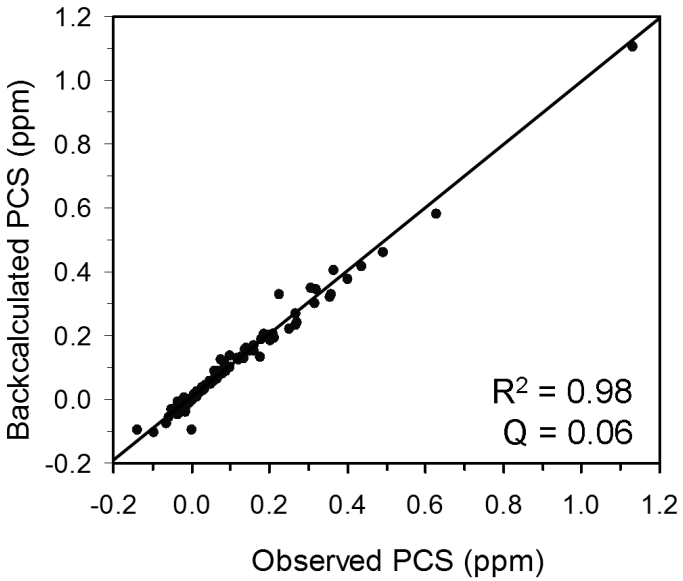
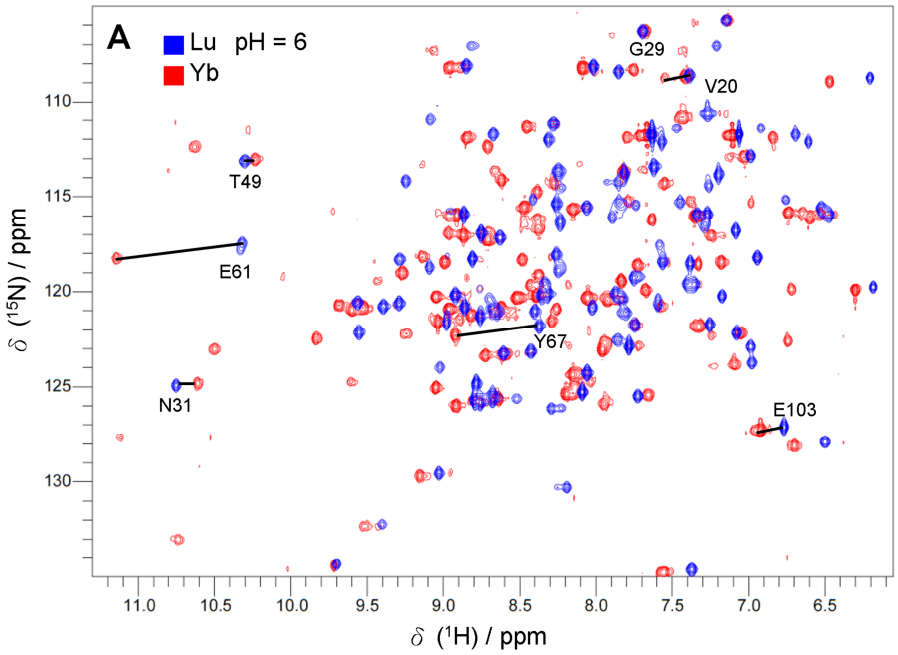
Design and synthesis of paramagnetic probes for structural biololgy

