Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Waveguide-based Chemo- and Biosensors: Complex Emulsions for the Detection of Caffeine and Proteins

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Contents

1. Materials and Methods

All chemicals were purchased from Sigma-Aldrich and were used as received, unless otherwise noted. The emulsion droplet oils, dibutylphthalate and methoxyperfluorobutane, were obtained from Sigma-Aldrich and SynQuest, respectively. The artificial active surfactants for the sensing of caffeine and concanavalin A were synthesized according to the procedures detailed below. DI water was used for the preparation of the continuous phases.

NMR spectra were recorded using a Bruker Avance 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm), spectra were referenced with TMS for ^{1}H NMR. Deuterated solvents were obtained from Cambridge Isotope Laboratories Inc. Mass spectral data were obtained with a *Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer* (FT-ICR-MS) at the MIT Department of Chemistry Instrumentation Facility. Samples were ionized with the electrospray technique (ESI). ATR-FTIR spectra were obtained using a Thermo Scientific Nicolet 6700 FTIR with a Ge crystal for ATR. Absorption spectra were obtained using an Agilent Cary 4000 UV/Vis spectrophotometer. Bright-field images were taken with a *Zeiss Axiovert 200 Inverted Microscope* and a *Zeiss AxioCam HRc* camera. Test experiments with the emulsions were observed on an *Amscope* Binocular Stereo Microscope. For the optical real-time detection of small morphology changes the complex emulsions were placed onto the hypotenuse side of a UV fused silica right-angle prism, purchased from Thorlabs. Light intensities were recorded using a fiber optic mount. A collimated laser diode module (λ = 405 nm; 4.5 mW; Ø: 11 mm; Thorlabs) with a round beam profile was used as the excitation source/light beam. An AvaSpec-ULS2048 StarLine Fiber-optic Spectrometer (Avantes) was used for recording the emission intensities. For the preparation of monodispersed complex emulsion droplets the following equipment was purchased from Dolomite Microfluidics: two Mitos P-Pumps, Basic, and the Remote partnered with an external Remote Chamber 30 mL, Telos® High Throughput Droplet System, Telos 2 Reagent Chip (100µm), 1.6mm O.D. x 0.25mm I.D. FEP tubing, End Fittings and Ferrules, Linear Connector Funnel with FEP Tubing, 1/16" x 0.8mm, 10 meters, and T-Connector.

2. Fabrication of Complex Emulsions

Emulsification was conducted at temperatures above the critical solution temperature (28 °C) of the dispersed phase solvents, typically a 1:1 mixture of dibutylphthalate and methoxyperfluorobutane. The continuous phase consisted of 1 wt. % solution of SDS and Zonyl-300 in varying ratios. Cooling the emulsion droplets below the critical solution temperature induces phase separation inside the droplets, as described previously.[1] Emulsions were fabricated using a microfluidics device. This procedure allows for the formation of droplets with highly uniform morphology, composition, and size. Emulsion droplets

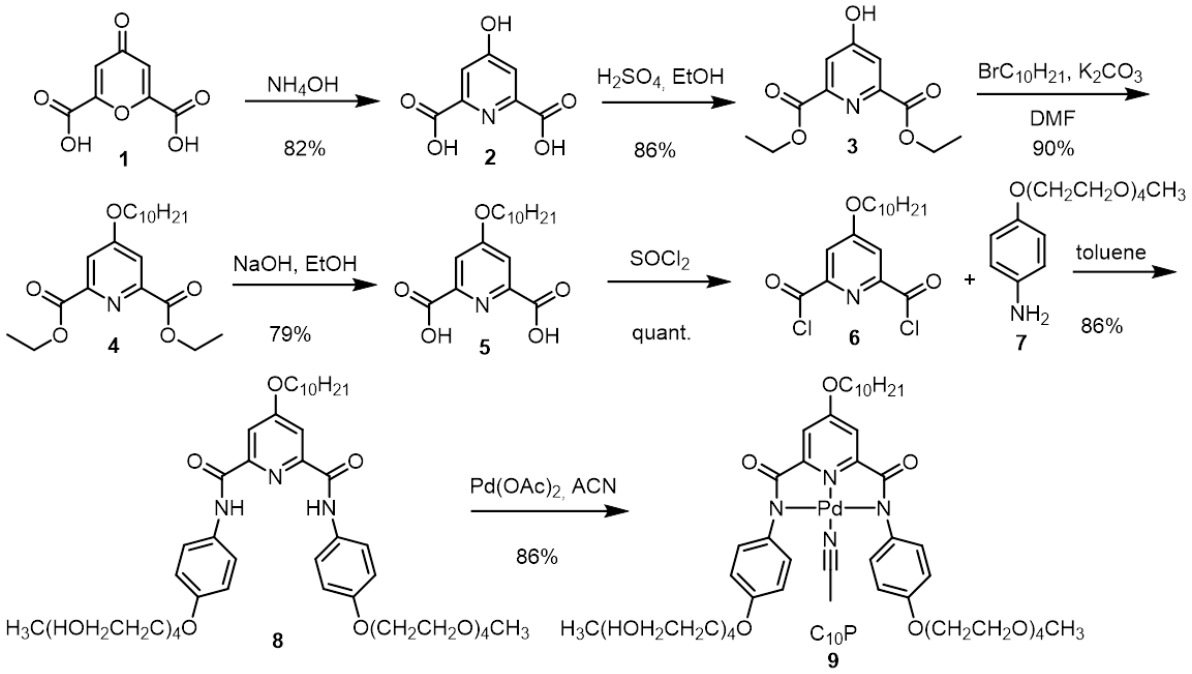
were fabricated using a Dolomite Microfluidic Setup inside a laminar flow hood using a Telos 2 Reagent Chip (100μm). Two Mitos P pressure pumps, one for the mixed droplet phases and one for the continuous phase, were used for controlling the flow rate. After heating the droplet phase above the upper critical solution temperature, the fluids were driven by pressurizing the two individual droplet and continuous chambers with N_2 providing a pulseless, stable flow to the flow focusing chip (pressures: droplet phase: 300 mbar; continuous phase: 320 mbar). The droplet phase was split into two crinkled adjacent flow resistors which provide additional flow stability and mixing. For the fabrication of dyed complex emulsions, perylene (1.5 mM) was dissolved in the hydrocarbon phase prior to emulsification.

