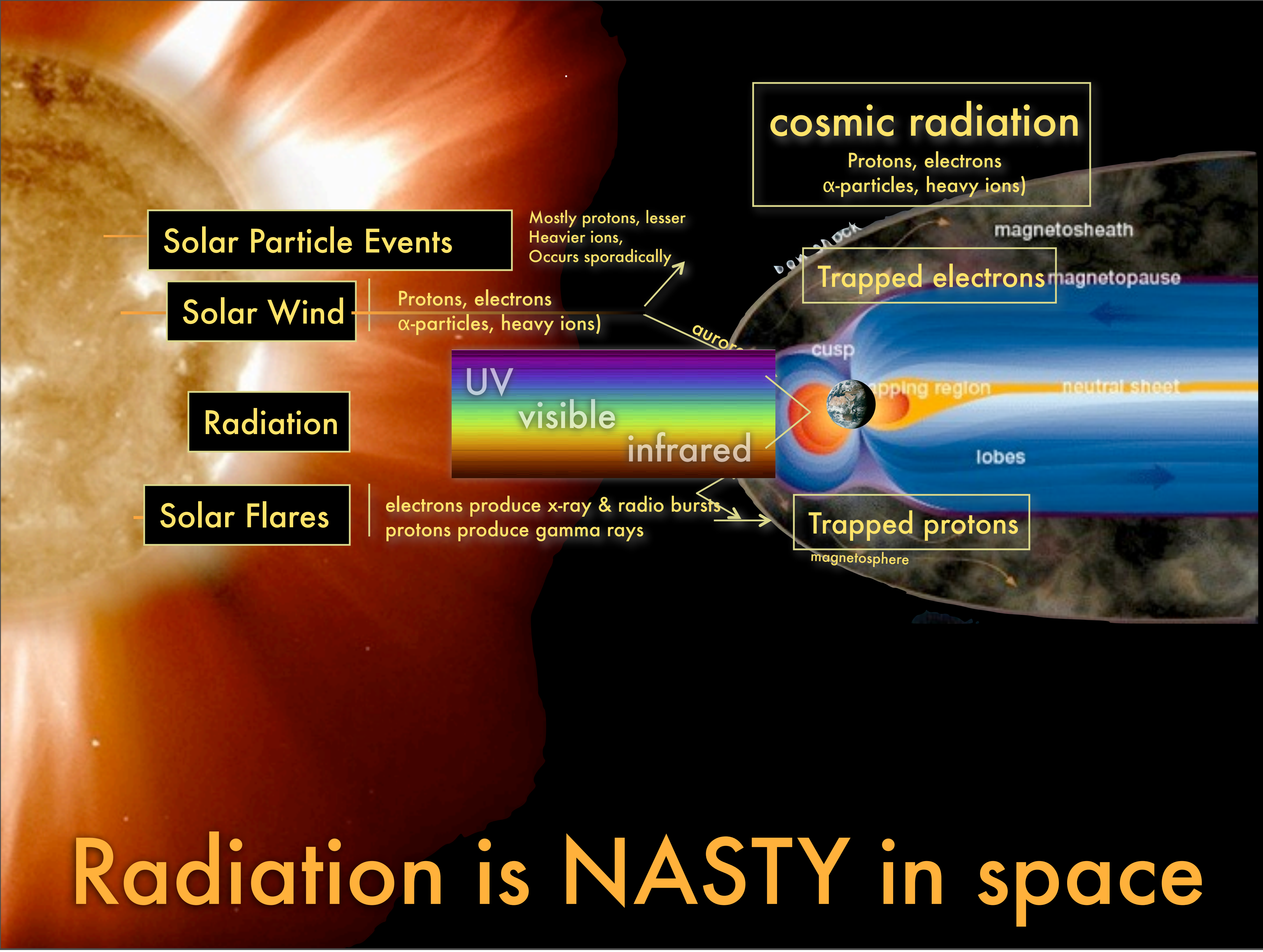


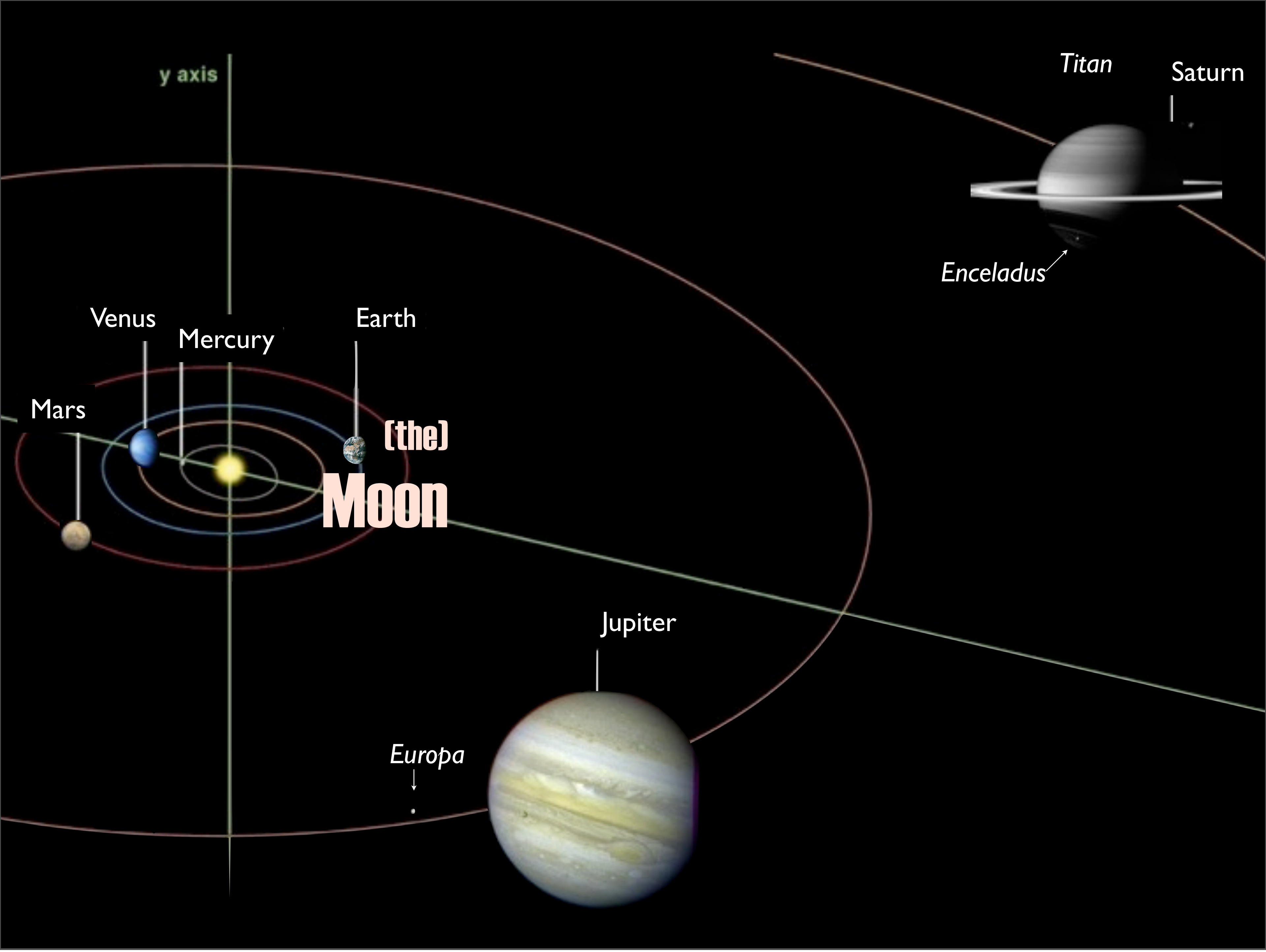
Radiation

The energy spectrum

UV Radiation: direct and indirect effects




cosmic radiation
Protons, electrons
α-particles, heavy ions)
Solar Particle Events
Mostly protons, lesser
Heavier ions,
Occurs sporadically
Solar Wind
Protons, electrons
α-particles, heavy ions)
Radiation
UV
visible
infrared
Solar Flares
electrons produce x-ray & radio bursts
protons produce gamma rays
magnetosheath
Trapped electrons
magnetopause
cusp
neutral sheet
lobes
Trapped protons
magnetosphere
Radiation is NASTY in space


