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Water driven adsorption of amino acids on the (101) anatase TiO_2 surface: an ab-initio study

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Arg, Lys and Asp amino acids are known to play a critical role in the adhesion of RKLPDA engineered peptide on the (101) surface of the titania anatase phase. To understand their contribution to the peptide adhesion, we have considered the relevant charge states due to protonation (Arg and Lys) or deprotonation (Asp) occurring in neutral water solution, and studied their adsorption on the (101) anatase TiO_2 surface by ab-initio total energy calculations based on density functional theory. The adsorption configurations on the hydrated surface are compared to those on the dry one considering also the presence of the hydration shell around amino acid side-chains. This study enlighten the way how water molecules mediate the adsorption of charged amino acids showing that protonated amino acids are chemically adsorbed much more strongly than de-protonated Asp. Moreover it is shown that the polar screening of the hydration shell reduces the adsorption energy of the protonated amino acids by a small extent, thus evidencing that both Arg and Lys strongly adhere on the (101) anatase TiO_2 surface in neutral water solution and that they play the major role for the adhesion of the RKLPDA peptide.

Interfaces between biomolecules and inorganic materials are now receiving an increasing attention in various fields of application ranging from nanotechnology to medicine and pharmacology [1,2]. The recent progresses in combinatorial biological techniques have permitted to select sequences of amino acids possessing specific affinities to inorganic targets [1]. A full understanding of the specific affinities observed requires the atomic scale study of the processes involved such as the charge and hydrogen bond type interactions found in some studies [3–6]. We focused our attention on the Titania surface as it is of fundamental importance to develop biocompatible and bioinspired nanosctuctured materials. The RKLPDA peptide (Arg–Lys–Leu–Pro–Asp–Ala, minTBP-1) has been demonstrated to display a large and selective affinity to TiO_2 [3]. Experimental mutational analyses have indicated that the charged residues of Arg and Asp have a role in the specific binding indicating that the recognition of the substrate is dominated by electrostatic interactions [3]. The mutation of the neutral Pro reduces the peptide-surface binding affinity [3] likely because it is known that it plays as alpha-helix breaker and generate kinks in the peptide chains [7] conferring structural rigidity and bended conformations [8]. This suggests that the recognition is based on a complex interplay of electrostatic interactions and conformational patterns. Surprisingly, the depletion of a positively charged group by substituting the Lys with the neutral Ala residue, increases the affinity of the minTBP-1 binding to TiO_2 [3]. This unexpected behavior is puzzling, being possibly related to the competition of the two adjacent positively charged groups (Arg–Lys) [9,10]. The selectivity and specificity to Titania substrate has been supposed to be driven by two specific electrostatic bonds: the first between the Arg residue and an acidic site of the surface and the second one between the Asp residue and a basic site on the Ti oxide surface [10]. The substitution of Arg with the positively charged Lys residue decreases the adhesion of the peptide [10] that may be due to the presence of an uncharged polar group in the Arg that can form hydrogen bonds with the polar surface groups.

Classical Molecular Dynamics (MD) simulations suggested that the surface recognition is mediated by water layers at the interface and by the ability of the amino acids side chains to sense the molecular solvent structure at the surface-water interface [8,11,12]. Recently, ab-initio calculations have been used to model the water adsorption on various TiO_2 surfaces both of the rutile and the anatase phases [13–17] while the peptide-TiO_2 surface interactions have been studied concerning the rutile phase mostly through classical MD [18–20] with suitable ab-initio derived force fields [18] but also by first principles MD [21,22] and, more recently, through total energy ab-initio calculations [23]. In particular, DFT calculations have indicated that, contrarily to previous MD results [24], the RGD peptide adheres on the rutile TiO_2 (110) surface through the Asp carboxyl group instead of the Arg side chain. In this case, the

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RGD peptide was considered in its neutral state and the role of the solvent was not investigated. For these reasons an accurate characterization of amino acids adhesion is needed taking into account explicitly the water molecules that deeply affect the selectivity and the strength of adhesion.