3. Waveguide Read-Out

In order to realize an optical read-out of small morphological changes, as-prepared complex emulsion droplets were placed onto the hypotenuse side of a right-angle glass prism (Thorlabs, borosilicate right angle glass prism; 20mm) . First, 200 μL of pure surfactant solution was placed onto the surface of the prism using a micropipette. Then, 20 μL of droplets were added into the surfactant solution on the surface. Due to gravity, the monodisperse droplets started spreading and ultimately aligned in a perfect monolayer, with the fluorocarbon phase facing downwards. A collimated laser beam (λ = 405 nm; laser diode: Thorlabs) was coupled into the glass prism and directed such that it hits the hypotenuse side of the glass prism (area of the droplets) at an angle of ~ 70°, which is above the critical angle, resulting in TIR of the laser beam at the interface between the glass slide (n_g = 1.47) and the aqueous surfactant solution (n_W = 1.33) containing the droplets. An optical fiber (NA: 0.22) was mounted in a distance of 2.0 cm above the droplet monolayer. The optical fiber was connected to an AvaSpec-ULS2048 StarLine Fiber-optic Spectrometer (Avantes) for recording the light intensities (integration time 3 msec; averaging 50 spectra). For the determination of the calibration curves (*i.e.*, the emission intensity as a function of the droplet morphology) of dyed emulsion droplets, pre-fabricated monodisperse droplets were placed in surfactant solutions containing different ratios of SDS and Zonyl prior to the deposition on the prism. The recorded emission intensities were normalized to the highest light intensity measured for droplets in the double emulsion state.

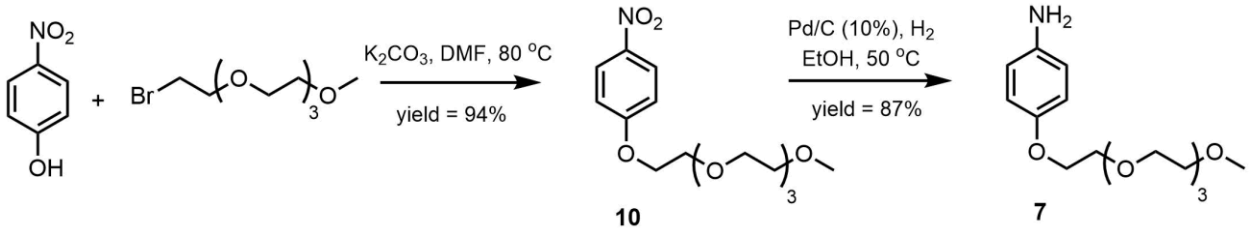
4. Synthesis of Pincer Surfactant

The synthesis of Pd-pincer surfactant $C_{10}P$ followed a modified literature procedure:[2]



Synthesis of tetraethylene glycol functionalized anilines:

13-(4-nitrophenoxy)-2,5,8,11-tetraoxatridecane (10)



Triethylene glycol 2-bromoethyl methyl ether (1.56 g, 5.75 mmol, 1.0 equiv.) was added to a solution of 4-nitrophenol (800 mg, 5.75 mmol, 1.0 equiv.) and potassium carbonate (1.19 g, 8.63 mmol, 1.5 equiv.) in dimethylformamide (DMF) and stirred at 80 °C for 12 hours. The solvent was removed under reduced pressure and the residue extracted with dichloromethane (DCM, 3x20 mL). The combined organic layers were dried over magnesium sulfate ($MgSO_4$) and concentrated *in vacuo* to give the product 10 as an orange oil (1.78 g, 5.4 mmol, Yield: 94 %).

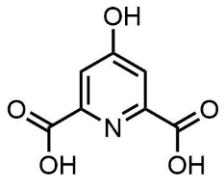
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, *J* = 9.2 Hz, 2H), 6.98 (d, *J* = 9.3 Hz, 2H), 4.26 – 4.18 (m, 2H), 3.96 – 3.86 (m, 2H), 3.75 – 3.71 (m, 2H), 3.66 (dddd, *J* = 11.6, 9.3, 4.0, 1.9 Hz, 10H), 3.56 – 3.52 (m, 2H), 3.37 (s, 3H).

4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)aniline (7)

A solution of **10** (1.07 g, 1 equiv.) and 10 wt% Palladium on carbon (108 mg, 10 wt%) in Ethanol (EtOH) was heated to 50 °C and stirred for 3 h under hydrogen atmosphere. The mixture was allowed to reach room temperature again and filtered through a short plug of silica. The solvent was removed under reduced pressure to give **7** as a light orange oil (830 mg, 3.25 mmol, Yield: 87%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.79 – 6.71 (m, 2H), 6.68 – 6.59 (m, 2H), 4.08 – 4.01 (m, 2H), 3.84 – 3.78 (m, 2H), 3.72 (ddd, *J* = 5.7, 3.5, 1.2 Hz, 4H), 3.70 – 3.62 (m, 10H), 3.57 – 3.52 (m, 2H), 3.37 (s, 3H).

Synthesis of 4-hydroxypyridine-2,6-dicarboxylic acid (2)

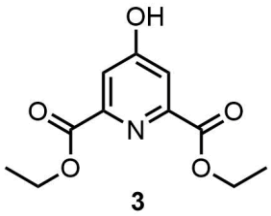


2

Chelidonic acid (1 g, 5.43 mmol, 1 equiv.) was added to an ammonia solution (28-30 %, 11 mL, 25 equiv.) and cooled to 0 °C in an ice bath. The mixture was heated to 100 °C for 6 h, then the solution was cooled to room temperature again. Hydrochloric acid was added carefully until no more precipitation of the product occurred. The solid was filtered, washed with ice water and dried in the vacuum oven at 55 °C overnight to give **2** as a white solid (807 mg, 4.41 mmol, Yield: 82 %).

No signals observed in ^{1}H NMR spectrum in D_2O, Chloroform-*d* or DMSO-*d.*

Synthesis of diethyl 4-hydroxypyridine-2,6-dicarboxylate (3)

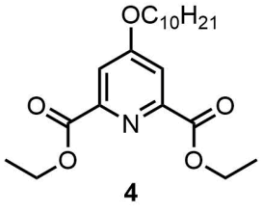


3

2 (1.8 g, 9.8 mmol, 1 equiv.) was dissolved in 20 mL ethanol and 0.45 mL of concentrated sulfuric acid (8.5 mmol, 0.85 equiv.) added. The mixture was refluxed for 12 h and then the solvent evaporated. The residue was dissolved in a mixture of DCM and $NaHCO_3$ solution (1:1) and the water phase extracted with 3x15 mL DCM. The combined organic layers were dried over $MgSO_4$ and the solvent removed to give **3** as a beige solid (2.03 g, 8.5 mmol, Yield: 86 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.12 (s, 2H), 4.47 (q, *J* = 7.1 Hz, 4H), 1.42 (t, *J* = 7.1 Hz, 6H). HRMS (ESI): calc. for $C_{11}H_{13}NO_5$ $[M-H]^-$ 238.0721, found 238.0733.

