Target Identification of Natural Products

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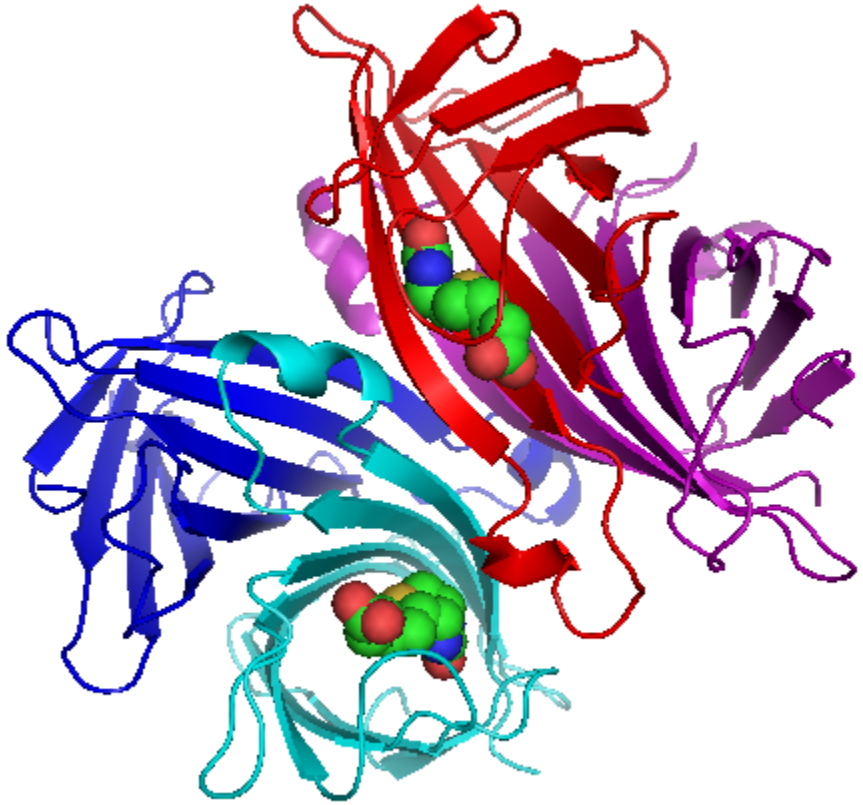
4/26/2016

