

Digital Biology, Fall 2014

Project Suggestions and Reading List

September 30, 2014

The course will explore a solvent-centric view of dehydrons as opposed to the accepted structure-centric view that has been the primary view of dehydrons in the past. The initial phase of the class will cover the basics of protein biophysics and present the ideas behind dehydrons and their role in structural interactions. The material will include basic bioinformatics techniques for examining protein structure. After this introductory period, the class will be based primarily on student presentations.

The dehydron is a structural defect in gene products (especially proteins) and represents a key concept in the understanding of biological interfaces. Its unique physico-chemical properties have inspired a multi-scale theory of biological water while making the dehydron a pivotal factor in protein associations. In this course, we intend to examine dehydrons from a solvent-centric perspective, rather than adopting the structure-centric view that most people are familiar with. This shift in view point entails several advantages as one strives to understand the functional properties of dehydrons. In particular, a solvent-centric view leads to the striking conjecture that dehydrons may behave as catalytic elements, often stimulating, enabling and even promoting enzyme function. We want to study the involvement of dehydrons in biochemical events after we have already delineated its involvement in protein associations, the hallmarks of biological activity.

The new solvent-centric view of dehydrons also yields an interesting interpretation of solvent-as-material in biological associations, with dehydrons playing a significant role in causing material defects. This could have significant implications for materials engineering involving solvents in other biomaterials and beyond. These ideas and projects have been developed in collaboration with Ariel Fernández Stigliano.

The course will require

- presentation in class of one significant paper,
- the execution of a research project, and
- presentation in class of your results.

To promote coherence of the class, it is suggested that you choose a project from the following list, as well as a paper for the presentation that is closely related to the project. Students are encouraged to form groups to work on a single project. Students may choose other projects subject to approval of the instructor.

1 Projects

The following are suggested projects for the course. The first six projects relate to a possible role of dehydrons in phosphorylation, and the following two are closely related to one of the six.

Direct inspection of protein structure shows that dehydrons tend to cluster around residues implicated in catalytic function and protein association. The latter property comes as no surprise due to the dehydration propensity of dehydrons and has been widely studied, as will be explained at the beginning of the class. However, the possibility of a dehydron-based catalyst or a dehydron-based stimulator/enabler of protein function has not been explored. Why are phosphorylation sites cluttered with dehydrons? Why are dehydrons ubiquitous at the interfaces of transcription factors? These are the types of questions that, when properly addressed may shed light on the unique functional properties of dehydrons, i.e. on their participation in chemical events. In essence, we want to explore such functional properties from a solvent-centric perspective. That is we want to understand what is the dehydron doing to the surrounding solvent in order to prepare it for catalysis. Because we seek to delineate the participation of dehydrons in chemical events, we may ultimately need to resort to a quantum mechanical treatment [1, 2, 3] of the dehydron-decorated catalytically active site.

1.1 Deprotonation dynamics

A first step in phosphorylation of sidechains is deprotonation. For example, Figure 1 shows how this might work with the removal of the proton from the terminal OH group on threonine. Deprotonation of the tyrosine terminal (phenolic) hydroxyl enables the latter to perform the nucleophilic attack on the terminal phosphoester linkage of ATP, the step that enables the Tyr phosphorylation. So, the question becomes: which nearby chemical or quasi-chemical entity promotes that deprotonation? What has this deprotonation process to do with the fact that there are dehydrons near a functionally competent Tyr? In what way may it be that dehydrons contribute to promote this deprotonation?

The extent of wrapping changes the nature of hydrogen bonds [4] and the structure of nearby water [5]. Hydrogen bonds that are not protected from water have different dynamics [4]. Figure 5 of [4] shows the striking difference of water residence times for well wrapped versus underwrapped hydrogen bonds.

To deprotonate THR, a water molecule has to approach the OH group (1) close enough and (2) in the correct orientation, with the water oxygen adjacent to the hydrogen on threonine. The project is to

- determine the distribution of **distances** and **angles** for waters near threonine sidechain hydrogens and see if there is a difference between well wrapped THR and underwrapped THR.
- Do the same analysis for other sidechains that get phosphorylated.

This could be done by doing molecular dynamics from scratch or by looking at an existing set of trajectories [6].

1.2 Phosphorylation and wrapping

Another way to examine the role of dehydrons and phosphorylation is by bioinformatics. See if there is a correlation between phosphorylation sites and nearby dehydrons.

