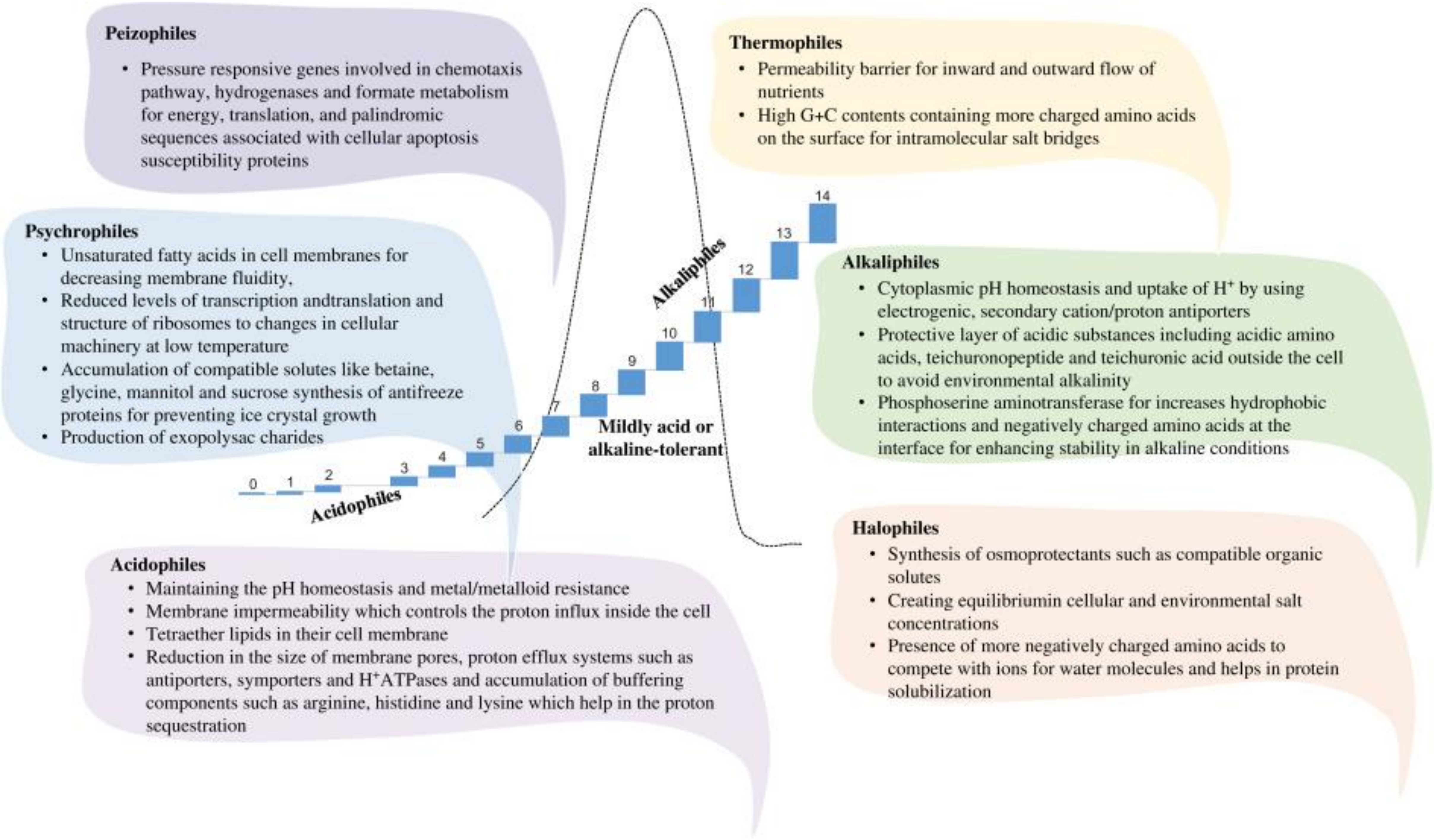
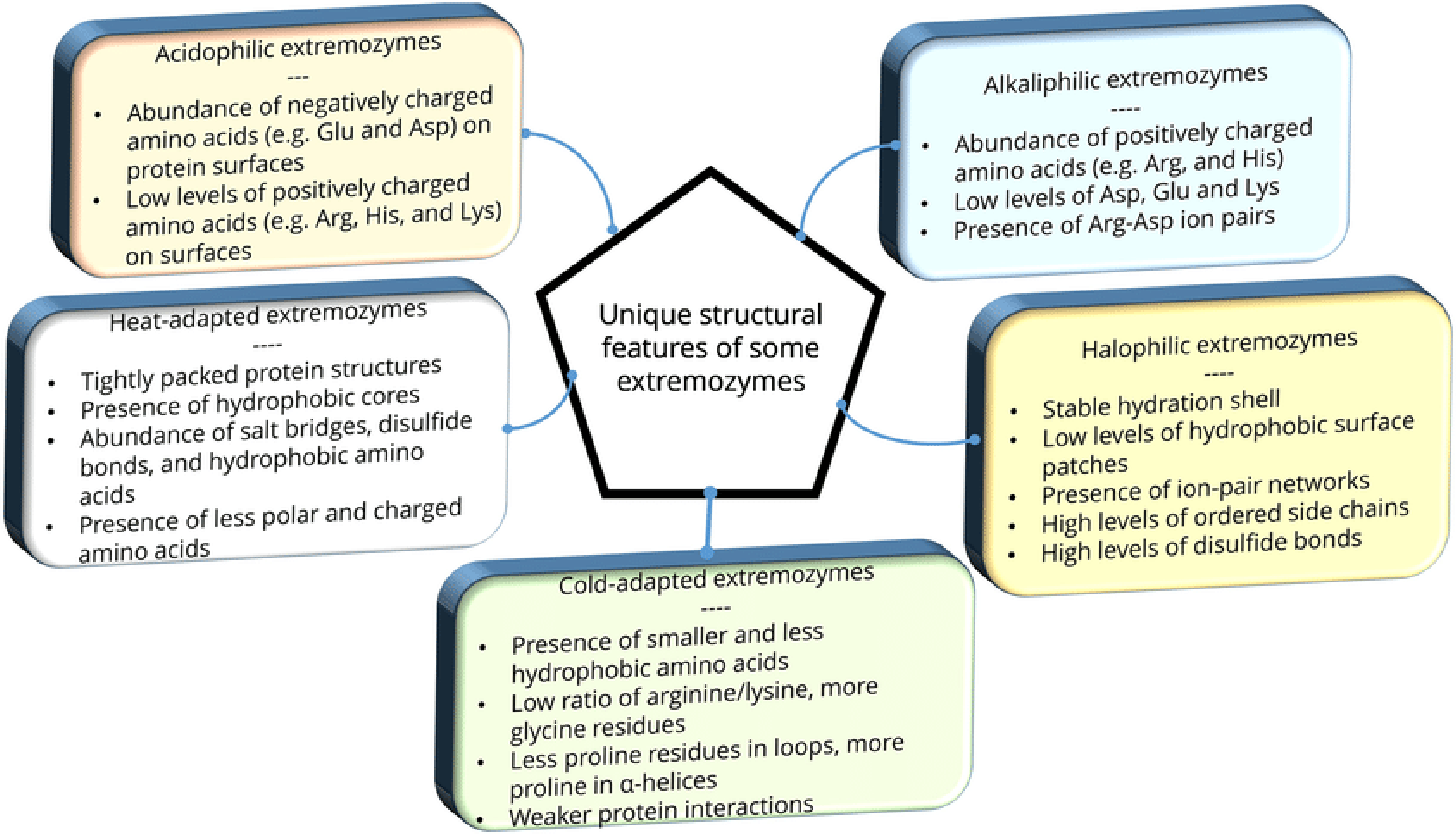
Applications of Extremophiles


