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Supporting Information

Self-suppression from Metabolin with a Precursor in Pathology Crystallization of Gout

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Experimental Procedures

Materials. Uric acid (>99%), xanthine (>98%), guanine (>99%), adenine (>98%), hypoxanthine (>99%), acetamide(>98%), n-butyl amide(>98%), decanamide(>98%), thymine(>99%), 5-fluorouracil (>99%), 2,4-diaminopyrimidine (>98%), 2-imidazolidone (>98%), 2-mercaptoimidazole (>98%), 1,3,7-trimethyluric acid (>98%) and 9-methyluric acid (>95%) were obtained from Sigma Aldrich and used without purification. Aqueous solutions were prepared using deionized water (18.2 MΩ) purified with a Nanjing Yipuyida purification system.

Monosodium urate monohydrate (MSUM) crystallization. MSUM crystals were obtained by adding 1.250 mL 1 M NaOH, 168.1 mg uric acid, desired concentration of additives and 818.6 mg NaCl to stirred 100 mL solutions of deionized H_2O, following up adjusting pH value to 7.4 using 1M sodium hydroxide solution. After heating under reflux at 100 °C for 10 min, the mixture was gravity filtered using filter paper (Whatman Grade 1 filters, >11 μm pores), and the solution was allowed to cool slowly to 37 °C. The container was then sealed to prevent evaporation and exposure to airborne particulates and stored for 72 hours at 37 °C without stirring. The precipitate was collected by vacuum filtration (Whatman Grade 1 filters, >11 μm pores) and was washed three times by deionized H_2O, then the sample was dried at 37 °C prior to analysis. Meanwhile, the relative mass yields of MSUM crystals were obtained by dividing the mass of MSUM crystals (collected from growth solution by filtration with additives) by the mass of MSUM crystals collected from growth solution without additives.

Characterization method. Powder X-ray diffraction (PXRD) was conducted on a Rigaku Ultima IV diffractometer (Japan) with Cu K_α target at scanning step of 10 degrees/min. All the samples were grained for one minute in an agate mortar prior the examination. The morphology of the crystals was observed by both scanning electron microscope (SEM) and optical microscope. The SEM pictures were taken on a Hitachi S-4800 Ⅱ field emission scanning electron microscope, the sample was fixed on the pillars and conductive coated by gold before measurement. The optical images were collected on

Olympus DP73 microscope (Japan) with appropriate magnification. Atomic force microscopy (AFM) was performed with a Bruker Instruments Dimension Icon. Measurements were performed in tapping mode in air or PeakForce tapping mode in a cell designed to contain liquids for in situ imaging. The cell contained two ports for inlet and outlet flow to maintain constant supersaturation during continuous imaging. The measurements were performed in PeakForce QNM in Fluid mode using Veeco SCANASYST-FLUID probe with a spring constant of 0.7 N/m (triangular, 70 μm length, 10 μm width). MSUM crystals with needle-like morphologies prepared by the procedure described above were transferred onto an AFM specimen disk coated with partially cured (1 hr) UV-curable optical cement. The (010) face of the part of MSUM crystals aligned parallel with the specimen disk so that the long needle axis of MSUM was aligned parallel to the fast scan x-direction by AFM. The partially cured polymer with the adhered crystals was cured completely by additional UV radiation (150 min) prior to analysis. The mounted MSUM crystals were etched slightly by immersion in deionized water for 45 seconds at 60 ^{o}C to remove amorphous impurities that may be present on the surface. HPLC analysis was conducted on an Agilent 1100 (Agilent Technologies, CA, USA) with an: Agilent XDB-C_{18} column (4.6mm×250mm,5.0μm). The optimum separation was obtained with mobile phase (v/v/v/v; water: methanol: glacial acetic acid: 10% ammonium hydroxide solution in water = 879/100/15/6). The flow rate was 1.0 mL/min and the column temperature was maintained at 30 ^{o}C, and the UV detector wavelength used was 254 nm. The solution of each standard was individually prepared at a concentration of 2 mM in 0.02 M NaOH solution because of the low solubility in neutral solutions.

Induction Time Measurement. The induction times of MSUM nucleation from solutions in the absence and in the presence of a given additive were measured at 23 °C.[1] Each experiment for induction time was carried out in a 100 mL glass jacketed beaker at 23 °C. In each run, a 25 mL MSUM solution was prepared in a properly capped glass bottle at an elevated temperature of 95 °C, with a given concentration and the given additive. This solution was kept agitated at 95 °C for 10 min to make sure all solids were fully dissolved. Then it was quickly transferred to a 100 mL jacketed beaker controlled at 23 °C, agitated with a magnetic stirrer at 60 rpm. The solution was recorded by using a

high definition web camera. The onset of nucleation was observed as an exceedingly rapid change from a clear to a cloudy solution. And the induction time was defined as the period from the moment at which the solution was transferred to the 100 mL jacketed beaker to the moment at which the cloud point was detected.

