Electronic supplementary information

Fluorescence identification of arthropathic calcium pyrophosphate single crystals using alizarin red S and xanthene dipicolylamine Zn^{II} complex

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Synthesis of MSU crystal

MSU crystals were prepared as described by Denko and Whitehouse (1976). Typically, 2.00 g of uric acid was weighed and added to 400 ml of deionized water under ambient air. The solution was continuously stirred and heated to 60°C for 30 minutes, then an appropriate volume of 2 M NaOH was added to adjust pH of the solution to 8.9 to obtain the clear solution. Then the solution was cooled down to room temperature and allowed to crystallize for 18 hours at room temperature. The obtained white powder was filtered through Whatman grade 1 filter paper and dried in hot-air oven at 180°C for 2 hours. The obtained MSU crystal was 1.84 g (yield 72.5%) and kept in a sealed glass vial stored inside a desiccator at room temperature.

Denko, C.W., & Whitehouse, M.W. (1976). Experimental inflammation induced by naturally occurring microcrystalline calcium salts. The Journal of Rheumatology, 3(1), 54-62.

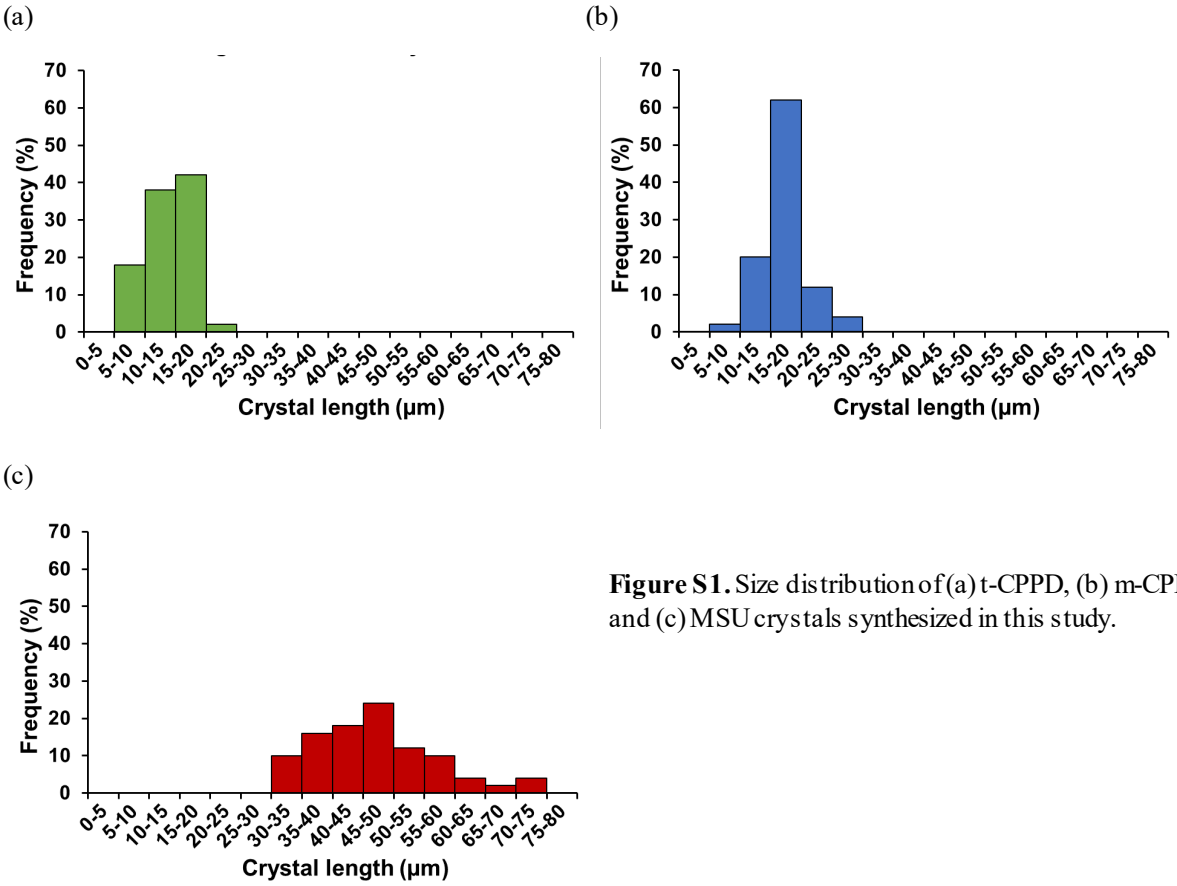


Figure S1. Size distribution of (a) t-CPPD, (b) m-CPPD and (c) MSU crystals synthesized in this study.

