## Supplementary data for

## Real-Time Kinetic Analysis and Detection of Glycated Hemoglobin A1c using Quartz Crystal Microbalance-Based Aptasensor

<u>Yossawadee Sriondee</u><sup>a</sup>, Pasara Vijitvarasan<sup>b</sup>, Arunothai Rattanachata<sup>d</sup>, Hideki Nakajima<sup>d</sup>, Sukunya Oaew<sup>c\*</sup>, Sarawut Cheunkar<sup>a\*</sup>

<sup>a</sup>Biotechnology Division, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, 10150, Thailand <sup>b</sup>Nakhonsawan campus, Mahidol University, Nakhonsawan, 60130, Thailand <sup>c</sup>Biochemical Engineering and Systems Biology Research Group, National Center for Genetic Engineering and Biotechnology, National Science and Technology, Development Agency at King Mongkut's University of Technology Thonburi, Bang Khun Thian, Bangkok, 10150, Thailand <sup>d</sup>Synchrotron Light Research Institute, Nakhon Ratchasima, 30000, Thailand

\*E-mail: <u>sarawut.che@mail.kmutt.ac.th</u> \*E-mail: <u>sukunya.oae@biotec.or.th</u>



**Fig. S1** Denatured polyacrylamide gel electrophoresis of HbA1c aptamers. 59-base aptamer desalt grade (lane 1), 72-base aptamer desalt grade (lane 2), 59-base HPLC grade (lane 3), 72-base HPLC grade (lane 4)



**Table S1** Secondary structure simulation of each aptamer at the lowest  $\Delta G$  value

Lane	Area	Mean	Min	Max	Integrated	Difference
					Density	Integrated
						Density
1	64	65.649	46.50	70.965	4201.536	1557 934
2	64	41.308	37.98	45.728	2643.712	1337.824
3	64	87.673	56.925	94.157	5611.072	1091 622
4	64	56.710	52.519	61.658	3629.440	1981.032

 Table S2
 Summary of the analyzed data using the Plot profile function from ImageJ.

The gel image (Fig. 2) was analyzed by the Plot profile function of ImageJ software. The integrated intensity of each lane was calculated from the product of the area (64 pixels for all lanes) and the mean gray value.

![](_page_3_Figure_0.jpeg)

**Fig. S2** Atomic force microscope (AFM) images of mixed SAMs-modified QCM substrates under different pretreatment processes: (a) bare gold (Au) on a quartz crystal, (b) normal preparation conditions (without pretreating the mixture), (c) ultrasonic pretreatment of the mixture for 1 min, and (d) ultrasonic pretreatment with horizontal shaking at 100 rpm during incubation overnight. The insets represent the height profile of the surface corresponding to the blue lines.

![](_page_4_Figure_0.jpeg)

**Fig. S3** QCM signals of non-specific adsorption of BSA (blue), Hb (black), and HbA1c (red) on aptamer functionalized surfaces.

![](_page_5_Figure_0.jpeg)

Fig. S4 The equilibrium characterization by using the saturation binding curve. Fitting the mass and HbA1c concentrations with the one-site specific binding model gave the  $K_D$  of 69.19 nM.

![](_page_6_Figure_0.jpeg)

**Fig. S5** A representative QCM measurement of spiked samples in real human plasma. The diluted human plasma was first introduced for 10 min to establish a baseline, followed by the injection of spiked sample.