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# Arginine interactions with anatase TiO$_2$ (100) surface and the perturbation of $^{49}$Ti NMR chemical shifts – a DFT investigation: relevance to Renu-Seeram bio solar cell

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**Abstract** Density functional theoretical calculations have been utilized to investigate the interaction of the amino acid arginine with the (100) surface of anatase and the reproduction of experimentally measured $^{49}$Ti NMR chemical shifts of anatase. Significant binding of arginine through electrostatic interaction and hydrogen bonds of the arginine guanidinium protons to the TiO$_2$ surface oxygen atoms is observed, allowing attachment of proteins to titania surfaces in the construction of bio-sensitized solar cells. GIAO-B3LYP/6-31G(d) NMR calculation of a three-layer model based on the experimental structure of this TiO$_2$ modification gives an excellent reproduction of the experimental value (-927 ppm) within +/- 7 ppm, however, the change in relative chemical shifts, EFGs and CSA suggest that the effect of the electrostatic arginine binding might be too small for experimental detection.

**Keywords** Amino acid binding · DFT · NMR simulation · TiO$_2$

Dedicated to Prof. Paul von Rague Schleyer on the occasion of his 80th birthday

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## Introduction

Interaction of TiO$_2$ with the mutants of bacteriorhodopsin (bR) is important in the design of efficient biosensitized solar cells (BSSC). Application of biologically-derived, inexpensive light harvesting protein sensitizers for sensitizing titanium dioxide (TiO$_2$) nano particles and fibers, instead of the traditional, toxic and expensive chemical based dyes in dye-sensitized solar cells (DSSCs), is an attractive proposition.

Protein-sensitized solar cells emulate the design of DSSCs except that the dye molecules are replaced by light harvesting biomolecules as sensitizer. The impetus for this replacement comes from potentially high efficiency of conversion that light harvesting protein complexes promise, their ability to self-assemble on a surface [1], their low cost of production by biotechnology and room temperature processing and their long lifetimes even at higher temperature (100°C) [2]. An interesting candidate for a

protein sensitizer is bacteriorhodopsin (bR) which is a trans-membrane protein found in the archae *Halobacterium salinarum* [3]. Absorption of photon in bR occurs principally through the chromophoric group and the charge separation that occurs eventually leads to shedding of electrons. Upon absorption of light, there is a shift of electron density from the β-ionone ring of the chromophore toward the Schiff base, which is comparable to a 2.6 Å displacement of a single electron down the polyene chain [4] and is in the opposite direction of the initial photovoltage spike that is observed in bR [5]. This short duration charge shift is a critical component of the primary event because it sets in motion events that lead to the photoisomerization of the chromophore [6].

TiO$_2$ has been in the focus of numerous investigations since the demonstration of the original DSSC photovoltaic cell [7]. Researchers have proposed the possibility of using hybrid architecture of nanoparticles and nanofibres [8–12] to facilitate better electron transport. Given the advancement in electrospinning for generating large surface-to-volume ratio nanofibres in larger-scale [13], the electrospun TiO$_2$ nanofibres have been shown to increase solar energy efficiency up to 6.2 % in DSSC [14].

The basic hypothesis for the design of such a BSSC, for which we propose the name Renu-Seeram cell is that bR is attached by chemisorption to TiO$_2$, and upon photoexcitation ejects electrons into the conduction band of semiconducting metal oxide completing the circuit and electrons flow from counter electrode to the conductive ITO glass plate. An iodide electrolyte recharges bR.

In order to shed some light on this interaction, we set out to investigate computationally the binding of arginine, which has been identified as a potential terminal amino acid in bR, on the (100) (or identical by symmetry the (010)) surface of TiO$_2$ anatase [15] in order to derive a model for the interaction of TiO$_2$ to bR mutants.

## Methods

All calculations were carried out using the Gaussian 03 [16] suite of programs. Density functional theory (DFT) with the hybrid B3LYP functional [17–19] in combination with Pople's 6-31G(d) [20, 21] was used for the optimizations of the ground state geometries of a TiO$_2$ anatase model, arginine and a complex of arginine on anatase and the simulation of the chemical shifts. The default convergence criteria and integration grid of the program were used. The nature of all found local minimum structures was confirmed by the lack of an imaginary harmonic vibrational frequency [22]. Absolute magnetic shieldings were determined with the GIAO (gauge-including atomic orbital) method [23–25] as implemented in Gaussian 03. The obtained shielding