Peizophiles
• Pressure responsive genes involved in chemotaxis pathway, hydrogenases and formate metabolism for energy, translation, and palindromic sequences associated with cellular apoptosis susceptibility proteins
Thermophiles
• Permeability barrier for inward and outward flow of nutrients
• High G+C contents containing more charged amino acids on the surface for intramolecular salt bridges
Psychrophiles
• Unsaturated fatty acids in cell membranes for decreasing membrane fluidity,
• Reduced levels of transcription andtranslation and structure of ribosomes to changes in cellular machinery at low temperature
• Accumulation of compatible solutes like betaine, glycine, mannitol and sucrose synthesis of antifreeze proteins for preventing ice crystal growth
• Production of exopolysac charides
Alkaliphiles
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Acidophiles
Mildly acid or alkaline-tolerant
Alkaliphiles
• Cytoplasmic pH homeostasis and uptake of H+ by using electrogenic, secondary cation/proton antiporters
• Protective layer of acidic substances including acidic amino acids, teichuronopeptide and teichuronic acid outside the cell to avoid environmental alkalinity
• Phosphoserine aminotransferase for increases hydrophobic interactions and negatively charged amino acids at the interface for enhancing stability in alkaline conditions
Halophiles
• Synthesis of osmoprotectants such as compatible organic solutes
• Creating equilibriumin cellular and environmental salt concentrations
• Presence of more negatively charged amino acids to compete with ions for water molecules and helps in protein solubilization
Acidophiles
• Maintaining the pH homeostasis and metal/metalloid resistance
• Membrane impermeability which controls the proton influx inside the cell
• Tetraether lipids in their cell membrane
• Reduction in the size of membrane pores, proton efflux systems such as antiporters, symporters and H+ATPases and accumulation of buffering components such as arginine, histidine and lysine which help in the proton sequestration


Unique structural features of some extremozymes
Acidophilic extremozymes

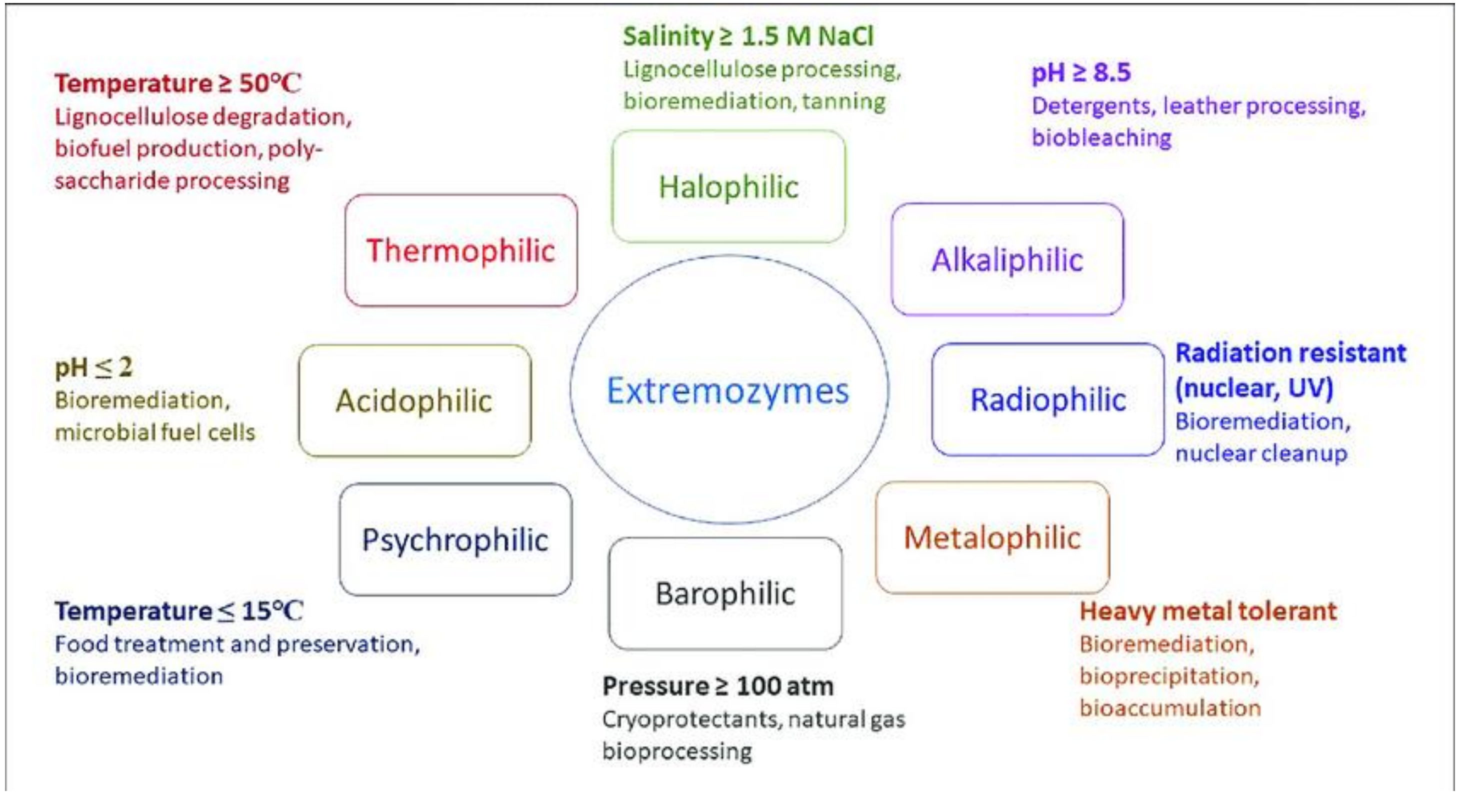
• Abundance of negatively charged amino acids (e.g. Glu and Asp) on protein surfaces
• Low levels of positively charged amino acids (e.g. Arg, His, and Lys) on surfaces
Alkaliphilic extremozymes

• Abundance of positively charged amino acids (e.g. Arg, and His)
• Low levels of Asp, Glu and Lys
• Presence of Arg-Asp ion pairs
Heat-adapted extremozymes

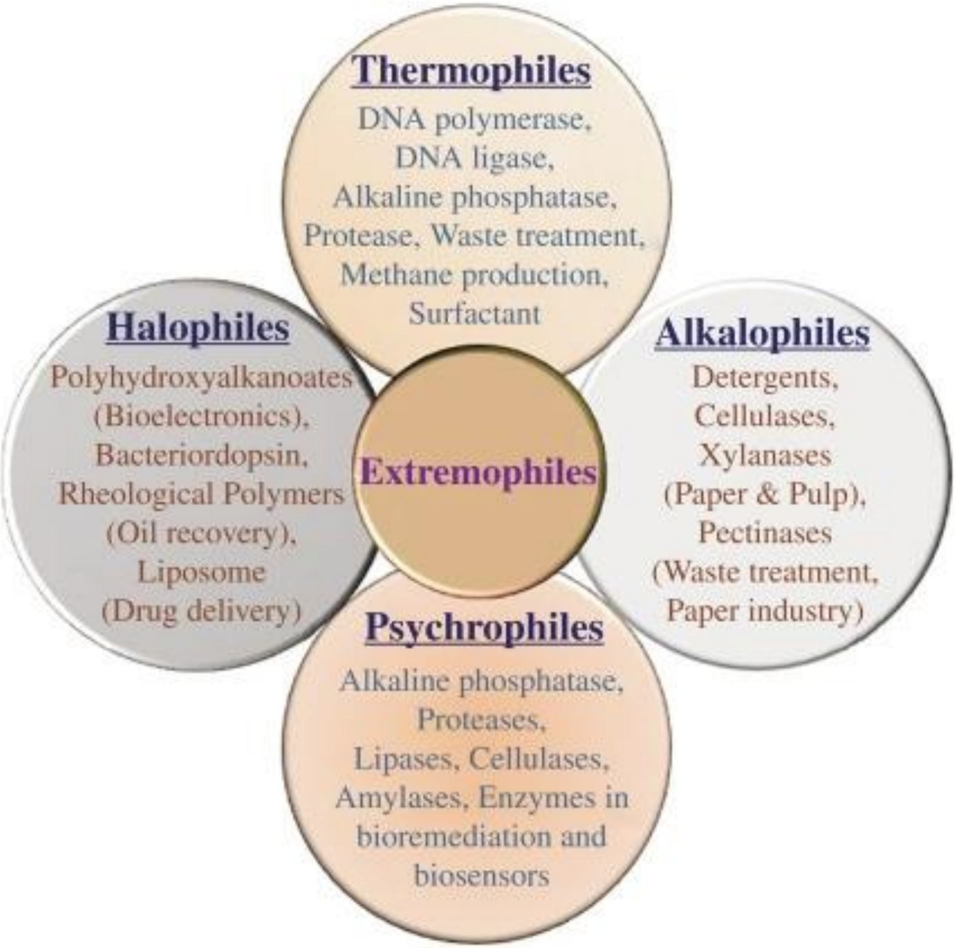
• Tightly packed protein structures
• Presence of hydrophobic cores
• Abundance of salt bridges, disulfide bonds, and hydrophobic amino acids
• Presence of less polar and charged amino acids
Halophilic extremozymes

