Non-existence of error correction in plausible prebiotic amyloids

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Nanda *et al.* have claimed that equilibrating networks of peptides can 'correct errors' by decreasing the proportion of side-chain products and D-amino acid configuration. Their system involved highly concentrated peptides having sequences able to template the reactions by forming β-sheets. The three amino acids used would not have been present prebiotically. Most of the individual experiments led to the wrong results, but a MATLAB simulation indicated that some combinations of chemically modified and activated peptides could favour the linear peptides. *This was not validated experimentally.* The mathematical model had been expertly calibrated for an unrealistically high concentration of peptides (lower concentrations gave the wrong results) and limited to 0–2.5 minutes. Examination of the computed predicted trends confirmed what was already known from the laboratory results: the results (which affected only a single amino acid residue in the final peptide), were *at best a transient artefact* which would have disappeared within minutes, long before the two peptides would have condensed.

In a review article on prebiotic amyloids, key advocate Maury wrote, in 2018:

> "The prebiotic relevance of the β-sheet networks and assemblies was recently highlighted ... by Nanda *et al.* [21] who demonstrated error correction within replication networks through the emergence of short polymers exhibiting selective autocatalytic properties."[1]

Error correction is a fundamental property of living cells, and the emergence of *replication networks* under prebiotic conditions would indeed be major news. Selective autocatalytic networks involving processes relevant to life would also be a significant breakthrough.

Therefore, examination of the referred paper titled "Emergence of native peptide sequences in prebiotic replication networks", by Nanda *et al.*, published in the prestigious journal *Nature Communications*, seemed justified.[2] Had significant discoveries been made?

Experiment 1: reaction of peptides E and N alone

This is another study involving synthetic peptides which produced large β-sheet complexes under the right laboratory conditions.[3–5] In the current experiments, two complementary polypeptides were designed, labelled E (Electrophile) and N (Nucleophile).[2] The three amino acids used were phenylalanine(Phe), glutamic acid (Glu), and proline (Pro).

The E isomers contained five residues plus capping groups:

E isomers: ABA-Glu-Phe-Glu-Phe-Glu-COSR

where ABA = 4-acetamidobenzoate, and the end carboxylic acid of glutamic acid had been activated to a thioester, SR = 4-mercaptophenylacetic acid (MPAA).

The N peptide contained seven residues plus a protecting capping group at the end: carboxylic acid:

N peptide: NH_2-Phe-Glu-Phe-Glu-Phe-Glu-Pro-$CONH_2$

E contained a chiral α-carbon and two -COOH positions which the nucleophile could attack, via the reaction:

$$E + N \rightarrow 2, 2^D, 2_\gamma, 2^D{}_\gamma \quad (1)$$

where the superscript D referred to the D-enantiomer at the α-position and no superscript to the L-enantiomer. The γ subscript refers to the side-chain position, otherwise the end-position is meant. The four isomers formed are shown in figure 1.

Ligation to form product 2 and 2^D were driven by the highly reactive glutamic acid thioester (-SR) on E, which condensed with the free Phe amino position of nucleophile N. Only a trace amount of the thioester group on E migrated to the γ position on $E^D{}_\gamma$ and none to E_γ as shown in figure 6 in their Supplementary file.[2]

Product peptide 2 formed β-sheets as designed, since it possessed alternating hydrophobic and hydrophilic residues and a homochiral backbone. Multiple copies led to amyloid fibril structures consisting of antiparallel peptide bilayers, and, with enough time, large hollow tubes were formed.[2]

An important observation was that the thioester group led to rapid racemization $E \rightleftharpoons E^D$ of the glutamic acid chiral α-carbon. According to figure 6 in their Supplementary file, the L-Glu residue would have only required ≈ 1 month to

