A system-level viewpoint on the chemical origins of life

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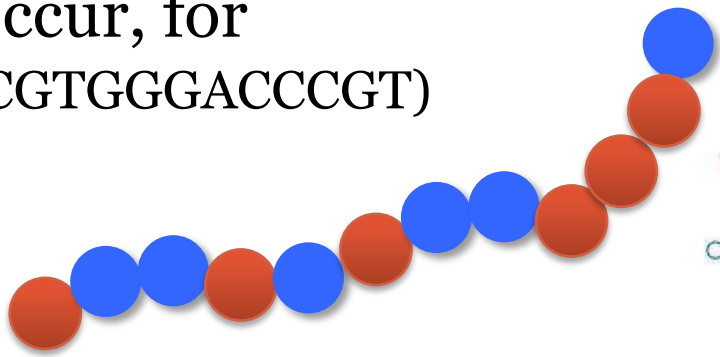
Goal

- Design and demonstrate prebiotically plausible minimal systems that can
  - Polymerize
  - Store information
  - Transfer information
  - Catalyze reactions (or other functions)
  - Undergo selection and evolution

- Ideally, we would like to find a common environment in which these steps would occur, for
  - Nucleic acid polymers (e.g. ACGTGGGACCCGT)
  - Peptides (e.g. KLVFFAE)
  - Other important biopolymers



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What does engineering have to do with origins of life?

- Research goal: Design a polymer system that can evolve.
- Design: a methodical series of steps that engineers use in creating functional products and processes
  - Wikipedia, “engineering design”
- Design involves tradeoffs
- Design involves specifying an objective

Design principles

- Robust function
  - Modularity
  - Redundancy
  - Feedback
    - Positive: amplification and selection
    - Negative: regulation and robustness
- Complex function
  - Diversity (division of labor)
  - Cooperation

Emergence?

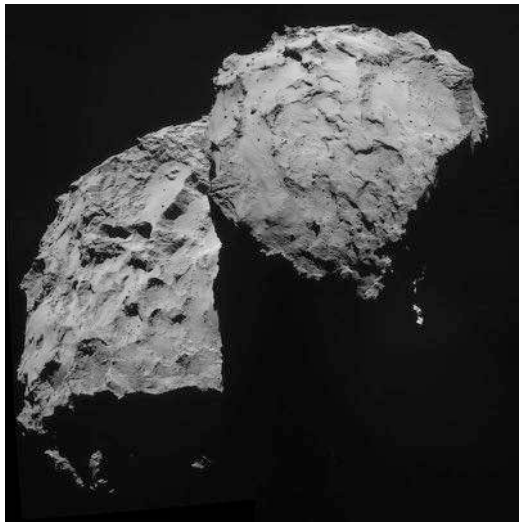
Optimization and Evolution

- Optimization
  - Maximizing an objective by adjusting design variables within constraints
- Evolution (Darwinian)
  - Change in the heritable characteristics of a population over successive generations
- Similarities
  - Genetic algorithms for engineering design
- Differences
  - Evolution: Selection by the environment
  - Optimization: Engineer specifies an objective function

The Organic Inventory

Rosetta Spacecraft - Philae Lander – Atmospheric MS Data

Name	Formula	Molar mass (u)	MS fraction	Relative to water
Water	H_2O	18	80.92	100
Methane	CH_4	16	0.70	0.5
Methanenitrile (hydrogen cyanide)	HCN	27	1.06	0.9
Carbon monoxide	CO	28	1.09	1.2
Methylamine	CH_3NH_2	31	1.19	0.6
Ethanenitrile (acetonitrile)	CH_3CN	41	0.55	0.3
Isocyanic acid	HNCO	43	0.47	0.3
Ethanal (acetaldehyde)	CH_3CHO	44	1.01	0.5
Methanamide (formamide)	$HCONH_2$	45	3.73	1.8
Ethylamine	$C_2H_5NH_2$	45	0.72	0.3
Isocyanomethane (methyl isocyanate)	CH_3NCO	57	3.13	1.3
Propanone (acetone)	CH_3COCH_3	58	1.02	0.3
Propanal (propionaldehyde)	C_2H_5CHO	58	0.44	0.1
Ethanamide (acetamide)	CH_3CONH_2	59	2.20	0.7
2-Hydroxyethanal (glycolaldehyde)	CH_2OHCHO	60	0.98	0.4
1,2-Ethanediol (ethylene glycol)	$CH_2(OH)CH_2(OH)$	62	0.79	0.2



Goesmann, F., Rosenbauer, H., Bredehöft, J. H., Cabane, M., Ehrenfreund, P., Gautier, T., ... & Ulamec, S. (**2015**). Organic compounds on comet 67P/Churyumov-Gerasimenko revealed by COSAC mass spectrometry. *Science*, *349* (6247).

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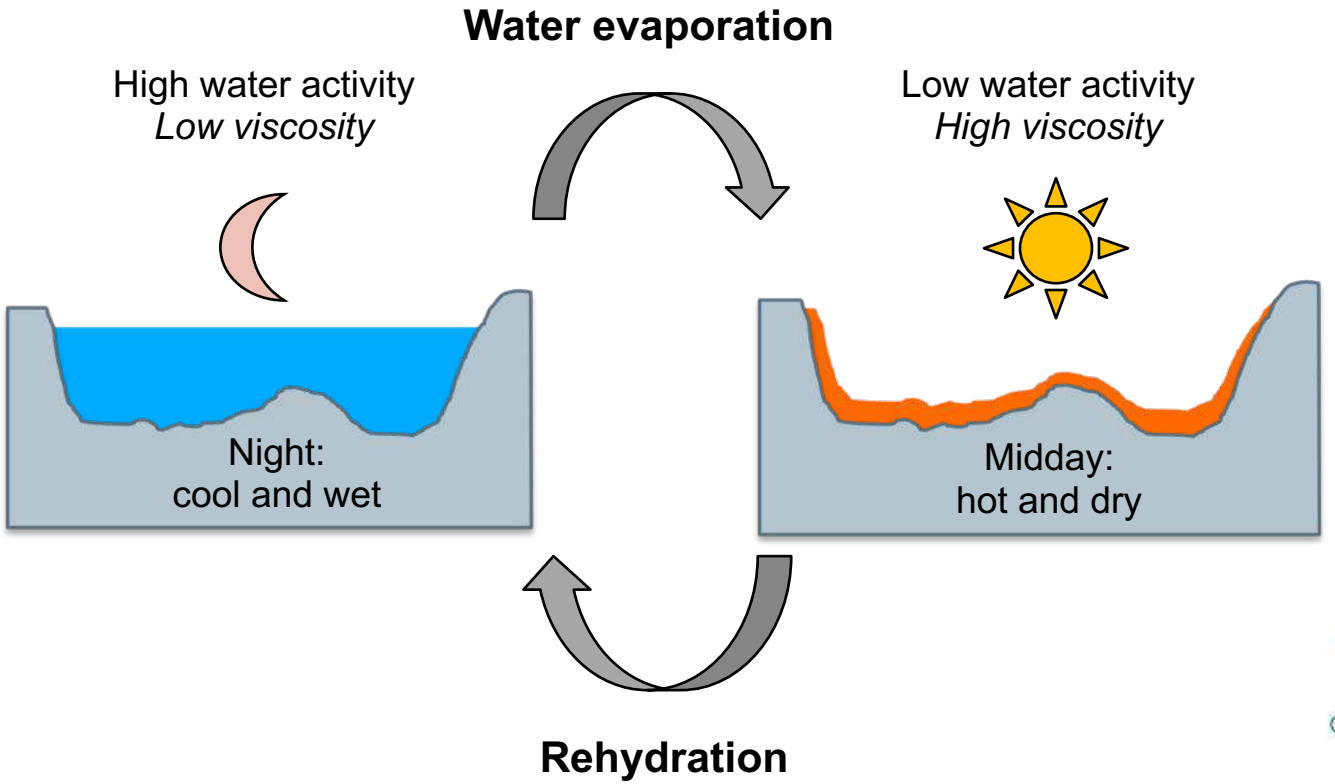
Need to address outstanding problems

- The water problem
  - Biopolymers are formed through condensation reactions, which are not favored in water.
- The strand inhibition problem
  - Heating drives duplex separation, but cooling promotes formation over replication.
- The single-winner scenario
  - Selection for the fastest replicator does not lead to a productive outcome.
    - Need to select for function.
    - Selection and evolution require sustained diversity.

