

# Rapid crystallization of glycine using metal-assisted and microwave-accelerated evaporative crystallization: the effect of engineered surfaces and sample volume

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## Abstract

Metal-Assisted and Microwave-Accelerated Evaporative Crystallization (MA-MAEC), is a new approach to crystallization of drug compounds, amino acids, DNA and proteins. In this work, we report our additional findings on the effect of engineered surfaces and sample volume on the rapid crystallization of glycine. With the use of hydrophilic functionalized surfaces and the MA-MAEC technique, glycine crystals ~1 mm in size were grown in 35 seconds with 100% selectivity for the  $\alpha$ -form. The use of moderately hydrophobic surfaces resulted in the growth of glycine crystals only at room temperature. An increase in volume of initial glycine solution (5-100  $\mu$ L) resulted in an increase in crystal size without a significant increase in total crystallization time. Raman spectroscopy and powder X-ray diffraction results demonstrated that the glycine crystals grown on engineered surfaces were structurally identical to those grown using conventional evaporative crystallization.

**Keywords:** Crystallization, amino acids, glycine, silver island films, plasmonic nanostructures, metal-assisted and microwave-accelerated evaporative crystallization

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## 1. Introduction

Crystallization of amino acids has been the subject of numerous reports, where new techniques and platforms including polarized laser light irradiation in solution [1], and engineered surfaces such as nanoscale cylindrical pores [2], platforms for micro-droplet solvent evaporation and self-assembled monolayers (SAMs) of alkane thiols on patterned surfaces [3-5] through solvent evaporation, just to name a few, were described. Most notably, Trout et. al. investigated the role of surface chemistry and morphology of porous polymer surfaces on heterogeneous nucleation of aspirin [6]. The effect of functional groups, such as, carboxyl, hydroxyl, tertiary amide and amine and phenyl ring was found out to have a profound effect on the nucleation and growth of aspirin crystals [6]. These studies demonstrated that the use of engineered surfaces clearly plays an important role in crystal nucleation and growth. However, the crystallization of amino acids and small molecules by conventional evaporative crystallization still takes several hours or longer

to complete and require the use of complex platforms. In this regard, there is still a need for new or improved crystallization techniques and platforms, which afford for rapid and selective crystallization of materials of interest.

Recently, Aslan Research Group has developed a new crystallization technique, called Metal-Assisted and Microwave-Accelerated Evaporative Crystallization (MA-MAEC) [7], which is based on the combined use of metal nanostructures deposited onto glass slides via facile chemical route and low power microwave heating. In the MA-MAEC technique, the accelerated crystallization is attributed to the thermal gradient created by selective microwave heating of the sample solution on the metal nanostructures but not the metal nanostructures themselves. The thermal gradient creates a driving force for the mass transfer of molecules in solution towards the metal nanostructures that act as heterogeneous nucleation sites; hence the technique includes the name of metal-

assisted crystallization. In our initial publication [7], the proof-of-principle of the MA-MAEC technique was demonstrated using a model amino acid, i.e. glycine. Since then, the MA-MAEC technique has been applied to other amino acids; L-arginine acetate [8] and L-alanine [9] and drug molecules; acetaminophen [10]. We envision that the MA-MAEC technique can be applied to the crystallization of biological macromolecules (i.e., DNA, proteins, etc.). Currently, high-throughput technologies have been employed for the crystallization of biological macromolecules, where more experiments can be carried out with small volume of samples to determine the optimum crystallization conditions. However, crystallization of biological macromolecules still takes a very long time (in the order of days or weeks). In this regard, there is still a need for a technique that affords for better control over the crystallization process, in terms of producing high-quality crystals of peptides and proteins in a significantly shorter time scale (in minutes to a few hours for rapid understanding of the structure-property relationships of protein-based materials. In this regard, the knowledge obtained in our current efforts to crystallize small biological molecules will be utilized in our future efforts the crystallization of biological macromolecules.

In this work, we investigated the effect of engineered surfaces and sample volume on rapid crystallization of glycine using the MA-MAEC technique. In this regard, the heterogeneous nucleation sites (i.e., silver island films, SIFs) for the glycine crystals were engineered via a surface modification procedure that includes the attachment of Self-Assembled Monolayers (SAMs) of alkane thiols with -COOH (hydrophilic) and -CH<sub>3</sub> (moderately hydrophobic) and an organic compound with -NH<sub>2</sub> (moderately hydrophilic) functionality onto SIFs. Our findings indicate that the use of hydrophilic surfaces in MA-MAEC based crystallization resulted in the rapid (~35 sec) crystallization of glycine and the increase in the volume (5-100  $\mu$ L) of initial glycine solution had no significant effect on the total crystallization time. Characterization of the glycine crystals was carried out via Raman spectroscopy and powder X-ray diffraction (XRD) measurements, which demonstrated that glycine crystals formed on surface engineered SIFs using MA-MAEC technique and room temperature evaporation, are identical in nature.

