Electronic supplementary information

Cascade Cycling of Nicotinamide Cofactor in a Dual Enzyme Microsystem

Rui Lv,‡ Sen Lin,‡ Shiyong Sun, * Huichao He, Fei Zheng, Daoyong Tan, Biaobiao Ma and Meng He

Materials and methods

Materials

The tourmaline ultrafine microparticles were obtained from Xinjiang, Aletai, China. Nonionic surfactant Span 60, hydrogen peroxide (30 wt. %), 3,3',5,5'-tetramethylbenzidine (TMB) and horseradish peroxidase (HRP) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Nicotinamide adenine dinucleotide (NAD^+, purity≥98 %), nicotinamide adenine dinucleotide hydride (NADH, purity ≥98 %), alcohol dehydrogenase (ADH), glucose oxidase (GOx) and bovine serum albumin (BSA) were purchased from Sigma. Anhydrous ethanol, hydrochloric acid, sodium phosphate dodecahydrate ($Na_3PO_4 \cdot 12H_2O$), toluene, tetraethoxysilane (TEOS), glucose and Tris(hydroxymethyl) methyl aminomethane (Tris-HCl) were purchased from Macklin Chemical Reagent Co., Ltd. Ultrapure water (Resistance 18.45 $M\Omega \cdot cm^{-1}$) was used in experiments. All other chemicals not mentioned were analytical grade and used without further purification.

Surface modification

The suspension (5 g, 10 % suspension in toluene) of tourmaline microparticles (TM) was mixed with Span 60 (0.25 g). Then, the dispersion was incubated at 90 °C for 24 h under magnetic stirring at 1200 rpm. The precipitated pellets were collected by centrifugation and washed three times with ethanol to remove free surfactant. The resulting TM were resuspended in toluene prior to further experiments.

Preparation of ADH/GOx-containing TM microsystem

The TM water-in-oil colloidosomes were prepared by a Pickering emulsion method. In general, an aqueous solution containing 100 μL ADH or FITC-labeled ADH (ADH-FITC) (100 $U \cdot mL^{-1}$) and 100 μL GOx or Rhodamine B-labeled GOx (GOx-RB) (200 $U \cdot mL^{-1}$) were added into 2 mL of 1 $mg \cdot mL^{-1}$ Span60 modified tourmaline in toluene. The mixture was homogenized for one minute to form water-in-oil emulsions by a homogenizer ((F10, Fluko, Berlin, Germany) at 10, 000 rpm.

Subsequently, the surface of the water-in-oil colloidosomes was cross-linked by hydrolytic reaction with 60 μL of TEOS for 24 h. The water-in-water TM colloidosomes or ADH/GOx@TM microsystem were prepared after toluene was removed by ethanol washing and transferred into the continuous water phase.

Peroxidase-like catalytic activity of ADH/GOx@TM microsystem

The catalytic oxidation of the colorimetric substrates (TMB) was performed in the presence of H_2O_2. In a typical reaction, 150 μL of 0.5 $mg \cdot mL^{-1}$ TMB in ethanol and ADH/GOx@TM microsystem were mixed together in 1.8 mL buffer (10 mM sodium phosphate; 100 mM Tris-HCl; pH 7.2) with various colloidosomes concentrations. Then 50 μL H_2O_2 was added into the mixture to initiate the catalytic reaction. The absorption intensity of the oxidized TMB was measured by UV-visible absorption band at 655 nm in given time intervals.

Catalytic cascade reactions of NAD^+/NADH cycling within ADH/GOx@TM microsystem

The stock solutions of NAD^+ (1 $mmol \cdot L^{-1}$) and NADH (1 $mmol \cdot L^{-1}$) were prepared in a buffer containing 10 mM sodium phosphate and 100 mM Tris-HCl at pH 7.2. For generation of NADH, 300 μL NAD^+ solution and ADH/GOx@TM were mixed in 1.6 mL buffer. Then, 100 μL ethanol (50 % in buffer) was added into the mixture to initiate the enzymatic reaction at 37 °C. The concentration of NADH generated was measured by the increasing UV-visible absorption band at 340 nm at the indicated time intervals. For oxidation of NADH, 100 μL glucose was added into the mixture. The residual NADH concentration was measured by the decreasing UV-visible absorption band at 340 nm at the indicated time intervals. Then, 100 μL ethanol (50 % in buffer) was added into the mixture again to initiate NAD^+/NADH cycling. The concentration of NADH generated was measured by the increasing UV-visible absorption band at 340 nm at the indicated time intervals.

