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High Z Metal Carbonyls for Imaging and Microspectroscopy

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The power of microscopic and crystallographic techniques in structure-function analysis of multi-protein complexes can be greatly enhanced by the use of high Z or heavy atom labels introduced at specific sites [1]. Heavy metal cluster complex labels that can be derivatized for site-specific covalent attachment to macromolecules have clear advantages over heavy metal salts [1,2]. We have commercialized combined fluorescent and heavy metal probes that enable collection of complimentary sets of data, from fluorescence and electron microscopy, for correlative microscopic studies of biological targets at different spatial resolutions [3]. We herein report combined heavy metal probes that will enable detection and spatially resolved chemical analysis of biological samples.

Recently, we and others reported construction of electron density maps of tetra-iridium (Ir_4) cluster labeled virus capsids and ribosome particles by cryo-EM and X-ray crystallography respectively at medium to high resolution [1, 4]. These Ir_4 clusters contain carbonyl ligands that exhibit extremely intense infrared (IR) absorptions in the region where most of the biological materials do not absorb (Fig. 1b). This unique feature of metal carbonyls has led to the development of non-isotopic IR spectroscopic immunoassays [5]. We recently demonstrated that functionalized heavy atom carbonyl cluster labeled reagents can be used for bio-sensing/bio-recognition using Fourier transform infrared (FTIR) spectroscopy, albeit with lower detection sensitivities than competing technologies [6]. To demonstrate the use of heavy metal carbonyl clusters as dual labels for electron microscopy and FTIR microspectroscopy, we conjugated the previously reported Ir_4 label [4] to freshly reduced goat anti-rabbit F(ab') fragments following standard protocols [7]. The Ir_4 labels are clearly visible in dark-field scanning transmission electron micrograph (STEM) of the Ir_4-labeled F(ab') conjugate (Fig. 2a). The Ir_4-F(ab') conjugate was captured by 100 ng of covalently immobilized rabbit IgG on IR reflective slides following reported procedures [6,8]. The Ir_4 labels could be localized at 10x10 micron resolution by FTIR microspectroscopy (Fig. 2b).

Since IR microspectroscopic imaging has been used to distinguish chemical differences between healthy and diseased tissues [9], we envisage the use of metal carbonyl cluster labels for rapid localization of diseased tissues. Subtraction of the Ir-$_4$ label spectrum from that of localized targets will decipher chemical information and underlying chemistry which other methods, such as light or fluorescence microscopy, do not. The Ir_4 labels reported here have active Raman vibrations. Since Raman microspectroscopy has also been used to distinguishing healthy and diseased tissues, we are investigating the use of metal carbonyls as labels for Raman microspectroscopy.

References

[1] S. Weinstein et al., J. Structural Biol. 127 (1999) 141.
[2] J. Thygesen et al., Structure 4 (1996) 513.

[3] R. D. Powell and J. F. Hainfeld, in: Gold and silver staining: techniques in molecular morphology, Hacker G.W., and Gu, J. (Eds): CRC Press, Boca Raton, FL (2002) 107.
[4] N. Cheng et al., J. Structural Biol. 127 (1999) 169.
[5] D. Osella et al., Bioconjugate Chem., 10, (1990) 607.
[6] V. Joshi et al., Advance ACS abstracts 226 (2003) U 683.
[7] http://www.nanoprobes.com/LGuide1.html
[8] R. Moller et al, Nucleic Acids Res., 28, 91 (2002).
[9] D. L. Wetzel and S. M. LeVine, Science 285 (1999) 1224.

