1 **Microbial life in the open ocean: a universe of tiny cells separated by empty space**
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14 ABSTRACT
15
16 Marine microbes are fundamental components of food webs and the biogeochemical cycles that
17 maintain the habitability of the planet. In the oligotrophic open ocean, these microscopic
18 organisms live in a dilute environment separated from other cells by large distances at the
19 microscale while surrounded by very few essential nutrient molecules. For ubiquitous sub-
20 micron sized and non-motile microbes, cellular growth requirements for hundreds of millions (or
21 more) of nutrient molecules are sustained predominantly by rapid molecular diffusion.
22 Characterizing the interactions of cells and molecules in the "empty space" of the ocean remains
23 central to understanding the drivers and consequences of oceanic biogeochemical cycles.
24
25 TEXT
26 Microscopic examination of a drop of seawater reveals the presence of millions of small cells,
27 including Bacteria, Archaea, Protists, as well as viruses (Figure 1A). That microscopic view
28 involves a filtration and concentration step that masks the remarkable distances between
29 individual cells *in situ* in the oligotrophic ocean. At the microbial scale, hundreds of micrometers
30 separate cells that themselves are less than a micrometer in size (Figure 1B). Despite their
31 microscopic size and relative isolation, marine microbes catalyze chemical transformations at
32 rates that are critical for maintaining the habitability of the planet. In the open ocean, but at a
33 microbial scale, not only are cells (and viruses) distantly distributed, so are the organic
34 compounds and inorganic nutrients that constitute the molecular building blocks of life. Recent
35 studies have shown that there are multiple interactions between microorganisms, microorganisms
36 and viruses (*1*), and microorganisms and molecules that occur at high daily rates despite
37 extremely dilute concentration of nutrient molecules and large, relative distances between
38 bacteria, algae, grazers and viruses of the sea. It is remarkable that every day, nitrogen molecules
39 for example, must be supplied from a volume of seawater orders of magnitude greater than an
40 individual nonmotile bacterial or cyanobacterial cell, in order to support microbial growth. By
41 reviving a historical perspective combined with simple analyses and modeling of physical
42 processes, we suggest that viewing the open ocean microbial world through the interwoven
43 threads of space, time, and diffusion is critical for understanding how microbial interactions
44 shape the biogeochemical cycles of one of the largest habitats on Earth.
45