Synthesis of diethyl 4-(decyloxy)pyridine-2,6-dicarboxylate (4)



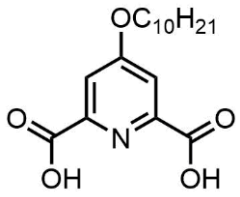
4

Diethyl 4-(decyloxy)pyridine-2,6-dicarboxylate **3** (340 mg, 1.42 mmol, 1 equiv.) was added to a mixture of 1-bromodecane (345 mg, 1.56 mmol, 1.2 equiv.) and K_2CO_3 (392 mg, 2.84 mmol, 2 equiv.) in DMF and the solution heated to 80 °C for 12 h. The solvent was removed in vacuo and the residue dissolved in 10 mL water and 10 mL DCM. The aqueous layer was extracted with DCM (3x10 mL), the combined organic layers were dried over $MgSO_4$ and the solvent removed. The residue was dried under vacuum overnight to remove residual DMF and yielded **4** as a beige solid (487 mg, 1,28 mmol, yield = 90%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75 (s, 2H), 4.45 (q, *J* = 7.2 Hz, 4H), 4.29 – 3.99 (m, 2H), 1.81 (p, *J* = 6.8 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 8H), 1.36 – 1.06 (m, 12H), 0.86 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ = 167.14, 164.91, 150.23, 114.43, 114.40, 69.13, 62.45, 31.97, 29.60, 29.39, 29.34, 28.85, 25.94, 22.77, 14.30, 14.20. HRMS (ESI): calc. for $C_{21}H_{33}NO_5$ $[M+H]^+$ 380.2431, found 380.2428. IR:

2925, 2854, 1748, 1720, 1594, 1567, 1445, 1405, 1372, 1342, 1245, 1155, 1104, 1031, 880, 862, 786, 728, 705, 660, 649, 643, 638, 632, 618, 610 cm^{-1}.

Synthesis of 4-(decyloxy)pyridine-2,6-dicarboxylic acid (5)

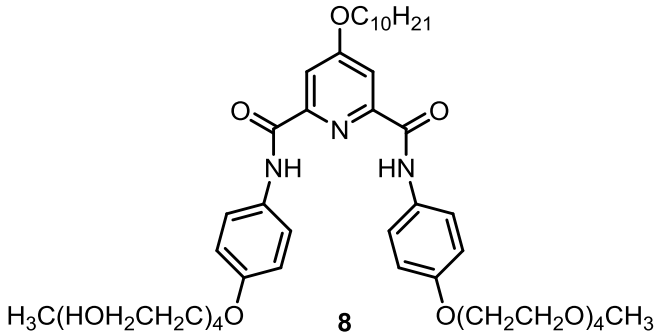


5

To a solution of **4** (487 mg, 1.28 mmol, 1 equiv.) in 8 mL EtOH and 4 mL water, solid NaOH (205 mg, 5.13 mmol, 4 equiv.) was added and the mixture was refluxed for 3 h. The solution was stirred for another 12 h at room temperature, then the solvent was removed *in vacuo*. Water was added (5 mL) and the white solid precipitated by slow acidification with 30 % hydrochloric acid to pH = 2. The solid was filtered, washed with cold water and dried in the vacuum oven over night to give **5** as a white powder (328 mg, 1.01 mmol, Yield: 79 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (s, 2H), 4.26 (t, *J* = 6.6 Hz, 2H), 1.74 (q, *J* = 7.2 Hz, 2H), 1.41 (d, *J* = 7.2 Hz, 2H), 1.35 – 1.15 (m, 12H), 0.94 – 0.73 (m, 3H). ^{13}C NMR spectrum could not be obtained due to the insolubility of the compound. HRMS (ESI): calc. for $C_{17}H_{25}NO_5$ $[M-H]^-$ 322.1660, found 322.1664. IR: 2920, 2855, 1728, 1633, 1602, 1575, 1467, 1426, 1401, 1379, 1306, 1231, 1176, 1114, 1035, 911, 887, 815, 792, 698, 646, 637, 619 cm^{-1}.

Synthesis of pincer ligand (8)



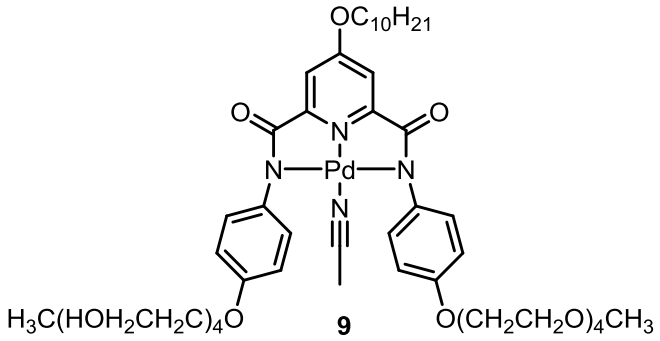
8

Under N_2-atmosphere 4-(decyloxy)pyridine-2,6-dicarboxylic acid **6** (260 mg, 803 µmol, 1 equiv.) was refluxed in 580 µL thionyl chloride ($SOCl_2$, 8.04 mmol, 10 equiv.) and 2 mL dry DCM for 2 h until the

reaction mixture was clear. Excess $SOCl_2$ was removed in vacuo and 109 mg of acid chloride **6** (302 µmol) redissolved in 15 mL dry toluene. Tetraethylene glycol functionalized anilin **7** (287 mg, 605 µmol, 2 equiv.) was added and the reaction refluxed for 12 h. The solvent was removed under reduced pressure to give the ligand **8** as a brown gel (322 mg, 260 µmol, yield = 86 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.60 (s, 2H), 7.90 (s, 2H), 7.64 (d, *J* = 8.5 Hz, 4H), 6.92 (d, *J* = 8.5 Hz, 4H), 4.24 – 4.02 (m, 6H), 3.84 (q, *J* = 7.2, 6.0 Hz, 4H), 3.67 (td, *J* = 18.1, 16.3, 4.9 Hz, 20H), 3.53 (t, *J* = 4.7 Hz, 4H), 3.35 (s, 6H), 1.82 (h, *J* = 6.9 Hz, 2H), 1.44 (q, *J* = 7.3 Hz, 2H), 1.28 (d, *J* = 10.7 Hz, 12H), 0.86 (q, *J* = 13.9, 9.9 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ = 168.36, 161.33, 156.10, 151.12, 130.69, 122.14, 115.15, 111.66, 72.03, 70.94, 70.89, 70.75, 70.72, 70.63, 70.59, 69.88, 69.30, 67.83, 59.14, 32.00, 29.64, 29.42, 29.39, 28.87, 25.95, 22.79, 14.24. HRMS (ESI): calc. for $C_{47}H_{71}N_3O_{13}$ $[M+H]^+$ 886.5060, found 886.5033. IR: 2925, 2871, 1727, 1677, 1592, 1529, 1511, 1454, 1416, 1349, 1300, 1247, 1174, 1104, 1065, 1036, 945, 878, 831, 750, 690, 665, 656, 643, 634, 614 cm^{-1}.

