Supporting Information For

Week-long operation of electrochemical aptamer sensors: new insights into degradation mechanisms and solutions for self-assembled monolayer stability in biofluid at body temperature.

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Materials and Methods

Materials. Sulfuric acid (96%, p.a.), Sodium hydroxide (98%, pellets), Pulverized phosphate buffered saline (PBS, pH 7.4), Tris-EDTA solution (TE buffer; pH: 8), Bovine Serum, tris(2-carboxyethyl) phosphine hydrochloride (TCEP; 98%), sodium azide (99.5%), 1,6-d6-mercapto-1-hexanol (MCH; 98%) and 8-mercapto-1-octanol (MCO; 97%) were obtained from Sigma Aldrich (USA). 2-hydroxy-2methylpropiophenone (photo-initiator, purity: >96%) was purchased from TCI Chemicals. [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (monomer, purity: >95%) was obtained from Chem-Impex INC, USA. Absolute ethanol (100%; anhydrous) was purchased from Fisher Scientific (USA). Vancomycin hydrochloride (94.6%) and ethylene glycol-dimethacrylate (cross-linker, purity: >98%) were obtained from Alfa Aesar. Polishing kits, including microcloth polishing pads, 0.3 μm alumina slurry, and 0.05 μm alumina slurry, were obtained from Buehler (USA). Gold disk (d: 2mm) working electrodes, silver/silver chloride (3M KCl) reference electrodes and platinum wire (d: 150 μm) counter electrodes were purchased from CH Instruments (Austin, TX). The 3'-methylene blue and 5'-thiol modified oligonucleotide sequences were synthetized by Integrated DNA Technologies (IDT, USA).

The thiolated zwitterionic blocking layer compound (2-methacryloyloxyethyl phosphorylcholine, MPC) was synthesized according to previous literature.[1,2]

The specific aptamer sequences utilized in this study are as follows:

Target Analyte	5' Modification	Sequence	3' Modification
Vancomycin[3]	Thiol [OH-$(CH_2)_6$-S-S$(CH_2)_6$]-	CGA GGG TAC CGC AAT AGT ACT TAT TGT TCG CCT ATT GTG GGT CGG	Methylene Blue -[MB]
Cortisol[4]	Methylene Blue [MB]-	GGA CGA CGC CAG AAG TTT ACG AGG ATA TGG TAA CAT AGT CGT	Thiol -[$(CH_2)_6$-S-S$(CH_2)_6$-OH]
L-Phenylalanine[5]	Thiol [OH-$(CH_2)_6$-S-S$(CH_2)_6$]-	CG ACC GCG TTT CCC AAG AAA GCA AGT ATT GGT TGG TCG	Methylene Blue -[MB]

If not otherwise stated, all electrode potentials are reported versus a Ag/AgCl (3 M KCl) reference electrode.

Methods:

Sensor preparation. Sensors were prepared according to common procedures previously reported.[6] Briefly, gold disk electrodes were first mechanically polished with 0.3 and 0.05 μm alumina slurries respectively. This step was followed by 5 min of ultrasonication in absolute ethanol and in deionized (DI) water respectively to remove residual alumina from the electrode surface. Electrochemical cleaning was performed in a standard three-electrode electrochemical cell consisting of a gold disk working electrode, platinum counter electrode, and Ag/AgCl reference electrode by running 700 cyclic voltammetry scans in 0.5 M NaOH from -1 V to -1.6 V at a scan rate of 1 V/s and subsequently 150 scans in 0.5 M H_2SO_4 solution from 0V to 1.6 V at 1 V/s. Once the electrochemical cleaning was complete, electrodes were thoroughly rinsed with DI water, dried in a nitrogen stream (99.999% purity), and used for subsequent functionalization with aptamer.

