# A Constant Flux of Diverse Thermophilic Bacteria into the Cold Arctic Seabed

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less of the level of taxonomic resolution. In this regard, genetic analyses have shown the existence of widespread, although disjunct, distribution patterns for several cryptic species of the marine diatom *Skeletonema* (*23*). Similar results have been reported for other microbial plankton groups, such as picoeukaryote algae and foraminifera (*17*, *24*). Alternatively, our results could reflect the convergence or parallelism of morphological traits among genetically unrelated taxa in disjunct oceanic regions.

Despite enormous environmental variability linked to glacial-interglacial climates of the Pleistocene, our analysis reveals that marine diatom communities have evolved slowly through gradual changes over the past 1.5 My of Earth's history (Fig. 2 and table S2). These patterns of community stability for extensive periods of geological time are probably associated with the great dispersal ability of marine diatoms (*25*, *26*) and highlight the potential of microbial plankton communities for recovering from past and future climatic variations. This conclusion implies that there are few or no biogeographical traces of historical climate change in contemporary communities of marine diatoms.

Models of evolution of species commonly assume that tectonic barriers and water mass fronts act as effective isolating mechanisms (*9*). This is a necessary condition that precedes the delineation of biogeographic provinces sensu stricto (*6*, *9*) and controls the development of global species richness. Our analysis, however, indicates that, even at the largest spatial scale, the geographic distribution of marine planktonic diatoms does not seem to be limited by dispersal. These results, together with recent genetic evidence for high rates of inter- and intra-oceanic gene flow in planktonic protists and widespread oceanic distributions of cryptic "sibling" species (*17*, *23*, *27*), suggest that the geographic isolation of marine diatoms cannot be maintained for long periods. Our results strongly support the hypothesis that environmental selection rather than dispersal dominates diatom community structure. To the extent that marine diatoms are a model microbial taxonomic group, our results imply that the biodiversity and macroevolutionary patterns at the microbial level fundamentally differ from those of macroscopic animals and plants, negating the idea that all living things follow similar ecological and evolutionary rules (*6*).

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**Supporting Online Material**

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Materials and Methods
Fig. S1
Tables S1 and S2
References

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# A Constant Flux of Diverse Thermophilic Bacteria into the Cold Arctic Seabed

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Microorganisms have been repeatedly discovered in environments that do not support their metabolic activity. Identifying and quantifying these misplaced organisms can reveal dispersal mechanisms that shape natural microbial diversity. Using endospore germination experiments, we estimated a stable supply of thermophilic bacteria into permanently cold Arctic marine sediment at a rate exceeding $10^8$ spores per square meter per year. These metabolically and phylogenetically diverse *Firmicutes* show no detectable activity at cold in situ temperatures but rapidly mineralize organic matter by hydrolysis, fermentation, and sulfate reduction upon induction at 50°C. The closest relatives to these bacteria come from warm subsurface petroleum reservoir and ocean crust ecosystems, suggesting that seabed fluid flow from these environments is delivering thermophiles to the cold ocean. These transport pathways may broadly influence microbial community composition in the marine environment.

Microbial diversity surveys have revealed that species richness is determined by many low-abundance taxa—the so-called rare biosphere (*1*–*3*). In the ocean, certain members of this relatively unexplored biosphere comprise a dormant microbial "seed bank" that can be transported passively over great distances (*1*). Quantitatively tracking the migration of indicator taxa can highlight key factors that influence patterns of biogeography and may help evaluate the extent to which microorganisms exhibit a cosmopolitan distribution (*4*). Endosporulation allows certain bacteria to persist as dormant cells in hostile environments, explaining discoveries of viable thermophilic *Firmicutes* in inhospitably cold habitats (*5*–*10*). Quantitative studies of this phenomenon are scarce, and the origin and distribution of thermophiles in cold environments remain enigmatic (*6*–*11*). Thermophilic sporulating taxa such as certain *Desulfotomaculum* spp. may constitute only 0.001% of marine microbial populations (*8*, *12*). Like the rare taxa, spores are less prone to viral lysis or predation, and are not detected by traditional diversity surveys (*1*, *2*). A spore-forming *Desulfotomaculum* strain that can only grow between 26° and 47°C was recently isolated from permanently cold Svalbard fjord sediment in the Arctic (*10*). The present study assessed thermophile diversity, abundance, and distribution in Svalbard sediments to reveal insights into mechanisms governing biogeography in the marine environment.

