A General Strategy for the Bioorthogonal Incorporation of Strongly Absorbing, Solvation-Sensitive Infrared Probes into Proteins

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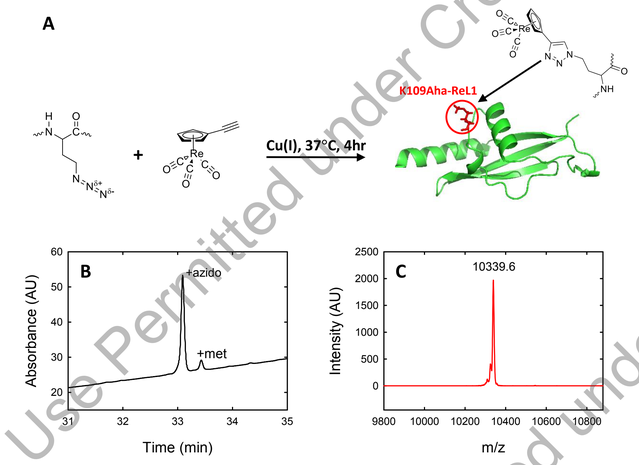
Abstract

A high sensitivity metal carbonyl based IR probe is described which can be incorporated into proteins or other bio-molecules in very high yield via Click chemistry. A two step strategy is demonstrated. First, a methionine auxotroph is used to incorporate the unnatural amino acid azidohomoalanine at high levels. Second, a tricarbonyl (η^5-cyclopentadienyl) rhenium(I) probe modified with an alkynyl linkage is coupled via the Click reaction. We demonstrate these steps using the C-terminal domain of the ribosomal protein L9 as a model system. An overall incorporation level of 92 % was obtained at K109, which is a surface exposed residue. Incorporation of the probe into a surface site is shown not to perturb the stability or structure of the target protein. Metal carbonyls are known to be sensitive to solvation and protein electrostatics through vibrational lifetimes and frequency shifts. In this paper, we report that the frequencies and lifetimes of this probe also depend on the isotopic composition of the solvent. Comparison of the lifetimes measured in H_2O versus D_2O provides a probe of solvent accessibility. The metal carbonyl probe reported here provides an easy and robust method to label very large proteins with an amino acid specific tag that is both environmentally sensitive and a very strong absorber.

Bioorthogonal Incorporation of Metal Carbonyl IR Probes

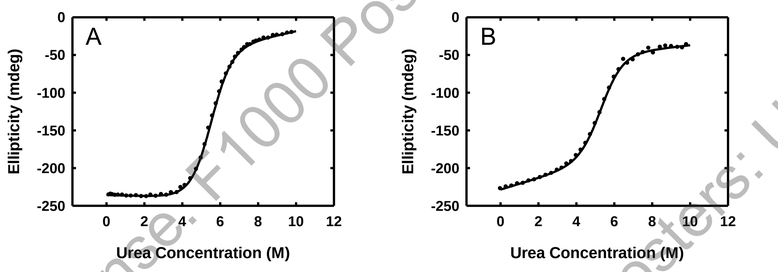


A) Azidohomoalanine (Aha) is incorporated using methionine auxotrophs and the probe attached using the click reaction. The site chosen for substitution is residue 109 of the protein CTL9, located in a loop.

B) LC trace from the LC-MS analysis of a tryptic digest indicates an incorporation of Aha of 92%. The LC trace is shown for the peptide which contains residue 109.

C) MALDI-TOF mass spectroscopy confirms the identity of the metal carbonyl labeled product. The expected m/z is 10340.0 and the observed value is 10339.6.

