Mechanisms of Panthenol Interaction with Hair Keratin:

Insights from Spectroscopy and Molecular Modeling Simulation

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Abstract (Maximum of 200 words)

Panthenol has been shown to penetrate inside hair and to improve hair strength, but the exact interactions with protein structures have not been well studied. In this work we confirm panthenol penetration into hair protein structures and use several NMR methods to understand the protein-panthenol interaction. The direct binding evidence between panthenol and hair is demonstrated across different hair types (Asian, Caucasian, and African American). 3D docking and molecular dynamics simulations show panthenol forms hydrogen bonds with major amino acids of hair keratin representative structure. In conclusion, both experiment and computational modeling work indicates that the bonds between panthenol and hair is the likely the source of its hair strength benefit.

Keywords: Panthenol, bonding, NMR, modeling and simulation

Introduction.

Incorporation of small molecules into hair is a topic of high interest in the cosmetics industry. Hair coloring and bleaching products rely on dye precursors and oxidants to diffuse into hair, then both lighten melanin and form chromophores to result in a color transformation. Similarly, the change of hair shape through perming involves penetration of thioglycolate to break disulfide bonds, followed by hydrogen peroxide to reform these bonds and create the desired new shape. In both cases, new covalent bonds are formed.

There is also a group of molecules that penetrate hair and are claimed to provide benefits, such as moisture control, strength and repair. It is proposed that these small molecules create bonds with hair through ionic bonds, hydrogen bonds, or hydrophobic interactions. In this study we wanted to understand the specific molecular interactions and develop measurement and modeling methods to measure bonding between the molecule and hair.

The molecule we investigated was panthenol, a pro-vitamin of pantothenic acid commonly referred to as pro-vitamin B5. It is frequently used in the hair care industry and has been reported to deliver both skin and hair benefits. Panthenol is a small, highly water-soluble molecule (molecular weight = 205 g/mol, aqueous solubility 103 g/L), making it very likely to penetrate hair. Given that panthenol is highly hydrophilic (Log P = -1.15), we would expect it to partition into protein regions of hair and interact via hydrogen bonds[1].

Visualization of panthenol penetrating inside hair was done by NanoSIMS imaging, a method which offers high spatial resolution (~50 nm) and good sensitivity, especially with labeled atoms within the molecule, such as ^{2}H or ^{13}C[1]. Panthenol was synthesized with deuterium (d_6) and diffused into hair. Cross-sections of hair were then cut and the ^{2}H signal mapped across the hair cross-section to enable its visualization in hair. This method has previously been used to measure hair structure changes, such as melanin bleaching[2], the location of calcium[3], and distribution of dye molecules[4].

Solid-state NMR was selected as the method to measure panthenol interactions with hair. It is a powerful spectroscopic tool that has been utilized for elucidating structures and interactions between atoms. Previous studies have investigated protein structures, including amorphous and crystalline regions in keratins[5], as well as disulfide bond content and cuticle structures in wool[6]. A recent study in hair used changes in ^{1}H T_1 relaxation times of small molecules, including methanol, ethyl acetate and isoamylacetate, to quantify fiber interactions[7].

Molecular modelling was used to obtain information about the binding affinity of panthenol with the hair keratin protein. 3D docking and molecular dynamics (MD) simulations techniques were conducted for understanding the various interactions between hair keratin and panthenol. The atomistic coiled coil heterodimer hair keratin structure was formed by acidic K35 (Type I) and basic K85 (Type II) proteins[8]. This atomistic heterodimer hair keratin model is used as a starting point for 3D docking and molecular dynamics simulation.

Materials & Methods

Hair Preparation

Asian hair:

Two gram, 15 cm Asian untreated hair (i.e., no chemical treatment) and 4g, 20cm bleached Asian hair were purchased from International Hair Importers & Products Inc. (Glendale, NY). The protocol used to bleach the Asian hair was two cycles of a powder persulfate bleach (Wella Blondor) mixed in a 1:2 ratio with 6% hydrogen peroxide developer and left for 45 mins at 40 ˚C and then rinsed.

Caucasian hair:

Two gram, 15 cm Caucasian untreated hair (i.e., no chemical treatment) was purchased from International Hair Importers & Products Inc. (Glendale, NY). The protocol used to bleach the Caucasian hair was 1 cycle of hydrogen peroxide for 35 min at room temperature, and then rinsed.

