Application of isothermal titration calorimetry and column chromatography for identification of biomolecular targets

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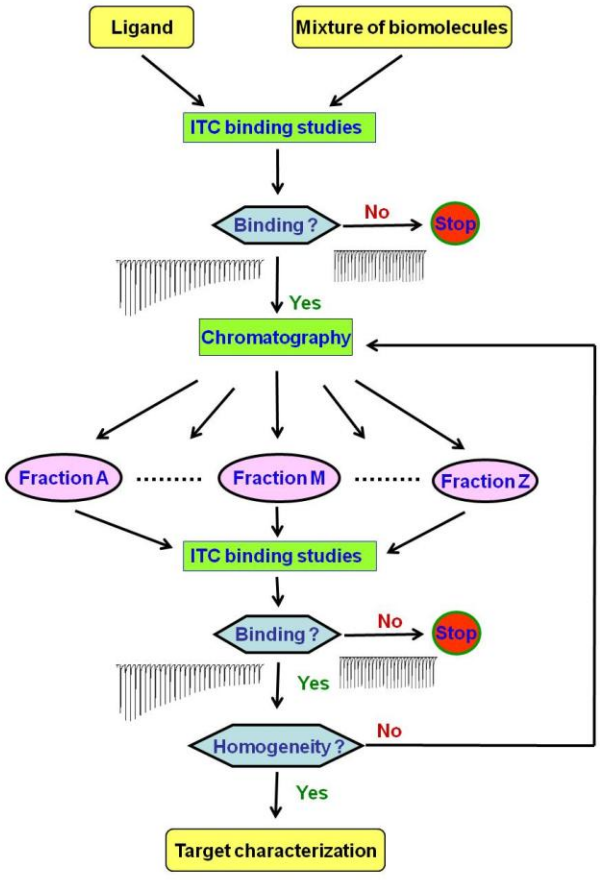
The traditional approach to discover biomolecular targets is (i) to identify the proteins (enzymes and receptors) that have a key role in the biological effect of interest and (ii) to find a potential target site through various genetic approaches, including transgenic animals and site-directed mutagenesis. After target confirmation, a small-molecule agonist or antagonist is searched. There are, however, circumstances in which a compound elicits a therapeutic effect, but its molecular target is unknown. In such cases, it is important to find the target protein to better understand the mechanism of action and its implications. This warrants the identification of the target from a complex mixture of proteins (such as cell lysate and plasma) for a given lead compound. Several approaches have emerged to accomplish this. These methods involve immobilization and modification of ligands that impose significant limitations. In addition, when the ligand is a macromolecule such as a protein, stability becomes a major problem, and detailed information regarding the interaction between the ligand and its partner must be available in order to correctly orient the ligand. To address these problems relating to the identification of an unknown target, we have designed and developed a protocol that combines conventional column chromatography (e.g., gel filtration, ion exchange) with isothermal titration calorimetry (ITC) as a tracking indicator[1]. The ITC tracking method is based on the concept that the compound could recognize its target even when the target is in a complex mixture.



This protocol describes a method for identifying unknown target proteins from a mixture of biomolecules for a given drug or a lead compound. The first step involves the use of ITC to confirm the binding of ligand to a component in the biomolecular mixture. Subsequently, the biomolecular mixture is fractionated by chromatography, and the binding of the ligand with individual fractions (or subfractions) is verified by ITC. The iteration of chromatographic purification on the fractions combined with ITC results in identifying the target protein. This method is useful when the target protein or ligand is unknown and/or not amenable to labeling, chemical modification or immobilization. This protocol has been successfully used by our team[1] and by others[2] to identify both low-abundance and highly abundant target proteins present in biomolecular mixtures. With this protocol, it takes approximately 3–5 days to identify the target protein from a mixture. With this strategy, we have demonstrated the identification of several targets from crude snake venom, which consists of hundreds of proteins and peptides, as a model biomolecular mixture.

References

1. Zhou, X., Sun, Q., Kini, R.M. and Sivaraman, J. *Protein Sci.* **17**, 1798–1804 (2008).
2. Kandeel, M., Miyamoto, T. and Kitade, Y. *Biol. Pharm. Bull.* **32**, 1321–1327 (2009).