Chapter 1

G protein-directed ligand discovery with peptide combinatorial libraries

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Introduction

G protein-coupled receptors (GPCRs)[1] relay diverse extracellular signals to intracellular signal transduction pathways through heterotrimeric G proteins (*1, 2*). While drug discovery efforts have primarily focused on GPCRs, ligands for intracellular G proteins that directly modulate signaling have been increasingly regarded as potential drugs (*3-5*). Short peptides, both naturally occurring and synthetically derived from segments of GPCRs, G proteins, and effectors, have been used extensively to map critical interaction sites and antagonize or activate G proteins (*4-6*). While successful, most of these peptides are weak modulators of signaling, exhibiting their activities at μM to mM concentrations. Combinatorial methods have the potential of substantially increasing the potency of known ligands and identifying novel peptides with new functions from diverse, random libraries (*7, 8*). Here, we review several examples of *in vitro* selection applied to the isolation of peptide modulators of G protein signaling.

G protein signaling cycle

In the classical G protein signaling model, an inactive GPCR is coupled to a GDP-bound, $G_{\alpha\beta\gamma}$ heterotrimer (Figure 1). $G_{\beta\gamma}$ binds tightly to G_{α}-GDP, which enhances coupling of the inactive heterotrimer to specific GPCRs and acts as a guanine nucleotide dissociation inhibitor (GDI) by preventing GDP release (*9*). Activation by an extracellular agonist causes the GPCR to act as a guanine nucleotide exchange factor (GEF), exchanging GDP for GTP in the G_{α} subunit. GTP-binding to G_{α} induces $G_{\beta\gamma}$ release and subsequently both

[1] Abbreviations: GAP, GTPase-activating protein; GDI: guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; GoLoco, $G_{\alpha i/o}$-Loco interaction; GPCR, G protein-coupled receptor; GPR, G protein regulatory; GTPγS, guanosine 5'-*O*-(3-thiotriphosphate); MBP, maltose-binding protein; RGS, regulator of G protein signaling; RT-PCR, reverse transcription-polymerase chain reaction.

G_α-GTP and $G_{\beta\gamma}$ can interact with downstream effectors. The intrinsic GTPase activity of G_α results in the eventual hydrolysis of GTP, leading to reformation of the inactive $G_{\alpha\beta\gamma}$ heterotrimer and re-coupling to the receptor. GTPase-activating proteins (GAPs) accelerate the hydrolysis of G_α-GTP, leading to shorter activation times and/or lower basal activities. This simple model of G protein signaling has grown increasingly complex because of (1) the numerous regulatory proteins that modulate or attenuate signaling by acting as GEFs, GDIs, or GAPs and/or by directly competing with receptor, G protein, or effector interactions (*10, 11*); (2) the immense diversity and crosstalk of signal transduction pathways controlled by heterotrimeric G protein activation (*2, 12*); and (3) the growing number of intracellular receptor partners discovered that activate signals through means other than classical G protein pathways (*13*).

In humans, there are 20 distinct, but highly homologous, G_α subunits that are divided into four classes based on their sequence and function: (1) $G_{i/o}$, (2) G_s, (3) $G_{q/11}$, and (4) $G_{12/13}$ (*2*). Despite their similarity, the G_α families can elicit different functions and have distinct and sometimes overlapping specificities for their binding partners (*2*). There are currently 6 known G_β and 11 G_γ subunits, making a large number of $G_{\beta\gamma}$ heterodimers possible. Each of the G_α and $G_{\beta\gamma}$ pairs can interact with a wide variety of effectors. While classical drugs targeting GPCRs usually antagonize natural agonist responses, direct G protein ligands can potentially modulate individual effector pathways, alter signals specifically from particular G protein classes or subclasses, and/or modify the kinetics of G protein signaling. Hence, there is a large degree of selectivity that can be

conferred by drugs that directly interact with G proteins or interfere with G protein signaling (*5*).

