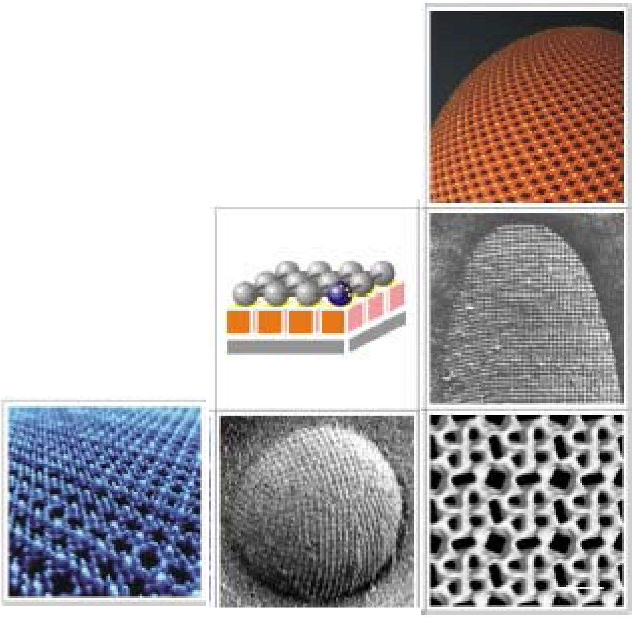
Drug delivery

Protein based

Drug delivery systems

S-layer proteins as basic building blocks in a biomolecular construction kit



Outline

- Development of a (bio)molecular construction kit
- Description of S-layer proteins (basic principles)
- S-layers as nano-scale patterning elements for life- and non- life science applications (selected exmples)

Which are the basic building blocks in a biomolecular construction kit

- Biological molecules (e.g. Proteins, Lipids, Glycans, Nucleic acids)
- Chemically or Genetically Modified Molecules
- Chemically Synthesized Molecules

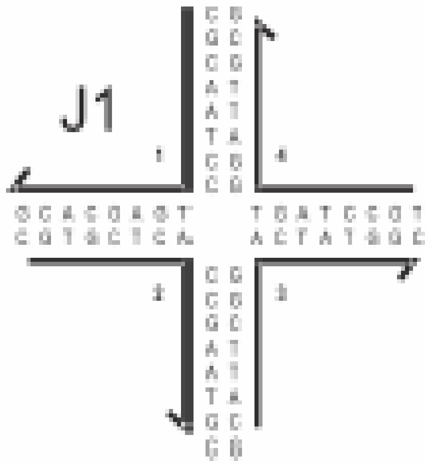
Key capabilities

- Self-assemble
- Molecular recognition
- Adaptable and evolved structure & function
- Dynamic structures

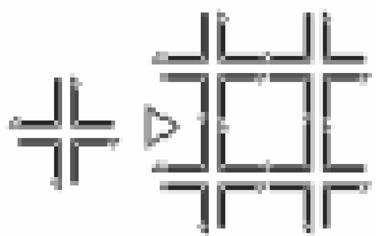
Basic Structures (Patterning Elements) for Generating Complex Supramolecular Structures

- DNA
- Monomolecular crystalline bacterial cell surface layers (S-layers)

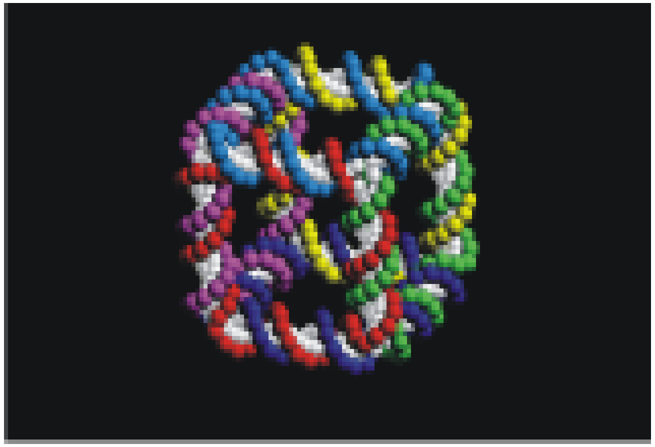
Nanoscale Assembly and Manipulation of Branched DNA (Ned Seeman, NY University)

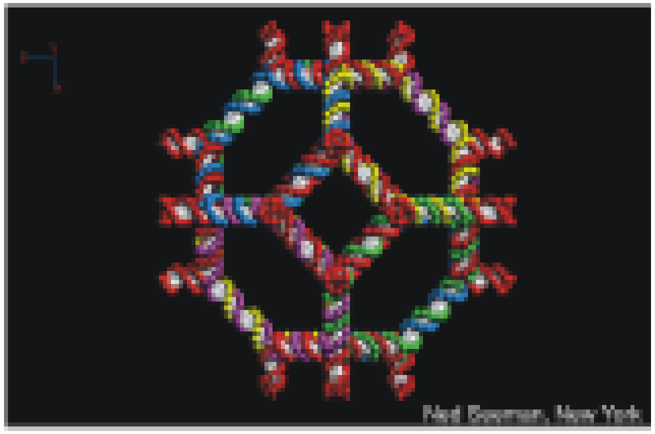


basic structure: junction J1



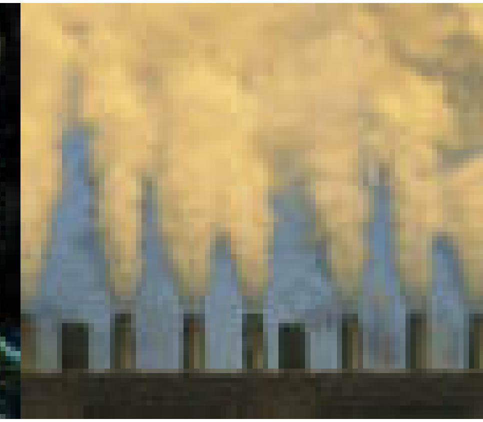
main features of "nanoscale assembly"





http://seemanlab4.chem.nyu.edu/homepage.html





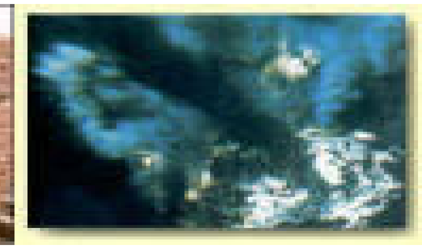
EXTREMOPHILES

NATURE’S ULTIMATE SURVIVORS

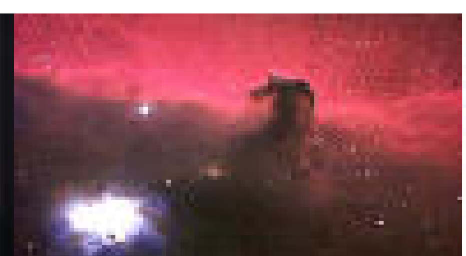
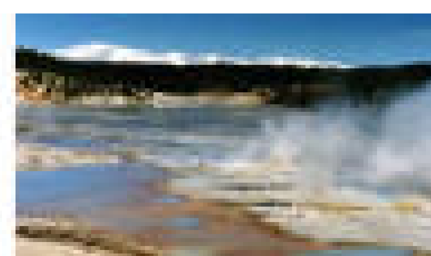
EXTREMOPHILES

I. What are they?
II. Types of Extremophiles
III. Extreme Prokaryotes
IV. Extreme Eukaryotes
V. Extreme Viruses
VI. Evolution of Extremophiles
VII. Biotechnological Uses
VIII. Industrial Uses
IX. Extraterrestrial Extremophiles?

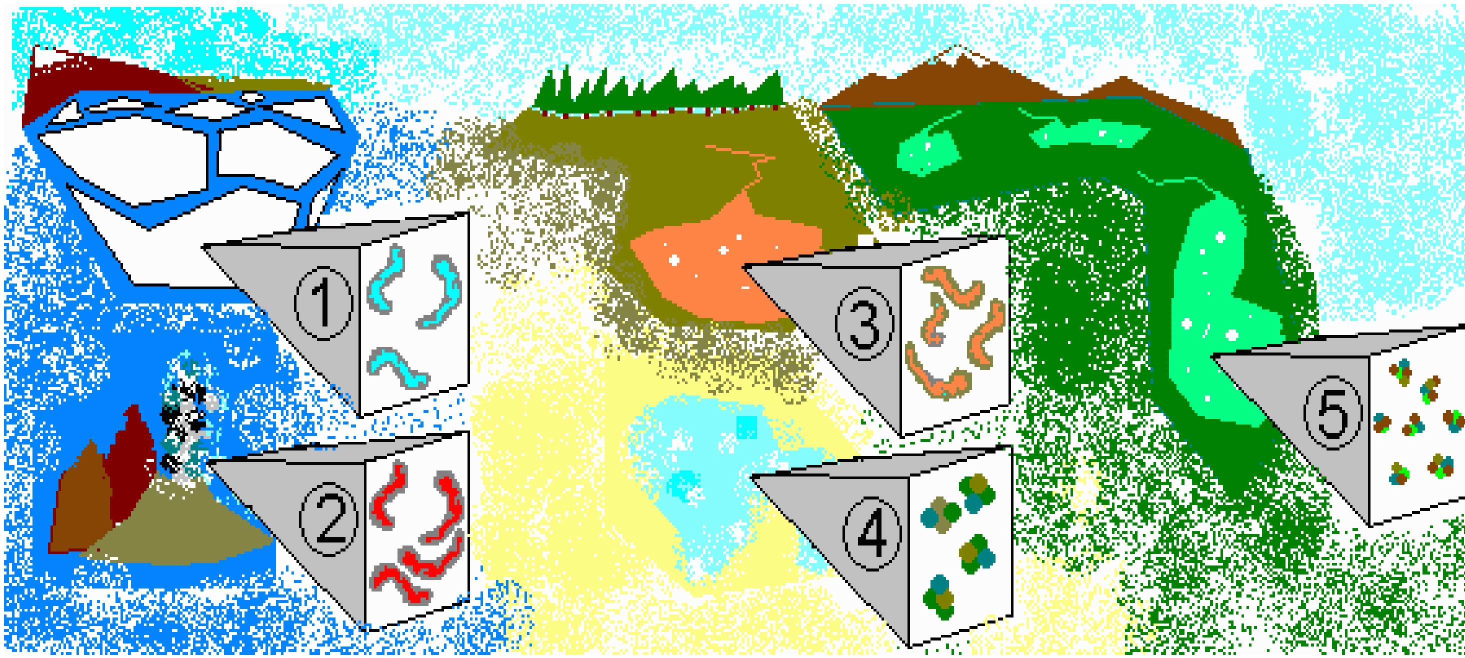
What are Extremophiles?



Extremophiles are microorganisms— whether viruses, prokaryotes, or eukaryotes— that survive under harsh environmental conditions that can include atypical temperature, pH, salinity, pressure, nutrient, oxic, water, and radiation levels



Types of Extremophiles



1) Psychrophiles
Microbes that live in cold environments like sea ice and the arctic and antarctic ice packs.

2) Thermophiles
Microbes that live in very hot environments like deep sea vents and volcanic lakes.

3) Alkaliphiles
Microbes that live in basic environments like soda lakes.

4) Halophiles
Microbes that live in very salty environments like salt lakes and salt mines.