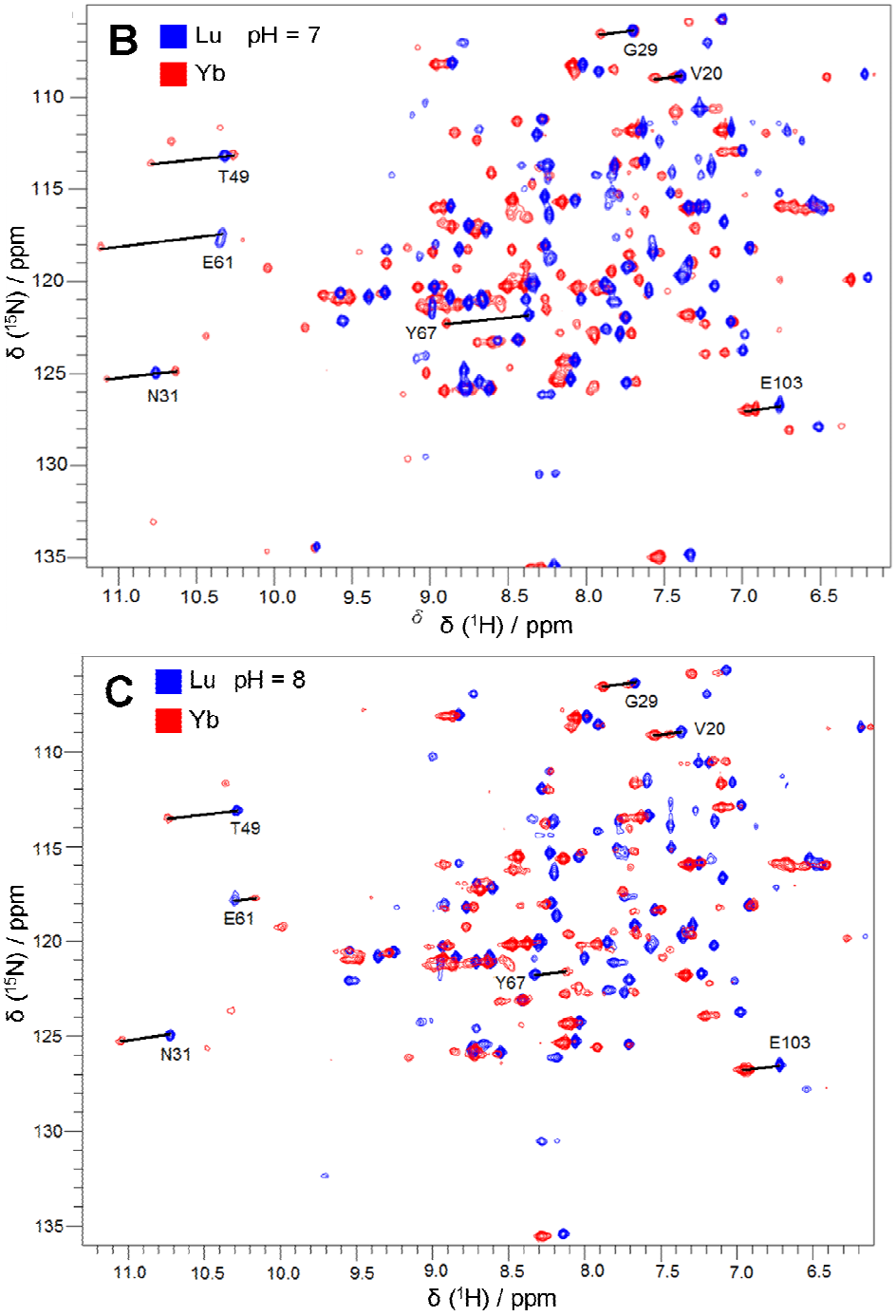
Liu, W.

Appendices

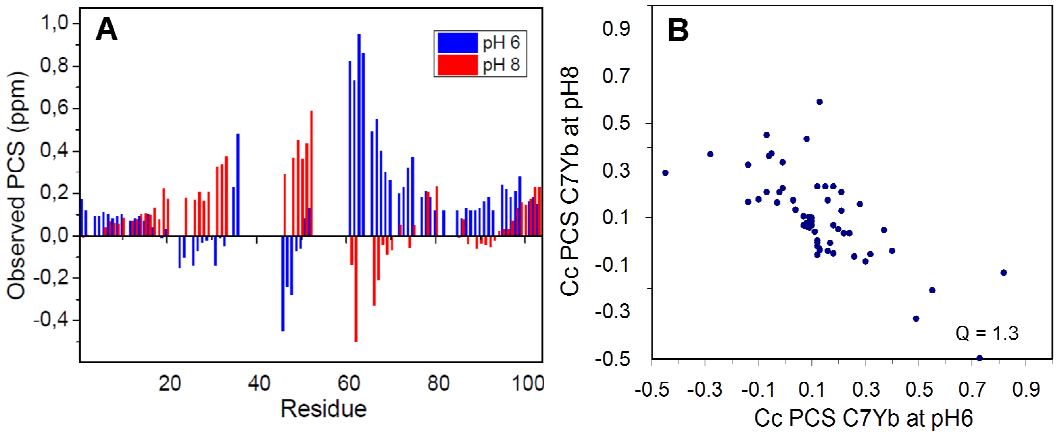


Appendix 1. Experimentally observed PCSs of Paz E51C/E54C Yb-CLaNP-7 plotted against the back-calculated PCSs (Q = 0.06). The solid line represents a perfect correlation.

