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Multiresolution non-covalent interaction analysis for ligand-protein promolecular electron density distributions

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Abstract

Nowadays, the topology of electron density (ED) distributions, as well as the non-covalent interaction (NCI) analysis of reduced density gradient (RDG) distributions, are extensively used to characterize intermolecular contacts. Here, topological and NCI-based analyses are combined for the multiresolution study of the intermolecular interactions occurring in a drug-protein system (PDB access code: 3UNK). The method involves the search for the critical points (CP) of the well-known PASA model developed by Carbó-Dorca and co-workers, and the minima in the corresponding RDG grids. The CP-based representation of the intermolecular contacts enriches the geometry-based interactions found using a Web server. The stability of the intermolecular interactions is studied by following their corresponding CP trajectory in space at several degrees of the ED smoothing, and by the evaluation of the local potential energy density (LPDE). It is observed that several RDG minima are located at the meeting point of CP trajectories initiated in the unsmoothed ED distribution. At high smoothing levels, the accumulation of ED charges at the ligand-protein interface progressively emerges and is detected through the study of the Laplacian values at the CPs and RDG minima. In parallel, the distance-dependency of the LPED values is less and less clear while the density-dependency is favored. The CP networks and their descriptors are seen as a signature of the ligand-protein arrangement, which is proposed to be further used in the characterization of ligand-protein stackings obtained from, e.g., crystal structures, docking calculations, Molecular Dynamics simulations, and pharmacophore designs.

Keywords

Promolecular electron density; Smoothing; Reduced density gradient; Critical points; CD2K-ligand interactions

1. Introduction

The Quantum Theory of Atoms-in-Molecules (QTAIM), first initiated by Bader and co-workers in the 70's to evaluate molecular properties [1], is one of the most disseminated analysis tools used to characterize molecular interactions from electron density (ED) distributions. In the framework of the QTAIM, a molecular system is defined as a set of atomic basins, each characterized by a maximum value in the ED distribution [2,3]. At the contact area of the basins that are associated with chemically bonded atoms, the ED distribution involves a saddle point, also named a bond critical point (BCP). The description of such BCPs, which often involves the analysis of their Laplacian values, is crucial for the identification of the interatomic interactions [4-6].

Besides quantum mechanical approaches, ED distributions can be conveniently obtained using promolecular descriptions. Within such ED models, all atoms are independent and are thus often considered as electrically neutral. Several studies have focused on the small differences between promolecular and quantum mechanical ED distribution functions [7-9]. Nevertheless, a precise knowledge of the redistribution of the ED that results from chemical bonding can yield information to distinguish specific interactions occurring, e.g., in crystal structures [10,11]. Saleh et al. reported that there is a good correspondence between weak non-covalent interactions (NCI) found in promolecular and experimental multipolar ED distributions, while larger differences may occur at the level of stronger interactions [12].

Topological analyses have also been applied to the treatment of experimental maps, through a multipolar description of the ED distribution [4,13]. Nevertheless, a recent work suggests that critical point (CP)-based ED analyses, when applied to experimental ED distributions, might not always compete with ED results obtained by quantum chemistry methods [14]. The author discusses the scientific domains that might benefit from a CP analysis of experimental ED maps. Macchi et al. also suggest that new challenges in the analyses and interpretation of experimental ED maps should involve a synergy between various approaches, as illustrated, e.g., in the design of drug molecules [15]. As an example, Rzęsokowska et al. used selective criteria such as the presence of a number of bonding NCI areas between ligands and the 5-HT_7 receptor [16].

The Bader's QTAIM has been applied to the analysis of intra- and inter-molecular interactions. More recently, the so-called NCI approach has complemented the Bader's method and showed that regions of space that are characterized by minimal values of the reduced

density gradient (RDG) distribution also depict interactions [9,17]. From RDG iso-contours, non-bonding and bonding regions of the 3D space can be visualized, as reviewed by Contreras-García and Yang [18]. Such regions do not necessarily coincide with QTAIM CPs, especially in the case of long-range interactions such as weak hydrogen bonds which are characterized by low ED and non-zero ED gradient values [19]. Nevertheless, as reported by Saleh et al., all CPs are surrounded by a low-RDG iso-surface and there is a value below which the RDG iso-surface is associated with only one CP [12]. The shape of such regions has also been observed to be related to the interaction strength [13].

CPs and NCI interaction analyses have been combined to provide a detailed picture of non-covalent interactions [20]. Intermolecular interactions, especially those occurring in crystal systems, are also often analyzed using the so-called Hirshfeld partitioning scheme, which consists in dividing space into regions characterized by high values of the ratio of the promolecular ED distribution over the procrystal ED distribution [21]. The 0.5 iso-contour of such a ratio distribution can be color-coded according to, e.g., the distance between the iso-surface and the nearest atom, or electrostatic potential values, to emphasize intermolecular interactions [22]. In addition, Pendás et al. showed that Hirshfeld surfaces are approximations of the QTAIM interatomic surfaces [23]. The NCI and Hirshfeld approaches provide a continuous description of the interactions from 3D scalar fields. Nevertheless, their discretization facilitates the description of the interaction networks.