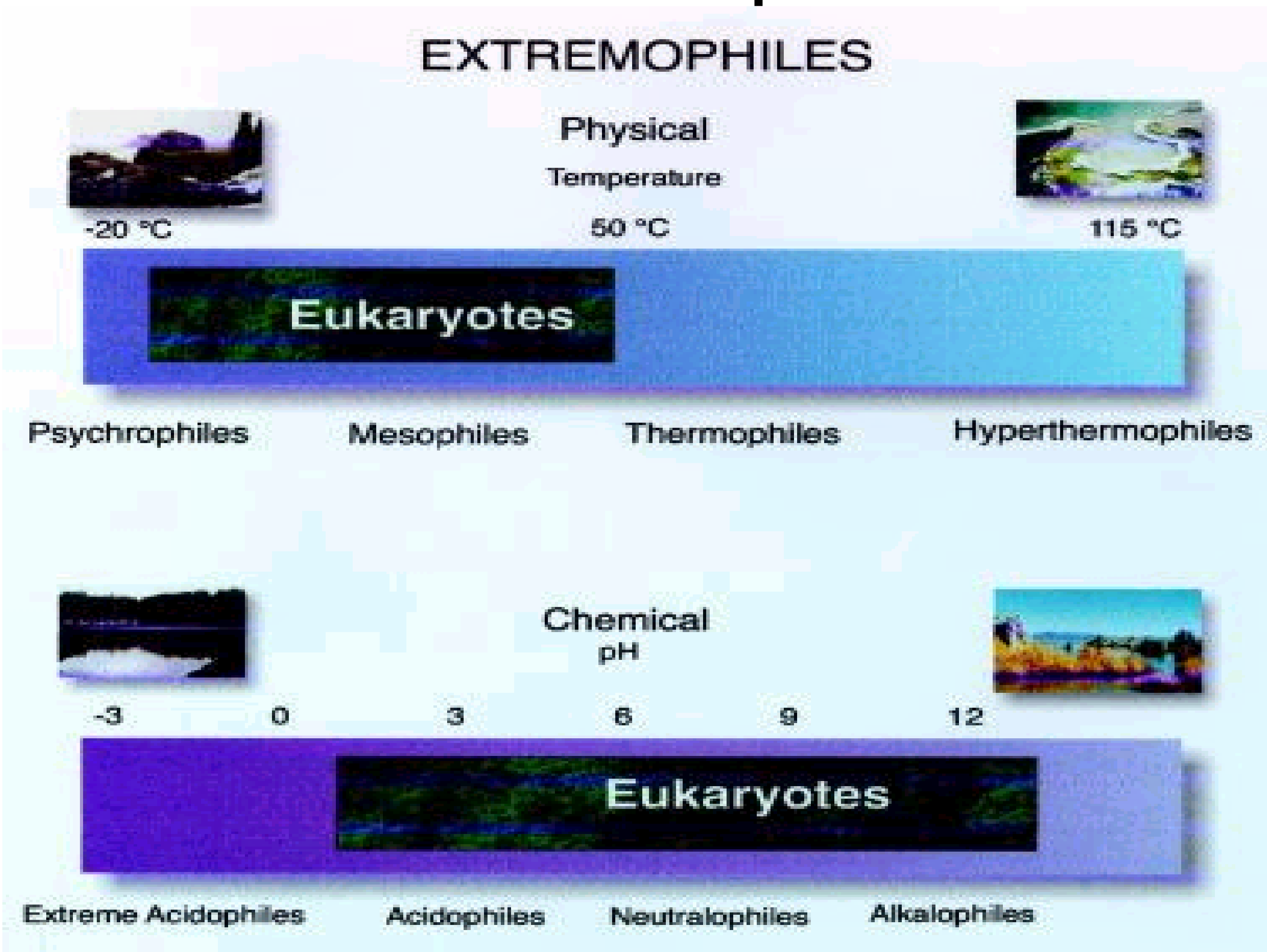
5) Acidophiles
Microbes that live in acidic environments like sulphur springs.

Types of Extremophiles

Other types include:

- **Barophiles -survive under high pressure levels, especially in deep sea vents**
- **Osmophiles –survive in high sugar environments**
- **Xerophiles -survive in hot deserts where water is scarce**
- **Anaerobes -survive in habitats lacking oxygen**
- **Microaerophiles -survive under low-oxygen conditions only**
- **Endoliths –dwell in rocks and caves**
- **Toxitolerants -organisms able to withstand high levels of damaging agents. For example, living in water saturated with benzene, or in the water-core of a nuclear reactor**

Environmental Requirements



Surviving the Extremes

Extremophiles Can Survive:

- 113 to 200 °C
- - 15 °C
- pH < 0.0
- pH > 11
- 1200 atmospheres
- 0% oxygen
- 20-40 million years dormancy
- 2 1/2 years in space, etc.

EXTREME PROKARYOTES
Hyperthermophiles

HYPERTHERMOPHILES at the base of the tree of Life

Eubacteria:

Aquifex pyrophilus	85° C
Thermotoga maritima	80° C

Archaebacteria:

Acidianus infernus	88° C
Pyrodictium abyssi	105° C
Pyrococcus furiosus	100° C

DATA FROM STETTER (1994)

-Members of domains Bacteria and Archaea

-Held by many scientists to have been the earliest organisms

-Early earth was excessively hot, so these organisms would have been able to survive

Morphology of Hyperthermophiles

- **Heat stable proteins that have more hydrophobic interiors, which prevents unfolding or denaturation at higher temperatures**

- **Have chaperonin proteins that maintain folding**

- **Monolayer membranes of dibiphytanyl tetraethers, consisting of saturated fatty acids which confer rigidity, preventing them from being degraded in high temperatures**

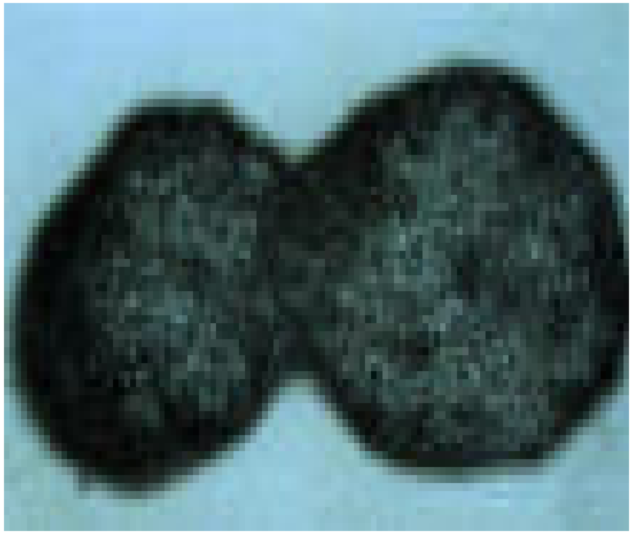
- **Have a variety of DNA-preserving substances that reduce mutations and damage to nucleic acids, such as reverse DNA gyrase and Sac7d**

- **They can live without sunlight or organic carbon as food, and instead survive on sulfur, hydrogen, and other materials that other organisms cannot metabolize**

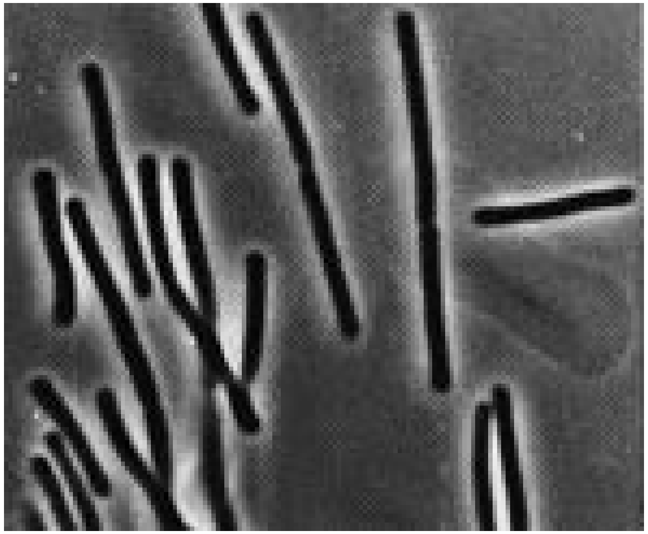
The red on these rocks is produced by *Sulfolobus solfataricus,* near Naples, Italy

Some Hyperthermophiles

Frequent habitats include volcanic vents and hot springs, as in the image to the left

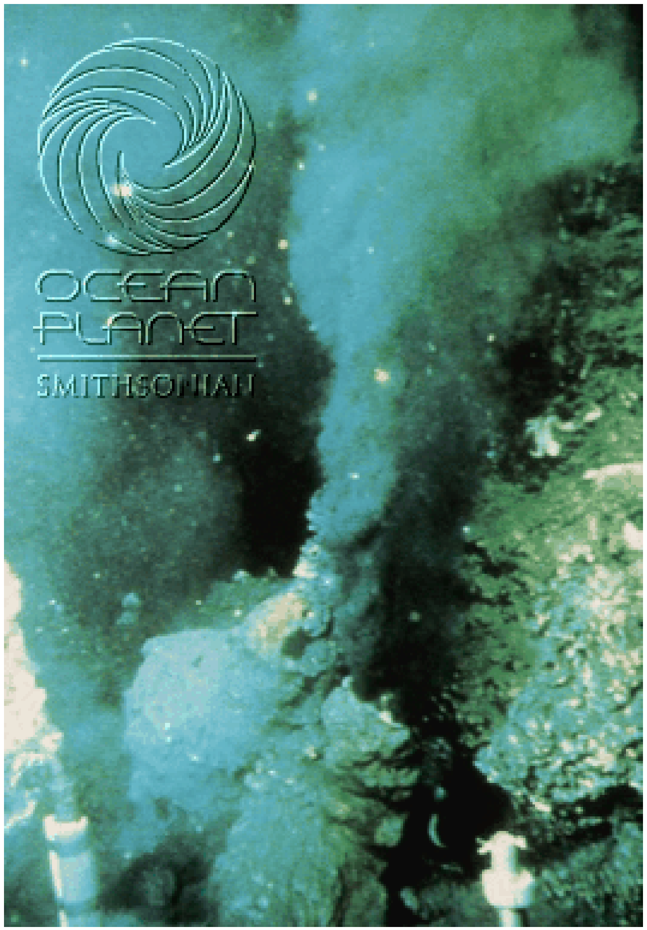


Pyrococcus abyssi **1μm**



Thermus aquaticus **1μm**

Deep Sea Extremophiles



A **black smoker, a** submarine hot spring, which can reach 518- 716°F (270-380°C)

The **deep-sea floor** and **hydrothermal vents** involve the following conditions:

low temperatures (2-3º C) – where only **psychrophiles** are present

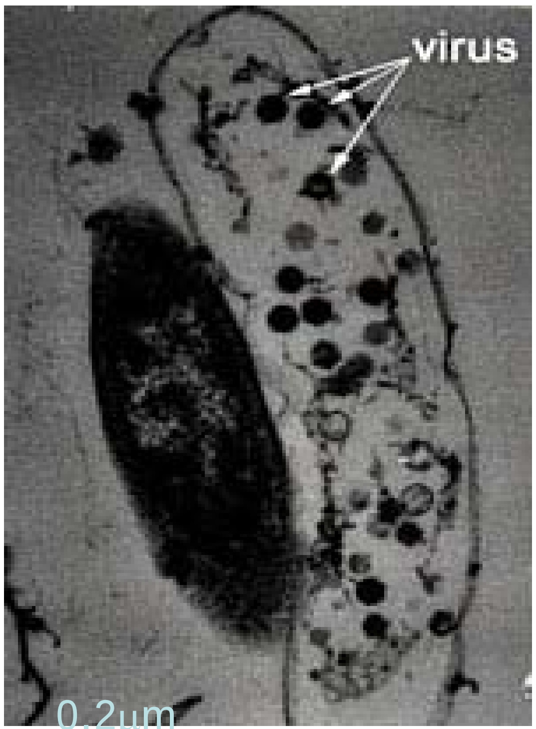
low nutrient levels – where only **oligotrophs** present

high pressures – which increase at the rate of 1 atm for every 10 meters in depth (as we have learned, increased pressure leads to decreased enzyme-substrate binding)