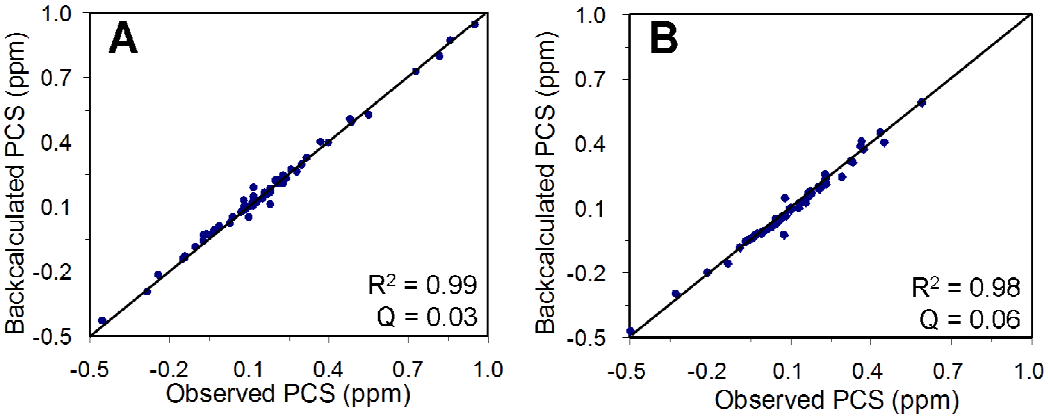




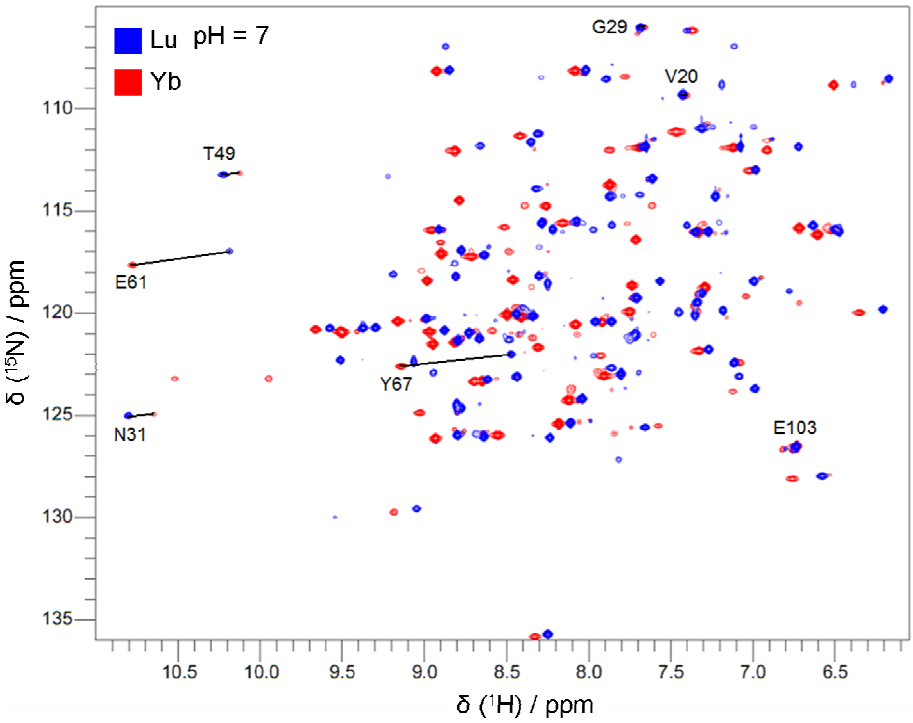
Appendix 2. pH dependence of CLaNP-7. [^{15}N, ^{1}H]-HSQC spectra of Cyt *c* N56C/L58C attached to Lu-CLaNP-7 (blue) and Yb-CLaNP-7 (red) at pH = 6.0 (A), pH = 7.0 (B) and pH = 8.0 (C). Several PCSs are indicated with solid lines.