African American hair:

One gram African American untreated hair (i.e., no chemical treatment) was purchased from International Hair Importers & Products Inc. (Glendale, NY).

Materials Panthenol Synthesis

^{2}H panthenol was synthesized from 3-aminopropan-1,1,2,2,3,3-d_6-1-ol and D-pantolactone. ^{1}H-NMR analysis of the product (10mg in 1ml d_6-DMSO) showed the product to be 97% d_6-D-panthenol and 3% D-pantolactone. 95.3% yield.

Hair Treatment

For NMR and NanoSIMS studies all testing hair was soaked in a 10% solution of either panthenol or d_6-panthenol for one hour and then rinsed for 10 secs to remove excess surface panthenol. Asian and Caucasian hair were treated with 1 cycle of soaking and rinsing. African American hair was treated 2 cycles of soaking and rinsing. Hair was dried and balanced at 22˚C and 45% RH overnight.

NanoSIMS

Control hair and hair soaked in 10% solution of ^{2}H panthenol (~50 fibers) were embedded in ice and sectioned at a thickness of 10 µm. A NanoSIMS 50L instrument (CAMECA, France) was used for imaging as previously described[9].

Solid-state NMR

A Bruker Avance III HD NMR spectrometer (Bruker Corporation, Billerica, MA, USA) at 14.1 T was used with a 3.2 mm MAS probe. For both ^{1}H-^{13}C and ^{1}H-^{2}H experiments, a +1 Hartmann Hahn condition is used for cross polarization (CP) transfer, a 90-100 ramp was used on proton channel. 80 kHz High power decoupling (SPINAL64) is used during detection. ^{2}H T_2 measurement is performed using standard spin-echo pulse sequence, a maximum 800 µs echo time were used. All spectra were processed using the Topspin software package and referenced to the unified scale using IUPAC recommended frequency ratios relative to the ^{13}C adamantane(s) methylene resonance (δ = 37.77 ppm).

Modeling and simulation

To understand the binding affinity and interaction of panthenol with hair keratin, the 3D docking analysis was performed using the Schrodinger Maestro13.7 packages and MD simulations were performed using GROMACS version 2022 software[10]. The developed atomistic heterodimer hair keratin structure was used for both 3D docking and MD simulations. The hair keratin protein was prepared using the wizard tool in Maestro version 13.7. and panthenol were prepared using LigPrep. The OPLS 2005 force field was used for optimization, which produced the optimized and energy minimized heterodimer hair keratin protein as well as low energy panthenol structure for docking. The Glide module was used to perform docking of panthenol for predicting the binding affinity with hair keratin protein[11]. For the MD simulations, panthenol molecules were parameterized using CGenFF. The system was set up by incorporating 200 panthenol molecules along with heterodimer hair keratin protein, and it was solvated with the TIP3P water model. The system was first subjected to energy minimization using the steepest-descent algorithm, followed by equilibration at 310 K in a 200 ns NVT ensemble, where positional restraints of 1000 kJ mol^{-1} nm^{-2} were applied to the protein backbone. This was followed by an additional 200 ns NPT ensemble. Finally, a 50 ns production MD simulation in the NPT ensemble was conducted, during which system coordinates, velocities, and energies were recorded every 100 ps. The non-bonded interaction energies and hydrogen bonds between the hair keratin protein and panthenol were calculated using GROMACS.

Results & Discussion

Asian hair treated with 10% panthenol was used for NanoSIMS measurement. The ^{2}H signal was normalized to the ^{1}H signal to avoid artifacts due to sputtering differences across different parts of the hair. Figure 1 shows 30 µm x 30 µm images of two representative hair fiber cross-sections with both the ^{1}H and $^{2}H/^{1}H$ ratio image shown. The green, yellow and red regions in the image below show the presence of panthenol. The images confirm clear penetration of d_6-panthenol throughout the fiber, within both the cuticle and cortex. It is difficult to identify the exact location of panthenol, but it is clearly dispersed through the hair cross-section. Panthenol is likely located in the protein matrix region due to its hydrophilicity. It can be hypothesized that panthenol would hydrogen-bond via its OH and NH groups more readily in protein parts of the cuticle or cortex. Panthenol concentration is lower in the medulla supporting its partitioning into the non-lipid parts of hair.

