Supporting Information

Accelerating and Breaking Adaptive Nano-Colloids (< 100nm) Into Unsteady State Operation Via Push-Pull Effects

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Experimentals

Chemicals were purchased from Sigma-Aldrich and TCI Chemicals. Glucose oxidase assay kit were purchased from Megazyme and performed with the corresponding protocol.

Synthesis

Synthesis of Iron(III) oleate precursor

Under a nitrogen atmosphere, 10.8 g Iron(III) chloride hexahydrate and 36.5 g sodium oleate was dissolved in a mixture of 80.0 mL ethanol, 140.0 mL hexane and 60.0 mL MilliQ water. After degassing for 20 min, the reaction was heated and refluxed for four hours. The organic phase was washed three times with MilliQ water, dried over MgSO3 and the solvent was evaporated to obtain the Iron(III) oleate precursor.

Synthesis of 28 nm-sized Fe_3O_4-nanospheres

In a heating-up method, different sized monodomained-Fe_3O_4 nanoparticles can be obtained by using solvents with different boiling points. In a typical synthesis, 4.5 g Iron(III) oleate precursor and 0.71 g oleic acid were added to 25.0 g of trioctylamine within a three-neck flask, heated up to 60 °C and dried under vacuum for 30 min. Afterwards, the reaction mixture was heated to reflux (365 °C) with a constant heating rate of 3.3 K/min and stirred for 30 min before cooling down to room temperature. The obtained nanoparticles were precipitated by adding 50.0 mL ethanol and purified via centrifugation for 10 times with a mixture of toluene:ethanol by increasing the amount of toluene with each washing step from 1:7 to 1:3.

Synthesis of CS nanoparticles and 1,3-Bistri(isopropoxysilyl)-benzene-5-acetylene were synthesized according to reference.[1]

Functionalization of isotropic-clicked CS@GOx nanoparticles

A total of 125.0 mg mercaptoundecanoic acid and 1.0 mg benzildiemethyl acetal (DMPA) were added to 3.0 mg of particles in 3.0 mL ethanol in a quartz tube and placed under a UV lamp (365 nm, 100 W) for 10 min. The clicked particles were washed several times by centrifugation in ethanol and redispersed in ethanol. The carbonic acid groups were activated by stirring a solution of the dispersed particles,

34.5 mg N-Hydroxy-succinimide (NHS) and 9.3 mg 1-Ethyl-3-3(3-dimethylaminopropyl) carbodiimide (EDC) for 30 min. After centrifugation for one time and redispersion in 0.5 mL MES puffer (pH 6.5) the activated particles can be coupled to the enzyme.

2.0 mL GOx solution (2.5 mg/mL in MES puffer) was added to the activated nanoparticles. After stirring at RT over night the particles were washed 10x in water by centrifugation and redispersed in water. The particles were stored at 4 °C.

Assembly of CS nanoparticles

For the monolayer assembly, 100 μL of a 1.0 mg/mL CS nanoparticle dispersion in ethanol were slowly dried on a 2x2 cm Si substrate with a 50 nm amorphous ZnO layer in a covered petri dish.

Functionalization of anisotropic CS@Pt|GOx particles

The assembled particles were sputtered with platinum (10 sec, 30 mA). To obtain the right amount of particles for further measurements the synthesis was repeat with six substrates. The obtained particles were removed from the substrate by addition of 150.0 μL 0.01 M HCl and purified by centrifugation in ethanol and redispersed in ethanol.

The dispersed particles were added to 65.0 mg mercaptoundecanoic acid and 1.0 mg benzildiemethyl acetal (DMPA) in 2.0 mL ethanol and placed under a UV lamp (365 nm, 100 W) for 5 min. The clicked particles were washed several times by centrifugation in ethanol and redispersed in MES puffer (pH 6.5). The carbonic acid groups were activated by stirring a solution of the dispersed particles, 34.5 mg NHS and 9.3 mg EDC for 30 min. After centrifugation for one time and redispersion in 0.5 mL MES puffer (pH 6.5) the activated particles were added to 2.0 mL GOx solution (2.5 mg/mL in MES puffer). After stirring at RT over night the particles were washed 10x in water by centrifugation and redispersed in water. The particles were stored at 4 °C.

