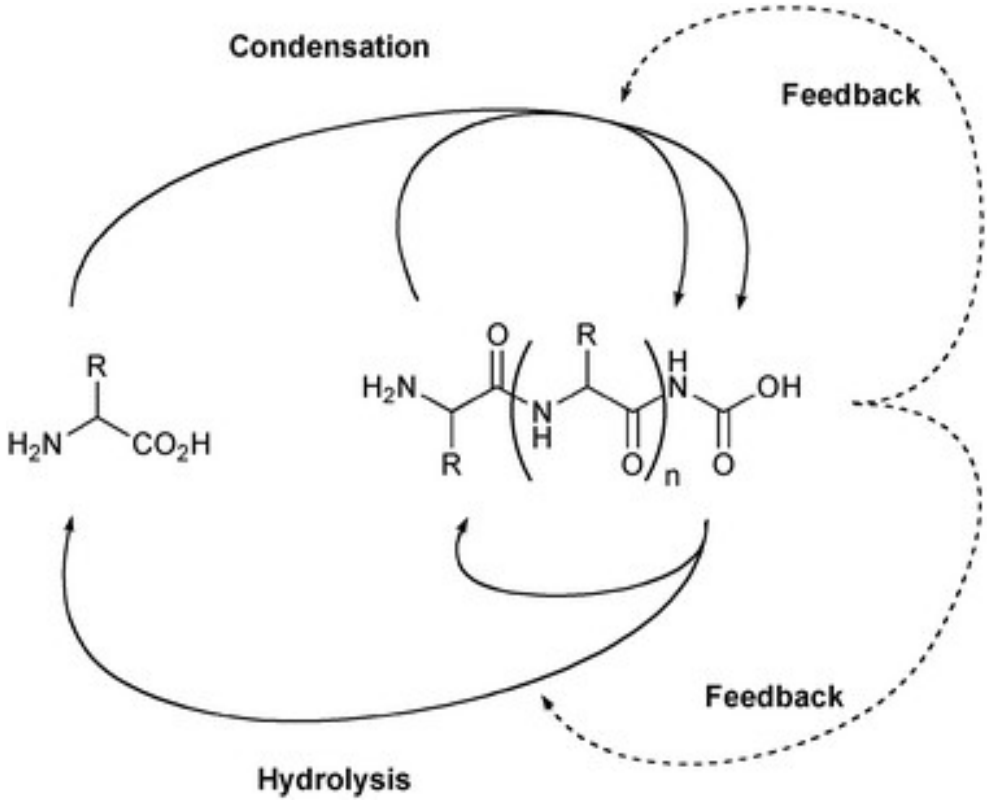
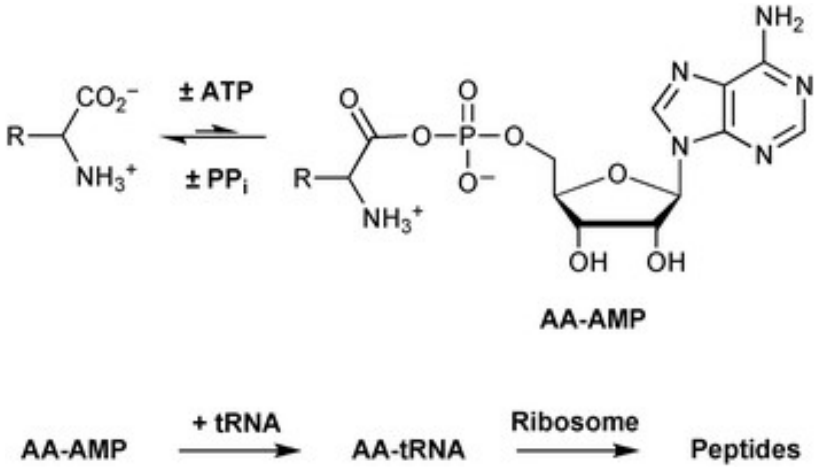
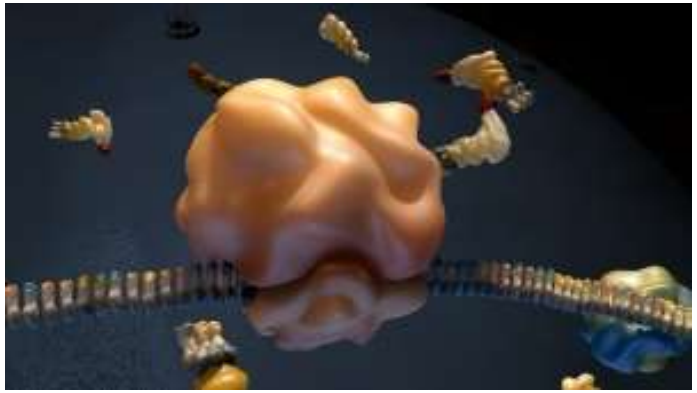
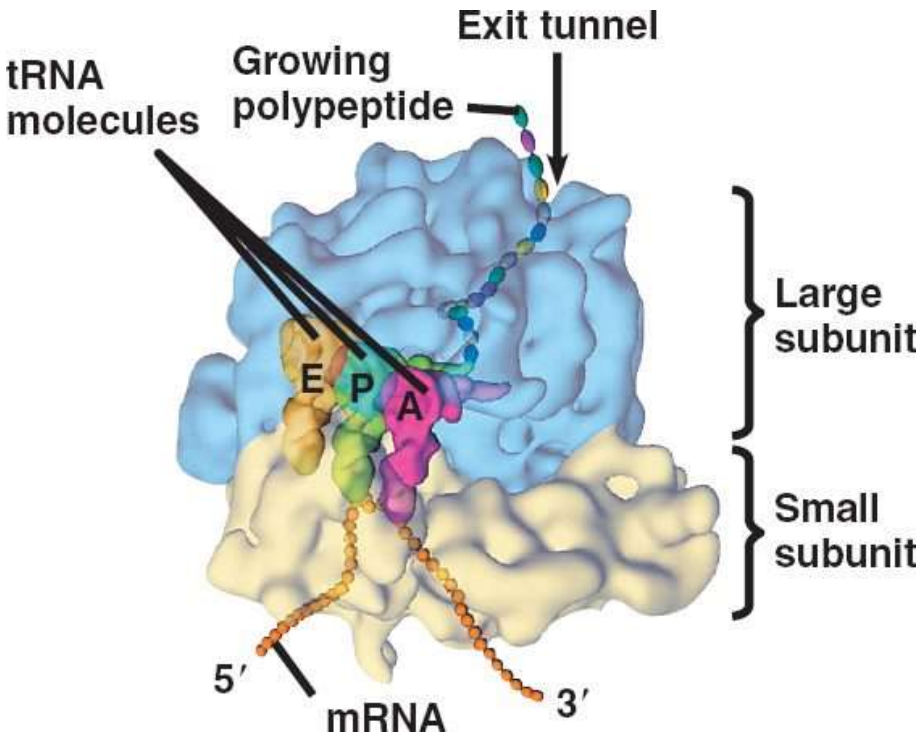
# *Condensation of aminoacids into peptides*