Upon arrival, the lyophilized pellet of modified aptamer was diluted down to a 100 µM stock solution using TE buffer and kept at -20 ˚C until use. Preparation of aptamer working solution was performed by first mixing an aliquot of the 100 µM aptamer stock solution with equal volume of 0.5 M TCEP dissolved in Milli-Q water. The mixture was set aside for 1hr to ensure complete reduction of any disulfide aptamer molecules. The obtained solution was diluted to an intermediary concentration of ~4 uM with 1xPBS/2 mM $MgCl_2$ buffer and the concentration confirmed via the absorbance measured at 260 nm using a Nanodrop UV/Vis Spectrophotometer. This solution was subsequently diluted to 500 nM with 1XPBS/2 mM $MgCl_2$ buffer and used for functionalization of the polished and cleaned gold-disk electrodes. A 20 µL droplet of the reduced 500 nM aptamer solution was drop casted over the cleaned gold electrode surface and left to incubate for 1hr in a light-protected and humidity-controlled chamber. The aptamer functionalized electrodes were rinsed with DI water and incubated overnight at room temperature in 5 mM MCH, MCO, or MPC solutions prepared in 1x PBS. The functionalized sensors were copiously rinsed with DI water prior to measurement.

Sensor modification with antibiofouling coating. The surfaces of gold disk electrodes were electrochemically roughened according to a previously published protocol.[7] Briefly, mechanically polished and electrochemically cleaned gold disk electrodes were immersed in 5 M NaOH solution and subject to 20 ms long alternating potential steps of -5 V and +0.8 V (vs. Hg/Hg_2SO_4 (sat. Na_2SO_4)) respectively, for a total duration of 6000s in an electrochemical cell consisting of a cylindrical Kapton®-carbon counter and saturated reference electrodes. Once the roughening was completed, the electrodes were carefully rinsed with copious amounts of DI water and the active surface area was determined by cyclic voltammetry in 0.5 M sulfuric acid solution.

The obtained nanostructured gold surface was used to prepare aptamer sensors according to the above-described procedure. Modification of the sensors with an antibiofouling zwitterionic polybetaine-based hydrogel[8] was performed by drop-casting 1 µL of the aqueous mixture (reference) consisting of monomer/cross-linker/photo-initiator (2.8g/1.8µl/36µl respectively dissolved in 1 ml of DI water) over the sensor and exposing it to UV light (λ: 280-450 nm, Bluewave LEDPrime UVA, Dynamax, USA) for 45 min. In the next step, the polysulfobetaine coated sensors were immersed in 1XPBS and left overnight to hydrate. The obtained sensors were used as prepared.

Electrochemical measurements: All electrochemical measurements were performed by a CHI 620E potentiostat (Austin, Texas) connected to a 64-channel multiplexer in a standard three-electrode system with aptamer/alkylthiolate functionalized gold disk electrodes serving as working electrodes, a platinum counter electrode, and a Ag/AgCl (3 M KCl) reference electrode at either 4˚C or 37˚C under temperature and humidity-controlled conditions. Cyclic voltammograms were recorded in 1X PBS in a potential window from -0.1 V to -0.5 V at a scan rate of 100 mV/s. For determination of the electron-transfer rate constant, a set of cyclic voltammograms was recorded at different scan rates ranging from 5 mV/s to 100 V/s. Square-wave voltammetry was performed in a potential window from -0.1 V to -0.5 V at 25 mV amplitude over the range of frequencies reported either in 1X PBS or undiluted bovine serum. For longevity experiments, bovine serum was spiked with sodium azide to a final concentration of 0.02% wt. to prevent growth of microorganisms over multiple day time periods.

