Electronic Supplementary Material (ESI) for CrystEngComm.

Supporting Information

Proteins crystallization in short-peptide supramolecular hydrogels. A versatile strategy towards biotechnological composite materials.

Mayte Conejero-Muriel, Rafael Contreras-Montoya, Juan J. Díaz-Mochón,* Luis Álvarez de Cienfuegos* and José A. Gavira.*

E-mail: jgavira@iact.ugr-csic.es; juandiaz@ugr.es; lac@ugr.es

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Table S1. Summarized the average crystal size (±SD) of lysozyme, glucose isomerase and thaumatin grown in agarose and hydrogels **1**, **2**, **3** and **4** expressed in µm· µm. Three to seven crystals were used to calculated the average.

	Lysozyme	Glucose Isomerase	Thaumatin
Ag	608.32±89.84 x 506.66±133.88	258.69±3.076 x 199.99±6.144	459.99±85.11 x 222.22±10.18
1	344.34±78.58 x 251.3±63.94	48.1±6.43 x 39.80±7.19	143.47±45.65 x 69.56±5.75
2	394.48±184.35 x 208.00±46.34	195.64±34.51 x 199.99±51.26	427.48±108.89 x 203.03±82.59
3	167.82±24.13 x 143.47±27.65	346.12±43.42 x 325.13±26.58	392.98±51.11 x 177.19±10.95
4	489.56±48.47 x 402.60±86.33	209.44±24.07 x 170.55±17.43	143.47±4.34 x 59.39±2.48

Table S2. Summarized X-ray data collection configuration for each protein.

Data Acquisition	**Lysozyme**	**Glucose Isomerase**	**Thaumatin**	**Insulin**	**Formamidase**
Beam-line	Xaloc (ALBA)	Xaloc (ALBA) ID23-1 (ESRF)	Xaloc (ALBA)	Xaloc (ALBA)	Xaloc (ALBA)
Detector type	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M
Wavelength (Å)	0.9794	0.9794	0.9794	0.9794	0.9794
Distance (mm)	128.17	128.127	128.127	298.61	128.127
Exposure time (s)	0.2	0.2	0.2	0.2	0.5
Oscillation (^{o})	0.25	0.25	0.25	0.25	0.5

Table S3. Summarized the data collection conditions and final statistical values of lysozyme crystals grown in hydrogels **3, 3b** and **4** (data in brackets correspond to high resolution shell).

Gel type	**3 (1)**	**3 (2)**	**3 (3)**	**3b (1)**	**3b (2)**	**3b (3)**	**4 (1)**	**4 (2)**	**4 (3)**
Data Statistics									
Space group	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2
Unit cell a=b, c (Å)	*78.85, 37.08*	*78.75, 37.07*	*78.48, 37.10*	*78.94, 37.09*	*78.97, 37.09*	*79.0, 37.14*	*78.43, 37.09*	*78.09, 37.2*	*78.33, 37.15*
Resolution (Å)	39.42-1.10	39.37-1.05	39.24-1.00	39.47-1.00	39.49-1.00	39.50-1.00	39.21-1.10	39.04-1.15	39.17-1.25
(High shell)	(1.12-1.10)	(1.07-1.05)	(1.02-1.00)	(1.02-1.00)	(1.02-1.00)	(1.02-1.00)	(1.12-1.10)	(1.17-1.15)	(1.27-1.25)
Unique reflections	48012 (2359)	54885 (2629)	63010 (3046)	63734 (3081)	63788 (3096)	63904-3105	47511 (2304)	41465 (2021)	32616 (1596)
R-merge * (%)	3.9 (69.2)	4.2 (67.2)	4.1 (78.3)	4.3 (50.2)	4.9 (37.0)	4.6 (36.1)	4.0 (52.4)	5.0 (80.0)	5.1 (61.1)
I/σ(I)	42.2 (6.0)	39.8 (5.4)	41.6 (4.2)	40.9 (6.6)	41.9 (9.4)	43.1 (9.3)	41.8 (6.3)	29.6 (3.7)	31.9 (5.5)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (99.8)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Redundancy	24.5 (24.9)	24.6 (24.0)	24.2 (22.4)	24.3 (22.7)	24.2 (22.8)	24.3 (22.6)	24.4 (24.5)	23.7 (23.8)	24.1 (25.5)
B-factor (Å^2)	11.3	10.9	10.8	9.0	7.7	8.3	12.2	13.0	12.6
Mosaicity	0.09	0.07	0.11	0.08	0.08	0.11	0.11	0.20	0.15