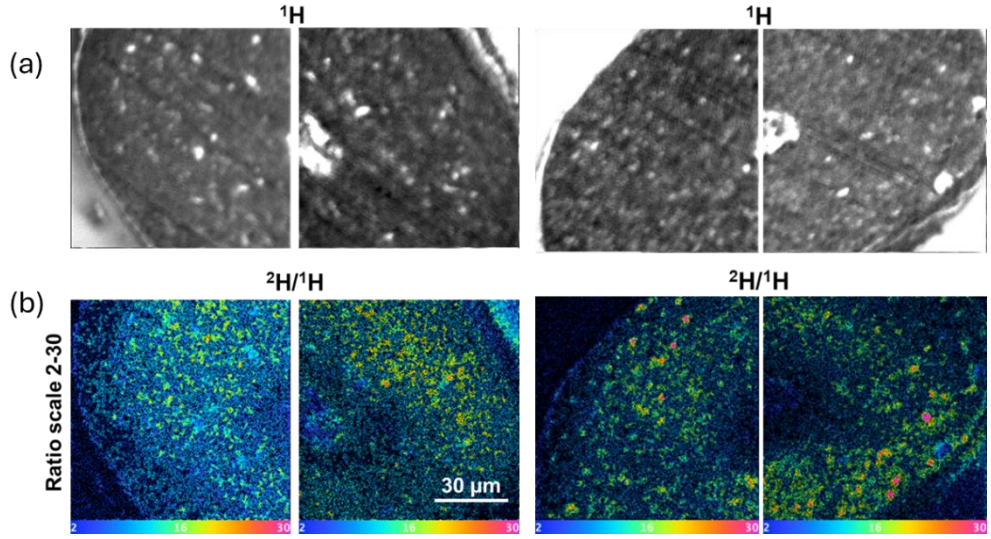

Figure 1 – NanoSIMS relative concentration images of d_6-panthenol inside hair
(a) are secondary ion images, (b) are concentration images of ^{2}H signal from panthenol

Solid-state NMR was used to investigate interactions between panthenol and hair at a molecular scale. All three hair types (Asian, Caucasian, and African American hair) were analyzed with this method. The intention of the test design is for reproducibility. We want to confirm the binding property between panthenol and hair is the same amongst different hair types.

In the first experiment ^{1}H to ^{13}C cross polarization (CP) spectra were measured (Figure 2 for Asian hair, Figure 3 for Caucasian hair, and Figure 4 for African American hair). During the CP experiment all protons are polarized but only carbon atoms within 5 Å to the polarized protons are recorded in the final detection. Figure 2(a), Figure 3(a), and Figure 4(a) show control hair, while Figure 2(b), Figure 3(b), and Figure 4(b) display hair treated with 10% panthenol. Compared to the control, the panthenol-treated hair exhibited additional peaks between 110 ppm and 140 ppm, corresponding to signals from aromatic groups. As panthenol does not contain any aromatic groups we assume these groups are part of the hair protein interacting with panthenol. The increased aromatic sensitivity could be due to aromatic groups being surrounded by panthenol, leading to additional signal transfer from panthenol to the hair protein's aromatic groups due to proximity effects. Alternatively, it may be explained by restricted ring dynamics due to panthenol-hair interactions[12,13,14].