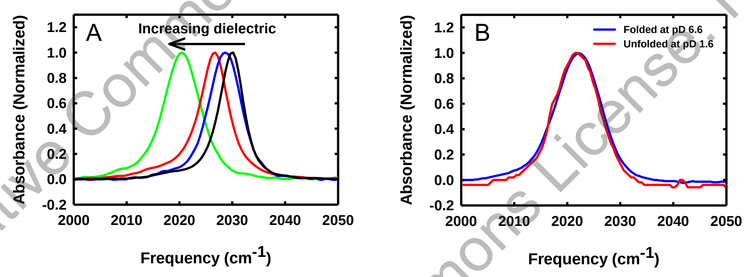
The Probe Does Not Significantly Modify the Stability of the Protein



	ΔG° (kcal mol^{-1})	m (kcal mol^{-1} M^{-1})	T_m (°C)*	ΔH° (at 78°C) (kcal mol^{-1})*
(A) Wild-type	6.00 ± 0.11	1.09 ± 0.02	78.1 ± 0.3	68.9 ± 2.0
(B) K109ReL1	5.48 ± 0.27	1.03 ± 0.05	74.2 ± 0.2	65.4 ± 1.7

* These values were determined from thermal denaturation experiments (not shown)

The Frequency of the Metal Carbonyl Probe is Sensitive to Environment

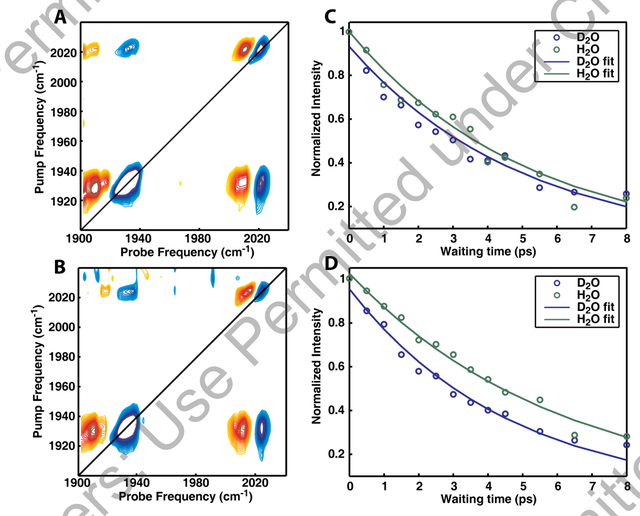


Solvent	Band position (cm^{-1})	FWHM (cm^{-1})	Dielectric constant
1:1 CH_2Cl_2:CCl_4	2029.8	4.7	9.1,2.2*
isopropanol	2028.6	5.7	17.9
acetonitrile	2026.5	6.0	37.5
DMSO	2020.4	6.6	46.7

* The dielectric constant of neat CH_2Cl_2 is 9.1 and that of neat CCl_4 is 2.2

B. The vibrational frequency is similar in the folded and unfolded states, as is expected for a solvent exposed site.

Two-dimensional IR Spectra of the Labeled Protein Differ in H_20 and D_20



(A) 2D IR Spectrum in H_2O, (B) 2D IR Spectrum in D_2O, (C) Intensity of the diagonal peak of the symmetric stretch (circles) with single exponential fits (lines), (D) Intensity of the diagonal peak of the asymmetric stretch (circles) with single exponential fits (lines)

	Symmetric stretch		Asymmetric stretch	
	Frequency (cm^{-1})	Lifetime (ps)	Frequency (cm^{-1})	Lifetime (ps)
H_2O	2021	5.17 ± 0.64	1930	6.10 ± 0.48
D_2O	2024	5.04 ± 0.66	1932	4.66 ± 0.57

Conclusions

- We have presented a general method for the high-level incorporation of a metal carbonyl IR probe that is broadly applicable.
- The band position is sensitive to the environment and solvent isotope effects can be exploited to interrogate accessibility.
- The methodology presented will facilitate IR-based investigations of protein ligand interactions and protein conformational changes, as well as provide site-specific structural information.
- The strategy can be used with Cys labeling, enabling structural and environmental probes at two sites simultaneously via multicolor experiments.

References

Peran, I.; Oudenhoven, T.; Woys, A. M.; Watson, M. D.; Zhang, T. O.; Carrico, I.; Zanni, M. T.; Raleigh, D. P. General Strategy for the Bioorthogonal Incorporation of Strongly Absorbing, Solvation-Sensitive Infrared Probes into Proteins. *J. Phys. Chem. B* **2014**, *118*, 7946-7953.

Acknowledgements

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