Synthesis of pincer complex C_{10}P (9)



9

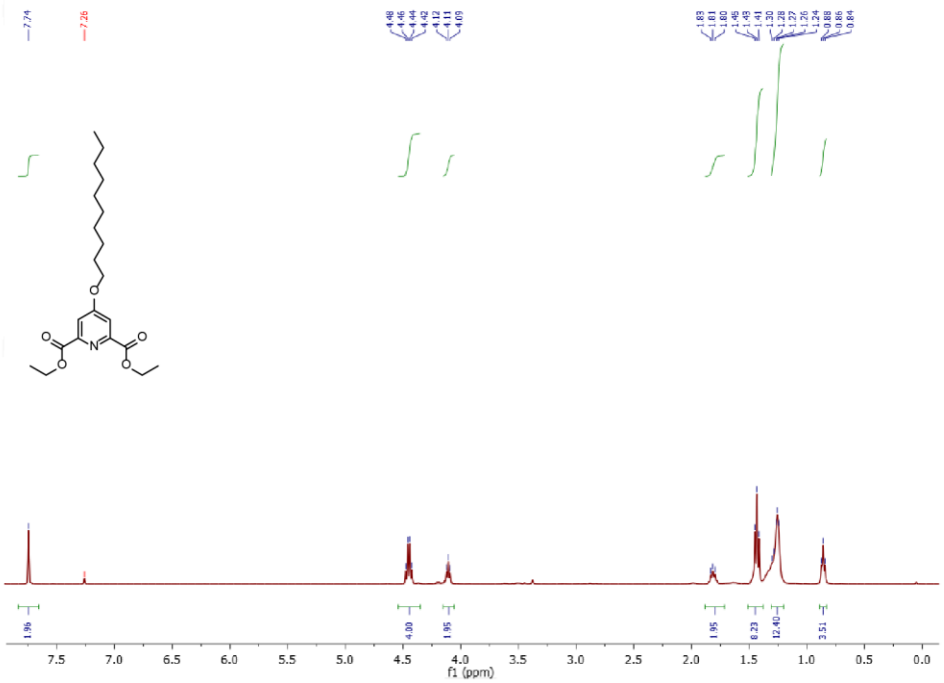
Under N_2 atmosphere ligand **8** (300 mg, 242 µmol, 1 equiv.) and $Pd(OAc)_2$ (59.8 mg, 266 µmol, 1.1 equiv.) were suspended in acetonitrile (10 mL). The mixture was stirred at 35 °C for 12 h and then filtered through a 0.45 µm Nylon syringe filter. The solvent was removed *in vacuo* and the residue washed three times with Et_2O/hexane to give pincer complex **9** as a brown solid (289 mg, 208 µmol, Yield: 86%).

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.22 – 7.11 (m, 6H), 6.80 (d, *J* = 8.4 Hz, 4H), 4.15 (t, *J* = 6.4 Hz, 2H), 4.07 (t, *J* = 4.7 Hz, 4H), 3.76 (q, *J* = 4.8 Hz, 4H), 3.67 – 3.49 (m, 20H), 3.49 – 3.40 (m, 4H), 3.27 (s, 6H), 1.78 (dd, *J* = 16.1, 8.8 Hz, 2H), 1.42 (t, *J* = 7.6 Hz, 2H), 1.29 (m, 12H), 0.88 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, Acetonitrile-d_3) δ = 170.64, 169.47, 156.41, 154.93, 141.10, 128.42, 114.77, 112.27, 72.52, 71.25, 71.10, 71.07, 71.05, 71.00, 70.90, 70.87, 70.38, 68.60, 58.94, 32.65, 30.29, 30.07, 29.98, 29.37, 26.42, 23.41, 14.45. HRMS (ESI): calc. for $C_{49}H_{72}N_4O_{13}Pd$ $[M+Na]^+$ 1053.4044, found 1153.4090. IR: 2926, 1726, 1604, 1534, 1504, 1450, 1380, 1295, 1242, 1209, 1107, 1092, 945, 829, 778, 734, 654, 640, 628, 610 cm^{-1}.

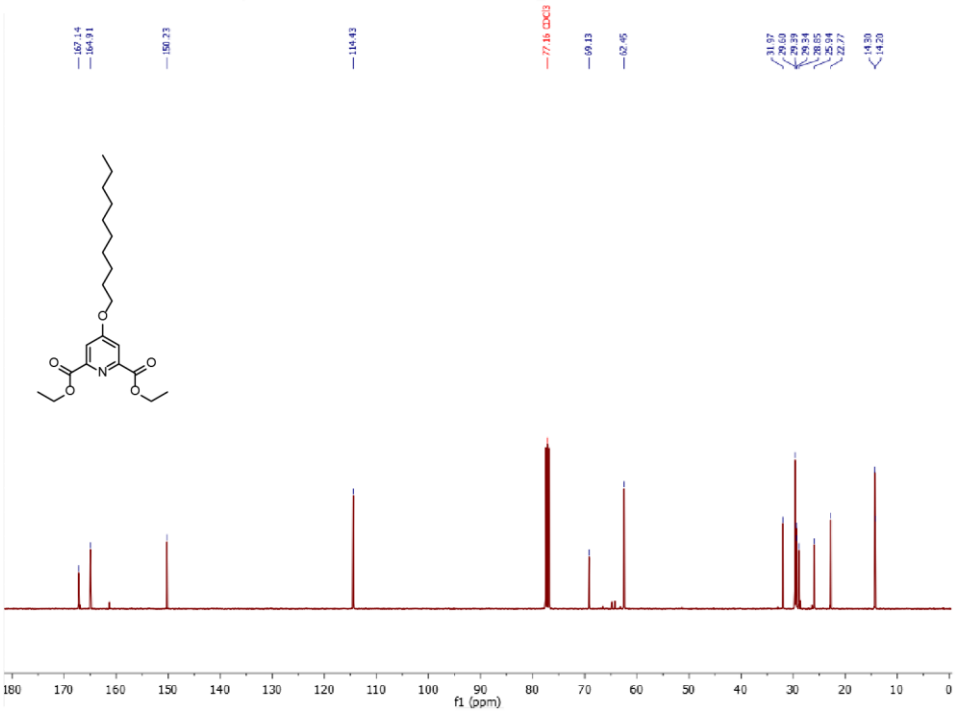
Spectral characterization of compounds:

diethyl 4-(decyloxy)pyridine-2,6-dicarboxylate (4)

^{1}H NMR in chloroform-*d*:



^{13}C NMR in chloroform-*d*: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164.91
150.23
114.43
77.16 CDCl3
69.13
62.46
31.97
29.60
29.39
29.34
28.85
25.94
22.77
14.39
14.20
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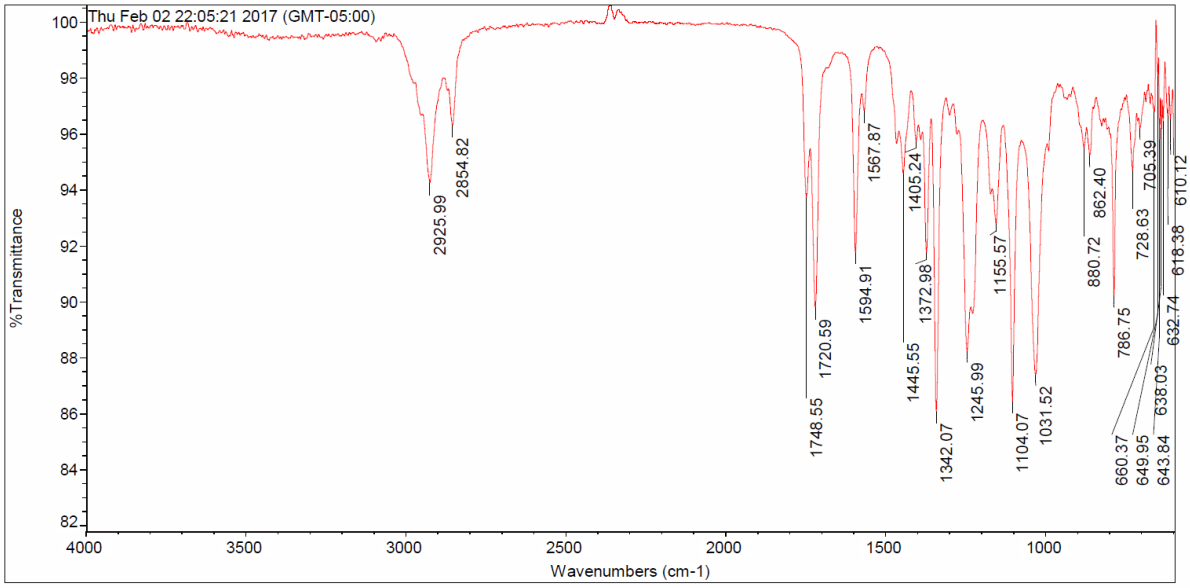
f1 (ppm)

IR:


Thu Feb 02 22:05:21 2017 (GMT-05:00)
%Transmittance
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1720.59
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1567.87
1445.55
1405.24
1372.98
1342.07
1245.99
1155.57
1104.07
1031.52
880.72
862.40
786.75
728.63
705.39
660.37
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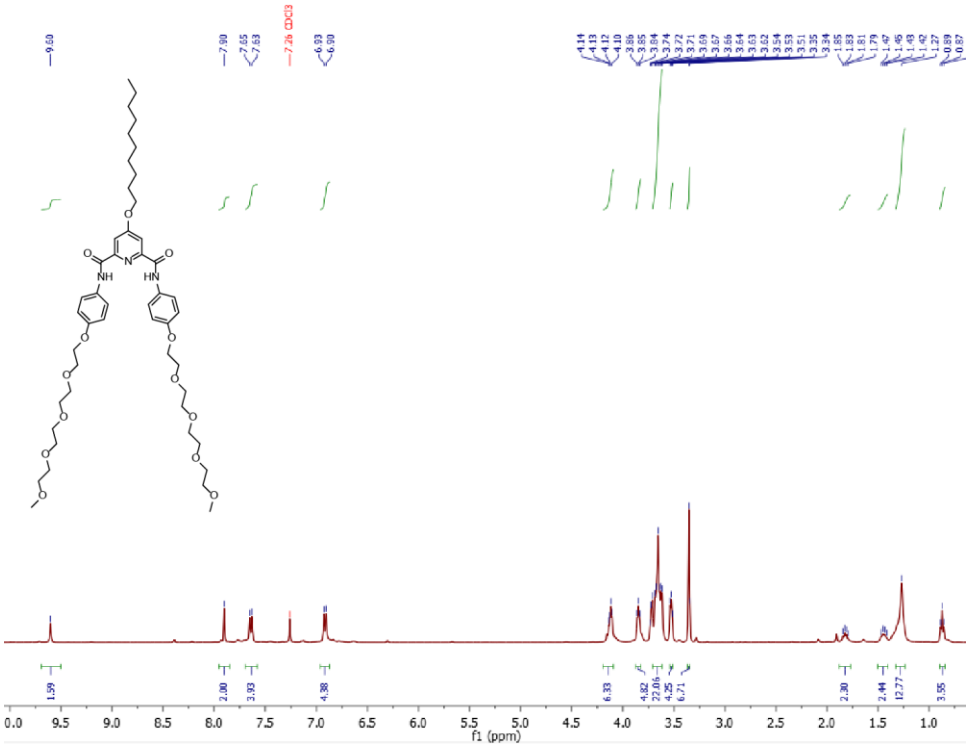
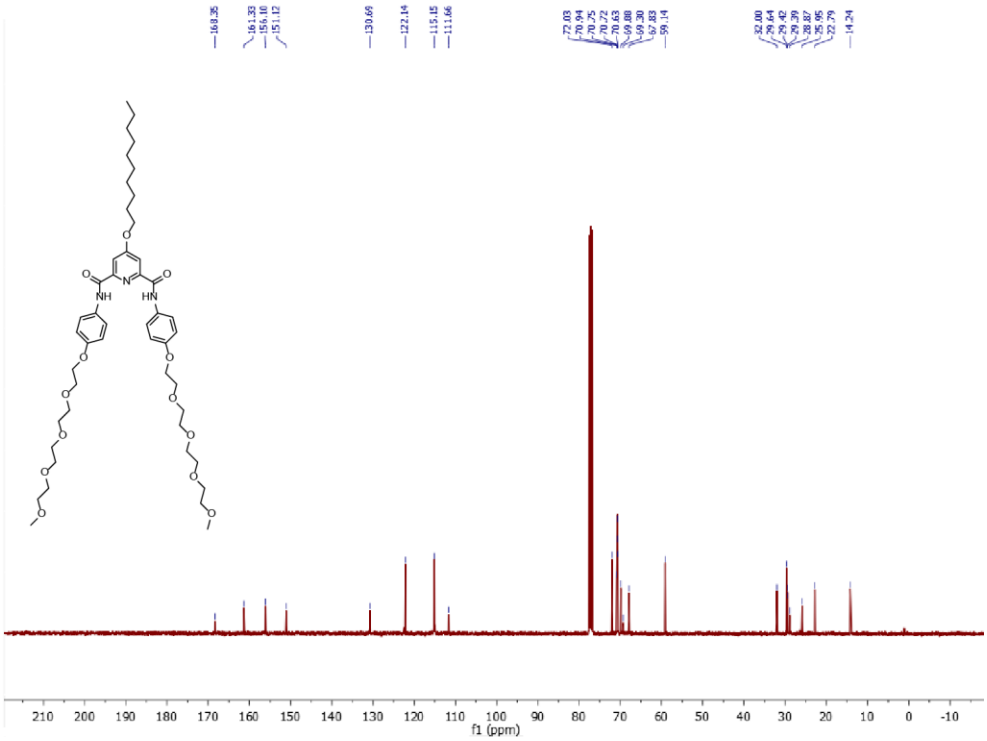
643.84
638.03
632.74
618.38
610.12