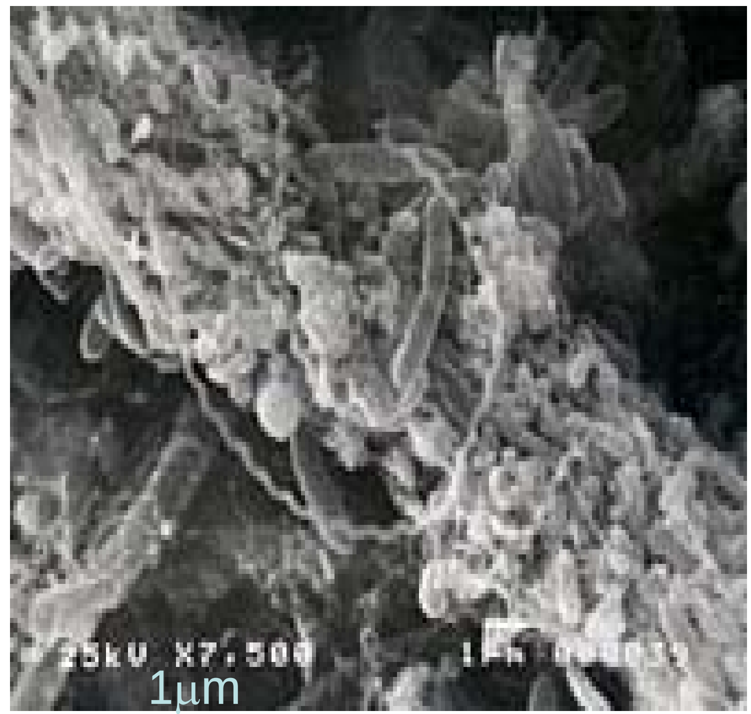
barotolerant microorganisms live at 1000-4000 meters

barophilic microorganisms live at depths greater than 4000 meters

Extremophiles of Hydrothermal Vents



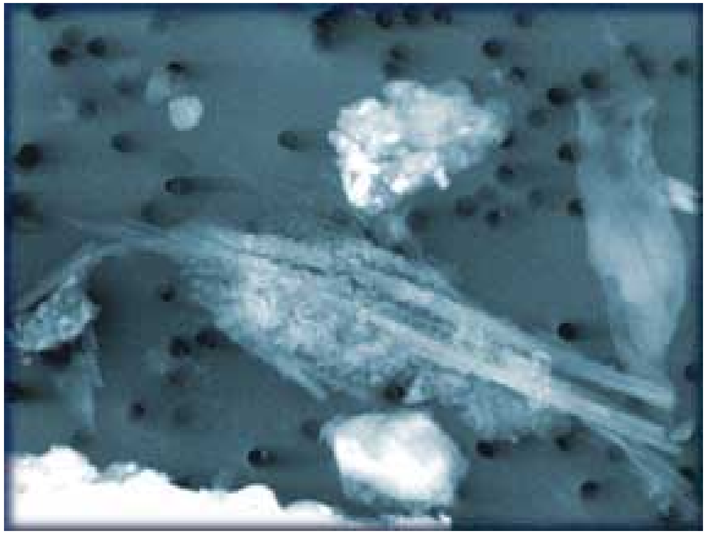
A cross-section of a bacterium isolated from a vent. Often such bacteria are filled with viral particles which are abundant in hydrothermal vents



A bacterial community from a deep-sea hydrothermal vent near the Azores

Natural springs which vent warm or hot water on the sea floor near mid-ocean ridges. Associated with the spreading of the earth's crust. High temperatures and pressures

Psychrophiles



Some microorganisms thrive in temperatures well below the freezing point of water, such as in Antarctica

Some researchers believe that psychrophiles live in conditions mirroring those found on Mars

Psychrophiles possess:

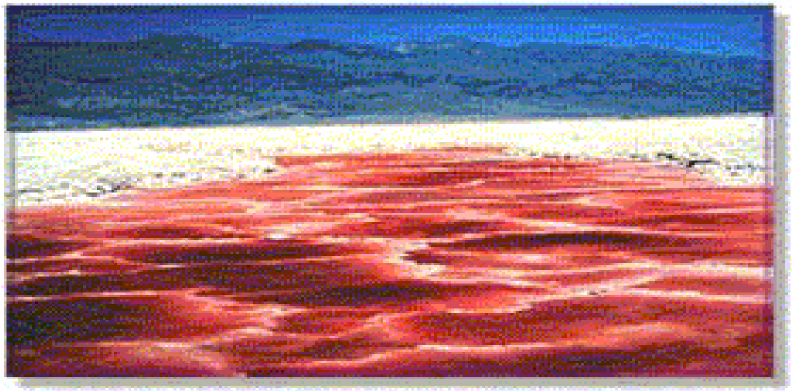
-proteins rich in α-helices and polar groups which allow for greater flexibility

-“antifreeze proteins” that maintain liquid intracellular conditions by lowering freezing points of other biomolecules

-membranes that are more fluid, containing unsaturated *cis*-fatty acids which help to prevent freezing

-active transport at lower temperatures

Halophiles



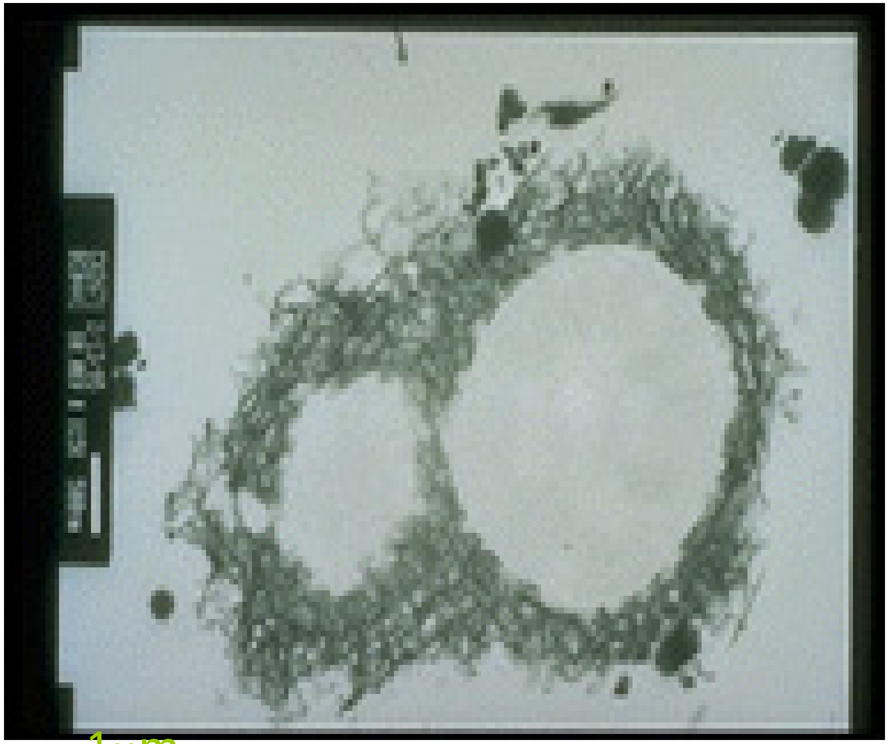
The vivid red brine (teaming with halophilic archaebacteria) of Owens Lake contrasts sharply with the gleaming white deposits of soda ash (sodium carbonate). The picturesque Inyo Range can be seen in the distance.

-Divided into mild (1-6%NaCl), moderate (6-15%NaCl), and extreme (15-30%NaCl)

-Halophiles are mostly obligate aerobic archaea

How do halophiles survive high salt concentrations?
-by interacting more strongly with water such as using more negatively charged amino acids in key structures
-by making many small proteins inside the cell, and these, then, compete for the water
-and by accumulating high levels of salt in the cell in order to outweigh the salt outside

Barophiles



A sample of barophilic bacteria from the earth's interior

-Survive under levels of pressure that are otherwise lethal to other organisms

-Usually found deep in the earth, in the deep sea, hydrothermal vents, etc

-scientists believe that barophiles may be able to survive on the Moon and other places in space

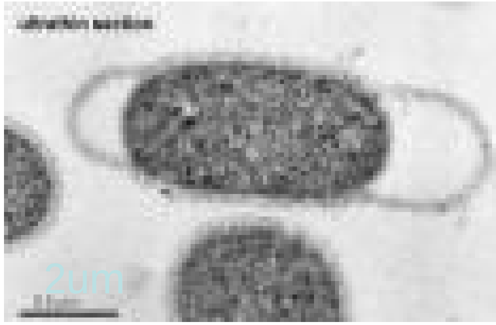
Xerophiles

Extremophiles which live in water-scarce habitats, such as deserts

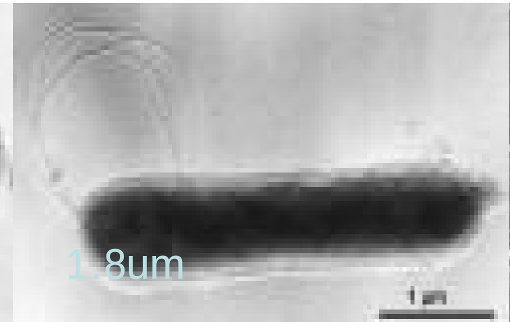
Produce **desert varnish** as seen in the image to the left

Desert varnish is a thin coating of Mn, Fe, and clay on the surface of desert rocks, formed by colonies of bacteria living on the rock surface for thousands of years

