Design of a directed molecular network using DNA double strand to accelerate peptide autocatalysis

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ABSTRACT: An attempt to accelerate known autocatalysis formation of T1N3 peptide and to increase its yield using DNA double strand specific binding by attaching each strand to T1N3 template and to one of the reactants by click chemistry.

PREVIEW

The attempt to construct a self-replicating system in the molecular level is a growing field in chemistry, biology and physics for the past two decades. The simple autocatalysis models created in the lab can eventually contribute to a more holistic understanding of complex biological systems that participate in the catalysis of self-replication. The system studied here is a synthetic autocatalysis system of coiled coil peptide researched separately for a few years, the kinetic constants for the reaction are known as well as yields of the wanted product and protocol procedure. The following experiments are the attempt to create and even more complex autocatalysis system and to gain more control and even improvement in the rate and yield of the reaction compared to the less complicated peptide only system. The new system designed here is based on previous peptide only autocatalysis system improved with the introducing of DNA oligomers to exploit DNA's single strand high affinity to react with complementary strand to achieve a double strand helix.

A rationally designed from the bottom up complex system combined with different molecular entities, amino acids and nucleic bases, that work together in order to catalysis a self-replication process is a step forward in the research of creating synthetic models to achieve understanding and insight on complex replication processes occurs in biological systems.

EXPERIMETIAL WORK

The purpose of the project is to try and increase the formation of the T1N3 peptide from the E1 moiety, an electrophilic peptide fragment bearing a C-terminal thiolester and a nucleophilic peptide, N1, possessing an N-terminal cysteine residue. The fragment ligation to form the T1N3 peptide is an autocatalysis process where T1N3 facilitate the reaction by creating a peptide coiled coil assembly of two T1N3 (figure 1). The coiled coil structure is in equilibrium conditions with the single peptide and a background reaction of the reactants, E1 and N3, without the template is also taking place.

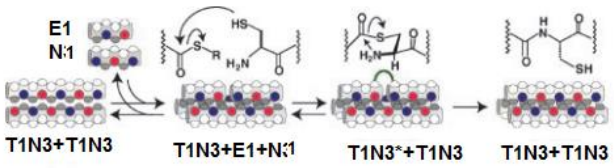

Figure 1- Schematic representation of the template-directed peptide fragment ligation preorganize on the complementary peptide T1N3 to form a coiled-coil.[1]

The following presentation is a summary of the preliminary results of the rational design of a peptide-DNA structure where a peptide and DNA single strand are attached via click reaction, with an Alkyne modified DNA and an Azide modified peptide. The idea is to use DNA double strand to accelerate the reaction time and to increase the product yield by exploiting DNA's specific binding to create a double strand structure.

Two single strand DNA with an Alkyne modification (figure 2) were purchased from 'base-click', each strand was modified on the opposite ends of the strand. FTA_As a 15 bases long strand without the modification, modified on 3' end. FTA_Ds a 25 bases long was modified on the 5' end. Additionally, two thymine (T) bases were added between the strand and the modification to leave sufficient space and not to disturb the double strand formation or the click reaction with bulky base groups. Each strand contains 44% G or C bases in order to maximize bonding affinity and to make sure the double strand formation will reach high yields. The strands were designed to be in different lengths but complementary along the 15 first bases in order to be able to conduct a strand migration reaction if wanted in further research, and by thus

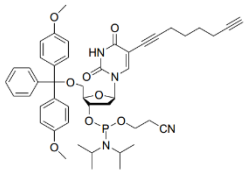

Figure 2 – Alkyne modification. C8-Alkyne-dU-CEP [2]

removing the effect of the DNA double strand allowing the coiled coil disassemble without the interference of the double strand.

