Nucleic acid-peptide Chimera in the Early Chemical Evolution

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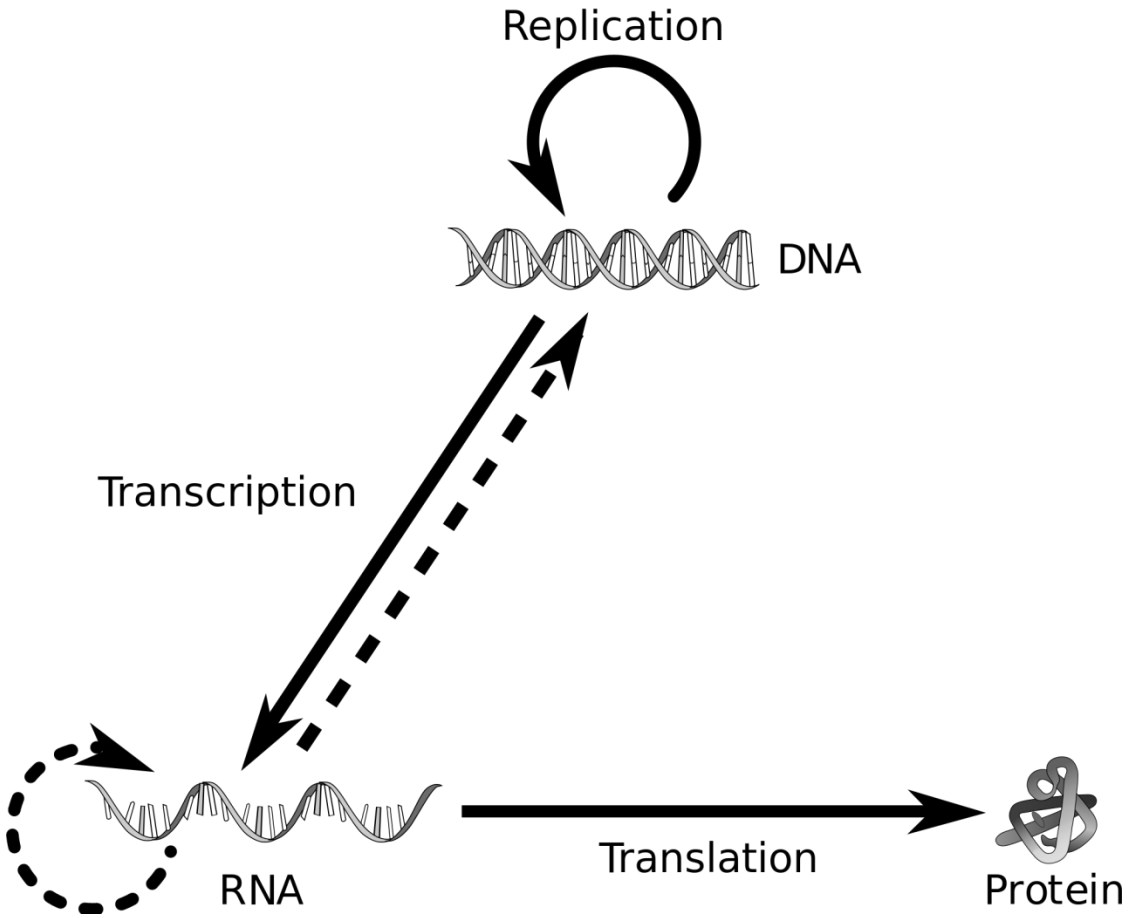
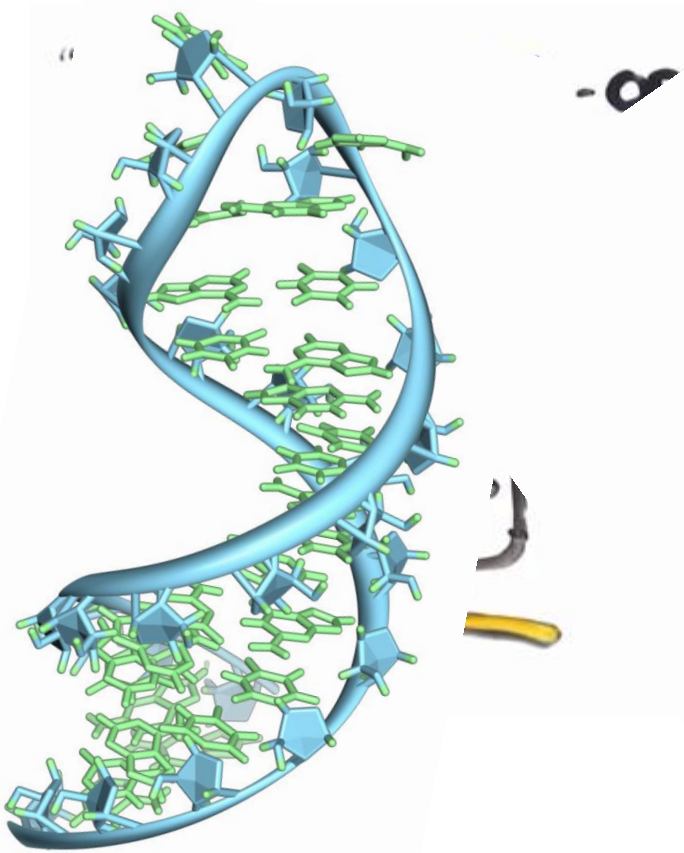
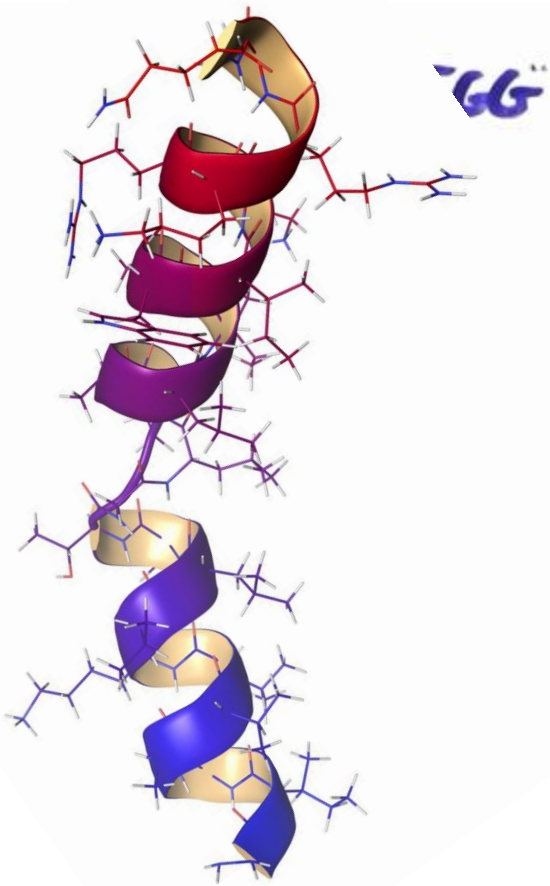
Central dogma of biology