Pincer ligand (8)

^{1}H NMR in chloroform-*d*:



^{13}C NMR in chloroform-*d*:



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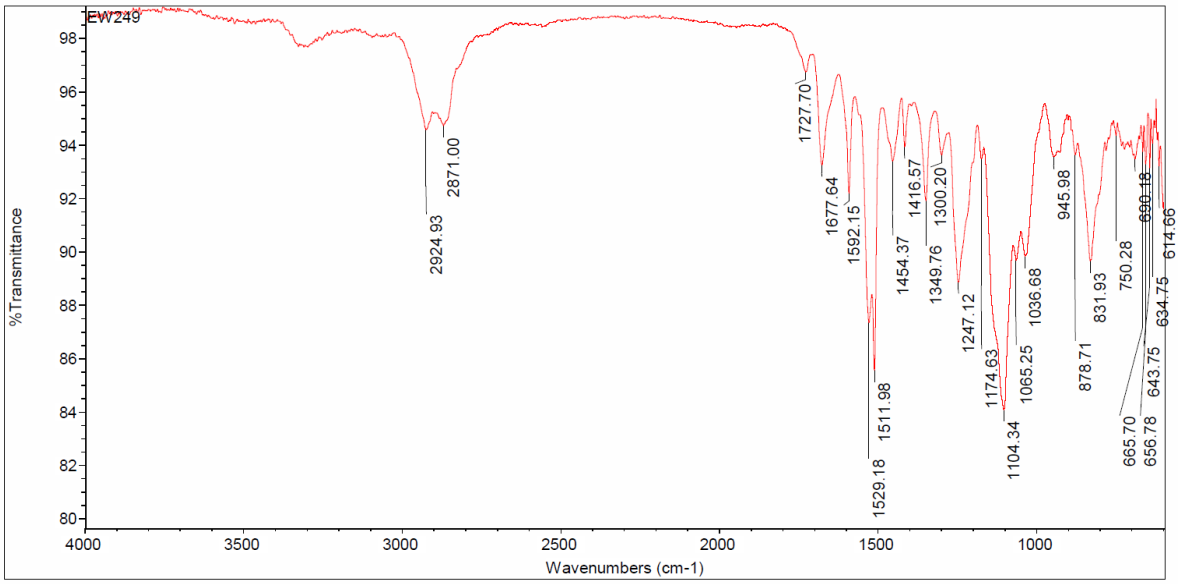
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1677.64
1592.15
1529.18
1511.98
1454.37
1416.57
1349.76
1300.20
1247.12
1174.63
1104.34
1065.25
1036.68
945.98
878.71
831.93
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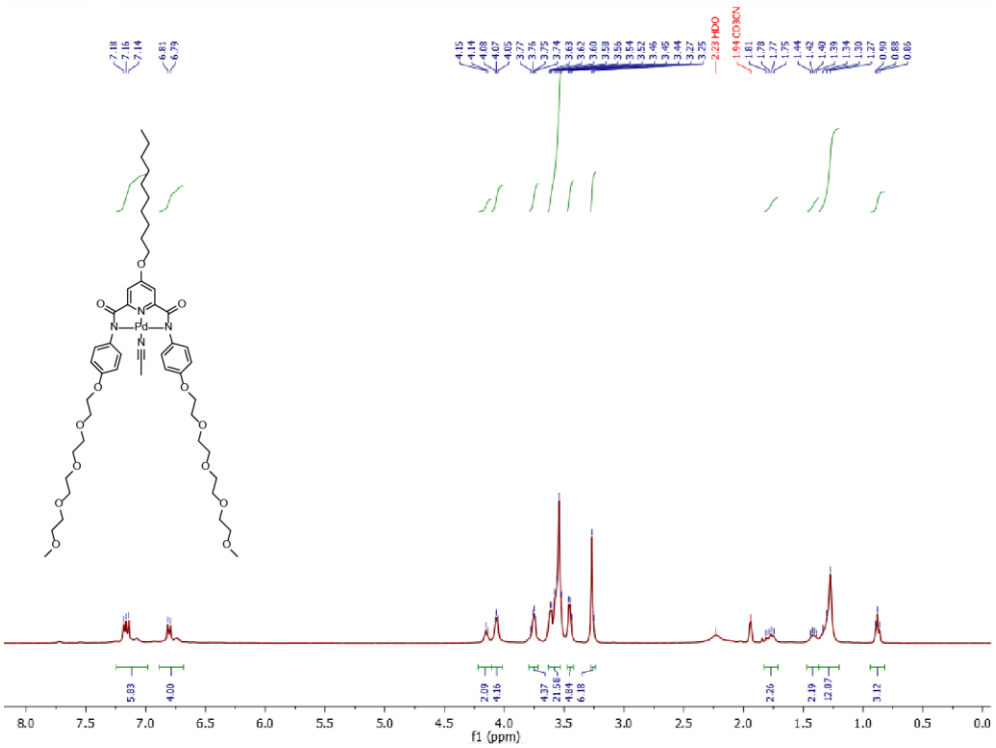
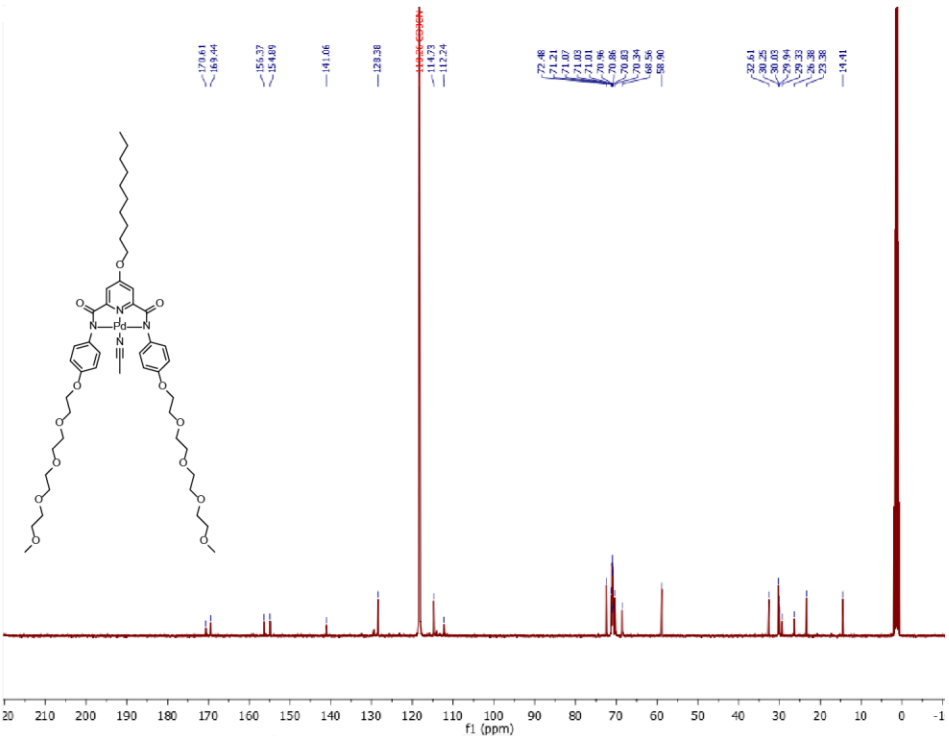
665.70
656.78
643.75
634.75
614.66

