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NICKEL-NTA-NANOGOLD BINDS HIS-TAGGED PROTEINS

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Six histidines added to expressed proteins have been a boon for rapidly purifying them from the expression organism lysate, since it was found that the 6x-His tag specifically binds (reversibly) to columns containing Ni^{+2} [1] . The nickel is chelated to the column with nitrilotriacetic acid (NTA), which is similar to EDTA. Since many proteins now have His-tags, and cells can be transfected to produce His-tagged proteins, it is of interest to have a gold label that also binds specifically to this tag. One application is identifying a specific protein against a background, for example in cell sections to see the localization of an expressed protein, much like green fluorescent protein; however, the gold label is visible in the EM for higher resolution studies; it can also be silver or gold enhanced to visualize it in the light microscope, or on blots. Another application is to use it as a molecular domain or subunit label for high resolution single particle analysis. Here, since the position of the His-tag is known, e.g., the amino terminus, that part of the molecule can be locally labeled and useful for cryoEM reconstructions. It would also serve as a high visibility fiducial mark for orienting single particles at low dose, perhaps permitting extension of the resolution obtainable by such techniques. For multi-subunit complexes, a particular subunit may be labeled to specifically identify it in the complex. In x-ray or electron protein crystallography, the label could be used to improve contrast or be used as a phasing aid. Another difference in this type of label is that the Ni-NTA group is quite small compared to an antibody, thus bringing the gold particle much closer to the 6x-His-tag giving higher resolution labeling with less ambiguity or floppiness. The smaller size will also improve its diffusion into cells or tissues.

Two papers have appeared using Ni-NTA-Nanogold [2,3]. These were based on first generation constructs. Several potential problems may occur with this type of probe. Since each Ni atom can only bind two histidines (even in column use), there should be three Ni-NTA groups per gold to fully complex a 6x-His tag and provide maximum affinity. Preparing a gold particle with exactly 3 Ni-NTA groups in the optimal conformation is a challenge and if additional groups are present, this can lead to aggregation of His-tagged proteins in solution, which was observed in some cases. Reducing the number of Ni groups lowers the gold's affinity. The linker arm from the gold to the NTA group may also be varied to find the optimal structure. A number of different Ni-NTA-gold cluster constructs were therefore synthesized varying the linker and the number of NTA groups. Although further work is in progress, some of these preparations have produced high binding to His-tagged proteins in solution without aggregation. Control samples were the same protein, but without the 6x-histidine residues, and these showed virtually no labeling. An example is shown in Fig. 1.

References

[1] E. Hochuli et al. J Chromatogr, 411 (1987) 177.
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[3] C. Buchel C et al. J Mol Biol. 312 (2001) 371.
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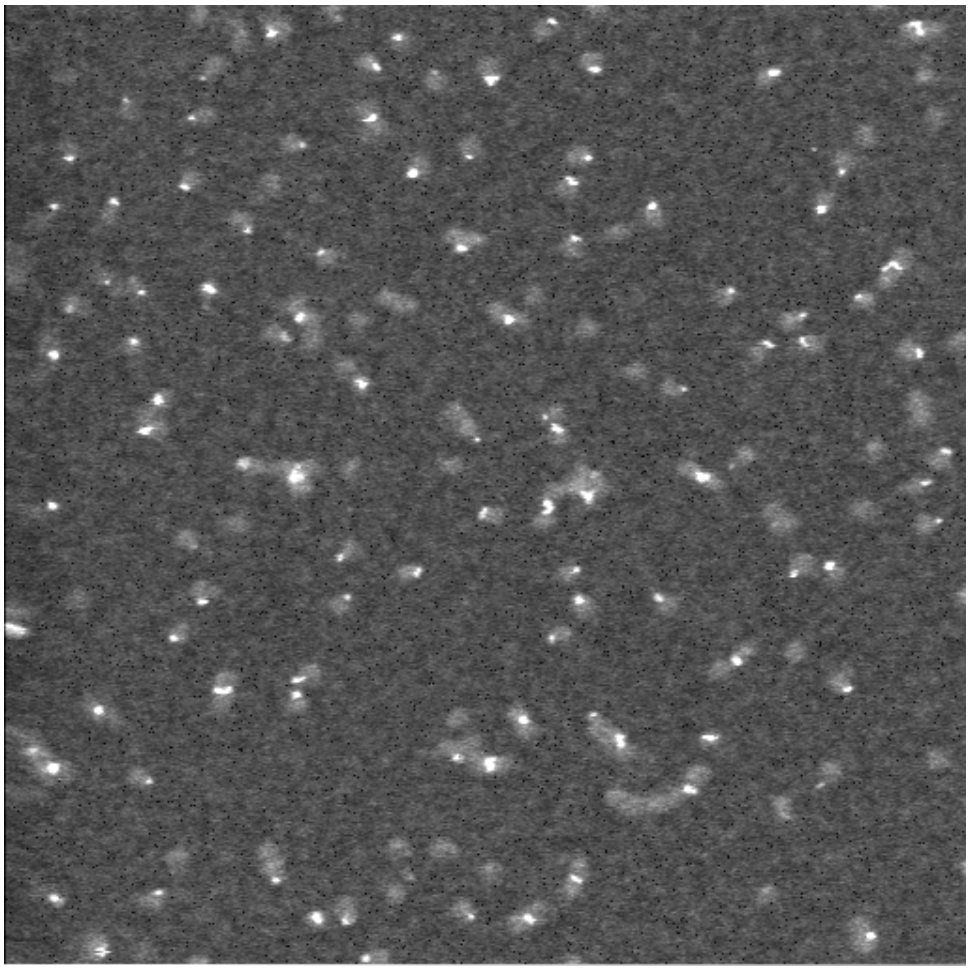

Fig. 1. Knob protein from adenovirus cloned with 6x-His tag, labeled with Ni-NTA-Nanogold, column purified from excess gold, and viewed in the Brookhaven STEM unstained. 256 nm full width.

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