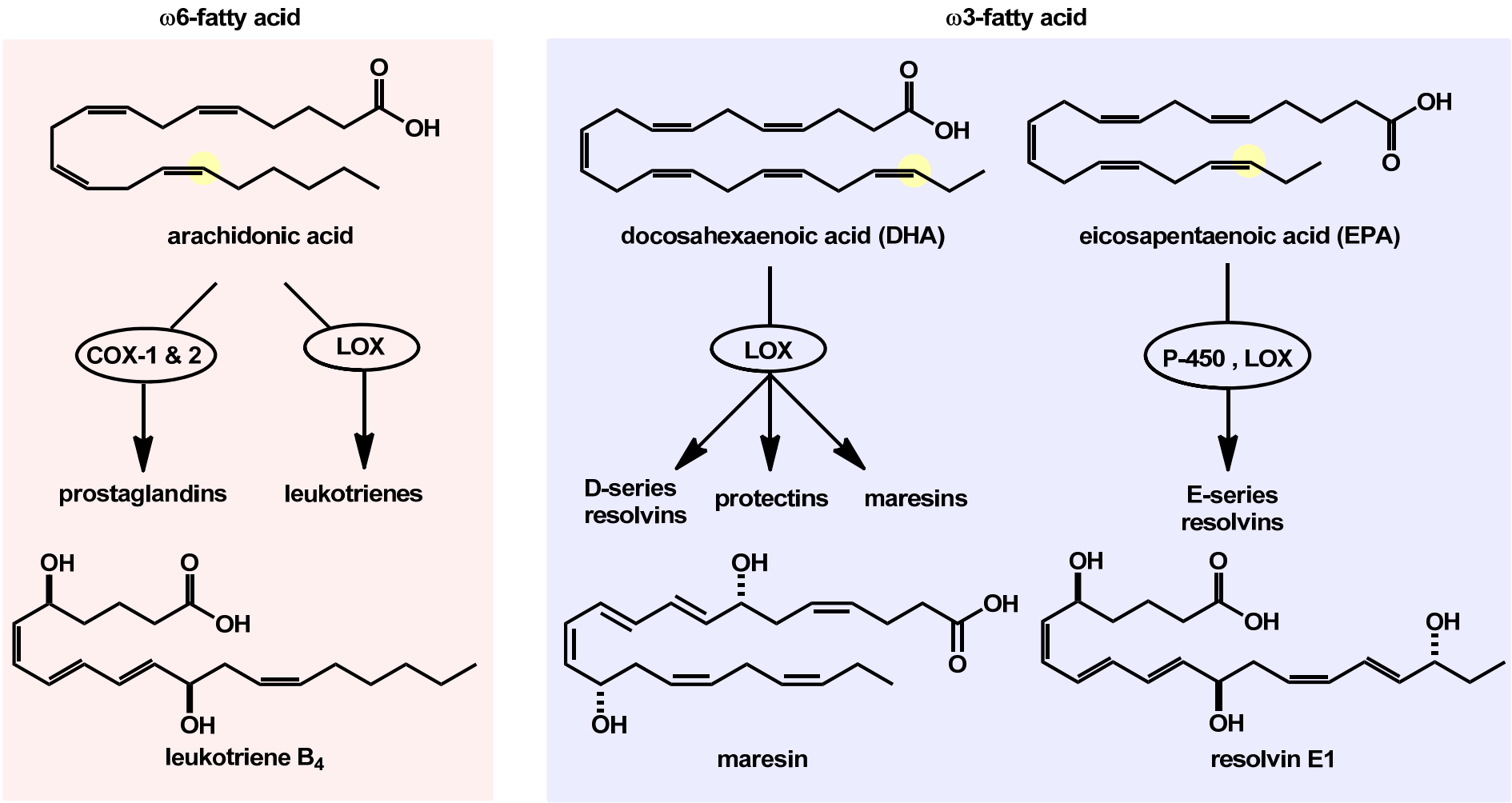
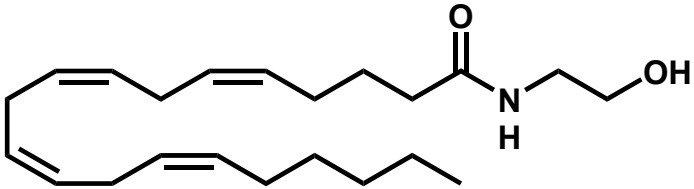
Endogenous Lipid Mediators



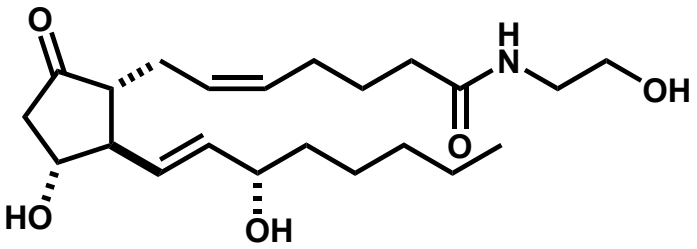
(a) Serhan, C. N.; Chiang, N.; Van Dyke, T. E. *Nat. Rev. Immunol.* **2008**, *8*, 349.
(b) Serhan, C. N.; Petasis, N. A. *Chem. Rev.* **2011**, *111*, 5922

Arachidonoyl Mediators

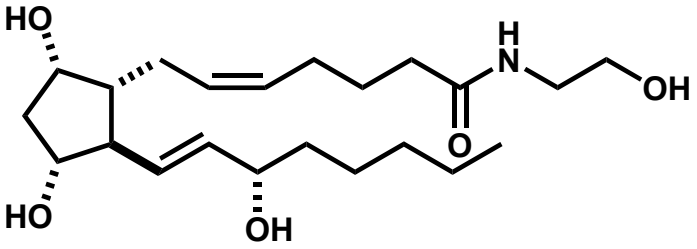


anandamide (AEA)

endogenous ligand of cannabinoid receptor



prostamide E

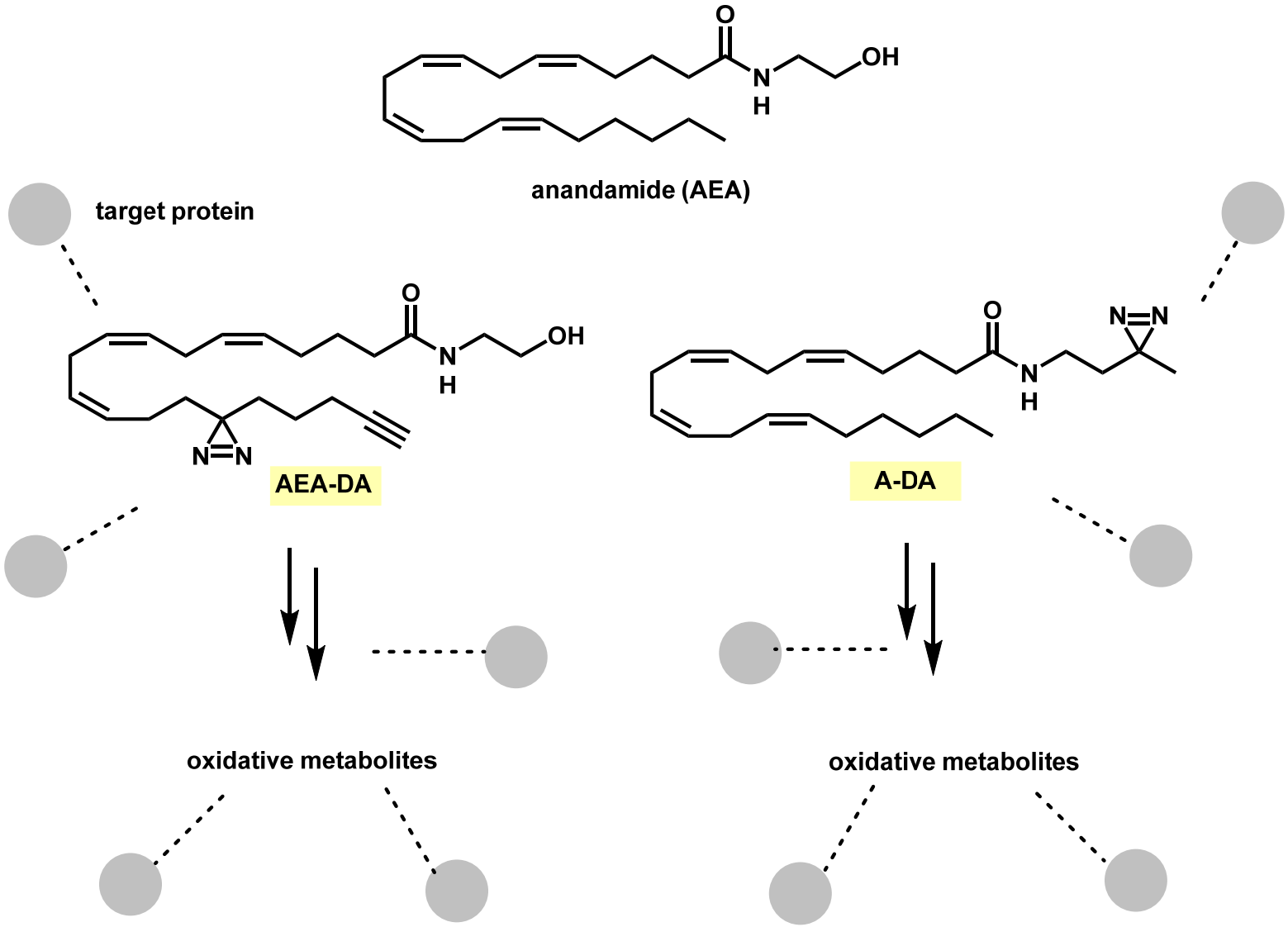


prostamide F

inflammatory mediators

The chemical mediators inspire the development of drugs and identification of target proteins involved in eicosanoid signaling.

