Supplementary Materials for
Amorphous aggregates with a very wide size distribution play a central role in crystal nucleation

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Table S1. *Fitting parameters for the distributions shown in Fig. 1(d) of the main text.*

|	A_1	τ_1	β_1	A_2	τ_2	β_2	R^2
20°C	0.617668	2.33869×10^{-6}	0.89539	0.112	0.000266301	0.82	0.999915
30°C	0.60059	1.86572×10^{-6}	0.895285	0.143	0.000247653	0.86	0.999838
40°C	0.612798	1.41361×10^{-6}	0.89475	0.119	0.000164014	0.84	0.999891
50°C	0.667717	1.04892×10^{-6}	0.832901	0.0916	0.000139757	0.92	0.999805
60°C	0.670747	8.44093×10^{-7}	0.865552	0.052	0.000104783	0.93	0.999585
70°C	0.73944	5.7833×10^{-7}	0.817092	0.0176	0.000114066	0.91	0.999589

Table S2. *Aggregate formation in supersaturated solution. Summary of aggregate formation and laser-induced nucleation in the investigated amino acids and peptides demonstrating the general nature of the formation of aggregates and their role in laser-induced nucleation.*

|	Solubility in H_2O at 298 K[1]	Prepared in D_2O/H_2O at 293 K (pD/pH)[a]	Aggregate seen under microscope	Laser-induced nucleation
L-histidine	0.0436 g/mL	0.13 g/mL (7.72)	Y	Y
L- phenylalanine	0.028 g/mL	0.05 g/mL (6.23)	Y	Y
L-threonine	0.0979 g/mL	0.2 g/mL (6.20)	Y	N
L-arginine	0.1959 g/mL	0.3 g/mL (11.63)	N	N
L-alanine	0.1663 g/mL	0.18 g/mL (5.74)	Y	Y
L-lysine	0.2466 g/mL	0.5 g/mL (10.72)	Y	Y
L-serine	0.3657 g/mL	0.4 g/mL (6.00)	Y	Y
L-proline	1.3 g/mL	1.7 g/mL (7.44)	Y	N
L-glutamic acid	0.0425 g/mL	0.13 g/mL (7.02)	Y	Y
Ala-Ala	0.29 g/mL[2]	0.3 g/mL (5.74)	Y	Y
Gly-Gly	0.195 g/mL[2]	0.2 g/mL (5.89)	Y	Y
Gly-Gly-Gly	0.061 g/mL[2]	0.1 g/mL (5.78)	Y	Y

[a]Values of pD/pH are measured at 293 K after preparation of solutions.
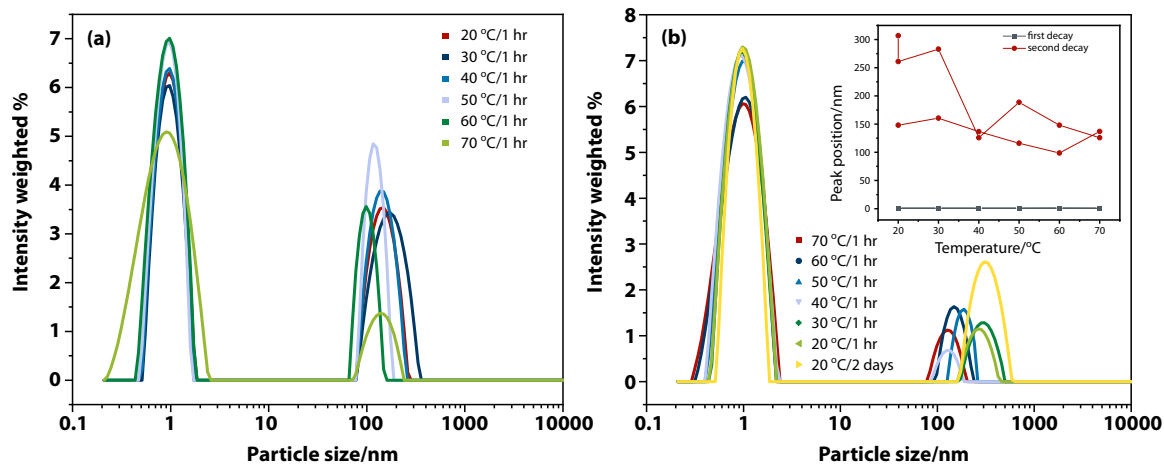
Fig. S1. Intensity weighted size distribution of the intensity correlation functions shown in Fig. 1. **(a)** Warm up from 20°C to 70°C; **(b)** Cooling down from 70°C to 20°C. The inset shows the changes in the peak position as a function of temperature.
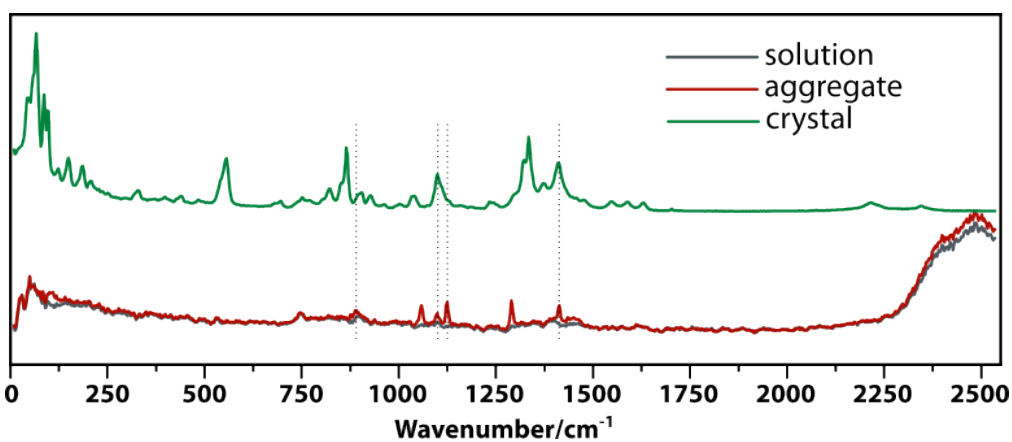