Concept for the environment

Coupling between the chemistry and the environment determines system level behavior



Environmental cycles

- Prebiotically plausible (e.g. daily, tidal, seasonal)
- Types include hot/cold, wet/dry, pH swings
- Drives non-equilibrium behavior through the influx of solar energy
- Induces reversible phenomena over the course of the cycle
  - polymerization and hydrolysis
  - duplex formation and separation

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Non-aqueous solvents

- Many organics present in the prebiotic inventory
- Solvents possibly created from non-volatile organics, after water evaporation
- Features
  - Drive condensation polymerization forward
  - Control differential mobility via viscosity
  - Promote intramolecular folding by suppressing intermolecular interactions

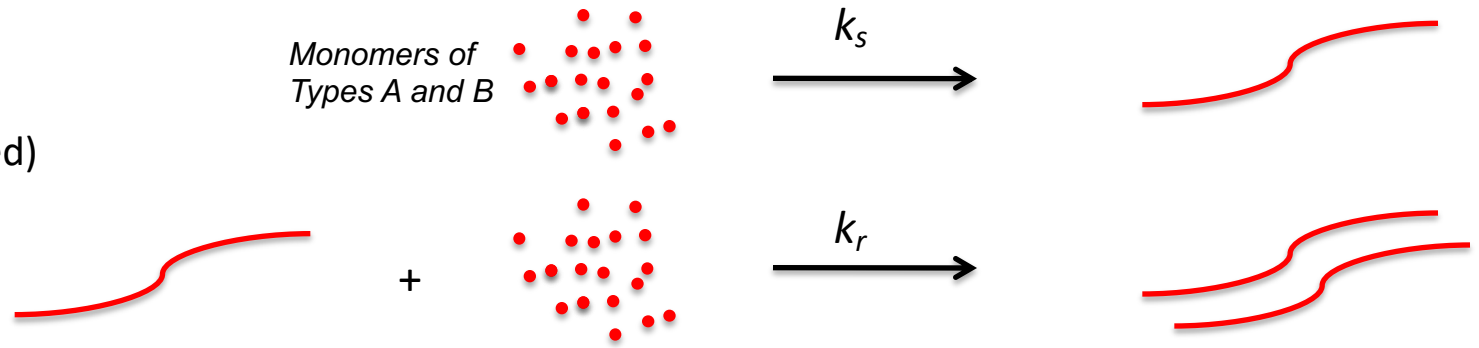
System-level behavior

- The interaction between the environment and the chemistry is critical to prediction of performance.
- Modeling approach
  - Use mathematical modeling to predict behavior and design promising experiments
  - It is difficult to predict the tradeoffs between simultaneously occurring phenomena without a mathematical model.
- A case study
  - The first functional biopolymer could have been the monomer synthetase.
  - Selection is not based on the inherent replication rate, but rather on the local resources in the environment.

A model of chemical evolution

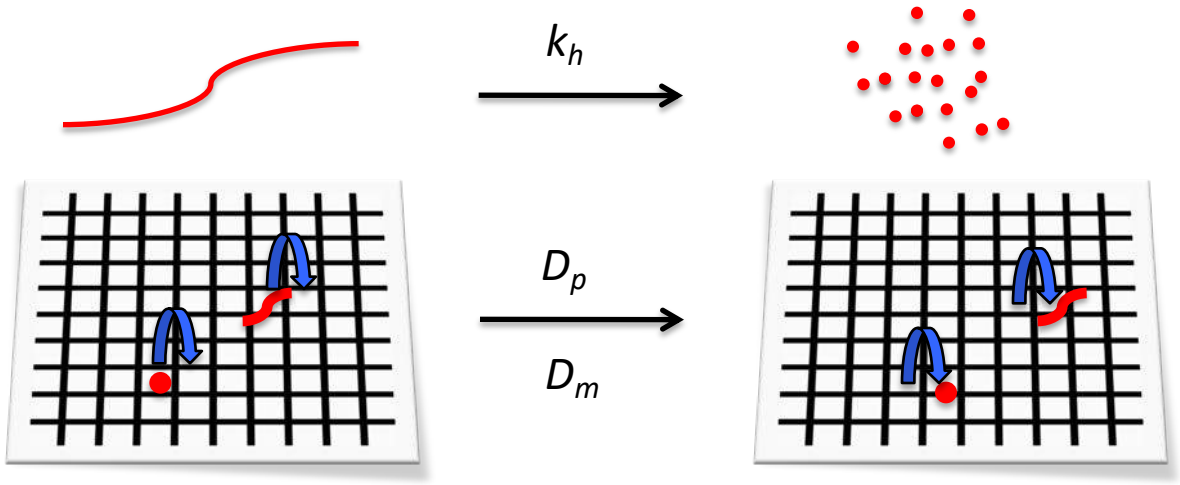
Five events and parameters define the system dynamics.

Day Phase
(dehydrated)



Rates of polymer formation are dependent on local resources.

Night Phase
(hydrated)



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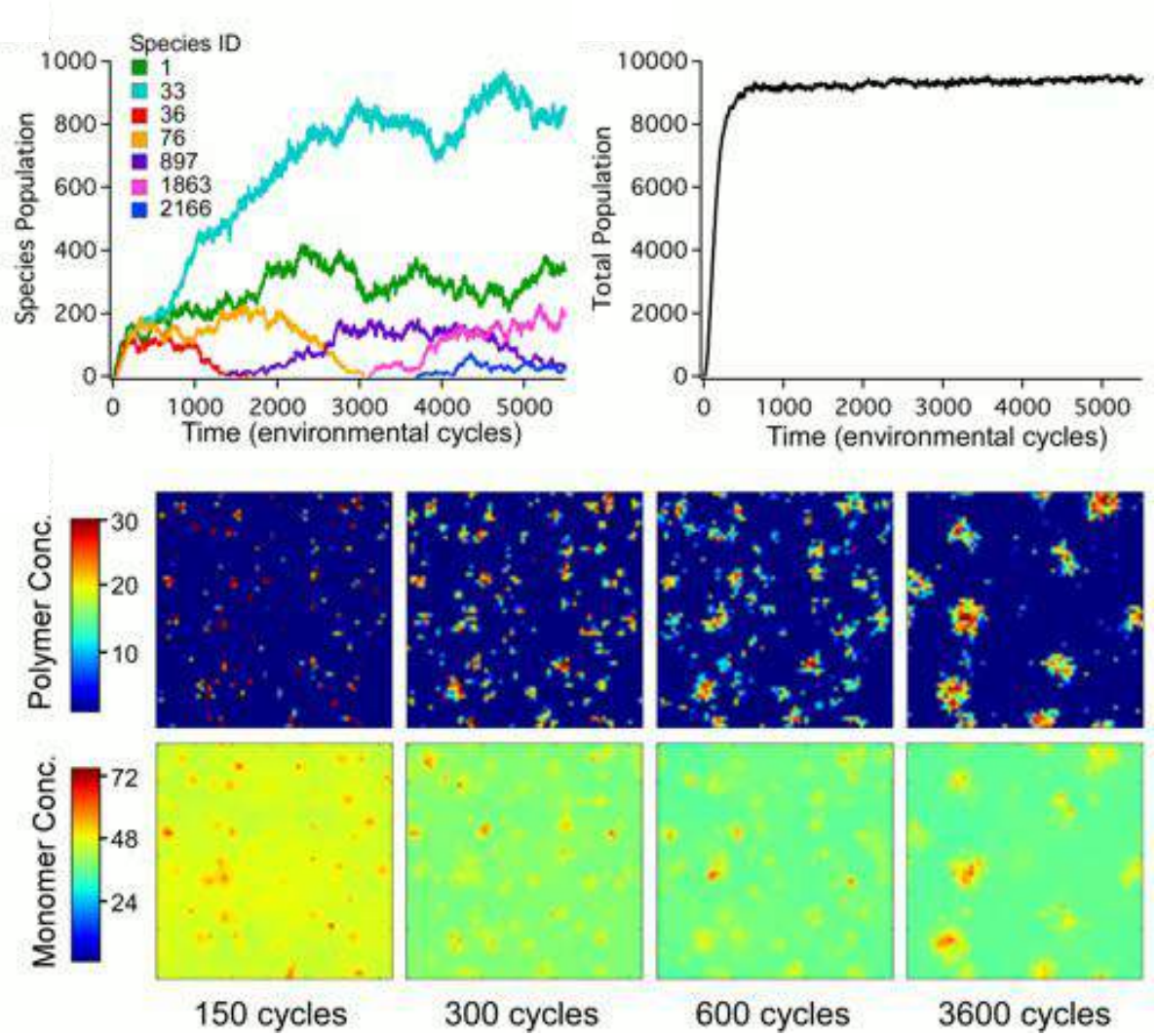
Sequence evolution of the pool



