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Original Research Article

HYDROPHOBIC MAPPING OF *CHLOROBIUM TEPIDUM*, THE ENERGY GENERATING BACTERIA

Anindita Roy Chowdhury[1*] and Anushka Kothari[2]

[1]Department of Physics, School of Basic and Applied Sciences, GD Goenka University, Gurgaon, Haryana, India.

[2]Department of Computer Science and Engineering, School of Engineering, GD Goenka University, Gurgaon, Haryana, India

Abstract: Among the four major forces responsible for the three dimensional structure of a protein, hydrophobic force is the dominant one. A protein is functional only after it attains its three dimensional structure. In this age of rising popularity of alternate energy resources, it is known that the bacteria, *Chlorobium tepidum* is capable of harvesting energy. In this paper, the authors aim to investigate the hydrophobic mapping of protein sequences of *Chlorobium tepidum*.

Keywords: *Chlorobium tepidum*, hydrophobicity, structure of protein, Fauchere and Pliska scale, PDB

Introduction: A proteome is the complete set of proteins thought to be expressed by an organism and encoded by a genome. Proteomics is in broad terms the total protein content of a cell or of an organism. It helps in understanding of alteration in protein expression during different stages of life cycle. It also helps in understanding the structure and function of different proteins and their interlinked interaction in an organism. A minor defect in protein structure, its function or any alteration in expression pattern can be detected easily using proteomic studies.

For Correspondence:
anindita.c4@gmail.com.
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Proteomics employ bioinformatics to better understand the protein structures through computational investigation, thus, reducing the time of investigation because of the *in silico* approach. Characterization of protein profile reveals information for the understanding of various aspects related to the protein, like disease, therapy, energy etc. Among the various parameters related to the structure of a protein, hydrophobicity plays a key role in its structure determination. (Frigaard *et al.*, 2002; Kellner, 2000). *Chlorobium tepidum* is a gram-negative bacterium of the green-sulphur phylum. Originally, it was isolated from high sulphide hot springs in New Zealand. Chlorobia are obligate anaerobic photolithoautotrophs and are in aquatic environments, where anoxic layers containing reduced sulphur compounds are exposed to light. The genus *Chlorobium* includes six species of which *C. tepidum* is the only thermophile,

growing optimally at 48°C (Eisen, *et al.*, 2002; Charnock *et al.*, 1991).

Chlorobium tepidum is known to be capable of generating energy from light. This microbe has a relatively small genome and is easy to grow in lab and has interesting biological systems for photosynthesis, and also for processing nitrogen and sulphur. Scientists are hopeful that these microbes may be used as alternative sources of energy (Wahlund and Madigan, 1993). To be functional, proteins need to attain a three dimensional structure which would be governed by the hydrophobicity of the macromolecule (Wahlund, T M and Madigan M T, 1992). The authors, thus, aimed to investigate the hydrophobic mapping of protein sequences of *Chlorobium tepidum* to get a deeper insight into the main factor governing the built-up of the protein structure.

Methodology: In this paper, the authors have approached the investigation the *in silico* way. As a first step, protein sequences of *Chlorobium tepidum* were downloaded from Protein Data Bank (PDB) on March 17, 2018 with not more than 30% sequential identity. The Protein Data Bank (Protein Data Bank, 1971) archive is the single worldwide repository of information about the 3D structures of large biological molecules, including proteins and nucleic acids; being the primary database, the protein sequences were downloaded from here. The number of sequences conforming to the search criteria was thirteen with which further investigation was carried out. The biological processes associated with these sequences varied from Oxidation Reduction Process to carbon fixation, tRNA processing and photosynthesis.

To determine the hydrophobic strength of these sequences of the selected bacteria, a hydrophobic scale is required (Hanson and Tabita, 2003). Different hydrophobic scales have emerged with their unique characteristics (Kyte and Doolittle, 1982; Cornette, 1987). The authors have followed Fauchere-Pliska (1983) hydrophobicity scale, primarily because it is an experimental scale, and was effective in multiple biophysical and pharmacological investigations (Margalit, 1987). This scale reflects hydrophobicity in the context of denatured proteins and/or small synthetic peptides (Frigaard *et al.*, 2003; Wahlund and Madigan, 1995).

