Versatile One-pot Synthesis of Gold Nanoclusters and Nanoparticles Using 3,6-(dipyridin-2-yl)-(1,2,4,5)-tetrazine.

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Supplementary information

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1. General experimental

Commercially available solvents and reagents were used without further purification. For the spectroscopic and HPLC studies, the solvents were purchased from Carlo-Erba Reagents (Dasit group) with a spectroscopic or a HPLC grade.

Steady states spectroscopies: A quartz cell of 10 mm path length has been used for solution measurement. **Absorption spectra** were recorded on a Cary-5000 spectrophotometer from Agilent Technologies. **Fluorescence spectra** were recorded on a FluoroLog 3 (FL3-221) spectrofluorimeter from HORIBA JOBIN-YVON, equipped with an excitation Xe bulb (450 W, spectral domain 200-800 nm). Emission and excitation spectra were corrected, and the absorbance at the excitation wavelengths were kept below 0.1.

^{1}H (399.78 MHz) and ^{13}C-NMR (100.53 MHz) spectra were recorded at room temperature (292-295K) on a JEOL ECS-400 spectrometer (399.78 MHz). Chemical shifts (δ) are given in parts per million using tetramethylsilane (TMS) as the internal standard for the ^{1}H, and the residual peak of solvent for ^{13}C.[1,2] The UDEFT sequence,[3] parametrized with a 5s relaxation delay, was used in addition to the usual ^{13}C decoupling sequence to enhance the carbon signals due to the very low concentration of the Au NCs sample obtained after HPLC. Attribution of the peaks was realized with complementary 2D experiments (CoSy, HMQC). The data were recorded with Delta NMR Processing and Control Software v 4.3.6.

Analytical HPLC separations were performed with Shimadzu Prominence UFLC equipped with 2 pumps LC-20AD, an oven CTO-20AC, a thermostatic module SPD-M20A for the UV-Vis absorption detection (photodiode array 190-800 nm), and a communication bus CBM-20A. The samples were filtered before injection through a cellulose acetate membrane and injected *via* a Rheodyne 7725i injection valve and a 20 µL injection loop. The solvents used were degassed under sonication every day. The data were recorded with LabSolutions, version 5.71, from Shimadzu.

Transmission Electron Microscopy (TEM) imaging is performed with a JEOL 1011 (100 kV) microscope by putting a drop of solution on a carbon-coated copper grid.

Scanning electron microscopy (SEM) images associated with **energy dispersive spectroscopy (EDS)** were performed with a JEOL-5510LV. Samples were prepared by drop casting the solution on silicon wafers, after centrifugation and extraction of the precipitate with chloroform to get rid of the excess of thiols.

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[1] H. E. Gottlieb, V. Kotlyar, A. Nudelman ; *J. Org. Chem.* **1997**, *62*, 7512-7515.

[2] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg ; *Organometallics* **2010**, *29*, 2176-2179.

[3] M. Piotto, M. Bourdonneau, K. Elbayed, J.-M. Wieruszeski, G. Lippens ; *Magn. Reson. Chem.* **2006**, *44*, 943-947.

Mass Spectrometry (MS) analyses were performed using an UltrafleXtreme mass spectrometer (Bruker Daltonics, Bremen). Acquisitions were performed in reflector or linear positive ion mode. The laser intensity was set just above the ion generation threshold to obtain peaks with the highest possible signal-to-noise ratio without significant peak broadening. The mass spectrometer is externally calibrated using PEG1500 and PEG4500. All data are processed using the program FlexAnalysis (Bruker Daltonics, Bremen). Trans-2-[3-(4-ter-Butylphenyl)-2-propenylidene] malonitrile (DCTB, used as the matrix for MALDI-TOF MS) of the highest grade available is purchased from Sigma Aldrich and used without further purification. Sample for MALDI analysis was a solution of nanoclusters in ethanol. The matrix solution was prepared at a concentration of 80 mM in ethanol (20 mg/mL). The sample was prepared by mixing the sample solution with matrix solution at a volume ratio of 1:1.

X-ray photoelectron spectroscopy (**XPS**) was conducted with a K-Alpha+ system (ThermoFisher Scientific, East-Grinsted, UK) fitted with a micro-focused and monochromatic Al Kα X-ray source (spot size of 400 µm, 1486.6 eV). Spectrometer pass energy was 150 eV for the survey and 40 eV for the narrow high resolution regions. The samples were prepared by depositing several drops of the solution on a silicon wafer. TEM images were recorded prior to XPS analysis to ensure that individual nanoclusters are actually present.

Finally, some data for HPLC and NMR were manipulated using Igor Pro 7, version 7.0.5.2, developed by Wavemetrics, Inc. and Origin for spectrophotometry and spectrofluorometry.