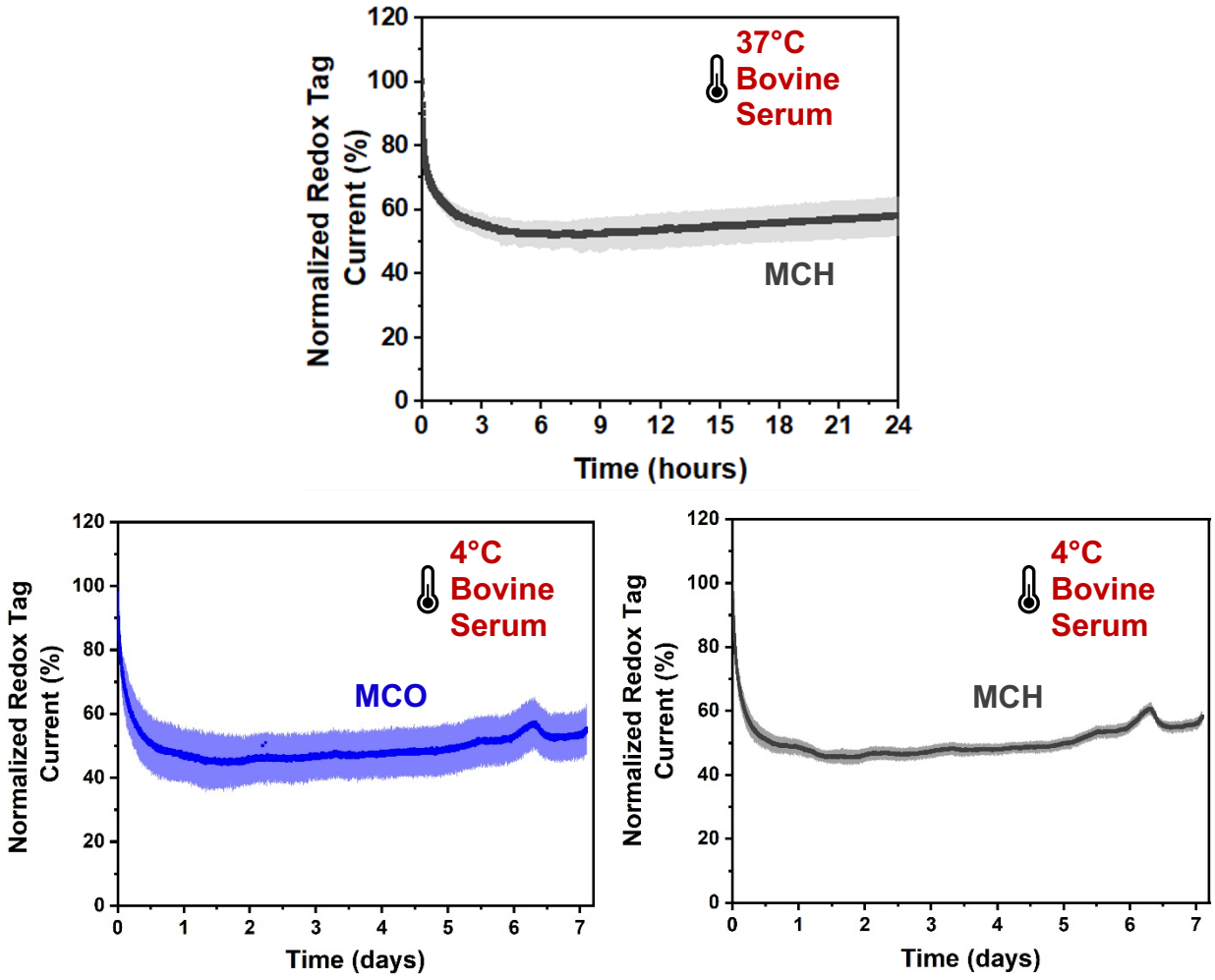

Figure S1: The "exponential" phase typically seen at 37 °C over the first ~3 hours[9] is extended over 24-48 hours at 4 °C, indicating that a similar biofouling process is occurring, albeit kinetically slower, due to the lower temperature.