In vitro selection with combinatorial libraries

Naturally occurring peptides, as well as peptides derived from portions of GPCRs, G proteins, and effectors, have been used effectively to study the interactions between these proteins (*6*). These peptides are able to modulate G protein signaling in different ways (e.g., by antagonizing G protein interactions or directly stimulating GDP exchange in G_α subunits). Methods for the directed evolution of peptides can both optimize these ligands for higher affinity and activity or isolate novel sequences with desired properties from random libraries (*7, 8*). A typical selection experiment involves (1) construction of a DNA library, (2) expression to produce a peptide library where members are physically linked to their nucleic acid sequences, (3) affinity selection against an immobilized target to retain functional peptides, and (4) amplification of the recovered nucleic acid sequences to produce an enriched library (Figure 2). Typical selection libraries examine 10^8 to 10^9 unique molecules, whereas totally *in vitro* methods that do not require an *in vivo* transformation step can access even greater pool complexities ($>10^{13}$).

Receptor-G protein interface

While a complete structural characterization of GPCR-G protein coupling and activation has not yet been described, biochemical analyses have established that the receptor-G_α interface involves several regions on G_α, including the N- and C-termini, and the intracellular loops and C-terminus of the GPCR (*10, 14*). Synthetic peptides corresponding to the last 11 amino acids in the C-terminus of a number of G_α subunits

have been shown to block G protein-receptor coupling with low potency (μM IC_{50} values), as well as stabilize active forms of the GPCR, presumably by mimicking the conformational effects of heterotrimeric G proteins (*4-6, 10*). These C-terminal-derived peptides generally demonstrate receptor selectivity similar to the full-length G_α subunit.

To enhance the potency of a rhodopsin-binding peptide derived from the C-terminus of $G_{t\alpha}$ (transducin), a "doped" library was constructed using the "peptides-on-plasmids" approach (*15*). In this selection method, peptides are expressed as LacI fusions, which bind stably to *lacO* DNA sequences on the plasmid encoding the peptide. Peptide-LacI-plasmid complexes were affinity purified on activated rhodopsin and recovered plasmids encoding functional peptides were subsequently amplified (*16*). Selected peptides were significantly more potent than the wild-type sequence and the amino acid conservation highlighted several critical residues (Table I). Subsequent work demonstrated that the $G_{t\alpha}$ peptide analogs are able to modulate high and/or low affinity states of the A_1 adenosine receptor and reduce GPCR signaling responses in a receptor-selective fashion (*17*). These results suggest that selections targeting other GPCRs may be able to produce specific ligands, even though many receptor-G_α contacts are shared.

Interestingly, while amino acid conservation was not observed in the random region of the library (Table I), full-length, 15-residue peptides were significantly more potent than synthetic, C-terminal 11-mers derived from the selected sequences (*16*), suggesting that the structural context of the synthetic peptides is important for the high affinity interaction with rhodopsin. Indeed, recombinant N-terminal MBP-peptide fusions were several orders of magnitude more potent than their synthetic peptide counterparts. These

fusion proteins may display the selected peptides in a context similar to the LacI fusion used in the peptides-on-plasmids approach.

The receptor-G protein interface can also be disrupted using peptides derived from the intracellular loops and C-terminus of the GPCR, which presumably bind to G proteins and prevent coupling (*6, 14*). Thorough analyses of these peptides and optimization using combinatorial libraries have not yet been demonstrated. Previously, peptides derived from different intracellular regions of rhodopsin were shown to inhibit G protein coupling (*18*). These peptides demonstrated synergistic inhibition; the addition of multiple peptides dramatically decreased G protein coupling by binding to multiple contact sites on the G_α subunit. Hence, selection libraries based on protein scaffolds that present several receptor-derived loops, thereby mimicking the intracellular face of a GPCR, may be more effective for isolating more potent ligands.

