Supporting information

Inline monitoring of high ammonia concentrations in methanol with a customized 3D printed flow cell

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1. Optical sensing

Sensor manufacturing:

The sensor cocktail is mixed according to the procedure described by Klimant et al.[1] First, the *OHBut* indicator dye (Figure S1 **a**, 0.25 mg, 0.25wt%) is dissolved in a hydrogel D4 (100 mg, 10wt%) solution in tetrahydrofuran (THF). Second, the non-volatile acid 4-dodecylbenzenesulfonic acid (DBSA, 0.6 mg, 60 µL of a 10 mg mL^{-1} solution in cyclohexane) is added to protonate the dye's hydroxy group. Due to this, the dye molecules are now in their 'ON'-state and shows fluorescence (see Figure S1 **b**). Third, silanized Egyptian Blue (15 mg) is added into the cocktail to act as an internal reference material. The mixture is stirred with a Vortex mixer intensely for 5 min. After this, the sensor cocktail is knife-coated (25 µm wet film) on a dust free PEN foil. Immediately after coating, the hydrophobic PES layer is laid on top of the sensing layer as protection.

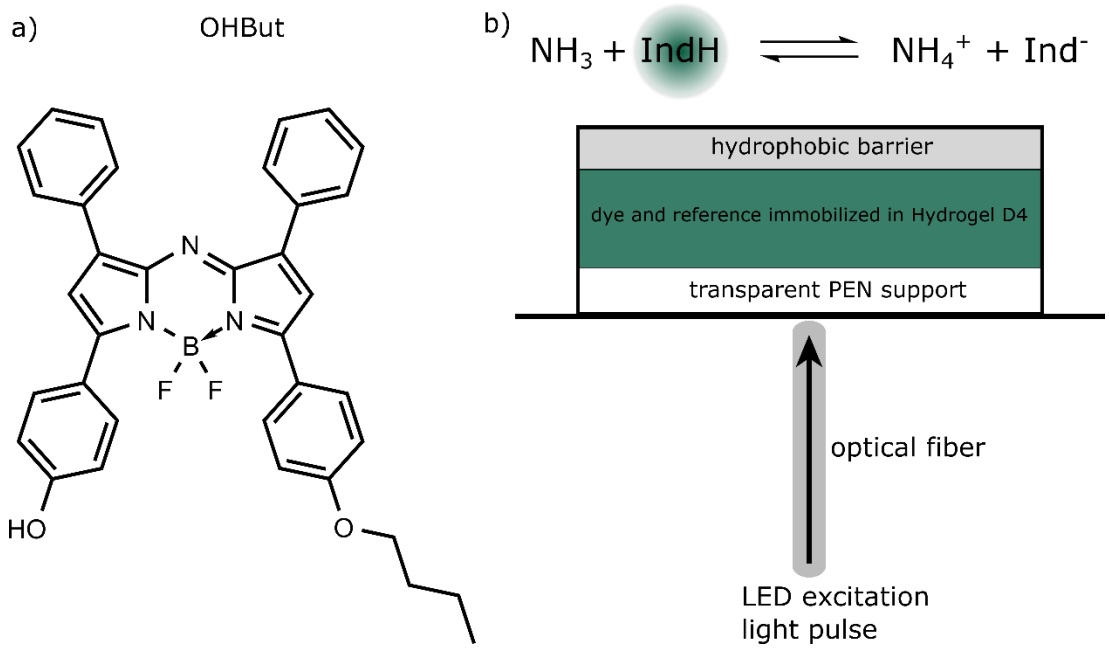

Figure S 1 a) Molecular structure of the OHBut dye. b) optical ammonia sensor scheme shows a layer by layer construction. The dye shows fluorescence in its protonated state. This fluorescence is shut off when NH_3 deprotonates the hydroxy group of the dye.

The used aza-BODIPY dye (4-(7-(4-butoxyphenyl)-5,5-difluoro-1,9-diphenyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,5,2]triazaborinin-3-yl)phenol or *OHBut*) has an apparent pK_a value of 8.90 (measured in a solution of EtOH:H_2O 1+1) and was synthesized in-house as described previously. The structural confirmation of this dye was already published in literature.[1] Silanized Egyptian Blue, which serves as inert reference dye, was used as microcrystalline powder and was

prepared as described elsewhere.[2] This compound is photochemically inert and matches in its spectrochemical properties with the used indicator dye. Therefore, both can be excited with the same excitation source (red LED) and read out with the same instrument (FirestingO_2 from Pyroscience, Aachen, Germany).

The host polymer was a polyurethane hydrogel. This HydroMed D4, was purchased from AdvanSource biomaterials (Wilmington, USA). 4-Dodecylbenzenesulfonic acid (DBSA) was purchased from Sigma Aldrich (St. Louis, USA). A superphobic PES 0.2 µm membrane (PES layer) was a project contribution from EMD Millipore Corporation (Billerica, MA, USA). It is used as protection shield on top of the sensing layer. Due to its hydrophobic nature no ionic species can pass through this membrane.[3] Additionally, its light-scattering properties enhance the sensing signal making the measurement data more reliable. The poly(ethylene naphthalate) (PEN) foil Teonex support, was purchased from Pütz (Tanusstein, Germany). The two solvents tetrahydrofuran (THF) and cyclohexane (CH) were purchased from Carl Roth GmbH (Karlsruhe, Germany). All chemicals and materials used for inline experiments are described in section 4 “Set up”.

