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ACCEPTED MANUSCRIPT

Specific chemiluminescent protocol for dual-site recognition of

Streptococcus mutans utilizing strong affinity between teicoplanin

and Gram-positive bacteria

Xiaoxiao Su, Mengyao Wang, Yue Wu, Yong He, Zhifeng Fu*

Key Laboratory of Luminescence and Real-Time Analytical Chemistry (Ministry of

Education), College of Pharmaceutical Sciences, Southwest University, Chongging 400716,

China

*Corresponding author. Tel: +86 23 6825 0184; Fax: +86 23 6825 1048

E-mail address: fuzf@swu.edu.cn (Z.F. Fu)

Abstract

A novel dual-site recognition protocol was developed for chemiluminescent (CL) detection of

Streptococcus mutans (S. mutans) based on a designed antibiotic-affinity strategy. Teicoplanin,

a broad-spectrum antibiotic against Gram-positive bacteria, was adopted to functionalize

magnetic particles and recognize S. mutans utilizing the strong affinity between this agent and

D-Alanyl-D-Alanine peptide moieties in the bacterial cell wall. To achieve ideal specificity

for S. mutans detection, rat immunoglobulin G2a (rat IgG2a) tagged with horseradish

peroxidase (HRP) was used as the second recognition agent and signal tracer since Fab region

of rat IgG2a could bind with *streptococcal* protein G highly expressed in the cell wall of S.

mutans. Thus HRP-tagged sandwich complex of teicoplanin/S. mutans/rat IgG2a was formed

on the magnetic particles, followed by a CL quantification of S. mutans based on a

HRP-catalyzed luminol-H₂O₂-p-iodophenol CL reaction. This dual-site recognition protocol

showed a linear range of 1.0×10^2 - 1.0×10^6 CFU mL⁻¹ and a detection limit of 33 CFU

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