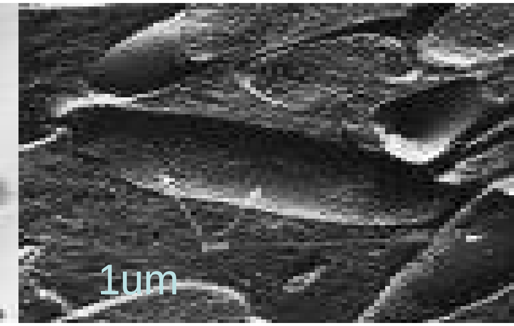
SOME COMMON GENERA OF PROKARYOTE EXTREMOPHILES



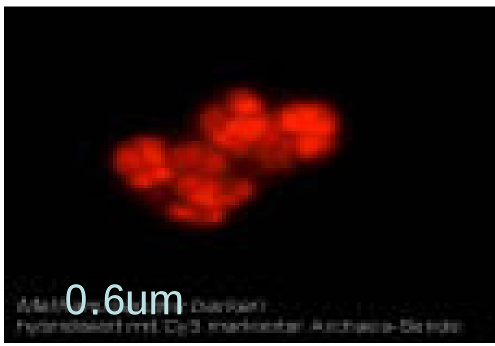
Thermotoga



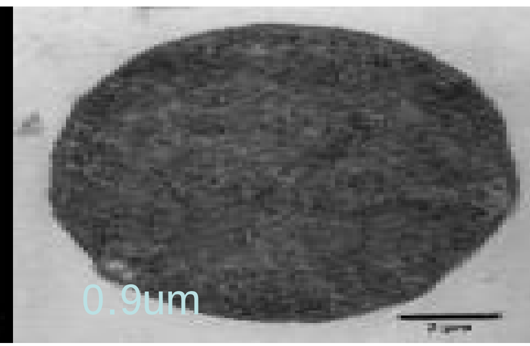
Aquifex



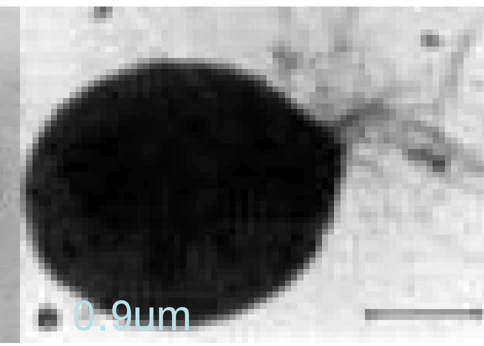
Halobacterium



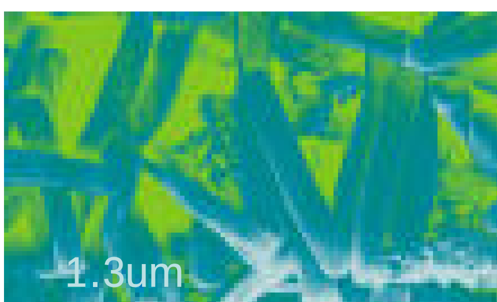
Methanosarcina



Thermoplasma

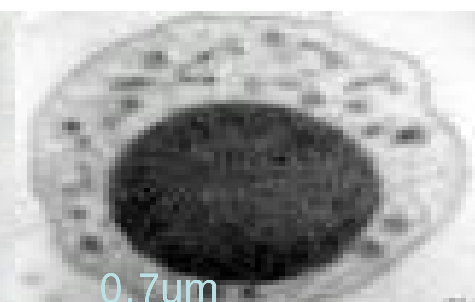


Thermococcus



Thermoproteus

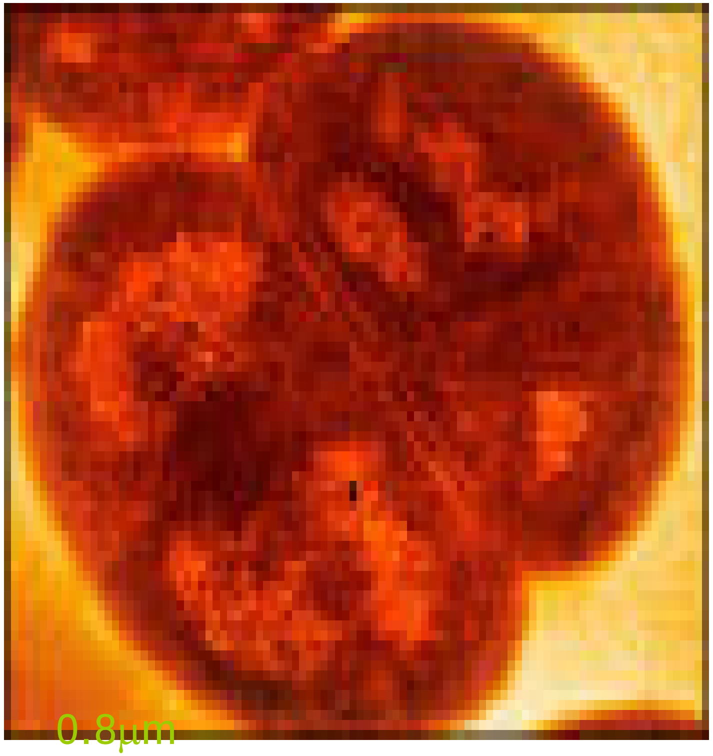
Pyrodictium



Ignicoccus

Deinococcus radiodurans

The Radiation Resistor

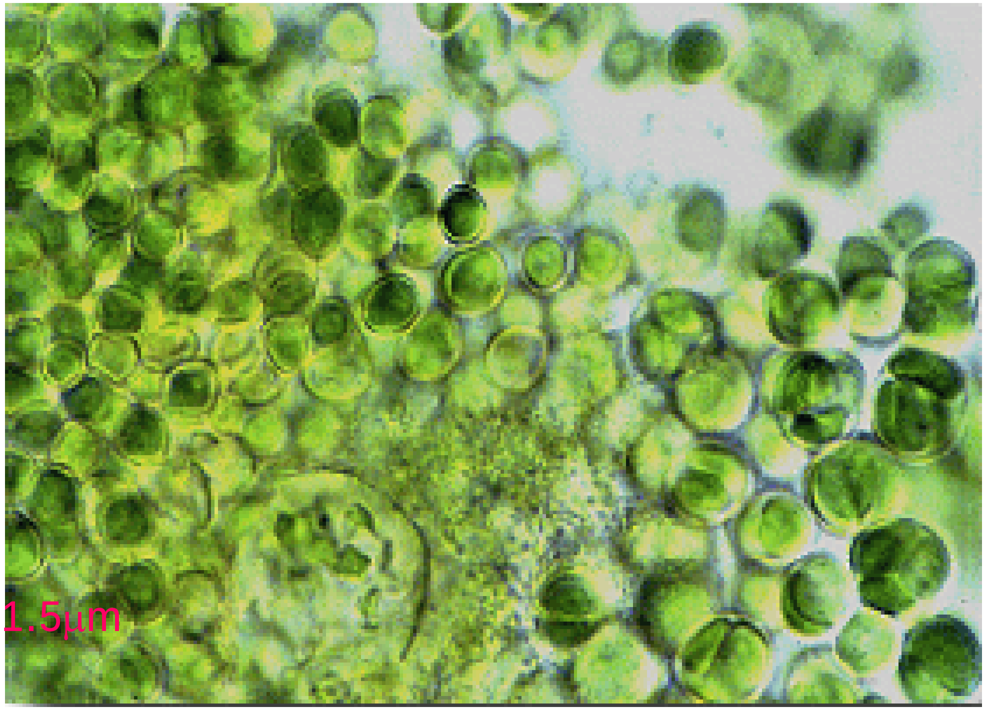


-Possesses extreme resistance to up to 4 million rad of radiation, genotoxic chemicals (those that harm DNA), oxidative damage from peroxides/superoxides, high levels of ionizing and ultraviolet radiation, and dehydration

-It has from four to ten DNA molecules compared to only one for most other bacteria

-Contains many DNA repair enzymes, such as RecA, which matches the shattered pieces of DNA and splices them back together. During these repairs, cell-building activities are shut off and the broken DNA pieces are kept in place

Chroococcidiopsis
The Cosmopolitan Extremophile

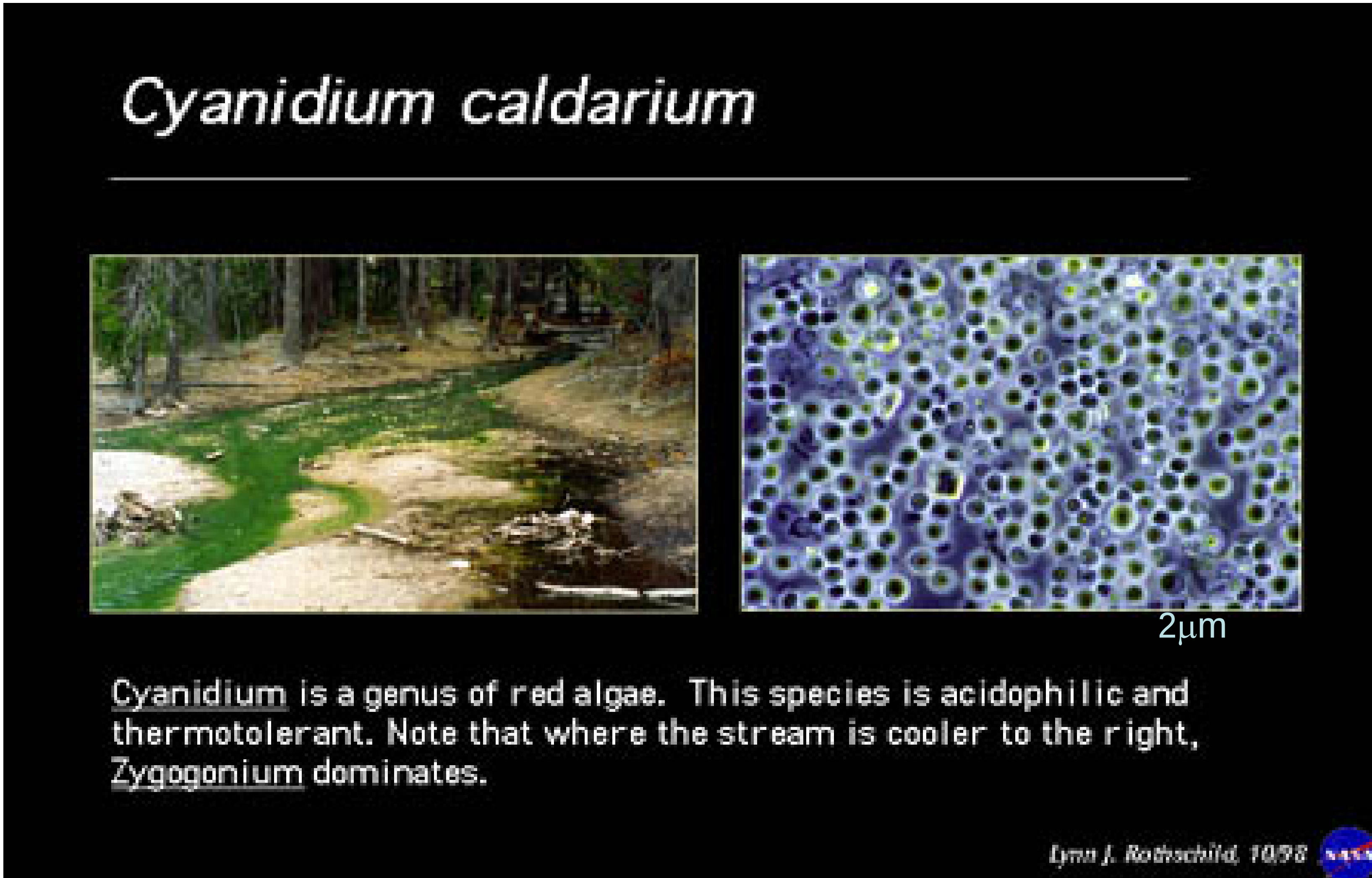


-A cyanobacteria which can survive in a variety of harsh environments, such as hot springs, hypersaline habitats, hot, arid deserts throughout the world, and in the frigid Ross Desert in Antarctica

-Possesses a variety of enzymes which assist in such adaptation

EXTREME EUKARYOTES

THERMOPHILES/ACIDOPHILES



EXTREME EUKARYOTES

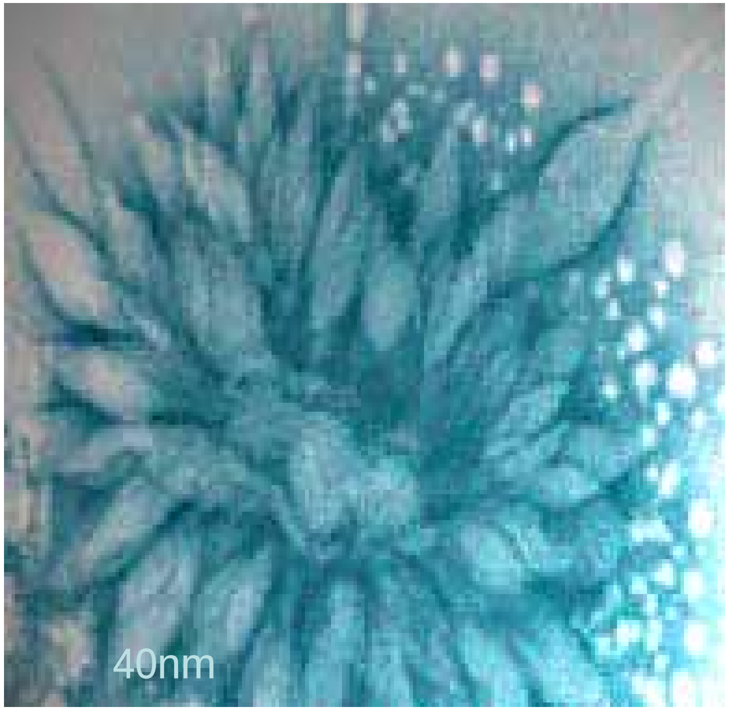
ENDOLITHS

Quartzite from Johnson Canyon, California. Sample shows green bands of endolithic algae. Rock is 9.5 cm wide

-Endoliths (also called hypoliths) are usually algae, but can also be prokaryotic cyanobacteria, that exist within rocks and caves