All chemicals and substances were used without additional purification.

Dual-Lifetime Referencing (DLR) measurements:

Fluctuation of the excitation source, performance of the detector and electronic interferences are factors which influence fluorescence intensity measurements. Therefore, we have chosen another sensing method which is less prone to such interferences. This method is called dual-lifetime referencing (DLR). Here, the overall phase shift (dphi) is measured. This dphi is based on the fluorescence intensity ratio of an analyte-sensitive dye with a short lifetime and an inert reference which has a comparable long lifetime. Due to this, both have to be excited simultaneously with a modulated light pulse. Conversion of this dphi into its corresponding cotangent values is directly proportional to the fluorescence intensity.[4]

All calibration measurements and further experiments of the DLR-referenced ammonia sensors were performed with a commercially available FirestingO_2 reader (PyroScience GmbH, Aachen, Germany) in combination with plastic optical fibers (Ø 1 mm, length 1 m, Ratioplast-Optoelectronics GmbH, Lübbecke, Germany). Settings for the calibration measurements were used as the following: LED intensity 60%, measuring time of 10 ms, amplification of 400x, a modulation frequency of 2000 Hz and a measuring interval of 1 second.

2. Calibration and measurement data

Batch experiments

The set-up for these measurements can be found in literature.[3] This in batch measured data shows the sensor behaviour in methanolic NH_3 solutions. As already published, such sensors can be fitted with a logarithmic Boltzmann fit as you can see in the following graph (Figure S2). Plotting the cot(dphi) against the NH_3 concentration yields a sigmoid curve (Figure S2) which is further fitted by the following Equation S1:

$$y = A2 + \frac{A1 - A2}{1 + 10^{\frac{\log(x) - x_0}{dx}}} \quad \text{(Eq. S1)}$$

Thereby, y is the value of the normalized cot(dphi), A1 and A2 represent the respective upper and lower plateau, x defines the concentration of the calibration point, x_0 is the point of inflection and dx the slope of the fitting curve.[3,5]

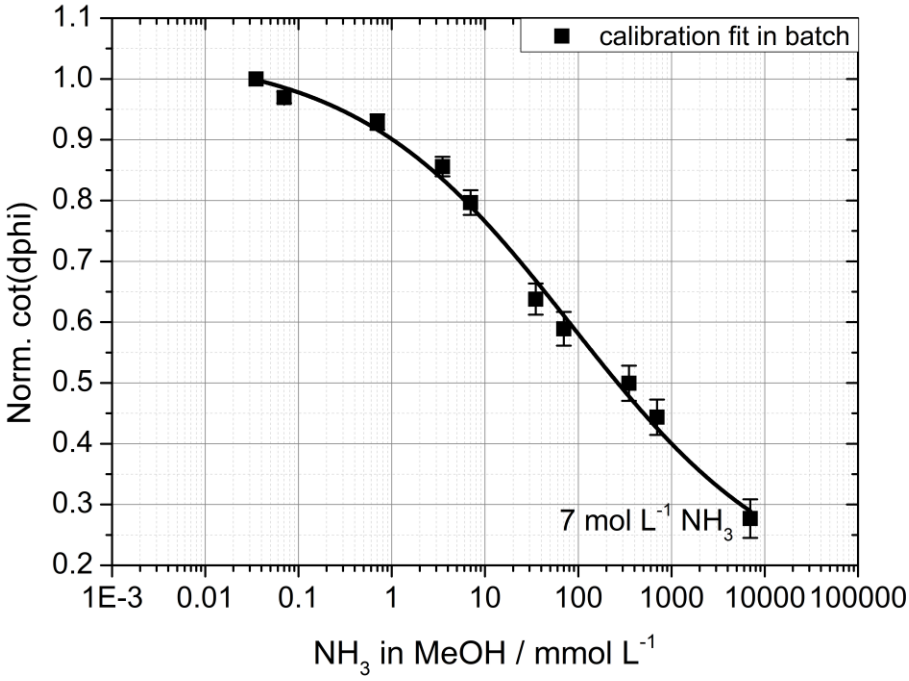

Figure S 2 Calibration curve of the novel optical ammonia sensor at 25°C measured in batch. Fitting parameters: A1 = 1.0448, A2 = 0.1442, x_0 = 1.9286, dx = 1.1610, R^2 = 0.9911.