一、Biochemical methods

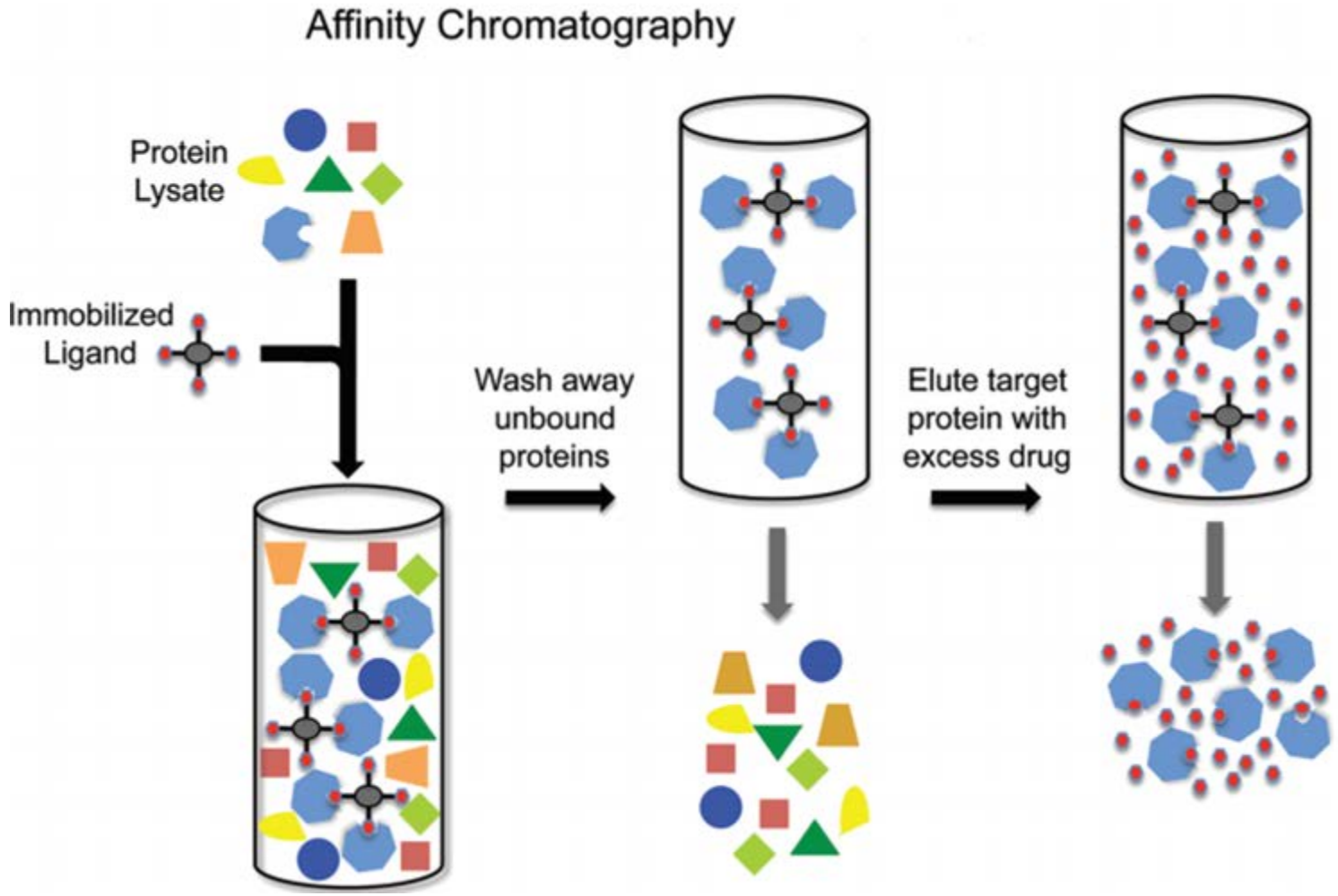
1.Introduction to the Biotin-Avidin System (BAS)



Streptavidin（链霉亲合素）: composed of four same subunits, each of which can bind with one molecule of biotin

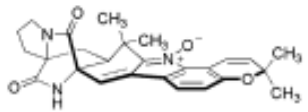
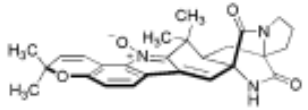
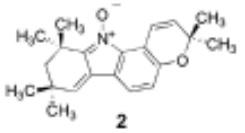
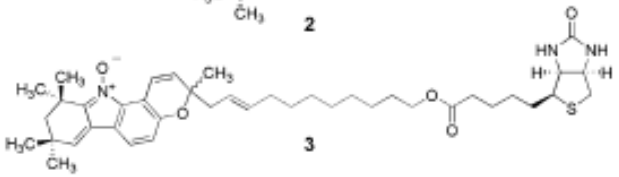
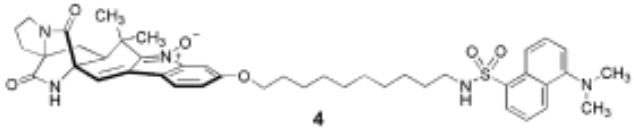
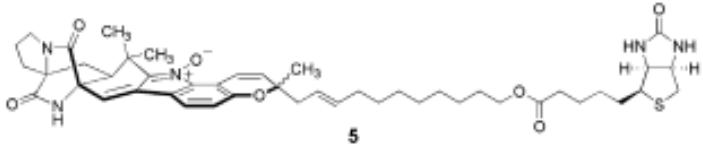
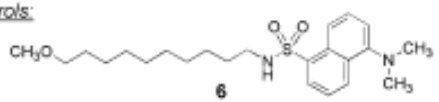
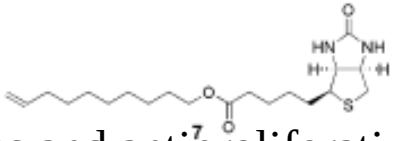
Tetrameric structure of streptavidin with two bound biotins

