



Universiteit  
Leiden  
The Netherlands

## **Transient interactions between photosynthetic proteins**

Hulsker, R.

### **Citation**

Hulsker, R. (2008, May 21). *Transient interactions between photosynthetic proteins*. Retrieved from <https://hdl.handle.net/1887/12860>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/12860>

**Note:** To cite this publication please use the final published version (if applicable).

## Summary

The biological processes that are the basis of all life forms are mediated largely by protein-protein interactions. The protein complexes involved in these interactions can be categorised by their affinity, which results in a range from static (high affinity) to transient (low affinity) complexes. Electron transfer complexes, which have to combine high turn-over with specificity are typically transient complexes. They achieve their high turn-over rates by high dissociation rate constants, while the association rates are comparable or higher to those found for static protein complexes. Together this results in the weak binding that characterises transient protein complexes.

The transient complexes under investigation in this work are all part of the photosynthetic redox chain, in which electrons are transferred from membrane bound cytochrome *f* (*cyt<sub>f</sub>*) to either plastocyanin (Pc) or cytochrome *c<sub>6</sub>* (*cyt<sub>c6</sub>*). The active site of these proteins contains a metal ion, and in the case of the cytochromes a haem, coordinated by several side chains of the protein.

In Chapter II, we study the protonation of a histidine copper ligand in plastocyanin at low pH, which inactivates the protein. This behaviour is observed for all plastocyanins, with one exception. Fern plastocyanin was thought not to protonate, because of the presence of a  $\pi$ - $\pi$  stacking interaction. With the use of nuclear magnetic resonance (NMR) spectroscopy we were able to show that the particular histidine does show protonation, although at a lower  $pK_a$  than expected. Mutations F12L and G36P that were expected to influence this behaviour did in fact not change the  $pK_a$ . Curiously, the crystal structure of both the wild type (wt) and G36P fern Pc shows a deprotonated histidine. The apparent discrepancy between crystallographic and NMR data can be explained by the presence of crystal contacts around the histidine. The proximity of the neighbouring asymmetric unit probably favours crystallisation of the deprotonated form. This example shows the advantages of solution state NMR, which can be used for the investigation of proteins and particularly transient protein complexes.

Studies of the Pc - *cyt<sub>f</sub>* complex and other transient protein complexes has led to a two-step model for complex formation. According to this model, free proteins associate to

form a dynamic encounter complex, followed by a well-defined state, which is capable of electron transfer. The effect of the mutation of two otherwise conserved residues, located in the hydrophobic patch surrounding the active site of *P. hollandica* Pc is investigated in Chapter III. While the structure of the wt Pc - *cyt<sub>f</sub>* complex could be determined by docking with NMR restraints, derived from chemical shift perturbations and pseudocontact shifts (PCS), this was not possible for the complex with mutant Pc. From a decrease in size of both the chemical shift perturbations and the PCS it could be concluded that the mutant complex is more dynamic than the wt Pc - *cyt<sub>f</sub>* complex. The movements in the complex were simulated by creating an ensemble of structures using the wt structure as a starting point. Comparison of the simulated and observed averaged NMR parameters shows that the mutations cause the complex to shift from the well-defined state towards the encounter state. This provides a way to examine the movements and dynamics in the encounter state of a transient protein-protein complex.

A similar approach is used in Chapter IV to study the dynamics in the *cyt<sub>c6</sub>* - *cyt<sub>f</sub>* complex. The use of paramagnetic relaxation enhancement (PRE) was introduced here, which provides information additional to that from chemical shift perturbations and PCS. For this purpose five separate spin labels were placed on the surface of *cyt<sub>f</sub>*, causing stronger relaxation of the *cyt<sub>c6</sub>* nuclei in the vicinity of the spin label. When PRE derived restraints from several positions of spin labels are used in docking calculations, converged structures are found, but with different orientations, depending on the combination of PRE data used. Furthermore, the violations for each of these determined structures remain large, suggesting that the complex is dynamic and cannot be described by a single structure. As in Chapter III ensembles of structures were created, which in this case could not decrease the violations of the PRE restraints. This leads to the conclusion that the *cyt<sub>c6</sub>* - *cyt<sub>f</sub>* complex cannot be described by an evenly distributed ensemble of structures and that the ensemble is most likely characterised by preferred orientations. The use of methods that do not require a starting structure, such as Brownian dynamics, might be more successful in precisely describing the encounter state of this complex.

Electrostatic interactions between the proteins forming a complex play an important role in the association and orienting of the proteins. An additional requirement for the formation of a transient complex is that electrostatic interactions should not prevent the fast dissociation of the proteins. For this reason the balance of electrostatics, hydrophobics and other surface properties that characterise the complex interface has been the subject of many studies. In Chapter V, charged peptides are used to study the role of electrostatics in protein-protein association. For this purpose  $^{15}\text{N}$ -labelled *S. pratensis* Pc was produced and its backbone amides were assigned. The addition of positively charged tetra-lysine peptide to Pc, which is known to inhibit electron transfer to *cyt f*, causes chemical shift perturbations at the negatively charged patches. Surprisingly, the number of affected residues and the size of the chemical shift perturbations indicate the complex between tetra-lysine and Pc is relatively dynamic. The addition of hydrophobic residues to the peptide does not significantly change the binding of the peptide supporting the idea that surfaces of electron transfer proteins are designed to interact weakly but specifically with their partners.

The dilute solutions in which proteins and protein complexes are often studied are different from the cellular environment in which these proteins normally function. This issue has been addressed by the use of macromolecular crowders, which mimic the presence of large amounts of other macromolecules in high concentrations. In Chapter VI the transient Pc - *cyt f* complex is studied by NMR in the presence of crowders. The use of several crowders was tested on a small globular protein, which revealed that Ficoll70, a highly branched polymer of sucrose, and dextran T70, a synthetic linear polysaccharide interfere least with the NMR experiments. The addition of up to 20% Ficoll70 to the Pc - *cyt f* complex did not result in significant differences of both binding constant or binding map. This supports the idea that high molecular weight crowders such as Ficoll70 are not uniformly distributed in solution and form a 'porous' medium in which relatively small proteins can move as they would in dilute solution. This raises the question whether high molecular weight crowders offer the most appropriate model of the cellular environment.

This work describes the investigation of several aspects of transient protein-protein interactions that are applicable to many other transient complexes. The use of NMR as a

tool of choice for these studies has proven it an invaluable technique that provides insight in rules that govern the world of transient complex formation. New approaches such as PRE and the creation of ensembles of structures will hopefully lead to a full understanding of dynamics and the variety of ways that transient complexes use dynamics to perform their function in the cell.