# *Biochemical condensation of aminoacids into peptides*

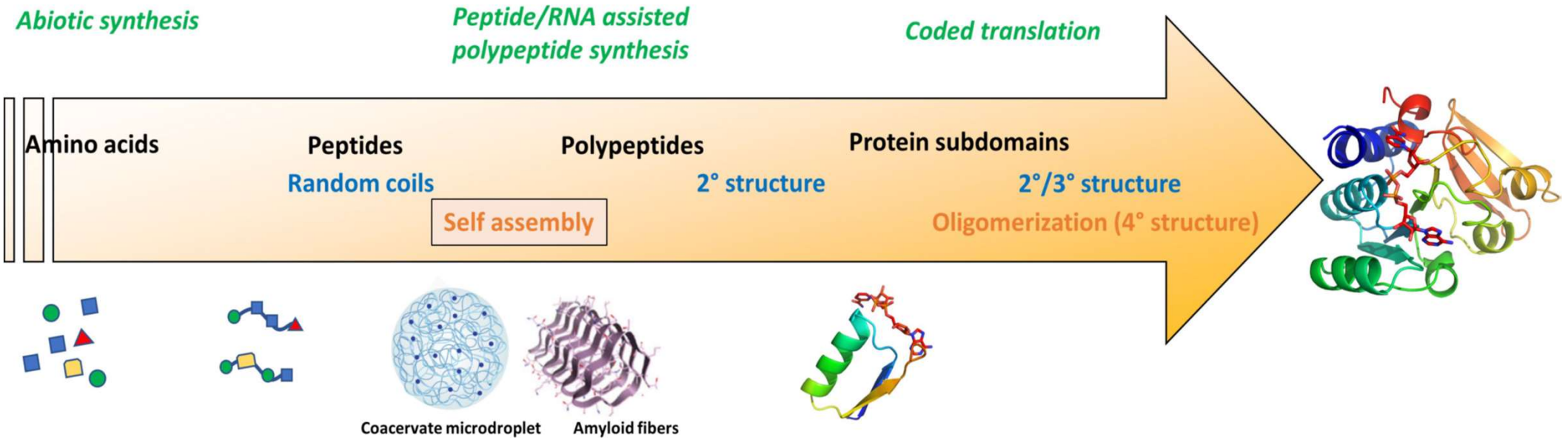




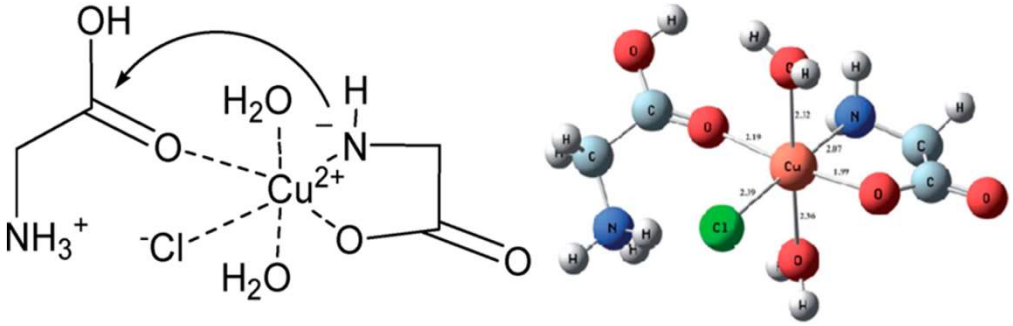

*Nature Publishing Group, www.nature.com/nrg/multimedia*

# *From abiotic aminoacids to modern proteins*




D. Despotovic, D. S. Tawfik *ChemSystemsChem* **2021**, *3, e2100002*

# *Spontaneous vs. assisted dehydratation*

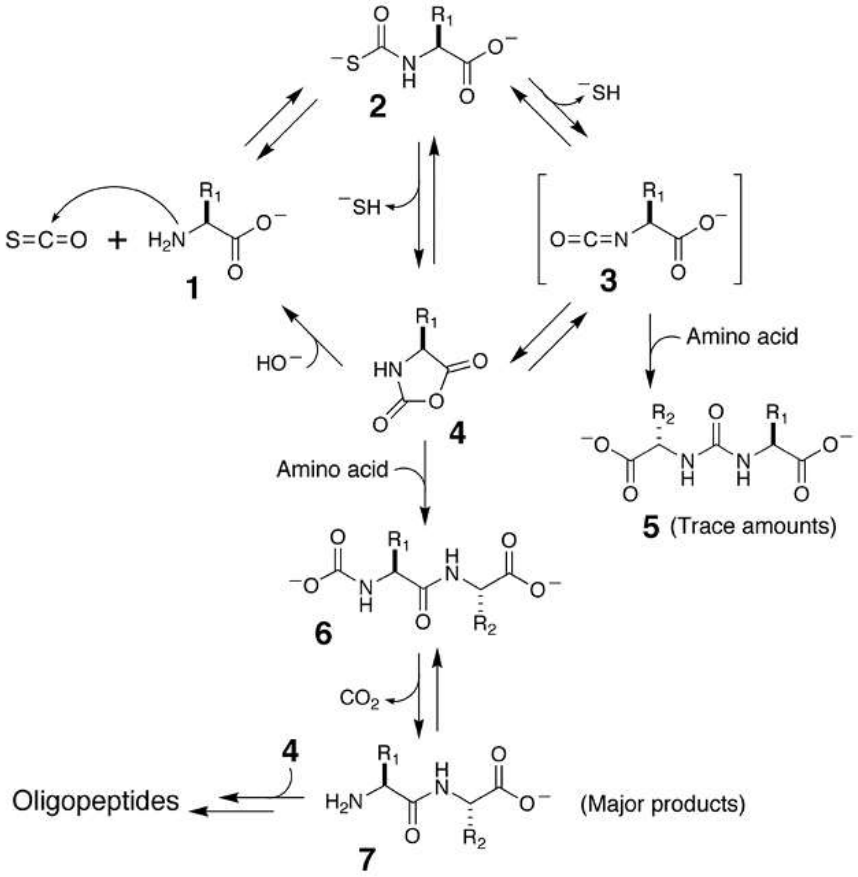


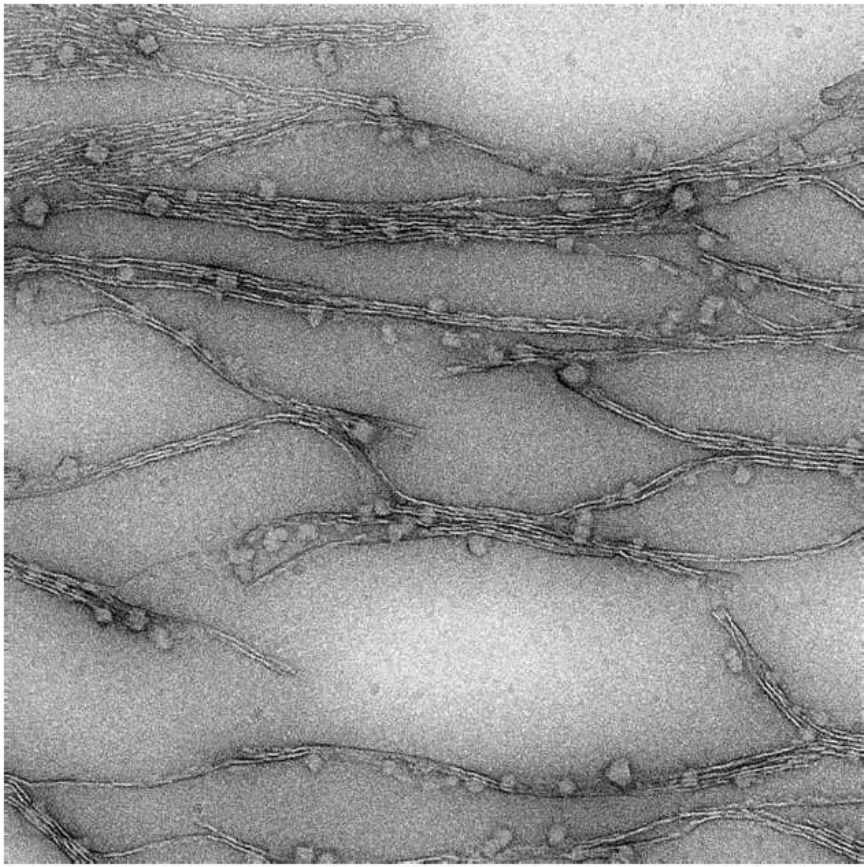

Rode, B. M.; Fitz, D.; Jakschitz, T. Chem. *Biodiversity* **2007**, *4*,2674.

| Activating agent | Hydrolysis/ hydration product | $\Delta G^{o\prime}$/ kJ mol$^{-1}$ |
|---|---|---|
| $NH_2CONH_2$ | $CO_2 + NH_3$ | $-16^a$ |
| COS (g) | $CO_2 + H_2S$ | $-17^a$ |
| Pyrophosphate | Phosphate | $-19^b$ |
| CO (g) | $HCO_2H$ | $-16^a$ |
| HNCO | $CO_2 + NH_3$ | $-54^a$ |
| HCN | $HCO_2H + NH_3$ | $-75^a$ |
| RCN | $RCO_2H + NH_3$ | $-80^c$ |
| $NH_2CN$ | Isourea | $-83^d$ |
| HNCNH | Isourea | $-97^d$ |
| HCCH (g) | $CH_3CHO$ | $-112^a$ |

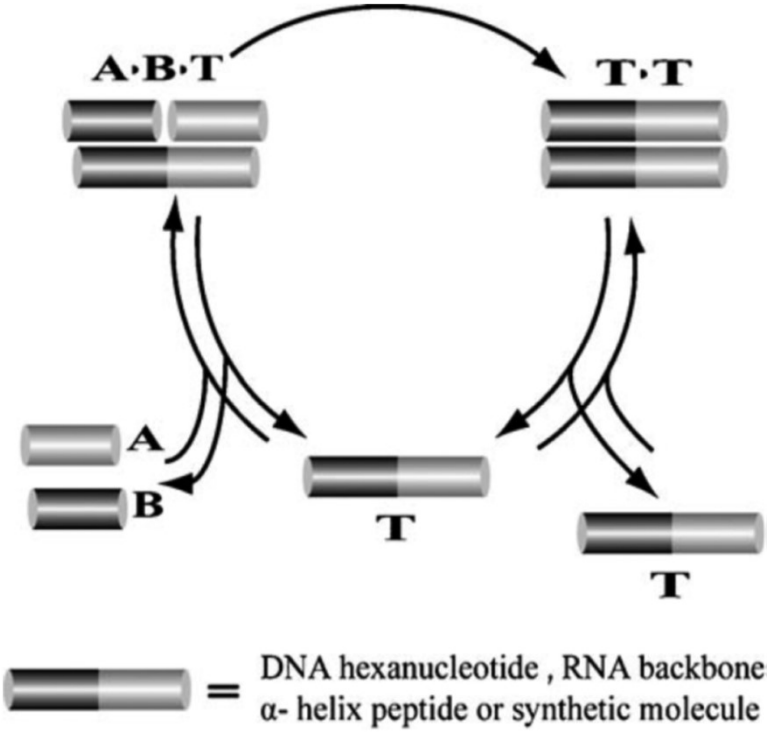
Danger, G.; Plasson, R.; Pascal, R. *Chem. Soc. Rev.* **2012**, *41*, 5416.

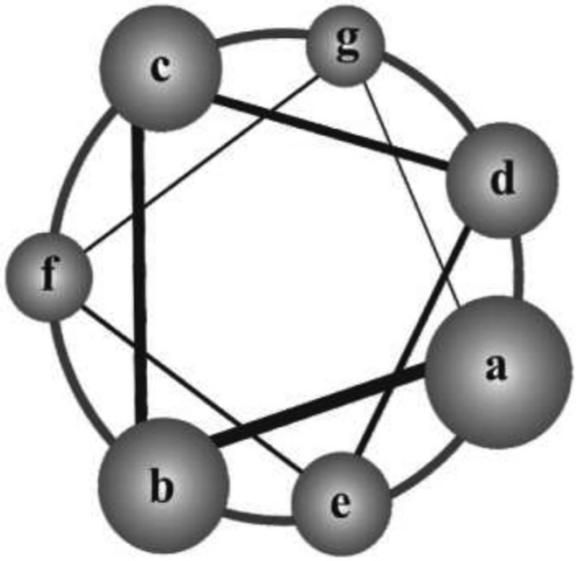
# *Condensation of aminoacids into peptides*

