Freezing the non-classic crystal growth of a coordination polymer using controlled dynamic gradients

Marta Rubio-Martínez, a Inhar Imaz, a Neus Domingo, a Afshin Abrishamkar, b,c Tiago Sotto Mayor, b René M. Rossi, b Carlos Carbonell, a Andrew J. deMello, c David Amabilino, d Daniel Maspoch, a,e Josep Puigmartí-Luis a,b

a Institut Catala de Nanociencia i Nanotecnologia, ICN2, Esfera UAB, Campus UAB, 08193 Bellaterra, Spain.

b Empa, Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland.

c Institute of Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Vladimir-Prelog-Weg 1, CH-8093 Zurich, Switzerland.

d School of Chemistry, The University of Nottingham, University Park, NG7 2RD, UK.

*eInstitució Catalana de Recerca i Estudis Avançats (ICREA), 08100 Barcelona, Spain. E-mail: daniel.maspoch@icn.cat

Manually engineered self-assembled structures have for many years been investigated under equilibrium conditions so that their most stable forms are reached, 1,2,3,4,5 until recently. There has been a growing interest in obtaining and studying self-assembled structures out-of-equilibrium, 6,7,8,9 The main reason is that non-equilibrium forms – which in most cases are formed transiently and under a constant influx of energy 10– can offer a broad number of intriguing opportunities towards the development of new smart materials and systems with advanced functionalities. 11 For example, transient and/or steady-state self-assembled structures generated far from equilibrium are the basis of many sophisticated functions present in nature, e.g. DNA replication and/or cell division. Nonetheless, the controlled synthesis and study of intermediate self-assembled structures is still a major bottleneck for modern science, which undoubtedly represents a frontier for new advancements in materials development and technology.

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In the realm of self-assembled structures, crystals are a particularly important class of self-assembled materials; they represent the best case scenario to induce large range order with control at the molecular level. In contrast to living systems, where energy dissipating processes allow the appearance of adaptive and emergent functionalities, crystalline ensembles are frequently studied in their thermodynamic stable forms, where final structures will be determined by chemical equilibria, diffusion and mass transport processes. Even though crystals, once they are form, are static structures that can be, in general, inherently kinetic self-assemblies. Hence, the route employed by chemist and nanotechnologist to understand and fine tune the self-organization process of crystalline matter consists in changing and adjusting the functional groups incorporated in their constituent units. This heuristic approach based on crystal engineering has proved efficient in order to control, to some extent, the self-assembly of molecular components into intricate functional structures. While there has been a tremendous interest in rationalizing the crystallization process through the modification of the functional groups present in the molecular building blocks, in general there is a lack of methodologies and/or processes by which out of equilibrium species can be isolated and studied. In this respect, it is important noting that to achieve non-equilibrium crystal forms can be useful not only to “undress” the self-assembly process of crystalline matter but is highly required as out of equilibrium structures are the key that can unlock the rationalization of new artificial materials and systems with advanced functionalities.

As crystallization is an inherently kinetic self-assembly process, dynamic controlled processing technologies such as microfluidics – where molecules can react under controlled diffusion conditions – can advantageously be used to undergo controlled out-of-equilibrium processes. For example, controlled reaction-diffusion systems such as a hydrodynamic flow focusing set-up can be utilized in this respect owing to the precise control over concentration and mass transport as a function of time that this technology can offer. That is, under hydrodynamic flow focusing conditions, the width of the reaction zone between two-reagent streams – where the diffusive mixing occurs – can be precisely controlled. Here, we show that reaction and/or diffusion-limited microfluidic environments can induce concentration gradients that facilitate the formation of novel and unprecedented ordered out-of-equilibrium structures in the crystallization process of a coordination polymer (CP). We show that in contrast to macroscopic reaction settings, the competition between crystallization and the dissolution

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regime created by the dynamic gradients present inside continuous hydrodynamic flow focusing microfluidic environments can lead to the isolation out-of-equilibrium crystal states.

