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Protein Crystallization during Short-Duration Microgravity Exposure: Preliminary Results from the GEN-2 Capsule Aboard the MAPHEUS-17 Mission

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Abstract

Protein crystallization under microgravity conditions provides an opportunity to investigate crystal formation under altered mass-transport conditions, with potential implications for structural biology, pharmaceutical development, and bioengineering. However, the feasibility of initiating protein crystallization during short-duration microgravity exposure, particularly in the absence of pre-existing crystal seeds, remains insufficiently characterized. Here, we investigated the crystallization of purified hen egg-white proteins during a seven-minute microgravity phase aboard the MAPHEUS-17 sounding rocket using the GEN-2 capsule. Five sodium chloride (NaCl) concentrations were evaluated as precipitants, each tested with and without 0.1 M sodium acetate buffer. Protein and precipitant solutions were stored separately before flight and brought into contact during launch, enabling crystallization to initiate without pre-grown seed crystals. Following payload recovery, light microscopy screening revealed crystalline structures in six space-flown samples, with a preliminary count exceeding 100 structures approximately 5 µm in size. No crystalline structures were detected in the ground controls under the same screening conditions. The identity and structural properties of the observed crystals have yet to be confirmed, and further analyses are underway to assess their suitability for X-ray diffraction. These preliminary findings demonstrate the feasibility of initiating crystallization experiments during a brief sounding-rocket microgravity phase and provide a basis for systematic investigations of crystallization kinetics, experimental conditions, and crystal properties under short-duration microgravity.

Introduction

Protein crystallization is a key technique in macromolecular structural biology, enabling the determination of three-dimensional structures by X-ray crystallography. However, obtaining well-ordered crystals suitable for structural analysis remains challenging for many proteins [1,2]. Crystal formation and growth are governed by multiple factors, including protein concentration, supersaturation, precipitant composition, pH, temperature, and mixing conditions. These parameters influence nucleation and subsequent crystal growth, determining whether a supersaturated solution produces ordered crystals, amorphous precipitates, or aggregates [1,2]. Controlling nucleation and growth is therefore essential for reproducible crystallization and the production of crystals suitable for structural studies.

Microgravity provides an experimental environment in which the effects of buoyancy-driven convection and sedimentation are substantially reduced. By altering mass transport and the distribution of dissolved molecules, these conditions may influence the transport of protein molecules to growing crystal surfaces and thereby affect crystal growth, morphology, and structural order [2,3].

Previous sounding-rocket experiments have demonstrated that protein crystallization can occur during short periods of microgravity exposure. The TEXUS 3 mission, conducted in 1981, produced β-galactosidase crystals during approximately six minutes of microgravity and demonstrated the relevance of diffusion-dominated transport under reduced-convection conditions [4]. Similarly, the MASER 3 experiment investigated the crystallization of ribonuclease A and pancreatic trypsin inhibitor during 7

min 15 s of microgravity. The flight samples yielded more and larger crystals than the corresponding ground references, and the ribonuclease A crystals produced X-ray diffraction data to a resolution of 1.06 Å [5]. To facilitate crystallization within the limited flight duration, the MASER 3 experiment employed macro- and micro-seeding techniques [5]. These experiments demonstrate the potential of short-duration microgravity platforms for protein crystallization, while also highlighting the importance of experimental design when interpreting crystal formation under such conditions [2,3,6,7].

An important question is whether crystalline structures can be detected following short-duration microgravity exposure when crystallization is initiated without pre-existing crystal seeds. In such experiments, the observed outcome may depend on the precipitant concentration, buffer composition, and other aspects of sample preparation and handling, in addition to the microgravity environment itself [2,3,5,6,7]. Moreover, studies of protein aggregate transport under microgravity conditions highlight the need to distinguish crystal-like morphology from confirmed crystal identity [8]. Systematic evaluation of these parameters is therefore necessary to assess the feasibility of seed-free crystallization during brief sounding-rocket missions.

In this study, we investigated the crystallization of purified hen egg-white protein material using the GEN-2 capsule aboard the MAPHEUS-17 sounding rocket. Five sodium chloride (NaCl) concentrations were tested, each prepared with or without sodium acetate buffer, and no pre-existing crystal seeds were introduced. Following recovery, the samples were examined by light microscopy to identify and document structures consistent with crystalline morphology across the tested conditions. The objective was to assess whether such structures could be detected after short-duration microgravity exposure and to compare their occurrence across the experimental conditions. Subsequent X-ray diffraction analysis is planned to assess crystal identity, structural order, and diffraction quality.