G protein activators

Random peptide libraries have been an effective tool in the isolation of novel sequences with desired properties. Recently, $G_{i\alpha 1}$ was directly targeted in a phage display selection using a commercially available, 7-mer (X_7) peptide library (*19*). In phage display, peptide sequences are expressed on the surface of filamentous phages and selected against an immobilized target (*20*). Three classes of peptides with short consensus motifs were identified from the selection (Table I). Because the consensus sequences were short, database searches identified many (250 to 1000) proteins containing the motifs, only a few of which were implicated or known to be involved in signal transduction (*19*).

Curiously, the authors did not report any direct binding assays to assess peptide affinity or specificity for G_α subunits. However, two of the peptide classes demonstrated the ability to increase the rate of binding of GTP to G_α i, o, and s (*19*). These peptides bear little similarity to other known G protein activators that have cationic, amphipathic structures such as mastoparan (*21*). Whether the selected peptides act specifically as GEFs has yet to be determined, though in a reconstituted GPCR membrane assay, the active peptides were shown to increase the sensitivity of A_1 adenosine receptor agonist-binding to GTP, most likely due to an increase in the equilibrium level of G_α-GTP present in the reconstituted system (*19*).

G_α-$G_{\beta\gamma}$ interface

Activation of effectors by either G_α-GTP or $G_{\beta\gamma}$ is effectively blocked by formation of the GDP-bound heterotrimer, $G_{\alpha\beta\gamma}$. Hence, individual effectors most likely share overlapping binding sites at the G_α-$G_{\beta\gamma}$ interface. Extensive mapping of key residues for effector binding on $G_{\beta\gamma}$, for example, has shown that various signaling partners for $G_{\beta\gamma}$ rely on different subsets of residues for interaction (*22*). Hence, by targeting different sites on or adjacent to the G_α-$G_{\beta\gamma}$ interface, individual pathways might be affected.

Phage display peptides against $G_{\beta\gamma}$

Recently, phage display was used to identify peptides that bind to $G_{\beta\gamma}$ (*23*). A variety of libraries were used, both linear and constrained with disulfide bridges (Table I). Approximately 250 copies of peptide were displayed per phage, permitting the recovery of peptides with even very low affinity due to avidity effects (though higher affinity

peptides may be more difficult to isolate due to the narrower dynamic range of binding). The authors cleverly modified $G_{\beta\gamma}$ with an amine-specific biotinylation reagent in the presence of G_{α}, thereby "protecting" the G_{α}-$G_{\beta\gamma}$ interface from modification. After G_{α} was removed by affinity chromatography, biotinylated $G_{\beta\gamma}$ was immobilized on streptavidin and used as the selection target.

The selected peptides were grouped into four families, one of which had significant homology to peptides derived from phospholipase C-β (PLC-β) and to a short motif in phosducin that binds to G_{β} subunits (*23*). Peptides from all four families appeared to bind to a single site on $G_{\beta\gamma}$ based on competition experiments, suggesting a "hot spot" for binding interaction (*24, 25*). One synthesized peptide was shown to actively promote G_{α} dissociation from $G_{\beta\gamma}$, presumably through a non-competitive, allosteric effect (*26, 27*). Intriguingly, the peptide inhibited activation of PLC-β by $G_{\beta\gamma}$, but not $G_{\beta\gamma}$-mediated inhibition of voltage-gated calcium channels or adenylyl cyclase (*23*). Subsequent studies were also able to demonstrate an *in vivo* response to the application of the peptides, which presumably resulted from the disruption of heterotrimers and activation of downstream MAP kinase pathways in the absence of receptor activation (*26*).

The more recent description of an N-terminal, single-site biotinylation tag on G_{β} (*26*) suggests that homogeneously oriented, immobilized $G_{\beta\gamma}$ could be used in the future as a selection target. This may provide access to additional protein interaction sites that were blocked by biotinylation, due to protection of only the G_{α}-binding surface. Various sets of effectors might also be useful as competitors during selection experiments to identify rare peptides with highly specific functions.

mRNA display with the GoLoco/GPR motif

mRNA display is a completely *in vitro* method for selection where individual peptides are covalently coupled to the 3'-end of their encoding mRNA, resulting in stable RNA-peptide fusions (*28*). Pools of fusions are selected for binding against an immobilized target and recovered sequences are amplified by RT-PCR. The G protein regulatory (GPR) or GoLoco motif binds selectively to $G_{i/o\alpha}$ subunits and acts as a GDI (*29, 30*). mRNA display libraries, based on the C-terminal half of a GPR consensus sequence (*31*), were selected against $G_{i\alpha1}$ specifically biotinylated at the N- or C-terminus (*32*). A strongly conserved motif was identified and the dominant peptide after selection (named R6A) demonstrated high affinity (60 nM) and GDI activity for $G_{i\alpha1}$ (Table I).

