

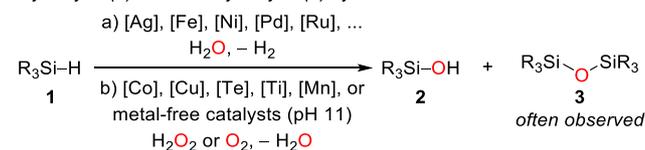
# Selective Enzymatic Oxidation of Silanes to Silanols

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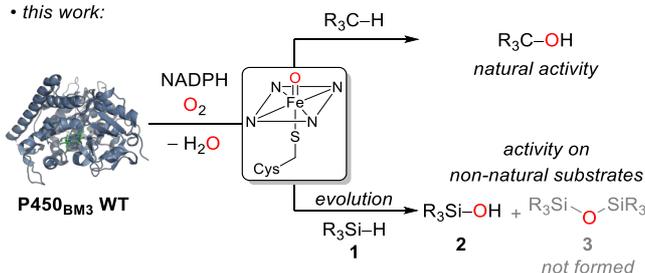
**Abstract:** Compared to the biological world's rich chemistry for functionalizing carbon, enzymatic transformations of the heavier homologue silicon are rare. We report that a wild-type cytochrome P450 monooxygenase (P450<sub>BM3</sub> from *Bacillus megaterium*, CYP102A1) has promiscuous activity for oxidation of hydrosilanes to make silanols. Directed evolution enhanced this non-native activity and created a highly efficient catalyst for selective silane oxidation under mild conditions with oxygen as terminal oxidant. The evolved enzyme does not touch C–H bonds also present in the silane substrates, nor does this biotransformation lead to disiloxane formation, a common problem in silanol syntheses. Computational studies reveal that catalysis proceeds through hydrogen atom abstraction followed by radical rebound, as observed in the P450's native C–H hydroxylation mechanism. Enzymatic silane oxidation now extends Nature's already impressive catalytic repertoire.

Silanols are widely used<sup>[1]</sup> to build silicone polymers<sup>[2]</sup>, which are found in an enormous number of modern lifestyle products. Silanols are also synthetic intermediates in organic synthesis<sup>[3]</sup>, catalysts,<sup>[4]</sup> and serve as functionally interesting isosteres for bioactive molecules.<sup>[5]</sup> The selective and mild synthesis of silanols has received substantial attention and remains of high interest.<sup>[1]</sup> Protocols that start from functionalized precursors such as chlorosilanes<sup>[6]</sup> or use strong oxidants<sup>[7]</sup> in combination with hydrosilanes often result in disiloxane formation, lead to other side reactions, or generate waste products in substantial amounts. Direct catalytic hydrosilane oxidation is an attractive alternative, and several methods have been developed, most of which rely on precious metals,<sup>[8]</sup> base metal catalysts<sup>[9]</sup> or metal-free transformations<sup>[10]</sup> are rare.

• hydrolytic (a) and non-hydrolytic (b) synthesis of silanols:



• this work:



**Scheme 1.** Synthesis of silanols (top) and native vs. evolved activity of P450s (bottom).

In most cases, these are hydrolytic processes, i.e. water is the oxidant, and stoichiometric amounts of dihydrogen are released (Scheme 1, top, a). This process is also exothermic which presents a safety issue when dihydrogen is evolved. Furthermore, contamination with disiloxane is often unavoidable, as the silanol product can react with the activated hydrosilane instead of water and some catalysts can also catalyze silanol self-condensation to yield disiloxanes. A limited number of protocols use other oxidants like oxygen or hydrogen peroxide together with base metals<sup>[9d-g]</sup> or even metal-free, though very basic, conditions (Scheme 1, top, b).<sup>[10]</sup> Few reported selective syntheses of silanols employ environmentally friendly catalysts, proceed under mild reaction conditions, and avoid competing side reactions.<sup>[1]</sup> We hypothesized that a biocatalytic protocol employing nature's versatile oxidation catalysts could be a valuable complement to existing synthetic methods.

