Enhancing Drug Residence Time by Shielding of Intra-Protein Hydrogen Bonds: A Case Study on CCR2 Antagonists

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Supporting Information

ABSTRACT: The target residence time (RT) for a given ligand is one of the important parameters that have to be optimized during drug design. It is well established that shielding the receptor–ligand hydrogen bond (H-bond) interactions from water has been one of the factors in increasing ligand RT. Building on this foundation, here we report that shielding an intra-protein H-bond, which confers rigidity to the binding pocket and which is not directly involved in drug–receptor interactions, can strongly influence RT for CCR2 antagonists. Based on our recently solved CCR2 structure with **MK-0812** and molecular dynamics (MD) simulations, we show that the RT for this and structurally related ligands is directly dependent on the shielding of the Tyr120–Glu291 H-bond from the water. If solvated this H-bond is often broken, making the binding pocket flexible and leading to shorter RT.

KEYWORDS: CCR2, GPCR, water networks, drug residence time, buried hydrogen bonds

Over the last couple of years, the importance of the chemokine receptor family for various disease conditions of the immune system became apparent.¹ Belonging to this family, the CC chemokine receptor 2 (CCR2) has been investigated thoroughly as it is responsible for monocyte and macrophage attraction.² This is triggered by activation through its cognate ligand CCL2, being a key player in inflammatory response.² The CCR2/CCL2 axis has been implied in various disease conditions such as diseases directly caused by immune cell infiltration, for instance psoriasis,³ rheumatoid arthritis,⁴ and atherosclerosis.⁵ Furthermore, crucial roles of CCR2 in neurodegeneration, metabolic diseases, pain perception, and cancer have been described.^{6,7} This has made this subfamily of G-protein coupled receptors (GPCRs) apparently ideal drug targets and moved them into the focus of pharmaceutical research. For many chemokine receptors highly potent ligands have been identified so far, and two small molecule inhibitors for CCR5 and CXCR4 even made it to market.⁸ Not surprisingly, quite a number of clinical trials have been conducted on CCR2 antagonists;^{7,9} however, so far no drug has made it to market. Thus, the quest for improved CCR2 antagonists is ongoing, which is also reflected in the number of patents on novel CCR2 ligands every year. A patent search reveals that since 2005, on average 16 new documents relating to CCR2 are published every year (according to a search in Clarivates Integrity database <https://integrity.clarivate.com/>).

One of the reasons for not being able to find efficacious drugs for CCR2 may lie in the high redundancy of the chemokine receptor/chemokine system per se,¹ requiring

polypharmacological inhibitors.^{7,9} Another explanation is that present antagonists do not show the required profile for a sustained blockage of the CCR2 receptor to yield clinical efficacy.⁷ One of the examples of failed drugs against CCR2 is **MK-0812** (shown in Figure 1B), which has been tested in phase II for the treatment of relapsing-remitting multiple sclerosis (clinicaltrials.gov, identifier NCT00239655). The failure of **MK-0812** has been attributed to its inability to cause sustained receptor occupancy. Recent reports from the Heitman group^{11,12} show that, although being of sub-nM potency, the receptor residence time (RT) of **MK-0812** is only 1.5 h, which is not ideal for such a drug. Their thorough structure–activity relationships (SAR), as well as structure kinetics relationships (SKR) studies on this structural class, resulted in **15a** (shown in Figure 1B), which is less potent compared to **MK-0812** but has a much longer receptor RT of 11.9 h. Furthermore, these investigations also revealed that minor changes in the structures, such as the omission of a halogen atom do not change the K_i but cause a substantial change in RT (e.g., **8** with a RT of 0.4 h).^{11,10} Figure 1A shows the corresponding simplified energy profiles for the three compounds and reveals that the free energy barrier of the transition state of **15a** is nearly ~2 kcal/mol higher as compared to the other two compounds.