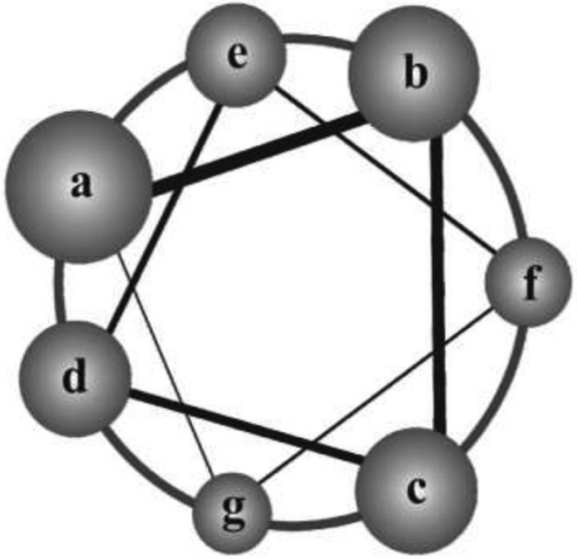


# *Peptide self-replication*

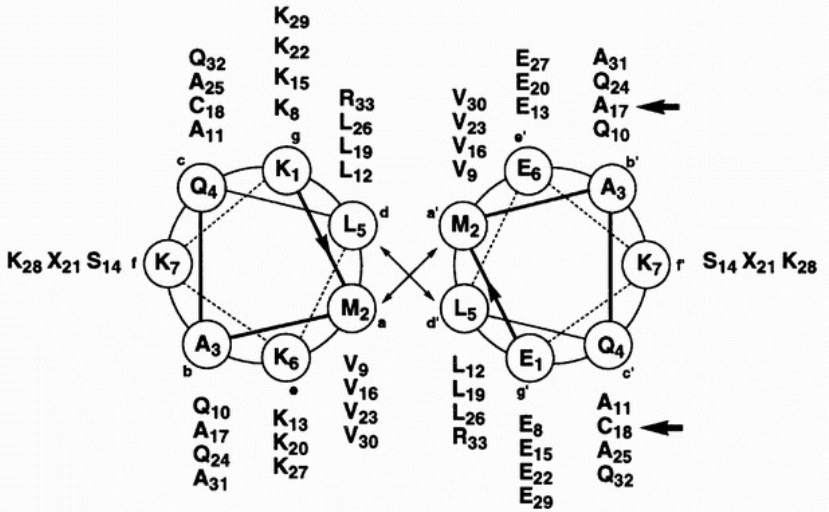



K. Severin, D. H. Lee, A. J. Kennan and M. Reza Ghadiri *Nature* **1997**, *389*, 706-709

# *Peptide self-replication*




E: Ar-**K**MAQL**KKK**VQAL**K**S**K**VA-CL**K**X**K**VQAL**KKK**VAQR-$CONH_2$

E*: Ar-**K**MAQL**KKK**VQAL**K**S**K**VA-SL**K**X**K**VQAL**KKK**VAQR-$CONH_2$

$S_1$: Ar-EMAQLEKEVQALESEVA-COSBn

$S_2$: Ar-EMAQLEKEVQALESEVA-$COS(CH_2)_2CONH_2$

$S_3$: Ar-EMAQLEKEVQALESEVA-CONHOH

$S_4$: Ar-**R**MKQLEE**K**VYELLS**K**VA-COSBn

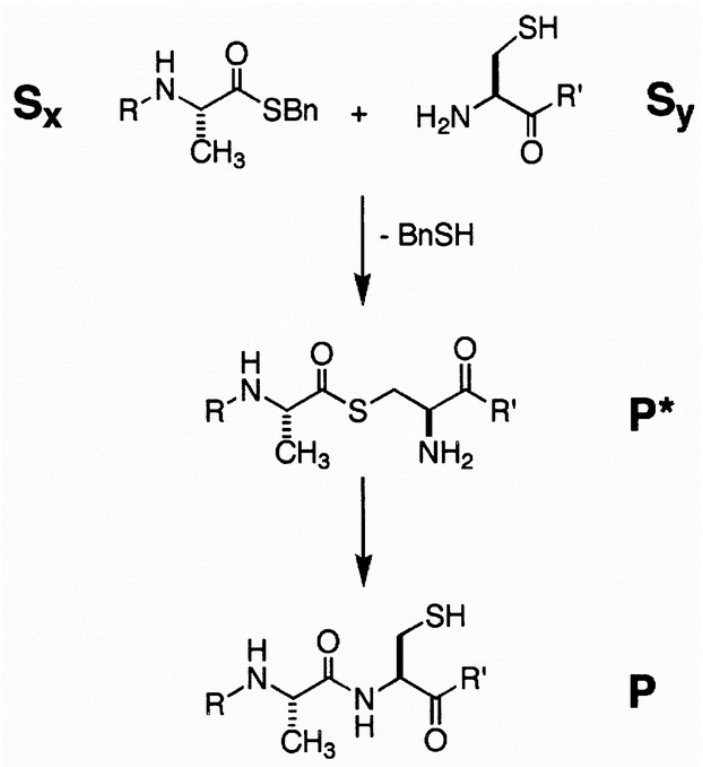
$S_5$: Ar-**K**MAQL**KKK**VQAL**K**S**K**VA-COSBn

$S_6$: $H_2N$-CLEXEVQALEKEVAQR-$CONH_2$

$S_7$: $H_2N$-CLEXEVARLKKLVGE-$CONH_2$

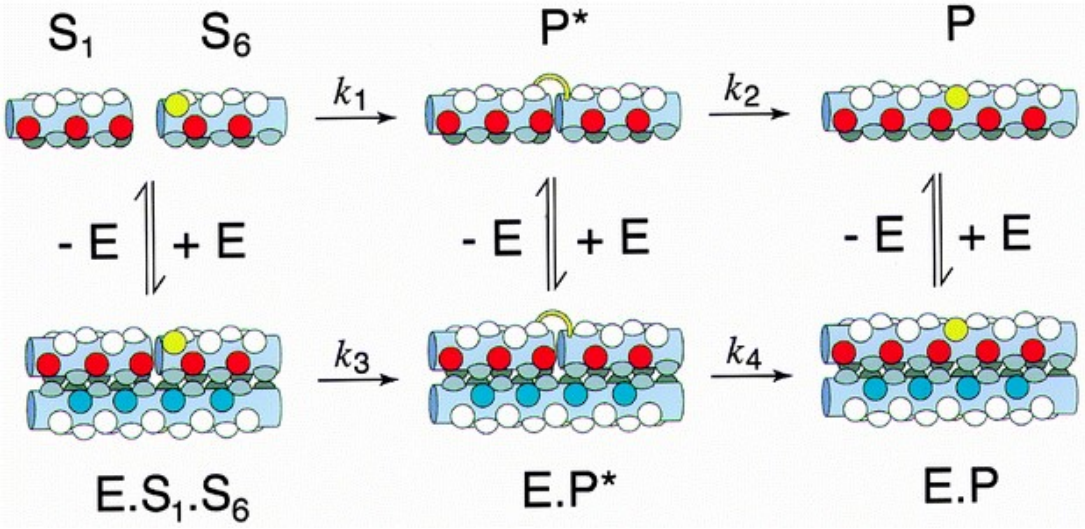
$S_8$: $H_2N$-(*d*-C)LEXEVQALEKEVAQR-$CONH_2$

