**Supporting Material**

# Insights into Molecular Recognition of Lewis$^X$ Mimics by DC-SIGN Using NMR and Molecular Modelling

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**Table S1**. Chemical shifts (500 MHz, 25 ºC, HDO residual at 4.7 ppm) for **3** and **4**.

| Mimic 3 | | Mimic 4 | |
|---|---|---|---|
| Protons | δ $^1$H (ppm) | Protons | δ $^1$H (ppm) |
| H-1 F | 5.41 | H-1 F | 5.440 |
| H-2 F | 3.990 | H-2 F | 2.89 |
| H-3 F | 3.75 | H-3 F | 3.73 |
| H-4 F | 3.61 | H-4 F | 3.57 |
| H-5 F | 3.53 | H-5 F | 3.45 |
| Me-F | 0.68 | Me-F | 0.66 |
| H-$1_{ax}$ C | 2.990 | H-$1_{ax}$ C | 2.990 |
| H-$2_{eq}$ C | 4.46 | H-$2_{eq}$ C | 4.37 |
| H-$3_{ax}$ C | 1.66 | H-$3_{ax}$ C | 1.64 |
| H-$3_{eq}$ C | 1.89 | H-$3_{eq}$ C | 1.88 |
| H-$4_{ax\text{-}eq}$ C | 1.47 | H-$4_{ax\text{-}eq}$ C | 1.45 |
| H-$5_{ax}$ C | 1.32 | H-$5_{ax}$ C | 1.32 |
| H-$5_{eq}$ C | 1.69 | H-$5_{eq}$ C | 1.69 |
| H-$6_{ax}$ C | 1.67 | H-$6_{ax}$ C | 1.67 |
| H-$6_{eq}$ C | 1.77 | H-$6_{eq}$ C | 1.77 |
| H-2 Py | 8.72 | | |
| H-4 Py | 8.06 | | |
| H-5 Py | 7.550 | | |
| H-6 Py | 8.61 | | |
| | | H-2 Ph | 7.04 |
| | | H-4 Ph | 6.98 |
| | | H-5 Ph | 7.29 |
| | | H-6 Ph | 7.12 |

**Table S2**. **3** Coupling Constants for key protons of cyclohexyl ring of **3.**

| Protons of Ring C | Φ | Exp. $^3$J (Hz) |
|---|---|---|
| H-1ax-H-6ax | 180º | 9.7 |
| H-1ax-H-6eq | 60º | 4.1 |
| H-2eq-H-3ax | 60º | 4.0 |
| H-2eq-H-3eq | 300º | 6.0 |