Results and Discussion

Phase-contrast microscopy identified crystalline structures in six space-flown samples: 1/2, 2/1, 2/2, 4/1, 5/1, and 5/2. The preliminary screening indicated that the positive samples collectively contained more than 100 crystals approximately 5 µm in size (Fig. 1).

No crystals were detected in the ground-control samples during the initial screening, whereas crystalline structures were detected in multiple space-flown samples.These findings indicate that crystalline structures can become detectable in this experimental system over a short flight-associated timescale.

One distinctive property has been observed exclusively in the space-flown samples containing visible, protein crystal-like structures: a localized increase in viscosity along the inner wall of the test tube, where the material is significantly more viscous than the bulk solution. This material forms an adherent film that remains attached to the glass surface and stretches into threads when disturbed. Unlike the bulk solution, it does not readily drain away when the test tube is lifted out of the vial.

Samples that yielded positive results during the initial light microscopic screening were selected for further characterization. X-ray-based structural analysis is ongoing. Quantitative image analysis and a complete mapping of sample identifiers to experimental conditions will also be required to assess the influence of precipitant concentration and buffer composition.

Conclusions

Crystalline structures were detected in six space-flown samples following the approximately seven-minute microgravity phase of the MAPHEUS-17 mission, whereas no crystals were observed in the ground-control samples during the initial screening. These findings establish a preliminary experimental basis for investigating rapid crystallization in short-duration microgravity experiments.

The molecular identity of the crystals and their structural quality remain to be confirmed. Future experiments using the GEN-3 platform are planned to extend the duration of microgravity exposure, potentially to days in orbit. Such experiments could help distinguish processes occurring during initial nucleation from those associated with subsequent crystal growth.

Overall, this study provides an initial observation of rapid crystallization-associated structures following short-duration microgravity exposure. Confirmation of their molecular identity and improved control of experimental variables will be essential for determining the underlying mechanisms and the potential value of this approach for macromolecular crystallography.

Materials and methods

The experiment investigated five NaCl concentrations: 0.8, 1.2, 1.6, 2.0, and 2.4 M. For each concentration, two precipitant formulations were prepared: NaCl (S9888, Sigma-Aldrich) dissolved in distilled water., and NaCl dissolved in a solution supplemented with 0.1 M sodium acetate (1.06268, EMSURE) buffer. The corresponding space-flown and ground-control conditions were prepared for comparison. The complete experimental matrix therefore comprised ten precipitant formulations (Table 1).

Table 1
Experimental conditions for flight and ground-control samples, including NaCl concentrations and buffer compositions.

Condition	NaCl concentration	NaCl mass	Final volume	Formulation
Sample 1/1	0.8 M	935 mg	20 mL	NaCl in water
Sample 1/2	0.8 M	935 mg	20 mL	NaCl + 0.1 M sodium acetate buffer
Control 1/1	0.8 M	935 mg	20 mL	NaCl in water
Control 1/2	0.8 M	935 mg	20 mL	NaCl + 0.1 M sodium acetate buffer
Sample 2/1	1.2 M	1.40 g	20 mL	NaCl in water
Sample 2/2	1.2 M	1.40 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Control 2/1	1.2 M	1.40 g	20 mL	NaCl in water
Control 2/2	1.2 M	1.40 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Sample 3/1	1.6 M	1.87 g	20 mL	NaCl in water
Sample 3/2	1.6 M	1.87 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Control 3/1	1.6 M	1.87 g	20 mL	NaCl in water
Control 3/2	1.6 M	1.87 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Sample 4/1	2.0 M	2.34 g	20 mL	NaCl in water
Sample 4/2	2.0 M	2.34 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Control 4/1	2.0 M	2.34 g	20 mL	NaCl in water
Control 4/2	2.0 M	2.34 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Images (Supporting Materials)				
Space-flown samples.				
Binocular Image of Space-flown sample 1/1				