tensors were referenced against titanium tetrachloride to yield relative chemical shifts. This approach has been used successfully for the determination of chemical $^{49}$Ti shifts previously [26]. The simulations of NMR tensor parameters were performed using the SIMPSON program [27]
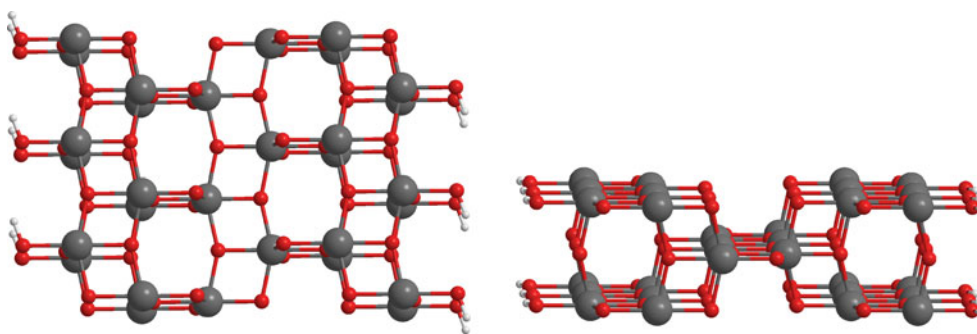
## Results and discussion

When a protein such as bR or its mutants is brought onto a surface such as anatase or ZnO, it will be either covalently attached or chemisorbed. It is therefore reasonable to expect subtle electronic perturbations of $^{49}$Ti chemical shift anisotropy (CSA or $\Delta\sigma$) and the quadrupole coupling, C$_q$, which provide useful tools to probe subtle interactions at the atomic level. It should be possible to compare the DFT derived changes in electric field gradient and CSA with experimental values [28].

TiO$_2$ surfaces have been thoroughly studied, both experimentally and theoretically [29–40]. Also, the adsorption of small molecules on TiO$_2$ surface has been reported in the literature recently [41–52]. Based on previous experience with the calculation of $^{49}$Ti NMR chemical shifts [25], we have started to explore a suitable model for the TiO$_2$ (100) surface on which the bR is attached [15]. This surface is one of the two major cleavage planes, together with the (101) surface. It possesses small grooves formed by oxygen atoms on either side of the groove along with both Ti and O atoms at the bottom. Several repeat unit cells are required for reliable and consistent data, since there are relative chemical shifts in the range of several hundred ppm for individual Ti atoms. The model employed herein to represent the (100) surface of anatase consists of 30 TiO$_2$ units, terminated with 12 hydrogen atoms (Fig. 1). It has been taken from the crystal structure of TiO$_2$ anatase [53] and the positions of all surface and added hydrogen atoms are kept fixed during the optimization, only allowing the amino acid to move freely on the surface.