RNA World hypothesis

Protein World hypothesis

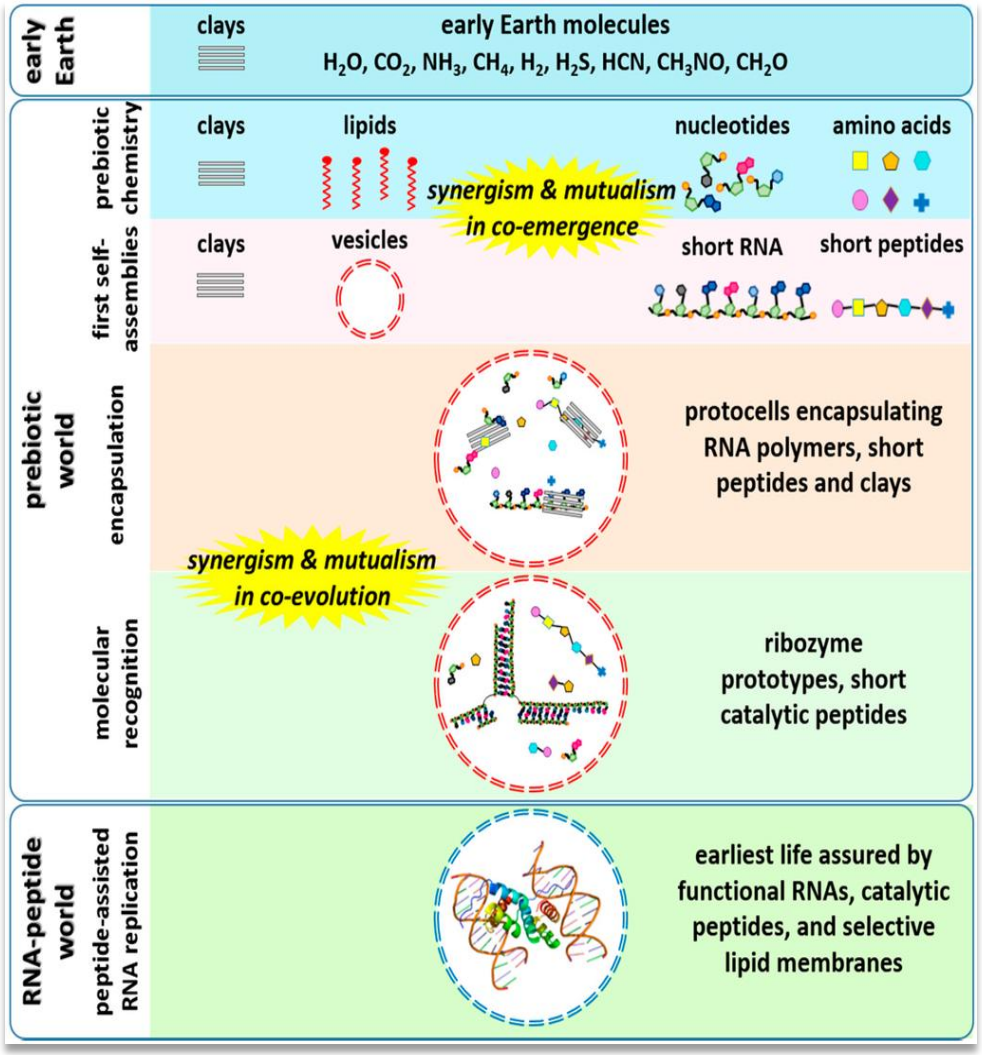




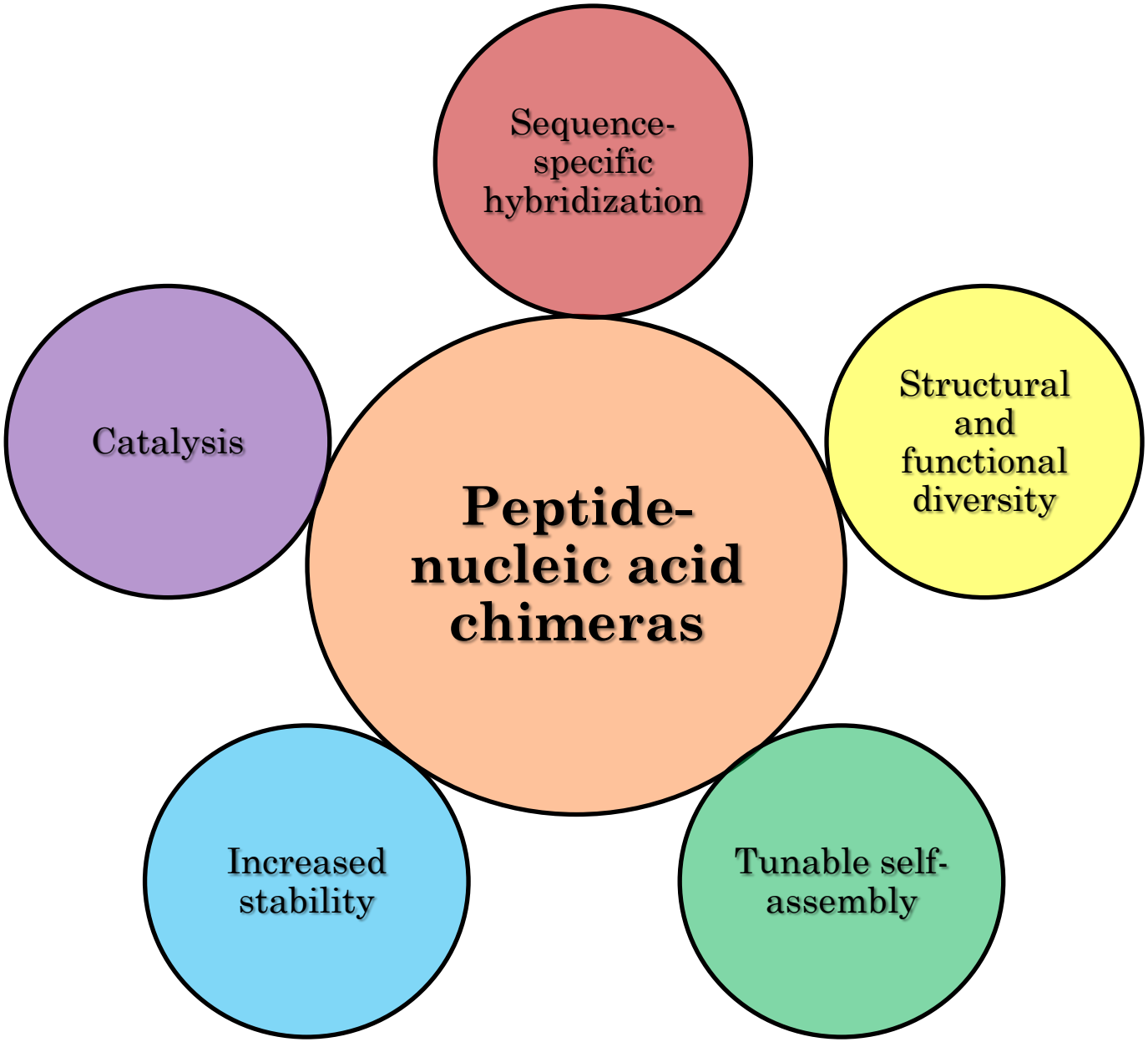
Co-evolution of proteins and nucleic acids

- **Amino acids or short peptides attached to the RNA could have been involved in stabilization of primitive ribozyme**
- **tRNA with short peptides attached could have served as a ribozyme with enhanced catalytic activity**
- **Presence of peptides in the ribozymes environment might have induced selection at the level of RNP enzymes**
- **Amyloid protein fibers could provide a protective support for nucleic acids and replicating nucleic acids stimulate fiber growth**