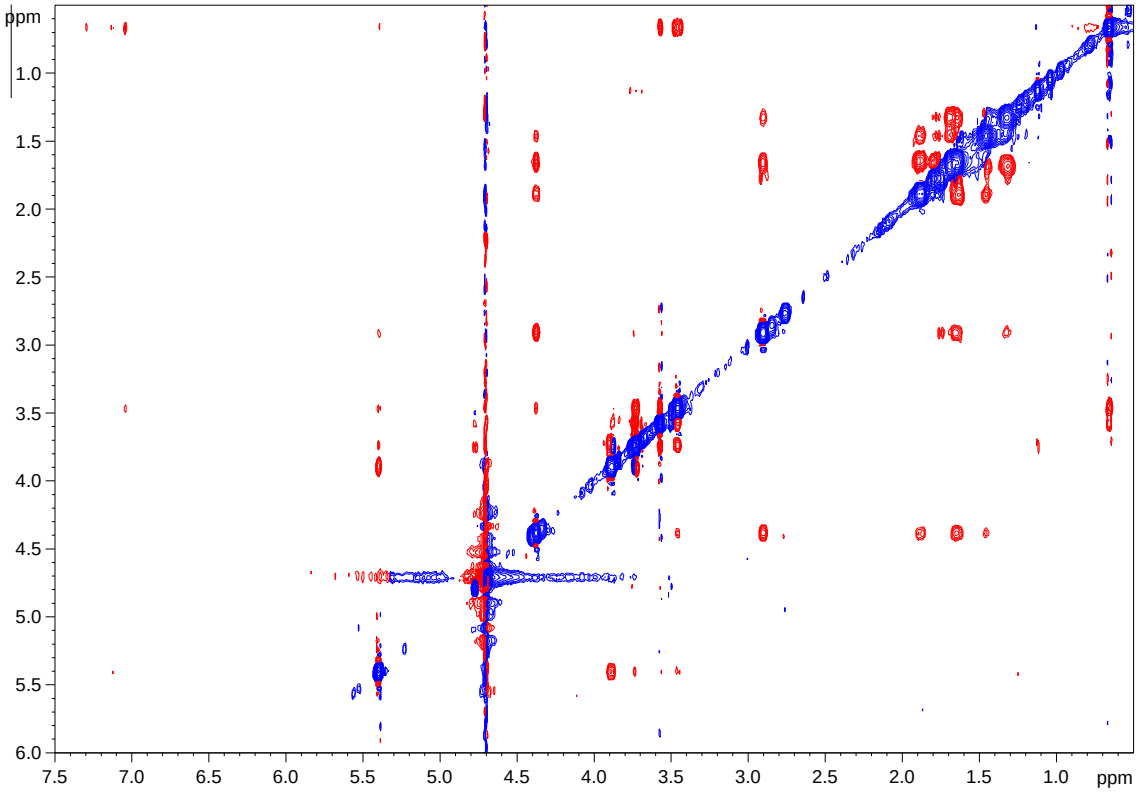



**Fig.S1** NOESY of **3** (2 mM 25 ºC, 500 MHz, 0.4 ms)

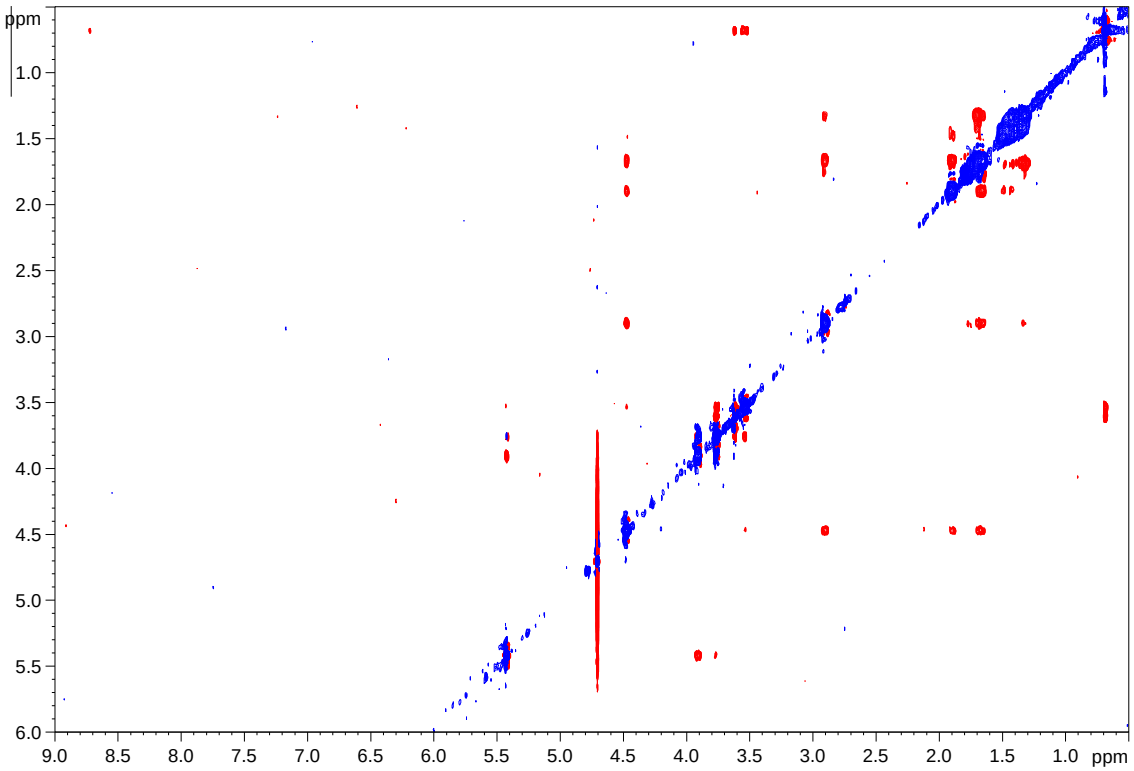



**Fig. S2** NOESY of **4** (1mM 25 °C, 500 MHz, 0.4 ms)

## S1.- Molecular Modelling Calculations

### S1.1.- Conformational Search

A Monte Carlo Multiple Minimum (MCMM)[1] conformational search of **3**, performed using MacroModel-AMBER* force field[2, 3] and the GB/SA water solvation model,[4] yielded 32 conformers within 3 kcal/mol from the global minimum. All conformers presented the chair conformation of the **C** ring which is in agreement with the experimental J-coupling data**.** In contrast**,** out of the 32 solutions only one structure showed interatomic distances consistent with the experimental NOE data. This conformer and the global minimum were used as the starting point of two separate Monte Carlo/ Stochastic Dynamics (MC/SD) simulations. The monitored interatomic distances converged towards values that were again inconsistent with the observed set of NOE contacts (Table 1). In search for an appropriate computational model, conformational searches using MM3* and OPLS_2005 rather than AMBER* were performed starting from the AMBER* global minimum. Conformers arising from MM3*[5, 6] multiple minimization, showed interatomic distances in agreement with NOE data, but also showed a number of low energy solutions in which the **C** ring adopted a chair conformation inverted relative to that determined from J-coupling data. A conformer populations which excluded these "wrong chair" conformations was obtained by performing a MC/SD Dynamic simulation with MM3* starting from the AMBER* minimum and disallowing ring opening. A multiple minimization run of the snapshots collected during the simulation gave a population of minima (MC+ MC/SD) consistent with the MM3* potential energy surface for the "correct chair" conformers. The distances monitored during this constrained dynamics and those calculated from the corresponding set of minima were consistent with experimental data (Table 1). The best agreement, however, was obtained using the OPLS_2005 force field.[7, 8] A multiple minimization gave rise to 26 conformers (within 3 kcal/mol) whose interprotonic distances matched with the experimental ones. The OPLS_2005 global minimum was used to perform a MC/SD simulation and again the results were in good agreement with the NMR data. (Table 1).

