

**W0647**

**Novel Cell-Free Expression System for Synthesis of Proteins used in Structural Analyses.** Jean-Pascal Viola, Uritza von Groll\*, Annette Zacharias\*, Steve Tétreault†, Christian Houde†, Frank Schäfer\*, QIAGEN Research & Development, †QIAGEN Canada Inc., Montreal, Quebec, CA H3K 1G6, \*QIAGEN GmbH, 40724 Hilden, Germany.

A structural genomics project is a multidisciplinary process including several working steps. One of the most time-consuming steps is expression of labeled protein in amounts sufficient for structural analysis by either X-ray crystallography or NMR.

The dramatic reduction in the time required to obtain proteins of interest makes cell-free expression systems an attractive alternative to conventional methods. Moreover, they do not require specialized equipment, use simple procedures, and offer the ability to express proteins that may be poorly expressed in in vivo systems. By employing large-scale reactions and adding an affinity tag to the expression construct, sufficient Se-Met or SI-labeled protein for a thorough structural determination can be synthesized and purified to homogeneity in a single working day.

We present a new technology, which following a small-scale construct evaluation step, can be used for expression of milligram amounts of functional proteins in a 2 hour procedure, and demonstrate its application to X-ray crystallography and NMR structural analysis of several prokaryotic and human proteins.