Chemical Probe for Global Mapping Lipid-Binding Proteins



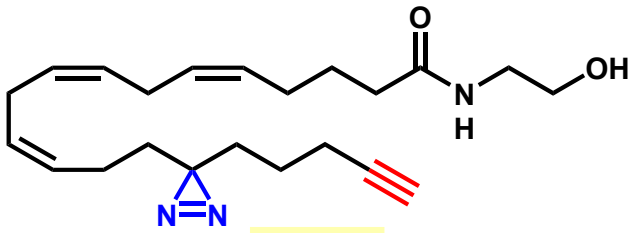
Niphakis, M. J.; Lum, K. M.; Congnetta, A. B., III; Correia, B. E.; Ichu, T.; Olucha, J.; Brown, S. J.; Kundu, S.; Piscitelli, F.; Rosen, H.; Cravatt, B. F. *Cell* **2015**, *161*, 1668.

Contents of the Main Paper

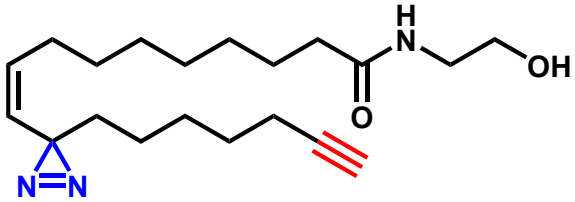
Lipid chemical probes were used for

1. mapping lipid-protein interactions
2. making landscape of lipid-binding protein based on the SILAC and LC-MS/MS
3. identification of target and off-target proteins of known drugs
   by competed experiment using SILAC (ligand-based approach to identify drug target engagement)
4. HTP ligand screening for the lipid-biding protein (protein-based approach to identify drug target engagement)
5. identification of the binding site of the lipid probe and the ligand

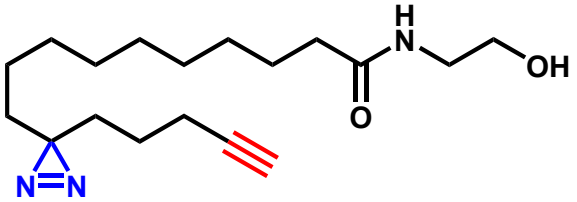
1. Chemical Probe for Mapping Lipid-Binding Proteins



AEA-DA

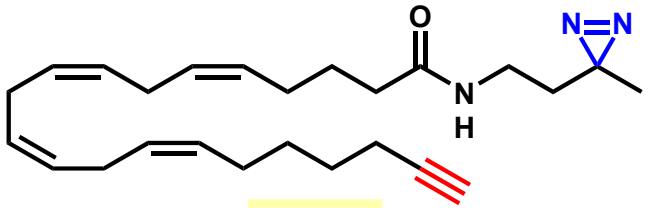


OEA-DA



PEA-DA

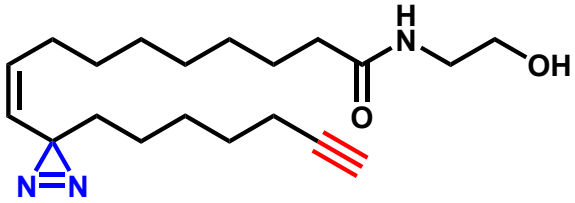
palmitoyl (18:0)



A-DA

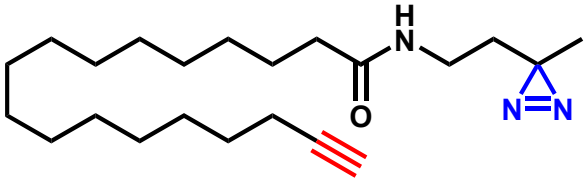
arachidonoyl (20:4) series

polyunsaturated fatty acid probe



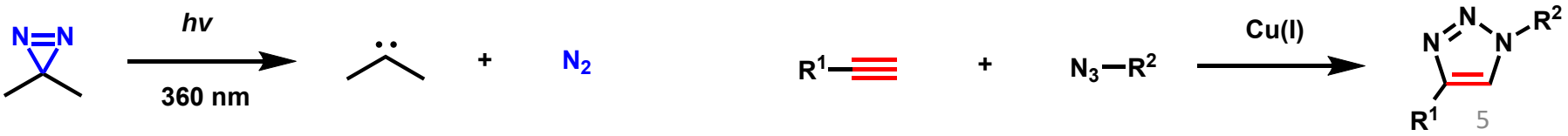
O-DA

oleoyl (18:1) series

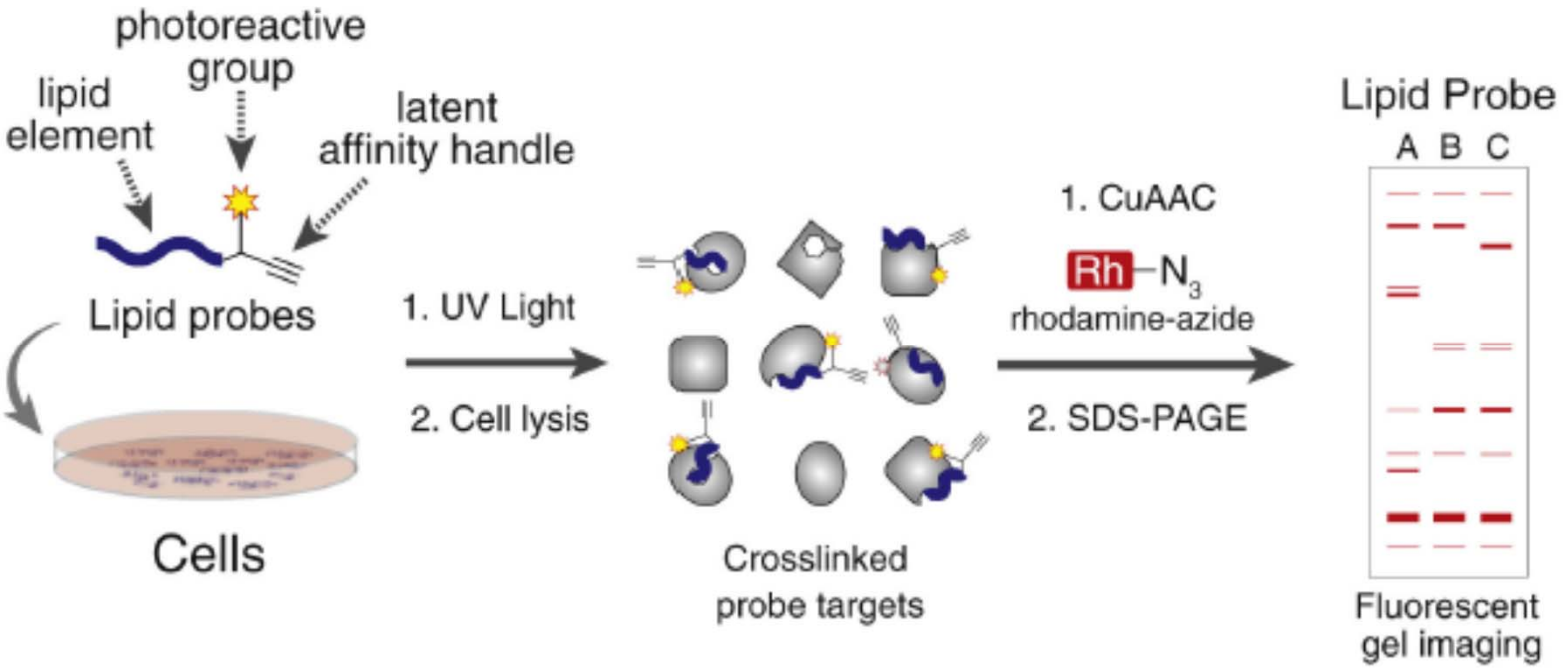


S-DA

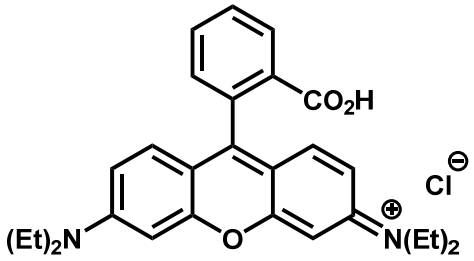
stearoyl (18:0)



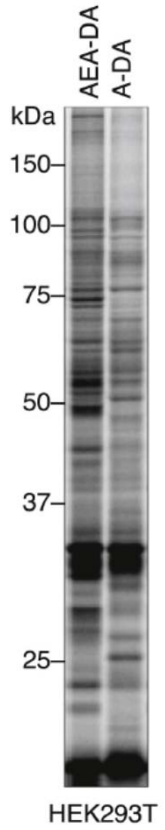
1. Experimental workflow for gel-based profiling of lipid-binding proteins