$S_9$: $H_2N$-GLEXEVARLKKLVGE-$CONH_2$



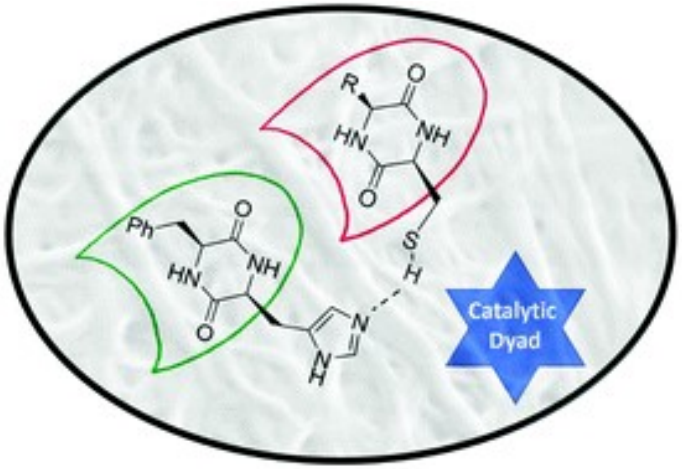

K. Severin, D. H. Lee, A. J. Kennan and M. Reza Ghadiri *Nature* **1997**, *389*, 706-709

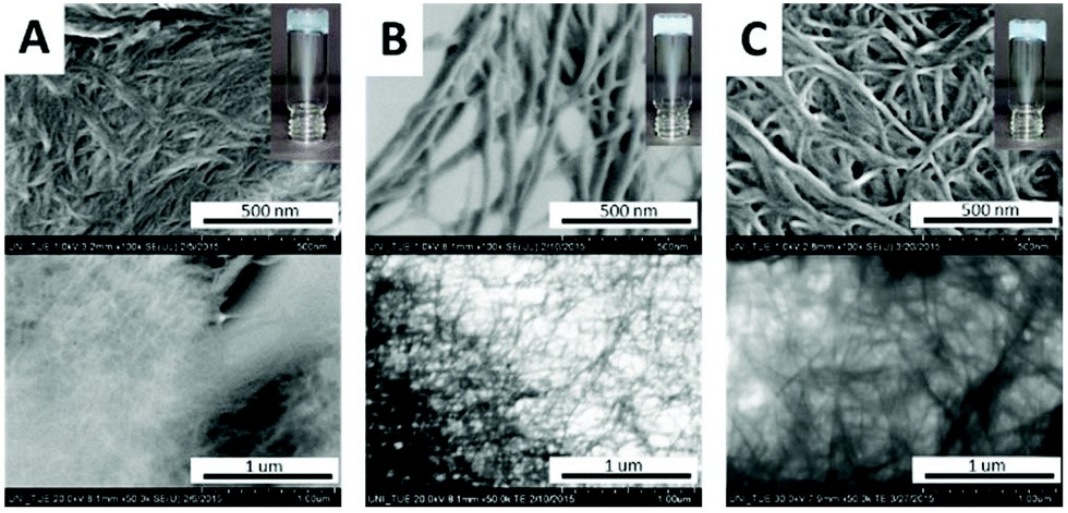
# *Peptide self-replication*

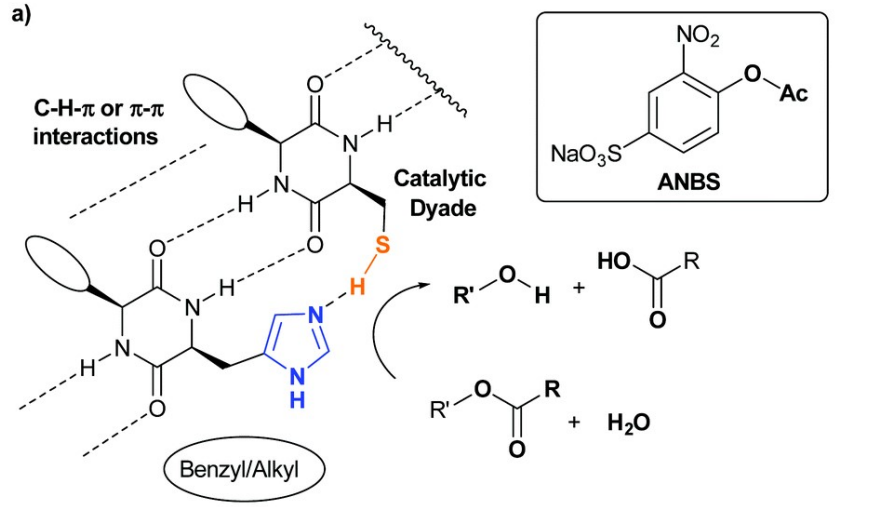



K. Severin, D. H. Lee, A. J. Kennan and M. Reza Ghadiri *Nature* **1997**, *389*, 706-709

# *Minimalistic enzyme-like peptidic self-assembly*



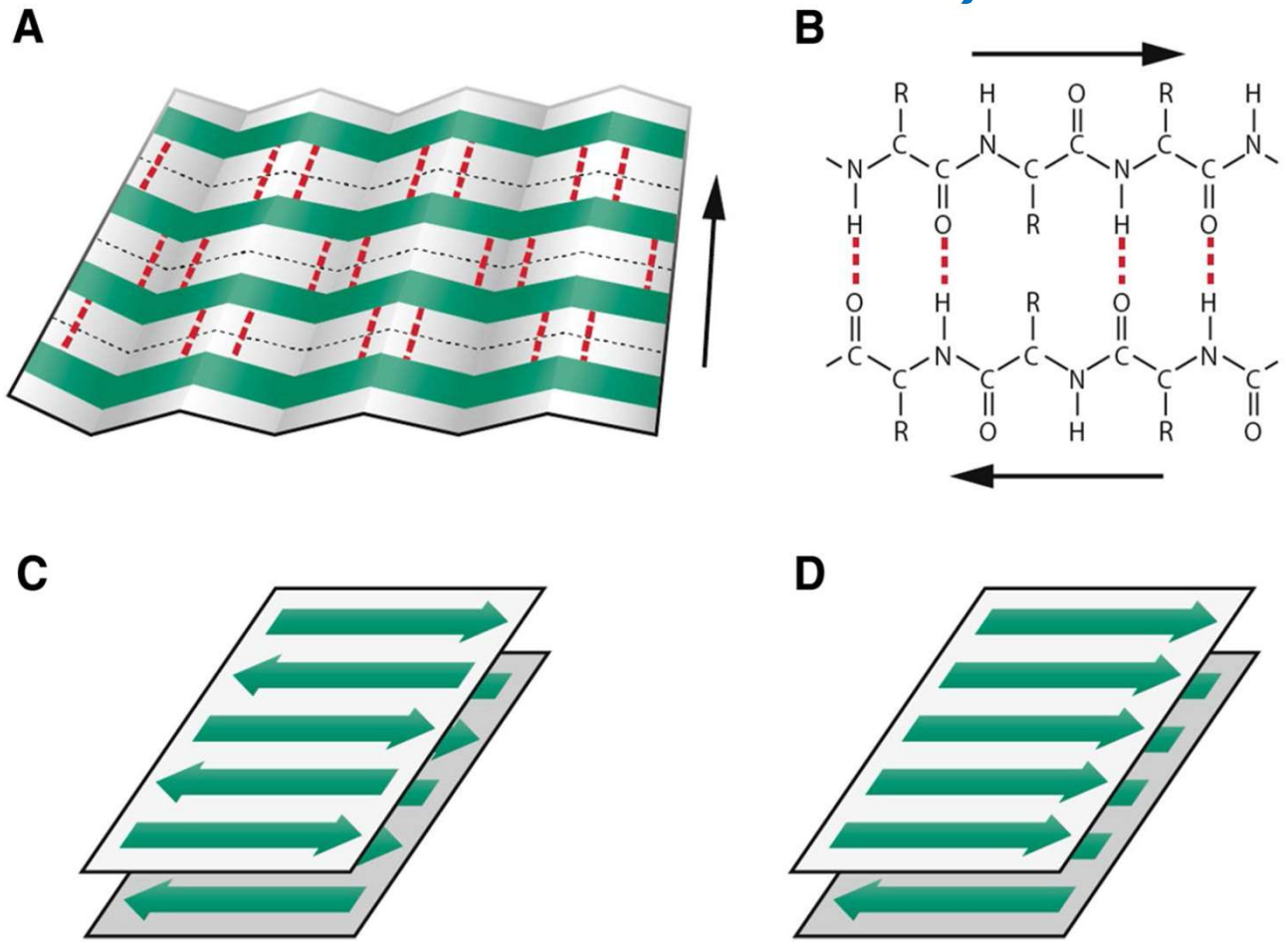




A catalytic turnover is exclusively observed in the self-aggregated state for heterologous mixtures (blends) of His- and a Cys-containing cyclic dipeptides.

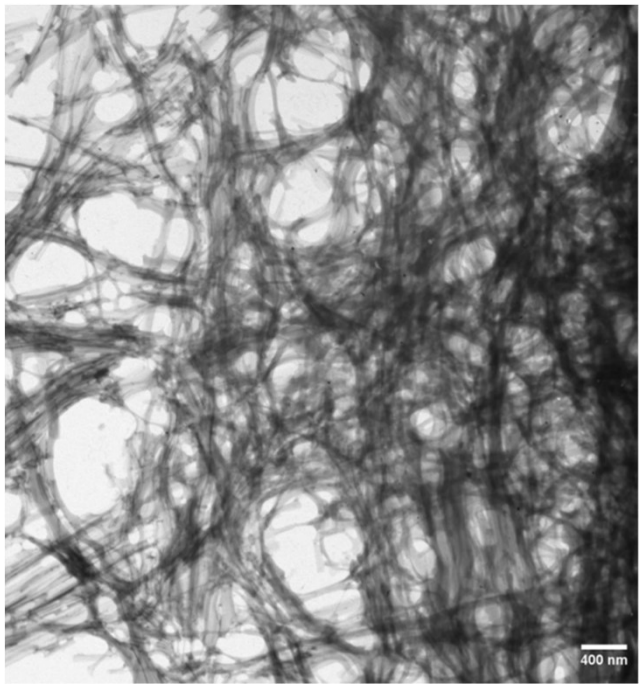
A. Kleinsmann and B. Nachtsheim *Org. Biomol. Chem.* **2020**, *18*, 102-107

# *Amyloid world*




Typically, the repeating unit of the amyloid fibrils consists of two tightly packed layers of β-sheets with side chains within the bilayers forming a dry interdigitating zipper interface.

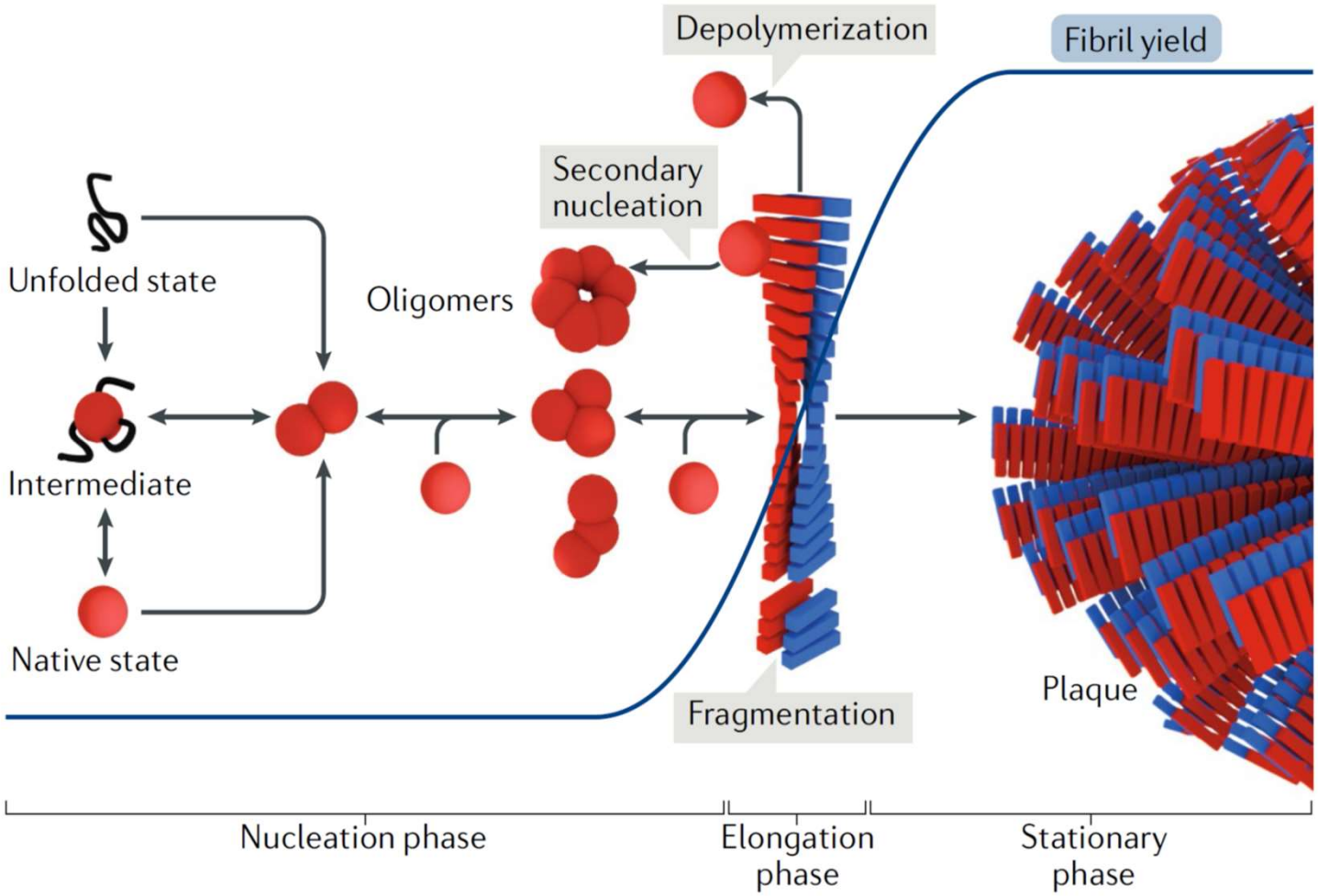
C. P. J. Maury *Cellular and Molecular Life Sciences* **2018** *75,* 1499–1507




Electron micrograph of a polymorphic fibrillar amyloid network self-assembled from a prebiotically relevant 9-mer peptide (EGGSVVAAD) in aqueous environment.

Maury CPJ, Liljeström M, Zhao F,
*J Biol Res* **2012**, *18,* 332–335

# *Amyloid world*




**Schematic of amyloid formation.** Native proteins are in dynamic equilibrium with their less-structured, partially folded and/or unfolded states. One of these states initiates amyloid fibril formation by assembling into oligomeric species. Oligomeric species can then assemble further to form higher-order oligomers, one or more of which can form a fibril nucleus, which, by rapidly recruiting other monomers, can nucleate assembly into amyloid fibrils.