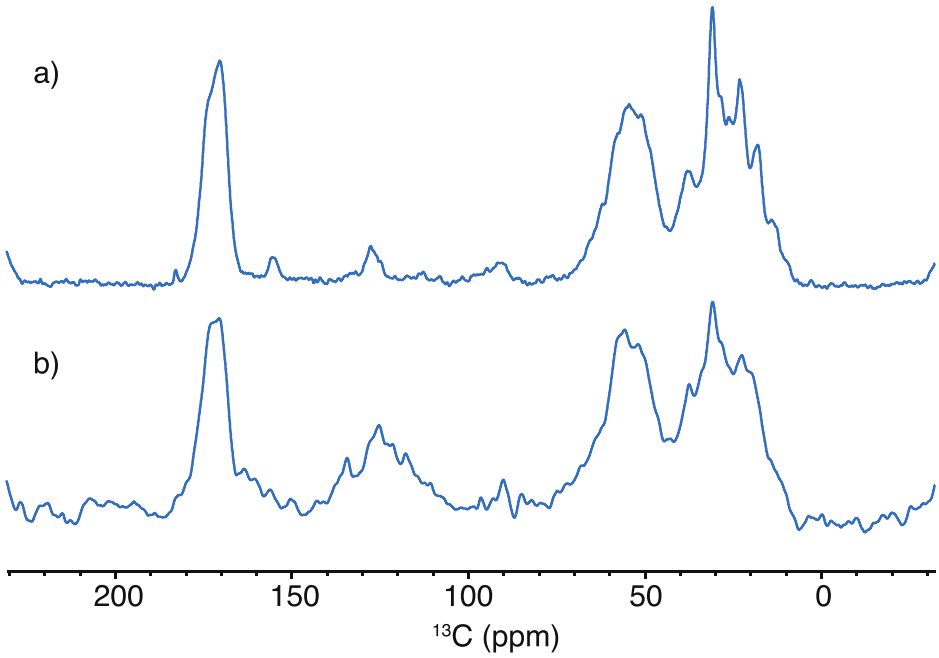

Figure 2 – ^{13}C NMR of (a) Asian hair and (b) Asian hair soaked in 10% panthenol. Measurement is performed at a 600 MHz NMR spectrometer and 12 kHz magic angle spinning (MAS) was used.

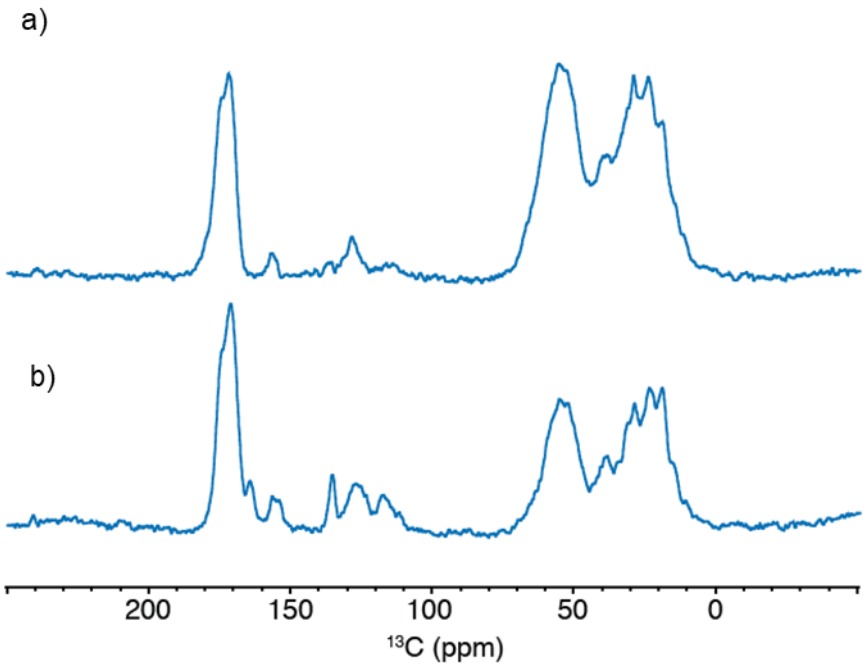

Figure 3 – ^{13}C NMR of (a) Caucasian hair and (b) Caucasian hair soaked in 10% panthenol. Measurement is performed at a 600 MHz NMR spectrometer and 12 kHz magic angle spinning (MAS) was used.