In the frame of ligand-protein complexes, intermolecular interactions can easily be accessed through the use of Web servers which provide lists of atom pairs involved in ligand-protein interactions based on the 3D atom locations. In the present paper, we use the PLIP Web server [24] to help in the identification of ED BCPs. We also combine the CP and the NCI description levels to rationalize interactions in terms of discrete points and their evolution with the smoothing of the ED distribution function. The present work proposes, as a proof-of-concept, an original application of ED analysis methods to interpret ligand-protein interaction networks through a multiresolution approach.

In the next Section, the biological system selected as a test case is presented. Then the computational methods, used to smooth the PASA ED distribution function and to search for its CPs are described, as well as the approach to locate NCIs in an RDG distribution. The results are first reported at the atomic level, and then at larger smoothing degrees.

2. Materials and Methods

2.1 Biological System

The approach described in the paper is applied to a 729 non-hydrogen atoms inhibitor-CDK2 system (PDB access code: 3UNK [25]), which is known to involve, notably, two intermolecular hydrogen bonds (Hbond) and a salt bridge (Fig. 1a). It also involves a halogen bond as described in [26]. The ligand-protein interactions were probed and labeled using the Web server PLIP [24]. Thirteen so-called hydrophobic interactions, two Hbonds, one halogen bond, and one salt bridge were found (First column of Table 1 and Fig. 1b). Particularly, the halogen bond is formed between the ligand and Gln131 of the receptor, both Hbonds involve Leu83, and the salt bridge occurs with Lys89.

2.2 Smoothing of the electron density distribution

In the present work, the molecular ED distribution function $\rho_t(\boldsymbol{r})$ is calculated as a summation over independent atomic contributions $\rho_{a,t}(\boldsymbol{r}-\boldsymbol{R}_a)$ modeled using the PASA parameters [27]. The subscript *t* stands for the smoothing degree of the ED function, and takes the value of zero at the atomic description level. Gaussian expressions for the atomic ED distribution functions are particularly convenient as gradient and Laplacian values can be easily calculated. As an example, the Gaussian functions derived from the crystallographic Cromer-Mann structure factor parameters have previously been employed to build ED distributions of protein systems and to analyze substrate-enzyme interactions at the atomic level [28,29]. Working with promolecular ED representations limits the time required for the ED calculation. To further reduce the computation time, the ED is calculated using only the protein residues having at least one atom located at a distance ≤ 10 Å from the ligand. Moreover, only heavy atoms are considered in the calculations.

Smoothed ED distribution functions are seen as deformed version of $\rho_0(\boldsymbol{r})$, and are expressed as solutions of the diffusion equation according to the formalism presented by Kostrowicki et al. [30]:

$$\rho_{a,t}(\boldsymbol{r}-\boldsymbol{R}_a) = Z_a \sum_{i=1}^{5} \alpha_{a,i} \left(1+4\beta_{a,i}t\right)^{-3/2} e^{-\beta_{a,i}|\boldsymbol{r}-\boldsymbol{R}_a|^2/(1+4\beta_{a,i}t)} \quad (1)$$

where $\beta_{a,i} = 2\varsigma_{a,i}$ and $\alpha_{a,i} = w_{a,i}\left(\frac{\beta_{a,i}}{\pi}\right)^{3/2}$ (2)

where $\zeta_{a,i}$ and $w_{a,i}$ are the original PASA parameters [27]. In this context, the smoothing parameter t is seen as the product of a diffusion coefficient with time, and is also directly related to the overall atomic mean square displacement property used in crystallography [28].

2.3 Critical points of the electron density distribution

CPs are points where the gradient of the ED distribution is zero. Four kinds of CPs can be identified, based on the number of negative eigenvalues of their corresponding Hessian matrix (a matrix built on the local second derivatives of the ED). A peak or maximum in the ED is characterized by three negative eigenvalues ($\lambda 1 \leq \lambda 2 \leq \lambda 3$), written (-,-,-). A pass is defined by the set of signs (-,-,+), i.e., the second eigenvalue is negative too. It is located between two peaks, not necessarily covalently bound [31]. The last two kinds of CPs, the pales or ring CPs, and the pits or cage CPs, are described by the eigenvalue signs (-,+,+) and (+,+,+), respectively, i.e., the second eigenvalue is positive. As mentioned in the Introduction, the Laplacian $L = \sum_{i=1}^{3} \lambda_i$ that is associated with a CP is an important property in the characterization of the interactions. The sign $\lambda 2$ is also considered as an indicator of the bonding or non-bonding character of the CP [4].

Previously, we described an algorithm to locate the peaks in a molecular ED distribution function expressed as a summation over atomic Gaussian functions [28]. We have extended the approach to locate the other kinds of CPs using the iterative refinement relationship:

$$\boldsymbol{r}_{CP(\mathrm{n}+1)} = \boldsymbol{r}_{CP(\mathrm{n})} - \delta\, \boldsymbol{H}_n^{-1}\, \nabla\rho(\boldsymbol{r}) \qquad (3)$$

where $\boldsymbol{r}$ is the position vector of the refined CP, n is the iteration step, δ is the allowed displacement, and $\boldsymbol{H}_n$ is the Hessian matrix at step n. The algorithm works in two stages, as described hereafter.