2. Process of Affinity Chromatography

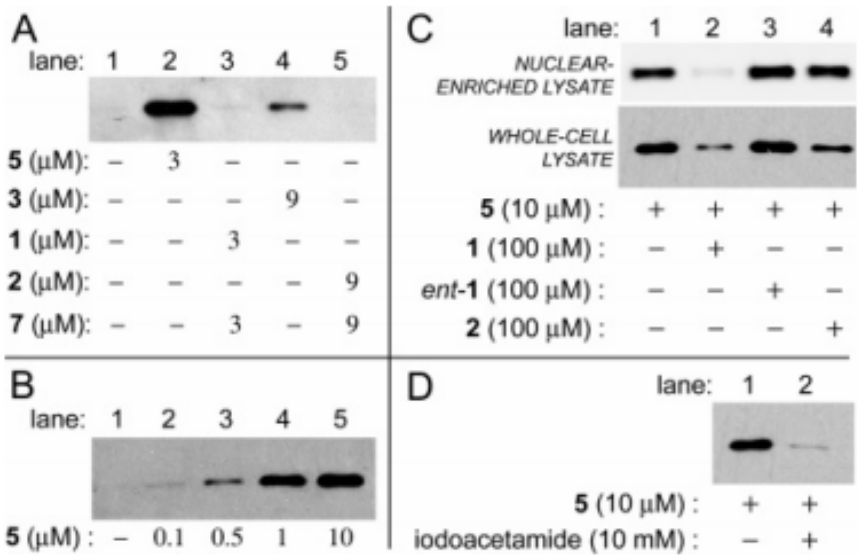


Brett L., Richard W.O., Jing H. *Chem. Biol.* **6**, 34–46 (2011).

3. Target Identification of Natural Product Avrainvillamide

Compound	GI_{50} T-47D	GI_{50} LNCaP
 (+)-avrainvillamide (**1**)	0.33 μM	0.42 μM
 (−)-avrainvillamide (*ent*-**1**)	0.91 μM	1.4 μM
 **2**	8.4 μM	5.2 μM
 **3**	7.0 μM	5.6 μM
 **4**	0.25 μM	0.77 μM
 **5**	0.66 μM	3.3 μM
Controls:  **6**	> 25 μM	> 25 μM
 **7**	> 25 μM	> 25 μM

Structures and antiproliferative activities of inhibitors, activity-based probes, and control compounds