46 Marine microbes were largely ignored in early considerations of marine food chains until
47 epifluorescence microscopy showed that microbes were very abundant, typically almost 1
48 million bacteria cells per milliliter of surface seawater. The concept of the "microbial loop (*2*)
49 made explicit the roles of microbes in nutrient recycling and funneling matter and energy into the
50 protists and larger organisms of the oceanic food web. It was not possible to measure these
51 processes at the scales relevant to cells, but only in large volume water samples that measure
52 integrated rates of metabolism and growth of billions of cells. Early studies suggested that ocean
53 algae (phytoplankton) grew at or near maximal growth rates despite extremely low
54 concentrations of inorganic nutrients (*3*). One explanation was that microscale heterogeneities, or
55 "patches" such as those made by grazing protists or zooplankton, provided localized high
56 concentrations of nutrients that could be rapidly taken up by phytoplankton to support high
57 growth rates in the ocean (*4*). This view of the microbial world as patchy and heterogeneous was
58 extended to organic molecules and heterotrophic microbes (*5*) and, more recently, to tractable
59 microscale experimental systems with bacteria (*6*). This conceptualization of what the microscale
60 world looks like – a complex milieu of microbes and organic molecules in "hotspots" (*5*) – has
61 become a common way of presenting the oceanic microbial world. These hotspots can result
62 from exudation of organic molecules from active photosynthetic phytoplankton cells, which has
63 been elegantly discussed in the context of the "phycosphere" (*7, 8*). Such microscale
64 heterogeneities can be exploited by motile or particle-bound microorganisms that can rapidly
65 take up localized elevated concentrations of organic matter (*9*). Yet, it is important to recognize
66 that for many, if not most, of the microbial cells in the open ocean habitat, such hotspots or
67 patches are "football fields" away, if considered on a human scale.
68
69 In the open ocean, bacterial and cyanobacterial communities are dominated by nonmotile cells of
70 two ubiquitous species – *Prochlorococcus marina* and *Pelagibacter ubique*. *P. marina* is the
71 smallest free-living autotroph on the planet, with abundances typically on the order of a hundred
72 million per milliliter and are responsible for the daily production of ~25% of the world's oxygen.
73 *P. unique* is the most abundant microbe in the ocean, a heterotrophic bacterium essential to the
74 microbial loop that can be 25-50% of total microbial abundances. To emphasize the remarkable
75 balance between cellular needs and supply, consider the rates of nutrient fluxes and uptake needs
76 for growth by single cells. Ammonium ions, the preferred inorganic source of nitrogen (N) for
77 most phototrophic algae, are typically at 10 nanomolar concentrations or less. Thus, ammonium
78 molecules are distributed at a distance of about 0.6 micrometers, similar to the cross-sectional
79 dimension of an open ocean bacterial or cyanobacterial cell (Figure 1B), which means that only a
80 handful of molecules are near each cell. A seawater volume equivalent to a large microbial cell
81 of radius 0.5 micrometers (~0.5 μm^3) would contain only a few (<5) molecules of ammonium.
82 However, the N requirement for microbial cell division is much greater than in the water
83 displaced by the cell; e.g. one *Prochlorococcus* cell requires 4 x 10^8 N atoms per day to divide
84 (*10*). In other words, in order to reproduce, a microbial cell needs to harvest the ammonium from
85 *hundreds of millions of times* its cell volume.
86
87 Two mechanisms that could expose individual cells to this number of ammonium molecules
88 from a large volume of surrounding seawater are active swimming or passive movement (via
89 Brownian motion) (*9*). A microbe of radius 0.25 micrometers swimming for one day at a velocity
90 of 30 $\mu m\ s^{-1}$ would access ~0.5 nanoliters containing ~3 x 10^6 ammonium molecules, which is
91 <1% of the daily requirements. In contrast, Brownian motion is much, much faster– the

92   diffusivity of molecules ($D_{mol} = 10^{-5}$ cm^2 s^{-1}), is 1000 times greater than that of the Brownian
93   motion of the microbial cells. Thus, molecular diffusion of nutrients leads to far more frequent
94   encounters than would active motion of the cells themselves.
95
96   The stirring number – defined by $(lv)/D_{mol}$, where D_{mol} is the molecular diffusivity of the
97   ammonium ions (*11*) – provides a benchmark for comparing the encounter efficiency of
98   swimming to that of molecular diffusion. At low stirring numbers, there is little to no
99   enhancement of nutrient uptake by swimming. For ammonium at 10 nanomolar concentration
100 with intermolecular spacing of $l = 0.6$ μm, molecular diffusion is >50 times more efficient than is
101 cellular swimming at $v = 30$ μm/s. Molecular diffusion alone generates a potential flux of ~2x10^9
102 N atoms per day per cell – four-times the daily N requirement for *Prochlorococcus*. Thus,
103 diffusive processes alone can fuel the growth and productivity of abundant, free-living
104 unicellular microorganisms in the open ocean. This demonstrates why lack of motility, and free-
105 living cells, in a dilute environment is ecologically successful in oligotrophic oceans.
106
107 Although such "simple" calculations can resolve the problem of how individual cells can grow in
108 the nutrient-limited open ocean, they explain how these cellular-scale processes cascade to
109 transform ecosystem functions, including global biogeochemical cycles. Sources of nutrients are
110 primarily recycling by bacteria (either free-living cells or in particles) or grazers (protistan
111 micrograzers or metazoan zooplankton) that decompose organic matter and liberate ammonium.
112 Early studies (*4*) on inorganic nutrients, and more recent studies on organic matter (*8*) have
113 analyzed how microscale heterogeneities of elevated concentrations of inorganic or organic
114 nutrients (patches or hotspots) can affect microbial growth and activity. Small scale patches of
115 elevated concentrations of nutrients, e.g., on the order of a few to hundreds of micrometers in
116 spatial extent, presumably left as a result of lysis by viruses or grazing and excretion by grazers,
117 could facilitate significantly higher uptake rates by microorganisms (*4*). However, early
118 modeling studies (*12*) contended that such patches of inorganic nitrogen diffuse too quickly and
119 are too short-lived to be effective in inorganic nutrient uptake by open ocean nonmotile
120 microbes. Our re-analysis based on recent estimates of micrograzer sizes, which are smaller than
121 those used by Jackson (*12*) , suggests that a 3 micrometer diameter micrograzer swimming at 100
122 μm per second and feeding and recycling ingested particulate nitrogen at a rate commensurate
123 with 1 doubling per day, might leave a plume of remineralized nitrogen of only 5-80 nanomolar
124 (assuming that the plume was not dispersed). The elevated concentrations of nutrients in
125 microscale patches such as micrograzer plumes are insufficient to supply dispersed non-motile
126 cells such as *Prochlorococcus* and do not nearly overcome the >50-fold advantage of molecular
127 diffusion relative to swimming to patches in the oligotrophic ocean. Similarly, the nonmotile
128 heterotrophic *Pelagibacter ubique* depends on diffusion for the organic molecules needed for
129 food. The nature of molecular diffusion also provides a mechanistic explanation for why the
130 most abundant organisms in the open ocean, *Prochlorococcus* and *Pelagibacter,* are non-motile
131 (*13*). In essence, swimming towards evanescent hotspots or phycospheres is not the dominant
132 mechanism for supporting productivity of small cells living in nutrient-depleted environments –
133 the situation most common in the open ocean.
134
135 The example of nitrogenous nutrients and *Prochlorococcus* demonstrates the importance of
136 considering space, time and diffusion in understanding major microbial processes of the dilute
137 ocean. But there are multiple implications of these types of small-scale processes in the open