In the present article we have studied by ab-initio Density Functional Theory (DFT) the adsorption of amino acids, taken from the RKLPDA sequence as the most probable responsible of its adhesion on the (101) anatase surface, namely Arg ($C_6H_{14}N_4O_2$) and Asp ($C_4H_7NO_2$), or that have been indicated to play a role such as Lys ($C_6H_{14}N_2O_2$). The role of water in the adsorption process is considered explicitly in three ways:

- the surface is hydrated and a specific hydration pattern has been chosen according to the literature [13].
- Arg, Lys and the Asp amino acids were modeled in their corresponding charge states due to the protonation (Lys and Arg) and de-protonation (Asp) of the side chains occurring in water solutions.
- the amino acids are solvated by the corresponding hydration shells around the side chains.

Our DFT scheme adopts a generalized gradient approximation (GGA) of the electron exchange and correlation energy using Perdew-Burke-Ernzerhof formula (PBE) [25], as implemented in the QUANTUM-ESPRESSO package [26]. Ultra-soft pseudopotentials (US-PPs) [27] have been used for all the atomic species and have allowed the usage of energy cut-off values of 60 Ry and 400 Ry for the wave functions and the electron density; the lattice parameters of the anatase bulk phase computed in this way are close to the experimental data. The (101) anatase TiO_2 surface has been modeled by using a slab geometry laying in the *xy* plane composed by 1x3x3 unit cells and 108 atoms; the supercell has been fully optimized resulting in a size of 10.42x11.23x25.03 Å^3 and includes a vacuum region 16.2 Å thick. The artificial electric field across the slab induced by the periodic boundary conditions has been corrected following Ref. [28] and a (2x2x1) Monkhorst-Pack *k*-point grid [29] for the Brillouin zone sampling has been employed. All the structures considered have been fully optimized using the BFGS algorithm [30] to find the ground state configurations; in particular, charged structures containing either protonated or de-protonated amino acids have been fully optimized in a uniform charged background to make the supercell neutral. To calculate the adsorption energy of charged structures, the total energy of the ground state configurations has been calculated on a neutral supercell obtained by adding to the ground state configuration a counter-ion placed in the vacuum region far from both the amino acid and the surface (see below). This in order to avoid all the difficulties related to the Ewald sums in such inhomogeneous systems. To find the right number of water molecules of the hydration shells and their localization around the amino acid side chain (see below), we have simulated both isolated Arg and Lys molecules in water environment using Classical MD and DFT. Classical MD simulations have been performed using NAMD [31] and CHARMM force field with water molecules treated as TIP3P charge structures. Then, the configuration obtained has been further relaxed in the DFT context to get the ground state adsorption configuration of the hydrated amino-acids. In all cases, long range forces have been included through a semi-empirical correction term [32].

As a final test, we have performed ab-initio molecular dynamics (AIMD) run for the Arg case to verify the reliability of the hydration scheme here adopted. In particular we have performed a Car-Parrinello molecular dynamics [33] of the system containing the amino acid, the hydrated surface and as many water molecules as needed to fill the super cell at the room temperature water density. The AIMD has been performed for more than 5 ps in a canonical ensamble at 300 K with the electron fictitious mass kept at 400 a.u. and the timestep at 1 a.u. initially and then at 5 a.u..

From the slab geometry, we have obtained that the (101) TiO_2 surface energy is E_s= 0.523 J/m^2 and the topmost surface layer is made of two-fold and five-fold coordinated oxygen O^{2f} and titanium Ti^{5f} atoms, respectively.

The hydrated surface is shown in Fig. 1 and the hydration scheme has been chosen as the ground state one among the various hydration patterns already studied by other authors [13]. The hydration pattern considered implies that in the supercell adopted there are two water molecules adsorbed on the top surface of the slab, both of them lying on a horizontal plane parallel to the slab surface (see Fig. 1). The water molecules are stably adsorbed on the anatase surface through one Ti^{5f}–O dative bond and two hydrogen bonds involving two O^{2f} surface oxygens, the adsorption energy per water molecule being E_a= -0.73 eV, in good agreement with previous calculations found in the literature, namely E_a= -0.72 eV (PW91) and E_a= -0.75 eV (PBE) [14].