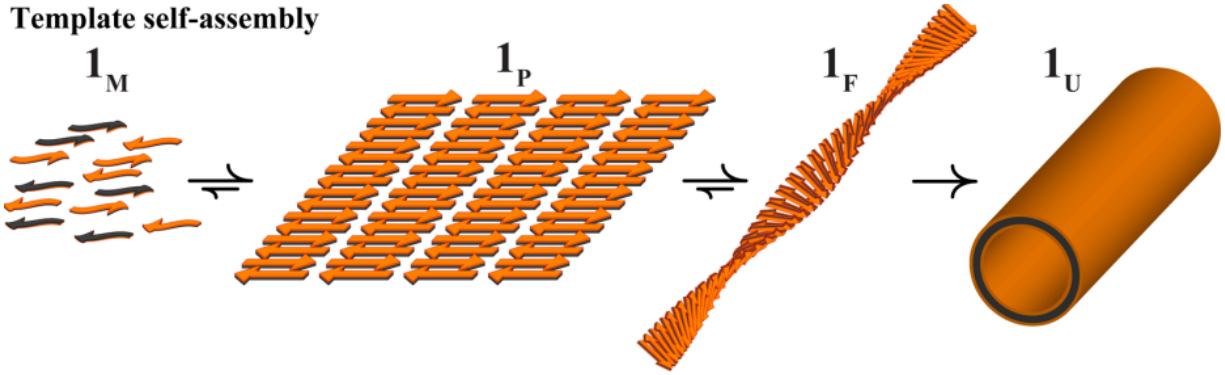
Prebiotic world



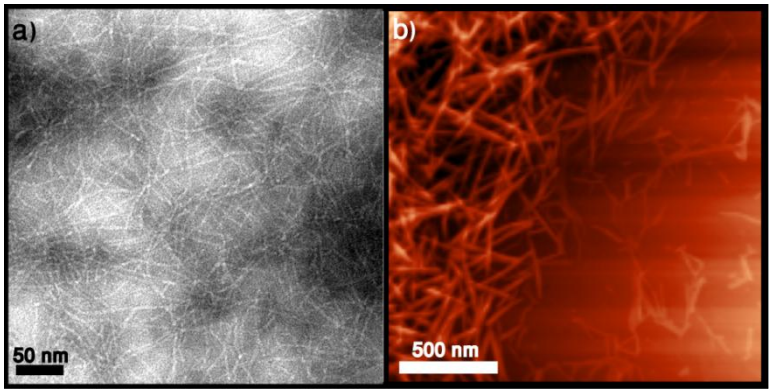
H. Kaddour and N. Sahai, *Life* **2014**, *4*(4), 598-620.

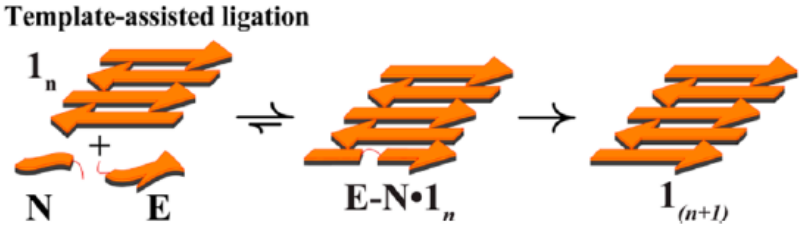

Sequence-specific hybridization
Catalysis
Structural and functional diversity
Peptide-nucleic acid chimeras
Increased stability
Tunable self-assembly

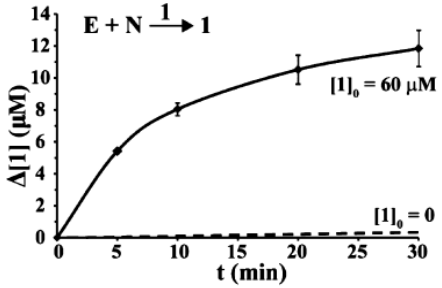
Synthetic amphiphilic peptides



Dynamic self-assembly of monomers (1_M) to anti-parallel ß-pleated sheets (1_P), fibers (1_F), and finally nanotubes (1_U).

