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Symmetry breaking and chiral amplification in prebiotic ligation reactions

Min Deng[1], Jinhan Yu[1] & Donna G. Blackmond[1]✉

The single chirality of biological molecules is a signature of life. Yet, rationalizing how single chirality emerged remains a challenging goal[1]. Research has commonly focused on initial symmetry breaking and subsequent enantioenrichment of monomer building blocks—sugars and amino acids—that compose the genetic polymers RNA and DNA as well as peptides. If these building blocks are only partially enantioenriched, however, stalling of chain growth may occur, whimsically termed in the case of nucleic acids as "the problem of original syn"[2]. Here, in studying a new prebiotically plausible route to proteinogenic peptides[3–5], we discovered that the reaction favours heterochiral ligation (that is, the ligation of L monomers with D monomers). Although this finding seems problematic for the prebiotic emergence of homochiral L-peptides, we demonstrate, paradoxically, that this heterochiral preference provides a mechanism for enantioenrichment in homochiral chains. Symmetry breaking, chiral amplification and chirality transfer processes occur for all reactants and products in multicomponent competitive reactions even when only one of the molecules in the complex mixture exhibits an imbalance in enantiomer concentrations (non-racemic). Solubility considerations rationalize further chemical purification and enhanced chiral amplification. Experimental data and kinetic modelling support this prebiotically plausible mechanism for the emergence of homochiral biological polymers.

The single chirality of the molecules composing the genetic polymers in modern biology has fascinated scientists and laymen alike since Pasteur's first painstaking separation of the enantiomorphic crystals of a tartrate salt in 1848[6]. Although much progress has been made in developing prebiotically plausible chemical routes to RNA, DNA and peptides, syntheses of these extended chain molecules have often simply assumed the availability of homochiral building blocks. Our laboratories have a long-standing interest in probing the origin of biological homochirality[1], in extensive studies of chemical and physical approaches to symmetry breaking and chiral amplification including asymmetric autocatalysis[7], crystallization[8,9] and kinetic resolutions leading to the enantioenrichment of amino acids and sugars[10–14]. A key next step is to explore whether a non-enantiopure monomer pool of L and D precursors can be used in viable processes for building homochiral polymer chains (that is, successive ligation of monomers of identical chirality).