Inline experiments

Before using the syringe pump (Lambda VIT-FIT, Lambda Instruments GmbH, Baar, Switzerland) with a single use plastic 20 mL syringe (BBraun, Melsungen, Germany) a calibration of this system had to be performed. Water was used as solvent. Since the used pump relies on a force-feeding concept which is based on the volume of the solvent, there is no difference whether this pump is calibrated with water or methanol. Due to this, flow rate of methanol and the flow rate of water can be set equal. In the following equations the terminology flow rate MeOH is used. With the corresponding linear fit parameters, a correlation between syringe settings and real flow rate was achieved (Figure S3). Using these data, the real ammonia concentration in the system after mixing was calculated.

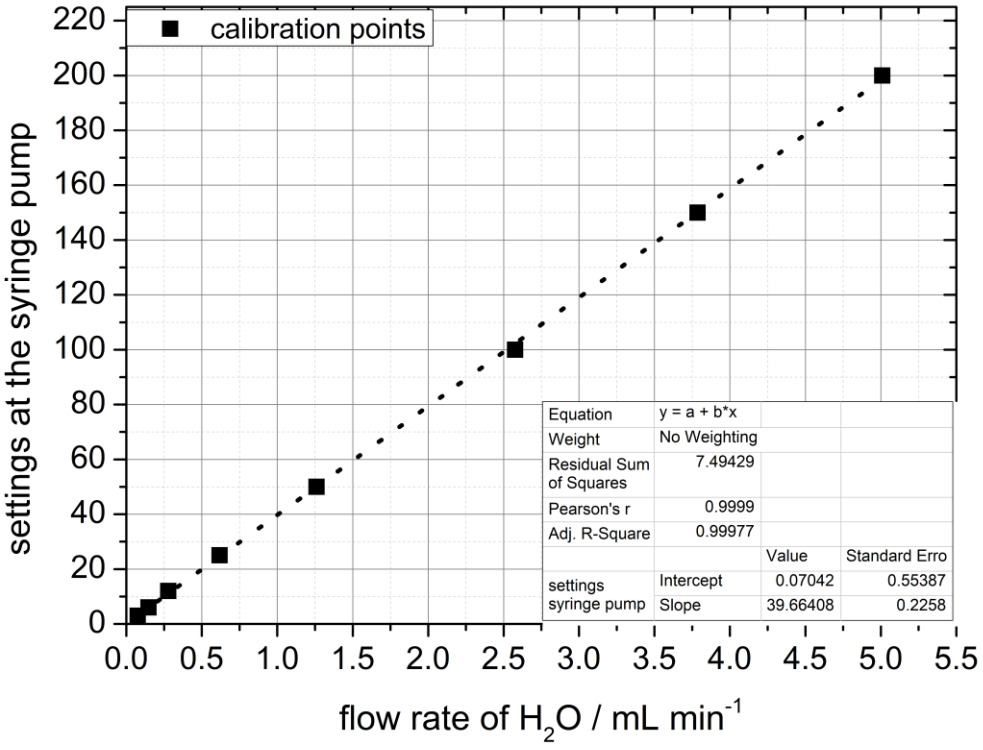

Figure S 3 Calibration of the syringe pump with water up to a flow rate of 5 mL min^{-1}. Pump settings can be chosen in hole numbers between 0 and 999 which represents the feeding value per time of the pump.

The mathematical calculation steps for a flow rate of 1 mL min^{-1} MeOH at the main stream can be seen in the following and yield into Figure S3:

The calculation pathway is shown with the example flow rate of 0.5 mL min^{-1} NH_3 in MeOH (theo. fr NH_3).

$$y = a + b * x \qquad \text{(Eq. S2)}$$

Within this Equation (Eq. S2), y is the setting at the syringe pump, b is the slope, x is the flow rate in mL min^{-1} and a is the axis section (see Figure S3). In this case b is approx. 39.664 which means that at 0.5 mL min^{-1} flow rate of the NH_3 starting solution, the settings at the syringe pump have to be set to 19.83. This is not a possible value to choose at this system because only hole numbers (0 – 999) can be used as setting for the feeding speed of the pump. Therefore, the closest possible setting had to be chosen which is 20. With Equation S3, the theoretical setting at the pump (pump set theo.) can be calculated. Additionally, the real flow rate at a pump setting of 20 (flow rate of H_2O/MeOH, fr real in Equation S4) has to be calculated via Equation S4. The last parameter which is needed for the calculation of the real ammonia concentration (real c NH_3) is the corresponding dilution factor (df real, Equation S5). It is represented by the quotient of flow rate MeOH and fr real. With these parameters the real ammonia concentration at each chosen calibration point can be calculated via Equation S6. The concentration of the ammonia containing methanol starting solution was 7 mol L^{-1} which is a commercially available and commonly used solution ($c_{start\ solution\ NH3}$).