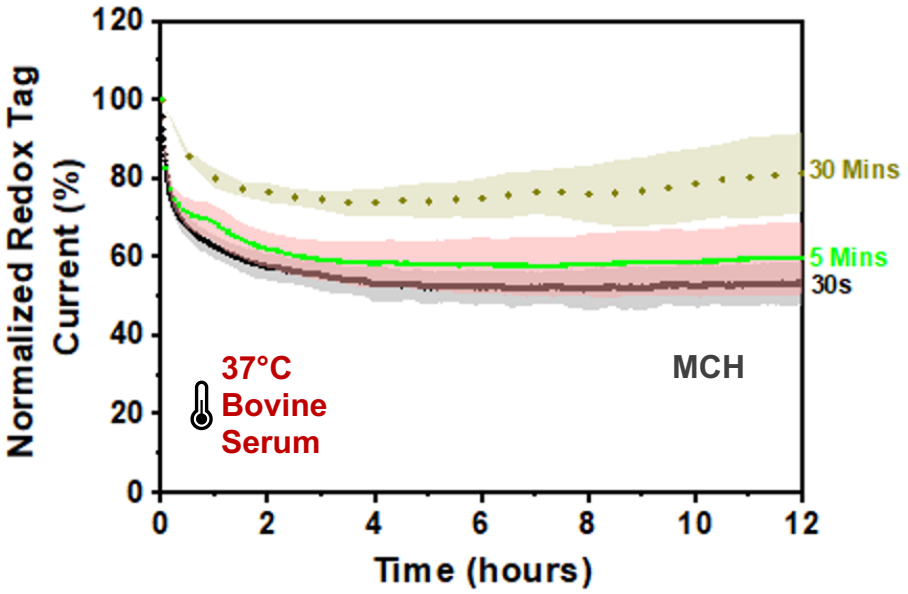

Figure S2: Scan dependent biofouling of MCH-based sensors scanned at periodicities of every 30 seconds, every 5 mins, and every 30 mins in serum at 37 °C

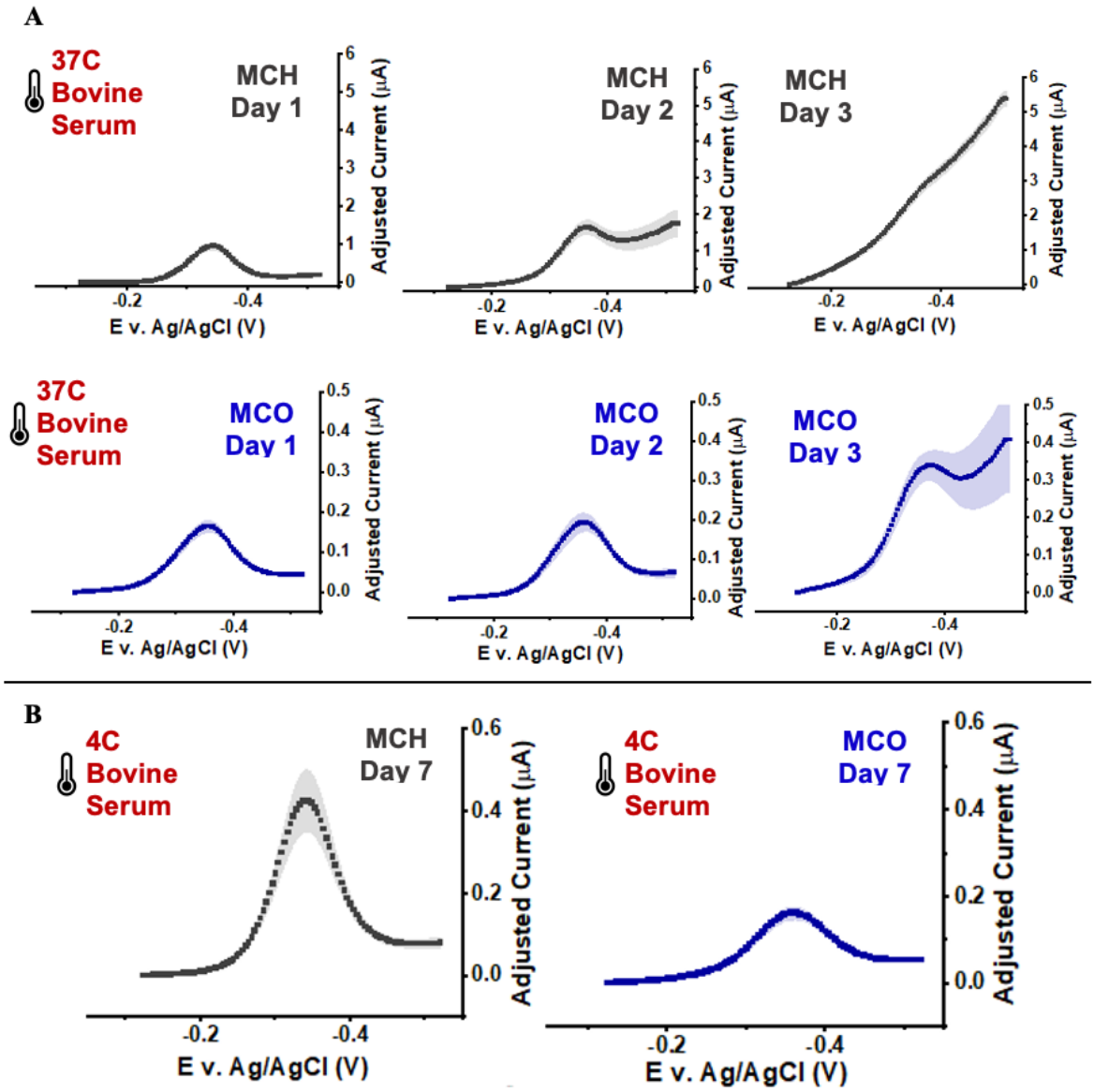

Figure S3: (A) Voltammograms from MCH and MCO sensors scanned once-per-day in serum at 37 °C. Sensors did not last past 3 days for either MCH or MCO based sensors. (B) Results from day 7 for MCH and MCO sensors scanned once-per-day in serum at 4 °C