$k_s = 10^{-7}$

$k_r = 10^{-4}$

$k_h = 0.1$

$k_p = 0.001$

$k_m = 10.0$

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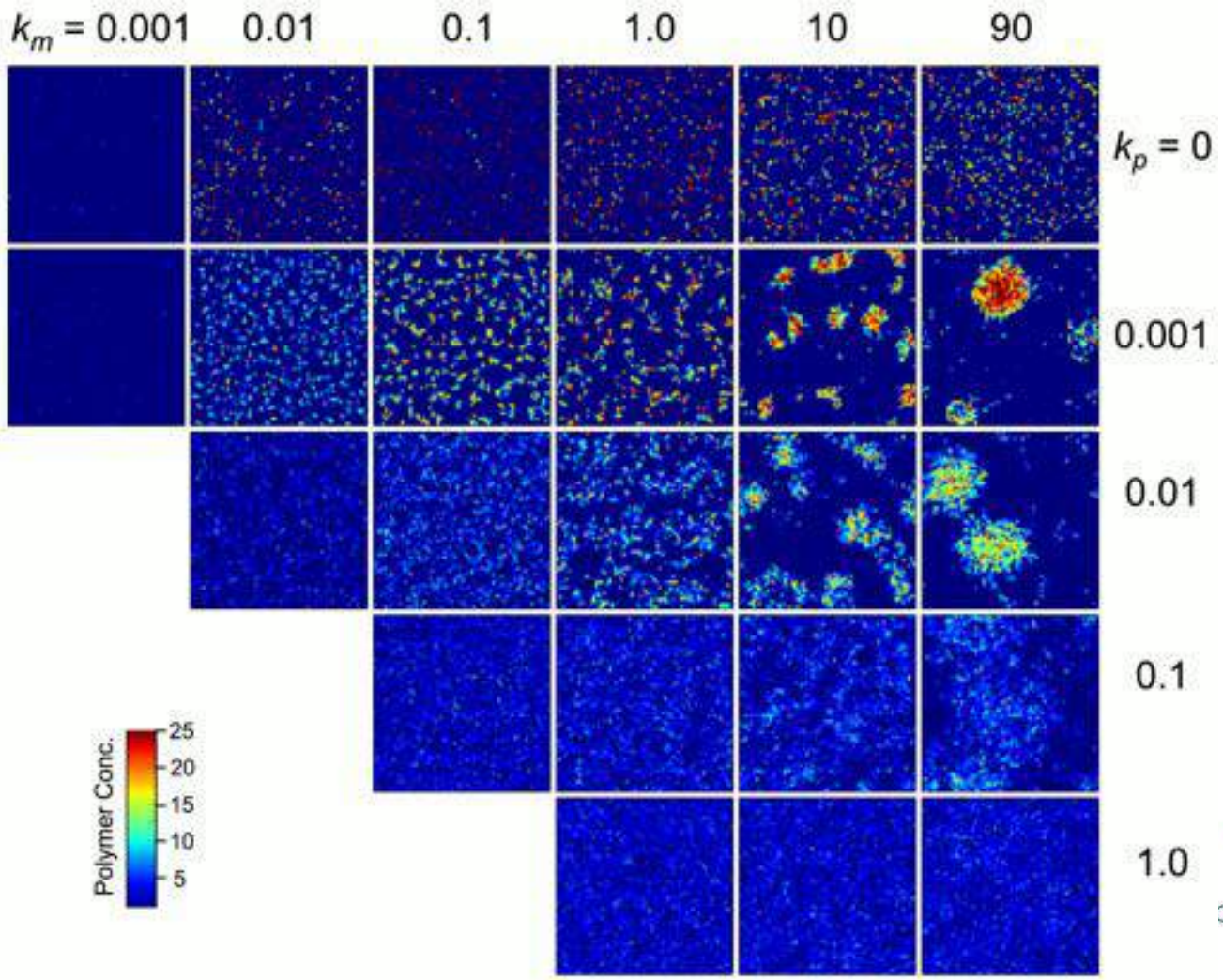
Spatial patterning



$k_s = 10^{-7}$

$k_r = 10^{-4}$

$k_h = 0.1$

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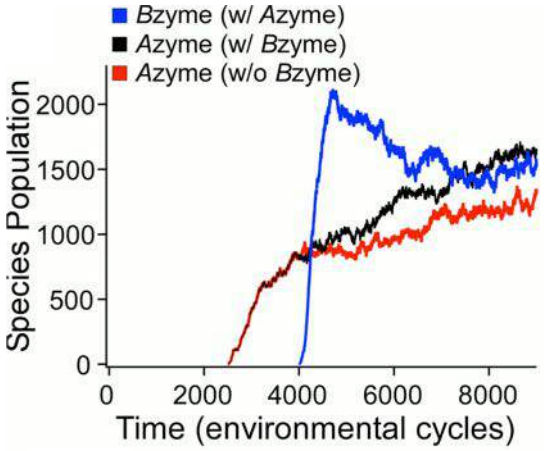
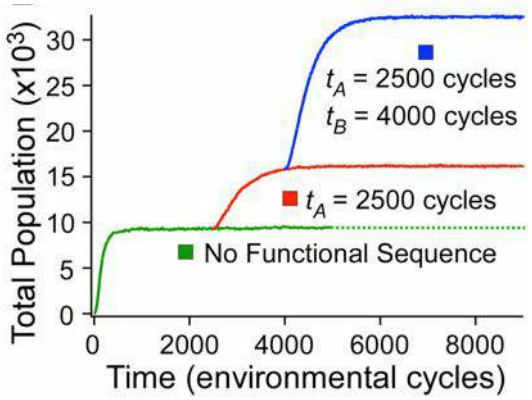
Cooperativity over time and space





