INSPECTOR POCKET

An open source software to detect protein binding sites
Analyses of examples

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Analyses of examples

In this analysis, examples of binding sites identified by Inspector Pocket, dedicated to detecting protein binding sites, will be explored. Understanding these binding sites is crucial for various applications in drug discovery, protein engineering, or molecular biology.

To demonstrate the capabilities of our software, we will examine three different protein structures representing diverse biological functions and structural characteristics. Each example will highlight the features and intricacies of binding sites identified by Inspector Pocket. The identified pockets will be compared with previously reported binding sites of the selected structures. All examples can be found inside the file Examples_for_analysis in InspectorPocket github repository [1].

1. Protein Kinase A (PKA):

Protein Kinase A, commonly referred to as PKA, plays a pivotal role in cellular signaling pathways, regulating essential cellular processes. Structurally, PKA comprises multiple domains, with a catalytic domain being central to its function in phosphorylation.

One of the critical features of PKA is its binding sites, which are strategically located within its structure. These binding sites facilitate interactions with crucial molecules such as ATP and substrate peptides. ATP, the primary energy carrier in cells, binds to PKA to provide the necessary phosphate groups for phosphorylation reactions. Substrate peptides, on the other hand, are targeted by PKA for phosphorylation, thereby regulating their activity and function within cellular signaling networks. These binding interactions are fundamental for orchestrating PKA-mediated cellular responses and modulating downstream signaling cascades essential for cellular homeostasis. [2]

To perform this analysis, the crystal structure of human cAMP-dependent protein kinase A (catalytic alpha subunit) was used (PDB ID: 4WB5). Six different pockets were detected by InspectorPocket (Figure 1A), spread within the enzyme structure. The main pocket (Figure 1B), number one, covers all the space occupied by the original ligand of the structure: adenosine triphosphate (ATP) .

Regarding the other pockets found in the analysis of Protein Kinase A (PKA), besides Pocket 1, five additional pockets were identified. These pockets represent potential

binding sites on PKA, each with its unique set of residues. As depicted in Figure 1A, the positioning of these pockets suggests locations suitable for accommodating ligands.

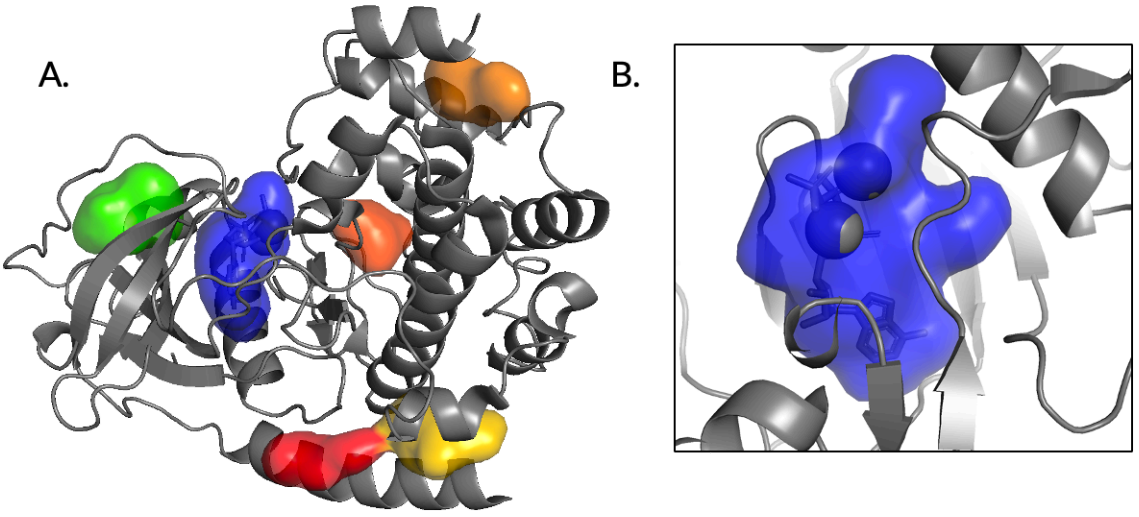

Figure 1. *Structure of human cAMP-dependent protein kinase A (catalytic alpha subunit) painted in gray (PDB ID: 4WB5). (A) The pockets detected by Inspector Pocket represented each in a different color as surfaces. (B) Pocket number one is represented as a blue surface. It can be seen how this pocket encompasses the entire volume occupied by the native ligand of the structure, ATP.*

2. Beta-2 Adrenergic Receptor (β2AR):

The Beta-2 Adrenergic Receptor (β2AR), a significant member of the G protein-coupled receptor (GPCR) family, holds paramount importance in mediating cellular responses to various ligands, notably adrenaline. Structurally, β2AR exhibits a distinctive seven-transmembrane helical bundle architecture, which serves as a scaffold for ligand recognition and subsequent signal transduction.

