Critical Steps Toward Protocells

NASA Astrobiology Institute Proposal

Submitted March 6, 2003, by

Los Alamos National Laboratory
Argonne National Laboratory
Reed College
University of Copenhagen, Denmark
University of Leipzig, Germany
Fraunhofer Institute, Germany

[Diagram of protocell processes]

1 PI and point of contact; Steen Rasmussen, Los Alamos National Laboratory, +1-505-665-0052, steen@lanl.gov
# Table of content

**Executive summary**

Summary of personal, commitments, and costs

Research and management plan.................................................................1

1. Introduction.........................................................................................1

2. Physicochemical approaches of the step-by-step assembly of Protocells........5
   2.1 Self-reproduction of micelles and vesicles......................................5
   2.2 Template directed PNA polymerization in the lipid phase.................8
   2.3 PNA directed surfactant and (functional) PNA formation
       in the lipid phase (from lipid- and PNA precursors).........................13
   2.4 Full system assembly .................................................................17
   2.5 Alternative implementations .......................................................21

3. Thermodynamics and kinetics of the step-by-step assembly of Protocells:
   Theoretical and computational approaches........................................23
   3.1 Multi scale simulation approach...............................................24
   3.2 The lipid aggregation process.....................................................27
   3.3 Loading (solubilization) of the lipid aggregates............................31
   3.4 Thermodynamics and kinetics of the proto-metabolism...................33
   3.5 Thermodynamics and kinetics of the proto-genes.........................37
   3.6 Thermodynamics and kinetics of the full system replication:
       Life-cycle of the Protocell.........................................................41

4. Conclusion.........................................................................................46

5. Technological, ethical, and moral implications of Protocells......................47

6. Management plan...............................................................................51

7. Plan for strengthening the Astrobiology community................................57

8. References.........................................................................................63
Executive Summary: Critical Steps Toward Protocells

NASA’s Goals and Objectives. The proposed research aims to understand the chemical processes that will create a very basic, primitive form of life, which we call “Protocells”. This research directly addresses NASA’s first fundamental question—“How does life begin and evolve?”—and two specifically stated goals under that question: (i) how life arose on earth, and (ii) organization of matter into living systems.

The proposed research addresses these questions by combining chemical raw materials under conditions in which they self-assemble into living systems, i.e., systems involving the coordinated mutual dependence of compartmentalization, metabolization, and inheritance. This exemplar will be invaluable guide to the origin of terrestrial life and to the transition to life in extra-terrestrial environments.

The proposed Institute is in a strong position to play a key role in assembling molecular self-replicating systems for main three reasons: (i) Los Alamos and Argonne National Laboratory, together with an external University team, have just developed and published by far the simplest existing Protocell design. (ii) Most of the chemical mechanisms needed to realize this novel design have been independently implemented in vitro. (iii) Advances in the theory and simulation of molecular self-assembly at Los Alamos have now reached the point that they can guide and propel experimental realizations of the first bottom-up Protocells.

Background and Significance. The proposed Institute explores a set of simple processes that bridge between nonliving and living matter, thus expanding our understanding of the connection between physics, chemistry, material science, and biology. The necessary components and most of the reactions that would support a Protocell have already been identified and studied both experimentally and in simulations. Our team has done significant parts of this work. The next critical steps toward creating Protocells are to assemble the individual components into functional subsystems, which are eventually integrated into a Protocell. This work requires a unique interdisciplinary team, world-class experimental and computational facilities, and cross-disciplinary coordination, all of which Los Alamos and Argonne are superbly situated to provide. Most of the above referenced university research groups are already part of NASA’s Astrobiology network; some are our competitors and others are our collaborators. The proposed Institute will help focus all of these ongoing efforts.

Science and Technology Goals. The proposed Protocell is much simpler than the smallest bacterium. It consists of just three critical physicochemical aggregates (a proto-container, proto-genes, and a proto-metabolism) that self-assemble into a single cooperative structure; see figure 1. We propose to realize this system both in detailed multi-scale simulations and in the laboratory.

Tasks and Probable Accomplishments. The critical steps toward the creation of a Protocell consist of a specific set of tasks. Although the tasks all have various connections, most can be pursued simultaneously (Tasks 1, 2, 3, 4, 6, and 7). But the final creation of a fully autonomous Protocell (Task 5) crucially depends on the prior completion of other tasks.