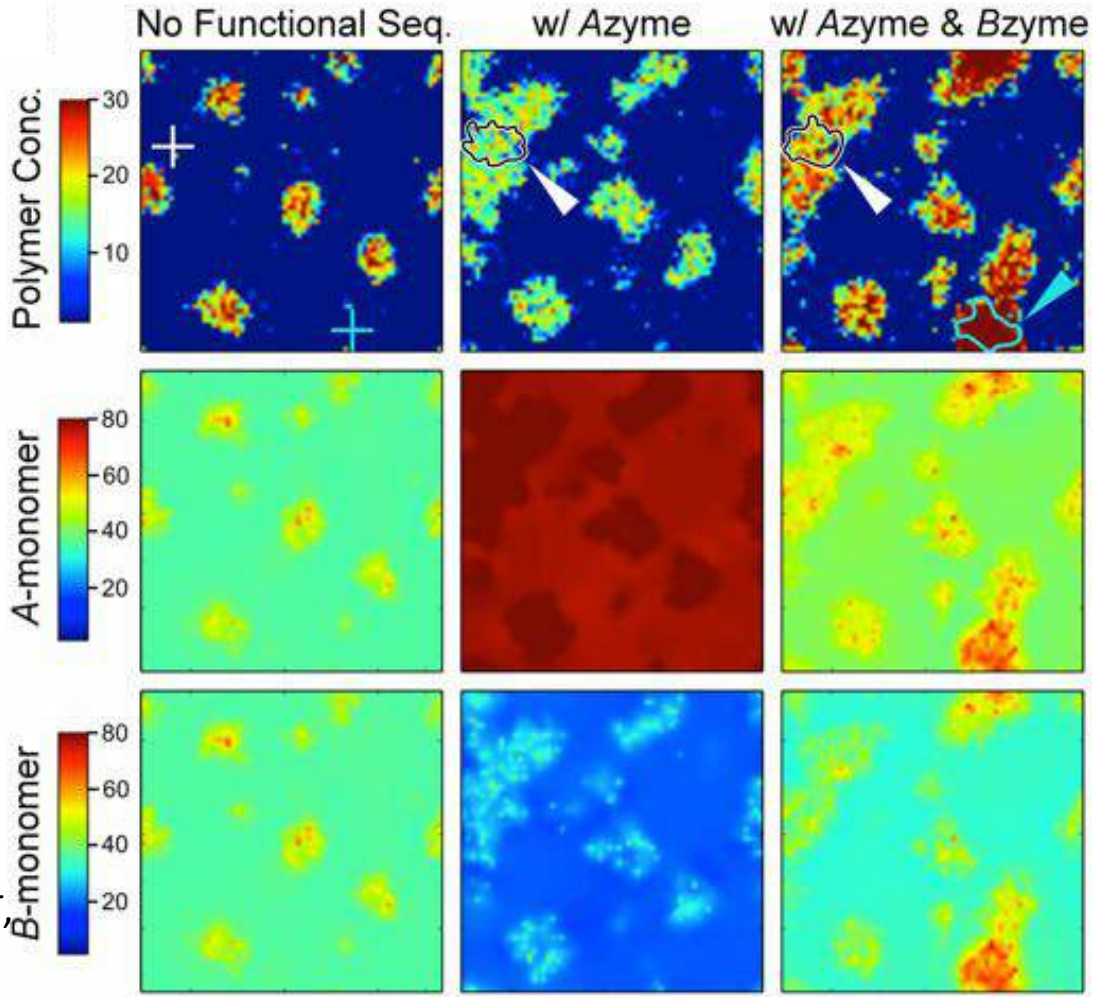
$k_s = 10^{-7}$

$k_r = 10^{-4}$

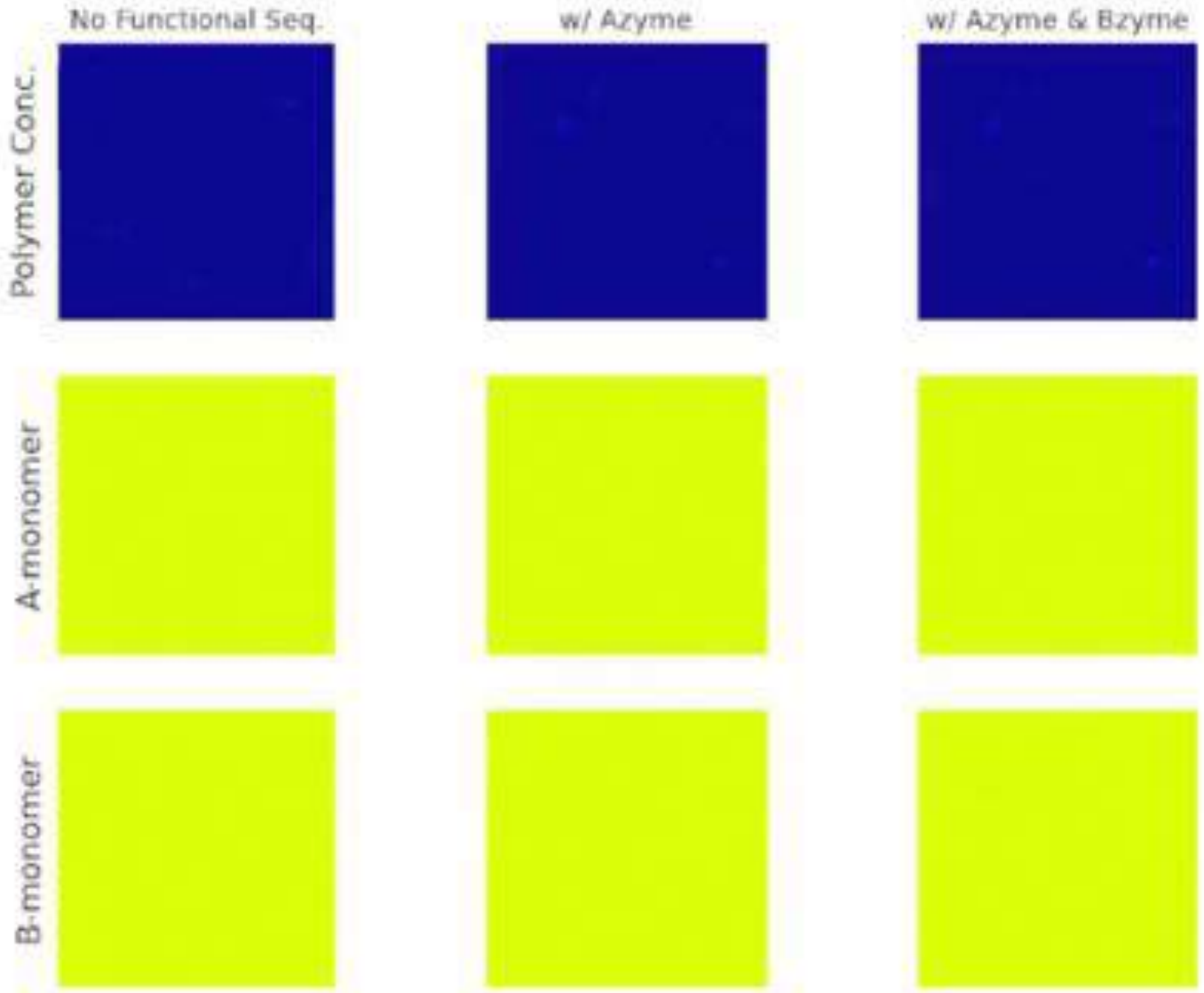
$k_h = 0.1$

$k_p = 0.01$

$k_m = 1.0$



Walker, Grover, Hud, *PLoSONE*, 7:e34166 (2012).


No Functional Seq.
w/ Azyme
w/ Azyme & Bzyme
Polymer Conc.
A-monomer
B-monomer

New insights

- Optimal system behavior occurs at “sweet spots.”
  - Some reversibility of polymerization is critical for the generation of diversity, but with too much, heredity is lost.
  - Some diffusion of species is needed for resource allocation, but limiting diffusion promotes diversity and selection.
- Cooperative networks could emerge step-wise.
- Emergent clustering
  - Did not expect to see clusters since the polymers have no explicit interaction in the model.
  - Clustering is driven by monomer recycling.
  - Limited diffusion on surfaces could provide early compartmentalization prior to lipid-based protocells.

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Features to be included in real systems

- Backbone reversibility and monomer recycling
- Environmental cycling
  - Drive condensation polymerization and hydrolysis
  - Drive duplex separation and replication
- Limited diffusion to bias mobility
- Selection based on function

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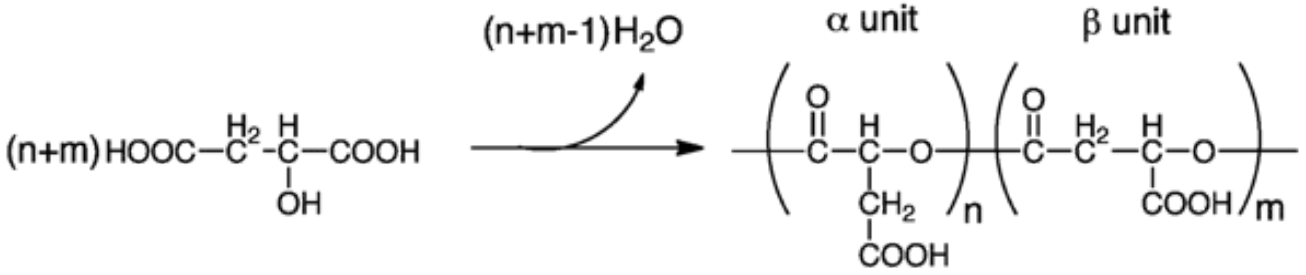
Reversible linkages: a candidate

- Hypothesis: Polyesters are prebiotic precursors to peptides
  - Ribozome catalysis of α-hydroxy acid coupling
  - Polymer interactions defined by sidechains, not backbone hydrogen bonds
  - Ester bond polymerizes more readily than amide bond
- Polyesters can be synthesized under day/night cycles



Orgel, L. E., "Some consequence of the RNA world hypothesis." *Origins of Life and Evolution of the Biosphere*, **33**(2), 211-218 (2003).

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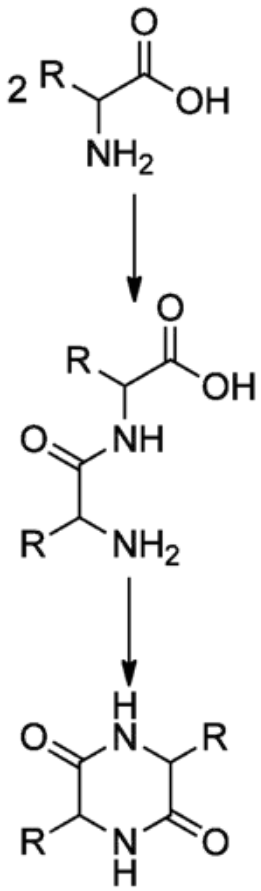
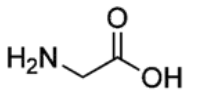
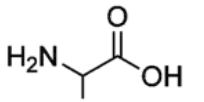
Amino and hydroxy acids

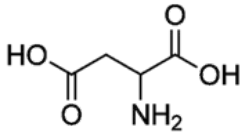


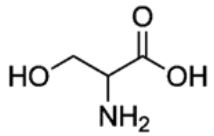
- Peptide problem
  - Bond formation free energy: 2-4 kcal/mol
  - Diketopiperazine sink
- Ester solution
  - Bond formatino free energy: -1 kcal/mol
  - Cyclization is reversible

α-amino acids

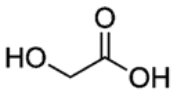

glycine

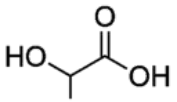

alanine

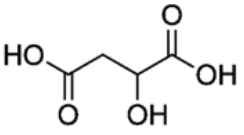

aspartic acid

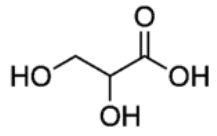

serine

α-hydroxy acids


glycolic acid


lactic acid


malic acid


glyceric acid

Martin RB. *Biopolymers* (1998)
Williams RJ *et al.*, JACS (1928)
Houk KN *et al.*, JOC (2007)

