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SUPPORTING INFORMATION

Preferential solvation of carbohydrates in water-trifluoroethanol mixtures: A solvent detected heteronuclear NMR approach

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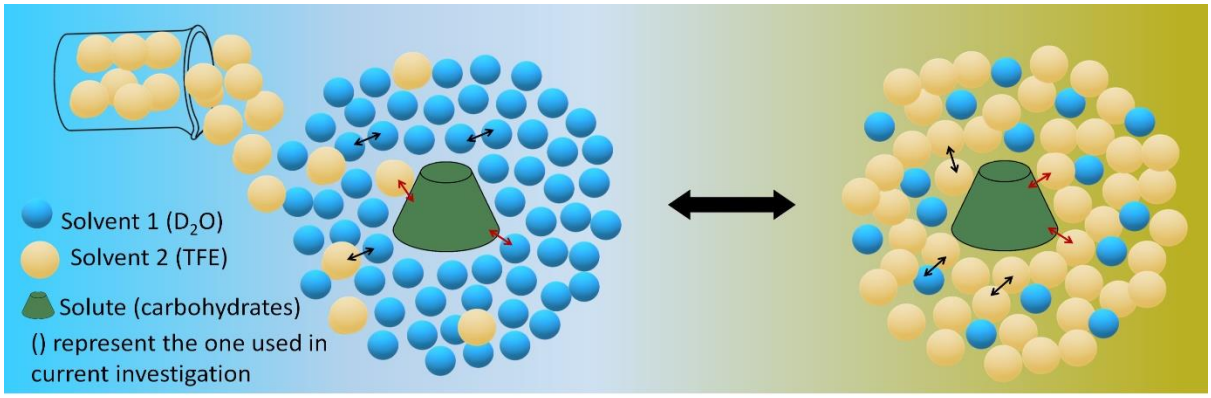


Scheme 1. Pictorial representation of preferential solvation of solute (carbohydrate) by solvent 2 (TFE) over solvent 1 (D2O) in a TFE:D_2O cosolvent system. Preferential solvation is expected due to (a) dielectric enrichment (b) solute-solvent interaction through hydrogen bonding (c) solvent 1-solvent 2 hydrogen bonding (d) self-association of a solvent giving rise to local inhomogeneity due to formation of microdomains. The double headed arrows represent the possible interactions among different constituents of the solution.

Preferential solvation (PS) of a solute is expected when the solute is dissolved in a solvent mixture of varied polarity. The solute interacts with each solvent component differently resulting in selective solvation by a certain solvent component. Obviously PS depends on the composition of the cosolvent mixture. Both specific and non-specific solute-solvent interactions are considered to be important in case of preferential solvation[1]. It is reported in literature that a solute induces local microscopic inhomogeneity in a multicomponent solvent mixture. Further, there are several non-bonded interactions among the solute and solvent components that ultimately result in preferential solvation of the solute. Some of these interactions are listed in the literature as a) specific solute-solvent association such as hydrogen bonding or electron donor-acceptor interaction; b) non-specific solute-solvent interaction due to dielectric enrichment in the solvent shell; c) solvent-solvent hydrogen bonding allowing both association of same solvent molecules as well as synergistic effect due to association of two different solvent molecules giving rise to mixed species; d) creation of micro-domains due to self-association of organic solvent molecules surrounded by water and vice-versa in an organic-aqueous solvent mixture; e) possible molecular recognition of a certain moiety of the solute by the solvent molecules due to electrostatic and van der Walls interactions[2].

In the present case we have investigated preferential solvation of Glucose and β-CD (5mM both) in similar compositions of TFE:D_2O cosolvent system. In TFE:D_2O cosolvent mixture, solvent microheterogeneity happens due to existence of micro-domains of TFE surrounded by water, and water solvated by TFE. The self-association of TFE molecules allows it to behave as a nanocrowder[3]. In case of glucose the microstructure of TFE molecules replaces all the water molecules from the solvation sphere of glucose resulting in preferential solvation of glucose by TFE[3]. On the other hand in case of β-CD similar nanocrowder effect of TFE prevails along with a possibility of TFE entering the β-CD cavity. It might be envisaged that TFE interacts with the β-CD cavity through its fluoroalkyl and hydroxyl groups. Inside the cavity the fluorinated alkyl group ($-CF_3CH_2$) will interact

with the inner cavity hydroxyl groups while the TFE hydroxyl group (–OH) will remain outside the β-CD cavity and will be able to interact with the water molecules present outside[4].

