SUPPLEMENTARY MATERIAL TO

In silico studies of phycobilins as potential candidates for inhibitors of viral proteins associated with COVID-19

VESNA B. JOVANOVIĆ[1], MILAN R. NIKOLIĆ[1] and SRĐAN Đ. STOJANOVIĆ[2]*

[1]*University of Belgrade, Faculty of Chemistry, Department of Biochemistry and Centre of Excellence for Molecular Food Sciences, Belgrade, Serbia and* [2]*University of Belgrade, Institute of Chemistry, Technology and Metallurgy – National institute of the Republic of Serbia, Department of Chemistry, Belgrade, Serbia*

HYDROGEN BONDS

Classical[1]

Conventional hydrogen bond interactions between a hydrogen bond donor atom and an acceptor atom can exist. Atoms of element types N, O, P, and S are considered as classical hydrogen bond donor atoms, and hydrogen atoms are considered as hydrogen bond donors if connected to such atoms. In this case, the heavy atom is no longer considered as a hydrogen bond donor. Atoms of element types N, O, P, and S are also hydrogen bond acceptor atoms if at least one electron lone pair is present (for example, NH3 but not NH4$^+$). However, an sp^2-hybridized N atom with at least one electron lone pair is not considered a hydrogen bond acceptor atom if it is connected by a single bond to another sp^2-hybridized atom. Additionally, atoms of element type F, Cl, Br, and I are also considered hydrogen bond acceptor atoms.

If both atoms are N or O, the distance criterion d between the heavy donor and acceptor atoms is 3.4 Å by default (*Strong H-bond D(H)...A (max dist)*). Otherwise the distance criterion is 3.8 Å (*Weak H-bond D(H)...A (max dist)*). If there is an explicit hydrogen atom, the distance dh between the hydrogen atom and the acceptor atom must be less than the specified distance criterion -0.7 Å (that is, 2.7 A or 3.1 Å). See Fig. 1-S and Fig. 2-S.

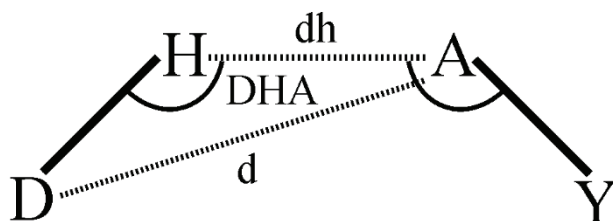* Corresponding author. E-mail: srdjanst@chem.bg.ac.rs

Fig. 1-S. Hydrogen bond when an explicit hydrogen exists. The angles and distances used to determine if an interaction exists are shown.
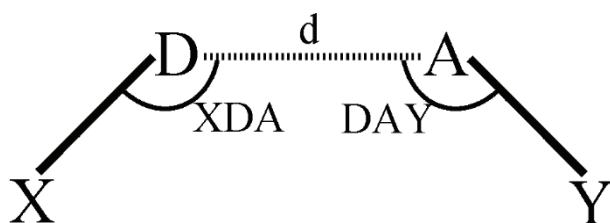

Fig. 2-S. Hydrogen bond when an explicit hydrogen does not exist. The angles and distances used to determine if an interaction exists are shown.

By default, angle constraints are checked to determine whether a hydrogen bond is present. For a general hydrogen bond X-D-H...A-Y, where D is the hydrogen bond donor heavy atom, H is the explicit donor hydrogen if present, A is the hydrogen bond acceptor atom, X is any non-hydrogen atom attached to the donor atom, and Y is any atom attached to the acceptor atom. The angles that can be constrained are X-D-A, D-H-A, D-A-Y, and H-A-Y. These must all lie within the minimum (90° by default) and maximum (180° by default) values of the following angle parameters: *Hydrogen-bond X-D-A angle*, *Hydrogen-bond D-H-A angle*, *Hydrogen-bond D-A-Y angle*, and *Hydrogen-bond H-A-Y angle*.

In the case of an sp hybridized donor atom, its hydrogen atom will be colinear with the D-X bond, so any interaction should lie closer to this vector. Therefore, a stricter minimum angle constraint *Hydrogen Bond with Sp donor X-DA* (135° by default) is applied.