First analyze explicitly phosphorylated residues PTR etc. in PDB files to see how frequently their phosphate groups are in desolvation spheres of underwrapped mainchain hydrogen bonds.

- Keep a count of how many dehydrons are near a given PTR etc.

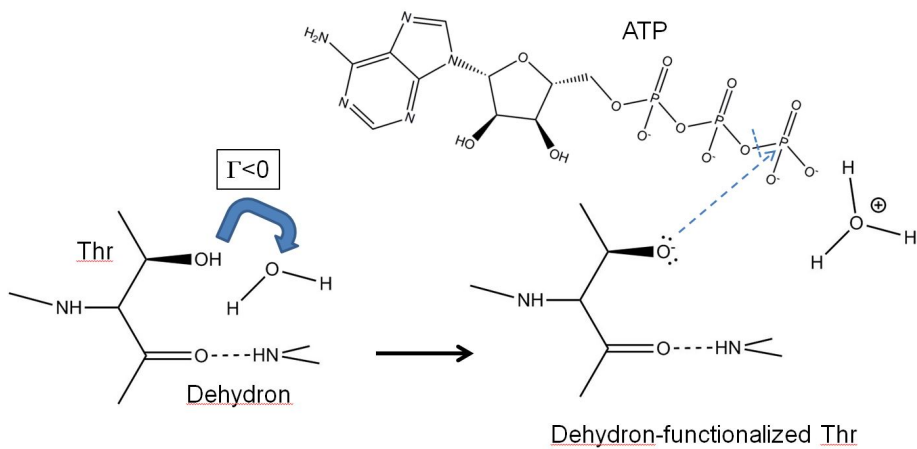


Figure 1: Phosphorylation process for threonine. Figure due to Ariel Fernández Stigliano [private communication].

- Further, plot the frequency of appearance of PTR etc. in desolvation domains as a function of wrapping.
- Compare this with nonphosphorylated residues Tyr, Ser, Thr.

Use wrappa to determine desolvation domains, amount of wrapping, and candidate dehydrons.

Next, if time permits, survey the various software tools available on the web to predict phosphorylation sites, e.g., <http://www.phosphosite.org/> and www.phosida.com. Pick one or more of them to predict phosphorylation sites as needed. Using the software to predict phosphorylation sites, consider wrapping around all predicted phosphorylation sites versus wrapping around all other Tyr, Ser, Thr. Count the number of dehydrons near a given site (for a given PTR etc. how many desolvation domains is it in?).

1.3 Hydroxide ions at surfaces

Another way to promote deprotonation of a sidechain atom is for a proton-deficient water molecule (hydroxide) to approach. So the question is: do dehydrons attract hydroxide ions?

A recent paper [7] says that hydroxide and hydronium ions behave like amphiphilic surfactants that stick to hydrophobic hydrocarbon surfaces, and

the effect is more pronounced for the hydroxide than for the hydronium ion. According to [7], hydroxide and hydronium ions near a hydrophobic interface stick to a hydrophobic hydrocarbon surface with their hydrophobic side, consistent with several experimental observations that hydrophobic surfaces in contact with water acquire a net negative charge. One reason that this could be the case is that, at a hydrophobic interface, water molecules must lose at least one binding partner. However, the hydroxide ion only has 3 binding partners, so there is nothing to lose. Thus hydroxide ions make an ideal intermediary between bulk water and a hydrophobic surface.

This picture is consistent with what is known in materials engineering: “impurity atoms ... dissolve in the boundary” between grains [8, p. 20]. Do “impurities” such as hydroxide collect even more strongly at more singular points in the water structure such as occur at dehydrons?

- Perform molecular dynamics simulations with hydroxide ions to see where they tend to accumulate more.
- Measure typical density in bulk, at hydrophobic surfaces, and near dehydrons.

There is a more general question of diffusion at protein surfaces that we explore in project 1.8

1.4 Kinases and wrapping

Kinases are proteins that are often implicated in cancer. By searching in the literature, examine constitutively active kinases that are often carcinogenic. Many oncogenic mutations in such kinases are in the phosphorylation sites and some occur in their periphery, yet the latter are seldom studied since it is not clear how to interpret them by currently accepted standards. Sidechain mutations often cause significant changes in wrapping without causing changes to three-dimensional structure, and thus they can have significant changes in function without raising red flags. A mutation that would lead to misfolding (or at least nonfolding) might cause the protein to be immediately degraded. Thus sidechain mutations that lead to changes in wrapping can often be the most dangerous.

