simple model indicates that the optimization of several experimental handles may lead to the assembly of large-scale structures. For example, maximizing the length and monodispersity of the SWNT ropes, as well as controlling dielectric screening and SWNT charge density, are all identified as key handles for improving this process. Furthermore, the frequency of the orienting AC field represents a variable that has not yet been optimized. We found little frequency dependence to this process between 10^4 Hz and 10^6 Hz. This is likely because the intertube Coulomb repulsions are dominant in this regime. However, at much higher frequencies, our calculations suggest that the polarizability of the tubes should become important, at which point the circuit pitch may decrease as it approaches the limit for infinite rods (Figure 2). We are currently designing experiments at $>10^9$ Hz deposition frequencies to explore this effect. We remark that none of these experimental handles to circuit fabrication require the use of lithographic techniques. Thus, relatively inexpensive routes toward fabricating designed circuits with characteristic dimensions on the order of a few nanometers may be possible.

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Protein Affinity Labeling Mediated by Genetically Encoded Peptide Tags**

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Affinity labeling is a powerful method by which protein residues in the immediate vicinity of a labeled ligand can be identified. Most such experiments employ latently photoactivatable groups such as benzophenones[1] or azides[2] attached covalently to a ligand of interest. When photolyzed, the resultant intermediate inserts into a carbon–hydrogen bond of the protein which results in the covalent coupling of the molecule of interest with its receptor. These experiments generally have been employed to identify active site residues proximal to the site of ligand or substrate binding.[3, 4]

Traditional affinity-labeling techniques have limitations when the ligand of interest is a protein and the goal is to identify the binding partners of that protein. With the exception of small proteins that contain only a single nucleophilic cysteine,[5] it is difficult to produce site-specifically labeled proteins by standard conjugation chemistry. An appealing solution to this problem would be to develop a genetically encoded peptide tag capable of binding an affinity-labeling reagent with high specificity. We demonstrate here that the complex of Ni^{II} with the tripeptide NH_2-glycine-

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glycine-histidine (NH_2-GGH) or the hexahistidine peptide (His_6) can mediate biotinylation of proximal proteins by an oxidatively triggered reaction. This method should be useful for labeling and identifying the binding partners of a given protein.

NH_2-GGH · Ni^{II} is a stable complex[6] that, when activated by a peracid,[7] mediates several oxidative reactions[8–10] including protein cross-linking, a process that is thought to be initiated by NH_2-GGH · Ni^{III}-mediated oxidation of tyrosine.[11, 12] In analogy with previous work with antibody–horseradish peroxidase conjugates,[13, 14] it was anticipated that oxidative activation of the nickel center in the presence of high levels of free biotin-tyramine would lead to the formation of a tyramine radical. Subsequent radical coupling to suitable groups on the surfaces of nearby proteins would mark them with biotin (Scheme 1).

To test this, NH_2-GGH-ecotin,[12] a homodimeric macromolecular serine protease inhibitor, was incubated in the presence of Ni^{II}, the peracid magnesium monoperoxyphthalate (MMPP), and biotin-tyramine. One tenth of the sample was then separated by SDS-PAGE, transferred onto a nitrocellulose membrane, and probed with avidin, which binds biotin tightly and specifically (see Supporting Information for a detailed protocol). Four major biotinylated species were detected which had the mobilities expected of the ecotin monomer, dimer, and two multimers. This indicates that both biotinylation and protein cross-linking[11, 12, 15–17] had occurred (Figure 1, lane 1). Biotin-tyramine, Ni^{II}, MMPP, and the NH_2-GGH tag were each required for labeling (Figure 1, lanes 2–5). Quantification of the level of biotinylation revealed that, on average, three to four molecules of biotin-tyramine were coupled to one molecule of ecotin (see Supporting Information).

After we had demonstrated that the NH_2-GGH · Ni^{II} complex can support the basic reaction, we investigated the more interesting possibility of labeling a binding partner of the tagged protein. Ecotin is known to inhibit trypsin with a K_i in the nanomolar range.[18] When the GGH-ecotin labeling experiment was repeated in the presence of trypsin, the protease was also biotinylated (Figure 2, lane 2). Biotinylated

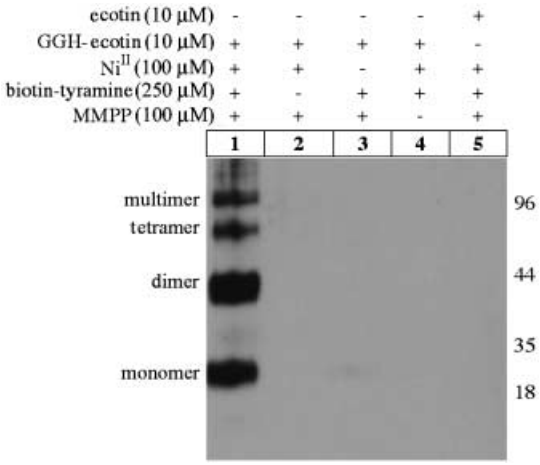


Figure 1. The NH_2-GGH tag supports the self-labeling of a tagged protein in the presence of Ni^{II}, biotin-tyramine, and MMPP (lane 1). The GGH tag, Ni^{II}, biotin-tyramine, and MMPP are all required for oxidative labeling (lanes 2–5). One tenth of the sample was separatated by SDS-PAGE, transferred to nitrocellulose, and probed with avidin-HRP.

