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ATP-Affinity chromatography to enrich protein kinases and associated proteins expressed in developing oilseed rape

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Protein kinases and associated proteins like 14-3-3 regulate many cellular processes. These proteins are usually expressed at very low levels depending on development and environmental factors. Identification of these proteins, which are largely under-represented in widely used gel-based (such as two-dimensional gel electrophoresis) and gel-free (multi-dimensional protein identification technology) proteomics approaches, will help in understanding the signaling and metabolic pathways involved in regulating the levels of storage reserves in oilseed plants. Here, we have applied an affinity-based ATP-binders resin purification strategy in combination with sodium dodecyl sulfate polyacrylamide gel electrophoresis and liquid chromatography tandem mass spectrometry on developing seeds (four week after flowering) of oilseed rape (*Brassica napus* L. Reston) to enrich and identify protein kinases and associated proteins. A total of 32 high-confident proteins were identified. Many of these proteins are known ATP-binding proteins including fructokinase and heat shock proteins as well as 14-3-3 proteins (known to interact with and modulate the activity of many kinases) and components of the 20S proteasome. Bioinformatic analysis of identified proteins and application of this approach to other oilseed plants, such as *Arabidopsis* and soybean, are in progress. This research was supported by NSF grants DBI-03324418 and DBI-0445287.

