Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information

Intrinsic fluorescence of nucleobase crystals

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Materials

The nucleobases powders of Adenine, Thymine, Cytosine, Uracil, Guanine, Theobromine, Theophylline, Caffeine, Xanthine and Hypoxanthine were purchased from Sigma-Aldrich. The 5-fluorouracil and 5-methylcytosine nucleobases were purchased from Alfa Aesar. Purity of all nucleobases was at least 97%.

Crystallization of nucleobases

Crystallization of each nucleobase was achieved by preparing a supersaturated, aqueous solution at the following concentrations: adenine, thymine, uracil and cytosine – 10 mg/ml, guanine – 50 mg/ml, theobromine – 5 mg/ml, theophylline – 20 mg/ml, caffeine – 50 mg/ml, xanthine – 10 mg/ml, hypoxanthine –50 mg/ml, 5-fluorouracil – 20 mg/ml and 5-methylcytosine – 25 mg/ml. For each solution, the sample was heated to 90 °C

with vigorous shaking and was allowed to cool down to room temperature. In all experiments, ddH_2O was used except for guanine and xanthine, which were dissolved in 1M HCl solution. Crystallization experiments were performed at 25 °C. Crystals were formed within 1-4 days. Powder X-ray Diffraction patterns (PXRD) of the nucleobases is presented in Fig S2 (ESI†).

Fluorescence spectroscopy measurements

Solid-state fluorescence spectra were recorded using a Horiba Jobin Yvon Fluorolog-3 spectrofluorometer at various excitation wavelengths as shown in Fig 3. Emission was recorded between 320 and 750 nm at 25 °C. Emission and excitation slits were set between 3-5 nm depending on the sample intensity. Measurements were performed on a quartz slide (AdValue Technology) 76.2 x 25.4 x 1 mm) with a dry sample of each nucleobase. For this purpose, the crystals were deposited on the slide in solution, followed by careful blotting with a filter paper and allowing the samples to further dry in a chemical hood. Fluorescence was measured using the front-face detection (sample was placed vertical to the light source and a 22.5 $^{\circ}$ correction was applied).

Fluorescence imaging

Epifluorescence microscopy measurements

Each nucleobase was deposited on a glass slide and covered with a coverslip to avoid movement of the crystals during the measurements. Images were acquired using five different excitation/emission filters as described in Fig. S2 (ESI†). All images were acquired using a Nikon Ti-E inverted motorized microscope. For each nucleobase, the exposure time was set to maximize the fluorescent signal.

Confocal imaging

All confocal images were taken using a Leica SP8 Lightning confocal microscope with a Leica Application Suite X (LAS X) software. The samples were excited using a 405 nm laser, the laser intensity was set to 50% and the gain was set to 500 for all images. All images were taken at a 5X magnification.

Fluorescence lifetime Imaging Microscopy (FLIM)

Fluorescence lifetime imaging was acquired using a LSM 7 MP 2-photon microscope (Carl Zeiss, Weimar, Germany) coupled to the Becker and Hickl (BH) simple-Tau-152

