Supporting Information

The Crystallization of Proteins at Ultra-low Supersaturations Using Novel 3D Nanotemplates

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Preparation of 3D Nanotemplate surfaces:

Unless stated separately, all chemicals used in this study were purchased from Sigma Aldrich Co., Dorset, UK and used without further purification. For preparation of surface having pore diameter 3-4nm sol-gel based methods reported previously in the literature was used[1]. In a typical synthesis, 16ml 2N HNO_3 (Sigma Aldrich Co., Product Code. 438073) was mixed with 54ml DI water and stirred for 30 minutes at room temperature. Once HNO_3 and water was well mixed, 56ml tetraethoxyorthosilicate (TEOS) (Sigma Aldrich Co., Product Code. 131903) was added to the solution and stirred at 350 rpm at room temperature until the solution becomes clear. Clear sol was then transferred to the soda-lime glass bottle moulds and hermitically sealed. The aging of the sol was carried out in a two step method. The temperature of the sol was raised from room temperature to 60^{o}C and sol was aged at this temperature for 48hours. In second step, the temperature of sol was increased from 60^{o}C to 130^{o}C and the sol was aged for 72hours. The samples so, aged were calcinated at 600^{o}C in a tube furnace under nitrogen environment to obtain mesoporous glass surfaces with 3-4nm pore diameter.

Further increase in the pore diameter was obtained using widely reported sacrificial template strategies[2]. Two triblock co-polymer surfactants; Pluronic P123 (Sigma Aldrich Co., Product Code 435365) and Pluronic F127 (Sigma Aldrich Co., Product Code P2443) were used as

sacrificial templates. In a typical synthesis, 1gm of Pluronic F127 (or 4gm of Pluronic P123) and 2.5gm KCl (Sigma Aldrich Co.) was added in 60ml 2M HCl (BDH Chemicals) solution. 120ml DI water was added in this mixture and the solution was stirred at room temperature for 24hours. After 24hours, 5ml TEOS was added and solution was stirred for 24hours. The sol so obtained was hydrothermally aged at 120^{o}C under autogeneous pressure for 48hours. The aged samples were filtered, washed with DI water and air dried for 24hours at ambient temperature. Dried samples were calcinated at 550^{o}C under nitrogen environment for 5hours to remove organic templates.

The mesoporous surfaces prepared, post calcinations, were characterized quantitatively using nitrogen sorption based methods, and qualitatively using transmission electron microscopy. Micromeritics ASAP Tristar 3000 (Micromeritics Instrument Corporation, USA) volumetric adsorption system was used to measure nitrogen adsorption and desorption isotherms. The adsorption, desorption isotherms and the nature of the hysteresis obtained were analyzed to determine the pore opening size, shape of the pores and nature of pore structures. JEOL 2010 (JEOL Ltd., Japan) (0.23nm resolution, $\pm$ 30^{o} tilt, 80-200kV) transmission electron microscope was used to analyze the samples prepared. The samples were dispersed in ethanol and subsequently deposited on a carbon-coated copper grid. The results obtained with the TEM measurements were used observe the pore arrangement and measure pore diameter.

Functionalization of Surfaces

Unless stated separately, all chemicals used in this study were purchased from Sigma Aldrich Co., Dorset, UK and used without further purification. In the present research in order to prepare the monolayer of the functional groups on the surface of the mesoporous silica, the

protocol proposed by Feng *et al.* was adopted and optimized[3]. 1.5gm of the mesoporous silica was suspended in 125ml of water. The mixture was continuously refluxed at 100^{o}C for 4hours. After cooling to room temperature, the solid was removed by filtration and dried in a vacuum oven at 60^{o}C for 24hours. Such hydrated samples was suspended in the 250ml toluene and stirred vigorously at room temperature for 2 hours followed by addition of 2.5ml organo-silane solution. After addition of organo-silane solution, the mixture was refluxed for 24hours at 100^{o}C. After cooling to room temperature, the reaction mixture was filtered and copiously washed with ethanol/ toluene to remove any unreacted silanes and dried under vacuum at 110^{o}C. The silane reagents used for functionalizing surface are listed in **Table S1** with the corresponding terminal groups.

Table S1 Details of the reagents used for preparation of functionalized monolayer on nanoporous glasses

	Functional Group	**Silane Reagent**
-	Dodecyl	Dodecyltriethoxy silane (Sigma Aldrich, UK) (Cat No. 44237)
-	Phenyl	Triethoxyphenylsilane (Fluka Analytical, Germany) (Cat No. 79223)
-	Methyl/Chloro	Dichlorodimethylsilane (Sigma Aldrich, UK) (Cat No. 440272)
-	Amino	(3-Aminoproply) triethoxysilane (Sigma Aldrich, UK) (Cat No. 281778)

Contact angle measurement was employed to characterize the wettability of the functionalized mesoporous surfaces. A Krüss Drop Shape Analyser DSA 10 (Krüss GmbH, Germany) was used for contact angle measurement. Water was used as probe liquid and advancing and receding contact angles were measured on the surface at ambient conditions. The tangent method was used to fit the shape of the droplet on both side of the droplet and analysis of the contact angle.