## 2 Methods and Materials

**Materials.** Sodium hydroxide, ammonium hydroxide, hydrochloric acid (HCl), D-glucose, 1-undecanethiol (1-UDET) (98%), 11-mercaptopundecanoic acid (11-MUDA) (95%), hexamethylenediamine (HMA) (98%), Ethanol (200-proof), and glycine (99.5%) were obtained from Sigma Aldrich. Ethanol (195-proof) was obtained from Pharmco Products Inc. All chemicals were used without further modification.

**2.1 Methods.** Preparation of SIFs. SIFs were deposited onto blank glass microscope slides by allowing them to soak in a heated silver nitrate/D-glucose solution as

previously described [11].

**2.2 Preparation of Solutions.** A 3.2 M aqueous solution of glycine was prepared by dissolving an appropriate amount of glycine solution slowly in water. Stirring and slight heating (30 °C) were applied to aid the preparation of this concentrated solution. The solution was then allowed to equilibrate to room temperature and then separated into three aliquots. The pH of the glycine solution was adjusted using 1 M NaOH and 1 M HCl to pH 4, 6 and 9. 0.5 mM solutions of 11-MUDA, and HMA were prepared by dissolving the appropriate amounts of solid in 200-proof ethanol. A solution of 0.5 mM of 1-UDET was prepared by pipetting the appropriate quantity of UDET into 200-proof ethanol and mixing until the solution was completely mixed.

**2.3 Preparation of Chemically Engineered Surfaces.** In order to investigate the effect of chemically engineered SIFs, SIFs were soaked in about 25 mL of 0.5 mM solution of 11-MUDA, HMA and 1-UDET separately, for 3 hrs. These slides, except for UDET-modified SIFs, were then removed for the respective solution and washed thoroughly using ethanol (195-proof)-deionized water-ethanol washing sequence to remove any unattached molecules. (All ethanol used for washing was 195-proof). For UDET-modified SIFs washing with deionized water stripped the monolayer and SIFs from the slide, subsequently, multiple ethanol washes were done to ensure this surfaces stayed intact.

**2.4 Glycine Crystallization.** In the experiments for the investigation of the effect of engineered surfaces on the rapid crystallization of glycine, the following procedure was carried out: a fixed volume (20  $\mu$ L) of freshly prepared glycine solution (pH 4, 6 or 9) at 30 °C was pipetted onto blank glass slide (control experiment) or blank SIFs or surface engineered SIFs and were either heated in a conventional microwave oven (Frigidaire, Model No: FCM09Z03KB, 0.9 cu.ft, 900 W output power, 100% power level) or incubated at room temperature (24  $\pm$  1 °C, control experiment). The total time taken for the solution to completely evaporate was recorded.

Glycine crystals were characterized by optical microscopy, Raman spectroscopy (iRaman, BW Tek, Inc, USA) and powder XRD. XRD data for glycine crystals placed in a capillary tube with thin walls (0.02 mm) were collected using an in-house X-ray generator (MicroMax 7, Rigaku/MS, The Woodlands, TX) and a Raxis4<sup>++</sup> image plate detector (Rigaku/MS), which is housed at the Core Facilities of the Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy. The distance between the detector and samples were kept constant at 75 mm. The radiation source was CuK (wavelength: 0.54 nm). The 2-D XRD data was collected at 0°  $\leq$   $\delta$   $\leq$  120° at values of 0°  $\leq$  2 $\theta$   $\leq$  40°.

The contact angles for the three glycine solutions (pH

4, 6 and 9) on the three engineered surfaces that contain SAMs of HMA, 11- MUDA and 1-UDET were measured using a Kruss Drop Shape Analysis System (DSA) Model Number 10 Mk2.

