

# Molecular Modeling of HEV Core Protein and Active Compounds from Northeast Folk Medicine

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## Abstract

The main etiological agent, which is considered to cause acute hepatitis is the Hepatitis E virus. Northeast India has a huge reservoir of medicinal plants for treating jaundice using folk medicine (ITK). The current study focuses on model 32 sequences of HEV core protein submitted in GenBank (KJ879461-KJ879492) and to evaluate the docking pattern with 10 selected active compounds (Glycyrrhizin, Lignans, Piperine, Wedelolactone, Galactomannan, Zingerone, Catechin, Gallic acid, Vasicinone) which are found in various medicinal plants species. Using Open Babel, the protein sequences, as well as the structures, were first converted to PDB format. The Gene Bank provided these sequences [protein sequence id: AIH14833-AIH14864]. The sequences were analyzed by PROTPARAM for chemical compositions and RaptorX for structure. Finally, PASS was applied for toxicity determination and ADME for screening the safety. The Raptor X and PROTPARAM analysis showed stable protein structures of HEV core protein. The analysis categorically showed the composition of C, H, N, O, and S in the studied sequences in a ratio of 108: 171: 35: 36: 1. However, the best results were found in Bhui-amla (Lignans) with the highest docking score of 6944 against sequence ID AIH14838. Lipinski Rule was carried out for all the active compounds and was found to be excellent. The docking score and minimum energy associated show efficient activity of the studied compounds against HEV protein and generates baseline scientific data on the use of folk medicine and the possibility of their commercial utilization.

**Keywords:** HEV core protein North East India, Folk medicine, RAMPAGE, PROTPARAM, RaptorX, Rasmol

## Introduction

The main etiological agent, which is considered to cause acute hepatitis is the Hepatitis E virus (Narayana *et al.*, 2020; Albureikan, 2020). Annually and worldwide, it was estimated that 20 million cases occur, causing the rates of mortality in pregnant women to reach 28%. Hepatitis E is increasing nowadays. HAV and HEV are constantly present at a high rate among the general

public and affect all age groups equally. Globally, it is estimated that 2.3 billion people are infected by Hepatitis E virus (HEV), which is a significant international public health problem (Das, 2014). During the third trimester, high mortality among pregnant women is the significant difference between HEV and other causes of acute viral hepatitis (Smith *et al.*, 2016).

HEV belongs to the family *Hepeviridae* and genus *Orthohepevirus*. Four of the species that have been defined are the ones that infect carnivores (Orthohepevirus C), soricomorphs, rodents and birds (Orthohepevirus B), and bats (Orthohepevirus D) (Knowles *et al.*, 2011). The Orthohepevirus A comprises seven genotypes that infect human (HEV 1, 2, 3, 4 & 7), wild boar (HEV-3, 4, 5 & 6), pig (HEV- 3 & 4), deer (HEV-3), rabbit (HEV-3), mongoose (HEV-3), yak (HEV-4) and camel (HEV-7) (Sridhar *et al.*, 2017). Annually, it was estimated that 2 million cases of hepatitis E occur in India alone in comparison with the estimated 1.4 million cases of hepatitis A. There have been consistent epidemiological characters of Hepatitis E since its first reported outbreak in New Delhi. The prevalence was found to be highest in young adults and women were reported to have high mortality rates especially in the third trimester of pregnancy (Smith *et al.*, 2014).

Northeast India is showing a shift in hepatitis A viral seroepidemiology. Adults are affected equally by hepatitis E. Unknown herbal medications and non-ABCE AVH and ALF are very common in this region (Hughes *et al.*, 2010). Major viral causes are constituted by HAV and HEV. Higher Mortality level can be found in females and the young ones that belong to the productive section of the society (Das, 2014; Das *et al.*, 2016). Northeast India has a huge reservoir for treating Jaundice by folk Medicine (ITK). It is important to understand the effectiveness of the active compounds of these traditional medicines and their potential use.