*R-merge = $\Sigma_{hkl}\Sigma_i \left| I_i(hkl) - (I(hkl)) \right| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $(I(hkl))$ is the weighted average intensity for all observations i of reflection hkl.

Table S4. Summarized the data collection conditions and final statistical values of glucose isomerase crystals grown in hydrogels **3, 3b** and **4** (data in brackets correspond to high resolution shell).

Gel type	**3 (1)**	**3 (2)**	**3b**	**3b (2)#**	**3b (3)#**	**4 (1)**	**4 (2)**	**4 (3)**
Data Statistics								
Space group	I 2 2 2	I 2 2 2	I 2 2 2	I 2 2 2	I 2 2 2	I 2 2 2	I 2 2 2	I 2 2 2
Unit cell a, b, c (Å)	92.79, 98.58, 102.39	93.51, 99.49, 103.30	*93.21, 98.88, 102.6*	*92.82, 98.11, 102.45*	*92.82, 97.98, 102.18*	*92.93, 98.59, 102.64*	*93.15, 98.59, 102.84*	*92.88, 98.75, 102.58*
Resolution (Å) (High shell)	46.39-1.37 (1.39-1.37)	71.66-1.70 (1.73-1.70)	46.61-1.05 (1.07-1.05)	40.79-1.00 (1.02-1.00)	46.41-1.00 (46.41-1.00)	46.46-1.00 (1.02-1.00)	46.58-1.00 (1.02-1.00)	46.44-1.00 (1.02-1.00)
Unique reflections	98347 (4821)	53201 (2785)	218305 (10727)	244995 (11525)	246581 (11939)	251657 (12292)	252585 (12271)	251979 (12410)
R-merge * (%)	11.0 (94.1)	18.3 (94.3)	4.9 (81.3)	3.6 (20.4)	4.1 (51.0)	3.9 (44.6)	4.2 (77.5)	5.0 (58.3)
I/σ(I)	14.6 (3.2)	8.8 (2.5)	11.6 (1.4)	20.5 (5.3)	14.0 (2.0)	29.9 (5.0)	25.1 (3.2)	23.4 (3.9)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	99.7 (99.8)	98.2 (94.2)	99.2 (97.8)	99.9 (99.3)	99.9 (98.9)	100.0 (100.0)
Redundancy	12.2 (12.0)	10.2 (8.8)	4.9 (4.7)	4.4 (4.2)	4.4 (4.0)	12.5 (11.7)	12.5 (11.8)	12.5 (11.7)
B-factor ($Å^2$)	11.5	12.4	8.8	6.8	6.9	8.2	8.4	8.2
Mosaicity	0.25	0.46	0.17	0.04	0.06	0.08	0.10	0.09

*R-merge = $\Sigma_{hkl}\Sigma_i \left| I_i(hkl) - (I(hkl)) \right| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $(I(hkl))$ is the weighted average intensity for all observations i of reflection hkl.

Data collected at ESRF ID23-1

Table S5. Summarized the data collection conditions and final statistical values of thaumatin crystals grown in hydrogels **1, 2, 3** and **4** (data in brackets correspond to high resolution shell).