Functionalization of anisotropic-clicked CS@GOx nanoparticles

The assembled particles were placed in a petri dish containing a solution of 32.5 mg mercaptoundecanoic acid and 0.5 mg benzildiemethyl acetal (DMPA). The UV lamp (365 nm, 100 W) was positioned directly above the petri dish and turned on for 2 min. To obtain the right amount of particles for further measurements the synthesis was repeat with six substrates. The obtained particles were removed from the substrate by addition of 150.0 μL 0.01 M HCl and purified by centrifugation in ethanol and redispersed in ethanol. The carbonic acid groups were activated by stirring a solution of the dispersed particles, 34.5 mg NHS and 9.3 mg EDC for 30 min. After centrifugation for one time and redispersion in 0.5 mL MES puffer (pH 6.5) the activated particles can be coupled to the GOx.

2.0 mL GOx solution (1.25 mg/mL in MES puffer) was added to the disperses particles. After stirring at RT over night the particles were washed 10x in water by centrifugation and redispersed in water. The particles were stored at 4 °C.

Glucose oxidase assay kit

Reactions:

(1) β-D-Glucose + O_2 + H_2O $\xrightarrow{\text{GOx}}$ D-glucono-δ-lactone + H_2O_2

(2) H_2O_2 + p-hydroxybenzoic acid + 4-aminoantipyrine $\xrightarrow{\text{POD}}$ quinoneimine dye + 4 H_2O

POD: peroxidase

If the sample contains Glucose oxidase (GOx) the quantitative formation of the quinoneimine dye complex takes place and this can be measured in the UV/Vis at 510 nm.

Analytical Methods

FT-IR spectra were obtained with a *Perkin-Elmer Spectrum 100* and a *Bruker Vertex 70v*. Sputter coating were performed with an *ATC Orion 5 UHV* sputter system and a Hitachi MC1000 ion sputter coater. UV lamp for click reactions with 365 nm and 100 watts. The samples were places with 0.5 cm distance. SEM and STEM images were acquired with a *Regulus 8230* from *Hitachi*. EDX mapping were obtained with an EDX Oxford Ultim Max 100mm² windowless. X-ray diffraction were performed in transmission with a *StadiP* from *Stoe* using Cu-K$_\alpha$ radiation. TEM images were obtained with a *Hitachi HT7800* (120 kV). UV/Vis spectra were acquired using an *Agilent Cary60* and *Cary4000*. SQUID measurements have been acquired using a *Quantum Design MPMS3* magnetometer. DLS measurements were acquired with a *Malvern Zetasizer ZMV 2000*. NTA measurements were acquired with a *Malvern Nanosight LM10*. For the measurements, 50 µL of sample dispersion without and with the fuel was pipetted on the substrate of the sample chamber and covered with a microscope cover slip. A glucose gradient was received by heating up 100 mM glucose and agar agar in 1 mL H_2O. 25 µL of the warm solution was pipetted on a microscopy slide and slowly gelled via cooling. The microscopy slide was put upside down on the NTA sample holder and 25 µL of the sample dispersion was added, additionally. Measurements in external magnetic fields were done with a NdFeB magnet (40x10x5 mm, magnetic field density: 580 mT) positioned with 0.3 cm distance to the sample. The magnetic field density at the observed particle window (1x1mm) in the NTA is ~300 mT. The samples were prepared without fuel, with fuel or with magnet and were directly measured. The tracking trajectory of single nanomotors were obtained by manual tracking via a self-written scrip in python. Based on the trajectory a total pathway could be obtained by calculating the mean square displacement (MSD) by msdanalyzer in MatLab.[2]