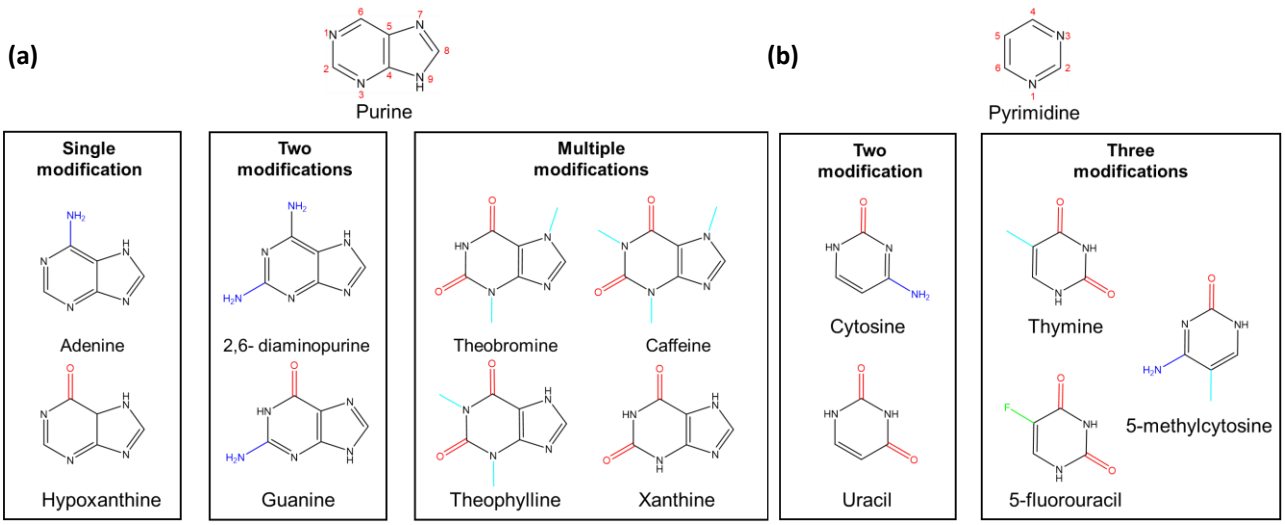
system. In order to excite the samples, a Chameleon Ti: Sapphire laser system with a repetition rate of 80 MHz was used. Images were acquired using a Zeiss 20 x 1 NA water-immersion objective. A Zeiss dichroic mirror (LP 760) was used to separate the excitation and emission light. Emission light was collected via a hybrid GaAsP detector (HPM-100-40, BH, Berlin Germany). The histogram presented in this work refers to the entire lifetime image. Pseudo colored lifetime images were generated by assigning a color to the value of average fluorescence lifetime τm in each pixel. Emission light was collected via a hybrid GaAsP detector (HPM-100-40, BH, Berlin, Germany) with a CFP-YFP bandpass filter (LP510 BP520-560).

Fluorescence kinetics measurements

All Samples were heated to 90°C in a 20ml glass vial until the solutions turned transparent (not longer than 20 minutes). The hot solutions (90 °C) of all nucleobases were placed in a black, clear, and flat-bottom 96-well microplate (Greiner). Temperature was maintained by keeping the 96-well plate on a hot incubator while transferring the solutions. The hot 96-well plate was placed in the plate reader and was let to cool down during measurement. Following excitation at 405 nm, nucleobase solutions emission data at 450 nm were measured over time and recorded using a TECAN Infinite 200 PRO plate reader. Gain was set to 1900.

Powder X-ray diffraction (PXRD)

Samples of nucleobases crystals were dried in a desiccator. The X-ray diffraction pattern was collected by using a Bruker's D8 Discover Diffractometer; the set-up was a θ: θ Bragg-Brentano geometry, the source was a copper anode and the detector was a LYNXEYE XE linear detector. The diffraction patterns were collected between 0 and 80 °2θ with step 0.02°2 θ for 1 second per step.



Scheme S1. Molecular structures of the nucleobases used in the study classified according to their chemical structure: **(a)** Purines. **(b)** Pyrimidines. For easier identification, chemical groups are denoted by different colors- amine (blue), methyl (green), carbonyl (red).