y axis
Titan
Saturn
Enceladus
Venus
Mercury
Earth
Mars
(the)
Moon
Jupiter
Europa

Ionizing Radiation

D. radiodurans

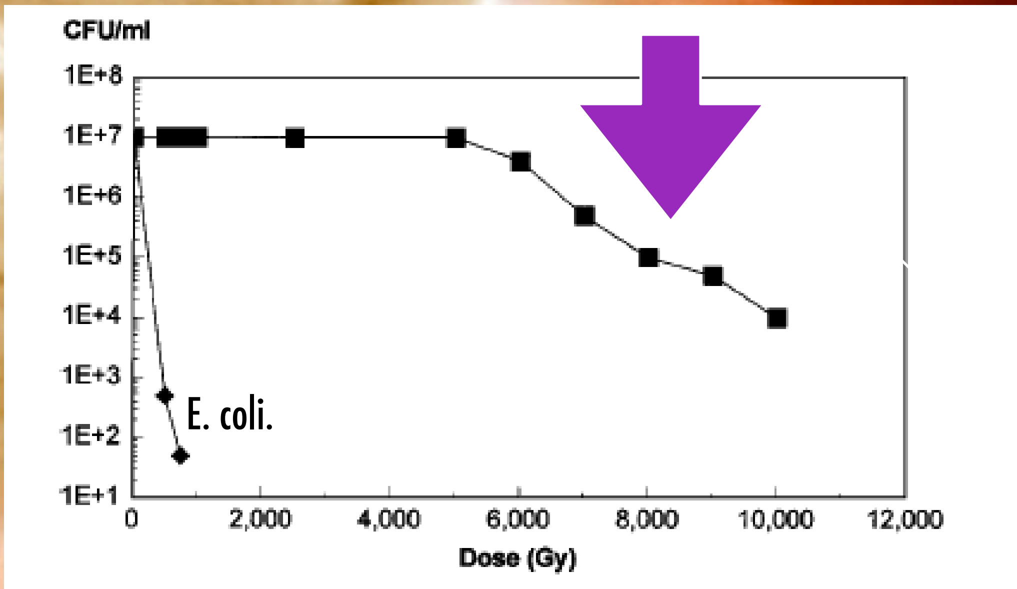


Figure 2 Representative survival curve for *D. radiodurans* R1 (*squares*) and for *E. coli* B/r (*diamonds*) following exposure to γ radiation.

Figure 2. Representative survival curve for D. radiodurans R1 (squares) and for E. coli.