Gel type	**1 (1)**	**1 (2)**	**1 (3)**	**2 (1)**	**2 (2)**	**2 (3)**	**3 (1)**	**3 (2)**	**4 (1)**	**4 (2)**	**4 (3)**
Data Statistics											
Space group	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2
Unit cell a=b, c (Å)	*57.91, 150.33*	*57.92, 150.27*	*57.99, 150.47*	*57.84, 50.27*	*57.87, 150.38*	57.87, 150.28	57.89, 150.32	57.86, 150.42	*57.99, 150.50*	*58.07, 150.59*	*57.96, 150.36*
Resolution (Å) (High shell)	45.88-1.05 (1.07-1.05)	45.87-1.05 (1.07-1.05)	45.93-1.05 (1.07-1.05)	45.83-1.00 (1.02-1.00)	45.86-1.05 (1.07-1.05)	45.85-1.03 (1.05-1.03)	45.86-1.10 (1.12-1.10)	45.86-1.35 (1.37-1.35)	45.93-1.32 (1.34-1.32)	45.98-1.35 (1.37-1.35)	45.90-1.35 (1.37-1.35)
Unique reflections	119964 (5831)	119594 (5738)	120426 (5886)	138131 (6712)	119837 (5836)	126759 (6196)	104543 (5107)	57193 (2767)	61376 (2964)	57640 (2765)	57357 (2761)
R-merge * (%)	4.9 (73.6)	5.8 (99.3)	7.0 (82.7)	4.5 (83.0)	5.1 (74.2)	5.5 (99.8)	6.3 (86.3)	8.8 (74.3)	7.1 (83.2)	8.0 (80.1)	7.9 (78.6)
I/σ(I)	33.8 (5.0)	30.1 (4.2)	23.7 (4.0)	36.3 (4.4)	32.9 (5.3)	30.2 (3.6)	28.7 (4.6)	27.7 (6.2)	28.3 (5.0)	25.2 (5.1)	28.1 (6.0)
Completeness (%)	100.0 (100.0)	99.8 (98.7)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Redundancy	24.6 (24.3)	24.6 (24.2)	24.4 (23.9)	24.5 (23.1)	24.6 (24.0)	24.4 (22.5)	24.7 (24.8)	24.9 (24.8)	24.7 (24.6)	24.4 (24.6)	24.7 (25.0)
B-factor ($Å^2$)	9.1	8.6	8.6	8.8	9.1	8.9	9.0	8.7	10.4	11.3	10.0
Mosaicity	0.08	0.07	0.09	0.06	0.07	0.08	0.09	0.08	0.10	0.15	0.09

*R-merge = $\Sigma_{hkl}\Sigma_i \left| I_i(hkl) - (I(hkl)) \right| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $(I(hkl))$ is the weighted average intensity for all observations i of reflection hkl.

Table S6. Summarized the data collection conditions and final statistical values of insulin crystals grown in hydrogels **1, 2** and agarose (data in brackets correspond to high resolution shell).