138 ocean microbial world. Submicrometer-sized viruses are 10s of micrometers apart (*14*),
139 eukaryotic algae and micrograzers 100s of micrometers apart and interactions occur over
140 relatively large microscale distances (e.g. (*15*)). Although we used nitrogen as an example, the
141 relationship of space, time, and diffusion applies to many other aspects of the microbial loop.
142 The spatial distributions of phosphorus-containing molecules and iron, both essential nutrients,
143 are much greater. Furthermore, recent studies suggest that metabolic exchanges between species
144 are important in microbial interactions in the marine microbiome (*16*) but such exchanges are
145 also controlled by the time-space considerations described here. The secretion or exchange of
146 molecules in the dilute open ocean ("public goods") is problematic because of the great dilution
147 in time and space in the oligotrophic ocean. Instead, mutualisms – cell-to-cell collaboration –
148 that reshape the environment and provide energetic advantages to organisms may help explain
149 long-term evolutionary adaptations linking the behavior of ubiquitous autotrophs, like
150 *Prochlorococcus*, and heterotrophs like *Pelagibacter* (*17*).
151
152 The non-motile marine microbes at the base of the ocean food chain, like *Prochlorococcus* and
153 *Pelagibacter*, transform and sustain ocean life. Such organisms may be small, even relative to
154 other ocean microbes, and non-motile but they are hardly simple. Adaptive release of
155 extracellular compounds, the distribution of ecologically distinct subspecies across different
156 light, temperature, and mixing regimes ("ecotypes"), and day-night synchronization of activity
157 (*18*) suggest some of the dynamic processes that contribute to the evolutionary fitness of these
158 tiny microbes that drive biogeochemical cycles of the oligotrophic oceans. Many questions
159 remain, including the interactions between viruses and grazers, the extent of the leakiness of the
160 microbial loop and, in turn, the export of carbon to the deep ocean. Even without microscale
161 complexity, chemotaxis and motility, it is essential to understand how the abundant