$$pump\ set\ theo. = \ theo.fr\ NH_3 * b = 0.50\ mL\ min^{-1} * 39.664 = 19.83 \quad \text{(Eq. S3)}$$

$$fr\ real = \frac{setting\ pump}{pump\ set\ theo.} * theo.fr\ NH_3 = \frac{20}{19.832} * 0.50\ mL\ min^{-1} \approx \approx 0.504\ mL\ min^{-1} \quad \text{(Eq. S4)}$$

$$df\ real = \frac{flow\ rate\ MeOH}{fr\ real} = \frac{1.00\ mL\ min^{-1}}{0.504\ mL\ min^{-1}} = 1.983 \quad \text{(Eq. S5)}$$

$$real\ c_{NH_3} = \frac{c_{start\ solution\ NH_3}}{1 + df\ real} = \frac{7\ mol\ L^{-1}}{1 + 1.983} \approx 2.35\ mol\ L^{-1} NH_3 \quad \text{(Eq. S6)}$$

Table S1 shows all relevant mathematical quantities for the eight calibration points. Thereby, the real ammonia concentration (real c(NH_3) is rounded due to statistical significance.

Table S 1 relevant parameters for the calculation of the NH_3 concentration of the calibration points

plateau number	aimed fr NH_3 mL min^{-1}	pump set theo.	setting pump	fr real mL min^{-1}	df real	real c(NH_3) mol L^{-1}
0	0	0	0	0	0	**0**
1	0.05	1.983	2	0.0504	19.832	**0.3**
2	0.1	3.966	4	0.101	9.916	**0.6**
3	0.2	7.933	8	0.202	4.958	**1.2**
4	0.4	15.87	16	0.403	2.479	**2.0**
5	0.8	31.73	32	0.807	1.240	**3.1**
6	1	39.66	40	1.008	0.992	**3.5**
7	2	79.39	80	2.017	0.496	**4.7**
8	4	158.7	160	4.034	0.248	**5.6**

At high ammonia concentrations, where the sensor operates at its upper dynamic limit, a one-phase exponential decay function can be used as fitting function (Eq. S7):

$$y = y_0 + A1 * exp^{\frac{-x}{t}} \quad \text{(Eq. S7)}$$

Thereby, y is the value of the normalized cot(dphi), y_0 represents the offset, A1 is the amplitude, x defines the analyte concentration of the respective calibration point and t is the time constant.

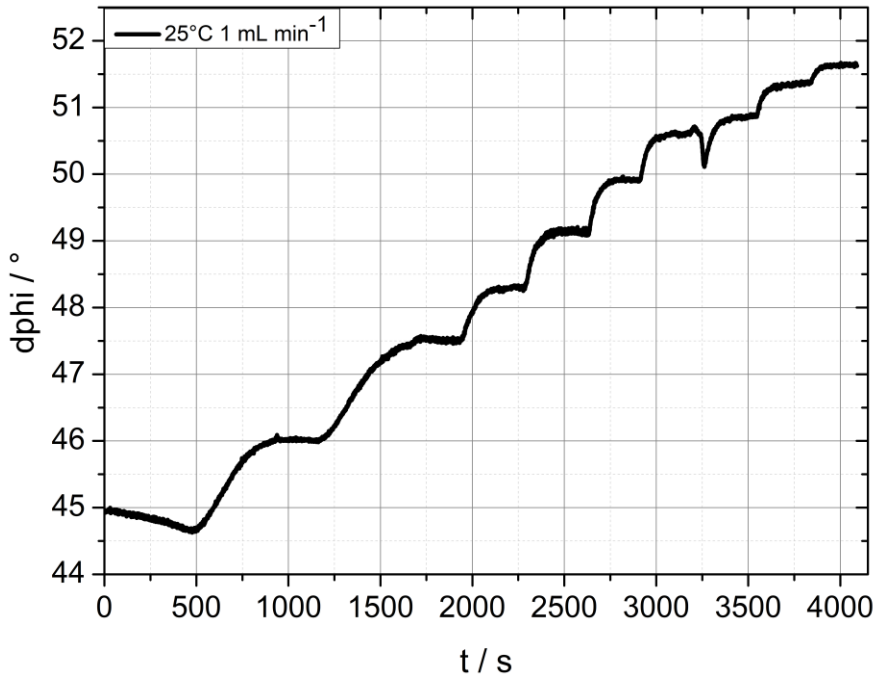

Figure S 4 Sensor response curve within a calibration at 25 °C with a flow rate of MeOH of 1.0 mL min^{-1}.

Figure S4 shows the sensor response data at 25 °C. At 3250 s a wrong syringe pump setting was first chosen. Therefore, the signal is decreasing very fast. After adjusting the settings to the right value, the sensor signal is rising again and afterwards stabilizing at the expected value.