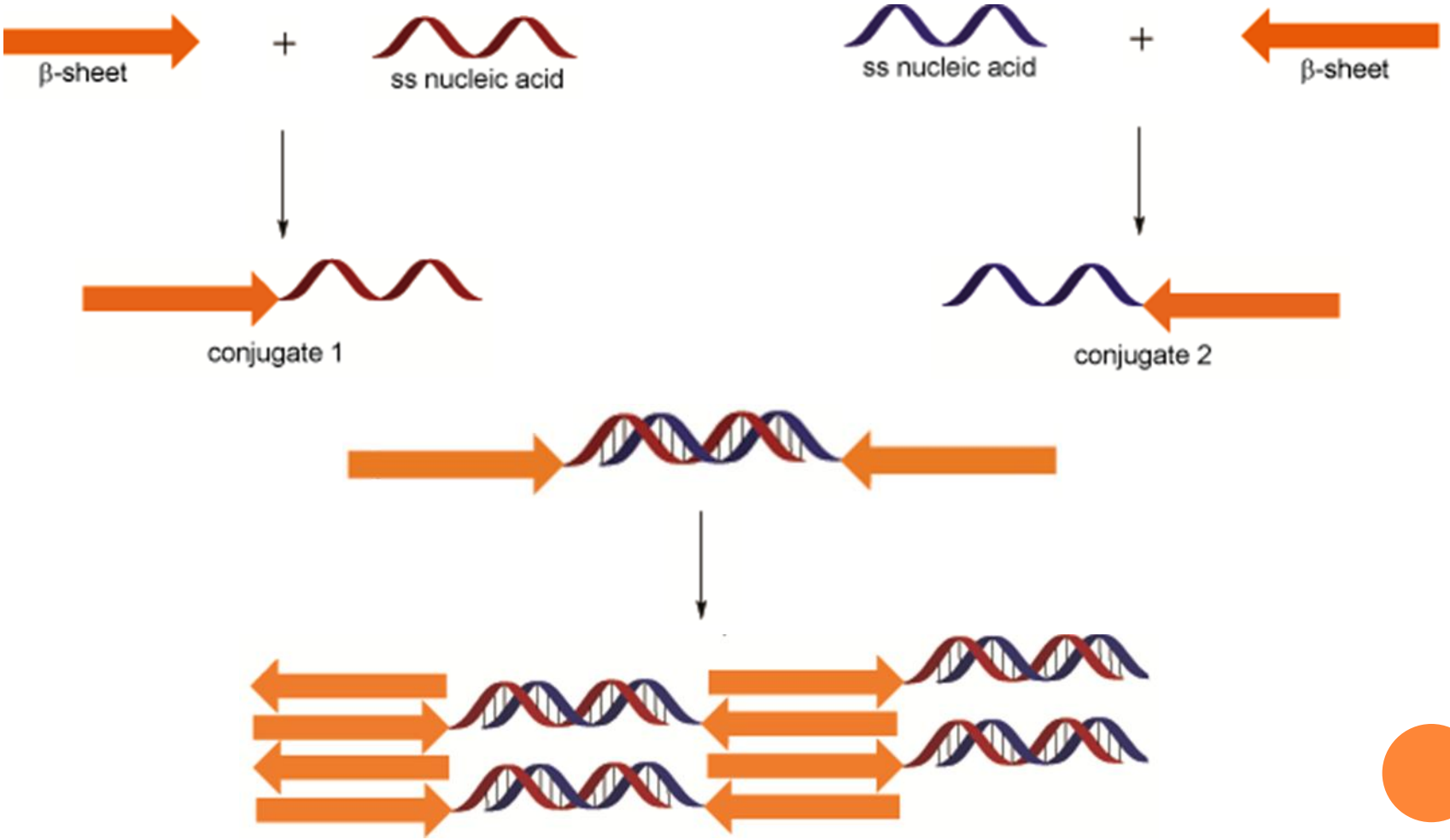




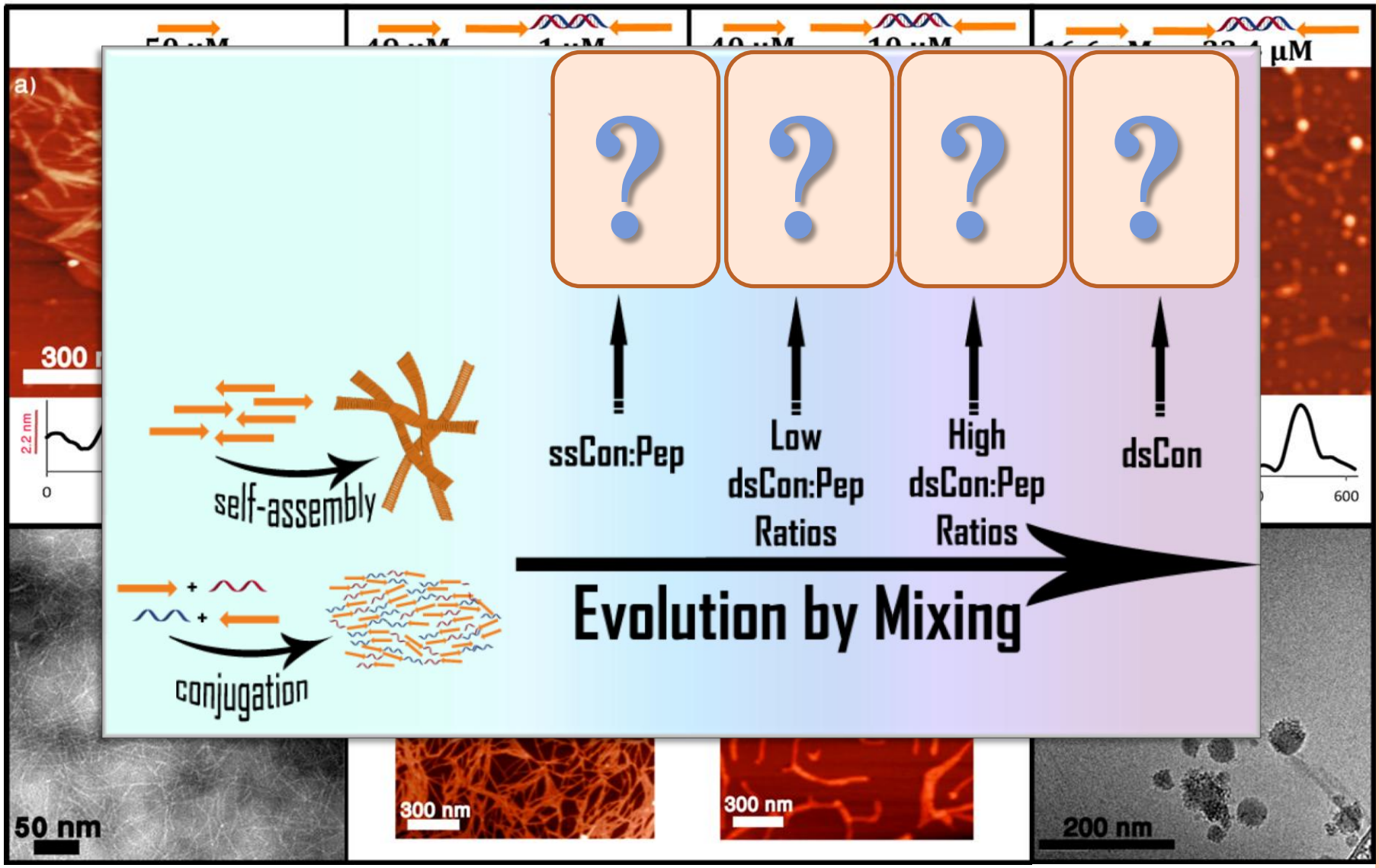


B. Rubinov, N. Wagner, M. Matmor, O. Regev, N. Ashkenasy and G. Ashkenasy, *ACS nano*, 2012, **6**, 7893-7901.

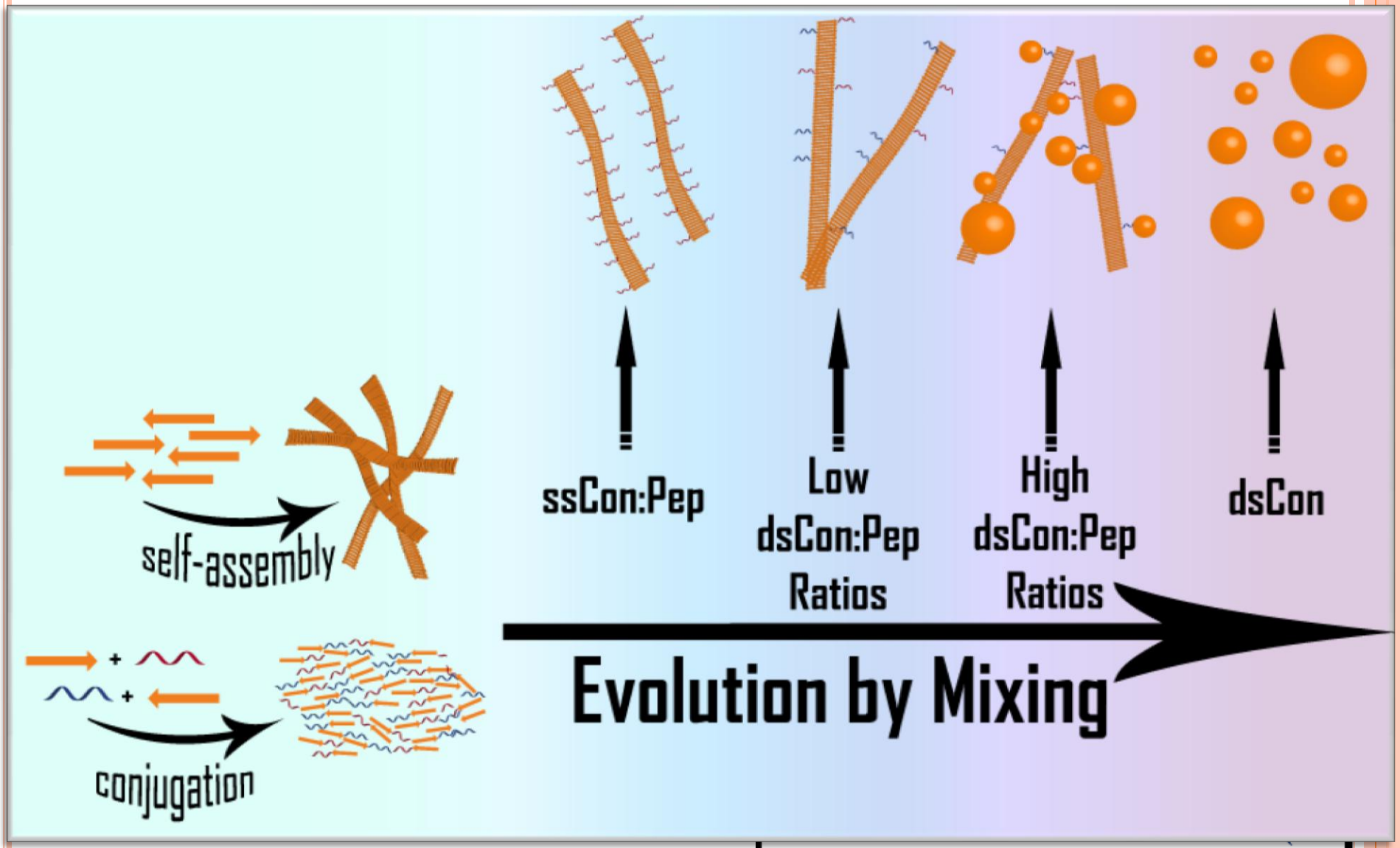