Stage 1. Search for the CPs at t = 0 bohr^2. At scale $t = 0$, all atoms of a molecular structure are considered as the local maxima (peaks) of the promolecular ED distribution function. They are consequently used as the starting points of the search procedure described by Equation 3. Once all peak positions are obtained, the starting locations of the passes are set at the mid-distance points of all peak-peak pairs, and are then refined using Equation 3. Similarly, the starting locations of the pales are set at mid-distance points of all pass-pass pairs, and are then refined. It is followed by the refinement of the pit locations, which are initially set at mid-distances of all pale-pale pairs.

Stage 2. Search for the CPs at t > 0 bohr². Starting from the set of all refined CPs obtained at t = 0 bohr², and as t increases from 0 to a given maximal value, each CP is considered to continuously move along a path to reach a location in the three-dimension (3D) space where $\nabla\rho_t(\boldsymbol{r}) = 0$. On a practical point of view, it consists in following the trajectory of the CPs obtained at t on the ED distribution surface calculated at $t + \Delta t$. At each t, the refinement process expressed in Equation 3 stops when a lower limit $|\nabla\rho(\boldsymbol{r})| = 10^{-7}$ e/bohr⁴ is reached. Once all CP locations are found, close CPs are merged if their inter-distance is lower than the initial value of δ, set equal to 1.73 10^{-3} bohr. In the present work, only CPs located at distances between 0.8 and 3.0 Å from the ligand are studied to focus on the intermolecular interactions only. The progressive loss of details in the ED distribution of the drug-CD2K complex with t, as well as the simultaneous decrease in the number of CPs, are illustrated in the Online Resource 1 at various levels of smoothing.

2.4 Minima of the reduced density gradient distributions

In the NCI approach, the ED distribution is analyzed so as to identify extended regions of space where the RDG function $s(\boldsymbol{r})$ approaches zero [9,10,32]:

$$s(\boldsymbol{r}) = \frac{|\nabla\rho(\boldsymbol{r})|}{2(3\pi^2)^{1/3}\rho(\boldsymbol{r})^{4/3}} \quad (4)$$

Equation 4 shows that QTAIM CPs, where $|\nabla\rho(\boldsymbol{r})| = 0$, lead to values of zero for the function $s(\boldsymbol{r})$. Particularly, the passes and pales which are characterized by negative and positive second derivatives $\lambda 2$ of $\boldsymbol{H}$, point to bonding and non-bonding sites, respectively. More extended regions where such interactions also occur are characterized by low but non-zero RDG regions that surround QTAIM CPs [12]. Contreras-García and coworkers analyzed the link between QTAIM CPs and $s(\boldsymbol{r})$ minima of small molecules. They showed that $s(\boldsymbol{r})$ iso-surfaces can reveal steric interactions not detected as QTAIM CPS, and they illustrated the evolution of $s(\boldsymbol{r})$ along the potential energy curve of a Hbonded complex [33]. Later, mathematical relationships between the complex topology of $s(\boldsymbol{r})$ and the topology of $\rho(\boldsymbol{r})$ were detailed by Boto et al. [34].

The NCI are most commonly detected using the visualization of 2D $s(\boldsymbol{r})$-sign($\lambda 2)\rho$ plots, where they appear as spikes [9] (Fig. 2). The acronym "sign($\lambda 2)\rho$" stands for ρ multiplied by

the sign of $\lambda 2$. Three-dimension iso-contours of $s(\boldsymbol{r})$ can also be plotted to visually locate the corresponding NCIs [10].

To search for the RDG-based NCIs, we adopted a flood-filling type algorithm, which allows to numerically locate holes in a grid. A flood-filling type approach has previously been used to locate the CPs in ED overlap regions [35]. In practice, the RDG region that is submitted to such an algorithm is defined by two $s(\boldsymbol{r})$ cut-off values, a lower and upper limit rdg_{low} and rdg_{high}. We used a lower limit $rdg_{low} = 0$ while the upper limit rdg_{high} varies with the smoothing degree of the RDG. Additionally, the regions of space that are probed satisfy two distance cut-off values, d_{low} and d_{high}, defined between grid points and ligand atoms, beyond and below which holes will be searched for. These values are set to 1.35 and 2.50 Å, respectively (Table 2).

In the present work, RDG grids were built with a grid step of 0.1 Å. Prior to the hole search, the RDG grid point values are replaced either by values of 0 at locations satisfying all cut-off criteria (rdg_{low}, rdg_{high}, d_{low}, and d_{high}), or 1 otherwise. The flood-filling algorithm is a particular case of a depth-first search algorithm. Considering one grid point with a value "0" at a time, one looks for a neighboring grid point with the same value of "0". If found, the neighborhood of that point is examined, and the procedure is iteratively carried out until no more "0" grid points are found. In such a case, another grid point with a value "0" is selected, and the procedure is again applied. Each time a grid point is met, its value is replaced by "2", preventing it to be considered again as a member of a hole or as a starting point for a hole search. Once a hole is found, the grid point with the minimal RDG value is reported. To avoid duplicates with CPs, RDG minima below a distance of 0.5 Å from a CP are deleted.

Two distinct hole searches are carried out, one for the RDG grid where points are characterized by $\lambda 2 < 0$ and the other for the grid where $\lambda 2 \geq 0$. Indeed, the search for holes in a single grid, regardless of the sign of $\lambda 2$, does not always allow to identify all spikes in $s(\boldsymbol{r})$-sign$(\lambda 2)\rho$ plots. Fig. 2a and 2b show that a number of spikes occurring between sign$(\lambda 2)\rho = 0$ and 0.05 e/bohr^3 are not detected when the search is carried without differentiating RDG points according to their $\lambda 2$ sign.