Gel type	**Ag (1)**	**Ag (2)**	**Ag (3)**	**1 (1)**	**1 (2)**	**1 (3)**	**2 (1)**	**2 (2)**	**2 (3)**
Data Statistics									
Space group	H3		H3	H3	H3	H3	H3	H3	H3
Unit cell a=b, c (Å)	*81.48, 33.71*	*81.52, 33.70*	*81.30, 33.68*	*81.60, 33.67*	*81.55, 33.65*	*81.75, 33.71*	*81.63, 33.71*	*81.52, 33.65*	*81.69, 33.62*
Resolution (Å)	40.74-1.50	40.76-1.50	40.65-1.50	40.80-1.50	40.77-1.50	40.88-1.50	30.43–1.55	40.76-1.55	40.84-1.55
(High shell)	(1.53-1.50)	(1.53-1.50)	(1.58-1.50)	(1.53-1.50)	(1.53-1.50)	(1.53-1.50)	(1.58-1.55)	(1.58-1.55)	(1.58-1.55)
Unique reflections	13011 (524)	12841 (462)	12870 (493)	13195 (581)	13084 (539)	13112 (543)	10955 (496)	11849 (475)	11982 (507)
R-merge * (%)	2.1 (4.5)	2.7 (8.0)	3.2 (19.3)	2.5 (7.2)	3.0 (20.3)	4.7 (13.8)	5.0 (17.7)	3.6 (16.3)	12.1 (24.7)
I/σ(I)	36.4 (12.3)	27.0 (8.1)	19.3 (3.6)	30.7 (9.6)	20.3 (3.2)	17.8 (5.5)	13.6 (4.5)	19.2 (5.1)	7.1 (3.4)
Completeness (%)	97.3 (77.9)	96.0 (69.0)	96.8 (74.0)	98.5 (86.5)	97.9 (80.6)	97.4 (79.6)	90.1 (81.6)	97.9 (78.5)	98.7 (84.8)
Redundancy	3.9 (2.3)	3.9 (2.6)	3.9 (2.4)	3.8 (2.0)	3.7(1.9)	3.7 (2.0)	2.9 (1.9)	4.0 (2.9)	3.8 (2.8)
B-factor ($Å^2$)	12.0	14.0	15.7	11.6	14.8	10.7	14.5	16.5	12.0
Mosaicity	0.09	0.24	0.21	0.17	0.33	0.22	0.20	0.23	0.23

*R-merge = $\Sigma_{hkl}\Sigma_i \left| I_i(hkl) - (I(hkl)) \right| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $(I(hkl))$ is the weighted average intensity for all observations i of reflection hkl.

Table S7. Summarized the data collection conditions and final statistical values of formamidase crystals grown in hydrogel **1** (data in brackets correspond to high resolution shell).

Gel type	**1 (1)**	**1 (2)**	**1 (3)**
Data Statistics			
Space group	P622	P622	P622
Unit cell a=b, c (Å)	*159.04, 149.27*	*160.67, 151.30*	*158.87, 149.69*
Resolution (Å)	49.16-2.80	49.68-2.70	49.12-2.70
(High shell)	(2.95-2.80)	(2.83-2.70)	(2.83-2.70)
Unique reflections	27481 (3975)	32162 (4200)	30482 (4014)
R-merge* (%)	17.9 (98.9)	20.5 (90.2)	18.4 (80.9)
I/σ(I)	10.5 (2.1)	9.1 (2.6)	15.4 (4.0)
Completeness (%)	98.7 (99.4)	99.9 (100.0)	98.4 (99.1)
Redundancy	8.3 (8.3)	7.9 (8.2)	13.9 (14.2)
B-factor ($Å^2$)	37.5	27.1	28.2
Mosaicity	0.16	0.15	0.14

*R-merge = $\Sigma_{hkl}\,\Sigma_i \left| I_i\,(hkl) - (I(hkl)) \right| / \Sigma_{hkl}\Sigma_i\, I_i\,(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and ($I(hkl)$) is the weighted average intensity for all observations i of reflection hkl.

Figure S1. Time-lapse dissolution experiments of lysozyme (A), glucose isomerase (B) and thaumatin (C) crystals obtained in solution.