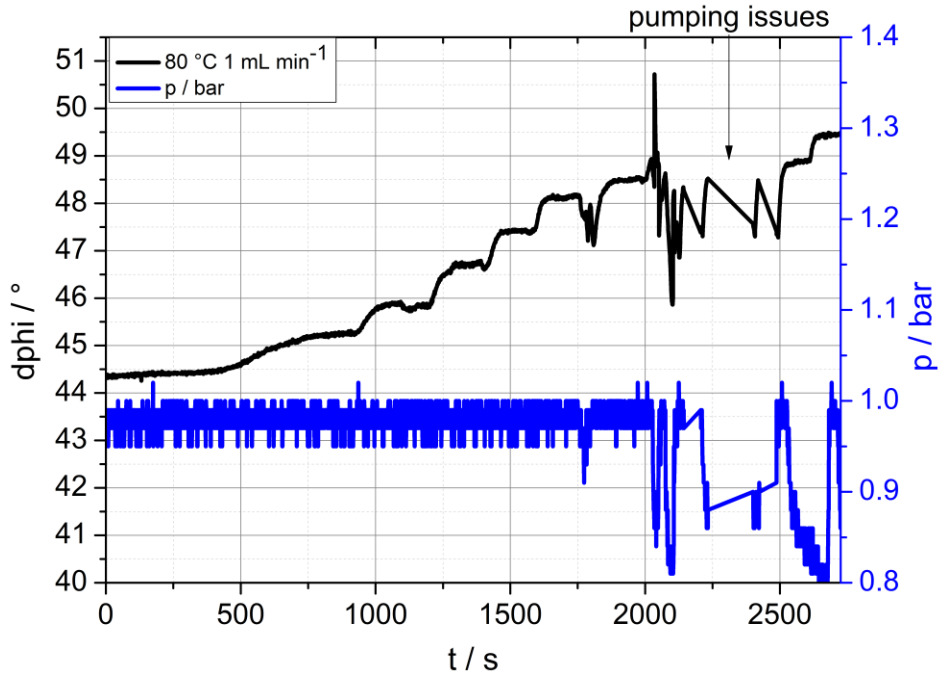

Figure S 5 Sensor response curve within a calibration at 80 °C with a flow rate of MeOH of 1.0 mL min^{-1}.

Figure S5 shows the sensor response to different ammonia concentrations at 80 °C. At higher concentrations, the syringe pump did not work properly due to the fact that the pump reached its maximal force of operation which can be applied without bending the polypropylene material of this syringe (160 N). Therefore, it shut off several times (between 2000 and 2500 s). After fixing this problem by using a new syringe, the highest two ammonia concentrations were measured. To prevent this issue for experiments in future, a stainless steel syringe can be used with a maximal force of 600 N for the used pump.

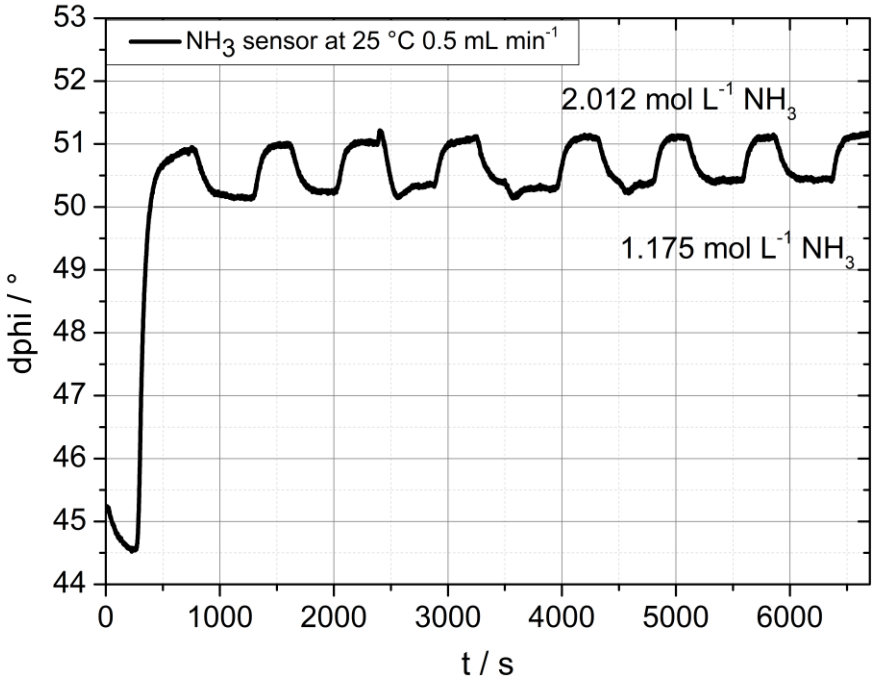

Figure S 6 Reversibility measurements at 2.012 and 1.175 mol L^{-1} NH_3 at 25 °C and with a flow rate 0.5 mL min^{-1} MeOH.

Figure S6 shows a reversibility experiment at 25 °C. Therefore, the sensor was exposed alternating to two different concentrations of ammonia. The aim of this experiment is to show the stability and reversibility of the sensor. Here, it can be seen, that the sensor shows a small drift during this experiment at lower ammonia concentration but hardly any difference at the higher concentration.