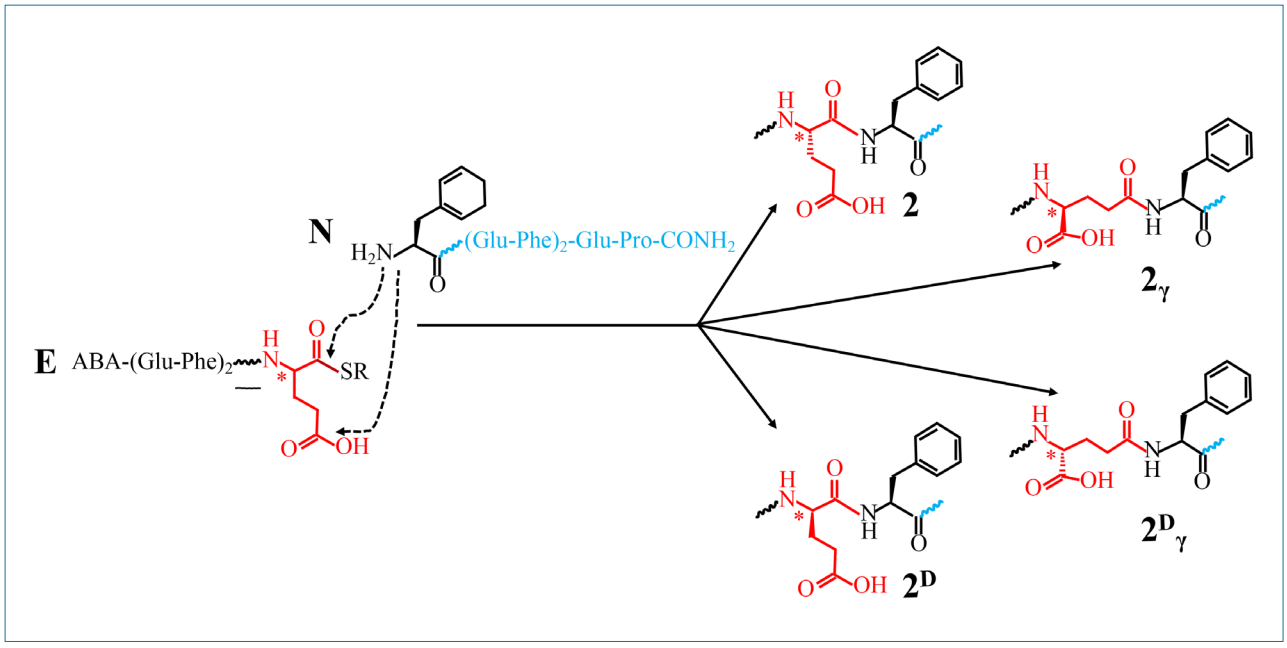

Figure 1. Ligation reaction of peptides **E** (200 μM) and **N** (300 μM) leading to four isomeric products.[2] The glutamic acid involved in the peptide bond is shown in red. ABA = 4-acetamidobenzoate; SR = 4-mercaptophenylacetic acid (MPAA). Artwork by R. Truman based on structures from ref. 2.

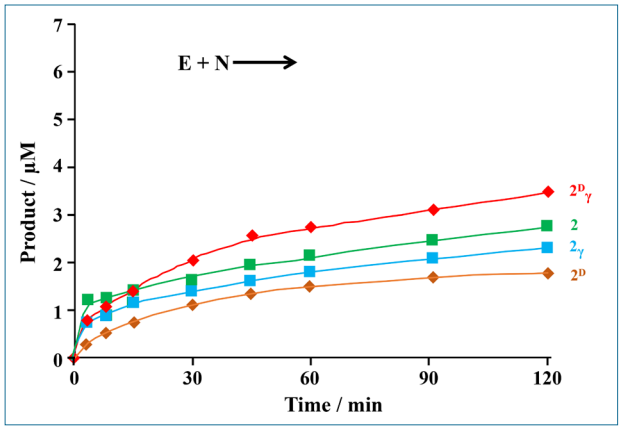

Figure 2. Yield of the four isomers over time from the reaction of peptides E (200 μM) and N (300 μM). Redrawn with slight modification from figure 1a in ref. 2.

become racemic under very mild conditions (75 μM E or E^D in 200 mM MOPS buffer (pH = 7) at room temperature).

Even more startling, examining the reaction E + N → revealed that after 120 hours, the major product was not the hoped-for linear product E having an L-Glu residue, but rather the γ side-chain enantiomer, $2^D{}_\gamma$ having the D-enantiomer as shown in figure 2. Recall that only the end carboxyl group had been activated and not the sidechain one, which nevertheless ended up reacting preferentially.

Figure 2 identifies one critical flaw in this paper: the experiments (and later computer simulation) must be discontinued after about 5 minutes to obtain primarily the desired product 2.

Pure D-enantiomer also led to $2^D{}_\gamma$ preferentially:

$$E^D + N \rightarrow 2^D{}_\gamma \text{ (major product).} \quad (2)$$

As expected, activated γ-COOH also reacted with N to produce primarily $2^D{}_\gamma$:

$$E^D{}_\gamma + N \rightarrow 2^D{}_\gamma \text{ (major product)} \quad (3)$$

$$E_\gamma + N \rightarrow 2^D{}_\gamma \text{ (major product).} \quad (4)$$

In all cases (1) – (4), much less linear peptide 2 was produced, whether starting with D-enantiomer (E^D or $E^D{}_\gamma$) or L-enantiomer (E or E_γ)! The details are shown in figure 5 of their Supplementary file.