The R6A peptide was subsequently minimized to a 9-residue sequence that retained high affinity and GDI activity and also competed with $G_{\beta\gamma}$ for binding to $G_{i\alpha1}$ (*32*). This 9-mer sequence retained only two residues from the original GPR consensus motif and, based on subsequent analysis, most likely exerts its effects though a different mechanism than the GPR consensus peptide. Recent results have demonstrated that the minimal peptide is able to bind to different G_α subunits representing all four G protein families.[2] Hence, this peptide acts as a core motif for G protein binding and most likely interacts with a conserved region in all G_α subunits. By starting with doped libraries based on this consensus sequence, peptides could be selected against various G_α subunits to produce peptides with class- and/or subclass-specificity.

[2] Ja and Roberts, manuscript in preparation.

G_α specificity using adapter peptides

The high sequence and structural similarity between the various G_α subunits makes it difficult to isolate small ligands that can distinguish between G protein classes. When comparing G_α subunits, it is evident that the helical domain represents the best target for developing class-specific molecules because of the high variability between all four G protein classes (Figure 3, left). This has been shown for RGS9, a GAP which differentiates between $G_{t\alpha}$ and $G_{i\alpha1}$ by recognizing subclass-specific residues in the helical domain (*33*). The crystal structure of a $G_{i\alpha1}$:RGS14-GoLoco complex revealed how a short peptide could selectively bind to a G_α subunit (*34*). The poorly conserved region C-terminal to the GoLoco motif makes numerous contacts with residues in the helical domain of $G_{i\alpha1}$ that differ in $G_{o\alpha}$, thereby imparting increased affinity and subclass specificity (Figure 3, right). RGS14 specificity has recently been extended to $G_{i\alpha1}$ over $G_{i\alpha2}$, which is remarkable due to the high protein sequence identity (88%) between these two isoforms (*35*).

The GoLoco peptide essentially acts as an efficient payload delivery system for directly affecting G protein interactions. While the GoLoco consensus sequence (the "payload") interacts with regions that interfere with nucleotide exchange and $G_{\beta\gamma}$-binding, the C-terminal region acts as an adapter peptide that delivers the required functional groups to a specific G_α target. Indeed, when replacing the RGS14-GoLoco peptide C-terminus with a sequence derived from Pcp2 (a GoLoco protein that acts on $G_{o\alpha}$ rather than $G_{i\alpha}$), the specificity of the RGS14-GoLoco-Pcp2 chimera is switched (*34*). Hence, it may be possible to design class-specific G_α ligands using various adapter

peptides to deliver small molecules or functional peptide motifs that modulate signaling activity.

While the G_α helical domain is an attractive target for designing specific ligands, several selective peptides have been characterized that likely interact primarily with the Ras-like domain. A GoLoco/GPR consensus peptide that binds to $G_{i\alpha}$ without the presence of the extended, non-conserved C-terminal region retains a strong preference for $G_{i\alpha}$ over $G_{o\alpha}$ subunits (*31, 36*). Assuming that the consensus peptide binds similarly to the strongly related RGS14-GoLoco peptide used in the $G_{i\alpha1}$ complex crystal structure (*34*), specificity for $G_{i\alpha1}$ over $G_{o\alpha}$ most likely results from different conformations of the G_α binding surface rather than the identity of specific residue contacts (Figure 3, right). While $G_{i\alpha1}$ has been extensively characterized by crystallography, structures of other $G_{i/o\alpha}$ isoforms are not yet available. These structures may reveal subtle conformational differences of interaction sites that establish subclass specificity between these strongly related proteins.