The search for prebiotic routes that ligate amino acid building blocks of increasing structural complexity has a long history. Early work used COS or CS_2 to polymerize amino acids through formation of N-carboxyanhydrides[15]. Alternative approaches include wet–dry cycling[16] or mineral catalysis[17,18]. References 4,5 reported an N-acylcysteine-catalysed peptide ligation cycle using α-amidonitriles as reaction partners to amino acids. Reference 19 demonstrated chiroselectivity in a template-directed peptide replicator system. Reference 20 discussed the problem of enantiomeric cross-inhibition, and ref. 21 conjectured that random breaking of molecular mirror
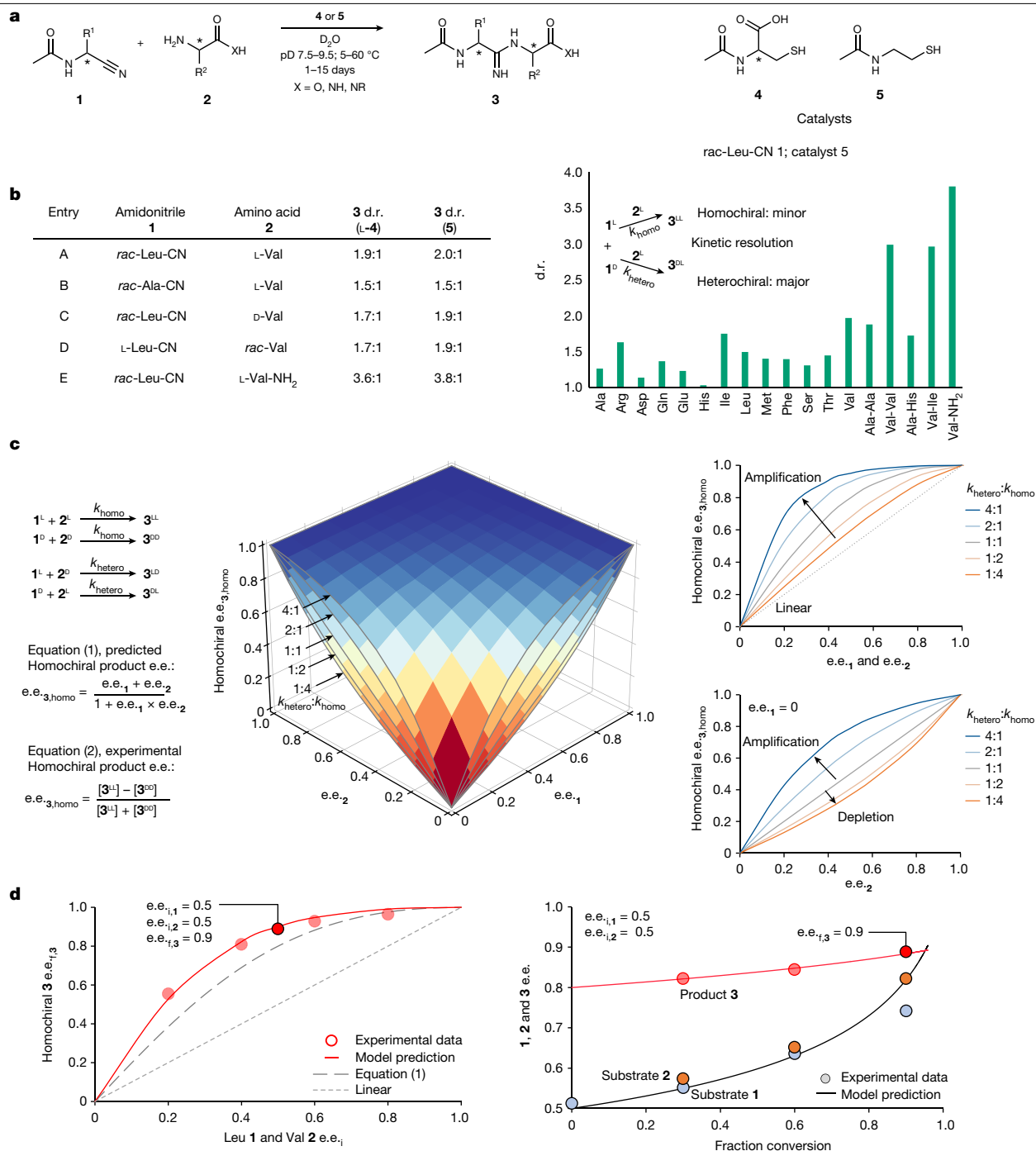
symmetry would be an intrinsic consequence of the combinatorial synthesis of oligonucleotide libraries starting from racemic (equal concentrations of L and D) precursors. Reference 22 studied the separation of homochiral and heterochiral oligopeptides due to physical property differences. References 23–25 synthesized cross-chiral RNA polymerases that can undergo exponential amplification.

Although refs. 3–5 did not report the stereochemistry of the dipeptide product in their catalytic peptide ligation, our work finds that the heterochiral product is preferred. We show that this scenario in fact leads to both symmetry breaking and chiral amplification for both reactants and homochiral ligation products in complex reaction mixtures, even when only a single component is initially non-racemic. Concomitant implications for prebiotic plausibility and generality are discussed.

Diastereoselectivity in prebiotic peptide ligation

The catalytic peptide ligation[3–5] between amidonitrile **1** and amino acid **2** (Fig. 1a), with our optimized reaction conditions, gives dipeptide product **3** with two stereogenic centres denoted by asterisks. The product diastereomeric ratio (d.r.), is defined as the relative concentrations of products **3** exhibiting opposite (heterochiral) and identical (homochiral) stereochemistry in the two stereocentres. Initial reactions were carried out using one enantiopure and one racemic reactant (Fig. 1b). Similar d.r. values are obtained using either racemic amidonitriles **1** and enantiopure amino acids **2**, or the converse (Fig. 1b,

[1]Department of Chemistry, Scripps Research, La Jolla, CA, USA. ✉e-mail: blackmond@scripps.edu