Even though silicon is the second most abundant element in the Earth's crust, the silicon chemistry of the biological world is limited. Diatoms, for example, can incorporate *ortho*-silicic acid as stabilizing units in skeletons and materials; this process is usually based on the hydrolysis or alcoholysis of Si–O bonds.<sup>[15,16]</sup> Also, biocatalytic approaches for the formation of silicon polymers and small molecules are limited to the formation of Si–OR bonds through condensation reactions.<sup>[16]</sup> Although enzymatic manipulations of functional groups in neighborhood or remote to the silicon in various organosilicon compounds have been reported,<sup>[17]</sup> the direct biocatalytic functionalization of silicon centers remained unknown until this group engineered a thermostable cytochrome *c* in 2016 to catalyze carbene insertion into Si–H bonds *in vitro* and *in vivo*.<sup>[18,19]</sup> The ubiquitous cytochrome P450 enzymes catalyze a range of oxidative reactions using atmospheric oxygen as terminal oxidant.<sup>[11,12]</sup>

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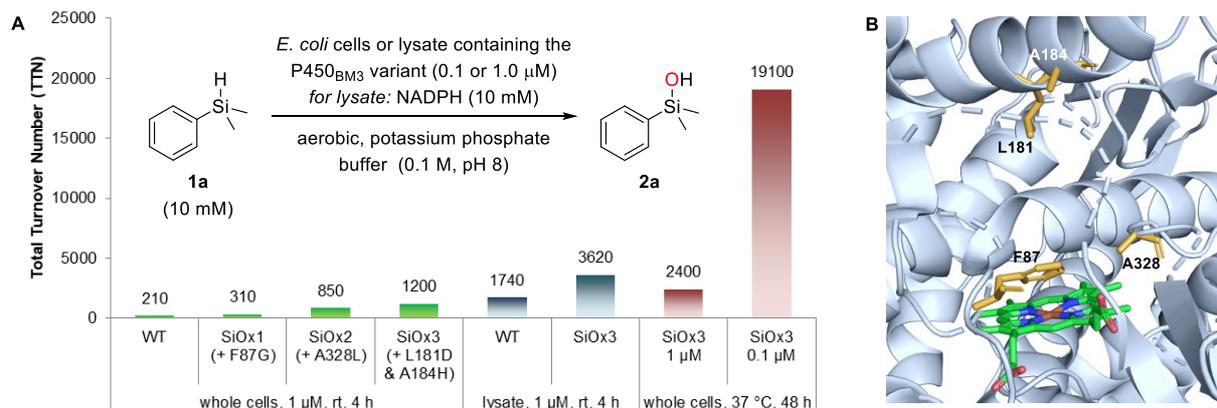
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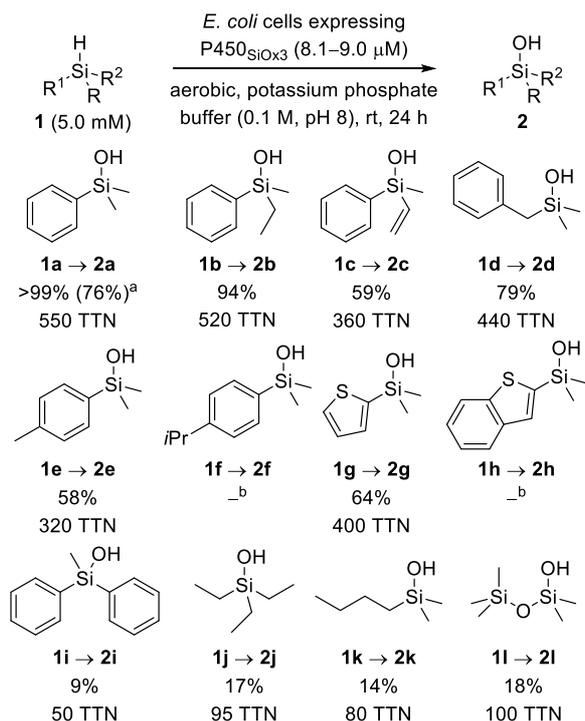
**Figure 1.** Activity of P450<sub>BM3</sub> variants in whole cells (green) or lysate (blue) over the course of directed evolution and at optimized conditions (red), given as total turnover number for silanol production. See the Supporting Information for experimental details. B) Structure of wild-type cytochrome P450<sub>BM3</sub> (PDB:1JPZ)<sup>[22]</sup> showing bound heme cofactor. Amino acid residues mutated during evolution are highlighted in orange (A = alanine, F = phenylalanine, L = leucine; NADPH = nicotinamide adenine dinucleotide phosphate).