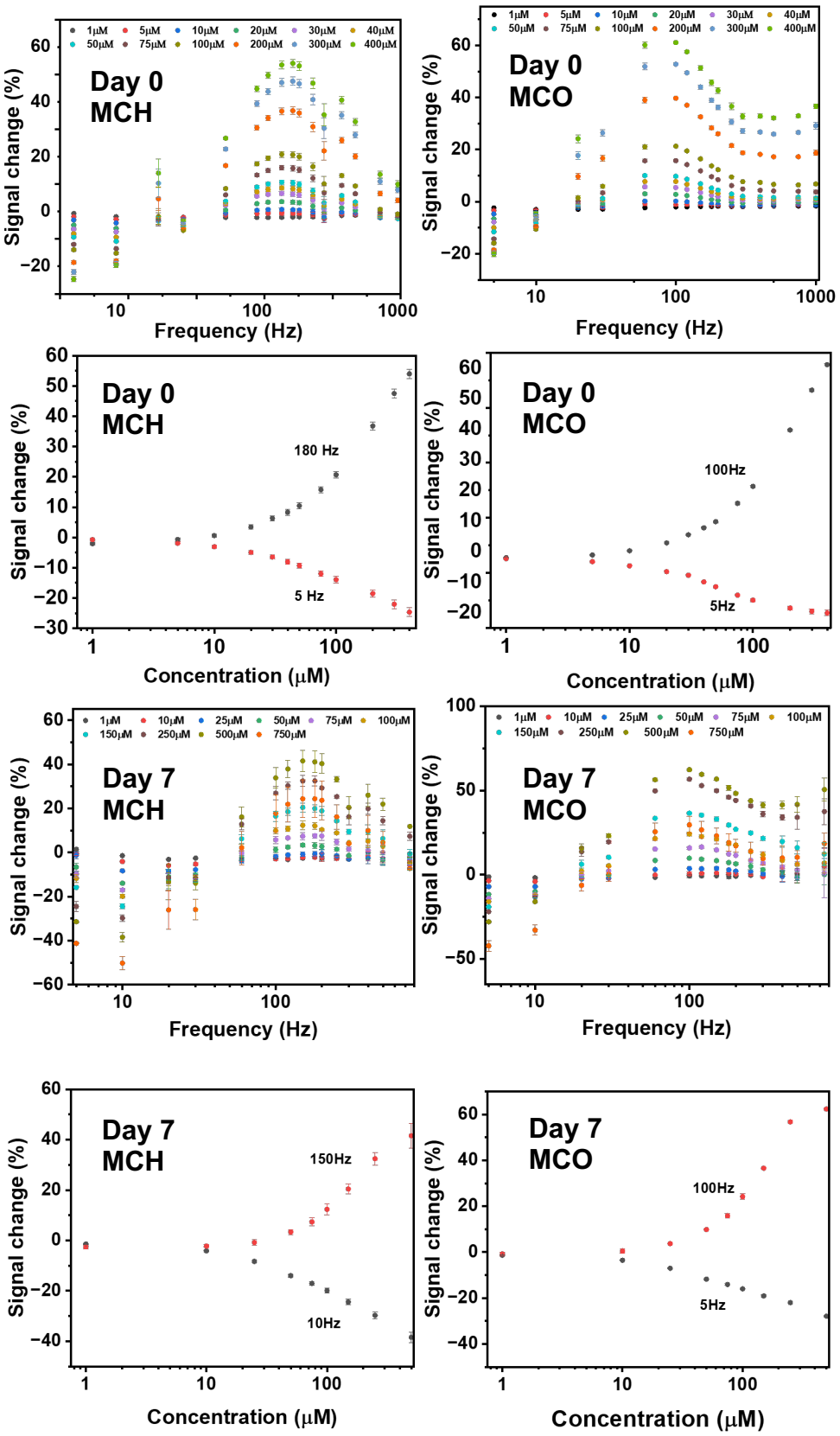

Figure S4: Titrations at 37°C in PBS from Day 0 to Day 7 of repeated scanning

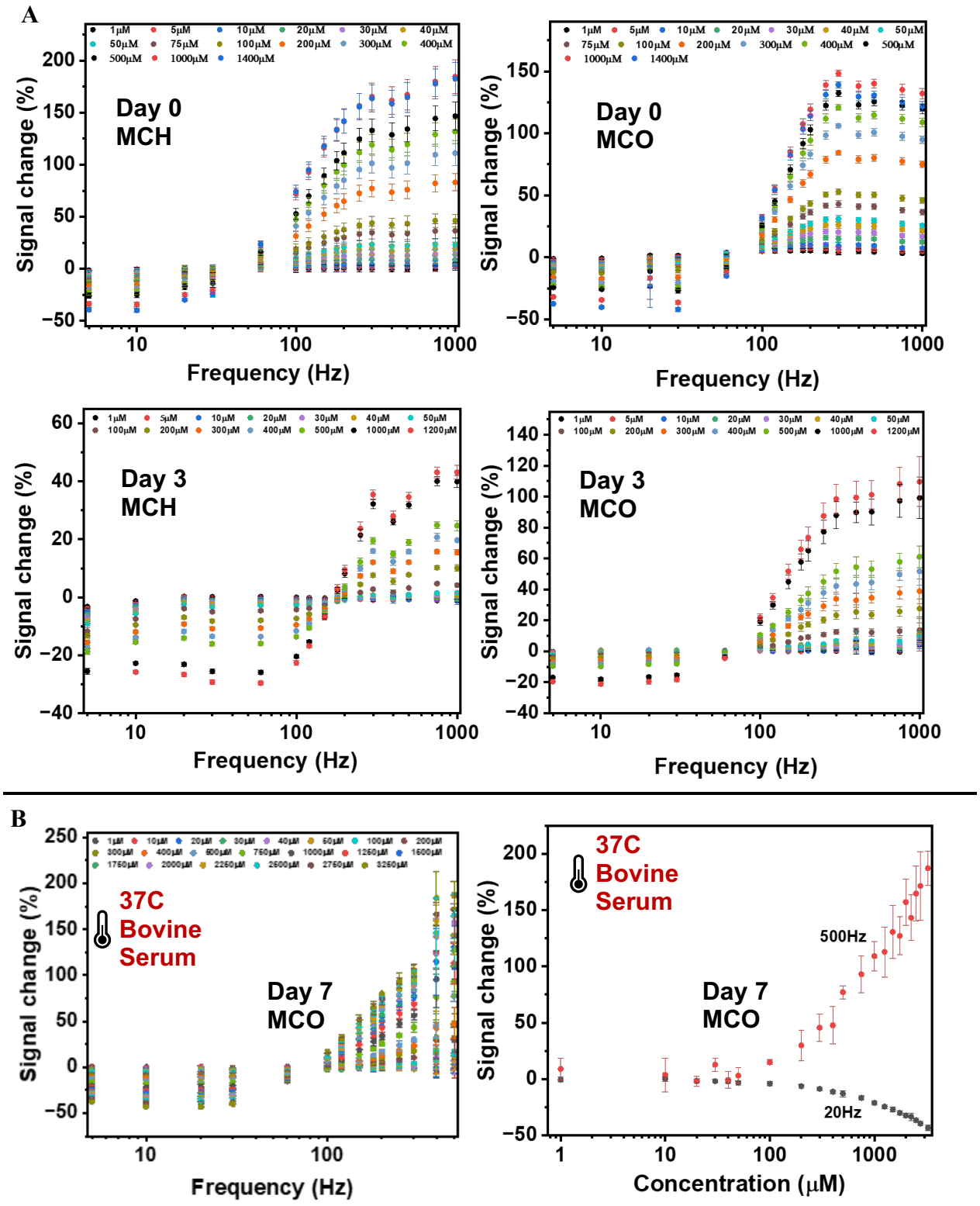

Figure S5: (A) Change in frequency response after 3 days repeatedly scanning in 37 °C serum. (B) Day 7 titration results for MCO sensors repeatedly scanned in 37 °C serum.