Quantum Chemical Calculations. Density functional theory (DFT) calculations have been applied using a Gaussian 09 package.[2] The (010) surface of MSU, on the basis of the optimized unit cell, was kept frozen and the inhibitor was allowed to fully relax in our calculations. For the optimized molecular geometries, there are no vibrational fre-quencies, which indicate that the optimized geometries are stable. Binding energies of the complexes are calculated using a B3LYP functional. A Gaussian-type 6-311++G(d,p) basis set is used for geometry optimization and accounted for solvent effects through the SMD solvation model (the solvent is water). The binding energy of inhibitors and uric acid to crystal surfaces is defined as:

$$BE=E_{inhibitor+MSUM} - E_{inhibitor} - E_{MSUM}$$

where E_x represents the total electronic energy of species x.

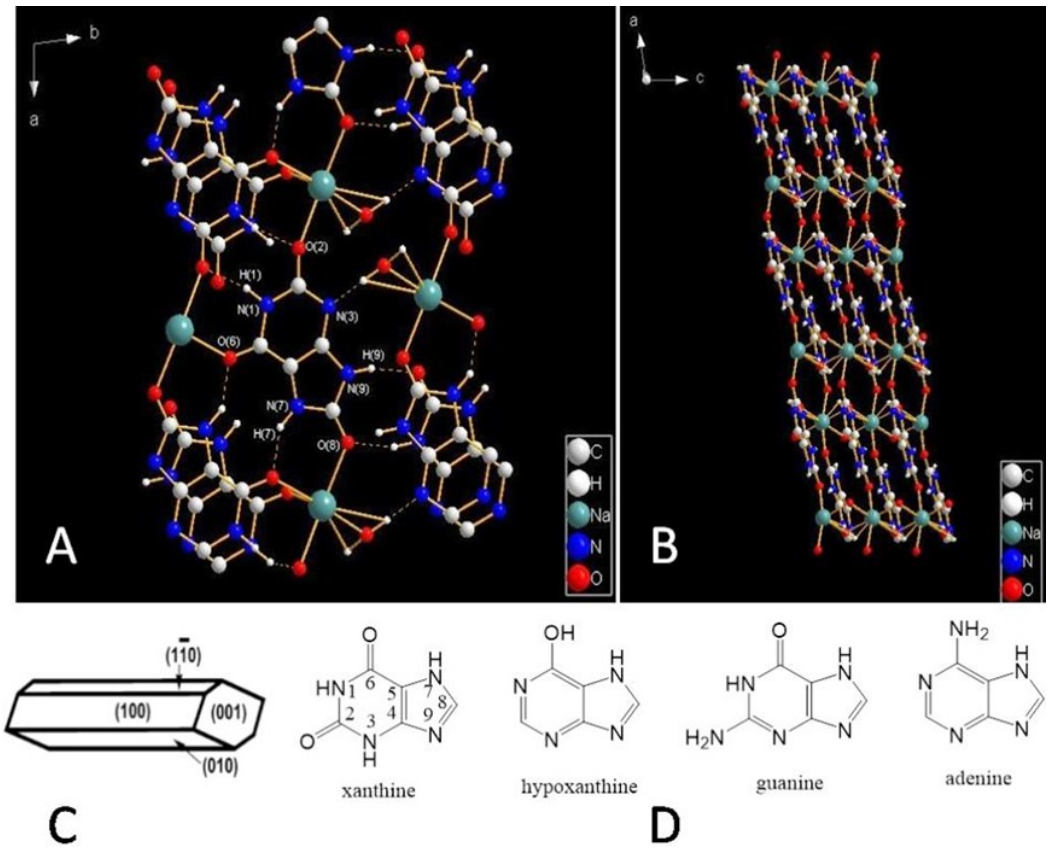


Figure S1. A) A view down c*, indicating hydrogen bonding and sodium ion coordination. B) Packing diagram of MSUM viewed along the b axis. C) A macroscopic MSUM crystal with typical faces are indicated. D) The structure of purine derivatives

Table S1. The ratio of relative mass yield (RM) of a seeded crystal nucleus solution to unseeded crystal nucleus solution in the presence of various concentrations of purine derivatives and 10 mM MSUM.[α,β]

Entry	Concentration of xanthine	Time		
		24h[γ]	48h	72h
1	0	102.2%	100.4%	99.9%
2	0.5mM	107.5%	102.3%	101.2%
3	1mM	114.2%	106.4%	103.4%
4	1.25mM		320.3%	112.1%