M. G. Iadanza *et al. Nature Rev. Mol. Cell Biol.* **2018**, *19*, 755-773

# *Amyloid world*

**The amyloid replicator**

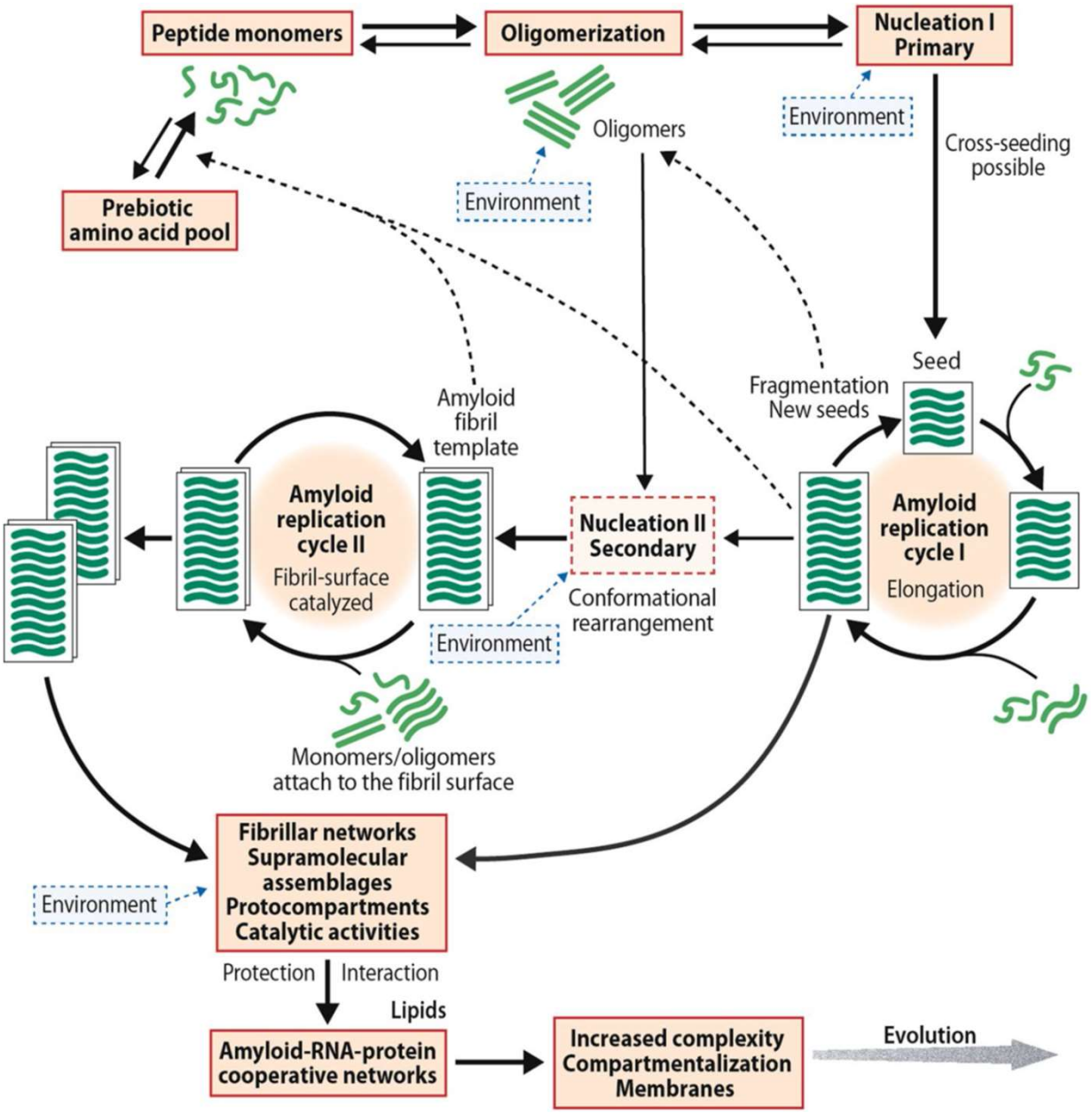
the primordial information system was based on structurally stable catalytic and self-replicating β-sheet amyloid conformers

In contrast to native peptides which are easily denatured, and whose functionality requires longer peptide sequences, short peptides may express diverse catalytic, replicative, and informational properties when adopting the amyloid conformation.

The same peptide monomer can generate functionally and structurally different amyloid conformers of which one or several can propagate and make new copies of itself/themselves
→ evolvability of the system

The environmentally adapted changes in the amyloid architecture can then be replicated and the pool of the fittest variants can expand.

C. P. J. Maury *Cellular and Molecular Life Sciences* **2018** *75,* 1499–1507



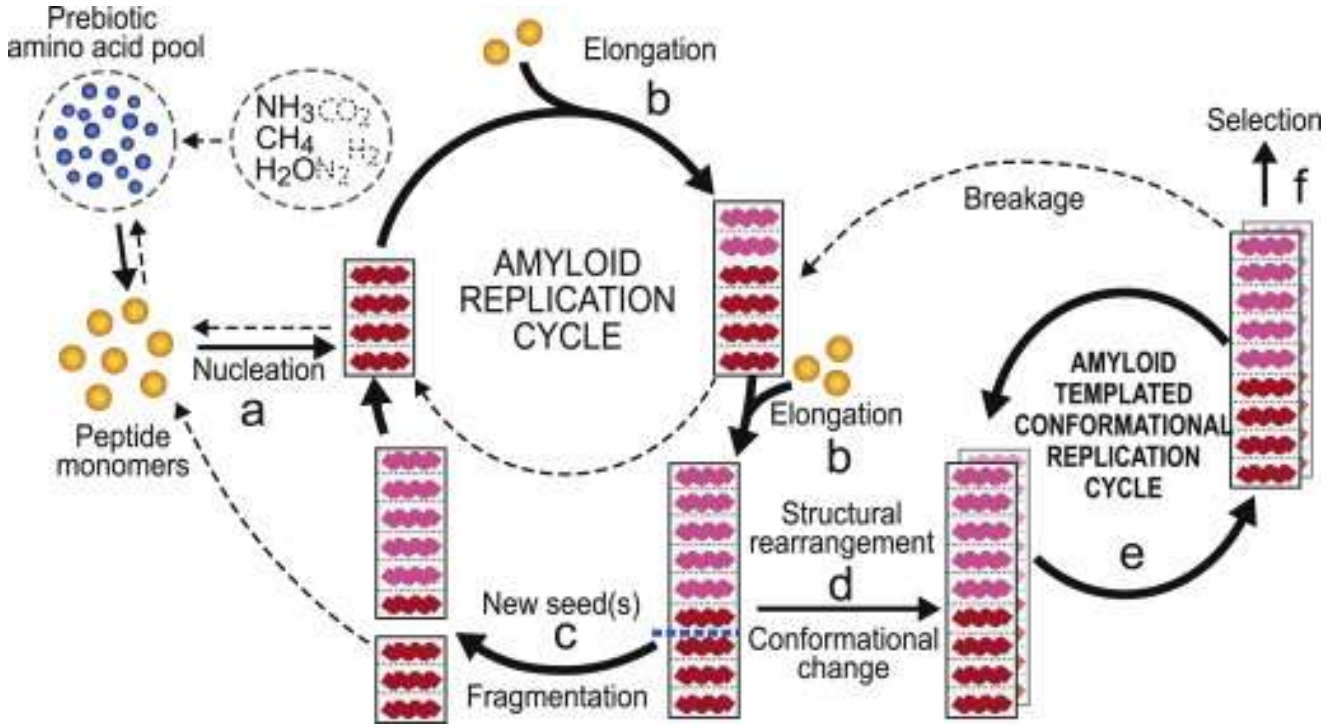

# *Amyloid world*

An initial **slow nucleation** process is followed by a **fast polymerization** phase where peptide monomers are added to the growing end of the protofilament. **Fragmentation** generates new seeds that can initiate **repeated replication cycles.** The same peptide monomer can give rise to different amyloid structures and molecular rearrangements are possible. Specific conformational changes can be replicated in the fibril/protofibril-catalyzed cycle II. Amyloid is also able to **direct the synthesis of its own constituent peptides.** The β-sheet conformers and ribonucleotides interact dynamically and cooperatively, and the amyloid-based supramolecular fibrillar assemblies can function as a primitive metabolic apparatus catalyzing the formation metabolite precursors.

C. P. J. Maury *Cellular and Molecular Life Sciences* **2018** *75,* 1499–1507

# *Amyloid world*

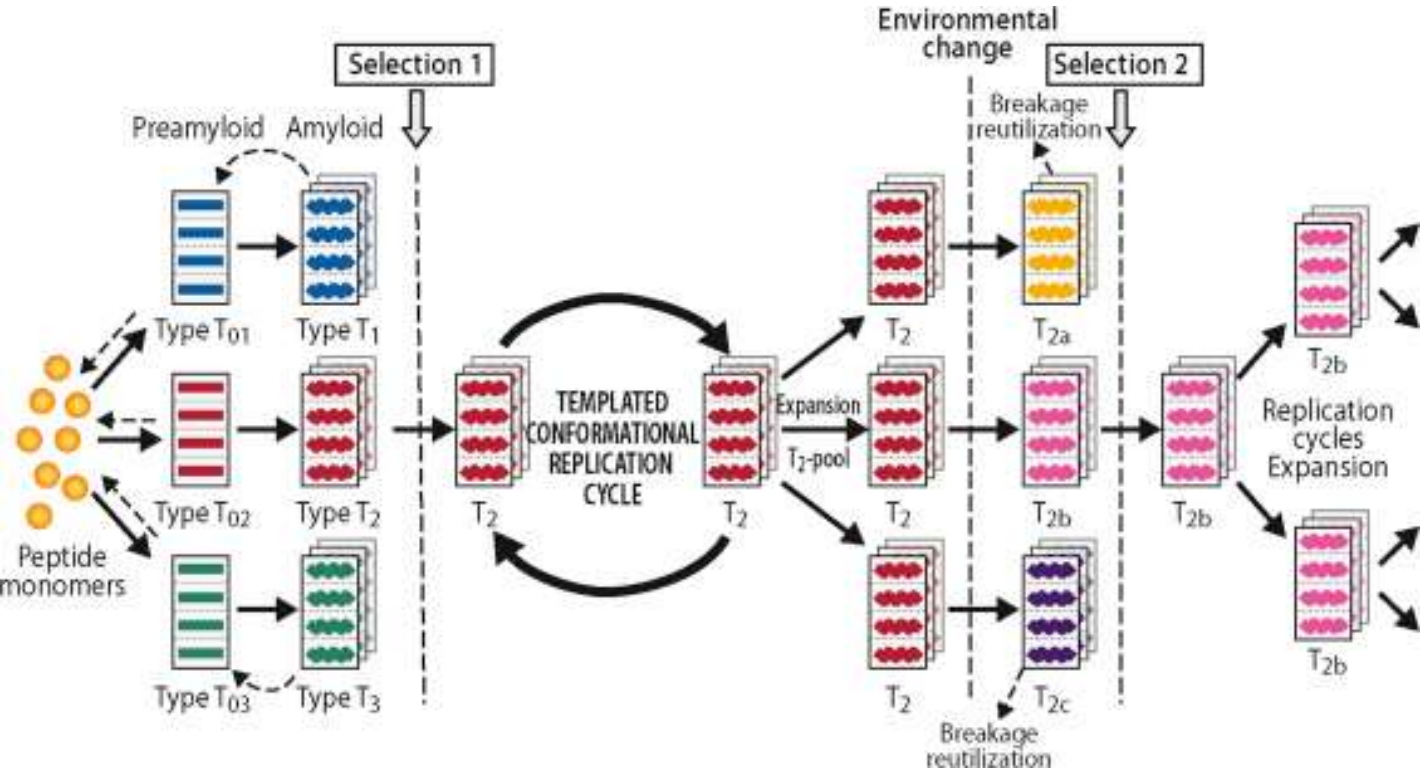
*Schematic representation of the self-replicating cycles of amyloid.*




