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Labelling IL-18 with alkaloids: towards the use of cytokines as carrier molecules in chemotherapy

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Abstract

Recent *in vivo* models demonstrated that immune checkpoint cancer therapy can be improved by injecting interleukine (IL)-18, a proinflammatory cytokine of the IL-1 family. Aiming to enhance the beneficial action of cytokines in cancer treatments, we used here docking, non-covalent interaction analysis and molecular dynamics simulation to determine their ability for embedding alkaloid-based drugs. According to our simulations, three alkaloids with anticancer activity, e.g., paclitaxel, vincristine and vinorelbine, are efficiently retained in the central cavity of IL-18. The reported results pave a new synthetic route for designing novel bifunctional carrier-cargo systems with enhanced anticancer activity.

Keywords: immune checkpoint blocking therapy, cancer, interleukin, docking, molecular dynamics, non-covalent interactions

1. Introduction

2 More than ten years ago, Cragg and Newman published their seminal
3 paper “Plants as a source of anti-cancer agent”, an updated review over
4 the applications of the traditional medicine within the framework of current

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5 clinical therapies [1]. As discussed by these authors, the use of plant-derived
6 extracts as anticancer agents has attracted an increasing attention with a
7 particular focus on alkaloids, which are known exhort a strong anticancer
8 activity [2]. Indeed, alkaloid-based drugs has been proposed for the treatment
9 of several disorders including carcinomas, lung, prostate and breast cancer
10 [3]. Although the biological mechanism of alkaloids is rather than complex
11 at the cellular level [4], the inhibition of the microtubulin function is now
12 accepted to be the key step in the their anticancer activity [5]. Unfortunately,
13 that reaction is not site-specific and alkaloids show severe side effects due to
14 their high cytotoxic in healthy cells.

15 To circumvent chemotherapy risks, novel drugs can be encapsulated into
16 a carrier molecule [6], that is, the formation of a host-guest complex that
17 avoid an early activation of embedded drug while simultaneously concentrat-
18 ing their action in the tumor area [7]. Such approach is also possible for
19 alkaloids. As Cragg and Newman stated, “the ability to attach agents to
20 carrier molecules directed to specific tumors, shows promise for effectively
21 targeting highly cytotoxic natural products to the tumors while avoiding
22 their toxic side effects on normal tissues” [1]. In the same vein, an attractive
23 idea was recently proposed by Yadav an co-workers, who used theoretical
24 simulations to determine the ability of trastuzumab (Herceptin[®]) to embed
25 three alkaloids: paclitaxel (Taxol[®]), vincristine (Oncovin[®]) and vinorel-
26 bine (Navelbine[®]) [8]. Trastuzumab is a humanized monoclonal antibody
27 that selectively binds to the HER2/neu over-expressed protein in breast can-
28 cer patients [9, 10]. In this case, both carrier and cargo molecules posses
29 anticancer activity, so that they can be defined as bifunctional drugs [11–14].

30 Computational simulations can be used to broaden the chemical space
31 of carrier molecules beyond traditional antibodies. Here we aim to assess
32 the use of interleukin (IL)-18 as carrier molecule. IL-18 is a proinflamma-
33 tory cytokine of the IL-1 family that improves immune checkpoint blocking
34 cancer therapy [15]. Although its biological mechanism of action is still
35 under debate, the beneficial effects of IL-18 might be associated to the ac-
36 tivation of cytotoxic pre-mNK cells, low accumulation of regulatory T cells,
37 and suppression of soluble inhibitor secretion of IL-10 and TGF β [15]. The
38 present contribution provides the first comprehensive insights on the use of
39 this cytokine as carrier material for paclitaxel, vincristine and vinorelbine
40 anticancer drugs. More specifically, blind docking (BD), molecular dynamics
41 (MD) simulations and non-covalent interaction (NCI) analyses are carried
42 out to assess the distribution of potential binding sites across IL-18 protein

43 surface and the stability of the IL-18 embedded alkaloids. Our theoretical
44 predictions can help to improve immune checkpoint cancer immunotherapy
45 based on novel IL-18 bifunctional carrier-cargo systems.