The results of a flood-filling algorithm are strongly dependent on the cut-off values. The lowest rdg_{high} is, the larger the number of holes is likely to be found. Indeed, large holes are split in a number of smaller ones, and NCIs with high values of $s(\boldsymbol{r})$ are less likely to be found. Finally, the minimum values of $s(\boldsymbol{r})$ stay constrained to be located at grid points only.

While the current work focuses on the analysis of the $s(\boldsymbol{r})$ distribution function, it has been suggested that the square of $s(\boldsymbol{r})$ leads to more stable NCI descriptors. Indeed, regions of very low RDG values are characterized by $s^2(\boldsymbol{r})$ values close to zero (Online Resource 3). At $t = 0$ bohr^2, the local variation of $s^2(\boldsymbol{r})$ in the vicinity of an RDG minimum has a larger magnitude (Online Resource 3) while, at larger values of t, the function $s^2(\boldsymbol{r})$ is less and less corrugated. Particularly, at $t = 3.0$ bohr^2, the $s^2(\boldsymbol{r})$ fluctuations are of the order of 10^{-7} while $s(\boldsymbol{r})$ is still characterized by three shallow minima. It is thus expected that the use of $s^2(\boldsymbol{r})$ for the search of RDG minima might be less sensitive to the grid interval.

3. Results

Below, we present the results for a drug-CD2K complex system (PDB access code: 3UNK [25]) using the parameters reported in Table 2. Passes and pales are especially focused on as they depict bonding and non-bonding interactions. The identification of the CPs and RDG holes obtained at the atomic level ($t = 0$ bohr^2) is achieved through a comparison with the interactions identified using the Web server PLIP [24]. Then, the evolution of the CP network and the RDG holes with t is discussed in terms of interaction stability.

3.1 At the atomic level ($t = 0.0$ bohr^2)

The PLIP results, as well as the number of passes detected using the merging/clustering algorithm obtained for the system 3UNK are presented in Table 1 and Online Resource 4. The Online Resource 5 illustrates the distribution of the passes, pales, and RDG holes around the ligand. As observed from the Online Resource 4, all Laplacian values L of the ligand-protein intermolecular passes are positive, which characterize NCIs, as reviewed by Koritsanszky and Coppens [4]. Almost all passes lie on a straight line joining two atoms, as shown by the ligand-pass-protein angle values around 170 to 180° (Online Resource 4). All but one of the 13 so-called hydrophobic interactions discovered by the program PLIP show a correspondence with a CP. Contrarily, the CG(Leu134)-C14 PLIP hydrophobic interaction is detected only as an RDG hole (Online Resource 4). Among the passes associated with a PLIP interaction, pass #18 involves the atom OD1 of Asp145, rather than CB as reported in the PLIP results. It is characterized by values of ρ and L, equal to 0.0131 e/bohr^3 and to 0.0749 e/bohr^5, respectively, which are greatly larger than the other values obtained for the so-called hydrophobic interactions. It is explained by the short ligand-protein distance of 2.79 Å (Online Resource 4

and Fig. 3). Indeed, the CP properties depend strongly on the overlap of the atomic ED distributions, and thus on the interatomic distances. Fig. 3 illustrates the correlation that occurs between L and the distance between the ligand and protein atoms associated with the passes. Here, the distance is calculated as the sum over the pass-ligand and pass-protein separation lengths. Finally, one pass (pass #16) corresponds to two PLIP hydrophobic contacts, occurring at the level of the atom pairs C18-CE2(Phe80) and C19-CD2(Phe80) (Fig. 4). The pass is surrounded by an RDG iso-contour that is consistently characterized by negative $\lambda 2$ values.

Several CD2K residues detected by PLIP as closely interacting with the ligand form a more complex network than first expected by the geometry-based Web server, as confirmed by the presence of stacking passes that cannot be associated with any PLIP interaction (Table 1). Particularly, Ile10 and Leu134 are surrounded by three passes while PLIP detects only two hydrophobic interactions. In Phe82, a stacking interaction complements the hydrophobic PLIP interaction, and slight differences occur at the level of Lys33 where two contacts are detected, both as passes and with PLIP. However, the two methods assign different (but spatially close) atoms to the interactions, i.e., the PLIP and CP-based interaction patterns involve the atom pairs CB-C14 and CD-C19, and CB-C18 and CE-C19, respectively. Thus, geometry-based interactions can slightly differ from ED topology-based methods due to the presence of the molecular environment. In addition to the protein residues identified by PLIP, several other residues are also detected as interacting with the ligand, such as Glu81, His84, Asp86, Lys89, and Asn132. As an example, the chlorine atom that forms a halogen bond with Gln131 is also involved in two stacking interactions, with Asn132 and Leu134 (Table 1).