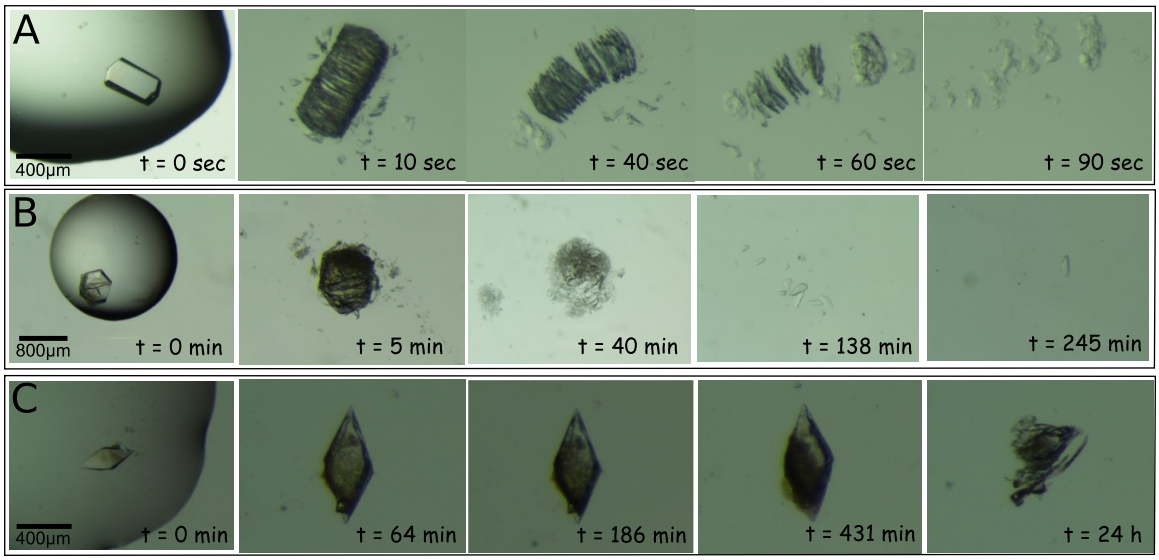

Figure S2. Time-lapse dissolution experiments of glucose isomerase crystals obtained in agarose (A), hydrogel **1** (B), **2** (C), **3** (D) and **4** (E). First column shows cleaned crystals in a 2 µL isotonic precipitant solution. Note the bar-size scale at the bottom left of the first pictures.