Design of the NA-peptide system



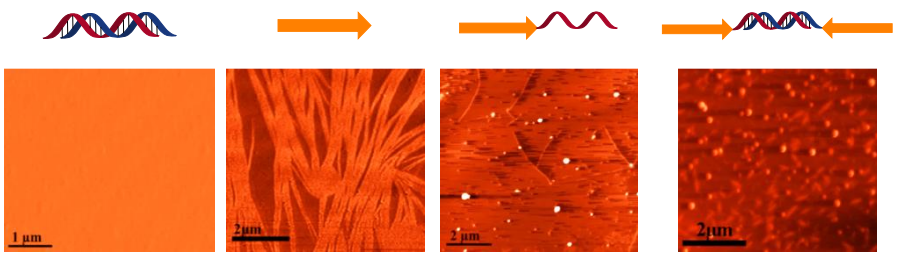
Morphological pathway



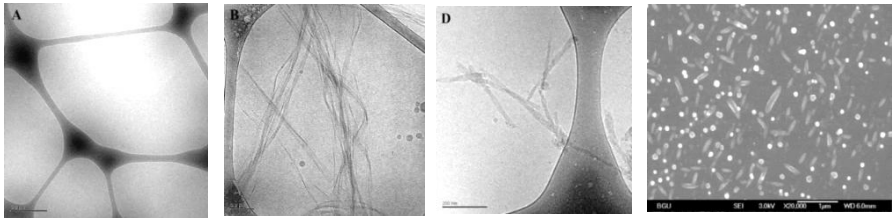
dsCon – structural characterization



PEP-RNA CONJUGATES SELF-ASSEMBLY

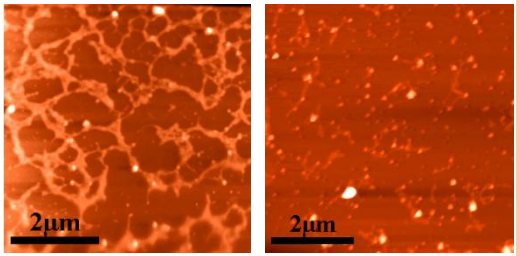


AFM images obtained for 50 µM concentration of non-conjugated RNA, 50 µM peptide, 50 µM RNA1-pep conjugate and 50 µM dsRNA-pep (10 mM phosphate buffer, pH = 7).