## 2.5 Effect of Sample volume.

To investigate the effect of sample volume on crystallization time, four different volumes, 5, 20, 10, 50 and 100  $\mu\text{L}$  of glycine solutions were pipetted onto HMA-, MUDA- and UDET-modified SIFs. The time taken for the complete evaporation of the solvent, during microwave heating and incubation at room temperature, was recorded. In eight control samples, the crystallization of glycine of sample volumes 5, 20, 10, 50 and 100  $\mu\text{L}$  were carried out on blank glass slides. Half of the samples were microwave heated and the other half were incubated at room temperature. For the glycine solution volumes of 50 and 100  $\mu\text{L}$ , silicon wells were placed on the slides to prevent the glycine solution from spilling off surfaces.

## 3 Results and Discussions

In our initial previous publication [7], we demonstrated the proof-of-principle of our new crystallization technique, MA-MAEC, using glycine, blank glass slides and unmodified SIFs. In the current work, we furthered our understanding of crystallization of glycine by studying the effect of different functional groups on SIFs, pH and volume of the initial solution. It is necessary to comment on the chemical nature of glycine for the sake of better understanding of the use of MA-MAEC based crystallization of glycine. Glycine is the simplest amino acid containing one carboxylic and amine groups and thus is frequently employed as a model amino acid in the demonstration of new crystallization techniques and tools. Glycine, like all amino acids, form peptide chains by a condensation reaction between an amine group and carboxylic group on two different amino acids to form an amide bond. Based on the pH of the glycine solution, these groups could be protonated or deprotonated, allowing electrostatic interactions to occur and different forms of glycine crystals are formed [3]. Taking the chemical nature of glycine (i.e., the presence of carboxylic and amine groups) into account, three functional groups,  $-\text{COOH}$ ,  $-\text{NH}_2$  and  $-\text{CH}_3$  were introduced to the SIFs using SAMs of MUDA, HMA and UDET respectively (Scheme 1). The assembly of glycine on each of these engineered surfaces is thought to be different:

i) The assembly of monolayer of glycine on MUDA-modified SIFs, which presents functional  $-\text{COOH}$  groups is expected to take place via electrostatic interactions between the  $\text{NH}_2$  groups of the glycine molecule and  $-\text{COO}^-$  (depending on the pH of glycine solution) groups on SIFs.

ii) The assembly of monolayer of glycine onto HMA-modified SIFs occurs via the interactions between the  $-\text{COO}^-$  groups of the glycine molecule and  $-\text{NH}_2$  groups













on SIFs.

iii) The assembly of initial glycine molecules onto UDET-modified SIFs is due to hydrophobic interactions between  $-\text{CH}_2/\text{CH}_3$  groups.

Subsequent glycine molecules assemble onto the monolayer of glycine molecules via electrostatic interactions between both the carboxylic and amine groups of glycine on all surface engineered SIFs. The functional groups on SIFs are thought to lower the interfacial energy between SIFs and glycine crystals, which promotes the heterogeneous nucleation of glycine crystals on SIFs. The presence of  $-\text{COOH}$ ,  $-\text{NH}_2$  and  $-\text{CH}_3$  groups on SIFs results in hydrophilic, moderately hydrophilic and moderately hydrophobic surfaces, respectively. It is important to characterize these surfaces to confirm that the surface modification process was carried out as intended. The hydrophilic/hydrophobic nature of the engineered surfaces was assessed by measuring the contact angle of a drop of 3.2 M glycine solution at pH 4, 6 and 9, and was shown in Table 1. The contact angle for all glycine solutions on a blank glass slide and SIFs were less than  $10^\circ$  (i.e., hydrophilic surface) and  $26$ – $29^\circ$  (moderately hydrophilic). Surface modification of SIFs with MUDA results in the presence of  $-\text{COOH}$  terminal functional groups (as depicted in Scheme 1), which are known to create a hydrophilic surface. The contact angle of glycine solutions on MUDA-modified SIFs is similar to that of blank glass (less than  $10^\circ$ ) at pH 4 and increases to  $14^\circ$  at pH 9, which proves the conversion of moderately hydrophilic SIFs into hydrophilic surfaces. On HMA-modified SIFs, the contact angle of glycine solutions is between  $38^\circ$ – $48^\circ$ , which implies a moderately hydrophilic surface due to the presence of  $-\text{NH}_3^+$  groups on SIFs. The attachment of UDET onto SIFs, which results in the presence of  $-\text{CH}_3$  terminal functional groups on SIFs, further increases the contact angle of glycine solutions up to  $58^\circ$  (moderately hydrophobic).