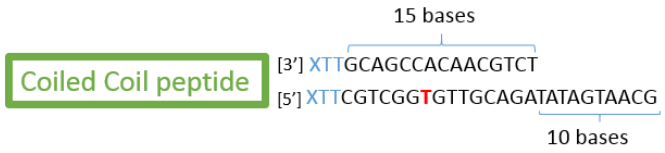

Figure 3 - DNA sequences. X=Alkyne modification

The synthesis of the peptide was done according to the protocol, a ABA residue was added before the azide modification in order not to interfere with the formation of the peptide. Until now only T1N3 template with the azide modification was synthesized, in the following weeks an E1 electrophile with an azide will be synthesized.

The purpose of the binding between DNA strand and peptide is to minimize the time a template and one of the reactants takes to meet in the sample and to stabilize the template and reactant construction, by thus reducing the reaction time and increasing T1N3 amounts.

The Click reaction between the template and FTA_As was conducted according to the protocol and method used in previous click reactions between DNA and peptide. The protocol is attached at the end.

PERLIMANRY HPLC RESULTS

All measurements were performed using Water's 2695 HPLC and Water's 2996 full array detector. The column in use is C18 Clarity 3µm Oligo-RP, LC Column 30x2mm. Detection of peptide segments is measured under 270nm wavelength. DNA or DNA-peptide is measured under 260nm. The mobile phase is TEAA 1x pH 7 (detailed at the end) and acetonitrile. DNA samples are kept in TE buffer and peptide samples in water. Flow rate is 1.5mL/min. Materials were quenched before injection to pH ~2 in 20% Acetic Acid in order to maintain conditions at the experimental stage. All samples are added TEAA 1x to 100uL volume prior to injection.

Reactants retention time:

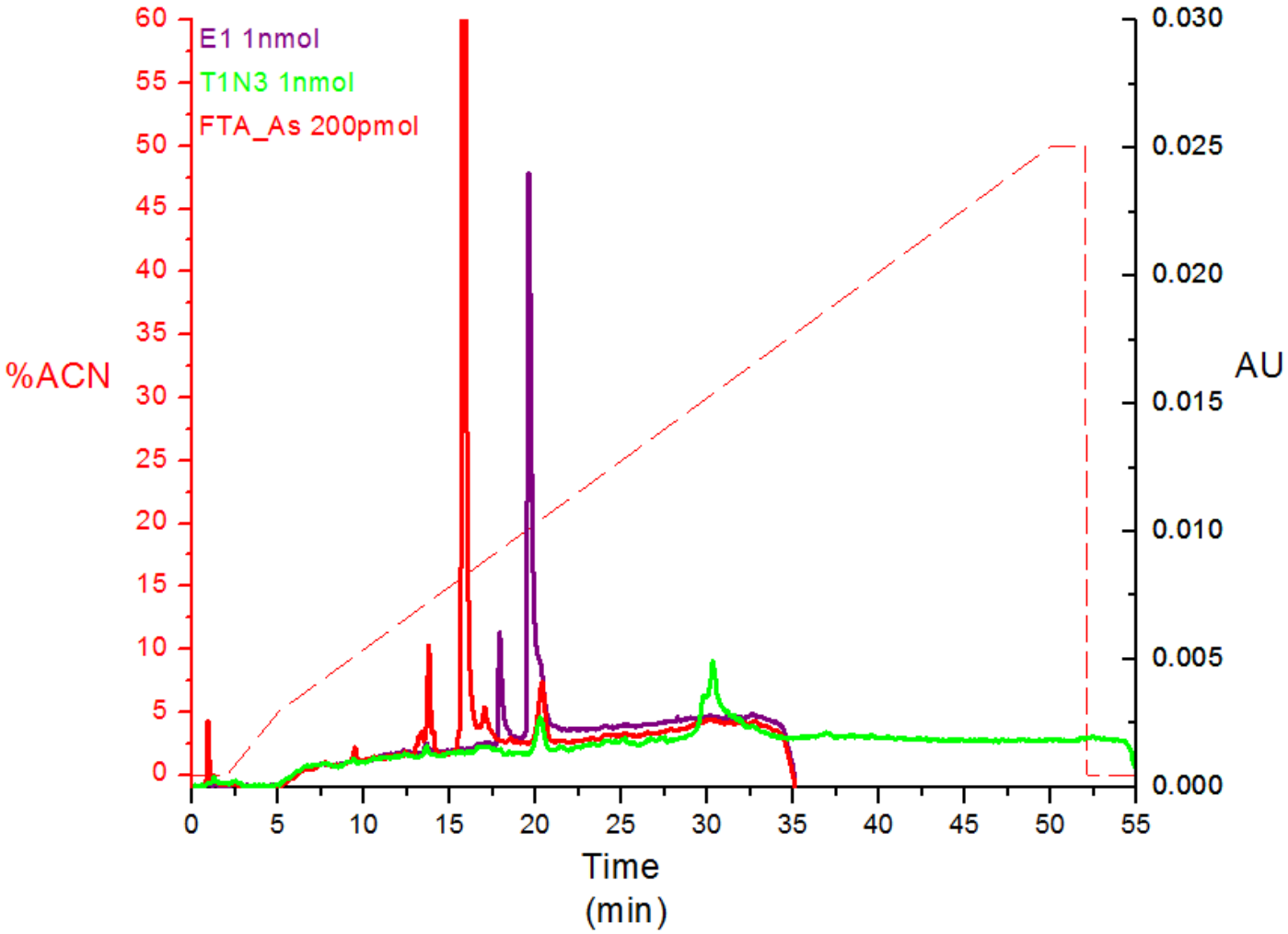

Figure 4 - E1 has a specific two pick layout as a result of the ABA tag. T1N3 was not quenched as a result a wide pick.

Click reaction between T1N3 and FTA_As after 3hr:

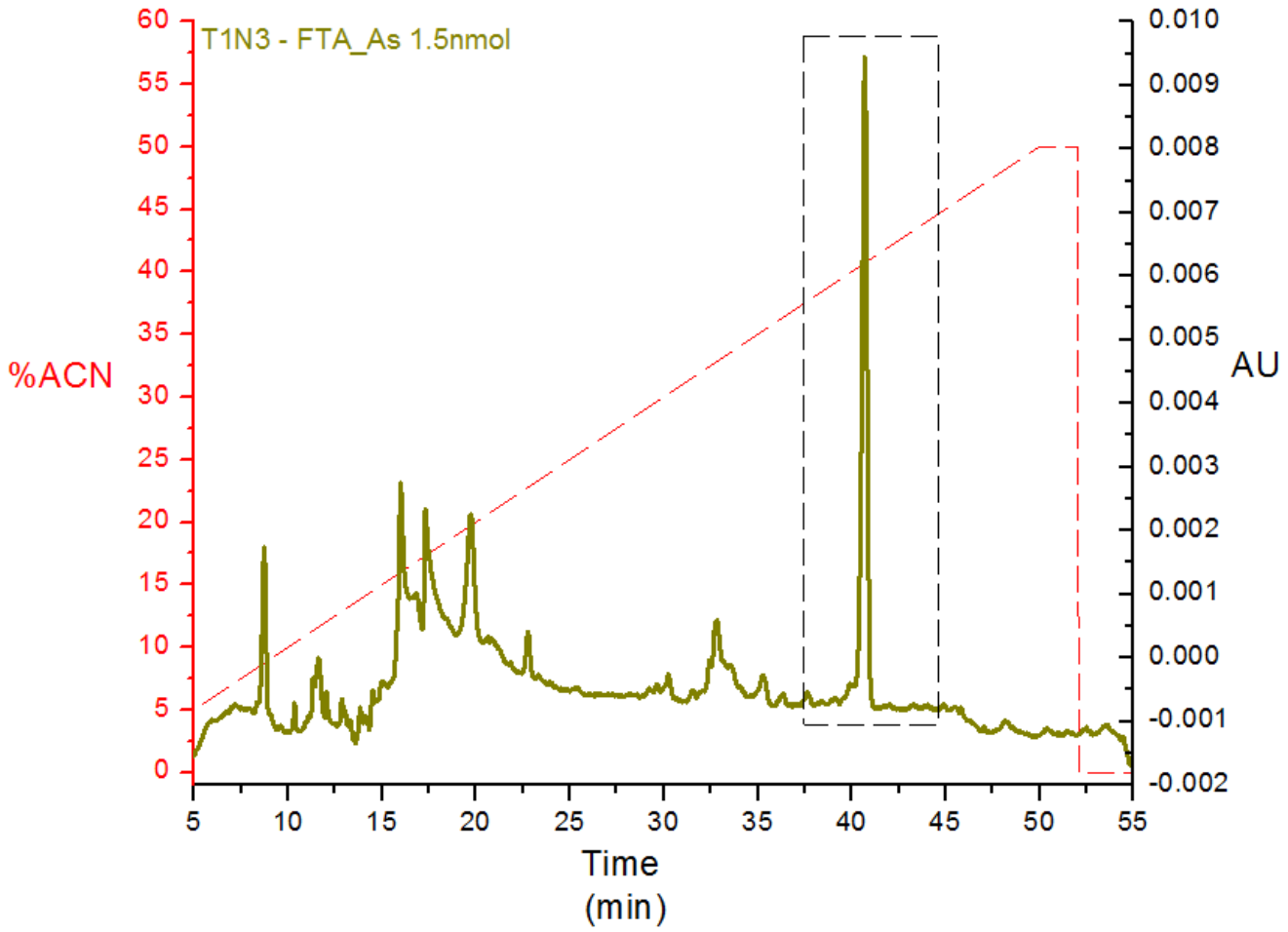

Figure 5 - Pick was collected and re-injected to verify product. Sodium Ascorbate absorb at 260nm, thus 5 first minutes were discarded. A sample of the product will be mass spectra to verify Mw.

E1 + N1 background reaction:

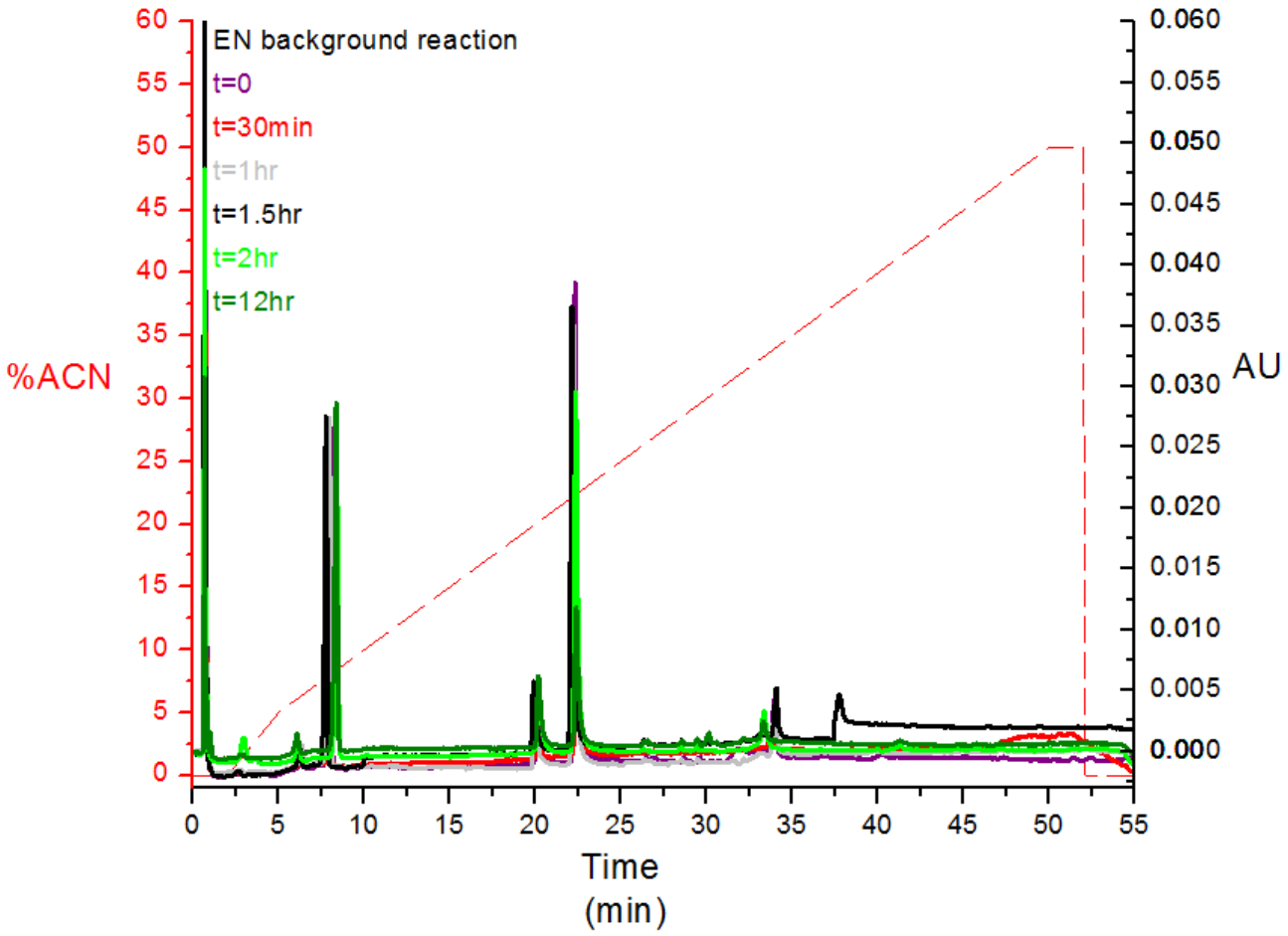

Figure 6 – EN background reaction is conducted in order to reduce T1N3 concertation in the final experiment from the autocatalysis reaction All samples were quenched prior to injection in order to stop the reaction. According to the area of E1 there is no obvious decrease in its amounts. The experiment will be conducted again in higher concertation to verify the results.

DEDUCTIONS

Peptides have lower absorbance coefficient than DNA, thus use of higher concertation in experiments has to be taken into account and to minimize DNA modified strand waste.

A new method of verifying Mw or product analysis has to be introduce. HPLC alone would not give conclusive results.

Click chemistry materials (especially Sodium Ascorbate) has to be taken in consideration when using HPLC at 260nm wavelength.

There is a need to improve acetonitrile gradient program to fit reactants and products to save time and receive better results (maybe a column changes).

FURTHER WORK IN COMING WEEKS

EN background reaction has to done again, in higher amounts and a more control setting.

Synthesizing E1 Azide modified and click with FTA_Ds. Receive data of products from HPLC and some other technique to verify Mw of product.

Conducting autocatalysis reaction without the DNA double strand as a control experiment.

Conduct the experiment with the DNA in different concentrations and reaction time and to compare with the control experiment.

SUPPLEMTRY DATA

Peptides:

Name	**Sequence**	**Molecular weight**
E1	Aba-**RVARLEKKV SALEKKVA**-COSR	2210
N1	**CLEYEVARLKKLVGE-**CONH2	1750
T1N3	N3-**$A_\beta A_\beta$RVARLEKKVSAL EKKVAGLEK(**Aba**)EVARLKKLVGE**-CONH2	3919

Buffers:

TEAA 1x

Materials	Volume percentage
Glacial HOAc	5.7
Triethylamine	13.9
H_2O	80.4

TE

Materials	Concertation (mM)
Tris-HCl	1
EDTA	0.1

Click reaction protocol:

Solvent is TDW-TBuOH (50:50)

Materials	Concertation (mM)
$CuSO_4$	1
TBTA	1.5
Na Ascorbate	40
Alkyne	0.1
Azide	0.1

REFERNCES

1. 'Design of a directed molecular network', PNAS, Gonen Askenasy, 15, 2004.
2. 'BaseClick' product list.