KBSI

Korea Basic Science Institute

CUPID (Cell-based Un-/identified Protein Interaction Discovery)

Protein-protein Interactions (PPIs) as a Drug Target

❖ Unlike genes of the genome, there are numerous cellular functions of proteins that are carried out by proteins interacted with other proteins.

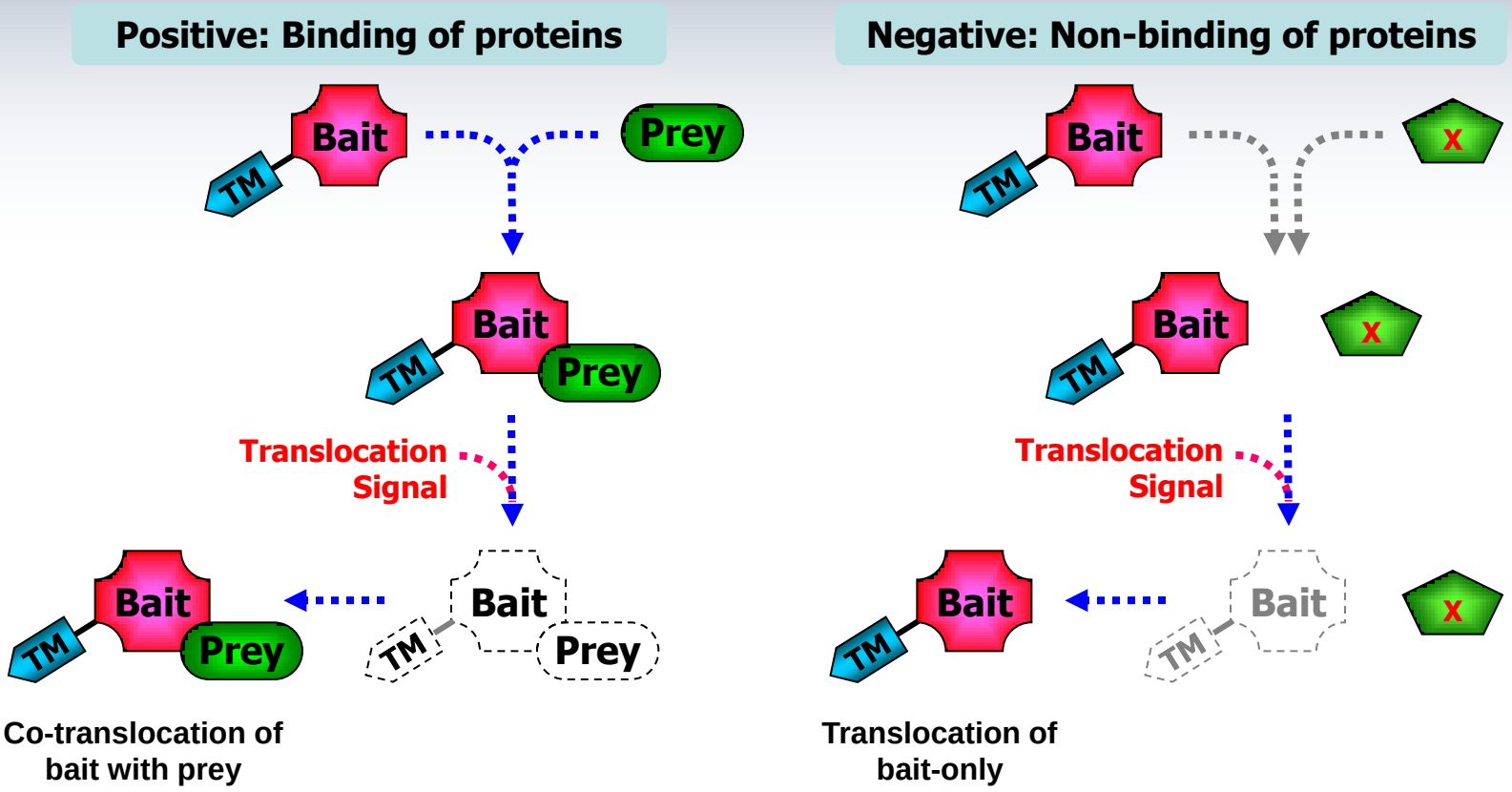
❖ Development of targeting PPI technology is critical in enhancing or shutting down key downstream events of signal transduction pathways and then modulate cellular functions.