**Table 1.** List of active compounds against HEV core protein and their source

Sl. No.	Scientific Name	Common name	Active compounds
1	Glycyrrhiza glabra	Liquorice	Glycyrrhizin
2	Phyllanthus niruri	Bhui-amla	Lignans
3	Piper longum	Long pepper	Piperine
4	Trigonella foenum graecum	Fenugreek seeds	Galactomannan
5	Eclipta alba	Bhringraj	Wedelolactone

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6	Cajanus cajan	Pigeon pea, Arhar	Cajarin
7	Camellia sinensis	Green tea	Catechin
8	Lawsonia inermis	Henna leaf	Gallic acid
9	Justicia adhatoda	Malabur nut	Vasicinone
10	Zingiber officinale	Ginger	Zingerone

We have selected a list of active compounds against HEV core protein and their source as shown in **Table 1**. Glycyrrhizin is a glycoside obtained from roots and stolon of Liquorice (*Glycyrrhiza glabra*). It helps the liver to detoxify drugs and is used for the treatment of liver disease. Glycyrrhizin exhibits activities like antihepatotoxic activity (Amagaya *et al.*, 1984; Cosmetic Ingredient Review Expert Panel, 2007) while phyllanthin and hypophyllanthin belong to the lignan (*Phyllanthus niruri*) category and have been shown to possess hepatoprotective and antigenotoxic activities (Dahanayake *et al.*, 2020). The major plant alkaloid Piperine, which is found in *P. longum* Linn (Long pepper) has bioavailability enhancing activity for some drugs nutritional and some substances and is known to exhibit a hepatoprotective activity apart from exhibiting a toxic effect against hepatocytes (Panahi *et al.*, 2015; Matsuda *et al.*, 2008; Shukla *et al.*, 2011). Wedelolactone (7-methoxy-5, 11, 12-trihydroxy-coumestan) is a natural plant product, which is primarily synthesized by the members of the Asteraceae family (Ding *et al.*, 2017; Kaushik-Basu *et al.*, 2008). WDL is abundantly found in the plant genus *Eclipta* (or *Bhringaraj*). It is an acrid, bitter herb medicine traditionally used extensively for the prevention of liver damage due to alcohol overdose and jaundice and for hair and skin health (Singh *et al.*, 2001; Patel *et al.*, 2008; Roy *et al.*, 2008). Also, in India, it was also used for the treatment of infective hepatitis (Singh *et al.*, 2001; Patel *et al.*, 2008; Roy *et al.*, 2008). *Cajanus cajan* is a perennial member of the family Fabaceae with the presence of two globulins, cajanin, and concajanin (Zu *et al.*, 2010). It has been used widely for many years for treating dysentery, sores, skin irritations, measles, jaundice, diabetes, hepatitis and many other illnesses; for expelling bladder stones and stabilizing menstrual period (Zu *et al.*, 2010). Likewise, the rare risk of hepatotoxicity in a few individuals have been associated with Catechins of green tea extract (Teschke *et al.*, 2014). On the other hand, in many regions, *Lawsonia inermis* (Henna) is a shrub or small tree cultivated as commercial dye crop and an ornamental (Muthumani *et al.*, 2010) is as astringent, hypertensive, jaundice, and against a headache, sedative, and leprosy (Muthumani *et al.*, 2010; Saadabi, 2007). Vasicinone was isolated from the leaves of *Justicia adhatoda* and its crude extract has been reported to have hepatoprotective activity (Sarkar *et al.*, 2014). The major pungent compounds in *Zingiber officinale* (Ginger) of rhizome extract consists of potentially active gingerols, which can be converted to shogaols, zingerone, and paradol (Govindarajan and Connell, 1983; Jolad *et al.*, 2004). Lastly, hepatoprotective activity were noticed in the Seeds of fenugreek, which were annual herbs (Kaviarasan *et al.*, 2007).

In the discovery of new mechanism-or structure-based drugs, drug design assisted by computer have allowed many success stories by new molecular modeling approaches, which are driven by these fast-developing computational platforms (De Ruyck *et al.*, 2016). Molecular modeling tools are extensively used in drug designing. These tools consider 3D molecular structures and their relevant physicochemical properties. The research work aimed to investigate the efficacy of the prevalent medicines against the HEV virus using CADD(www.bioinfo3d.cs.tau.ac.il) (Computer-aided drug designing and to comment on the promising compound of NE folk for future use.