These transformations typically proceed via a reactive, high-valent iron-oxene intermediate (Compound I, Scheme 1, bottom),<sup>[13]</sup> which undergoes, for example, hydroxylations or epoxidations with C–H or C=C bonds.<sup>[14]</sup> We envisioned that we could use the natural promiscuity of P450s and repurpose a native enzyme for the efficient and selective oxidation of hydrosilanes using directed evolution. To our knowledge, an enzyme that catalyzes this reaction has not been described.

We chose cytochrome P450<sub>BM3</sub> (CYP102A1) from the soil bacterium *Bacillus megaterium*<sup>[12]</sup> to investigate Si–H oxidation activity. This self-sufficient monooxygenase is a 118-kDa enzyme with its NADPH-dependent reductase domain fused to the C-terminus of the heme domain, and it has served as robust scaffold for directed evolution of hydroxylation activity in previous work.<sup>[20]</sup> We tested the ability of wild-type P450<sub>BM3</sub> to oxidize dimethylphenylsilane (**1a**) in *Escherichia coli* (*E. coli*) cells under aerobic conditions (Figure 1A). The enzyme delivered product **2a** with 210 TTN in 2.1% yield at 1.0 μM protein concentration. A negligible amount of **2a** was observed in control reactions under anaerobic conditions and otherwise identical setup, indicating that oxygen serves as the oxidant (see Supporting Information). We then used directed evolution by sequential rounds of saturation mutagenesis at selected amino acid residues to increase the enzyme's activity for hydrosilane oxidation. Mutations at a number of amino acid residues in P450<sub>BM3</sub> are known to affect oxidation activity.<sup>[20]</sup> We chose F87 as a first target for directed evolution due to its close proximity to the heme cofactor (Figure 1B).<sup>[21]</sup> A single site-saturation mutagenesis (NNK) library was screened in whole *E. coli* cells, from which we identified improved variant P450<sub>SiOx1</sub> (containing mutation F87G) with a 1.5-fold improvement in both yield and TTN of product **2a** (Figure 1A). Subsequently, we targeted residues T327 and A328 with double site-saturation mutagenesis using the 22-codon trick.<sup>[23]</sup> These residues are close to the iron cofactor in the distal heme-binding pocket, and mutations at these positions have influenced the performance and selectivity in previous engineering studies.<sup>[24]</sup> A double site-saturation mutagenesis strategy was chosen in an attempt to discover potential epistatic interactions between these sites. Screening identified only a single beneficial mutation, A328L,

which further increased the yield of silanol **2a** to 8.5% (850 TTN) using 2<sup>nd</sup>-generation variant P450<sub>SiOx2</sub>. A further round of double site-saturation mutagenesis at residues L181 and A184<sup>[25]</sup> led to the discovery of mutations L181D and A184H in P450<sub>SiOx3</sub>, which improved the TTN to 1,200.