2. Synthesis of Au NCs

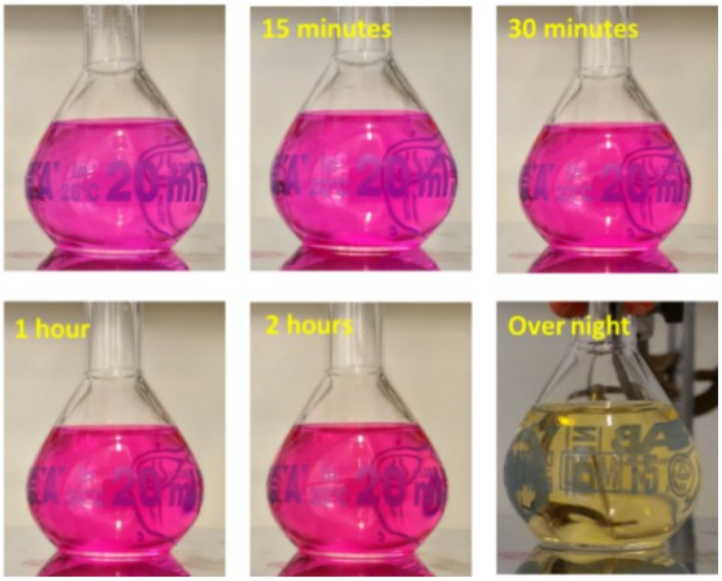


Figure S1 : Color evolution for the binary mixture DDT+bptz (in absence of Au).

3. Electron microscopy imaging

3.1. TEM

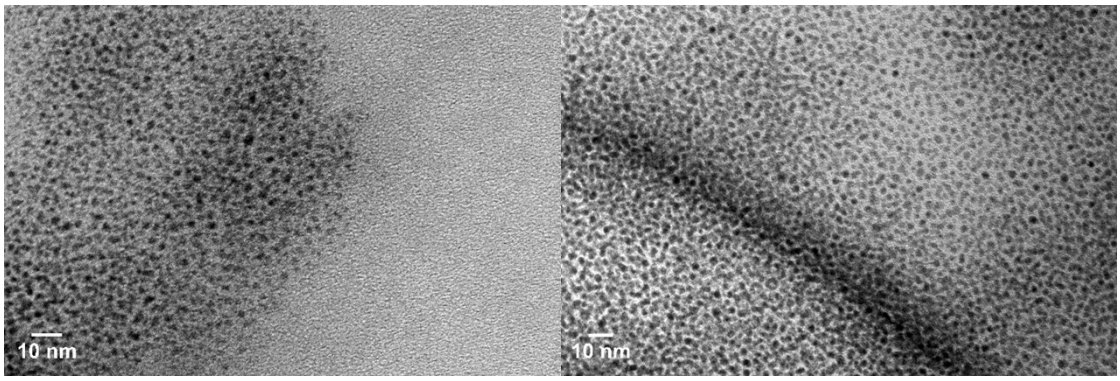


Figure S2 : TEM snapshots of Au NCs (same conditions as in fig. 2, other areas).

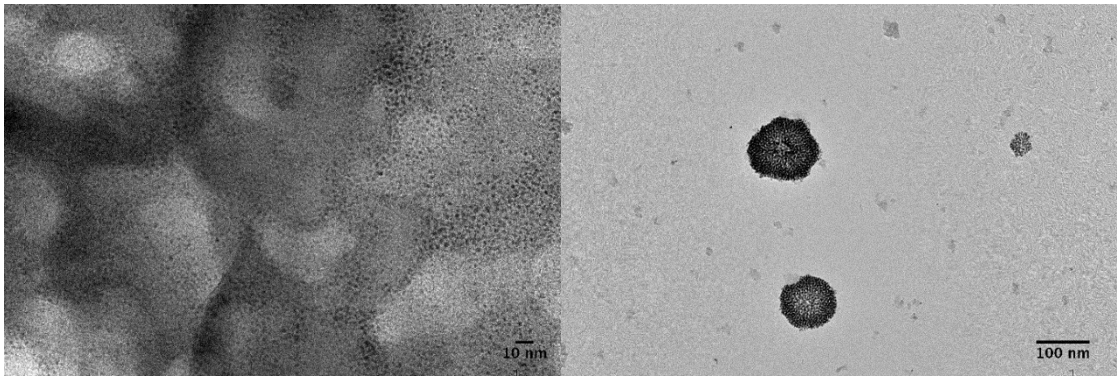


Figure S3 : TEM pictures of sample 3 (see table 2) synthesized by mixing DDT, bptz and gold salt in proportions 2:1:1 and showing the simultaneous presence of NCs and NPs.