Pristine sediment was sampled from Smeerenburgfjorden (80°N; fig. S1) and incubated over an experimental temperature range (*13*), which revealed two distinct sulfate-reduction regimes

(Fig. 1A). The first has a temperature optimum ($T_{opt}$) of 22°C and a maximum ($T_{max}$) of 32°C, consistent with earlier studies in Svalbard sediments (*14*, *15*) where the in situ temperature is –2° to +4°C year-round. This temperature-activity profile is characteristic of psychrophilic sulfate-reducing bacteria (SRB) that are well adapted to cold conditions (*14*, *16*), which explains their activity and abundance (up to 16%) in this environment (*17*). A second $T_{opt}$ of 56°C (Fig. 1) indicates a previously unrecognized thermophilic community with a $T_{max}$ above 60°C. Pasteurization at 80°C killed the psychrophiles but did not adversely affect the thermophiles (Fig. 1B), indicating that the latter exist as endospores in situ and only germinate after heating. This was supported by successful polymerase chain reaction targeting the spore-forming SRB genus *Desulfotomaculum* only if the sediment was incubated at 50°C before DNA extraction.

Psychrophilic and thermophilic communities were investigated further by incubating homogenized surface sediment at 50°C (*13*). Sulfate reduction rates (SRR) quickly dropped to below the detection limit (Fig. 2A) due to stimulation and subsequent death of vegetative psychrophiles as the sediment warmed up (compare to Fig. 1A). Thermophilic SRR increased exponentially between 20 and 96 hours. The transition from SRR below detection (up to 16 hours) to the onset of the exponential phase (at 20 hours) suggests a lag phase during which conditions became favorable for germination of thermophilic SRB spores. Endospore germination requires appropriate nutrients and substrates (*18*) such as volatile fatty acids (VFA) that are produced by microbial fermentation. VFA are typical electron donors for SRB and were generated rapidly during incubation at 50°C (Fig. 2B), creating conditions suitable for thermophilic sulfate reduction.

Several lines of evidence demonstrate that the VFA production was biologically mediated and not due to thermal alteration of the sediment organic matter. VFA did not accumulate in autoclaved sediment incubated in parallel at 50°C (concentrations never exceeded 0.3 mM). Amendment with the fluorescently labeled polysaccharides pullulan or arabinogalactan revealed extracellular enzymatic hydrolysis in sediment incubated at 50°C, whereas no hydrolysis was detected in sediment-free controls. Clone library analyses of 16*S* ribosomal RNA (rRNA) genes revealed enrichment at 50°C of different bacterial groups related to *Desulfotomaculum*, *Caminicella*, and *Caloranaerobactor-Clostridiisalibacter-Thermohalobacter* lineages within the *Firmicutes* phylum (Fig. 3) [see supporting online material (SOM) and table S3]. The latter lineages are represented by thermophilic anaerobes that convert carbohydrates and proteins to VFA, such as *Caminicella sporogenes* (*19*). Hydrolysis and fermentation at 50°C were therefore likely due to thermophilic spores that germinated at high temperature in the presence of complex natural organic substrates in the sediment.

VFA production at 50°C stimulated growth of a thermophilic *Desulfotomaculum* population

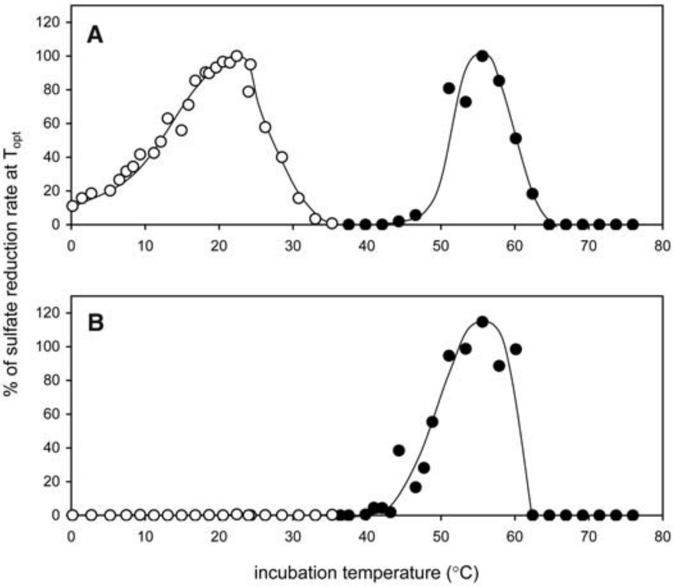

**Fig. 1.** Temperature-gradient incubations. SRR were measured in Smeerenburgfjorden sediment samples incubated between 0° and 76°C. Distinct SRB populations were active between 0° and 32°C, and between 41° and 62°C. Replicates were either untreated (**A**) or pasteurized for 1 hour at 80°C (**B**) before incubation. Open and closed symbols correspond to experiments below and above 35°C, respectively. Incubations in the thermophilic range resulted in much higher SRR (fig. S3); therefore, data are plotted as the percentage of the maximum SRR in (A) at either $T_{opt}$. Pasteurization killed the psychrophilic community and resulted in slightly higher activity by the thermophilic community [115% at the $T_{opt}$ (B)].