**S1.2 Docking**

The preparation of the protein was performed using the methodology previously described by Bernardi's group[9] (see also ref 10 in main text). This basically used the standard PPREP protocol, but the final minimizaton of the protein was performed using MacroModel and the GB/SA water model. This step allowed to optimize H-bonding of the crystallopgraphic water molecules conserved from the 1SL5 complex. One of the ligand structures employed for docking was the global minimum from the multiple minimization of the structures stored during MC/SD Dynamics (MM3*). A rigid docking (Fig. S3) was performed by superimposing the fucose ring of mimic **3** with the fucose residue of Lewis$^X$ as it is in the crystallographic structure (pdb entry 1SL5). Then, semi-flexible docking was performed by Glide (Grid-Based Ligand Docking with Energetics) program,[10-12] that uses a hierarchical series of filters to perform a systematic search for possible locations of the ligand in the protein active site. Ligand conformational flexibility is handled by a conformational search. All docked poses generated maintain the interactions between the $Ca^{2+}$ atom and two hydroxyl groups of the fucose residue, mostly, hydroxyl groups 3 and 4, as in the case of the crystal structure. Only one of the poses was able to explain the key inter residue NOE signals experimentally observed (Fig. S4). Further docking studies were performed starting from the same structures and using QM-polarized-docking protocol of Glide[10, 13]By this protocol, ligands are docked by Glide, then charges on the ligand induced by the protein are calculated with Qsite and a set of the best ligand poses are redocked. In this way the polarization of the charges on the ligand by the receptor is accounted for and redocking of the ligands with these new charges can result in improved accuracy. The ten final poses obtained were not consistent with the experimental data and, in addition, five of them showed a reversed chair of the **C** ring. Hence, based on experimental data, we excluded all docked poses obtained, with the exception of the one arising from the semi-flexible docking that fitted well with experimental evidences. This complex (Fig. S4) was our starting point to explore the existence of multiple binding modes using the CORCEMA-ST protocol[14, 15] that, indeed, allow to refine the bound-conformation of a weakly binding ligand positioned within the binding site of a target protein and to obtain quantitative analysis of STD NMR data. The procedure requires as data input the pdb coordinates for the bound and free protein, as well as some NMR and kinetics parameters. From the docking pose which presents a coordination of $Ca^{2+}$ with hydroxyls 3 and 4, we built 3 other models of interaction and minimized them using MacroModel. The complexes, taking VAL 351 on the right as a reference and an orientation as in Fig. S8, were: one showing the interaction between the $Ca^{2+}$ atom and hydroxyl groups 4 and 3 of the fucose residue, another with hydroxyl groups 2 and 3, and another one by hydroxyls 3 and 2. To resume, we named the above-mentioned structures as O3-O4, O4-O3, O2-O3 and O3-O2.

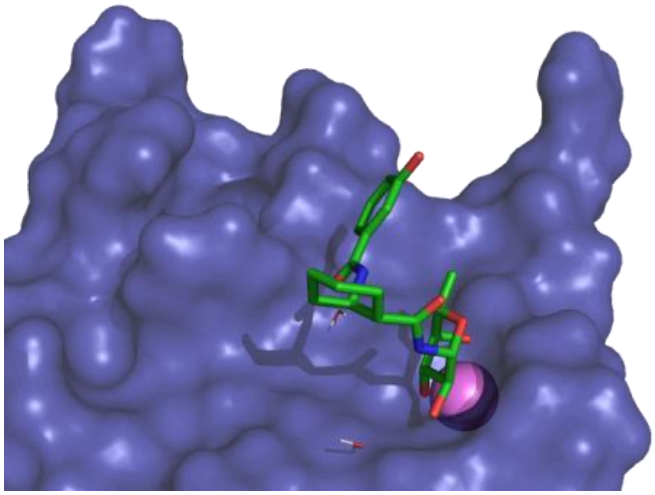


**Fig. S3** Rigid docking pose of mimic **3** in the binding pocket of DC-SIGN. The structure of the ligand corresponds to the global minimum (ΔE= 0.0 Kcal mol $^{-1}$) from multiple minimization of the structures obtained after MC/SD (MM3).