We also compared the performance of wild-type P450<sub>BM3</sub> and the final variant P450<sub>SiOx3</sub> in *E. coli* lysate, which requires the addition of the cofactor NADPH in stoichiometric amounts (Figure 1A, blue bars). Turnover numbers were generally higher in lysate than in whole cells, which we ascribe to the absence of a diffusion barrier for the substrate and product through the cell wall and the limited availability of NADPH in whole cells. The final variant P450<sub>SiOx3</sub> proved superior in lysate as well, with 3,620 TTN (36% yield of **2a**) compared to 1740 (17% yield) for wild-type P450<sub>BM3</sub>. We next investigated the effect of changing various conditions on the production of **2a** (Figure 1A, red bars; see Supporting Information). Running the reaction for 48 h at 37 °C substantially increased the yield, now delivering silanol **2a** with 2,400 TTN and also indicating that the protein retains activity over this time. Furthermore, the yield of **2a** was almost as high at a lower catalyst loading (0.1 μM protein concentration), resulting in remarkably high TTNs of 19,100. Even though these conditions show the potential of variant P450<sub>SiOx3</sub>, the yield of **2a** obtained in this setup is not synthetically useful. Increasing the protein concentration to 8.1–9.0 μM and lowering the substrate concentration to 5.0 mM, we were able to achieve the transformation of **1a** to **2a** in quantitative yield as determined by GC analysis (76% isolated yield after 72 h at 37 °C; Scheme 2). Similarly, ethyl-substituted silanol **2b** was formed in high yield. The conversion of vinylsilane **1c** to silanol **2c** was lower, but chemoselectivity was good, and epoxidation of the double bond was not observed.<sup>[26]</sup> Both **2b** and **2c** are silicon-stereogenic, but as we expect configurational lability on the silicon center of acyclic silanols under aqueous, slightly basic conditions, we did not attempt to monitor enantioselectivity.<sup>[27]</sup>

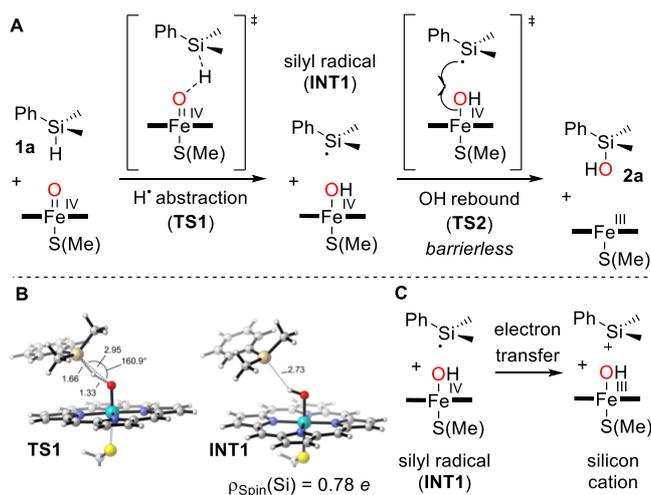


**Scheme 2.** Substrate scope of P450<sub>SiOx3</sub>. GC yields are given as average of triplicate runs (see the Supporting Information for further details). <sup>a</sup> Isolated yield after 72 h at 37 °C in parentheses. <sup>b</sup> No conversion to the silanol observed.

Silanol **2d** was obtained in good yield as well, again showing that a certain degree of steric flexibility is accepted close to the silicon center. Conversely, steric congestion of the aromatic ring was limited: whereas a methyl group in *para*-position of the aryl substituent in **2e** was still tolerated, increasing the steric demand thwarted the reaction and isopropyl-substituted **2f** was not formed. For both **1d** and **1e**, C–H oxidation in the benzylic positions did not occur.<sup>[28]</sup> The performance of the catalyst on electronically similar hydrosilanes **1g** and **1h** again indicates a certain size limitation. The smaller thiophenyl substituent was accepted and **2g** was obtained in 64% yield; we did not observe the formation of **2h**. Methylphenylsilane (**1i**) as well as aliphatic hydrosilanes **1j** and **1k** reacted selectively to the corresponding silanols **2i–k**, albeit with lower yields. Notably, siloxane **1l** was oxidized to **2l**, even though uncatalyzed background hydrolysis occurred in significant amounts (see Supporting Information). It is worth noting that, if moderate or low yields of the silanols were obtained, they are a consequence of incomplete conversion of the starting hydrosilane. Products of competing C–H or C=C oxidation were not observed, nor was the formation of disiloxanes.