162 microorganisms in the dilute habitat of the open ocean interact, and how they interact with
163 hotspots and motile microorganisms. To do so requires that we recognize how dilute spatial
164 distributions and molecular diffusion, at scales relevant to marine microbes, act in ways that may
165 not seem intuitive to us [non-microscopic humans], yet are critical for understanding how the
166 oceans and the global Earth system work. Recent discoveries, new techniques for measuring
167 rates of chemical transformations at the microscale, genetic and genomic analyses of single cells
168 and visualization and experimentation at the scale of milliliters rather than liters, make it possible
169 to examine the microscale processes in the open ocean that affect oceanic biogeochemical
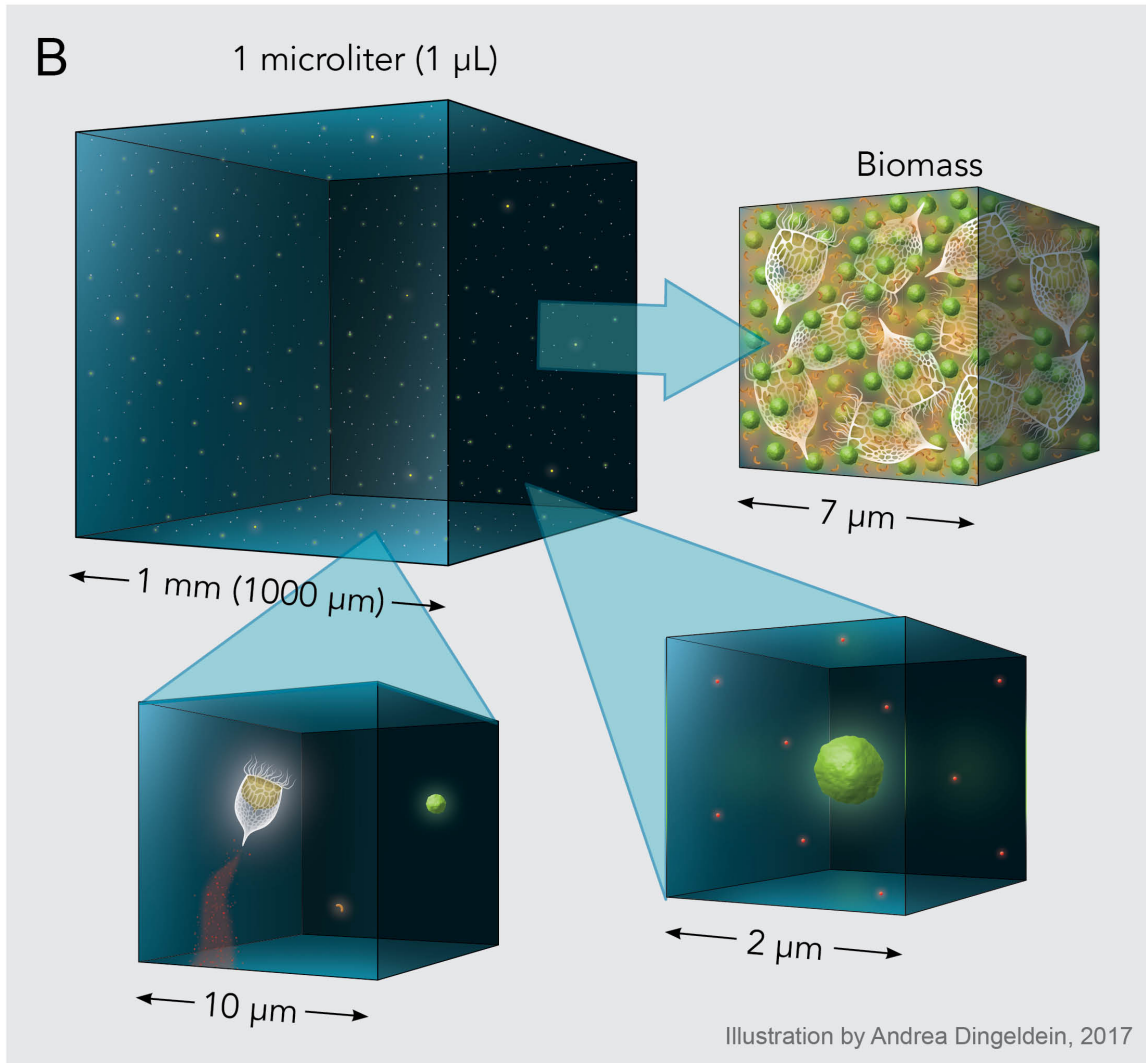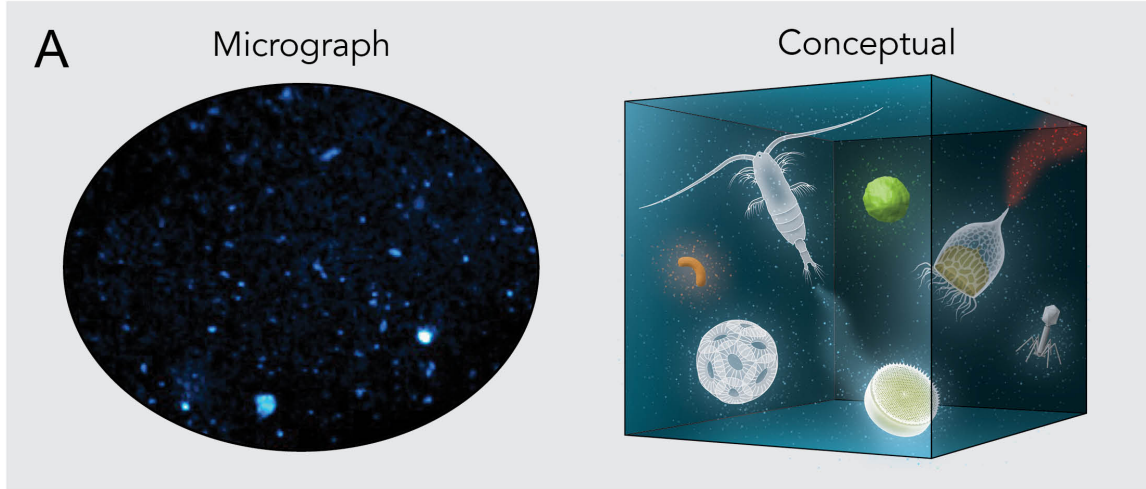170 processes at the ecosystem scale.
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185   Figure 1. The components, sizes and distances in the microbial engine that drives Earth's
186   biogeochemical cycles in the open ocean. A. Conceptual representation of microbial components
187   that are not to scale but emphasize biological complexity, and a typical epifluorescence
188   microscopic image of marine microbes from 0.2 nanoliter of seawater concentrated onto a
189   polycarbonate filter. B. Scaled representation of microbe size and distances between them in the
190   open ocean microbial world. Upper left: Scaled microliter with typical *Prochlorococcus*
191   concentrations. *Prochlorococcus* cells are magnified approximately 3X, in order to be visualized
192   at the same scale. Upper right: All of the microbial biomass from 1 µL (1 cubic millimeter, or
193   microliter) fits in a 7 micrometer cube, just a fraction of space of a microliter. Lower left: Protist
194   micrograzer in relation to size of prey (*Prochlorococcus* and other microbes) and plume of
195   nutrients that are recycled for microbes. Lower right: *Prochlorococcus* cell with recycled
196   ammonium molecules (not to scale), showing the minute fraction of ammonium molecules
197   available relative to the daily needs of a single cell for cell division.