46 2. Computational Details

47 The initial model for the host system was built up by using the crystal
48 structure recently resolved by Tsutsumi and co-workers, which was deposited
49 at Protein Data Bank (PDB) [16] with code 1WO2 [17]. Hydrogen atoms
50 were added and and cap termini were included with the Protein Preparation
51 Wizard module implemented in Maestro [18]. Protonation states of all side
52 chains were defined at pH=7 using PROPKA 3.1 [19]. The three selected
53 alkaloids (paclitaxel, vincristine and vinorelbine) were subsequently docked
54 into the refined IL-18 structure using a BD approach [20], where multiple
55 parallel and independent docking simulation were performed around geo-
56 metric centers of all residues. BD simulations provide first insights into the
57 ligand interaction pattern with IL-18, namely, which residues of the pro-
58 tein (not restricted to a particular cavity, but considering the whole protein
59 surface) are involved, and how these interactions contribution to the total
60 protein-ligand interaction energy [21]. All individual docking calculations in
61 the BD approach were carried out with AutoDock Vina [22]. As discussed
62 elsewhere [22], the scoring function implemented in composed by two electro-
63 static terms (specified as *gauss1* and *gauss2*), and repulsive, hydrophobic,
64 hydrogen bonds (H-bonds) and an entropic term linearly dependent on the
65 number of rotatable bonds of the ligand.

66 The top BD pose of each alkaloid-based drug, namely, the one with the
67 highest value of the scoring function, can be indentified as the most favourable
68 binding site and was retained for the further analysis/simulations. To this
69 end, NCIPLOT code was applied to identify the nature of the chemical in-
70 teractions established between the drug and the carrier molecule [23, 24].
71 That scheme provides a qualitative description of the main chemical con-
72 tacts present in a host-guess system by combining the electron density ρ over
73 all atoms and the associated reduced gradient ∇ as follows:

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

74 which enables to visualize weak interactions as colored isosurfaces of the
75 reduced density gradient, s [25].

76 The resulting IL-18-embedded drug model systems were eventually lo-
77 cated in a orthorhombic water box by defining a buffer distance of 10 Å in
78 X, Y and Z directions by using the simple point charge (SPC) model [26].
79 Sodium cations were added to counterbalance the total electric charge. Ad-
80 ditional sodium and chloride ions were added to reproduce the physiological
81 NaCl salt concentration of 0.15 M. The whole solvated host-guest systems
82 were intially minimized for 2000 steps with the steepest descent scheme by
83 imposing a convergence threshold of 1.0 kcal/mol/Å. The solvated systems
84 were next relaxed with a multi-step protocol that includes a solute-restrained
85 minimization, free-restrain minimization, NVT simulation of 24 ps at T=10
86 K, NPT simulations at T=10 K and P=1 atm. For the production phase,
87 the temperature was set to 300 K with the Nosé-Hoover algorithm with
88 a relaxation time of 1.0 ps [27, 28]. Pressure was set at 1 bar using the
89 Martyna–Tobias–Klein barostat with an isotropic coupling and a relaxation
90 time of 2.0 ps [29]. The RESPA integrator was used to integrate equations
91 of motions with a 2.0 fs time step for bonded and close interactions and a 6.0
92 fs time step for farther interactions [30]. A cutoff of 9 Å was imposed to all
93 non-bonded interactions. Van der Waals interactions were assesing using a
94 cut-off radius of 9 Å and the electrostatic part was defined using the Particle
95 Mesh Ewald (PME) [31] method with a tolerance limit of 10^{-9} . All MD
96 simulations were carried out with the OPLS-2005 force field as implemented
97 in Desmond code [32].