Fig. 1 | Catalytic peptide ligation and chiral amplification under prebiotically relevant conditions. a, Reaction between amidonitriles **1** and amino acids, amino amides or peptides **2** catalysed by thiols **4** or **5**. The stereogenic centre is denoted by an asterisk. **b**, Left: selected results for different substrates and catalysts. For all entries: 400 mM of **1** (except entry D, 100 mM); 100 mM of **2** (except entry D, 400 mM); 400 mM of catalyst **4** or **5**; 5 °C; D₂O; pD = 9 (except entry E, 8.5). L-Val-NH₂ is an amino amide of L-Val. d.r. = heterochiral:homochiral **3**. Right: d.r. for different amino acid, amino amide and dipeptide substrates **2** reacting with *rac*-Leu-CN **1** using catalyst **5**. **c**, Left: e.e. of homochiral dipeptide product **3**, e.e.$_{3,homo}$, written in terms of the

initial e.e. values of substrates **1** and **2** (equation (1)) or homochiral product concentrations **3**^{LL} and **3**^{DD} (equation (2)); see Supplementary Information for derivation of equation (1). Middle: 3D simulations of homochiral product **3** e.e. for reaction of equimolar **1** and **2** for a range of k_{hetero}:k_{homo} values and initial e.e. values of **1** and **2** from 0 ≤ e.e. ≤ 1. Right: selected 2D slices from the 3D plot. **d**, Experimental data and model simulations for the reaction of **a** using Leu-CN **1** and Val **2** at different e.e. values. Symbols: experimental data; solid lines: model simulations. Left: homochiral product **3** e.e. as a function of initial e.e. values of **1** and **2**. Right: temporal e.e. values for **1** and **2** and homochiral **3** as a function of conversion in the case shown in the left plot with e.e.$_{i,1}$ = e.e.$_{i,2}$ = 0.5.

entries A and D). Similar d.r. values are also observed regardless of whether the stereochemistry of the enantiopure reaction component is D or L (Fig. 1b, entries B and C). These last two observations confirm that the reaction proceeds as a kinetic resolution of the racemic reaction partner.

Diastereoselectivities d.r. ≤ 4:1 in product **3** are observed, varying with the nature of the substrates (Fig. 1b). The reaction does not occur in the absence of a thiol catalyst. Most strikingly, the achiral catalyst **5** gives diastereoselectivities similar to those observed with the enantiopure catalyst L-**4** (Fig. 1b, left), implying that selectivity is largely

substrate controlled. The fact that the catalyst need not be chiral enhances the potential prebiotic viability of the system, as the provenance of a catalyst's enantioenrichment is no longer a consideration.