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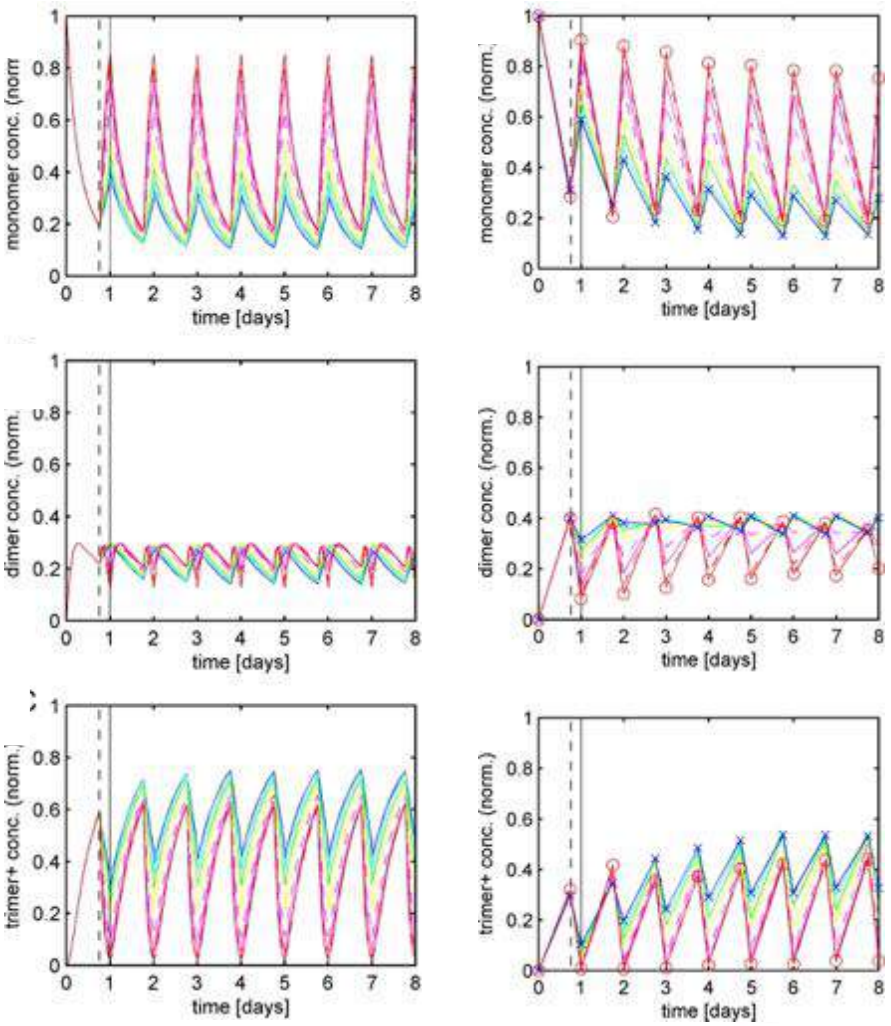
Polymerization under cycling

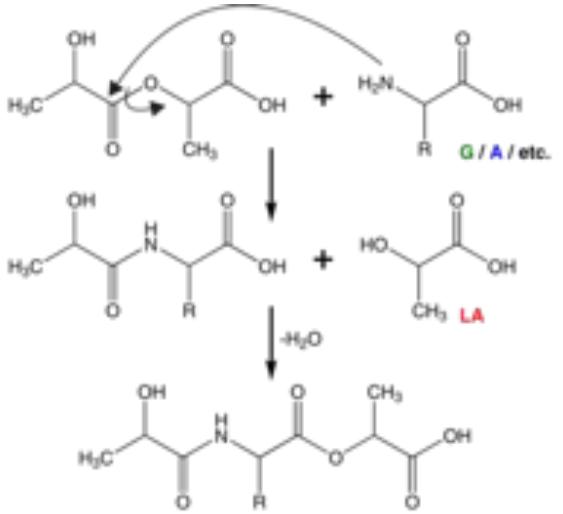
- 8 cycles of 1 day each
  - 18 hours open/dry
  - 0.5 hours rehydrate and sample
  - 5.5 hours capped
- 25 mM L-malic acid
  - No buffering/pH control
- Characterization by GPC
- Cross-over behavior seen in dimer
  - Monomer-dimer exchange
  - Dimer-trimer exchange
- Ratchet followed by cyclic steady state

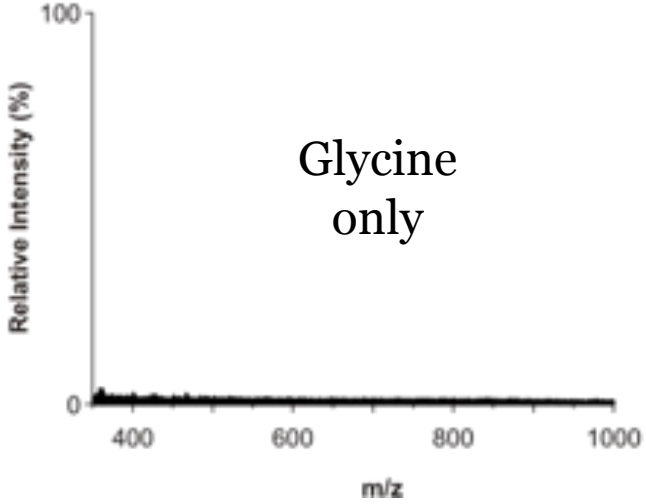
Mamajanov, *Macromolecules* (2014)