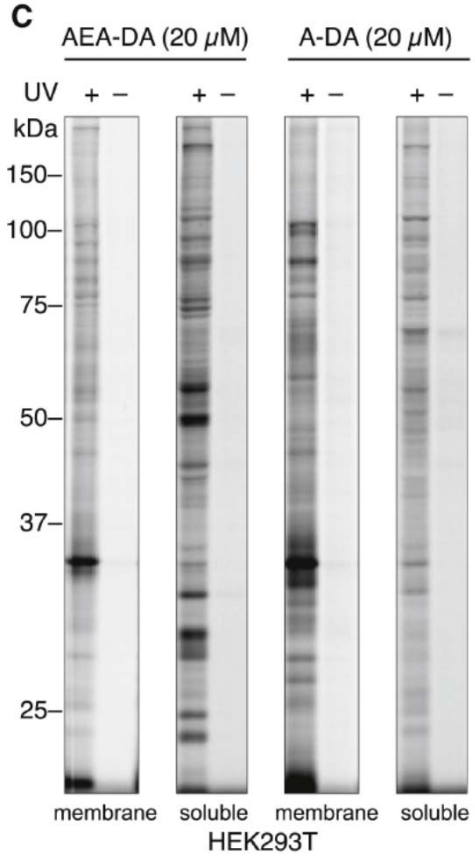
rhodamine



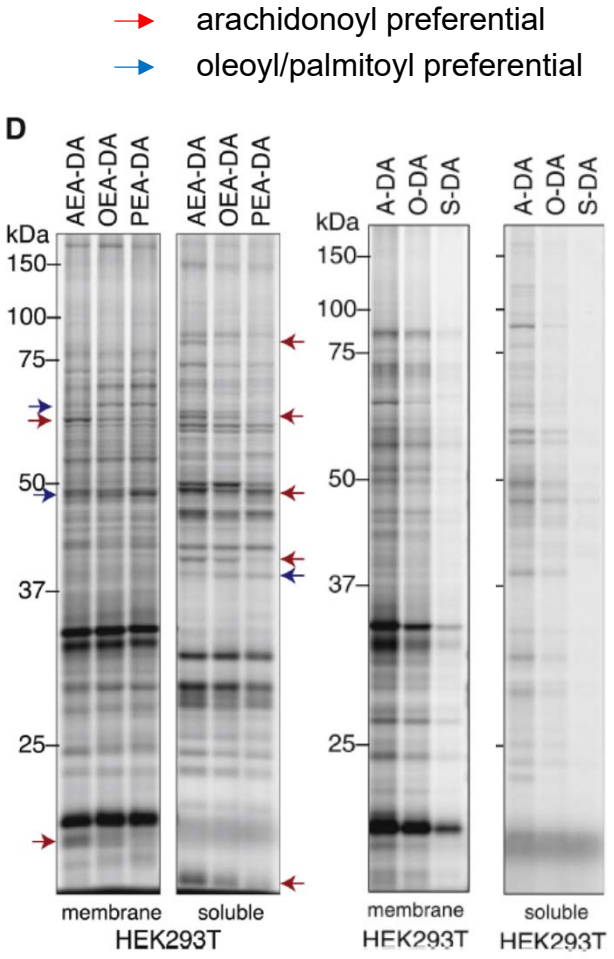
1. Chemical Proteomic Probes for Mapping Lipid-Binding Proteins in Cells



AEA-DA and A-DA probes showed distinct protein labeling profiles.



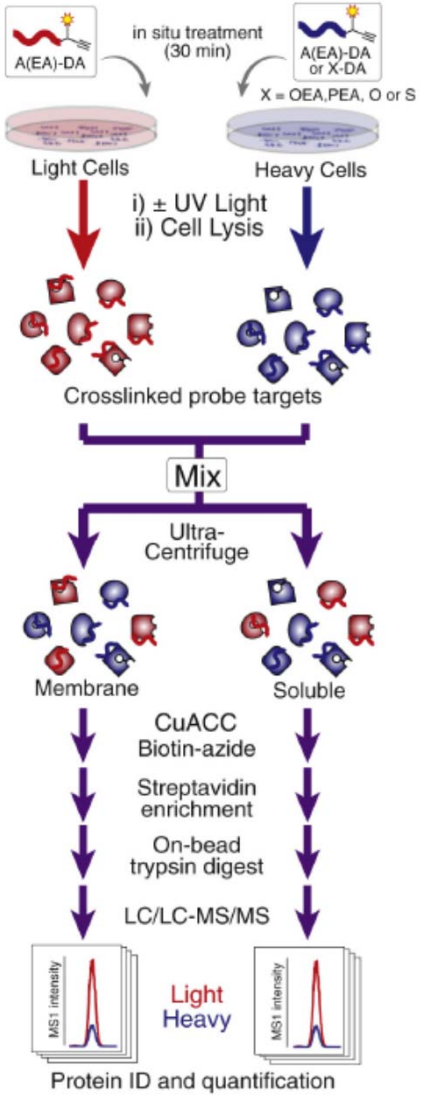
Probe labeling of membrane and soluble proteins depends on UV irradiation of cells, confirming reversible binding interactions between the probes and cellular proteins

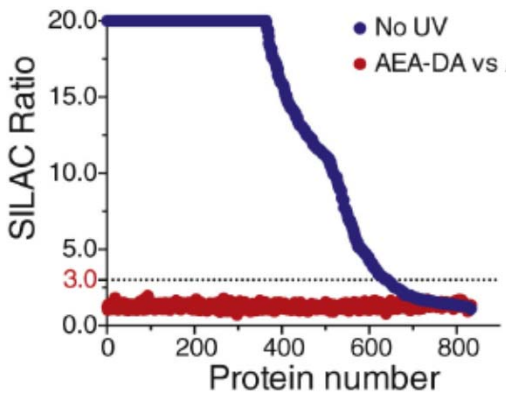


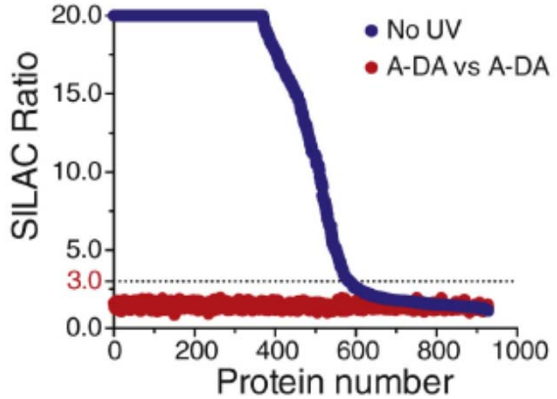
AEA-DA and A-DA showed more extensive proteomic labeling profiles compared to OEA-DA, O-DA, PEA-DA, S-DA lipid probes.

2. MS-based proteomic studies on mapping the proteins that interact with the arachidonoyl lipid probes in HEK293T cell

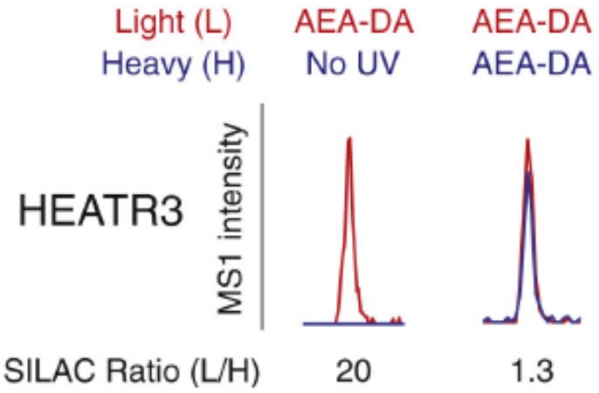
Schematic flow of the SILAC experiment[1)] SILAC: stable isotope by labeling amino acid in cell culture







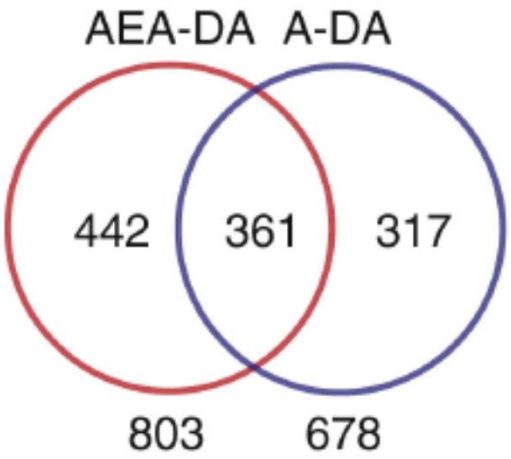
More than 1,000 proteins in total were enriched from HEK293T



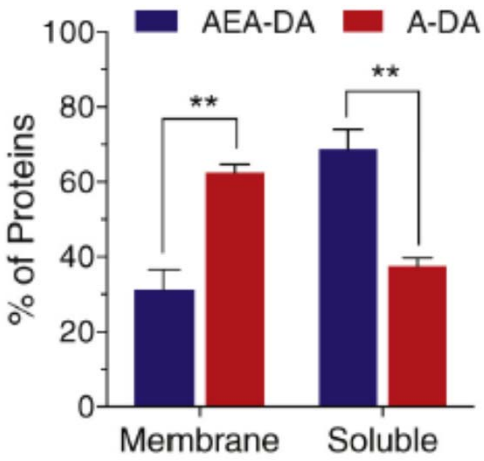
1) Hulce, J. J.; Cognetta, A. B.; Niphakis, M. J.; Tully, S. E.; Cravatt, B. F. *Nature Method* **2013**, *10*, 259.