To explain this selectivity, we investigated the mechanism of the P450-catalyzed Si–H oxidation in further detail using density functional theory (DFT) calculations of a truncated computational model (Figure 2A, see Supporting Information for computational details). This model consists of an iron–porphyrin pyrrole core and methanethiol as axial ligand, mimicking the heme cofactor bond to a cysteine in the enzyme active site. The calculations indicate that the oxidation of dimethylphenylsilane (**1a**) proceeds through a transition state (**TS1**, Figure 2A–B) in which hydrogen atom transfer (HAT) from the hydrosilane to an Fe<sup>IV</sup>-oxene intermediate generates a silyl radical (**INT1**, Figures 2A–B). This intermediate

subsequently undergoes a barrierless process (**TS2**) where hydroxyl rebound occurs to yield the final silanol product **2a**. The hydrogen atom abstraction corresponds to the rate-limiting step of the overall reaction ( $\Delta G^\ddagger = 18.1 \text{ kcal}\cdot\text{mol}^{-1}$ ). We also explored the possibility of silyl cation formation, which might be formed through electron transfer (ET) from the silyl radical to the porphyrin–Fe–OH species (Figure 2C). This step was found to be thermodynamically unfavorable ( $\Delta G = 10.2 \text{ kcal}\cdot\text{mol}^{-1}$  for **1a**); in contrast, the OH rebound step is barrierless (see Figure S1), and hence silyl cation formation is unlikely. We found similar energy barriers for the rate-limiting hydrogen atom abstraction when using benzyldimethylsilane (**1d**,  $\Delta G^\ddagger = 19.6 \text{ kcal}\cdot\text{mol}^{-1}$ ) or a simple siloxane (HMe<sub>2</sub>Si–O–SiMe<sub>2</sub>H,  $\Delta G^\ddagger = 17.7 \text{ kcal}\cdot\text{mol}^{-1}$ ) as substrate. This is expected, since C–Si bonds are longer than C–C bonds, and orbital overlap and resonance stabilization by phenyl of the radical intermediate at the silicon center is less effective (see Figure S5).<sup>[29]</sup> Thus, Si–H-bond strengths are less prone to vary substantially with the substitution pattern.<sup>[30]</sup> We concluded that the limitations we observe in the substrate scope are related to potential issues with binding the substrate in a catalytically competent pose and steric effects, which the truncated DFT model does not account for, and not to intrinsic Si–H-bond strengths or electronic effects.



**Figure 2.** A) Computed mechanism for Fe<sup>IV</sup>-oxene catalyzed oxidation of **1a** to **2a**. B) DFT-optimized, lowest energy and rate-determining H atom abstraction, transition state **TS1** and radical intermediate **INT1** (quartet electronic state). The spin density localized at the Si atom ( $\rho_{\text{spin}}(\text{Si})$ ) in **INT1** is shown. C) The endergonic ( $\Delta G = 10.2 \text{ kcal}\cdot\text{mol}^{-1}$ ) electron transfer for the silyl cation formation pathway reinforces the conclusion that the radical oxidation pathway is the most plausible. Key distances are given in Å and angles in degrees.

Overall, the Si–H-oxidation mechanism described here is similar to the mechanism for native P450-catalyzed C–H oxidation,<sup>[12]</sup> and it involves the same Fe<sup>IV</sup>-oxene catalytic intermediate as well as sequence of H-atom abstraction and rebound. The high chemoselectivity observed for Si–H oxidations over C–H can be directly attributed to the lower bond dissociation energy (BDE) of the Si–H bond as compared to the sterically accessible C–H bonds from the Si–Me groups (see Figure S5). This DFT study confirms that the heme cofactor of P450 enzymes can adapt its native mechanism to oxidize hydrosilanes, and it also explains the absence of disiloxane side products which would require the

activation of the hydrosilane to invite attack of the produced silanol.

In summary, we have engineered a fully genetically encoded catalyst capable of functionalizing silicon-containing compounds inside and outside cells, expanding our ability to manipulate this element in living systems. Three rounds of directed evolution created a highly active variant with four mutations from wild-type P450<sub>BM3</sub>. Even though current yields are limited for sterically hindered and trialkyl silanols, our work demonstrates that P450s are easily evolvable for unnatural hydrosilanes. Furthermore, this renewable, iron-based biocatalyst shows high selectivity for forming silanols instead of disiloxanes; it also favors Si–H over the native C–H and C=C oxidation. This ability to enzymatically oxidize hydrosilanes in concert with previously identified biocatalysts for C–Si bond formation<sup>[18]</sup> could eventually offer access to a range of useful organosilicon compounds in engineered microbial systems and open more sustainable paths to producing the silicon-containing molecules that have become ubiquitous in our modern world.