Experiment 2: reaction of E and N with a template

Close analogues of the four isomer products 2, 2^D, 2_γ, and $2^D{}_\gamma$, labelled *i*-2, *i*-2^D, *i*-2_γ, and *i*-$2^D{}_\gamma$, were synthesized.[2] All contained the same twelve residue sequences. The isomer structure and chirality matched pairwise (e.g., 2 with *i*-2; 2^D with *i*-2^D). The only difference was the chemical cap added to the N-end amino group: 4-acetamidobenzoate (ABA) for the 2x isomers vs 4-iso-butylamide benzoate (IBA) for the *i*-2x isomers. Both series had their C-end carboxyl -OH replaced by a stabilizing -NH_2 cap to stabilize and hinder chemical reactions there.

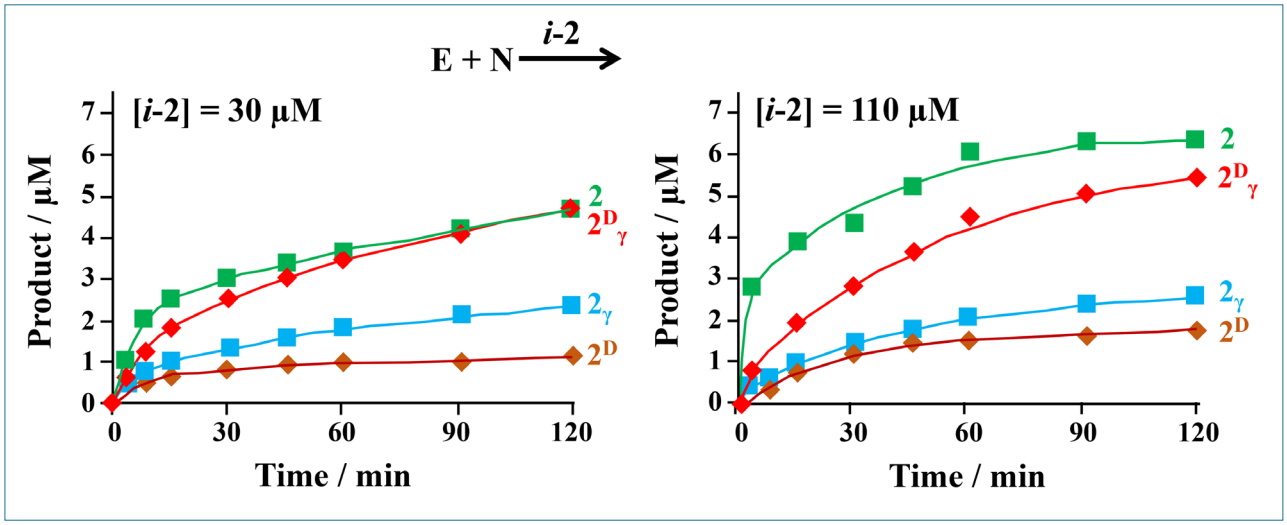

Figure 3. Yield of the four isomers over time from the reaction of E (200 µM) with N (300 µM) in the presence of template *i*-2. Left panel: [*i*-2] = 30 µM. Right panel: [*i*-2] = 110 µM. Redrawn with minor alternations from figures 6a and 6b in ref. 2.

The researchers knew that both 2 and *i*-2 could form amyloid fibrils but did not explain why a different cap was used for the new template peptides nor justify this for origin-of-life purposes.

The E and N peptides were reacted in the presence of template peptide *i*-2, leading to the proportion of four isomers and very low yields shown in figure 3.

Comparing figures 2 and 3 shows that the yield for 2 and 2^D_γ were somewhat higher in the presence of *i*-2, but the template had little effect on the yield of 2^D and 2_γ.

Two important observations from figure 3 were not emphasized by the authors: 1) Yields [2] > [2^D_γ] after 120 mins, *only at the higher concentration of template i*-2; 2) After 120 mins, *the yield of 2 had levelled off* whereas 2^D_γ was continuing to increase at both of the concentrations. After only about four hours, the 'undesired' 2^D_γ would have dominated if the concentration of 2^D_γ indeed continued to increase.