Characterization

The morphology of the prepared colloidosomes were observed by optical microscopy (DM 2000, Leica, Germany), scanning electron microscopy (Ultra 55, Zeiss, German) and transmission electron microscopy (Libra 200, Zeiss, Germany). The confocal laser scanning microscopy (CLSM) images of ADH-FITC and GOx-RB within the microsystem were obtained on an inverted CLSM (TCS SP8, Leica, Mannheim, Germany). The mineral phase of tourmaline was characterized by X-ray diffractometer (X pert pro, Panalytical, Netherlands). The elemental components were measured by X-ray fluorescence spectrometer (Axios, Panako, Netherlands). The Fe L-edge spectra of near

edge X-ray absorption fine structure of tourmaline particles was carried out with the soft X-ray spectromicroscopy beamline (BL08UA) of the Shanghai Synchrotron Radiation Facility. The particle size distribution of tourmaline articles was characterized by particle size analyzer (Plus 90, Brookhaven, USA). The spectra of UV-visible absorption were analyzed by a micro-spectrophotometer with integrated cuvette (Nanodrop 2000C, Thermo Scientific Instruments, USA).

Table S1. The elemental components of tourmaline particles by XRF. The content of FeO was calculated by dichromate titrimetric method. The molar Fe(II) to Fe(III) ratio is 2.3.

Al_2O_3	SiO_2	B_2O_3	CaO	MgO	LOI_{1000}	TFe_2O_3	FeO	Fe_2O_3
39.12	31.47	6.34	0.58	1.07	2.88	13.93	8.72	4.23
MnO	**Na_2O**	**P_2O_5**	**SO_3**	**SrO**	**F**	**TiO_2**	**K_2O**	**BaO**
0.21	1.37	0.10	0.16	0.04	0.50	1.08	0.86	0.01

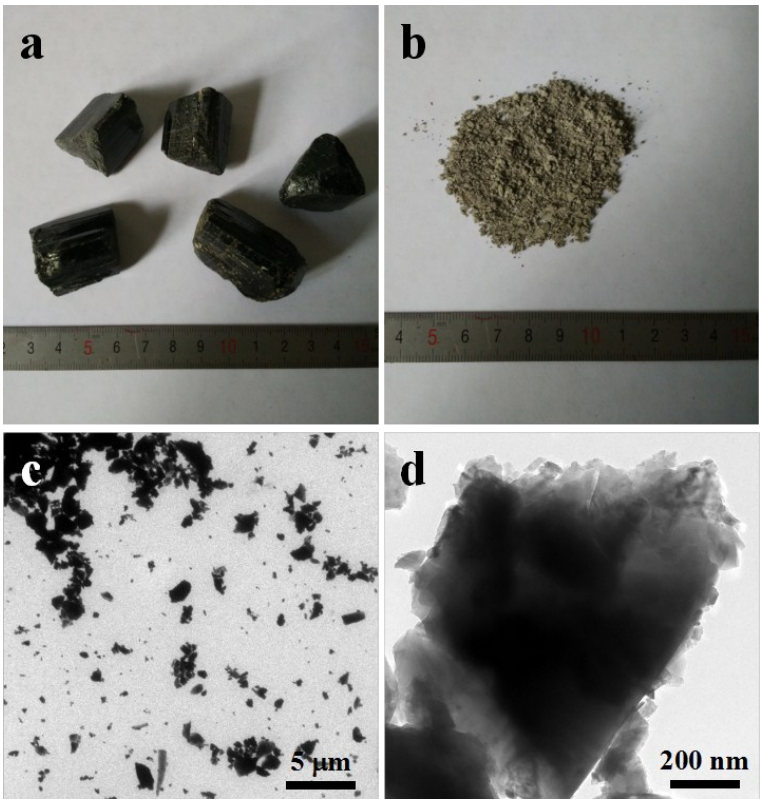

Figure S1. (a) the crystals of tourmaline; (b) the small size fraction used in experiments. (c, d) TEM images of tourmaline particles. (c) low magnification image; (d) high magnification image.