[α] The method for preparing a seeded crystal nucleus solution: the 5mg ground MSUM crystals, as the crystal nucleus, were pre-added to the solution in different concentrations of xanthine and 10mmol MSUM

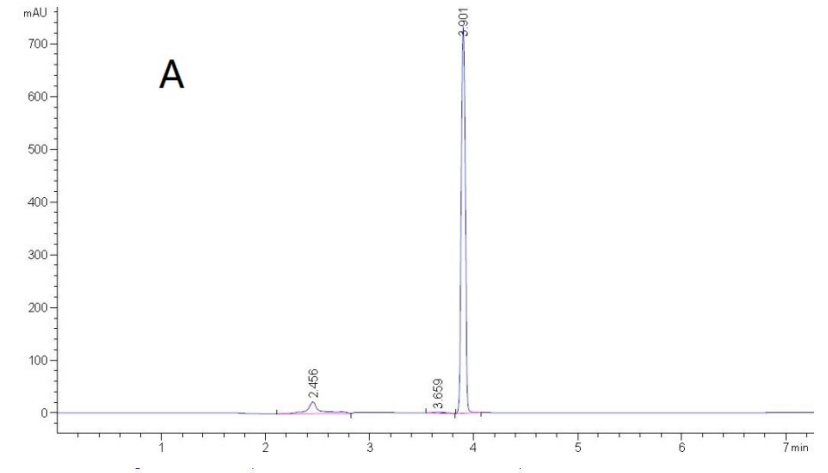
[β] The ratio of relative mass yield (RM) of a seeded crystal nucleus solution to unseeded solution is defined as:

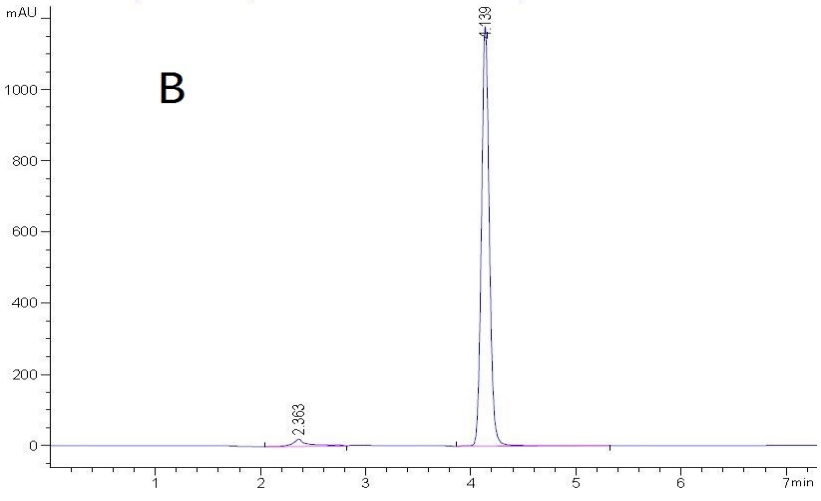
$RM = (Weight_{seed} / (5 + Weight_{unseed})) 100\%$

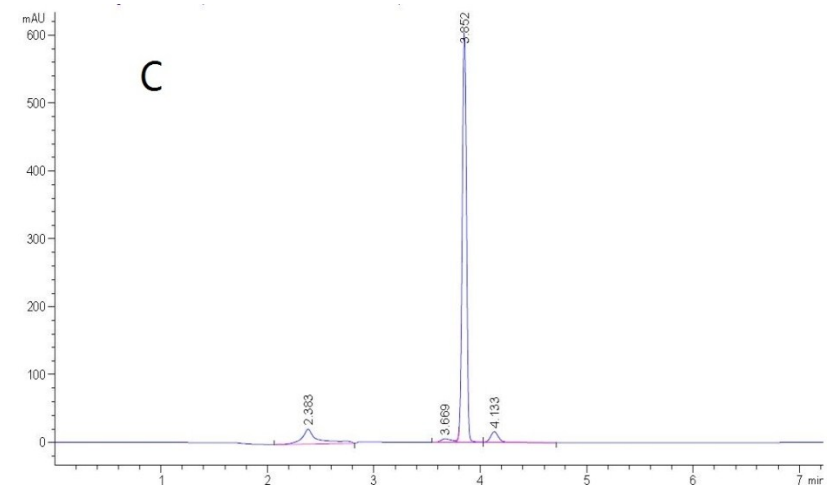
$Weight_{seed}$: The measured crystal weights (mg) of MSUM crystals were obtained after crystallization for a certain time in a seeded solution in the presence of various concentrations of xanthine and 10 mM MSUM.