From the vast number of CPs\textsuperscript{22,23} that could be employed in our investigations, we demonstrate this concept by choosing a two-dimensional CP with formula [Cu(4,4'-bpy)](NO\textsubscript{3})\textsubscript{2} (hereafter \textbf{1}; where 4,4'-bpy is 4,4'-bipyridine), which is built up from connecting \(\mu_2\)-oxo-bridged and \(\mu_2\)-NO\textsubscript{3}-bridged Cu(II) chains through 4,4'-bpy linkers (Figure 1a). Two main reasons were considered when choosing \textbf{1} as the best-case scenario in our investigations: i) \textbf{1} can be easily crystallized by performing a diffusion of an ethanolic solution of 4,4'-bpy into an aqueous solution of Cu(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O; or, by mixing the two solutions with or without stirring, and ii) \textbf{1} crystallizes in the form of plate-like crystals (Figure 1b); a common crystal habit in the field and from which little knowledge regarding self-assembly and/or crystal growth development has been gleaned so far.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{a) Crystal structure of \textbf{1}. b,c) Representative TEM images showing the plate-like crystals synthesized through conventional mixture of reactants (b) and laminar flow microfluidics at FRR of 0.1 (c). The scale bars are 2 \(\mu\)m.}
\end{figure}

Figure 2a shows the planar microfluidic device employed herein that consists of four input channels and one outlet. The two inlet channels located at the sides, the focusing streams, are used to inject a pair of sheath flows (flow (1) and flow (4)) and the two other channels located at the middle are employed to supply the reagent solutions, one containing the Cu(NO\textsubscript{3})\textsubscript{2}·6H\textsubscript{2}O (flow (2)) and the second one containing the 4,4'-bpy linker (flow (3)). This microfluidic device was imprinted in polydimethylsiloxane.

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(PDMS) and was covered by a glass cover slide (see the Supplementary Information for further details). We defined the four input channels and corresponding flow rates (in µL/min) as: [flow (1): Q₁, flow (2): Q₂, flow (3): Q₃, flow 4: Q₄]. Using this device, all reactions were done by injecting a water flow at Q₁, an aqueous solution of Cu(NO₃)₂·6H₂O (100 mM) at Q₂, an ethanolic solution of 4,4’-bpy (100 mM) at Q₃, and an ethanol flow at Q₄ via a syringe pump system. The reactant concentrations were optimized to guarantee a rapid crystallization process avoiding the clogging of the microfluidic device employed in our investigations. We studied the crystallization process of 1 by changing the flow-rate ratio (FRR) inside the microfluidic platform. The FRR is defined as the ratio of flow between the focusing streams and the reagent fluids (i.e. FRR = Q₁+Q₄/Q₂+Q₃). Variations in the FRR clearly induced changes in the reaction-diffusion conditions present in the main microfluidic channel where the crystallization process took place (vide infra). In all cases, crystals of 1 were immediately formed at the interface between both Cu(II) ions and 4,4’-bipy streams once these four solutions were injected. The resulting crystals were carefully collected and filtered at the end of the main channel to avoid out of chip reactions. Subsequently, the structures formed were further characterized by Field-Emission Scanning (FE-SEM), Transmission Electron Microscopy (TEM), and X-Ray Powder Diffraction (XRPD).

**Figure 2.** a) Schematic illustration of the microfluidic device and the configuration of the reactant and sheath flows employed in our investigations. b) XRPD of the simulated pattern of 1 (black) and 1 synthesized at FRR of 0.1 (dark blue), 1 (violet), 2 (blue), 4 (green) and 5 (red). Peaks marked with asterisks correspond to the precipitation of free 4,4’-bpy ligand. c) Sequence of TEM images of crystals of 1 fabricated in the microfluidic device at the different FRR with three different magnification levels, showing the different trapped crystalline phases from needles to hollow frames to plate-like crystals (left to right). The scale bars are 5 µm (top row), 2 µm (middle row) and 1 µm (bottom row).
In an initial experiment, crystals of 1 were prepared at FRR of 0.1. Figures 1c, 2c (right) and 3b (right) show typical FE-SEM and TEM images of the resulting crystals, showing the formation of square plate-like crystals that were very similar to those synthesized either by conventional diffusion or simple mixing in a macroscopic vial (Figure 1b). These results are expected because as the FRR decreases the width of the diffusive mixing zone at the interface of two reagent streams will increase, and hence, the reaction area created for the structures to assemble is enlarged, which reproduces, to some extent, conventional diffusion studies in a flask. These crystals had side dimensions in the range of 2.80 ± 0.52 µm. In addition, the simulated (derived from the single crystal structure of 1) and experimental (resulting from the crystals synthesized at FRR of 0.1) powder X-ray diffraction (PXRD) patterns are consistent (Figure 2b), confirming that 1 synthesized via microfluidics is structurally identical to 1 prepared through conventional mixing or diffusion, and that it can be obtained as phase pure; even though the precipitation of small amount of free 4,4′-bpy ligand could also be detected.