## Materials and Methods

### Retrieving the Gene bank HEV Sequences

A total of 32 HEV sequences which were submitted by the author of this manuscript [KJ879461-92<sup>1</sup>] were converted into FASTA and the protein sequences were converted into PDB format using open babel.

### Selection of active compounds

A total of 10 active compounds were selected which are present in the folk medicine used to treat jaundice in North East India and whose source parts are abundant in nature. The 2D structures of these active compounds were retrieved in SDF form using NCBI and were further converted mol2, mol, and pdb format.

### Structure prediction

RaptorX was carried out for predicting the structures of HEV protein sequences. Protparam was done for computing the physical and chemical parameters of these protein sequences.<sup>2</sup>

### 3D structure analysis

RASMOL was used for visualization of 3D structures of the studied protein. Using Rampage, to understand the stability of these proteins, Further Ramachandran plots were accessed.<sup>3</sup>

### Molecular Docking

Molecular docking was carried out by the PATCHDOCK server where the PDB format of protein sequences and selected active compounds was taken.

### Toxicity test

PASS (Prediction of activity spectra for substances) was carried out for prediction of toxicity of selected active compounds while ADME for screening the safety of the selected active compounds (active compounds were in mol format).

<sup>1</sup> <http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>.

<sup>2</sup> <http://raptorx.uchicago.edu/StructurePrediction/predict>

<sup>3</sup> <https://sourceforge.net/projects/openrasmol/>

Potentiality as Drug

Lipinski Rule of 5 was done to evaluate drug-likeness. Results were displayed using RASMOL.

Results and Discussion

Table 2. Docking Profile of All the Active Compounds (De Ruyck et al., 2016)

Common name	Scientific name	Active compounds	Protein ID	Best score
Liquorice	Glycyrrhiza glabra	Glycyrrhizin	AIH14839	5464
Bhui-amla	Phyllanthus niruri	Lignans	AIH14838	6944
Long pepper	Piper longum	Piperine	AIH14839	5606
Fenugreek seeds	Trigonella foenum graecum	Galactomannan	AIH14834	3170

Bhringraj	Eclipta alba	Wedelolactone	AIH14839	6728
Pigeon pea, Arhar	Cajanus cajan	Cajanine	AIH14846	6756
Green tea	Camellia sinensis	Catechin	AIH14853	5388
Henna leaf	Lawsonia inermis	Gallic acid	AIH14842	2290
Malabur nut	Justicia adhatoda	Vasicinone	AIH14863	6132
Ginger	Zingiber officinale	Zingerone	AIH14839	3368

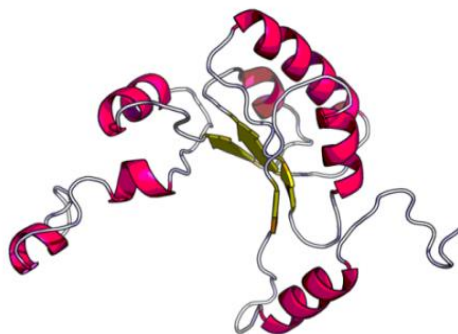
Table 2 shows the best result found from molecular docking of the active compounds against the proteins sequence which were collected from Gene Bank carried out using Patch DOCK. The best result was seen in Bhui-amla (Lignans) with the highest docking score of 6944. A table of docking profile of Bhui-amla against all the protein sequences is given in Table 3.