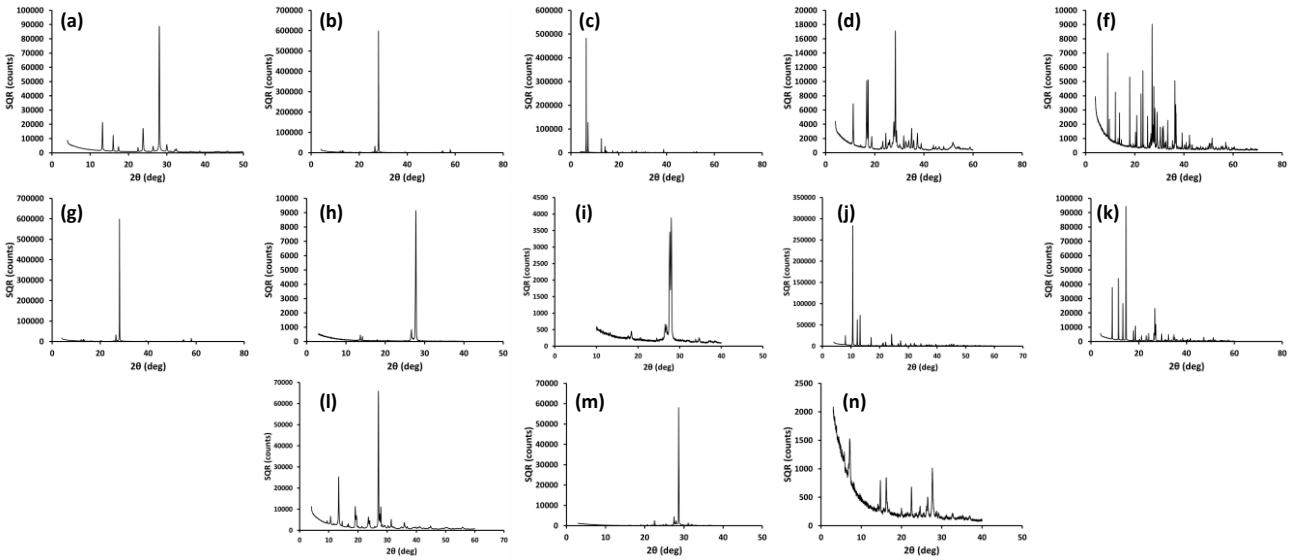


Fig. S2 PXRD analysis of the studied nucleobases. (a) Adenine. (b) Cytosine. (c) Thymine. (d) Uracil. (e) Guanine. (f) 2,6-Diaminopurine. (g) Xanthine. (h) Hypoxanthine. (i) Caffeine. (j) Theophylline. (k) Theobromine. (l) 5-Fluorouracil. (m) 5-Methylcytosine.

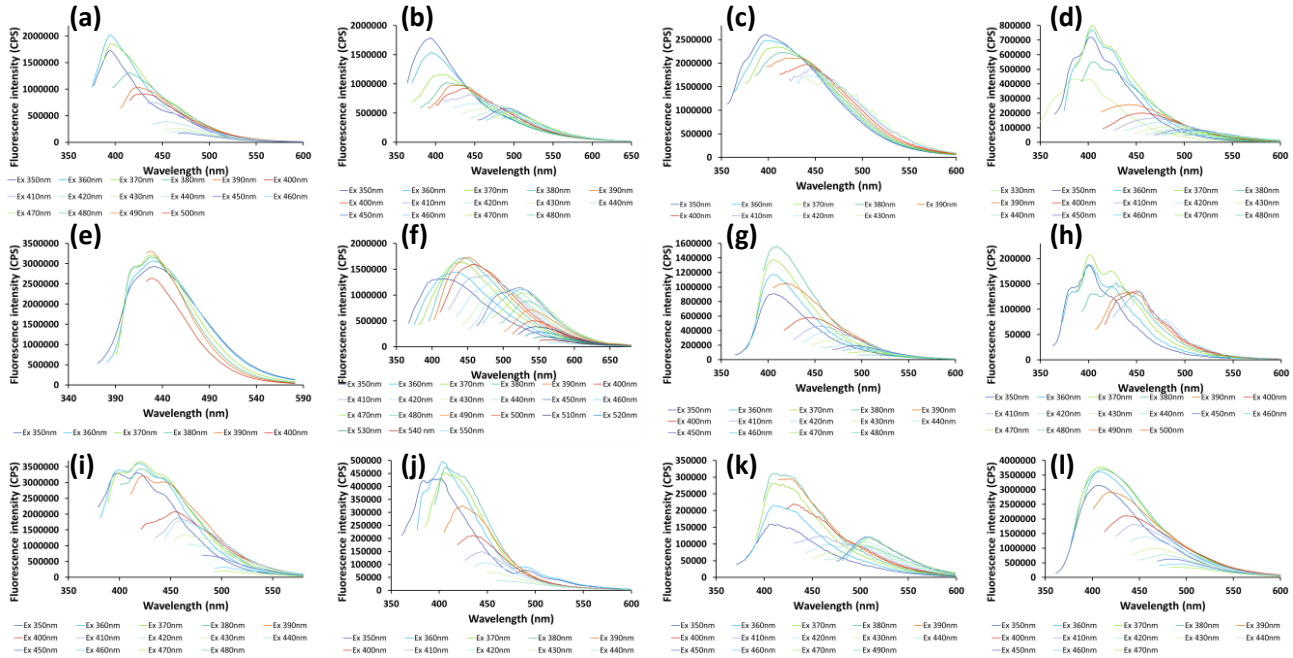


Fig. S3 Solid-state emission spectra of nucleobase crystals at different excitation wavelengths. (a) Cytosine. (b) Thymine. (c) Uracil. (d) Guanine. (e) 2,6-Diaminopurine. (f) Xanthine. (g) Hypoxanthine. (h) Caffeine. (i) Theophylline. (j) Theobromine. (k) 5-Fluorouracil. (l) 5-Methylcytosine.

