Corrected: Author correction

SYNTHETIC BIOLOGY

Foldamers wave to the ribosome

Ribosomes have now been shown to accept certain initiator tRNAs acylated with aromatic foldamer–dipeptides thereby enabling the translation of a peptide or protein with a short aromatic foldamer at the N-terminus. Some foldamer–peptide hybrids could be cyclized to generate macrocycles that present conformationally restricted peptide loops.

Alanna Schepartz

In 1907 Leo Henry Baekeland synthesized the first chemical polymer in his basement in Yonkers, New York, USA. Working alone, Baekeland mixed formaldehyde with phenol and baked the mixture at 140 degrees in a sealed tube. The resulting material was "insoluble in all solvents, does not soften. I call it Bakalite [sic]". Since the days of Baekeland, chemists and polymer scientists have innovated to develop molecular building blocks and elongation chemistries that can specify polymer length, composition, branching, size distribution and much more. The impact of these chemical polymers on our everyday life is profound. And the number of potential building blocks is virtually limitless.

Yet despite this enormous impact and potential, it is still impossible to synthesize a non-α-peptide chemical polymer whose monomer sequence is both diverse and truly defined in the same manner as proteins, RNA or DNA. Sequence-defined chemical polymers could, in theory, store molecular-level data, define anti-counterfeiting tags, fine-tune bulk materials and even accelerate drug discovery. But the reality is that nobody really knows what transformative properties and applications could emerge from polymers with protein-like sequence definition, because molecules of this type have never been prepared. Diverse and truly sequence-defined oligomers and foldamers (even long ones[1]) can be prepared using solid-phase synthesis and other methods, including DNA-templated chemistry[2] or molecular machines[3], but not polymers.

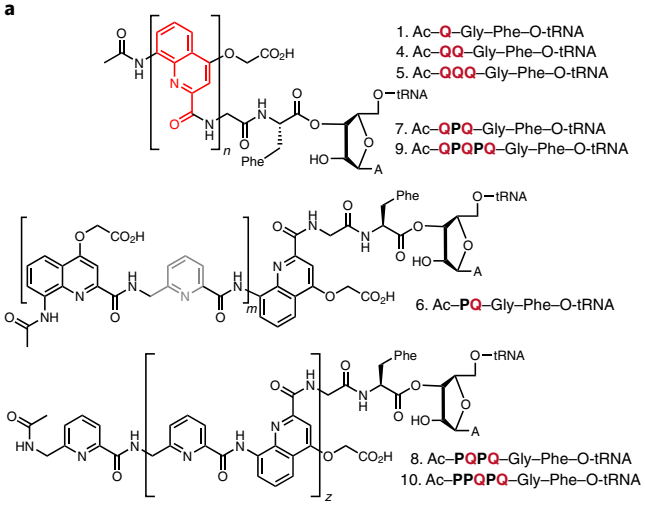
What keeps sequence-defined chemical polymers in an imaginary state is that, as far as we know, in cells, ribosomes naturally form bonds between L-α-amino acids only. Ribosomes capable of promoting templated bond formation between other, more diverse monomer backbones would catalyse a tremendous step forward for polymer science, as they would allow scientists to use mRNA to encode materials in the same way that we currently encode software or DNA. And if these designer ribosomes could operate in living cells, they could support the evolution of new materials in the same way that we currently evolve biochemical polymers and living organisms. The concept builds on a firm foundation: using engineered enzymes and translation factors, wild-type ribosomes can incorporate >200 different unnatural α-amino and hydroxy acids[4] site-specifically into proteins, in vitro, in cells and even in animals[5]. N-Alkyl and D-α-amino acids and β-amino acids have been incorporated into peptides using cell-free in vitro systems[6]. There are even examples in which β-amino acids have been incorporated into a protein, in vitro[7] and in a living cell[8], by designer ribosomes whose peptidyl transferase centre (PTC) has been remodelled.

Writing in *Nature Chemistry*, a team led by Hiroaki Suga and Ivan Huc[9] take one step closer to this goal by demonstrating that in vitro, wild-type *Escherichia coli* ribosomes accept certain initiator tRNAs acylated with aromatic foldamer–dipeptide appendages that are not small (Fig. 1a). Using genetic code reprogramming approaches, the team could fabricate a protein or peptide of which the N-terminus carried an aromatic foldamer — a foldamer–protein hybrid. No bonds to or from a foldamer monomer are formed by the ribosome; the foldamer–protein hybrids result from translation beyond the foldamer–dipeptide appendage. This process requires accommodation of the foldamer appendage within the ribosome PTC and ultimately within the exit tunnel. What is unique about these appendages is their propensity to fold, and how the stability of this fold influences the extent of translation of foldamer–peptide and foldamer–protein hybrids. Inspired by previous work on amphotericin–peptide macrocycles[10], the team now show that appropriately designed foldamer–peptide hybrids can cyclize and that the conformational stability of the resulting product is influenced by foldamer identity. Thus the entities produced could possess some of the favourable attributes of hydrocarbon-stapled peptides[11], and it would be quite interesting if they also could traffic efficiently into the cell interior. Incorporation of a semi-rigid organic template — whose structure is not so different from an aromatic foldamer — into a macrocycle was recently shown to endow that product with remarkable thermal stability[12].