Subsequently, as shown in images placed in Table 1, initial glycine solution spreads over a larger area on blank glass slides and MUDA-modified SIFs (due to

**Table 1.** Contact angles of glycine solution of pH 4, 6, and 9 on blank glass slides and surface engineered SIFs. The insets show the digital image of the drop of glycine on the respective surfaces. The contact angles for SIFs were 26.0, 28.5, 29.0, respectively.

pH Glycine	Contact angle (glycine drop)		
	4	6	9
Glass (Blank)	< $10^\circ$ 	< $10^\circ$ 	< $10^\circ$ 
HMA	$38^\circ$ 	$43^\circ$ 	$48^\circ$ 
MUDA	< $10^\circ$ 	$13^\circ$ 	$15^\circ$ 
UDET	$47^\circ$ 	$53^\circ$ 	$58^\circ$ 

**Table 2.** Summary of results for the crystallization of glycine from 20 ml initial solution using MA-MAEC technique and control experiments.

pH	Microwave Heating Crystal morphology Time (3.2 M Glycine)			Room Temperature Crystal morphology Time (3.2 M Glycine)		
	HMA	MUDA	UDET	HMA	MUDA	UDET
4	N/A <sup>#</sup>	-	-	$\alpha$	$\alpha$ : $\gamma$	$\alpha$ : $\gamma$
	30 ± 6 sec	26 ± 3 sec	132 ± 17 sec	15 ± 1 min	21 ± 2 min	38 ± 5 min
6	$\alpha$	$\alpha$	-	$\alpha$ : $\gamma$	$\alpha$	$\alpha$ : $\gamma$ : $\beta$
	45 ± 1 sec	35 ± 3 sec	130 ± 17 sec	26 ± 1 min	17 ± 2 min	34 ± 0 min
9	$\alpha$ : $\gamma$	-	-	$\alpha$ : $\gamma$	$\alpha$ : $\gamma$	-
	68 ± 8 sec	30 ± 0 sec	60 ± 0 sec	37 ± 0 min	20 ± 0 min	62 ± 0 min

**Note:** Reported times refer to the complete evaporation of solvent. In most cases, glycine crystal appears before the complete evaporation of the solvent. Morphology of “as prepared” glycine crystals was determined from optical images using a minimum of 100 crystal count. Significant differences in the shapes of glycine polymorphs allows for visual determination to be carried out.

their hydrophilic nature) than HMA- and UDET-modified SIFs that are more hydrophobic. It is thought that the hydrophilic/hydrophobic nature of SIFs [9] will have significant effect on the crystallization of glycine. The more hydrophilic MUDA-modified SIFs and moderately hydrophilic HMA-modified SIFs afford for a quicker solvent evaporation time due to faster heat transfer to SIFs and more dispersed glycine crystals. The use of hydrophobic UDET-modified SIFs reduces the surface area of the initial glycine solution exposed to, hence the time for complete evaporation of solvent is increased and glycine crystals are grown on a smaller surface.

Table 2 summarizes the crystallization time and morphology of glycine crystals grown from 3.2 M glycine solutions at pH 4, 6, and 9 on HMA-, MUDA- and UDET-modified SIFs at room temperature and microwave heating (results for glass slides not shown to avoid repetition of previously published data). The morphology of the glycine crystals was determined by observing a minimum of ~100 crystals throughout the surface of SIFs using optical microscope images and SEM images (Fig. 1 and Fig. S1, Supporting Information). It is important to note that we did not attempt to quantify the purity of the glycine crystals from optical images due to the semi-quantitative nature of this technique. Fig. 1 also shows that the size of the glycine crystals grown on all surface engineered SIFs using microwave heating visually appears larger than those grown on the identical surfaces at room temperature. The size range for glycine crystals grown on all engineered SIFs will be discussed later in the text in conjunction with the effect of volume of initial glycine solution.

At pH 4, the glycine molecule exists as a positively charged ion due to the protonated amine group ( $-\text{NH}_3^+$ ). Since the isoelectric point of glycine is at about pH 6, it is a zwitterion and at pH > 6, its carboxylic group is deprotonated ( $-\text{COO}^-$ ). At room temperature (i.e. no

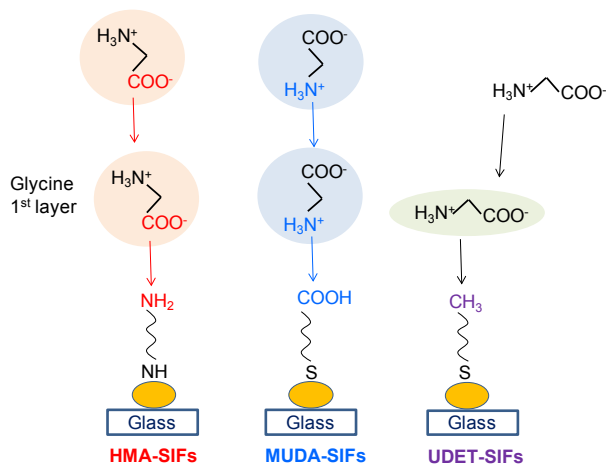
**Table 3.** The effect of sample volume on the length of glycine crystals grown from 3.2 M, pH = 6 initial solution at room temperature (RT) and using MA-MAEC technique.