Condition	NaCl concentration	NaCl mass	Final volume	Formulation
Sample 5/1	2.4 M	2.80 g	20 mL	NaCl in water
Sample 5/2	2.4 M	2.80 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Control 5/1	2.4 M	2.80 g	20 mL	NaCl in water
Control 5/2	2.4 M	2.80 g	20 mL	NaCl + 0.1 M sodium acetate buffer
Images (Supporting Materials)				
Space-flown samples.				
Binocular Image of Space-flown sample 1/1				

Purified hen egg-white protein solution and the corresponding precipitant formulations were used in the crystallization experiments. Hen egg white was separated from the yolk, diluted with an equal volume of distilled water, and gently stirred for 1 h at 4°C. The diluted sample was dialyzed against distilled water using dialysis tubing with a 1 kDa molecular-weight cut-off, with four changes of the external water at 4°C to remove low-molecular-weight compounds and salts [9]. For crystallization experiments, the protein solution was adjusted to pH 4.5 using sodium acetate buffer.

A 100 µL aliquot of the appropriate precipitant solution was loaded into a 1 mL (8mm OD X) glass microwave reaction vial (CG-4920, Chemglass), which was positioned inside each sample vial. The inner reaction vial served as a reservoir for the precipitant, preventing premature mixing with the protein solution, minimizing handling errors, and maintaining liquid containment during pre-launch handling and ascent. Subsequently, 100 µL of the hen egg-white protein solution was added to the corresponding sample vial, separately from the precipitant-containing inner vial. The experimental design relied on the mixing of the two solutions during launch, thereby initiating crystallization in association with the flight sequence.

The GEN-2 capsule was assembled and sealed at ambient pressure (1 atm) and room temperature at 04:21 local time on 1 October 2026, as part of the late-access procedures during the launch campaign. The flight and ground-control samples were installed in the capsule’s modular internal compartments. The final assembly mass was verified against the engineering model, yielding a total mass of 666 g, including the capsule and experimental samples.

At 04:45, the GEN-2 module was handed over for integration with the SSC Space Shared Module and subsequent installation aboard the MAPHEUS-17 sounding rocket (Fig. 3).

The payload was successfully launched at 08:50 local time aboard the DLR MAPHEUS-17 mission from Esrange Space Center, Sweden. The ground-control samples were shaken to mix the solutions at the

launch facility in temporal association with the rocket launch. The vehicle reached an apogee of 291 km, and the experiment experienced seven-minute microgravity phase. Atmospheric reentry began at 08:57, with the vehicle reaching a reported speed of approximately Mach 7. Landing occurred at approximately 09:00, when the external temperature was reported to be approximately 4°C.

Helicopter recovery operations were completed at 12:20, and the GEN-2 module was returned to the launch facility. The module was disassembled and the samples were retrieved at approximately 12:45.

Following recovery, the samples were cooled and initially maintained at approximately 4°C in their mother liquor for preliminary screening. They were subsequently stored at approximately – 19°C pending further analysis. Initial sample screening was performed using a binocular microscope to identify visible precipitates and candidate crystalline structures.

For subsequent phase-contrast microscopy, small aliquots were collected directly from the sample vials using an inoculation loop and transferred as droplets to an imaging dish. Imaging was performed using a Leica THUNDER widefield microscope (Leica Microsystems, Wetzlar, Germany). Phase-contrast microscopy was used to visualize structures in the samples based on optical phase differences [10].

Images were acquired at nominal magnifications of 20× and 40×, and z-stack images were collected at 20× to examine the distribution and morphology of structures across successive focal planes. Loop-based sampling was selected to minimize disturbance of the remaining sample; however, the small aliquot examined may not have been representative of the entire sample population.

Declarations

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Author Contributions

B. Matyas: Conceptualization, Investigation, Methodology, Project administration, Writing the original draft. P. Knaus: Methodology, Validation, Writing, review and editing. Both authors have read and approved the final version of the manuscript.

Data availability

A link to the complete microscopy image dataset (approximately 250 GB) will be provided upon request during the peer-review process or following initial editorial acceptance. The dataset includes:

- Sample microscopy images

- Control sample microscopy images (including null results)

- Space-flown sample microscopy images

- Raw image files

Competing interests

The authors declare that they have no competing interests that could have influenced the work reported in this paper.