Cryo-TEM images obtained for 500 µM concentration of non-conjugated RNA, 50 µM peptide, 50 µM RNA1-pep conjugate and SEM microscopy of dsRNA-pep (10 mM phosphate buffer, pH = 7).

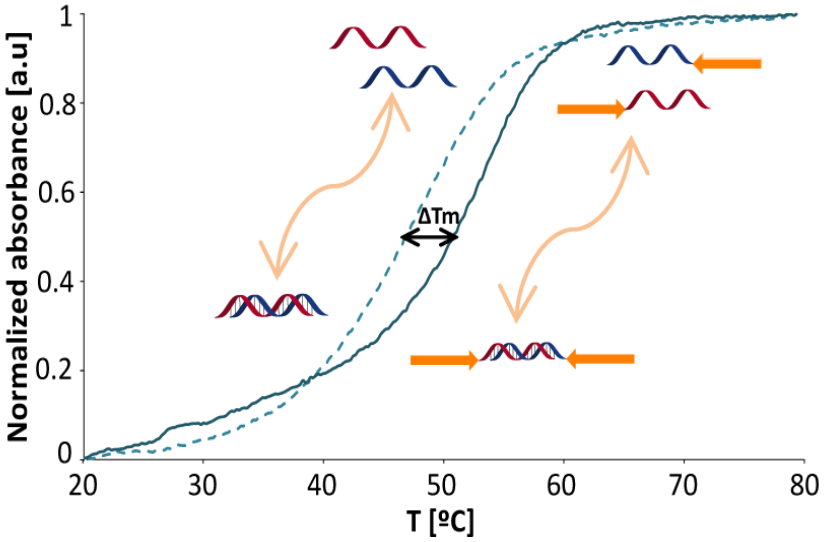
→	40µM	16.6µM
→ ←	10µM	33.2µM



AFM images obtained for 50 µM total concentration of peptide and dsRNA-pep mixtures (10 mM phosphate buffer, pH = 7).

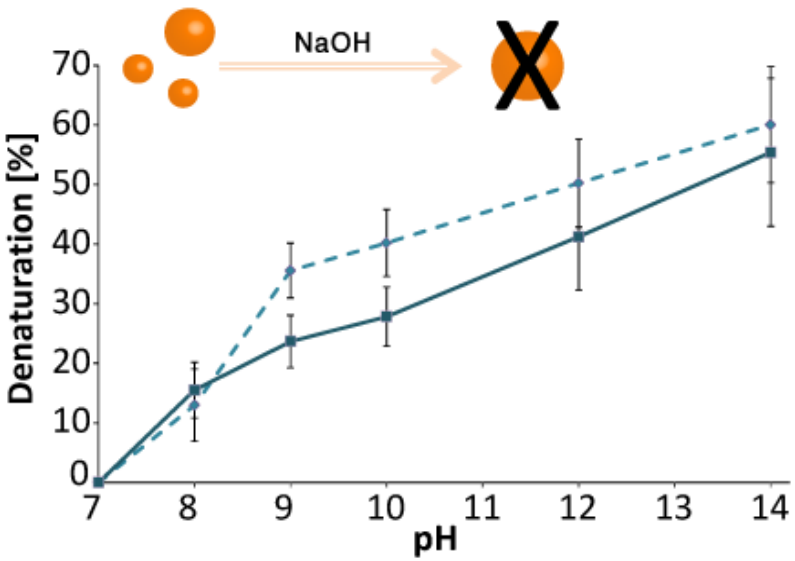
Stability of NA-pep conjugates

Thermal Denaturation



Thermal melting of DNA-pep chimeras and non-conjugated dsDNA as a control. Tm was found using the change in absorption at 260 nm upon transition from duplex to single strands. The graph shows the change in UV-absorption at 260 nm upon a change in temperature, averaged from three down and up scans.

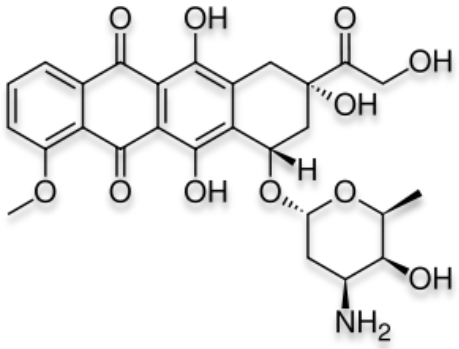
Chemical Denaturation



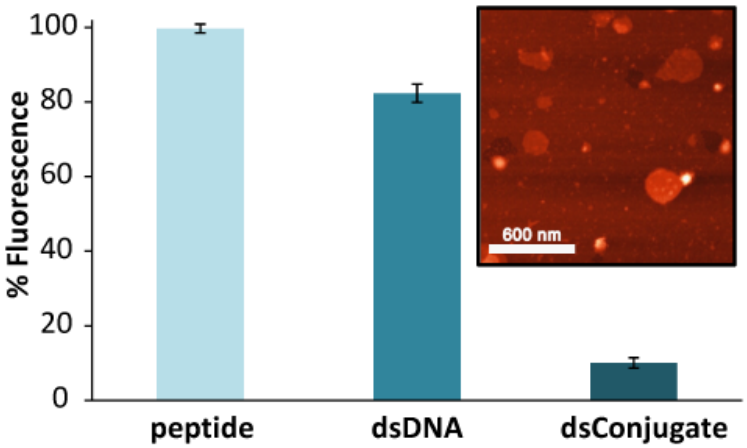
Chemical denaturation of DNA helix leads to the separation of complementary strands that can be followed by an increase in absorption at 260 nm, similarly to the Tm determination.