❖ Limitations of Conventional Drug Target Approaches

- Difficulty of optimizing PPI-pairs between bait and prey (FRET, BiFC)
- Difficulty of utilizing traditional PPI technologies to identify protein-protein interaction site, peptides targeting PPI sites, and small molecule binding site on/in a PPI
- Difficulty in characterizing involvement of more than two proteins in the complex
- Requirement of antibodies (IP), purification steps (GST-pull down), and other substrates
- Requirement of expensive instruments and technologies such as analytical ultracentrifugation (AUC), surface plasmon resonance (SPR), nuclear magnetic resonance (NMR), X-ray crystallography, and fluorescence correlation spectroscopy (FCS).

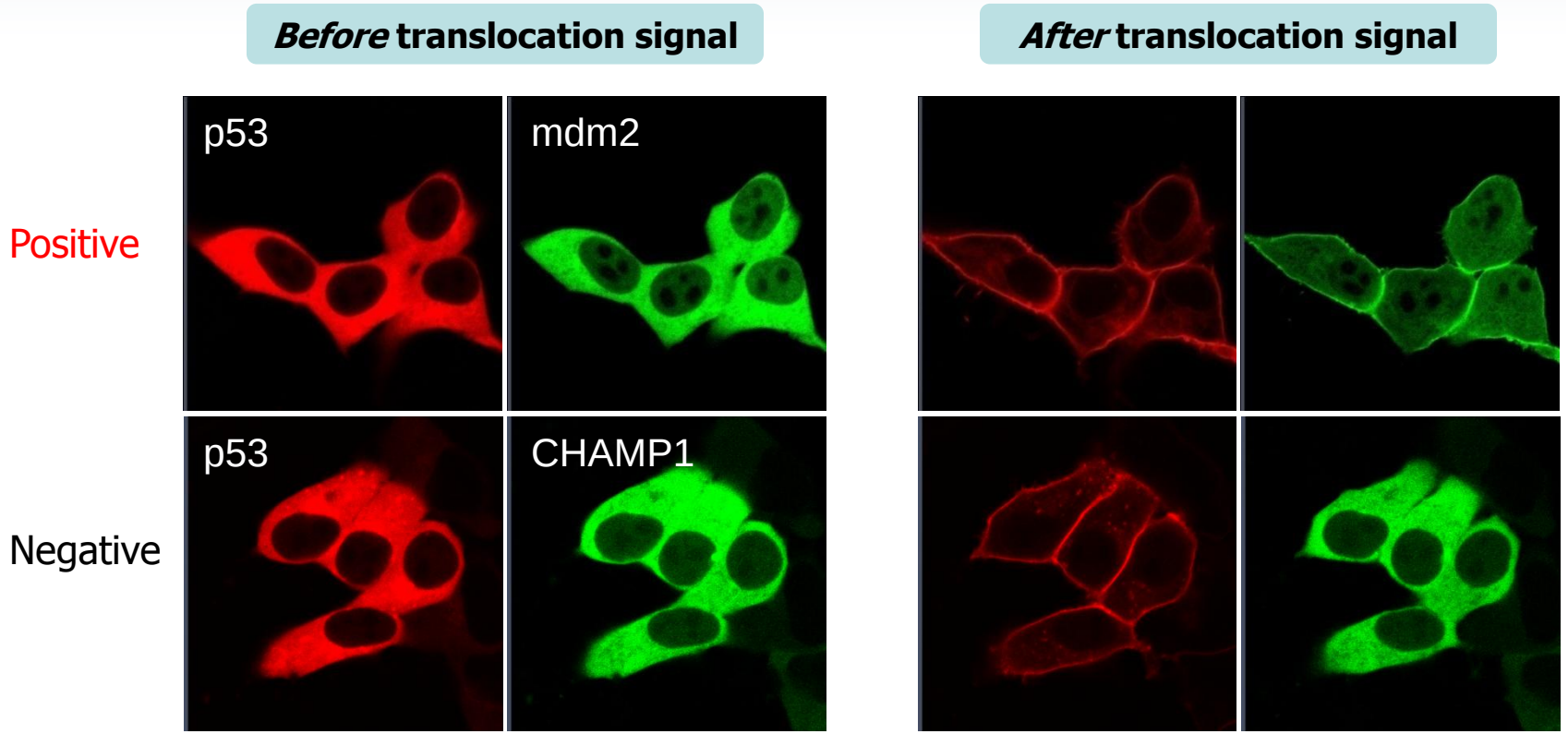
Concept of *CUPID* Technology

Active co-translocation event of bait and prey reflects the binding status of a bait-prey complex (protein-protein interaction)



Result Section

- Positive: Active co-translocation of bait (p53, red) and prey (mdm2, green) to cellular membrane reflects the binding status of the complex.
- Negative: Only bait (p53, red) is translocated to cellular membrane while prey (CHAMP1, green) stays in cytoplasm.



Advantages of ***CUPID***

- **Native Environment:**
  - ✓ No need for excessive artificial synthetic substrate
  - ✓ Intact Cellular Context: Authentic physiological conditions
  - ✓ No purification of bait/prey proteins
  - ✓ No need antibodies for bait or prey
