**2P417**

**Prediction of hydrogen-bonding sites between Protein and Ligand by Molecular Dynamics Simulation**

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Hydrogen-bonding site is one of the most important pharmacophores in drug discovery. This site is known as a target site of drug design and it can be used for de novo design with a compound generator. In general, hydrogen-bonding sites are generated by statistical hydrogen-bond distributions derived from the database. This approach shows good prediction for some cases, but not for other cases. Alternatively, a large number of probes (e.g., methanol, amide, etc.) are placed in a binding site, and energy of each of them is minimized. The regions where the minimized probes cluster are inferred to be favorable interaction sites. However, they result in too many energy minima on the surface of protein, and it is difficult to determine which of these minima is actually important. We present here a new method for predicting hydrogen-bonding sites between protein and ligand by using water molecule as a probe based on molecular dynamics (MD) simulations. First, the system is divided into 1 x 1 x 1 Å³ cubes. Then we assign a hydrogen-bond to cubes within 3.5 Å from the N, O, and S atoms of the protein surface. We use the angle cutoff of 120°(X-H+Y) for hydrogen-bond, where X and Y denote a donor and acceptor, respectively. After this, we calculate hydrogen-bond frequencies between protein and a water molecule observed in the trajectory of MD simulation and use them to predict the hydrogen-bonding sites between protein and ligand. Details of the results will be presented in the poster.

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**Hydrophilic and hydrophobic effects on the function of an allosteric protein in solution**

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Changes in solution have a substantial impact on the structure and function of many, if not all proteins. We have studied the effect of three electronucleotides (compounds 1, 2, and 3), on the function of the allosteric protein hemoglobin (Hb). These solutes were both amphiphilic (carrying both hydrophilic and hydrophobic moieties) and amphipophilic (displaying characteristics of both strong acid and strong base), yet they are not considered detergents. In the presence of compound 1 -the solute with the least degree of hydrophilicity, when compared to stripped Hb, we observed a significant decrease in both the oxygen affinity, and K_{a_{max}} (association constant for the first oxygen), and to a less extent, K_{a_{mp}} (association constant for the last oxygen), resulting in an increase in cooperativity. Striking effects were observed in the presence of compound 2 -the solute with intermediate degree of hydrophilicity. K_{a_{max}} increased significantly compared to that for stripped Hb, causing an increase in oxygen affinity. K_{a_{mp}} on the other hand, remained unaltered. Further, the oxygenation curve resembled that of a dimeric system, yet cooperativity remained surprisingly high. In the presence of compound 3 -the solute with the strongest hydrophobic moiety, K_{a_{max}} remained as high as when in the presence of compound 2. However, K_{a_{mp}} decreased considerably, resulting in a decrease in cooperativity. These effects suggested the existence of some mechanism of modulation on the Hb system in its fully ligated form.

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**Calorimetric titration study of F-actin solution with potassium chloride**

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Using a microwave dielectric spectroscopic method, we have revealed hyper-mobile water around F-actin, which has a much higher mobility than that of bulk water [1]. This water is considered to be identical to the water around water-structure breakers such as I and Br [2], because the dielectric relaxation frequencies of both waters are equivalent. The hyper-mobile water may be induced by a strong negative electric field around F-actin. If so, shielding of the electric field by adding KCl to F-actin may cause a decrease in the degree of hyper-mobile water. As the hyper-mobile water decreases, the density of hydrogen-bond among the water molecules may increase to the level of bulk water. To detect an exothermic heat by this hydrogen-bond formation, we here performed calorimetric titration of F-actin solution with KCl.

The F-actin dissolved in 50 mM KCl was titrated with an aliquot of 3 M KCl until the concentration reached 350 mM. The large endothermic heats (270-340 cal/mol of KCl) were corrected for the comparable heats of dilution of KCl into the buffer, and plotted against KCl concentration. Unlike typical titration curve, the exothermic heat reached the maximum (-7 cal/mol of KCl) around 150 mM KCl, and slightly decreased beyond this concentration. This is distinct from a titration curve of water-structure making protein (albumin) with KCl, for which exothermic heats gradually decreased with increasing KCl concentration.


**2P420**

**Two-colored emission from an intermediate \(M_{420}\) of firefly luciferin in deoxygenated DMSO under some content rates of water and t-ButOK**

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We have investigated the reaction process of chemiluminescence of firefly luciferin (Ln) in dimethyl-sulf oxide (DMSO) added potassium t-butoxide (BuOK). This light emitter is interpreted to be an exited oxyluciferin (OxyLn)') formed by the decomposition of diotaxetanone generated from oxygenation of Ln. We produced intermediates of Ln before forming the dioxygenate by removing the oxygen dissolved in DMSO. The intermediate with the absorption maximum at \(420\) nm (called \(M_{420}\)) shows light emissions by pouring oxygen gas into its solutions. Under some combinations of water content rate of DMSO and t-ButOK concentration, the time-resolved spectra of \(M_{420}\) indicate green- and red- colored light emissions with the maxima at 510 and 620 nm, respectively. Under a water content rate 0.05% DMSO and 10mM t-ButOK, green-, red-, and again green-colored light emissions are successively observed, while in the dehydrated DMSO, green-colored light shows the same decay curve as the red one. The products (called \(N_{420}\) and \(P_{420}\) formed after the green- and red-colored light emissions show the absorption and the fluorescence peaks at \(420\) nm, \(560\) nm, and at \(535\) nm, \(620\) nm respectively. Furthermore, the intensity of \(M_{420}\) chemiluminescence decreases with the increase in water content rate of DMSO. We thus conclude that \(M_{420}\) emits simultaneously red- and green- colored light, which process can be effected on water content rate of DMSO.