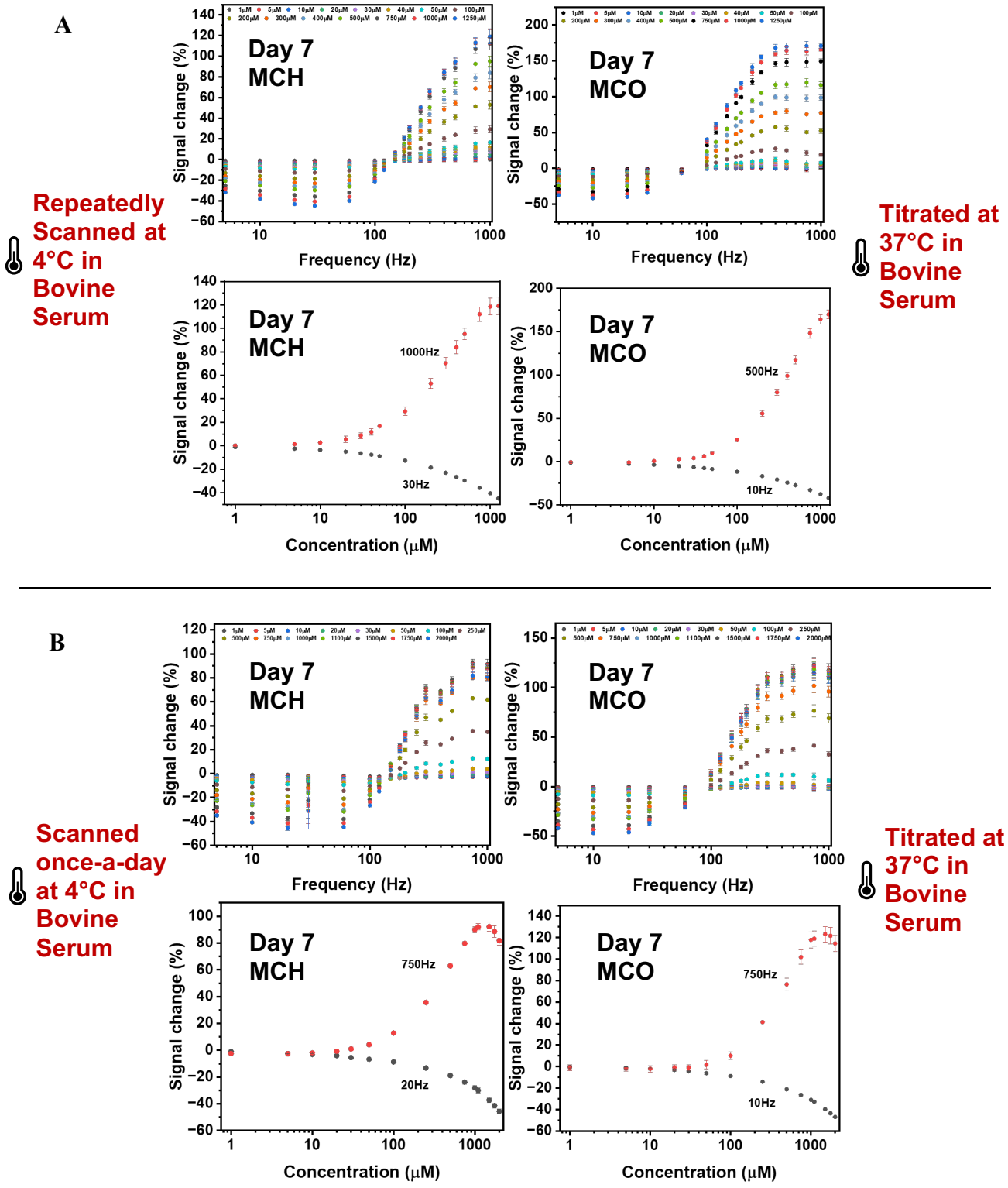

Figure S6: (A) Sensors repeatedly scanned at 4°C for 7 days in serum and subsequently titrated at 37°C show similar sensor responses to those titrated at 37°C on Day 0 (B) Sensors scanned 1x day in serum at 4°C for 7 days and subsequently titrated at 37°C are a bit less sensitive than those titrated at 37°C on day 0 indicating a scan dependance on biofouling and therefore on sensor response