A C++ program designed to determine the hydrophobic strength of protein sequences (Chowdhury *et al.*, 2011) was executed on the fasta format sequences of *Chlorobium tepidum*. The output files showed the specific hydrophobic calculations related to each protein sequence. The hydrophobic strength attributed to aromatic hydrophobic and aliphatic hydrophobic content including the calculation due to other hydrophilic residues were determined. Relative variation of the contributions were assessed. Based on frequency of appearance of the amino acid residue and their respective scale value, hydrophobic content of each of the twenty amino acids in each protein sequence were determined along with their total contribution as per their category. Besides measuring each amino acid's hydrophobic contribution towards their category specific hydrophobic content, hydrophobic content per unit length of each amino acid was also determined. The results are tabulated and also plotted in the following section.

Results and Discussion: On executing the C++ program on the fasta format PDB sequences of specific criteria, the contribution of every amino acid in their respective category was determined. The total aromatic hydrophobic (TAroH%) and total aliphatic hydrophobic content (TAliH%) present per unit length in each of the protein sequences is shown in Fig.1. It can be observed that irrespective of the specific nature of the sequences, the aliphatic hydrophobic content is high in each one. For the whole set of sequences, TAroH% varies within a minimum of 3.6% to a maximum of 17.16% whereas TAliH% varies in the range of 33.92% and 55.52%.

As the number of hydrophobic amino acids in aliphatic category is more than that in aromatic category, the probability of their appearance in every sequence would be comparatively higher. Furthermore, hydrophobic contribution of aliphatic amino acids per unit length of the protein sequence in the enzyme classes is more

than that of aromatic residues. This is likely for additive values of seven aliphatic versus four aromatic residues.

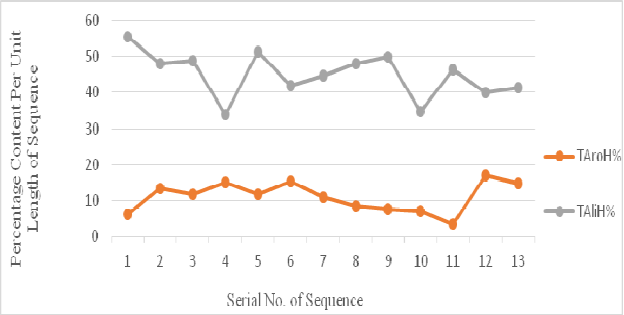

Fig. 1: Variation of Aromatic and Aliphatic Hydrophobic Content Percentage in *Chlorobium tepidum*

Table 1 and 2 reveals deeper insight of aromatic hydrophobic contribution. Fig. 2 graphically shows the variation of contribution of different hydrophobic amino acids.

Table 1 and Fig. 2 shows that except for the tenth sequence, in all other sequences, among all the other aromatic residues, the contribution of phenylalanine is the highest and the least contribution is from histidine. It is notable, here that the frequency of appearance of phenylalanine in the protein sequences being highest in this category makes it the maximum contributor to aromatic hydrophobic content though its hydrophobic scale value is not the highest. In case of histidine, its least hydrophobic scale value and frequency of occurrence in the protein sequences makes it contribute the minimum to total aromatic hydrophobic content.

Table 1: Contribution of Different Aromatic Residues to Total Aromatic Hydrophobic Content in *Chlorobium tepidum*

S. No.	PDB ID	Seq Len	Number (W/F/Y/H) of Hydrophobic Aromatic Residues and their Hydrophobic Contribution (Contr.) in Total Aromatic Hydrophobic Content (WH%/FH%/YH%/HH%) in Sequence (Seq) of different Length (Len)							
			Tryptophan		Phenylalanine		Tyrosine		Histidine	
			No. (W)	Contr. (WH%)	No. (F)	Contr. (FH%)	No. (Y)	Contr. (YH%)	No. (H)	Contr. (HH%)
1	>1GUZ:A	310	1	11.68	6	55.73	6	29.89	4	2.70
2	>1YKW:A	435	5	19.20	19	58.04	12	19.66	14	3.11
3	>2BD0:A	244	1	7.83	11	68.51	6	20.04	8	3.62
4	>2KRU:A	63	1	23.71	3	56.59	1	10.12	7	9.59
5	>2QLC:A	126	1	15.01	6	71.65	1	6.40	8	6.94
6	>3A9F:A	92	1	15.83	6	75.58	1	6.76	2	1.83
7	>3AB1:A	360	4	22.99	9	41.15	13	31.88	12	3.98
8	>3CP8:A	641	1	4.11	19	62.13	16	28.06	24	5.70
9	>3GEE:A	476	0	0.00	15	73.34	8	20.98	16	5.68
10	>4J20:A	88	1	36.17	0	0.00	4	61.74	1	2.09
11	>5DA8:A	545	0	0.00	6	54.68	9	43.99	2	1.32
12	>5H8Z:A	365	8	28.73	19	54.29	10	15.32	8	1.66
13	>5LCB:A	59	1	25.83	3	61.65	1	11.02	1	1.49