Fig. S2. Raman spectrum of threonine aggregates in solution compared to the surrounding solution and crystal.
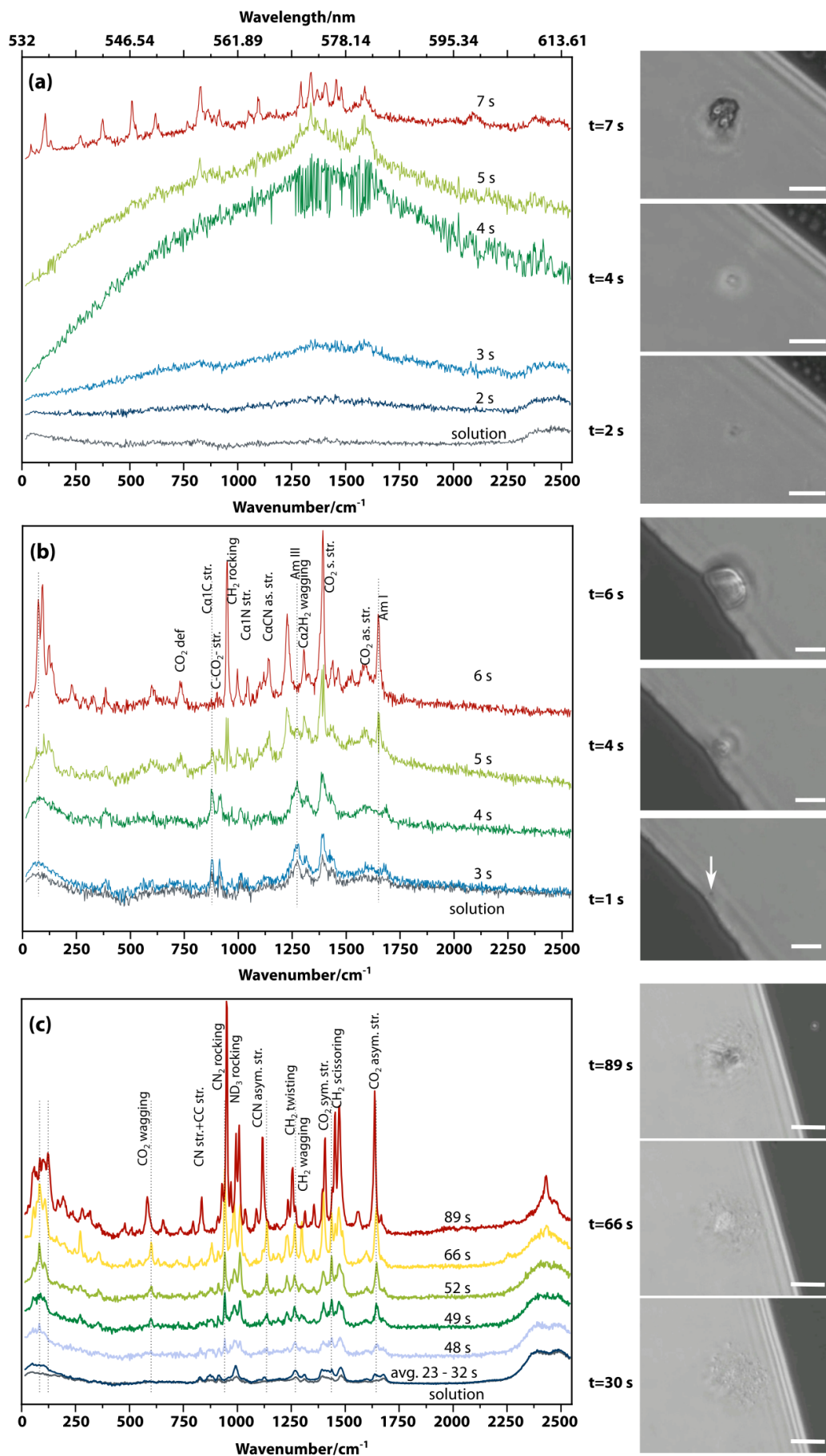
Fig. S3. Amorphous aggregates are essential to laser-induced nucleation in amino acid and peptide solutions. Aggregate-assisted laser-induced nucleation from solution: **(a)** alanine, **(b)** Gly-Gly and **(c)** Gly-Gly-Gly. Shown are time-dependent Raman and luminescence spectra on the left, along with phase-contrast microscopy images at selected times on the right (scale bars, 5 μm). The burst of luminescence in (a) at approximately 4 s and in (c) at approximately 66 s is likely due to crystalloluminescence.[3]