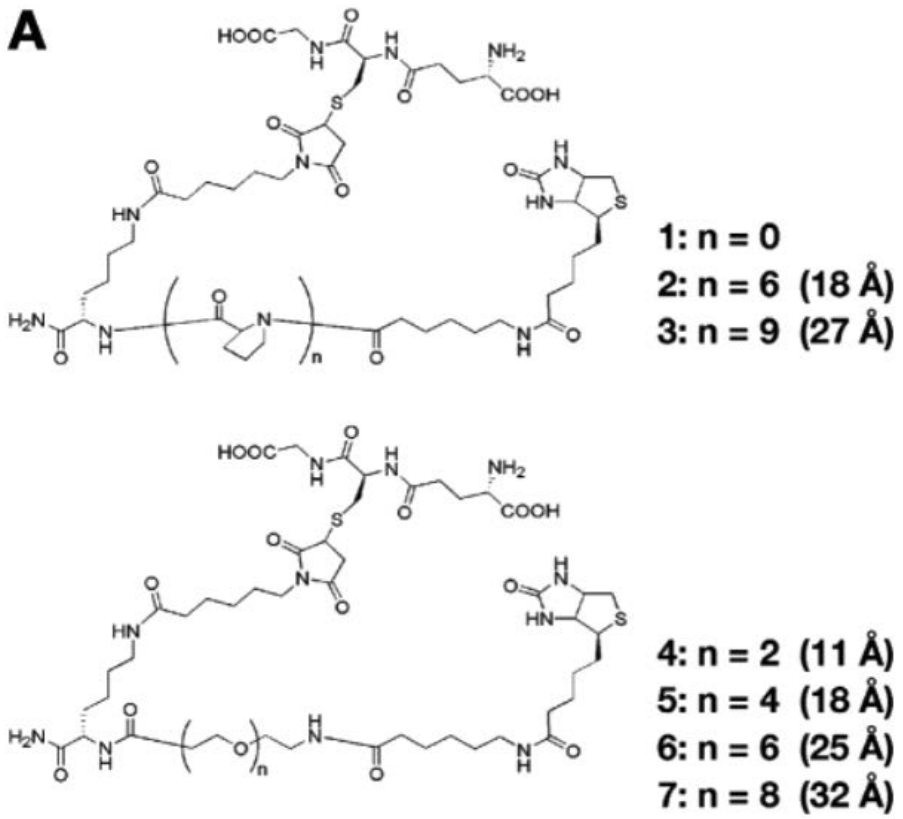


Western-blot detection of nucleophosmin after affinity-isolation and PAGE

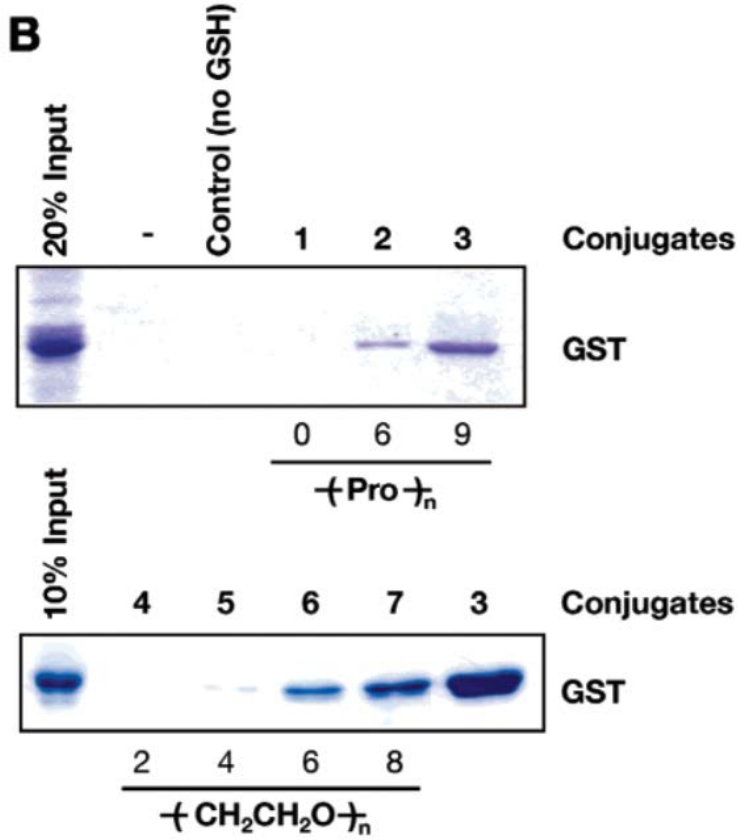
Jeremy E. W., Romain S., Andrew G. M.
J. AM. CHEM. SOC. **129**, 14444-14451 (2007).