Homochiral amplification via heterochiral selectivity

Synthesis of authentic samples of the diastereomeric products **3** revealed that the major products of the ligation reaction are the heterochiral 3^{DL} or 3^{LD} species. As the stereochemistry of the enantiopure reactant is retained in the product **3**, as was also found previously[3-5], this result indicates that L-amino acids preferentially ligate with the D-amidonitrile and vice versa. Although heterochiral preference might suggest that the reaction is an unlikely candidate for the emergence of homochirality in peptide chains, our next experiments hinted at several separate chiral amplification mechanisms that come into play when both reaction partners are present in non-racemic, non-enantiopure form. For example, in the reaction of 20% enantiomeric excess (e.e.) L-Leu-CN **1** with 20% e.e. L-Ala or 20% e.e. L-Val amino acids **2** (as fractional values: $e.e._{i,1} = e.e._{i,2} = 0.2$, where the subscript i denotes initial value), the homochiral peptide ligation product **3** was observed with amplified percent e.e. values of 40% and 43%, respectively. This experimental result may be rationalized in part by the kinetic model in Fig. 1c, left, in which simulations[26] of the elementary bimolecular reaction rate steps describe the formation of two sets of enantiomeric dipeptide products, homochiral 3^{LL} and 3^{DD} and heterochiral 3^{LD} and 3^{DL}. The intrinsic e.e. of homochiral product **3**, $e.e._{3,homo}$, may be predicted in terms of the initial fractional e.e. values of substrates **1** and **2**, $e.e._1$ and $e.e._2$ (equation (1)) or, alternatively, given in terms of the homochiral product concentrations of 3^{LL} and 3^{DD} (equation (2)) shown in Fig. 1c, left. Simulations carried out of reactions using equimolar **1** and **2** for a variety of initial substrate **1** and **2** e.e. values with various k_{hetero}:k_{homo} ratios are shown in the three-dimensional (3D) plot in Fig. 1c, middle (see Supplementary Video 1), and in Fig. 1c, right, for two cases extracted from that plot. Ligation between two non-enantiopure, non-racemic substrates leads to chiral amplification in the homochiral product $e.e._{3,homo}$ compared to that of the monomer substrates (Fig. 1c, top right). This result is rationalized as a multiplicative effect: the reaction rate between substrates **1** and **2** of the same chirality is overall second order ($[1^L] \times [2^L]$ and $[1^D] \times [2^D]$). If both **1** and **2** are present with e.e. values towards L, the multiplicative concentration driving force produces the LL-dipeptide product with $e.e._{3,homo}$ that is amplified over both monomer substrate e.e. values. Chiral amplification is observed in all cases in which both substrates **1** and **2** exhibit non-racemic, non-enantiopure e.e. values towards the same hand, regardless of the diastereoselectivity of the reaction, albeit with smaller magnitude for homochiral preference. When one substrate is initially present as racemic, however (Fig. 1c, bottom right), homochiral preference results in a depletion, rather than an amplification, of product e.e. Thus, counter-intuitively, the preference for heterochiral selectivity in the ligation reaction aids, and homochiral selectivity hinders, enantioenrichment of the homochiral ligation reaction product.

Temporal chiral amplification

Further experimental studies reveal a second chemical mechanism for chiral amplification that operates in the case of heterochiral preference. Figure 1d, left, shows that the experimental homochiral product **3** e.e. values (red circles) for the reaction of *rac*-Leu-CN **1** and L-Ala **2**, which exhibits heterochiral diastereoselectivity of k_{hetero}:k_{homo} = 2:1, exceeds the amplification predicted from equation (1). Consider the reaction between 50% e.e. L-Leu-CN **1** and 50% e.e. L-Val **2**, highlighted with a darker red circle in Fig. 1d, left. Figure 1d, right, shows the temporal $e.e._{3,homo}$ for this case, which compares experimental data (symbols) to the kinetic model simulation (solid lines) with excellent agreement. The reaction initially gives the multiplicative amplification to 0.8 (80%)