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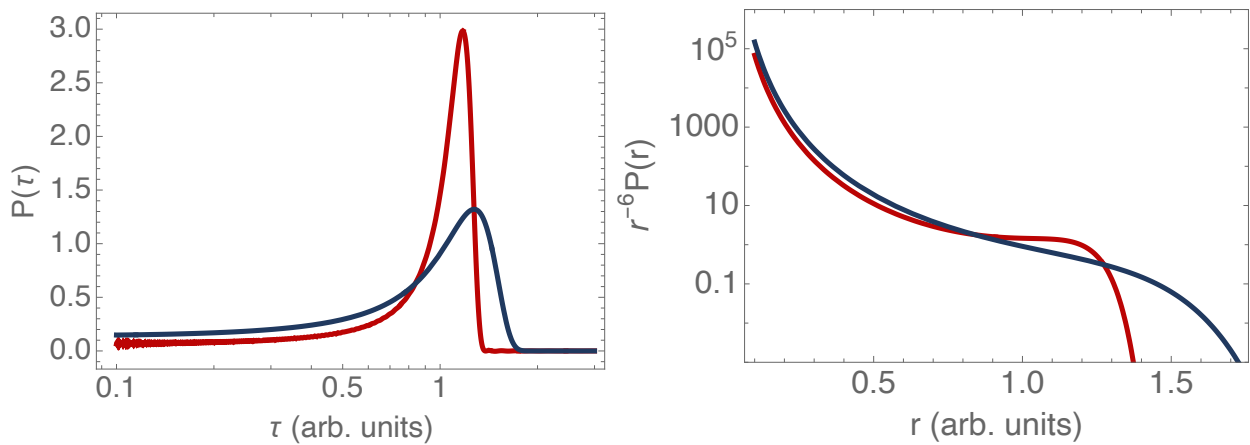
Fig. S4. Relaxation time *vs*. particle size distribution. **(left)** The relaxation-time (τ) distribution corresponding to a stretched exponential with overall relaxation time $\tau_0 = 1$ for stretch parameters $\beta = 0.95$ (red) and $\beta = 0.9$ (blue). The relaxation time is related to the particle size through the Stokes-Einstein law, $r \propto \tau$. **(right)** Particle size (r) distribution for the same parameter values (see main text for explanation).
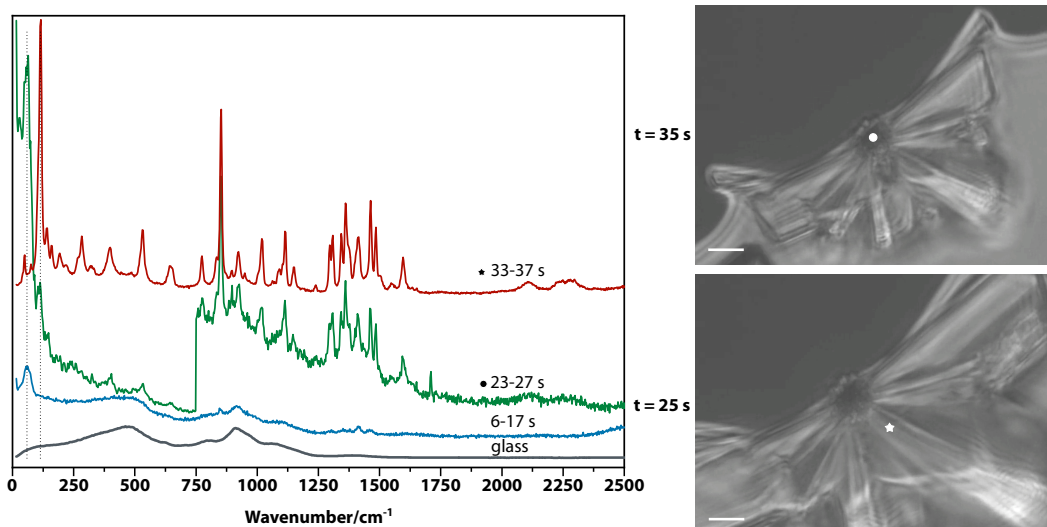
Fig. S5. Aggregate-assisted laser-induced nucleation of alanine from solution. Averaged Raman spectra are shown on the left, along with microscopy images at selected times on the right. The green spectrum is 5× magnified from 750 – 2500 cm^{-1} for comparison with better clarity; dot and star signs indicate the locations where spectra are taken. Scale bar, 5 µm.

