Electronic supplementary information (ESI)

A microfluidic capacitive immunosensor system for human cartilage chitinase-3-like protein 2 (hYKL-39) quantification as osteoarthritis marker in synovial joint fluid

Wethaka Chaocharoen[a,c], Araya Ranok[a,c], Wipa Suginta*[,a,c,d] and Albert Schulte*[,b,c,d]

[a]School of Biochemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand. E-mail: wipa@sut.ac.th

[b]School of Chemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand. E-mail: schulte@sut.ac.th

[c]Biochemistry - Electrochemistry Research Unit, Schools of Chemistry and Biochemistry, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

[d]Center of Excellence on Advanced Functional Materials, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

Corresponding authors:

Assoc. Prof. Dr. Wipa Suginta; E-mail: wipa@sut.ac.th; Tel.: ++ 66 (0) 44 22 6187;

Fax: ++ 66 (0) 44 22 4185

Assoc. Prof. Dr. Albert Schulte; E-mail: schulte@sut.ac.th; Tel.: ++ 66 (0) 44 22 6187;

Fax: ++ 66 (0) 44 22 4185

1. hYKL-39 immunosensor fabrication.

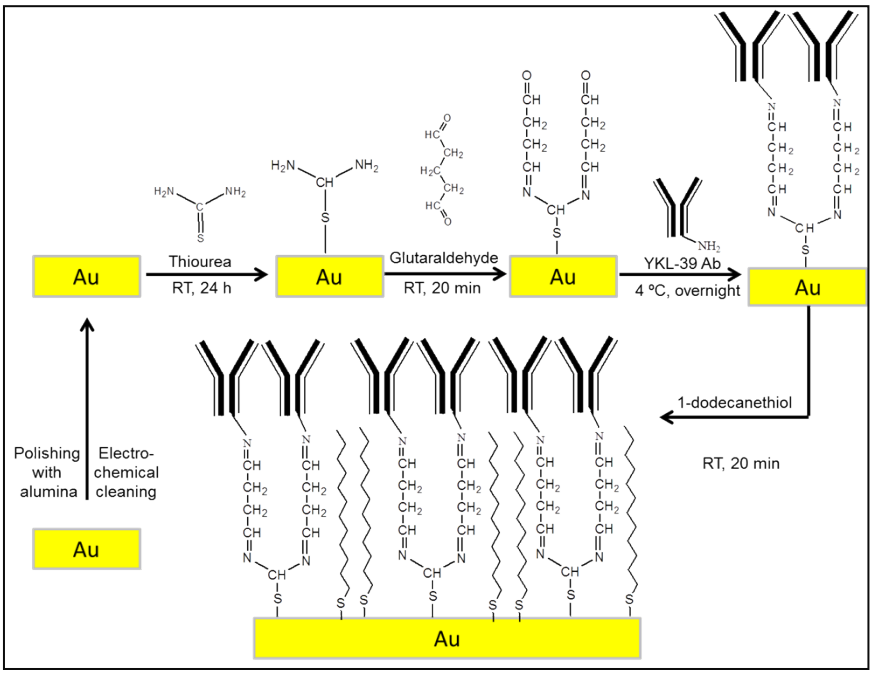

Figure S1: A diagram showing the sequence of steps involved in the preparation of capacitive hYKL-39 immunosensors. The procedure begins with sensor surface cleaning. The cross-linking strategy then takes advantage of the formation of a thiourea monolayer via self-assembly and subsequent glutaraldehyde (GA)-assisted covalent antibody fixation. Gap-filling with linear dodecane thiol blocks remaining conductive gold surface and ensures proper insulating behavior, which is needed for capacitive detection of hYKL-39.