$e.e._{3,homo}$ predicted by equation (1), which then increases with reaction progress to 0.9 (90%) $e.e._{3,homo}$ at 90% conversion. As the heterochiral species (3^{LD} + 3^{DL}) sequester monomers from each substrate in equal proportions (1^L + 2^D and 2^L + 1^D), a larger proportion of the minor enantiomer of each substrate is continually directed towards these heterochiral chains, causing the e.e. of homochiral product **3** to increase as the reaction proceeds. The e.e. values of remaining substrates **1** and **2** also increase over the course of the reaction. Equation (1) for $e.e._{3,homo}$ remains valid in cases in which k_{hetero}:k_{homo} ≠ 1, with the proviso that instantaneous $e.e._t$ values of $e.e._1$ and $e.e._2$ are used (see Supplementary Information).
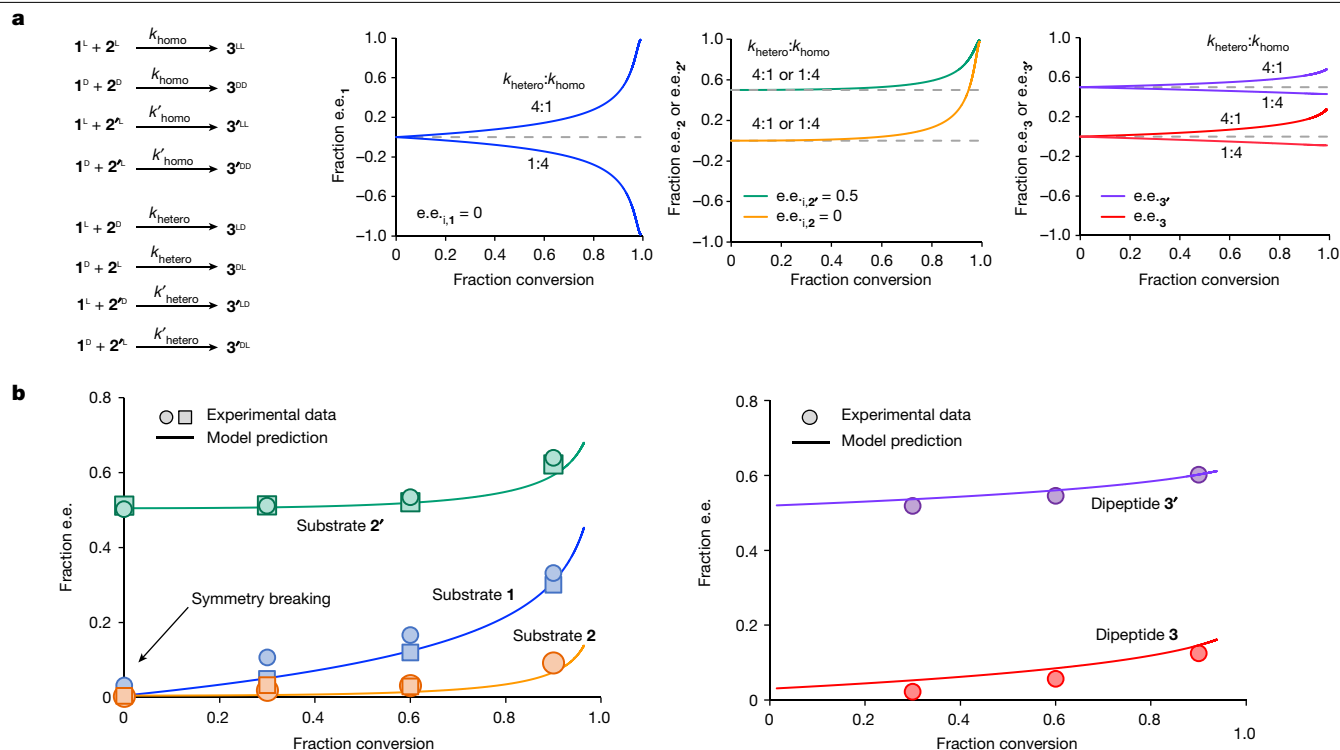
These results demonstrate two distinct chemical chiral amplification mechanisms that augment each other to provide robust enantioenrichment in the ligation of near-racemic reaction partners, arising from the multiplicative kinetic concentration dependences given by equation (1) and from the heterochiral diastereoselective preference that enhances e.e. over the course of the reaction. The model bears resemblance to the Frank model for spontaneous asymmetric synthesis[27] in that it is the "mutual antagonism" of the heterochiral ligation that provides the driving force for chiral amplification in the homochiral products.

Symmetry breaking and chirality transfer in complex mixtures

We next considered the likely possibility that prebiotic mixtures would be more complex than a single reaction by extending the kinetic model to the case shown in Fig. 2a, in which one racemic amidonitrile, *rac*-**1**, can ligate with two different amino acids, *rac*-**2** and 50% e.e. L-**2′**, and **2′** is the sole species with an initial imbalance of left- and right-hand monomers. When heterochiral selectivity is preferred, all homochiral products exhibit chiral amplification towards the LL-dipeptide, and all of the remaining racemic monomeric building blocks show symmetry breaking and enantioenrichment towards L over the course of the reaction. For the case of homochiral preference, by contrast, substrate *rac*-**1** and both products **3** and **3′** exhibit depletion of e.e. The two amino acid substrates **2** and **2′** both show chiral amplification under either homochiral or heterochiral preference.