In the hydration model of the (101) anatase surface here adopted, only the first water layer is considered that, according to the recent literature, is the one that mediate the adsorption as both classical and first principled MD simulations evidence [8,11,19]. In particular, the major role played by the first water layer is related to the local water density of the first layer in the presence of peptides at the surface [19]. Moreover, also first principles MD simulations evidence that the adsorption configurations are mediated by at most two water molecules [21,22]. Hence, there is a large consensus in the literature that the amino acid adsorption on the (101) anatase surface in water solution is mediated by one or at most two water layers and this is exactly what occurs in our model because we consider just the first hydration layer of the (101) anatase

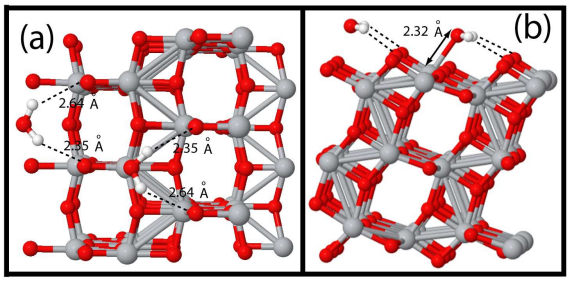

Fig. 1 (Color online) Fully relaxed configurations of the hydrated (101) anatase TiO_2 surface: top (a) and side (b) views.

surface and the closest solvation shell of the amino acids (see below).

The ground state adsorption configurations of protonated Lys and Arg and de-protonated Asp on the dry (101) anatase surface are reported in Fig. 2. Lys is adsorbed through two hydrogen bonds (with a large electrostatic component) between two O^{2f} surface oxygens and two hydrogens of the -NH_3^+ side chain terminal group. Similarly Arg is adsorbed on the dry surface through two hydrogen bonds between hydrogens belonging to the protonated side chain -$C(NH_2)_2^+$ terminal group and two O^{2f} oxygens which are stronger than the ones for Lys. On the contrary, negative charged de-protonated Asp is adsorbed at two Ti^{5f} atoms via two Ti–O bonds. The adsorption energies have been calculated including a counter-ion (OH^- for the Lys and Arg molecules, and H_3O^+ in the Asp case) in the relaxed adsorption configuration as:

$$E_{ads} = E_T - E_S - E_{amino} - E_{CI} \quad (1)$$

where E_{amino} and E_{CI} are the total energies of the isolate amino acid and its corresponding counter-ion, E_T is the total energy of the system and E_S is the slab energy.

On the hydrated surface, the amino acid adsorption is mediated by the water molecules, as shown in Fig. 2. The protonated side chains of Lys and Arg stick on the hydrated surface through a water molecule that is detached from the original Ti^{5f} adsorption site and stays close to the protonated terminal groups of the side chains, with the two H–O water bonds oriented upwards: in the Lys case they are aligned along a N–H bond of the protonated -NH_3^+ terminal group while in the Arg case the water oxygen, the bisector of the $\widehat{HOH}$ angle and the C atom of the protonated -$C(NH_2)_2^+$ terminal group are aligned; in the last case, the region between the two amine groups is electron-depleted and attracts the oxygen lone-pairs thus causing the rotation of the water molecule as shown in Fig. 2. In neutral water solution, Asp has the carboxyl group of the side chain de-protonated into -COO^-. As a consequence its ground state adsorption configuration on the hydrated surface, reported in Fig. 2(f) is a water-hydrogens mediated adsorption with the water oxygens still bonded to