The binding sites on the β2AR exhibit a complex molecular architecture that facilitates the recognition and interaction with a diverse array of ligands. These binding sites are primarily situated within the transmembrane domain of the receptor, comprising specific amino acid residues that form complementary binding pockets for ligand engagement. [3]

To perform the analysis for the β2AR, Human B2-adrenergic G protein-coupled receptor (PDB ID: 2RH1) was used. Six different pockets were detected by InspectorPocket (Figure 3A). Five out of six predicted pockets were positioned within the transmembrane domain of the protein structure. Note that pocket number one (Figure 3B) covers all the space occupied by the inverse agonist Carazolol. [4] Moreover, pocket number five corresponds to the binding site occupied by alpha-D-glucopyranose-(1-4)-alpha-D-glucopyranose (Figure 3C). The other potential binding sites detected seem to be positioned in cavities where other ligands have been

reported in other GPCRs. For instance, between pocket number one and four (painted in blue and dark orange respectively), eticlopride (a selective dopamine antagonist) has been reported to be bound in human dopamine D3 receptor. [5]

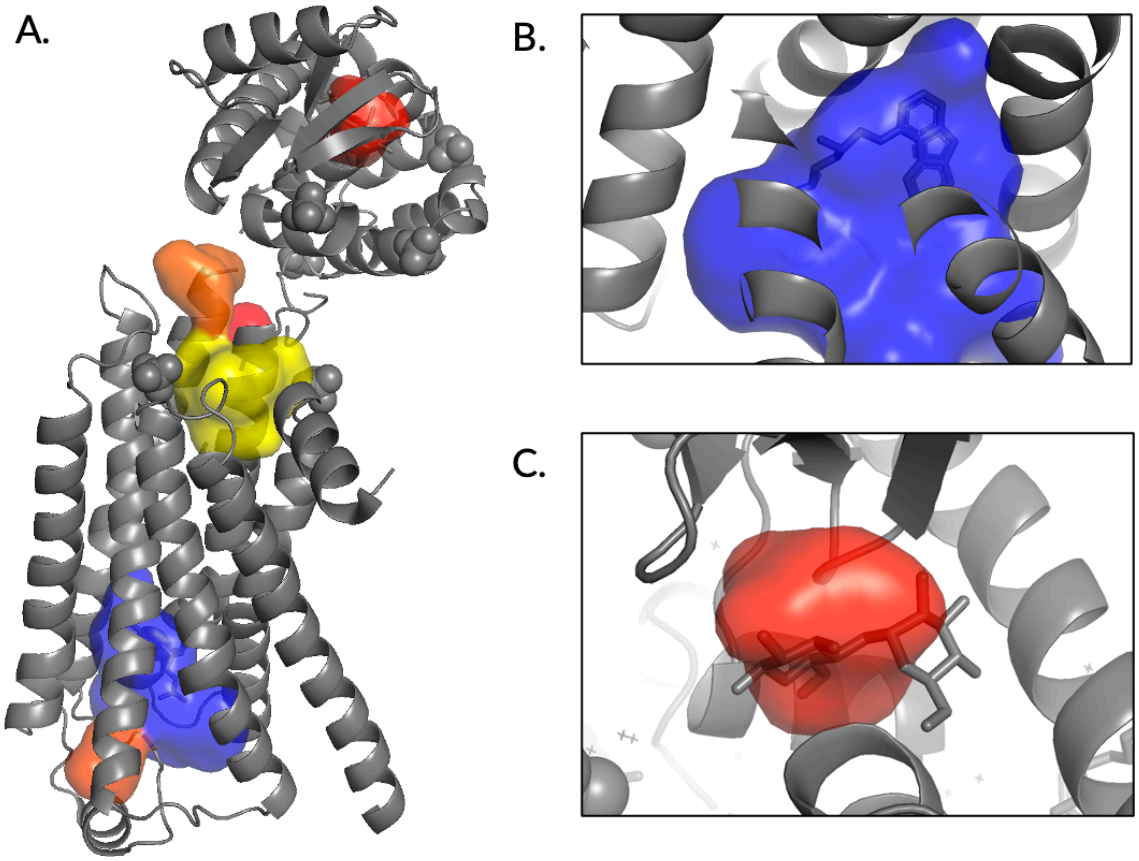

Figure 2. Human B2-adrenergic G protein-coupled receptor (PDB ID: 2RH1) depicted in gray. (A) All pockets found by InspectorPocket are represented as surfaces. Note the majority concentrate in the seven helix bundle. (B) Pocket number 1 is represented as a blue surface; this observation illustrates how the pocket spans the entirety of the space previously occupied by Carazolol. (C)Pocket number five is associated with the binding site where alpha-D-glucopyranose-(1-4)-alpha-D-glucopyranose is situated.