3.2. SEM-EDS

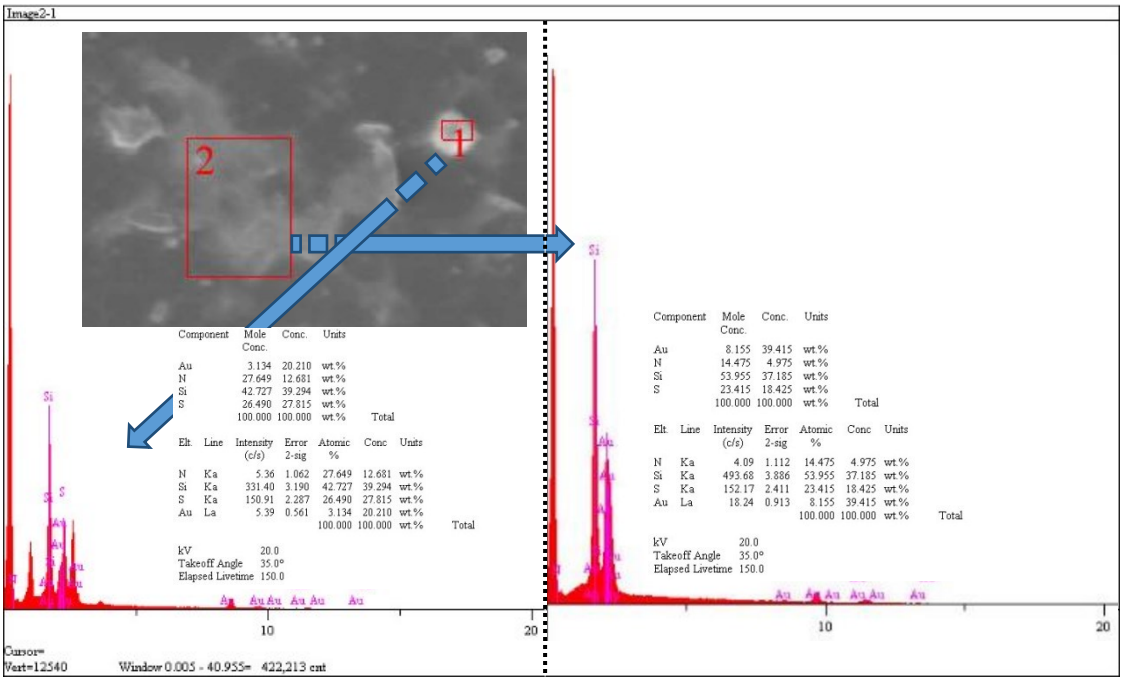


Figure S4: SEM pictures and EDS spectra of two areas (1 left; 2 right) after coating a silicon wafer with Au clusters.

4. UV-vis Absorption

4.1. UV-Vis absorption spectra for the NCs preparation

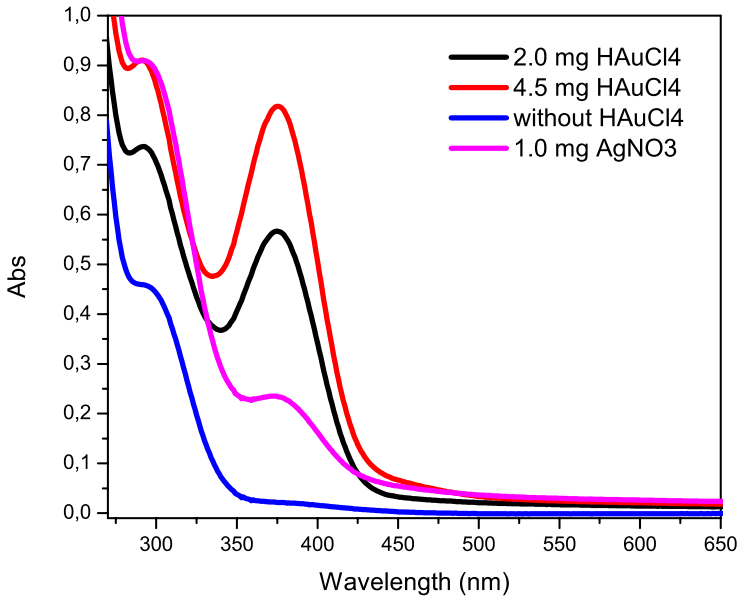


Figure S5: UV-vis absorption spectra of the crude solution 6 hours after starting the reaction between bptz, DDT and $HAuCl_4$ for various amounts of gold salt in the initial mixture. The pink curve is related to a mixture where the gold salt was replaced by a silver salt.

4.2. UV-Vis absorption kinetic monitoring of AuNCs synthesis

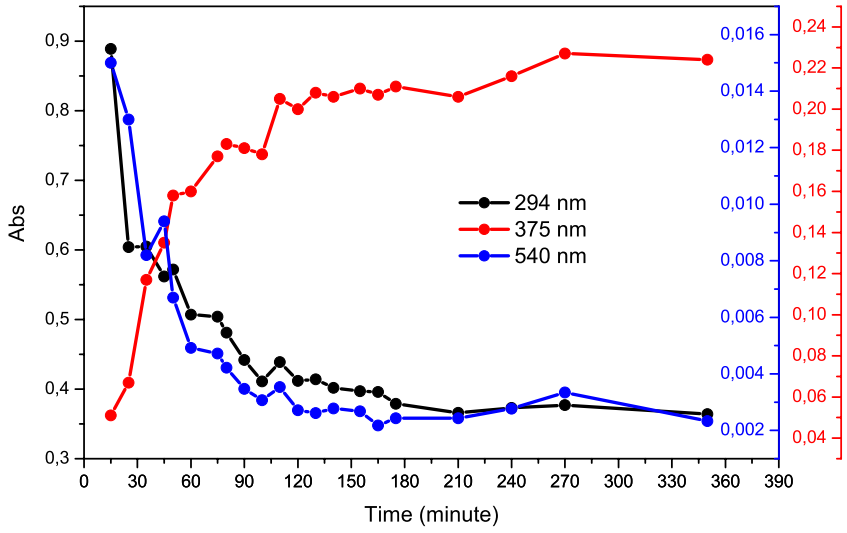


Figure S6: Absorption kinetics monitoring at various wavelengths vs. synthesis time of Au NCs.