• Stable hydration shell
• Low levels of hydrophobic surface patches
• Presence of ion-pair networks
• High levels of ordered side chains
• High levels of disulfide bonds
Cold-adapted extremozymes

• Presence of smaller and less hydrophobic amino acids
• Low ratio of arginine/lysine, more glycine residues
• Less proline residues in loops, more proline in α-helices
• Weaker protein interactions



Salinity ≥ 1.5 M NaCl
Lignocellulose processing, bioremediation, tanning
Temperature ≥ 50°C
Lignocellulose degradation, biofuel production, poly-saccharide processing
pH ≥ 8.5
Detergents, leather processing, biobleaching
Halophilic
Thermophilic
Alkaliphilic
pH ≤ 2
Bioremediation, microbial fuel cells
Acidophilic
Extremozymes
Radiophilic
Radiation resistant (nuclear, UV)
Bioremediation, nuclear cleanup
Psychrophilic
Metalophilic
Barophilic
Temperature ≤ 15°C
Food treatment and preservation, bioremediation
Heavy metal tolerant
Bioremediation, bioprecipitation, bioaccumulation
Pressure ≥ 100 atm
Cryoprotectants, natural gas bioprocessing



Thermophiles
DNA polymerase, DNA ligase, Alkaline phosphatase, Protease, Waste treatment, Methane production, Surfactant
Halophiles
Polyhydroxyalkanoates (Bioelectronics), Bacteriordopsin, Rheological Polymers (Oil recovery), Liposome (Drug delivery)
Extremophiles
Alkalophiles
Detergents, Cellulases, Xylanases (Paper & Pulp), Pectinases (Waste treatment, Paper industry)
Psychrophiles
Alkaline phosphatase, Proteases, Lipases, Cellulases, Amylases, Enzymes in bioremediation and biosensors

Extremophiles secrete a wide range of extracellular hydrolytic enzymes which are useful to human kind. These enzymes primarily include proteases, lipase, amylases, cellulases, chitinase and xylanases.

The pH, salinity and thermal stability of the enzymes make them suitable for an array of industrial processes.

HARVESTING EXTREMOZYMES

One approach – as long as can be cultured – gene obtained, cloning into domesticated hosts – pure enzymes isolated – tested

Second approach – By pass the need to grow

Isolate DNA – rDNA – deliver random stretches into host – one insert/cell – without knowing the function of gene – DNA libraries – screen for colonies – look for evidence of activity –

Metagenomics -

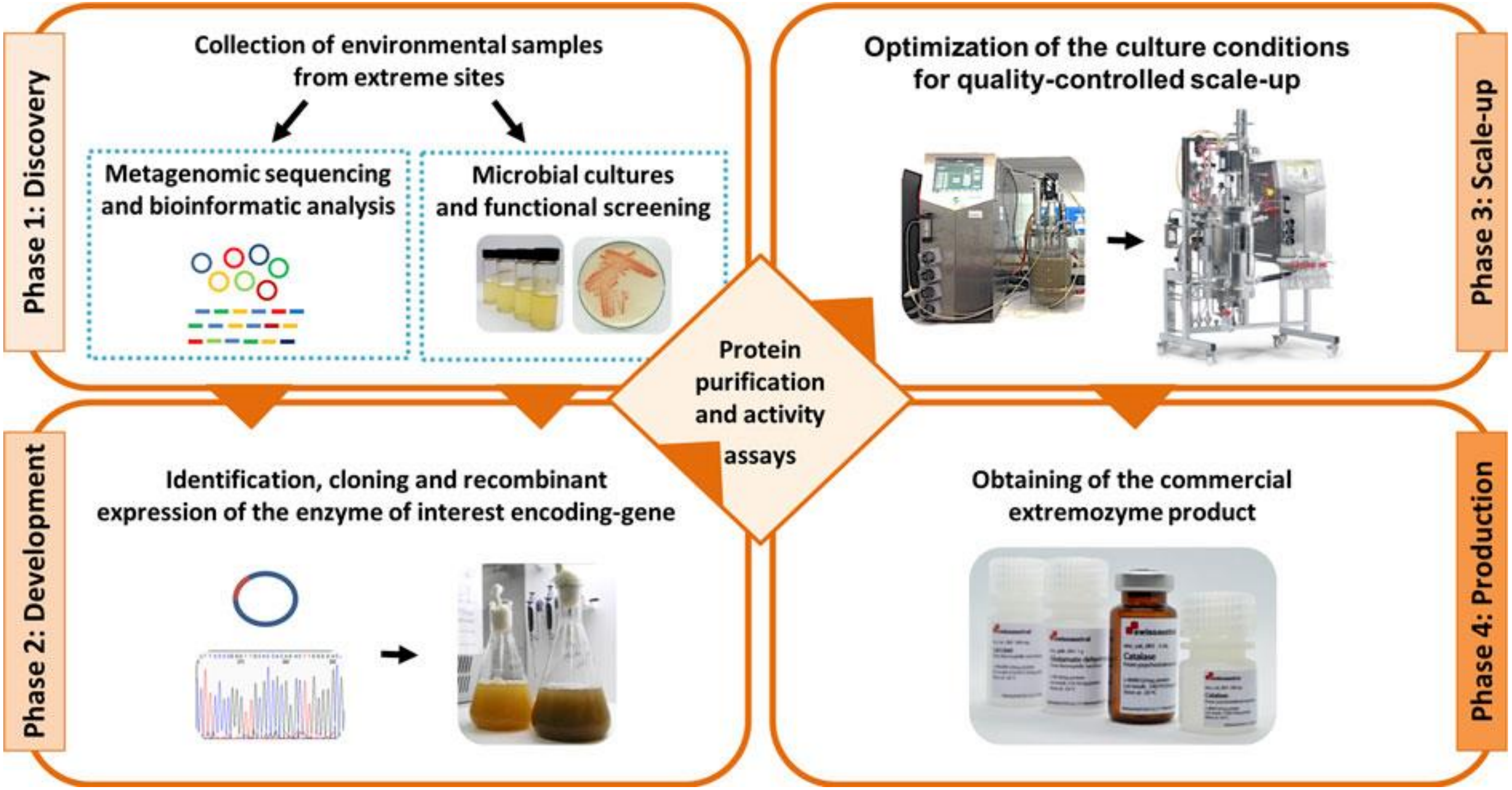
Industrial enzyme technology – began to modify extremozymes – if degrades protein at high temp- alter the enzyme to work at acidic or saline conditions

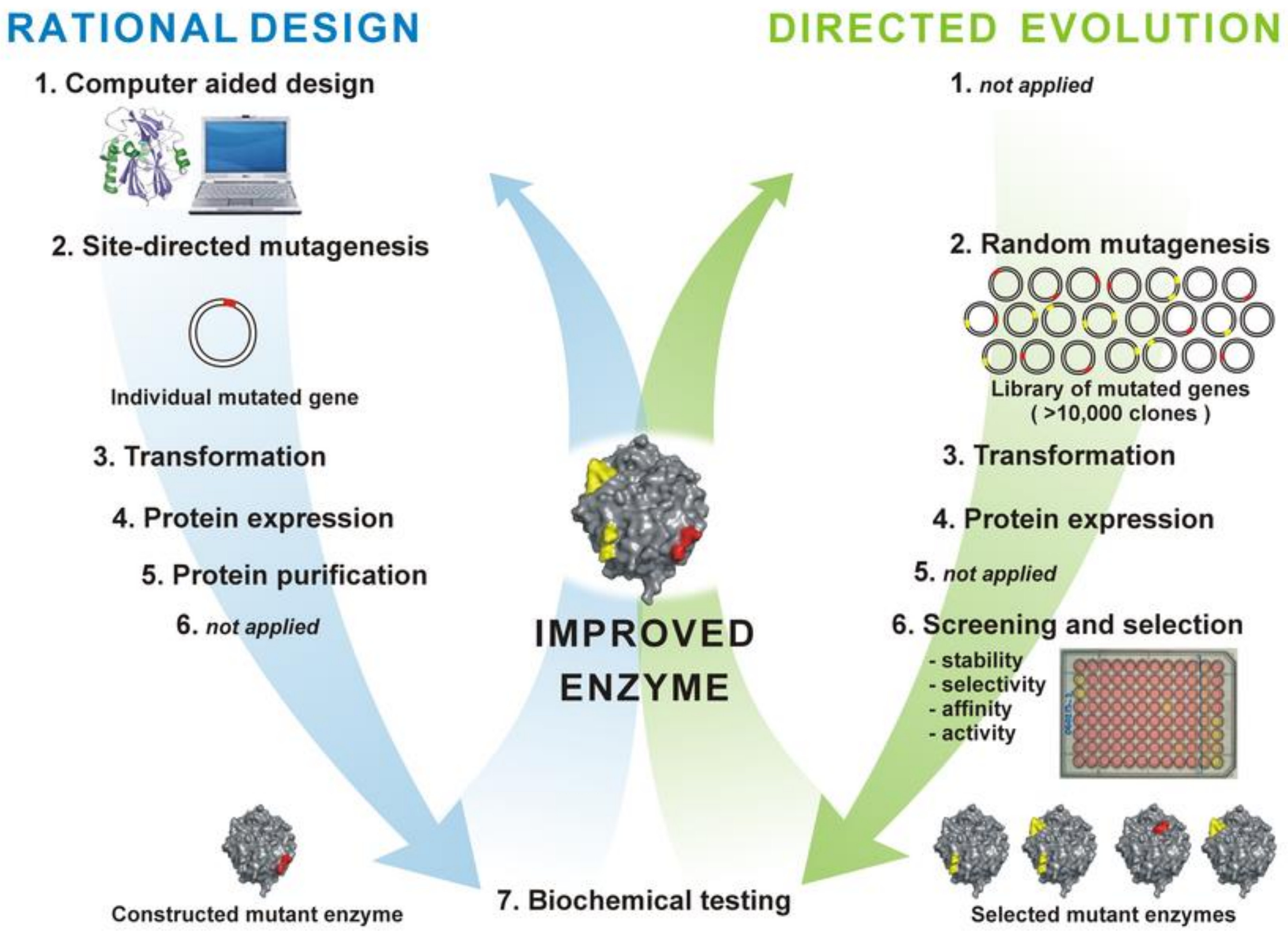
Two approaches – rational design and directed evolution