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Figure 1. (A) Thermodynamic and kinetic parameters of MK-0812, 8, and 15a (Vilums et al.,¹⁰) as well as a simplified energy barrier defining the free energy differences of the ligand bound state ΔG_{BS} as well as the free energy differences of the transition state ΔG_{TS} . (B) Structures of the molecules under investigation (X = N for MK-0812 and X = C for 8 and 15a) as well as quantification of the free energy differences in comparison to MK-0812.

The receptor RT has become a parameter of increasing importance in drug design.^{13–17} Contrary to establishing SAR and optimizing pharmacokinetics (PK) of a compound, deriving SKR is more subtle and not straightforward.^{18,19} If it comes to computational prediction of RT, quite often very complex molecular mechanisms come into play; some of the recent studies shed light on this phenomenon by molecular dynamics (MD) methods.^{19–21} The involvement of water molecules in binding and unbinding events has been found by various groups to be of uttermost importance.^{20–25} Schmidtke et al.²³ have thoroughly investigated the role of water in drug/receptor dissociation in a very illustrative way on model systems. They found that buried H-bonds between receptor and ligand, if shielded from water, can contribute to long RT. Furthermore, they showed that shielding can be achieved in a deeply buried pocket or in a highly curved protein surrounding where the H-bond is not accessible by water due to steric constraints by performing MD studies on different shapes of the acetonitrile receptor.²³ Pearlstein et al.²⁵ investigated the role of water in the binding dynamics of p38 α MAP kinase inhibitors and identified the rate-limiting step in ligand dissociation to be the transfer of water from bulk solvent to the inhibitor binding site. In a different approach, Krimmer et al.²⁶ investigated the role of surface water networks and their influence on ligand binding strength as well as dissociation rates for thermolysin inhibitors. They found that optimal surface water network geometry leads to higher affinity and to slower off-rates.

Sparked by this body of recent work and based on our previous work in the field^{15,21,27} we set out to investigate the currently unknown causality behind the difference in the RT of 15a and MK-0812 on the CCR2 receptor. Recently, we solved the structure of MK-0812 bound CCR2A,²⁸ and this lays the basis for the explanation of reported SAR and is prerequisite for computationally investigating the SKR. If it comes to the explanation of SKR, static X-ray structures on their own are not sufficient as the association/dissociation kinetics strongly depend on the dynamic features of the receptor. Therefore, we employed unbiased microsecond long MD simulations and focused our analysis on the difference in the water networks for the bound ligands to investigate differences in SKR.

To identify differences in the molecular interactions of MK-0812 and 15a with CCR2, we begin with performing unbiased 1 μ s long MD simulations with 5 replicates for each system. For CCR2/MK-0812, the recently solved crystal structure with few edits (see method section) was used as the starting point. Due to the high structural similarity of MK-0812 and 15a, the latter could be manually docked into the receptor based on the X-ray structure (Figure 2A). These structures were then placed in the hydrated POPC membrane bilayer.

Analysis of water densities around the ligands in the binding pocket of CCR2 revealed one major difference for MK-0812 and 15a. As shown in Figure 2B, the difference lies in extra water density near Tyr120^{3,32}, which is H-bonded to

Figure 2. (A) Overlay of ligands (simulation snapshot). MK-0812 (in red), 15a (in yellow), and 8 (in blue) in CCR2 binding pocket. (B) Overlay of water density mesh of MK-0812 (red) and 15a (yellow); the “extra-water” density region for MK-0812 is shown in the green circle. (C) Overlay of water density mesh of MK-0812 (red) and 8 (blue); the “extra-water” density is shown in the green circle. (D) Water network in the protein binding site for MK-0812 and (E) for 15a.

Glu291^{7,39}, which in turn forms the key ionic interaction with the ligands.²⁸

Apart from this single difference in water density for **MK-0812** and **15a**, the water density in other areas is similar. Thus, we focused our attention on the region with differences in the water density for further investigation. The case we have at hand is somewhat puzzling and unique in a way that, in previously reported studies where the relationship between long RT and accessibility of water has been established, water molecules have been in direct contact with protein–ligand H-bonds.^{23,29} However, in our case the extra water density is detected near the residue Tyr120 which is not directly involved in ligand–protein binding.