Several peptide activators of G_α subunits have also been studied. Mastoparan and its analogs demonstrate varying specificities for the $G_{i/o\alpha}$ and $G_{s\alpha}$ families (*21, 37, 38*). Competition binding studies suggest that mastoparan interacts with the C-terminus of $G_{i\alpha}$ (*39*). A 14-residue peptide derived from the IGF-II receptor preferentially activates $G_{i\alpha2}$ over $G_{i\alpha1}$ and $G_{i\alpha3}$, though the binding site is unknown (*40*). From these examples it is evident that class-specific peptide modulators of G protein signaling targeting the Ras-like domain can be developed, though the molecular design and mechanism of achieving this specificity is much less clear.

Future directions

The increasingly complex model for G protein signaling drives the need for new tools for probing G protein structure and function. Selection techniques have already enabled the discovery of novel peptide ligands with unique properties. Targeting of different G protein states (nucleotide-free (*41*), GDP, GDP-AlF_4^- (*42, 43*), or GTPγS) may facilitate the isolation of various G protein modulators that act as GEFs, GDIs, or GAPs. Similar effects may be achieved by targeting natural G protein regulators (e.g., RGS proteins (*44, 45*)). Assaying the effect *in vivo* of potential signal modulators will be crucial toward their use as drugs or drug leads. The utility of direct peptide modulators of G protein signaling is illustrated in the targeted expression of a C-terminal $G_{q\alpha}$ peptide that inhibited G_q-signaling in a murine model of cardiac pressure overload, thereby protecting the mice against subsequent myocardial hypertrophy (*46*). Hence, if peptide ligands can overcome the plasma membrane barrier and avoid proteolysis, they may indeed be useful as drugs *in vivo*.

Of the techniques used for peptide selection against G protein targets, mRNA display will be a significant tool for the rapid isolation of potent ligands. mRNA display has significant advantages over other peptide selection techniques, including access to higher complexity and monovalent display of library members, resulting in the identification of high affinity sequences (*47*). Access to extremely large libraries, comprising $>10^{12}$ molecules, most likely led to the successful isolation of high affinity $G_{i\alpha1}$-binding peptides that contain a critical mutation in the peptide constant region (*32*). The recent incorporation of unnatural amino acids into mRNA display libraries using sense (*48, 49*) and nonsense (*50*) suppression schemes provides further molecular diversity to explore.

Additionally, mRNA display libraries of peptide-drug conjugates (*51*) may be useful in the selection of molecules consisting of nucleotide analogs or other G protein-interacting ligands covalently coupled to peptides optimized for selectivity.

New discoveries of GPCR and G protein activation through non-traditional means continue to add complexity to the classical G protein signaling model (*13*). A number of diverse proteins (e.g., arrestins, GPCR kinases, and small GTP-binding proteins) have been found that associate with activated GPCRs and may represent additional targets for selection. Inhibition of G proteins may attenuate these alternate modes of signaling and demonstrate whether targeting G proteins for pharmaceutical purposes will be viable. Numerous molecules that interact with proteins involved in G protein signaling, including peptides derived from receptors, effectors, and G proteins, as well as natural peptides and, increasingly, designed small molecules, represent a rich source of potential starting points for selection libraries (*3-6, 52*).

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Tables

Table I. Peptide selections against G protein-related targets.

