**Topic 2**

The primordial soup

**WS 2016**

The molecular origins of life

Zibi Pianowski

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**When life originated on Earth?**

**Hadean Eon** (4600 Ma – 4000 Ma)

- 4600 Ma – Earth formation
- 4500 Ma – Theia collides Earth → Moon
  *Earth’s axis of rotation stabilized, which allowed abiogenesis*
- 4460 Ma – oldest known lunar rock - Lunar sample 67215, Apollo 15
- 4404 Ma – oldest known material of terrestrial origin – zircon mineral (Australia) – isotopic composition of oxygen suggests presence of water on the Earth’s surface
- 4374 Ma – the oldest consistently dated zircon

**Archean Eon** (4000 Ma – 2500 Ma)

- 4031 Ma – formation of the Acastia Gneiss - the oldest known intact crustal fragment on Earth
- 4100 Ma – „remains of biotic life” found in zirconites (Australia)
- 3900 Ma – 2500 Ma – cells remaining prokaryotes appear
  *first chemoautotrophes: oxidize inorganic material to get energy, CO₂ – carbon source*
- 3700 Ma – oldest evidences for life – biogenic graphite in Isua greenstone belt (Greenland)
- c.a. 3500 Ma – lifetime of the Last Universal Common Ancestor (LUCA)
  *split between bacteria and archaea*
- 3480 Ma – oldest fossils – microbial mat (bacteria and archaea) fossils – sandstone, Australia
- 3000 Ma – photosynthesizing cyanobacteria evolved – water used as reducing agent → production of oxygen → oxidation of iron into iron ore (FeOₓ) (banded iron)
- 2500 Ma - free oxygen in atmosphere → Great Oxygenation Event („Oxygen catastrophe“) → extinction of most anaerobic organisms

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*If life arose relatively quickly on Earth ... then it could be common in the universe.*
The origin of life on Earth

• 384-322 BC – Aristotle – *abiogenesis*: spontaneous generation of life forms from unanimated matter (flies from old meat, mice from dirty hay)

• 1665 AC – Robert Hooke (microscope) – discovery of bacteria – considered a proof for spontaneous generation (bacteria division was not observed by then)

• 1668 – Francisco Redi – *biogenesis*: every life comes from another life

• 1861 – Louis Pasteur – bacteria do not grow in sterilized nutrient-rich medium, unless inoculated from outside; abiogenesis under current conditions regarded as impossible and therefore disproven

*Panspermia* – idea that life came to Earth from elsewhere in the Universe (e.g. Extremophilic organisms hibernated and traveling inside meteorites) – Anaxagoras (400ts BC), Berzelius, Kelvin, von Helmholtz, Arrhenius...; *Pseudo-panspermia* – biorelevant molecules delivered from outside of Earth (meteorites)

The origin of biorelevant molecules on Earth

„atmospheric oxygen prevents the synthesis of certain organic compounds that are necessary building blocks for the evolution of life“

1. The early Earth had a chemically reducing atmosphere.
2. This atmosphere, exposed to energy in various forms, produced simple organic compounds ("monomers").
3. These compounds accumulated in a "soup" that may have concentrated at various locations (shorelines, oceanic vents etc.).
4. By further transformation, more complex organic polymers - and ultimately life - developed in the soup.

„Primordial soup“

„Biopoeiesis“ – prebiotic oceans as „hot diluted soup“ under anoxic conditions: e.g. CO\textsubscript{2}, NH\textsubscript{3}, H\textsubscript{2}O

Life arose through the slow evolution of chemical systems of increasing complexity

Basic classes of biomolecules

- Aminoacids
- Lipids
- Carbohydrates (sugars)
- Nucleotides
- Nucleosides (sugar+nucleotide)

Vital chemical reactions

Aminoacid polymerization → ribosome
nucleotide polymerization → DNA/RNA polymerases

Experimental prebiotic organic chemistry

- Prebiotic chemistry deals with reactive substances (like HCN) often at concentrations much higher than probable in prebiotic environments
- Prebiotic experiments usually performed with very small number of pure substrates
- Early protometabolic processes might have used a broader set of organic compounds than the one contemporary biochemistry

- No evidences/fossils from that early Earth we try to SPECULATIVELY fit different examples of chemical reactivity into an EXPECTED OUTCOME which we know as contemporary biochemistry