2. Analysis of lipid probe targets

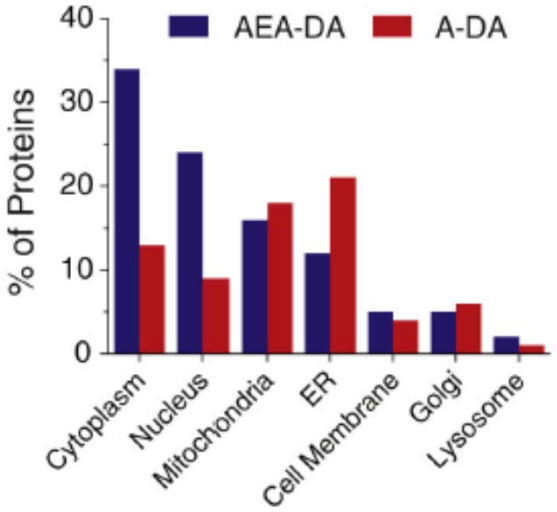
Diagram of shared and unique protein targets of AEA-DA and A-DA probes in HEK293T cell



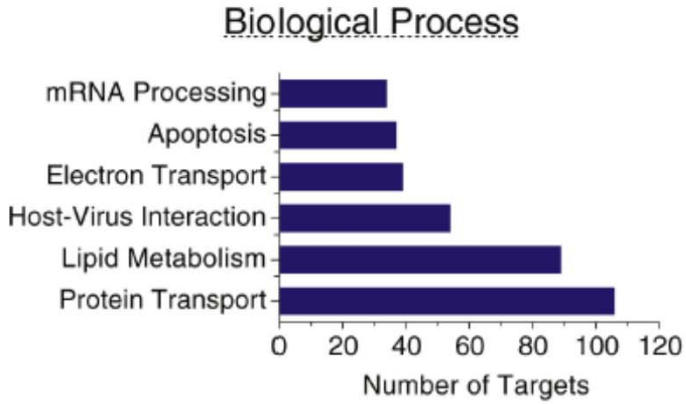
membrane and soluble distribution



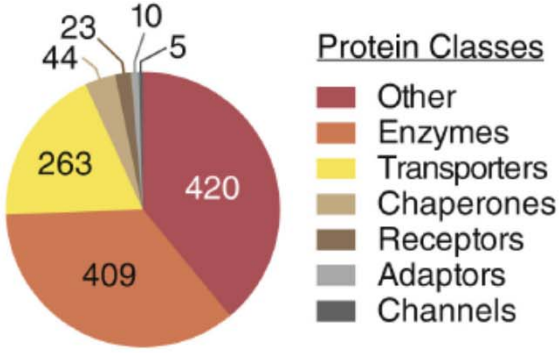
subcellular distribution



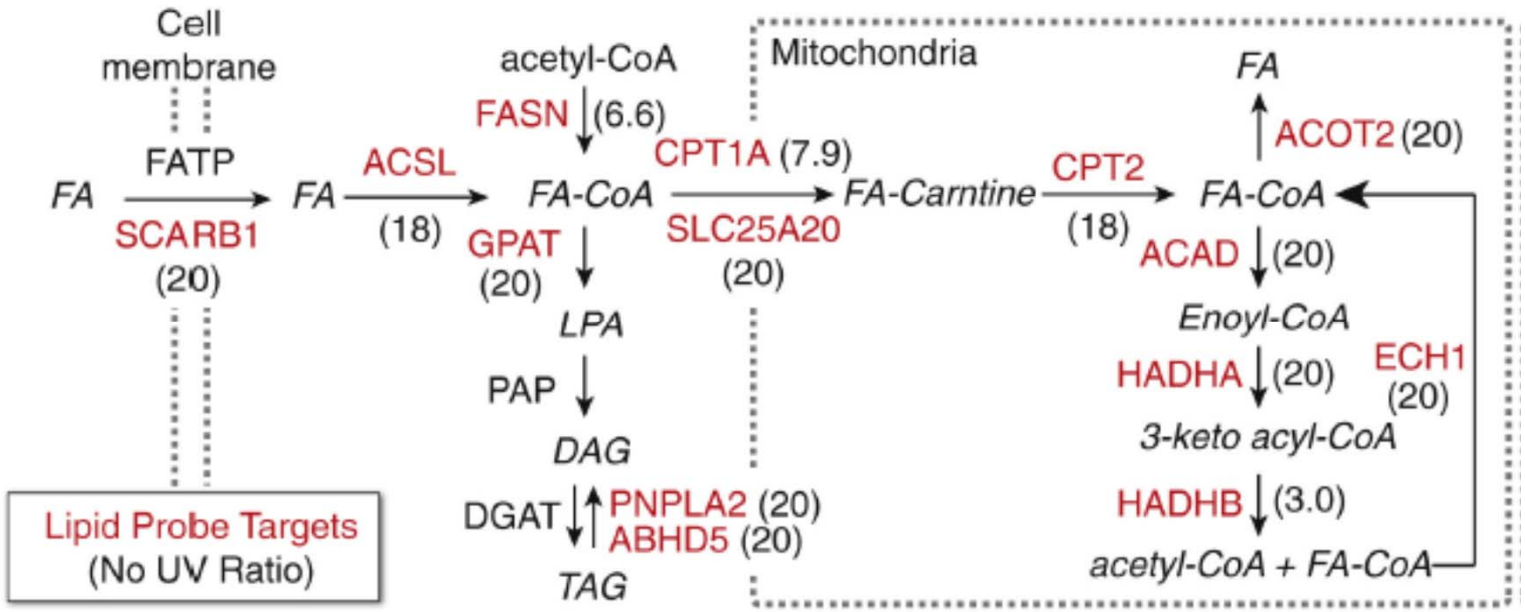
involvement in specific biological processes



protein class distribution



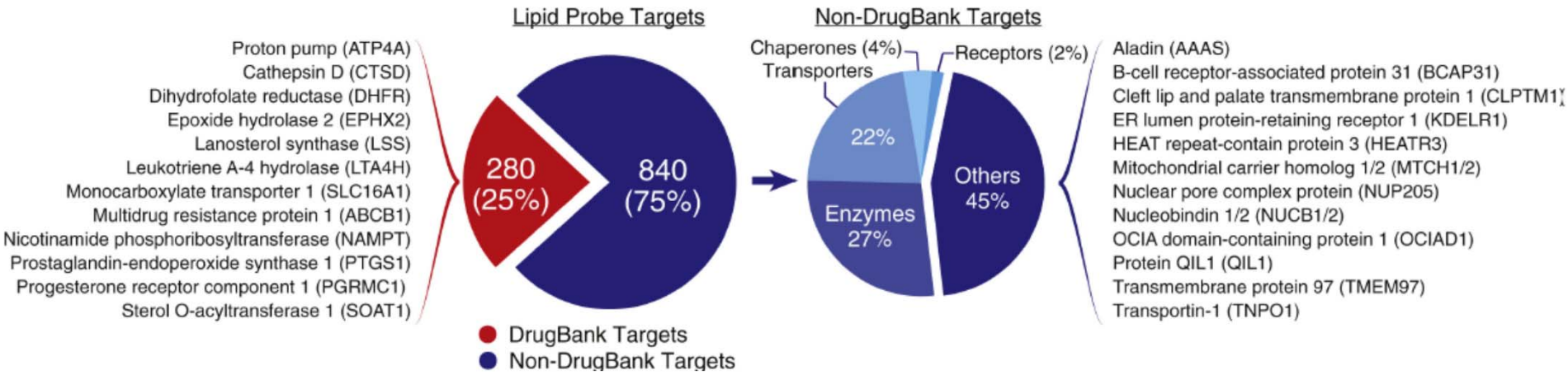
2. Diagram highlighting lipid probe targets (red) in major fatty acid metabolic pathways



SILAC ratios from probe-versus-no UV experiments are indicated in parentheses next to gene names

SCARB1: fatty acid uptake
SLC25A20: transport
FASN, PNPLA2: biosynthesis
ACAD, HADHA: catabolism

Categorization of lipid probe targets based on distribution in DrugBank and analysis of non-DrugBank targets by protein classes considered ligandable or not