4. Improvements to Affinity Chromatography

1). Increase the distance between glutathione and biotin

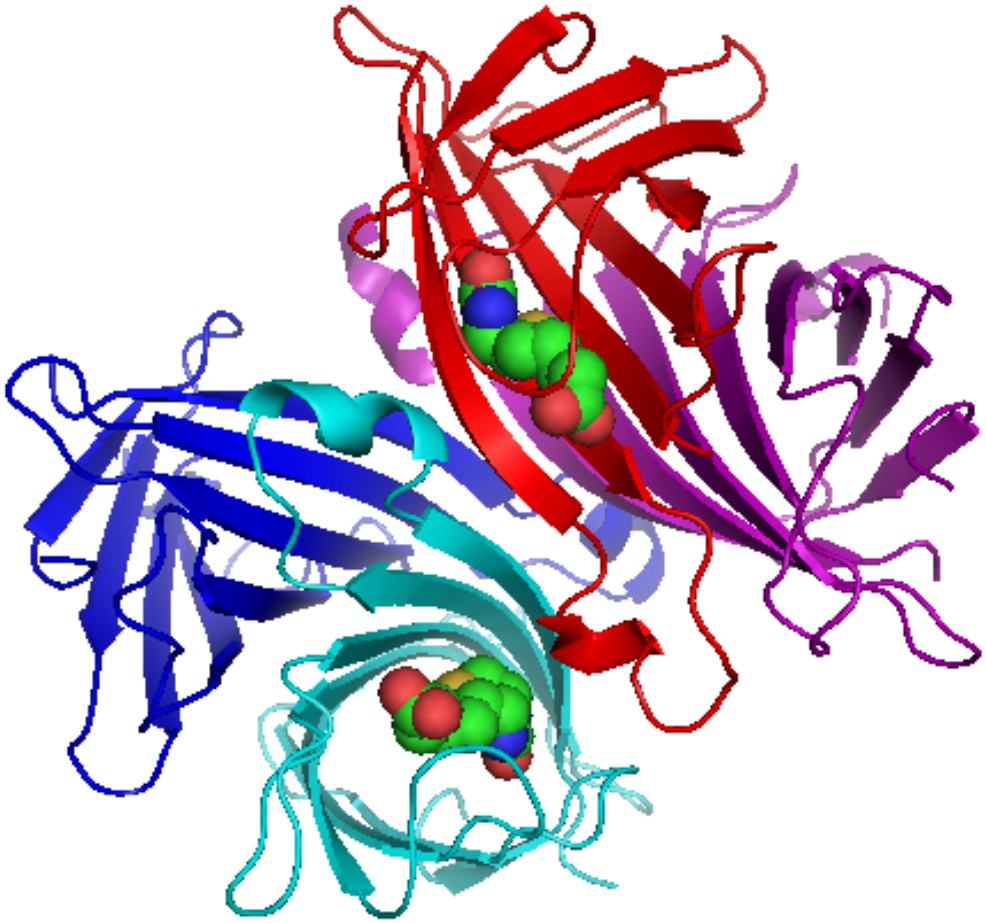


A: Design of biotinylated glutathione with a polyproline linker



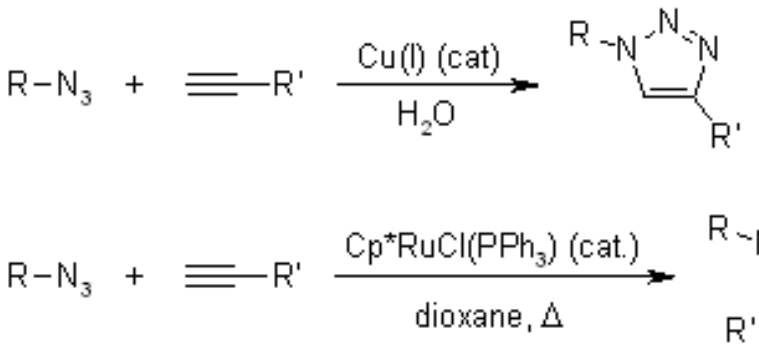
B: Affinity purification of GST from bacterial lysates

Shin-ichi S., Youngjoo K., Shinji K., *et al*. *J. AM. CHEM. SOC.* **129**, 873-880 (2007)