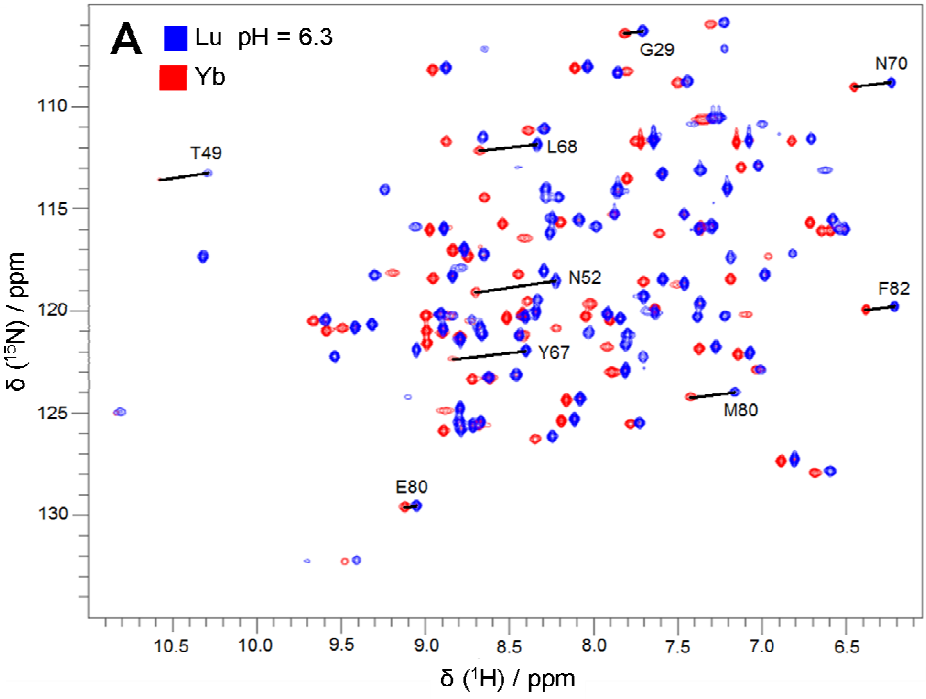
Appendix 3. Comparison of the PCSs at different pH. (A) The experimentally observed PCSs at pH 6 (blue) and 8 (Red); (B) Experimentally observed PCSs of Cyt *c* N56C/L58C Yb-CLaNP-7 at pH 6 plotted against the observed PCSs at pH 8 (Q = 1.3).

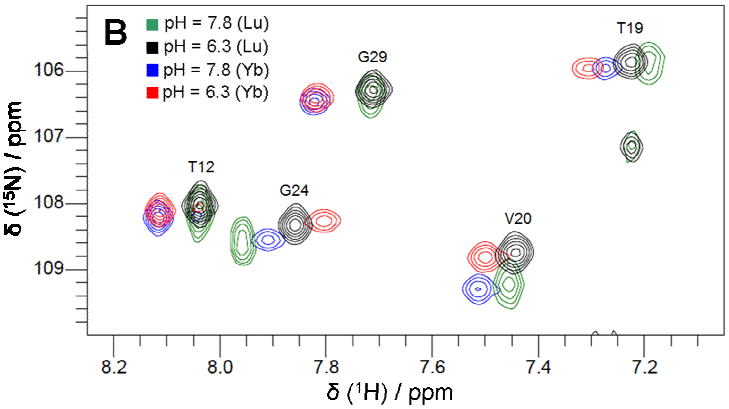


Appendix 4. Experimentally observed PCSs of Cyt *c* N56C/L58C Yb-CLaNP-7 plotted against the back-calculated PCSs at pH = 6 (A, Q = 0.03) and pH = 8 (B, Q = 0.06). The solid line represents a perfect correlation