All intermolecular passes which are not associated with a PLIP interaction are characterized by low values of $s(\boldsymbol{r})$, $|\lambda 2|$, and L, which illustrate the weak character of such ligand-protein contacts. The ED values range from 0.0006 to 0.0043 e/bohr^3 (Online Resource 4). Contrarily, both Hbond passes are characterized by larger values of $\rho(\boldsymbol{r})$, $s(\boldsymbol{r})$, $|\lambda 2|$, and L. Particularly, their values $\lambda 2$ are the most negative ones, -0.0100 and -0.0138 e/bohr^5. The salt bridge has high $s(\boldsymbol{r})$ and $|\lambda 2|$ values, while the halogen bond is associated only with a moderate L value of 0.0305 e/bohr^5 (Fig. 3 and Online Resource 4).

Sixteen intermolecular RDG holes were detected by the flood-filling algorithm (Online Resource 4). Since the corresponding value of $s(\boldsymbol{r})$ can be rather high, some of them are actually not associated with spikes in the 2D $s(\boldsymbol{r})$-sign($\lambda 2$)ρ plots (Fig. 2). Eight of those points are characterized by negative values of $\lambda 2$, and are thus considered as bonding NCIs. In terms of their numbers, bonding and non-bonding NCIs almost contribute equally to the ligand-protein

binding. As already mentioned, the PLIP contact CG(Leu134)-C14 which was left undetected as a CP, actually corresponds to an RDG hole (hole #4 in Online Resource 4). The RDG hole corresponding to the atom pair O(His84)-C07 (hole #9) is seen as an approximation of pass #9 from which it is separated by a distance of 0.51 Å only.

As for the non-bonding RDG holes, the pales, which are characterized by positive values of $\lambda 2$, were also investigated. Thirty-nine intermolecular pales were found (Online Resource 4) and are identified in terms of the closest ligand and protein atoms. They are all characterized by a low ED value, below 10^{-4} e/bohr^3, and $\lambda 2$ values below 0.0068 e/bohr^5 compared to, e.g., the intramolecular pales of the ligand rings with $\lambda 2 > 0.08$ e/bohr^5 (Online Resource 4). They allow the building of a pass-pale network that defines interaction rifts between the ligand and the protein (Fig. 5), wherein the high node density regions characterize the interaction pattern of the system.

The local energy associated with Hbonds has often been carried out from the values of ρ and L at the corresponding BCPs. It has indeed been shown that the local kinetic energy density $G(\boldsymbol{r})$ at a pass can be approximated by [36]:

$$G(\boldsymbol{r}) = \frac{3}{10}(3\pi^2)^{\frac{2}{3}}\rho(\boldsymbol{r})^{\frac{5}{3}} + \frac{1}{6}\nabla^2\rho(\boldsymbol{r}) \quad (5)$$

while the local expression of the virial theorem relates $G(\boldsymbol{r})$ and the local potential energy density (LPED) $V(\boldsymbol{r})$ to the Laplacian of the ED by:

$$2G(\boldsymbol{r}) + V(\boldsymbol{r}) = \frac{1}{4}\nabla^2\rho(\boldsymbol{r}) \quad (6)$$

Espinosa et al. used Equations 5 and 6 to evaluate the LPED values of a set of Hbond BCPs [37]. Later, Contreras-García et al. applied the formalism to Hbonds in small molecules and studied the influence of the exponent of ρ, in Equation 4, on the binding energy curves [33]. Spackman however reported on the low reliability of intermolecular interaction energy values evaluated from the set of BCPs in experimental ED distributions [38]. Nevertheless, the author confirmed the clear relationship that exists between $V(\boldsymbol{r})$ at the BCP and the atom-atom separation length. Thus, we have tentatively calculated $V(\boldsymbol{r})$ at the ligand-protein passes and pales to visualize trends in the intermolecular interaction strength. Fig. 6a shows that the LPED value of the passes converges to zero at long distances due to the zeroing of all parameters such

as the ED, the gradient, and the Laplacian. All pales yield LPED values close to zero, consistently with their non-bonding character. An exponential relationship can be fitted, $V = -2.79\ e^{-2.09d}$ ($R^2 = 0.96$), which emphasizes the strong influence of the ligand-protein distance. The four passes occurring at short distances, below 2.9 Å, and at LPED values below -0.03 e/bohr^5, originate from the two Hbonds (passes #2 and #5), the salt bridge (pass #21), and one short-range stacking contact (pass #18) which corresponds to an interaction site located between the chloro-phenyl moiety and the side chain of Asp145. The halogen bond is characterized by a LPED value of -0.0029 a.u. Ayoub et al. showed that the strength of a Hbond is linearly dependent on the density value at the BCP [39]. In the present calculations, the plot of $V(\boldsymbol{r})$ as a function of the density value at the BCPs obtained at $t = 0.0$ bohr^2 suggests that a part of the LPED variability can also be explained by a linear relationship, i.e., $V = -0.77\ \rho + 0.00$ ($R^2 = 0.97$) (Fig. 6b).

RDG holes can be spatially close, as illustrated by the bonding and the non-bonding ones (holes #1 and #10), that are close to the strong hydrophobic interaction site (Fig. 4 and Online Resource 4). While the corresponding pass (pass #18) is located between atoms C20 and OD1(Asp145) separated by a distance of 2.8 Å, the RDG holes occur at a position slightly displaced towards the atom pair C19-OD2(Asp145) with a separation length of 4.1 Å. The corresponding two-colored RDG iso-contour indicates a balance between stabilizing and destabilizing interactions at such a separation distance value [40].