These modelling results were confirmed in experiments (Fig. 2b) for either *rac*-Leu-CN or 3% e.e. L-Leu-CN **1** reacting competitively with amino acids *rac*-Leu **2** and 50% e.e. L-Val **2′**. Note again that the solid lines represent not a fit to, but a prediction of, the experimental e.e. values. A key feature of the model in cases in which heterochiral selectivity is dominant is that symmetry breaking will occur, and chiral amplification will evolve, from an imbalance in enantiomers in a single component of multicomponent competitive ligation reaction mixtures, even when all other reaction partners and the catalyst are racemic. If the sole non-racemic reaction partner has an imbalance towards the L enantiomer in these heterochirally diastereoselective reactions, all substrates and products will also be enriched towards the L enantiomer as the reaction proceeds. This simple mechanism provides a prebiotic means for chiral amplification and chirality transfer across a range of racemic amino acid monomers engaged in competitive catalytic peptide ligation. An important implication of this model is that prebiotic amino acid and peptide enantioenrichment need not be addressed one compound at a time, using different physical and chemical strategies for chiral amplification tailored to each individual molecule. Such a chirality transfer mechanism perhaps predates strategies used by modern biology, in which key amino acids use enzymatic transamination to produce many of the other non-essential amino acids.

We envision that the kinetic model developed here may be extended to reactions forming other genetic polymers (for example, in the ligation of partially enantioenriched RNA or DNA nucleotide monomers). An extension of our peptide ligation model (see Supplementary Information) predicts that several published mechanisms for

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Fig. 2 | Ligation reactions in complex mixtures. a, Rate equations and simulation results for competitive reactions of substrate **1** with either substrate **2** or **2'** for different cases of k_{hetero}:k_{homo} with e.e.$_{i,1}$ = 0; e.e.$_{i,2}$ = 0 and e.e.$_{i,2'}$ = 0.5 (50%). The simulations were carried out with $k_{homo} = k'_{homo}$ and $k_{hetero} = k'_{hetero}$ with ratios as shown on the plots. The initial concentrations of [**1**]:[**2**]:[**2'**] were 2:1:1.

The dashed lines show 1:1 diastereoselectivity. **b**, Experimental data and model prediction for the reaction of Leu-CN **1** (200 mM) with Leu **2** (50 mM) and Val **2'** (150 mM); e.e.$_{i,1}$ = 0 (squares) or 3% e.e. (circles), e.e.$_{i,2}$ = 0; e.e.$_{i,2'}$ = 50%; catalyst **5** (400 mM), pD = 9.5, 5 °C, D_2O.

enantioenrichment in the Powner–Sutherland synthesis of the single RNA monomer D-cytidine[10,11,28] may be sufficient for the emergence of homochirality in all ribose-based nucleotide polymer chains. The model may also help to address questions about prebiotic RNA–protein interactions[29].

