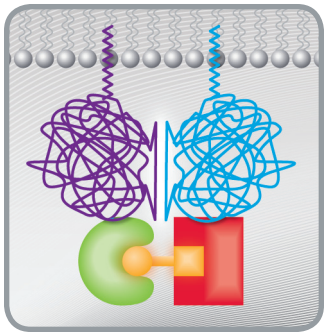
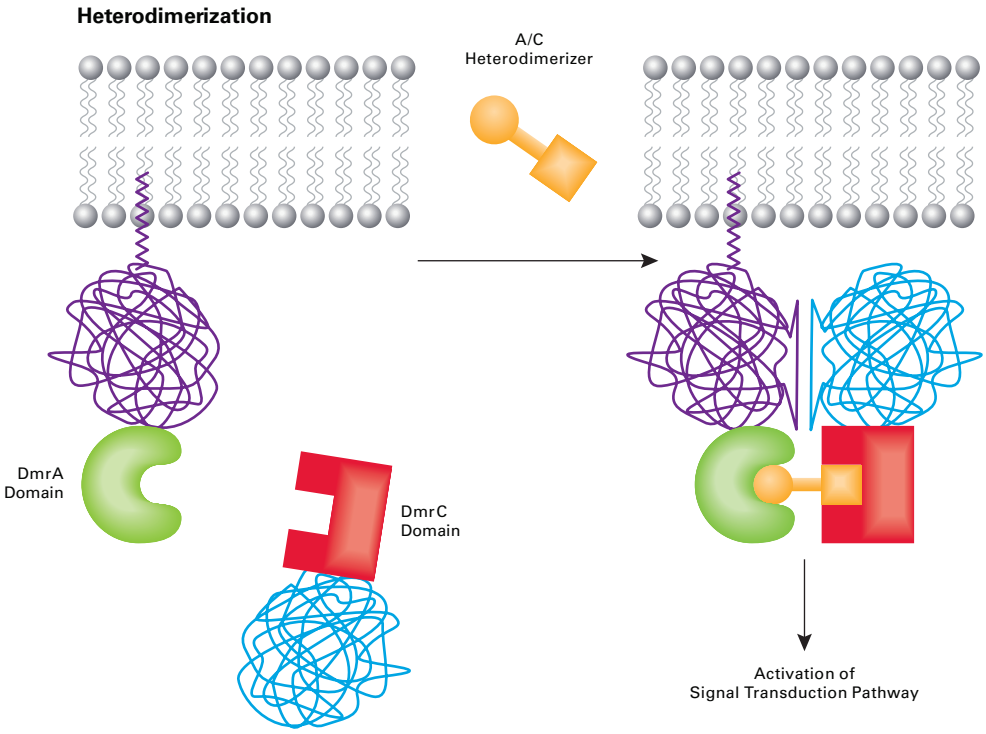
Inducible Heterodimerization Citations

Clontech's **iDimerize™ Inducible Heterodimer System** and **A/C Heterodimerizer** were previously available from ARIAD Pharmaceuticals, Inc. as the ARGENT Regulated Heterodimerization Kit and AP21967. The Inducible Heterodimerization System can be used to create and control specific interactions between two different proteins.





Fusion proteins are created which contain the DmrA (green) and DmrC (red) dimerization domains respectively. The two proteins do not interact until the A/C heterodimerizer (AP21967) is added. This cell-permeable ligand induces the fusion proteins to interact and activates downstream events.

Products

ARIAD/ARGENT Product	Clontech Product	Cat. #	Package Size
ARGENT Regulated Heterodimerization Kit	iDimerize Inducible Heterodimer System	635067	each
	Lenti-X™ iDimerize Inducible Heterodimer System	635074	each
AP21967	A/C Heterodimerizer	635057 635056 635055	500 µl 5 x 500 µl 5 mg

Each system contains a vector set and 500 µl (0.5 mM) ligand.