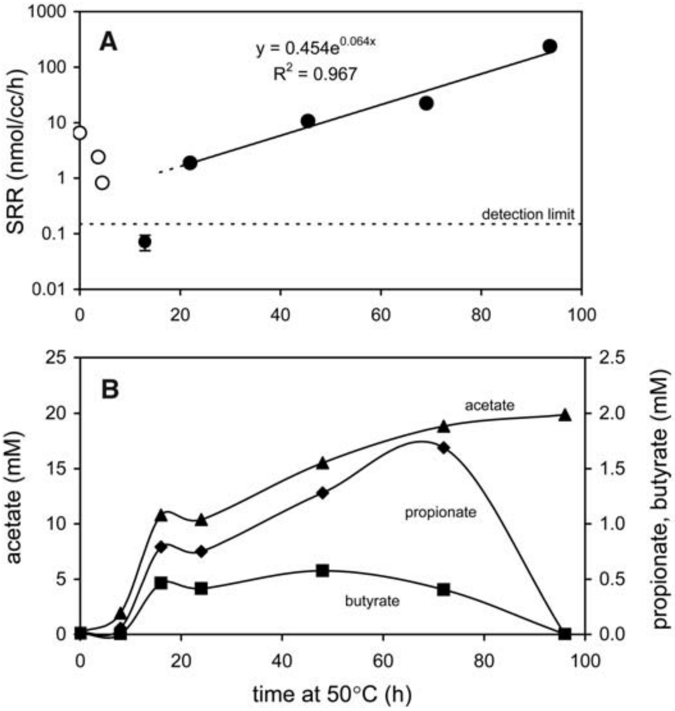

**Fig. 2.** Sediment incubation at 50°C. SRR (**A**) and concentrations of VFA (**B**) in Smeerenburgfjorden sediment (0- to 3-cm depth) incubated at 50°C. SRR represent 4- to 8-hour incubations with $^{35}SO_4^{2-}$ radiotracer. Open symbols correspond to the psychrophilic SRB community that was killed as the temperature increased to beyond their $T_{max}$ (compare to Fig. 1A). SRR were below detection during the 10 to 16 hours time interval (*n* = 2; vertical bar: standard error). Sulfate reduction by 20 to 24 hours indicates germination of *Desulfotomaculum* spores, which subsequently catalyzed an exponential increase in SRR. These data constrain the earliest onset of the exponential phase to 16 hours, as indicated by the dashed extension of the exponential trendline. The exponential phase corresponded to the consumption of butyrate and propionate, which increased steeply, together with acetate (B), before the onset of thermophilic sulfate reduction.

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(Fig. 3), resulting in an exponential increase in SRR (Fig. 2A). The *Desulfotomaculum* cell density at the onset of sulfate reduction can be estimated by dividing the bulk rate (1.3 nmol $\mathrm{cm^{-3}}$ $\mathrm{hour^{-1}}$ at 16 hours based on the exponential function in Fig. 2A) by a mean cell-specific sulfate reduction rate of 2.0 fmol $\mathrm{cell^{-1}}$ $\mathrm{hour^{-1}}$ [based on 33 pure cultures during exponential growth (*20*), and representative of thermophilic *Desulfotomaculum* spp.; see SOM]. This calculation estimates a population of $6.3 \times 10^5$ SRB $\mathrm{cm^{-3}}$ at 16 hours, which corresponds to the in situ spore density given that SRB growth did not occur before 16 hours (Fig. 2A).