S1: Characterization of core-shell (CS) nanoparticles.
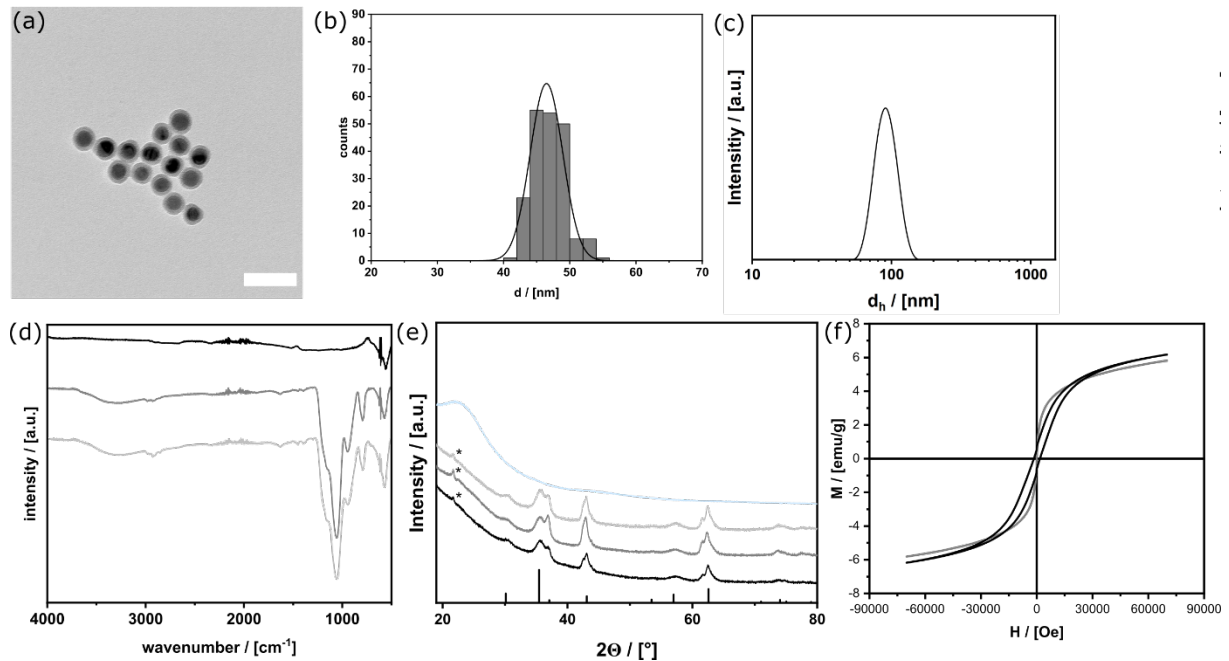
a: TEM image of CS nanoparticles, scalebar 100 nm.

b: Histogram counted from TEM images (200 nanoparticles), diameter = 46.5 nm, PDI = 5.4%.

c: DLS measurement of CS nanoparticles; measured with intensity distribution.

d: IR spectra of magnetite cores (black), magnetite-silica nanoparticles (gray) and magnetite-silica-organosilica (CS) nanoparticles (light gray) with characteristic signal of Fe-O vibration at 570 cm^{-1} and Si-O vibration at 1060 cm^{-1}.

e: PXRD of magnetite cores (black), magnetite-silica nanoparticles (gray), magnetite-silica-organosilica (CS) nanoparticles (light gray), pure silica-organosilica nanoparticles (light blue) and reference of magnetite (black). The marked reflexes refer to artefact from sample preparation for PXRD.

f: SQUID measurement, M(H) at 5 K (black) and 300 K (grey) with typical superparamagnetic behavior above blocking temperature.