The well-established B3LYP/6-31G(d) level of theory has been chosen because of its previously demonstrated superior performance in the $^{49}$Ti NMR determination [26]. Herein, relative chemical shifts of the Ti atoms of this model are computed compared against the available experimental NMR data. In our model there are four titanium atoms which have a "complete" oxygen-saturated environment – in the middle layer of the side-on view of Fig. 1 – which can serve as representatives of the bulk titanium atoms. These Ti atoms are calculated to have isotropic chemical shifts (relative to TiCl$_4$) between -921 and -922 ppm, in excellent agreement with the experimental value for anatase of -927 ppm [26]. Shifts of those titanium atoms on the surface which have five oxygen contacts are in the range of -450 to -530 ppm.
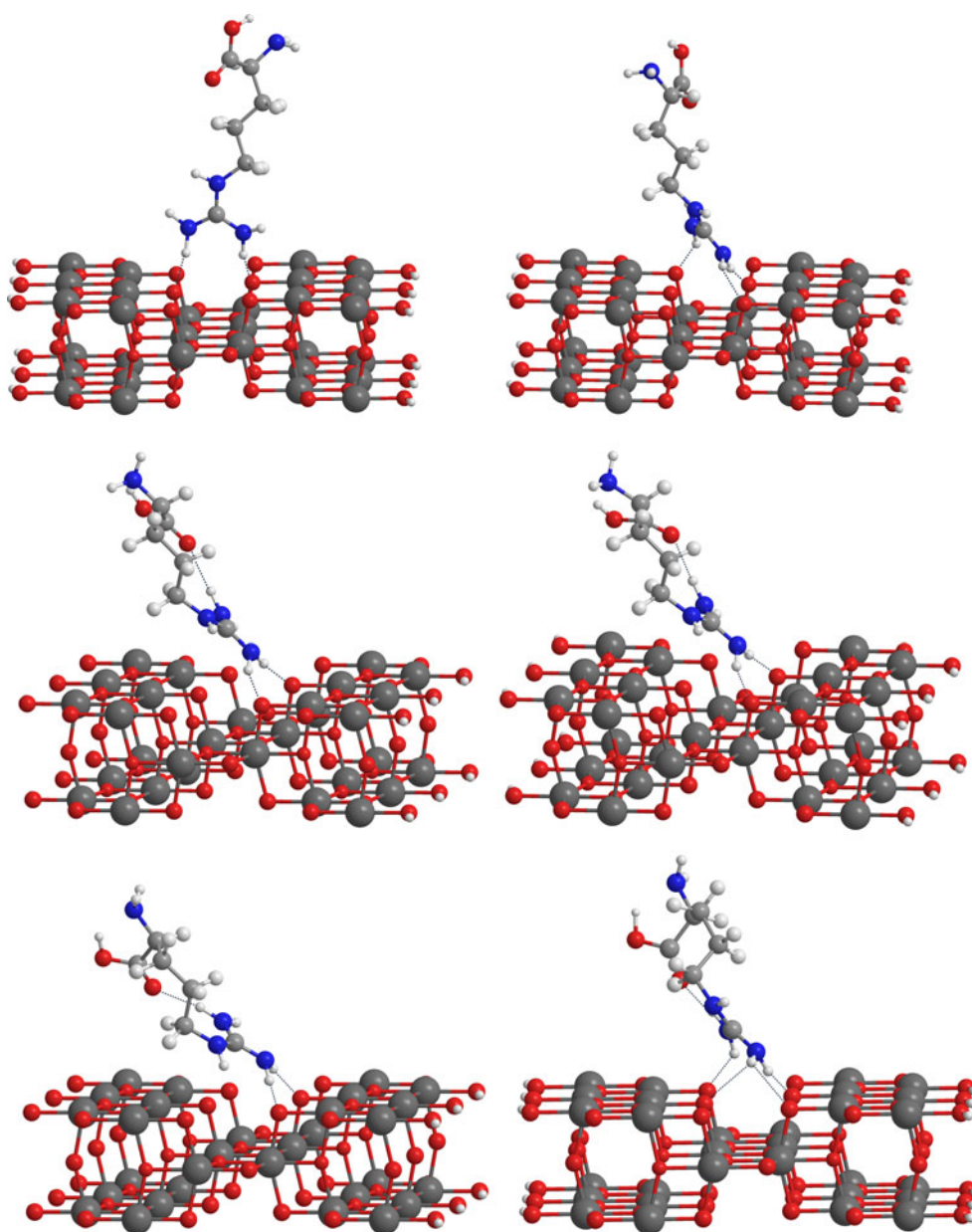
**Fig. 1** Model of (100) TiO$_2$ anatase surface, top (left) and side (right) views; oxygen atoms in red, titanium atoms in gray



In a previous study, the positively charged arginine and lysine amino acid residues have been identified as potential binding groups as they reside at the cytoplasmic surface of bR [15]. For this reason we have chosen arginine as a model for bR in this binding investigation. Different orientations of arginine on the surface have been modeled with the most stable ones reported herein (Fig. 2): In structure 1, arginine possesses two hydrogen bonds

**Fig. 2** Different orientations of arginine on the anatase (100) surface model: 1 (top left), 2 (top right), 3 (middle left), 4 (middle right), 5 (bottom left) and 6 (bottom right). Hydrogen bonds are shown

bridging the groove, originating from each of the terminal nitrogen atoms and pointing toward the central oxygen atoms in our model. This orientation is stabilized relative to the separated TiO$_2$ surface and arginine by about 160 kJ mol$^{-1}$. The largest part of this energy can be attributed to attractive electrostatic interaction between the positively charged arginine N terminus and the surface oxygen atoms. The importance can be seen in the increasing tilting of arginine toward the surface in the following structures 2-5, thus bringing the third partially charged nitrogen atom of the guanidinium group closer to the negatively charged oxygen atoms. Structure 2 exhibits this slightly tilted position across the groove and has a third hydrogen bond which lowers the energy by an additional 60 kJ mol$^{-1}$. Orientations 3 and 4 are quite similar to each other, being stabilized by 250 and 254 kJ mol$^{-1}$, respectively. Both trade the docking across the gap formed by the surface oxygen atoms in favor of one intramolecular and two hydrogen bonds to oxygen atoms on the same side of the groove.