Table 3. Docking Profile of Lignans (Bhui-amla) against Studied Proteins

Sl No.	Protein Id	Receptor	Score	Area	Ace	Transformation
1.	AIH14833	302746	5648	739.80	-32.25	-2.53 -0.19 0.51 2.61 -9.79 36.36
2.	AIH14834	302747	5530	703.00	-210.62	2.43 -0.00 -3.07 15.51 -5.29 -6.45
3.	AIH14835	302748	5146	578.70	-123.17	1.69 -0.09 -2.36 21.35 8.25 4.24
4.	AIH14836	302749	5562	709.00	-113.13	1.61 0.96 0.81 -11.85 6.03 3.89
5.	AIH14837	302752	6108	825.90	-260.98	1.31 0.71 -3.01 12.88 1.16 -9.33
6.	AIH14838	302753	6944	831.50	-114.26	-1.99 -0.31 -1.46 -6.10 9.99 2.38
7.	AIH14839	302754	6340	884.60	-333.88	1.63 0.30 1.22 0.59 -4.97 20.89
8.	AIH14840	302810	5186	744.10	-74.80	-0.36 -0.46 -1.76 -7.58 12.69 9.78
9.	AIH14841	303094	5820	759.60	-45.67	-2.54 1.39 -1.38 -3.86 10.31 11.80
10.	AIH14842	303095	6260	838.30	-251.68	1.67 1.29 -2.98 10.70 -19.85 6.95
11.	AIH14843	303096	6236	766.60	-171.93	-1.12 -0.40 -2.98 2.93 1.19 11.87
12.	AIH14844	303097	5360	695.80	-148.29	0.21 1.34 -2.27 2.43 -9.77 1.37
13.	AIH14845	303098	5614	836.40	-240.54	0.72 0.41 -1.83 13.59 12.30 29.08
14.	AIH14846	303100	6034	793.40	-152.09	-0.53 -0.79 -3.11 14.23 8.76 9.75
15.	AIH14847	303101	4840	570.80	-168.84	0.62 0.79 0.65 12.37 7.51 0.43
16.	AIH14848	303102	5564	689.20	-188.51	2.96 -0.71 -0.79 8.17 9.54 43.99
17.	AIH14849	303106	5624	746.40	-243.42	1.41 0.80 -1.23 5.66 11.82 34.67
18.	AIH14850	303107	5210	670.80	-180.86	0.65 0.59 -0.64 -6.50 21.27 -0.89
19.	AIH14851	303108	4958	652.90	-51.76	0.35 0.62 -1.71 6.08 -15.28 -0.49
20.	AIH14852	303110	5544	654.50	-121.53	2.24 1.25 -0.14 -10.61 21.53 23.16
21.	AIH14853	303153	5598	639.00	-158.99	-1.88 1.27 -0.07 -6.88 -21.86 9.13
22.	AIH14854	303157	6196	800.20	-229.59	-1.45 1.25 2.91 -0.43 7.45 27.75
23.	AIH14855	303160	4726	546.40	-202.31	2.90 -0.40 2.00 0.73 -3.79 38.21
24.	AIH14856	303163	5602	738.10	-229.32	0.21 -1.30 -0.64 -11.11 16.05 8.81
25.	AIH14857	303166	5090	653.10	-202.47	-2.31 0.06 2.94 22.03 5.18 20.97
26.	AIH14858	303167	6626	846.50	-233.11	-1.88 -0.85 -1.38 -5.79 14.16 20.40
27.	AIH14859	303168	5636	741.80	-176.59	-0.76 -0.35 1.99 30.32 -6.17 23.72
28.	AIH14860	303169	5314	701.60	-2.43	-1.09 0.23 0.84 9.69 -16.01 16.19
29.	AIH14861	303170	5504	683.00	-186.13	2.75 -0.78 0.97 3.92 7.25 10.50
30.	AIH14862	303172	4748	560.90	-44.75	-0.41 0.16 -1.06 -6.56 13.97 3.68
31.	AIH14863	303173	6240	755.00	-232.47	0.03 0.04 -1.59 8.30 9.47 6.44
32.	AIH14864	303174	5028	656.70	14.03	1.55 1.18 -0.09 0.77 12.54 10.46