Supplementary note
Classical nucleation theory (CNT)

The basic Gibbs equation

Homogeneous crystal nucleation begins with the growth of a nucleus with radius r. The free energy is the sum of a term proportional to the surface area and a term proportional to the volume. Thus, the change in free energy for a spherical nucleus is given by[4]

$$\Delta G(r) = \frac{4}{3}\pi r^3 \Delta G_f + 4\pi r^2 \gamma_{sl}, \qquad (S1)$$

Where ΔG_f is the change in Gibbs free energy per unit volume of the solid phase *vs.* the liquid/solution phase (the free energy of formation) and γ_{sl} the interfacial energy (or surface free energy or interfacial tension). A typical interfacial energy is about 10^{-3} J/m^2. ΔG_f can be written in terms of the latent heat (heat of fusion) as

$$\Delta G_f = -\frac{\Delta H_f \Delta T}{T_f}, \qquad (S2)$$

where $\Delta T = T_f - T$ is the degree of supercooling (supersaturation) and T_f the melting (liquidus) temperature. It can also be written in terms of the supersaturation as

$$\Delta G_f = -\frac{k_B T}{\Omega} \ln S, \qquad (S3)$$

where Ω is the molecular volume in the crystal (volume of 1 mol of substance), and S the supersaturation here defined by

$$S \equiv \frac{C}{C_S}, \qquad (S4)$$

where C is the concentration of the solute and C_S the saturation concentration or solubility. The molecular volume can be calculated from

$$\Omega = \frac{M}{1000\rho N_A} \qquad (S5)$$

(in m^3/molecule), where M is the molar mass (in g/mol), and ρ the density of the crystal (in kg/m^3). Thus, when the concentration is equal to the equilibrium concentration ($S = 1$), $\Delta G_f = 0$, whereas in a supersaturated solution ($C > C_0$), ΔG_f is negative favouring nucleation.

Hence, the equation for the change in free energy for a spherical nucleus with radius r (Eq. (S1)) can be written in terms of the supersaturation as

$$\Delta G(r) = -k_B T \frac{4\pi r^3}{3\Omega} \ln S + 4\pi r^2 \gamma_{sl}. \qquad (S6)$$

The number of molecules contained in the nucleus is

$$n_{nucleus} = \frac{4\pi r^3}{3\Omega}. \qquad (S7)$$

Thus, the change in free energy per nucleus can also be written as

$$\Delta G(n_{nucleus}) = -k_B T n_{nucleus} \ln S + 6^{2/3}\pi^{1/3}\left(n_{nucleus}\Omega\right)^{2/3}\gamma_{sl}. \qquad (S8)$$

Here, we propose that the free energy of formation can be written as

$$\Delta G_f / \Delta G_f^0 = (1-\delta)e^{-r/\lambda} + \delta \qquad (S9)$$

where λ is the fractal correlation length and δ accounts for the possibility that the free energy of formation decays to a finite value. Using Eq. (S9) with (S3) and (S6), we get the expression

$$\Delta G(r) = -k_B T \frac{4\pi r^3}{3\Omega}\left[(1-\delta)e^{-r/\lambda} + \delta\right]\ln S + 4\pi r^2 \gamma_{sl} \qquad (S10)$$

Typical parameters for an organic molecule are those of glycine, which has a molecular volume of 0.107 nm^3. Typical interfacial tensions range from about 0.1 to 10 mJ/m^2. A reasonable supersaturation is S=1.1, so a reasonable value for ΔG_f is -3.9 10^6 J/m^3. Some typical average numbers are given in the table below.

