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論　文　内　容　要　旨

（NO．1）

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学位論文の 題　　目	Development of fluorogenic peptide probes carrying internally-incorporated cyanine dyes as FID indicators for dsRNAs （RNA 二重鎖を標的とする FID インジケーター: シアニン色素を内部に組み込んだ蛍光性ペプチドプローブの開発）		

論　文　目　次

In **Chapter 1**, she expounded on dsRNA-targeting probe scene that serves as the backdrop for both Chapters 2 and 3. She pointed out that new analytical methods for double-stranded RNA (dsRNA) need to be developed to overcome problems such as poor selectivity or perturbation / destruction of the native dsRNA structure. To that end, she designed two fluorogenic peptide probes – the small molecule – PNA oligomer conjugate (SPOC) probe and the light-up peptide indicator (LUPI). These two probes both have a thiazole orange (TO) fluorophore that is internally-incorporated into the probe. These two probes were designed to be used as fluorescent indicators in a fluorescent indicator displacement (FID) assay to screen for new small molecules that could potentially act as drugs targeting dsRNA. The mechanism for the FID assay as well as characteristics of good FID indicators were also discussed.

Chapter 2. The first dsRNA target she chose the bacterial ribosomal RNA acceptor site (rRNA A-site). The bac rRNA A-site is the proofreading centre of the bacterial ribosome, and thus has been an attractive drug target for many decades. It contains an internal loop flanked by dsRNA regions. In this chapter, she designed the small molecule – PNA oligomer conjugate (SPOC) probe to simultaneously bind to the internal loop and the dsRNA region by a simple conjugation strategy between ATMND-C_2-NH_2 (internal loop binder) and a tFIT probe. The SPOC probe was expected form a triplex with the bacteria rRNA A-site through Hoogsteen base-pairing, with the ATMND moiety binding to the internal loop.

This conjugation strategy, although simple, worked impressively. Notably, the SPOC probe was able to bind to the dsRNA target even at a neutral pH, which is an unfavourable condition for Hoogsteen base-pairing. This was because the conjugated ATMND moiety was able to act as an anchor, facilitating binding even at pH 7.0. The probe was measured to have a dissociation constant (K_d) of 190 ± 72 nM and bound specifically to the bac rRNA A-site over the corresponding human rRNA A-sites. Lastly, she showed how the SPOC probe had potential as an FID indicator by conducting a mock FID assay, where the degree of displacement was in good correlation with the reported K_d value of the test compounds.

However, the SPOC probe design has its drawbacks. The lack of information about small molecules that can specifically bind to RNA structural features as well as the need for a homopurine stretch of RNA for Hoogsteen-base pairing limits the dsRNA targets that SPOC can be applied to.

Thus, in **Chapter 3**, she turns to a new probe design – LUPI. This time, instead of targeting a dsRNA, she targeted a ribonucleoprotein (RNP) complex (RNA + RNA-binding protein, RBP). As such, LUPI was designed to be a peptide indicator based on a well-known RNP complex– the HIV-1 Tat protein – TAR RNA model. The Tat protein binds to the TAR RNA to greatly enhance viral transcription. Without the Tat-TAR interaction, gene expression and replication are adversely affected. Hence, finding Tat-TAR inhibitors is an attractive therapeutic strategy. Unlike SPOC, LUPI is made up of amino acids, with its internally-incorporated TO acting as a surrogate amino acid, something that has never been reported before. Its amino acid sequence is based on the arginine rich motif (ARM) of the Tat protein, the part that is responsible for binding to TAR RNA.

By comparing LUPI with three other probes, one of which was the original Tat peptide fluorescent indicator, she showed that LUPI has superior light-up response, binding affinity (K_d = 1.0 ± 0.6 nM) and selectivity towards the TAR RNA over two other unrelated RNAs. Moreover, in a mock FID assay with five test compounds, only the test compound with a K_d comparable to LUPI's was able to displace it, highlighting LUPI's ability to sieve out super-strong binders.

Finally, in **Chapter 4**, she summarized her findings for the SPOC and LUPI probes as well as provide some thoughts about how these two probes can be tailored for other dsRNA targets, as well as other possible ways to develop the probe designs even further.

別　紙

論文審査の結果の要旨

本博士論文は、全４章から構成されており、FID アッセイ（Fluorescent Indicator Displacement Assay）の蛍光プローブとして、RNA 二重鎖構造を標的とする三重鎖形成ペプチド核酸（peptide nucleic acid, PNA）あるいは短鎖ペプチドの設計・合成と機能解析に関する研究成果が述べられている。

第１章では、RNA 二重鎖構造を標的とする分析手法を概観するとともに、本博士論文の主目的である FID インジケータを開発する意義・重要性が記述されている。

第２章では、バクテリアリボソーム RNA（rRNA）の A-site を標的として、三重鎖形成 PNA と小分子とのコンジュゲート型プローブ(small molecule-PNA oligomer conjugate, SPOC)を提案、中性条件下においても、SPOC プローブが三重鎖を形成して rRNA A-site に結合し、優れた結合力・選択性・蛍光応答機能を発現することを見出している。その結合力は中性条件下においても解離定数 K_d =190 ± 72 nM（pH 7.0, 25°C）に達し、rRNA A-site を標的とする非アミノグリコシド系化合物として最強と言ってよい。また、結合選択性も優れており、バクテリア rRNA A-site に対して明確な結合選択性を発現する。さらに，off-on 型の明瞭な蛍光応答を示し（λ_{em} = 537 nm, I/I_0 = 68-fold）、FID 法における蛍光インジケータとして活用できることを示している。小分子を PNA に連結することで、中性条件化における三重鎖形成を実現した意義は大きく、三重鎖核酸形成のボトルネック「酸性条件」を克服する有用な方法論になると期待できる。

第３章では、ヒト免疫不全ウイルス I 型（HIV-1）TAR (trans-activation responsive region) RNA を標的として、Tat (Trans-activator) ペプチドの basic domain を活用した LUPI (Light-up peptide indicator) を提案、LUPI が極めて強力な結合力と結合選択性を発現することを見出している。その結合力は、K_d =1.0 ± 0.6 nM（pH 7.4, 25°C）に達し、Tat ペプチド自身に匹敵する。また、LUPI の off-on 型の light-up 応答は特筆に値するもので、ほぼ無蛍光の状態から蛍光強度が 108 倍増加する（λ_{em} = 540 nm, ϕ_{free} = 0.00567, ϕ_{bound} = 0.614）。ここで得られた最も重要な知見は、蛍光色素として組み込んだチアゾールオレンジ（thiazole orange, TO）が擬似アミノ酸残基として機能することで、light-up 応答機能を付与しうる新規分子デザインとして、種々のペプチドに適用できると期待できる。

第４章では、本研究で得られた知見を総括するとともに、今後の展望が述べられている。

以上の研究成果は、論文提出者が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、LEE, En Ting Tabitha 君提出の博士論文は、博士（理学）の学位論文として合格と認める。