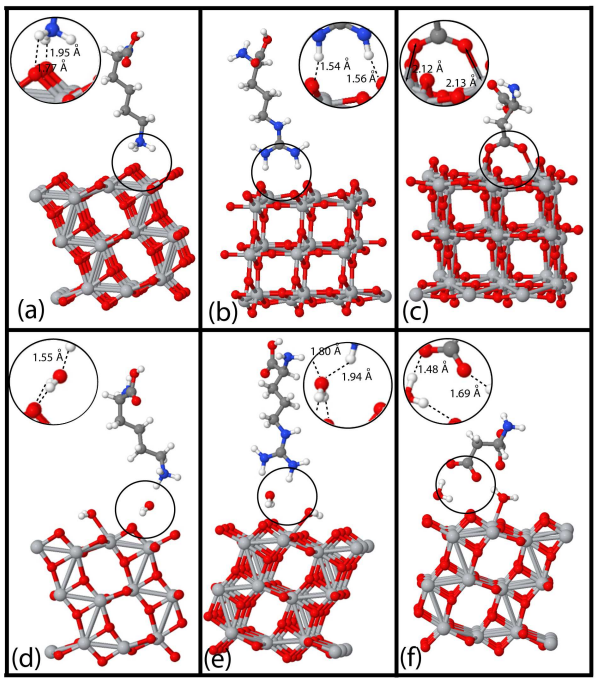

Fig. 2 (Color online) Ground state adsorption configurations for Lys (a, d), Arg (b, e) and Asp (c, f) on the (101) anatase TiO_2 surface. Adsorption on the dry and the hydrated surfaces are reported in the upper and lower panels respectively.

the Ti^{5f}. Two hydrogen bonds are formed between the two oxygens of the -COO^- group and the hydrogens of two water molecules on the surface; hence the water molecules that mediate the adsorption still stick on the surface through one O-Ti^{5f} and one H-O^{2f} bonds. The amino acids adsorption energies on the hydrated surface have been calculated again through the Eq. 1 where E_S must be replaced by $E_{S+(H_2O)_2}$ (i.e. the energy of the hydrated slab) and are reported in the Table 1. While on the dry surface Arg is just nearly 65 meV more stable than Lys, on the hydrated one Arg adsorption results favored by nearly 220 meV with respect to Lys (in Table 1). The reason of this difference (in spite of the same charge) is related to the kind and the number of bonds formed on the dry and the hydrated surface. On the dry surface, Lys stays attached with two H–bonds that, although they are enforced by the charge of the side chain, are relatively weak [see Fig. 2(a)]. On the hydrated surface instead, Lys is adsorbed via only one O–H bond involving the water oxygen that, being detached from the surface, is oriented properly to form a much stronger bond with a larger electrostatic contribution [see Fig. 2(d)]. Arg adsorption on the hydrated surface is also weakened, but at a smaller extent with respect to Lys because adsorption is still mediated by two hydrogen bonds slightly weaker than the ones occurring on the dry surface (the O^{2f}-H bonds described

above) [see Fig. 2(b)(e)]. Thus, the Arg adsorption is weaker on the hydrated surface because of a bond softening with respect to the dry surface rather than a change in the number of adsorption bonds as occurs in the Lys case. The de-protonated Asp adsorption is largely weakened on the hydrated surface with respect to the dry one because of the formation of two H-bonds between the -COO^- and the water hydrogens on the hydrated surface: indeed some energy is lost to detach one hydrogen per water molecule from the surface so that the adsorption energy in the end is reduced with respect to the dry surface in spite of the strong bonds formed by the Asp with the water molecules.

The adsorption on Arg, Lys and Asp amino acids on the hydrated surface is thus largely mediated by the water adsorbed on the (101) anatase surface; the mechanism evidenced indicates that water molecules, linking the charged amino acids to the surface, are partially detached from the surface. Hence a question arises whether or not the amino acid adsorption may favor the desorption of the system composed of the amino acid itself and the water molecules that mediate the adsorption. Therefore we have calculated the relevant adsorption energies that casts:

$$E_{ads} = E_T - E_{S+(H_2O)_{2-n}} - E_{amino+(H_2O)_n} - E_{CI} \quad (2)$$

where n is the number of water molecules that mediate the amino acid adsorption on the hydrated surface ($n = 1$ for Lys and Arg, $n = 2$ for Asp), $E_{S+(H_2O)_{2-n}}$ is the energy of the surface plus the residual water molecules adsorbed on it, $E_{amino+(H_2O)_n}$ is the total energy of the charged amino acid plus n water molecules that are eventually detached from the hydrated surface and E_{CI} is the energy of the isolated counter ion. The adsorption data, reported in Table 1, show that this phenomenon does not occur for Asp because its adsorption energy with two water molecules is quite larger than the adsorption energy of Asp alone; for Lys and Arg, the adsorption energy of the amino acid plus one water molecule are still larger in absolute value than the one for the amino acid alone: however, while the desorption of the Lys+H_2O system from the hydrated surface require nearly 450 meV more than the desorption of Lys from the hydrated surface, the two phenomena seem to compete more closely in the Arg case because Arg+H_2O system requires nearly 250 meV more energy than the Arg alone. The stability of the amino acids adsorption on the hydrated surface has been also checked looking at a more complicated phenomenon that, together with the desorption of the hydrated amino acids, involves also further adsorption of water molecules on the anatase surface at the adsorption site previously occupied by a water molecule: in water ambient, indeed, the empty anatase surface adsorption site may be rapidly occupied by another water molecule. The adsorption energy is thus calculated as:

$$E_{ads} = E_T + E_{(H_2O)_n} - E_{S+(H_2O)_2} - E_{amino+(H_2O)_n} - E_{CI} \quad (3)$$

where E_T is the total energy of the system including the counter ion, $E_{(H_2O)_n}$ is the energy of the n extra water molecules that are needed to replace the waters eventually detached from the surface with the amino acid, $E_{S+(H_2O)_2}$ is the total energy of the fully hydrated surface, $E_{amino+(H_2O)_n}$ is the total energy of the amino acid plus the waters eventually detached from the surface and E_{CI} is the energy of the isolated counter ion; the calculated values are reported in the fourth row of Table 1. Except for the Asp case, the adsorption energy is still quite large being approximately E_{ads}=-1.3 eV for both Lys and Arg (with a Arg slightly favored over Lys by about 30 meV) indicating that the adsorption is stable for Lys and Arg even if the dynamical equilibrium of water adsorbed on the surface is considered. On the contrary, the Asp adsorption energy drops markedly if such a dynamical equilibrium is considered.

The role of water environment has been considered till now only in two aspects: the protonated/de-protonated states of the side-chain terminal groups and the hydration pattern of the (101) anatase surface. However in aqueous solution, the amino acid itself is hydrated; anyway due to the fact that the carboxyl and the amino groups should be involved in the formation of the peptide bonds and that the adhesion properties here addressed pertain the side chain, we are just interested in the way the side chains are hydrated in neutral water solution. We have limited the study of the fully hydrated amino acid side chain just to Arg and Lys that, up to the previous results, contribute significantly to the RKLPDA adhesion on the anatase surface. Therefore we have preliminarily studied the solvation of the two amino acids in water solution at the right density at RT by classical MD; the results indicate that the hydration shells of solvated Arg and Lys include 19 and 12 water molecules respectively that are up to 5 Å away from the last carbon atom in the terminal group of the side chain. In the Arg case there are five regions where the water molecules stay most frequently during the MD run: four near the -$C(NH_2)_2^+$ group and one lying along the NH bond just above the terminal group. In the Lys case there are five regions of the hydration shell close to the -$C(NH_3)^+$ terminal group where the molecules stay most frequently. Then the two amino acids including the relevant solvation shell with 19 (Arg) or 12 (Lys) water molecules, have been treated in the DFT context and the ground state configurations of the adsorbed and desorbed systems have been obtained.

Structural optimization by DFT total energy calculations of the previous configurations resulted in an adsorption energy of E_{ads}= -2.654 eV and E_{ads}= -2.953 eV. The large adsorption energy values measured are due to the formation of various hydrogen bonds chains of the solvated amino acids on the hydrated surface, and involving the protonated side chain, various water molecules, and the O^{2f} atoms on the surface. However the formation of such a network of hydrogen bonds is

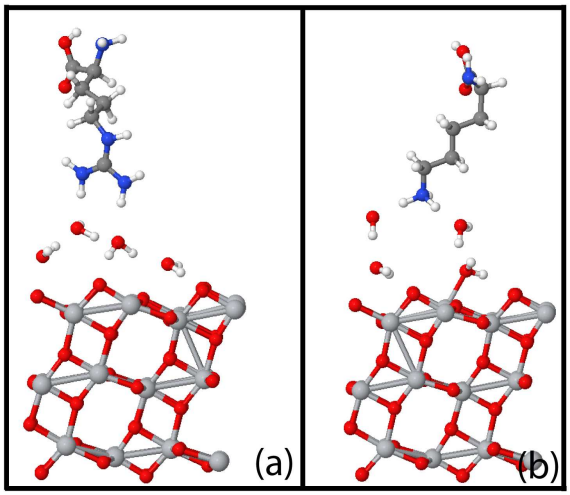

Fig. 3 (Color online) Ground state adsorption configurations of protonated and hydrated Arg (a) and Lys (b) on the hydrated (101) anatase TiO_2 surface. The hydration core shell of the amino acids have been reduced to include only two water molecules in order to avoid artificial hydrogen bond chains (see the text).