On further investigation, it can be observed that 1GUZ, 2BD0, 2KRU, 3AB1, 5H8Z are involved with oxidation-reduction process; 5LCB, 5H8Z, 2KRU are involved in photosynthesis; 3CP8, 3GEE are for tRNA processing whereas 1YKW is for carbon fixation, 5DA8 for protein folding and the remaining sequences, i.e. 2QLC, 3A9F, 4J20 do not have any specific biological process; however 3A9F and 4J20 are associated with heme binding.

Accordingly, some similarity can be noted in the quantification of the hydrophobicities.

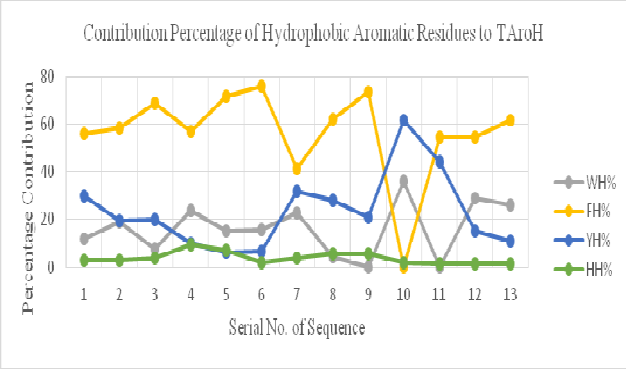

Fig. 2: Percentage Contribution of Individual Hydrophobic Aromatic Amino Acids to Total Aromatic Hydrophobic Content (TAroH) in *Chlorobium tepidum*

Among the several functional sub-categories of protein sequences, it can be observed that for the protein sequences involved in photosynthesis, the quantification of contribution of phenylalanine, tryptophan and tyrosine are almost similar, i.e. within a variation of 5%; though contribution of histidine is quite similar for 5H8Z and 5LCB, it is at 8% variation for the third sequence, 2KRU. Interestingly, it can be noted that for the sequences involved with tRNA processing, hydrophobic contribution of histidine is quite similar whereas unlike the other sequences tryptophan is their least aromatic hydrophobic contributor.

Table 2: Contribution of Different Aromatic Residues Per Unit Length of the Sequence to Total Aromatic Hydrophobic Content in *Chlorobium tepidum*

S No	PDB ID	Seq Len	Number (W/F/Y/H) of Hydrophobic Aromatic Residues and their Hydrophobic Percentage Contribution (Cont.) Per Unit Length (Len) (WHL/FHL/YHL/HHL) in each Sequence (Seq.)							
			Tryptophan		Phenylalanime		Tyrosine		Histidine	
			No. (W)	Cont. Per Unit Len (WHL)	No. (F)	Cont. Per Unit Len (FHL)	No. (Y)	Cont. Per Unit Len (YHL)	No. (H)	Cont. Per Unit Len (HHL)
1	>1GUZ:A	310	1	0.73	6	3.46	6	1.86	4	0.17
2	>1YKW:A	435	5	2.59	19	7.82	12	2.65	14	0.42
3	>2BD0:A	244	1	0.92	11	8.07	6	2.36	8	0.43
4	>2KRU:A	63	1	3.57	3	8.52	1	1.52	7	1.44
5	>2QLC:A	126	1	1.79	6	8.52	1	0.76	8	0.83
6	>3A9F:A	92	1	2.45	6	11.67	1	1.04	2	0.28
7	>3AB1:A	360	4	2.50	9	4.48	13	3.47	12	0.43
8	**>3CP8:A**	641	1	0.35	19	5.31	16	2.40	24	0.49
9	**>3GEE:A**	476	0	0.00	15	5.64	8	1.61	16	0.44
10	>4J20:A	88	1	2.56	0	0.00	4	4.36	1	0.15
11	**>5DA8:A**	545	0	0.00	6	1.97	9	1.59	2	0.05
12	>5H8Z:A	365	8	4.93	19	9.32	10	2.63	8	0.28
13	>5LCB:A	59	1	3.81	3	9.10	1	1.63	1	0.22