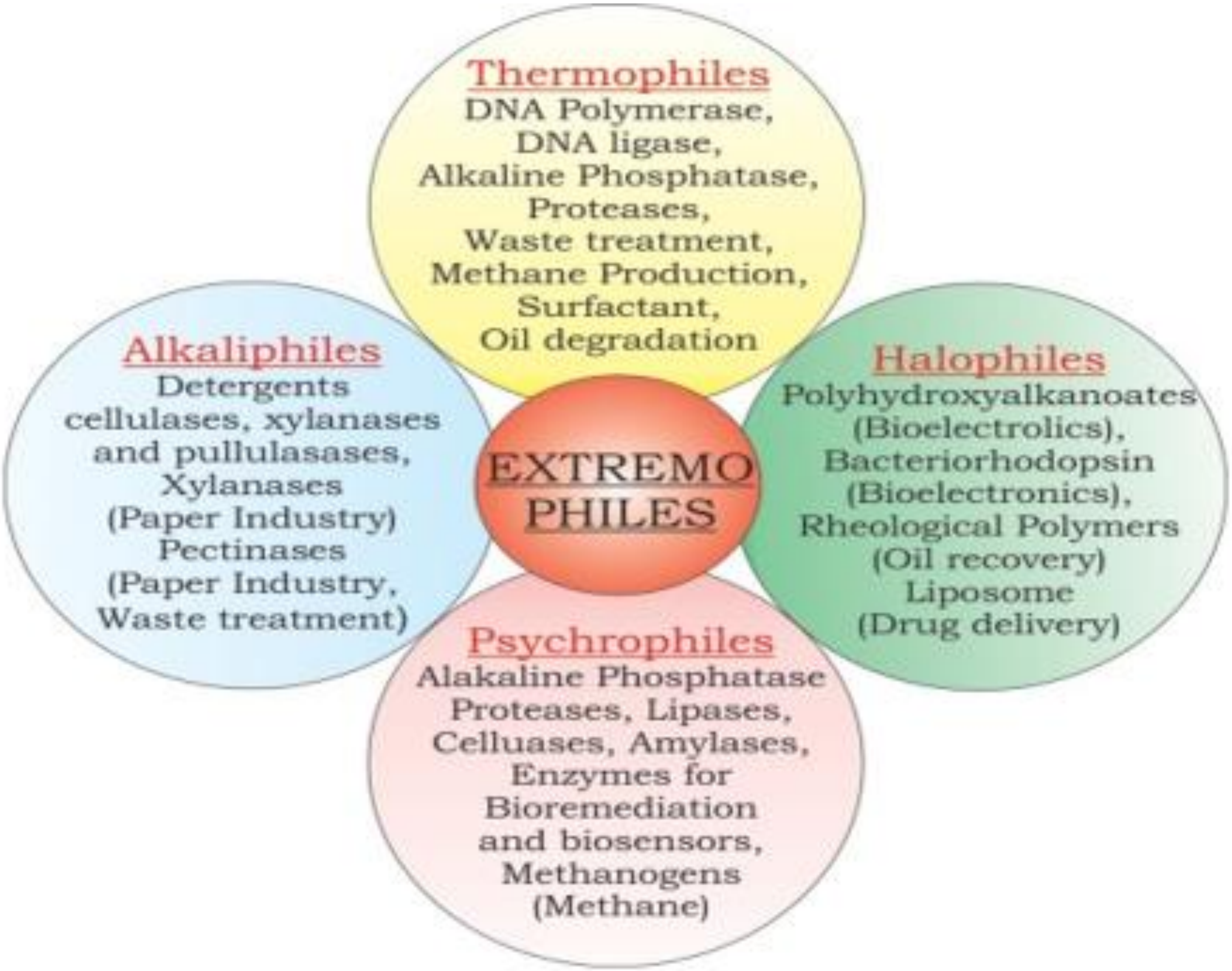
Extremophile-derived enzymes, or extremozymes, are able to catalyze chemical reactions under harsh conditions, like those found in industrial processes, which were previously not thought to be conducive for enzymatic activity.
Extremozymes also include cellulases, proteases, pectinases, keratinases, lipases, esterases, catalases, peroxidases, and phytases. Owing to the unusual properties of these classes of enzymes, they are expected to fill the gap between biological and chemical industrial processes.

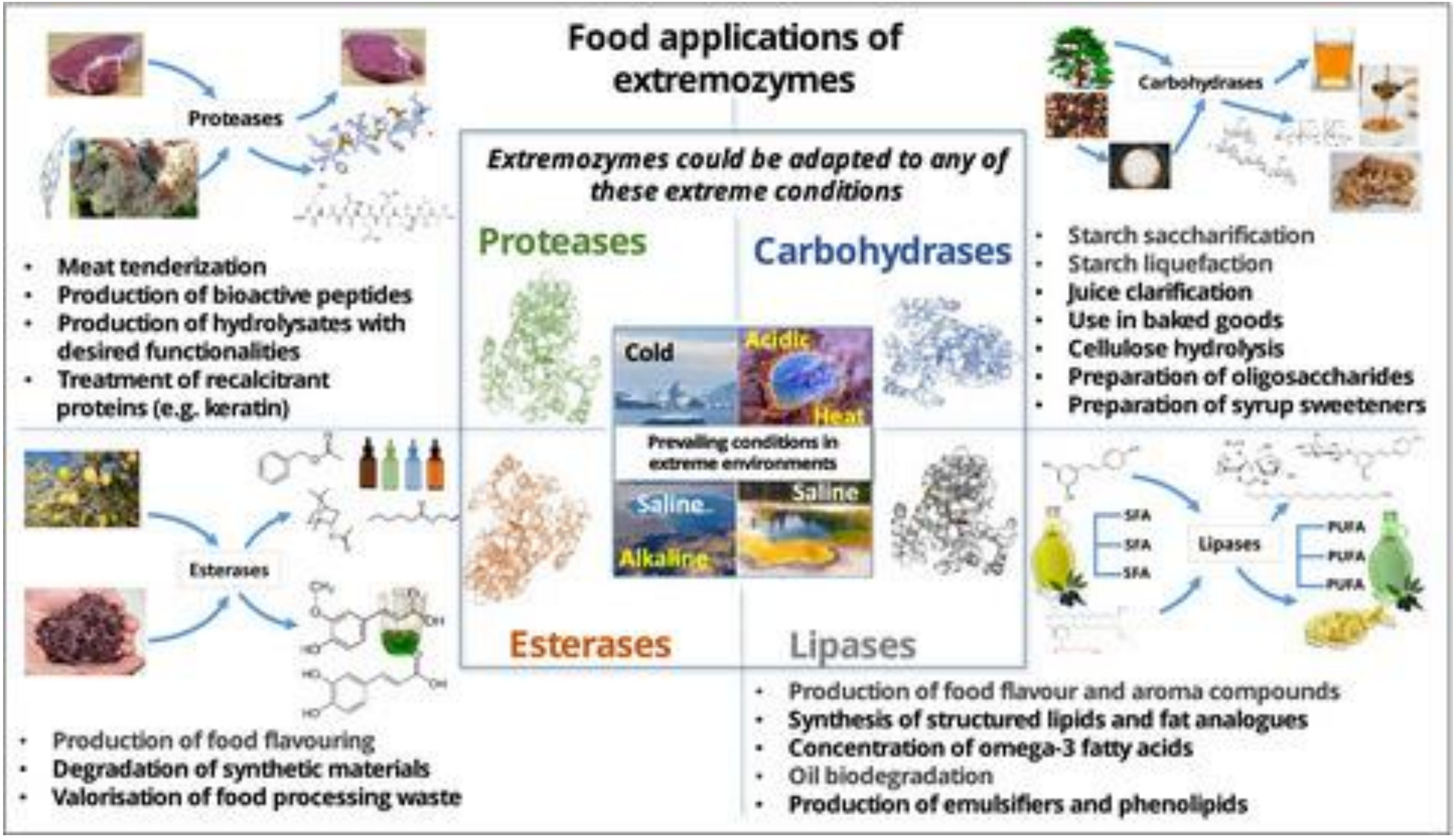
Why Extremozymes?