Volume/ mL	Length of crystals / $\mu\text{m}$ Microwave Heating			Length of crystals / $\mu\text{m}$ Room Temperature		
	HMA	MUDA	UDET	HMA	MUDA	UDET
5	90-322	93-800	72-553	68-174	62-389	63-500
10	92-558	97-957	80-512	48-288	40-358	51-612
20	110-710	101-1020	116-788	52-400	90-350	70-785
50	177-1010	112-1200	152-1020	119-450	120-354	81-1250
100	64-950	110-574	N/A <sup>#</sup>	100-510	112-450	162-1650

microwave heating), for a fixed volume of 20  $\mu\text{L}$  solution of glycine at pH 4, 6 and 9, the total crystallization time (i.e., complete evaporation of solvent) on MUDA-modified SIFs was 21, 17 and 20 min, respectively. Glycine crystals grown on MUDA-modified SIFs were mostly  $\alpha$ -form (Fig. 1). The total evaporation time was drastically reduced with the introduction of microwave heating (i.e., MA-MAEC): 26-35 sec, which corresponds to a 30- to 48-fold decrease in crystallization time as compared to room temperature crystallization. Microwave heating also afforded for the increase in the purity of the glycine crystals: ~100% pure  $\alpha$ -glycine crystals were grown from initial glycine solution at pH = 6, which provided the best results for MA-MAEC-based crystallization of glycine in terms of production time and purity. Microwave heating of glycine solution at pH = 4 and 9 did not yield any discernible glycine crystals on surface engineered SIFs.

At room temperature and on moderately hydrophilic surfaces of HMA-modified SIFs, the total crystallization times were longer than that observed from MUDA-modified SIFs: 15, 26 and 37 min for initial glycine solution of pH 4, 6, and 9, respectively. Glycine crystals grown on HMA-modified SIFs were mostly  $\alpha$ - and  $\gamma$ -form (Fig. 1). The introduction of microwave heating of glycine solutions on HMA-modified SIFs resulted in complete evaporation of the solvent in 30-68 sec, which corresponds to a 30- to 34-fold decrease in crystallization time as compared to room temperature crystallization. On HMA-modified SIFs, ~100% pure  $\alpha$ -glycine crystals were grown from initial glycine solution at pH = 6 similar to MUDA-modified SIFs. The use of UDET-modified SIFs for the crystallization of glycine at room temperature resulted in the growth of glycine crystals from initial glycine solution at pH = 4 and 6, which was completed in 38 and 34 min, respectively. No discernible glycine crystals were observed at room temperature when the pH of the initial glycine solution was increased to 9. Although the solvent evaporated at quicker times (60-132 sec) using microwave heating, no glycine crystals were grown on UDET-modified SIFs. This observation can be attributed to the faster evaporation of solvent in combination with the lack of nucleation sites for glycine crystals. From these results one can deduce that the crystallization of glycine using MA-MAEC technique is more effective on MUDA- and HMA-modified SIFs.