Notice to Purchaser

Your use of these products and technologies is subject to compliance with any applicable licensing requirements described on the product's web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

2011 Citations

Broermann, A. *et al.* (2011) *J. Exp. Med.* **208**(12):2393–2401. Dissociation of VE-PTP from VE-cadherin is required for leukocyte extravasation and for VEGF-induced vascular permeability *in vivo.* Vascular permeability and leukocyte extravasation were strongly inhibited when VE-PTP and VE-Cadherin were heterodimerized in two mouse injury models.

Dossena, S. *et al.* (2011) *J. Biol. Chem.* **286**(47):40659–40670. The molecular and functional interaction between ICln and HSPC038 proteins modulates the regulation of cell volume. Dimerization of HSPC038 and ICLn increases swell-induced chlorine current (Iclswell) during hypotonic shock.

Hagan, C. R. *et al.* (2011) *Mol. Cell Biol.* **31**(12):2439–2452. Ck2-dependent phosphorylation of progesterone receptors (PR) on Ser81 regulates PR-B isoform-specific target gene expression in breast cancer cells. Progesterone Receptor B was inducibly expressed in breast cancer cells in the absence of exogenously added progestins, to demonstrate that some PR-B regulated genes are regulated ligand-independently.

2010 Citations

Boeckeler, K. *et al.* (2010) *J. Cell Sci.* **123**(Pt 16):2725–2732. Manipulating signal delivery - plasma-membrane ERK activation in aPKC-dependent migration. FKBP-paxillin dimerization was induced at the leading edge in NRK cells, using AP21967.

Grünberg, R. *et al.* (2010) *Nucleic Acids Res.* **38**(8):2645–2662. Building blocks for protein interaction devices. The ARGENT Regulated Heterodimerization Kit was combined with FRET to demonstrate the feasibility of parts-based protein synthetic biology and modular design.

Irannejad, R. and Wedegaertner, P. B. (2010) *J. Biol. Chem.* **285**(42):32393–32404. Regulation of constitutive cargo transport from the trans-Golgi network to plasma membrane by Golgi-localized G protein βγ subunits. Constitutive or inducible targeting of G protein beta gamma subunits to the Golgi, but not other subcellular locations, causes phospholipase C- and protein kinase D-dependent vesiculation of the Golgi in HeLa cells.

Lavieu, G. *et al.* (2010) *Proc. Natl. Acad. Sci. USA* **107**(15):6876–6881. Induction of cortical endoplasmic reticulum by dimerization of a coatomer-binding peptide anchored to endoplasmic reticulum membranes. HeLa cells expressing fluorescent fusions of cortical endoplasmic reticulum (cER) and Ist2 were treated with AP21967 to change protein localization from a cytosolic ER pattern to a peripheral pattern typical of the cER.

Varma, D. *et al.* (2010) *Proc. Natl. Acad. Sci. USA* **107**(8):3493–3498. Development and application of *in vivo* molecular traps reveals that dynein light chain occupancy differentially affects dynein-mediated processes. Chemically induced dimeric "traps" for the dynein light chains LC8 (Dynll1) and TcTex1 (Dynlt1) are specific and effective.

2009 Citations

Miller, R. A. (2009) *Methods Mol. Biol.* **535**:315–331. Ligand–regulated peptide aptamers. A detailed protocol for the creation of a peptide aptamer plasmid library, followed by selection in a yeast two-hybrid system and screening for ligand-regulated interactions.

Zimnik, S., Gaestel, M., and Niedenthal, R. (2009) *Nucleic Acids Res.* **37**(4):e30. Mutually exclusive STAT1 modifications identified by Ubc9/substrate dimerization-dependent SUMOylation. FKBP fusion proteins of STAT1, p53, CRSP9, FOS, CSNK2B, HES1, TCF21 and MYF6 were coexpressed with Ubc9-FRB *in vivo* and treated with AP21967 to induce SUMOylation.

2008 Citations

Geda, P. *et al.* (2008) *Yeast* **25**(8):577–594. A small molecule–directed approach to control protein localization and function. Rapamycin was used to control the subcellular localization of the calcineurin-dependent transcription factor Crz1p, and to determine that nuclear translocation is insufficient for transcription activation.