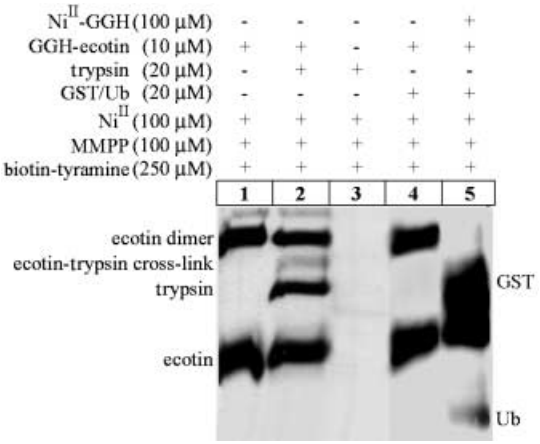
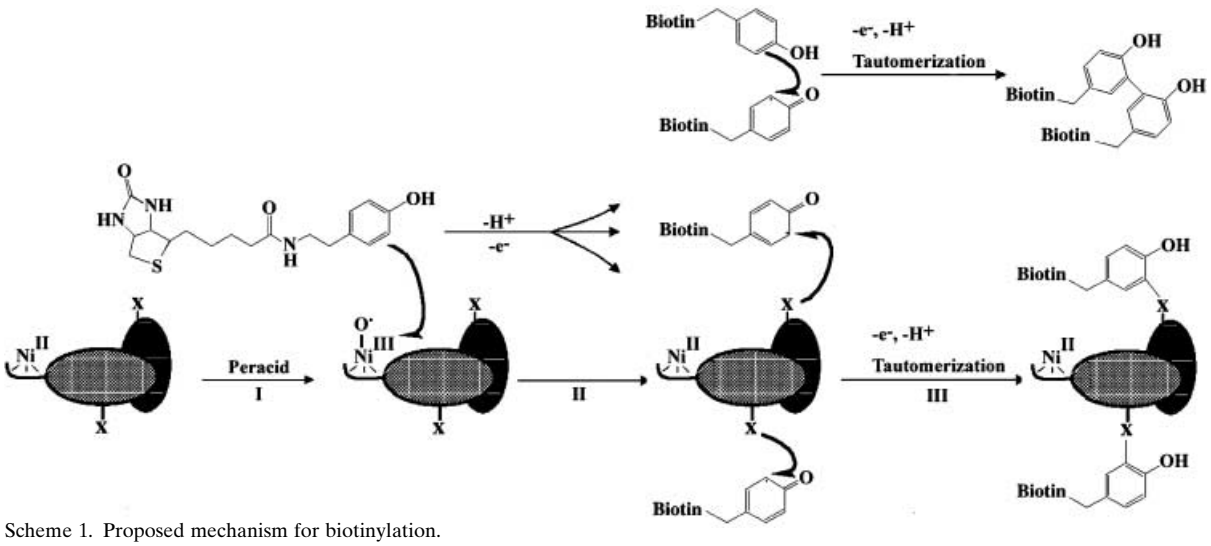


Figure 2. The NH_2-GGH tag supports the localized labeling of a tagged protein's binding partner but not to noninteracting proteins. One tenth of the sample was separated by SDS-PAGE, transferred to nitrocellulose, and probed with avidin-HRP.



Scheme 1. Proposed mechanism for biotinylation.

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trypsin was not detected in the absence of GGH-ecotin, which rules out the possibility of self-labeling as a result of spurious metal-binding sites on the protease (Figure 2, lane 3).

For this methodology to be useful for probing protein–protein interactions, labeling must be limited to proteins that are in close proximity to the NH_2-GGH · Ni^{II} tag. To probe this point, glutathione *S*-transferase (GST) and ubiquitin (Ub) were included in the reaction along with GGH-ecotin. Only GGH-ecotin was labeled (Figure 2, lane 4), which indicates that the affinity-labeling chemistry is localized. However, when exogenous NH_2-GGH-COOH · Ni^{II} was added to the solution, all three proteins were labeled (Figure 2, lane 5). This observation confirmed that the lack of biotinylation of GST and Ub did not result from their lack of suitable reactive groups on their surfaces.