5. Fluorescence monitoring

5.1. Emission spectra

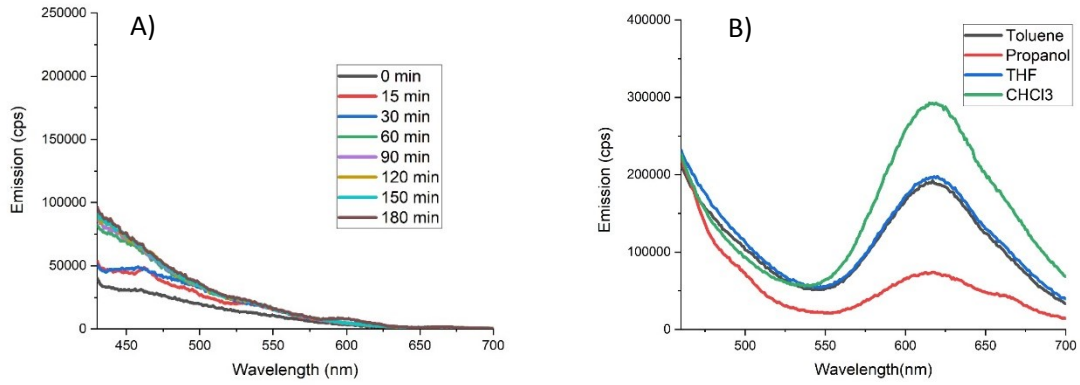


Figure S7 : A) Emission spectra (λ_{exc} = 368 nm) at various reaction times for the mixture DDT + bptz (in absence of gold). B) Emission spectra (λ^{exc} = 368 nm) for various extraction solvents of Au NCs after centrifugation.

5.2. Emission kinetics

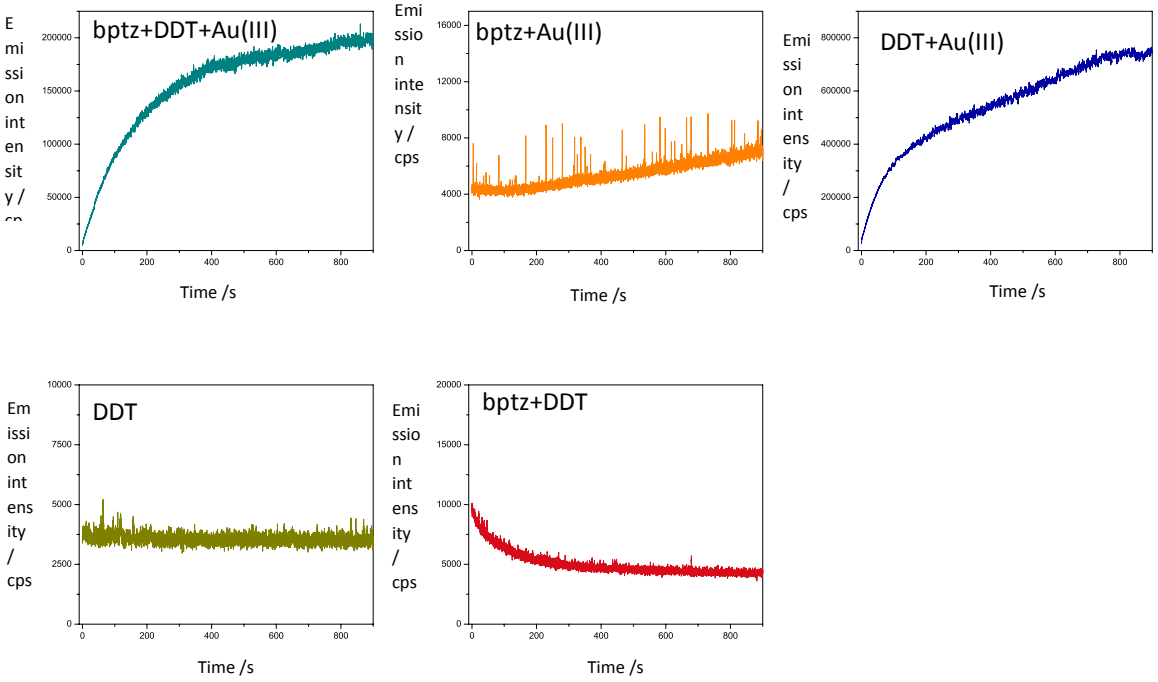


Figure S8 : Emission intensity (λ^{em} = 630 nm, λ^{exc} = 368 nm) as function of reaction time for various mixtures of tetrazine (bptz), 1-dodecanethiol (DDT) and $HAuCl_4$ (Au(III)) in ethanol.