Pincer complex C10P (9)

^{1}H NMR in acetonitrile-*d*:



^{13}C NMR in acetonitrile-*d*:



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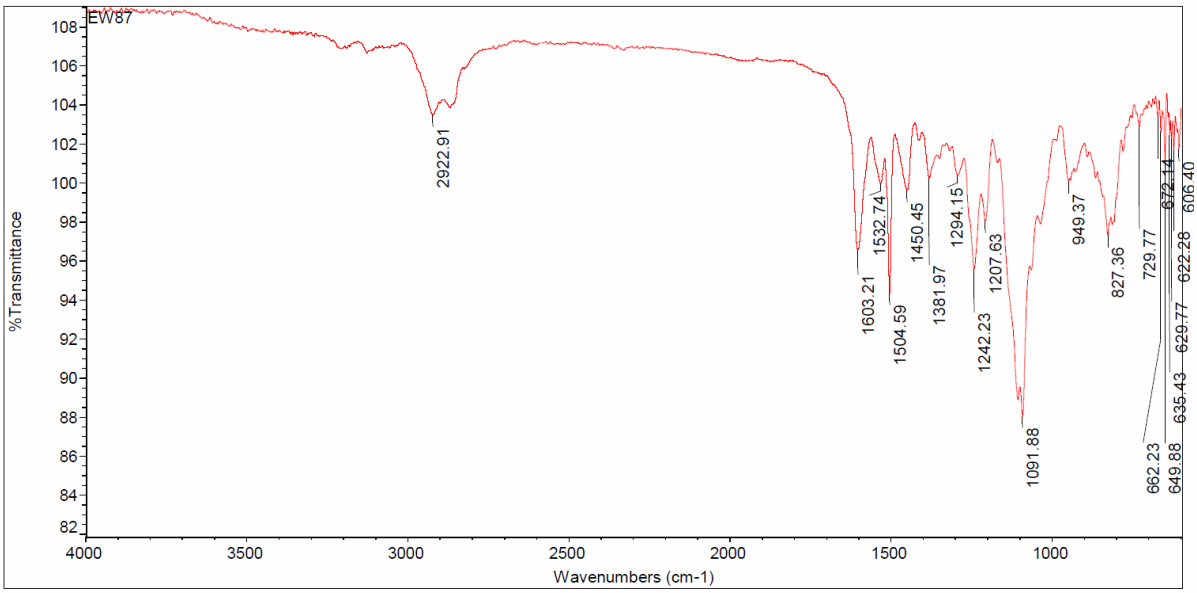
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1207.63
1091.88
949.37
827.36
729.77
672.14
662.23
649.88
635.43
629.77
622.28
606.40

5. Sensing of Caffeine

5.1 Qualitative demonstration using optical scattering

Caffeine detection with $C_{10}P$:

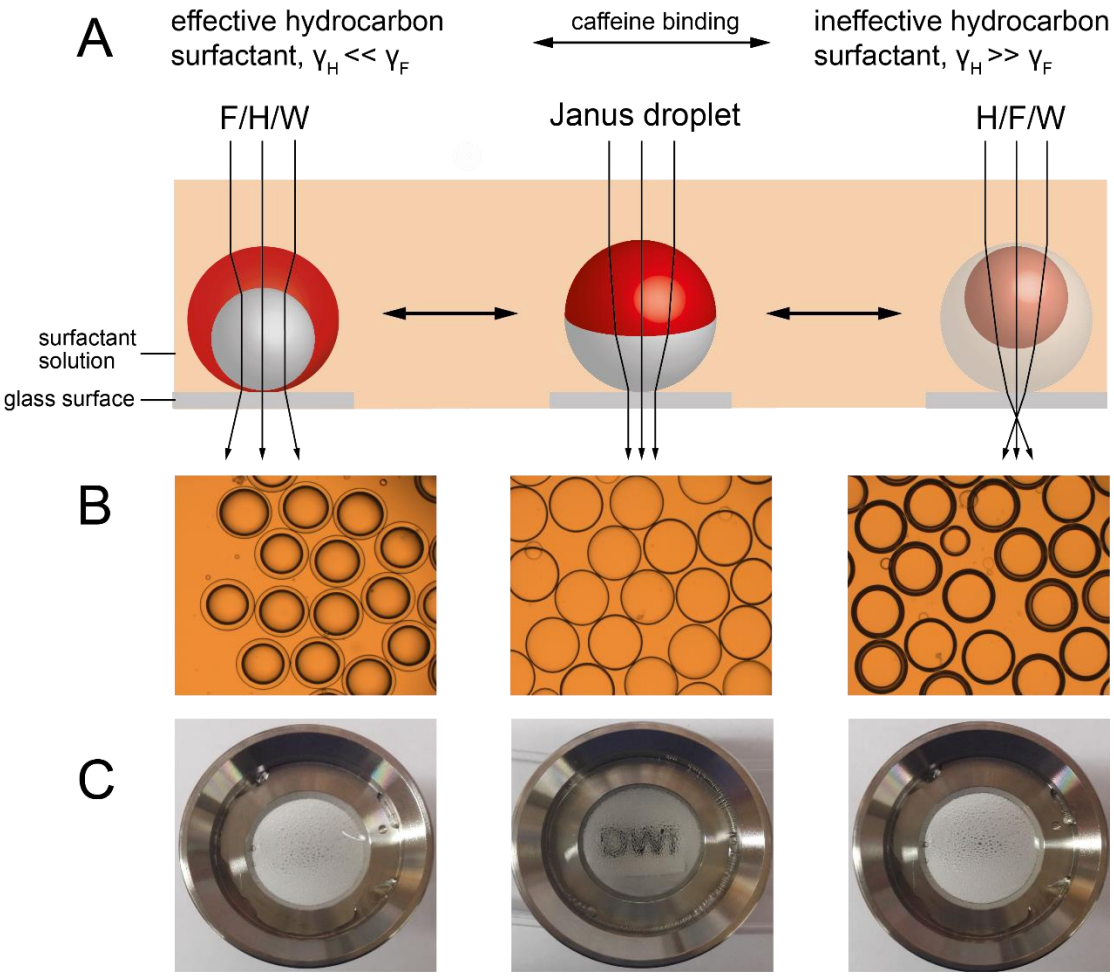

Figure S1: Qualitative demonstration of the working principle of the pincer complex based on the lens effect. Transmission changes upon change in the morphology of the droplets. (**A)** Schematic drawing of the morphology of the droplets and their transmission. (**B**) Images of the droplets in double emulsion and Janus state. (**C**) Change of transmission from double emulsion to Janus state morphology in droplet solution. The Logo underneath the sample plate only becomes visible if droplets are in Janus morphology and transmissive.