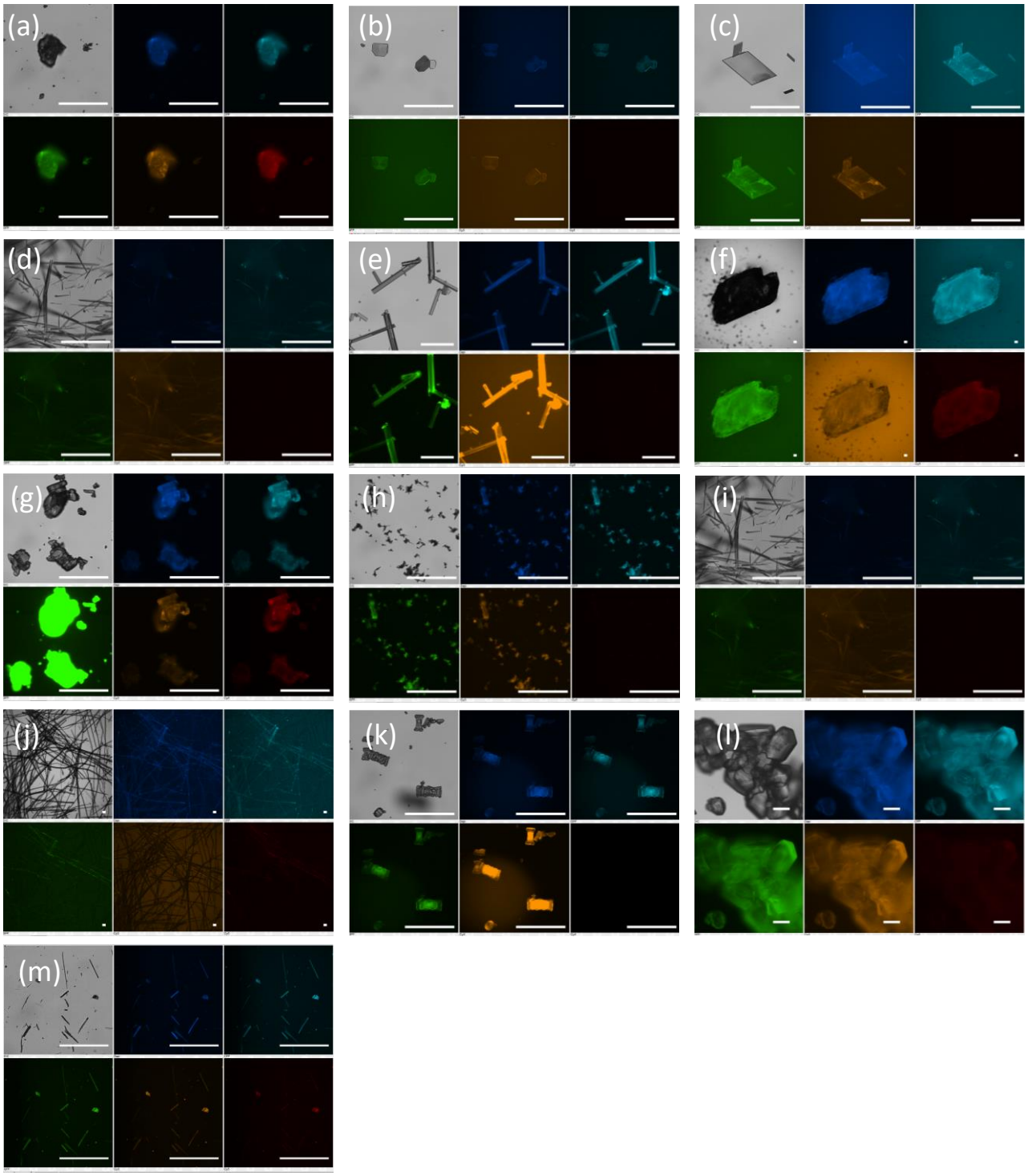


Fig. S4. Differential interference contrast (DIC) and fluorescence images of the same microscopic field of the different nucleobase crystals. Each fluorescence image was acquired using different excitation and emission filters: DAPI, CFP, GFP, Cy3 and Cy5. Pseudo-colors represent the corresponding emission colors. (a) Adenine. (b) Cytosine. (c) Thymine. (d) Uracil. (e) Guanine. (f) 2,6-Diaminopurine. (g) Xanthine. (h) Hypoxanthine. (i) Caffeine. (j) Theophylline. (k) Theobromine. (l) 5-Fluorouracil. (m) 5-Methylcytosine. Scale bars: 100 μm.