Exponential increases in SRR were also measured across a series of intact sediment cores (0- to 23-cm depth) incubated at 50°C (*13*). These data indicate similar numbers of thermophilic *Desulfotomaculum* spores, on the order of $10^5$ $\mathrm{cm^{-3}}$, at all depths (fig. S2). Thermophiles thus constitute a stable component of the rare biosphere (~0.01%) in this Arctic marine habitat (*17*). Abundant taxa in Smeerenburgfjorden sediment are psychrophilic *Cytophaga*, *Flavobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria* (*17*) that catalyze hydrolysis, fermentation, and sulfate reduction during degradation of organic matter (*15*, *21*). We induced the same processes at 50°C, which stimulated SRR about 30-fold higher than in sediment incubated at 4°C (fig. S3). It is interesting that the thermophilic bacterial community has metabolic potential that mirrors the dominant metabolic processes occurring in situ. However, these thermophilic spores make no contribution to local biogeochemical cycling (Fig. 1). Our experience with thermophiles cultivated from Smeerenburgfjorden that have no detectable metabolic activity below 25°C supports this. Thermophilic spores must therefore be supplied externally and can be considered akin to particles accumulating in the sediment.

To estimate the particle flux of thermophilic spores into the sediment, we determined an age model by measuring the $^{210}$Pb activity as a function of depth (see SOM). This revealed a sediment accumulation rate of 0.19 cm $\mathrm{year^{-1}}$ in Smeerenburgfjorden (figs. S2 and S4). Given a stable abundance of about $10^5$ spores $\mathrm{cm^{-3}}$ with depth (fig. S2), the constant supply of thermophiles to this site is $2 \times 10^8$ $\mathrm{m^{-2}}$ $\mathrm{year^{-1}}$. Other fjords along the west coast of Svalbard have comparable thermophilic SRB densities in surface sediments (see SOM; fig. S1). The fjords and the adjacent coastal shelf in this area cover about 1000 $\mathrm{km^2}$ (fig. S1), hence an extrapolation of our estimate corresponds to an annual supply of ~$10^{17}$ thermophiles in this Arctic region. These large numbers highlight not merely the occurrence of thermophiles in the Arctic but rather their unexpected quantity and the consistency of their flux. The warm, anaerobic environment where these bacteria originate must have sufficient geographic distribution, magnitude, and source strength to support the population sizes indicated by our data.

A limited number of marine habitats meet these criteria. Deeply buried sediments hundreds of meters below the sea floor represent warm habitats for anaerobic microbes (*22*). Advective flow of hydrocarbons or other fluids from these sediments can penetrate the sea floor (e.g., at mud volcanoes) and transport microscopic particles or cells up into the cold ocean (*23*). Seafloor pockmarks and active cold seeps are known around west Svalbard (*24*) (fig. S1) and are often