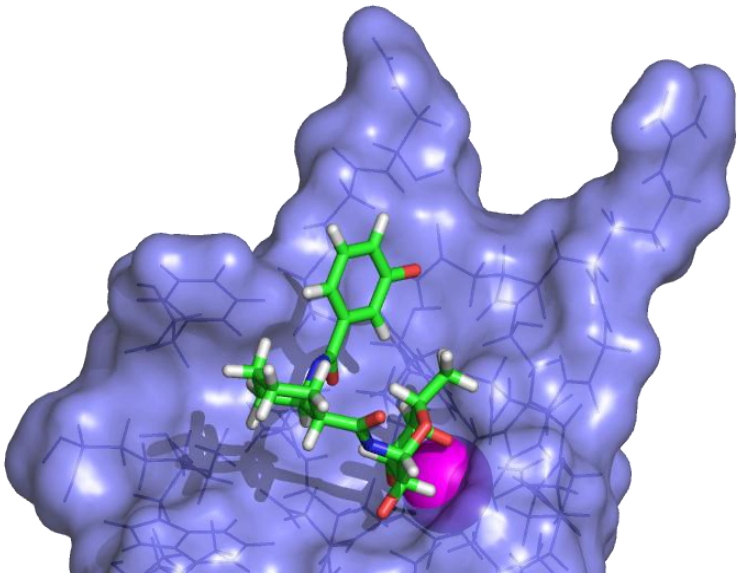
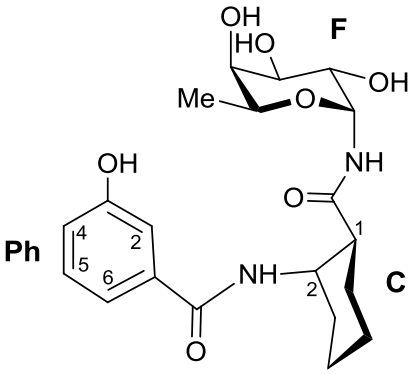


**Fig. S4** Best docking pose by semi-flexible docking of mimic **3** in the binding pocket of DC-SIGN, fitting with the experimental evidences.

## S2. - STD Experimental Growth Rates




**3**

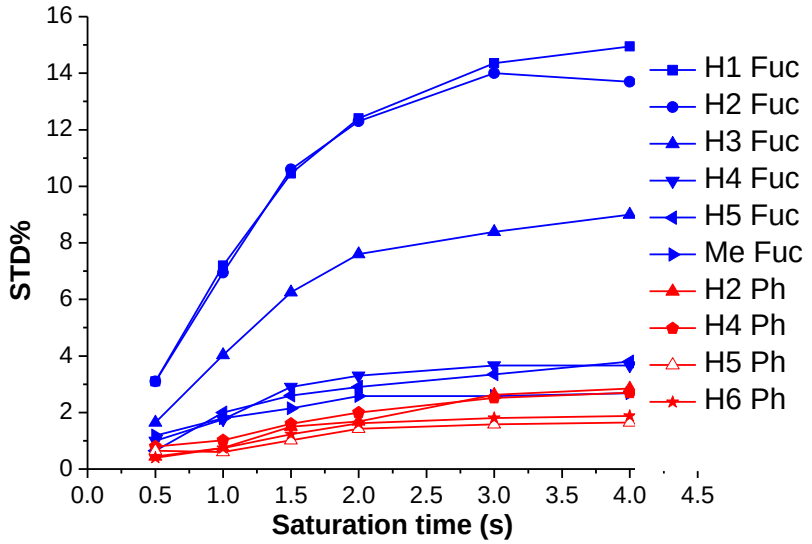
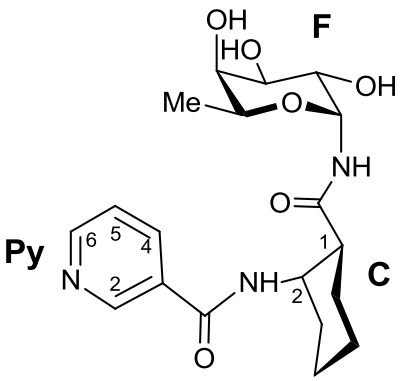



**Fig. S5** STD growth curves of **3** as function of saturation time. In blue, protons belonging to fucose, in red those of the aromatic ring.