Haruki, H., Nishikawa, J., and Laemmli, U. K. (2008) *Mol. Cell* **31**(6):925–932. The anchor-away technique: rapid, conditional establishment of yeast mutant phenotypes. The nucleus of *Saccharomyces cerevisiae* was depleted of 43 different proteins of interest (involved in protein import, RNA export, transcription, sister chromatid cohesion, and gene silencing) via rapamycin-dependent heterodimerization. The expected defective growth phenotypes were obtained on rapamycin plates, and specific mutant phenotypes were obtained within minutes.

Luik, R. M. *et al.* (2008) *Nature* **454**(7203):538–542. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. STIM1 oligomerization (controlled with AP21967) was used to deplete calcium stores and control calcium entry at ER-PM junctions.

Ng, Y. *et al.* (2008) *Cell Metab.* **7**(4):348–356. Rapid activation of Akt2 is sufficient to stimulate GLUT4 translocation in 3T3–L1 adipocytes. Drug-inducible activation of Akt2 is sufficient to stimulate GLUT4 translocation in 3T3-L1 adipocytes, to an extent similar to insulin.

Rosenfeldt, G. *et al.* (2008) *Mol. Plant* **1**(1):4–14. Chemically induced and light-independent cryptochrome photoreceptor activation. Cryptochrome signal transduction in plants can be chemically induced in a dose-dependent fashion and uncoupled from the light signal, to study the role of photoreceptors in darkness or in signaling cross-talk.

Villalobos, V., Naik, S., and Piwnica-Worms, D. (2008) *Methods Mol. Biol.* **439**:339–352. Detection of protein-protein interactions in live cells and animals with split firefly luciferase protein fragment complementation. Drug-inducible split luciferase fusions were used to measure protein-protein interactions *in vivo.*

2007 Citations

Grabocka, E. and Wedegaertner, P. B. (2007) *Mol. Pharmacol.* **72**(4):993–1002. Disruption of oligomerization induces nucleocytoplasmic shuttling of leukemia-associated rho Guanine-nucleotide exchange factor. Oligomerization of leukemia-associated RhoGEF (LARG) with AP21967 prevented nucleocytoplasmic shuttling and retained LARG in the cytoplasm.

Greenstein, A. E. *et al.* (2007) *J. Biol. Chem.* **282**(15):11427–11435. Allosteric activation by dimerization of the PknD receptor Ser/Thr protein kinase from *Mycobacterium tuberculosis.* Rapamycin-inducible *M. tuberculosis* PknD kinase domain dimers were used to study how extracellular signals activate bacterial receptor Ser/Thr protein kinases and stimulate phosphorylation.

Jullien, N. *et al.* (2007) *PLoS ONE* **2**(12):e1355. Conditional transgenesis using Dimerizable Cre (DiCre). A rapamycin-controlled dimerizable Cre recombinase system was used to regulate Cre activity in mouse liver, heart, kidney, and muscle.

Li, B. *et al.* (2007) *Carcinogenesis* **28**(3):572-583. Conditional Akt activation promotes androgen-independent progression of prostate cancer. A mouse model for prostate-specific iAKT activation with AP21967 demonstrates that active Akt plays a functional role in androgen-independent progression of prostate cancer.

Lukacs, V. *et al.* (2007) *J. Neurosci.* **27**(26):7070–7080. Dual regulation of TRPV1 by phosphoinositides. A dimerizable membrane phospholipid was used to study transient receptor potential vanilloid 1 (TRPV1) ion channel regulation.

Miller, R. A., Binkowski, B. F., and Belshaw, P. J. (2007) *J. Mol. Biol.* **365**(4):945–957. Ligand–regulated peptide aptamers that inhibit the 5'–AMP–activated protein kinase. Small molecule ligand control over the presentation of peptide aptamers that interact with and inhibit AMPK.

Pratt, M. R., Schwartz, E. C., and Muir, T. W. (2007) *Proc. Natl. Acad. Sci. USA* **104**(27):11209–11214. Small-molecule-mediated rescue of protein function by an inducible proteolytic shunt. A system for the inducible rescue of a variety of proteins—including proteases, kinases, and transcription factors—which maintains native structure and function.

