

# Reverse Chemical Proteomics in the Discovery of Cellular Receptors for natural Products

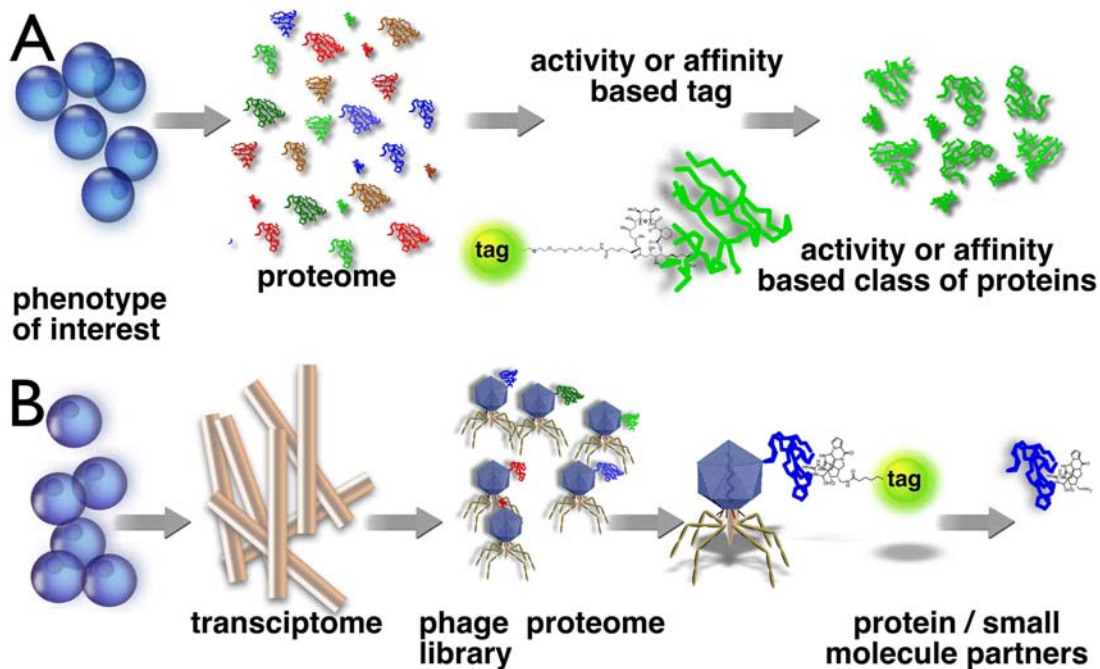
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As reactions in Nature are biased towards function, it follows that every natural product must have a biological receptor; only if one accepts this premise can the enormous biochemical expense of producing natural products be rationalised. Even though natural products may not have co-evolved with human proteins, they have emerged in nature to interact with biomolecules. As Jerrold Meinwald succinctly put it, “Natural products have evolved to interact with something, and that something may not be so different from human proteins” [1]. This assertion is supported by a recent survey [2], which found that 61% of the 877 new small molecule chemical entities introduced as drugs worldwide during 1981-2002 were either natural products, natural product derivatives or natural product mimics. The percentages were even higher when considering only the antibacterial (79%) and anticancer (74%) compounds. These figures are not surprising as natural products cover a far greater area of chemical space than synthetic compounds, and have property distributions that are similar to those of drugs currently in use [3]. For instance, when compared to synthetic compounds, natural products, on average, have higher molecular weights, incorporate fewer nitrogen, halogen or sulfur atoms, but more oxygen atoms, and are sterically more complex, with more bridgehead atoms, rings, and chiral centres.

Clearly, natural products are a rich source of drugs and drug leads. However, even when a natural product is found to exhibit biological activity, the cellular target and mode of action of the compound are rarely identified. This is also true of many natural products that are currently in clinical trials or have already been approved as pharmaceuticals. The absence of a definitive cellular target for a biologically active natural product hinders the rational design and development of more potent therapeutics. Therefore, there is a great need for new techniques to facilitate the rapid identification of cellular targets for biologically active natural products.

Chemical proteomics (Fig. 1A) is a powerful tool for isolating and identifying cellular receptors for biologically active natural products, thereby facilitating subsequent rational drug design, and often providing valuable information regarding underlying biochemical and cellular processes. The key to chemical proteomics is the construction of an affinity probe. In reverse chemical proteomics (Fig 1B), the starting point is a transcriptome of a phenotype of interest. A cDNA library is cloned into an expression system and the resulting tagged proteome is screened against a small molecule. To date, only phage display has been used in a reverse chemical proteomics context, although other methods, such as bacterial [4] and yeast [5] cell-surface display, are also possible, but need to be

developed further. If an entire cDNA library is cloned into a phage display vector, each phage particle will contain a different gene insert and will express the protein encoded by that gene on its surface. The displayed proteins often behave as if they were free in solution, so phage displaying the target protein can be rescued from an entire library using an immobilised natural product, as per standard chemical proteomics experiments. However, the real power of phage display comes from the fact that rescued phages can be amplified by transfection into *E. coli* and then subjected to another round of affinity selection with the immobilised natural product.



**Figure 1.** Schematic representation of chemical proteomics and reverse chemical proteomics. In chemical proteomics (A), one uses a small molecule (e.g. marine natural product) to construct an affinity or activity probe to isolate specific proteins or a family of proteins. In reverse chemical proteomics (B), we start with the transcriptome, which is cloned into an amplifiable vector (e.g. a virus) that expresses a single protein from the proteome on its surface. A tagged natural product can be used to probe the tagged proteome in an iterative manner.

The first report of reverse chemical proteomics being used to identify the cellular receptors for a natural product was by Austin *et al.* [6, 7], who used biotinylated the immunosuppressant FK506 immobilised on an avidin-derivatised agarose resin to isolate FKBP1a from a T7 phage-displayed human brain cDNA library by affinity chromatography after six rounds of selection.

In this lecture, we will report on our work to improve the method. As a control experiment, we have used olefin metathesis to attach a long bintinylated PEG linker to FK506 and use streptavidin derivatised microtitre plates to isolate FKBP2 and FKBP3 after only three rounds of selection against two human brain cDNA libraries. Both these

proteins are known receptors for FK506. In further experiments we have derivatised the marine natural products palau'amine and kahalalide F and successfully isolated a ribosomal protein as the cellular receptor for the anticancer kahalalide F from several human tumor libraries.

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