Crystallization of Proteins:

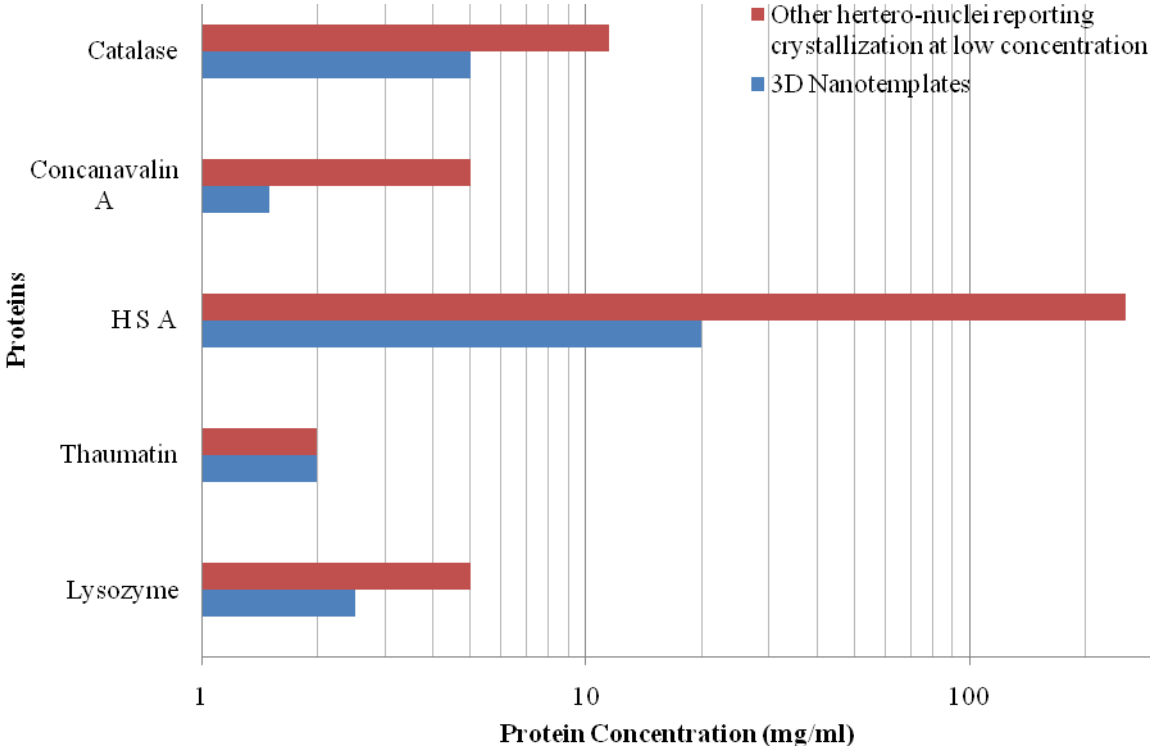
Unless stated separately, all chemicals used in this study were purchased from Sigma Aldrich Co., Dorset, UK and used without further purification. Vapor diffusion crystallization methods were used in this study for protein crystallization. For setting up the hanging drop vapor diffusion experiment, VDX 24 well crystallization plates were used. Wells of the crystallization plate was filled with 750µl of precipitant solution. The top raised edge of each well was applied with high vacuum silicon grease. Equi-volume droplet (5µl) of protein and precipitant was created on the mesoporous template surfaces, which was deposited onto a 19mm soda-lime glass coverslip (VWR, UK). The coverslips were carefully inverted and sealed on the top of the reservoir. These crystallization cells were then carefully placed in the temperature controlled incubator (Surface Measurement Systems, UK) at the prescribed temperature and the crystallization behavior was monitored. Details of the proteins used in this study can be obtained from **Table S2**.

Single crystal X-ray data was collected on crystals obtained on the surfaces of the 3D Nanotemplates. A Rigaku single crystal X-ray diffraction equipment was used for data collection, which was equipped with the Rigaku rotating anode x-ray generator operating at 40kV and 30mA. The data was recorded using CCD detector and indexed integrated and scaled with the open source analysis software from CCP4 (Collaborative Computational Project No. 4) and XtalView.

Table S2 Comparative analysis of the Protein Source used in the present study and the same used in the study referred in *Column 4* and *Column 6* of Table 3 of main text.

Protein	**Current Study – 3D Nanotemplates**		**Compared with Literature Reports (Nucleant with disordered porosity)** *(Column 4 Table:3)*		**Compared with Literature Reports (Nucleants with different surface chemistry)** *(Column 6 Table:3)*	
	Purchased From (Product Code)	*Purity*	*Purchased From (Product Code)*	*Purity*	*Purchased From (Product Code)*	*Purity*
Lysozyme	Sigma Aldrich (L6876)	≥90%	Sigma Aldrich (L6876)	≥90%	Sigma Aldrich (L6876)	≥90%
Thaumatin	Sigma Aldrich (T7638)	No data available	Sigma Aldrich (T7638)	No data available	Sigma Aldrich (T7638)	No data available
HSA	Sigma Aldrich (A1653)	≥96%	Not Crystallized before		Sigma Aldrich (A3782)	≥99%
Con A	Sigma Aldrich (C2010)	Highly Purified	Not Crystallized before		Sigma Aldrich (C7275)	Highly Purified
Catalase	Sigma Aldrich (C9322)	Highly Purified	Sigma Aldrich (C9322)	Highly Purified	No Comparable Data available	

Figure S1 Comparative analysis of lowest protein concentration reported in current study with the same reported as lowest protein concentration in the past.



References:

(1) Ahmad, M.; Jones, J. R.; Hench, L. L. *Biomedical materials* **2007**, *2*, 6.

(2) Zhao, D.; Feng, J.; Huo, Q.; Melosh, N.; Fredrickson, G. H.; Chmelka, B. F.; Stucky, G. D. *Science* **1998**, *279*, 548.

(3) Feng, X.; Fryxell, G. E.; Wang, L.-Q.; Kim, A. Y.; Liu, J.; Kemner, K. M. *Science* **1997**, *276*, 923.