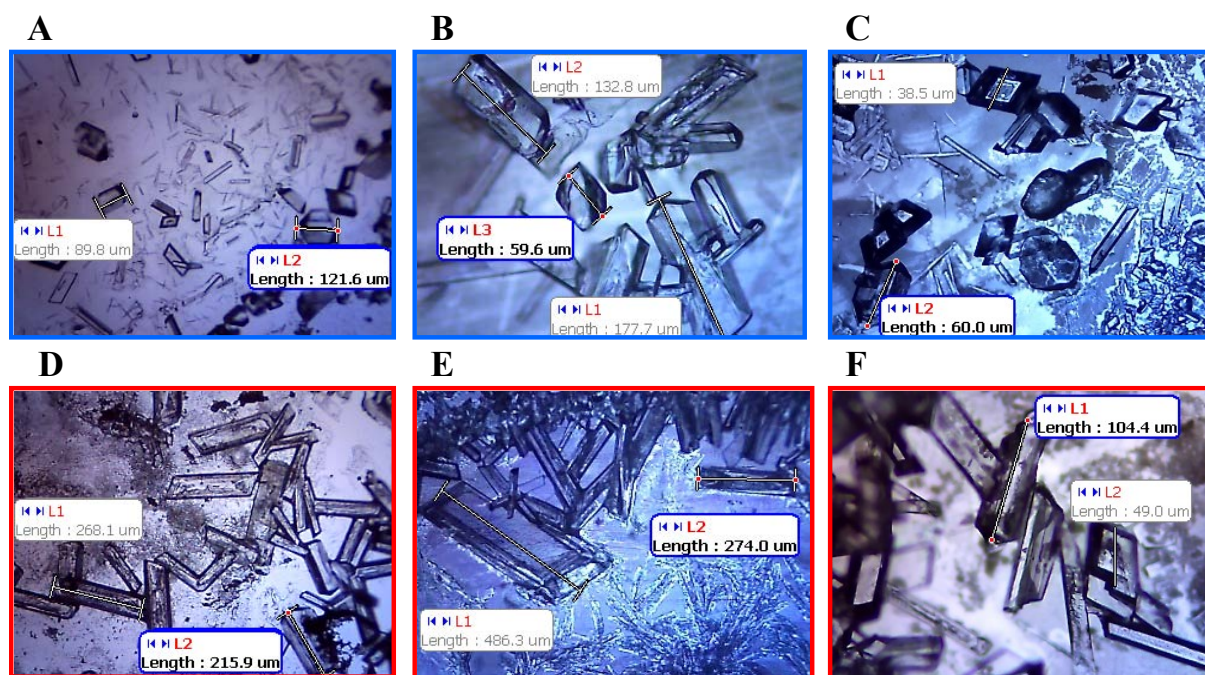


**Scheme 1.** Schematic depiction of proposed attachment mechanisms of glycine to surface engineered SIFs

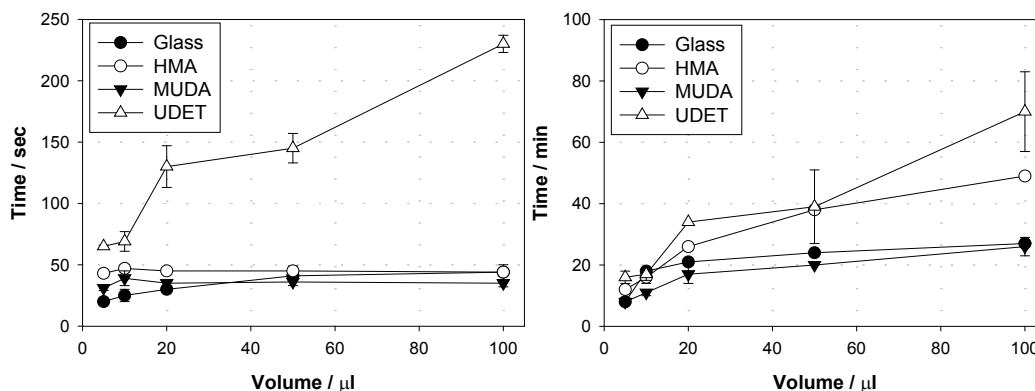
As it was described in our previous publications [7-9], the growth of amino acid crystals occur during the evaporation of the solvent and the complete evaporation of the solvent is not required in MA-MAEC-based crystallization. Since amino acid crystals nucleate, attach and grow on surfaced engineered SIFs during MA-MAEC-based crystallization, one can harvest amino acid crystals by removing the solvent at any time. In this study, the solvent is completely evaporated to investigate the full effect of solvent evaporation. In order to demonstrate that glycine crystals are indeed grown during microwave heating, optical images of the initial solution on surface engineered SIFs were intermittently taken by stopping the microwave heating process and capturing the microscope image within ~5-10 sec each time. These images are shown in Fig. S2 (Supporting Information). Glycine

crystals were observed after 10 seconds of microwave heating of initial glycine solution on MUDA-modified SIFs, while the appearance of glycine crystals takes a minimum of 5 minutes at room temperature. It took 40 seconds for the complete evaporation of solvent using MA-MAEC technique without significant visual changes to the glycine crystals form 20 to 40 seconds.