On comparison of molecular structures and the chemical groups available for glucose and β-CD it is clear that for single glucose molecule, five –OH (hydroxyl) groups are available for interactions with the cosolvents while for one molecule of β-CD, two different types of –OH groups. There are fourteen –OH groups present outside the rim/cavity and seven –OH groups inside the cavity of β-CD that are available for interactions with the cosolvents. Hence, there will be a greater number of solute-solvent non-covalent interactions in β-CD compared to glucose. Also, solute-solvent van der Waals interactions will be more rigid in case of β-CD leading to reduced entropy (energy–entropy compensation) as compared to glucose where the solute-solvent interactions will be more fluctuating[5].

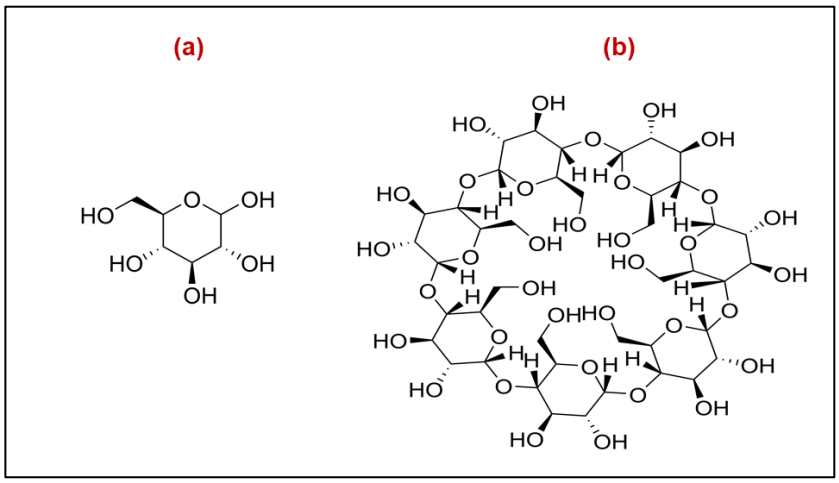
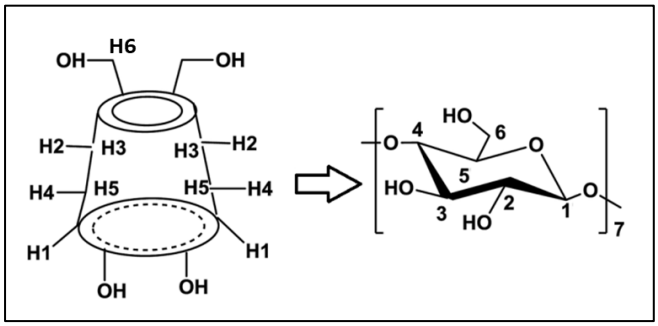


Figure S1. Molecular structure of (a) glucose (pyranose form) (b) β-Cyclodextrin (β-CD)

β-CD structure can also be represented as:



The interaction of the solute with the solvent molecules present in its immediate surrounding imparts modification of the physicochemical properties of the solute that further influences the interaction of these solute molecules with other molecules present in solution. The choice of a mixed solvent mixture rather than a pure solvent will allow fine-tuning of the solvent properties for the system under investigation.[6] Various methods such as calorimetry, infrared spectroscopy, molecular dynamics, adiabatic compressibility determined by ultrasonic velocity measurements and NMR are often exploited to analyze the solvation of various molecular entities in the presence of water or various cosolvent mixtures.[6-10]

β-CD is a well-known cyclic oligosaccharide made up of seven D-glucose units joined together through 1,4 glycosidic linkage.[11] Glucose molecules serve as monomer units for β-CD (the molecular structure of both are given in figure S1). Fluorinated alcohol such as TFE in TFE:D_2O cosolvent mixtures possess a unique ability to mimic the hydrophobic nature of biological systems and simulate cellular conditions. It has been used on several occasions to understand the solvation and conformational behavior of various proteins, peptides, as well as CD.[12-14] CDs are well known for their ability to form a large number of host-guest complexes by encapsulating a wide range of guest molecules completely or partially in their cavity.[15] Cosolvent can significantly influence the inclusion process and stability of the complexes. A number of earlier reports suggest that the addition of a third component, *i.e.,* TFE enhances the apparent association strength in CD-host complexes. The

molecules of TFE can act as a capable spacer in the complex formed by removing the water from the CD cavity.[16] Also, the addition of alcohol along with CD further increases the solubility of various poorly soluble drugs.[17,18]