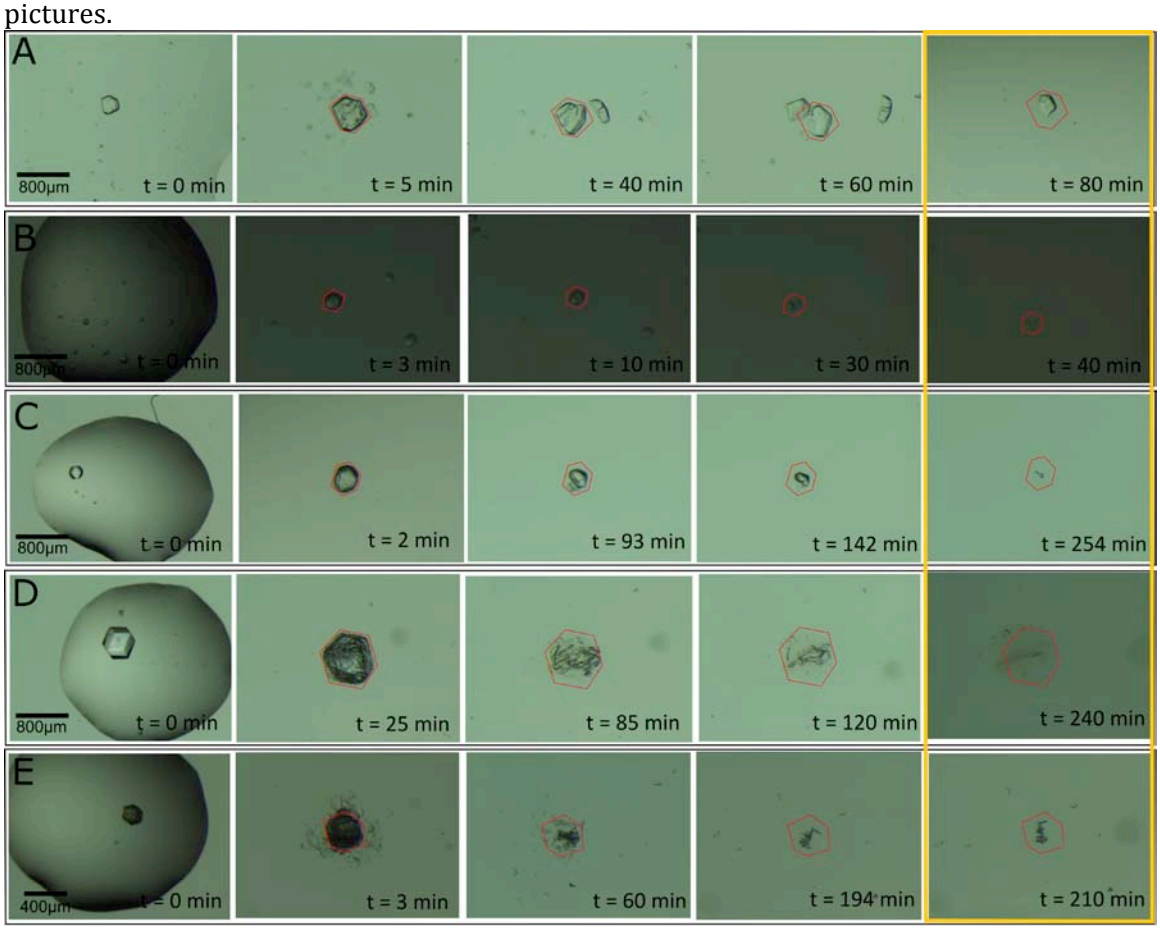

Figure S3. Time-lapse dissolution experiments of thaumatin crystals obtained in agarose (A), hydrogel **1** (B), **2** (C), **3** (D) and **4** (E). First column shows cleaned crystals in a 2 μL isotonic precipitant solution. Note the bar-size scale at the bottom left of the first pictures.