S2: Synthesis and Characterization of CS@Pt nanoparticles.
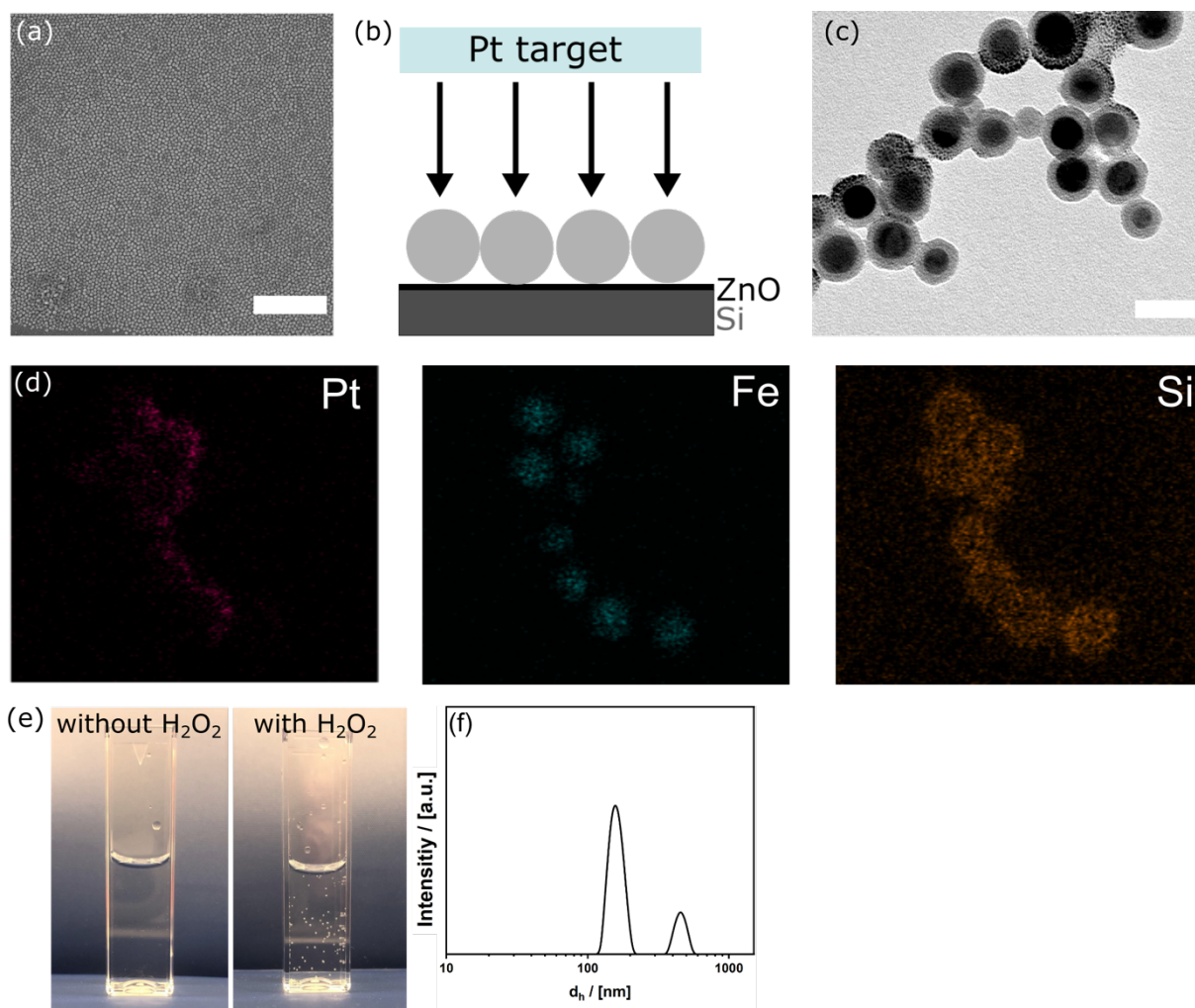
a: SEM image of CS nanoparticle monolayer on Si@ZnO wafer, scalebar 1 µm.
b: Schematic depiction of Pt sputter coating on upper particle hemisphere.
c: TEM image of CS@Pt nanoparticles, scalebar 50 nm.
d: EDX mapping of CS@Pt nanoparticles; mapping of Pt (pink), Fe (blue) and Si (orange).
e: Catalytic activity of Pt hemisphere. After adding H_2O_2 (3%, 0.5 mL) O_2 is formed.
f: Particle-size distribution determined from DLS data.

