the binding epitopes will be of crucial importance for the design of new selective and potent glycosyltransferase inhibitors.

Experimental Section

All spectra were acquired on a Bruker DRX 500MHz spectrometer with a 5-mm TXI probehead. The STD spectra were measured at 293 K with 2 k scans. The reference spectra were measured with 1 k scans. Saturation tranfer was achieved by using 40 selective gaussian pulses (duration 50 ms, spacing 1 ms). The protein envelope was irridiated at $\delta = 0$ (on-resonance) and $\delta = 40$ (off-resonance). Subsequent substraction was achieved by phase cycling.

The concentrations of β4Gal-T1 were 20 μM and 7 μM for UDP-Gal and UDP-Glc, respectively. Sample volume: 300 μL in a Shigemi tube. The concentration of UDP-Gal was 0.9 mM (UDP-Glc 1 mM). Buffer: 20 mM Tris, 20 mM NaCl, 10 mM $MgCl_2$ ph 7.4 (not corrected).

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[1] For an overview, see A. Varki, R. Cummings, J. Esko, H. Freeze, G. Hart, J. Marth, *Essentials of Glycobiology*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, **1999**.

[2] a) L. N. Gastinel, C. Bignon, A. K. Misra, O. Hindsgaul, J. H. Shaper, D. H. Joziasse, *EMBO J.* **2001**, *20*, 638–649; b) U. M. Unligil, S. Zhou, S. Yuwaraj, M. Sarkar, H. Schachter, J. M. Rini, *EMBO J.* **2000**, *19*, 5269–5280; c) L. N.Gastinel, C. Cambillau, Y. Bourne, *EMBO J.* **1999**, *18*, 3546–3557; d) U. M. Unligil, J. M. Rini, *Curr. Opin. Struct. Biol.* **2000**, *10*, 510–517.

[3] M. Mayer, B. Meyer, *Angew. Chem.* **1999**, *111*, 1902–1906; *Angew. Chem. Int. Ed.* **1999**, *38*, 1784–1788.

[4] a) J. Klein, R. Meinecke, M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **1999**, *121*, 5336–5337; b) M. Vogtherr, T. Peters, *J. Am. Chem. Soc.* **2000**, *122*, 6093–6099; c) H. Maaheimo, P. Kosma, L. Brade, H. Brade, T. Peters, *Biochemistry* **2000**, *39*, 12778–12788.

[5] M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 6108–6117.

[6] Recombinant bovine β4Gal-T1 (M_W 38 kDa) and synthetic UDP-Gal (M_W 610.3 Da) were purchased from Calbiochem.

[7] N. J. Kuhn, S. Ward, W. S. Leong, *Eur. J. Biochem.* **1991**, *195*, 243–250.

[8] a) M. M. Palcic, O. Hindsgaul, *Glycobiology* **1991**, *1*, 205–209; b) L. J. Berliner, R. D. Robinson, *Biochemistry* **1982**, *21*, 6340–6343.

[9] a) T. Endo, Y. Kajihara, H. Kodama, H. Hashimoto, *Bioorg. Med. Chem.* **1996**, *4*, 1939–1948; b) Y. Kajihara, T. Endo, H. Ogasawara, H. Kodama, H. Hashimoto, *Carbohydr. Res.* **1995**, *269*, 273–294; c) G. Srivastava, O. Hindsgaul, M. M. Palcic, *Carbohydr. Res.* **1993**, *245*, 137–144; d) H. Yuasa, O. Hindsgaul, M. M. Palcic, *J. Am. Chem. Soc.* **1992**, *114*, 5891–5892.

[10] a) H. Debray, J. Montreuil, *Carbohydr. Res.* **1989**, *185*, 15–26; b) F. Fukumori, N. Takeuchi, T. Hagiwara, H. Ohbayashi, T. Endo, N. Kochibe, Y. Nagata, A. Kobata, *J. Biochem.* **1990**, *107*, 190–196; c) T. Weimar, T. Peters, *Angew. Chem.* **1994**, *106*, 79–82; *Angew. Chem. Int. Ed.* **1994**, *33*, 88–91.