6. XPS

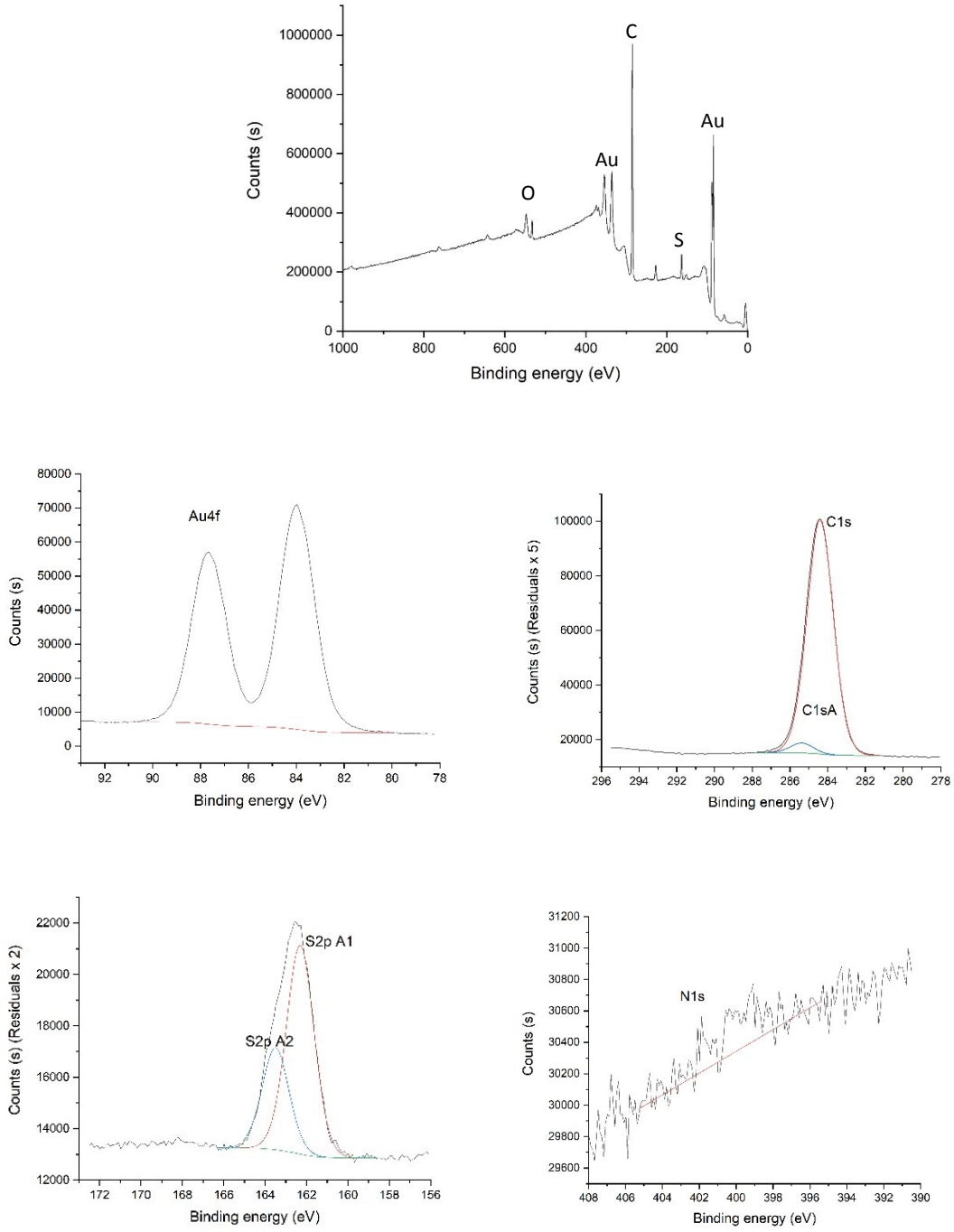


Figure S9: XPS wide spectrum (top) and spectra of Au4f, C1s, S2p, N1s elements (bottom).

7. HPLC separations

7.1. Analytical HPLC separations

Chromatogram in MeOH:H_2O (80:20)