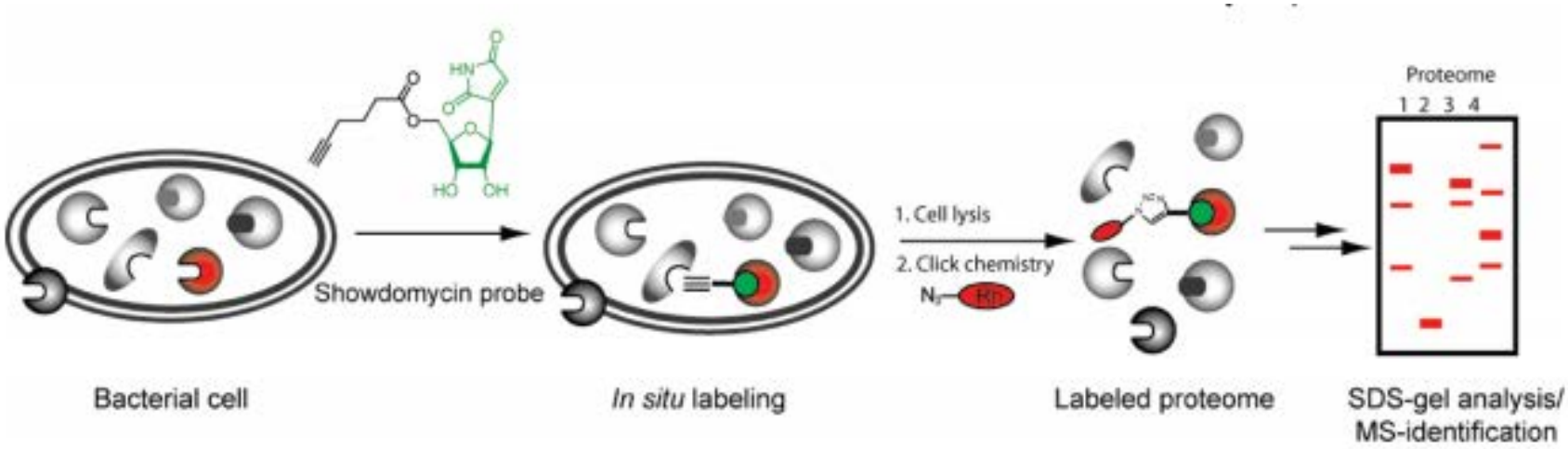


Explanation: the distance from the bound biotin molecule to the surface of avidin is as long as 7 Å and the polyproline helix can make the small molecule away from the biotin-avidin complex

2) Make use of the click chemistry



Click chemistry



Thomas B. and Stephan A.
J. AM. CHEM. SOC. **132**, 6964–6972 (2010)

5. Drug Affinity Responsive Target Stability (DARTS)

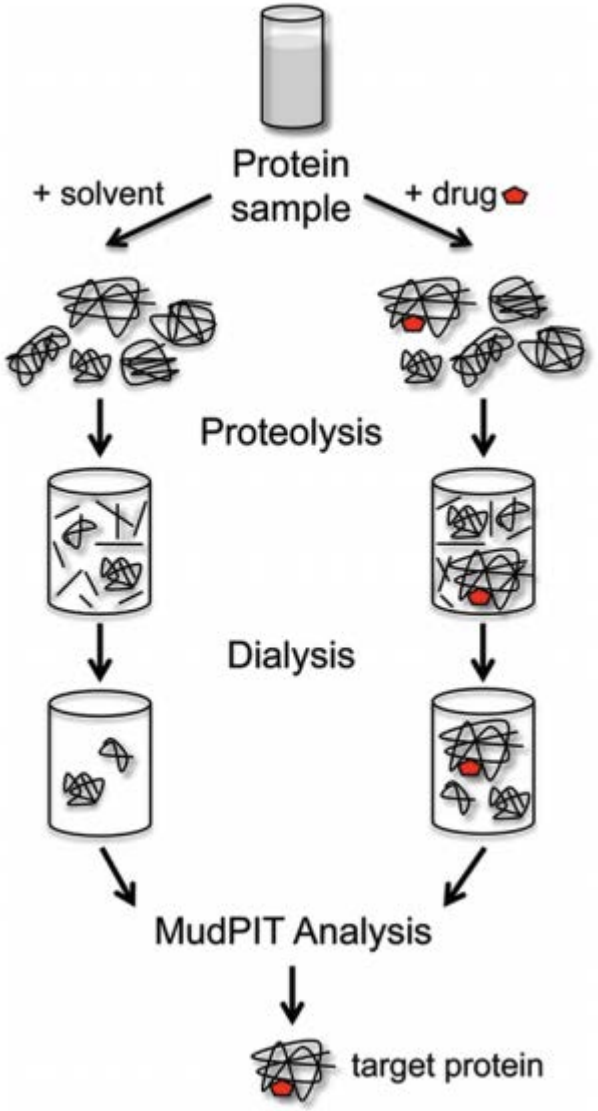
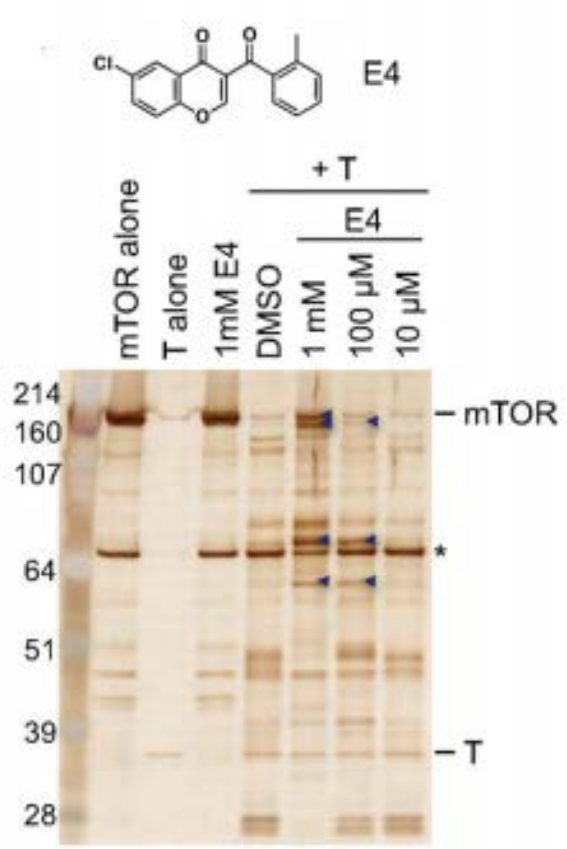