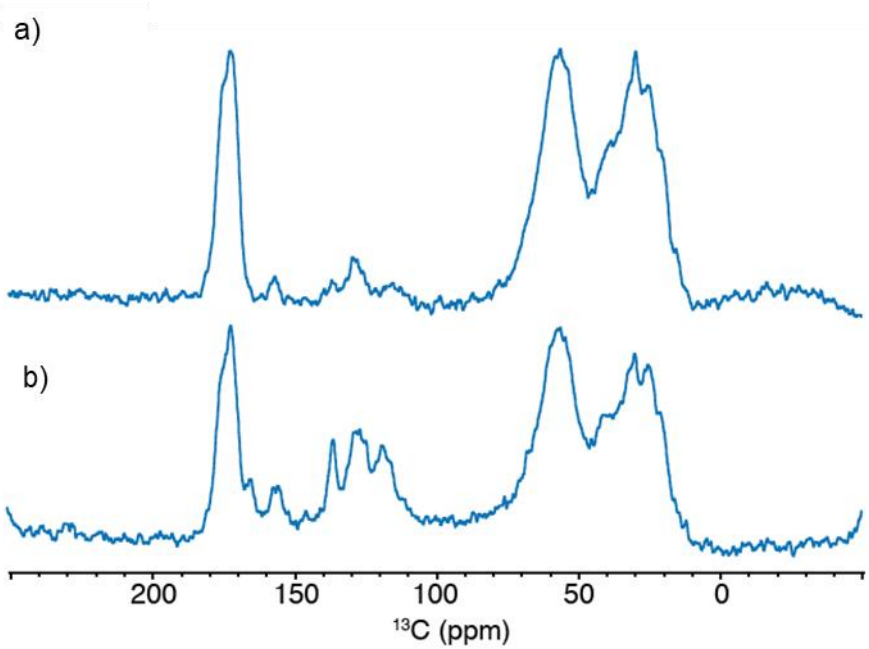

Figure 4 – ^{13}C NMR of (a) African American hair and (b) African American hair soaked in 10% panthenol. Measurement is performed at a 600 MHz NMR spectrometer and 12 kHz magic angle spinning (MAS) was used.

Results from the second experiment are concluded for all Asian, Caucasian, and African American hair. Asian hair result is depicted in Figure 5. In all hair types tested, two dimensional ^{1}H-^{13}C and ^{1}H-^{2}H heteronuclear correlation (HETCOR) spectra were shown for panthenol-soaked hair. Peaks from ^{1}H-^{13}C transfer are shown in blue and ^{1}H-^{2}H transfer are shown in red. The position of these peaks is in the aromatic carbon region supporting the initial experiment that panthenol is interacting with aromatic groups of the hair. The proton from panthenol interacting with hair is the N-H proton. Thus, from this second study there is evidence of a 'shared' proton between panthenol and hair at a distance that would be expected for a bonding interaction[15,16]. Since the interaction is between aromatic groups of protein and panthenol amides, it is either a hydrogen bond or an interaction of -N-H- with the aromatic ring. The NMR method has limitations in identifying interactions between the hydroxyl groups of panthenol and hair through hydrogen bonding to other protein groups, such as carbonyl groups. Since both hair proteins and panthenol contain carbonyl groups, it is challenging to differentiate NMR signals between the two. It is anticipated that panthenol will also create other interactions beyond the ones identified in this NMR experiment.