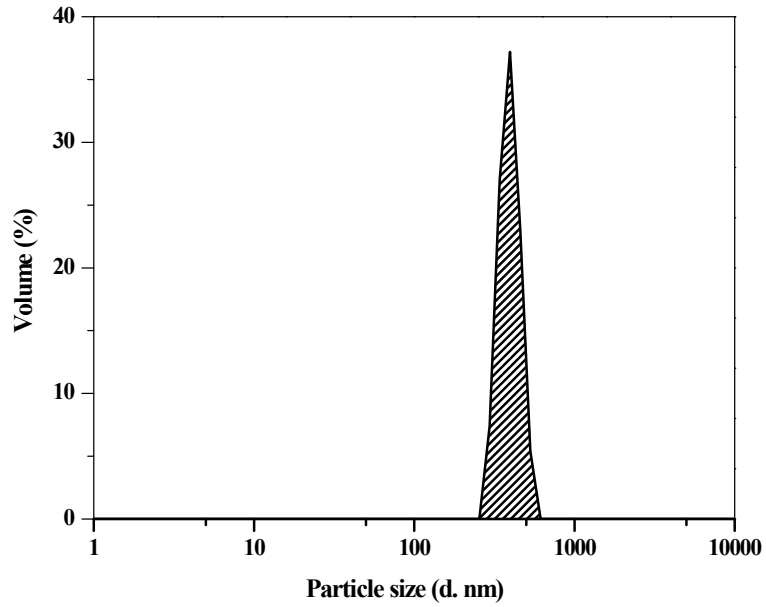

Figure S2. The particle size distribution of tourmaline particles. The mean size is ca. 390 nm.

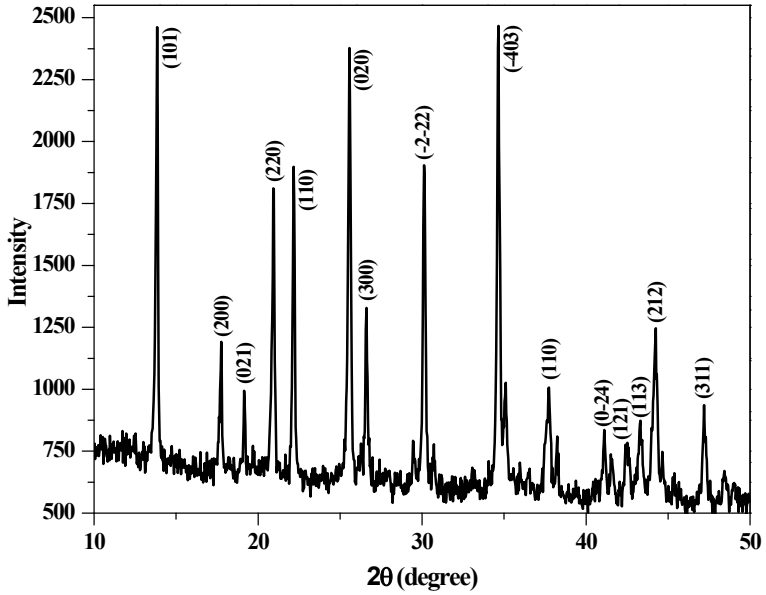

Figure S3. X-ray diffraction pattern (XRD) of tourmaline particles.