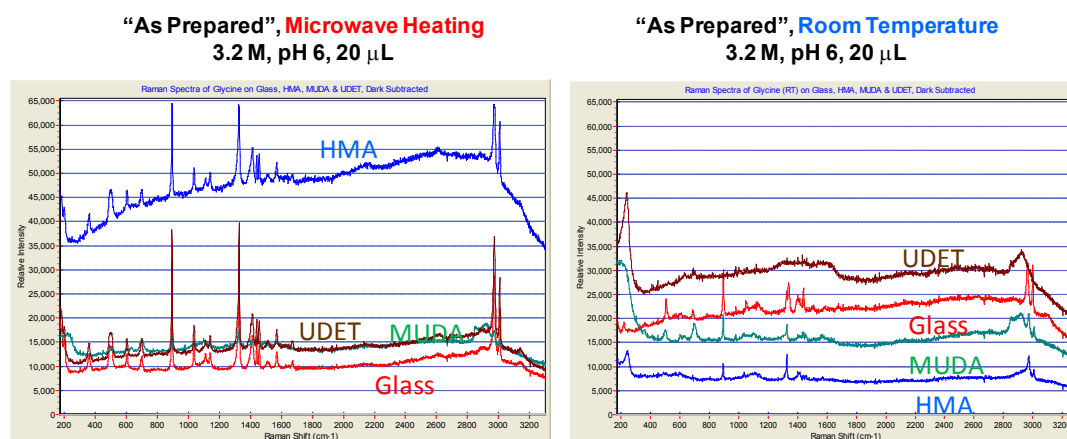
In our publications on MA-MAEC-based crystallization of amino acids to date [7-9], the volume of the initial amino acid solution was set to 20  $\mu$ L. In this work, the effect of the volume of initial glycine solution on the growth of glycine crystals was also investigated. In this regard, initial glycine solution volumes up to 20  $\mu$ L (5, 10 and 20  $\mu$ L) were used without modification to the surface engineered SIFs deposited onto glass slides (dimensions: Length x width = 75 x 25 mm<sup>2</sup>). For volumes greater than 20  $\mu$ L, commercially available silicone isolator with a single well were used to accommodate the initial glycine solution on surface engineered SIFs. Subsequently, 5, 10, 20, 50 and 100  $\mu$ L (largest possible volume for the silicone isolator used) of pH 6 glycine solution was pipetted onto the surface engineered SIFs and the total time taken for complete evaporation of solvent at room temperature and with microwave heating was recorded and is shown in Fig. 2. At room temperature, there was a slight increase in the total time for complete evaporation of solvent on MUDA-modified SIFs as the volume of the initial glycine solution was increased. The increase in total time was more pronounced on UDET- and HMA-modified SIFs. For UDET-modified SIFs, the total evaporation time increased from 19 minutes for 5  $\mu$ L of initial glycine solution to 70 minutes for 100  $\mu$ L of initial glycine solution. In MA-MAEC-based crystallization experiments, the total evaporation time was constant for



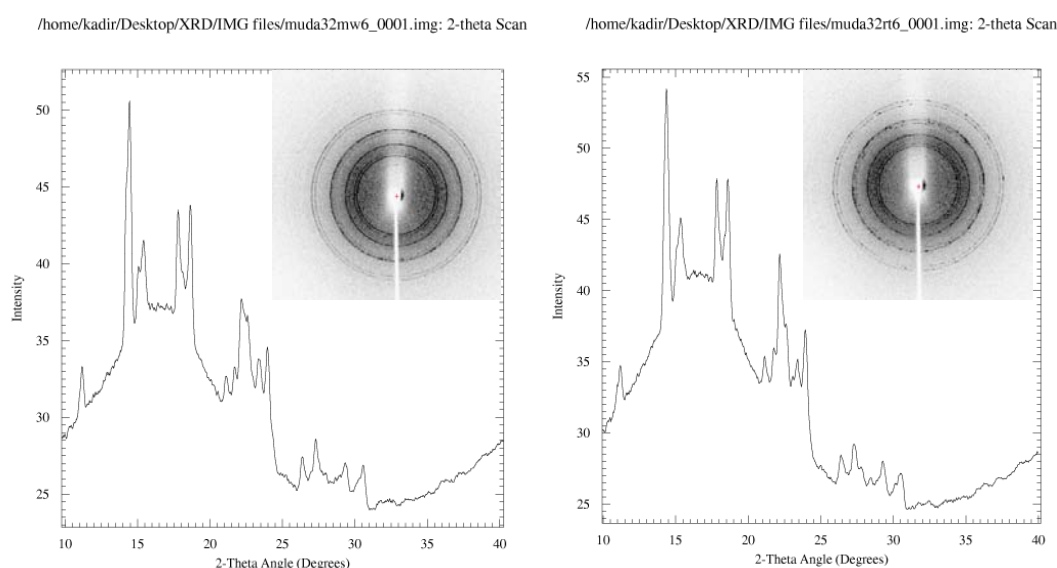
**Fig. 1.** Optical images of glycine crystals grown from 3.2 M solution at pH 6 on HMA-, MUDA-, and UDET-modified SIFs at room temperature (A, B and C, respectively) and using MA-MAEC technique (D, E and F, respectively).