An initial **slow nucleation phase (a)** is followed by a kinetically **fast elongation phase (b)** where monomers (or oligomers) are added sequentially to the growing end of the protofiber. **Breakage** of the fiber results in **new seeds (c)** and repeated replication cycles. Importantly, molecular **rearrangements** and conformational changes in amyloid may occur **(d)** that, by a **templated *conformational* replication mechanism (e),** can faithfully be transmitted to other amyloid conformers. The pool of the environmentally fittest variant(s) then **expands (f)**.

C. P. J. Maury *J. Theor. Biology* **2015** *382,* 292-297

# *The amyloid world model*




From one type of prebiotic peptide monomer a spectrum of amyloid conformers may be formed ($T_1$, $T_2$, $T_3$). By templated conformational replication, the pool of the environmentally fittest type ($T_2$, Selection 1) rapidly expands. A change in the environment (e.g., pH, temperature, radiation) induces conformational changes in $T_2$ ($T_{2a}$, $T_{2b}$, $T_{2c}$). The fittest conformer ($T_{2b}$) is selected (Selection 2) and undergoes templated conformational replication cycles expanding the ($T_{2b}$) pool. The environmentally less suitable variants are decomposed and recycled. The environment-induced variations in the amyloid conformations combined with faithful replication of the selected amyloid conformers (variants) and repeated selection cycles allow evolution to occur.

C. P. J. Maury *J. Theor. Biology* **2015** *382,* 292-297

# *Amyloid world*

## Catalytic activity

*Amyloids can catalyze their own formation, but also other chemical reactions.*

Small, 7-residue amyloid-forming peptides form efficient catalysts of **ester hydrolysis**.
Other studies have demonstrated amyloid-related **aldolase**, **ATPase** , and **carbonic anhydrase** activities, as well as copper-mediated **oxygen activation**.

The catalytic functions are fibril/protofibril-dependent:
the corresponding nonaggregated peptides are catalytically inactive.

C. P. J. Maury *Cellular and Molecular Life Sciences* **2018** *75,* 1499–1507

amino acid condensation under prebiotic conditions readily leads to peptide amyloids

J. Greenwald, M. P. Friedmann, R. Riek, *Angew. Chem. Int. Ed*. **2016**, *55*, 11609–11613

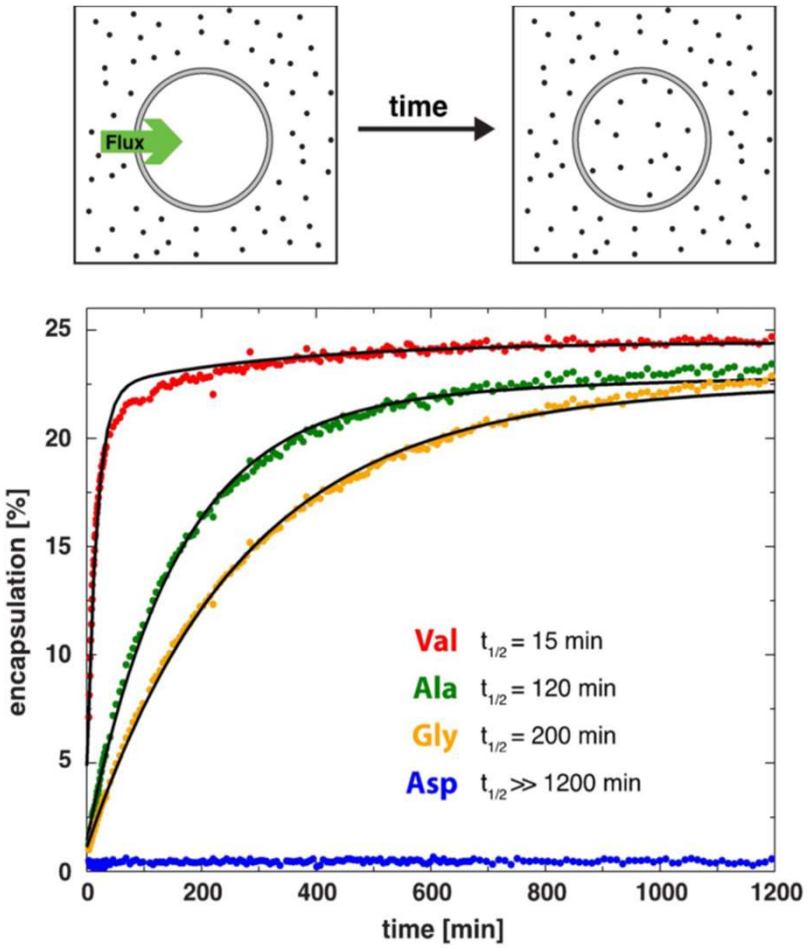
amyloids are capable of directing the sequence-selective and stereo-selective synthesis of peptides

M. P. Friedmann, V. Torbeev, V. Zelenay, A. Sobol, J. Greenwald, R. Riek, *PLoS One* **2015**, *10*, e0143948

# *Amyloid world*

Vesicles formed from either simple fatty acids or lipids can support the spontaneous internal formation of amyloids from de novo synthesized peptides via an external supply of activated amino acids.

*Permeation of amino acids into oleic acid vesicles.*

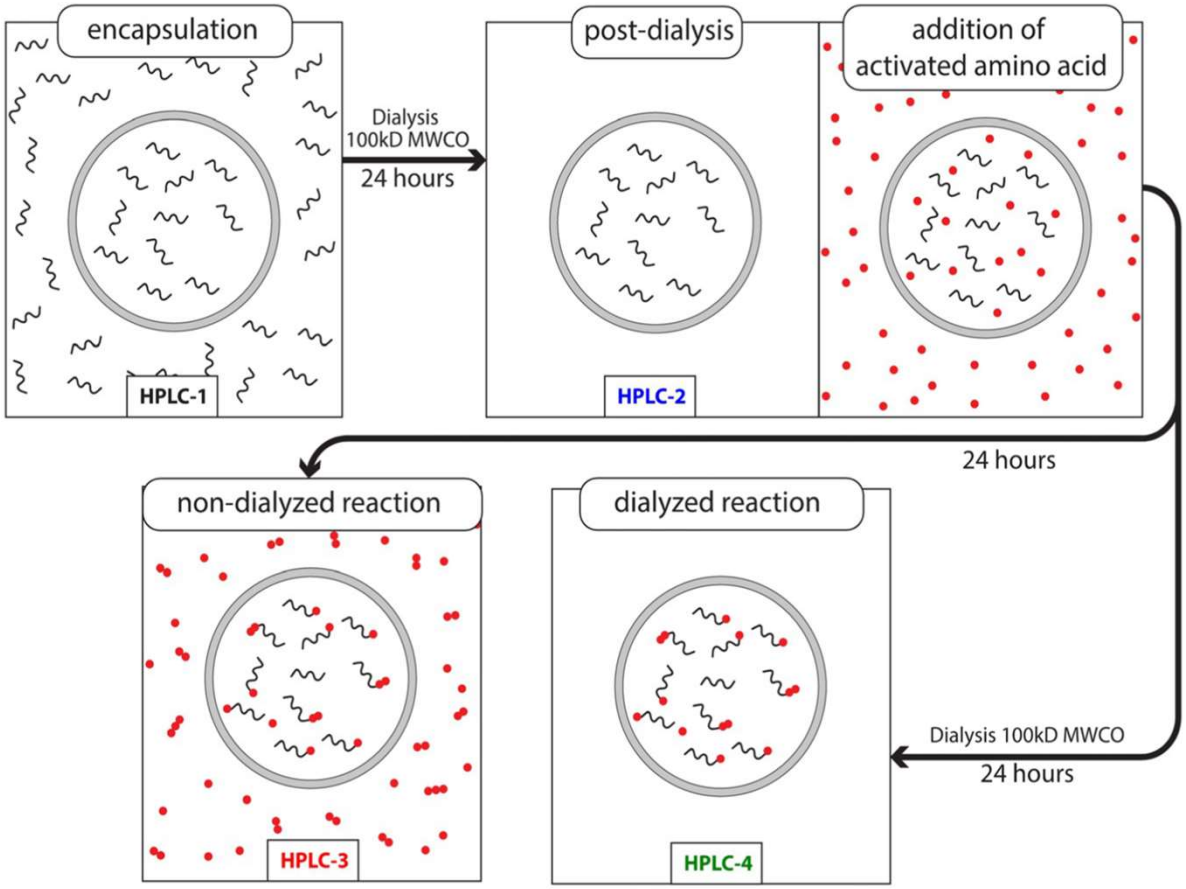



Spontaneous formation of amyloids is achieved when activated amino acids are supplied to the outside of the vesicles under conditions of continuous dilution or the presence of an external competing reaction, either of which prevents the accumulation of detectable peptides outside the vesicles

W. Kwiatkowski, R. Bomba, P Afanasyev, D. Boehringer, R. Riek, and J. Greenwald *Angew. Chem. Int. Ed.* **2021**, *60*, 5561 – 5568

# *Amyloid world*

*Peptide bond formation on the inside of oleic acid vesicles*




A peptide is encapsulated inside of a vesicle,

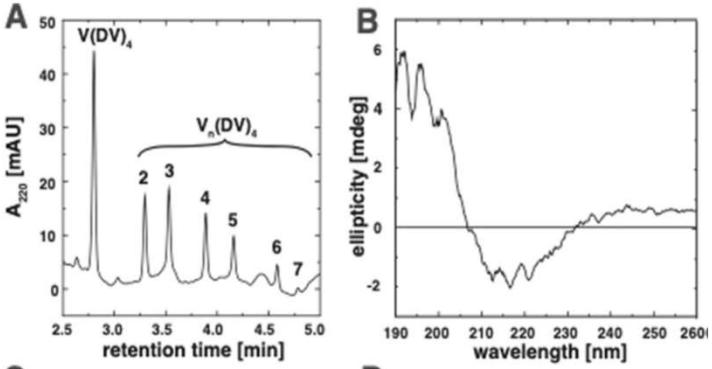
a membrane-permeable amino acid is activated with CDI and added to the vesicle.