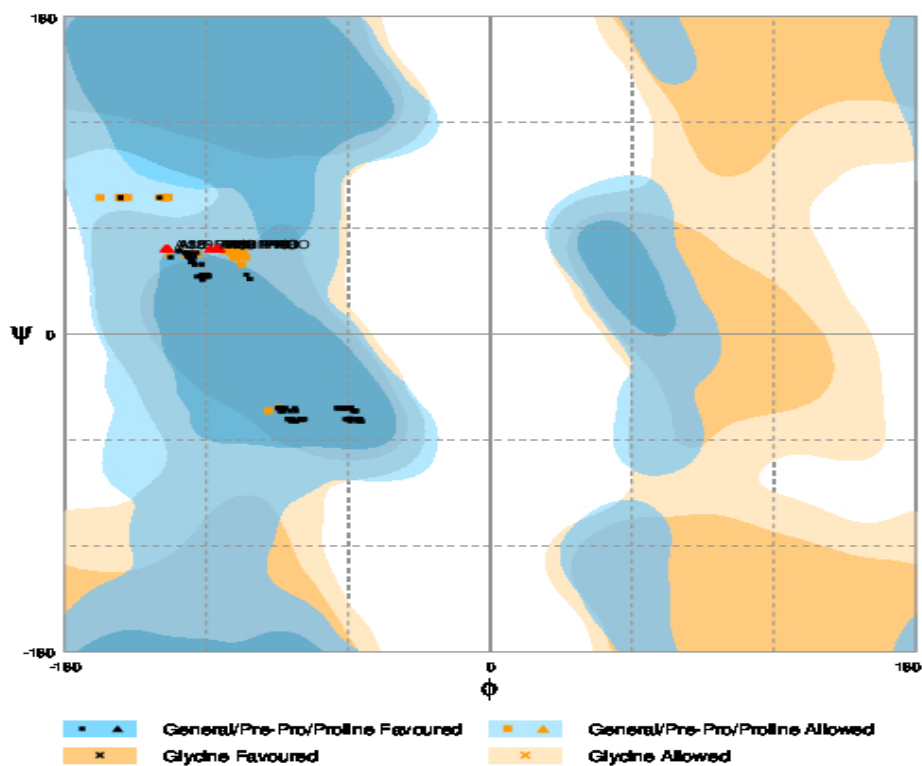
Without close homolog's in the Protein Data Bank (PDB), **raptorx** is a protein structure prediction server developed by the Xu group, which excels at predicting 3D structures for protein sequences<sup>4</sup>. Raptorx helps in the structural prediction of the protein sequences

which is been retrieved from Gene Bank under accession number KJ879461-92. There are 32 sequences present and a single representative structure has been represented in **Figure 1**.



**Figure 1.** A representative protein structure found by RaptorX (AIH14833)

The **Ramachandran plots** were constructed for each protein sequence. For a clear view, one of the Ramachandran plots has been displayed in **Figure 2**.



**Figure 2.** Ramachandran Plot for a given protein sequence(AIH14838)

<sup>4</sup> [www.bioinfo3d.cs.tau.ac.il](http://www.bioinfo3d.cs.tau.ac.il)

The figure shows that the red regions correspond with conformations. These are the allowed regions, where there are no steric clashes like the alpha-helical and beta-sheet conformations. The yellow areas show the allowed regions if slightly shorter Van der Waals radii are used in the calculation, i.e. the atoms are allowed to come a little closer together. This brings out an additional region that corresponds to the left-handed alpha-helix<sup>5</sup>.

RasMol is a computer program written for molecular graphics visualization. It is mainly used to explore and depict biological macromolecule structures, such as those found in the Protein Data Bank<sup>6</sup>. RASMOL was used to visualize all the results as well as all the structure of all the protein sequences.

**Table 4.** Result of Lipinski Rule for All the Active Compounds

Compound	Mass	HBD	HBA	cLOGP	Molar Refractivity
Glycyrrhizin	912.000000	0	0	0.000000	0.000000
Lignans	912.000000	0	0	0.000000	0.000000
Piperine	342.000000	0	3	9.319986	139.000488
Galactomannan	576.000000	0	0	0.000000	0.000000
Wedelolactone	364.000000	10	7	6.725702	116.919746
Cajanine	364.000000	7	6	8.785706	135.272186
Catechin	342.000000	5	6	6.780993	123.389862
Gallic acid	202.000000	4	5	3.909029	68.664085
Vasicinone	242.000000	9	2	3.086290	82.836876
Zingerone	230.000000	4	3	5.821792	93.607079

Drug-like and non-drug like molecules can be differentiated by **Lipinski Rule** of 5. It predicts a high probability of success or failure due to drug-likeness for molecules<sup>7</sup>. Lipinski Rule was carried out for all the active compounds. The results are given in **Table 4**.

Prot Param is a tool that computes the various chemical and physical parameters for a given protein sequence which is given by the user. The parameters which are computed include the extinction coefficient, atomic composition, theoretical PI, molecular weight, amino acid composition, have estimated half-life, instability index, aliphatic index, and grand average of hydrophobicity (**Table 5**)<sup>8</sup>.