2. Cyclic voltammetry tests in course of hYKL-39 immunosensor preparation

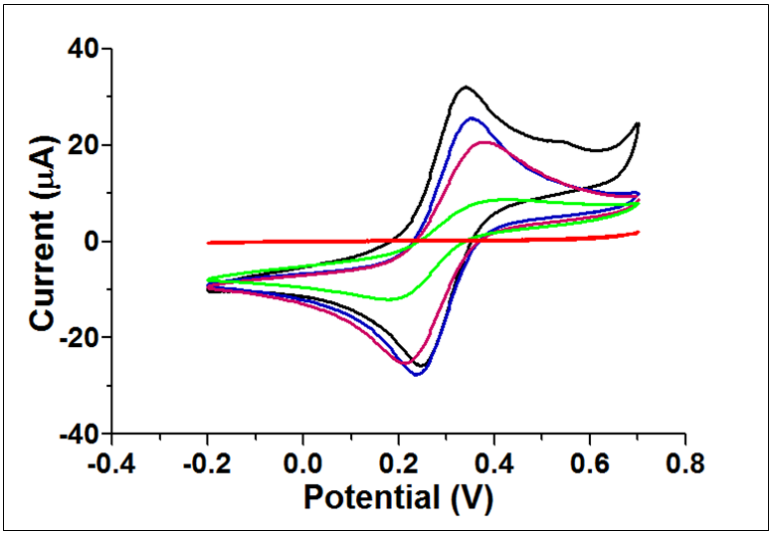

Figure S2: Cyclic voltammograms of 5 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution with a scan rate of 0.1V/s for the bare gold electrode (black curve), the electrode with a thiourea monolayer (blue curve), the electrode coated with GA-thiourea (pink curve), the electrode coated with anti-hYKL-39 GA-thiourea (green curve) and the same electrode after final treatment with 1- dodecanethiol (red curve). Reference electrode was an Ag/AgCl wire.

3. Electrochemical Impedance Spectroscopy (EIS) tests in course of hYKL-39 immunosensor preparation

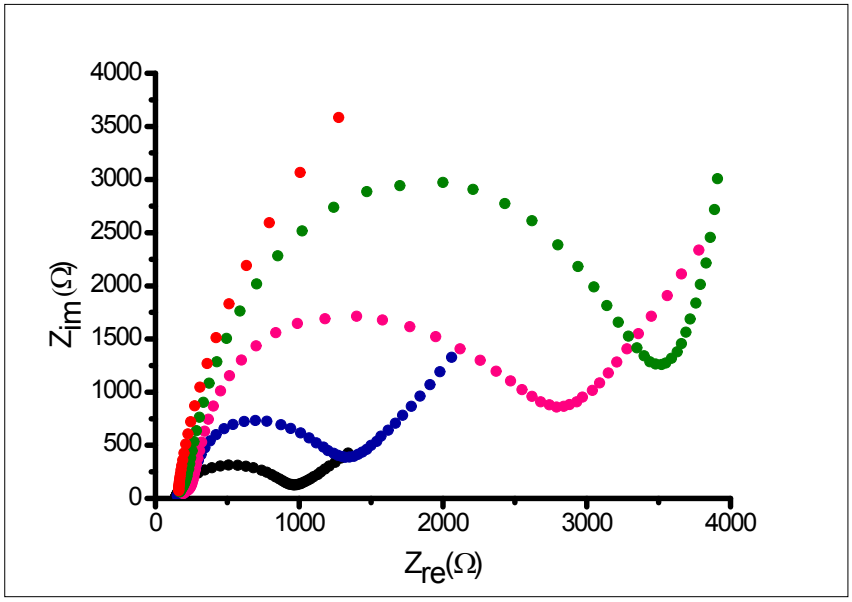

Figure S3: Potentiostatic EIS recorded with various sensor configurations for 5 mM $[Fe(CN)]_6^{3-}$ and 5 mM $[Fe(CN)]_6^{4-}$ in a 25 mM phosphate buffer, pH 7.0. Frequency range was 100 mHz to100 kHz and the agitation voltage had a 10 mV amplitude. Traces represent the Nyquist plots for the bare gold electrode (black curve), the electrode coated with a thiourea monolayer (blue curve), the electrode coated with GA-thiourea (pink curve), the electrode coated with anti-hYKL-39 GA-thiourea (green curve) and the same electrode after final treatment with 1- dodecanethiol (red curve).