3.2 Below the atomic level ($t > 0.0$ bohr^2)

Below the atomic level, the passes are less numerous (Table 2) and their location with respect to the closest ligand and protein atoms deviates from a straight line (Online Resources 4 and 6-9). The passes obtained at $t = 0.75$ bohr^2 do not differ very much from those observed at the atomic level (Table 1), while there is a drastic increase in the number of RDG holes observed at the selected cut-off level rdg_{high}. A tentative application of Equations 5 and 6 to calculate the LPED value of the CPs at $t = 0.75$ bohr^2 shows a behavior similar to the atomic level one, i.e., passes occurring at the shortest ligand-protein distances are associated with the most negative LPED values (Online Resource 10). In comparison with $t = 0.0$ bohr^2, the five most negative LPED values are now all associated with the expected strongest interactions, i.e., both Hbonds, the salt bridge, and the short-range hydrophobic chlorophenyl-Asp145 interaction. The halogen bond is now among the five most stable interaction sites (Table 3 and Online Resource 10).

All five most stable interaction sites actually appear as passes up to at least $t = 1.5$ bohr^2 (Table 1). At such a smoothing degree, the pass-pale network running along the ligand still occurs, as illustrated in Fig. 5 which displays the networks at $t = 0$ and 1.5 bohr^2. Similarly to intramolecular peaks studied previously, where t around 1.5 bohr^2 appeared to be a suitable smoothing factor to generate reduced molecular representations while retaining an adequate amount of structural information [28,41], the intermolecular passes obtained at the same smoothing value yield essential information about the ligand-protein interaction sites.

At $t = 3.0$ bohr^2, the Hbond characterized by the longest donor-acceptor distance is not detected any longer. The LPED ordering of the passes is changed, with the halogen bond being more stable than the salt bridge (Table 3 and Online Resource 10). Some stacking interactions are now seen as more stable than the salt bridge. The order obtained at $t = 3.0$ bohr^2 is the same as $t = 4.5$ bohr^2. As illustrated in Online Resource 10, the influence of the distance between the ligand and the protein atoms on the LPED is less and less clear as t increases, while the density-dependency of $V(\boldsymbol{r})$ is well retained. Indeed, at $t = 4.5$ bohr^2, the corresponding linear relationship is $V = -1.44\ \rho + 0.03$ ($R^2 = 1.00$).

Fig. 7a illustrates that eight passes observed at the atomic level undergo a displacement following a trajectory in the 3D space so that a correspondence can be found with the passes observed at $t = 4.5$ bohr^2 (Table 1). All those passes are seen as locators of stable interaction sites for the ligand within the protein structure, i.e., five stacking contacts, the N01-containing Hbond, the salt bridge, and the halogen bond. The location of the passes #2, #4, and #8 at $t = 4.5$ bohr^2, all associated with stacking interactions, is only slightly moving between 0 and 4.5 bohr^2 (Table 1). They all correspond to interaction sites between the protein and the chlorophenyl moiety, which is buried in the active site of the protein structure.

Besides the continuous pass trajectories depicted above, a second kind of behavior of the CP trajectories is observed. Particularly, two trajectories originating from a pass and from a pale merge at some value of t and then lead to an RDG hole. A specific example, involving pass #19, is displayed in Fig. 7a. However, the merge of a pass and pale trajectory does not necessarily lead to the creation of an RDG hole, as illustrated by the pathway of pass #10. Multiple trajectory meeting points can also be observed, such as the successive merge of an RDG hole and a pale trajectory, followed by the merge of the same pale trajectory with a pass trajectory (pass #13 in Fig. 7b). Finally, some RDG hole trajectories are seen as being independent on CP trajectories as observed in locations I, II, and III in Fig. 7a. On the whole, a great variability is found regarding the location of the RDG holes with the smoothing factor

t. They are present at all values of *t* and are distributed all around the ligand. At the highest values of *t*, 3.0 and 4.5 bohr^2, their number is strongly limited. One obtains similar numbers of bonding and non-bonding sites at all *t*, except at 0.75 bohr^2 where the non-bonding sites are more numerous (Table 2).

The pale Laplacian values are all positive at any value of *t* (Online Resources 4 and 6-9). Contrarily, some passes are characterized by negative values at $t = 1.5$ bohr^2 and higher, which indicates a charge concentration in the ED distribution (Fig. 8). At $t = 1.5$ bohr^2, the *L*-negative passes are those associated with the N01-Hbond, the short-range hydrophobic contact, and the salt bridge (Online Resource 7), i.e., three interactions that are classified among the strongest ones, as also observed at $t = 3.0$ bohr^2. At $t = 3.0$ bohr^2, only two non-PLIP stacking passes and one PLIP hydrophobic interaction are still characterized by positive values of *L* (Online Resource 8). Eventually, at $t = 4.5$ bohr^2, all pass *L* values are negative (Online Resource 9). It indicates a progressive accumulation of charges at the interface of the ligand and the protein, which is consistent with the more and more pronounced gap in the ED values, observed around $\text{sign}(\lambda 2)\rho = 0$ (Online Resource 11). At $t = 3.0$ bohr^2, three pairs of close RDG minima characterizing holes with negative and positive $\lambda 2$ are observed (Online Resource 8). The presence of such pairs is correlated with the pass-pale merge pattern illustrated in Fig. 7c for pass #19. At the RDG level of description, the comparison between the $s(\boldsymbol{r})$-$\text{sign}(\lambda 2)\rho$ plots shows that the gap in the $s(\boldsymbol{r})$-$\text{sign}(\lambda 2)\rho$ plots separating the bonding and non-bonding regions is more pronounced when *t* increases and the RDG values decreases (Fig. 2 and Online Resource 11). Indeed, while the ED values diminish as *t* increases, ED values close to zero are absent at the ligand-protein interface.