98 3. Results and discussion

99 Results obtained from BD simulations are summarized in Figures 1 and
100 2, which provides the location and energetics of the different poses clusters
101 across the IL-18 surface. According to the visual inspection of the best
102 poses, the three selected alkaloids fit into the central region of the IL-18
103 structure. The histograms included in the left panel show the distribution
104 of pose clusters. Pink bars represent the number of poses whose scoring
105 function value falls into the assigned interval. Blue bars are specified as
106 follows; the pose with the highest value of the scoring function is assigned a
107 blue bar. Next, the clash of poses with lower values of the scoring function
108 is calculated against the top pose. In case there is collision, pink color is
109 assigned, otherwise it means a new cluster of poses is found and a blue bar
110 is designated. This process is repeated until no more poses can be processed
111 and afterwards, all pose clusters have been located.

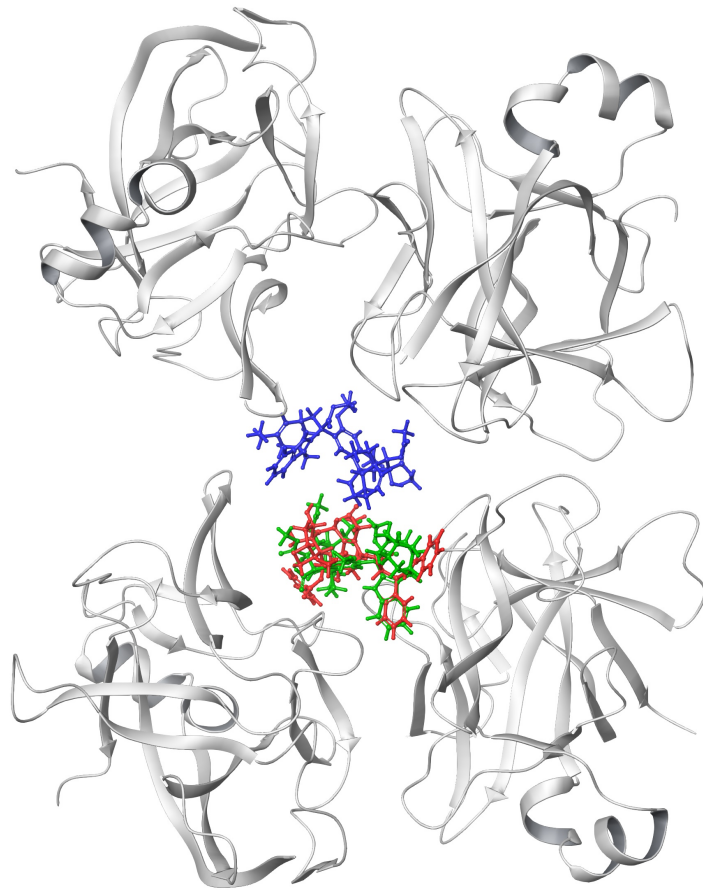
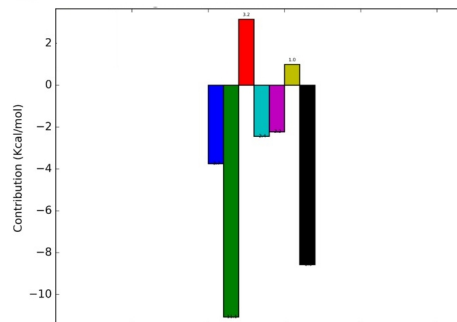
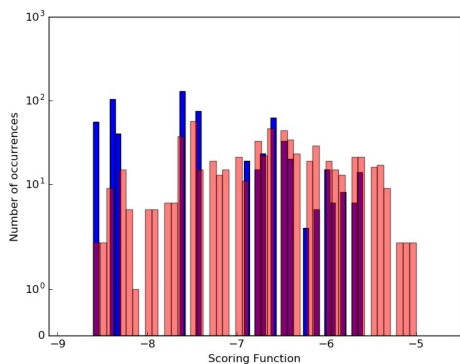


Figure 1: Conformations of the top docked poses. Ligands are shown as sticks: paclitaxel in red, vincristine in green and vinorelbine in blue. The structure of the IL-18 cytokine is displayed as gray cartoon.