- Most of the discussed transformations are performed by highly specific and evolved enzymes at high speed and efficiency – prebiotic chemistry is supposed to be much slower and less efficient, but more robust and diverse
Proteinogenic amino acids
genetically encoded

By Matteo Ferla

Biosynthesis of biogenic amino acids

Atmosphere composition for young terrestrial planets

<table>
<thead>
<tr>
<th>Reduced</th>
<th>Neutral</th>
<th>Oxidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>CH₄</td>
<td>CO, CO₂</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>NH₃</td>
<td>N₂</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>H₂O</td>
<td>H₂O, CO, CO₂</td>
</tr>
<tr>
<td>Hydrogen (H)</td>
<td>H₂, CH₄, NH₃, H₂O</td>
<td>H₂O</td>
</tr>
</tbody>
</table>

Harold Urey (1893-1981)
UCSD, Nobel prize 1934
Discovery of deuterium

Stanley Miller (1930-2007)
UCSD San Diego, CA, USA

Miller-Urey experiment - 1952

UCSD, Nobel prize 1934
Discovery of deuterium
**Products of the Miller-Urey experiment**

Cysteine and methionine also present, when H$_2$S is added to the reaction mixture.

**Strecker reaction**

Ammonia + Aldehyde $\rightarrow$ Imine + Hydrogen cyanide

$\text{NH}_3 + R - C = \text{OH} \xrightarrow{\text{heat}} R - C = \text{NH} + \text{H}_2\text{O}$

**Scheme 1. Synthesis of $\alpha$-Amino Acids through the Strecker Reaction**

$\text{HO} - \text{CO}_2\text{H} + \text{CH}_2\text{CN} + \text{NH}_3 \xrightarrow{\text{K}_2} \text{H}_2\text{NCO}_2\text{H}$
**α-Aminoacid production in the Miller-Urey experiment**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Yield (µM)</th>
<th>Amino Acid</th>
<th>Yield (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>440</td>
<td>Norleucine</td>
<td>6</td>
</tr>
<tr>
<td>Alanine</td>
<td>790</td>
<td>Isoleucine</td>
<td>5</td>
</tr>
<tr>
<td>α-Aminoisobutyric</td>
<td>270</td>
<td>Serine</td>
<td>5</td>
</tr>
<tr>
<td>Norvaline</td>
<td>61</td>
<td>Allantoicin</td>
<td>5</td>
</tr>
<tr>
<td>Aspartic</td>
<td>34</td>
<td>Proline</td>
<td>2</td>
</tr>
<tr>
<td>α-Aminoisobutyric</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>20</td>
<td>Threonine</td>
<td>1</td>
</tr>
<tr>
<td>Lysine</td>
<td>11</td>
<td>Alanosine</td>
<td>1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8</td>
<td>Ser-Lysine</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: Protogenic amino acids in bold type.