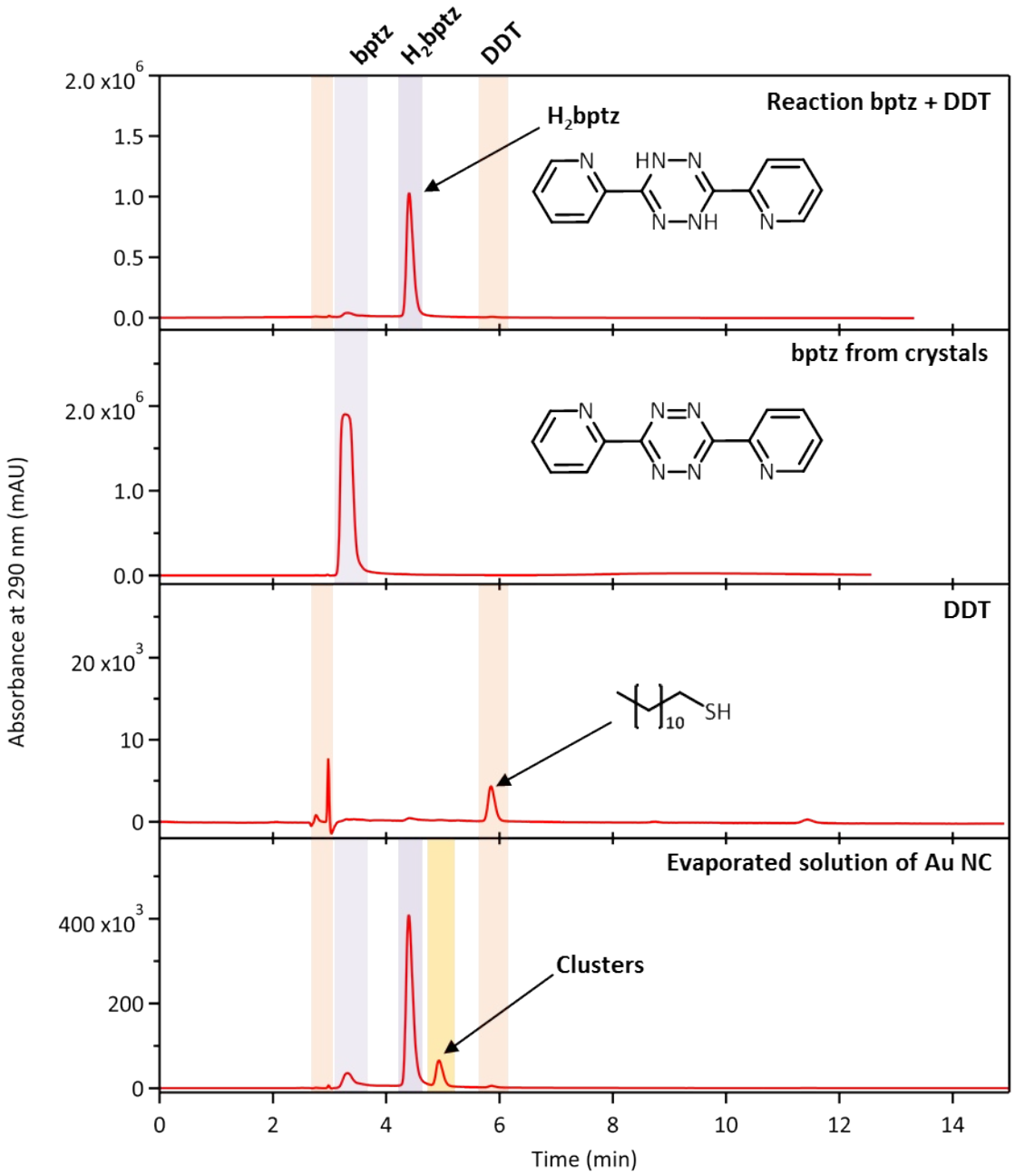


Figure S10: Analytical HPLC chromatograms obtained for various starting reactants and the ternary mixture in methanol:water 80:20. Column: Phenomenex C12; injection loop: 5µL; Pressure: 100 bar.

Detailed conditions

- Column: Phenomenex Luna C-18 (250x4.6 mm, 5µm, 100Å)
- Column and absorption cell temperature: 40°C (thermostatically controlled)
- Sample injection: Rheodyne 7725i with 5 µL* loop or 20 µL**

- Solvents: MeOH:H_2O 80:20* or 50:50** isocratic
- Flow: 1 mL min^{-1} (indicative pressure ≈ 100-110 bars)
- Wavelength detection: 290 nm, slits 4 nm
- The indicated elution time is not corrected of the dead time

7.2. Semi-preparative HPLC separation

Chromatogram in EtOH:H_2O (40:60)

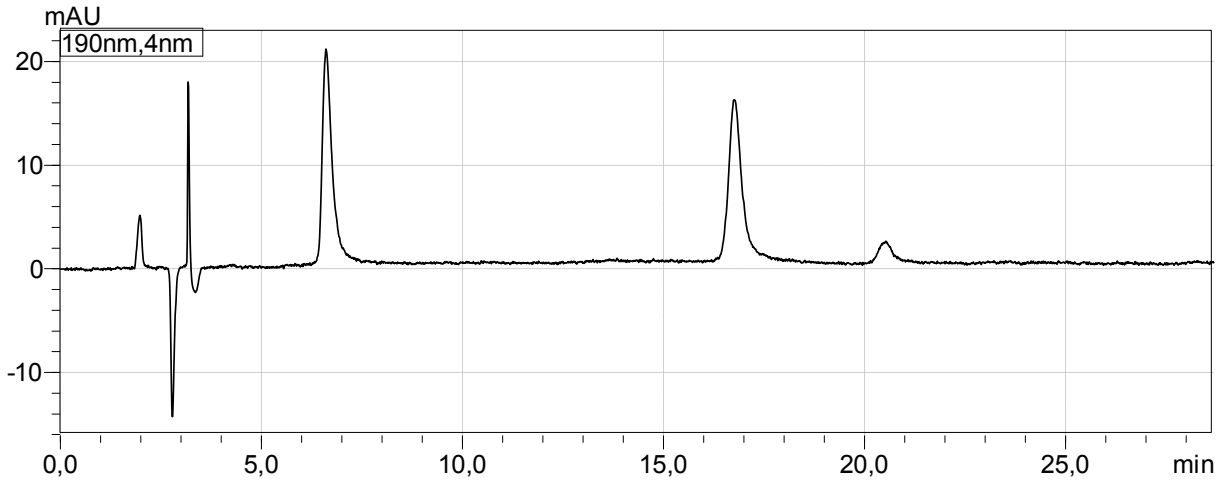


Figure S11: Semi-preparative HPLC chromatogram obtained from a filtered crude solution after AuNC synthesis.