S3: Synthesis and Characterization of GOx functionalized CS nanoparticles.
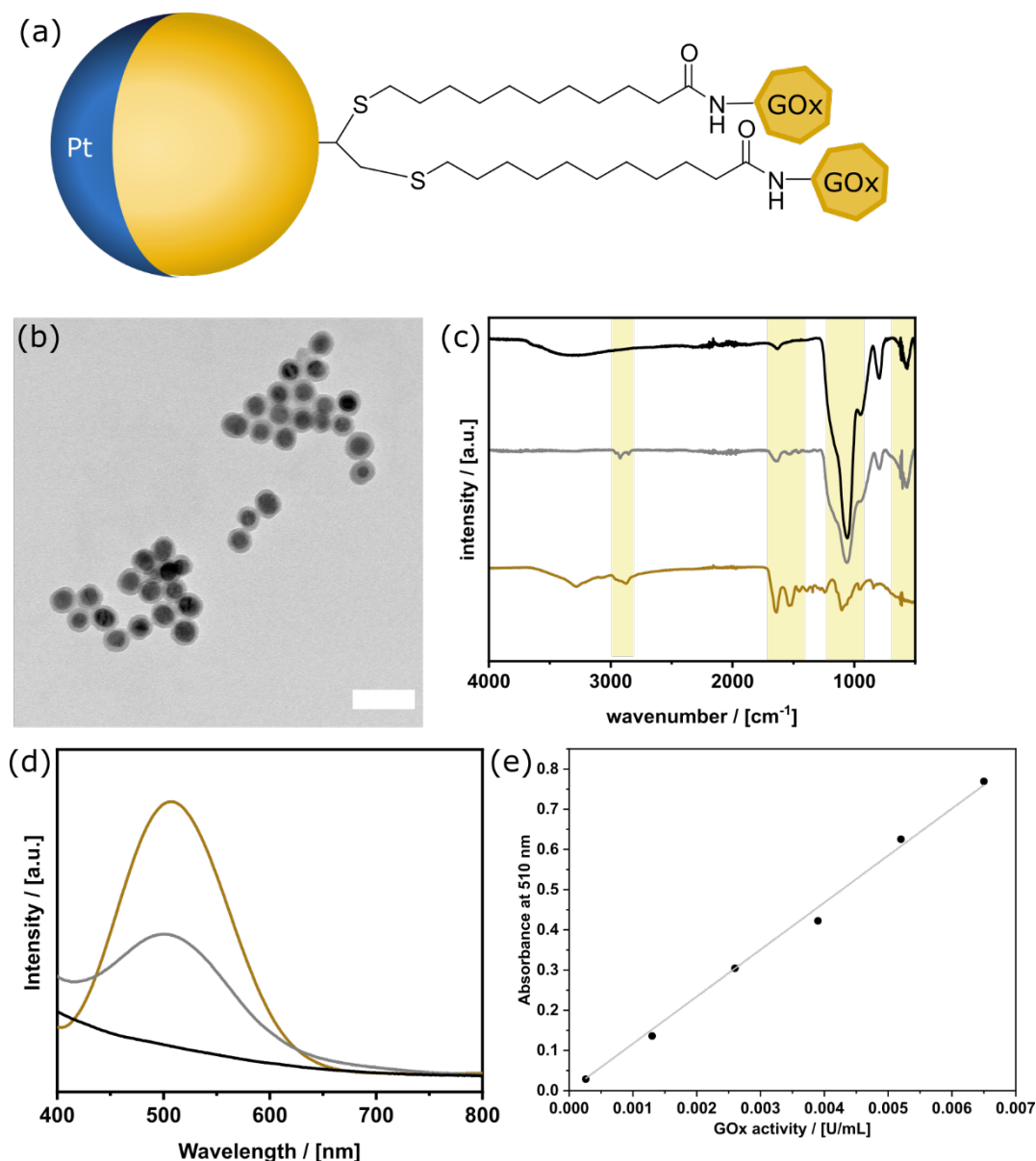
a: Schematic depiction of the of CS@Pt|GOx nanomotors showing the anisotropic modification and binding of glucose oxidase (GOx) via 11-mercaptoundecanoic acid and activation by NHS/EDC.
b: TEM image of isotropic functionalized CS@GOx nanoparticles, scalebar 100 nm.
c: IR spectra of CS nanoparticles (black), isotropic functionalized CS@GOx nanoparticles (gray) and pure GOx (brown) with characteristic signal of Fe-O vibration at 