-Often are exposed to anoxic (no oxygen) and anhydric (no water) environments

EXTREME VIRUSES



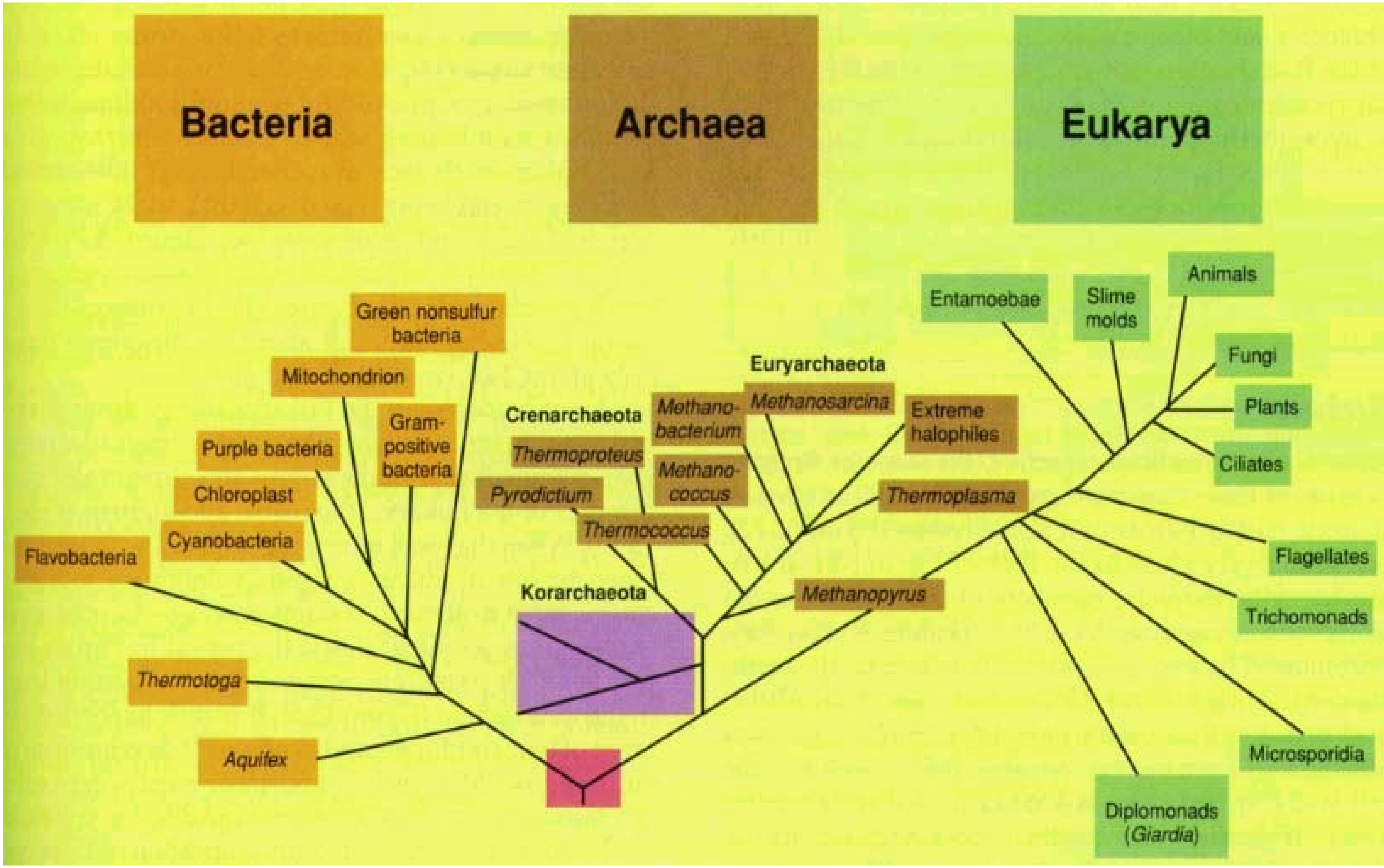
Virus-like particles isolated from the extreme environment of Yellowstone National Park hot springs

Viruses are currently being isolated from habitats where temperatures exceed 90°C

Instead of the usual icosahedral or rod-shaped capsids that known viruses possess, researchers have found viruses with novel propeller-like structures

These extreme viruses often live in hyperthermophile prokaryotes such as *Sulfolobus*

CLASSIFICATION OF EXTREMOPHILES



Extremophiles are present among Bacteria, form the majority of Archaea, and also a few among the Eukarya

PHYLOGENETIC RELATIONSHIPS

-Members of Domain Bacteria (such as *Aquifex* and *Thermotoga*) that are closer to the root of the "tree of life" tend to be hyperthermophilic extremophiles

-The Domain Archaea contain a multitude of extremophilic species:

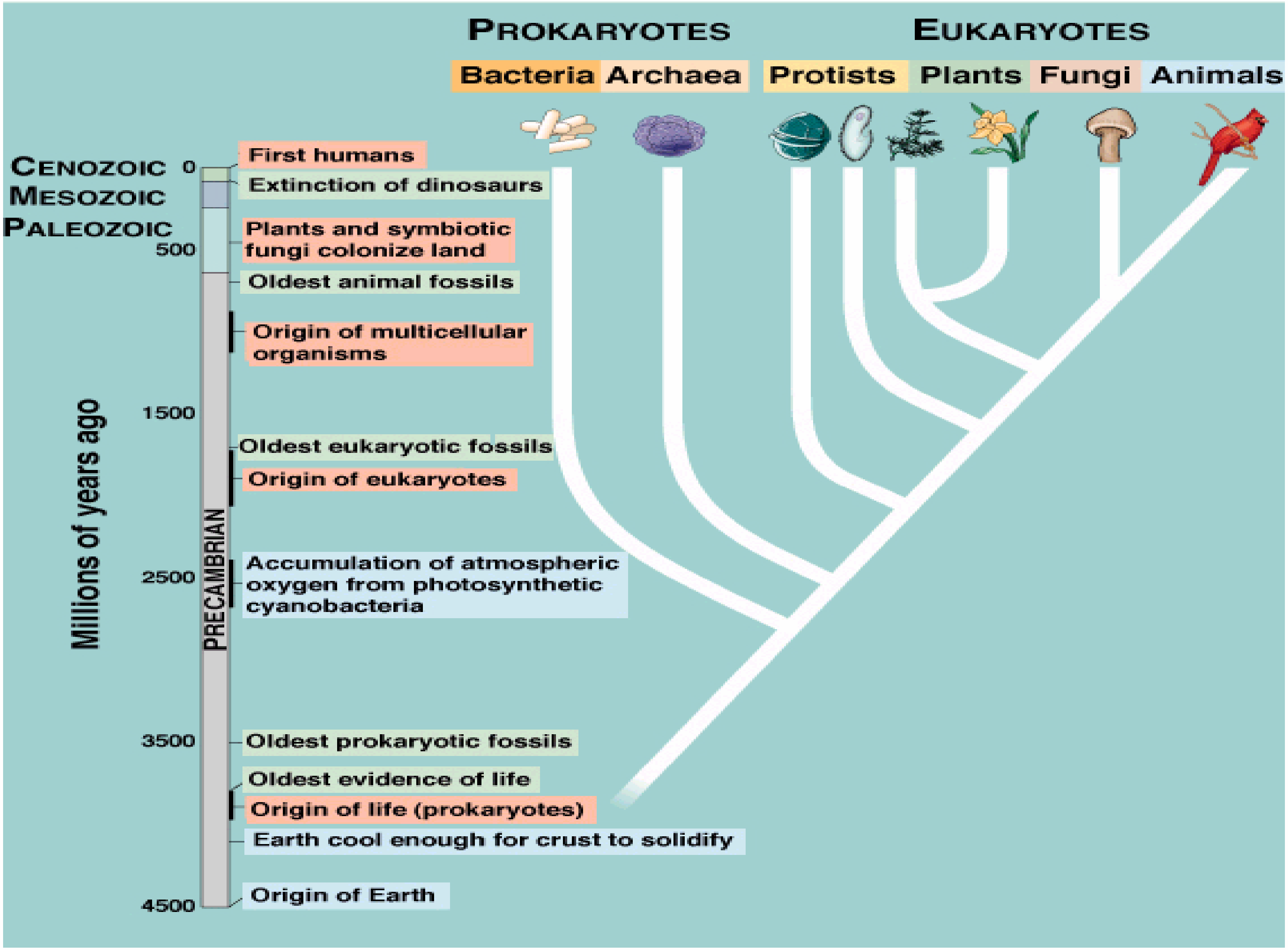
Phylum Euryarchaeota-consists of methanogens and extreme halophiles

Phylum Crenarchaeota-consists of thermoacidophiles, which are extremophiles that live in hot, sulfur-rich, and acidic solfatara springs

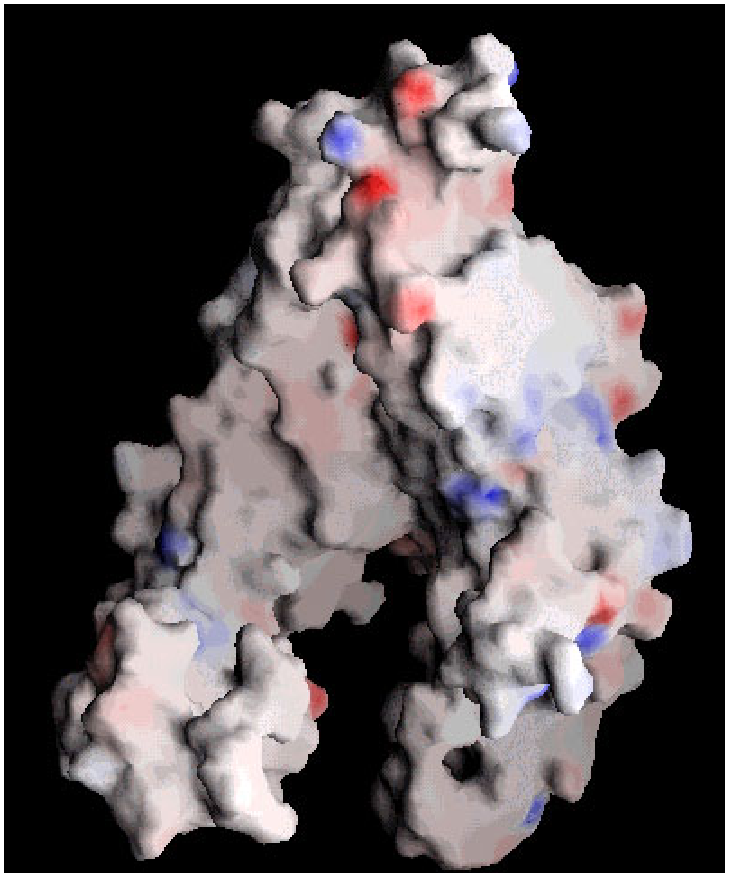
Phylum Korarchaeota-new phylum of yet uncultured archaea near the root of the Archaea branch, all are hyperthermophiles

-Most extremophilic members of the Domain Eukarya are red and green algae

Chronology of Life



Taq Polymerase

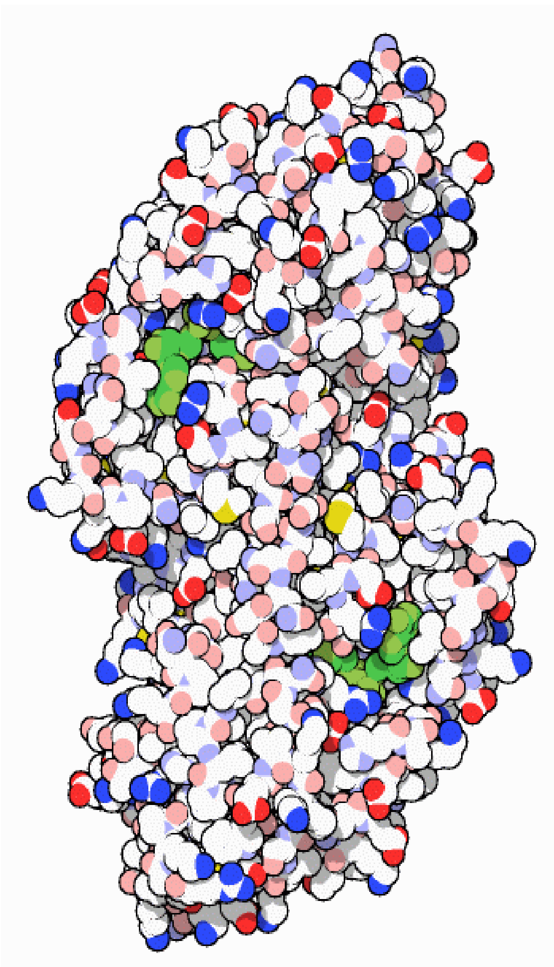


Isolated from the hyperthermophile *Thermus aquaticus*

Much more heat stable

Used as the DNA polymerase in the very useful Polymerase Chain Reaction (PCR) technique which amplifies DNA samples

Alcohol Dehydrogenase



-Alcohol dehydrogenase (ADH), is derived from a member of the archaea called *Sulfolobus solfataricus*

-It works under some of nature's harshest volcanic conditions: It can survive to 88°C (190ºF) - nearly boiling - and corrosive acid conditions (pH=3.5) approaching the sulfuric acid found in a car battery (pH=2)

-ADH catalyzes the conversion of alcohols and has considerable potential for biotechnology applications due to its stability under these extreme conditions