4. Parameter optimization for flow-based capacitive hYKL-39 immunosensing

Reach of a good quality of capacitive hYKL-39 immunosensing in a microfluidic device needs a careful adjustment of the type and concentration of regeneration and running buffer, the volume of the injected samples and the flow rate. In triplicate trials, the parameters were optimized in approach that changed the variable under inspection while the others were kept constant. In the following some remarks will be provided that represent the findings for the optimization trials of individual parameters and the optimal settings will be reported.

4.1 Choice and optimization of regeneration solution

A hYKL-39 sample injection triggers the binding of hYKL-39 to the immobilized antibody and thus is the cause of a decrease in the monitored sensor capacitance. Cleavage of the

antibody-antigen bond and antigen removal is required before next sample exposure to reset the immunosensor to initial capacitance value and reach the appropriate starting condition for another measurement. The efficiency of the requisite surface regeneration was judged on by a comparison of the capacitance changes of a hYKL-39 immunosensor for two sequential sample injections with identical analyte levels and regeneration applied in between. The performance of acidic, neutral and alkaline regeneration solutions is summarized in Figure S4 and Table S1. Apparently, the three tested acidic choices supported regeneration best. HCl of pH 2 with the highest recovery rate in the selection was chosen for further trials aiming on an identification of the best pH.

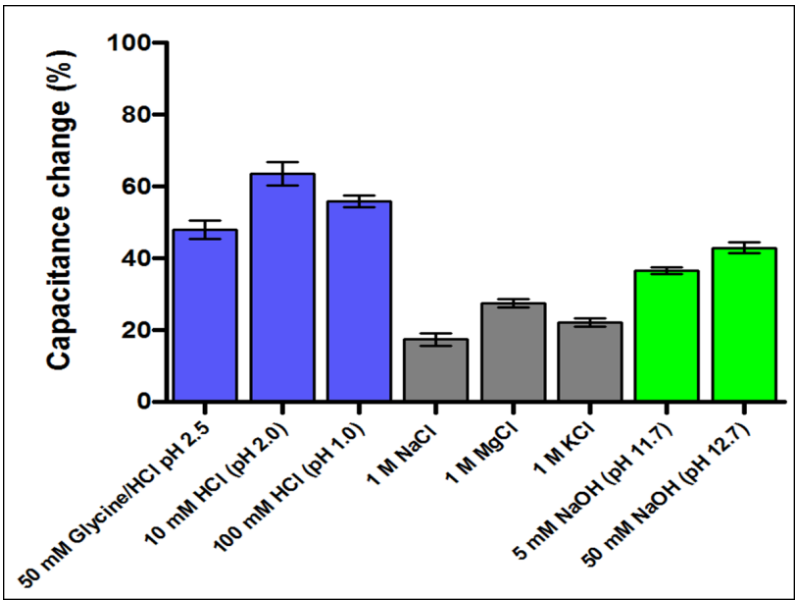

Figure S4: Percentage of the immunosensor capacitance recovery for three different types of regeneration solutions (low pH; blue, high ionic strength; gray, high pH; green); sample injections were of 10 µg/L of hYKL-39. Reference point (100%) is the capacitance change induced by the first sample injection.

Hydrochloric acid regeneration solutions were tested with their pH adjusted at values ranging from 1 - 3.5. As evident from Figure S5 and Table S1 the capacitance recovery was clearly the best for an HCl that had a pH of 2.5 and it was thus chosen as the routine regeneration solution chosen for all hYKL-39 capacitive immunosensing measurements.