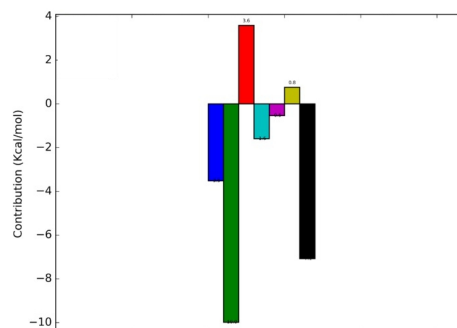
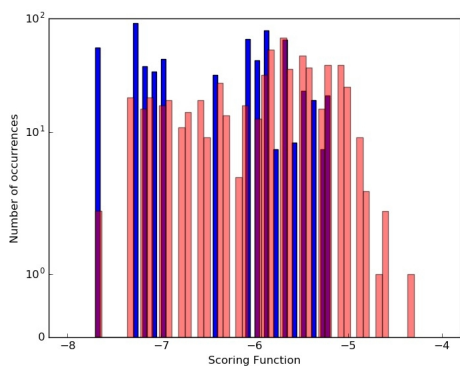
112 That outcomes hints that the cytokine is preferentially loaded into its
113 core structure, e.g., without altering its more external chemical region, the
114 biological activity is consequently preserved. consequently act as an efficient
115 carrier for any of these alkaloids. As one can see in Figure 2, the computed
116 top poses lie to the left end of the histogram with high scoring function values
117 (< -7 kcal/mol), which indicate a large affinity of the ligands. Figure 2 also
118 reveals that several pose clusters (blue bars) are found close to the top pose.
119 In all cases there are no big differences, regarding scoring function values,
120 between clusters, which means there is no clear binding site preference for
121 the compounds. The number of clusters goes around 20, so the protein would
122 in principle be able to transport more than 10 molecules at the same time.
123 Figure 2 also depicts the energetic analyses of the previously mentioned top
124 poses. We can see that main stabilizing interactions in all cases are related
125 with hydrogen bonds and favorable hydrophobic contacts. These theoretical
126 findings are relevant for our study since they suggest or confirm the utility
127 of this protein for carrier purposes.

128 Docking provides a cost-effective evaluation of the protein domain(s) in
129 interaction with drugs, a prerequisite for assessing all possible binding pock-
130 ets [33]. However, further calculations are necessary to fully understand the
131 biological mechanism of action. A NCI analysis is performed to provide a
132 qualitative picture of the drug binding modes of the top BD poses and, there-
133 fore, complete the results arising from docking. The NCI analysis is sum-
134 marized in Figure 3. In the selected color scheme the H-bonds are shown as
135 blue surfaces and weak attractive interactions are given as green regions. Let
136 us start with the paclitaxel drug. According to the computed surfaces, large
137 attractive interactions are established between this drug and IL-18 through
138 H-bonding with D:Ile80 and D:Lys135. This non-covalent interactions are
139 highlighted by blue surfaces in left panel of Figure 3. Additional dispersion
140 interactions appear on the benzenic moiety, that partially contribute to fix
141 paclitaxel in the binding site. For instance, one can observe a weak attrac-
142 tion with the lateral chain of residue D:Ile137. Additional interactions with
143 D:Ile81 can be also identified in the binding site. It is also remarkable the in-
144 teraction with B:Lys79. Paclitaxel is located very close to the latter residue
145 so that an steric clash appears, which is identified by red surface in the
146 NCI analysis. It is worth noting here that the major drawbacks of docking
147 protocols are the lack of protein flexibility (it is usually kept as a rigid en-
148 tity upon drug binding), which may lead to unreliable protein-drug contacts.
149 Middel panel of Figure 3 illustrates the interactions established between vin-