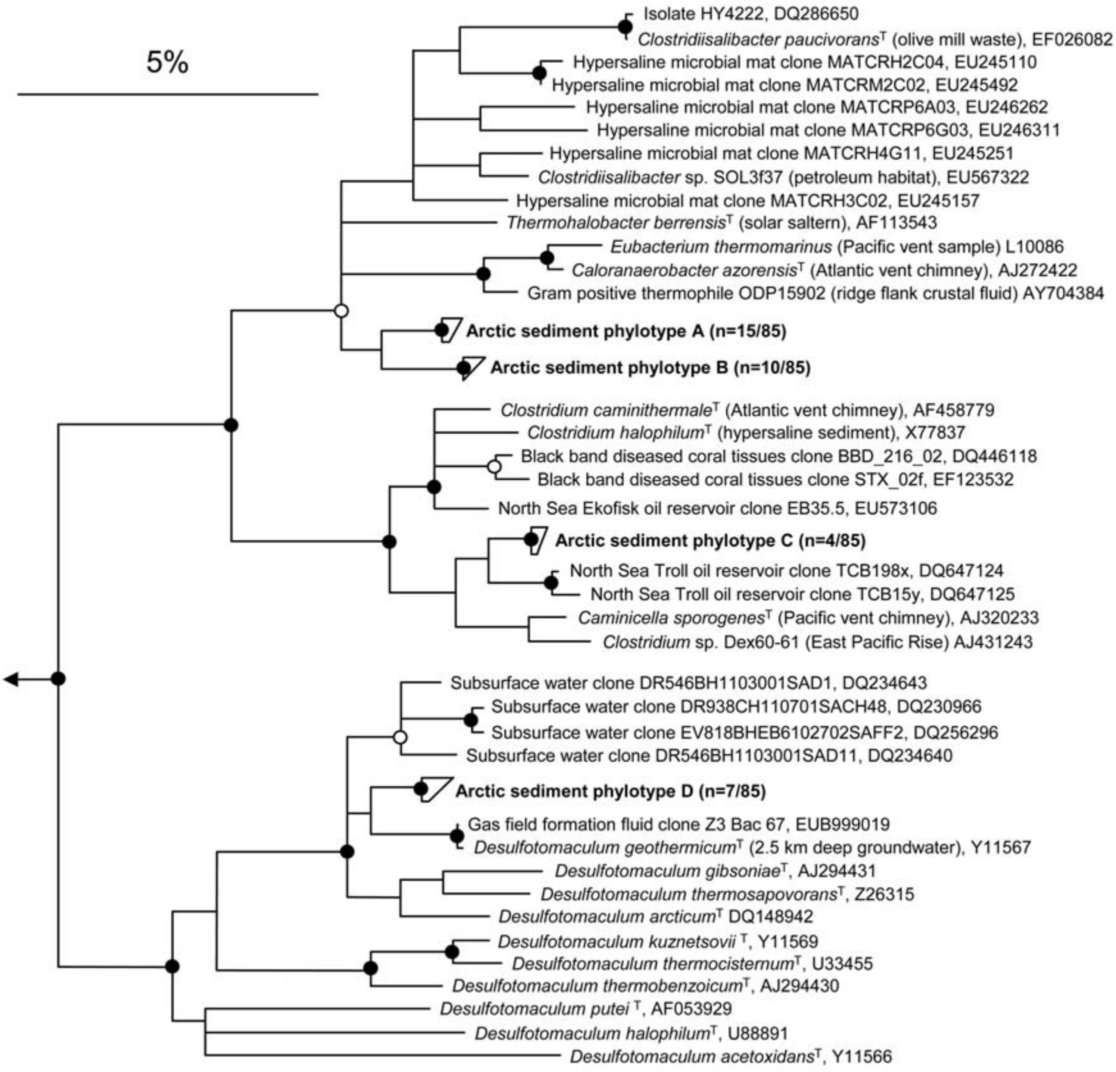
5%
Isolate HY4222, DQ286650
Clostridiisalibacter paucivorans$^{T}$ (olive mill waste), EF026082
Hypersaline microbial mat clone MATCRH2C04, EU245110
Hypersaline microbial mat clone MATCRM2C02, EU245492
Hypersaline microbial mat clone MATCRP6A03, EU246262
Hypersaline microbial mat clone MATCRP6G03, EU246311
Hypersaline microbial mat clone MATCRH4G11, EU245251
Clostridiisalibacter sp. SOL3f37 (petroleum habitat), EU567322
Hypersaline microbial mat clone MATCRH3C02, EU245157
Thermohalobacter berrensis$^{T}$ (solar saltern), AF113543
Eubacterium thermomarinus (Pacific vent sample) L10086
Caloranaerobacter azorensis$^{T}$ (Atlantic vent chimney), AJ272422
Gram positive thermophile ODP15902 (ridge flank crustal fluid) AY704384
Arctic sediment phylotype A (n=15/85)
Arctic sediment phylotype B (n=10/85)
Clostridium caminithermale$^{T}$ (Atlantic vent chimney), AF458779
Clostridium halophilum$^{T}$ (hypersaline sediment), X77837
Black band diseased coral tissues clone BBD_216_02, DQ446118
Black band diseased coral tissues clone STX_02f, EF123532
North Sea Ekofisk oil reservoir clone EB35.5, EU573106
Arctic sediment phylotype C (n=4/85)
North Sea Troll oil reservoir clone TCB198x, DQ647124
North Sea Troll oil reservoir clone TCB15y, DQ647125
Caminicella sporogenes$^{T}$ (Pacific vent chimney), AJ320233
Clostridium sp. Dex60-61 (East Pacific Rise) AJ431243
Subsurface water clone DR546BH1103001SAD1, DQ234643
Subsurface water clone DR938CH110701SACH48, DQ230966
Subsurface water clone EV818BHEB6102702SAFF2, DQ256296
Subsurface water clone DR546BH1103001SAD11, DQ234640
Arctic sediment phylotype D (n=7/85)
Gas field formation fluid clone Z3 Bac 67, EUB999019
Desulfotomaculum geothermicum$^{T}$ (2.5 km deep groundwater), Y11567
Desulfotomaculum gibsoniae$^{T}$, AJ294431
Desulfotomaculum thermosapovorans$^{T}$, Z26315
Desulfotomaculum arcticum$^{T}$ DQ148942
Desulfotomaculum kuznetsovii $^{T}$, Y11569
Desulfotomaculum thermocisternum$^{T}$, U33455
Desulfotomaculum thermobenzoicum$^{T}$, AJ294430
Desulfotomaculum putei $^{T}$, AF053929
Desulfotomaculum halophilum$^{T}$, U88891
Desulfotomaculum acetoxidans$^{T}$, Y11566