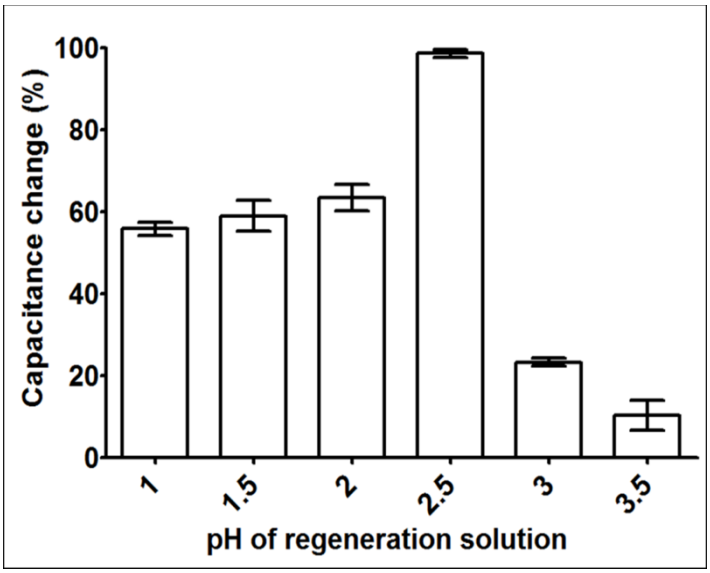

Figure S5: Percentage of the immunosensor capacitance recovery HCl regeneration solutions of various pH. Sample injections were of 10 µg/L of hYKL-39. Reference point (100%) is the capacitance change induced by the first sample injection.

Table S1: Efficiency of hYKL-39 removal from anti-hYKL-39 immobilized on the electrode studied by injecting 0.0001 g/L of hYKL-39 (3 replications).

Regeneration solution	Percentage of capacitance recovery
Low pH	
• 50 mM Glycine/HCl pH 2.5	47.9 ± 2.6
• 100 mM HCl (pH 1.0)	55.8 ± 1.6
• 31.6 mM HCl (pH 1.5)	59.0 ± 3.7
• 10 mM HCl (pH 2.0)	63.4 ± 3.3
• 3.2 mM HCl (pH 2.5)	98.7 ± 1.0
• 1 mM HCl (pH 3.0)	23.3 ± 1.0
• 0.3 mM HCl (pH 3.5)	10.3 ± 3.7
High pH	
• 5 mM NaOH	36.5 ± 0.9
• 50 mM NaOH	42.8 ± 1.8
High ionic strength	
• 1 M NaCl	17.4 ± 1.4

• 1 M KCl	22.1 ± 1.1
• 1 M MgCl	27.4 ± 1.1

4.2 Choice and optimization of running buffer

The performance of capacitive hYKL-39 immunosensing as function of the running solution is shown in Figure S6 for a Phosphate (PBS), HEPES and Tris-HCl buffer. All three choices allowed the analytical scheme to produce a linear response from 0.1 to 1000 µg/L; however, PBS produced the largest capacitance changes for a given antigen injection and offered also a slightly increased sensitivity compared to the two others. Running buffer of choice was thus PBS, which was then further tested at pH levels of 6.0, 6.5, 7.0, 7.5, and 8.0. The recorded capacitance change of hYKL-39 immunosensors in response to the injection of 10 µg/L of hYKL-39 increased with pH, reached a maximum at 7.0 and then decreased (Figure S7). Accordingly, a phosphate buffer of pH 7.0 was routinely used as the running solution in the flow system for hYKL-39 analysis.

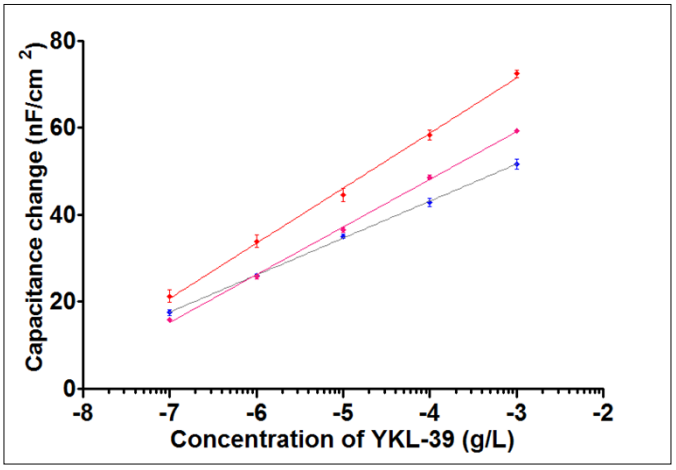

Figure S6: Capacitance change induced by the injection of hYKL-39 samples as function of analyte level. Measurements were made in PBS (red curve), HEPES (pink curve) and Tris-HCl (blue curve).