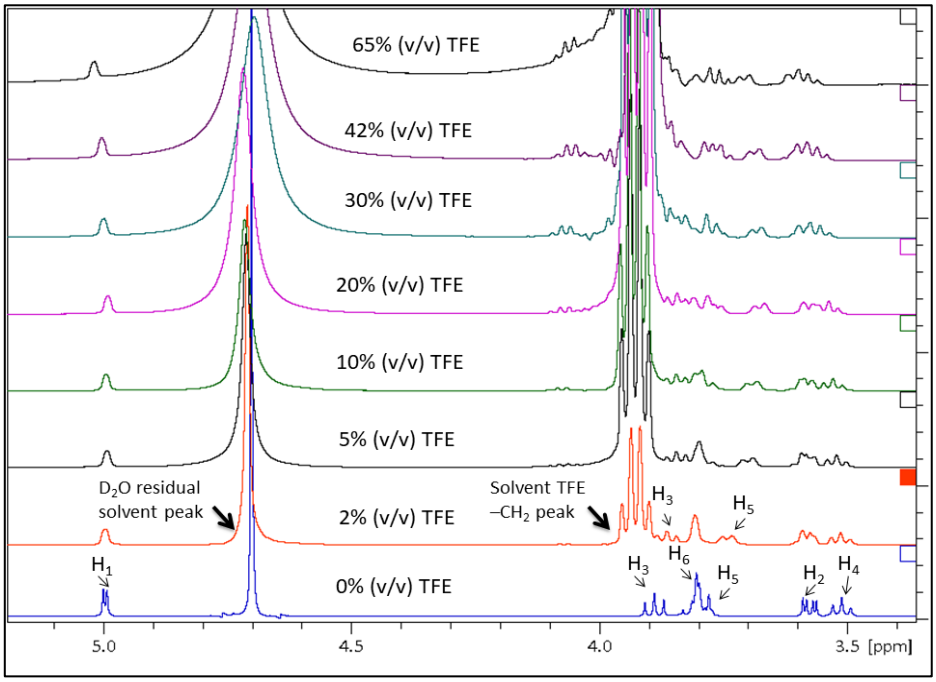


Figure S2. ^{1}H NMR spectra of β-CD in TFE: D_2O cosolvent mixture as a function of % (v/v) TFE composition at 298 K.

Table S1. Viscosity of the solution (a) TFE:D_2O (b) β-CD in TFE:D_2O (c) glucose in TFE:D_2O and the calculated hydrodynamic radius (r_H) for β-CD and glucose from the viscosity and diffusion coefficient using Stoke-Einstein's' Equation S1 at 298 K.

%(v/v) TFE in D_2O	Viscosity (η) (mPa.s)	*η (mPas)* in presence of *β-CD*	*η (mPas)* in presence of Glucose	r_H (Å) *β-CD*	r_H (Å) *Glucose*
0%	1.306	1.318	1.310	5.34	2.53
2%	1.320	1.343	1.334	5.57	2.56
5%	1.425	1.438	1.430	5.76	2.60
10%	1.565	1.579	1.577	5.95	2.87
20%	1.861	1.876	1.870	6.26	2.99
30%	1.988	2.105	2.041	6.40	3.06
42%	2.203	2.250	2.220	6.52	3.10
65%	2.385	2.427	2.409	6.66	3.07
80%	2.050	2.120	2.052	6.85	3.11
95%	1.672	1.751	1.713	……	…….