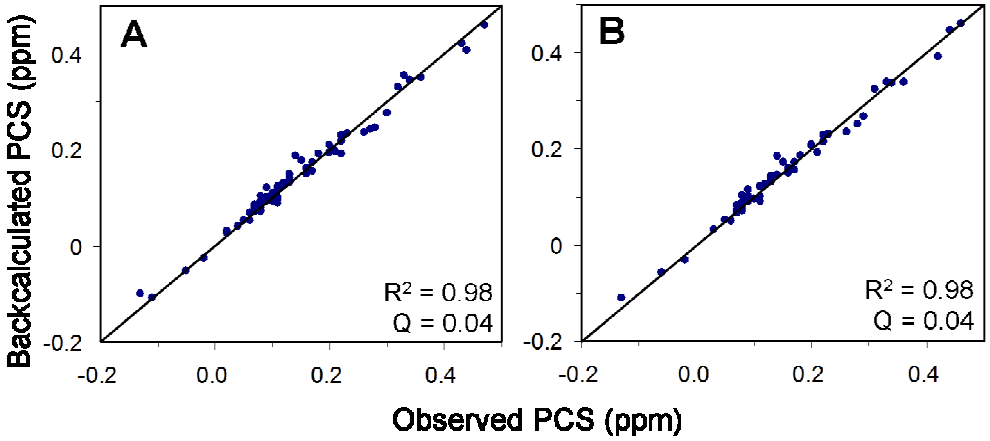


Appendix 5. CLaNP-5 linked to Cyt *c*. [^{15}N, ^{1}H]-HSQC spectra of Cyt *c* N56C/L58C attached to Lu-CLaNP-5 (blue) and Yb-CLaNP-5 (red) at pH = 7.0. Several PCSs are indicated with solid lines.

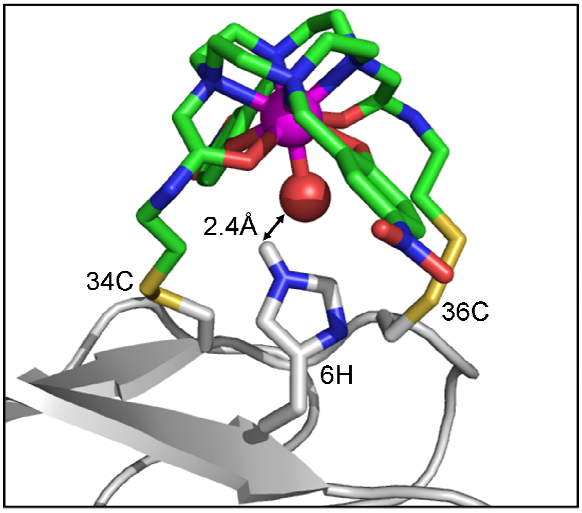




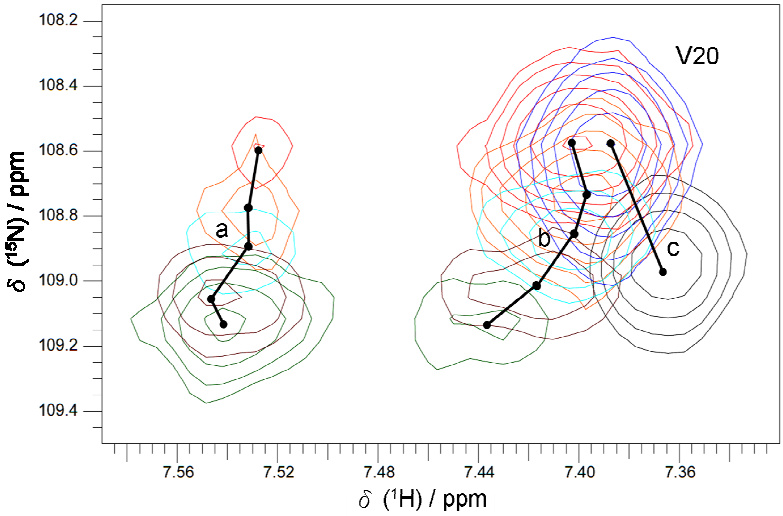
Appendix 6. Overlay of [^{15}N, ^{1}H]-HSQC spectra of Cyt *c* N56C/L58C/H39A attached to Lu-CLaNP-7 (blue) and Yb-CLaNP-7 (red) at pH = 6.3 (A); (B) Detail of [^{15}N, ^{1}H]-HSQC spectra of Ln-CLaNP-7 tagged ^{15}N-labeled Cyt *c* N56C/L58C/H39A. The spectra were recorded at pH = 6.3 (black, Lu; red, Yb) and 7.8 (green, Lu; blue, Yb).