Schwartz, E. C. *et al.* (2007) *Nat. Chem. Biol.* **3**(1):50–54. Post-translational enzyme activation in an animal via optimized conditional protein splicing. Protein trans-splicing can be used to modulate enzymatic activity in cultured cells and in *Drosophila melanogaster.*

Tokuo, H., Mabuchi, K., and Ikebe, M. (2007) *J. Cell Biol.* **179**(2):229-238. The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation. A dimer-inducing technique was used to show that the motor function of the two-headed form of myoX is critical for actin reorganization at the leading edge.

Yang, T. *et al.* (2007) *Nat. Chem. Biol.* **3**(12):795–804. Genetically encoded molecules for inducibly inactivating CaV channels. Rapidly manipulating Ca^{2+} signals in excitable cells with a membrane-targeted rapamycin-binding domain.

2006 Citations

Bayle, J. H. *et al.* (2006) *Chem. Biol.* **13**(1):99–107. Rapamycin analogs with differential binding specificity permit orthogonal control of protein activity. Several rapamycin analogs, including AP21967, can act as heterodimerizers in transcription and protein relocalization assays.

Bernad, R. *et al.* (2006) *J. Biol. Chem.* **281**(28):19378–19386. Nup214-Nup88 nucleoporin subcomplex is required for CRM1-mediated 60 S preribosomal nuclear export. A dramatic defect in export of preribosomes was found in Nup214-Nup88-depleted cells and rescued by derivatives of Nup214 lacking the FG-repeat domain.

Dube, D. H., de Graffenried, C. L., and Kohler, J. J. (2006) *Methods Enzymol.* **415**:213–229. Regulating cell surface glycosylation with a small-molecule switch. The modular domains of most Golgi-resident enzymes can be physically separated and fused to the small molecule binding proteins FRB and FKBP, which dimerize in the presence of rapamycin. In this way, rapamycin serves as a "switch" for enzyme activity.

Dudas, M. *et al.* (2006) *Dev. Biol.* **296**(2):298–314. Epithelial and ectomesenchymal role of the type I TGF-beta receptor ALK5 during facial morphogenesis and palatal fusion. Uses AP21967-mediated heterodimerization of ALK5 to show that ALK5 activity can be induced by interaction with multiple TGF-beta type II receptor kinases.

Fili, N. *et al.* (2006) *Proc. Natl. Acad. Sci. USA* **103**(42):15473–15478. Compartmental signal modulation: Endosomal phosphatidylinositol 3-phosphate controls endosome morphology and selective cargo sorting. Regulated dimerization was used to test whether local compartmental loss of a phosphoinositide disrupts the normal traffic of specific cargoes through endosomes.

Pecot, M. Y., and Malhotra, V. (2006) *Mol. Biol. Cell* **17**(12):5372–5380. The Golgi apparatus maintains its organization independent of the endoplasmic reticulum. The authors tested the hypothesis that Golgi enzymes constitutively recycle through the ER by using rapamycin to trap FKBP-tagged Golgi enzymes when they visit the ER.

Silvius, J. R. *et al.* (2006) *Mol. Biol. Cell* **17**(1):192–202. K-ras4B and prenylated proteins lacking "second signals" associate dynamically with cellular membranes. Uses AP21967-mediated heterodimerization to study the dynamics of membrane localization of prenylated or myristoylated proteins.

Suh, B. C. *et al.* (2006) *Science* **314**(5804):1454–1457. Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. Dimerizer was used to control the location of enzymes that quickly alter the phosphoinositide composition of the plasma membrane, in order to study KCNQ ion channels.

Varnai, P., and Balla, T. (2006) *Biochim. Biophys. Acta* **1761**(8):957–967. Live cell imaging of phosphoinositide dynamics with fluorescent protein domains. Review of imaging techniques for studying phosphoinositide dynamics in live cells.

Wehrman, T. S. *et al.* (2006) *Proc. Natl. Acad. Sci. USA* **103**(50):19063–19068. A system for quantifying dynamic protein interactions defines a role for Herceptin in modulating ErbB2 interactions. A system to monitor and quantitatively compare protein-protein interactions in intact mammalian cells.