$Weight_{unseed}$: The measured crystal weight (mg) of MSUM crystals was obtained after crystallization in an unseeded solution for a certain time in the presence of various concentrations of xanthine and 10 mM MSUM.

[γ] $Weight_{unseed}$ was equal to zero, which was obtained after crystallization for 24h in unseeded solution in the presence of 1.25mM xanthine and 10 mM MSUM.







#	[min]		[min]	[mAU*s]	[mAU]	%
1	2.383	BV R	0.1354	229.46417	22.05078	11.1019
2	3.669	BV E	0.1094	39.21095	5.35709	1.8971
3	3.852	VV R	0.0449	1713.69678	606.88605	82.9116
4	4.133	VB	0.0816	84.52452	16.01802	4.0894

Figure S2. HPLC analysis of a standard sample of A) MSUM and B) xanthine. C) HPLC analysis of MSUM crystals after washing three times with water following filtration in aqueous solutions containing 10 mM MSUM in the presence of 1mM xanthine.

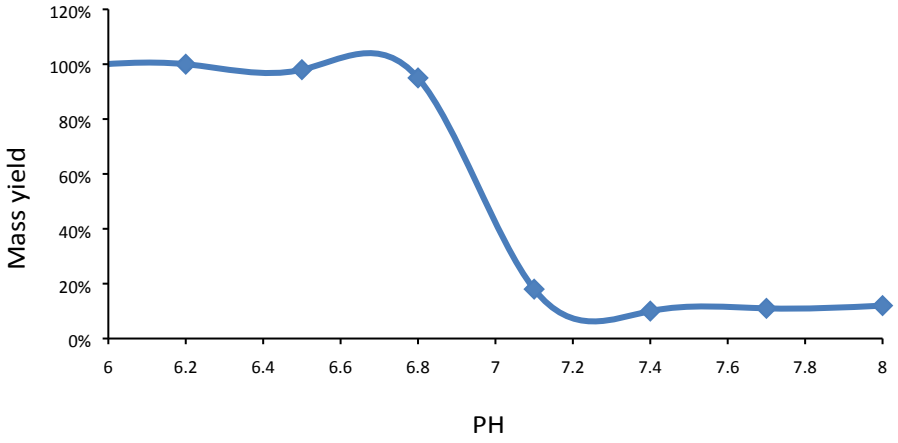


Figure S3. The relative mass yield of MSUM crystals obtained after crystallization for 72 h in the presence of 0.35 mM xanthine and 7 mM sodium urate in 0.01 M sodium phosphate buffer.[α]

[α] The measured values of crystallization mass yield of MSUM from a buffer solution of different pH values were set to 100 wt% and used as control samples.

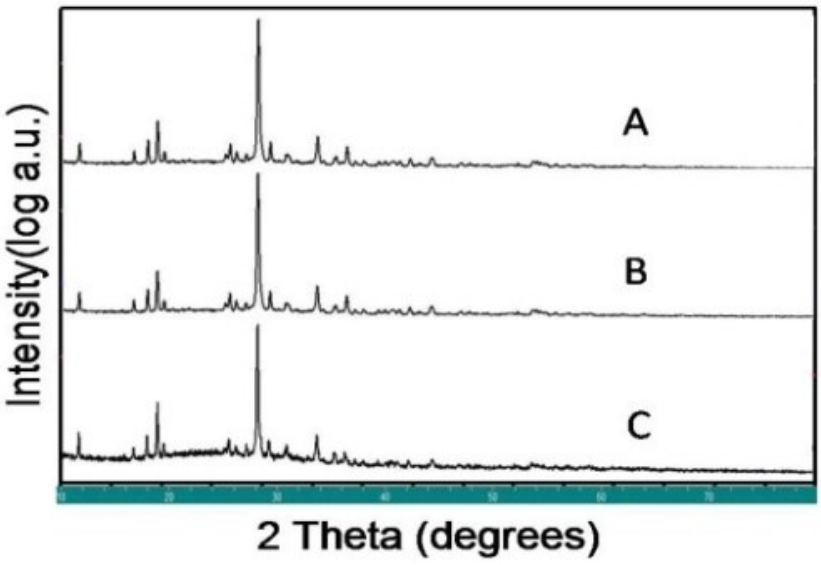


Figure S4. Powder X-ray diffraction patterns of MSUM crystals obtained after crystallization for 72 h in the presence of A)10 mM MSUM or B) 10 mM MSUM and 0.5 mM xanthine as well as C) 10 mM MSUM and 1 mM xanthine

References

[1] H. Guangjun, C. Pui, T. Reginald. *Cryst. Growth Des.,* 2014, **14**, 1406−1411. ,

[2] M. J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R.Cheeseman; et al. Gaussian 09; Gaussian Inc.: Wallingford, CT, 2009.