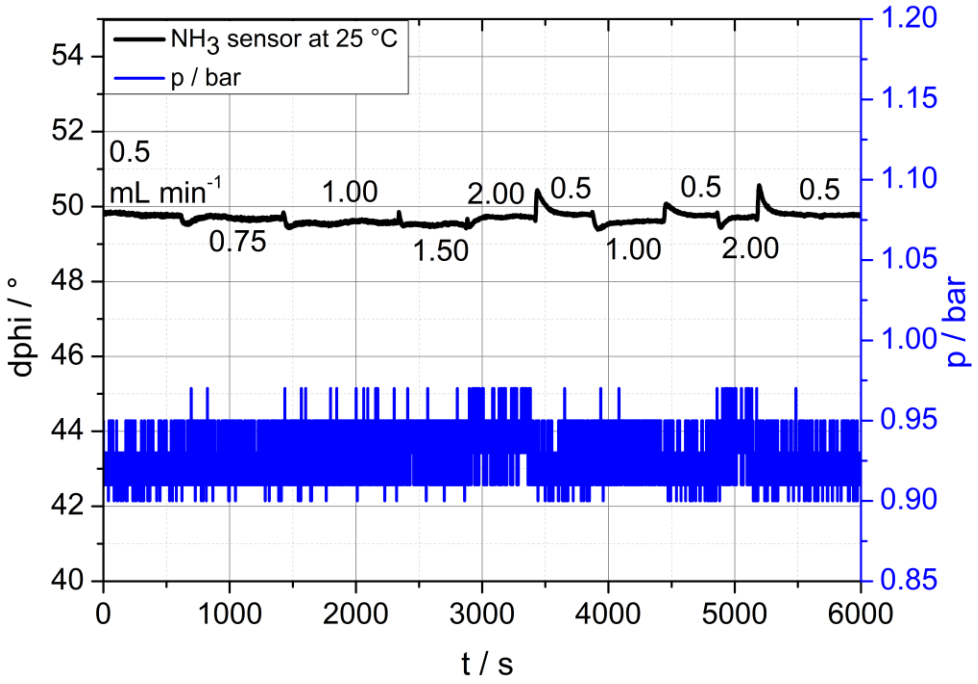

Figure S 7 Influence of varying MeOH flow rates between 0.5 – 2.0 mL min^{-1} on the sensor signal at 1.175 mol L^{-1} NH_3 at 25 °C and a set internal pressure.

Figure S7 shows the influence of varying flow rates (0.5 – 2.0 mL min^{-1}) on the sensor signal (black line). Thereby, only a small impact on the sensing signal is detected when changing the flow rate by changing the speed of the two pumps manually.

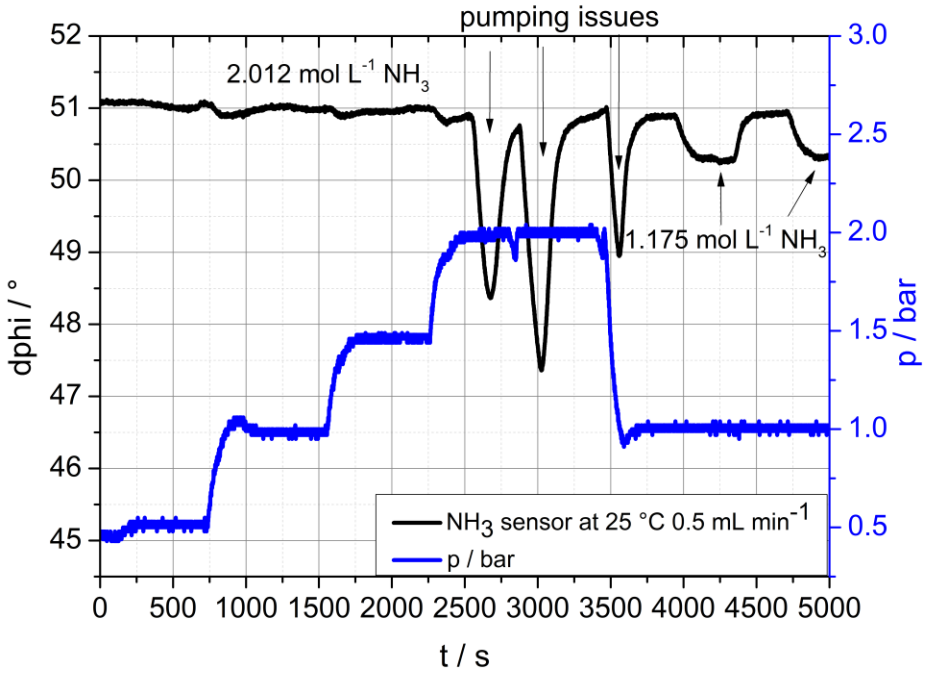

Figure S 8 Influence of internal pressure between 0.5 and 2.0 bar on the sensor signal at 2.0 mol L^{-1} NH_3 at 25 °C and 0.5 mL min^{-1} MeOH flow rate.