Zhan, L., Xiang, B., and Muthuswamy, S. K. (2006) *Cancer Res.* **66**(10):5201–5208. Controlled activation of ErbB1/ErbB2 heterodimers promote invasion of three-dimensional organized epithelia in an ErbB1-dependent manner: implications for progression of ErbB2-overexpressing tumors. Uses AP1510-mediated homodimerization and AP21967-mediated heterodimerization to compare the tumor-promoting activities of ErbB2 homodimers and ErbB1-ErbB2 heterodimers.

Zhu, S., Zhang, H., and Matunis, M. J. (2006) *Exp. Cell Res.* **312**(7):1042–1049. SUMO modification through rapamycin-mediated heterodimerization reveals a dual role for Ubc9 in targeting RanGAP1 to nuclear pore complexes. Uses AP21967 to study the function of small ubiquitin-related modifiers (SUMOs) by inducing them to heterodimerize with candidate substrates.

2005 Citations

Ameres, S. L. *et al.* (2005) *EMBO J.* **24**(2):358–367. Inducible DNA-loop formation blocks transcriptional activation by an SV40 enhancer. AP21967-mediated heterodimerization of DNA binding domains is used to reversibly alter DNA structure to study its role in controlling gene expression.

Binkowski, B. F., Miller, R. A., and Belshaw, P. J. (2005) *Chem. Biol.* **12**(7):847–855. Ligand-regulated peptides: a general approach for modulating protein-peptide interactions with small molecules. Uses the rapamycin-based heterodimerization in yeast to generate peptides that interact with protein targets in a ligand-reversible manner.

Graveley, B. R. (2005) *RNA* **11**(3):355–358. Small molecule control of pre-mRNA splicing. An inducible system in which a rapamycin derivative is used to heterodimerize an RNA binding domain and a splicing activation domain, in order to control pre-mRNA splicing.

Hatzivassiliou, E. G., Tsichritzis, T., and Mosialos, G. (2005) *J. Virol.* **279**(8):5215-5219. Induction of apoptosis by rewiring the signal transduction of Epstein-Barr virus oncoprotein LMP1 toward caspase activation. An inducible association of LMP1 and caspase-8 (by the heterodimerizing agent AP21967) causes apoptosis.

Inoue, T. *et al.* (2005) *Nat. Methods* **2**(6):415–418. An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. Inducible plasma membrane targeting using a rapamycin analog allows the rapid activation and inactivation of Rho GTPases.

Karpova, A.Y. *et al.* (2005) *Neuron* **48**(5):727–735. Rapid and reversible chemical inactivation of synaptic transmission in genetically targeted neurons. Neurotransmitter release from cultured neurons, brain slices, and the Purkinje neurons of transgenic mice was manipulated via AP20187- or AP21967-mediated control of synaptic vesicle fusion.

2004 Citations

de Graffenried, C. L. *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**(48):16715–16720. A small-molecule switch for Golgi sulfotransferases. Demonstrates that the activity and localization of Golgi-resident sulfotransferases can be put under heterodimerizer control.

Luker, K. E. *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**(33):12288–12293. Kinetics of regulated protein-protein interactions revealed with firefly luciferase complementation imaging in cells and living animals. A firefly luciferase protein fragment complementation system is developed and used to visualize rapamycin-induced protein-protein interactions *in vivo.*

Paulmurugan, R. *et al.* (2004) *Cancer Res.* **64**(6):2113–2119. Molecular imaging of drug-modulated protein-protein interactions in living subjects. A luciferase protein fragment complementation system was developed and used to visualize rapamycin-induced protein-protein interactions *in vivo.*

Pecot, M.Y., and Malhotra, V. (2004) *Cell* **116**(1):99–107. Golgi membranes remain segregated from the endoplasmic reticulum during mitosis in mammalian cells. Uses rapamycin-controlled protein localization to demonstrate that Golgi membranes remain separate from the endoplasmic reticulum during mitosis.