**Modifications of the Miller-Urey experiment**

<table>
<thead>
<tr>
<th>Researcher(s)</th>
<th>Year</th>
<th>Reactants</th>
<th>Energy source</th>
<th>Results</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller</td>
<td>1952</td>
<td>CH₄, NH₃, H₂O, H₂</td>
<td>Electric discharge</td>
<td>Simple amino acids, organic compounds</td>
<td>unlikely</td>
</tr>
<tr>
<td>Abelson</td>
<td>1963</td>
<td>CH₃CO₂H, NH₃, H₂O, H₂</td>
<td>Electric discharge</td>
<td>Simple amino acids, HCN</td>
<td>unlikely</td>
</tr>
<tr>
<td>Gold and Wreyerhoff</td>
<td>1952</td>
<td>CH₄, NH₃, H₂O</td>
<td>Ultraviolet light (0.1-3 ηm)</td>
<td>Simple amino acids (low yields)</td>
<td>unlikely</td>
</tr>
<tr>
<td>Baldauf, et al.</td>
<td>1967</td>
<td>formaldehyde, methylamines, acetic acid</td>
<td>Sunlight (photosynthesis)</td>
<td>Simple amino acids possible</td>
<td>possible</td>
</tr>
<tr>
<td>Parabokaya and Pasymski</td>
<td>1970</td>
<td>formaldehyde, nitrous acid</td>
<td>High pressure H₂ lamp (photosynthesis)</td>
<td>Simple amino acids possible</td>
<td>possible</td>
</tr>
<tr>
<td>Palm and Calvino</td>
<td>1965</td>
<td>CH₃CO₂H, NH₃, H₂O</td>
<td>Electron irradiation</td>
<td>Glycine, alanine, aspartic acid</td>
<td>unlikely</td>
</tr>
<tr>
<td>Harada and Fonseca</td>
<td>1964</td>
<td>CH₃CO₂H, NH₃, H₂O</td>
<td>Thermal energy (990-1200°C)</td>
<td>14 of the (essential) amino acids of proteins</td>
<td>unlikely</td>
</tr>
<tr>
<td>Otti</td>
<td>1967</td>
<td>CH₃CO₂H, NH₃, H₂O</td>
<td>Phosphatase</td>
<td>Simple amino acids possible</td>
<td>possible</td>
</tr>
<tr>
<td>Bar-Nun et al.</td>
<td>1979</td>
<td>CH₃CO₂H, NH₃, H₂O</td>
<td>High voltage</td>
<td>Simple amino acids</td>
<td>unlikely</td>
</tr>
<tr>
<td>Sagan and Khare</td>
<td>1979</td>
<td>CH₃CO₂H, NH₃, H₂O</td>
<td>Ultraviolet light (0.1-3 ηm)</td>
<td>Simple amino acids (low yields)</td>
<td>unlikely</td>
</tr>
</tbody>
</table>

**Aminoacid production under hydrothermal conditions**

- Ni(OH)₂/KCN/CO in alkaline aqueous conditions (80-120°C) \(\rightarrow\) α-amino and α-hydroxyacids
  - Huber, C.; Wächtershäuser, G. Science 2006, 314, 630-632

- Ca(OH)₂/Na₂CO₃/KCN/CO in alkaline (pH 9.1-12.9) aqueous conditions (145-280°C) \(\rightarrow\) α-amino and α-hydroxyacids (higher yields): glycine, alanine, serine, glycolate, lactate, glycerate
Extraterrestrial origin of biomolecules

Table 1. Soluble Organic Compounds in the Murchison Meteorite

<table>
<thead>
<tr>
<th>class of compounds</th>
<th>parts per million</th>
<th>mole*</th>
</tr>
</thead>
<tbody>
<tr>
<td>aliphatic hydrocarbons</td>
<td>&gt;35</td>
<td>140</td>
</tr>
<tr>
<td>aromatic hydrocarbons</td>
<td>15-28</td>
<td>67</td>
</tr>
<tr>
<td>polar hydrocarbons</td>
<td>&lt;120</td>
<td>117</td>
</tr>
<tr>
<td>carboxylic acids</td>
<td>&gt;300</td>
<td>49</td>
</tr>
<tr>
<td>amines</td>
<td>60</td>
<td>75</td>
</tr>
<tr>
<td>amines</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>hydroxylic acids</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>dicarboxylic acids</td>
<td>&gt;30</td>
<td>17</td>
</tr>
<tr>
<td>dicarboxylics</td>
<td>&gt;50</td>
<td>2</td>
</tr>
<tr>
<td>pyridine carboxylic acids</td>
<td>&gt;7</td>
<td>7</td>
</tr>
<tr>
<td>sulfonic acids</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td>phosphonic acids</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>N heterocycles</td>
<td>7</td>
<td>34</td>
</tr>
<tr>
<td>amines</td>
<td>13</td>
<td>59</td>
</tr>
<tr>
<td>amines</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td>polyols</td>
<td>30</td>
<td>19</td>
</tr>
</tbody>
</table>

Catalytic properties of amino acids - organocatalysis

Mannich reaction

Michael addition

Hydrocyanation

Robinson annulation

Aldol reaction

The origins of homochirality

Currently known biopolymers are homochiral

Structural propensity and catalytic activity strongly depends on the enantopurity

- Homochirality must have been involved early in the process of life formation
- Chiral monomers could be only partially enantioenriched

General cause of homochirality:
- the initial symmetry breaking + subsequent assymetry amplification:
  - The pairity violation
  - Stochastic symmetry disturbances

Electroweak interactions and the pairity violation principle cause L-aminoacids and D-sugars to be SLIGHTLY MORE STABLE than their enantiomers

The pairity violation – Wu experiment

Mirror image of the world does not behave exactly like the world itself because TIME GOES UNIDIRECTIONALLY

in a mirror, time will not go BACKWARDS!