Characterization of crystals

XRD measurements were performed with a Bruker AXS model D8 discover equipped with Cu radiation set at 40 mA current and 40 kV voltage. The crystals were scanned from 2-70° in the 2θ range with a scanning rate of 0.0116° per minute at 25°C. FT-IR spectrums of the crystals were characterized by a PerkinElmer frontier FTIR with 4 cm^{-1} resolutions and 16 scans per spectrum over the wavenumber range of 4000 - 400 cm^{-1}. Raman spectroscopy was performed to characterize the crystal using a Horiba XploRA PLUS confocal Raman microscope over the wavenumber range of 50-3400 cm^{-1} with laser source at 532 nm. TGA was performed with a PerkinElmer TGA 4000 in the temperature range of 30-500°C at a heating rate of 5°C per min.

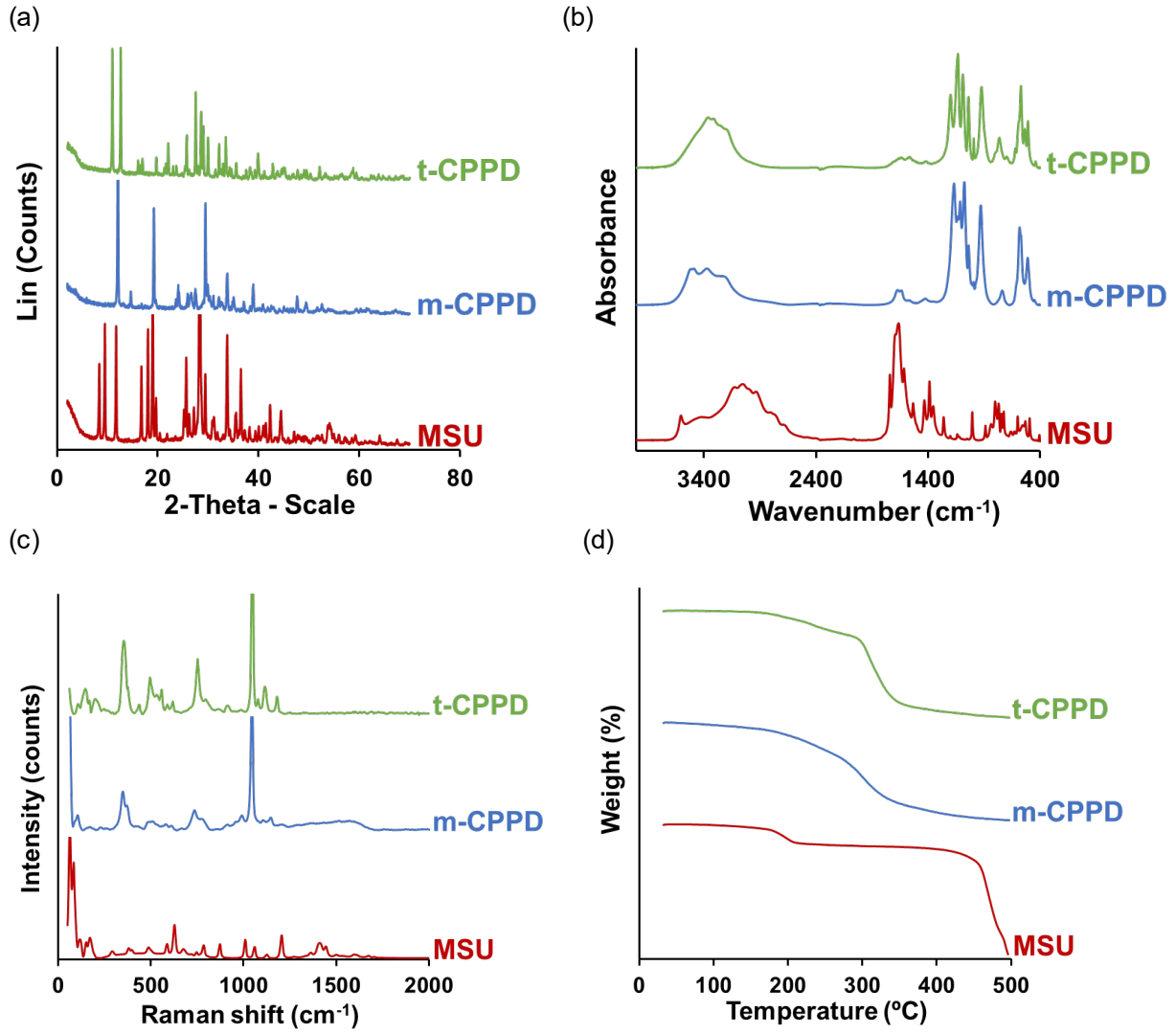


Figure S2. (a) XRD patterns, (b) FTIR, (c) Raman spectra and (d) TGA curves of t-CPPD (green), m-CPPD (blue) and MSU (red) crystals synthesized in this study.