T	298 K
Ω	0.1 nm^3 (10^{-28} m^3)
γ	1 mJ/m^2 (10^{-3} J/m^2)
$-\Delta G_f$	1 MJ/m^3 (10^6 J/m^3)

Reduction of S during crystal growth

When a crystal starts growing, the supersaturation parameter must of course decrease. The number of molecules available in the solution at the start is,

$$n_{solution,0} = C_0 10^3 V N_A \qquad (S11)$$

and hence the number of molecules in the (remaining) solution is

$$n_{solution,0} - n_{nucleus} = C_0 10^3 V N_A - \frac{4\pi r^3}{3\Omega}. \qquad (S12)$$

The concentration of the (remaining) solution is

$$C = \frac{n_{solution,0} - n_{nucleus}}{10^3 V N_A} = C_0 - \frac{4\pi r^3}{3000 V N_A \Omega}, \qquad (S13)$$

where C_0 is the initial concentration (C and C_0 both in M) and V the volume (in m^3). If the initial supersaturation is

$$S_0 = \frac{C_0}{C_S} \qquad (S14)$$

then the r-dependent supersaturation is

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$$S = S_0 - \frac{4\pi r^3}{3000 V N_A \Omega C_S} . \quad \text{(S15)}$$

When including this effect, one would expect the free energy to minimise for a crystal of such a size that it has reduced the supersaturation to S=1.

$$1 = S_0 - \frac{4\pi r^3}{3000 V N_A \Omega C_S} , \quad \text{(S16)}$$

which gives

$$r_{S=1} = \sqrt[3]{(S_0 - 1) C_S \frac{3000 V N_A \Omega}{4\pi}} . \quad \text{(S17)}$$

This turns out to be not quite the case: the minimum always occurs quite a bit before the value predicted by Eq. (S16). However, the free energy has a zero crossing at the value predicted by Eq. (S16). Thus, in Fig. 5, the minimum occurs for a crystal of radius ~0.9 mm where S = 1.049, while the supersaturation only decreases to S1 for r = 1.13 mm. The barrier peaks at the critical radius of ~1 nm.

References

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Movie S1.
Aggregate-assisted laser-induced nucleation of alanine from solution (0.18 g/mL in D_2O, 5 days old).
Movie S2.
Aggregate-assisted laser-induced nucleation of Gly-Gly from solution (0.2 g/mL in H_2O, 2 days old).
Movie S3.
Aggregate-assisted laser-induced nucleation of Gly-Gly-Gly from solution (0.1 g/mL in D_2O, 1 day old).
Movie S4.
Aggregate-assisted laser-induced nucleation of Ala-Ala from solution (0.3 g/mL in D_2O, 1 day old).
Movie S5.
 Aggregate-assisted laser-induced nucleation of serine from solution (0.4 g/mL in D_2O, 1 day old).
Movie S6.
Aggregate-assisted laser-induced nucleation of glutamic acid from solution (0.13 g/mL in H_2O, 2 days old).
Movie S7.
Aggregate-assisted laser-induced nucleation of phenylalanine from solution (0.05 g/mL in H_2O, 2 days old).
Movie S8.
Aggregate-assisted laser-induced nucleation of lysine from solution (0.5 g/mL in H_2O, 2 days old).
Movie S9.
Aggregate-assisted laser-induced nucleation of histidine from solution (0.13 g/mL in H_2O, 1 day old).
Movie S10.
Evaporation-driven spontaneous nucleation of Gly-Gly-Gly.
Movie S11.
Evaporation-driven spontaneous homogeneous nucleation of Gly-Gly-Gly (5× speed).
Movie S12.
Evaporation-driven spontaneous nucleation of histidine.
Movie S13.
Evaporation-driven spontaneous nucleation of alanine (5× speed).
Movie S14.
Evaporation-driven spontaneous nucleation of histidine.