

PROJECT TITLE

Study of different platforms for screening of nucleic acid markers associated with celiac disease using electrochemiluminescence

PROJECT SUPERVISORS: Dr. Mayreli Ortiz, Dr. Ciara O'Sullivan

e-mail: mayreli.ortiz@urv.cat, ciara.osullivan@urv.cat

Telephone: + 34 977 55 8576

Nanobiotechnology and Bioanalysis Group

Department of Chemical Engineering

Universitat Rovira i Virgili

Avinguda Països Catalans, 26

Tarragona 43007, Spain

Overview:

The overall objective of the project is to develop a generic platform capable of detection of nucleic acid markers associated with celiac disease with electrochemiluminescent (ECL) transduction.

Background and State of the Art:

Electrogenerated chemiluminescence (also called electrochemiluminescence and abbreviated as ECL) is a process whereby species generated at electrodes undergo high-energy electron-transfer reactions to form excited states that emit light. ECL has become a very powerful analytical technique and been widely used in the areas of, for example, immunoassay, food and water testing, and biowarfare agent detection,¹ highlighting the use of ECL as a powerful tool for ultrasensitive biomolecule detection and quantification. There are commercially available clinical chemistry analysers such as the Elecsys 1010 and 2010 by Roche Diagnostics which are prohibitively expensive for developing regions. Thus, high-throughput, miniaturized biosensors based on ECL technology capable of multiplexed detection with high sensitivity, low detection limit, and good selectivity and stability continue to attract the interest of the research community. Electrochemiluminescence (ECL) of tris(2,2'-bipyridyl)ruthenium(II) ($\text{Ru}(\text{bpy})_3^{2+}$) is a well-known detection method that provides high sensitivity with low background through generation of an optical signal triggered by an electrochemical reaction.² To trigger the optical signal, a sacrificial amine (usually tripropylamine, TPA) is oxidised at the electrode surface generating a radical that reduces the Ru(II) complex to Ru(I) which is further transformed into a Ru(II) excited state that generates the luminescence. In comparison with traditional laser-induced luminescence detectors, the instrumentation for ECL detection is substantially less complicated and less expensive as the excitation laser and optical filters are eliminated. Moreover, because the ECL reaction only occurs close to the surface of an electrode, the sample requirement is quite small. This makes ECL an ideal detection method for microdevices.³ For example, ECL detection has been utilized in DNA microarray chips, where immobilized oligonucleotide probes are used to screen blood samples for the presence of specific target sequences.⁴ This method has the drawback of the necessity to modify the target DNA in the sample with the ECL label, which is normally incorporated into the amplification step to generate sufficient copies of the target DNA for detection. The amplification/labelling step adds to the processing time but it is necessary in order

¹ Miao, W. *Chem. Rev.*, **2008**, *108*, 2506.

² *Electrogenerated Chemiluminescence*; Bard, A. J., Ed.; Dekker: New York, **2004**.

³ Cao, W.; Liu, J.; Yang, X.; Wang, E. *Electrophoresis* **2002**, *23*, 3683.

⁴ Miao, W.; Bard, A. J. *Anal. Chem.* **2003**, *75*, 5825

to achieve the required levels of sensitivity; however, this step could be eliminated if alternative detection methods with high sensitivity were available.

Coeliac disease is a gluten-sensitive enteropathy that affects as much as 1% of the population and patients with coeliac disease should maintain a lifelong gluten-free diet, in order to avoid serious complications and consequences. Therefore, it is essential to diagnose coeliac disease at the earliest possible conjuncture.

The Human Leukocyte Antigen system (HLA) is the name of the human major histocompatibility complex. In celiac disease, HLA typing can find application in establishing the genetic risk (predisposition).⁵ The association between certain HLA types and coeliac disease is one of the strongest known, and the linkage peak to this region has been very high in all the genome wide linkage studies, and it is clear that understanding the exact nature of the HLA association is not only essential in unravelling the genetics of coeliac disease, but also helpful to understanding the pathogenesis on cellular level and finally, and of relevance here, in developing the use of HLA testing in a clinical situation as an adjunct to diagnosis in coeliac patients.

Methodology:

Task 1: Synthesis and characterisation of bioconjugates.

Nucleic acid detection will be based on immobilized DNA probes carrying either ruthenium-bipyridyl or ferrocene moieties. These probes will be designed for the detection of coeliac disease susceptibility alleles of the HLA-DQA1 and HLA-DQB1 families.