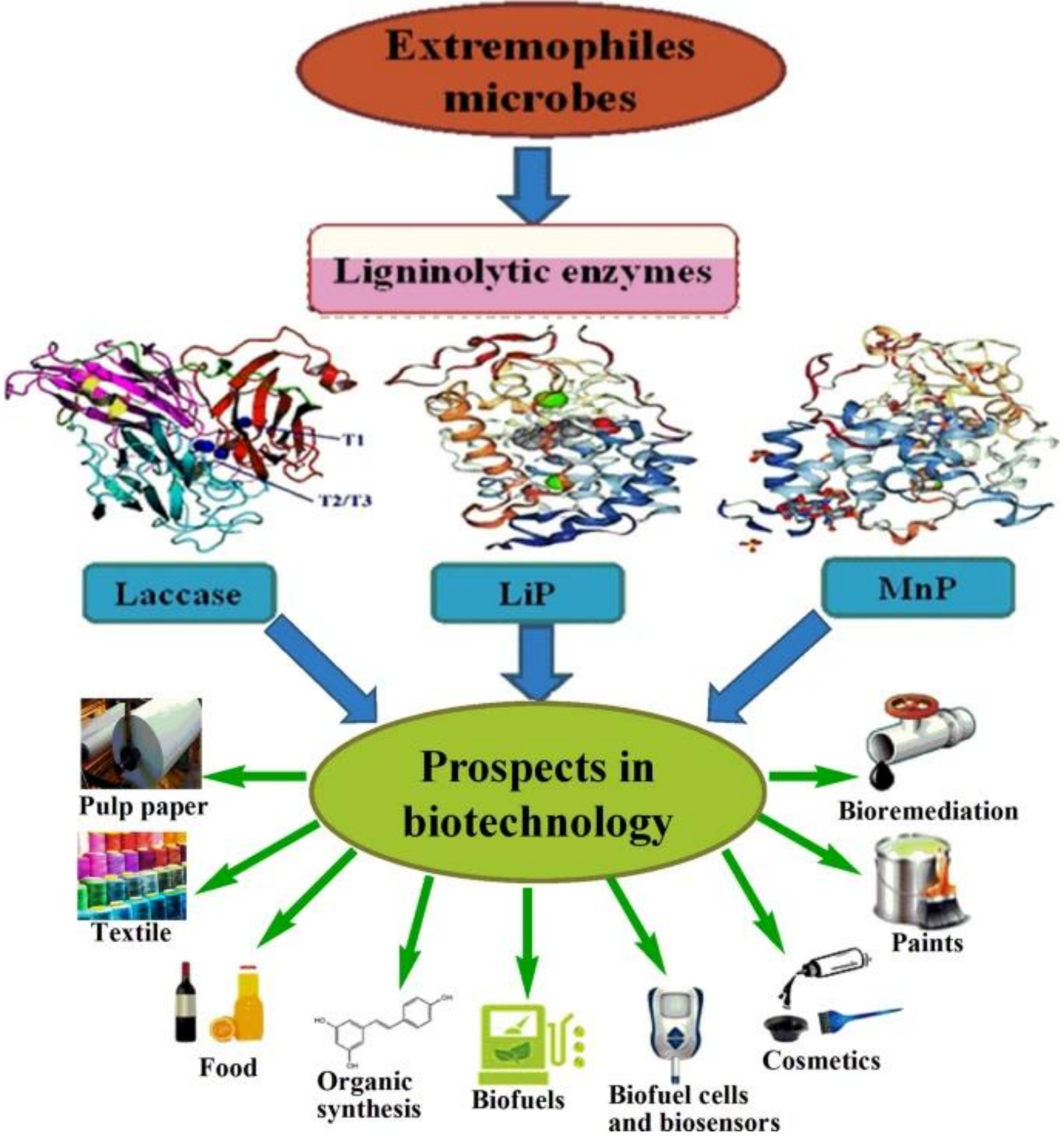
- What are extremozymes?
  - Enzymes isolated from organisms inhabiting unconventional ecosystems (Biotechnology (N Y). 1995 Jul;13(7):662-8)
- Extremozymes expand the limits of biocatalysis
  - The information acquired from the study of extremozymes makes it possible to modify enzymes to improve their ranges of stability and activity (for industrial and medical applications)
- However, the organisms living in extreme environments are hard to be cultured, so they are less well studied as compared to other organisms.
  - Vast majority of microbes uncultured –>99% of soil organisms; >50% in human gut; >99.9% in seawater and thus cannot understand the community as a whole.
- Metagenomics enables sequencing of an entire microbial community without the need to culture them


Phase 1: Discovery
Collection of environmental samples from extreme sites
Metagenomic sequencing and bioinformatic analysis
Microbial cultures and functional screening
Protein purification and activity assays
Phase 3: Scale-up
Optimization of the culture conditions for quality-controlled scale-up
Phase 2: Development
Identification, cloning and recombinant expression of the enzyme of interest encoding-gene
Phase 4: Production
Obtaining of the commercial extremozyme product
Catalase


RATIONAL DESIGN
DIRECTED EVOLUTION
1. Computer aided design
2. Site-directed mutagenesis
Individual mutated gene
3. Transformation
4. Protein expression
5. Protein purification
6. not applied
1. not applied
2. Random mutagenesis
Library of mutated genes
(>10,000 clones)
3. Transformation
4. Protein expression
5. not applied
6. Screening and selection
- stability
- selectivity
- affinity
- activity
IMPROVED
ENZYME
Constructed mutant enzyme
7. Biochemical testing
Selected mutant enzymes


Thermophiles
DNA Polymerase,
DNA ligase,
Alkaline Phosphatase,
Proteases,
Waste treatment,
Methane Production,
Surfactant,
Oil degradation
Alkaliphiles
Detergents
cellulases, xylanases
and pullulasases,
Xylanases
(Paper Industry)
Pectinases
(Paper Industry,
Waste treatment)
EXTREMO
PHILES
Halophiles
Polyhydroxyalkanoates
(Bioelectrolics),
Bacteriorhodopsin
(Bioelectronics),
Rheological Polymers
(Oil recovery)
Liposome
(Drug delivery)
Psychrophiles
Alakaline Phosphatase
Proteases, Lipases,
Celluases, Amylases,
Enzymes for
Bioremediation
and biosensors,
Methanogens
(Methane)


Food applications of extremozymes
Proteases
Carbohydrases
Extremozymes could be adapted to any of these extreme conditions
Proteases
Carbohydrases
Cold
Acidic
Heat
Prevailing conditions in extreme environments
Saline
Alkaline
Saline
Esterases
Lipases
Meat tenderization
Production of bioactive peptides
Production of hydrolysates with desired functionalities
Treatment of recalcitrant proteins (e.g. keratin)
Starch saccharification
Starch liquefaction
Juice clarification
Use in baked goods
Cellulose hydrolysis
Preparation of oligosaccharides
Preparation of syrup sweeteners
Esterases
SFA
SFA
SFA
Lipases
PUFA
PUFA
PUFA
Production of food flavouring
Degradation of synthetic materials
Valorisation of food processing waste
Production of food flavour and aroma compounds
Synthesis of structured lipids and fat analogues
Concentration of omega-3 fatty acids
Oil biodegradation
Production of emulsifiers and phenolipids



Adaptive mechanisms of extremophilic ligninolytic enzymes such as laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP).