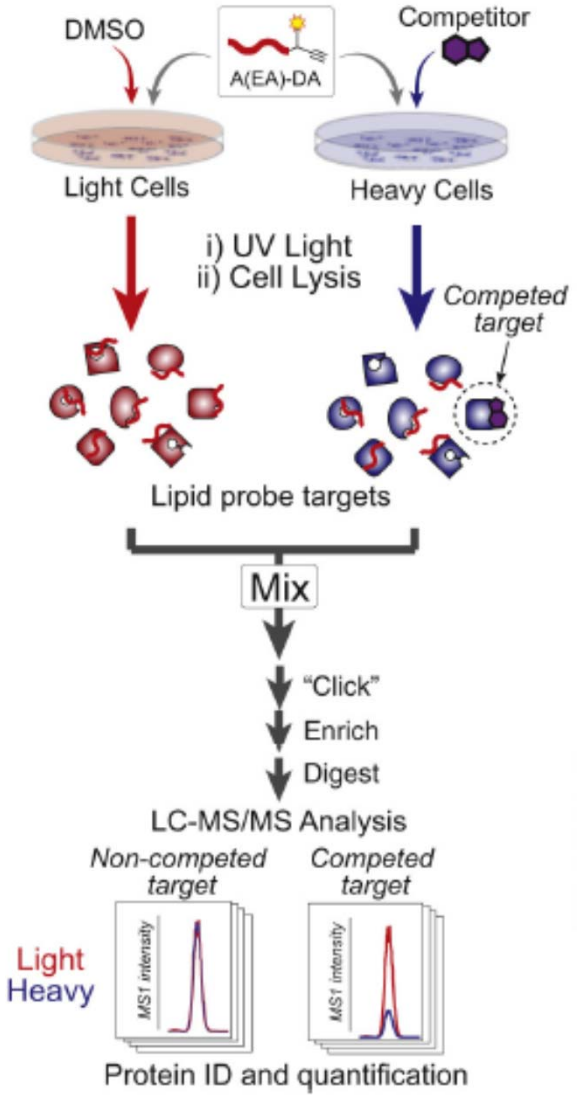


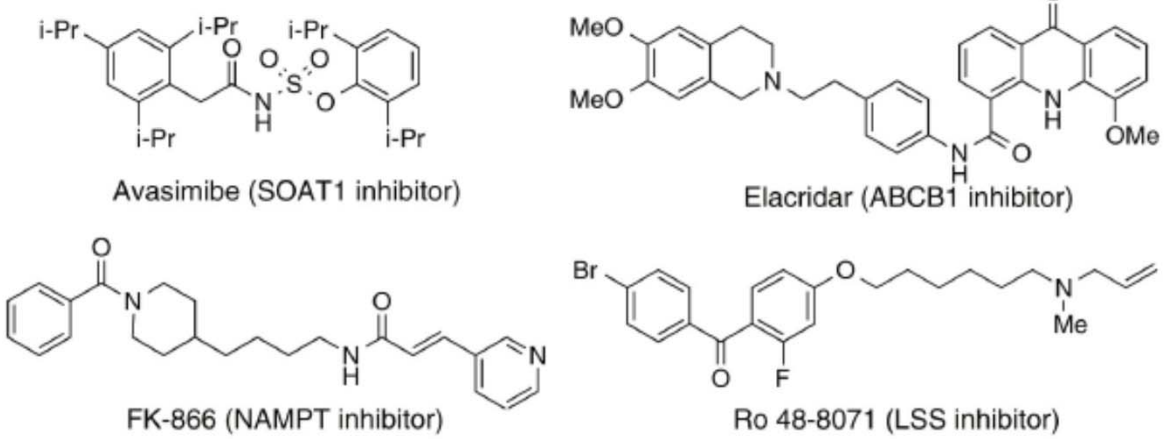
The lipid probes might facilitate the discovery of many additional proteins with the potential to bind small-molecule ligands.

For ligandable proteins known or identified herein, the lipid might provide a method to determine drug target engagement and the selectivity of these interactions in cells.

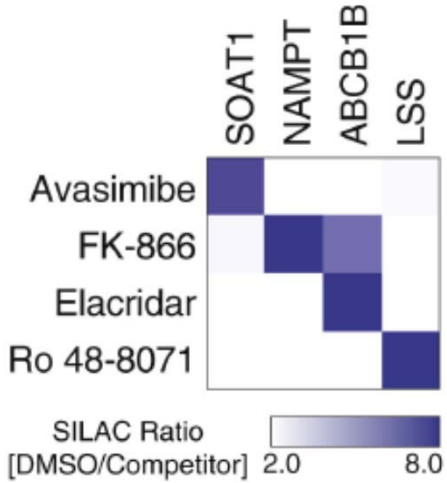
3. Diagram highlighting lipid probe targets (red) in major fatty acid metabolic pathways

Scheme for competitive profiling of ligands and lipid probes

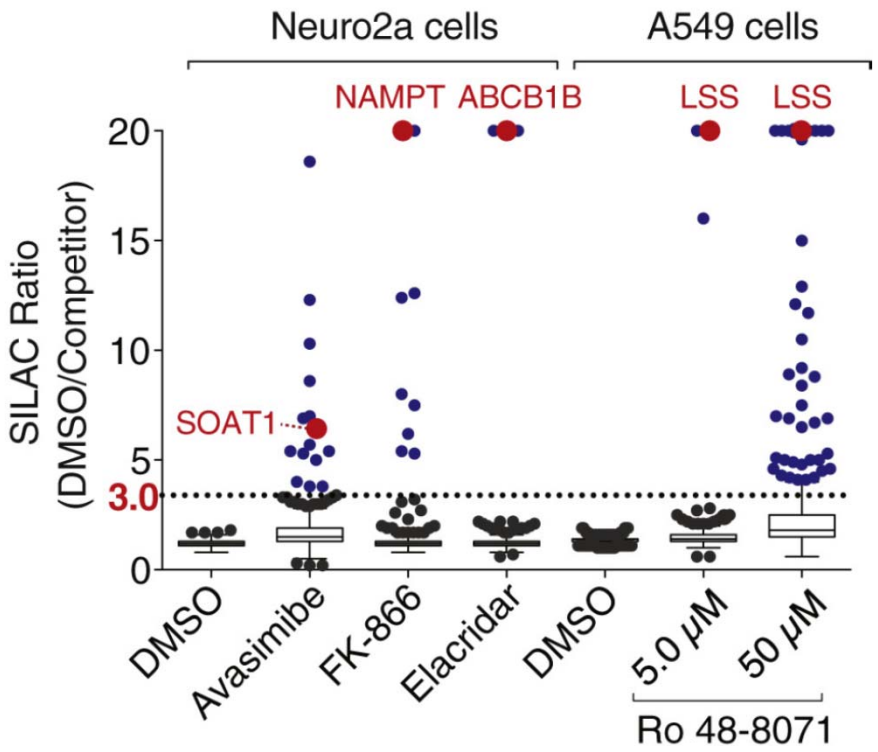




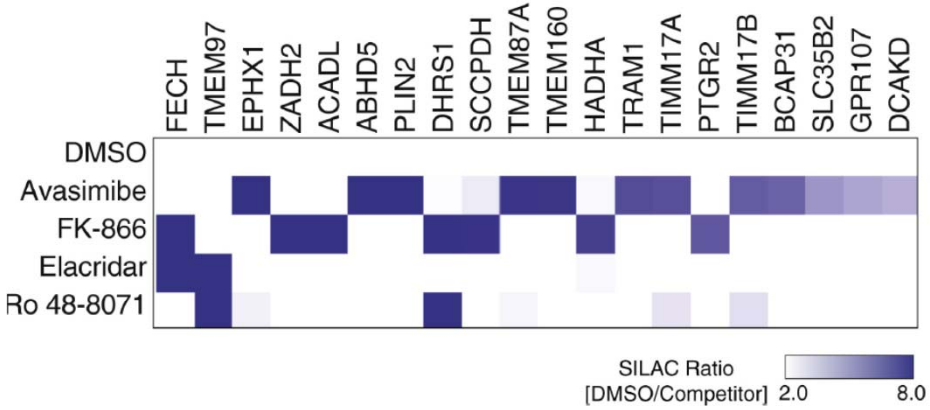
Heatmap showing SILAC ratios for primary targets of drugs in Neuro2a

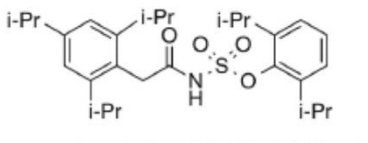


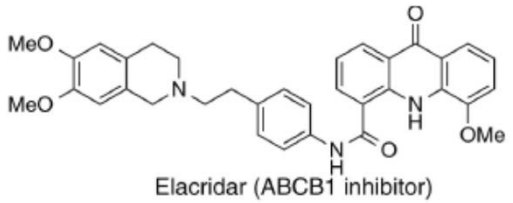
3. A greater survey of the lipid-interaction proteome to reveal a unique set of additional targets for each drug

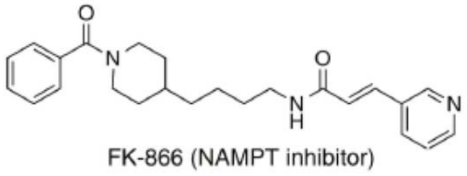


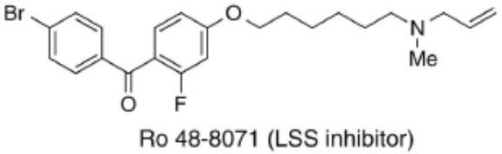
Heatmap of competed off-targets for tested drugs