Battista, 1997

Chroococcidiopsis

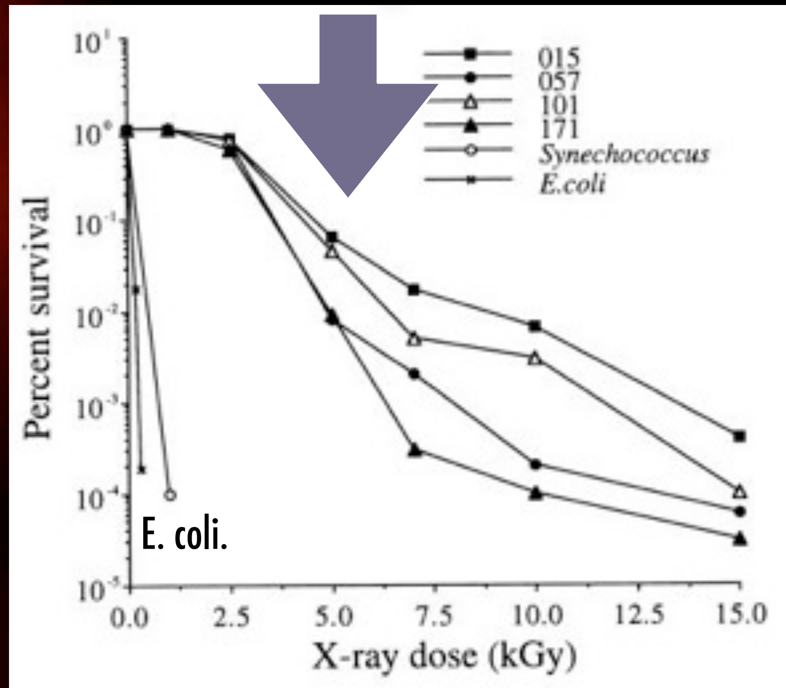


Figure 2. Representative survival curves for four Chroococcidiopsis strains and controls. The values are means based on two independent trials with three replicates per trial. Daniela Billi, E. Imre Friedmann, Kurt G. Hofer, Maria Grilli Caiola, and Roseli Ocampo-Friedmann. 2000. Ionizing-Radiation Resistance in the Desiccation-Tolerant Cyanobacterium Chroococcidiopsis. Appl Environ Microbiol. 66: 1489-1492

How *do they* do it ?

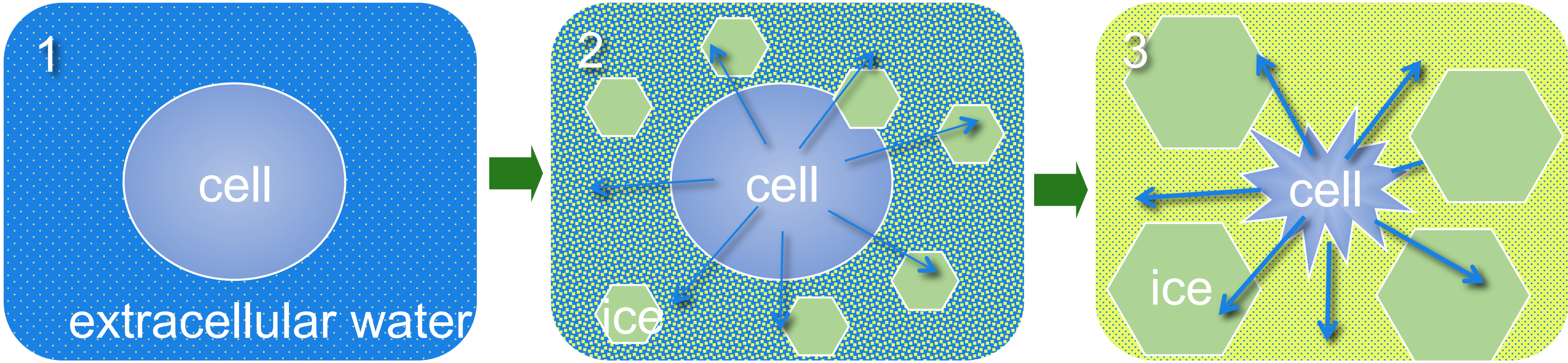
Life is Lazy

(it saves energy for other things)

pH: a perfect example of a (relatively) small adaptation

- pH is as –log10[H+]. At pH 0, [H+] = 1 M.
- Easiest is to keep internal pH near neutral.
- Acidophiles: maintain neutral pH. Strong proton pump or low proton membrane permeability?
- Alkaliphiles: internal pH 2+ units below medium. Need effective proton transport system. Serious problem if for membrane-bound ATP synthase system in bacteria.