The extremophilic ligninolytic enzyme adaptive features such as
different compositions of amino acids,
hydrophobic interaction,
surface charges,
tighter packing (compactness),
a deleted loop,
saturated/unsaturated fatty acid,
salt bridge, disulfide bridge, hydrogen bond,
several ions, and α-helical, as well as,
cysteinyl-tRNA synthetase enzyme to maintain their active stability for catalytic functionalities in extreme conditions.

USES OF EXTREMOPHILES

HYPERTHERMOPHILES (SOURCE)	USES
DNA polymerases	DNA amplification by PCR
Alkaline phosphatase	Diagnostics
Proteases and lipases	Dairy products
Lipases, pullulanases and proteases	Detergents
Proteases	Baking and brewing and amino acid production from keratin
Amylases, α-glucosidase, pullulanase and xylose/glucose isomerases	Baking and brewing and amino acid production from keratin
Alcohol dehydrogenase	Chemical synthesis
Xylanases	Paper bleaching
Lenthionin	Pharmaceutical
S-layer proteins and lipids	Molecular sieves
Oil degrading microorganisms	Surfactants for oil recovery
Sulfur oxidizing microorganisms	Bioleaching, coal & waste gas desulfurization
Hyperthermophilic consortia	Waste treatment and methane production

USES OF EXTREMOPHILES

PSYCHROPHILES (SOURCE)	USES
Alkaline phosphatase	Molecular biology
Proteases, lipases, cellulases and amylases	Detergents
Lipases and proteases	Cheese manufacture and dairy production
Proteases	Contact-lens cleaning solutions, meat tenderizing
Polyunsaturated fatty acids	Food additives, dietary supplements
Various enzymes	Modifying flavors
b-galactosidase	Lactose hydrolysis in milk products
Ice nucleating proteins	Artificial snow, ice cream, other freezing applications in the food industry
Ice minus microorganisms	Frost protectants for sensitive plants
Various enzymes (*e.g.* dehydrogenases)	Biotransformations
Various enzymes (*e.g.* oxidases)	Bioremediation, environmental biosensors
Methanogens	Methane production

USES OF EXTREMOPHILES

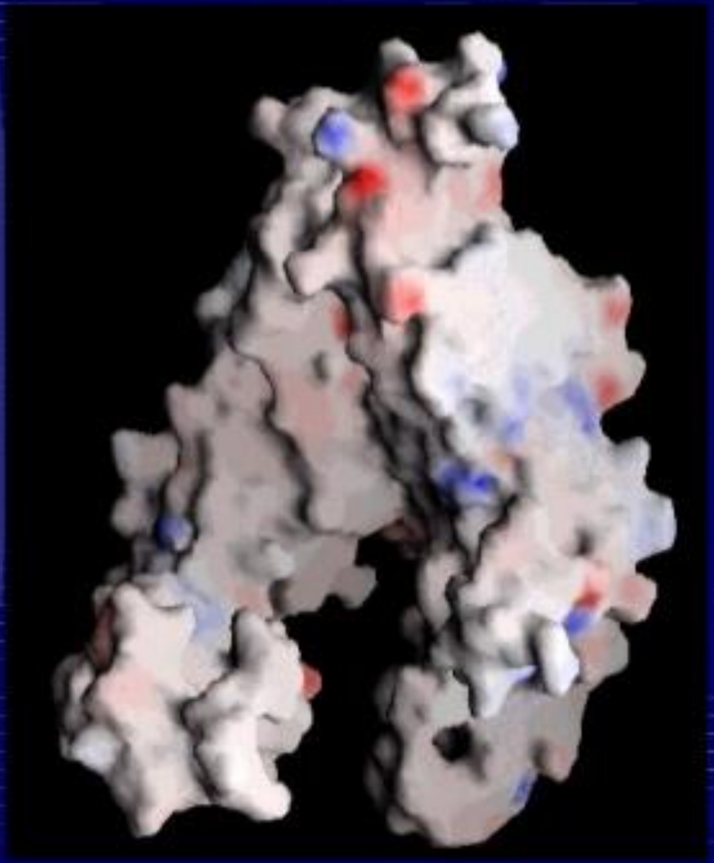
ALKALIPHILES (SOURCE)	USES
Proteases, cellulases, xylanases, lipases and pullulanases	Detergents
Proteases	Gelatin removal on X-ray film
Elastases, keritinases	Hide dehairing
Cyclodextrins	Foodstuffs, chemicals and pharmaceuticals
Xylanases and proteases	Pulp bleaching
Pectinases	Fine papers, waste treatment and degumming
Alkaliphilic halophiles	Oil recovery
Various microorganisms	Antibiotics

ACIDOPHILES (SOURCE)	USES
Sulfur oxidizing microorganisms	Recovery of metals and desulfurication of coal
Microorganisms	Organic acids and solvents

USES OF EXTREMOPHILES

HALOPHILES (SOURCE)	USES
Bacteriorhodopsin	Optical switches and photocurrent generators in bioelectronics
Polyhydroxyalkanoates	Medical plastics
Rheological polymers	Oil recovery
Eukaryotic homologues (*e.g. myc* oncogene product)	Cancer detection, screening anti-tumor drugs
Lipids	Liposomes for drug delivery and cosmetic packaging
Lipids	Heating oil
Compatible solutes	Protein and cell protectants in variety of industrial uses, *e.g.* freezing, heating
Various enzymes, *e.g.* nucleases, amylases, proteases	Various industrial uses, *e.g.* flavoring agents
g-linoleic acid, b-carotene and cell extracts, *e.g. Spirulina* and *Dunaliella*	Health foods, dietary supplements, food coloring and feedstock
Microorganisms	Fermenting fish sauces and modifying food textures and flavors
Microorganisms	Waste transformation and degradation, *e.g.* hypersaline waste brines contaminated with a wide range of organics
Membranes	Surfactants for pharmaceuticals