Table 2 that reveals the variation of the hydrophobic content per unit length also indicates similar result like Table 1; visual variation of the same is plotted in Fig. 3.

In Table 3, the detailed contribution of individual aliphatic hydrophobic content to total aliphatic hydrophobic content is observed; graphs of the same is in Fig. 4.

Table 3: Contribution of Different Aliphatic Residues to Total Aliphatic Hydrophobic Content in *Chlorobium tepidum*

S. No.	PDB ID	Seq Len	Number (I/L/C/V/P/A/M) of Hydrophobic Aliphatic Residues and Their Hydrophobic Contribution (Contr.) in Total Aliphatic Hydrophobic Content (IH%/ LH%/CH%/VH%/ PH%/AH%/MH%) in Sequence (Seq) of different Length (Len)													
			Isoleucine		Leucine		Cysteine		Valine		Proline		Alanine		Methionine	
			No. (I)	Contr. (IH%)	No. (L)	Contr. (LH%)	No. (C)	Contr. (CH%)	No. (V)	Contr. (VH%)	No. (P)	Contr. PH%	No. (A)	Contr AH%	No. (M)	Contr. MH%
1	>1GUZ:A	310	25	26.2	28	27.7	3	2.7	35	24.8	13	5.4	26	4.7	12	8.6
2	>1YKW:A	435	27	23.3	37	30.1	6	4.4	35	20.4	25	8.6	37	5.5	13	7.7
3	>2BD0:A	244	17	25.7	21	29.9	4	5.2	16	16.4	8	4.8	25	6.5	11	11.4
4	>2KRU:A	63	1	8.4	4	31.8	0	0.0	6	34.3	2	6.7	5	7.3	2	11.5
5	>2QLC:A	126	12	33.4	15	39.4	0	0.0	8	15.1	4	4.5	8	3.8	2	3.8
6	>3A9F:A	92	4	18.6	7	30.8	2	8.0	6	18.9	5	9.3	10	8.0	2	6.4
7	>3AB1:A	360	22	24.7	32	34.0	2	1.9	29	22.0	12	5.4	35	6.8	7	5.4
8	**>3CP8:A**	641	50	29.2	60	33.1	9	4.5	37	14.7	31	7.3	48	4.8	16	6.4
9	**>3GEE:A**	476	31	23.5	57	40.8	5	3.2	32	16.4	11	3.3	50	6.5	12	6.2
10	>4J20:A	88	2	11.7	2	11.1	2	10.0	5	19.9	3	7.0	16	16.2	6	24.1
11	**>5DA8:A**	545	40	28.4	43	28.9	0	0.0	48	23.1	14	4.0	68	8.3	15	7.3
12	>5H8Z:A	365	20	24.6	23	26.7	0	0.0	35	29.2	19	9.4	28	5.9	5	4.2
13	>5LCB:A	59	3	22.1	4	27.8	0	0.0	4	19.9	0	0.0	4	5.1	5	25.1

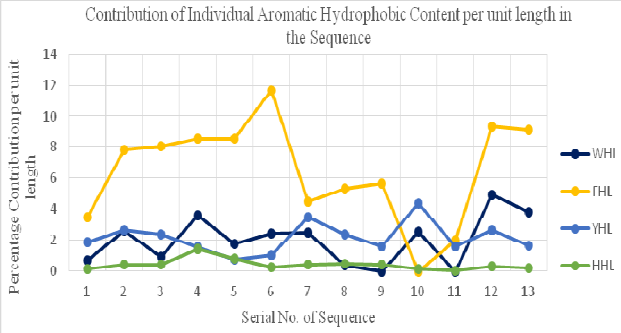

Fig. 3: Contribution of Individual Hydrophobic Aromatic Residues per unit length in the Sequence in *Chlorobium tepidum*

It can be observed that leucine emerges as the highest aliphatic hydrophobic contributor to both total aliphatic hydrophobic content and also per unit length. Cysteine shows up as the least contributor in this category. Hydrophobic contribution of leucine to all the three sequences involved with photosynthesis is quite similar, a maximum variation of 5% is only observed. A very close relationship exists among the hydrophobic contribution of proline and alanine.