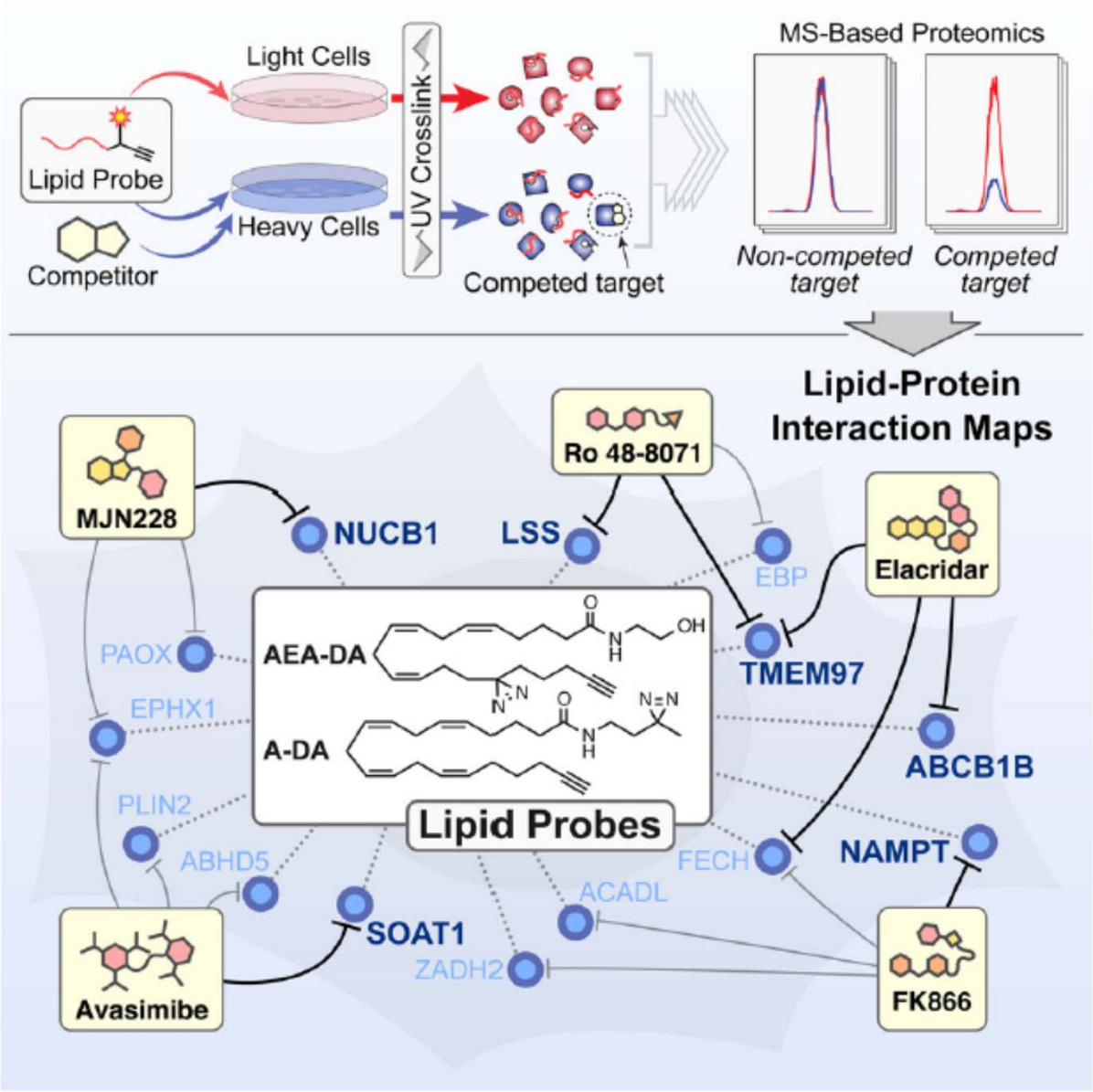








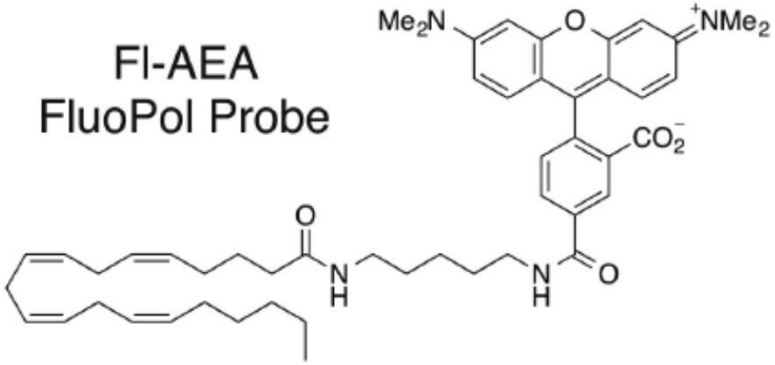
Graphical summary of in situ drug profiling with lipid probes



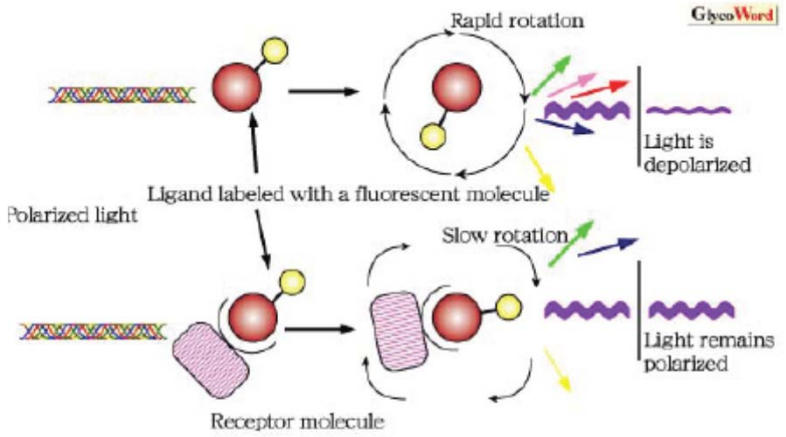
Investigating the proteome-wide interactions of known drugs provides one path for discovering ligand-binding proteins

Next challenge is
whether lipid probes can be adapted for the screening of large compound libraries to discover the selective ligand for the lipid-binding protein.

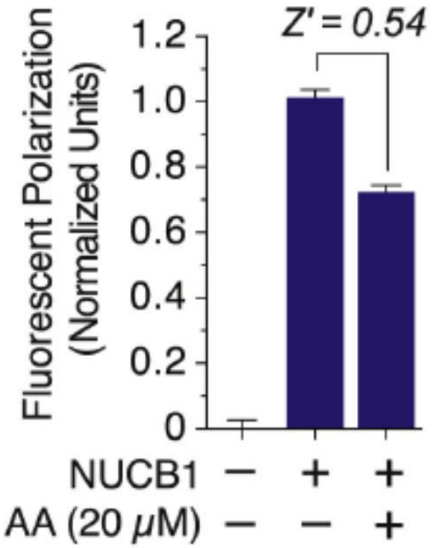
4. Fluorescent arachidonoyl lipid probe



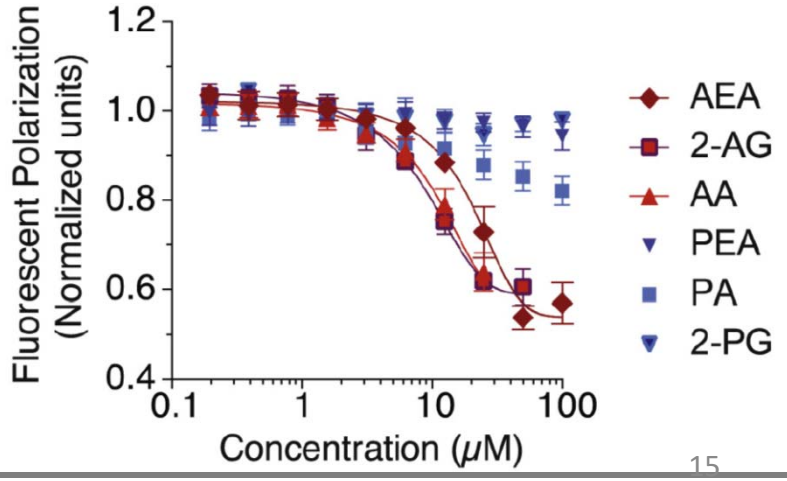
Principle of fluorescence polarization[1)]