[11] a) W. F. Broekaert, M. Nsimba-Lubaki, B. Peeters, W. J. Peumans, *Biochem. J.* **1984**, *221*, 163–169; b) E. J. M. Van Damme, A. Barre, P. Rougé, F. Van Leuven, W. J. Peumans, *Eur. J. Biochem.* **1996**, *235*, 128–137.

Watching the Photo-Oxidation of a Single Aromatic Hydrocarbon Molecule[**]

Thomas Christ, Florian Kulzer, Patrice Bordat, and Thomas Basché[*]

The ability to observe chemical reactions at the level of single molecules has been demonstrated by fluorescence microscopy.[1] Actually, every optical single-molecule experiment with organic fluorophores under ambient conditions is eventually terminated by a photochemical reaction, which converts the molecule into a state in which it no longer absorbs or fluoresces. Often this photobleaching of organic fluorophores depends on the presence of oxygen, the influence of which on the photophysics and -chemistry of the fluorophores has been elucidated in recent single-molecule experiments.[2] Although photobleaching quite generally is an important mechanism for the degradation of organic dye molecules under ambient conditions, understanding of this process is limited especially for the class of comparatively photo-stable dyes used in single-molecule spectroscopy or for technological applications. For single terrylene molecules in a *p*-terphenyl host crystal, we can unambiguously show that a self-sensitized photo-oxidation is the initial step or directly leads to the terminal photobleaching.

The fluorescence of single terrylene molecules in a *p*-terphenyl host crystal[3] was imaged by confocal microscopy. When following the fluorescence intensity as a function of time (emission time trace) under ambient conditions, various types of intensity changes are observed. Either the fluorescence signal drops to the background level irreversibly after a period of constant emission intensity (Figure 1 a) or it jumps to a new level after having dropped to the background level for a short period of time (Figure 1 c). As seen in Figure 1 c, the latter sequence of events in this case is also terminated by an irreversible drop of the fluorescence signal to the background level. In addition to the time traces, a complete sequence of emission spectra of the molecules under study is recorded simultaneously at regular intervals (5 s). For the molecule, the time trace of which is shown in Figure 1 a, all emission spectra are identical until the emission ceases and fully agree with an ensemble spectrum of terrylene in *p*-terphenyl. The spectra in Figure 1 d, which belong to the time trace in Figure 1 c, shows however that after this molecule resumes emission at ~53 s the spectrum has shifted by 40 nm to the blue. We attribute all the intensity and spectral changes to a photo-oxidation of terrylene or subsequent internal rearrangements of the primary photoproducts.

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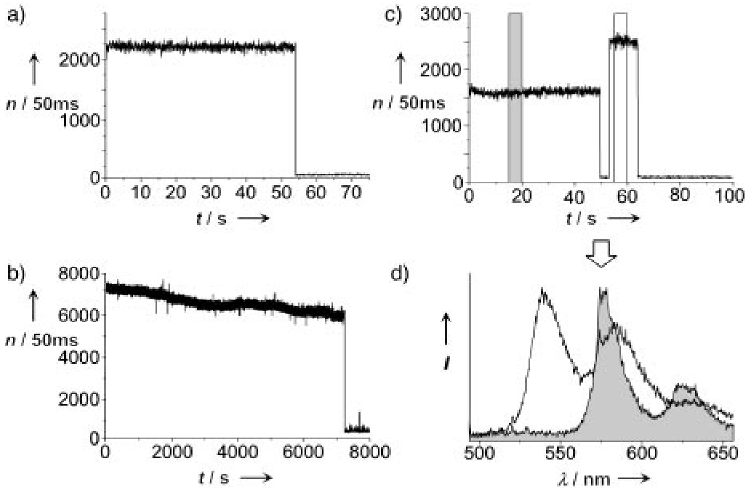

Figure 1. a) Fluorescence intensity of a single terrylene molecule in *p*-terphenyl as a function of time (emission time trace) in air ($\lambda_{exc} = 488$ nm; $I = 53$ kW cm^{-2}; $n =$ number of photo counts). At around 53 s the fluorescence signal irreversibly drops to the background level. b) Emission time trace of a single terrylene molecule in *p*-terphenyl under an argon atmosphere. In this case the emission ceases at around 7200 s after the molecule had emitted 2×10^{10} photons ($I = 53$ kW cm^{-2}). c) Emission time trace of another terrylene molecule in air ($\lambda_{exc} = 488$ nm; $I = 26.5$ kW cm^{-2}). After a dark-state transition the molecules resumes emission at a different intensity level. d) Fluorescence spectra taken during the time periods indicated in c). After the dark-state transition the spectrum is shifted by 40 nm to the blue.