Non Classical[1]

Carbon hydrogen bond interactions are considered weaker hydrogen bonds where the donor is a polarized carbon atom. A carbon atom is considered to be a donor if it is either in an acetylene group or if it is adjacent to an oxygen or nitrogen atom.

These interactions are determined using the same geometric criteria used for classical hydrogen bonds with the exception of the default distance criterion being 3.8 Å (*Weak H-bond D(H)...A (max dist)*).

Pi-Donor hydrogen bond interactions are hydrogen bonds that occur between hydrogen bond donor atoms and a Pi ring that functions as a hydrogen bond acceptor. The following tests are performed to find these interactions:

1. Hydrogen bond donor atoms are found.

2. C-H donors are excluded because they form Pi-Sigma interactions.

3. The distance between each donor heavy atom and the centroid of each Pi ring is tested to find those within the *Pi-Donor (max dist)* cutoff (4.2 Å by default).

4. The angle between the donor heavy atom-centroid vector and the normal to the ring plane should be less than the *Pi-Donor* angle (40° by default).

5. The positions of each of the donor's explicit or implicit hydrogen atoms are found. A heavy-H-centroid angle must exist that deviates from linearity by no more than the *Pi-Donor deviation* angle (40.0° by default).

Water[1]

The Water monitor is useful for identifying bridging water molecules (when a water molecule is hydrogen bonded to two different molecules such as a protein and a ligand or two domains of a protein) and visualize water networks. After all hydrogen bonds have been determined, the bonds that bridge chains are classified as *Water Mediated Hydrogen Bonds* and colored mid-blue. Other hydrogen bonds involving at least one water molecule are classified as *Water Hydrogen Bonds* and colored light-blue.

Salt Bridge[1]

Salt Bridge interactions are relatively strong non-bonded interactions between pairs of oppositely charged groups where hydrogen bonding also occurs. These interactions can be observed at a longer range than uncharged hydrogen bonds.Interactions are classified as salt bridges for pairs of atoms closer than 4.0 Å (*Salt bridge D(H)...A (max dist)*), where one atom is positively charged, one is negatively charged, and there is a hydrogen bond between them.

ELECTROSTATIC

Charge[2]

Attractive Charge interactions exist between atoms bearing opposite whole or fractional formal charges that are within the *Charge-charge (max dist)* cutoff (5.6 Å by default).

Salt Bridge interactions are also listed in the Electrostatic and Hydrogen bond categories since they can be considered members of both.

Pi-Charge[3,4]

Pi-Cation interactions can exist between a positively charged atom and the electrons of a delocalized Pi system. The following tests are performed to find them:

1. Cations are considered to be atoms that have a formal charge of at least +0.5. This allows the inclusion of delocalized cationic species such as lysine and arginine side chains.

2. The distance between a cation and the centroid of a Pi ring should be less than the *Pi-Cation (max dist)* cutoff (5.0 Å by default, see R in Fig. 3-S).

3. The angle between the cation-centroid vector and the normal to the ring plane should less than the *Pi-Cation* maximum angle (40° by default, see θ in Fig. 3-S).

Pi-Anion interactions are calculated similarly to *Pi-Cation* interactions, but only atoms with charges of -0.5 or less are considered.
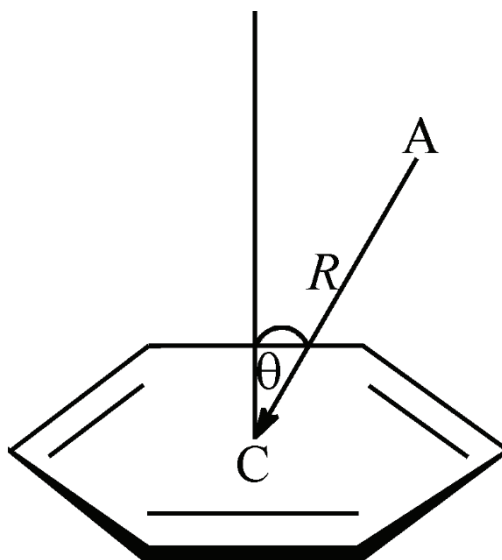

Fig. 3-S. Example of a Pi-atom interaction (for example, Pi-cation, Pi-anion, and so on). The important measurements are the angle θ and the distance R.