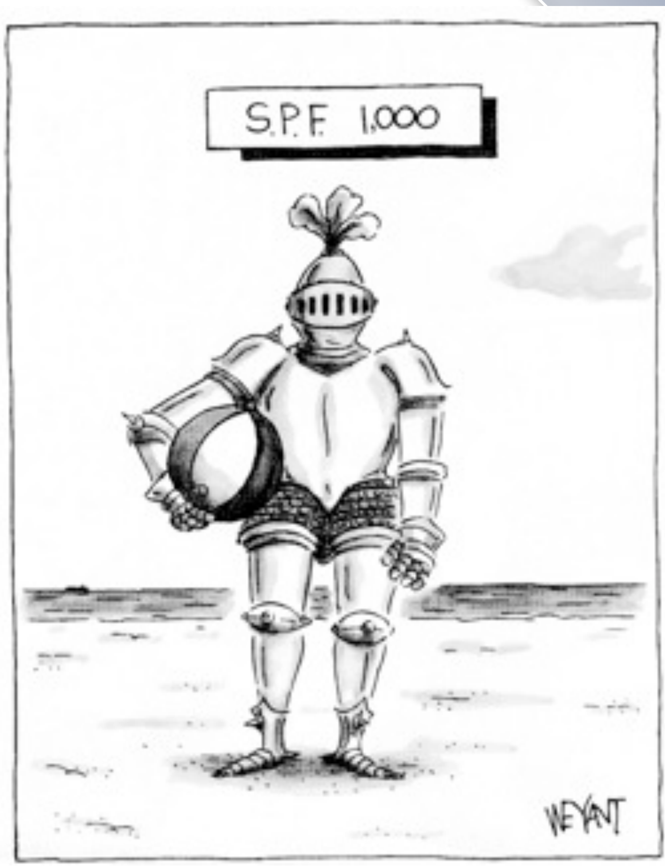
Freeze tolerant insects



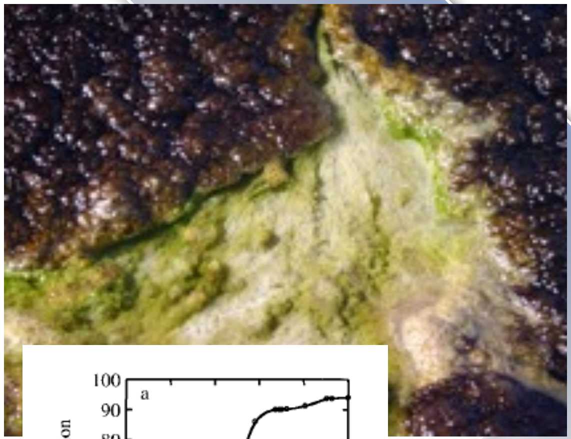
- Ice nucleation encouraged by ice-nucleating proteins in haemolymph, food / bacteria in the gut, and inoculative freezing.
- Cryoprotective measure include: freeze at high subzero temperature, prevention of intracellular freezing, small molecule cryoprotectants (e.g., glycerol), ice-active proteins inhibit re-crystallization.
- (from Wharton, Life at the Limits, fig. 5.5)

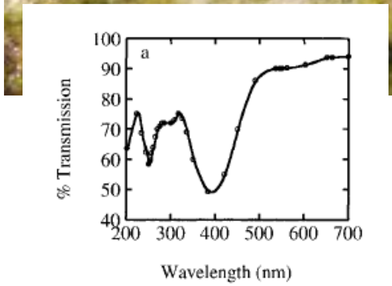
Ways to deal with UV damage...

Avoid damage



production of UV-absorbing pigments (i.e., scytonemin, MAAs, phycoerythrin)





production of quenchers, antioxidants, enzymes to neutralize radicals

Repair damage

"Biomining" the genome of extremophiles

- Why not take some of these evolutionary adaptations and transfer them to species of interest?
- Billi et al. did that by transferring desiccation tolerance to E. coli with Synechocystis sp. gene sucrose-6-phosphate synthase (SpsA)