To prove the assumption that photo-oxidation occurs, we have studied several hundred terrylene molecules in air, oxygen, and argon atmospheres at an excitation wavelength of 488 nm. In all cases, approximately 80 % of the molecules showed an irreversible drop of the fluorescence signal while for the remaining number of molecules (20 %) reversible intensity changes and spectral shifts were observed. When analysing the distributions of "on" times in air and oxygen atmospheres before an irreversible signal drop or before a reversible dark state (accompanied by a subsequent spectral shift) occurred, as exemplified in Figures 1 a or 1 c respectively, it is found that the average "on" time becomes much shorter in an oxygen atmosphere. When the measurements are done in a pure argon atmosphere, the "on" times are orders of magnitude longer: For the molecule shown in Figure 1 b the fluorescence signal ceased after ~7200 s, probably due to residual oxygen in the crystal.

The first step in the photo-oxidation of aromatic hydrocarbons is a reaction between electronically excited oxygen in its singlet state [O_2 ($^1\Delta_g$)] and the ground-state aromatic hydrocarbon.[4] Singlet oxygen can be formed by a self-sensitation process through interaction between an electronically excited state (T_1 or S_1) of the hydrocarbon and ground-state oxygen.[4] The self-sensitized photo-oxidation of linear acenes (for example anthracene) in solution is known to yield endoperoxides, which, depending on the illumination wavelength, can either revert to the parent compounds or rearrange through complex mechanisms into different secondary photoproducts.[5] On the other hand, knowledge about photo-oxidation pathways and products of rylenes is rather limited. For perylene in aqueous solution, it has been suggested that the primary reaction with singlet oxygen leads to the formation of either an endo- or an exoperoxide. As major secondary oxidation products 3,10-perylenequinone and 1,12-perylenequinone have been identified.[6]

In case of terrylene, we have performed quantum chemical calculations[7] on a number of possible product structures. Structure **I** in Figure 2 represents the only exoperoxide feasible and structure **II** an endoperoxide formed by attack of the central naphthalene unit. The quantum chemical calculations show that the exoperoxide is stable while they do not converge for the endoperoxide **II**. By computing the S_0-S_1 transitions energies of terrylene and its exoperoxide **I**, we find that the latter is blue shifted by approximately 100 nm. We therefore conclude that the main product (80 %) is structure **I**, which is shifted out of resonance by the fixed excitation wavelength of 488 nm. This shift leads to the irreversible signal drop of the fluorescence signal (see Figure 1 a). It could be argued that the irreversible dark states are due to photoproducts which do not fluoresce rather than being shifted out of resonance. This scenario is highly unlikely because dark photo-oxidation products which absorb light typically rearrange into fluorescent products.[8]

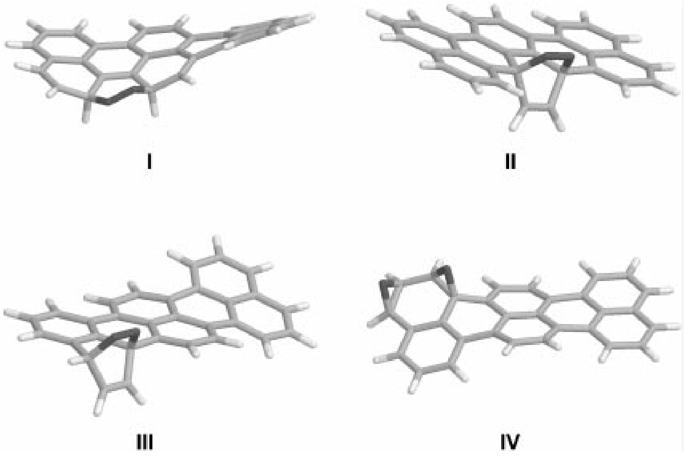

Figure 2. Ground-state structures of the exoperoxide **I**, two endoperoxides **II** and **III**, and a diepoxide **IV** of terrylene as obtained by semiempirical and ab initio quantum chemical calculations in vacuum. The dark segments in the structures indicate the oxygen containing rings. While **I**, **III**, and **IV** are predicted to be stable, this is not true for **II**.