Section : S1

The ^{1}H self-diffusion coefficient (*D*) for solute molecules is determined using BPPLED (Bipolar pulse pair and longitudinal eddy current) pulse sequence, and the hydrodynamic radius (r_H) is determined following the Stokes-Einstein equation S1 given in SI. The r_H values for carbohydrates showed an increase with increasing %(v/v) TFE concentration that confirmed the preferential coating[12] of the carbohydrates surface by TFE.

$$D = \frac{k_B T}{6\pi\eta r_H} \text{.....(S1)}$$

Here, k_B = Boltzmann constant, T=Temperature, η = viscosity

The hydrodynamic radius (r_H) of the carbohydrates is calculated from the above Stokes-Einstein equation S1. These variations of the r_H are shown in table S1 as a function %(v/v) TFE composition with respect to D_2O. The close inspection of table 1 reveals that the size of the both carbohydrates exhibits an overall tendency to increase with increasing TFE composition than in pure water. At high TFE concentrations (>10% TFE) size of both the carbohydrates becomes significantly larger than in water. These findings can be interpreted as preferential coating of the TFE molecules in the TFE: D_2O cosolvent mixture which covers effectively the surface of carbohydrates increasing their "apparent size". Also, the increase in β-CD size is of higher extent that that for glucose. This suggest additional tendency of β-CD to undergo preferential solvation or to form inclusion complex than the glucose.

The R_{2F} ratio in presence and absence of carbohydrates measured employing CPMG pulse sequence showed a similar trend as of R_{1F} but with greater magnitude (figure S3). It is consistent with the well-known fact that in the case of ^{19}F, R_{2F} is a better probe of molecular interaction than R_{1F}.[19] R_{2D}* values are determined from linewidth of D_2O resonances measured as full width at half maximum (FWHM, $\nu_{1/2(D)}=R_{2D}/\pi$).[20] It is further found to support the conclusions drawn from the R_{1D} ratio following the same trend with a higher magnitude of ratio.

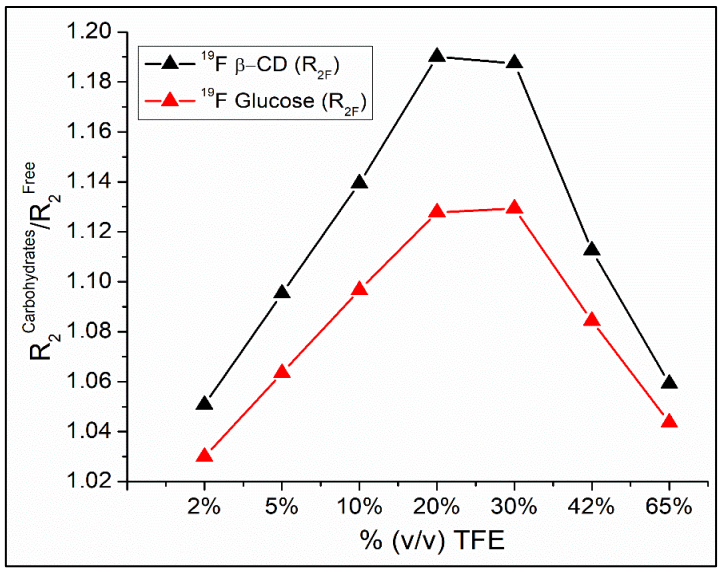


Figure S3. R_{2F} ratio for TFE (^{19}F-triangle) in presence of (a) β-CD (black) and (b) Glucose (red) to free cosolvent mixture as a function of %(v/v) TFE composition in D_2O at 298 K.

Table S2. Error bar of the data presented in Table 1 in the manuscript pertaining to extracted rotational correlation times (τ_c) and Relaxation rates (R_1) for solvent D_2O and TFE respectively. $\tau_{c(D)}$* : the value determined from solution viscosity for D_2O; $\tau_{c(F)}$*: the value determined from solution viscosity for TFE. T=298 K