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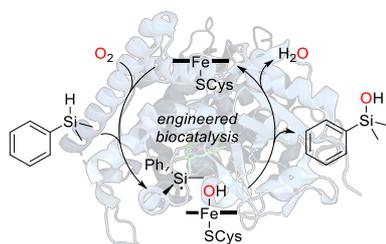
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- [1] a) M. Jeon, J. Han, J. Park, *ACS Catal.* **2012**, *2*, 1539–1549; b) V. Chandrasekhar, R. Boomishankar, S. Nagendran, *Chem. Rev.* **2004**, *104*, 5847–5910.
- [2] A. Colas, *Silicones: Preparation, Properties and Performance*; *Dow Corning, Life Sciences*, 2005.
- [3] a) S. E. Denmark, C. S. Regens, *Acc. Chem. Res.* **2009**, *41*, 1486–1499; b) M. Mewald, J. A. Schiffner, M. Oestreich, *Angew. Chem.* **2012**, *124*, 1797–1799; *Angew. Chem. Int. Ed.* **2012**, *51*, 1763–1765; c) S. E. Denmark, A. Ambrosi, *Org. Process Res. Dev.* **2015**, *19*, 982–994.
- [4] Especially silanediols in catalysis: a) K. M. Diemoz, J. E. Hein, S. O. Wilson, J. C. Fettinger, A. K. Franz, *J. Org. Chem.* **2017**, *82*, 6738–6747 and references cited therein. b) A. G. Schafer, J. M. Wieting, T. J. Fisher, A. E. Mattson, *Angew. Chem.* **2013**, *125*, 11531–11534; *Angew. Chem. Int. Ed.* **2013**, *52*, 11321–11324.
- [5] a) A. K. Franz, S. O. Wilson, *J. Med. Chem.* **2013**, *56*, 388–405; b) R. Ramesh, D. S. Reddy, *J. Med. Chem.* **2018**, *61*, 3779–3798.
- [6] J. A. Cella, J. C. Carpenter, *J. Organomet. Chem.* **1994**, *480*, 23–36.
- [7] Selected examples: a) P. D. Lickiss, R. Lucas, *J. Organomet. Chem.* **1995**, *521*, 229–234 (KMnO<sub>4</sub>); b) K. Valliant-Saunders, E. Gunn, G. R. Shelton, D. A. Hrovat, W. T. Borden, J. M. Mayer, *Inorg. Chem.* **2007**, *46*, 5212–5219 (OsO<sub>4</sub>); c) W. Adam, R. Mello, R. Curci, *Angew. Chem.* **1990**, *102*, 916–917; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 890–891 (dioxirans); d) L. H. Sommer, L. A. Ulland, G. A. Parker, *J. Am. Chem. Soc.* **1972**, *94*, 3469–3471 (peracids). See also Ref [1].
- [8] The use of Pt, Pd, Au, Ag, Ru and Ni as heterogeneous catalysts is reported. Leading reference: a) G. H. Barnes, Jr., N. E. J. Daughenbaugh, *J. Org. Chem.* **1966**, *31*, 885–887. See also Ref [1]. Hydrolytic, homogeneous catalysis: b) M. Lee, S. Ko, S. Chang, *J. Am. Chem. Soc.* **2000**, *122*, 12011–12012 (Ru); c) M. Shi, K. M. Nicholas, *J. Chem. Res. (S)*, **1997**, 400–401; d) M. Yu, H. Jing, X. Liu, X. Fu, *Organometallics* **2015**, *34*, 5754–5758 (both Rh); e) Y. Kikukawa, Y. Kuroda, K. Yamaguchi, N. Mizuno, *Angew. Chem.* **2012**, *124*, 2484–2487; *Angew. Chem. Int. Ed.* **2012**, *51*, 2434–2437 (silver); f) Y. Lee, D. Seomoon, S. Kim, H. Han, S. Chang, P. H. Lee, *J. Org. Chem.* **2004**, *69*, 1741–1743 (Ir); g) E. A. Ison, R. A. Corbin, M. M. Abu-Omar, *J. Am. Chem. Soc.* **2005**, *127*, 11938–11939 (Re).
- [9] Base metals in hydrolytic processes: a) E. Matarasso-Tchiroukhine, *J. Chem. Soc., Chem. Commun.* **1990**, 681–682 (Cr, no selectivity for the silanol); b) U. Schubert, C. Lorenz, *Inorg. Chem.* **1997**, *36*, 1258–1259 (Cu, low selectivity for the silanol); c) A. K. Liang Teo, W. Y. Fan, *Chem. Commun.* **2014**, *50*, 7191–7194 (Fe). A Te complex with O<sub>2</sub>: d) Y. Okada, M. Oba, A. Arai, K. Tanaka, K. Nishiyama, W. Ando, *Inorg. Chem.* **2010**, *49*, 383–385. A Ti zeolite with H<sub>2</sub>O<sub>2</sub>: e) W. Adam, H. Garcia, C. M. Mitchell, C. R. Saha-Möller, O. Weichold, *Chem. Commun.