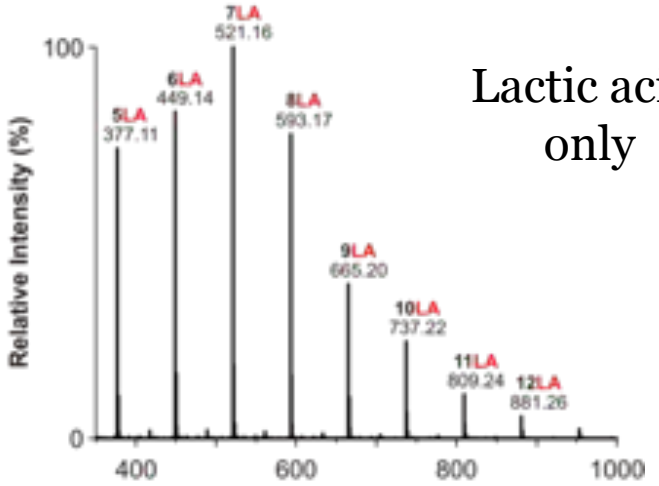
Dry T = 85° C, Wet T = 60-85° C

Ester-amide exchange: a path back to peptides

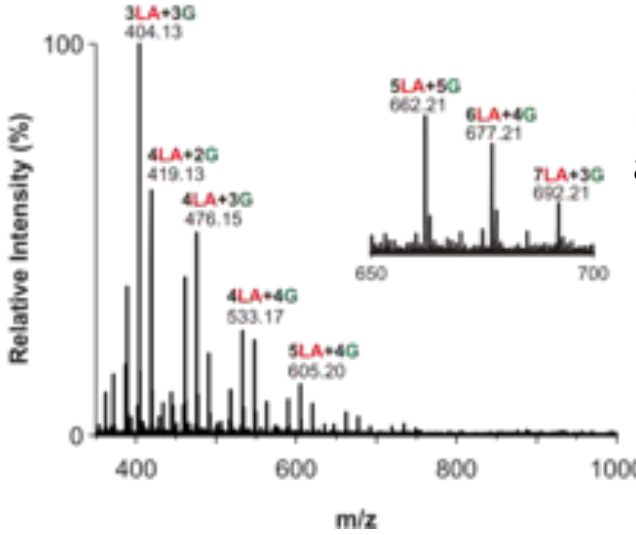




Glycine only



Lactic acid only



Lactic acid and glycine

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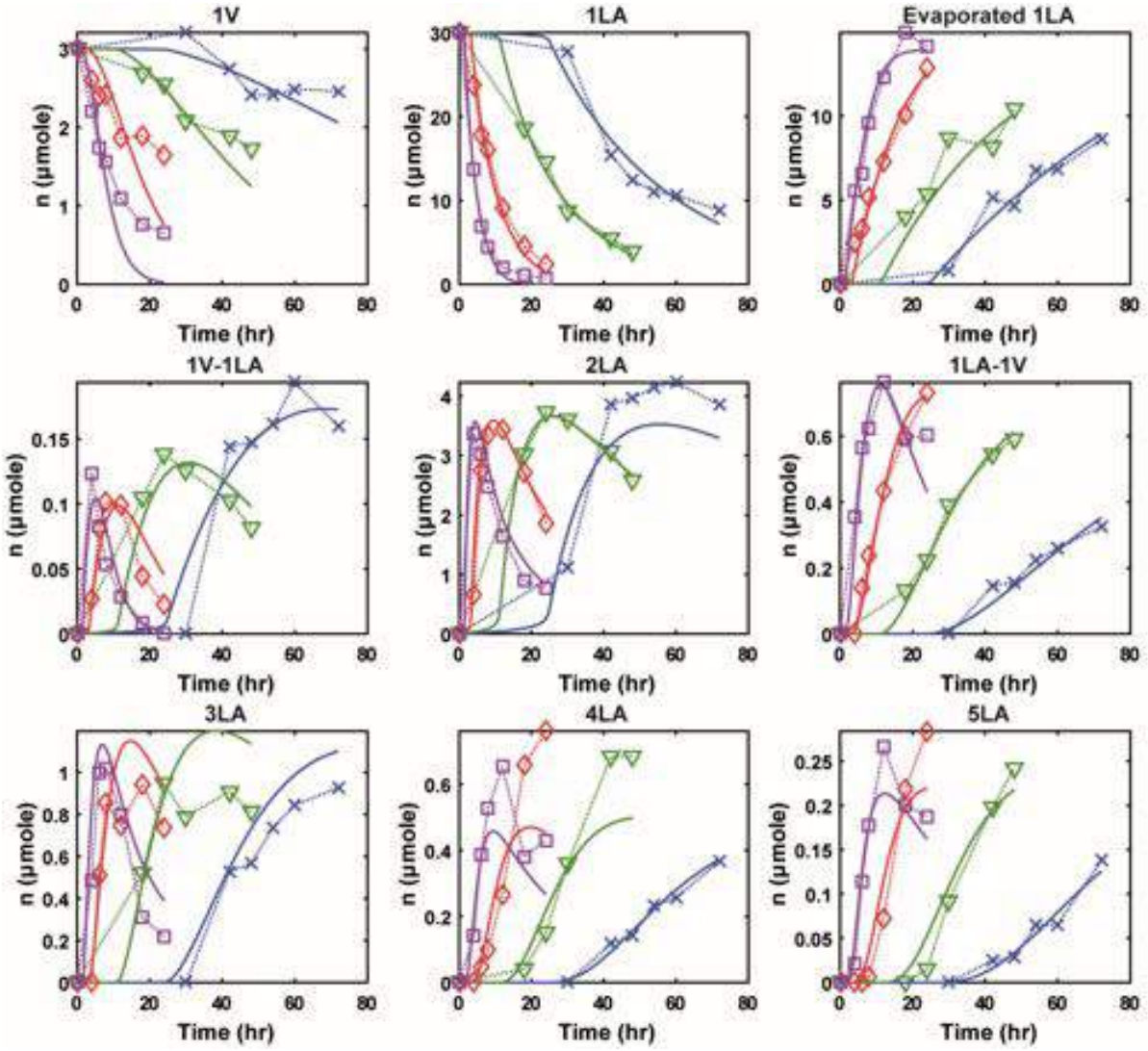
Forsythe, Yu, *Angewandte Chemie* (2015)

A system-level model



Our theoretical results and the experimental data achieved agreement qualitatively and quantitatively.

This allows us to pursue more in-depth understanding of the reaction.

Reaction at four different temperatures:
95 ^{0}C, 85 ^{0}C, 75 ^{0}C and 65 ^{0}C.

Yu, *Phys Chem Chem Phys* (2016)

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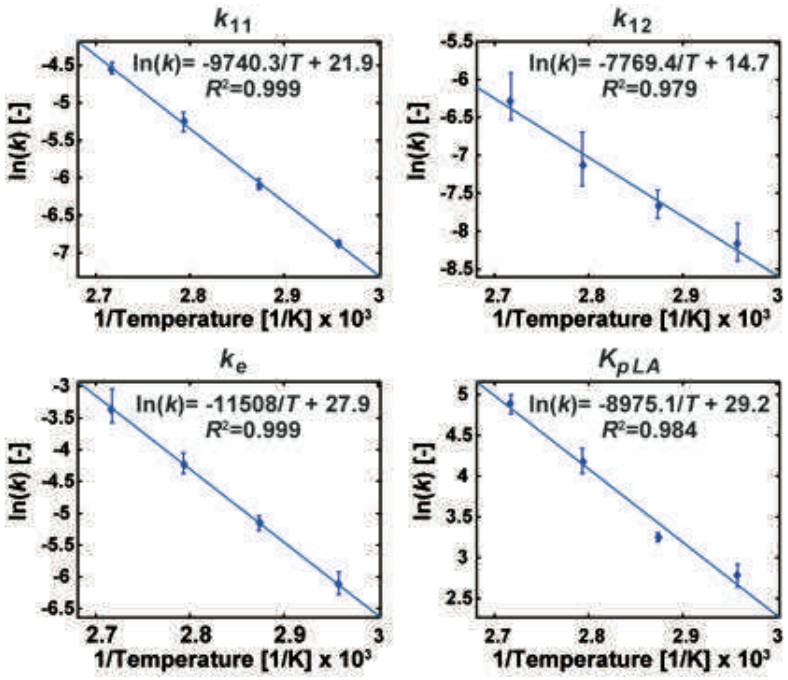
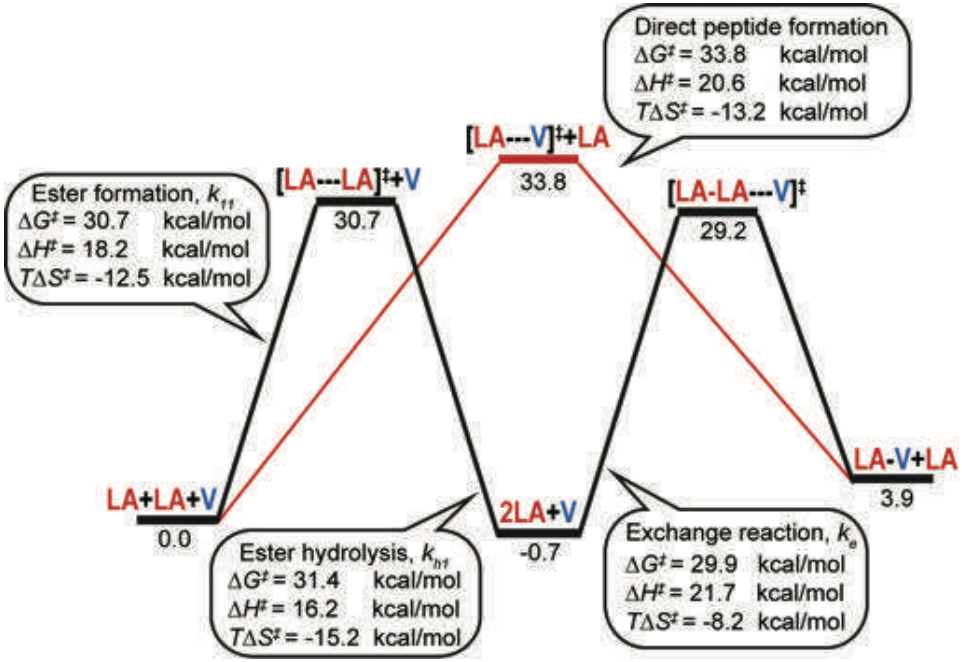
Analysis of the model





The rate constants follow the empirical Arrhenius equation even the reaction proceeds under nearly dry state.

Yu, *Phys Chem Chem Phys* (2016)

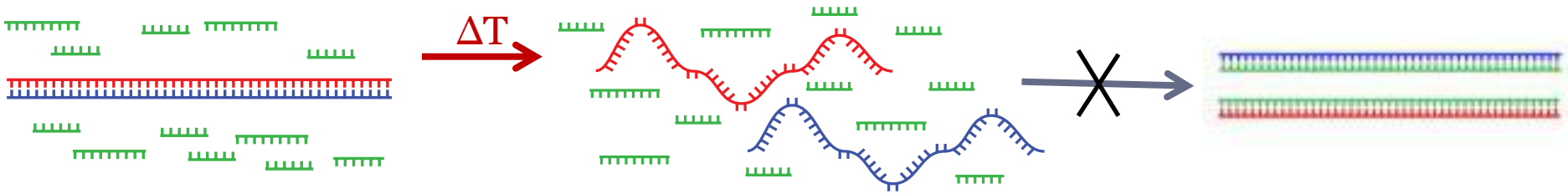
Calculation of reaction activation from the Arrhenius equation reveals the **ester-mediated pathway** is indeed more favorable than the direct amide bond formation.

New insights

- Original idea: Hydroxy acids could play the role of amino acids in prebiotic chemistry
- Turn in the road: Hydroxy acids may be the *catalyst* for amino acid polymerization
  - Ester-amide exchange with polyesters
  - Peptides form as the more stable product over time.
  - Depsipeptides could also be important co-polymer evolutionary intermediates.
- Life uses condensation polymers *because* they were able to recycle in water, and therefore evolve. Not a "problem."

The strand inhibition problem

- Before the appearance of coded proteins, duplex separation likely occurred by heating.