paclitaxel



vincristine



vinorelbine

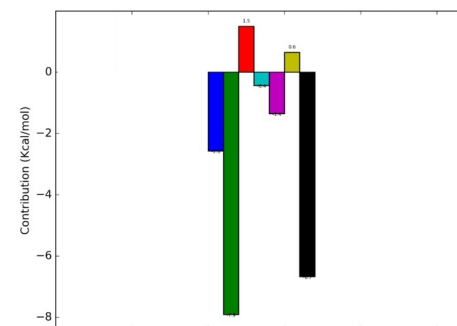
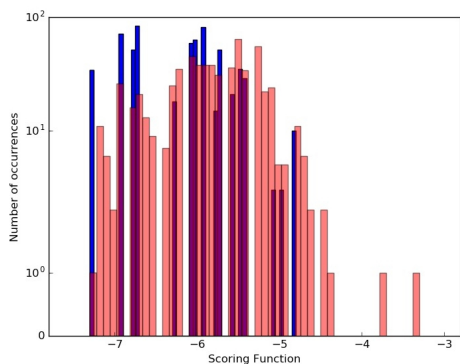


Figure 2: Left panel: Histogram of the distribution of values of the scoring function obtained after BD simulations for each of the three compounds over IL-18. Right panel: Energetic analysis of the top pose from each compound docked to IL-18. Representation of the values of the different energetic contributions to the predicted binding energy (kcal/mol) where depicted energetic contributions are; electrostatic interaction types Gauss1 and Gauss2 (blue and green color), repulsion forces (red color), hydrophobic interactions (light blue), hydrogen bonds (pink color), entropic contribution (yellow color) and total predicted binding energy (black). Paclitaxel, vincristine and vinorelbine are shown at top, middle and bottom, respectively.

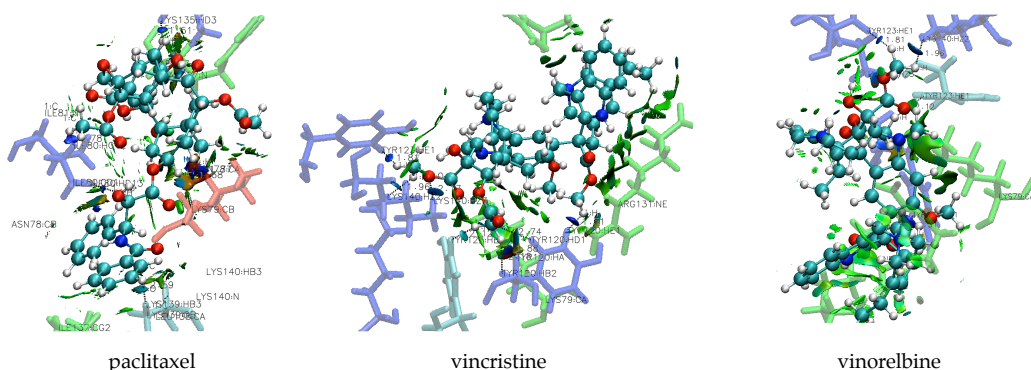


Figure 3: NCI analysis of main alkaloids-IL-18 residue non-covalent interactions in the binding pocket. Color scheme for paclitaxel binding site (left panel): D:Ile80 and D:Lys135 in blue; D:Arg27 and D:Ile137 in green, B:Lys79 in red. Vincristine binding site (middle panel): B:Lys79, D:Ile80 and D:Asn78 in blue; D:Lys135, D:Arg131, D:Ile137 in green. Vinorelbine binding site (right panel): B:Tyr123, B:Lis140, B:Tyr120 in blue, D:Arg131, D:Asp132 and B:Lys79 in green. Isosurfaces color code: the H-bonds are shown as blue surfaces and weak attractive interactions are given as green regions (threshold of $s = 0.5$ au).