Many oncogenic mutations in kinases are known, and these provide a rich data set to explore.

- See if perhaps there are mutations that create dehydrons in the periphery of catalytic sites.
- See if corresponding changes in phosphorylation rates correlate with nearby dehydrons.

1.5 Proton exchange

What is the rate of proton exchange for surface residues Ser or Thr or Tyr when decorated by dehydrons versus when they are not? We suspect it is much higher in the first case, if the dehydrons turn surrounding water into a chemical base (proton acceptor). Explore the NMR and other literature on this matter. Start with the web site

[http://nmrwiki.org/wiki/index.php?title=Amide_Hydrogen_Exchange_\(HX\)](http://nmrwiki.org/wiki/index.php?title=Amide_Hydrogen_Exchange_(HX))

or other sources on the web and then

- do a literature search to see what data exists that could address this question
- compare this data with wrapping data.

1.6 Dual roles

Dehydrons are known for their role as dehydration promoters. If there is a role of dehydrons as proton acceptors, as suggested in the above projects,

- what is the significance of the dual role as proton acceptors and dehydration promoters for enzyme catalysis?
- Are there examples of catalytic performance compromised by mutations that remove nearby dehydrons, and vice-versa?

The above projects suggest that a dehydron can be viewed as a chemical base, in the sense that it causes the surrounding waters to act as a chemical base, in addition to its accepted role as a promoter of its own dehydration. But each role is exerted at a different time. When the dehydron nanocavity is filled up with water, the dehydron can be viewed as a base, or rather, the water that envelops it is a base. When a binding partner approaches, the dehydron reveals itself as promoter of self-dehydration, as this property

translates into a mechanical attractive drag known as dehydronic field. As water is expelled upon association, the dehydron ceases to act as a base.

The basic nature is used to titrate local charges, disguising them upon protein associations. For example, the charge on amonium (Lys) or guanidium (Arg) in transcription factors is “disguised” through deprotonations induced by dehydrons. On the other hand, activation of nucleophilic groups for catalysis also requires deprotonation by dehydrons nearby.

As the dehydron also helps with the anchoring of the binding partner, it ceases to act as a base. Titrating the local charge is very important: it activates the enzymatic site or disguises the charges mitigating the dehydration cost that results upon association.

This dual role, if correct, would say that dehydrons contribute to functionalize sites both for protein recognition (binding) and for enzymatic activity. Mechanistically, it would say that water wants to leave the dehydron and in so doing it can take a proton with it.

The same dual role may also affect the concentration of dehydrons around Arg or Lys in protein-nucleic acid recognitions (i. e., transcription factors), so we expand on this topic in project 1.7.

1.7 Transcription factors and wrapping

Transcription factors are proteins that bind to specific DNA sequences. The key side chains in such proteins are the DNA-intercalating Arg and Lys. Arg and Lys have significantly different hydration demands, but in addition dehydrons can alter local solvation environments. In this way, dehydrons are often involved in lowering the dehydration cost in biomolecular associations.

- Examine the distribution of dehydrons around DNA-intercalating Arg and Lys sidechains by surveying PDB files that display proteins binding to DNA.

1.8 Diffusion at surfaces

In materials engineering it is known that “much faster diffusion in the boundary plane” between grains occurs than would be typical in bulk [8, p. 20]. Does the same principle apply in proteins? The high dehydron count for many toxins [9] insures that they will not stick to water, and so it is plausible that they would migrate rapidly to protein/water surfaces. Would this

explain the rapid diffusion of toxins apparently necessary for their nanomolar activity? Use molecular dynamics to study the diffusion rate of toxins and prions [4] compared with well wrapped proteins.

2 Reading list

You will need to present a paper for the course. Hopefully you will pick a paper related to your project that particularly resonates with you. A good choice might be one of the references in the list below related to your project. Of particular interest may be the following papers that have recently suggested that quantum mechanical simulations may be feasible using new insights. We give a brief description of them as follows.

Learning theory [1] is being used to improve standard models used for molecular systems.

Recent work [2, 3] suggests that high-dimensional problems like the Schrödinger equation admit accurate low rank approximations.

References

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