The reaction is then diluted though dialysis, thereby removing the membrane permeable and external components of the system.

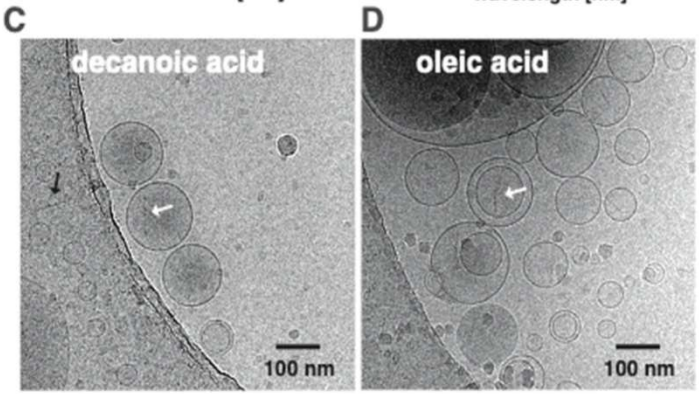
W. Kwiatkowski, R. Bomba, P Afanasyev, D. Boehringer, R. Riek, and J. Greenwald *Angew. Chem. Int. Ed.* **2021**, *60*, 5561 – 5568

# *Amyloid world*

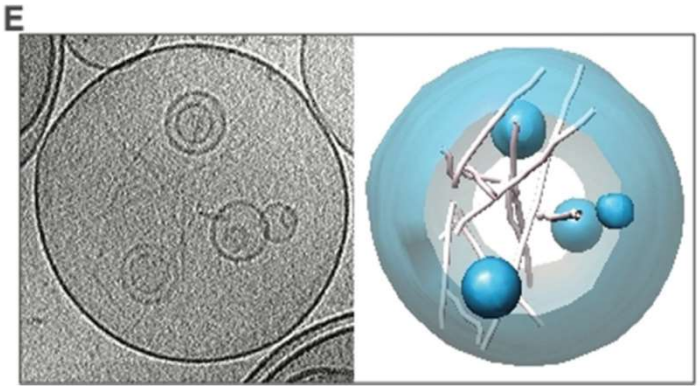
*Valine addition to $V(DV)_4$ and subsequent amyloid formation inside vesicles.*




CDI-activated Val was added to $V(DV)_4$-encapsulated peptide followed by a second addition reaction and dialysis step.



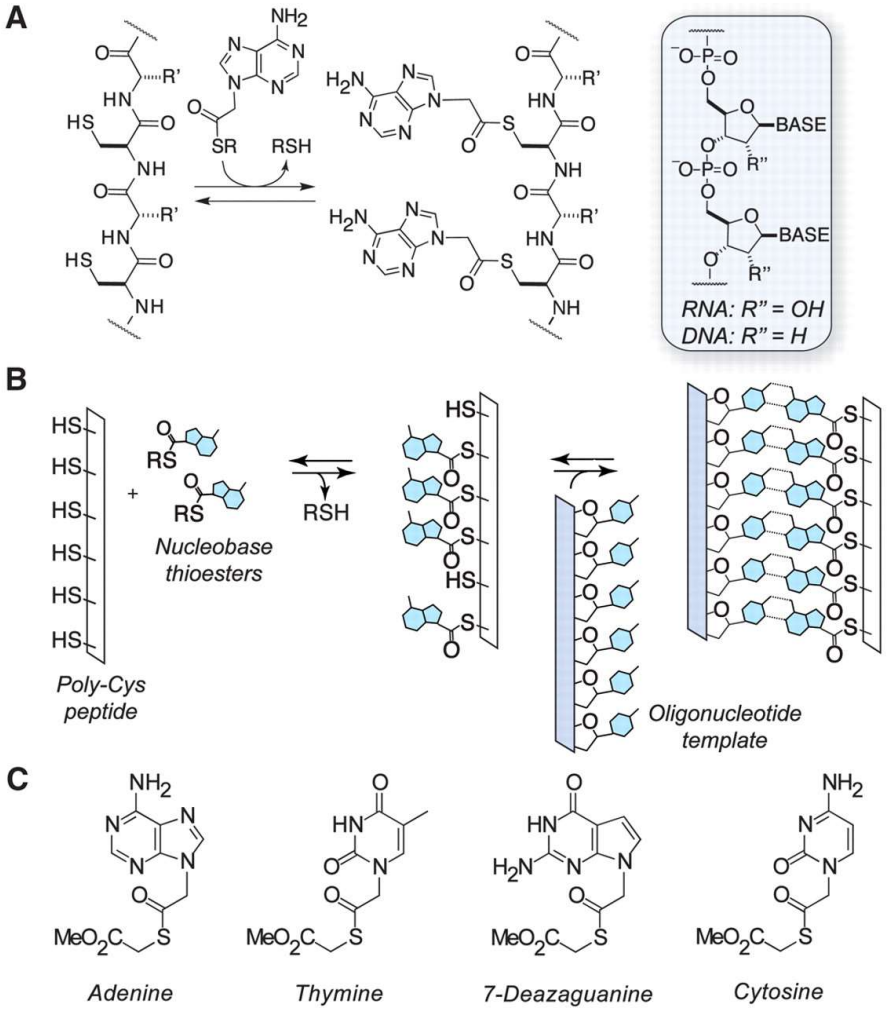

Cryo-EM images of decanoic acid and oleic acid vesicles revealing that the fiber aggregate occurs inside (example highlighted with white arrows) and only rarely outside of vesicles (example highlighted with black arrow).

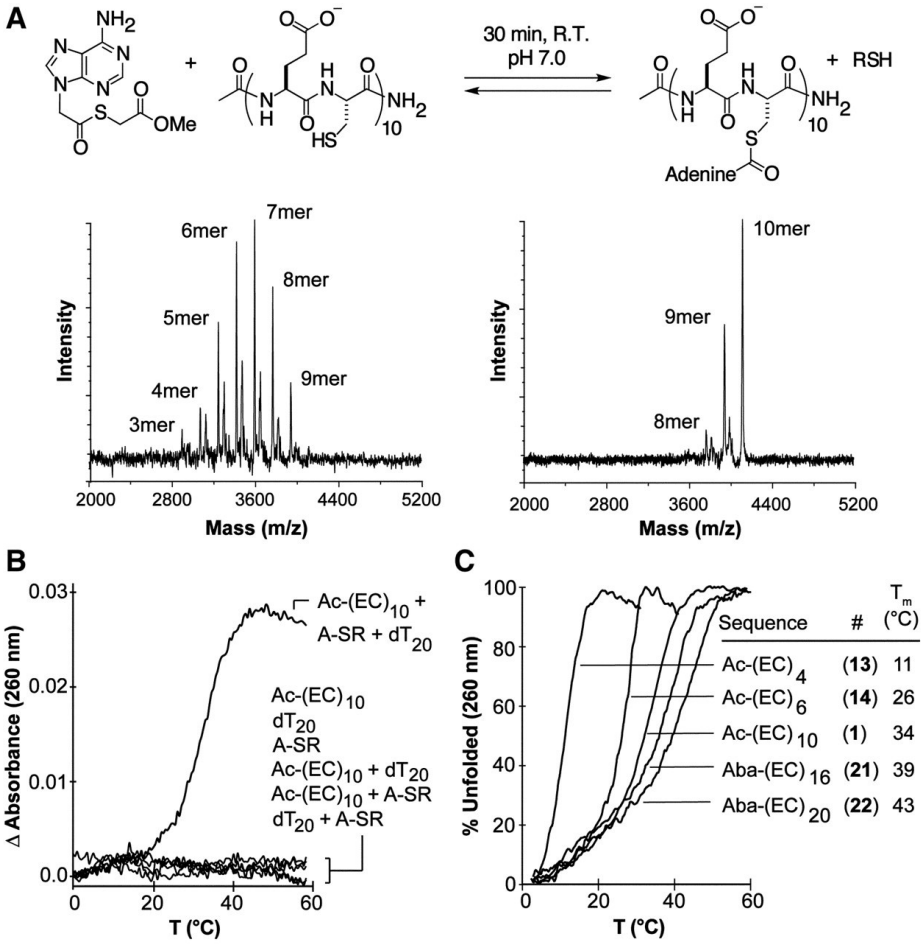



A projection from a cryo-electron tomogram reconstruction of a POPC vesicle with several fibers inside and a 3D model created from this data showing the vesicle and its contents.