* **1998**, 2609–2610. A Mn complex with H<sub>2</sub>O<sub>2</sub>: f) K. Wang, J. Zhou, Y. Jiang, M. Zhang, C. Wang, D. Xue, W. Tang, H. Sun, J. Xiao, C. Li, *Angew. Chem.* **2019**, *131*, 6446–6450; *Angew. Chem. Int. Ed.* **2019**, *58*, 6380–6384. Cu- or Co-catalysis with O<sub>2</sub>: g) A. V. Arzumanyan, I. K. Goncharova, R. A. Novikov, S. A. Milenin, K. L. Boldyrev, P. N. Solyev, Y. V. Tkachev, A. D. Volodin, A. F. Smol'yakov, A. A. Koriyukov, A. M. Muzafarov, *Green Chem.* **2018**, *20*, 1467–1471. For heterogeneous, hydrolytic Ni-catalysis, see ref. [8a].
- [10] Metal-free protocols, both at pH 11: a) D. Limnios, C. G. Kokotos, *ACS Catal.* **2013**, *3*, 2239–2243; b) A. T. Kelly, A. K. Franz, *ACS Omega* **2019**, *4*, 6295–6300.
- [11] P. Manikandan, S. Nagini, *Curr. Drug Targets.* **2018**, *19*, 38–54.
- [12] C. J. C. Whitehouse, S. G. Bell, L.-L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218–1260.
- [13] J. Rittle, M. T. Green, *Science* **2014**, *330*, 933–938.
- [14] a) E. M. Isin, F. P. Guengerich, *Biochim. Biophys. Acta.* **2007**, *3*, 314–329; b) P. R. Ortiz de Montellano, *Chem Rev.* **2010**, *110*, 932–948; c) S. Shaik, S. Cohen, Y. Wang, H. Chen, D. Kumar, W. Thiel, *Chem. Rev.* **2010**, *210*, 949–1017.
- [15] P. Treguer, D. M. Nelson, A. J. V. Bennekorn, D. J. DeMaster, A. Leynaert, B. Quéguiner, *Science* **1995**, *268*, 375–379.
- [16] M. B. Frampton, P. M. Zelisko, *Chem. Asian J.* **2017**, *12*, 1153–1167.
- [17] M. B. Frampton, P. M. Zelisko, *Silicon* **2009**, *1*, 147–163.
- [18] S. B. J. Kan, R. D. Lewis, K. Chen, F. H. Arnold, *Science* **2016**, *354*, 1048–1051.
- [19] The oxidation of hydrosilanes in rats has been reported almost fifty years ago, but the identification of the enzyme responsible for this remains elusive: R. J. Fessenden, R. A. Hartman, *J. Med. Chem.* **1970**, *13*, 52–54.
- [20] S. T. Jung, R. Lauchli, F. H. Arnold, *Curr. Op. Biotech.* **2011**, *22*, 809–817.
- [21] H. Li, T. L. Poulos, *Biochim. Biophys. Acta* **1999**, *1441*, 141–149. Whereas F87, A328 and L181 are important for substrate recognition, A184 plays a role in the thermostability of P450<sub>BM3</sub> variants. See Ref. [20].
- [22] D. C. Haines, D. R. Tomchick, M. Machius, J. A. Peterson, *Biochemistry* **2001**, *40*, 13456–13465.
- [23] S. Kille, C. G. Acevedo-Rocha, L. P. Parra, Z. G. Zhang, D. J. Opperman, M. T. Reetz, J. P. Acevedo, *ACS Synth. Biol.* **2013**, *2*, 83–92.
- [24] Examples for the mutation of site A328: a) G. Roiban, M. T. Reetz, *Chem. Comm.* **2015**, *51*, 2208–2224 and cited references. For a recent example, see: b) H. Zhou, B. Wang, F. Wang, X. Yu, L. Ma, A. Li, M. T. Reetz, *Angew. Chem.* **2019**, *131*, 774–778; *Angew. Chem. Int. Ed.* **2019**, *58*, 764–768. See also Ref. [20].
- [25] For the importance of sites 181 and 184, see for example: K. Zhang, B. M. Shafer, M. D. Denars II, H. A. Stern, R. Fasan, *J. Am. Chem. Soc.* **2012**, *134*, 18695–19704.
- [26] Double-bond epoxidation was for example observed in a manganese-catalyzed protocol with H<sub>2</sub>O<sub>2</sub> as oxidant, see Ref. [9f].
- [27] a) R. J. P. Corriu, C. Guerin, *J. Organomet. Chem.* **1980**, *198*, 231–320; b) C. Chuit, R. J. P. Corriu, C. Reye, J. C. Young, *Chem. Rev.* **1993**, *93*, 1371–1448.