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Figures

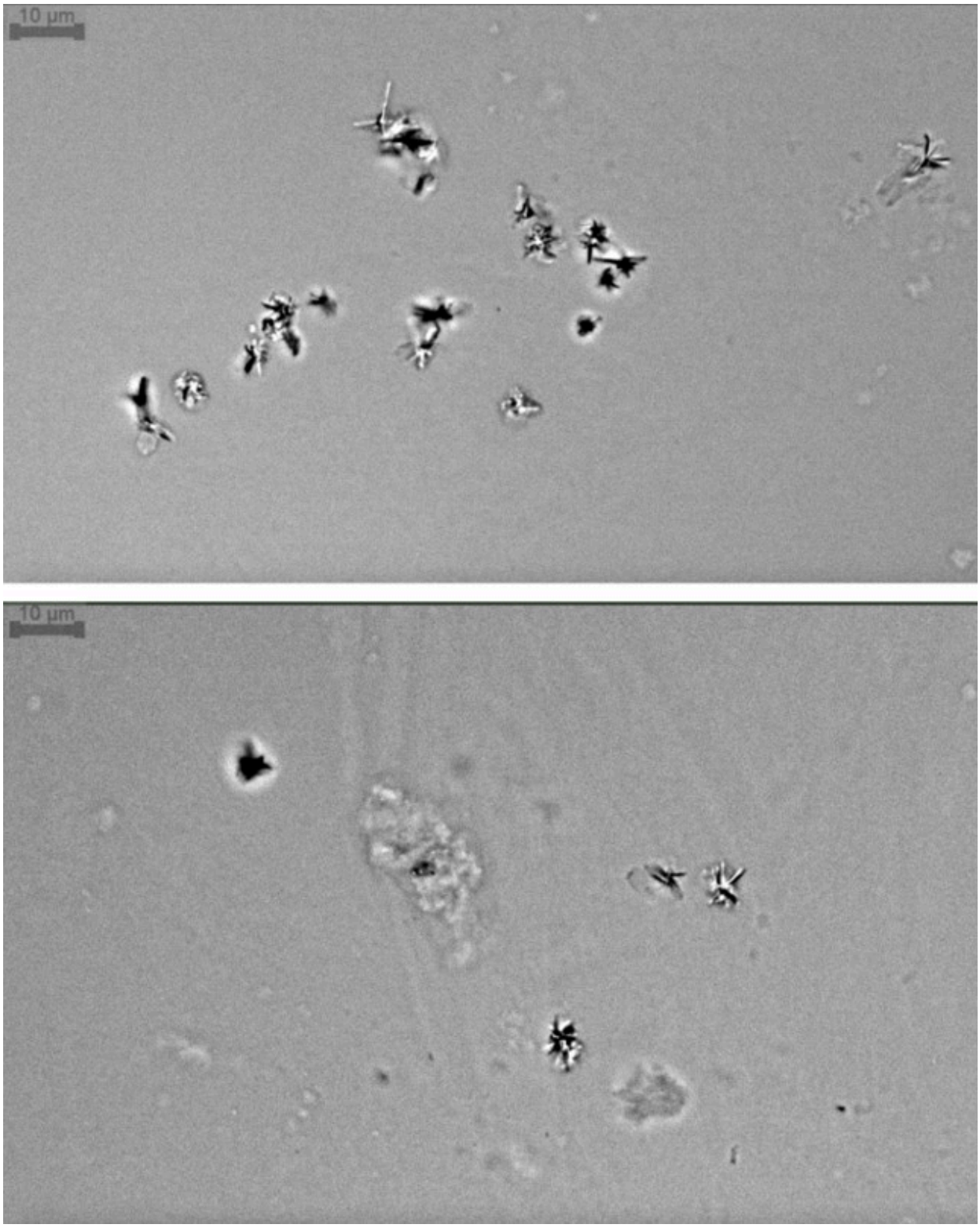


Figure 1

Representative structures approximately 5 µm in size observed in Sample 1/2 by light microscopy at 20× magnification using a Leica THUNDER widefield microscope (Leica Microsystems, Wetzlar, Germany).



Figure 2

Experimental setup of the Protein Crystallization Module (PCM) aboard the GEN-2 capsule. Left: flight samples; right: ground-control samples. The vials were secured to the sample holder using adhesive to minimize damage caused by shaking during liftoff and atmospheric re-entry. The GEN-2 capsule aeroshell components are shown in the background.