Library	Diversity	Target	Result[a]	Activity	Ref
X_4-IKENLKDCGLF[b]	2×10^9	Light-activated rhodopsin	X_4-hXXXLKDCGLF	IC_{50}[c] 140 nM	(*16*)
X_7	$20^7 = 10^9$	$G_{i\alpha1}$[d]	(i) aPXXaHP (ii) QXPXSXP (iii) LPaXXXH	EC_{50}[e] (i) 16 μM (ii) >1000 μM (iii) 17 μM	(*19*)
X_m[f] XCX_nCX $X_5CX_3CX_4$ $X_4CX_pCX_4$ X_8CX_8 XCX_{15} $X_{15}CX$ $XCCX_3CX_5C_4$GIEGRG	10^8–10^9 (each library)	$G_{\beta1\gamma2}$	(i) KAXXLLG (ii) KaXXaaG (iii) CEKRXGXXXC (iv) CX_5C	IC_{50}[g] (i) ~5 μM	(*23*)
MSQSKRLDDQR-X_6	$20^6 = 6 \times 10^7$ [h]	$G_{i\alpha1}$-GDP	MSQTKRLDDQLYWWEYL[i]	K_D[j] 60 nM	(*32*)

[a] Amino acid types: h = hydrophobic; a = aromatic or aliphatic. Multiple sequences represent consensus classes.

[b] Each residue in the constant region was mutated at a 50% rate.

[c] IC_{50} of competition with $G_{t\alpha}$ for binding to light-activated rhodopsin (Meta II). Activity is for the most potent, full-length, synthetic peptide. MBP fusion proteins were several orders of magnitude more potent (*16*).

[d] Selection buffer was apparently not supplemented with nucleotide. Hence, the G_α nucleotide state is unclear, though it probably consisted of a mix between GDP-bound and nucleotide-free subunits.

[e] EC_{50} of rate enhancement of GTPγS binding to $G_{i\alpha1}$.

[f] Subscripts m = 6, 15, or 30; n = 4, 6, 8, 10, or 12; and p = 4, 5, or 6.

[g] IC_{50} of $G_{\beta\gamma}$-mediated phopholipase C activation. Peptides also disrupt $G_{\alpha\beta\gamma}$ heterotrimer formation at similar concentrations (*26*).

[h] Selected peptides encoded critical mutations in the constant region. The presence of these mutations implies that the initial diversity of the library was actually higher than indicated. The total number of molecules in the initial mRNA display pool was approximately 10^{12}. Hence, at least 10^4 copies of each unique (random region) peptide were present. This over-representation, coupled with a finite error-rate during PCR amplification, is most likely what permitted access to extremely rare sequences derived from mutations in the constant region.

[i] Underlined region represents the minimal active peptide (K_D = 200 nM to $G_{i\alpha1}$).

[j] K_D for binding to $G_{i\alpha1}$-GDP. Peptides also exhibited GDI activity and competed with $G_{\beta\gamma}$ for binding.

Figures

Figure 1. Classical G protein signaling. An intracellular, GDP-bound $G_{\alpha\beta\gamma}$ heterotrimer is coupled to a membrane-spanning GPCR (R). $G_{\beta\gamma}$ acts as a GDI for G_{α}-GDP, inhibiting nucleotide exchange and maintaining the inactive state. Extracellular agonists cause the GPCR to act as a GEF, catalyzing the exchange of GDP for cytosolic GTP in the G_{α} subunit. G_{α}-GTP and $G_{\beta\gamma}$ subsequently dissociate and are free to signal downstream effectors (E1 and E2). Hydrolysis of G_{α}-GTP to the GDP-bound state, a reaction that is catalyzed by GAPs, results in reassociation with $G_{\beta\gamma}$ and re-coupling to the receptor. Potential modulators of G protein signaling can interfere with protein-protein interactions (e.g., receptor coupling of G proteins, $G_{\alpha\beta\gamma}$ heterotrimer formation, or effector-G protein) and/or act as GDIs, GEFs, or GAPs.

Figure 2. General strategy for the selection of functional peptides. Starting from a DNA construct encoding a peptide library (top left), a selection pool is generated using various methods that localize each peptide with its encoding nucleic acid sequence. Examples of selection methods described in this review are peptides-on-plasmids (*15*), phage display (*20*), and mRNA display (*28*). After the library is affinity-selected against an immobilized target, functional peptides are "amplified" from the recovered nucleic acid sequences (e.g., by PCR). These peptides can be identified by DNA sequencing of individual clones and/or used as the library for the next round of selection. Each round of selection generates a new library that is enriched for functional members, eventually resulting in a pool that is dominated by active peptides.