%(v/v) TFE in D_2O	R_{1D} (s^{-1})	$\tau_{c(D)}$ (ps)	τ_c* (ps)	R_{1F}[a] (s^{-1})	R_{1F}[b] (s^{-1})	$\tau_{c(F)}$[b] (ps)	$\tau_{c(F)}$* (ps)
0%	±0.03	±0.03	±0.04	-------	-------	-------	-------
0%+β-CD	±0.01	±0.01	±0.02	-------	-------	-------	-------
2%	±0.02	±0.02	±0.01	±0.006	±0.007	±0.48	±0.25
2%+β-CD	±0.01	±0.01	±0.03	±0.009	±0.005	±0.34	±0.29
5%	±0.02	±0.02	±0.03	±0.005	-------	-------	-------
5%+β-CD	±0.04	±0.04	±0.05	±0.007	-------	-------	-------
10%	±0.04	±0.04	±0.02	±0.005	±0.008	±0.55	±0.30
10%+β-CD	±0.03	±0.03	±0.04	±0.008	±0.009	±0.61	±0.28
20%	±0.05	±0.05	±0.04	±0.004	±0.005	±0.34	±0.36
20%+β-CD	±0.06	±0.06	±0.05	±0.007	±0.005	±0.34	±0.39
30%	±0.06	±0.06	±0.04	±0.004	-------	------	-------
30%+β-CD	±0.07	±0.07	±0.07	±0.006	-------	------	-------
42%	±0.08	±0.08	±0.05	±0.003	±0.003	±0.20	±0.25
42%+β-CD	±0.07	±0.07	±0.09	±0.004	±0.006	±0.41	±0.28
65%	±0.09	±0.09	±0.06	±0.002	±0.004	±0.27	±0.41
65%+β-CD	±0.08	±0.08	±0.10	±0.004	±0.003	±0.20	±0.39
80%	±0.10	±0.10	±0.07	±0.006	------	------	-------
80%+β-CD	±0.11	±0.11	±0.10	±0.007	-------	------	-------

Table S3. Values of measured Relaxation rate, R_{1D} for D_2O and R_{1F} for TFE at 11.7 T. Extracted rotational correlation times (τ_c) from ^2H R_{1D} (11.7 T) and ^{19}F R_{1F} (0.34 T) for solvent D_2O and TFE respectively in absence and presence of 5mM glucose. τ_c* represent the value determined from solution viscosity only for D_2O. T=298 K

%(v/v) TFE in D_2O + 5 mM glucose	R_{1D} (s^{-1})	$\tau_{c(D)}$ (ps)	τ_c* (ps)	R_{1F}[a] (s^{-1})
0%	2.39±0.03	2.40±0.03	2.29±0.02	---------
2%	2.56±0.02	2.58±0.02	2.35±0.03	0.244±0.007
5%	2.65±0.01	2.67±0.01	2.52±0.05	0.262±0.007
10%	2.79±0.03	2.82±0.03	2.78±0.04	0.279±0.006
20%	3.23±0.04	3.25±0.04	3.30±0.06	0.324±0.004
30%	3.42±0.06	3.44±0.06	3.60±0.05	0.344±0.004
42%	3.79±0.08	3.82±0.08	3.92±0.08	0.353±0.002
65%	4.17±0.09	4.20±0.09	4.25±0.07	0.385±0.003
80%	6.16±0.11	6.22±0.11	7.23±0.08	0.609±0.006

[a]: ^{19}F R_1 measurements at 11.7 T , [b]: ^{19}F R_1 measurements at 0.34 T

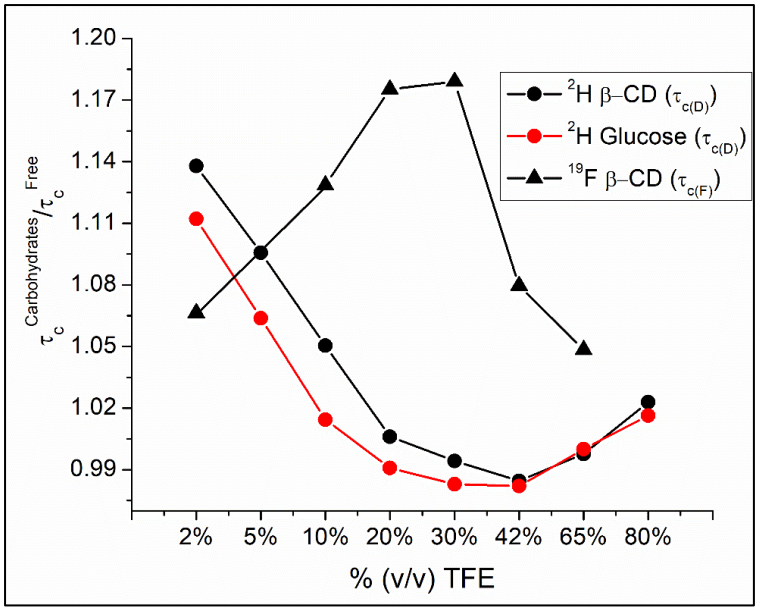


Figure S4: $\tau_{c(D)}$ and $\tau_{c(F)}$ ratio for D_2O (^{2}H-circle a 11.7 T) and TFE (^{19}F-triangle at 0.34 T) respectively in presence of (a) β-CD (black) and (b) Glucose (red) to free cosolvent mixture (without carbohydrates) as a function of % (v/v) TFE composition in D_2O at 298 K