3. Hemoglobin:

Hemoglobin is a vital protein found in red blood cells, responsible for transporting oxygen from the lungs to tissues throughout the body. Its structure consists of four subunits, each containing a heme group that binds oxygen molecules.

The structure of hemoglobin features a globular shape, allowing it to efficiently carry oxygen through the bloodstream. Each subunit of hemoglobin contains a unique binding site for oxygen, located within the heme group. This binding site consists of an iron ion coordinated by a porphyrin ring, which binds to oxygen molecules reversibly.

The binding sites on hemoglobin are critical for its physiological function, as they enable the protein to bind and release oxygen in response to changes in oxygen concentration. This allows hemoglobin to effectively transport oxygen from the lungs to tissues where it is needed for cellular respiration. [6]

To perform the analysis for hemoglobin, the structure of deoxy human hemoglobin (PDB ID: 1A3N) has been used. Nine different pockets were detected by InspectorPocket. The output pockets 2-5 predicted by InspectorPocket align with the known binding sites of hemoglobin (Figure 3A). The output pocket number 1 could also be interesting to be commented on. It corresponds to a sizable pocket (Figure 3B) identified within the tetramer's core, which is likely a consequence of the inter-monomer void. This insight prompts consideration for future refinements within InspectorPocket to preclude the prediction of binding sites across interchain regions. Four extra pockets spread in the structure have also been predicted as putative binding sites.

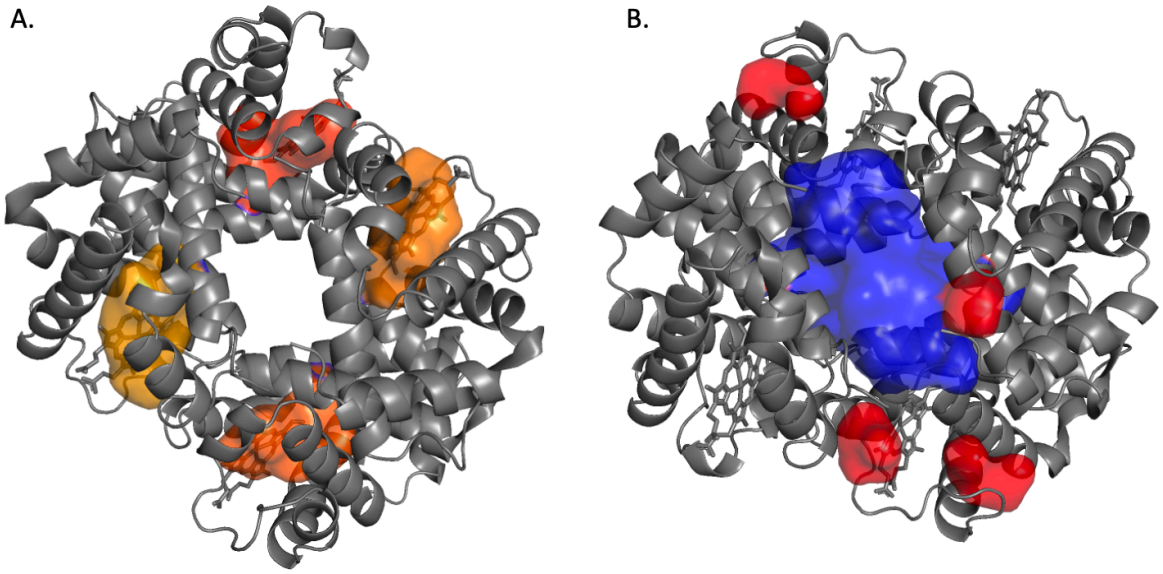

Figure 3. Deoxy human hemoglobin structure tetramer bound to 4 heme groups shown in gray (PDB ID: 1A3N). (A) The four pockets observed in the image in surface representation correspond to the binding sites of the heme groups in the tetramer, all of them have been identified by Inspector Pocket. (B) Additional found pockets by InspectorPocket. The prominent blue pocket located at the center of the tetramer may potentially arise from the presence of a void between the four monomers.

The list of residues predicted to be involved in each pockets are defined as pocket_report.txt in each example inside the Examples_for_analysis in InspectorPocket github repository.

REFERENCES

[1] https://github.com/Jordi-mp/InspectorPocket.git

[2] Morrison H. Chapter 29 - Protein kinase A. In: Morrison H, editor. Enzyme Active Sites and their Reaction Mechanisms. Academic Press; 2021. p. 173-178. ISBN 9780128210673. Available from: https://doi.org/10.1016/B978-0-12-821067-3.00029-5.

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[4] Vadim Cherezov et al.,High-Resolution Crystal Structure of an Engineered Human β2-Adrenergic G Protein–Coupled Receptor. Science 318,1258-1265(2007).DOI:10.1126/science.1150577.

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