Diagram of the DARTs method

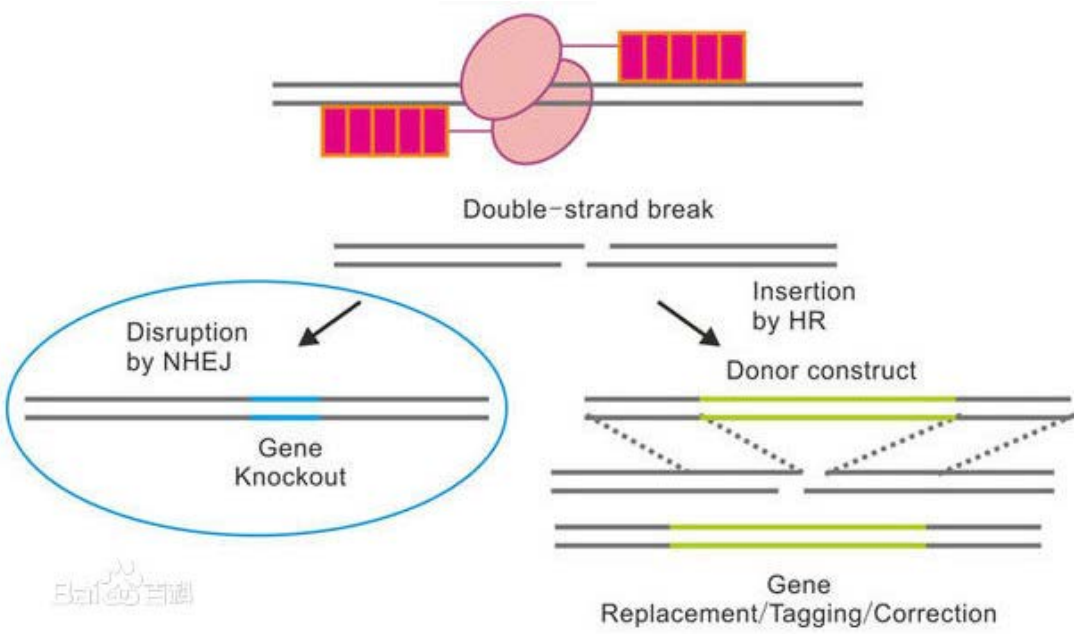


DARTS with a micromolar mTOR kinase inhibitor (E4).
T: thermolysin proteolysis; *****, nonspecific band