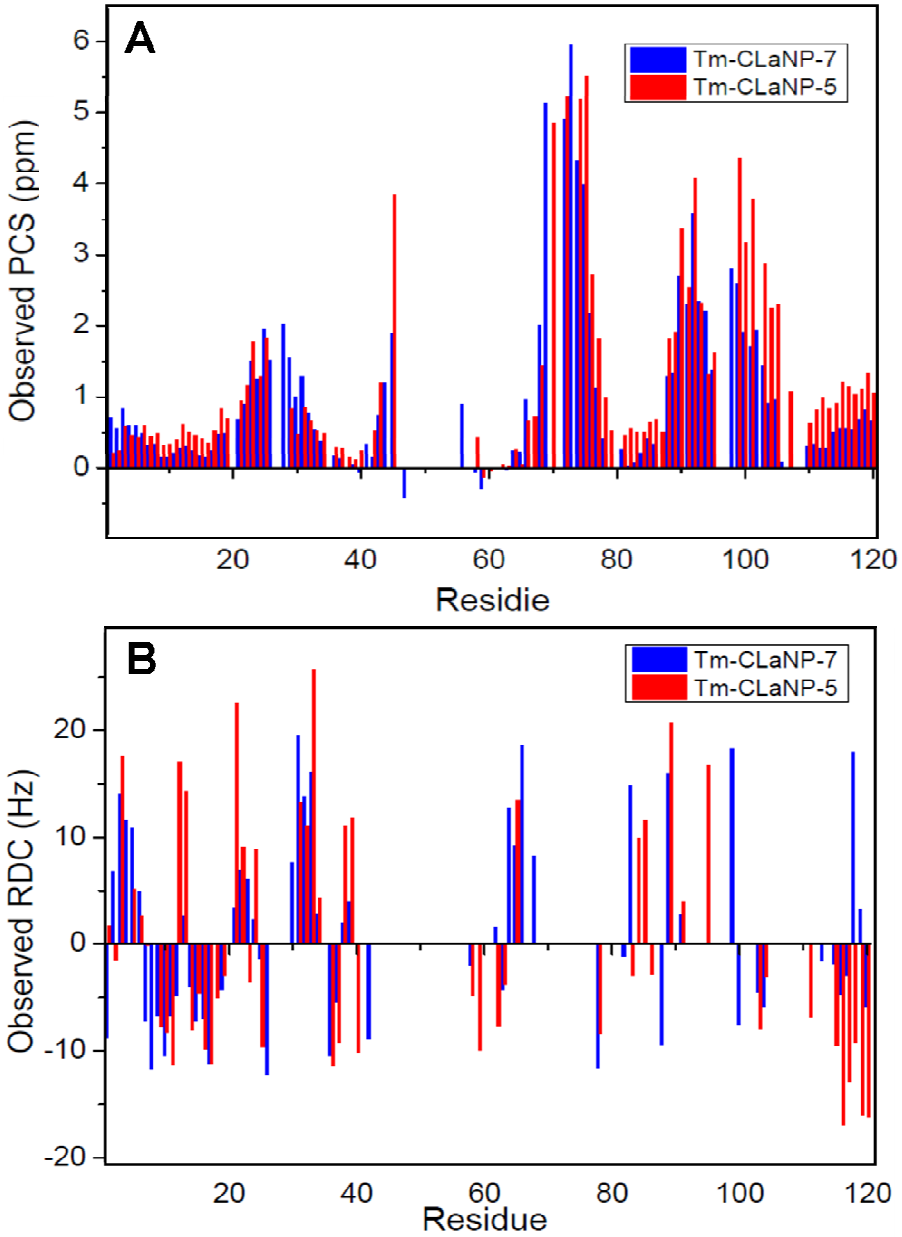
Appendix 7. Experimentally observed PCSs plotted against the back-calculated PCSs for Cyt *c* N56C/L58C/H39A Yb-CLaNP-7 at pH = 6.3 (A, Q = 0.04) and pH = 7.8 (B, Q = 0.04). The solid line represents a perfect correlation.



Appendix 8. Model of CLaNP-7 linked to C34, C36 and H6 of the I34C/V36C mutant of Paz. The position of Yb^{3+} is shown in magenta. The protein main chain is shown in grey. The Cys (I34C/V36C) and the His 6 side-chains are shown in CPK colors. The carbon atoms from CLaNP-7 are shown in green, nitrogen atoms in blue, oxygen atoms in red and sulfur atoms in yellow. The distance between the $H^{\varepsilon 2}$ of His 6 and the ligating oxygen atom is 2.4 Å.