Bacteriorhodopsin

-Bacteriorhodopsin is a trans-membrane protein found in the cellular membrane of *Halobacterium salinarium*, which functions as a light-driven proton pump

-Can be used for electrical generation

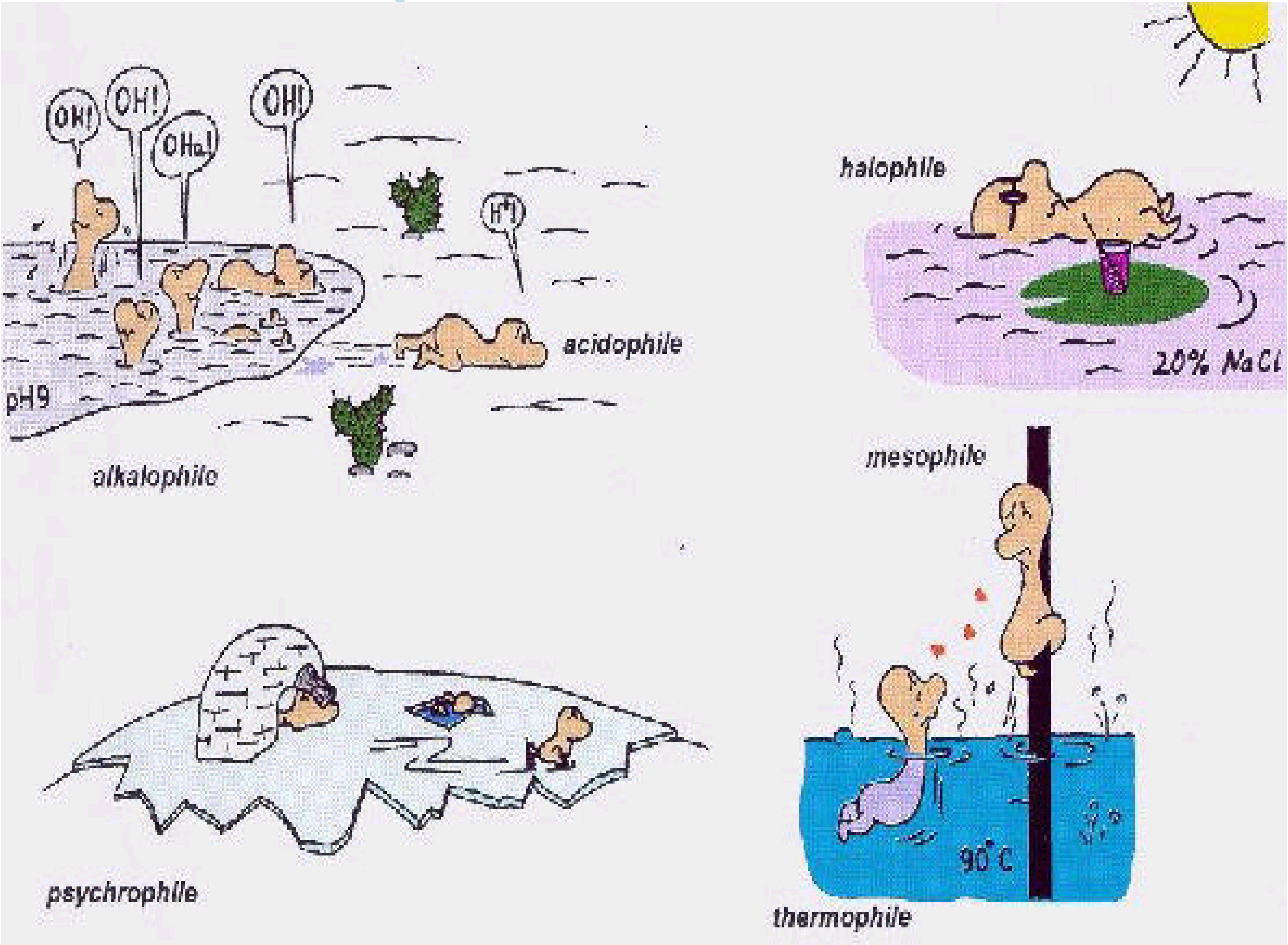
CONCLUSIONS

-Extremophiles are a very important and integral part of the earth’s biodiversity

They:

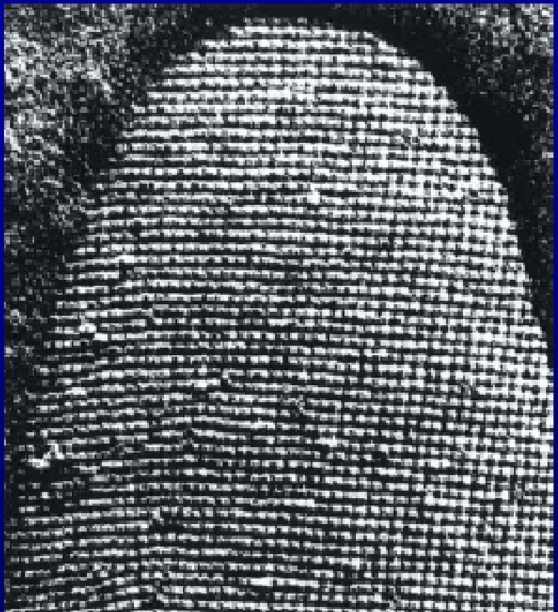
- reveal much about the earth’s history and origins of life
- possess amazing capabilities to survive in the extremes
- are proving to be beneficial to both humans and the environment
- may exist beyond earth

Questions?


OH!
OH!
OHa!
OH!
H+!
pH9
alkalophile
acidophile
halophile
20% NaCl
mesophile
psychrophile
90°C
thermophile

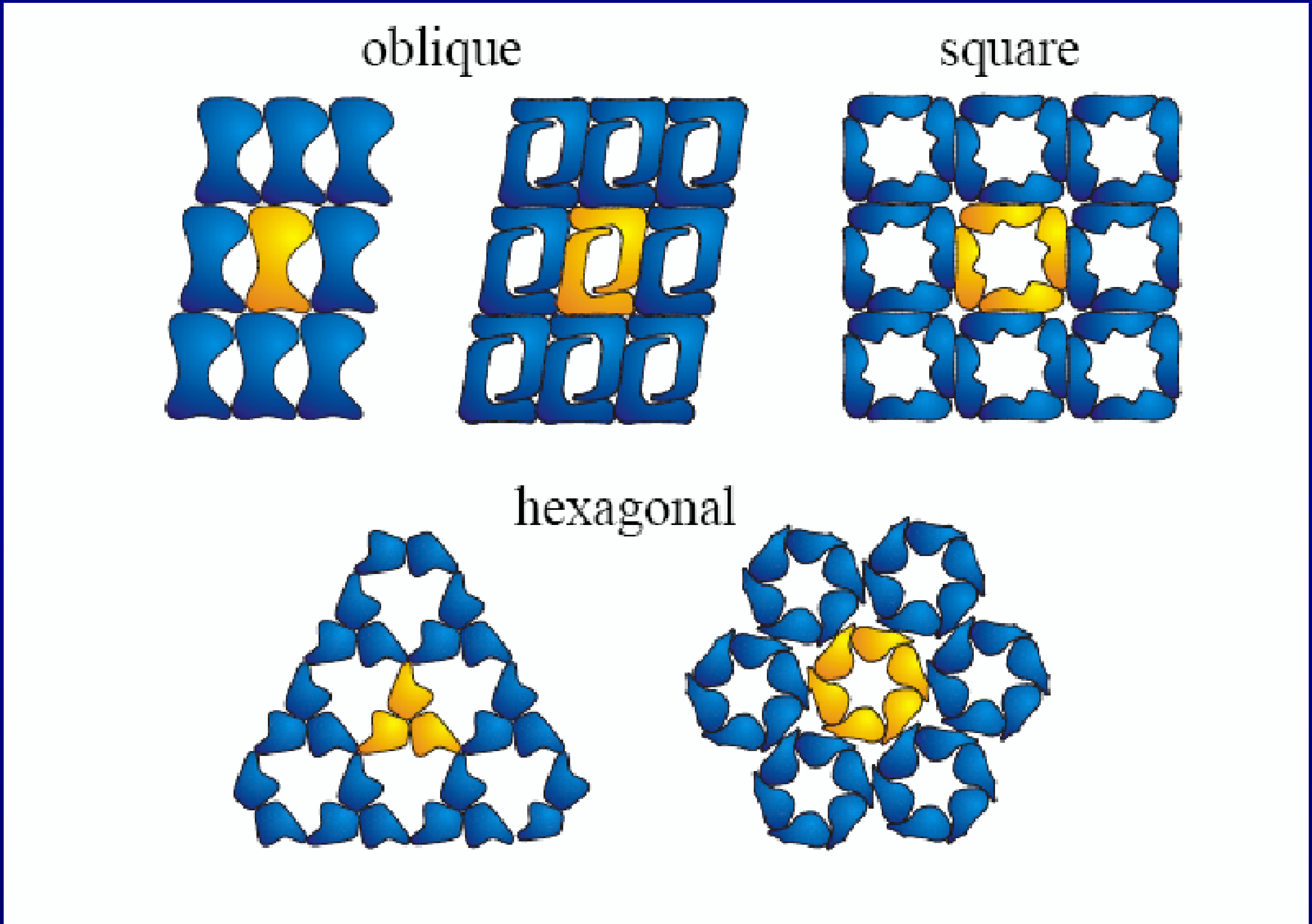
What are S-Layers ? (Surface Layers)

- The most common surface structure in prokaryotic organisms (bacteria, archaea).
- Highly ordered protein arrays composed of single protein or glycoprotein subunits.
- The simplest type of membranes developed during biological evolution.
- They completely cover the cell surface during all stages of bacterial growth and division.



Freeze-etched preparation of a cell

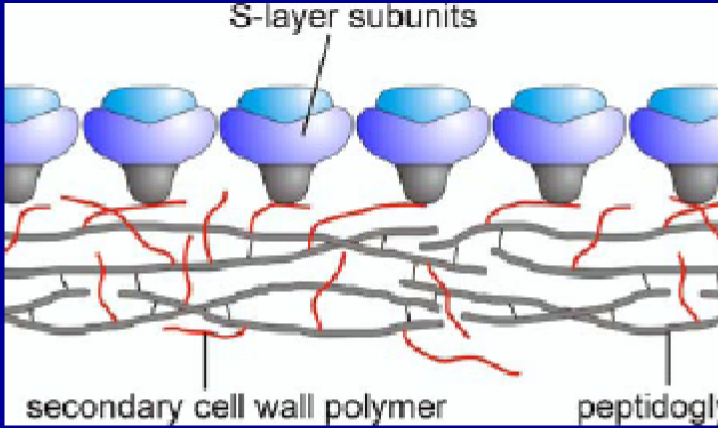
Lattice Types of S-layer Proteins



nding Mechanism of S-Layer Subunit

bacteria, the S-layer subunits are linked to each other and
underlying cell envelope layer by non-covalent interactio