Terrillon, S., and Bouvier, M. (2004) *EMBO J.* **23**(20):3950–3961. Receptor activity-independent recruitment of beta arrestin2 reveals specific signalling modes. Uses AP21967 to study the intrinsic signaling properties of beta-arrestin2 via the forced recruitment to vasopressin V1a or V2 receptors.

2003 Citations

Hoogenraad, C. C. *et al.* (2003) *EMBO J.* **22**(22):6004–6015. Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport. AP21967 was used to study the dramatic effect Bicaudal D has on the subcellular distribution of membrane organelles. The supplementary material includes videos showing AP21967-induced movement of organelles.

Jullien, N. *et al.* (2003) *Nucleic Acids Res.* **31**(21):e131. Regulation of Cre recombinase by ligand-induced complementation of inactive fragments. Rapamycin-activated, inducible Cre recombinase induced the association of two complementing fragments of Cre, which should be useful for the creation of conditional knock-out animals.

Kohler, J. J., and Bertozzi, C. R. (2003) *Chem. Biol.* **10**(12):1303–1311. Regulating cell surface glycosylation by small molecule control of enzyme localization. Rapamycin was used to control the Golgi localization and catalytic activity of alpha1,3 fucosyltransferase in living cells, thereby bringing sialyl Lewis^{X} production under small molecule control.

Mootz, H. D. *et al.* (2003) *J. Am. Chem. Soc.* **125**(35):10561–10569. Conditional protein splicing: a new tool to control protein structure and function *in vitro* and *in vivo.* Rapamycin and AP21967 were used to heterodimerize two halves of an artificially split intein in mammalian cells, to create a conditional protein splicing system.

Schlatter, S., Senn, C., and Fussenegger, M. (2003) *Biotechnol. Bioeng.* **83**(2):210–225. Modulation of translation-initiation in CHO-K1 cells by rapamycin-induced heterodimerization of engineered eIF4G fusion proteins. An inactive translational initiation factor, eIF4G, was reconstituted via rapamycin-induced heterodimerization.

So, C. W. *et al.* (2003) *Cancer Cell* **4**(22):99–110. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. AP21967-mediated dimerization of MLL was sufficient to enhance the self-renewal of hematopoietic progenitors.

Stankunas, K. *et al.* (2003) *Mol. Cell* **12**(6):1615–1624. Conditional protein alleles using knockin mice and a chemical inducer of dimerization. A loss-of-function GSK-3beta mutant was restored in the presence rapamycin or a rapamycin analog. This system may provide a general method of making conditional alleles to facilitate the study of protein function *in vitro* and *in vivo.*

2002 Citations

Abida, W. M. *et al.* (2002) *Chembiochem.* **3**(9):887–895. Receptor-dependence of the transcription read-out in a small-molecule three-hybrid system. Characterized a dexamethasone-methotrexate (Dex-Mtx) heterodimerizer in yeast.

Althoff, E. A., and Cornish, V. W. (2002) *Angew. Chemie Int. Ed. Eng.* **41**(13):2327–2330. A bacterial small-molecule three-hybrid system. Mtx-SLF was used to heterodimerize dihydrofolate reductase- and FKBP-containing fusion proteins and to construct a three-hybrid system that functions in bacteria.

Baker, K. *et al.* (2002) *Proc. Natl. Acad. Sci. USA* **99**(26):16537–16542. Chemical complementation: a reaction-independent genetic assay for enzyme catalysis. A dexamethasone-methotrexate (Dex-Mtx) heterodimerizer system was used to develop a general yeast-based screen for enzymatic activity.

Chang, D. W. *et al.* (2002) *EMBO J.* **21**(14):3704–3714. c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. Rapamycin-mediated heterodimerization and AP20187-mediated homodimerization were used to explore the role of c-FLIP-L in the CD95-mediated apoptotic signaling pathway.

Chen, M. *et al.* (2002) *J. Biol. Chem.* **277**(52):50761–50767. Activation of initiator caspases through a stable dimeric intermediate. AP20840 was used to heterodimerize different caspases in order to study their activation mechanisms.