  - ✓ No expensive equipment for experiment
- **Native Conformation of proteins:**
  - ✓ Full-length proteins with correct folding and post-translational modifications
  - ✓ Expressed in human cells
  - ✓ Avoiding denaturation of proteins by artificial buffer
  - ✓ No modification (truncation, deletion, etc) expression and/or purification of proteins
  - ✓ No optimization to avoid topological hindrance
- **Novel drug-target interactions can be elucidated:**
  - ✓ Simple detection of protein-protein interactions by conventional fluorescence microscopy
  - ✓ Real-time monitoring possible within a few minutes
  - ✓ Differentiation of different signaling cues
  - ✓ Elimination of false positive results from indirect reporter assays such as IP, Y2H, FRET, BiFC, and FCS

Applications what we have done with CUPID technology

Dimer bindings

- Cytoplasmic proteins:
  - ✓ Raf1 *vs*. MEK2, MEK2 *vs*. ERK2
  - ✓ ERK2 *vs*. p90RSK, KSR1 *vs*. 14-3-3
  - ✓ p38AIMP2 *vs*. complex-forming ARSs
  - ✓ NFkB *vs*. IkB
- Nuclear proteins:
  - ✓ p53 *vs*. mdm2, p53 *vs*. SV40 T antigen
  - ✓ RelA *vs*. p50

Multimer bindings

- 3-mer bindings:
  - ✓ NFkB/IkB (RelA/p50/IkB) *(Multiple Myeloma)*
  - ✓ AIMP1/AIMP2/KARS *(Lung Cancer, etc)*
  - ✓ NICD/RBPJk/MAM *(Branin Cancer, etc)*
- 4-mer bindings:
  - ✓ AIMP1/AIMP2/KARS/DARS
- 5-mer bindings:
  - ✓ AIMP1/AIMP2/KARS/DARS/RARS

Peptide-Protein bindings

- ✓ p53 domain *vs*. hdm2
- ✓ TAB1 domain *vs*. TAK1
  (Osteoclast, Bacterial Infection, etc)