Detailed conditions

- Column: Phenomenex Luna C-18 (250x10 mm, 5µm, 100Å)
- Column and absorption cell temperature: 20°C (room temperature)
- Sample injection: Rheodyne 7725i with 200 µL loop
- Solvents: EtOH:Water 40:60 isocratic
- Flow: 3mL/min (indicative pressure ≈ 150 bars)
- Wavelength detection: 190 nm, slits 4 nm
- Collected fractions of interest: 16-18' (product from bptz+DDT) and 18-21' (clusters)
- The indicated elution time is not corrected of the dead time

8. NMR

8.1. ^{1}H NMR in MeOD

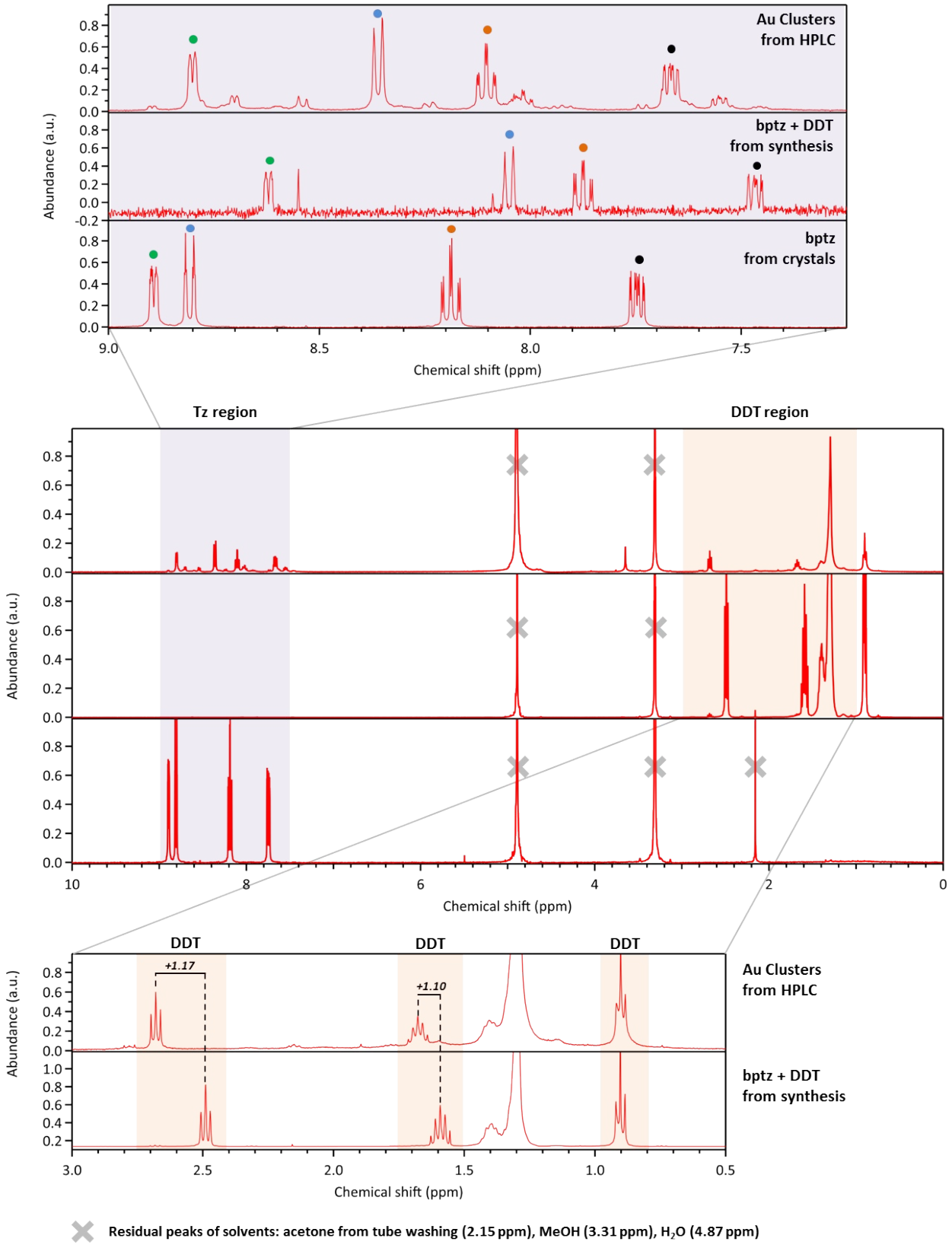


Figure S12: ^{1}H NMR spectra in MeOD of Au NCs, binary mixture bptz+DDT (H_2bptz) and bptz.