Purification and chiral amplification via crystallization

During these experimental reaction studies, we uncovered an additional physical mechanism that enhances the prebiotic relevance of these peptide ligation reactions. Reaction between monomers of *rac*-Leu-CN and L-Val proceeds with heterochiral selectivity of **3**^{DL}/**3**^{LL} = 2:1 (Fig. 3a, left). When both substrates are present in racemic form (Fig. 3a, right), the reaction proceeds with similar d.r. until about 70% yield, after which the solution-phase concentration drops precipitously, a highly insoluble cosolvate precipitates from solution, and the d.r. of the molecules remaining in solution undergoes an inversion from heterochiral to homochiral preference, with (**3**^{DL} + **3**^{LD})/(**3**^{LL} + **3**^{DD}) = 1:3.
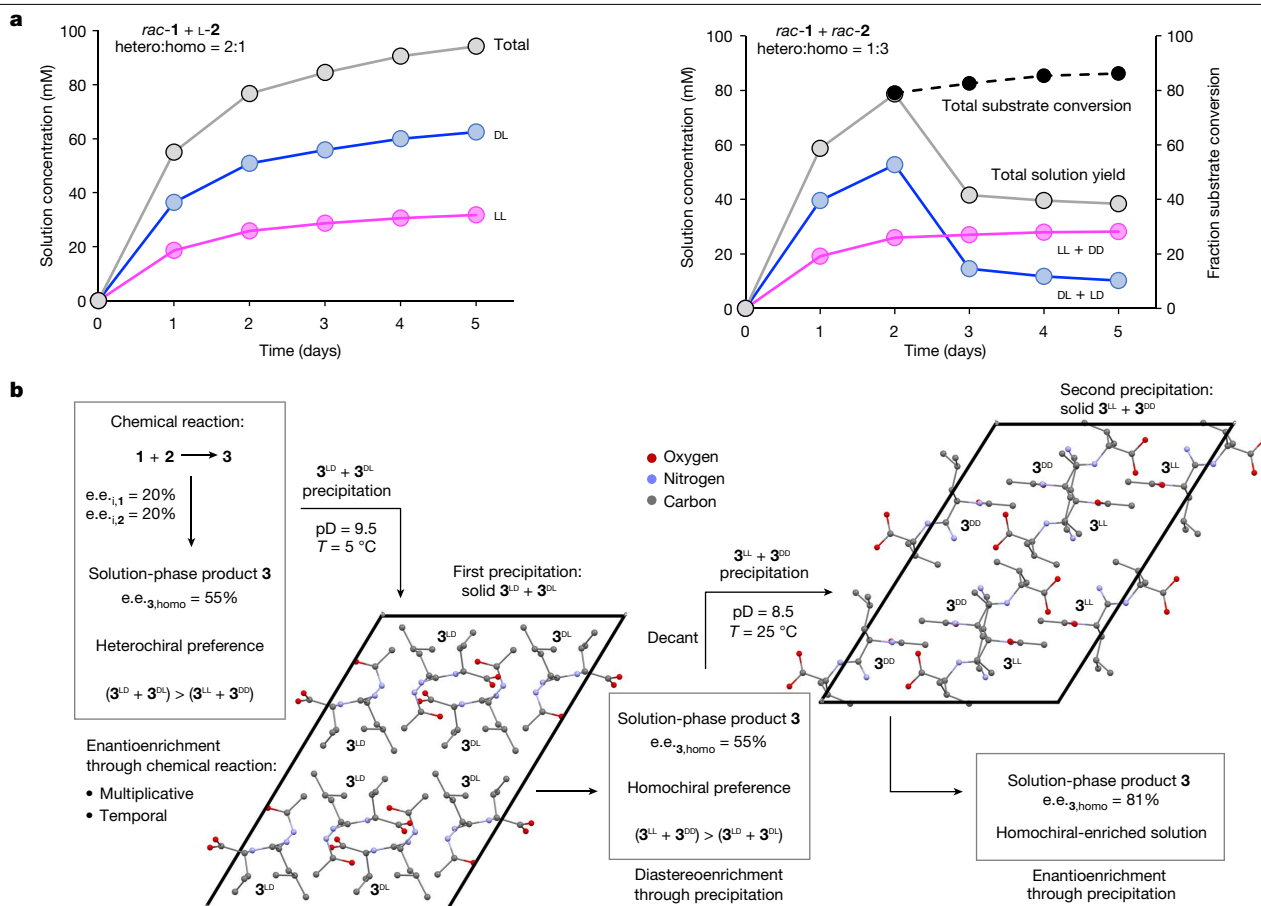
We obtained the structure of this cosolvate (**3**^{DL} + **3**^{LD}) crystal (Fig. 3b, left). All four substrate molecules—**1**^{L}, **1**^{D}, **2**^{L} and **2**^{D}—are required in the reaction for this compound to form. A similar reversal of solution-phase d.r. is observed when racemic mixtures of *rac*-Leu-CN **1** are used with mixtures of *rac*-Leu and *rac*-Val or *rac*-Ala and *rac*-Val, suggesting that in general the heterochiral dipeptide cosolvate (**3**^{DL} + **3**^{LD}) is less soluble than its homochiral dipeptide counterparts. Prebiotically relevant conditions for such ligation reactions using both **1** and **2** at very low e.e. values may thus benefit from cosolvate crystallization as an in situ means of chemically purifying the solution phase, removing heterochiral product **3** from solution in a physical process that does not alter the solution-phase product e.e. but preserves the chiral amplification of the homochiral product e.e. dictated by the two chemical processes described while producing a solution consisting primarily of homochiral dipeptides.