Figure S8 shows the influence of internal pressure towards the sensing signal. At 2.0 bar internal pressure the syringe pump shut down three times (see marked areas in the figure). After fixing this problem, the original ammonia concentration of 2.0 mol L^{-1} was achieved. At the end of this experiment the NH_3 concentration was lowered twice to 1.2 mol L^{-1}. This shows that the sensor is still working properly. In between, the sensor shows the same stable signal at 2.0 mol L^{-1} showing the reproducibility of the sensor signal.

3. Development of the flow cell

Figure S9 shows the development of the 3D printed flow cell. In picture **a)** the first schematic design is shown. This design was tested by printing the cell with a Fused Deposition Modelling printer (FDM, Anycubic Mega S from Anycubic, Shenzhen, China) using a black polylactic acid filament (PLA, Anycubic, Shenzhen, China). This version is shown in picture **b)**. After considering how the coolant tubes may be connected later, the final design was changed. It is shown schematically in picture **c)**. Thereby, the sides for coolant attachment were relocated. Now, the flow cell can stand more stable on a flat surface, however it required now additional support structures for 3D printing. This design was then 3D printed in its stainless-steel version (picture **d**). This version together with the connecting screw was used for all inline measurements described in this manuscript.

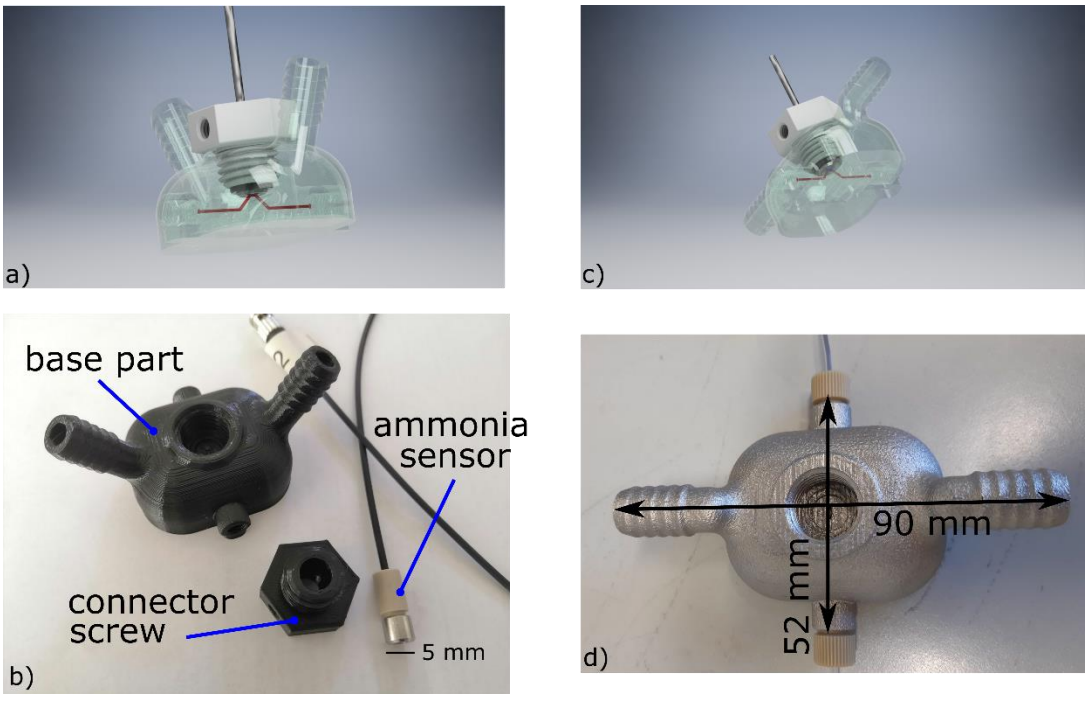

Figure S 9 Four pictures showing the development of the 3D printed flow cell. a) first design, b) first design 3D printed with the PLA filament, c) final design, d) final 3D printed cell made of stainless steel.

4. Set-up

The flow chart depicted in Figure S10 shows the experimental set-up being used for all inline experiments. All connections were made with standard 1/16” x 0.031” PTFE capillaries and their corresponding fittings. A commercial solution of ammonia in MeOH (Acros Organics, ca. 7N solution in methanol) was continuously mixed with MeOH (VWR Chemicals, reag. Ph. Eur. for HPLC) in a T-junction (Valco® Unions, bore size 0.75 mm) before entering the 3D printed flow cell equipped with the ammonia sensor. MeOH was pumped continuously with an HPLC pump (Knauer Azura P4.1S) equipped with a spring-loaded back pressure regulator (BPR 1, IDEX, 500 psi) to allow a smoother pumping. The 7 mol L^{-1} ammonia in MeOH solution was pumped with a syringe pump (Lambda VIT-FIT) equipped also with spring-loaded BPR (BPR 2, IDEX, 40 psi) to prevent degassing of the ammonia at room temperature. A dome-loaded backpressure regulator (BPR 3, Zaiput, BPR-10) was used to prevent bubble formation during the measurement as well as to allow variable pressure changes during the measurements. An additional pressure indicator (General Electric, DPI 104 - Digital pressure indicator) close to the ammonia sensor was used to monitor (see Figure S10 pressure control) the system pressure, to locate possible leakages, malfunction of equipment and dependency of the measured NH_3 on the system pressures. Data of this pressure indicator was translated internally to a voltage between 0 to 5 V and processed for the data acquisition via a microcontroller (Arduino Mega 2560). The measured voltage can be correlated to a pressure value between 0 – 20 bar. The microcontroller was programmed to send continuously its measured value via a serial communication to a PC where it was locally continuously saved into a txt file. The flow cell was temperature controlled by a thermostat (Fisherbrand™, Isotemp 6200 R20). Within this system, the syringe pump was used for the individual controlled addition of NH_3 to the system and had to be calibrated before its usage. Respective calibration data for this pump and calculations on eight different ammonia concentrations (see Table S1) in methanol are shown in Figure S3 and Equations S2 – 6.