FluoPol signal was suppressed by the competitor lipid AA

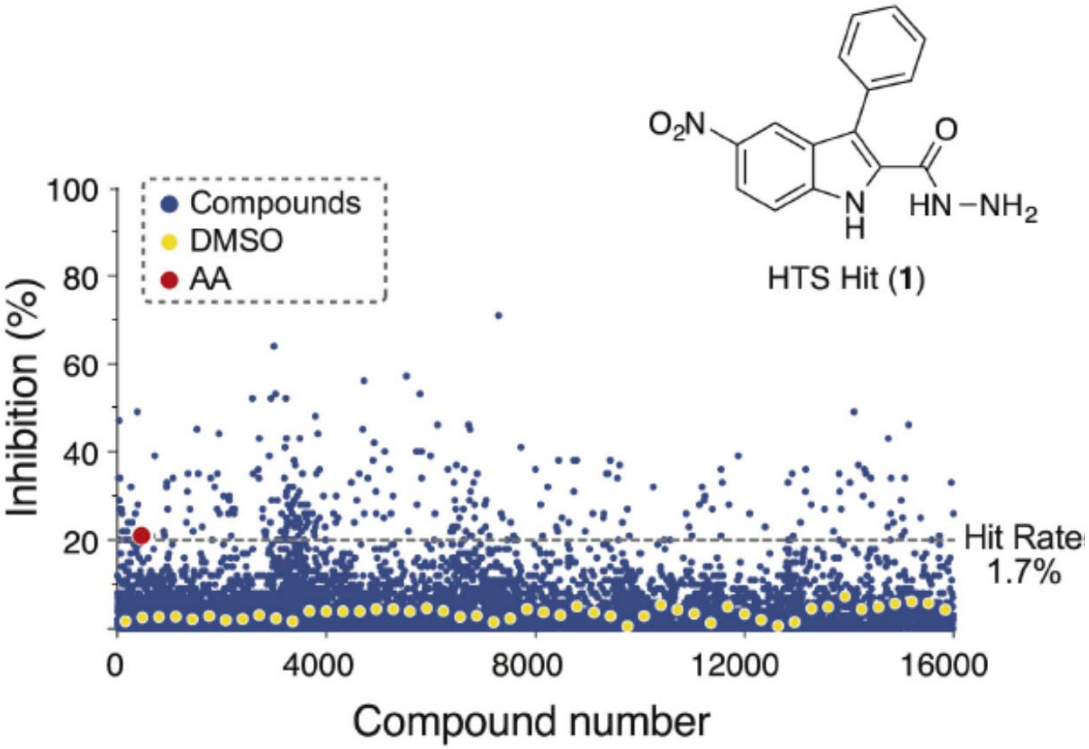


Concentration-dependent suppression of the NUCB1-FluoPol signal by arachidonoyl lipids

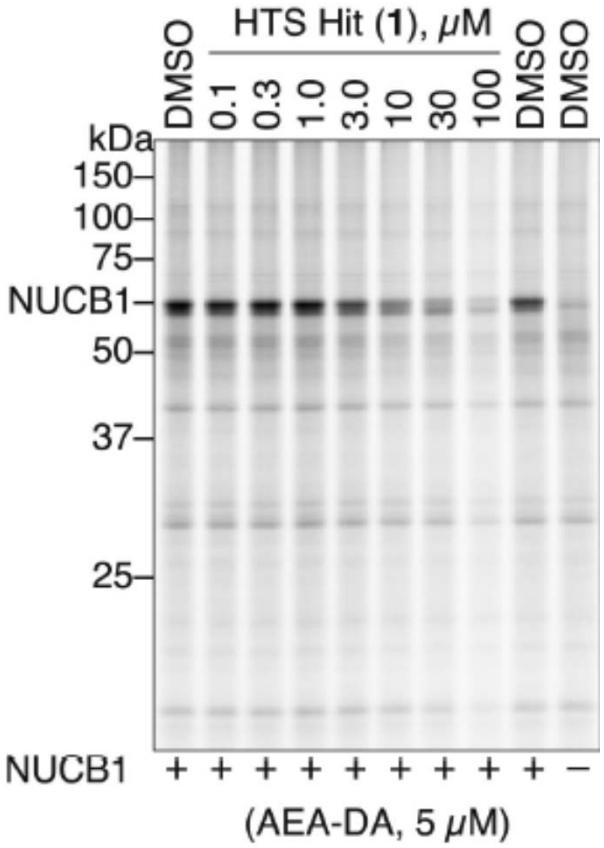


1) http://www.glycoforum.gr.jp/science/word/glycotechnology/GT-C06J.html

4. Adapting Lipid Probes for HTS to Discover NUCB1 Ligands



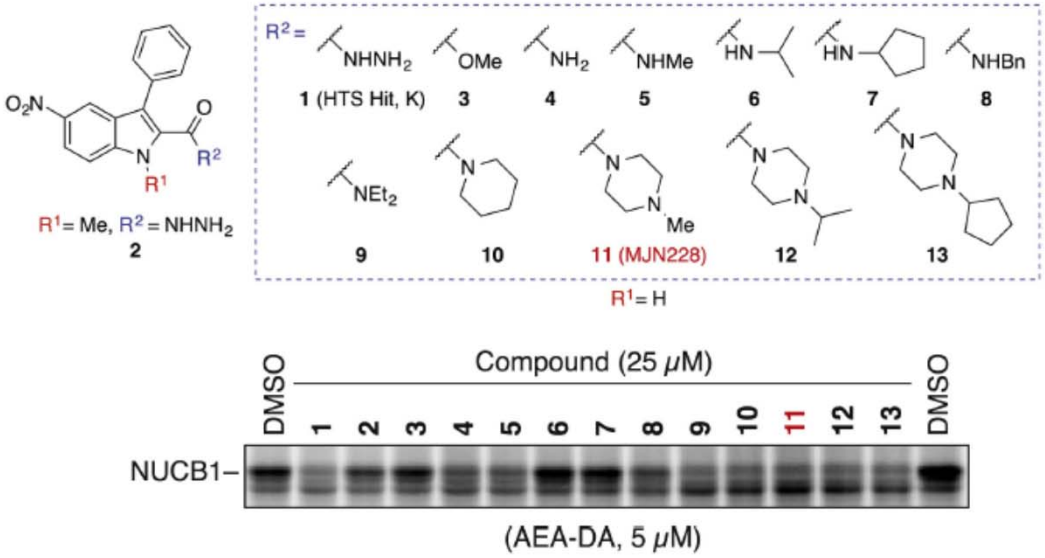
Concentration-dependent blockade of AEA-DA labeling of recombinant NUCB1 by HTS hit 1



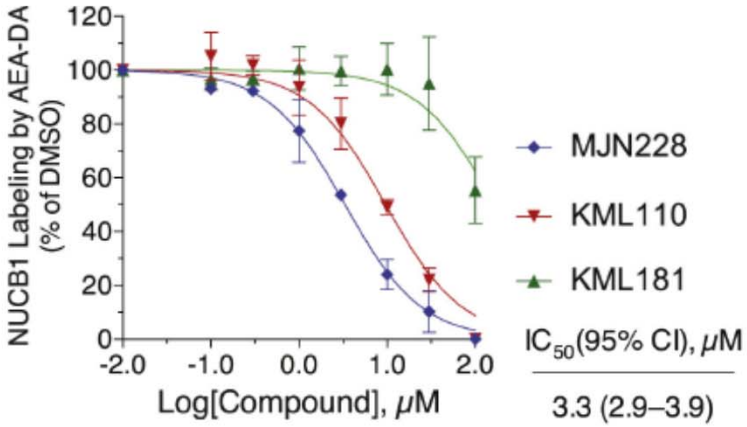
The FluoPol assay was used to screen 16,000 compounds.
100 compounds that produced a 20% or greater reduction in FluoPol signal was identified.
Gel-based competitive profiling with the AEA-DA probe against recombinant hNUCB1 was identified **1** as a strong competitor of NUCB1.

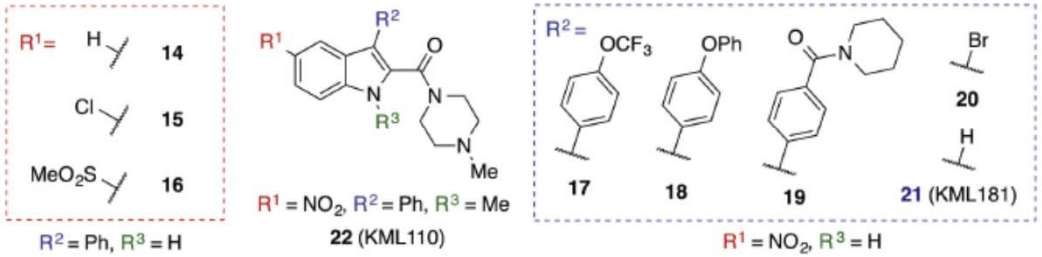
4. Optimization of Hit compound and their activities

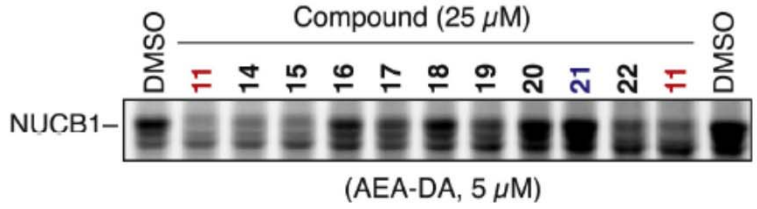
Structures and competition gel profiles of amide and ester analogs