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200

A

Micrograph

Conceptual

B

1 microliter (1 µL)

Biomass

← 7 µm →

← 1 mm (1000 µm) →

← 10 µm →

← 2 µm →

Illustration by Andrea Dingeldein, 2017

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Supplemental Calculations for "How microbes survive in the open ocean" by J. Zehr, J.S. Weitz, and I. Joint

Ammonium molecules per micron cubed Given ammonium concentration of ρ in units of nM then the number of ammonium molecules per micron cubed, x, is defined as

$$x = \overbrace{\rho}^{\text{[nanomoles/L]}} \times \overbrace{10^{-9}}^{\text{[moles/nanomoles]}} \times \overbrace{10^{-3}}^{\text{[L/cm}^3\text{]}} \times \overbrace{10^{-12}}^{\text{[cm}^3/\mu\text{m}^3\text{]}} \times \overbrace{6.02 \cdot 10^{23}}^{\text{[molecules/moles]}} = 0.6\rho. \tag{1}$$

Hence for $\rho = 10$ nM then $x \approx 6$ molecules/μm^3.

Distance between ammonium molecules Given molecular density, x, then the typical distance l between ammonium molecules is $l \approx x^{-1/3}$. For $x \approx 6$ molecules/μm^3, then $l \approx 0.5$ μm.