$$\text{Denaturation (\%)} = \left(\frac{Final\ A_{260} - Blank\ A_{260}}{\text{Initial } A_{260}} - 1\right) x200$$

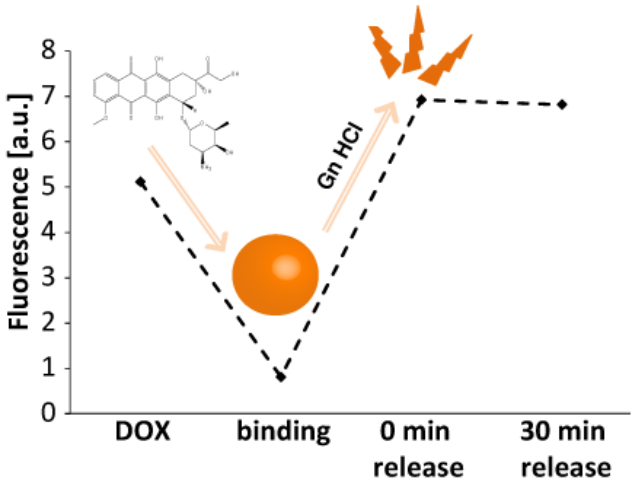
Function of NA-PEP conjugates: Dox binding



- Doxorubicin is a chemotherapeutic drug
- Popular research tool due to the inherent fluorescence
- Interacts with DNA by intercalation
- DOX fluorescence is quenched upon intercalation



Binding of 10 μM DOX in 1:1 ratio (inset showing the AFM image of spheres upon intercalation of DOX).

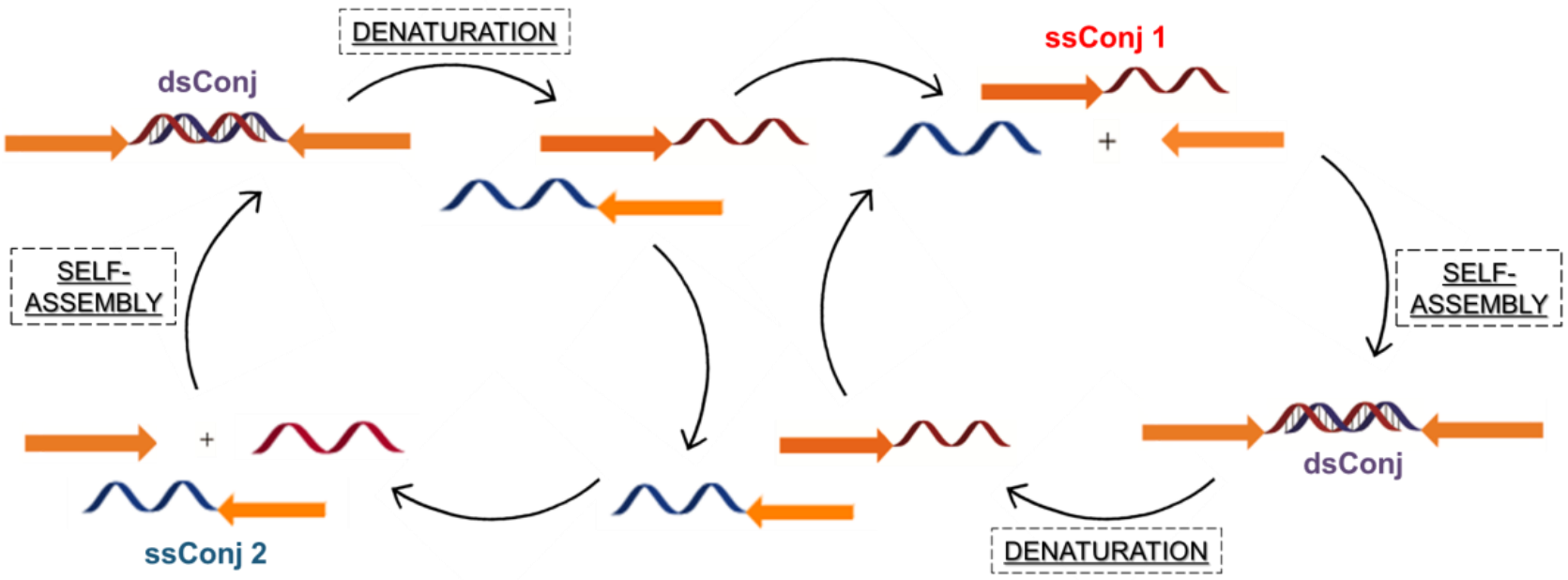


Binding of DOX to dsCon and release using 3 M guanidinium chloride as a denaturing agent.

Conclusions

- **We have successfully designed and synthesized novel peptide-DNA conjugates.**
- **We observed step-by-step morphological transition leading from peptide dominated fibrillar architectures into dsCon based spherical structures.**
- **Using a range of tools we studied assemblies formed by the dsCon and we revealed the formation of round spheres. According to presented statistical analysis of observed entities, we proposed the formation of lamellar spheres due to sequential assembly of the dsCon layers.**
- **In addition, we proved that dsCon exhibits increased stability towards elevated temperatures and pH, and serves as efficient binder for small molecules, such as Doxorubicin.**

FUTURE OUTLOOK:
NA-PEPTIDE SELF-REPLICATION



Acknowlegdments

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