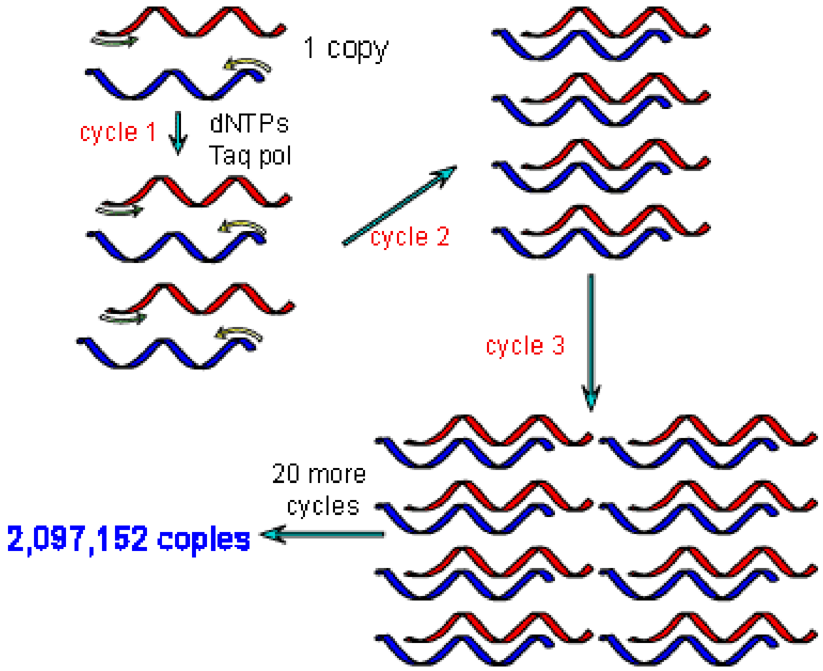
Taq Polymerase



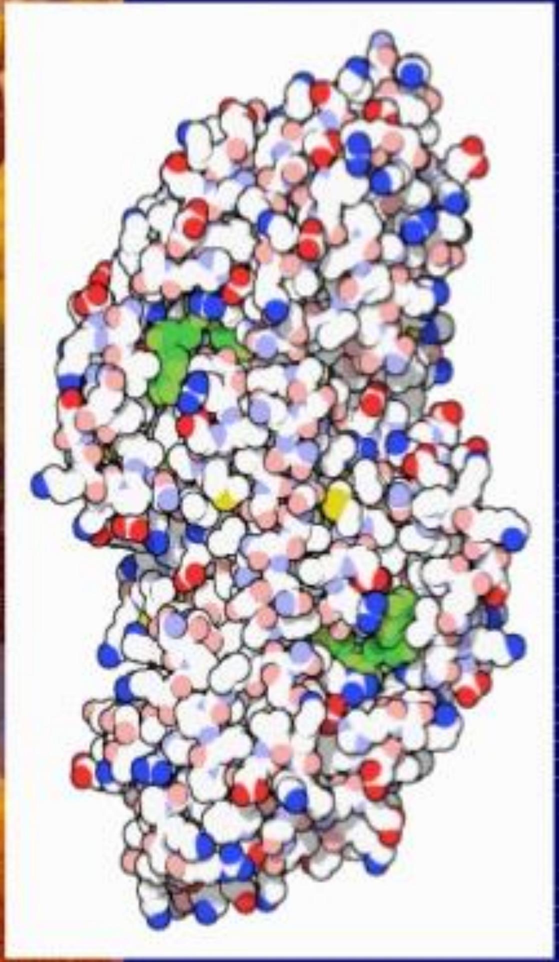
- Isolated from the hyperthermophile *Thermus aquaticus*
- Much more heat stable
- Used as the DNA polymerase in the very useful Polymerase Chain Reaction (PCR) technique which amplifies DNA samples

PCR

- In 1985, **Kary Mullis** invented the process known as **polymerase chain reaction** (**PCR**), in which a small amount of DNA can be copied in large quantities over a short period of time.
- By applying heat, the DNA molecule's two strands are separated and the DNA building blocks that have been added are bonded to each strand.



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

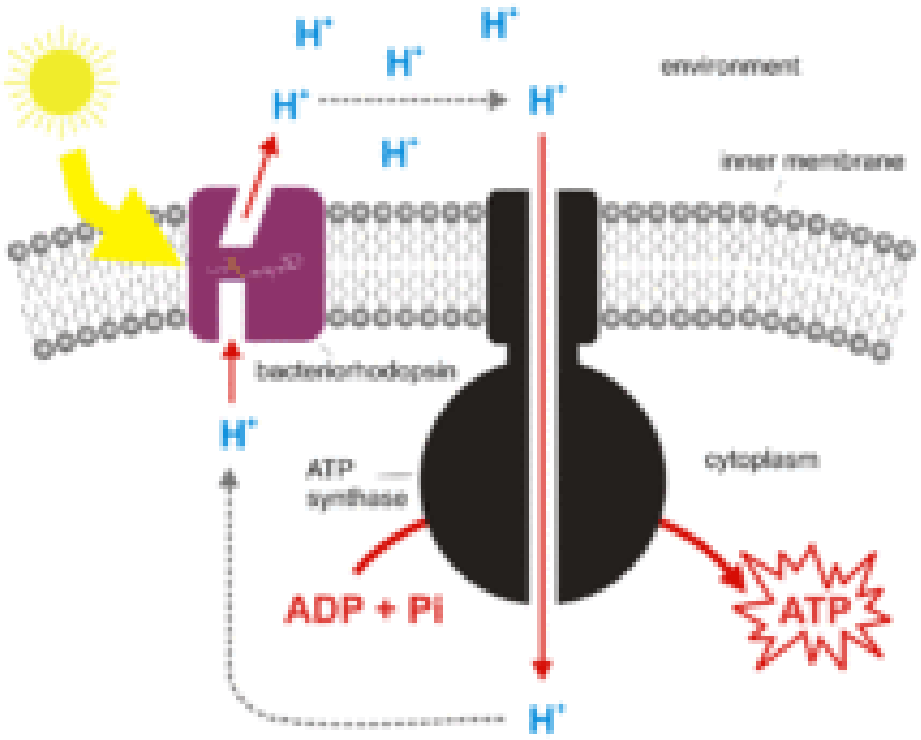
Bacteriorhodopsin

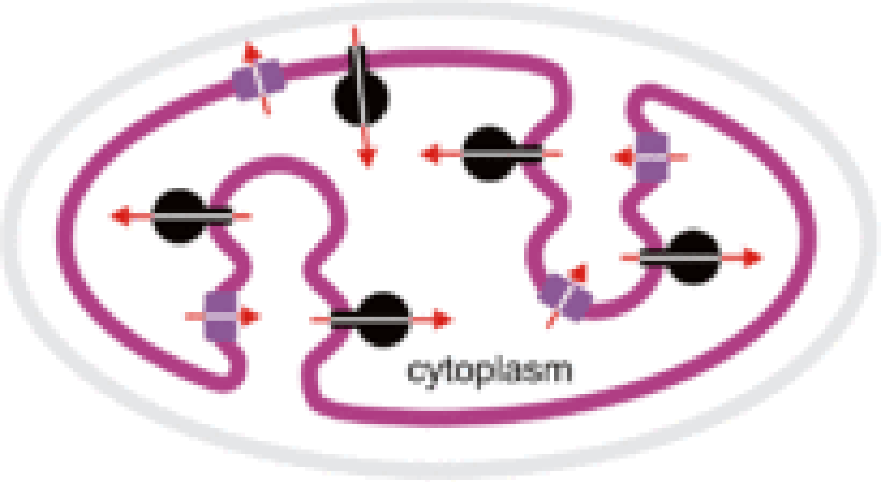
-Bacteriorhodopsin is a trans-membrane protein found in the cellular membrane of *Halobacterium salinarium*, which functions as a light-driven proton pump

-Can be used for electrical generation

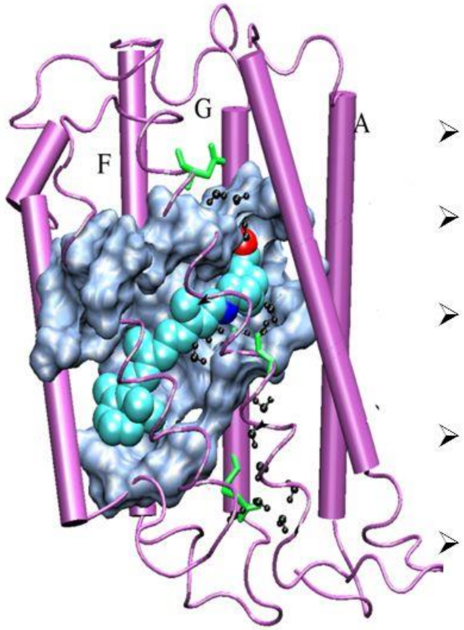
Bacteriorhodopsin

- **Bacteriorhodopsin** is a protein used by Archaea, the most notable one being *Halobacteria*. It acts as a proton pump; that is, it captures light energy and uses it to move protons across the membrane out of the cell. The resulting proton gradient is subsequently converted into chemical energy.
- The retinal protein **bacteriorhodopsin** is the major photosynthetic protein of the archaeon *Halobacterium salinarum*. It converts the energy of "green" light (500-650 nm, max 568 nm) into an electrochemical proton gradient, which in turn is used for **ATP production** by **ATP** synthase.
- The **bacteriorhodopsin** molecule is **purple** and is most efficient at absorbing green light (wavelength 500-650 nm, with the absorption maximum at 568 nm). **Bacteriorhodopsin** belongs to a family of bacterial proteins related to vertebrate rhodopsins, the pigments that sense light in the retina.





Bacteriorhodopsin -- bR



- The simplest ion pump in biology
- The simplest photosynthetic center
- The best characterized membrane protein
- Technological applications in molecular electronics
- The first membrane protein with a known atomic-detail 3D structures

Bacteriorhodopsin (BR), a seven α-helical protein taking in a chromophore molecule (Retinal), is a light-dependent proton pump. ... The incorporation of BR into electronic circuitry; leads to **applications** such as artificial retina, photochromic data storage, holographic memories, light batteries and information processing.