Nitrogen atoms in *Prochlorococcus* We use a consolidated estimate of $m_{Pro} \approx 10$ fg per *P. marina* cell following Bertillson et al. (2003), hence the number of nitrogen atoms per cell, x_{Pro}, is approximately

$$x_{Pro} = \overbrace{m_{Pro}}^{\text{[fg]}} \times \overbrace{10^{-15}}^{\text{[g/fg]}} \times \overbrace{(1/14)}^{\text{[moles/g]}} \times \overbrace{6.02 \cdot 10^{23}}^{\text{[molecules/mole]}} = 4.3 \times 10^7 m_{Pro}, \tag{2}$$

such that $x_{Pro} \approx 4.3 \times 10^8$ nitrogen atoms per cell.

Daily volume swept by swimming microbe The daily total volume of water, V (nL), explored by cellular swimming and neglecting the effects of diffusion, is approximated as the product of the cellular cross-section, πr^2 where $r \approx 0.3$ μm for *P. marina*, and the distance traveled $d = vt$, i.e., given a hypothetical value of $v = 30$ μm/sec,

$$V = \pi \overbrace{(0.3)^2}^{[\mu\text{m}^2]} \times \overbrace{30}^{[\mu\text{m/sec}]} \times \overbrace{86400}^{\text{[sec]}} \times \overbrace{10^{-12}}^{\text{[cm}^3/\mu\text{m}^3\text{]}} \times \overbrace{10^6}^{\text{[nL/cm}^3\text{]}} \approx 0.7\text{nL}. \tag{3}$$

Ammonium molecules per nL Given ammonium concentration of ρ in units of nM then the number of ammonium molecules per nL is

$$x_{nL} = \overbrace{\rho}^{\text{[nanomoles/L]}} \times \overbrace{10^{-9}}^{\text{[moles/nanomoles]}} \times \overbrace{6.02 \cdot 10^{23}}^{\text{[molecules/mole]}} \times \overbrace{10^{-9}}^{\text{[L/nL]}} = 6 \cdot 10^5 \rho, \tag{4}$$

such that for $\rho = 10$, then $x_{nL} \approx 6 \times 10^6$ (molecules/nL).

Diffusive flux of ammonium into microbial cells The daily maximum diffusive flux for a microbial cell of radius r given concentration ρ is $J \approx (4\pi Dr\rho \cdot 86400)$ where D is the diffusion rate of the molecule. We note that if $x \approx 6$ molecules/μm^3 then the number of molecules per cm^3 is $\approx 6 \times 10^{12}$, and

$$J = 4\pi \times \overbrace{10^{-5}}^{\text{[cm}^2/\text{sec]}} \times \overbrace{0.3 \cdot 10^{-4}}^{\text{[cm]}} \times \overbrace{6 \cdot 10^{12}}^{\text{[molecules/cm}^3\text{]}} \times \overbrace{86400}^{\text{[sec]}} \approx 2 \times 10^9. \tag{5}$$

Plume-generated remineralization of ammonium Consider a micrograzer of diameter 3 μm swimming at 100 μm/sec releasing a plume of diameter 20 μm, representing a volume $(10/0.3)^2 \times (100/30) \approx 4000$-fold higher than in Eq. (3), equivalent to 2800 nL in one day. Following Caron et al. (2017), we assume a micrograzer (or nanoflagellate) has $n_{grazer} \approx 50 - 200$ fg nitrogen per cell. Assuming an equivalent amount of nitrogen is remineralized this would correspond to a plume concentration:

$$\rho_{plume} = \frac{\overbrace{n_{grazer}}^{\text{[fg/nL]}}}{2800} \times \overbrace{10^{-15}}^{\text{[g/fg]}} \times \overbrace{1/14}^{\text{[moles/g]}} \times \overbrace{10^9}^{\text{[nL/L]}} \times \overbrace{10^9}^{\text{[nanomoles/mole]}} \approx 0.026 \cdot n_{grazer}, \tag{6}$$

such that ammonium concentrations in the plume would range from $\rho_{plume} \approx 1 - 5$ nM for micrograzers with nitrogen content ranging from 50-200 fg, respectively, if the plume did not disperse. These concentrations are less than background, however if the plume diameter were 6 μm and given content of 200 fg then the resulting plume concentration would be $\rho_{plume} \approx 60$ nM. Note that larger grazers releasing small, concentrated excretions could provide local opportunities for significant enhancement of uptake via active motility.

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