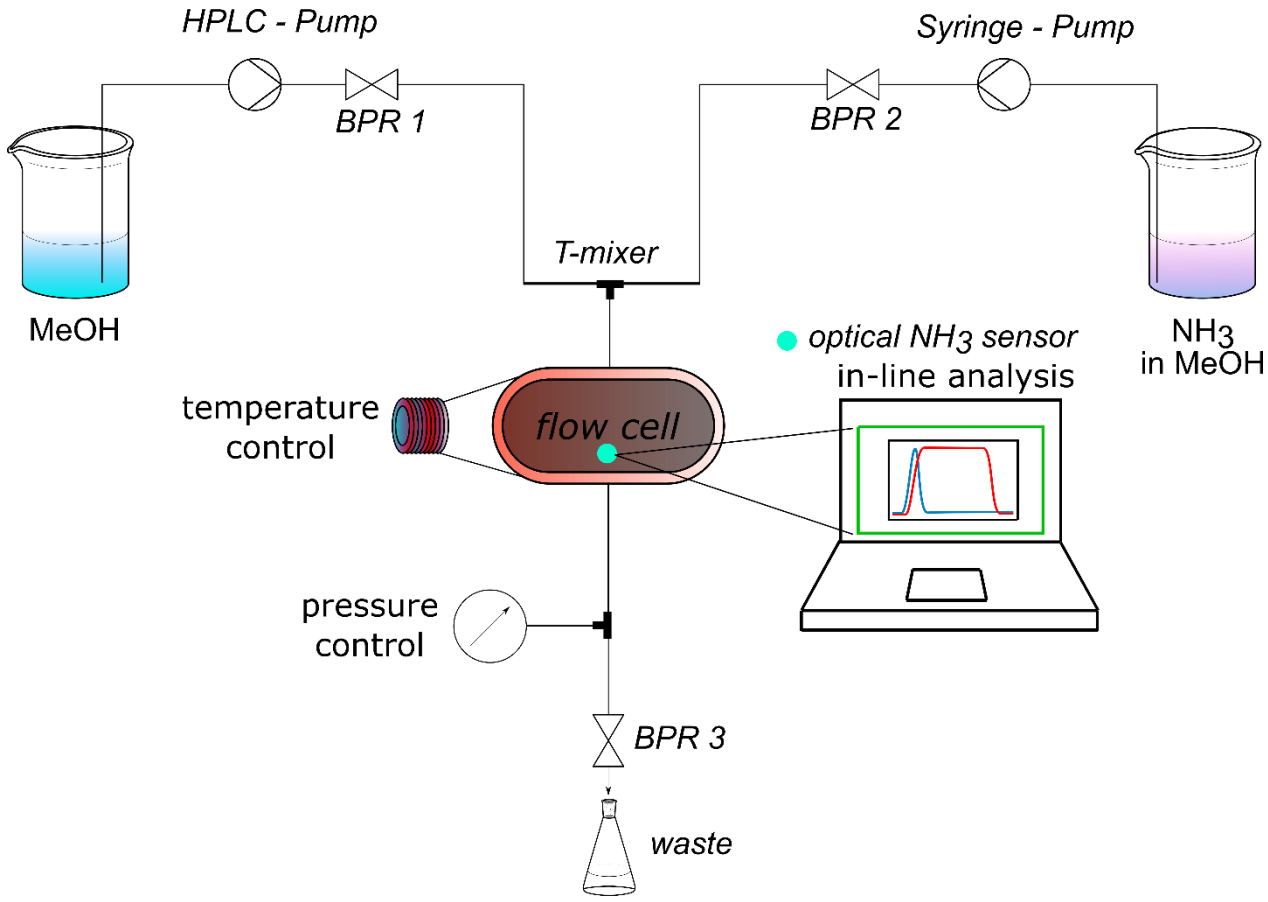

Figure S 10 Measurement set-up consists two solvent reservoirs (MeOH and NH3 in MeOH), and HPLC and a syringe pump, different backpressure regulators (BPR 1-3), two T-mixers, a temperature and pressure control system, the flow cell with an attached ammonia sensor, a read-out device (laptop) and the waste compartment.

5. References

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