2026 Summer NHL Project Descriptions

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Micelles for the Characterization and Treatment of Calcium Phosphate-based Kidney Stones

Kidney stones form naturally when excessive concentrations of certain minerals, such as calcium, are found in the blood. Numerous conditions can lead to kidney stone formation, including but not limited to dehydration, a chemical imbalance, or certain medical conditions such as Crohn's disease. Due to the variety of formation pathways of kidney stones, their composition in humans is also varied. Despite this, 70-80% of kidney stones have calcium as a principal component, with calcium phosphate acting as a nucleus for their formation. As such, targeting the kidney stones' calcium component, and in particular removing the calcium from the stones, may lead to their dissolution.

One possible means of treating kidney stones would be to use micelles possessing negatively charged hydrophilic head groups. Preliminary experiments have shown that these negatively charged micelles may interact with calcium, even in the presence of phosphate, with which it is well-known to mineralize, imitating the function of the calcium sequestering protein, fetuin-A.

To characterize this process, we use terbium lifetime measurements. As terbium is positively charged, like calcium, it also interacts with the micelles and, in the presence of phosphate, is protected from water, causing a change in its lifetime compared to when it is dissolved in water.

- Lead: Shane Scott, Munir Muhammad, Samir Pal

Competitive TR-FRET assay to dose small molecules

Collaboration with Bio-techne (www.bio-techne.com)

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The goal of this project is to develop a sensitive and specific biochemical assay platform for the quantification of residual small molecule reagents used in the generation of cell therapies. The designed competitive TR-FRET (Time-Resolved Förster Resonance Energy Transfer) assay could serve as an important quality control test for residual reagents, as well as a method to further optimize the reagents used in iPSC derived cell lines manufacturing.

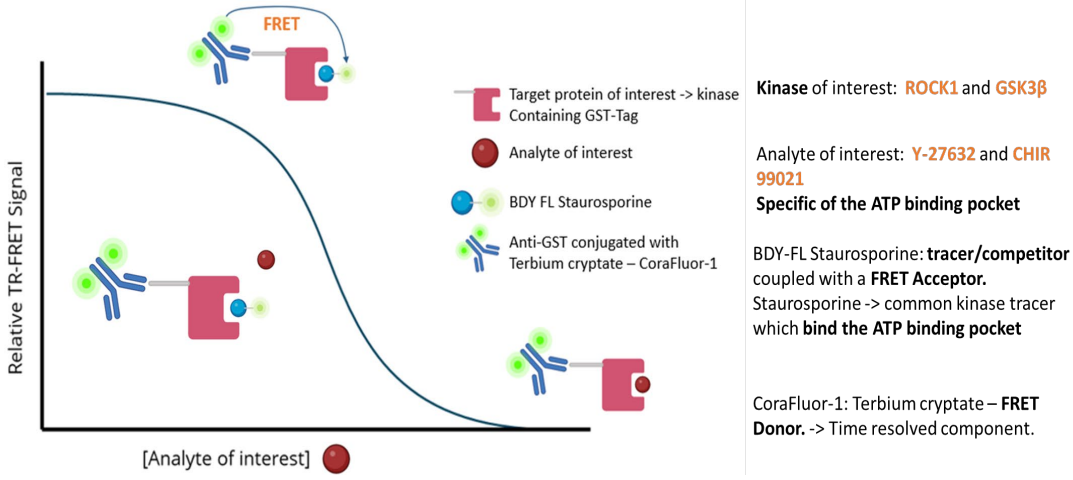
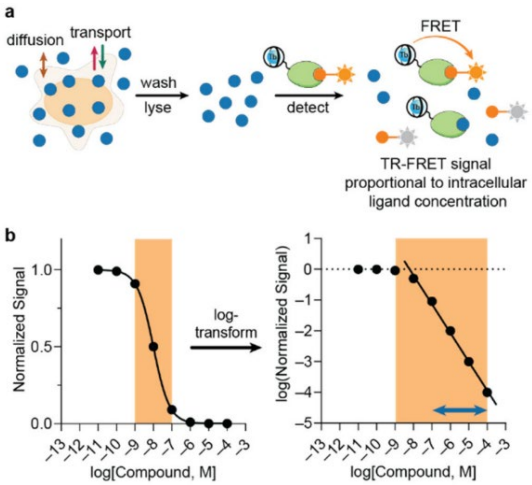


Figure 1 – Principle of the competitive TR-FRET assay platform.



One molecule is widely used to support stem cell differentiation and cryopreservation, improving cell survival. It is also being studied for preconditioning to enhance cell survival post-transplantation, with promising results observed in iPSC-derived (induced pluripotent stem cell) retinal cell transplantation in monkeys[1].

Figure 2 – Quantification of intracellular small molecule concentrations using a competitive TR-FRET assay[2].

An other molecule is commonly used to promote cell differentiation across various cell types. In a review article, CHIR 99021 was included in 20 out of 26 molecule cocktails to induce the direct reprogramming of host-harvested cells[3].

References:

(1) Ishida M, Sugita S, Makabe K, Fujii S, Futatsugi Y, Kamao H, et al. A ROCK Inhibitor Promotes Graft Survival during Transplantation of iPS-Cell-Derived Retinal Cells. Int J Mol Sci. 22 march 2021;22(6):3237.