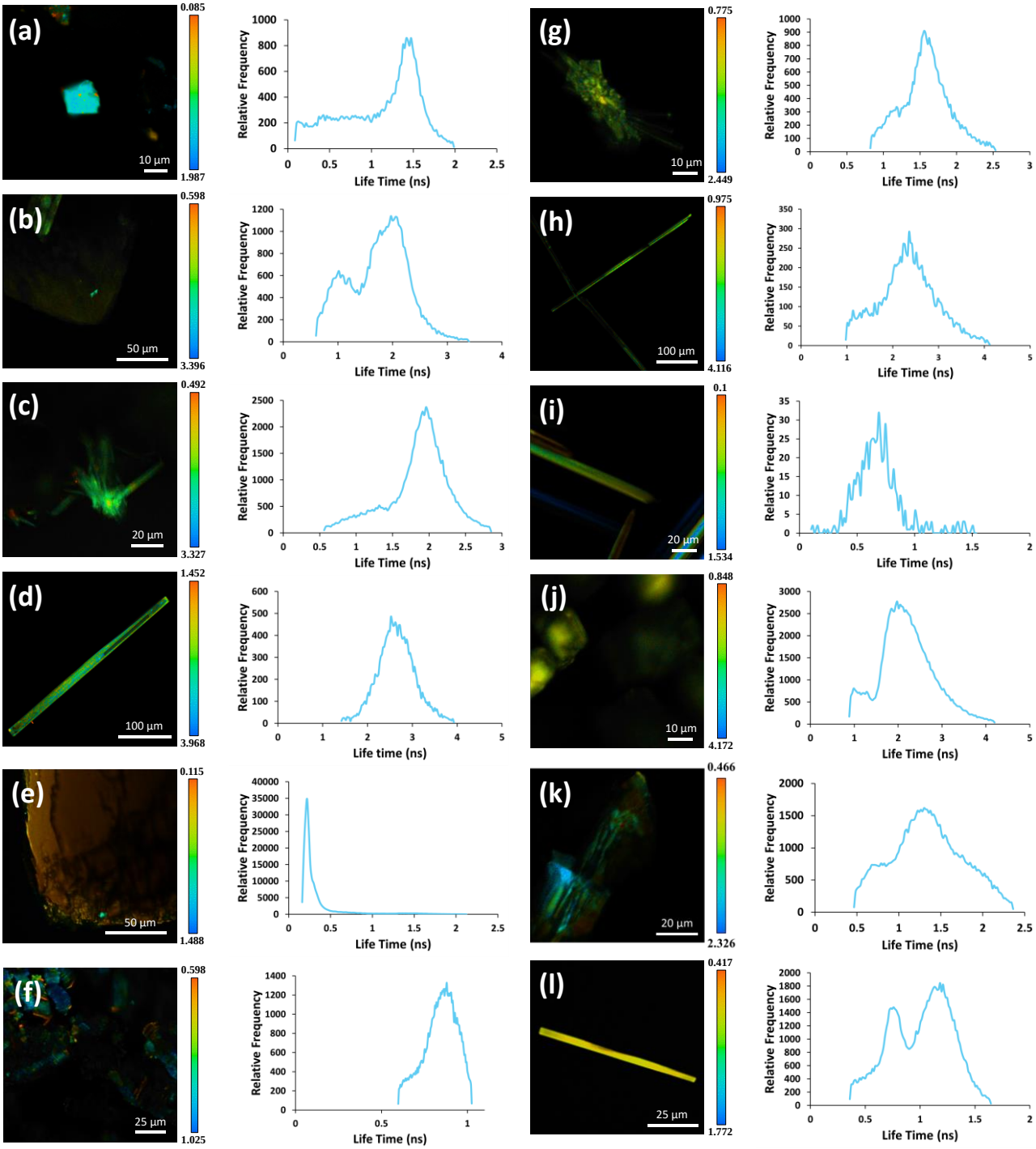


Fig. S5 Left: Fluorescence lifetime images of nucleobase crystals measured at room temperature. False colors represent the average lifetime at each pixel. Right: Frequency histogram showing the occurrence of the average lifetime calculated from a three-component fit. Two-photon excitation was at 780 nm, and detection at 450–500 nm. (a) Cytosine. (b) Thymine. (c) Uracil. (d) Guanine. (e) 2,6-Diaminopurine. (f) Xanthine. (g) Hypoxanthine. (h) Caffeine. (i) Theophylline. j) Theobromine. (k) 5-Fluorouracil. (l) 5-Methylcytosine.