W. Kwiatkowski, R. Bomba, P Afanasyev, D. Boehringer, R. Riek, and J. Greenwald *Angew. Chem. Int. Ed.* **2021**, *60*, 5561 – 5568

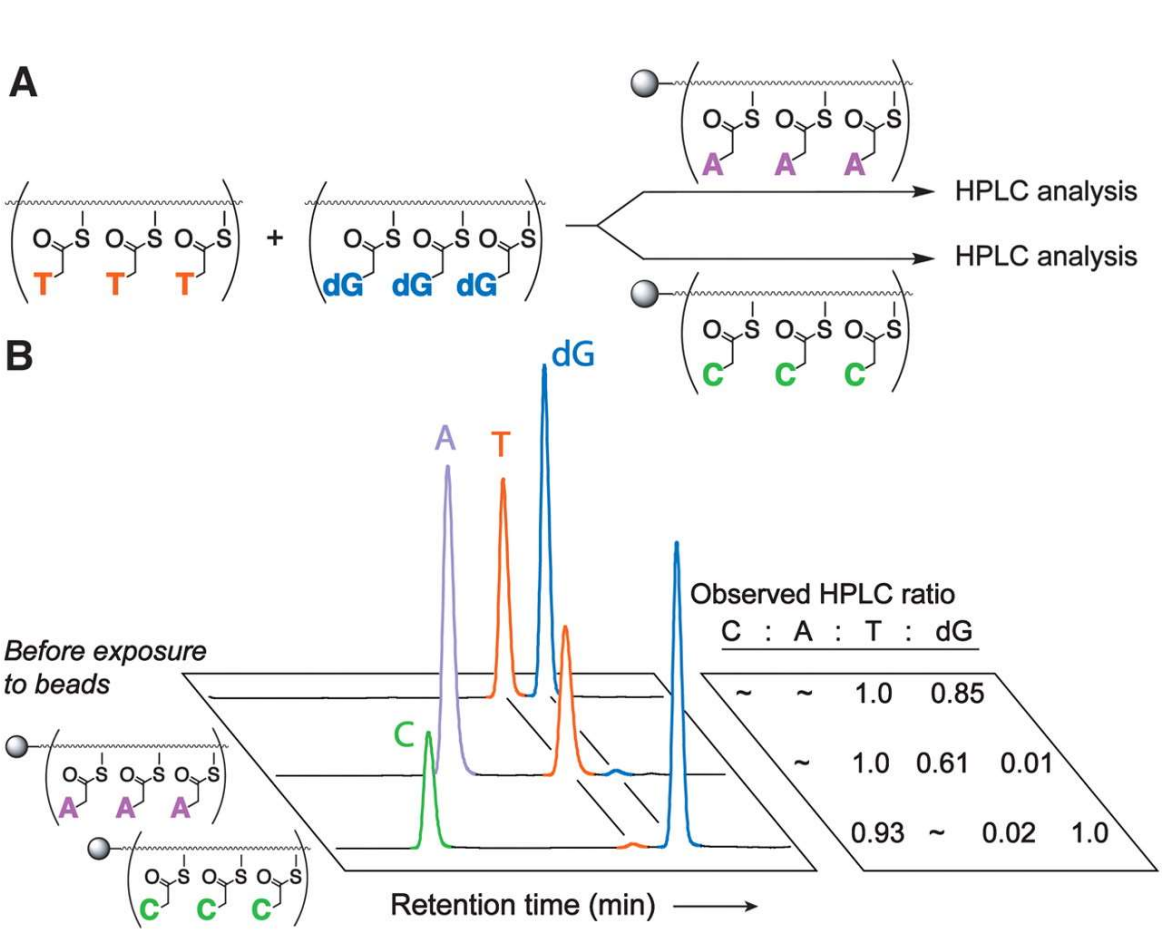
# *Dynamic oligonucleotide analogue sequence-specific assembly*

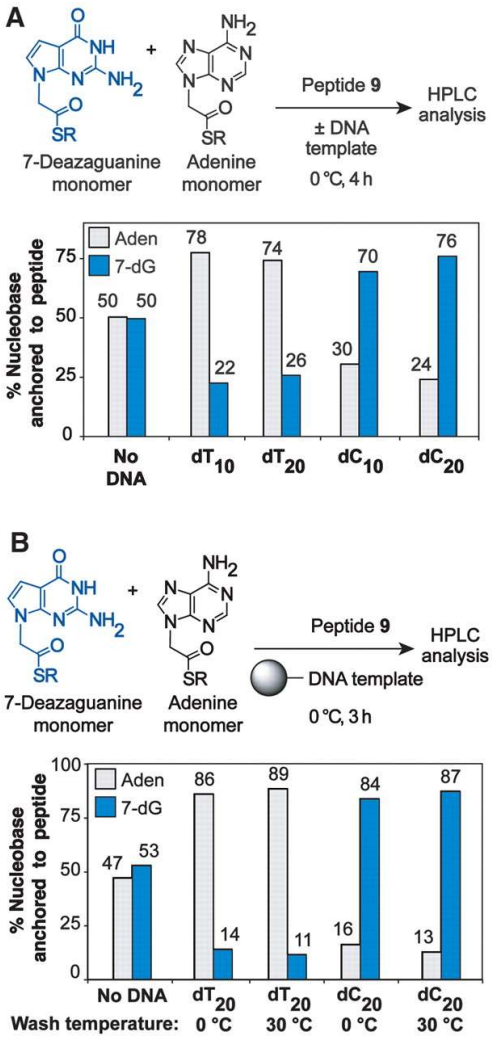


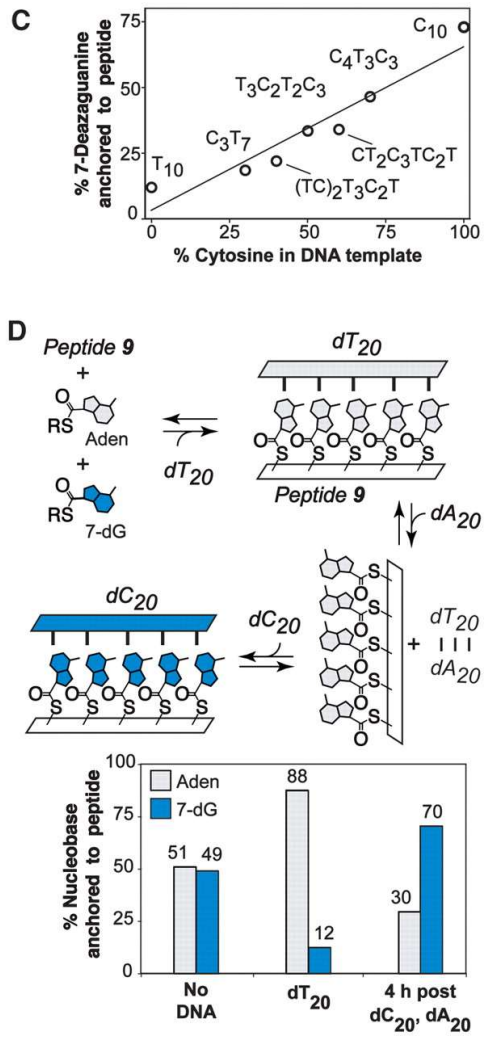




M. R. Ghadiri *et al. Science* **2009,** *325*, 73-77

# *Dynamic oligonucleotide analogue sequence-specific assembly*

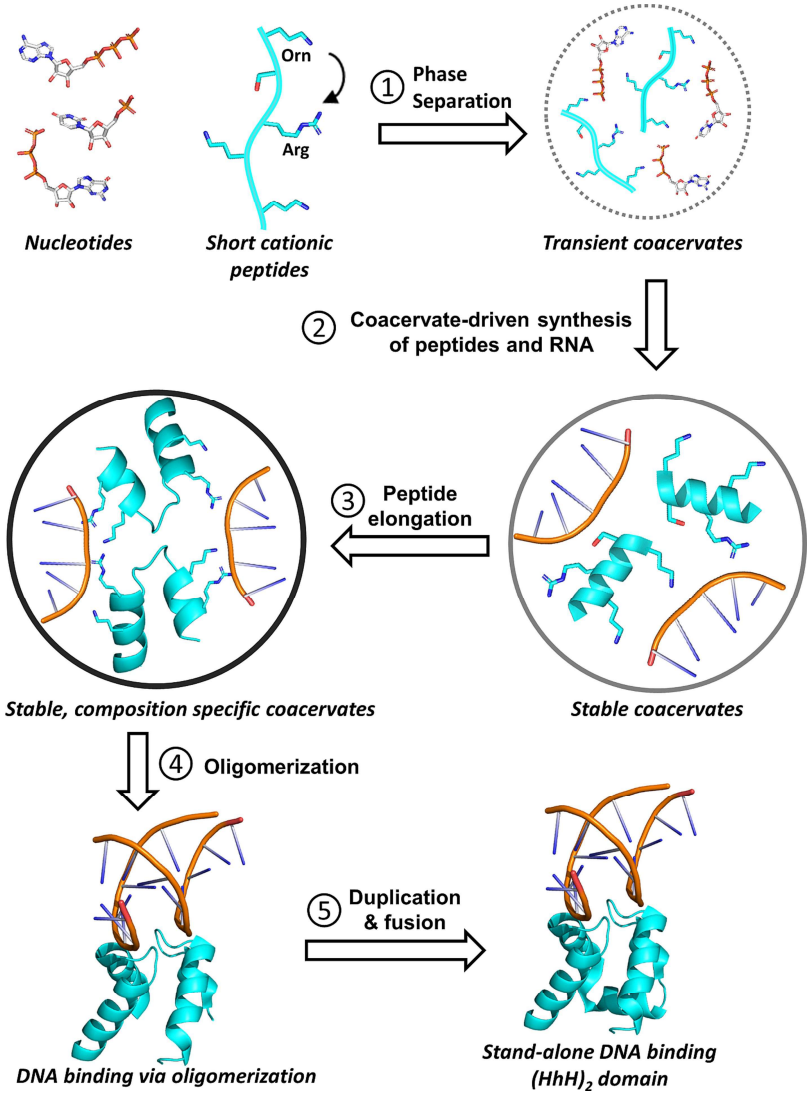



M. R. Ghadiri *et al. Science* **2009,** *325*, 73-77

# *From abiotic aminoacids to modern proteins*




**The evolution of nucleic acids binding proteins via phase separating intermediates.**

(1) An abiotic mixture of amino acids and short peptides, and of nucleotides and short nucleic acids enables the formation of coacervates that are relatively small and unstable. A critical component of these peptides is abiotically formed cationic amino acids such as ornithine.

(2) Coacervate formation drives the synthesis of longer peptides and nucleic acids, eventually modification of ornithine to arginine.

(3) Peptide compositions that promote faster formation and/or larger droplets are enriched. These compositions also promote the acquisition of helical structures that in turn bind phospho-ligands with higher affinity, thereby endowing higher droplet stability.

(4) Oligomerization enables sequence-specific polypeptides of >20 residues to bind nucleic acids in solution (by virtue of avidity, of having multiple binding sites per assembly). Depicted here is a dimer, following the known (HhH)2 domain; however, in principle, higher oligomeric states can serve as intermediates.

(5) Duplication and fusion of these polypeptides yields an independently-folding protein domain that binds nucleic acids with high affinity and selectivity (*e. g.*, double-versus single-stranded, or DNA versus RNA).

D. Despotovic, D. S. Tawfik *ChemSystemsChem* **2021**, *3, e2100002*