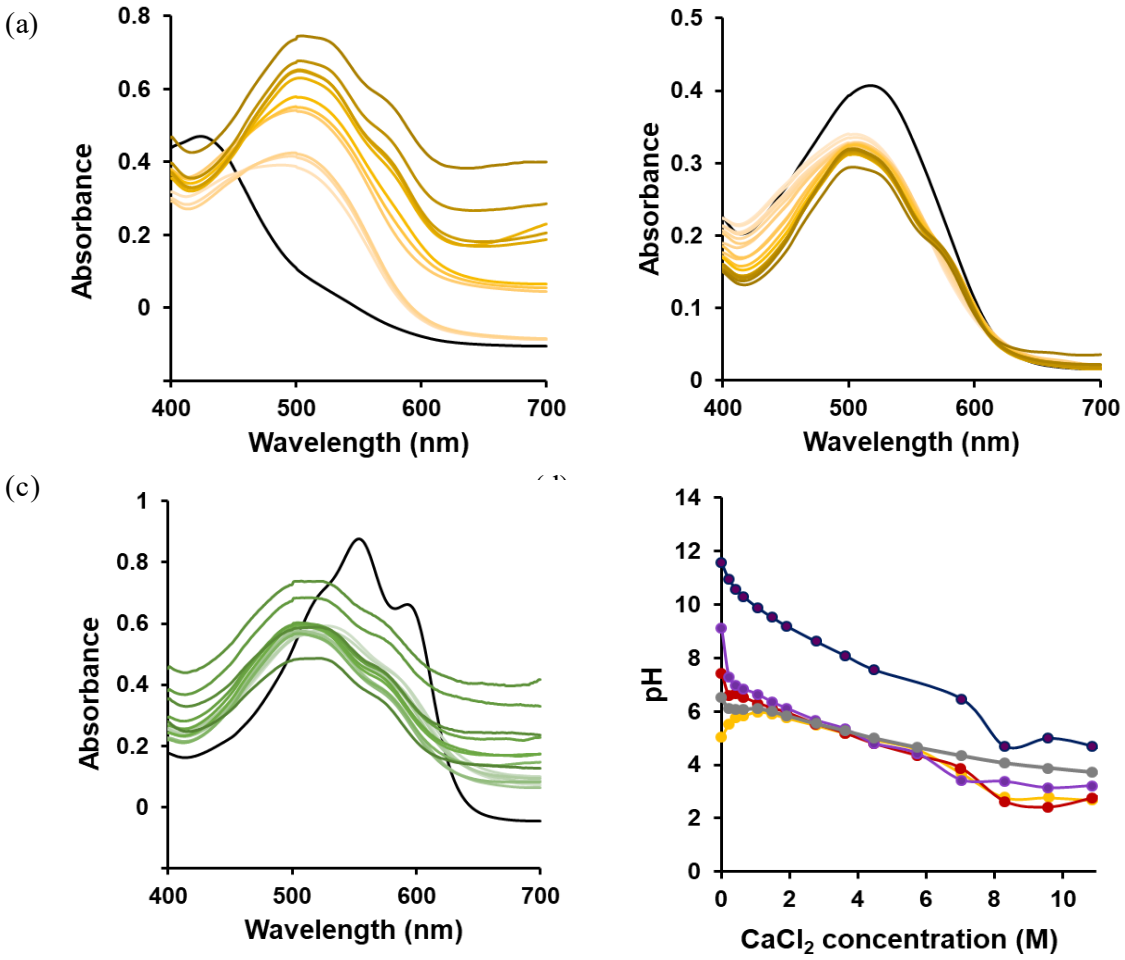
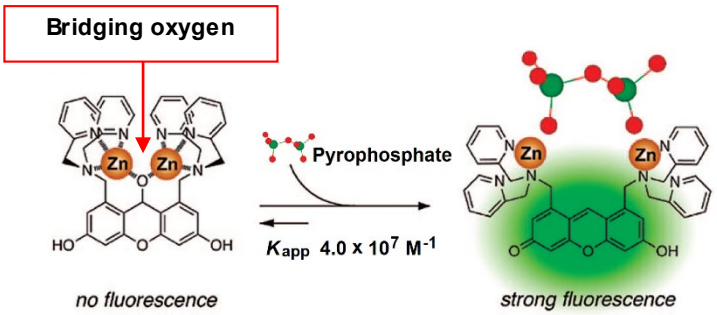