The team then examined the effect of using the other three templates *at only the high concentration of 100 µM* and *for no longer than 120 mins*. After 120 mins, in all cases 2^D_γ was the major product; using template *i*-2^D_λ led to about twice as much 2 as 2^D_γ whereas [2] ≈ [2^D] when using templates *i*-2^D and *i*-2^D_γ. These results are shown in figure 9 of their Supplementary file.

Experiment 3: reaction of E isomers and N in four templates

The four thioester isomers E, E^D, E_γ, and E^D_γ, were reacted with N mixed with 100 µM of templates *i*-2^D, *i*-2_γ, or *i*-2^D_γ.[2] Only some of the twelve combinations were tested and, of these, not all four 2x isomer products were reported. All except one produced 2^D_γ as the major product after 120 mins (see figures 9–12 of their Supplementary files). In other words, no *backbone correction* resulted.

A complex *MATLAB* model predicted backbone correction; i.e., [2] > [2^D_γ] for some combinations according to their Supplementary figure 14 (E^D + N; E_γ + N; E + N + 2; E + N + 2^D_γ; E^D + N + 2; E^D + N + 2_γ). But also the opposite, [2^D_γ] > [2], was found in other cases like E^D_γ + N, according to figure 8 in their main text. However, the special conditions were *0–2.5 minutes and 250 µM each peptide*. Critically, the observation pointed out above for figure 2 was already apparent for the simulated behavior within 2.5 minutes: the yield of 2 begins to level off but that of 2^D_γ continues to increase linearly.

Discussion and conclusions

Peptide 2 was desired since it conserved an L-glutamic acid and avoided the side-branch reaction.

A *MATLAB* model using more than 40 equations (see Supplementary figure 13) predicted that the backbone correction mechanism could occur for some combination of reactants and templates (but not others) under seriously unrealistic prebiotic conditions. This is another example of an expertly tailored origin-of-life experiment having been designed and interpreted to produce the result desired.[6] Critically, why were the predictions from the incomprehensibly complex *MATLAB* model not simply validated by mixing the components simulated? This should have been an easy experiment, no simulation was necessary.

Conclusion 1. The expertly designed system could have, *at best*, affected the chirality and side-chain reaction of a single artificially activated amino acid within a large peptide, in very low yield.

Reaction not realistic in a pre-life Earth

The detailed 2023 review by Kobayashi *et al.* on the major sources of prebiotic amino acids showed no Phe, Glu, or Pro being formed.[7] Analysis of the Murchison meteorites showed no Phe or Pro, and Glu only in ppb levels.[8] Forming peptide bonds in water is very endothermic, and the five- and seven-residue E and N peptides would not have formed in measurable amounts, far less as amphiphilic sequences co-located in high concentration. Over time, the entire sequences would have racemized, especially after glutamic acid had been converted to thioester, as mentioned above, preventing stable β-sheets from forming.[9]

If the reaction E + N → could have occurred prebiotically in high concentration, the proportion of any template present would have been negligible, and the authors admit that the side-branch D-enantiomer 2^{D}_{γ} would have been the major product. If any template had been present, it would have been 2^{D}_{γ} or *i*-2^{D}_{γ}, resulting in an ever more preferential yield of 2^{D}_{γ}, as shown in figure 9 of their Supplementary file.

Had instead some of the best 'template' *i*-2 been present, it would have been in miniscule proportion. Furthermore, figure 3 shows that after 120 hrs more 2^{D}_{γ} than 2 would have been produced anyway.

Conclusion 2. Experimental conditions were selected to optimize the desired peptide 2, whereas natural conditions would have produced more product having both the wrong stereochemistry and side-branch reaction.

The term 'error correction' is misleading

The authors wrote that

> "Finally, we note that our results are in line with the old notion that the emergence of a primitive replicator was crucial and potentially sufficient for the origin of life further down the road."

Suppose this replicator had managed to *remove errors*, leading to an identical sequence. How could anything homogeneous, like identical crystals with no 'errors' left, be, or become, living?

The proposed β-sheets responsible for the "correction of the evolutionary drift towards non-functional heterogeneous mixtures" would have led to only insoluble amyloids, not the necessary variety of proteins having a vast range of tertiary structures.

Error only has meaning with reference to a purpose. Flawed biochemicals are *erroneous* if a biological function is hindered, not because they deviate from some templating, homogenous structure. To their credit, the authors did mention that post-replication error correction after DNA polymerization and removal of defective proteins in cells proceed in completely different manners than their equilibrating network. But it confuses matters to talk about error correction, which has no functional meaning.

Conclusion 3. A mechanism that forces peptides to adopt a homogenous shape has no relevance to error correction in living systems.

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