5.2 Microscopy Read-Out

We visualized the change in morphology of the complex emulsions when caffeine was added using inverted optical microscopy. Similar to the qualitative demonstration using optical scattering, we started with the Janus droplets in a mixture of $C_{10}P$ and Zonyl surfactants. We then obtained optical micrographs upon the step-wise additions of caffeine. To produce a quantitative readout from the micrograph, we implemented an image processing program that detects the radius of the droplets (R_{out}) and the radius of the inner phase (R_{in}) and calculates the ratio between the two radii (R_{in}/R_{out}), **Figure S3**.

At the starting Janus condition, the value of the ratio between the radii is close to unity, **Figure S3B**. And upon the addition of caffeine, the complex emulsions transform from Janus droplets toward double emulsions (with the organic phase encapsulated), thus reducing the value of the inner phase radius and the ratio, **Figure S3C**. We chose to measure the ratio between the two radii rather than just the radius of the inner phase to eliminate the variability arising from polydispersity in size of the droplets. **Figure S3D** shows the gradual decrease in the ratio of the radii with higher concentration of caffeine, as expected when the Janus emulsions transform into double emulsions.

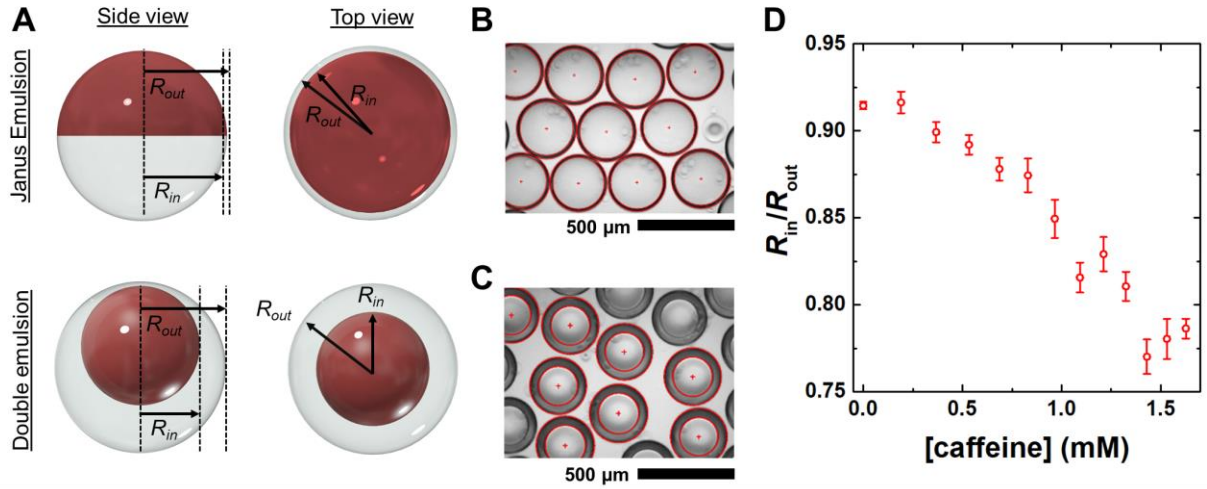

Figure S2: Analysis of the optical micrographs of the changes in droplet morphology upon the addition of caffeine. (A) Side view and Top view schematic diagrams of the droplets in Janus and double emulsion states, depicting the radius of the droplet (R_{out}) and the inner radius (R_{in}). The ratio of the radii is near unity in Janus emulsions and approaches 0.8 for double emulsions. Top view optical micrographs of Janus emulsions with $C_{10}P$ and Zonyl surfactants (B) and double emulsions after the addition of 1 mM of caffeine (C). (D) The ratio between R_{in} and R_{out} as a function of the concentration of caffeine.

5.3 Waveguide Read-Out

For sensing of caffeine, droplets were prepared using the as-synthesized Pd-pincer complex $C_{10}P$ as the organic surfactant instead of SDS. Before recording the calibration curves, droplets were added to a surfactant solution containing a 95:5 ratio of $C_{10}P$:Zonyl (overall surfactant concentration 0.1 wt.% and 1.0 wt. %, respectively). The ratio of the surfactants in the aqueous phase ($C_{10}P$ and Zonyl) was strategically chosen such that the droplet morphology at the starting point (*i.e.*, no caffeine present) resulted in an almost encapsulated double emulsion state resulting in a close to the maximum emission intensity. Subsequently, for sensing, vials with the respective surfactant solution mixture containing different concentrations of caffeine in an overall volume of 1 mL were prepared. Then 100 μL of the pre-pepared complex emulsions were added. The emulsions were agitated in a Labnet Vortemp 56 incubator (shaking speed of 150 rpm at rt) for 10 min prior to measuring the emission intensities as described above. In a typical setup we used 1 wt.% surfactant concentrations, which allows for the precise detection of caffeine concentrations below 0.5 mM, a concentration regime of interest for the monitoring of caffeine content in coffee. The decrease of the surfactant effectiveness and thus a decrease in the interfacial tensions resulted in a step-wise decrease of the light intensity measured above the droplet monolayer. At low concentrations, the calibration curve is relatively linear and provides precise detection of caffeine (R^2=0.99). A decrease of the surfactant concentrations from 1 wt.% down to 0.1 wt.% results in an increase of sensitivity towards the detection of caffeine in the concentration regime between 0 and 50 μM (R^2=0.98). The procedure was repeated five times for each caffeine concentration.

6. Sensing of ConA

In order to monitor the emission intensity upon droplet agglutination, we first prepared droplets using a mannose surfactant, as reported previously.[3] In brief, for the continuous phase, ManC14 and Zonyl FS-300 were used to stabilize and generate emulsions with Janus morphology. The two surfactants were dissolved in HEPES buffer solution (10 mM, containing 1 mM $CaCl_2$, and 1 mM $MnCl_2$, pH = 7.5) separately with concentration of 0.01% by weight. The final volume ratio between ManC14 solution and Zonyl FS-300 solution was kept at 3:1 to generate Janus emulsions. The Janus emulsions were subsequently added to different vials with the same surfactant solution but containing different concentrations of concanavalin A (ConA). The solutions were then swirled gently in a Labnet Vortemp 56 incubator (shaking speed of 150 rpm at rt) for 6 h. Then, the droplets were deposited onto the prism and the light intensities were recorded as described above.

7. References

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