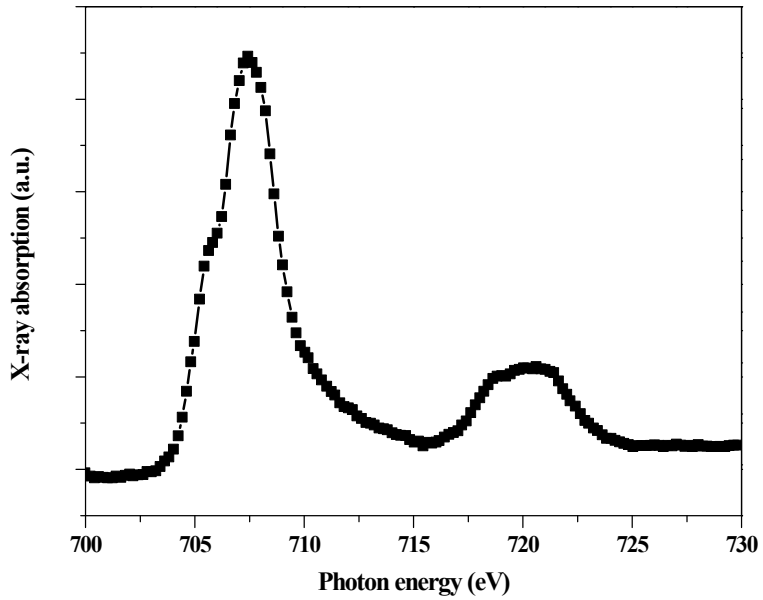

Figure S4. Fe L-edge spectrum of near edge X-ray absorption fine structure of tourmaline particles by soft x-ray synchrotron radiation spectroscopy that indicates the structural Fe in tourmaline in the forms of bivalence and trivalence.

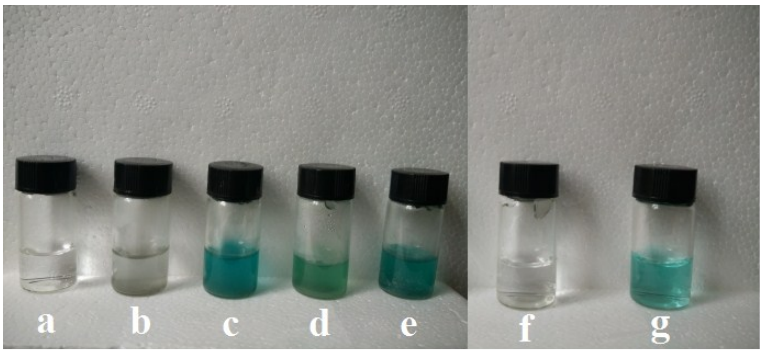

Figure S5. Photographs of sample tubes showing that the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB, colorless) to TMB diimine (blue) is catalyzed by tourmaline microparticles, ADH/GOx@TM microsystem or Horseradish Peroxidase (HRP) in bulk water. (a) TMB and H_2O_2 showing no product of TMB diimine; (b) TMB and tourmaline particles showing no product of TMB diimine in the absence of H_2O_2; (c) TMB, H_2O_2 and tourmaline particles showing blue product of TMB dimine; (d) TMB, H_2O_2 and ADH/GOx@TM microsystem showing blue product of TMB dimine; (e) TMB, glucose and ADH/GOx@TM showing blue product of TMB dimine; (f) TMB and HRP showing no product of TMB dimine in the absence of H_2O_2; (g) TMB, H_2O_2 and HRP showing blue product of TMB dimine. Reactions undertaken with TMB (1 mM, 200 uL), H_2O_2 (30 wt. %, 100 μL), glucose (1M, 200 μL), ADH/GOx@TM (0.1 mg·mL^{-1}, 500 μL) or HRP (10 μg·mL^{-1}, 500 μL).