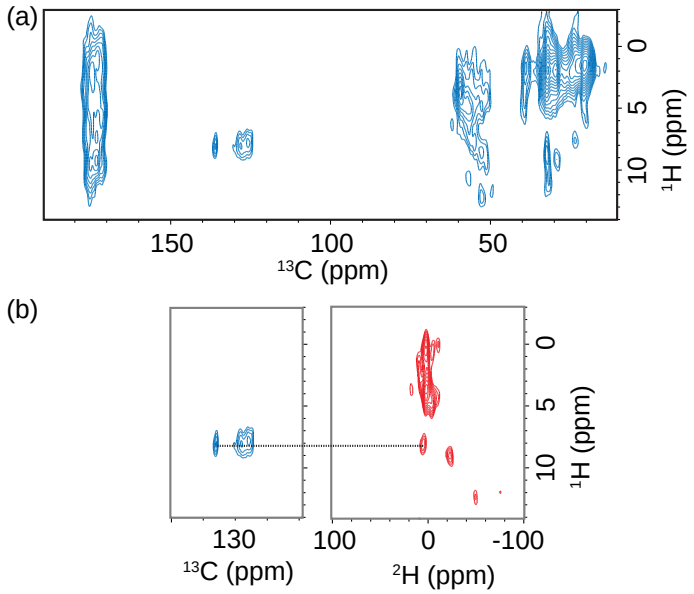

Figure 5 – 2-dimensional NMR of Asian hair soaked in 10% panthenol. ^{1}H-^{13}C correlation (blue) and ^{1}H-^{2}D correlation (red) were shown. (a) ^{1}H–^{13}C correlation spectrum of hair soaked in 10% panthenol; (b) aromatic region of the ^{1}H–^{13}C correlation spectrum of hair soaked in 10% panthenol and the ^{1}H–^{2}D correlation spectrum of hair soaked in 10% ^{2}H panthenol. All three –CH_2 groups in panthenol were deuterated in the ^{2}H panthenol. In (b), amide proton chemical shifts are aligned in ^{1}H-^{13}C and ^{1}H-^{13}C correlated spectra, signal transfers were observed from amide protons to carbons from hair and deuterium from panthenol. Measurement is performed at a 600 MHz NMR spectrometer and 12 kHz magic angle spinning (MAS) was used for ^{1}H-^{13}C spectra and 17 kHz MAS was used for 1H-2D spectra.

The two NMR studies demonstrate similar binding properties for panthenol across all 3 hair types. This makes sense, because all human hair, fundamentally, is made with the same chemical compositions.

Modeling and simulation

The 3D docking of panthenol with hair keratin protein showed a good docking score of -6.74 and quite a good number of hydrogen bonding and hydrophobic interactions with hair keratin (Figure 6a, 6b). As shown in figure 6b, panthenol is making H-bond interactions with Glu677, Lys684, Asp199, while making hydrophobic interactions with Val196, Val681, Phe680, and Leu195.

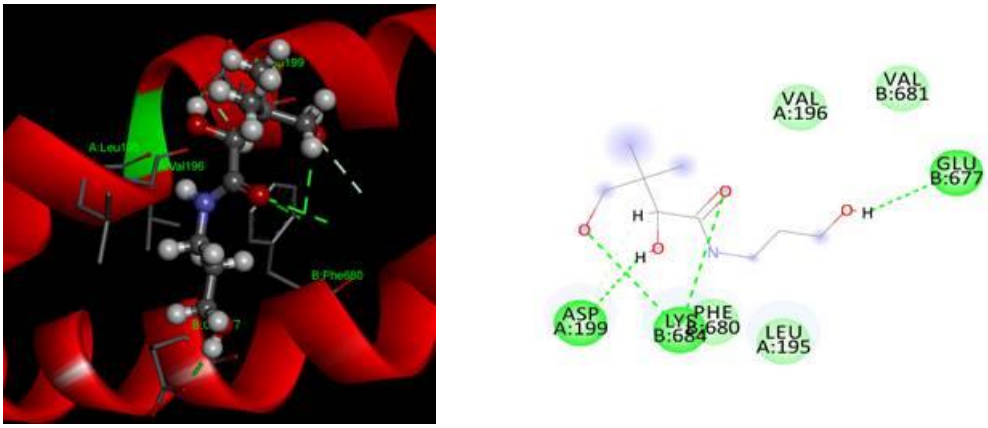

Figure 6: (a) Residues and hydrogen bond contacts (green dotted line), (b) the 2D template representing the types of contacts formed between panthenol and hair keratin protein

The average binding energy between keratin and panthenol was evaluated over the 50 ns MD simulations trajectory using the GROMACS tool. The electrostatic interaction energy was calculated to be –1042.81 kJ·mol^{-1}, while the van der Waals interaction energy was –1714.96 kJ·mol^{-1}, indicating strong and favorable binding between panthenol and the keratin surface. The average number of hydrogen bonds was computed over the final 10 ns (40–50 ns) of the simulation using gmx hbond. The average number of intra-protein hydrogen bonds in the keratin structure was 672.89 in the control system and 678.64 in the panthenol-bound system, suggesting minimal disruption to the native hydrogen-bonding network upon ligand binding. An average of 25.93 hydrogen bonds were formed between keratin and panthenol. Residue-type analysis showed that panthenol interacted most frequently with polar residues (10.29), followed by basic (5.70), acidic (4.85), aliphatic (3.94), aromatic (1.62), and sulfur-containing residues (1.29).

Conclusion

We created a collection of advanced spectroscopic measurement capabilities, accompanied with cheminformatics (molecular modeling), to decipher that panthenol forms hydrogen bonds inside hair at its protein region, which is the hypothesized mode of action for panthenol's ability to strengthen hair.

Acknowledgements

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Conflict of Interest

None of the authors have any conflict of interest.

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