

W0038

Phasing on an In-House X-Ray Source Using the Site-Specific Incorporation of *p*-Iodo-L-Phenylalanine Into Proteins. Glen Spraggon¹, Jianming Xie², Lei Wang², Ning Wu², Ansgar Brock¹ & Peter G. Schultz^{1,2} ¹Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Dr., San Diego, CA 92121, ²The Scripps Research Inst., 10550 North Torrey Pines Rd., La Jolla, CA 92037

Studies have shown that the single wavelength anomalous dispersion (SAD) method may be used to derive phases from native proteins on in-house X-ray sources. Unfortunately, the weak anomalous signals derived from sulfur in proteins results in the need for highly redundant data, restricting the use of in-house SAD phasing. Recently, a method was developed to genetically encode unnatural amino acids in *E.coli* and yeast. We now show that this technology can be used to site-specifically incorporate *p*-iodo-L-phenylalanine (iodoPhe) into proteins in response to an amber TAG codon for in-house SAD experiments. To illustrate this, the crystal structure of bacteriophage T4 lysozyme with Phe53 mutated to iodoPhe was determined. The iodoPhe residue, present in the hydrophobic core of the protein, did not perturb the protein structure and produced an anomalous signal comparable to that routinely obtained at synchrotrons with SeMet substituted protein. Accurate experimental phases were derived using considerably less data than needed for the equivalent experiment with native sulfurs.