- However, at lower temperatures, the template duplex reforms before oligonucleotides have the opportunity to associate with the single stranded templates.
- Synthesis on a template strand is inhibited by the presence of the complementary template strand.

Szostak, *Journal of Systems Chemistry* (2012)

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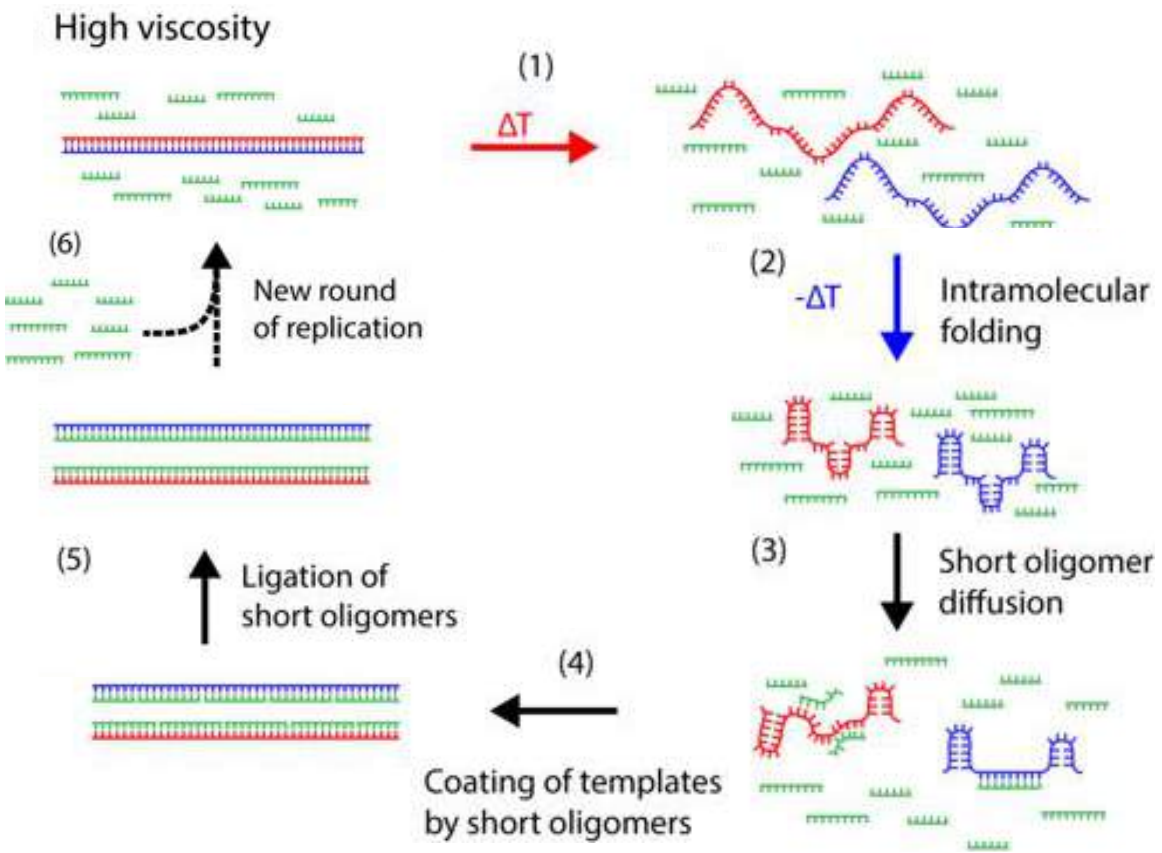
Hypothesis

Thermal cycling in viscous environments can be utilized to overcome strand inhibition and promote template-directed nucleic acid synthesis.

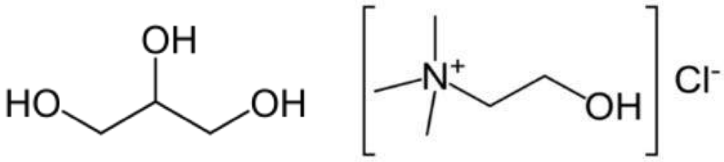


Viscosity-mediated replication can promote copying from long (gene-length) templates of arbitrary sequence.

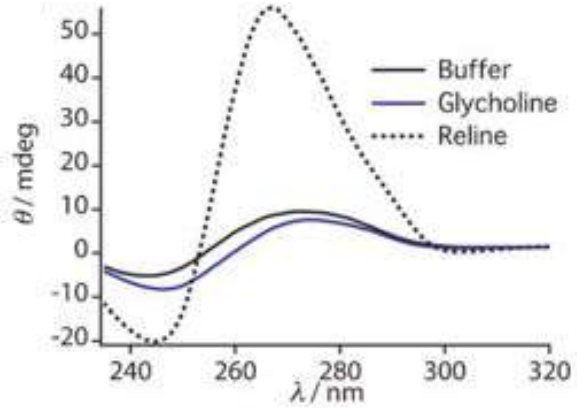
He, *Nature Chemistry* (2017)

Choosing a viscous environment

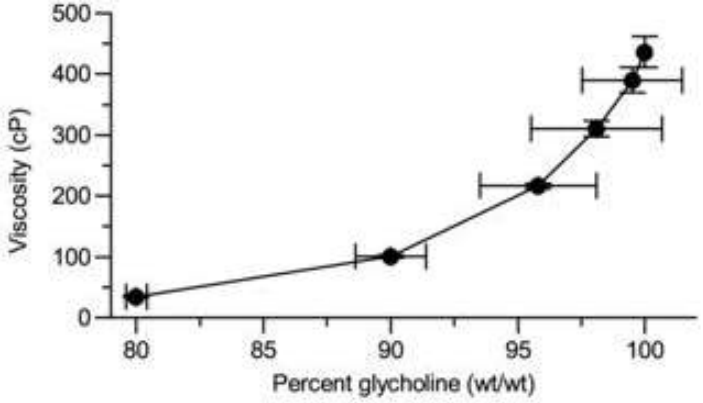
Glycholine: glycerol and choline chloride in a 4:1 molar ratio



B form DNA is retained



Highly hygroscopic and miscible with water



Does not depress duplex melting temperatures as severely as reline

Melting temperatures of DNA duplex species				
Species	**Base Pairs**	**%GC**	**T_m in Aqueous Buffer (°C)**	**T_m in Glycholine (°C)**
Hairpin	7 bp stem, 3 nt loop	41	79.5	44.7
32-mer duplex	32	47	72.2	49.0
3 kb duplex	2957	50	88.0	50.7

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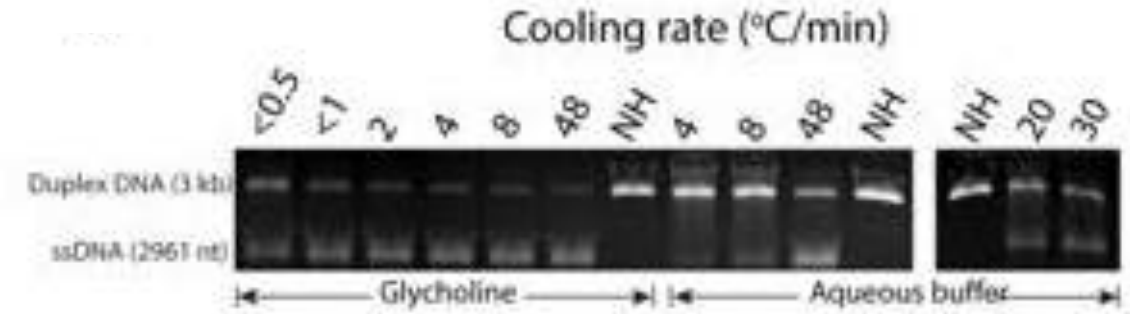
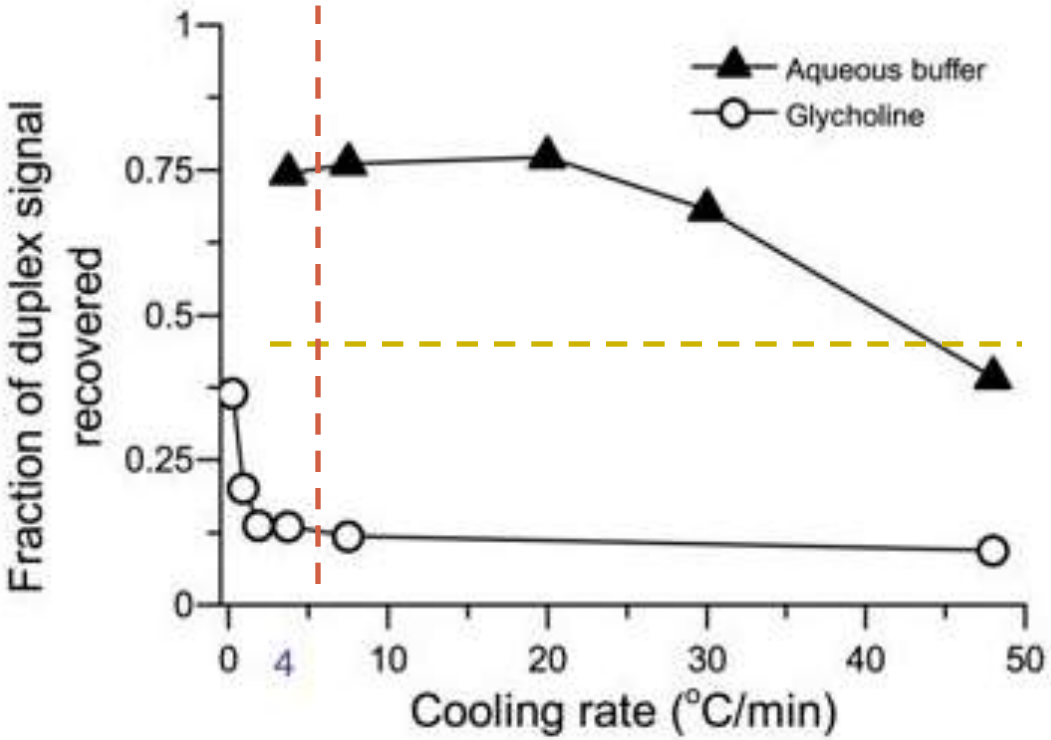
Tuning kinetic trapping with cooling rate