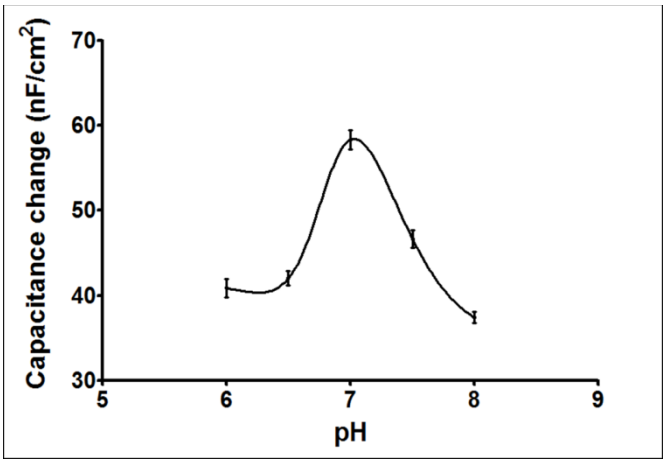

Figure S7: Plot of the capacitance change induced by the injection of hYKL-39 samples vs. PBS pH.

Since the ionic strength of buffer solutions is known to be dependent on buffer concentration hYKL-39 quantification was attempted in PBS, pH 7, however, of 5, 10, 25, 50 and 100 mM concentration. Figure S8 confirms that for a given sample injection the maximal capacitance change was recorded by the hYKL-39 immunosensor in 25 mM PBS of pH 7.0, while lower and higher buffer concentrations produced smaller responses. Choice for analytical applications in this study was thus 25 mM PBS, pH 7.

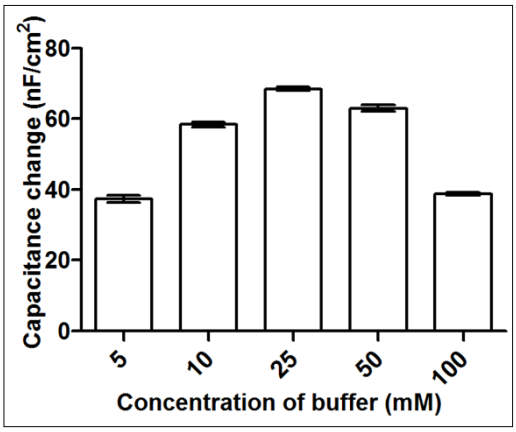

Figure S8: Capacitance change induced by the injection of hYKL-39 samples as function of the concentration of the PBS-based running buffer, pH 7.0.

4.3 Optimization of sample volume

The injected sample volume was expected to have an impact on the quality of the analytical response of the capacitive hYKL-39 immunosensing scheme. As shown in Figure S9, 500 µL sample injections produced the largest capacitance change. However, responses with 200, 300, and 400 µL were similar and to save reagent and analysis time,

200 µL was made the compromise for use with the proposed electrochemical hYKL-39 analysis.

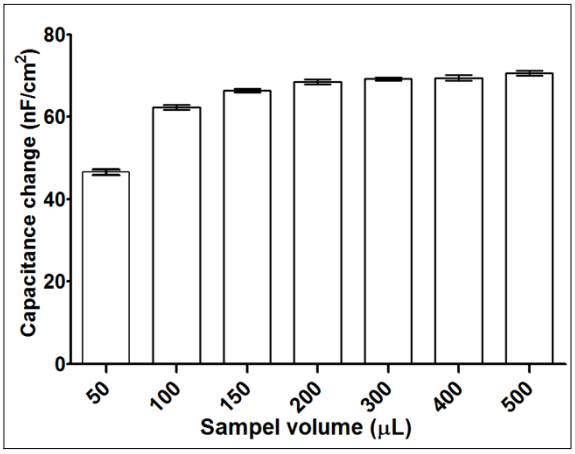

Figure S9: Capacitance change induced by the injection of hYKL-39 samples vs. sample volume.