Bacteriorhodopsin properties and engineering applications

- **Proton pump function** (draws photons from inside the cellular membrane to the outside)

  → Changes light to an electrical current
  Characteristics: ultra-fast directional responses (< 5 picoseconds), differential responses
  Applications: artificial retinas, direction sensors, robot vision

- **Photochromism** (changes color when exposed to light, reverts back to the original color when light is extinguished)

  → Stores light intensity and complex amplitudes
  Characteristics: variable storage retention times (humidity levels, pH, electric fields, gene recombinations, etc.)
  High-density storage with a size on the order of molecules (> 5000 lpm), high speed (low temperature), rewritable
  Applications: real-time holography

- **Second-order and third-order nonlinearlity** (response is proportional to the second and third power of the input)

  Characteristics: rhodopsin (retinal) is a colorant, so no fluorescent pigments are needed (remains living)
  Applications: nonlinear spectrum (Raman scattering), nonlinear nanoimaging (Raman, SHG)
  Phase conjugate wave generation → quantum optics (strong correlation)

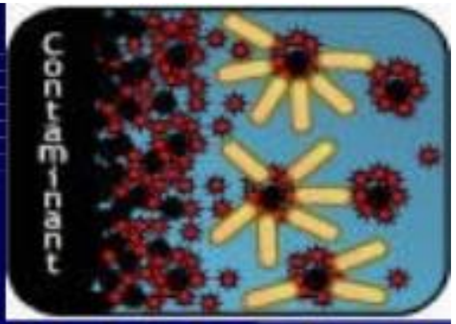
- **Photoinduced double refraction** (phase velocity varies according to direction)

  → Stores polarized light
  Applications: switching elements using polarized light information, security holograms

Holography is a technique that enables a wavefront to be recorded and later re-constructed. Holography is best known as a method of generating real three-dimensional images, but it also has a wide range of other applications. In principle, it is possible to make a hologram for any type of wave.

Applications of holography include information storage, recording of images in depth, the use of holograms as optical elements, and as a means of performing precise interferometric measurements on three-dimensional objects of any shape and surface finish

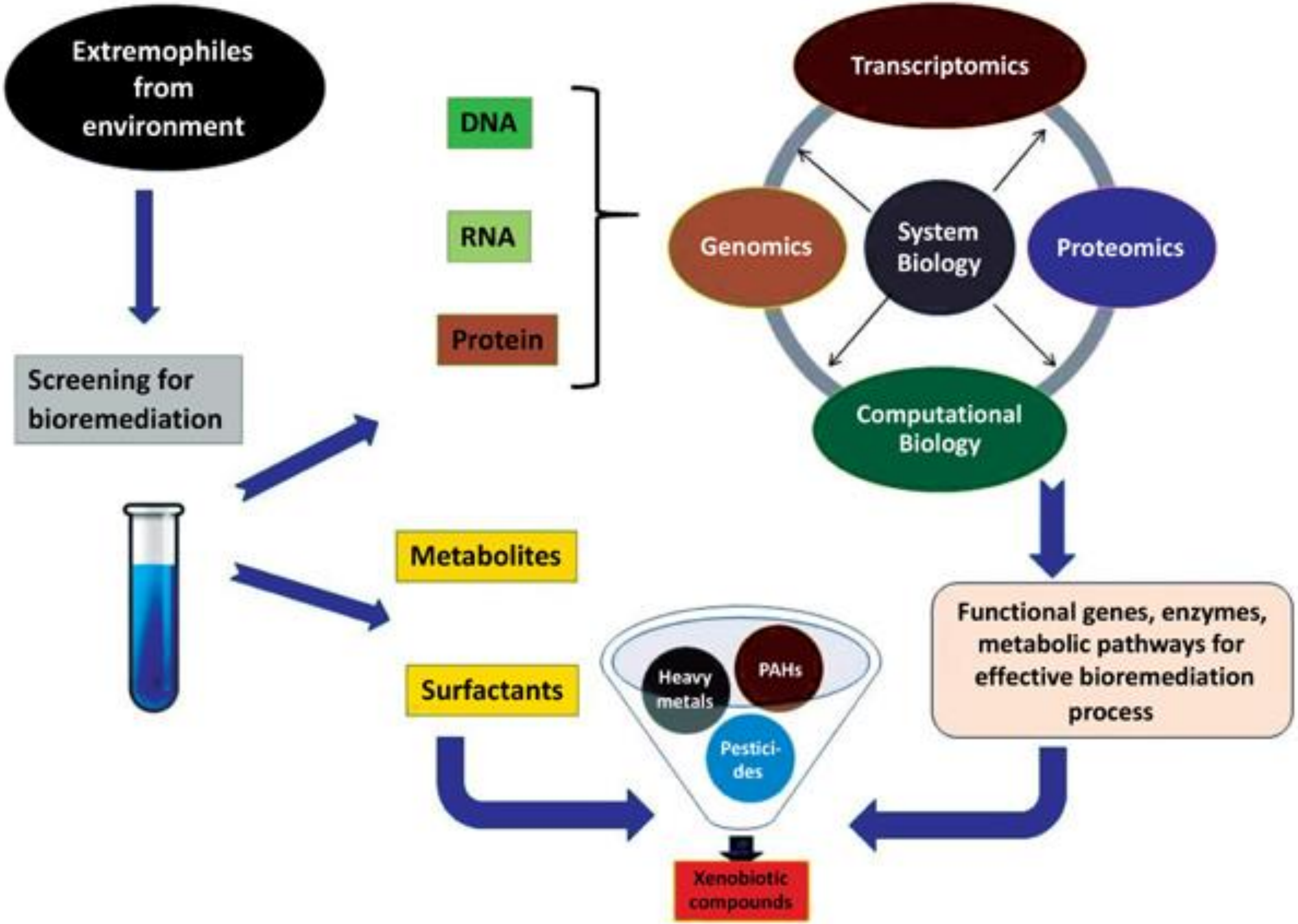
Bioremediation



- Bioremediation is the branch of biotechnology that uses biological processes to overcome environmental problems

- Bioremediation is often used to degrade xenobiotics introduced into the environment through human error or negligence

- Part of the cleanup effort after the 1989 Exxon Valdez oil spill included microorganisms induced to grow via nitrogen enrichment of the contaminated soil



Extremophiles from environment
Screening for bioremediation
DNA
RNA
Protein
Transcriptomics
Genomics
System Biology
Proteomics
Computational Biology
Metabolites
Surfactants
Heavy metals
PAHs
Pestici-des
Xenobiotic compounds
Functional genes, enzymes, metabolic pathways for effective bioremediation process

Psychrophiles as Bioremediators

- Bioremediation applications with cold-adapted enzymes are being considered for the degradation of diesel oil and polychlorinated biphenyls (PCBs)

- Health effects that have been associated with exposure to PCBs include acne-like skin conditions in adults and neurobehavioral and immunological changes in children. PCBs are known to cause cancer in animals

An End to Pollution?

New and innovative methods are being developed that utilize extremophiles for the elimination of pollution resulting from oil slicks, toxic chemical spills, derelict mines, etc

Applications

- Extremophilic enzymes have been model systems to study enzyme evolution, enzyme stability, activity, mechanism, protein structure, function, and biocatalyst under extreme conditions.
- Thermophiles have yielded stable α-amylase for starch hydrolysis, oxylonases for paper bleaching, and proteases for brewing and for detergent purposes.
- Alkaline active proteases, amylases, cellulases, mannanases, lipases, etc. are used in the formulation of heavy-duty laundry and dishwashing detergents as they are efficient in removing stains and allow effective low-temperature (30–40°C) washing.
- Some species of acidophilic microorganisms can be used not only to reduce mine water pollution but also to recover metals from acidic wastewater via selective biomineralization.

Applications

•Extroenzymes like Taq polymerase from *Thermus aquaticus* is an ideal for use in a polymerase chain reaction as it reduces the need for adding extra polymerase during the reaction.

•Cellulose for various extremophilic organisms has been used for the treatment of juices, color brightening in detergents, and treating cellulose-containing biomass and crops to improve their digestibility and nutritional quality.

•Similarly, halophiles are being exploited as a potential source of carotene, compatible solutes, glycerols, and surfactants for pharmaceutical use.

- Some extremophilic microorganisms may also comprise a large reservoir of novel therapeutic agents—for example, iron-binding antifungal compound, pyochelin isolated from halophilic species of *Pseudomonas*.
- A thermostable glucokinase from the thermophilic species, *Bacillus stereothermphiolus*, can be used as a glucose sensor for quick glucose assay.

Applications

- Alkaline active enzymes have got several notable applications in textile and fiber processing in processes like cotton scoring and blast fiber degumming.
- Alkaliphiles and their enzymes have been tried in various synthesis reactions with peptide synthesis being the most important one.
- Information on the microbial composition and biogeochemical cycling of extreme ecosystems also helps in understanding the global change, threats, and opportunities for living beings.
- Enzymes from extremophiles can also be used in bioremediation processes like toxifying wastewater and air and removing metallic waste from sewages and industries.
- Different barophilic enzymes are used for the production and sterilization of items at varied pressure conditions.