**4**

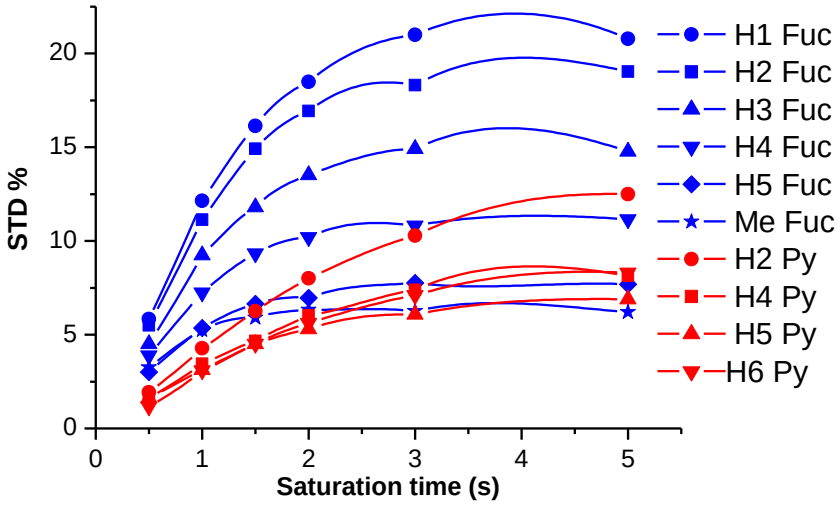



**Fig. S6** STD growth curves of **4** as function of saturation time. In blue, protons belonging to fucose, in red those of the aromatic ring. Notice the differences on growth rates between the pyridine ring and the fucose moiety.

## S3. - CORCEMA-ST Calculations

**Nomenclature of the complexes.** The complexes were built varying the coordination of $Ca^{2+}$ with fucose hydroxyls together with the orientation. Taking the VAL 351 on the right as a reference (orientation as in figures), were: one showing the interaction between the $Ca^{2+}$ atom and hydroxyl groups 4 and 3 of the fucose residue, another with hydroxyl groups 2 and 3, and another one by hydroxyls 3 and 2. To abridge, we named the above-mentioned structures as O3-O4, O4-O3, O2-O3 and O3-O2

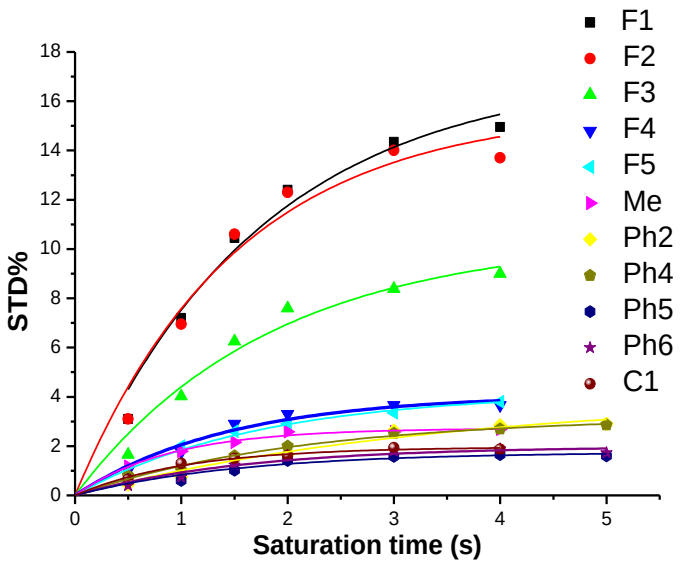
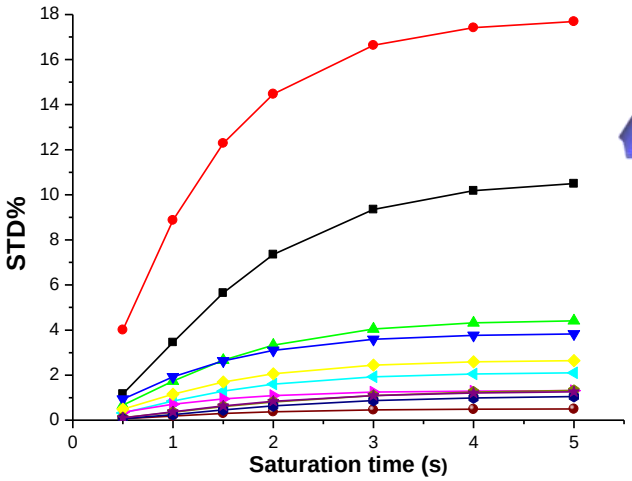
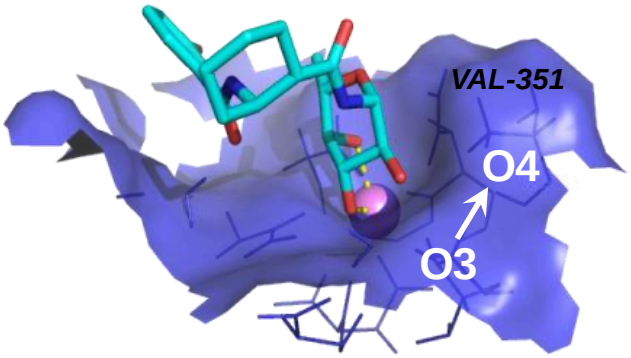