Clemons, P. A. *et al.* (2002) *Chem. Biol.* **9**(1):49–61. Synthesis of calcineurin-resistant derivatives of FK506 and selection of compensatory receptors. A variant of FK506 was used to control the activity of a transcription factor in yeast and mammalian cells.

de Wildt, R. M. *et al.* (2002) *Proc. Natl. Acad. Sci. USA* **99**(13):8530–8535. Isolation of receptor-ligand pairs by capture of long-lived multivalent interaction complexes. The rapamycin-dependent interaction of FKBP and FRAP was used to demonstrate the ability of the SAC (selection by avidity capture) approach to detect receptor-ligand interactions *in vitro.*

Galarneau, A. *et al.* (2002) *Nat. Biotechnol.* **20**(6):619–622. Beta-lactamase protein fragment complementation assays as *in vivo* and *in vitro* sensors of protein protein interactions. Rapamycin was used to heterodimerize two complementing fragments of beta-lactamase, demonstrating the system's ability to detect inducible protein interactions.

Li, B. *et al.* (2002) *Gene Ther.* **9**(4):233–244. A novel conditional Akt 'survival switch' reversibly protects cells from apoptosis. Akt was conditionally activated with a rapamycin analog (via membrane recruitment) in order to protect cells from apoptosis.

Mootz, H. D., and Muir, T. W. (2002) *J. Am. Chem. Soc.* **124**(31):9044–9045. Protein splicing triggered by a small molecule. A system for conditional protein splicing which uses rapamycin to heterodimerize two halves of an artificially split intein.

2002 Citations…continued

Scheid, M. P., Marignani, P. A., and Woodgett, J. R. (2002) *Mol. Cell Biol.* **22**(17):6247–6260. Multiple phosphoinositide 3-kinase-dependent steps in activation of protein kinase B. Kinase activation was studied using AP21967-mediated membrane recruitment of protein kinase B/Akt.

Wehrman, T. *et al.* (2002) *Proc. Natl. Acad. Sci. USA* **99**(6):3469–3474. Protein-protein interactions monitored in mammalian cells via complementation of beta-lactamase enzyme fragments. Two complementing fragments of beta-lactamase were heterodimerized with rapamycin, in order to detect inducible protein interactions in mammalian cells.

2001 Citations

Biggar, S. R., and Crabtree, G. R. (2001) *EMBO J.* **20**(12):3167–3176. Cell signaling can direct either binary or graded transcriptional responses. The heterodimerizer FK506 was used to understand how transcriptional activators cause changes in gene expression.

Koide, K. *et al.* (2001) *J. Am. Chem. Soc.* **123**(3):398–408. A synthetic library of cell-permeable molecules. Development of novel, synthetic heterodimerizers that penetrate cells and target known proteins.

Otto, K. G. *et al.* (2001) *Blood* **97**(11):3662–3664. Cell proliferation through forced engagement of c-Kit and Flt-3. Rapamycin-mediated heterodimerization of the signaling domains of c-kit and Flt-3 stimulated proliferation of cells normally dependent on IL-3 for growth.

2000 Citations

Biggar, S. R., and Crabtree, G. R. (2000) *J. Biol. Chem.* **275**(33):25381–25390. Chemically regulated transcription factors reveal the persistence of repressor-resistant transcription after disrupting activator function. Used rapamycin and FK506 as heterodimerizers to study the function of transcriptional repressors.

Castellano, F., and Chavrier, P. (2000) *Methods Enzymol.* **325**:285–295. Inducible membrane recruitment of small GTP-binding proteins by rapamycin-based system in living cells. Review of recent work using the rapamycin heterodimerizing system to study the function of signaling proteins by recruiting them to the membrane.

Castellano, F., Montcourrier, P., and Chavrier, P. (2000) *J. Cell Sci.* **113**(Pt17):2955–2961. Membrane recruitment of rac1 triggers phagocytosis. Rapamycin-mediated recruitment of Rac1 to the cell surface triggers phagocytosis.