150 cristine and IL-18. In this case, dispersion is much more favored compared
 151 to the results obtained for paclitaxel. More specifically, vincristine is very
 152 complementary along Arg131. In addition, the ligand is anchored through
 153 an important number of hydrogen bonds (blue surfaces) on another part of
 154 the pocket, which ensures a large interaction energy. Finally, NCI analysis
 155 shows similar result to vinorelbine, which forms H-bonds though B:Tyr123
 156 and B:Lis140 residues and a stable complementary along the benzenic core
 157 of the drug with D:Arg131. The hole created around the central pyridinic
 158 group is also complemented by Lis79 in an optimal way.

159 As noted above, docked structures might produce steric interactions with
 160 embedded drugs since the protein is not allowed to relax. Following the pro-
 161 posed methodology, MD simulations have been next performed to further
 162 refine “raw” docking structures through a full relaxation of the system. The
 163 combination of docking and MD approaches accounts for both drug and pro-
 164 tein flexibility to mimic the induced-fit effects in the structure of the binding
 165 site [34]. Accordingly, the three top BD poses (Figure 1) are equilibrated in
 166 water boxes and subjected to MD simulations during trajectories of 25 ns.
 167 Figure 4 shows all-heavy atoms root-mean-square displacement (RMSD) of



Figure 4: Time dependence of all-heavy atoms RMSD (in Å) of IL-18 in the presence of the selected alkaloid-based anticancer drugs during MD trajectories of 25 ns. Blue, red and green lines stand for paclitaxel, vincristine and vinorelbine molecules, respectively.

168 the host relative to its initial structure *vs.* time for selected alkaloids. All
169 MD simulations lead to a similar conclusion: the three selected alkaloid-based
170 anticancer drugs remain within the central cavity of IL-18, which hints that
171 the formed non-covalent interaction pattern is strong enough to stabilize the
172 cargo inside the host structure. A closer inspection reveals that three cargo-
173 host systems reach the equilibrium after 15 ns, with a RMSD saturation of
174 ca. 1 Å. Consequently, IL-18 interactions with the alkaloids were monitored
175 throughout the last 10 ns of simulation (15 – 25 ns) to obtain deeper insights
176 into the dynamic behavior of the binding sites.

177 As one can see in Figure 5, most of the interactions identified by NCI in
178 the docked structure are retained during MD simulations. According to the
179 produced MD trajectory, paclitaxel binding mode is dominated by H-bonds
180 interaction pattern with a large number of bonds with residues B:Asn78,
181 B:Lys140, D:Ile81, D:Lys135 and D:Leu138. It is worth noting that the clash
182 of paclitaxel with B:Lys79 disappears during the trajectory. Indeed, B:Lys79
183 significantly contributes to the stabilization of the binding site through a
184 water bridge. This interaction cannot reproduced by our initial docking
185 simulations as they do not include explicit water molecules. This finding
186 confirms the MD refinement as a key step in the computational protocol to
187 properly describe the *loading* process in the synthesis of novel host-cargo
188 systems.

189 4. Conclusions and outlook

190 Herein, we performed docking, non-covalent analysis and molecular dy-
191 namics simulations to assess the possible use of IL-18 as carrier molecule
192 for anticancer drugs. According to our predictions, IL-18 efficiently embeds
193 three alkaloids with anticancer activity, e.g., paclitaxel, vincristine and vi-
194 norelbine, in its central cavity, so that the biological activity of the cytokine
195 is preserved while allowing the selective transport of the embedded drugs.

196 Of course, a larger panel of anticancer drugs will be required to define the
197 most efficient anticancer drug that can be loaded into the structure of IL-
198 18 cytokine (i.e., by including metallodrugs, anthracyclines, antimetabolites,
199 etc). Simultaneously to perform such theoretical calculations, the cytokine as
200 carrier molecule can be already tested *in vivo* to validate their future clinical
201 applications.