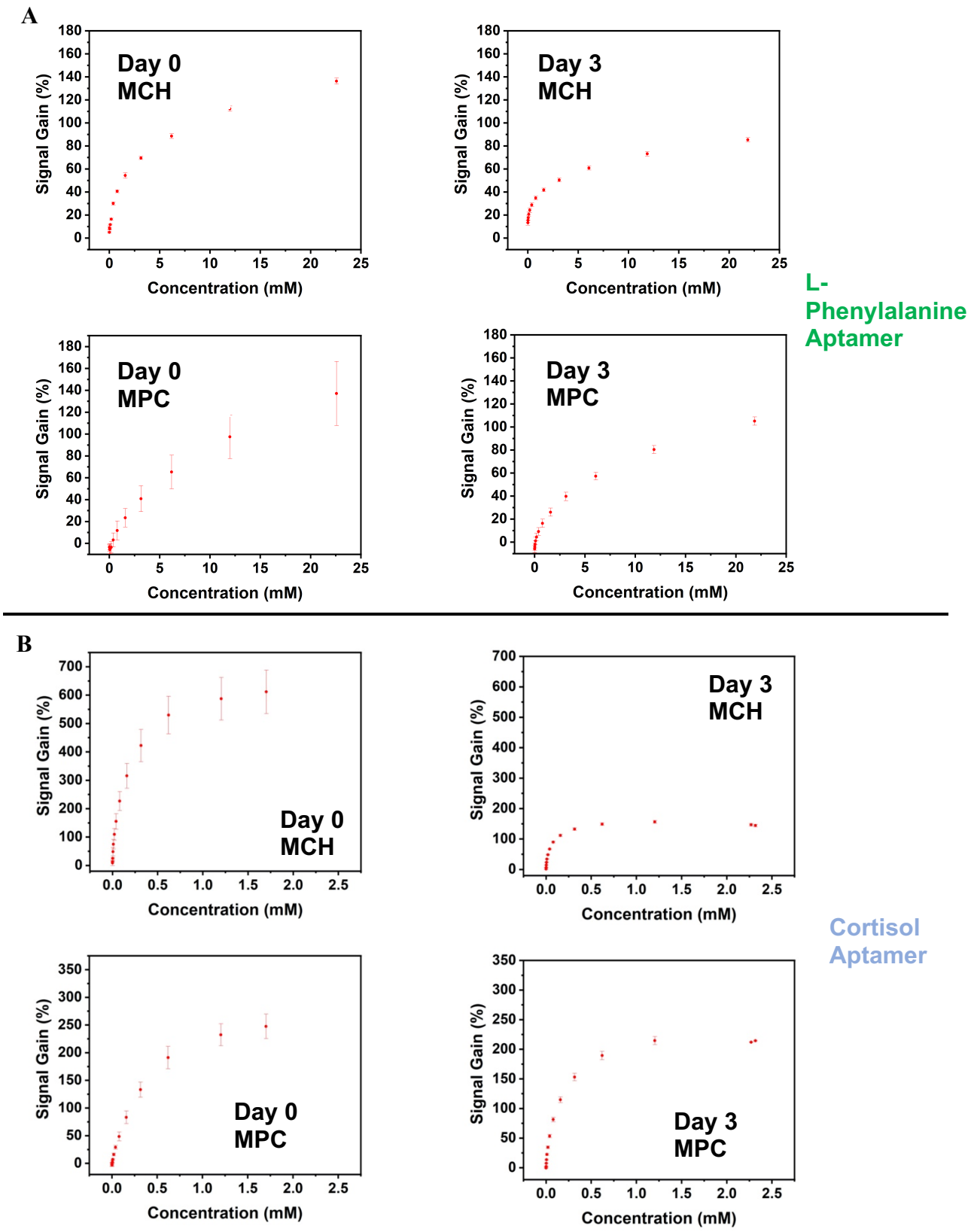

Figure S7: PC terminated monolayer (MPC) is capable of better preserving sensor response after 3 days of repeatedly scanned at 37°C in serum compared to MCH for (A) L-Phenylalanine and (B) cortisol aptamer sensors.

References:

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