1682 BILLI ET AL.

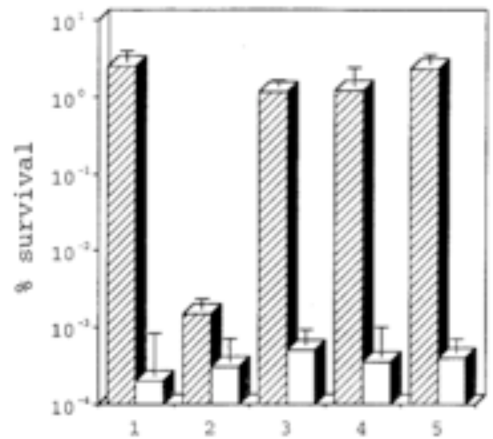


FIG. 2. *spsA* enhances survival of *E. coli* BL21DE3(pSpsA). The data points are means of three values, and the error bars indicate standard deviations based on more than 20 trials. Open bars, BL21DE3(pT7-7); cross-hatched bars, BL21DE3(pSpsA). Data set 1, freeze-drying in the light; data set 2, air drying in the light; data set 3, air drying in the dark; data set 4, chemical desiccation in the light; data set 5, chemical desiccation in the dark.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2000, p. 1680–16840099-2240/00/$04.00⊡0

Engineering Desiccation Tolerance in Escherichia coli

DANIELA BILLI,[1] DEBORAH J. WRIGHT,[1] RICHARD F. HELM,[1] TODD PRICKETT,1 MALCOLM POTTS,1* AND JOHN H. CROWE2
Vol. 66, No. 4
Virginia Tech Center for Genomics (VIGEN), Fralin Biotechnology Center, Virginia Polytechnic Institute and StateUniversity, Blacksburg, Virginia 24061,[1] and Department of Molecular and Cell Biology, University of California-Davis,Davis, California 95616[2]

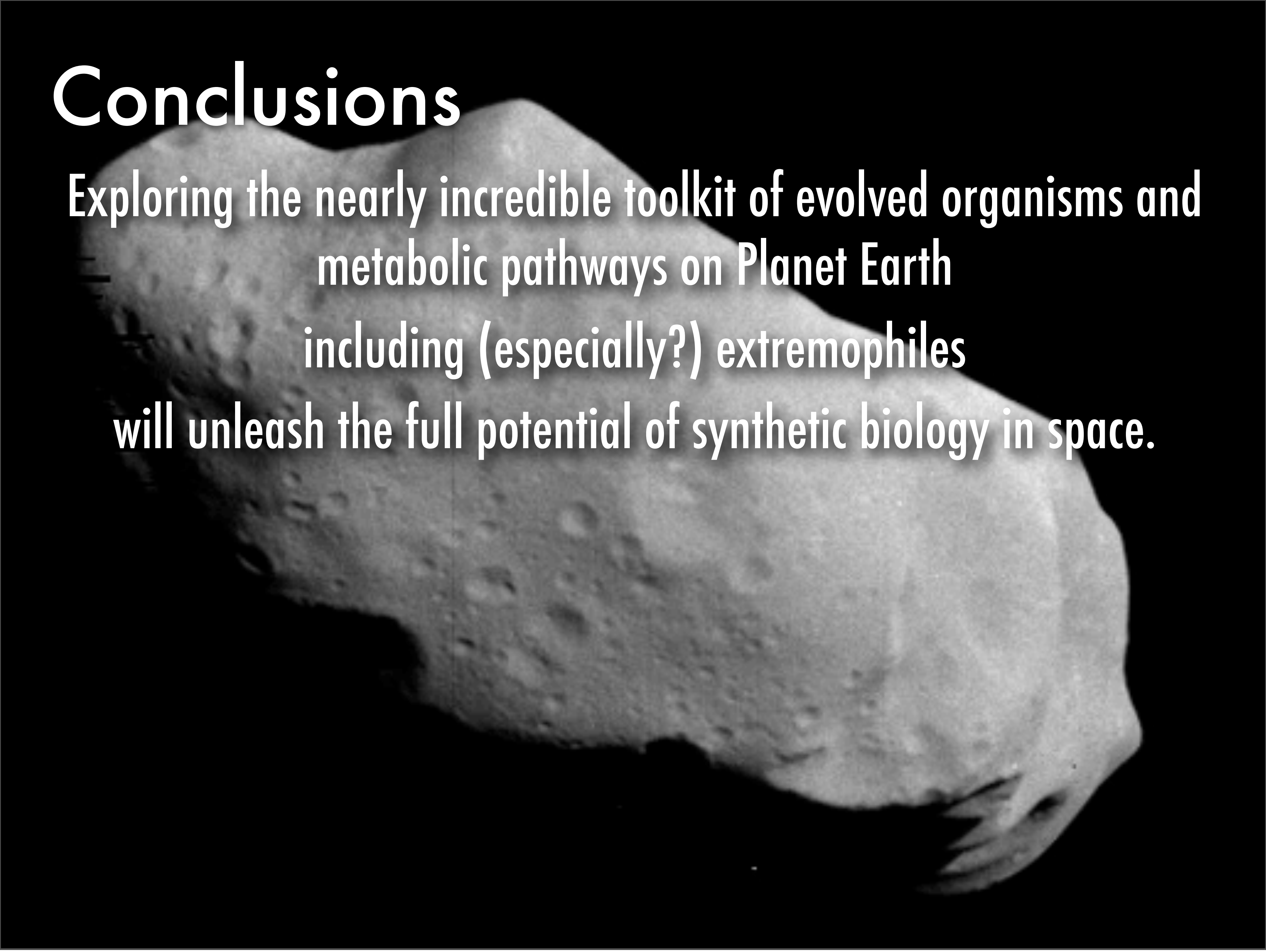
Recombinant sucrose-6-phosphate synthase (SpsA) was synthesized in Escherichia coli BL21DE3 by usingthe spsA gene of the cyanobacterium Synechocystis sp. strain PCC 6803. Transformants exhibited a 10,000-foldincrease in survival compared to wild-type cells following either freeze-drying, air drying, or desiccation overphosphorus pentoxide. The phase transition temperatures and vibration frequencies (PAO stretch) in phos-pholipids suggested that sucrose maintained membrane fluidity during cell dehydration.