HYDROPHOBIC

Pi-Hydrophobic[5,6]

Pi rings are defined as planar ring systems composed of sp^2 hybridized atoms. They include (but are not confined to) aromatic rings.

Pi-Pi stacked interactions are determined following the methodology of McGaughey et al.[5] Stacked and staggered Pi-Pi interactions are determined by the following tests:

1. The distance between the centroid of each Pi ring pair is determined to find those which fall within the cutoff distance (*Pi-Pi centroid (max dist)*, 6 Å by default, see Rcen, Fig. 4-S).

2. For these interactions, an atom from each ring must be within the (*Pi-Pi closest atom (max dist)*) cutoff, 4.5 Å by default, see Rclo, Fig. 4-S).

3. The angle θ between the normal of one or both rings and the centroid-centroid vector must be between 0° and ± the *Stacked Pi-Pi theta* angle cutoff (50° by default), and the angle γ between the normal to each ring must be between 0° and ± the *Stacked Pi-Pi gamma* cutoff angle (35° by default).

Pi-Pi T-shaped interactions occur if the following conditions are met:

1. The distance between the centroid of each pair of Pi rings is determined to find those which fall within the *Pi-Pi centroid (max dist)* cutoff distance (6 Å by default).

2. An atom from each ring should be within the *Pi-Pi closest atom (max dist)* cutoff (4.5 Å by default).

The angle ω between the normal of one or both rings and the centroid-centroid vector must be between 0° and ± the *T-Shaped Pi-Pi theta* angle cutoff (30° by default), and the angle γ between the normal to each ring must be within the *T-Shaped Pi-Pi gamma* angle cutoff (55° by default) or greater.
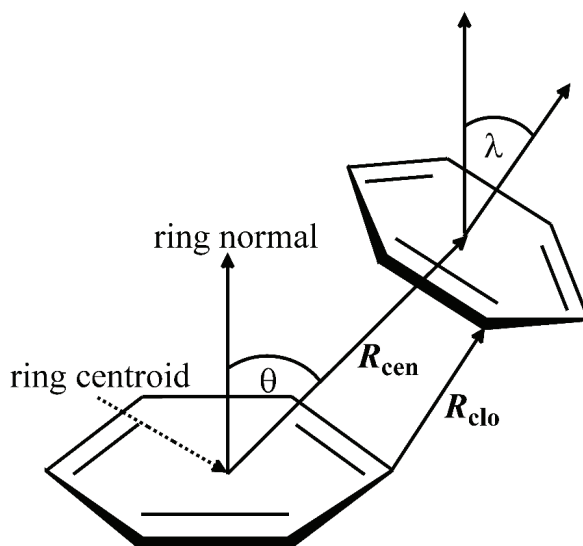

Fig. 4-S. Example of a Pi-Pi interaction. The distances and angles needed for an interaction are labeled.

Amide-Pi Stacked interactions occur between an amide group and a Pi ring if the following criteria are met:

1. The distance between the centroid of the amide group and the Pi rings falls within the *Pi-Pi centroid (max dist)* (6 Å by default).

2. An atom from each group should be within the *Pi-Pi closest atom (max dist)* (4.5 Å by default).

The angle ω between the normal of one or both groups and the centroid-centroid vector must be between 0° and ± the *Stacked Pi-Amide theta* angle cutoff distance (40° by default), and the angle γ between the normal to each must be between 0° and ± the *Stacked Pi-Amide gamma* angle cutoff (20° by default).

Alkyl Hydrophobic[2]

Alkyl groups are defined as the following non-polarized, non-Pi systems:

Predominantly aliphatic amino acid side-chains: These include Alanine, Valine, Leucine, Isoleucine, Methionine, Selenomethionine, Cysteine, Proline, atoms CB, CG, and CD of Lysine, and atoms CB and CG of Arginine.

Hydrophobic groups on ligands are conyiguous sets of atoms that are not adjacent to concentrations of charge (charged atoms or electronegative atoms). A group of atoms is considered hydrophobic if its surface area is equal or greater than the area of a methyl group multiplied by the *Surface area scale factor* (default 0.65), which corresponds to the surface area of a chlorine atom.