While the system considered above does not include any H atoms, an additional calculation was carried out for the drug-protein system containing polar H atoms. The presence of such atoms on the two ligand-Leu83 Hbonds yields slight differences in the CP locations at low values of *t* (Online Resource 12). Indeed, the superimposition of the CPs locations obtained for the H-free and H-containing systems shows that, at $t = 0$ bohr^2, the passes and pales are separated by a distance of 0.2 to 0.3 Å. Contrarily, starting at $t = 0.3$ bohr^2, the CP trajectories remain spatially very close. With and without polar H atoms, a first pass trajectory ends up at $t = 4.4$ and 4.1 bohr^2, respectively, while the second pass and the pale trajectories terminate at $t = 1.7$ bohr^2. The topology of the RDG distribution is affected in a more complex manner by the presence of H atoms. In the H-free system, a single bonding RDG hole appears at a central location between the two Hbonds, at $t = 4.2$ bohr^2 and beyond. With H atoms, the RDG hole is

extended towards the Leu83 moiety, and a second bonding hole appears at $t = 4.5$ bohr^2 in the vicinity of the ligand. Working without H atoms appears to smooth slightly faster the topology of the ED as t increases. It also simplifies the topology of the RDG distribution. Additionally, the properties of the CPs of the H-free system are characterized by slightly lower absolute values of ρ, λ_2, L, and LPED.

4. Conclusions

The interactions between a drug molecule and its protein receptor (PDB access code: 3UNK) were investigated using a critical point (CP) and a non-covalent interaction (NCI) analyses of the Promolecular Atomic Shell Approximation (PASA) of the electron density (ED) distribution function. The CPs, especially the passes (bond CPs) and pales (ring CPs), were located using the analytical expression of the PASA ED distribution function, while the NCI sites were identified as holes in reduced density gradient (RDG) grids. The study was carried out at several levels of ED smoothing, t. The interactions identified through a geometry-based Web server were used for the purpose of the CPs identification.

At the atomic level, i.e., $t = 0$ bohr^2, the CP analysis covers the set of interaction sites found by the geometry-based approach, i.e., the hydrogen bonds (Hbond), the halogen bond, the salt bridge, and all but one so-called hydrophobic interactions. The method completes the geometry-based analysis, yielding a set of additional stacking contacts. The set of passes and pales form an interaction network between the ligand and the protein, with dense node regions around specific ligand moieties such as aromatic groups and the halogen atom. The RDG holes complete the CP network as they focus on regions of space where weaker interactions occur. One of them is associated with the geometry-based hydrophobic interaction that did not correspond to a CP. An approximate evaluation of the local potential energy density (LPED) at the CPs shows that specific interactions like Hbonds, halogen bond, salt bridge, and short-range hydrophobic contacts are characterized by low LPED values. Additionally, all Laplacian values at the CPs are positive, which indicates that the CP sites correspond to charge-depleted regions of space.

Consistently with the increase of t, the numbers of CPs and RDG holes decrease. At $t = 1.5$ bohr^2, the dense node regions initially observed at $t = 0$ bohr^2 tend to be reduced to single node regions, thus simplifying the interaction network while preserving its description of the main interaction sites. Several low-LPED sites, i.e., a short-range Hbond, the halogen bond,

the short hydrophobic contact, and the salt bridge, are preserved up to the selected smoothing level of t = 4.5 bohr^2. At that level of smoothing, the LPED values are strongly linearly correlated with the pass density values. Besides the strong interaction sites, most of the weak sites are characterized by the merge of a pass and pale trajectories as t increases, to eventually yield RDG holes. The Laplacian values that are associated with the passes more and more often adopt negative values, first at the level of the strongest interactions and, at larger smoothing degrees, for all interaction sites.

Despite the lack of ED redistribution due to the presence of chemical bonds, the CPs and NCIs of a H-free promolecular ED allow to catch the main interaction sites between a ligand and its protein receptor due to the 3D packing. While the NCI approach spans large regions of the protein-ligand interface and visually allows to discriminate between bonding and non-bonding regions, a reduction of the RDG iso-surfaces to discrete points and their descriptors more clearly rationalize the ligand-protein interactions. However, it remains dependent on the grid point interval, and further analyses of modified RDG distributions, such as the square of the RDG distribution, will be considered.

The approach described in the paper, which is based on the single knowledge of the 3D structure and the promolecular ED parameters, is expected to be useful for the comparison of ligand-protein interaction sites and networks, e.g., in the evaluation of predicted or experimental ligand poses towards a given receptor. It allows to identify the strong interaction sites between the molecular partners. Although the smoothing of the ED can be seen as a method to mimic the overall atom fluctuations, a dynamics description of the CPs and NCIs might also be investigated from Molecular Dynamics trajectories. It will allow to determine how the descriptors of the interaction sites are sensitive to protein site fluctuations.