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- [28] For a comparison of Si–H and C–H bond strengths, see a) R. Walsh, *Acc. Chem. Res.* **1981**, *14*, 246–252; b) S. J. Blanksby, G. B. Ellison, *Acc. Chem. Res.* **2003**, *36*, 255–263 (BDE of a benzylic C–H bonds: 89.8 kcal/mol). Phenyl-substitution weakens the Si–H bond strength by ca. 2 kcal/mol; c) C. Chatgililoglu, *Acc. Chem. Res.* **1992**, *25*, 188–194 (BDE of Ph<sub>3</sub>Si–H: 84 kcal/mol, Et<sub>3</sub>Si–H: 91 kcal/mol). For reviews, see also: c) B. Tumanskii, M. Karni, Y. Apeloig in *Organosilicon Compounds Theory and Experiment (Synthesis)* (Ed.: V. Y. Lee), Elsevier, Academic Press, **2017**, pp. 231–294; d) B. Tumanskii, M. Karni, Y. Apeloig in *Encyclopedia of Radicals in Chemistry, Biology and Materials, Vol. 4* (Eds.: C. Chatgililoglu, A. Studer), J. Wiley, Chichester, **2012**, pp. 2117–2146.
- [29] a) W. S. Sheldrick in *The Chemistry of Organic Silicon Compounds, Vol. 1* (Eds.: S. Patai, Z. Rapport), Wiley, Chichester, **1989**, pp. 227–303; b) I. V. Alabugin in *Stereoelectronic Effects: A Bridge Between Structure and Reactivity, Vol. 1*, John Wiley & Sons, Hoboken, **2016**, 1–392.

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## Entry for the Table of Contents



**In rerum natura:** Wild-type cytochrome P450<sub>BM3</sub> catalyzes the oxidation of hydrosilanes to silanols both *in vivo* and *in vitro*. Directed evolution is used to generate an efficient and selective biocatalyst that delivers a broad range of aryl- and alkylsubstituted silanols. Computational studies reveal a sequence of H atom abstraction and OH rebound as the mechanism, in analogy to the native C–H hydroxylation activity.

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