Inhibitor Screenings

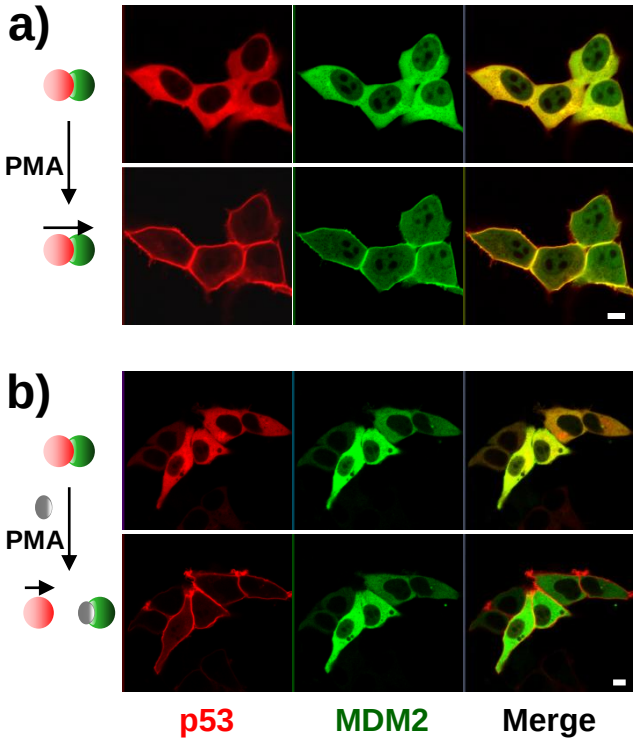
- ✓ Nutlin-3 for p53-hdm2 interaction
  (Hodgkin Lymphoma, etc)
- ✓ U0126 and PD98059 for ERK2-p90RSK
  (Breast Cancer, etc)

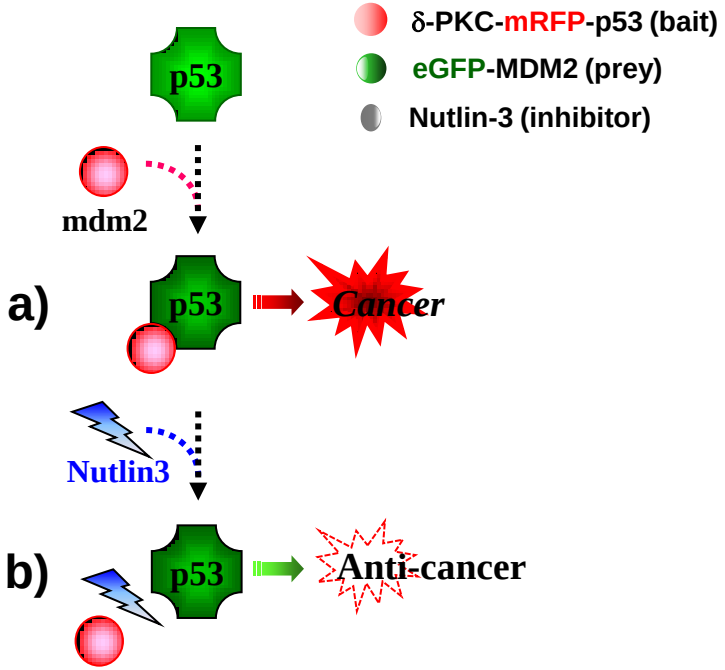
Compound-mediated Interaction & Its Inhibition

- ✓ Rapamycin-mediated mTOR-FKBP binding *(Immunosuppressant, etc)*
- ✓ Inhibition of Rapamycin-mediated mTOR-FKBP binding complex by FK506 *(Inflammation, etc)*

p53-mdm2 binding and their inhibition

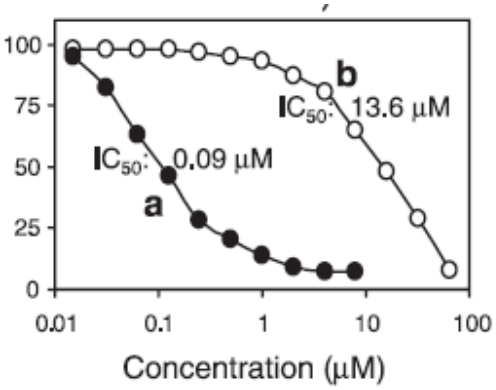
- The small molecular inhibitor Nutlin-3 is a cis-imidazoline analogue commonly used in anticancer studies that inhibits the interaction between p53 and mdm2