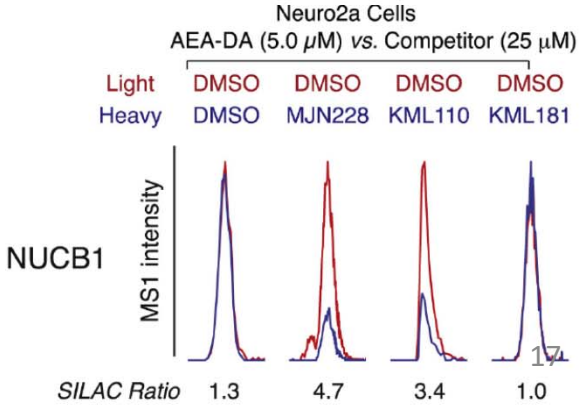
IC_{50} curves and values





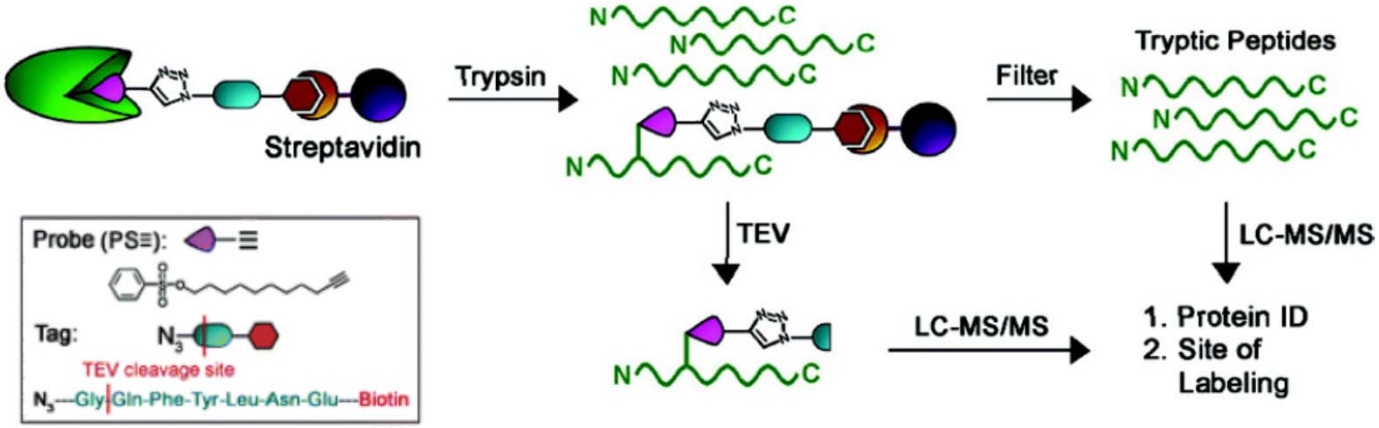


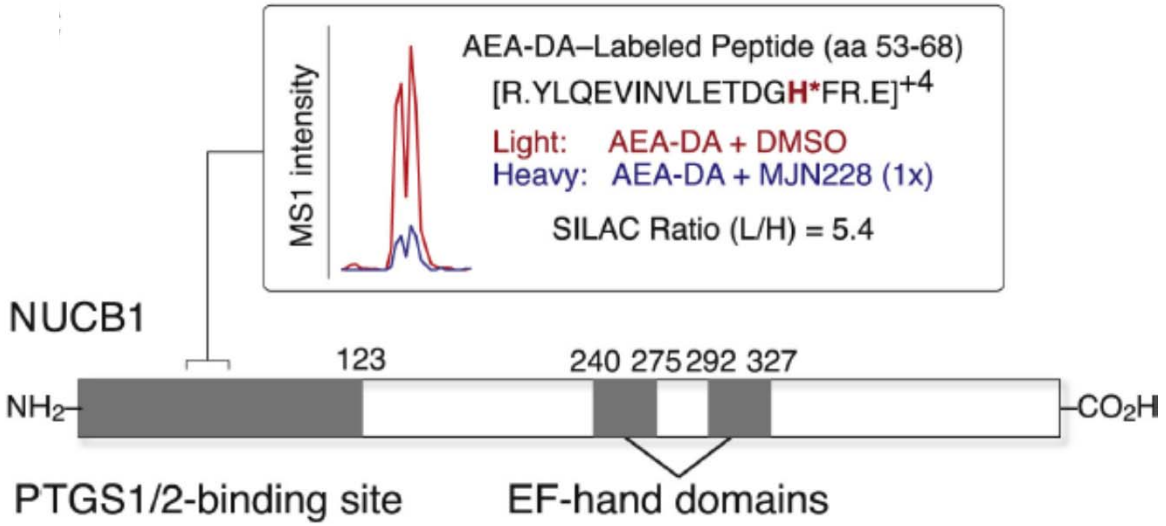
Blockade of AEA-DA probe labeling of endogenous NUCB1 in Neuro2a cells by MJN228 and KML110, but not KML181.



5. The identification of biding site of arachidonoyl probe and MJN228 to NUCB1

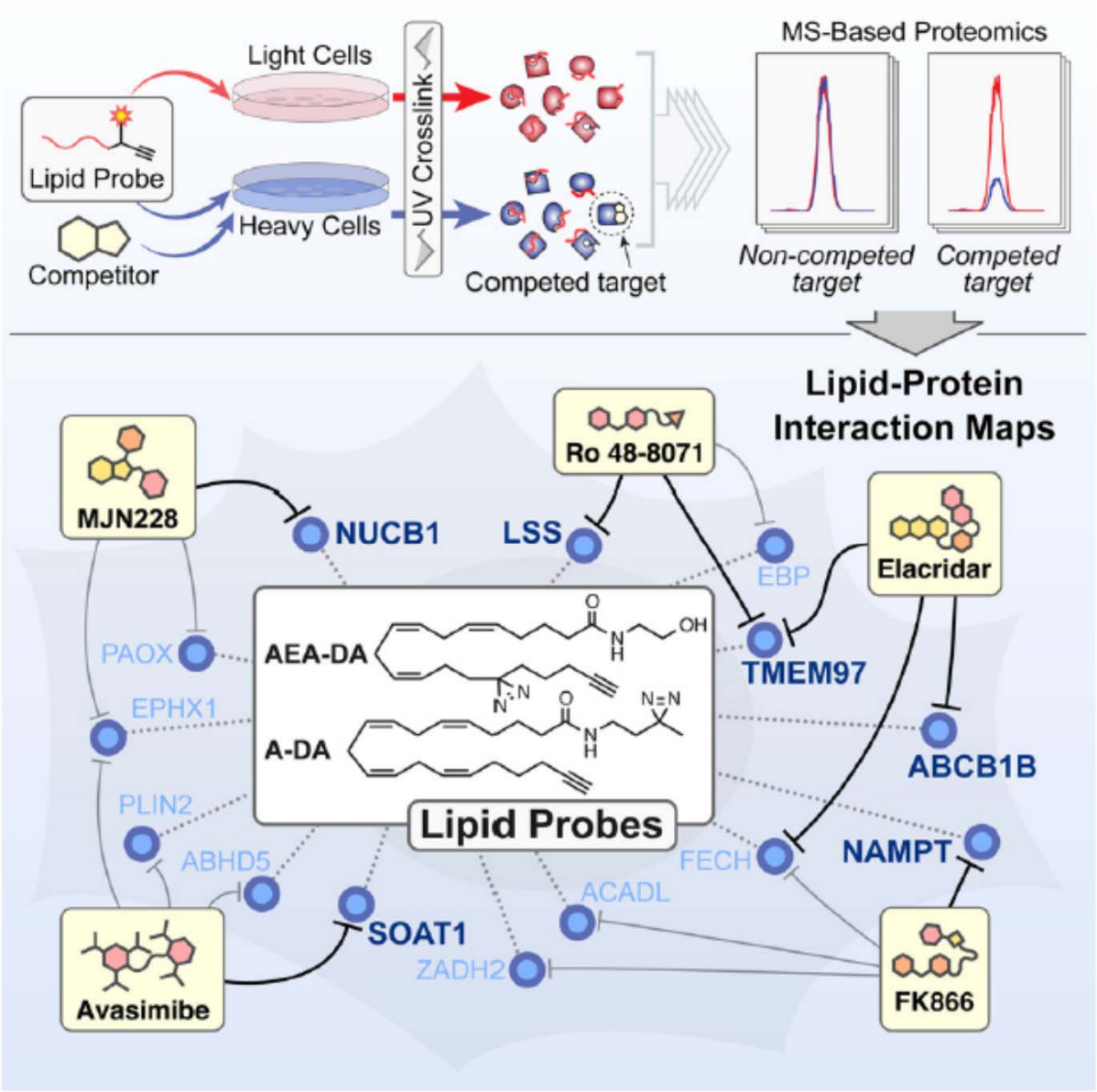
Schematic flow of Tandem Orthogonal Proteolysis Strategy[1)] TEV: tobacco etch virus protease





1) Speers, A. E.; Cravatt, B. F. J. Am. Chem. Soc. 2005, 127, 10018.

Conclusion



1. Chemical probes are utilized to for mapping lipid-binding protein.
2. Combination of lipid probes and SILAC provide a global map of lipid-binding protein.
3. Combination of lipid probes and SILAC is utilized to determine drug target engagement and the selectivity of these interactions.
4. Fluoroscent lipid probes is utilized for HTP screening of chemical libraries.
5. The use lipid probes in Tandem Orthogonal Proteolysis Strategy determines the binding site of the probe and optimized ligand.