4.4 Optimization of flow rate

The lower the flow rate the longer time antigen molecules are given to bind with antibody entities on the modified gold electrode. Hence, the capacitance response increased with a decreasing flow rate (Figure S10). The lowest tested flow rate of 50 µL/min gave the highest capacitance response; however, it demanded also the longest analysis time. As a suitable settlement between analytical response and measuring time, 100 µL/min was chosen as the routine flow rate for all quantification trials of this study.

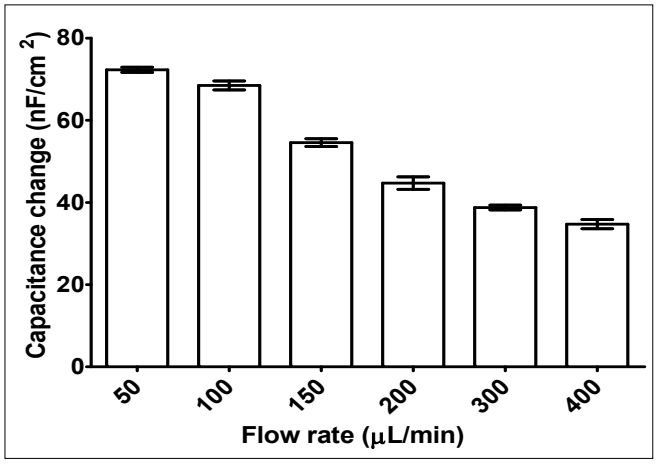

Figure S10: Dependence of capacitive hYKL-39 immunosensing on flow rate.

5. Sensor stability test

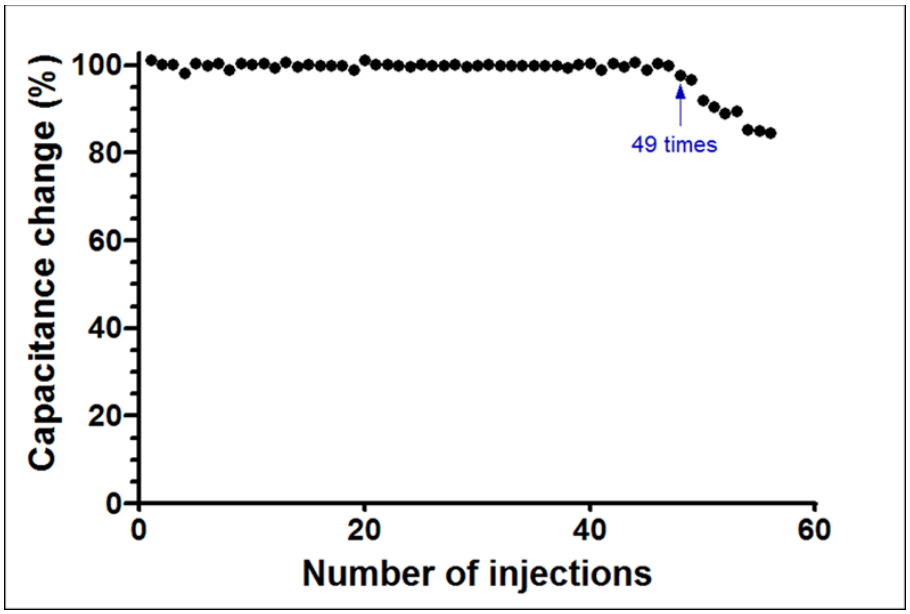

Figure S11: The capacitance response from an anti-hYKL-39 modified gold electrode for 57 repetitive injections of 200 µL volumes of a hYKL-39 solution (10 µg/L) with optimized regeneration and reconditioning steps between individual measurements.