Chien-Shiung Wu (1956) –

experiment on $^{60}$Co decay

Differentiation in left and right handedness is inherent property of weak interactions

The origins of homochirality

Circularly polarized light (CPL) from gamma ray bursts

K. Wiersma et al., Nature 509 201, 2014

Small enantiomeric excess can be obtained by enantioselective degradation of aminoacids with CPL

Up to 2.6% ee


Stochastic induction of assymetry – Frank model

Reactions (3) and (4) are autocatalytic

Open flux reactor in non-equilibrium stationary state

If a chiral dissipative structure catalyzes its own formation and inhibits formation of the opposite enantiomer, any stochastic symmetry breaking in the system will be amplified
External stimuli and autocatalytic processes

Deracemisation of non-natural and proteinogenic aminoacids was observed

autocatalytic Soai reaction – extreme chirality amplification

Organometallic reaction - NOT prebiotic

Scheme 9. Soai Autocatalytic Reaction

autocatalytic organic reactions

Meaningful transformations for the prebiotic syntheses of aminoacids and sugars

Organocatalysis – the origin of homochirality

Table 1. Enantiomeric concentration amplification of phenylalanine after two crystallizations from water

<table>
<thead>
<tr>
<th>Component</th>
<th>Initial ee, %</th>
<th>Final ee, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>10</td>
<td>90.0 ± 3.7</td>
</tr>
<tr>
<td>l</td>
<td>5</td>
<td>91.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>87.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>88.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>86.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>90.9 ± 0.3</td>
</tr>
</tbody>
</table>

Solutions with as little as 1% enantiomeric excess (ee) of l- or d-phenylalanine are ammobilized to 90% ee (a 95/5 ratio) by two successive evaporations to precipitate the racemate. Such a process on the prebiotic earth could lead to a mechanism by which meteoritic chiral α-alkyl amino acids could form solutions with high ee values that were needed for the beginning of biology.

Chirality amplification in biphasic systems

Reaction and solution behaviour as a function of the overall proline enantiomeric excess.

a. Product enantiomeric excess versus proline enantiomeric excess for the aldol reaction of equation

b. Solution proline enantiomeric excess (left axis, triangles) and solution proline concentration (right axis, diamonds) as a function of the overall enantiomeric excess for proline at 0.1 M


Oligomerization of activated aminoacids

(a) Packing arrangement of (d,l)-PheNCA crystal viewed along the a-axis, showing four rows of molecules in an enantiopolar arrangement. The polymerization of the d-molecules (in bold) occurs along the +b direction to yield oligo-d chains and that of the l-molecules along the −b direction to yield l-oligopeptides, as modeled in panel b. (ii) Computer model of two adjacent isotactic d- and l-oligopeptides assembled into a racemic parallel β-sheet formed within the lattice of the (d,l)-ValNCA crystal.


Condensation of aminoacids into peptides

Proposed route for chain elongation via formation of racemic antiparallel (ap) β-sheets comprising alternating oligo-d and oligo-l chains, both with the residues of the L-Phe-OMe, i, initiator at their C-terminals, as modeled on the basis of the (d,l)-PheNCA crystal structure, viewed down the c-axis. The arrows show the antiparallel direction of chain propagation of the growing NH₂ termini of the d- and l-chains.