Figure S3. The UV-visible spectrum of the ARS solution (73 μM) upon addition with $CaCl_2$ at initial pH of 5.0 (a), 7.5 (b) and 11.5 (c). Black line refers to ARS solution without addition of salts. Gradient line color from light to dark represents the concentration of $CaCl_2$ from 0 to 11 M. (d) pH change of the ARS solution (73 μM) during addition of $CaCl_2$ when the initial pH of the ARS solution was 5.0 (yellow), 7.4 (red), 9.0 (purple), 11.5 (indigo) and no ARS (gray).

Fluorescent sensing mechanism of XDZ complex

The fluorescent quenching of XDZ complex in the aqueous solution is based on the unique mechanism in which the bridging oxygen from water molecule disrupts a system of conjugated double bonds of xanthene ring as previously described (Ojida et al., J. Am. Chem. Soc., 2008, 130, 12095-12101; Wongkongkatep et al., Top. Curr. Chem. (Z), 2017, 375, 30). The presence of inorganic pyrophosphate in the solution removes the bridging oxygen and restores the conjugated double bonds system of xanthene ring, thus turns on the fluorescent emission in relation to the concentration of pyrophosphate added as shown below.



However, when the XDZ complex was used for staining the MSU crystals under dried condition, the bridging oxygen from water molecule of the quenched XDZ complex seemed to be removed during the drying process, resulting in the strong green fluorescence of XDZ complex at the surface of the MSU crystals due to the non-specific binding as shown in Figure S4.

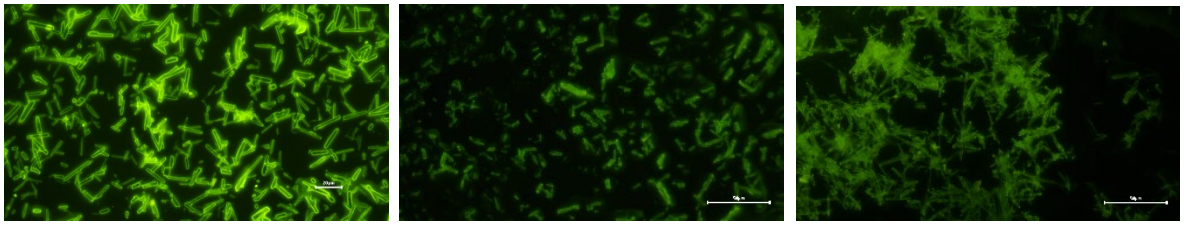
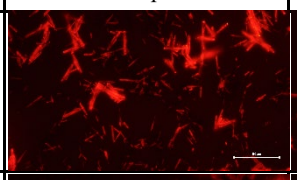
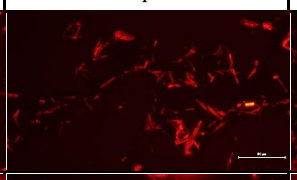
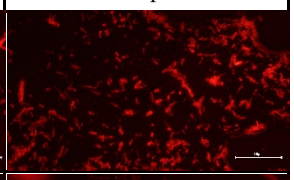
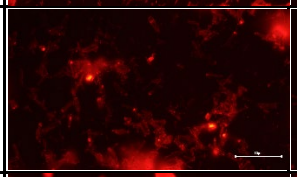
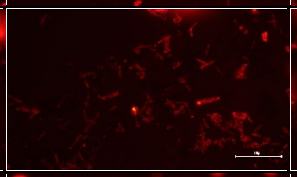
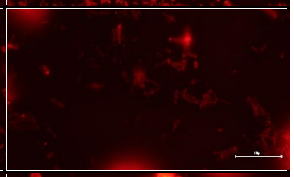
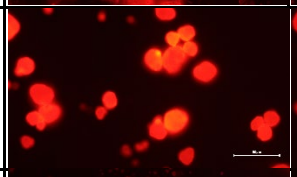
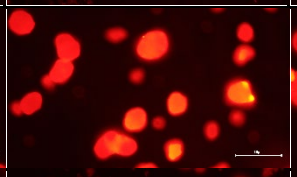
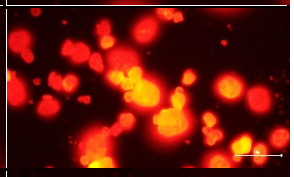
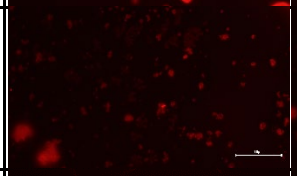
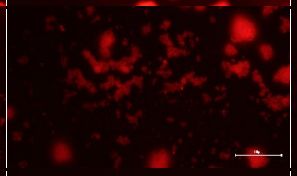
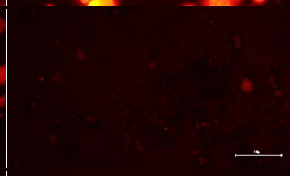
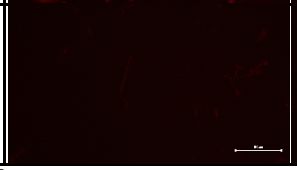
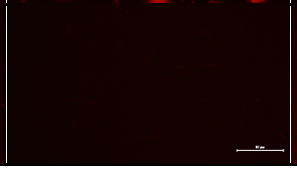
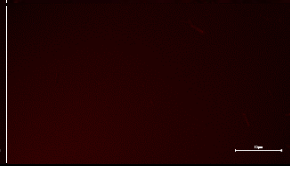