Appendix 9. Detail of [^{15}N, ^{1}H]-HSQC spectra of Ln-CLaNP-7 tagged ^{15}N-labeled Cyt *c* N56C/L58C. The spectra of Lu^{3+} tagged protein were recorded at pH = 6 (blue) and 8 (black). The spectra of Yb^{3+} tagged protein were recorded at pH = 6.0 (red), 6.6 (orange), 7.0 (cyan), 7.4 (brown) and 7.9 (green). Shifting resonances are connected by lines (a = basic PCS, b = acidic PCS, c = diamagnetic basic shift).



Appendix 10. Comparison of CLaNP-5 and CLaNP-7. The experimentally observed PCSs (A) and RDCs (B) of Paz E51C/E54C Tm-CLaNP-7 (blue) and Tm-CLaNP-5 (red) are shown for each Paz residue.

List of Publications

1. Dasgupta, S.; Hu, X.; Keizers, P. H. J.; **Liu, W. -M.**; Luchinat, C.; Nagulapalli, M.; Overhand, M.; Parigi, G.; Sgheri, L.; Ubbink, M. "Narrowing the conformational space sampled by two domain proteins with paramagnetic probes in both domains" *J. Biomol. NMR* **2011**, *51*, 253-263.
2. **Liu, W. -M.**; Keizers, P. H. J.; Hass, M. A. S.; Blok, A.; Timmer, M.; Sarris, A. J. C.; Overhand, M.; Ubbink, M. "A pH-sensitive, colorful, lanthanide-chelating paramagnetic NMR probe" *J. Am. Chem. Soc.* **2012**, *134*, 17306-17313.
3. Guan, J.-Y.; Keizers, P. H. J.; **Liu, W. -M.**; Loehr, F.; Skinner, S. P.; Heeneman, E. A.; Schwalbe, H.; Ubbink, M.; Siegal, G. D. "Small molecule binding sites on proteins established by paramagnetic NMR spectroscopy" *J. Am. Chem. Soc.* **2013**, *135*, 5859-5868.
4. Hiruma, Y.; Hass, M. A. S.; Kikui, Y.; **Liu, W. -M.**; Olmez, B.; Skinner, P. S.; Blok, A.; Kloosterman, A.; Koteishi, H.; Loehr, F.; Schwalbe, H.; Nojiri, M.; Ubbink, M. 'The structure of the Cytochrome P450cam-Putidaredoxin complex determined by paramagnetic NMR spectroscopy and crystallography' (*J. Mol. Biol.* **2013,** DOI: 10.1016/j.jmb.2013.07.006)
5. **Liu, W. -M.**; Overhand, M.; Ubbink, M. 'The application of paramagnetic lanthanoid ions in NMR spectroscopy on proteins' (*Coord. Chem. Rev.* **2013**, under revision)
6. **Liu, W. -M.**; Skinner, S. P.; Timmer, M.; Blok, A.; Filippov, D.; Overhand, M.; Ubbink, M. 'A two-armed lanthanoid-chelating paramagnetic NMR probe linked to proteins via thioether linkages' manuscript to be submitted (**2013**)

Curriculum vitae

Wei-Min Liu, who has always been interested in science, was born on 26th of August 1983 in Kaohsiung, Taiwan. When he was a high school student, he joined a "National and Private High School Science Fair" and got an award of excellent work. After high school, he was recommended to National Taiwan Normal University and obtained his bachelor degree in 2005. After his graduation, he began his master degree in the field of organic chemistry at National Taiwan University and started his first research project, which was correlative with drug discovery, under the supervision of Prof. Chao–Tsen Chen. He obtained his master degree in July 2007. After his master, he left the academic field and was a high school chemistry teacher to have a break from study and to find his own way for his future career. During this period, he supervised his students in their perparation and attendance of the high school science fair and realized his commitment to research. Therefore, he decided to come back the academic field. He started in September 2009 as a Ph.D student in the group of Protein Chemistry, Leiden Institute of Chemistry, under supervision of Prof. Dr. Marcellus Ubbink. During his Ph.D, he designed and synthesized several paramagnetic probes for the study of protein-protein and protein-ligand interactions in close collaboration with Dr. Mark Overhand in the Bio-organic synthesis group. The results of this research are described in this thesis.