Electronic supplementary material

PASA ED contours at t = 0.75, 1.5, 3.0, and 4.5 bohr^2 (ESM_1).
List of RDG holes (Figure 2) which correspond to QTAIM CPs (ESM_2).
Comparison of ρ, $s(\boldsymbol{r})$, and $s^2(\boldsymbol{r})$ one-dimension profiles (ESM_3).
Numerical details regarding the passes, pales, and RDG holes at t = 0 (ESM_4), t = 0.75 (ESM_6), t = 1.5 (ESM_7), t = 3.0 (ESM_8), and t = 4.5 bohr^2 (ESM_9).
Three-dimension distribution of the passes, pales, and RDG holes at t = 0.0 bohr^2 (ESM_5).

Distribution of the LPED as a function of the distances between the passes and the molecular structure, and as a function of the pass density value at t = 0.75, 1.5, 3.0, and 4.5 bohr^2 (ESM_10).

Plots of $s(\boldsymbol{r})$-$sign(\lambda 2)\rho$ and 3D distribution of the CPs and RDG holes at t = 0.75, 1.5, 3.0, and 4.5 bohr^2 (ESM_11).

Comparison of Hbond-related CP trajectories focused on the drug-Leu83 system with and without consideration of polar H atoms (ESM_12).

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Figure Captions

Fig. 1 (a) 2D structure of 4-({4-[(2-chlorophenyl)amino]pyrimidin-2-yl}amino)benzoic acid (PDB ID: 0BY, PDB access code: 3UNK) (**b)** PLIP intermolecular interactions between the ligand 0BY and the protein CD2K (Hbonds = *, salt bridge = **, halogen bond = ***, hydrophobic interactions = black lines). The non-PLIP ligand-protein interactions detected as passes are in shown using transparent gray lines. Residues of CD2K involved in the PLIP and non-PLIP interactions are represented with black and transparent sticks, respectively

Fig. 2 s(**r**)-sign($\lambda 2$,ρ) plots for the system 3UNK. Only data points corresponding to locations beyond 1.35 Å and below 2.50 Å from the ligand atoms are used (light gray crosses). Analysis at t = 0.0 bohr^2 of **(a)** a single RDG grid and **(b)** two RDG grids, with $\lambda 2 < 0$ and $\lambda 2 \geq 0$. RDG holes after removal of points close to CPs are shown with black dots. **(c)** RDG holes at t = 3.0 bohr^2 before (circles) and after (black dots) removal of points close to CPs. The labeled spikes also correspond to QTAIM CPs, as detailed in Online Resource 2

Fig. 3 Laplacian-distance plot corresponding to the intermolecular passes of the system 3UNK obtained at t = 0.0 bohr^2: Passes corresponding to PLIP interactions (plain circles), passes not associated with a PLIP interaction (open circles), Hbonds (+), halogen bond (triangle), salt bridge (square). The distance axis corresponds to the sum over the pass-ligand and pass-protein distances

Fig. 4 Superimposition of the protein CD2K, the ligand 0BY, two passes, and the RDG iso-contour level = 0.8 obtained using the merging/clustering algorithm at t = 0.0 bohr^2. Two RDG hole minima are shown with small spheres ($\lambda 2 > 0$ for RDG hole #10) and ($\lambda 2 < 0$ for RDG hole #1)

Fig. 5 Superimposition of the protein CD2K, the ligand 0BY (black sticks), and the pass-pale networks at t = 0 (black lines), and t = 1.5 (gray lines) bohr^2 (passes are shown with spheres). Distance cut-offs of 2.5 and 3 Å are used to connect the CPs at t = 0 and 1.5 bohr^2, respectively. Selected ligand atoms are shown with labeled spheres

Fig. 6 Local potential energy density V(**r**) associated with the passes (black dots) and pales (gray crosses) as a function of **(a)** the distance and **(b)** the BCP density for the system 3UNK at t = 0.0 bohr^2. The distance axis corresponds to the sum over the pass-ligand and pass-protein distances. Selected passes are labelled according to their PLIP identification

Fig. 7 (a) Superimposition of the ligand of the system 3UNK (black sticks), the intermolecular passes (small gray spheres) and pales (small orange spheres) from t = 0 to 4.5 bohr^2. Passes at t = 0.0 (red), passes at = 4.5 bohr^2 (blue), pales at t = 0.0 (white), pales et t = 4.5 bohr^2 (black) are shown. Numbers identifies passes at t = 0.0 (red) and 4.5 bohr^2 (blue) **(b)** Meeting of an RDG and a pale trajectories at t = 1.5 bohr^2, then with a pass trajectory at t = 3.5 bohr^2. The arrows point to the meeting locations **(c)** Meeting of the trajectory of pass #19 superimposed with the paired RDG hole minima #1 ($\lambda 2 < 0$ yellow sphere) and #5 ($\lambda 2 > 0$ green sphere) and the 0.03 RDG iso-contour at t = 3.0 bohr^2. The RDG iso-contour level = 0.03 is colored using $\lambda 2$ values of -0.01 e/bohr^5 (red) to 0.01 e/bohr^5 (blue). The reader is referred to the online version of the paper for the colored version of this figure

Fig. 8 Distribution of the Laplacian values of the PASA passes at various values of t for the 0BY-CD2K system (PDB access code: 3UNK)