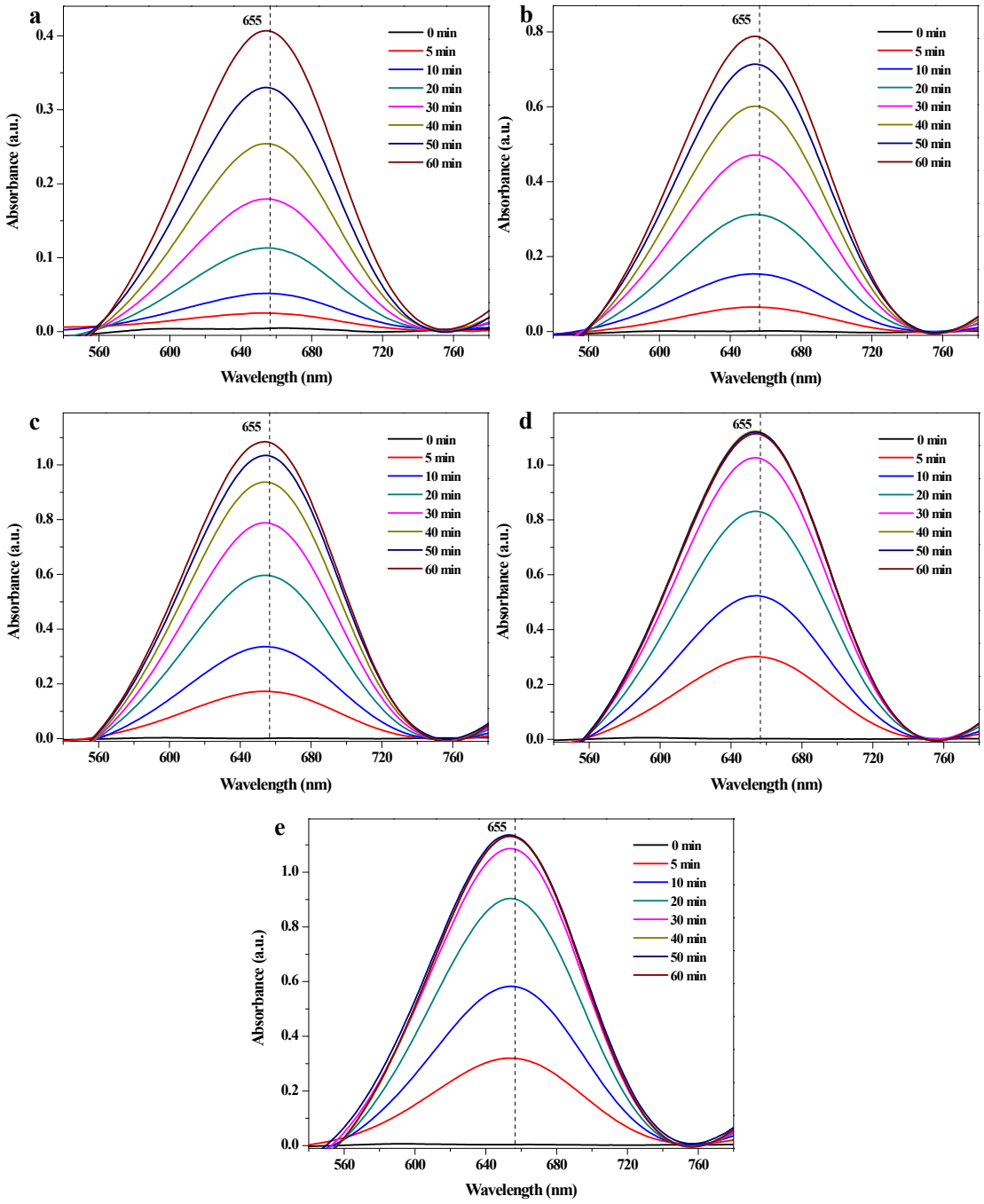

Figure S6. UV-Vis spectra showing increase in absorption intensity at 655 nm consistent with formation of TMB diimine in a reaction mixture containing ADH/GOx@TM varied from 0.1 to 1 mg·mL^{-1}, TMB (0.5 mg·mL^{-1}, 150 µL) and H_2O_2 (30 wt. %, 50 µL) at pH 7.2. (a) 0.1 mg·mL^{-1}; (b) 0.2 mg·mL^{-1}; (c) 0.5 mg·mL^{-1}; (d) 0.8 mg·mL^{-1}; (e) 1 mg·mL^{-1}.