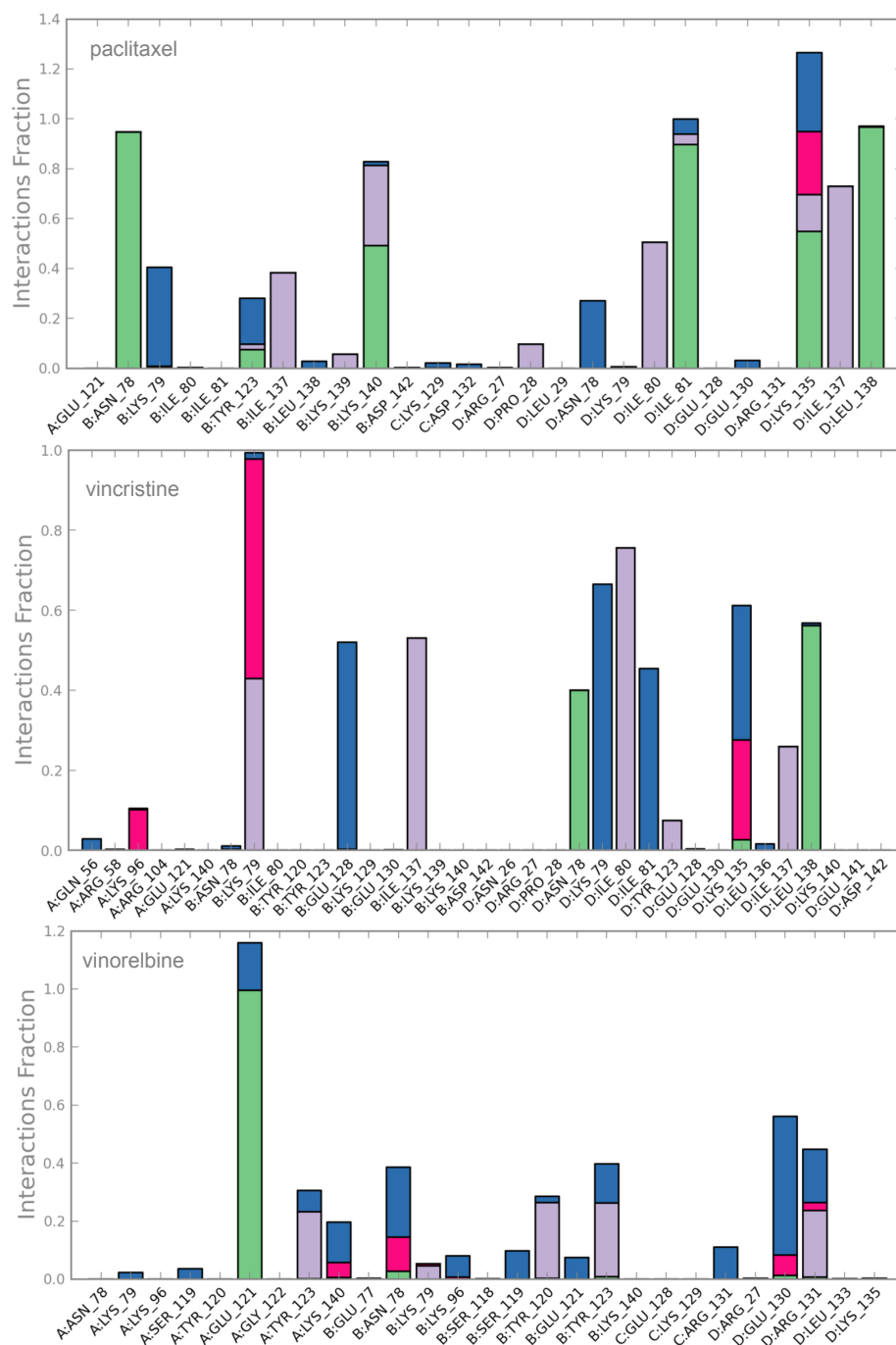


Figure 5: Decomposition of main protein-ligand interactions for IL-18 embedded alkaloids throughout the last 10 ns of MD simulations. Color code: H-bonds in green, dispersion interactions in pink, ionic contacts in pink and water bridges contribution in blue.

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