After filtration of the resultant solution containing enantioenriched homochiral product **3**, adjusting the pD and temperature of the supernatant allows a subsequent precipitation event of a second mixed solid containing equal amounts of **3**^{LL} and **3**^{DD} (Fig. 3b, right) preferentially removing a larger fraction of the minor component **3**^{DD} and thus enhancing the chiral amplification achieved in the chemical reaction[9]. The differential solubilities of the (**3**^{DL} + **3**^{LD}) and (**3**^{LL} + **3**^{DD}) crystals allows for two sequential crystallization processes that result in chemical and stereochemical purifications of the solution phase following ligation reactions containing all enantiomers of **1** and **2**. Figure 3b shows the workflow that ultimately yields 81% e.e. homochiral ligation product **3**^{LL} after two chemical amplification processes and two physical amplification processes from a starting point of 20% e.e. L-**1** and L-**2**.

Conclusions

We demonstrate that a recently reported thiol-catalysed peptide ligation reaction occurs preferentially between reaction partners with opposite stereochemistry. Paradoxically, this heterochiral selectivity provides a mechanism for enantioenrichment of both the homochiral dipeptide products and the remaining substrates. In addition, an achiral thiol catalyst provides selectivities similar to those observed using an enantiopure asymmetric thiol catalyst, lending further prebiotic plausibility to the model. The substantially lower solubility of the major heterochiral dipeptide products ultimately provides a solution containing essentially only enantioenriched homochiral dipeptides. Additional solution-phase enantioenrichment is obtained owing to a second precipitation process that preferentially removes the minor homochiral dipeptide from solution. Synergy between chemical and

Fig. 3 | Physical processes leading to chemical purification and chiral amplification of dipeptide product 3. a, Reaction of Leu-CN **1** (400 mM) and Val **2** (100 mM) with achiral catalyst **5** (400 mM) in D_2O at pD = 9.5. The solution-phase concentration (in mM) equals the percentage yield. Left: *rac*-Leu-CN **1** and enantiopure L-Val **2**. Right: *rac*-Leu-CN **1** and *rac*-Val **2**. **b**, Crystal structures of sequential cosolvate products **3** from the ligation reaction between *rac*-Leu-CN **1** and *rac*-Val **2** (heterochiral **3**^LD + **3**^DL and homochiral **3**^LL + **3**^DD). Experimental results and workflow are shown for solution-phase e.e._{3,homo} and product **3** d.r. after reaction and after each precipitation event starting from 20% e.e. L-Leu-CN **1** and 20% e.e. L-Val **2**.

physical processes provides a holistic approach to the single chirality that is a key feature in the emergence of functional genetic polymers from a prebiotic world presumably comprising racemic or near-racemic building blocks.

Prebiotic routes to rationalize the chirality of the 19 chiral proteinogenic amino acids have invoked one-by-one enantioenrichment protocols because most proposed mechanisms are constrained by the specific properties of the molecules treated. Our results suggest that the chiral amplification and chirality transfer model demonstrated here could have served as the primordial, ur-level mechanism for symmetry breaking, chiral amplification and chirality transfer emanating from a single, partially enantioenriched amino acid leading to the general prebiotic synthesis of proteinogenic peptide chains formed in complex mixtures containing multiple racemic reaction partners. The presence of a single non-racemic reactant—as is likely to be found in a prebiotic scenario—enables symmetry breaking in all other reaction partners and provides chiral amplification of all reaction products. These results provide a prebiotic network for symmetry breaking, enantioenrichment, solution purification and chirality transfer that could generally rationalize the emergence of homochirality in genetic polymers including peptides, RNA and DNA.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information,

acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-024-07059-y.

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Data availability

All source data are available in the Supplementary Information, including a video of the 3D plot in Fig. 1c. CoPaSi modelling files are available from the authors upon request. Crystallographic data for structures have been deposited at the Cambridge Crystallographic Data Centre, with numbers 2237664 (LD-DL) and 2238268 (LL-DD).

Author contributions D.G.B. conceived the project, verified kinetic modelling, constructed the figures and wrote the first draft of the manuscript. M.D. carried out all experimental work (including developing ^{13}C-NMR methodology for chiral analyses of complex product mixtures), developed the chiral amplification model and carried out kinetic modelling. J.Y. aided with critical chiral analyses. All authors contributed to discussion, interpretation of results and writing of the final version of the manuscript.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-024-07059-y.
Correspondence and requests for materials should be addressed to Donna G. Blackmond.
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