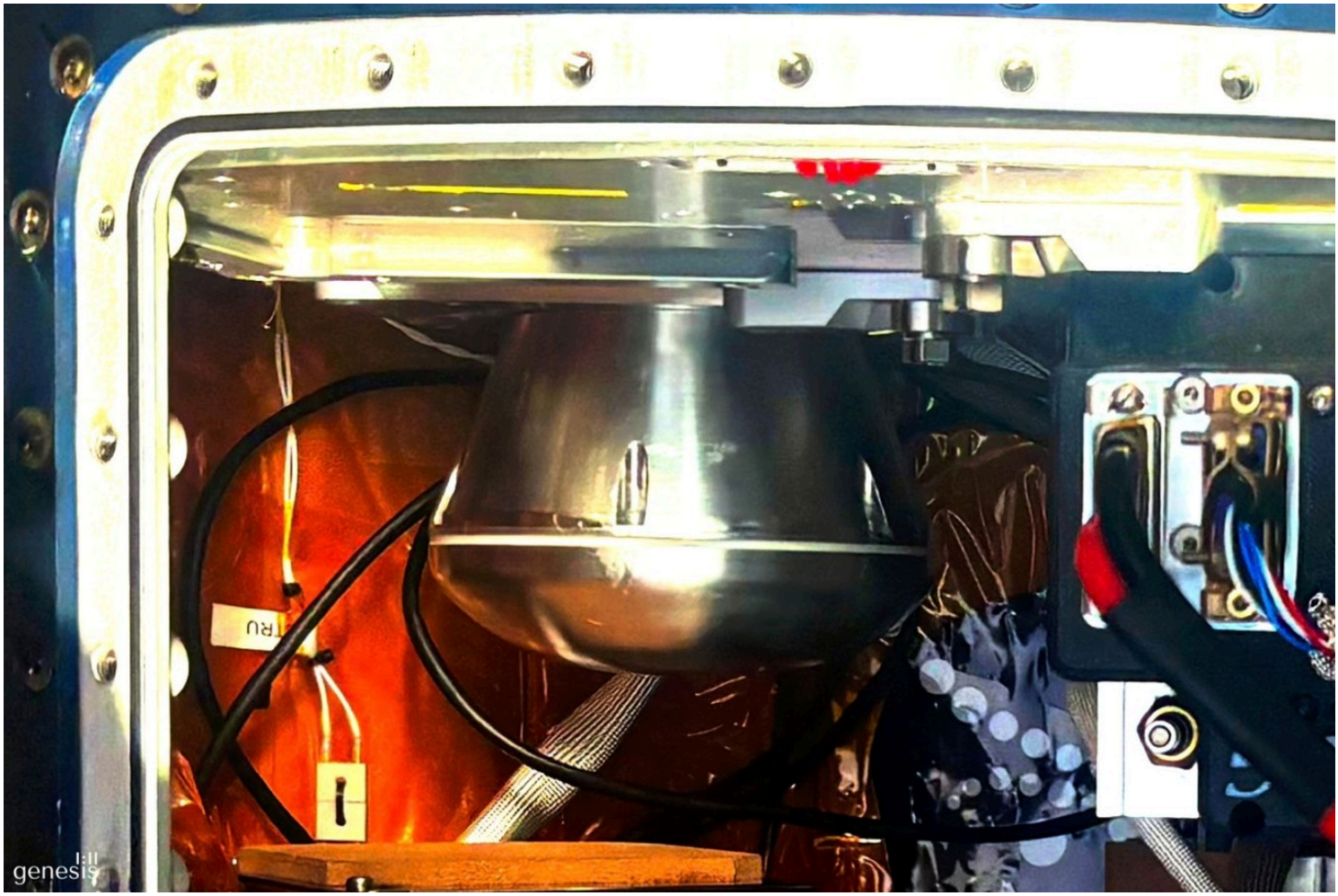


Figure 3

GEN-2 Capsule assembled and integrated into the SSC Space Shared Module aboard the 13 m-long DLR'S MAPHEUS-17 sounding rocket. The GEN-2 Capsule has an outer diameter of 11 cm, a mass of 0.7 kg, and occupies 1U on the rideshare platform.

Supplementary Files

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