Figure S4. XDZ-staining of t-CPPD, m-CPPD and MSU using dried drop technique. Scale bars represent 20 μm for t-CPPD and 50 μm for m-CPPD and MSU.

Table S1. Microscopic images of MSU and calcium associated crystals/aggregates stained with ARS under red fluorescence channel. All images were acquired at exposure time 350 ms, gain 500%.

Type of crystal	ARS pH 5	ARS pH 7.4	ARS pH 9
m-CPPD			
t-CPPD			
Hydroxyapatite			
Calcium oxalate			
MSU			

Scale bars represent 50 μm.

Table S2. Heat map and values of fluorescence obtained from ARS/XDZ two-step method.

Unknown sample No.	Red pixel values of ARS-stained unknown solid[a]	F/F_0 of XDZ and soaked solution[a]	Identification by 2-steps ARS/XDZ method[b]	Prepared compound[c]
1	29.8882	1.207645	negative	MSU
2	255	0.707139	Other calcium-containing compound	HA
3	166.2758	10.25313	CPPD	m-CPPD
4	70.2704	1.428873	Other calcium-containing compound	CO
5	0	39.08573	Other pyrophosphate-containing compound	NaPPi
6	0	1.176221	negative	BSA
7	23.0652	2.42822	negative	MSU
8	0	1.335608	negative	BSA
9	255	0.718928	Other calcium-containing compound	HA
10	0	0.723514	negative	HyA
11	0	0.722283	negative	HyA
12	132.766	21.80415	CPPD	t-CPPD
13	88.8542	5.908305	CPPD	m-CPPD
14	77.1412	1.309149	Other calcium-containing compound	CO
15	0	39.74467	Other pyrophosphate-containing compound	NaPPi
16	99.0648	17.14383	CPPD	t-CPPD
17	80.9664	21.5608	CPPD	t-CPPD
18	88.0286	1.302058	Other calcium-containing compound	CO
19	255	0.708404	Other calcium-containing compound	HA
20	61.4286	17.42701	CPPD	t-CPPD
21	0	39.93765	Other pyrophosphate-containing compound	NaPPi
22	0	0.736571	negative	HyA
23	248.522	0.724501	Other calcium-containing compound	HA
24	147.8572	8.146295	CPPD	m-CPPD
25	0	40.46338	Other pyrophosphate-containing compound	NaPPi
26	30.537	1.544191	negative	MSU
27	111.071	1.243405	Other calcium-containing compound	CO
28	126.8628	21.61589	CPPD	t-CPPD
29	23.6236	1.278182	negative	MSU
30	250.0526	0.713783	Other calcium-containing compound	HA
31	0	0.760937	negative	HyA
32	0	1.243893	negative	BSA
33	0	1.130504	negative	BSA
34	96.256	1.224595	Other calcium-containing compound	CO
35	132.5602	5.352301	CPPD	m-CPPD
36	159.735	10.30149	CPPD	m-CPPD
37	24.56066667	1.248632	negative	MSU
38	0	41.57253	Other pyrophosphate-containing compound	NaPPi
39	0	1.241871	negative	BSA
40	0	0.729137	negative	HyA

[a]Color scale:

ARS 0  255

XDZ 0.7  42

[b]Criteria for CPPD identification: ARS >50 and XDZ>3.

[c]BSA: bovine serum albumin; m-CPPD: monoclinic calcium pyrophosphate dihydrate; t-CPPD: triclinic calcium pyrophosphate dihydrate; CO: calcium oxalate; HA: hydroxyapatite; Hya: hyaluronic acid; MSU: monosodium urate; NaPPi: sodium pyrophosphate.