**Fig. 2.** The effect of sample volume on the total evaporation time for glycine crystals grown from 3.2 M, pH =6 initial solution at room temperature (RT) and using MA-MAEC technique on glass and surface engineered surfaces.



**Fig. 3.** Raman spectrum of glycine crystals grown on blank glass slides and engineered surfaces at room temperature and using MA-MAEC technique. "As Prepared" refers to glycine crystals attached to the surfaces.



**Fig. 4.** Powder XRD of glycine crystals grown on MUDA-modified SIFs at room temperature and using MA-MAEC technique. "Powdered Crystals" refers to glycine crystals harvested from the surfaces and crushed to form powder.

HMA- and MUDA-modified SIFs. On UDET-modified SIFs, the total evaporation time increased from 60 seconds for 5  $\mu\text{L}$  to 235 seconds for 100  $\mu\text{L}$  of initial glycine solution. In addition to the total evaporation times, the size of the glycine crystals grown from initial glycine solution with different volumes was investigated and summarized in Table 3. In almost all cases, the increase in the volume of initial glycine solution resulted in an increase the range of glycine crystal size. At room temperature, glycine crystals of  $\sim 1.65$  mm size (grown from 100  $\mu\text{L}$  of initial glycine solution) were observed on UDET-modified SIFs, which corresponds to a  $\sim 3$ -fold increase in size as compared to glycine crystals grown from 5  $\mu\text{L}$  of initial glycine solution. In MA-MAEC-based crystallization experiments, the size range of glycine crystals was also increased as the volume of initial glycine solution was increased up to 50  $\mu\text{L}$  (decreased for 100  $\mu\text{L}$  samples). The growth of larger glycine crystals from larger volume solution can be attributed to the availability of larger amount of glycine molecules for crystal growth.

Characterization of glycine crystals grown on surface engineered SIFs was done using Raman spectroscopy and powder XRD. Fig. 3 shows the Raman spectra of glycine crystals on surface engineered SIFs at room temperature and with microwave heating. The Raman peaks obtained from glycine crystals on the surface engineered SIFs were comparable with those grown on blank glass slides (control experiment) room temperature and microwave heating. This observation proves that the glycine crystals grown on surface engineered SIFs and microwave heating were identical to those grown using conventional technique (blank glass slides and room temperature). The crystals were also characterized using powder XRD. As shown in Fig. 4 and Fig. S3-S6 (Supporting Information), the diffraction peaks obtained from glycine crystals on MUDA-modified SIFs at room temperature and microwave heating were identical. This observation provides further evidence that the MA-MAEC technique produces glycine crystals, which are comparable with other methods. Hence, one can grow glycine crystals using the MA-MAEC technique and surface engineered SIFs in a fraction of the time of the conventional evaporative crystallization technique without compromising the glycine crystal structure. Aslan Research Group is currently working on the development of a new crystallization platform designed for microwave heating, where one can crystallize 21 different samples "on demand" and all at once. The findings of these efforts to increase the capacity of crystal production will be published in due course.

## 4 Conclusions

The optimization of MA-MAEC technique for rapid crystallization of glycine by investigating the effect of surface functionality through engineered SIFs as well as the sample volume is reported. The functionalization of SIFs affected the solvent evaporation time and crystal

morphology. Using MUDA- and HMA-modified SIFs, the total evaporation time was decreased to 35 and 45 seconds, respectively. UDET-modified SIFs did not result in growth of glycine crystals using the MA-MAEC technique. This study demonstrated that hydrophilic MUDA- and HMA-modified SIFs are favored for the growth of glycine crystals using the MA-MAEC technique. Sample volume (in the range of 5-100  $\mu\text{L}$ ) did not affect the total crystallization time but resulted in increase in the size of the glycine crystals. Raman and powder XRD data showed that glycine crystals grown on surface engineered SIFs using MA-MAEC technique were identical to those grown using conventional evaporative crystallization technique.

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