570 cm^{-1}, Si-O vibration at 1060 cm^{-1}, Amide I and II vibration at 1540 and 1600 cm^{-1} and CH vibration at 2800 cm^{-1}.
e: UV/Vis spectra of enzymatic assay with CS nanoparticles (black), isotropic functionalized CS@GOx nanoparticles (gray) and the standard solution (brown). CS nanoparticles without GOx functionalization shows no enzymatic activity.
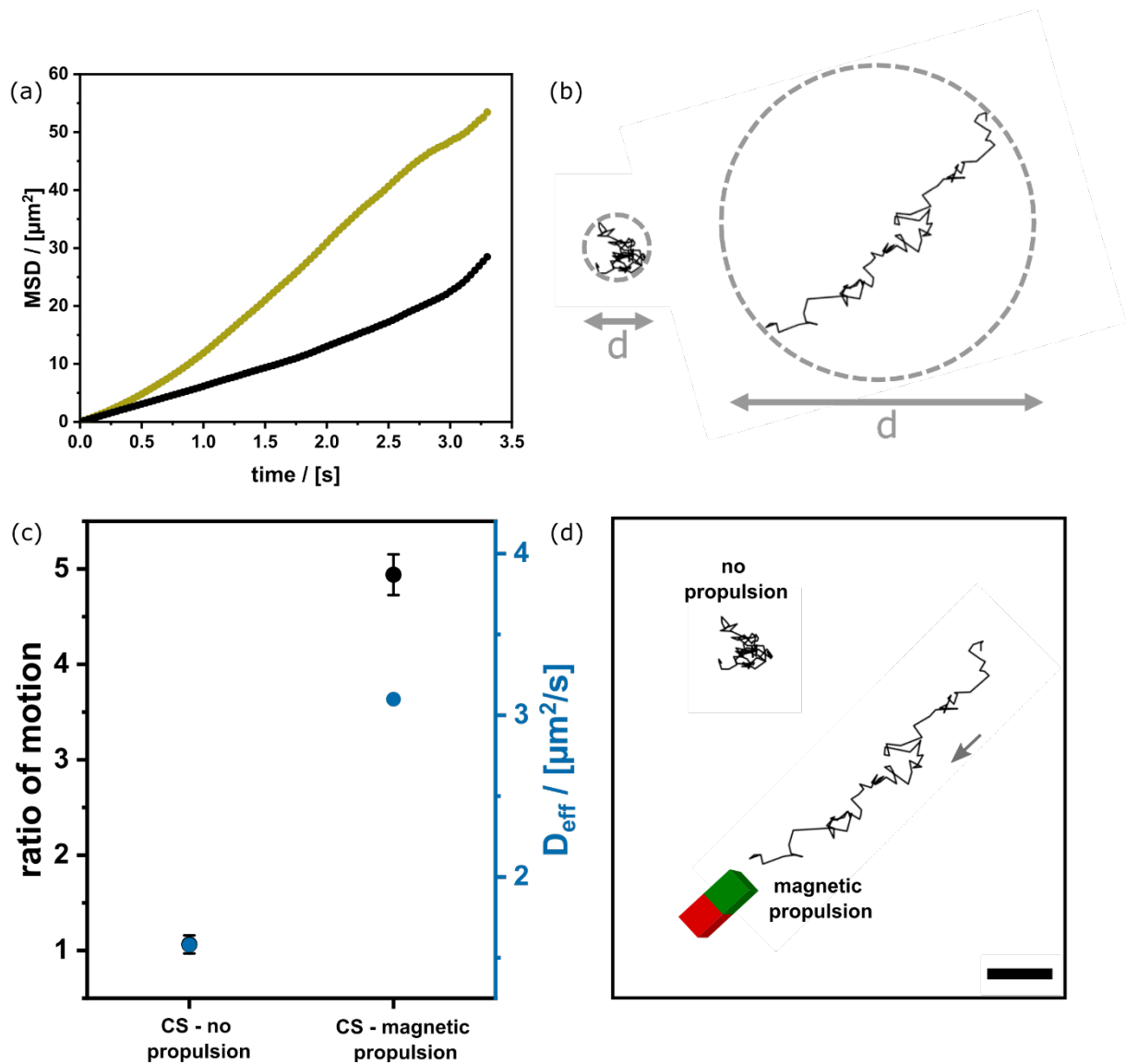e: Determination of enzymatic activity. One unit (U) is defined as the amount of enzyme which catalyzes 1 μmol D-glucose/min at pH 7,0 and 25 °C.