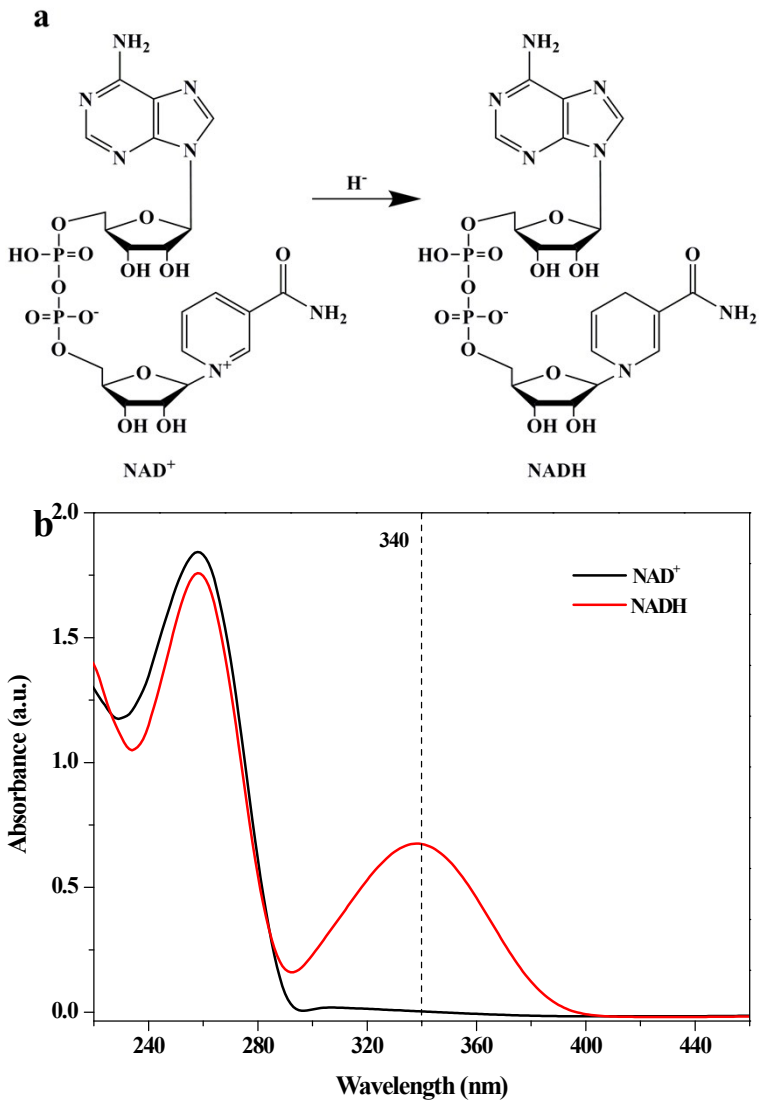

Figure S7. (a) Chemical formula and (b) UV-Vis spectra of NAD^+ and NADH.