As shown in Figure 2c, we then varied the FRR from 0.1 to 5 (i.e. between 6-34 ms, respectively) and observed the formation of numerous unprecedented non-equilibrium crystal architectures. In contrast to precedent work where blocking agents are used to study intermediate states during a crystal growth process,24 in our investigations the ultimate shape of all the structures generated purely depends upon the conditions established in the diffusive mixing zone inside the microfluidic device. By increasing the FRR, a rationalized reduction of the diffusive mixing zone where the crystallization takes place can be achieved, thus leading to diffusion-limited and kinetically controlled environments in which the formation of the most thermodynamic stable crystal forms can be avoided to some extent.25,26 For example, we observed the formation of needles at FRR of 5; needles that start to be perpendicularly connected through their edges at FRR of 4; hollow frames at FRR of 2; frames partially filled with a thinner layer at FRR of 1; and the above-mentioned square plate-like filled crystals at FRR of 0.1. Importantly, varying the total flow-rate without varying the FRR changed the residence time of crystallization process inside the microfluidic device and its throughput but had no significant effect in the habits and structures generated (see Figure S2).

The isolated needles obtained when conducting the crystallization process at FRR of 5 had an average length of 500 nm and a diameter of 20 nm. At lower flow-rate ratio, at FRR of 4, we detected the coexistence of identical needles with some structures formed by two or three needles perpendicularly connected from their edges. Interestingly, at FRR of 2, the presence of hollow frames was found to be predominant, which side dimensions ranged to 2.95 ± 0.71 µm with an edge around 200 nm of thickness. Further decreasing the FRR at 1 resulted in the filling of these hollow frames, forming the above-mentioned plate-like crystal when a FRR of 0.1 was used.

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Moreover, in contrast to other mechanistic studies where amorphous intermediate states of CPs are simply investigated by time-lapse SEM imaging analysis,\textsuperscript{27,28,29,30} in our investigations all the structures generated are crystalline, and therefore, XRPD studies can provide valuable insights to better understand and characterize the nanoscale self-organization of the building blocks in their isolated non-equilibrium forms. XRPD studies were essential to certify that all crystals generated under diffusion-limited and kinetically controlled microfluidic environments corresponded to 1. Indeed, as shown in Figure 2b, the XRPD patterns measured on all crystals obtained at the different FRR revealed a perfect match with that simulated from the crystal structure of 1. Note that XRPD patterns of the needles obtained at FRR = 5 and 4 show broad peaks, which are attributed to their lower crystallinity.

In order to shed light on the crystal growth mechanism that transforms the hollow frames (FRR = 2) to plate-like crystals (FRR = 0.1) (Figure 3a,b), we further analyzed different non-equilibrium crystal forms of 1 by atomic force microscopy (AFM). In the first stages of frame’s formation, we confirmed that the frames are completely hollow as no evidences of residuals were detected inside the area of the frames (Figure SI3). At the vertices of the frames, the perpendicular needles do not overlap but instead they completely interpenetrate (Figure SI4). The progressive filling of the internal area of the frames tends to occur by parallel growth of needles at a single level, as observed in Figure 3c For higher degrees of filling (Figure 3d), precise observation of the different filling levels shows that rods tend to align parallel at each level, and organize orthogonally in alternative levels. Some degree of interweaving appears in areas where full covering is not yet achieved and rods at subsequent levels fill up the gaps. At some point, coalescence among rods happens and the surface of the area inside the frames becomes uniform, ultimately forming the plate-like crystals.

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Figure 3. a) Schematic representation of the crystal growth of 1 that illustrates the progressive filling of internal area of hollow frames. b) FE-SEM images showing the crystal transformation from hollow frames to plate-like crystals. (c,d) AFM images showing the crystal growth of the internal area of the frames at initial (c) and progressive (d) filling stages.

The detailed mechanism leading to the formation of these ordered out-of-equilibrium structures remains unclear at present, however, the process seems to occur as to lower and/or eliminate high-energy facets in the structures generated; a possibility that has been previously reported by others studying shape-controlled growth of inorganic crystals. Based on precedent studies with inorganic crystals and taking into account the results presented above, we proposed a dynamic crystal growth process as shown the sequence in Figure 2c. It is likely that the needles isolated at high FFR can act as seeds for the assembly of the non-equilibrium trapped intermediate states generated, and then that those evolve to the final thermodynamic stable forms; the plate-like crystal structures. This possibility is in part supported by the XRPD studies; they prove that all the structures generated have an identical chemical connectivity. Furthermore, the AFM studies support our hypothesis, the growth process of 1 is dynamic and as indicated in Figure 3 the agglomeration and progressive filling of non-equilibrium forms can occur due to a parallel growth of needle-based structures. That is, the AFM results suggest that the early stage isolated seeds, i.e. the needle structures, organize at a single level and in a perpendicular fashion leading to the final plate-like crystalline morphologies observed in bulk and at FFR of 0.1 (Figures 1b and 1c, respectively).