The criteria for this type of interaction are met when if the groups' centroids are within the *Alkyl centroid (max dist)* cutoff (5.5 Å by default) and they have at least one pair of atoms within the same *Pi-Pi closest atom (max dist)* cutoff used for Pi-Pi interactions.

Mixed Pi/Alkyl Hydrophobic[7]

Pi-Sigma interactions (sometimes referred to as CH-π interactions) are weak interactions between a hydrogen and a Pi ring system. An interaction of this type must meet the following conditions:

1. The hydrogens acting as the donor can be implicit or explicit hydrogens. They must be connected to a nonaromatic carbon atom.

2. The distances between the hydrogen and the center of the Pi ring must be within the *Pi-Sigma (max dist)* cutoff (4.1 Å by default, see R in Fig. 5-S).

3. The C-H-centroid angle cannot deviate from linearity by more than the *Pi-Sigma deviation* angle (20° by default, see δ in Fig. 5-S) and the angle between the C-centroid vector and the normal to the ring plane is not more than the *Pi-Sigma* angle (45° by default, see θ in Fig. 5-S).

Pi-Alkyl interactions exist where the centroids of a Pi ring and an alkyl group are within the *Alkyl centroid (max dist)* cutoff (5.5 Å by default) and they

have at least one pair of atoms within the same *Pi-Pi closest atom (max dist)* cutoff as used for Pi-Pi interactions.
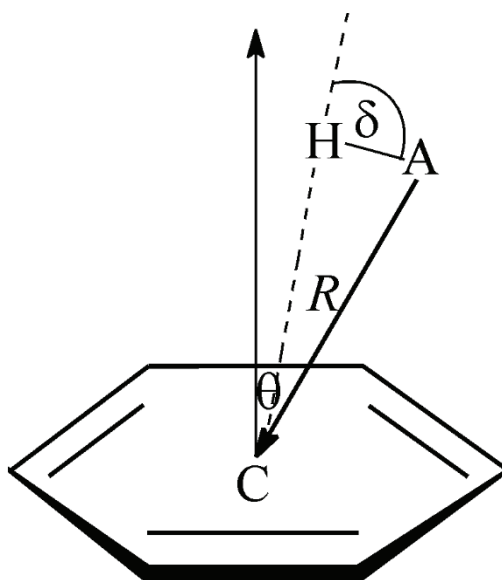

Fig. 5-S. Illustration of a Pi-Sigma interaction.

HALOGEN

Fluorine[1,8]

Halogen (Fluorine) interactions are carbon-bound halogen interactions (C-X...B-Y) with structural significance to weak hydrogen bonds. Interactions with carbon-bound fluorine are monitored and identified separately from those involving Cl, Br, and I. Additionally, halogen interactions (including F) with hydrogen donors are identified separately.

Fluorine interactions (C-F...B-Y) are identified and monitored with all hydrogen donors and the specific case where B is carbon, nitrogen, and oxygen. In all cases, a maximum distance criterion (*Fluorine non-bond (max dist)* is 3.7 Å by default) is used. When the interaction is with a hydrogen donor, the hydrogen bond angle criteria are used and the interaction is identified as both a fluorine and a hydrogen bond interaction.

Fluorine interactions with carbon and oxygen are limited to C=O moieties. Nitrogen interactions are limited to nucleophilic nitrogen. These interactions are also limited by the same maximum distance criterion (3.7 Å by default). However, no angle constraints are applied.

Cl, Br, I[1,8]

Halogen (Cl, Br, I) interactions have distance criteria defined as a fraction of the sum of the atoms' van der Waals radii (controlled by *Halogen (Cl, Br, I) VDW fraction (max)*). The default fraction is 1, using the full van der Waals distance as the cutoff. As with fluorine interactions with a hydrogen donor, halogen interactions with hydrogen donors use the hydrogen bond angle criteria to identify interactions.

Non-fluorine halogen interactions to carbon and oxygen are also limited to C=O moieties. Additionally, interactions with (B-Y) N-C, N-S, N-P, S-C, S-S and S-P are considered. A separate set of angle criteria are used in these cases (*Halogen (Cl, Br, I) bond X-B-Y angle* with a default between 75° and 180° and *Halogen (Cl, Br, I) bond C-X-B angle* with a default between 120° and 180°).

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