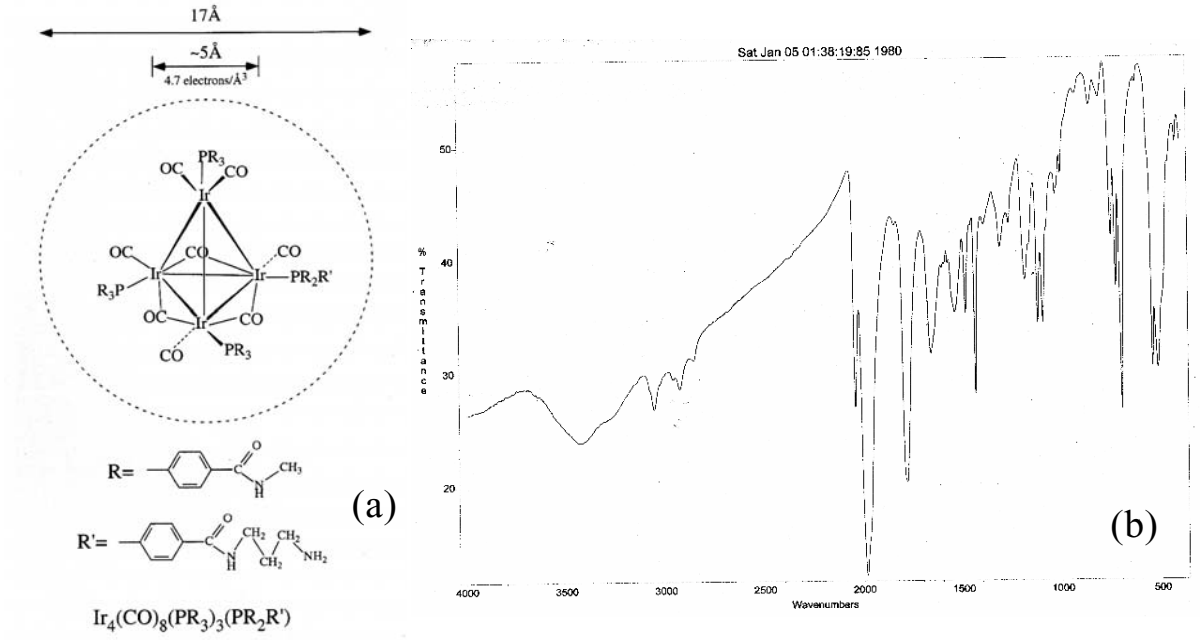

Fig. 1. Schematic diagram of the Ir_4 cluster label (a) and its IR spectrum (b).

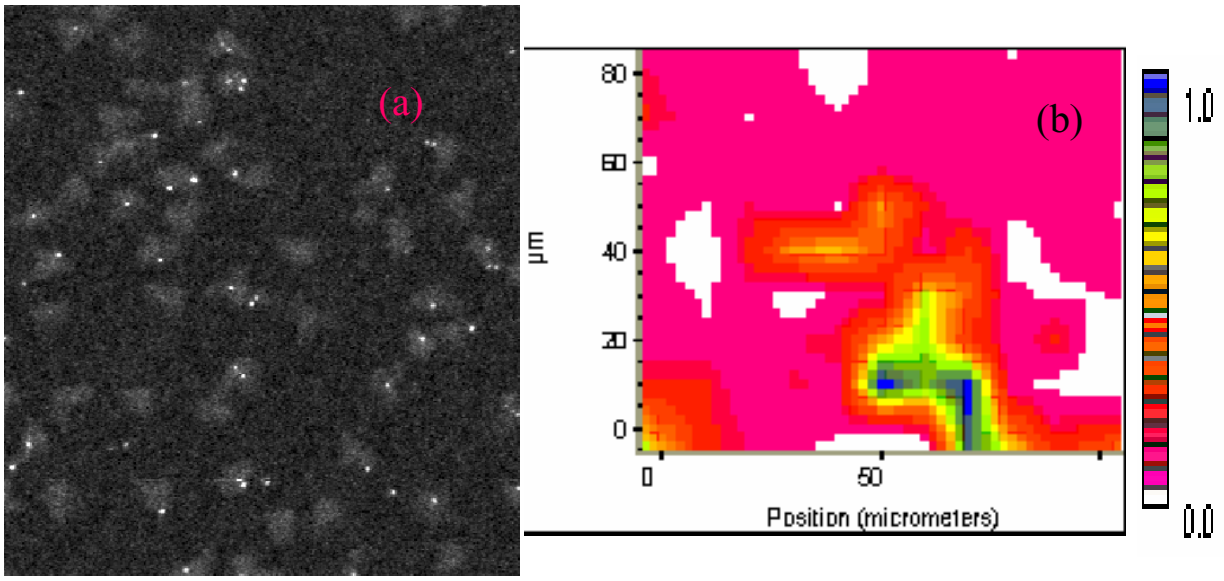

Fig. 2. a) STEM of Ir_4-F(ab') (full image width is 128 nm), and b) IR image of Ir_4-F(ab') captured by immobilized IgG (calculated as peak height ratio of 1990/2150 cm^{-1}; the blue colored regions indicate highest concentration).

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