In summary, we have shown that diffusion-limited and kinetically controlled growth regimes undergoing in microfluidic devices can provide valuable insights into crystallization processes. In
contrast to other methods where trapping of the structures generated during a polymerization process is achieved by taking aliquots in a controlled solvent-induced precipitation method, here we show, for first time, that hydrodynamic flow-focusing condition offered by microfluidic means can be a useful experimental tool for the generation and isolation of non-equilibrium forms. We believe that the microfluidic-based approach presented here should be of significant assistance to circumvent limitations generally ascribed to the isolation and studies of transient forms during crystallization processes. We have demonstrated that microfluidic dynamic processing provides an accessible range of out-of-equilibrium structures present during crystal growth. These results are encouraging as predictable chemical and physical properties in crystalline matter can only be achieved when methods that can precisely “undress” their self-assembly process can be established. Although the structures generated here exhibit no functionality, the technology presented constitutes a potential step for a wealth of new and improved materials where the rationalization of controlled chemical and physical properties may become reality.

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Supporting Information

Materials and methods

The reagents Cu(NO$_3$)$_2$·3H$_2$O and 4,4’-bipyridine (4,4’-bpy) were obtained from Sigma-Aldrich Co. High purity EtOH was purchased from Teknokroma. Deionised Milipore Mili-Q water was used in all experiments. Infrared (IR) spectra were performed on a FTIR Tensor 27 InfraRed spectrophotometer (Bruker) equipped with a Bruker Golden Gate diamond ATR (Attenuated Total Reflection) cell. Scanning electron microscopy (SEM) images were collected on a scanning electron microscope (ZEISS EI MERLIN FE-SEM) at acceleration voltages of 0.2-30 kV. Aluminium was used as support. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM 1400. The X-Ray EDX microanalysis was performed on an Oxford Instruments INCA energy SEM system. The measurements were performed at room temperature and a voltage of 120 kV. X-ray powder diffraction (XRPD) measurements were performed with an X’Pert PRO MPD diffractometer (Panalytical) especially configured for in-plane diffraction. Elemental analysis was performed with CHNS Thermo Scientific Flash 2000 model. Atomic force microscopy images were taken in amplitude modulation dynamic AFM mode, in pure noncontact conditions with an Asylum MFP3D system, using Pt-coated tips (Nanosensors PPP-EFM) and a resonant frequency around 70 kHz. Images were obtained using a scanning rate of 1 Hz and keeping the amplitude of oscillation constant at about 50 nm. For Kelvin probe force microscopy images, an AC voltage of 1 V amplitude was applied to the tip at a distance of 50 nm to the surface, and the surface potential function difference between the tip and the sample was obtained.

Fabrication of the microfluidic device

The microfluidic channels employed in this study were moulded into polydimethylsiloxane (PDMS, SYLGARD® 184 Silicone Elastomer Kit) using an SU-8 (2015, Microchem) master form fabricated with standard photolithographic techniques. Before attaching the cured and structured PDMS mould to a patterned electrode surface, inlet holes connecting to the microfluidic channels were punched with a Biopsy puncher. Non-bonded chips were used, which enabled peeling of the PDMS mould before the thermal treatment processes were conducted on the localized superstructures. The dimensions of the microchannels were 50 µm × 50 µm for the four input microchannels, and 250 µm × 50 µm for the main reactor channel. The total length of the main reactor channel was 9 mm.

Synthesis of 1 via mixture of reactants
In a typical experiment, an aqueous solution of Cu(NO$_3$)$_2$·6H$_2$O (100 mM) was added to a solution in ethanol of 4,4’-bpy (100 mM) with or without stirring. After few seconds, blue crystals of 1 were formed.

Anal. (%) Calcd. for C$_{20}$H$_{18}$Cu$_2$N$_6$O$_8$,NO$_3$,H$_2$O C, 35.45; H, 2.97; N, 14.47. Found: Cu, C, 35.67; H, 2.69; N, 14.28.

**Synthesis of 1 via diffusion**

In a typical experiment, a solution of 4,4’-bpy (100 mM) in ethanol and an aqueous solution of Cu(NO$_3$)$_2$·6H$_2$O (100 mM) were prepared. The solutions were then transferred carefully to a test tube generating an interface of water/ethanol between the two layers. After 4 days, dark blue crystals of 1 suitable for single-crystal XRD analysis started to form at the liquid-liquid interface. Anal. (%) Calcd. for C$_{20}$H$_{18}$Cu$_2$N$_6$O$_8$,NO$_3$,H$_2$O C, 35.45; H, 2.97; N, 14.47. Found: Cu, C, 35.11; H, 3.12; N, 14.28.