**Fig. 3.** Phylogenetic analysis of putative spore-forming thermophilic bacteria in Svalbard sediment. Clone libraries constructed before and after incubation at 50°C differed significantly due to the enrichment of thermophilic *Firmicutes* (see SOM, tables S2 and S3). Dominant phylotypes (i.e., >5% of clones) from the 50°C sediment library are indicated in boldface with relative abundances shown in parentheses. These phylotypes were not detected before 50°C incubation. The consensus tree of 16*S* rRNA gene sequences of selected *Firmicutes* includes next relatives for each phylotype (top 10) plus additional type strains (for *Desulfotomaculum*). Habitats of origin for closest relatives are indicated. Filled and open circles indicate lineages with >90% and 80 to 90% parsimony bootstrap support (100 resamplings), respectively. The scale bar indicates 5% sequence divergence as estimated from maximum-likelihood analysis.

associated with deep gas or oil-bearing deposits (*23*). 16*S* rRNA gene sequence comparisons revealed that taxa enriched in our 50°C experiments (Fig. 3) are most closely related to bacteria from subsurface petroleum reservoirs or oil production facilities (94 to 96% similarity; table S3). Another source of thermophiles could be nearby mid-ocean ridge spreading centers (fig. S1). Large volumes of fluids circulating through ocean crust (*25*) could transport cells away from warm anoxic niches in this seafloor habitat (*26*) and suspend them in abyssal currents. The closest relatives to the Arctic thermophiles also include an anaerobic thermophile isolated from deep, hot crustal fluid (94% similarity; table S3) (*27*).

Petroleum-bearing sediments and fractured ocean crust both host anaerobic heterotrophic microbial communities (*26*, *28*). Areas of discharge connecting these habitats to the water column are widespread, and both processes expel large volumes of fluid into the oceans (*23*, *25*). A combination of different point sources could explain the diversity and distribution of thermophilic taxa in Arctic sediments (Fig. 3 and fig. S1). Although our observations suggest that seabed fluid flow governs the biogeography of thermophilic spore formers, these passive dispersal mechanisms are unlikely to act only on these particular bacteria. Permeable conduits through sediments and ocean crust pass through several microbial niches with changing local temperature and geochemistry (*22*, *25*, *26*). Widespread seeding of the oceans by geofluids from deep biosphere habitats may therefore contribute broadly to the high microbial diversity observed in the marine environment.

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**Supporting Online Material**

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Tables S1 to S3

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# Three-Dimensional Structural View of the Central Metabolic Network of *Thermotoga maritima*

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**Metabolic pathways have traditionally been described in terms of biochemical reactions and metabolites. With the use of structural genomics and systems biology, we generated a three-dimensional reconstruction of the central metabolic network of the bacterium *Thermotoga maritima*. The network encompassed 478 proteins, of which 120 were determined by experiment and 358 were modeled. Structural analysis revealed that proteins forming the network are dominated by a small number (only 182) of basic shapes (folds) performing diverse but mostly related functions. Most of these folds are already present in the essential core (~30%) of the network, and its expansion by nonessential proteins is achieved with relatively few additional folds. Thus, integration of structural data with networks analysis generates insight into the function, mechanism, and evolution of biological networks.**

The advent of genome sequencing has enabled development of computational and experimental tools to investigate complete biological systems, but it has also highlighted the difficulty in integrating complex information for the hundreds to thousands of different molecules that compose even the smallest biological networks. Such integration presents many challenges, especially when assembling data from diverse fields, such as biochemistry and structural biology, that use different operational languages and conceptual frameworks. Biochemistry has traditionally focused on individual reactions and pathways, but recent advances in genomics have led to more rapid growth in the reconstruction and modeling of metabolic networks on a genome-wide scale (*1*–*3*). Thus, biochemical reactions, pathways, and networks can now be described in the context of entire cells, thereby enabling more realistic simulations of the behavior of metabolic networks in a growing number of organisms (*4*–*7*). Nevertheless, metabolism is still generally defined in terms of the chemical names and identity of substrates, products, and reactions. It does not explicitly consider the three-dimensional structures of its components, although such knowledge is required for a comprehensive understanding, not only of the individual reactions, but more importantly, of metabolic networks as a whole. Without such knowledge, we cannot rigorously define enzyme mechanisms or

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