The sequences engaged in oxidoreductase activity, 1GUZ, 2BD0, 2KRU, 3AB1, 5H8Z interestingly showed similar contribution for leucine and isoleucine. Leucine variation is within 5% and that for isoleucine is a mere 4%; hydrophobic contribution from cysteine, proline and alanine are also very close to each other. Hydrophobic contribution from cysteine, valine, alanine and methionine are respectively quite comparable with each other for the tRNA processing sequences, 3CP8 and 3GEE.

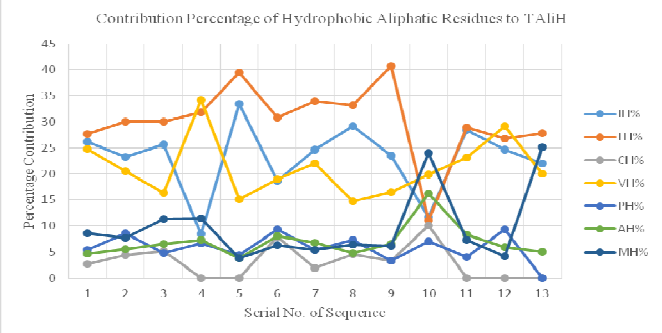

Fig. 4: Percentage Contribution of Individual Hydrophobic Aliphatic Amino Acids to Total Aliphatic Hydrophobic Content in *Chlorobium tepidum*

Fig. 4 above shows the relative variation of the different aliphatic hydrophobic components. Leucine emerges as the highest contributor and cysteine as the least. Though the hydrophobic scale value of neither leucine is the highest nor that of cysteine is the least, they are respectively the maximum and minimum hydrophobic contributor of total aliphatic hydrophobic content. This indicates that the frequency of occurrence of the respective amino acids in the protein sequences is respectively highest and least for leucine and cysteine.

Table 4 that reveals the variation of the aliphatic hydrophobic content per unit length also indicates parallel result; graphic variation of the same is plotted in Fig. 5.

Similar to Fig. 4 above, Fig. 5 indicates the maximum and minimum aliphatic hydrophobic contributor with an exception for tenth sequence; insignificant variation for the minimum contributor is noticed for sixth and eight sequence.

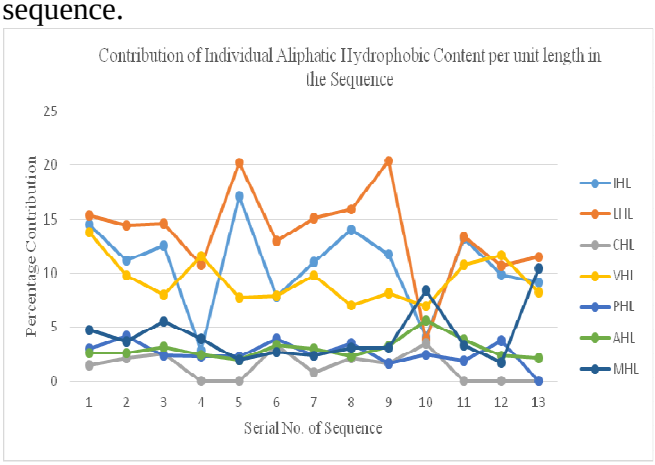

Fig. 5: Contribution of Individual Hydrophobic Aliphatic Residues per unit length in the Sequence in *Chlorobium tepidum*

The results indicate that the maximum and minimum contributors remain the same for both aromatic and aliphatic hydrophobic categories of *Chlorobium tepidum*. Similar trend has been reported for an entire sequence database of protein sequences from six different enzyme classes (Chowdhury *et al.*, 2011).