The β -cyclodextrin (β CD) derivatives will be prepared according to previously reported procedures.⁴ In general, the synthetic route will follow the sequence: activation of the hydroxyl groups of β CD, substitution by NH_2 and/or COOH groups and coupling to the probe. In the case of DNA- β CD- $[\text{Ru}(\text{bpy})_3]^{2+}$ the β CD will be previously modified with $[\text{Ru}(\text{bpy})_3]^{2+}$ and subsequently conjugated to DNA.

The conjugates will be purified using gel permeation chromatography GPC (NAP10 column, Sephadex-25) and reverse phase-HPLC. Characterisation will be carried out using NMR and MALDI-TOF.

Task 2: Evaluation of surface chemistry methodologies

In all cases, the biosensor surface will be optimised in terms of achieving maximal sensitivity while reducing non-specific interactions. Optimisation of DNA detection will look at (i) the immobilisation of thiolated DNA on gold electrodes followed by backfilling with a second alkanethiol, (ii) direct co-immobilisation of DNA in the presence of the backfilling agent. The effect of the ratios of one to the other will be measured. Several backfilling agents such as α,ω -mercaptoalcohols and carboxylic acids, as well as thiols bearing poly(ethylene glycol) units will be tested.

Task 3: Development of novel supramolecular DNA detection schemes based on ECL of ruthenium complexes and β -cyclodextrins.

The possibility of using β -cyclodextrins (β CD) to modulate the probe-to-quencher distance through supramolecular interactions will also be studied (Figure 1). Cyclodextrins are cyclic oligosaccharides composed of D-glucose units with the remarkable property to include hydrophobic guests in their central cavity through supramolecular interactions. They have found a broad spectrum of applications in sensing, chromatography, enzyme technology, etc. For this purpose, secondary probes carrying a β CD unit will be prepared. These probes will hybridise to a

⁵ a) Kaukinen, K.P.J., Mäki, M., Collin, P.: *Am J Gastroenterol* **2002**, 97, 695. b) Louka, A.S., Sollid, L.M. *Tissue Antigens* **2003**, 61, 105.

target already recognized by a Ru labelled immobilized probe in a 'sandwich'-type format. Finally, a quencher with the ability to form an inclusion complex with the β CD will be added (Figure 1a). The β CD-Ru distance will be minimized to allow an optimal Ru-quencher interaction. The number of steps in this kind of assay can be reduced if the immobilized probe is modified with a β CD moiety carrying the luminescent unit and the secondary probe incorporates the quencher (Figure 1b).

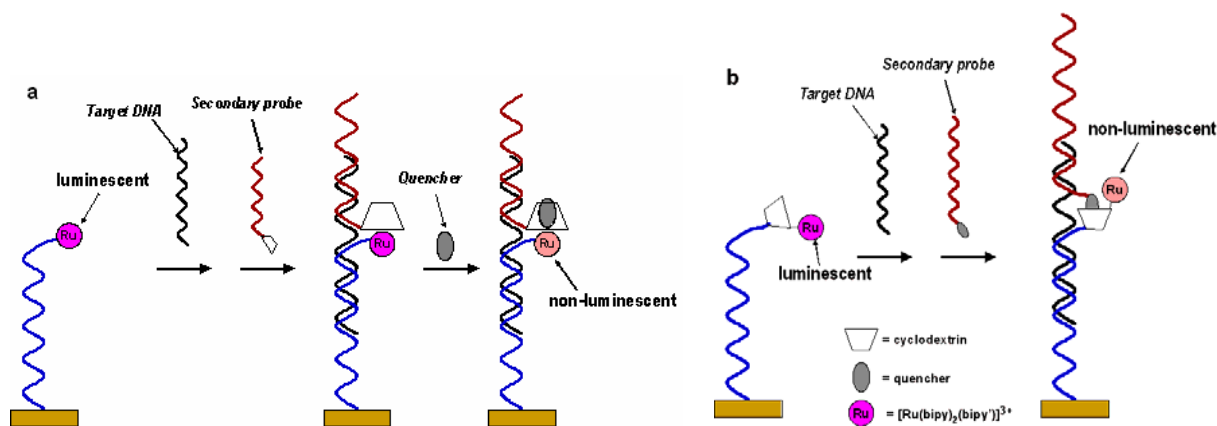


Figure 1. Cyclodextrin based detection of DNA sequences based on ECL quenching. The β CD moiety can be attached to the secondary (a) or to the primary probe (b).