Calculation of enzymatic activity:
CS@GOx isotropic = 1.5×10^{-3} μmol D-glucose in 1 min and 1 mL particle dispersion
CS@Pt|GOx = 4.4×10^{-4} μmol D-glucose in 1 min and 1 mL particle dispersion

S4: Magnetic propulsion mode.

a: MSD versus time of CS@Pt|GOx nanomotors in magnetic propulsion mode (green) and without propulsion (black). RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.
b: Visualization of RoM calculation by the ratio of diameter of a circle fitting to the motion trajectories. The RoM is a mean value of eight trajectories of each sample. Example shows CS particles without propulsion and under magnetic propulsion.
c: D_{eff} calculated by MSD and RoM of CS nanoparticles in the magnetic propulsion mode. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM
d: Trajectories (3s) of CS nanoparticles in absence of a magnetic field (no propulsion) and under magnetic propulsion. The grey arrow indicates the moving direction of the CS nanoparticles; scalebar 20 µm.

S5: Chemical propulsion mode.
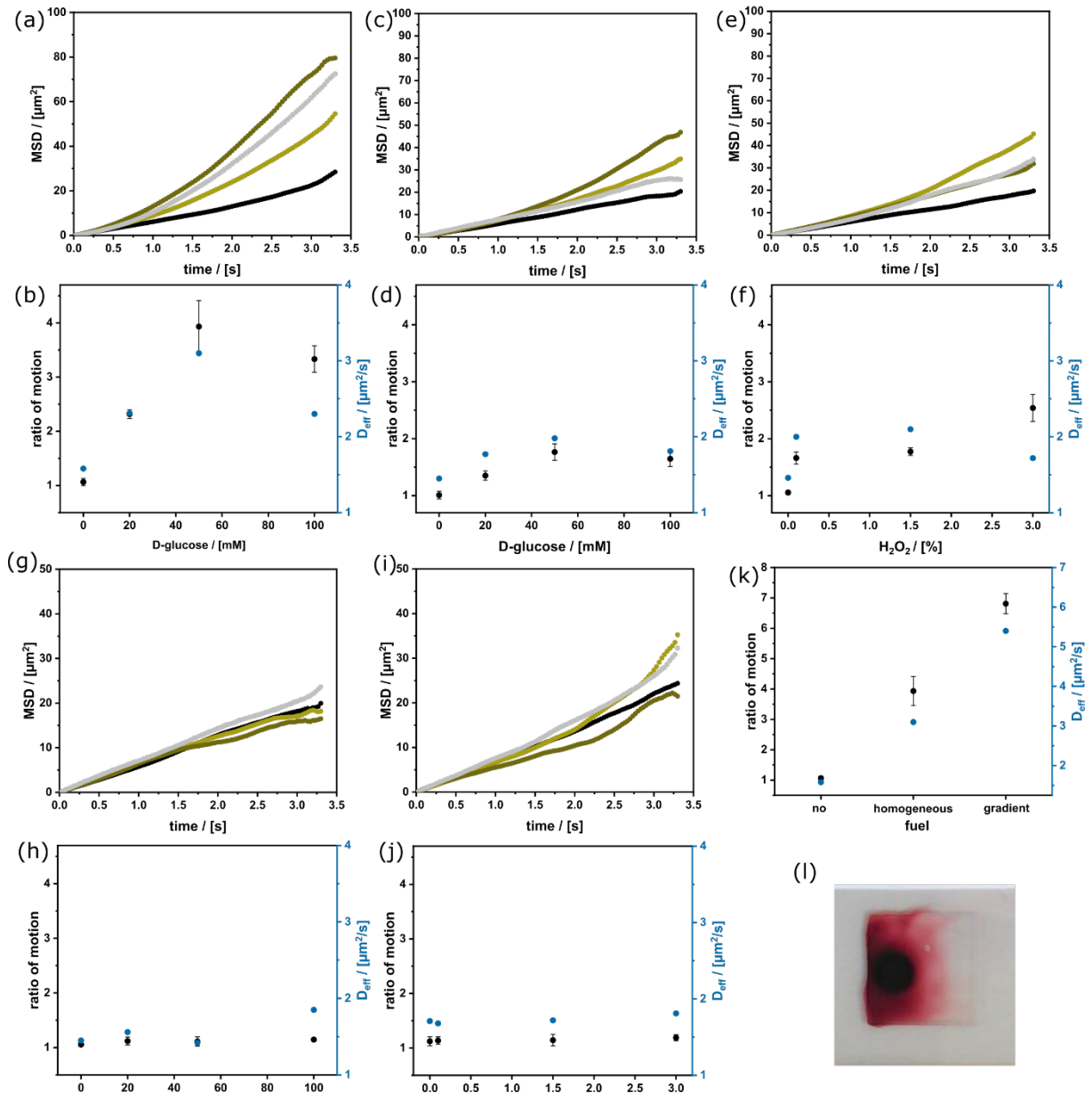
a: MSD of CS@Pt|GOx nanomotors dependent on D-glucose concentration, 0 mM (black), 20 mM (light green), 50 mM (dark green) and 100 mM (grey).