Conclusions

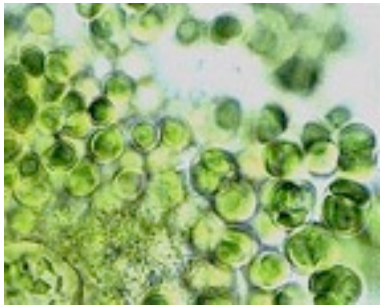
Exploring the nearly incredible toolkit of evolved organisms and metabolic pathways on Planet Earth

including (especially?) extremophiles

will unleash the full potential of synthetic biology in space.



Chroococcidiopsis



- Among the organisms that are known today," says Friedmann, "Chroococcidiopsis is most suitable" for (creating arable land on Mars).
- "Chroococcidiopsis is the constantly appearing organism in nearly all extreme environments," Friedmann points out, "at least extreme dry, extreme cold, and extremely salty environments."
- The pebbles provide an ideal microenvironment for Chroococcidiopsis in two ways. First, they trap moisture underneath them. Experiments have shown that small amounts of moisture can cling to the undersurfaces of rocks for weeks after their above-ground surfaces have dried out. Second, because the pebbles are translucent, they allow just enough light to reach the organisms to sustain growth.
- Friedmann envisions large farms where the bacteria are cultured on the underside of strips of glass that are treated to achieve the proper light-transmission characteristics. Before even as hardy a microbe as Chroococcidiopsis could be farmed on Mars, the planet would have to be warmed up considerably, to just below the freezing point.

- http://www.marsdaily.com/reports/Greening_The_Red_Planet.html

Sources of heritable novelty used by evolution

Source of novelty	Type	Examples
Genomic	Endogenous	Point mutations, gene/genome duplication, gene loss/loss of function, altered mutation rates, sexual recombination
	Exogenous – gene transfer	Uptake of DNA from the environment, viral transfer, interspecies gene transfer, sexual and parasexual processes, symbiosis
	Exogenous – environmental	Environmental mutagens (e.g., UV radiation)
Regulatory	Internal	Regulatory genes, nc (noncoding) RNA
	External	Environmental mutagens
	Multiple sources	Duplication of parts (e.g., segmentation, multicellularity)
Developmental	Timing	Neoteny
	Relative rates	Allometry
Physical	Compartmentalization	Organelles, multicellularity
	Non-genetic template	Cortical inheritance (e.g., in ciliates)