Section:S2

In writing Equation (3) of the manuscript , the extreme narrowing limit ($(\omega_F+\omega_H)^2\tau_c^2 \leq 0.01$)) has been applied to the well-known standard expression for relaxation arising from fluctuating dipolar interactions between spins, and the motional spectral density function $J(\omega_i,\tau_c)$ relevant for intramolecular relaxation mediated by random isotropic molecular tumbling[21] has been employed as expressed in Equation (S1).

$$J(\omega_i,\tau_c)=\left(\frac{\tau_c}{1+\omega_i^2\tau_c^2}\right).....(S1)$$

While writing equation 3 for ^{19}F relaxation for TFE, several approximations have been invoked:

i) ^{19}F nuclei in TFE are relaxed entirely by the dipole-dipole mechanism[22,23];

ii) any contributions from the shielding anisotropy have been neglected at the low magnetic field as discussed in manuscript;

iii) effect of spin-rotation has been omitted considering the fact that the contribution of this mechanism to R_1 is significant only at elevated temperatures (48 and 66°C) as demonstrated by an earlier ^{19}F relaxation study of neat TFE[24];

iv) negligible contribution of the intermolecular dipole coupling between ^{19}F of TFE and ^{2}H of D_2O as well as intramolecular interaction between ^{19}F and ^{1}H of -OH group of TFE due to longer spatial separation[22];

v) the cross-correlation effects in the ^{19}F three-spin system of the CF_3 group have been ignored since theoretical analysis of the analogous CH_3 system indicates that such effects are very small[25].

In summary, for the present case, as the relaxation measurements are carried out at low field and room temperature (*ca.* 26 °C) contributions of CSA and spin-rotation mechanism to R_1 would not be significant. Only the dipole interactions of ^{19}F with the other two F atoms of CF_3, as well as with the two protons in the adjacent CH_2 group have been considered as the active relaxation mechanisms.

Molecular radius of TFE: To determine the molecular radius (a) of TFE, equilibrium geometries of trifluoroethanol are optimized using Nwchem software and corresponding bond lengths are caculated using molecular orbitral theory at the second-order perturbation MP2 level with the 6-31G* basis set. a for TFE comes out to be 4.76 Å.

Experimental Details: : Samples containing various compositions of TFE *i.e.* 2%, 5%, 10%, 20%, 30%, 42%, 65%, and 80% (v/v) TFE with respect to D_2O are prepared in absence and presence of 5 mM β-CD and 5 mM glucose. Viscosity of the TFE-D_2O compositions in absence and presence of MLT or carbohydrates are determined with an Anton Paar MicroViscometer. The temperature is controlled at 25°C. For calibration the viscosity of distilled water at 25°C has been taken from the literature (η = 0.894 cP) as a reference[26]. All the samples are equilibrated at room temperature and are degassed with nitrogen before measurements. All ^{2}H NMR experiments for these samples are acquired on Bruker Ascend Widebore spectrometer (11.7 T) equipped with BBFO probe at 298 K, while ^{19}F relaxation rates of TFE were measured both at 11.7 T and at 0.34 T

(corresponding to *ca*. 13.7 MHz for ^{19}F and 14.6 MHz for ^{1}H)at 298 K. ^{19}F R_1 are measured employing standard inversion recovery with a set of upto 20 recovery periods ranging from 50 µs to 45 s while for ^{2}H R_1 measurements, 20 recovery periods ranging from 50 µs to 10 s are used. The ^{19}F & R_2 is measured at 11.7 T (500 MHz) using CPMG pulse sequences employing a range of spin echo repetition loops $(\tau-\pi-\tau)_n$ from 2 to 5000 with single spin-echo delay (τ) of 2 ms.

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