**Synthesis of 1 using laminar flow**

The syntheses of the different crystal morphologies of 1 were carried out in a planar microfluidic device that consists of four input channels and one outlet channel, imprinted in polydimethylsiloxane (PDMS) and covered by a glass plate. The solutions of reactants were injected via a syringe pump system at the different selected flows rates. We defined the flow rates (all µL/min) with the following abbreviations: flow (1), Q$_1$; flow (2), Q$_2$; flow (3), Q$_3$; and flow (4), Q$_4$. In a typical synthetic procedure, crystals of 1 were initially prepared by injecting an aqueous solution of Cu(NO$_3$)$_2$·3H$_2$O (100 mM) in Q$_2$ and an ethanolic solution of 4,4’-bpy (100 mM) in Q$_3$. Both were accomplished by an auxiliary flow with the corresponding solvents, Q$_1$ and Q$_4$.

**Numerical Simulations**: The 2D steady-state fluid flow and mass transport across the microfluidic device was simulated using a Finite Element approach, considering geometries and boundary-conditions as described in the manuscript. Diffusion coefficients of both reagents were assumed to be 10 −9 m $^2$ s $^{-1}$, in line with literature data for ethanol-water mixtures. [3] Density and dynamic viscosity of reagents and sheathed currents were assumed to be those of the corresponding pure solvents, i.e., 103 kg m $^{-3}$ and 8.9 $\times$ 10 $^{-4}$ Pa$\cdot$s for water-based currents and 789 kg m $^{-3}$ and 1.1 $\times$ 10 $^{-3}$ Pa$\cdot$s for ethanol-based currents, respectively.

**Single-Crystal**: X-ray single-crystal diffraction data for 1 was collected on the BM16 Spanish line of ESRF synchrotron in Grenoble (λ = 0.7901 Å). Data were indexed, integrated and scaled using HKL2000.
The H atoms have been included in theoretical positions but not refined. The low max value is due to the data collections that have been performed in the BM16 line with only a phi scan. The structure was solved by direct methods using the program SHELXS-97. The refinement and all further calculations were carried out using SHELXL-97. Empirical absorption corrections were applied in both cases with SCALEPACK.

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Table S1.1: Crystal and structure refinement data.

| Compound | 1 |
|----------|--|
| Empirical formula | \( \text{C}_{20}\text{H}_{18}\text{Cu}_2\text{N}_6\text{O}_8,\text{NO}_3,\text{H}_2\text{O} \) |
| Formula weight | 677.3 |
| Crystal system | triclinic |
| Space group | P-1 |
| CCDC ref | 1059883 |
| Unit cell dimensions | |
| \( a \) (Å) | 10.385(4) |
| \( b \) (Å) | 11.555(4) |
| \( c \) (Å) | 11.790(4) |
| \( \alpha \) (deg) | 73.91(3) |
| \( \beta \) (deg) | 84.08(3) |
| \( \gamma \) (deg) | 70.54(3) |
| \( V \) (Å\(^3\)) | 1281.6(7) |
| \( Z \) | 2 |
| \( F(000) \) | 686 |
| Ind refln (\( R_{int} \)) | 4905 (0.0199) |
| \( q_{max} \) (deg.) | 27.12 |
| Final R indices | R1 = 0.0528 |
| [\( I > 2\sigma(I) \)] | wR2 = 0.1553 |
**Figure SI.1:** Comparison of the experimental XRPD pattern (blue) of the microcrystals of 1 obtained by simple mixture of Cu(II) and 4,4’-bpy with the XRPD pattern simulated from its single-crystal structure (black).

![Figure SI.1](image1.png)

**Figure SI.2:** Concentration profiles of reagents at the microfluidic reactor exit, for increasing values of flow rate ratio (FRR = 0.1, 1, 2 and 5) and two values of total flow rate i.e. TFR=220 μL/min (a) and 1,200 μL/min) (b). Solid and dashed lines correspond to concentration profiles of Cu(NO$_3$)$_2$·6H$_2$O and 4,4’-bpy, respectively.

![Figure SI.2](image2.png)
Figure SI.3. AFM topography images of a hollow frame (a) and a partially filled frame (d) and corresponding Scanning Kelvin Probe Microscopy (SKPM) (b,e) and 3D AFM topography (c,f) images in colour scale. Note that SKPM, which is a technique that measures the surface potential function difference between the tip and the samples (and thus, is very sensitive to the material on the surface), confirms the absence of CP material inside the frame.

Figure SI.4 3D AFM topography images of a single frame, showing different perspectives for the vertices. Note that the vertices are interpenetrated.