For the molecules in which photo-oxidation led to detectable spectral shifts, the general sequence of events as tracked by the time course of fluorescence intensity and spectra was the same for all molecules investigated (see for example Figure 1 c,d). After a temporary dark state, the molecules resumed emission at a different intensity level and the complete spectrum was shifted. The spectral shifts were not uniform but showed a distribution. Besides the already mentioned 40 nm shift to the blue, stronger (70 nm) or weaker (10 nm) blue shifts and occasionally even red shifts (25 nm) have been observed. These data indicate that the photo-oxidation reaction of terrylene leads to a distribution of products. In the following, we will limit our discussion to the one shifted by 40 nm to the blue which was the most common product (overall yield: 7.6%) giving rise to a detectable spectral shift.

Endoperoxides of aromatic hydrocarbons are known to be nonfluorescent[8] but can revert into the parent compounds or rearrange into secondary fluorescent products under irradiation. Which of these reactions occur and which secondary products are formed strongly depends on the illumination wavelength.[8] As to the origin of a temporary dark state as exemplified in Figure 1c, we propose the formation of a nonfluorescent terrylene endoperoxide. In our experiments, all detectable temporary dark states were followed by secondary fluorescent photoproducts.

According to our model, the nonfluorescent endoperoxide must still absorb the laser light. This requirement is fulfilled if the endoperoxide is formed in one of the two outer naphthalene rings in the *para*-positions (structure **III**, Figure 2). Quantum chemical calculations[7] indicate that this structure is stable and leads to a blue shift of around 70 nm. (The calculations also have shown that only the *para*-endoperoxide is stable.) At this point, in addition to the quantum chemical calculations in vacuum, we have inserted several product structures into the *p*-terphenyl matrix and performed molecular dynamics (MD) simulations to estimate the solvation shifts. Although these quantities are usually very sensitive to the potentials, the shifts relative to pure terrylene are quite large and we could refer to an optimized model for terrylene in *p*-terphenyl.[9] For oxygen, well-known potentials like OPLS[10] were used. The shift of the endoperoxide **III** relative to terrylene was found between 75 and 100 nm,[11] which is in accordance with the quantum chemical calculations.

Our model is also corroborated by the observation that the Diels–Alder adduct of tetra-*tert*-butylterrylene and acetylenedicarboxylic acid diethylester, which is a structural analogue of **III**, yields an absorption blue shift of 70 nm by conservation of the overall spectral shape.[12] Structure **III** therefore can indeed be expected to still absorb at 488 nm.

In detailed studies by Rigaudy et al.[5], they showed for anthracene and some of its derivatives that the initial step of the photochemical rearrangement of the endoperoxides is the homolytic rupture of the O−O bond. This biradical intermediate rearranges mainly into a diepoxide, which can be stabilized by further subsequent rearrangements. Following these observations, the product shifted by 40 nm to the blue (Figure 1d) could correspond to the diepoxide (**IV**, Figure 2), which is a stable structure according to our calculations. The shifts calculated by quantum chemistry in vacuum[7] and MD[11] in the crystal amount to 70 and 30–55 nm, respectively, which strongly supports our model. While the diepoxide structure seems to be quite reasonable, at present we have no convincing evidence about the chemical structures of the additional secondary fluorescent products mentioned before.

After the secondary fluorescent photoproduct has formed in Figure 1c the emission ceases irreversibly after another 10 seconds, which we attribute to a further photo-oxidation event shifting the product out of resonance with the excitation wavelength. This assumption is based on the observation that the lifetime of any secondary photoproduct becomes, on average, much longer when after its formation the atmosphere is switched immediately to argon or the illumination is blocked.