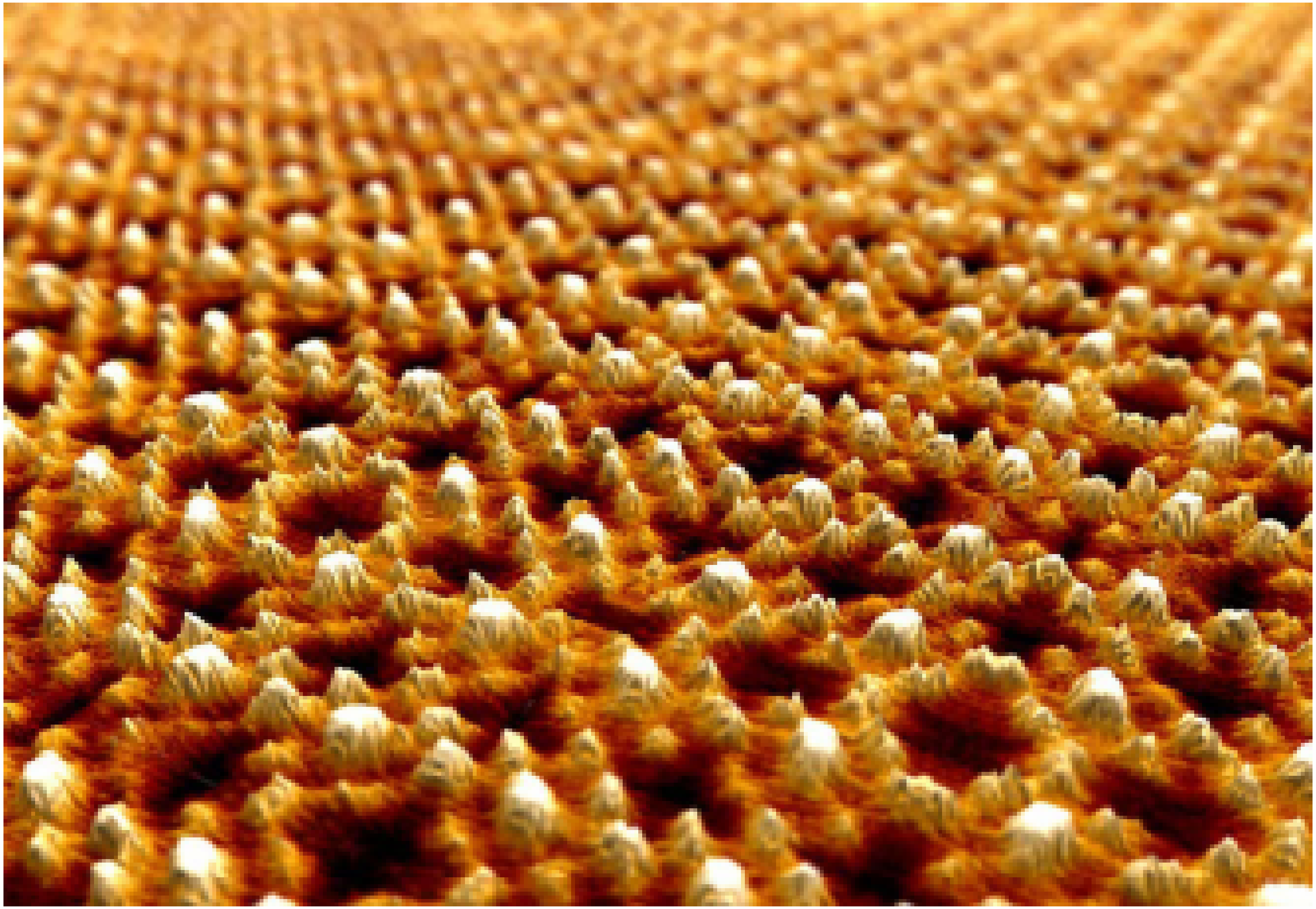
e N-terminal region of S-layer
teins from Gram-positive
anisms recognizes a distinct
e of secondary cell wall polymer
the proper binding site.



WPs are covalently linked to the peptidoglycan backbone.

ayer subunits can be extracted from cell wall fragments w
otropic agents (e. g. GHCl) and form self-assembly produ
er removal of the disintegrating agent.

Top-on view of the S-layer lattice of SbpA

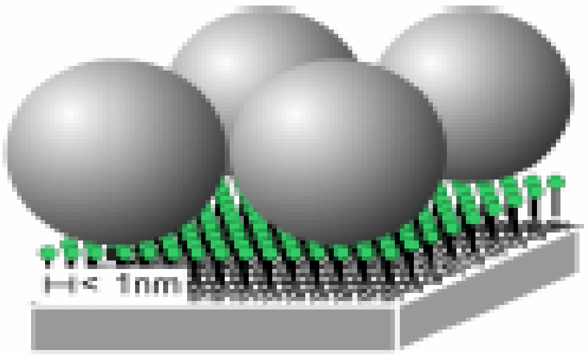


NanoBiotechnologcal applications of functional S-layer arrays

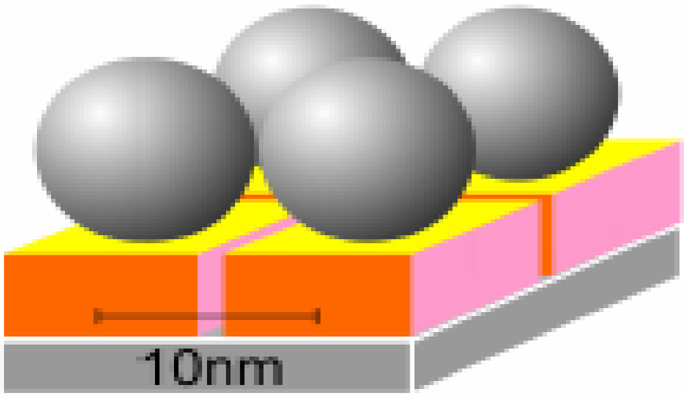
- Ultrafiltration membranes
- Matrices for a well defined binding of functional molecules and nanoparticles (biosensors, nano electronics and optics).
- Supporting structure for functional lipid membranes (planar membranes, liposomes and nanocapsules).
- Drug delivery and drug targeting systems (artificial viruses).

Strategies for modification and functionalization of solid supports

- Self-assembled monolayers (SAMs)



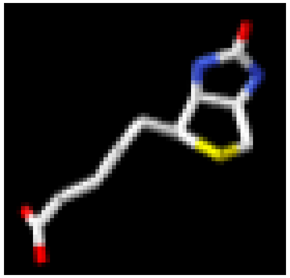
- S-layers lattices



Binding by Functional Domains on S-layer Fusion Proteins

SbsB / Streptavidin Fusion Proteins

Streptavidin (per subunit of the tetramer: 13 270 Da; 126 aa)

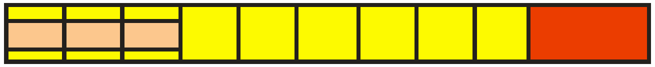


Biotin (244 Da)

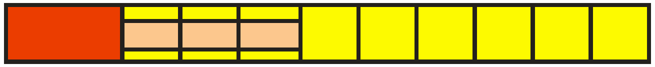
$K_a = 2{,}5 \times 10^{13}\ M^{-1}$

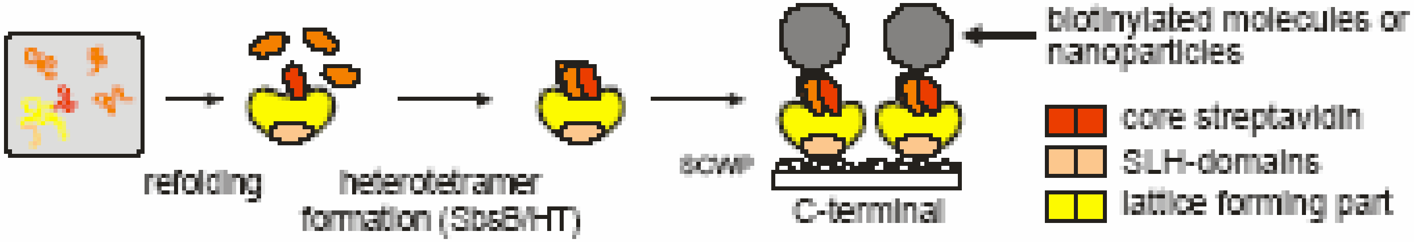
The highest affinity interaction between a protein and a ligand known in nature.

"C-terminal fusion proteins (BS1)"



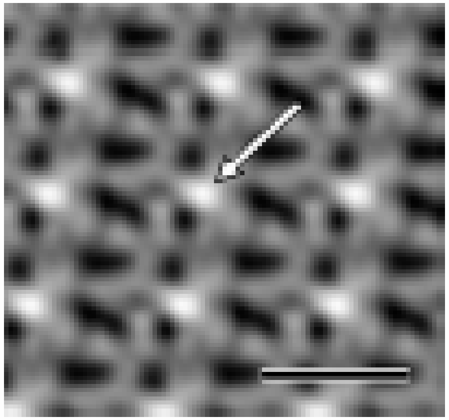
"N-terminal fusion proteins (S1B32)"



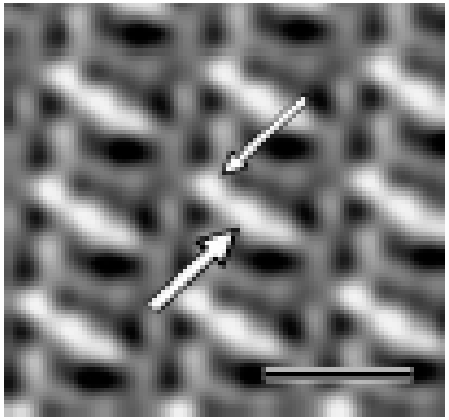


Moll *et al.*, PNAS, 99 (2002) 14646

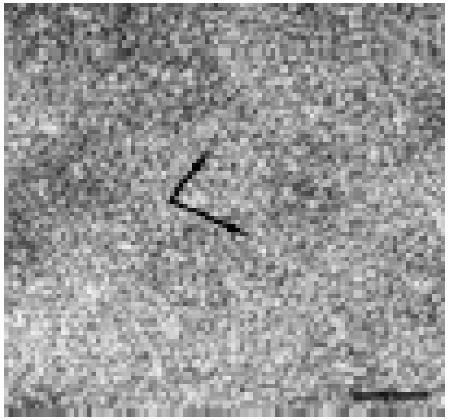
SbsB / Streptavidin fusion proteins



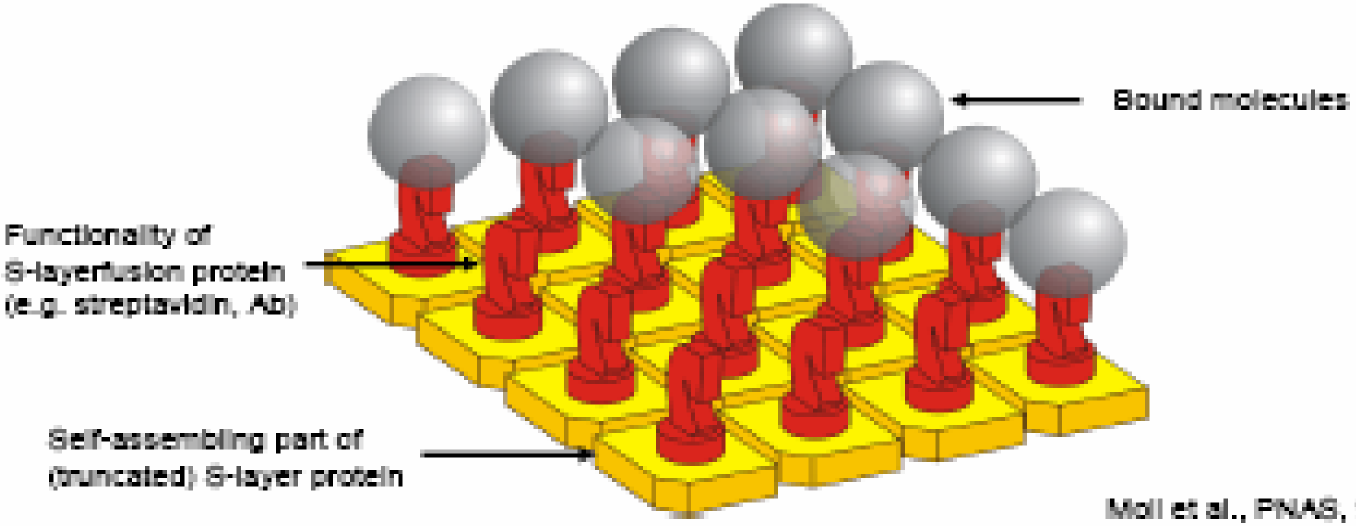
Native SbsB S-layer protein.
Bar, 10nm



SbsB/Stv fusion protein.
Bar, 10nm.



Biotinylated ferritin bound on
BS1. Bar, 100nm.