**Fig. S7** Experimental STD growth curves of **3** as function of saturation time.

A)

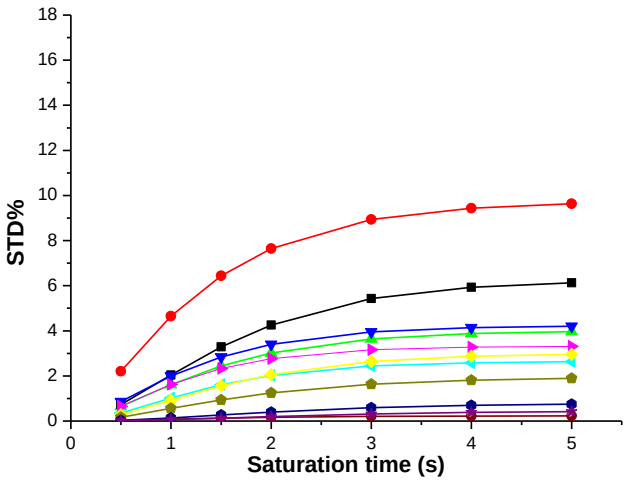
**O3-O4**

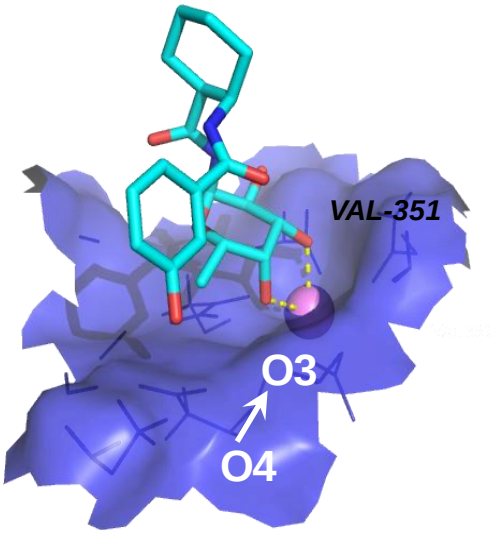


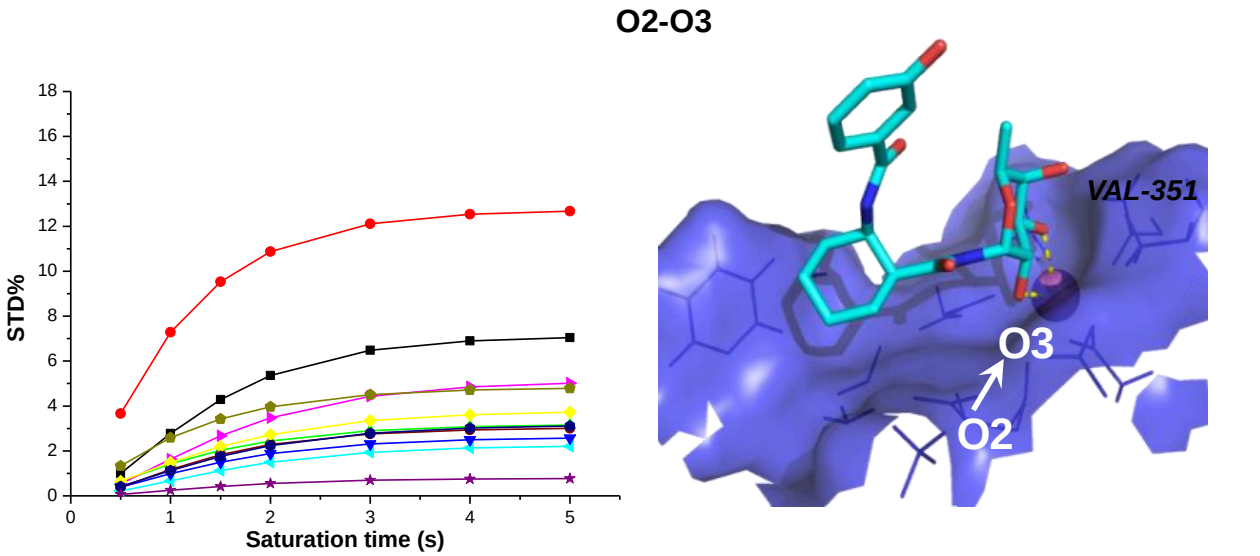

B)