The most stable arrangements are the last two presented in Fig. 2. They are 266 and 272 kJ mol$^{-1}$ respectively more stable than the separate molecules and adopt an orientation which allows additional weak hydrogen contacts across the groove. Structure 5 forms such a bond at about 2.6 Å from the previously uninvolved third nitrogen atom, in addition to the two strong bonds at about 1.9 Å, while the most stable structure 6 possesses four contacts at 1.7, 2.1, 2.4 and 2.4 Å (see also Fig. 3). Both orientations retain the intramolecular hydrogen bond. These data illustrate that the attachment at an oxygen-based channel is in fact the most likely position but it appears preferentially to form as many hydrogen bonding interactions with oxygen atoms on both sides of this trough as possible, even at the expense of their individual strength. In addition, the latter structures are
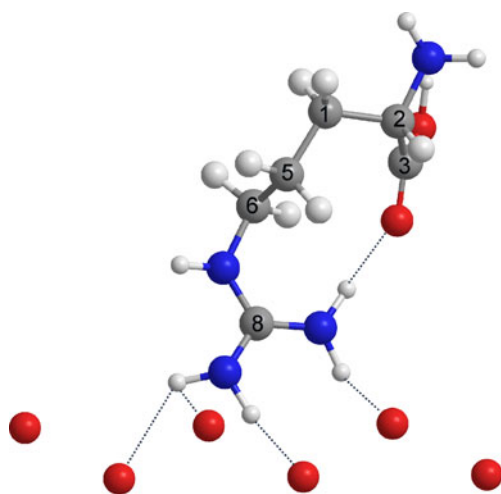


**Fig. 3** Numbering scheme for arginine used in Table 1. The groove oxygen atoms are also shown, allowing easier identification of the hydrogen bonds

**Table 1** Relative $^{13}$C chemical shifts (in ppm) of arginine before and after attachment to a TiO$_2$ anatase surface

| Atom number | Arginine | Arginine on anatase | Difference |
|---|---|---|---|
| 1 | 34.8 | 33.0 | −1.8 |
| 2 | 52.0 | 58.1 | 6.1 |
| 3 | 162.6 | 173.6 | 11.0 |
| 5 | 29.0 | 29.9 | 0.9 |
| 6 | 44.2 | 44.0 | −0.2 |
| 8 | 140.5 | 152.9 | 12.4 |

closer to the surface, leading to increased electrostatic stabilization.

Starting from this final orientation 6, we also derived the chemical shifts for both the loaded anatase ($^{49}$Ti) and the amino acid ($^{13}$C). The $^{13}$C chemical shifts of arginine under the influence of the anatase attachment are given in Table 1. One can clearly see that the carbon atoms which are close to a new hydrogen bond (atoms 3 and 8) are shifted to a higher field by up to 12 ppm.

The relative $^{49}$Ti chemical shifts of the four central Ti atoms with oxygen saturation (see above) are influenced by the arginine attachment by only a few ppm (between 1 and 9 ppm, values range from -913 to -928 ppm). This appears too small to be easily detectable in a standard NMR experiment. However, the surface titanium atoms are much more strongly influenced: the induced changes in relative chemical shifts range from -83 to +93 ppm.

For comparison we have simulated the experimental parameters previously obtained for bulk anatase with those predicted by quantum mechanical calculations using the most stable orientation 6 (Fig. 2). These data are depicted in Fig. 4 for each NMR active isotope of titanium. The experimental values [26] of quadrupole coupling constant, C$_q$, previously measured for anatase are 4.94 and 4.04 MHz for the 47 and 49 isotopes respectively. The asymmetry parameter for each was 0.06, as these systems are nearly axially symmetric one would expect it near zero. The chemical shielding anisotropy (CSA) was 117 ppm with an asymmetry parameter of 0.1 and all three Euler angles fixed at 0° (coincident principle axis systems). These parameters are contrasted with those for the model systems in Table 2. We define the shielding anisotropy as $\Delta\sigma = \delta_{33} - \frac{1}{2}(\delta_{11}+\delta_{22})$, where the elements of the shielding tensor are ordered as $|\delta_{33} - \delta_{iso}| \geq |\delta_{11} - \delta_{iso}| \geq |\delta_{22} - \delta_{iso}|$; and $\delta_{iso}$ is the trace of the tensor $\delta$.