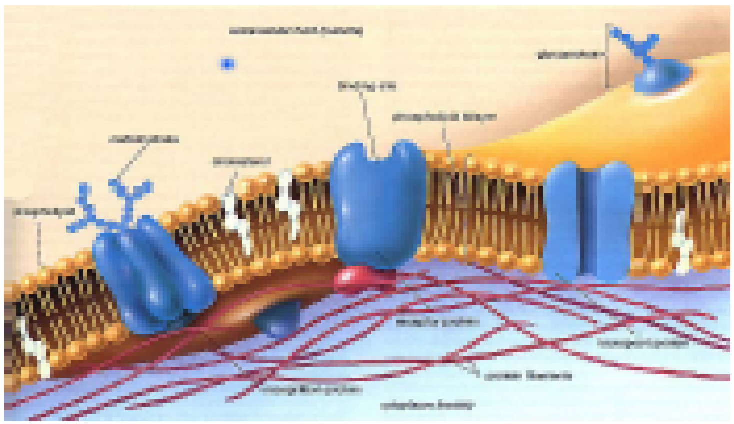
Moll et al., PNAS, 99 (2002) 14646

Applications of S-layer Fusion Proteins

- Sensing layers for label free detection systems

  (SPR, SAW, QCM-D)
- Functionalization of liposomes or lipid/plasmid particles as targeting and delivery systems
- Development of anti-allergic vaccines
- Stabilization of Langmuir lipid layers
- Metal binding structures (fusion of metal binding peptides)
- Binding of nanoparticles

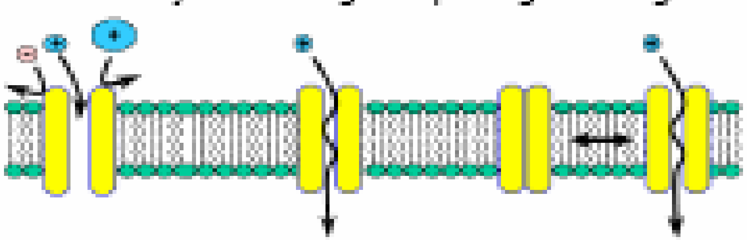
Biomimetic Cell Membranes



Cell membrane: fluid mosaic model

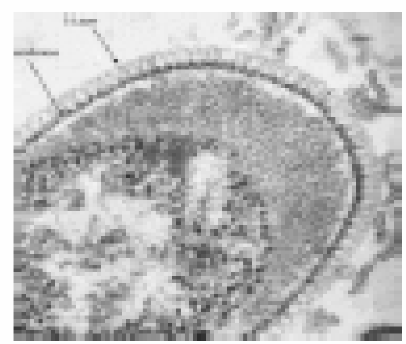
Specific membrane functions

1.selectivity 2.binding 3.opening / closing

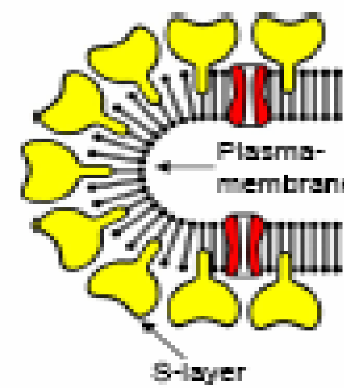


Archaeal cell envelope structure

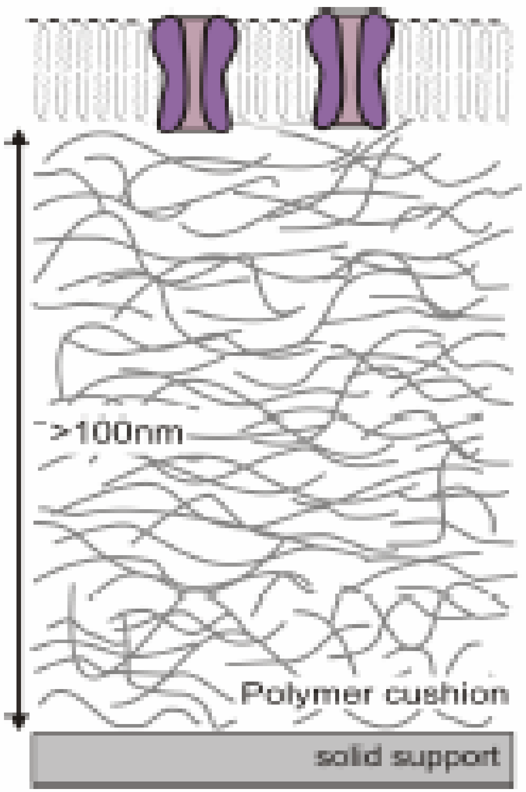
A supramolecular structure optimized in ~ 3,5 billions of years under extreme environmental conditions (120°C, pH 0, concentrated salt solutions, 1100 bar).



TEM of an archaeal cell



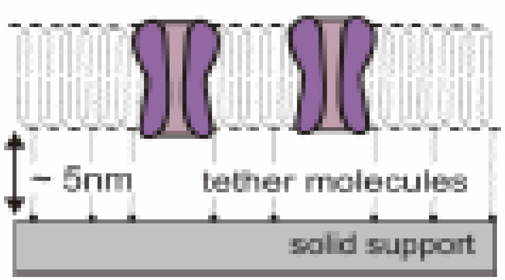
Biomimetic Lipid Membrane (Semifluid Membrane Model)



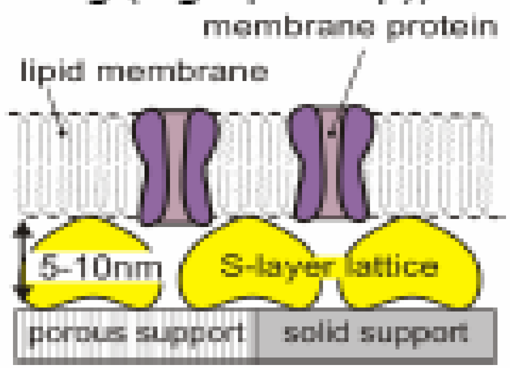
Sackmann, Science 271 (1996) 43

- S-layer stabilized solid supported lipid membranes
- S-layer acts as stabilizing structure, tethering structure and ionic reservoir
- Applications:

  Membrane Sensors, Lab-on-a-chip devices, High Throughput Screening (e.g. lipid chip), DNA sequencing



Schiller et al. Angew. Chem. Int. Ed. 42 (2003) 208



Schuster et al., Langmuir 17 (2001) 499
Schuster et al., Langmuir 19 (2003) 2392
Gufler et al. BBA 1661 (2004) 154

Application potential of S-layer-supported lipid membranes

- Exploiting functional lipid membranes at meso- and macroscopic scale:

  ~30 % of all proteins found in various organisms are membrane proteins

  > 50 % of the proteins interact with membranes

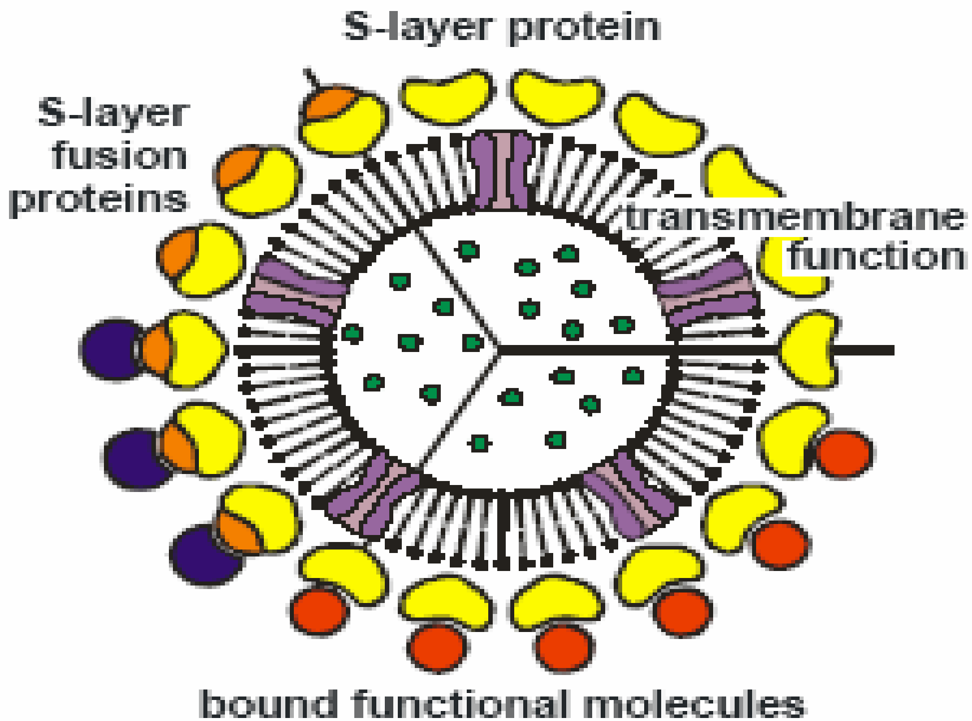
  ~ 15 % of the most sold drugs act on ion channels

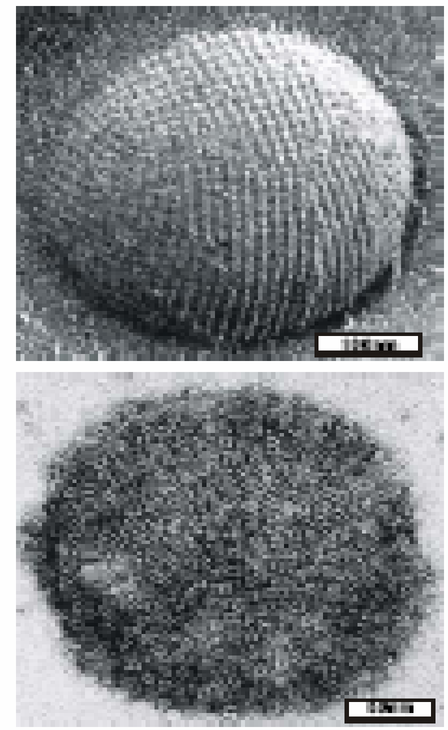
  ~ 60 % of the ethical drugs affect membrane proteins

- Linking silicon technology and solid state physics with biological systems (e.g. coupling cells to surfaces)

Biosensors, HTScreening, Diagnostics, Lab-on-a-chip

S-Layer coated liposomes





K0pc0 et al. BBA 1235 (1995) 263, Mader et al. BBA 1418 (1999) 106, Mader et al. BBA 1463 (2000) 142

Properties and Application Potential of S-layer coated liposomes

Properties:

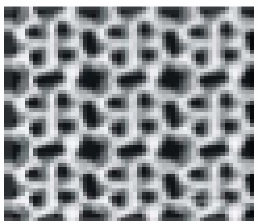
- Higher (mechanical and thermal) stability
- Monomolecular coating with functional molecules

Applications:

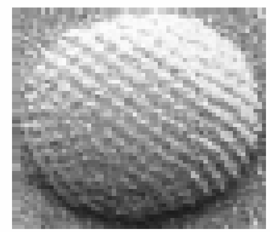
- Drug-targeting and drug-delivery
- Artificial viruses (inclusion of nucleic acid) for gene therapy

Summary

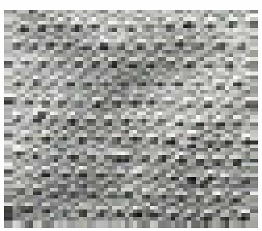
Basic research on structure, genetics, chemistry, morphogenesis and function of S-layers has led to a broad spectrum of applications in molecular nanotechnology and biomimetics:



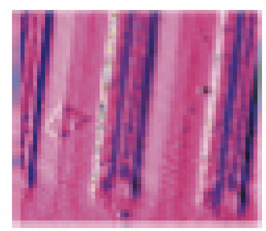
Isoporous ultrafiltration membranes



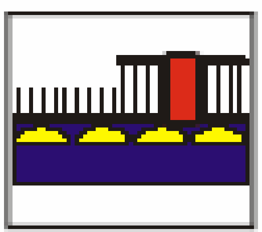
Vaccines, artificial viruses, drug delivery and targeting, gene therapy



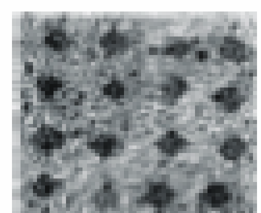
Matrices for controlled immobilization of molecules



Patterning by microlithographic procedures



Biomimetic lipid membranes



Nanoelectronic applications