To probe the issue of nonspecific labeling in a more demanding venue, biotinylation experiments were conducted with phage fd-tet[19] genetically modified to display GGH at the N-terminus of gpIII. Three to five copies of gpIII are displayed at one end of the filamentous phage particle, whereas 2800 copies of gpVIII constitute most of the rest of the phage surface.[20] Incubation of a GGH-gpIII fd-tet phage strain with Ni^{II}, biotin-tyramine, and MMPP resulted in the exclusive labeling of gpIII (Figure 3, lane 1). However, the incubation of the phage with exogenous NH_2-GGH · Ni^{II}, MMPP, and biotin-tyramine resulted in the biotinylation of both coat proteins (Figure 3, lane 2), which confirms that gpVIII can be labeled, albeit less efficiently,[21] by this chemistry.

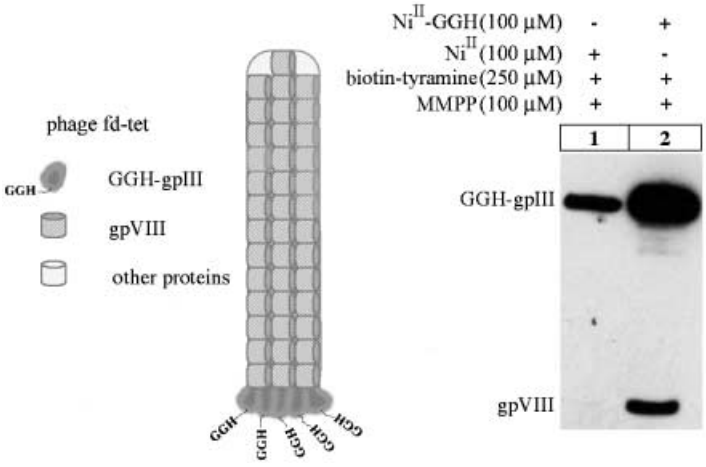

Figure 3. Affinity labeling of the phage fd-tet outer coat protein NH_2-GGHgpIII. 5×10^{11} phage particles were treated with biotinylation reagents. One tenth of the sample was separatated by SDS-PAGE, transferred to nitrocellulose, and probed with avidin-HRP.

The lack of detectable intramolecular labeling of gpVIII in the above experiment, despite its abundance, and the inability of NH_2-GGH-ecotin to label GST and Ub argue that NH_2-GGH · Ni^{II}-mediated biotinylation has a sufficiently short "range" that proteins not associated with the tagged protein are not biotinylated to a significant degree. This is likely a result of the dimerization of biotin-tyramine through a phenolic coupling mechanism if the radical form escapes into bulk solution (Scheme 1).

A limitation of the NH_2-GGH tag is that it binds Ni^{II} only when present at the N-terminus of a protein. This is because the terminal main chain amino group is directly involved in metal complexation.[22] While the GGH tag can be revealed on the N-terminus by site-specific proteolysis,[23] the utility of affinity labeling could be expanded if other more versatile peptide tags were able to mediate the biotinylation chemistry. The His_6 tag also binds Ni^{II}, albeit not as well as NH_2-GGH, and can do so when placed anywhere in a polypeptide, so long as protein function is not compromised by the insertion. Furthermore, His_6 · Ni^{II} complex, like NH_2-GGH · Ni^{II}, supports oxidative protein cross-linking,[16, 17] which suggests that it might be active in biotinylation as well.

A His_6-tagged derivative of the dimeric yeast Gal80 transcriptional repressor, where the tag represents residues 5–10 of the fusion protein, was expressed and purified.[24] A GST fusion protein with the C-terminal activation domain of Gal4 (GST-AD), Gal80's native binding partner,[25] was also isolated. Under the same conditions used for NH_2-GGH-Ni^{II}-mediated reactions, the His_6 tag also supports the localized oxidative biotinylation of a tagged protein and its target. As shown in lane 1 of Figure 4, both the monomeric

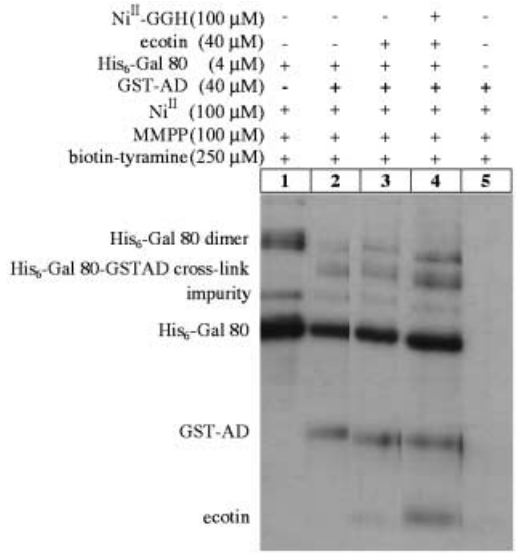

Figure 4. The His_6 tag mediates localized affinity labeling. Reactions were performed under the same conditions used for NH_2-GGH-Ni^{II}-mediated reactions. One tenth of the sample was separatated by SDS-PAGE, transferred to nitrocellulose, and probed with avidin-HRP.