It is clear from the data in Fig. 4 that the level of theory seems to overestimate both the electric field gradient (EFG) and the CSA. The $^{47}$Ti (nuclear spin 5/2) data is dominated by the quadrupolar coupling which is directly proportional to the EFG tensor and therefore shows the effects of this
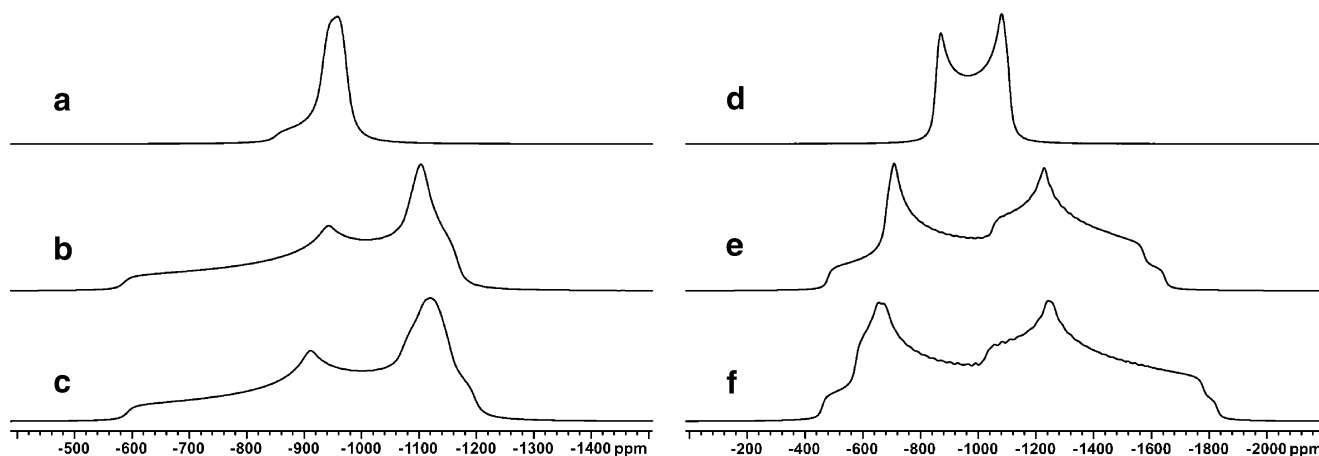
**Fig. 4** Ideal pulse simulations at 21.1 T (900 MHz for $^1$H) of solid-state NMR spectra for the experimental values for anatase (**a** + **d**), the model with no arginine on the surface (**b** + **e**), and the surface Ti atom with arginine bound (**c** + **f**) with the first column showing $^{49}$Ti and the second $^{47}$Ti NMR spectra

parameter, while in the $^{49}$Ti (nuclear spin 7/2) data it is evident that the CSA interaction dominates this lineshape at the simulated magnetic field. The key point is that the effects are similar for the models with and without the arginine present. The implication is that the electrostatics of the Ti atoms on the surface are not strongly impacted by the binding of the amino acid. Although it is possible with some effort to experimentally distinguish surface atoms from the bulk, measuring such a surface effect with NMR seems difficult, despite the above mentioned calculated changes in relative chemical shifts.

## Conclusions

The nature of the attachment of arginine on the anatase (100) surface is clearly based on electrostatic interaction together with hydrogen bonds. It appears that an increased number of these weak interactions lead to stabilization of the complex between our (100) anatase model and the amino acid, indicating that the attachment of bR onto a TiO$_2$ surface is possible in the Renu-Seeram Cell [54].

Additionally, the small energetic changes between some of the found orientations suggest that one can expect dynamic behavior of arginine on the surface. The correct DFT reproduction of $^{49}$Ti NMR chemical shifts for anatase gives confidence in the reliability of the employed approach for the evaluation of this property. Detailed computational investigation of both $^{13}$C (arginine) and $^{49}$Ti (TiO$_2$) NMR data show very little change in predicted relative chemical shifts upon binding. This is further confirmed by the simulation of the titanium spectra based on experimental and calculated data. It suggests that NMR investigation of the protein binding on titania nanoparticles seems unfeasible. Other techniques such as SERS on TiO$_2$ [55] might give additional insight into the attachment mode.

**Table 2** NMR tensor parameters for anatase, experimental and model systems

| System | $C_q$ (MHz) $^{47}$Ti/$^{49}$Ti | $\eta_q$ | $\delta_{iso}$ (ppm) | $\Delta\sigma$ (ppm) | $\eta_\sigma$ | $\alpha$ | $\beta$ | $\gamma$ |
|---|---|---|---|---|---|---|---|---|
| Expt [26] | 4.94/4.04 | 0.06 | −927 | 117 | 0.10 | 0 | 0 | 0 |
| Anatase TiO$_2$ (Fig. 1) | 7.5/6.1 | 0.66 | −923 | 446 | 0.19 | 0 | 86 | 180 |
| Orientation 6 (Fig. 2) | 8.4/6.8 | 0.68 | −923 | 422 | 0.19 | 188 | 87 | 182 |

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