Tyr120 forms a H-bond with Glu291,²⁸ and this bond can potentially be part of a water-involving H-bond network. Thus, we focused our attention on water interference with the Tyr120–Glu291 interaction and whether it can affect the Glu291–ligand interaction. Throughout the entire simulation, the salt-bridge between Glu291 and the positively charged amine group of the ligands is never broken for both **MK-0812** and **15a** (Supporting Information Figure 1); however, for the Glu291–Tyr120 H-bond this does not hold true. As shown in Figure 3B this H-bond is maintained throughout the

A Tyr120–Glu291 hydrogen bond for **MK-0812**

Figure 3. H-bond interactions in the CCR2 binding pocket. (A) Schematic showing broken H-bond between Glu291–Tyr120 due to insertion of water molecules in between for **MK-0812**. Water molecule highlighted by the green circle denotes the extra water density region identified for **MK-0812**. (B) H-bond between Glu291–Tyr120 vs time for **MK-0812**, **15a**, and **8**.

simulation time only for **15a** but not for **MK-0812**. For **MK-0812** this H-bond is disrupted repeatedly and autocorrelation analysis for this bond make-and-break is calculated to be ~ 10.4 ns (Figure 3B). A closer inspection revealed that the disruption of this H-bond (Tyr120–Glu291) happens in the course of the insertion of a bridging water molecule as shown in Figure 3A. Root mean-square-fluctuation (RMSF) analysis between the two compounds revealed that Tyr120 is flexible in the case of bound **MK-0812** in contrast to **15a** (See Figure 4A and 4B). This is due to the fact that water insertion displaces Tyr120

Figure 4. Tyr120–Glu291 interactions: (A) Schematic representation of Glu291 and Tyr120 heavy atoms showing conformational space explored by these residues from 1 μ s long MD simulations. (B) RMSF showing the flexibility of heavy atoms of Glu291 and Tyr120 for **MK-0812**, **15a**, and **8**.

away from Glu291 and makes Tyr120 to assume alternate conformations. In contrast, the H-bonded Tyr–Glu dyad is permanently maintained throughout the simulation for **15a**. Although as shown in Figure 2B and 2C, some water is always present near the Tyr120–Glu291 H-bond for both of the ligands, it is able to disrupt that H-bond only in the case of **MK-0812** (Figure 2D) bound CCR2, because of the formation of a dynamic water network due to extra water present near Tyr120.

These findings bring us to postulate the hypothesis that extra water density near Tyr120 as shown in Figure 3A (highlighted in the green circle) is responsible for the short RT of **MK-0812**, by disrupting the rigidity of the binding pocket and keeping it flexible. In order to test this hypothesis with further simulations, we pick an additional ligand from the study by Vilums et al.¹⁰ with the following criteria: (1) the chemical structure should be very similar to **15a**, (2) affinity should be similar to **15a**, and (3) RT should be much shorter than for **15a**, if possible closer to the RT of **MK-0812**. Compound **8** (see Figure 1) from their data set was chosen, as it fulfills all of the above-mentioned criteria and the only change in structure is the deletion of the Br in **15a**. The RT of **8** (21 min) is more than 30-fold shorter than that of **15a** (714 min), although the K_i is more or less identical. We then performed similar simulations and analysis for **8** as done before for the other ligands and compared the water densities in the binding pocket with **15a** and **MK-0812**. Due to the severe drop in RT for **8**, we expected a similar water density pattern as for **MK-0812**

because we postulated a similar underlying mechanism causing a faster off-rate. Closer inspection of water networks in the binding site around **15a** and **8** revealed a significant difference because the additional -Br group of **15a** disrupts the continuous water network which is present for **MK-0812** as shown in Figure 2D and 2E. It is interesting to note that the ability of the Br group in **15a** to disrupt the water network is due to its van der Waals volume rather than its polarizability. Modification of the Br substituent in **15a** to the equally sized CF₃ group retains the long residence time of the ligand, although the electronic features of the substituent are massively altered.¹⁰ The detailed comparison of all three ligands in the CCR2 binding site is shown in Supporting Information section 2.