b: D_{eff} calculated by MSD and RoM of CS@Pt|GOx nanomotors dependent on D-glucose concentration. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.

c: MSD of CS@GOx nanomotors dependent on D-glucose concentration, 0 mM (black), 20 mM (light green), 50 mM (dark green) and 100 mM (grey).

d: D_{eff} calculated by MSD and RoM of CS@GOx nanomotors dependent on D-glucose concentration. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.

e: MSD of CS@Pt nanomotors dependent on H_2O_2 concentration, 0 wt% (black), 0.1 wt% (light green), 1.5 wt% (dark green) and 3 wt% (grey).

f: D_{eff} calculated by MSD and RoM of CS@Pt nanomotors dependent on H_2O_2 concentration. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.

g: MSD of CS nanoparticles dependent on D-glucose concentration, 0 mM (black), 20 mM (light green), 50 mM (dark green) and 100 mM (grey).

h: D_{eff} calculated by MSD and RoM of CS nanopartilces dependent on D-glucose concentration. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.

i: MSD of CS nanoparticles dependent on H_2O_2 concentration, 0 wt% (black), 0.1 wt% (light green), 1.5 wt% (dark green) and 3 wt% (grey).

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j: D_{eff} calculated by MSD and RoM of CS nanopartilces dependent on H_2O_2 concentration. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.

k: D_{eff} calculated by MSD and RoM of CS@Pt|GOx nanomotors without propulsion, homogenously distributed fuel and gradient fuel. RoM: mean value of eight trajectories, error bars refer to standard deviation of RoM.

l: Image of dye gradient prepared with neutral red in agar agar to illustrate the glucose gradient used for chemical propulsion of nanomotors.

References

[1]   C. Lanz, M. Schlötter, N. Klinkenberg, P. Besirske, S. Polarz, *Angew. Chem. Int. Ed.* **2020**, *59*, 8902–8906.
[2]   J.-Y. Tinevez, Mean square displacement analysis of particle trajectories for MatLab, https://de.mathworks.com/matlabcentral/fileexchange/40692-mean-square-displacement-analysis-of-particles-trajectories, **2021**.