**Table 5.** Shows composition of different elements in the studied sequence (physical and chemical parameters for a given protein sequences)

Results of ADMET OF LIGNANS (Phyllanthus niruri)	
ID	Value
BBB	0.0100522
Buffer_solubility_mg_L	18.8721
Caco2	26.8396
CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Inhibitor
CYP_2D6_inhibition	Non
CYP_2D6_substrate	Non
CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Substrate
HIA	97.099394
MDCK	14.3364
Pgp_inhibition	Inhibitor
Plasma_Protein_Binding	76.666659
Pure_water_solubility_mg_L	9.94881
Skin_Permeability	-4.24331
SKlogD_value	1.740200
SKlogP_value	1.740200
SKlogS_buffer	-4.341610
SKlogS_pure	-4.619660

ADME was carried out for each active compound to check their skin permeability, buffer solubility, plasma protein binding, and pure water solubility, etc<sup>9</sup>.

Rasmol is a computer program for molecular graphics visualization. It is mainly used to explore and depict biological macromolecule structures, such as those found in the Protein Data Bank<sup>10</sup>. RASMOL was used to visualize all the results as well as all the structures of all the active compounds and protein sequences.

This method which has been adopted in the current study has been tried and tested for identifying active compounds for the treatment of jaundice. A study has got similarity with a study carried out by Xia *et al.*, 2011 where that target protein was HEV ORF2 protein. The procedure in their study mainly focuses on homology modeling and molecular docking. Also, the calculation of the binding domain and details of energy involved along with the configuration of hydrogen bonds are similar to our study (Xing *et al.*, 2011).

In a study, carried out by You *et al.*, 2014 that they predicted the epitope of 8H3 on E2S by epitope prediction software based on the

<sup>5</sup> <http://raptorx.uchicago.edu/StructurePrediction/predict>

<sup>6</sup> [www.mordred.bioc.cam.ac.uk](http://www.mordred.bioc.cam.ac.uk)

<sup>7</sup> <https://sourceforge.net/projects/openrasmol/>

<sup>8</sup> <http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>

<sup>9</sup> [www.web.expasy.org](http://www.web.expasy.org)

<sup>10</sup> [www.mordred.bioc.cam.ac.uk](http://www.mordred.bioc.cam.ac.uk)

combined approaches of ZDOCK. The study was to check a specific epitope of HEV E2S (You *et al.*, 2014).

In a study carried out by Xing *et al.*, 2011, where molecular docking of the HEV VLP crystal structure have shown that fab 224 covered three surface loops of the recombinant second open reading frame protein. Also determined the structure of a chimeric HEV VLP (Xing *et al.*, 2011).

In a study, carried out by Quintero-Gil C *et al.*, 2017 that they derived six antiviral peptides from the sequences of porcine Beta-Defensin-2 and bacteriocins Nisin and also generated Subtilisin by using in silico tools to propose new antiviral agents. And also, interactions between the HEV capsid protein and the six new antiviral peptide candidates were evaluated by molecular docking. (Quintero-Gil *et al.*, 2017)

## Conclusion

The selected active compounds have hepatoprotective activities of traditional medicinal plants of North-East India for treating jaundice. The present study shows that the docking score and energy associated shows the efficient activity of the studied compounds against HEV protein. Selected proteins were having 108: 171: 35: 36: 1 as the ratio for C, H, N, O, and S. All the selected proteins were rich in Leu (L), Arg (R), Ser(S), Pro (P), Glu (E). Ramachandran Plot refers to the  $\beta$ -sheet of the selected HEV protein. ADME results showed favorable results for the selected active compounds. Toxicity prediction of the selected active compounds was showing antitoxic and hepatoprotective properties. Therefore, in this study, we mainly tried to focus on the traditional herbs present in NE, India. The purpose of the study is to figure out the medicinal properties of the natural products present in the environment. The in silico analysis of these traditional herbs can be also useful. However, the current study finding needs to be validated by AUTODOCK results for carrying out animal model studies and human clinical trials.

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**Conflict of interest:** None

**Financial support:** None

**Ethics statement:** None

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