Comparing water densities in the binding site for **MK-0812** and **8**, as shown in Figure 2C, the water density overlays in perfect fashion, implying an identical water network around these two ligands despite their distinct structural differences. We then looked at the other signatures identified from the previous analysis, which were the stability of the H-bond between Glu291 and Tyr120 and the flexibility of the mentioned residues. As seen from Figure 3B (lower panel) for **8** bound CCR2, the H-bond is repeatedly disrupted throughout the simulation, with an autocorrelation time of ~11 ns for bond make-and-break. Similar to the case of **MK-0812**, the disruption is complemented by insertion of bridging water between Tyr120 and Glu291. RMSF analysis reveals also for **8** that Tyr120 is displaced away from the Glu291 and is more flexible like **MK-0812** (Figure 4B). These findings support our hypothesis that extra water density around Tyr120 is one of the key factors responsible for the short RT. We then performed a set of biased simulations, where the partial charges on the water molecules present near Tyr120, for **MK-0812** and **8** were set to zero so that they cannot form H-bonds (See Supporting Information section 3 for details). In these simulations, the Tyr120-Glu291 H-bond was not disrupted for the whole of the simulation time, thus supporting the finding that these “extra-waters” are responsible for imparting flexibility in the binding site.

The relationship between the kinetic stability of drug target complexes and shielded H-bonds from water has been comprehensively established in the previous studies.^{21–23,29} However, in all of them, water molecules responsible for shorter RT of ligands were directly interfering with protein–ligand interactions. Contrary to that, in this specific case of CCR2, we find that shielding of water from intra-protein interactions which confer the rigidity of the binding site is also an important factor in ligand binding kinetics. These observations also point toward a difference in the enthalpy and entropy in the binding of the discussed ligands. Recently the influence of ligand-dependent protein entropy changes on binding kinetics has been discussed for HSP90.³⁰ In this case, the authors observe ligand-dependent changes in secondary structure of the protein and correlate the entropy changes to dissociation rates. In our case, unfortunately, no isothermal titration calorimetry data for CCR2 ligands are available. Therefore, we have to speculate that the differences in binding site rigidity upon ligand binding lead to differences in the binding entropy and the equipotency of **15a** and **8** is due to entropy–enthalpy compensation.

In summary, our MD simulations reveal that the relatively short RT of **MK-0812** on CCR2 as compared to that of **15a** is due to extra water density around Tyr120. Tyr120 is present in

the binding pocket of CCR2, forming a H-bond to Glu291, but it does not directly interact with the ligands. This H-bond interaction can, in turn, be disrupted by insertion of water molecules present around Tyr120, which happens in the case of **MK-0812**. This finding was then confirmed by choosing another ligand (**8**) which has a short RT like **MK-0812** and is of similar structure as **15a**. However, it shows the same water density characteristic as **MK-0812**, especially we observe the extra water entity close to Tyr120. We find that the binding pocket residues, Tyr120 and Glu291, are flexible in the case for **MK-0812** and **8** binding as compared to **15a**. To conclude, we here show that an intra-protein H-bond, which is not directly involved in ligand binding, can nevertheless be modulated by ligand binding. If the ligand distorts the highly dynamic network, a rigidification of the binding pocket occurs and this causes significantly slower off-rates. In general, this opens a new opportunity for medicinal chemists to optimize RT for drugs, if such dynamic water networks can be identified. Until now, the shielding of ligand-protein interactions was known as design strategy; through this example on CCR2, we are extending this strategy to all H-bonds in the binding site, be it protein–ligand or protein–protein.

■ ASSOCIATED CONTENT

Supporting Information

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Methods and additional figures (PDF)

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Author Contributions

The study was designed by G.S., A.-K.A., and C.T., modeling, simulations, and analysis were performed by A.M., C.T., and D.S. The manuscript was written by A.M. and C.T. with help from G.S. and D.S.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; RT, residence time; PK, pharmacokinetics; MD, molecular dynamics; RMSF, root mean-square-fluctuation; GPCR, G-protein coupled receptor; POPC, 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine

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