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(2) Payne, N. C.; Mazitschek, R. A Lysate-Based TR-FRET Approach for the Facile Quantification of Cellular Drug Concentration. January 17, 2024. https://doi.org/10.26434/chemrxiv-2024-2v7k1.

(3) Takeda Y, Harada Y, Yoshikawa T, Dai P. Chemical compound-based direct reprogramming for future clinical applications. Biosci Rep. 29 june 2018;38(3):BSR20171650.

- Lead: Arthur Charasson

RCA-TG-FRET miRNA detection for endometriosis diagnosis

Collaboration with the Leonardi Research Group (https://mathewleonardi.com/research-and-publications)

Collaborations might evolve for this project.

Endometriosis is a chronic, inflammatory gynecological condition characterized by the presence of endometrial-like tissue outside the uterus, often causing debilitating pain.

According to Home and al.[1], it affects approximately 10% of women during their reproductive years. The diagnostic process for this condition is lengthy and painful: nearly 60% of women consult three or more clinicians before receiving a diagnosis, with an average delay of seven years from the onset of symptoms, allowing the disease to progress. A performant, non-invasive and rapid method to diagnose endometriosis does not exist. Laparoscopy, a chirurgical exploration of the peritoneal cavity, is the most common diagnosis method.

MiRNAs are seen as having significant potential for developing a non-invasive diagnostic method for endometriosis[2]. Several studies have highlighted their diagnostic potential, with some suggesting they could be as effective as laparoscopy, particularly in detecting the early stages of the disease, which are more difficult to identify[3,4].

This project aims to apply RCA-TR-FRET (Rolling Circle Amplification Time-Resolved Förster Resonance Energy Transfer) detection method for miRNA, previously developed by Niko Hildebrandt's lab[5], to the diagnosis of endometriosis. This approach has been shown to offer improved sensitivity and selectivity compared to the standard RT-qPCR.

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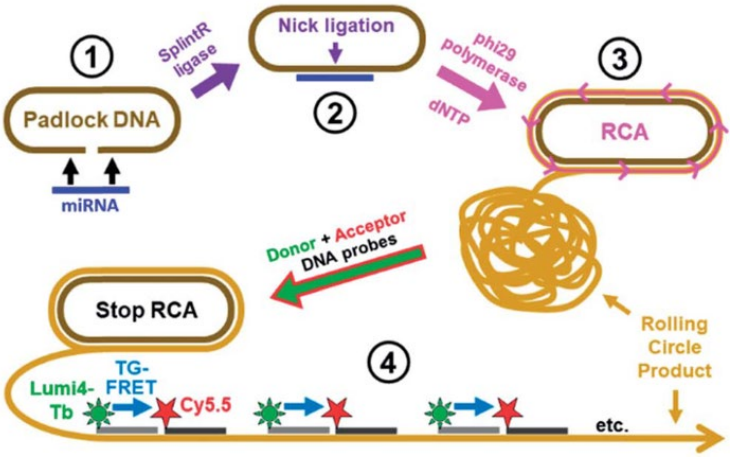

Figure 1: Principle of miRNA detection by amplified TG-FRET.[5] After specific recognition of miRNA by a padlock DNA (1), the DNA padlock nick is ligated over the miRNA target (2) and the miRNA becomes a primer for a polymerase to synthesize and displace (by RCA) complimentary DNA around the circularized padlock DNA (3). After stopping RCA, the rolling circle product (RCP) is incubated with Tb (Lumi4-Tb) donor and Cy5.5 acceptor labeled ssDNA, which hybridize to specific sequences that exist more than 1000-fold on the amplified RCP concatemer. The close distance of Lumi4-Tb and Cy5.5 in the RCP allows for Tb-to-Cy5.5 FRET, which is not possible if both are free in solution (not hybridized to the RCP). Thus, the TG-FRET signal can be used for quantifying miRNA without any washing or separation steps.

Collaborating with the Leonardi Research Group provides expertise on disease and access to patient samples from laparoscopic surgery performed by Dr. Leonardi.

References:

(1) Home AW, Missmer SA. Pathophysiology, diagnosis, and management of endometriosis. BMJ-British Medical Journal. 14 nov 2022;379:e070750.

(2) Ronsini C, Fumiento P, Iavarone I, Greco PF, Cobellis L, De Franciscis P. Liquid Biopsy in Endometriosis: A Systematic Review. Int J Mol Sci. 24 mars 2023;24(7):6116.

(3) Moga MA, Bălan A, Dimienescu OG, Burtea V, Dragomir RM, Anastasiu CV. Circulating miRNAs as Biomarkers for Endometriosis and Endometriosis-Related Ovarian Cancer—An Overview. Journal of Clinical Medicine. 23 mai 2019;8(5):735.

(4) Papari E, Noruzinia M, Kashani L, Foster WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertil Steril. juin 2020;113(6):123241.

(5) Qiu X, Xu J, Guo J, Yahia-Ammar A, Kapetanakis NI, Duroux-Richard I, et al. Advanced microRNA-based cancer diagnostics using amplified time-gated FRET. Chemical Science. 2018;9(42):8046-55.