Much more gradual cooling rates can be utilized to kinetically trap a 3 kb duplex as single strands in glycholine compared to aqueous buffer.

A cooling rate of 4°C/min was utilized in all subsequent experiments.

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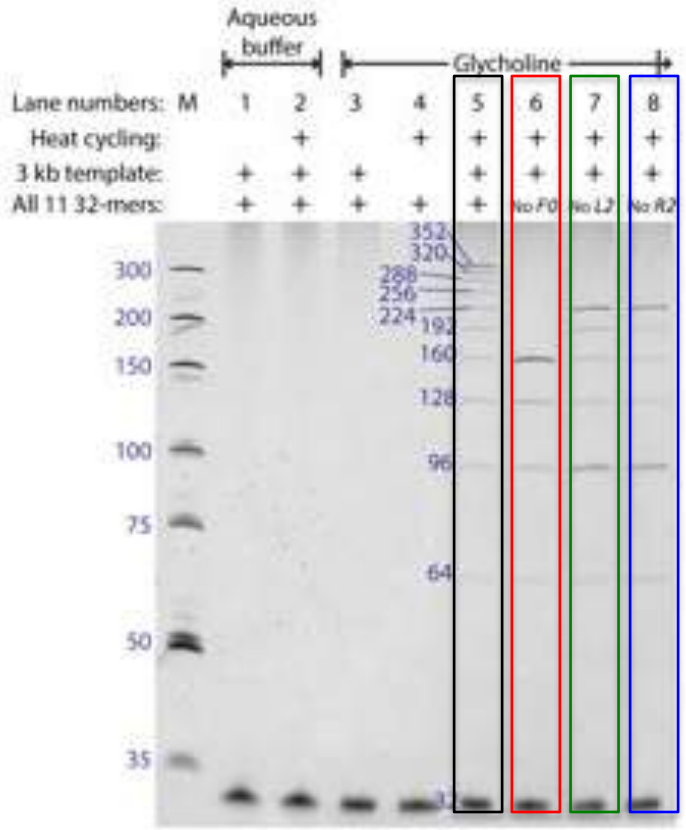
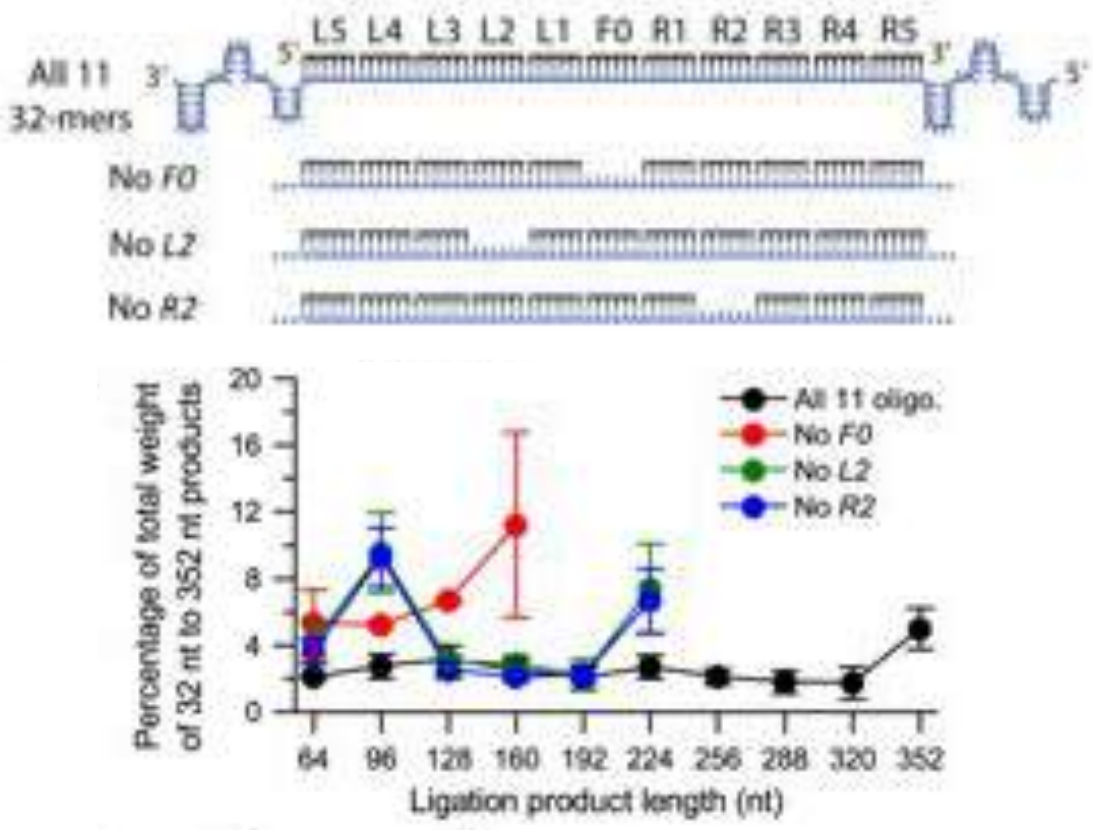
Ligation of assembled oligonucleotides

Ligation was carried out by T4 DNA ligase, added *after* the oligonucleotides have already been assembled on the template (overcoming strand inhibition).





Information transfer from a 3kb duplex template

He, *Nature Chemistry* (2017)

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Progression of ideas

- Earlier idea: Use non-aqueous solvents drive condensation polymerization, solving the water problem (Mamajanov, Angewandte Chemie (2010)).
- Original idea: Add viscogens to *aqueous* buffer to preferentially slow the reannealing of the duplex.
- Version 2: Use small molecule *non-aqueous* viscous solvents instead.
- Version 3: Desired behavior observed, perhaps more due to *intramolecular folding* than preferential diffusion.
- New insight: Viscous environments may help select for long strands and folded structures.
  - Under "typical" conditions, unfolded structures are easier to replicate because they are more accessible.

Closing remarks

- The Peptide World may be more accessible than has previously been thought, challenging the dominant RNA World Hypothesis.
- In an aqueous environment, condensation polymers may have been selected in life because they could evolve.
- Viscous environments may drive selection for folding and function.
- Research serendipity may (or may not) suggest that these systems mimic some key features of prebiotic environments and chemistries.

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Publications

- "A viscous solvent enables information transfer from gene-length nucleic acids in a model prebiotic replication cycle," C. Y. He, I. Gállego, B. Laughlin, M. A. Grover, N.V. Hud, *Nature Chemistry* (2017), DOI:10.1038/nchem.2628.
- "Kinetics of prebiotic depsipeptide formation from the ester–amide exchange reaction," S. Yu, R. Krishnamurthy, F. M. Fernández, N. V. Hud, F. J. Schork, M. A. Grover, *Phys. Chem. Chem.* Phys., **18**, 28441–28450 (2016), DOI: 10.1039/c6cp05527c.
- "Universal sequence replication, reversible polymerization and early functional biopolymers: A model for the initiation of prebiotic sequence evolution," S. I. Walker, M. A. Grover, N. V. Hud, *PLoSONE*, 7(4) e34166 (2012), DOI: 10.1371/journal.pone.0034166.
- "Surveying the sequence diversity of model prebiotic peptides by mass spectrometry," J. G. Forsythe, A. S. Petrov, W. C. Millar, S.-S. Yu, R. Krishnamurty, M. A. Grover, N. V. Hud, F. M. Fernandez, *PNAS* (2017), DOI: 10.1073/pnas.1711631114.

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