Table 4: Contribution of Different Aliphatic Residues Per Unit Length of the Sequence to Total Aliphatic Hydrophobic Content in *Chlorobium tepidum*

S. No.	PDB ID	Seq Len	Number (I/L/C/V/P/A/M) of Hydrophobic Aliphatic Residues and their Hydrophobic Percentage Contribution (Cont.) Per Unit Length (Len) (IHL/LHL/CHL/VHL/PHL/AHL/MHL) in each Sequence (Seq)													
			Isoleucine		Leucine		Cysteine		Valine		Proline		Alanine		Methionine	
			No. (I)	Cont. Per Unit Len IHL	No. (L)	Cont. Per Unit Len LHL	No. (C)	Cont. Per Unit Len CHL	No. (V)	Cont. Per Unit Len VHL	No. (P)	Cont. Per Unit Len PHL	No. (A)	Cont. Per Unit Len AHL	No. (M)	Cont. Per Unit Len MHL
1	>1GUZ:A	310	25	14.52	28	15.35	3	1.49	35	13.77	13	3.02	26	2.60	12	4.76
2	>1YKW:A	435	27	11.17	37	14.46	6	2.12	35	9.82	25	4.14	37	2.64	13	3.68
3	>2BD0:A	244	17	12.54	21	14.63	4	2.52	16	8.00	8	2.36	25	3.18	11	5.55
4	>2KRU:A	63	1	2.86	4	10.79	0	0.00	6	11.62	2	2.29	5	2.46	2	3.90
5	>2QLC:A	126	12	17.14	15	20.24	0	0.00	8	7.75	4	2.29	8	1.97	2	1.95
6	>3A9F:A	92	4	7.83	7	12.93	2	3.35	6	7.96	5	3.91	10	3.37	2	2.67
7	>3AB1:A	360	22	11.00	32	15.11	2	0.86	29	9.83	12	2.40	35	3.01	7	2.39
8	**>3CP8:A**	641	50	14.04	60	15.91	9	2.16	37	7.04	31	3.48	48	2.32	16	3.07
9	**>3GEE:A**	476	31	11.72	57	20.36	5	1.62	32	8.20	11	1.66	50	3.26	12	3.10
10	>4J20:A	88	2	4.09	2	3.86	2	3.50	5	6.93	3	2.45	16	5.64	6	8.39
11	**>5DA8:A**	545	40	13.21	43	13.41	0	0.00	48	10.74	14	1.85	68	3.87	15	3.39
12	>5H8Z:A	365	20	9.86	23	10.71	0	0.00	35	11.70	19	3.75	28	2.38	5	1.68
13	>5LCB:A	59	3	9.15	4	11.53	0	0.00	4	8.27	0	0.00	4	2.10	5	10.42

This possibly indicates the hydrophobic trend of protein sequences. Deeply looking into the hydrophobic details, the variations can be noted and a possible correlation with specific function can be hinted upon. Since functional implication is observed, it is obvious that the structural importance would be significant and hence, the existence of the hydrophobic amino acids is important. The significance of the presence of hydrophobic amino acids has been analyzed for these *Chlorobium tepidum* sequences, as well. Dalhus *et al.* (2002) stated the significance of histidine and tryptophan residues in a *Chlorobium tepidum* sequence. Similarly, Li *et al.* (2005) mentioned the significance of bioinformatic analysis of such sequences. Supangat *et al.* (2006) worked on the structure of this bacteria and commented on the bound between specific aromatic side chains. Muraki *et al.* (2010) also worked with *Chlorobium tepidum* and commented on the conserved C-terminal residues.

Wavelike characteristic of the energy transfer within the photosynthetic complex of *Chlorobium tepidum* has been evidenced by Engel *et al.* (2007). Through genome comparisons, genes were identified in *Chlorobium tepidum* that are highly conserved among photosynthetic species (Eisen *et al.*, 2002). For the structural configuration and hence the functional aspect of a protein molecule is indebted to hydrophobicity. Frigard and team (Frigard *et al.*, 2003) reviewed the structure, physiology and metabolism of this green sulphur bacterium. Examination of the hydrophobic character of protein sequences of *Chlorobium tepidum* thus, emerges as noteworthy.

Conclusion: In this paper, the total hydrophobic content due to the hydrophobic aromatic and aliphatic residues have been analyzed. Close investigation revealed the contribution of specific amino acids in the various protein sequences obtained for the light harvesting bacteria, *Chlorobium tepidum*. This may help in better realization of the ability of the bacteria in generation of energy.

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