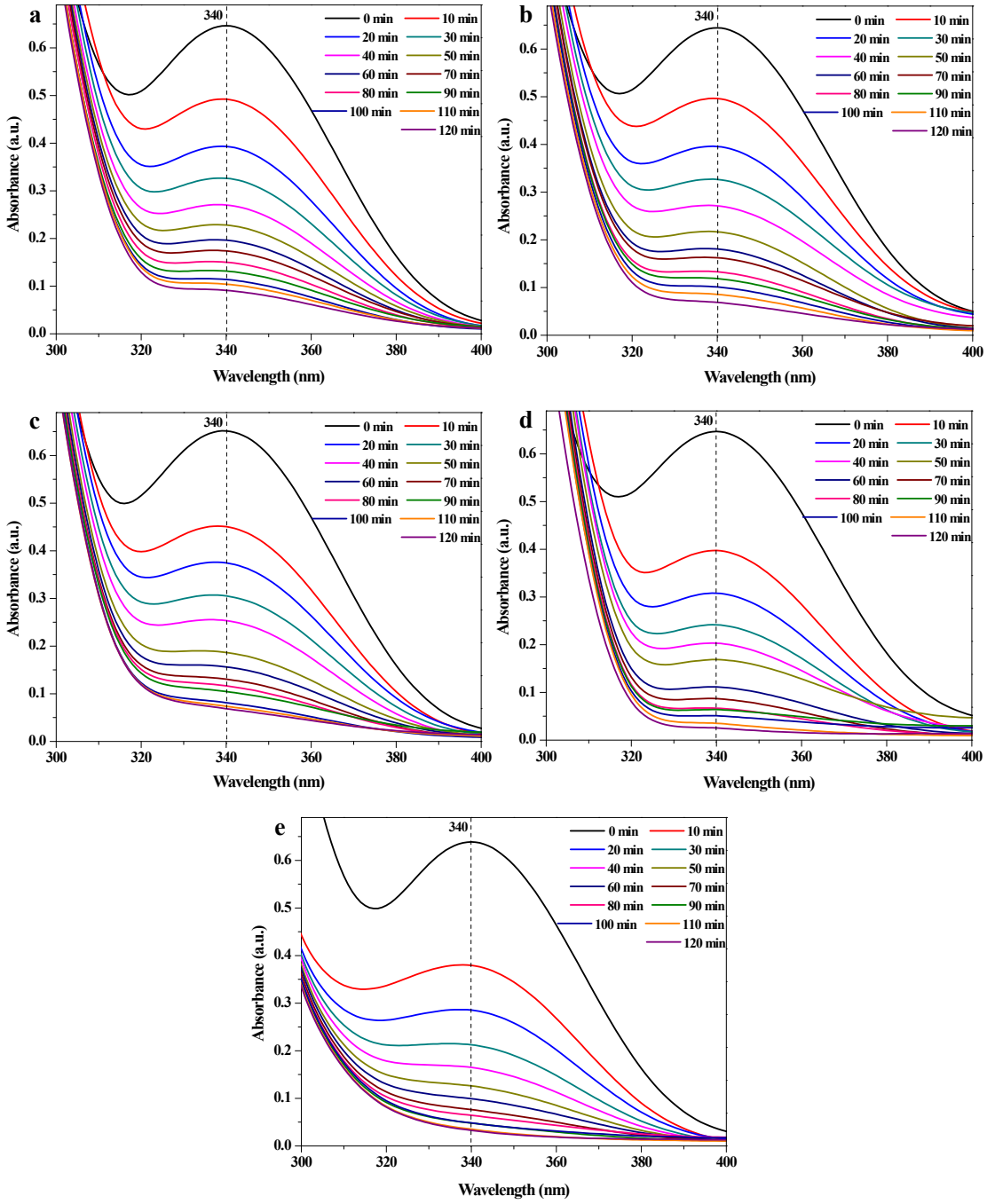

Figure S8. UV-Vis spectra showing decrease in absorption intensity at 340 nm consistent with oxidation of NADH to NAD^+ in a reaction mixture containing ADH/GOx@TM microsystem varied from 0.1 to 1 mg·mL^{-1}, NADH (1 mM, 200 µL) and H_2O_2 (30 wt. %, 40 µL) at 37 °C and pH 7.2. (a) 0.1 mg·mL^{-1}; (b) 0.2 mg·mL^{-1}; (c) 0.5 mg·mL^{-1}; (d) 0.8 mg·mL^{-1}; (e)1 mg·mL^{-1}.

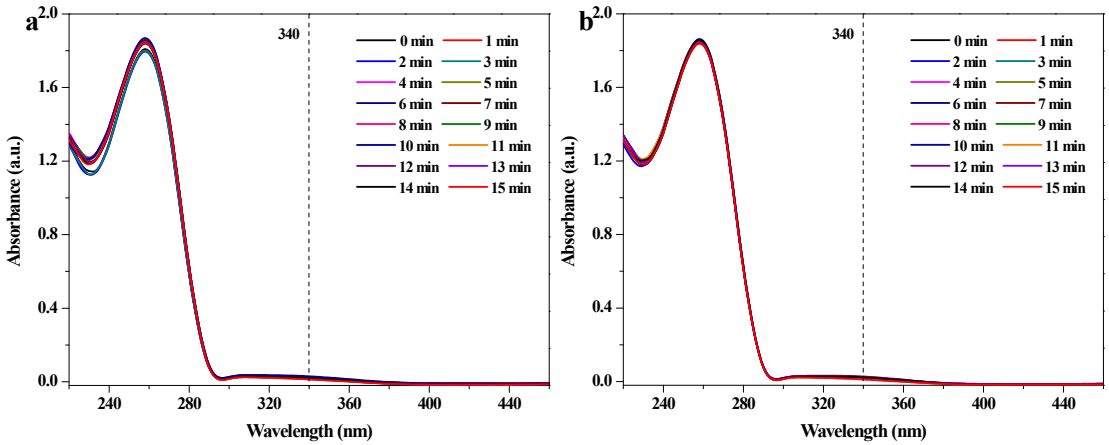

Figure S9. UV-Vis spectra of NAD^+ reduced to NADH under single factor condition. (a) without adding ADH; (b) without adding ethanol.