In some cases, multiple attacks of oxygen could be directly interrogated from intensity and spectral changes. In Figure 3, the formation of the secondary photoproduct, which again exhibits a 40 nm blue shift, is followed by a dark state at $\sim$29 seconds. After the molecule resumes emission, the spectrum is shifted by another 40 nm to give an overall blue shift (compared to terrylene) of about 80 nm. From the spectral shift, we deduce that the second singlet oxygen adds to the same naphthalene unit that was already attacked by the first oxygen molecule (**IV**, Figure 2). After passing a reversible dark state around 43 seconds the absorption is eventually shifted away from the excitation wavelength by what we believe is the addition of a third oxygen molecule.

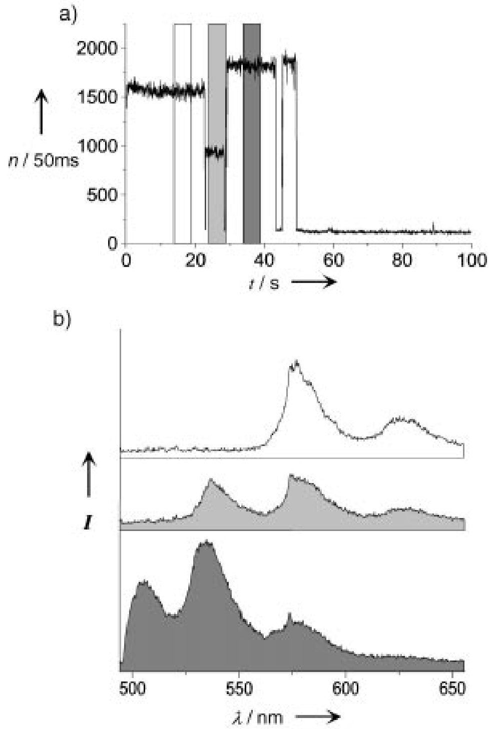

Figure 3. a) Emission time trace of a single terrylene molecule in *p*-terphenyl in air ($\lambda_{exc} = 488$ nm; $I = 26.5$ kW cm^2; n = number of photo counts). After the first two dark states, the molecule resumes emission at different intensity levels. Additionally a reversible dark-state transition occurs around 43 s, which is followed by a final signal drop. b) Fluorescence spectra taken during the time periods indicated in a). After the first dark period the spectrum shifts by 40 nm. The second dark-state transition is followed by a total blue shift of 80 nm. The spectral data below 510 nm are subject to convolution of the emission spectrum with the spectral response of the detection system.

By following fluorescence intensity and spectral changes, we have been able to watch the self-sensitized photo-oxidation of a single fluorophore. We have observed a number of photoproducts and reaction pathways not easily accessible by ensemble experiments, as exemplified by successive photo-oxidation of the same terrylene molecule. These results appear to be an important step towards an improved understanding of photobleaching and -degradation of aromatic hydrocarbons under ambient conditions. More generally, the study of chemical reactions at the level of single molecules promises novel insights into mechanisms, product distributions, and the reaction kinetics.[1]

Experimental Section

Thin crystalline platelets of *p*-terphenyl doped with terrylene were prepared by cosublimation. In a typical single-molecule experiment first an image ($10 \times 10\ \mu m^2$) was taken with an inverted confocal microscope by raster scanning the sample through the objective focus (numerical aperture: 0.9). Then single molecules were positioned in the focus and fluorescence time traces and spectra were recorded. By using a two-channel detection scheme, the fluorescence light was simultaneously detected by an avalanche photodiode (time traces) and imaged through a spectrograph onto a liquid N_2 cooled CCD (spectra). The microscope allowed the purging of the sample with oxygen, air, or argon.

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[1] a) M. M. Collinson, M. R. Wightman, *Science* **1995**, *268*, 1883–1885; b) H. P. Lu, L. Xun, X. S. Xie, *Science* **1998**, *282*, 1877–1882.

[2] a) O. Panzer, W. Göhde, U. C. Fischer, H. Fuchs, K. Müllen, *Adv. Mater.* **1998**, *10*, 1469–1472; b) L. Fleury, B. Sick, G. Zumofen, B. Hecht, U. P. Wild, *Mol. Phys.* **1998**, *95*, 1333–1338; c) J. Yu, D. Hu, P. F. Barbara, *Science* **2000**, *289*, 1327–1330.