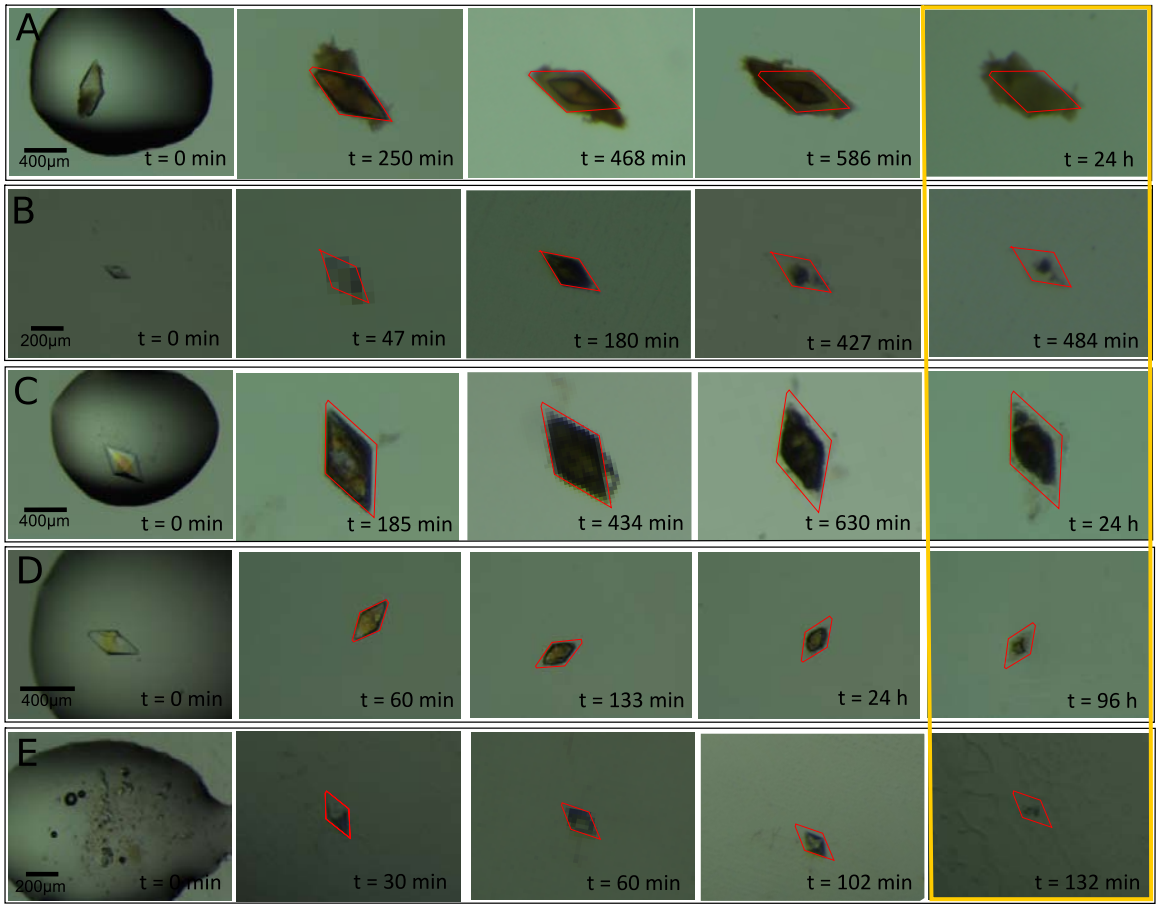

Table S8. Summarized dissolution experiments of lysozyme, glucose isomerase and thaumatin crystals grown in solution, agarose and hydrogels **1**, **2**, **3** and **4**. Average values for glucose isomerase and thaumatin are plotted in Figure S4 A and B respectively.

	Gel-free	**Ag**	**1**	**2**	**3**	**4**
Lysozyme	2.8 ±1.13min	5.5 ±0.91min	4.1 ±1.02min	4.125 ±1.10min	3.4 ±1.19min	139.6 ±52.44min
Glucose Isomerase	306 min	238 min	65 min	294 min	108.57 min	65 min
Thaumatin	24 h	24 h	12.9 h	24 h	96 h	2.2 h

Figure S4. Average dissolution time of glucose isomerase (A) and thaumatin (B) crystals grown in hydrogel free solution and in hydrogels including agarose.

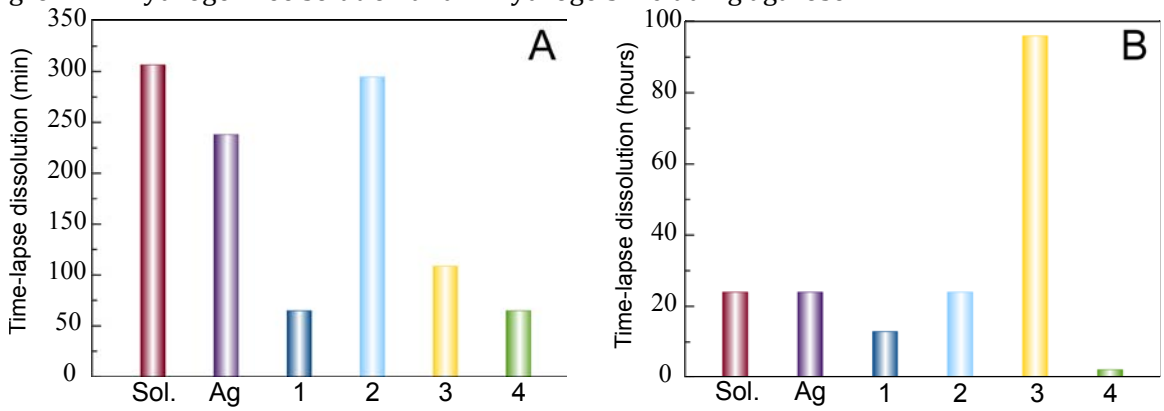

Figure S5. Crystals of lysozyme (a), thaumatin (b) and glucose isomerase (c-d) grown in hydrogels **4**, **2** and **3**, respectively in Eppendorf tubes by a two-layer counterdiffusion configuration. Picture (d) displays glucose isomerase crystals grown in gel **3** extracted to be fished-out for X-ray characterization.