Task 1. To better direct and understand the experimental systems, we propose to develop a multi-scale computational version of all key processes in the simple proto-organism. To do this we propose to develop a simulation framework for studying molecular self-assembly. This provides an up- and down-
Figure 1 Life-cycle of simple Protocell. Only ~ 5 nm in diameter and weighing about $4 \times 10^{-20}$ g, it is about $10^8$ times smaller than the smallest bacterium. (1) Self-assembly of the proto-organism components, the lipids, the PNA, and the sensitizer molecules. (2) Feeding the proto-organism with PNA- and lipid precursors and sensitizer molecules. The proto-organism swells up as it fills with precursor lipids. (4) As light energy provided, the phenyl group is fragments from the precursor PNA, forming PNA oligomers that can ligate once they are aligned by the template. (5) As the precursor lipids are turned into lipids (surfactants), the large aggregate becomes unstable and starts breaking up. Establishing a thermodynamic balance between hybridized and non-hybridized (double- and single-stranded) PNA allows a reasonable partition of the proto-genes between the two aggregates. This process may be the hardest experimental challenge. (6) The life cycle is complete as the proto-organism generates a copy of itself. The overall rate limiting steps are the PNA template-directed ligation process and the balanced lipid-PNA and PNA hybridization kinetics. See ref 10,11 for more details.

scaling computational capability reaching from Angstroms to millimeters and from femtoseconds to hours. Five existing simulation techniques 6,7,8,9 are integrated: ab initio calculations, molecular dynamics (MD), MD lattice gas, Ginzburg-Landau, and reaction kinetics. We will produce a complete 3-D description (“movie”) of the proto-organism and we will use this computational framework to predict system feasibility where possible to eliminate costly laboratory experiments, and to expand our understanding when predictions fail.

Thus, one of the deliverables is a novel multiscale theoretical and computational capability to address molecular self-assembly and charge transfer.

**Task 2.** We propose to study assembly and stability of micelles and vesicles (proto-containers) from simple lipids in the context of self-reproduction. This is the process depicted in Figure 1 (5–6). This work will be done both experimentally and in simulation.

**Task 3.** We propose to experimentally demonstrate template-directed replication of PNA peptide nucleic acid, PNA (proto-genes), at a lipid interface. We have studied this process theoretically and computationally 10,11 and we understand the kinetics. These experiments are simplifications of processes (4–5) in Figure 1, which decouples the metabolic process. Demonstrating this self-replication experimentally would constitute a major advance for the field.

**Task 4.** We propose to study a particular set of photo-fragmentation reactions (proto-metabolism), which both produce lipids and short functional PNA monomers (or short strings); see Figure 1 (4–5). We have already experimentally demonstrated 10 the production of lipids using this approach. It has also been demonstrated in a simpler experimentally setting 12 (and we have explained theoretically 13) that guanine-rich nucleobases in a stacked double helix could act as an electron relay chain that catalyzes this reaction. The breakthrough of combining these two reactions would establish a positive autocatalytic feedback between the three key elements in the proto-organism: the proto-container, proto-genes, and proto-metabolism.

**Task 5.** Once the former tasks have been accomplished, we propose to integrate all subsystem into a full, self-sustaining Protocell, to study its self-replication experimentally, and to compare its behavior with our simulation predictions (the 3-D “movie”).

**Task 6.** We propose to define and study the energy and entropy landscape for the sub-processes involved in bridging the transition between nonliving and living matter. Our
estimates of the free energy involved in one full Protocell replication cycle\textsuperscript{11} define the lower end of the universal scaling relations in biological systems\textsuperscript{14}.

**Task 7.** Pressing technological, social, and ethical issues are arising as the Astrobiology community comes increasingly closer to creating artificial life forms. We propose to address these issues and educate the public about them at the same time as we pursue our scientific objectives.

**Potential Impact of the Astrobiology Institute Investment.** Being able to create living systems from scratch will open new fields of science and technology and vastly expand our basic understanding of living systems. Developing a predictive multi-scale computational capability will also be of significant value for the scientific community at large; it could well become the canonical method for studying molecular self-assembly processes. Perhaps most importantly, demonstrating how life can self-organize from nonliving materials will be a significant step towards solving a central scientific question in the ancient puzzle about who we are and from where we came.