and dimeric cross-linked products of His_6-Gal80 were biotinylated in the presence of Ni^{II}, MMPP, and biotin-tyramine. More importantly, GST-AD was also biotinylated when it was included in the reaction (Figure 4, lane 2). A new species with the molecular weight of a GST-AD-Gal80 cross-linked product was also biotinylated. However, only trace amounts of untagged ecotin were labeled when it was included (Figure 4, lane 3). This provides evidence that the His_6 tag mediates localized affinity labeling. A control reaction with exogenous NH_2-GGH-COOH · Ni^{II} complexes again resulted in the biotinylation of all of the proteins present (Figure 4, lane 4). Likewise, the failure to detect biotinylated GST-AD

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products in the absence of His_6-Gal80 ruled out the possibility of GST-AD self-labeling (Figure 4, lane 5). Biotinylation was not detected when the His_6 tag was removed from Gal80, or when Ni^{II} MMPP or biotin-tyramine were omitted from the reaction (data not shown).

The detailed mechanism of the affinity-labeling reaction remains to be elucidated. However, it most likely proceeds through initial oxidation of the Ni^{II}-peptide complex by MMPP to form a high valent Ni^{III}–oxo species,[7] which subsequently abstracts an electron from the phenol group of a biotin-tyramine. The radical formed following loss of a proton from the radical cation could then couple to tyrosine or other suitably reactive amino acids on a tagged protein and any other proteins in the immediate vicinity. It is speculated that protein biotinylation is localized to the immediate region of the metal-binding tag by efficient quenching of the radical by excess biotin-tyramine in solution through phenolic coupling (Scheme 1).

In summary, we have demonstrated that two simple Ni^{II}–peptide complexes can mediate localized affinity-biotinylation reactions. This is a potentially useful tool for studying protein–protein interactions, particularly in the context of large complexes where it is difficult or impossible to label a particular protein uniquely with an azide- or benzophenone-containing reagent by using standard methods. Experiments in which this system is applied to such complex systems are underway.

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Expanding Divalent Organolanthanide Chemistry: The First Organothulium(II) Complex and the In Situ Organodysprosium(II) Reduction of Dinitrogen**

William J. Evans,* Nathan T. Allen, and Joseph W. Ziller

Although historically the organometallic chemistry of the divalent lanthanides has involved just three ions, Eu^{2+}, Yb^{2+}, and Sm^{2+}, these elements have provided an extensive series of unusual structures and reactions.[1] Recently, however, opportunities have arisen to expand divalent lanthanide chemistry to other metals in the series.[2] The isolation of the first molecular complex of divalent thulium, $[TmI_2(dme)_3]$ (dme = dimethoxyethane) in 1997,[2a] provided an analogue of the commonly used $[SmI_2(thf)_x]$,[3] a precursor to organosamarium(II) complexes such as $[(C_5Me_5)_2Sm(thf)_x]$.[4] Ionic metathesis reactions with $[TmI_2(dme)_3]$ could provide a direct route to divalent organothulium complexes, but the high reactivity of Tm^{2+} made this challenging (reduction potentials versus NHE: Tm^{2+} –2.3 V; Sm^{2+} –1.5 V).[5] In fact, since 1997 only one other Tm^{2+} derivative has been reported in the literature.[6]

The discovery of TmI_2 as an in situ organic reagent[7] initiated a study of the organometallic chemistry of Tm^{2+} showing why organothulium(II) complexes are so difficult to isolate. Nitrogen is not inert to these species; they react to form complexes such as $[\{[C_5H_3(SiMe_3)_2]_2Tm\}_2N_2]$, $[\{(C_5Me_5)_2Tm\}_2N_2]$, and $[\{(C_5H_4SiMe_3)_2Tm(thf)\}_2N_2]$.[8] Initial attempts to isolate organothulium(II) complexes under argon led to decomposition of the diethyl ether solvent to make ethoxide and oxide products.[8] Herein, we report that by choosing the proper combination of solvents, ligand, and reaction conditions, the first organometallic complex of Tm^{2+} has been isolated and structurally characterized.

Although the reaction of KC_5Me_5 with $[TmI_2(thf)_3]$ in Et_2O under argon leads to diethyl ether decomposition, the use of

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