8.2. ^{13}C NMR in MeOD

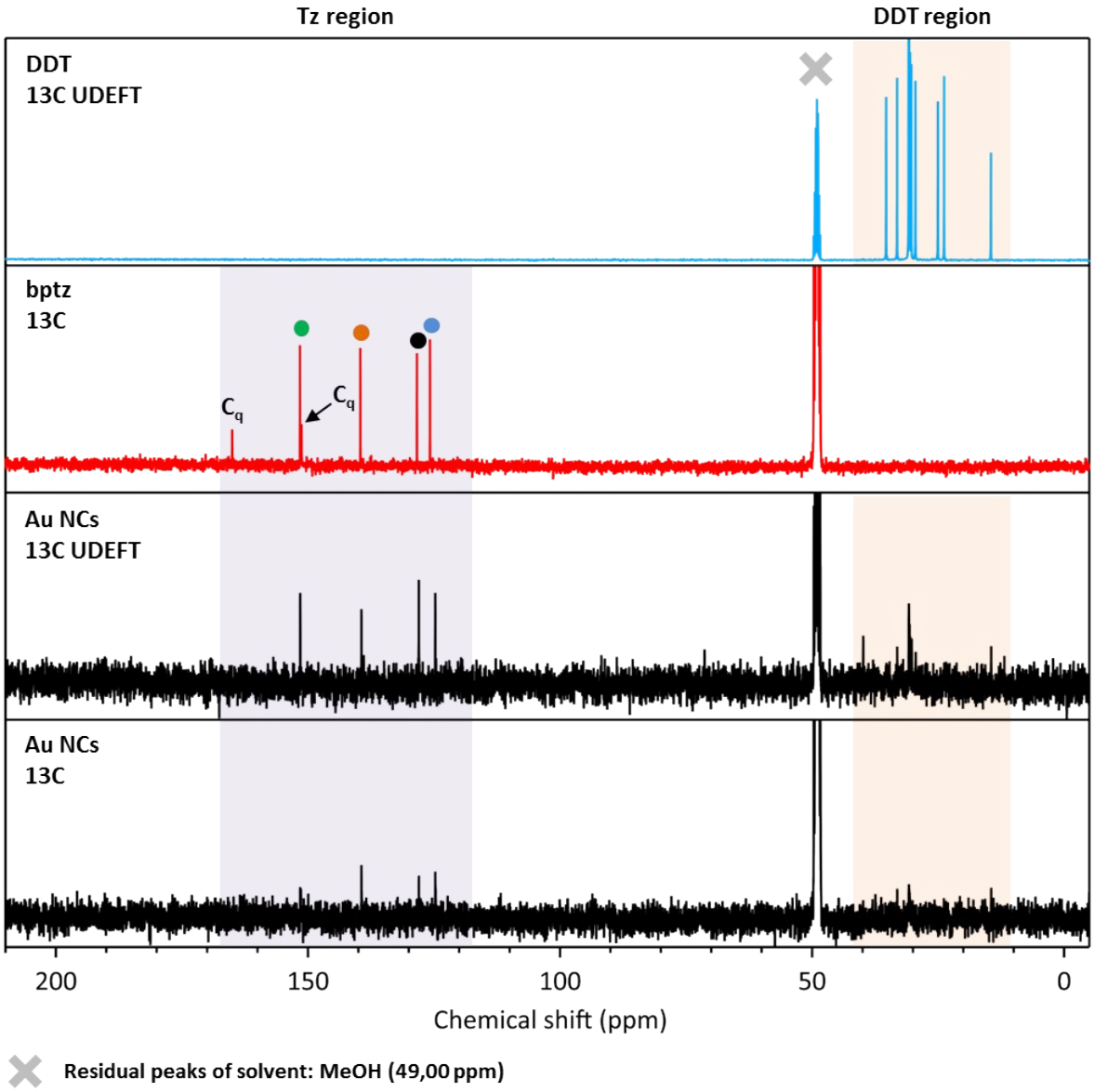
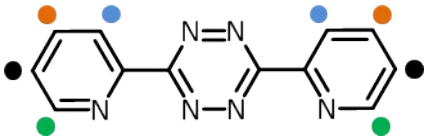


Figure S13: single pulse dec. and/or UDEFT ^{13}C and NMR spectra in MeOD of Au NCs, DDT and bptz.

8.3. Chemical shifts comparison in MeOD

Table S1 : Chemical shifts in MeOD of ^{1}H and ^{13}C (in ppm) for the binary mixture (H_2bptz) and Au NCs compared to bptz (chemical shift differences in red).



Signal	●		●		●		●	
Nucleus	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
bptz	8.89	151.5	8.81	125.8	8.19	139.6	7.75	128.3
H_2bptz (bptz+DDT)	8.62 ***-0.27***		8.05 ***-0.76***		7.87 ***-0.32***		7.47 ***-0.28***	
Au NCs	8.79 ***-0.10***	151.5 ***0.0***	8.36 ***-0.45***	124.7 ***-1.1***	8.10 ***-0.09***	139.4 ***-0.2***	7.66 ***-0.09***	128.0 ***-0.3***

^{13}C NMR of ***bptz***: 125.8 (*C*H), 128.3 (*C*H), 139.6 (*C*H), 151.2(C_q), 151.5 (*C*H), 165.0 (C_q).

UDEFT ^{13}C NMR of ***Au Clusters***: 124.7 (*C*H), 128.0 (*C*H), 139.4 (*C*H), 151.5 (*C*H). The C_q were not observed probably due to the low concentration of the sample.