**O4-O3**



C)




D)

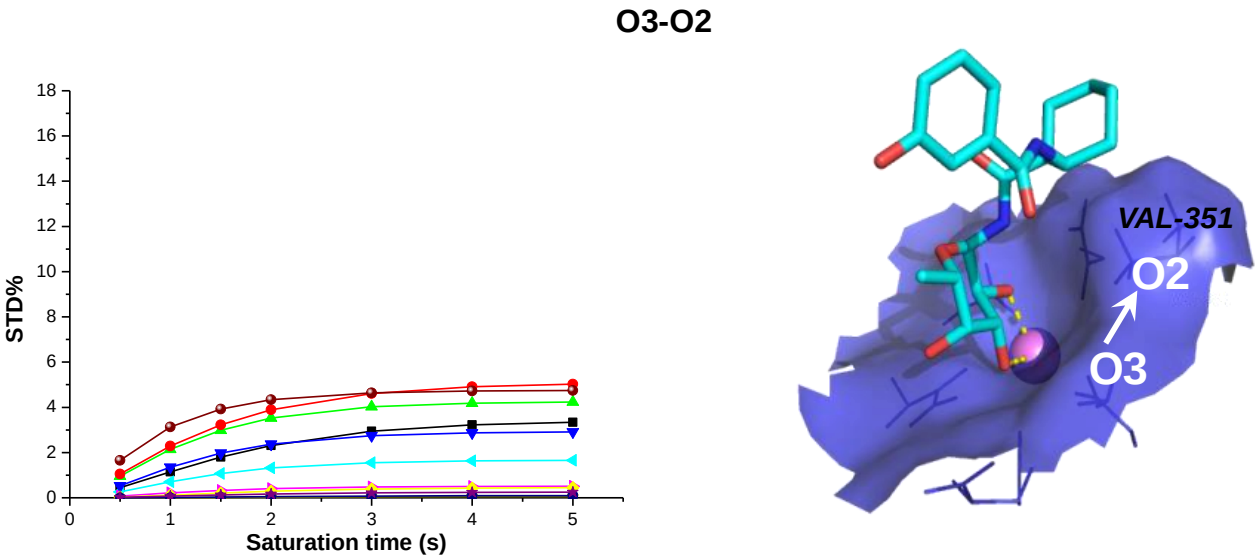



**Fig. S8** Predicted STD values by CORCEMA-ST for : A) model O3-O4 B) model O4-O3 C) model O2-O3 D) model O3-O2.

## References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