[3] F. Kulzer, F. Koberling, T. Christ, A. Mews, T. Basché, *Chem. Phys.* **1999**, *247*, 23–34.

[4] N. J. Turro, *Modern Molecular Photochemistry*, Benjamin/Cummings, Menlo Park, CA, **1978**.

[5] E. Afshari, H. D. Brauer, R. Schmidt, M. Lachgar, J. Rigaudy, *J. Photochem. Photobiol.* **1992**, *63*, 319–325.

[6] B. White, M. Nowakowska, J. E. Guillet, *J. Photochem. Photobiol. A* **1989**, *50*, 147–156.

[7] Semiempirical (AM1 and MNDO Hamiltonians) and ab initio calculations (4-31G basis) at the restricted Hartree–Fock (RHF) level have been performed for the ground state of photo-oxidation products of terrylene in vacuum with the MOPAC and GAMESS packages. Semiempirical calculations with configuration interaction (including single and double excitations) were employed to calculate electronic transition energies.

[8] R. Schmidt, H. D. Brauer, *J. Photochem.* **1986**, *34*, 1–12.

[9] P. Bordat, R. Brown, *Chem. Phys. Lett.* **2000**, *331*, 439–445.

[10] W. L. Jorgensen, D. S. Maxwell, J. Tirado-Rives, *J. Am. Chem. Soc.* **1996**, *118*, 11225–11236.

[11] To obtain the solvation shifts of the photoproducts by molecular dynamics simulations, in a first approximation we equated the calculated dispersion energy of the electronic ground state of terrylene in *p*-terphenyl with the known experimental solvation shift. This procedure gives us a rescaling factor which we can use to estimate the solvation shifts of the photoproducts (**III**) and (**IV**). (A more complete account of this approach is in preparation.)

[12] K.-H. Koch, PhD thesis, University of Mainz (Germany), **1990**.

Parity Violation Dominates the Dynamics of Chirality in Dichlorodisulfane**

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Nearly seventy five years ago, Friedrich Hund contemplated chirality by introducing the quantum mechanical tunnel effect.[1] He explained the origin of molecular chirality as de facto symmetry breaking[2] in relation to the small magnitude of the tunneling splitting $\Delta E_{\pm}$ between the delocalized lowest energy levels of well defined parity ($+$ and $-$), an explanation which held prior to the discovery of parity violation in 1956.[3, 4] Now, with electroweak quantum chemistry[5–7] taking the parity-violating nature of the weak interaction into account, we expect a small parity-violating energy difference ΔE_{pv} (about $10^{-13\pm3}\ J\,mol^{-1}$ for molecules consisting of lighter elements) between two enantiomers of a chiral molecule.[5–12] The discovery[6, 7, 10] that ΔE_{pv} is larger by an order of magnitude than previously anticipated has given new impetus to the search for molecular parity violation. Our early results[6, 7] have meanwhile been confirmed by further theoretical calculations.[10–12] For typical, stable chiral molecules with a high energetic barrier against interconversion between enantiomers, $|\Delta E_{pv}|$ should be as a rule much larger than the small tunneling splittings $|\Delta E_{\pm}|$ in the lowest vibrational states. In this case the molecular eigenfunctions have well defined handedness and molecular chirality arises from de lege symmetry breaking. Although this most striking situation is qualitatively predicted,[2, 5] there exists so far no such case for which a quantitative calculation has shown that $\Delta E_{pv} \gg \Delta E_{\pm}$ holds and thus chirality is dominated by de lege parity violation. Here, we present the first such example: dichlorodisulfane (ClSSCl, Figure 1).

Dichlorodisulfane has a chiral C_2-symmetric equilibrium structure with high barriers against interconversion between the *P*- and *M*-enantiomer (Figure 2) and has been previously investigated experimentally and by traditional (parity conserving) quantum chemical calculations on the general series X_2S_2.[13–18] We report here calculations of parity-violating potentials, the corresponding parity-violating energy differences, and the torsional tunneling splitting in the vibrational ground state for ClSSCl, which demonstrate that indeed parity violation dominates the dynamics of chirality in this molecule. Our theoretical findings on dichlorodisulfane and related compounds might prove crucial for experimental

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