Brett L., Rui H., Nao J., *et al*. *Proc Natl Acad Sci USA* **106**, 21984–21989 (2009).
Brett L., Richard W.O., Jing H. *Chem. Biol.* **6**, 34–46 (2011)

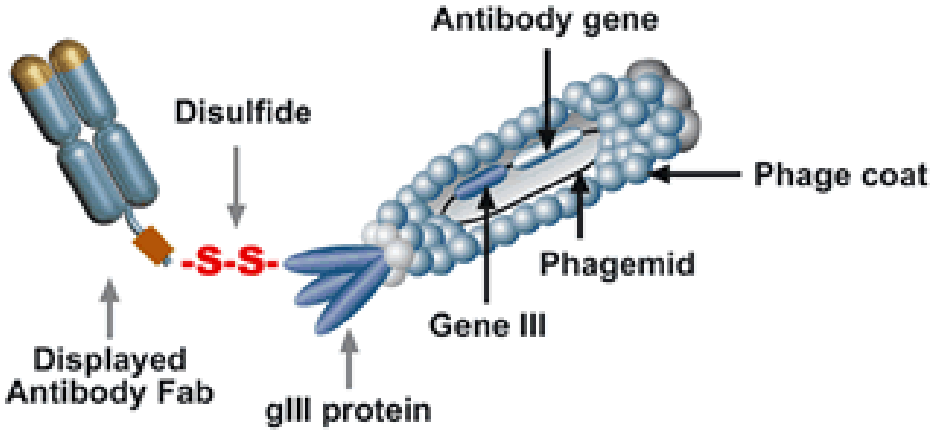
二、Genetic and genomics method

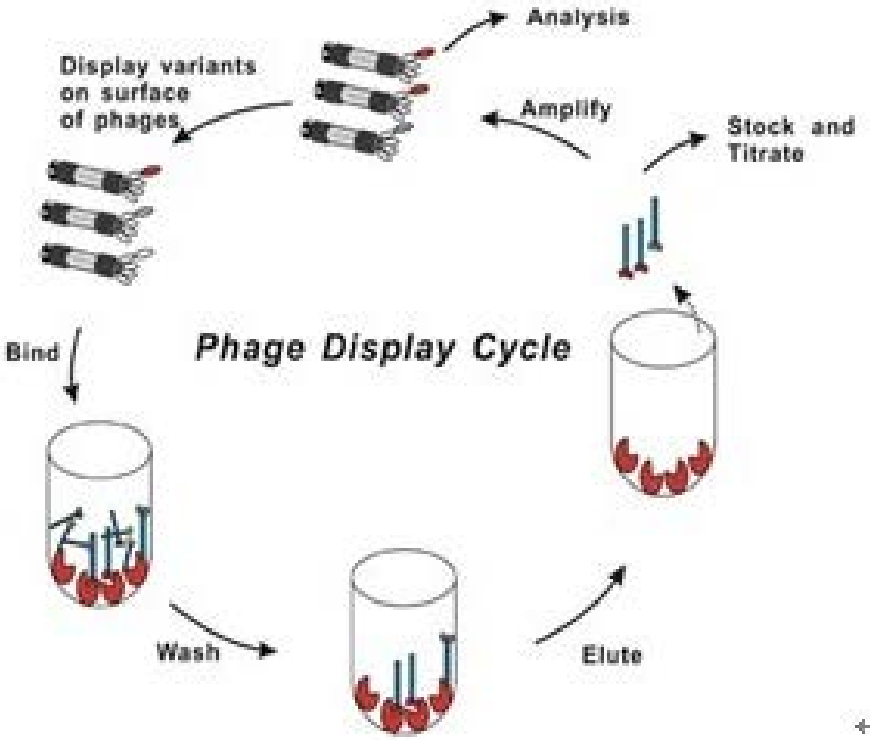
1.Gene knock-out, knock-down and overexpression



Knock-out: CRISPR/Cas9, TALEN, ZFN
Knock-down: RNAi

2. Phage display technique





Thank you for your attention!