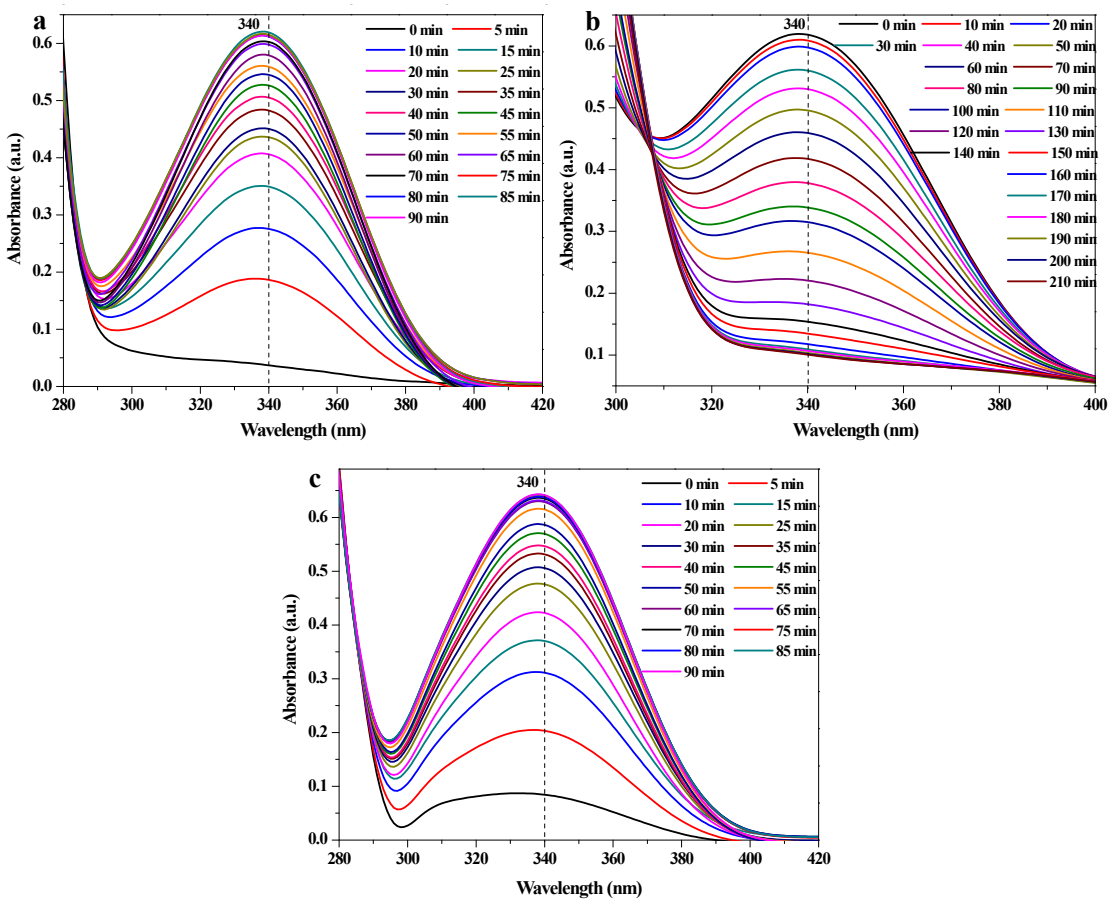

Figure S10. UV-Vis spectra showing NADH absorption intensity during cascade cycling of NAD^+/NADH within ADH/GOx@TM microsystem. (a)The reduction of NAD^+ to NADH by ADH; (b) The oxidation of NADH to NAD^+ by peroxidase-like TM membrane; (c) The regeneration of NADH from oxidized NAD^+. Reaction undertaken with ADH/GOx@TM microsystem(1 mg·mL^{-1}, 500 μL), NAD^+ (1 mM, 300 μL) at 37 °C and pH 7.2. Every time adding ethanol（50 %, 100 μL） for reduction reaction, adding glucose (1 M, 100 μL) for oxidation reaction.