Biochemical condensation of amino acids into peptides

Prebiotically relevant peptide condensation agents

<table>
<thead>
<tr>
<th>Entry</th>
<th>Activating agent</th>
<th>Hydrolysis product</th>
<th>$k \text{ mol}^{-1} \text{ s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NH$_2$CONH$_2$</td>
<td>CO$_2$ + NH$_3$</td>
<td>$10^6$</td>
</tr>
<tr>
<td>2</td>
<td>CO$_2$ (g)</td>
<td>CO$_2$ + H$_2$S</td>
<td>$10^7$</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoric acid</td>
<td>Phosphate</td>
<td>$10^8$</td>
</tr>
<tr>
<td>4</td>
<td>CO$_2$ (g)</td>
<td>HCO$_2$H</td>
<td>$10^6$</td>
</tr>
<tr>
<td>5</td>
<td>HNCO</td>
<td>CO$_2$ + NH$_3$</td>
<td>$10^7$</td>
</tr>
<tr>
<td>6</td>
<td>HCN</td>
<td>HCO$_2$H + NH$_3$</td>
<td>$10^8$</td>
</tr>
<tr>
<td>7</td>
<td>RNH$_2$</td>
<td>RNH$_2$H + NH$_3$</td>
<td>$10^7$</td>
</tr>
<tr>
<td>8</td>
<td>NH$_2$CN</td>
<td>Isocyanate</td>
<td>$10^7$</td>
</tr>
<tr>
<td>9</td>
<td>HNCH$_2$NH</td>
<td>Isourea</td>
<td>$10^7$</td>
</tr>
<tr>
<td>10</td>
<td>HCCN (g)</td>
<td>CH$_2$CHO</td>
<td>$10^7$</td>
</tr>
</tbody>
</table>


Condensation of amino acids into peptides

Salt-induced peptide formation (SPIF)

SIPF copper complex geometry with two glycine ligands, optimized by ab initio Hartree–Fock calculations.

A slow formation of NCAs from free amino acids and COS in the absence of oxidizing or alkylating agents has been reported and studied through theoretical chemistry investigations. However, it seems unlikely that COS ($D_{Go0} = 16.9 \text{ kJ mol}^{-1}$) could be able to generate NCA ($D_{Go0} \approx 60 \text{ kJ mol}^{-1}$) in spite of its cyclic structure.

A photochemical activation of thiocarbamate that could take place in a way similar to that of thioacetate in aqueous solution may provide an explanation to this observation. This potential photochemical reaction may also constitute an efficient pathway for the prebiotic formation of NCAs.

Diketopiperazines as intermediates for peptide condensation

Pathways for the formation of NCAs and further reactions including polymerization and interactions with inorganic phosphate (Pi), nucleotides (NMP), and RNA.

Condensation of amino acids into peptides

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Basic aminoacids for primitive genetic code?

Primordial genetic code might have involved only 4 „GNC“ codons:

- GGC for glycine
- GCC for alanine
- GAC for aspartic acid
- GUC for valine

Later, the „GNC“ code probably evolved into „SNS“ code (S = G/C, N = A, U, G, C) – 16 codons encoding 10 basic aminoacids (Gly, Ala, Asp, Val, Leu, Pro, His, Glu, Arg)

GADV-protein world

<table>
<thead>
<tr>
<th>α-helix</th>
<th>β-sheet (Val)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ala)</td>
<td>(Gly)</td>
</tr>
</tbody>
</table>

hydrophilic and hydrophobic structures

globular structures

catalytic activity (Asp)

Reduced aminoacid alphabet

9-aminoacid alphabet is sufficient to construct functional enzymes

Aminoacids: Asp, Glu, Asn, Lys, Phe, Ile, Leu, Met, Arg

AmoQ-structure and active site. A, the homodimeric EcCM is shown with a transition state analog inhibitor bound at its active sites; the two identical polypeptide chains are colored blue and pink for clarity. B, proposed interactions between residues in the evolved active site of the simplified enzyme and the transition state analog inhibitor, compound 1 (red), based on the x-ray structure of EcCM. Residues Glu⁷⁶ and Ser⁷⁸ in EcCM are substituted with Glu⁸⁸ and Asn⁸⁴ in the 9-amino acid enzyme. Residue numbers are referenced to EcCM.

Aminoacids - Summary

Prebiotic generation plausible – variants of the Miller-Urey experiment

Strecker-type of chemistry likely

Aminoacids are good catalysts, can perform various chemical transformations

The origin of homochirality in the Universe caused by the parity violation and stochastic fluctuations

Chirality amplification possible in numerous chemical reactions

Aminoacids can catalyse their own formation with chirality amplification and undergo physical enantioenrichment processes

Condensation of aminoacids into peptides plausible under prebiotic conditions using condensing agents

Simple peptides can exhibit broad structural variety, catalytically active enzymes can be constructed with reduced aminoacid alphabet