# Novel electrogenerated chemiluminescence peptide-based biosensing of protease

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Electrogenerated chemiluminescence (Electrochemiluminescence, ECL) occurs at/near the surface of the electrode as a result of electrochemical reactions and chemiluminescence reactions [1]. ECL methods have received considerable attentions in clinical diagnosis because of low background, high sensitivity and inexpensive instruments [2]. Recently, we have developed serial of ECL biosensing method for the detection of biomarker, such as small molecules [3], protein [4] and DNA[5]. Here, we reported a serial of novel ECL biosensing of protease biomarker in our lab. For example, we developed a highly sensitive and selective ECL biosensor for metallo-proteinase 2 (MMP-2) on the basis of iridium (III) solvent complex conjugating with histidine at the end of peptide and target-induced cleavage of peptide. The ECL probe was synthesized by coordination labeling ECL reagent (iridium (III) solvent complex, $[(ptz)_2Ir(DMSO)Cl]$, pbz=3-(2-pyridyl)benzoic acid, Ir1) on recognition element (a peptide, Biotin-GPLGVRGKHH) with high ECL efficiency and good storage stability (more than 6 months). This ECL biosensing strategy was proposed to determine MMP-2 via one pot-cleavage reaction and magnetic beads separation in the linear range of 1.0~10 ng/mL with the detection limit of 0.3 ng/mL. Further, the coordination - based site-specific labeling of histidine-rich peptide strategy for MMP-2 assay by incorporation of molecular recognition and signal probe in one step and using magnetic beads on the magnetic carbon electrode amplifying the detection signal, can simplify the experimental steps and avoid the interference of other substances in the sample. Moreover, the dissociation constant Kd between Ir1 labelled peptide and MMP-2 were calculated to be 7.8 ng/mL (0.11 nM) for peptide 1 and 8.3 ng/mL (0.12 nM) for peptide 2, indicate that high affinity of two kinds of peptides for MMP-2. In addition, the ECL biosensing method can be successfully applied for the detection of MMP-2 in serum sample with a 93.0±13.2 - 108.6±10.2 % of recoveries. These ECL biosensing methods provide a promising way for the determination of biological molecules in clinical diagnosis and point-of-care test of protease.

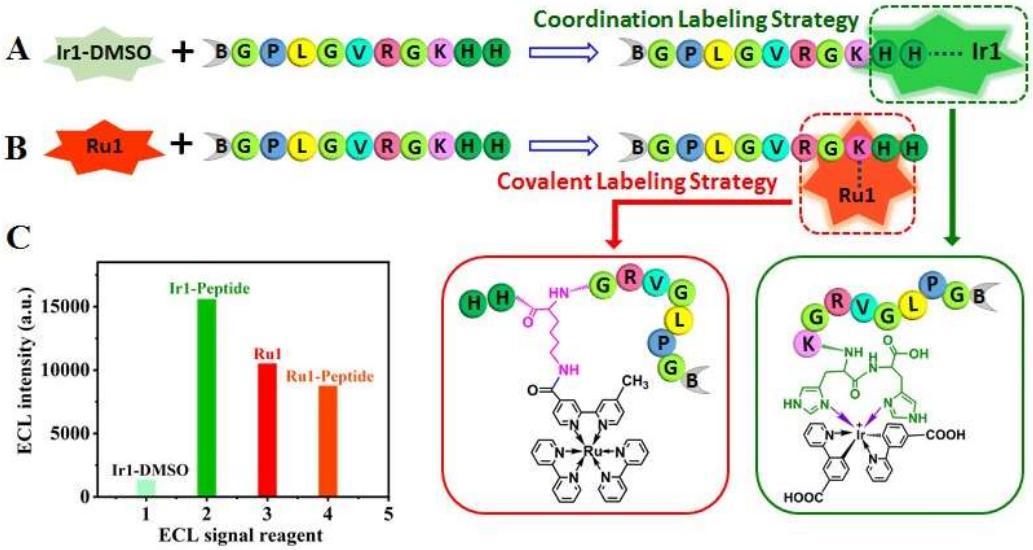


*Figure 1. (A) Schematic diagram of coordination labeling strategy between Ir1-DMSO and peptide, (B) Schematic diagram of covalent labeling strategy between IRu1 and peptide and (C) ECL intensity of 14 μM Ir1-DMSO, 1 μM Ru1, Ir1-peptide and Ru1-peptide ($C_{peptide}$ = 1.0 μM) in 0.1 M PBS (pH 7.40) containing 50 mM TPA Caption*

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