unlikely to occur in water solution at room temperature where the entropic terms should disrupt the hydrogen bonds chains involving more than two water molecules and thus we consider only the direct links between the surface water molecules and those ones closest to the protonated terminal group. Hence, the adsorption energy of the solvated amino acids on the hydrated surface have been revised considering only these direct links, two for both Arg and Lys (see Fig. 3), giving nearly equal values for Arg and Lys, respectively E_{ads}= -1.529 eV and E_{ads}= -1.524 eV (see Tab. 1).

It is worth noticing that for these configurations, both the (101) anatase surface and the amino acids are basically separated by two water layers. The adsorption energy data of the protonated and hydrated amino acids reveal that a considerable surface adhesion is still present and that the screening of the closest hydration shell reduces the adsorption energy of Arg to the same value as Lys that have the same charge states, thus showing that the presence of water makes the interaction studied basically of electrostatic nature.

The Arg adsorption has also been tested in the context of AIMD as specified above. The dynamical behavior observed confirmed that the hydration pattern here used, i.e. one water layer on the TiO_2 surface and one solvation water layer around the amino acid, catch properly the physics of the adsorption process. Indeed all the configurations observed throughout the entire AIMD were characterized by not more than two water molecules mediating the adsorption of the amino acid on the surface. Therefore the adsorption configurations we obtained with our hydration scheme was not affected by the presence of the additional water in the supercell. Moreover we have also verified that the lowest energy adsorption configurations obtained during the AIMD show the same distance between the amino acid and the surface as the one we have obtained in our static calculations confirming that our adsorption scheme is preserved also at room temperature in the time scale sampled (slightly more than 5 ps). Of course longer dynamical studies are desirable to check the validity of the above discussed picture for longer time scales where diffusion phenomena may occur.

Lastly the results here reported may be affected by the possible presence of surface or sub-surface defects such oxygen vacancies that may increase the adsorption energy of the water molecules in the hydration layer. On the basis of the above reported results, we may expect that, f.i, subsurface O vacancies may largely affect the O mediated amino-acid adsorption such as Arg and Lys because their adsorption proceeds through a partial water oxygen detachment from the surface. On the contrary the situation for Asp is not likely to change.

In summary the adsorption of protonated (Arg and Lys) and de-protonated (Asp) amino acids on both dry and hydrated (101) anatase TiO_2 surface has been studied using ab-initio calculations. On the dry surface the three amino acids have large adsorption energies, the protonated species being preferred over the Asp due to the stronger bonds involving Ti^{5f}. On the hydrated surface the adsorption energy values are slightly reduced but are still large to ensure the stability of the adsorption configuration. In this case the adsorption involves water molecules and the protonated species attach via a water-oxygen mediated adsorption while the Asp undergoes a weaker water-hydrogen mediated adsorption; Arg seems to show a larger affinity with respect to Lys at this stage but the the water screening of the protonated side chains reduces the adsorption energy of both Arg and Lys to the same value, evidencing that both of them may contribute at the same extent to the stable adhesion of the RKLPDA sequence.

Given these results further studies should focus on the Arg–Lys complex to clarify how the two protonated amino acids cooperate and why the substitution of one of them reduce the adhesion.

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Table 1 Adsorption energies E_{ads}(eV) of amino acids on the (101) anatase TiO_2 surface with and without water (first and second rows). Adsorption energies of the processes involving the desorption of surface waters (one for Lys and Arg, two for Asp) that mediate the adsorption on the (101) anatase TiO_2 surface according to to Eq. 2 and Eq. 3 are in the third and fourth rows respectively. Adsorption energy of partially solvated Lys and Arg in the fifth row (see text).

	LYS	ARG	ASP
dry (101)	-2.297	-2.362	-1.277
hydrated (101)	-1.703	-1.923	-0.427
Eq. 2	-2.151	-2.180	-1.690
Eq. 3	-1.296	-1.327	-0.233
solvated amino	-1.529	-1.524	

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