The foldamers studied here were short oligomers carrying **Q** and **P** monomers (Fig. 1a; the structure of a **Q** monomer is highlighted in red; the structure of a **P** monomer is highlighted in grey). It was observed that a tRNA carrying an Ac–**QQ**–Gly–Phe appendage (Fig. 1b, sequence 4) successfully initiated translation of a dodecapeptide whereas one carrying an Ac–**QQQ**–Gly–Phe appendage (sequence 5) did not. The team hypothesized that folding (too much of it) was the problem: **QQQ** is long enough to complete a full helical turn, whereas **QQ** is not. Increasing the concentration of initiation factor 2, which recruits initiator tRNA to the 30S initiation complex[13], did not improve translation initiated with sequence 5, whereas reducing foldamer conformational stability using a **P** monomer (shown in grey) in place of **Q**, as in Ac–**QPQ**–Gly–Phe (sequence 7), led to efficient dodecapeptide translation. The presence of a **P** monomer at the N-terminus of the foldamer seems essential, as even an initiator tRNA that was acylated with Ac–PP**QPQ**–Gly–Phe (sequence 10) performed well. tRNAs acylated with Ac–**PQPQ**–Gly–Phe and Ac–**PPQPQ**–Gly–Phe also effectively initiated translation of a 78-residue bacterial cell-wall protein, Protein G.

Taken together, these observations suggest that some amount of conformational flexibility within the foldamer appendage, especially at the N-terminus, is required to initiate translation using the tRNAs studied. It is possible that translation of tRNAs

a



b

Foldamer appendage	Initiation (foldamer–peptide)	Initiation (foldamer–protein)	Amide NH exchange	Diastereotopic ^{1}H NMR
Ac–**QQ**–Gly–Phe (4)	✓	ND	–	–
Ac–**QQQ**–Gly–Phe (5)	✗	ND	Slow	Yes
Ac–**PQ**–Gly–Phe (6)	✓	✓	–	–
Ac–**QPQ**–Gly–Phe (7)	✓	ND	Fast	No
Ac–**PQPQ**–Gly–Phe (8)	✓	✓	Fast	Partial
Ac–**QPQPQ**–Gly–Phe (9)	✗	ND	Slow	Yes
Ac–**PPQPQ**–Gly–Phe (10)	✓	✓	–	–

Fig. 1 | Engineered initiator tRNAs acylated with foldamer–dipeptide appendages can initiate translation of foldamer–peptide and foldamer–protein products. a, Structures of foldamer-dipeptide appendages used to initiate translation of foldamer–peptide and fordamer–protein hybrids. The foldamers used in this work consist of Q and P monomers; the structure of a Q monomer is highlighted in red whereas that of a P monomer is highlighted in grey. **b**, Table illustrating the complex relationships between foldamer identity, translation efficiency and the extent to which the isolated foldamer folds. A check indicates that a foldamer–peptide or protein product was observed; an X indicates that it was not. ND indicates that the result was not reported.

carrying inhibitory foldamer appendages could be aided by elongation-factor P (EF-P). Recent cryo-electron-microscopy structures of stalled ribosomes revealed how the binding of EF-P stabilizes the P-site tRNA and promotes bond formation[14], and very recent work has shown that EF-P can perhaps even persuade the ribosome to translate consecutive D-amino acids[15]. And, as the N-terminus of the longer foldamers may be approaching the well-characterized exit tunnel constriction, where a loop within ribosomal protein L22 approaches that within ribosomal protein L4[16], it is possible that mutations in these two ribosomal proteins or the associated 23S sequence in a designer ribosome could release the constriction enough to allow a well-folded foldamer to travel past, much as they facilitate translation through stalled sequences containing proline[17].

Perhaps most interesting are the relationships between the stability of an isolated foldamer in vitro and the efficiency of translation, which are complex and demand further study. Isolated foldamers containing the sequences **QQQ**, **QPQ**, **PQPQ** and **QPQPQ**, corresponding to foldamer appendages 5 and 7–9 (Fig. 1a) were compared on the basis of both amide NH exchange rates and the level of induced chirality evident in the corresponding ^{1}H NMR spectra (Fig. 1b). Based on these criteria, foldamers **QQQ** and **QPQPQ** form a stable helical fold, whereas **QPQ** and **PQPQ** do not. What is interesting is that an alternating sequence with a terminal P monomer appears essential: **PQPQ** is unfolded and an initiator tRNA carrying **PQPQ**–Gly–Phe initiates translation, whereas **QPQPQ** is folded and does not. Although some level of unfolding appears to be required to initiate translation of both dodecapeptide and protein G sequences, additional, detailed studies will be necessary to identify the structural requirements for both accommodation within the PTC and subsequent travel through the ribosome exit tunnel.

Despite the advances reported by Suga, Huc and co-workers, it is fair to say that the synthetic and chemical biology communities are still far from the goal of fabricating genetically encoded, sequence-defined polymers, especially those that can be evolved for a desired function. All evidence suggests this goal will require specialized orthogonal ribosomes[18,19] whose active sites have been remodelled to accommodate the linkages and chemistries most desirable in high-value materials such as polyurethanes, polyolefins, aramids, polyketides and even Nylon 6. For applications in vivo, it will also require orthogonal enzymes that load these unusual monomers on orthogonal tRNAs; specialized elongation factors that deliver the tRNAs to the ribosome; others that overcome what will almost certainly be a handicapped catalytic site for bond formation; genomically recoded organisms that enable an orthogonal translation system to operate in parallel[20]; novel synthetic base pairs that cooperate with the cellular machinery[21]; and structural and energetic models for allostery within an enormous ribonucleoprotein machine. The results of these investigations will not only provide a deep understanding of ribosome function and the diverse chemistries that can be supported by engineered translational systems, but also hopefully an array of functional polymers with previously unattainable properties that can be adopted industrially or that benefit society. ❐

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Published online: 22 March 2018
https://doi.org/10.1038/s41557-018-0036-5

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