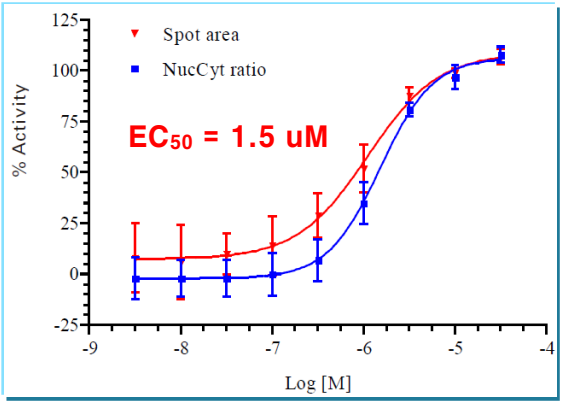


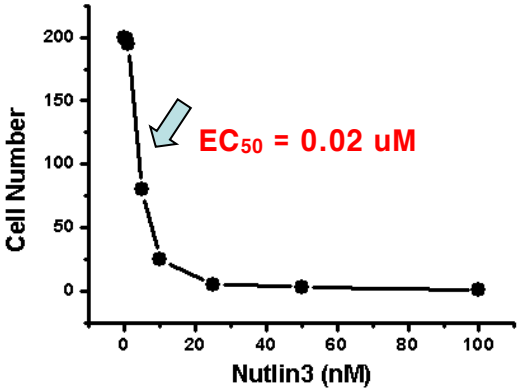


Comparison of Nutlin-3 inhibition curves from 3 different PPI technologies

- *In vitro* SPR assay : IC_{50} = 0.09 μM
- *In cell* GRIP assay : EC_{50} = 1.5 μM
- *In cell* CUPID assay : EC_{50} = 0.02 μM







Surface Plasmon Resonance;
(Science 303, 844-848, 2004)

GRIP Redistribution assay
(www.thermo.com/hcs)

CUPID anlaysis
(Angewante Chemie Intl. Ed. 50, 1314-1317, 2011)

Proposed Further Applications

1. Target Identification

- ✓ **Determination of target for unidentified protein-protein interaction partners or validation of specific protein-protein interaction pairs**

2. Inhibitor Screening

- ✓ **Determination of candidate chemical inhibitors for targeted protein-protein interaction pairs**

3. Peptide Inhibitor Screening

- ✓ **Determination of core-binding site (binding domain) and generation of peptide inhibitors**

4. Drug Repositioning

- ✓ **Exploration of unknown targets with known compound**

5. Complementation & Validation of Results from Conventional Methods

- ✓ **Confirmation and validation of targets for specific PPI pairs**

Validation of CUPID Technology

- **Patents and Paper**
  - ✓ **Korea, 10-0948767 (Mar. 12, 2010), registered**
  - ✓ **Korea, 10-2010-0037714 (Apr. 23, 2010), filed**
  - ✓ **USA, 12/547,943 (Aug. 25, 2009), filed**
  - ✓ **EU, 09168598.2 (Aug. 25, 2009) , filed**
  - ✓ **Japan, 2009-198750 (Aug. 28, 2009) , filed**
  - ✓ **Angew Chem Int Ed (2011) 50, 1314**

- **National Research Project**
  - ✓ **Development of drug target discovery systems using CLSM an FCS**
    (Korea Basic Science Institute, 2009-2010)
  - ✓ **Development of targeted drug screening system**
    (The Small & Medium Business Administration, 2009-2011)
  - ✓ **Development of UTOPIA system for high contents screening of CUPID technology**
    (Korea Basic Science Institute, 2010-2011)
  - ✓ **Pioneer research project**
    (Ministry of Education Science and Technology, PGA043, 2010-2014)