Griffith, E. C., Licitra, E. J., and Liu, J. O. (2000) *Methods Enzymol.* **328**:89–103. Yeast three-hybrid system for detecting ligand-receptor interactions. Detailed protocols for the use of the three-hybrid system to identify and characterize novel small molecule protein ligands.

Lin, H. *et al.* (2000) *J. Am. Chem. Soc.* **122**:4247–4248. Dexamethasone-methotrexate: an efficient chemical inducer of protein dimerization *in vivo.* Dex-Mtx is a novel alternative heterodimerizer which can dimerize DHFR- and glucocorticoid receptor-containing fusion proteins *in vitro.*

1999 Citations

Briesewitz, R. *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96**(5):1953–1958. Affinity modulation of small-molecule ligands by borrowing endogenous protein surfaces. Enhancing the affinity of small molecule inhibitors of target proteins with the heterodimerizer approach.

Castellano, F. *et al.* (1999) *Curr. Biol.* **9**(7):351–360. Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation. Rapamycin-mediated recruitment of Cdc42 or WASP to the cell surface triggers actin polymerization.

Muthuswamy, S. K., Gilman, M., and Brugge, J. S. (1999) *Mol. Cell Biol.* **19**(10):6845–6857. Controlled dimerization of ErbB receptors provides evidence for differential signaling by homo- and heterodimers. The homodimerizer AP1510 and the heterodimerizer rapamycin were used to study the differential signaling activities of ErbB1 and ErbB2 homodimers and heterodimers.

1998 Citations

Klemm, J. D., Schreiber, S. L., and Crabtree, G. R. (1998) *Annu. Rev. Immunol.* **16**:569–592. Dimerization as a regulatory mechanism in signal transduction. Reviews the natural role of protein dimerization in cellular processes and describes how dimerizers provide a general method to regulate such processes.

Stockwell, B. R., and Schreiber, S. L. (1998) *Curr. Biol.* **8**(13):761–770. Probing the role of homomeric and heteromeric receptor interactions in TGF-beta signaling using small molecule dimerizers. Explores the role of receptor oligomerization in TGF-beta signaling using a variety of homodimerizers and heterodimerizers, including rapamycin and FK1012.

1997 Citations

Graef, I. A. *et al.* (1997) *EMBO J.* **16**(18):5618–5628. Proximity and orientation underlie signaling by the non-receptor tyrosine kinase ZAP70. Explores the effects of membrane recruitment, orientation and oligomerization of ZAP-70 using the homodimerizer FK1012 and the heterodimerizer rapamycin.

Klemm, J. D., Beals, C. R., and Crabtree, G. R. (1997) *Curr. Biol.* **7**(9):638–644. Rapid targeting of nuclear proteins to the cytoplasm. Describes a general method to inducibly relocalize a nuclear protein to the cytoplasm by using rapamycin to heterodimerize a nuclear protein of interest and a protein containing a nuclear export signal.

Liberles, S. D. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94**(15):7825–7830. Inducible gene expression and protein translocation using nontoxic ligands identified by a mammalian three-hybrid screen. The rapamycin heterodimerizer system was modified to function with non-immunosuppressive analogs, and used to control the subcellular localization of a protein and the activity of a transcription factor.

Rossi, F., Charlton, C. A., and Blau, H. M. (1997) *Proc. Natl. Acad. Sci. USA* **94**(16):8405–8410. Monitoring protein-protein interactions in intact eukaryotic cells by beta-galactosidase complementation. Rapamycin was used to heterodimerize two complementing beta-galactosidase deletion mutants and to detect protein complexes within cells.

1996 Citations

Belshaw, P. J. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**(10):4604–4607. Controlling protein association and subcellular localization with a synthetic ligand that induces heterodimerization of proteins. An FKBP-cyclophilin heterodimerizer (FKCsA) was used to alter the subcellular localization of a fusion protein and control the activity of a transcription factor.

Licitra, E. J. and Liu, J. O. (1996) *Proc. Natl. Acad. Sci. USA* **93**(23):12817–12821. A three-hybrid system for detecting small ligand–protein receptor interactions. A chemical dimerizer "three-hybrid" system was used to identify and characterize novel small molecule-protein interactions.