The ability to create Protocells would create a threshold enabling technology, similar to the transistor, the sequencing of the human genome, or the promises of quantum computing. We do not yet know which application it will spawn first. However, by being the first, NASA and the National Laboratories will be in a good position to define how this technology should be developed in the future and to ensure that Protocells and their eventual progeny will serve humanity.

Beyond the production of Protocells, the proposed research will also give key guidance to the more general problems of engineering living systems. Protocells could in fact be the first example of what could literally be called “Living technology”.

There has never been greater need for living technology that captures life’s range of distinctive useful properties, such as autonomy, robustness, adaptation, simple intelligence, modular structuring, self-repair and self-replication. There is growing recognition that the creation of truly intelligent and adaptive physical artifacts such as robots depends on bridging the gap between nonliving and living matter\textsuperscript{15}. Conventional engineering is hitting a complexity barrier because it has been producing devices that are non-adaptive, brittle and costly to redesign.

NASA itself could be a primary beneficiary from these advances. The difficult context of space exploration directly encounters some of the least familiar and most hostile environments known to us. Understanding and controlling life’s creative powers will help us to design systems and environments that support continual and flexible improvement. Space stations and space colonies that are autonomously self-sustaining and self-maintaining, intelligently recycling materials and adapting to unpredictable environmental contingencies, are some of the future possible applications of Living Technology.

7) Kang et al., *PRE* 65 (2002) 036318
9) Rasmussen et al., *Artificial Life* 7 (2001) 329
10) Rasmussen et al., *Artificial Life*, in press (see www.lanl.gov/EES5/staff/steen/Protocells)
11) Rasmussen et al., *Origins of Life and Evol. of Biosphere*, in press (see www.lanl.gov/EES5/staff/steen/Protocells)
12) Yoo et al., *Phys Rev Lett*, 87 (2001) 198102
Summary of Personnel and Costs

Dr. Steen Rasmussen, Principal Investigator, Los Alamos
Responsibilities: Project guidance; progress reports, and lead of theory and computational efforts

Dr. Liaohai Chen, Co-Investigator, Argonne
Responsibilities: Lead of experimental investigations and experimental Protocell design

Dr. Mark Bedau, Co-Investigator, Reed College
Responsibilities: Lead for the ethical ramifications of Protocell technology

Dr. Norman Packard, Co-Investigator, Prediction Company and Los Alamos
Responsibilities: Co-lead of computational and theoretical tasks and oversight

Dr. Stirling Colgate, Collaborator, Los Alamos
Responsibilities: Thermodynamic properties (landscape) of transition to Protocells

Dr. Yi Jiang, Collaborator, Los Alamos
Responsibilities: MD and GL methods

Dr. John McCaskill, Collaborator, Fraunhofer, Germany
Responsibilities: Reaction kinetics and multi scale simulations

Dr. Peter Nielsen, Collaborator, U of Copenhagen, Denmark
Responsibilities: Lead PNA synthesis and PNA replication in lipid aggregates

Dr. Martin Nilsson, Collaborator, Los Alamos
Responsibilities: MDLG, MD, and multi scale methods

Dr. Kim Rasmussen, Collaborator, Los Alamos
Responsibilities: Charge transfer calculations

Dr. Peter Stadler, Collaborator, University of Lipzieg
Responsibilities: Reaction kinetics and mathematical fitness landscapes

Dr. Bryan Travis, Collaborator, Los Alamos
Responsibilities: GL and MDLG simulations

Dr. Sergei Tretiak, Collaborator, Los Alamos
Responsibilities: Quantum chemistry calculations

Dr. William Woodruff, Collaborator, Los Alamos
Responsibilities: Lipid properties, PNA lipid interactions, and metabolic energetics

Total cost/year and total Astrobiology Institute costs

<table>
<thead>
<tr>
<th>Year</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY-04</td>
<td>1,272 M</td>
</tr>
<tr>
<td>FY-05</td>
<td>1,364M</td>
</tr>
<tr>
<td>FY-06</td>
<td>1365M</td>
</tr>
<tr>
<td>FY-07</td>
<td>1,415M</td>
</tr>
<tr>
<td>FY-08</td>
<td>1,485M</td>
</tr>
<tr>
<td>Accumulated</td>
<td>6,901M</td>
</tr>
</tbody>
</table>