Figure 3. (left) Amino acid conservation between a representative G_α subunit from each family [human G_α i1, q, s (short-form), and 12], overlaid on a surface representation of $G_{i\alpha1}$. Gaps in the protein sequence alignment generally appear in surface loops (not shown). Amino acid differences between the G_α subunits are colored on a scale from gray (helical domain) or white (Ras-like domain), for highly conserved or identical residues, to red, for highly variable residues. GDP and Mg^{2+} are colored cyan and magenta, respectively. (right) Structure of $G_{i\alpha1}$-GDP in complex with the RGS14-GoLoco peptide (*34*). The GoLoco consensus domain (blue) and C-terminal region (yellow) make extensive contacts through the Ras-like and helical domains of $G_{i\alpha1}$. Non-identical amino acids between $G_{i\alpha1}$ and $G_{o\alpha}$ are colored in pink or red for conserved or non-conserved differences, respectively. Specific contact residues in the helical domain that differ between $G_{i\alpha1}$ and $G_{o\alpha}$ have been described previously (*34*). Residues in the Ras-like domain that may be important to the specific binding of a GoLoco/GPR consensus peptide (*31, 36*) that lacks the C-terminal region are marked. Protein alignments were performed using ClustalW (*53*) from human cDNA sequences obtained from the UMR cDNA Resource Center (http://www.cdna.org). Both structure images were made from Protein Data Bank file 1KJY (*34*) using PyMOL (http://www.pymol.org).

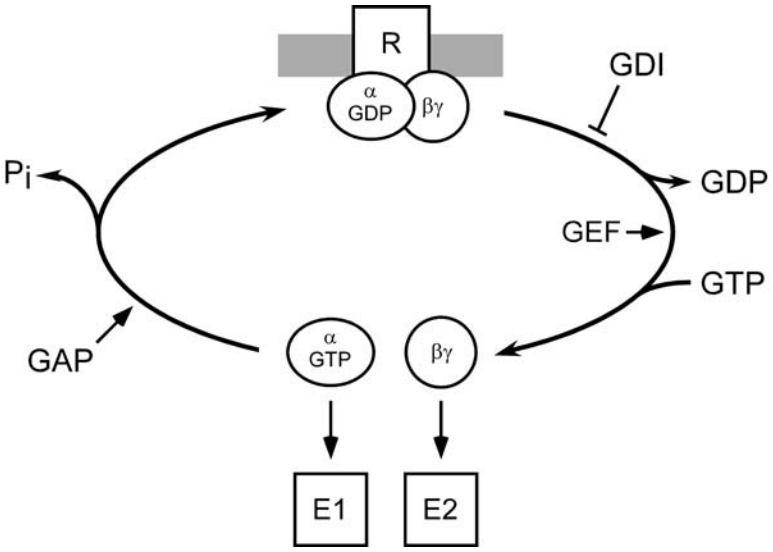

Figure 1

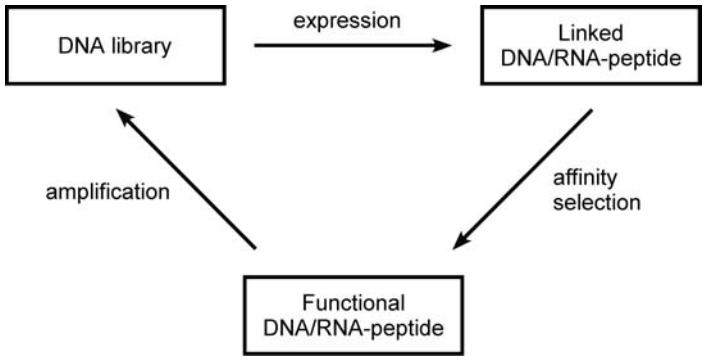
DNA library
expression
Linked
DNA/RNA-peptide
amplification
affinity
selection
Functional
DNA/RNA-peptide

Figure 2

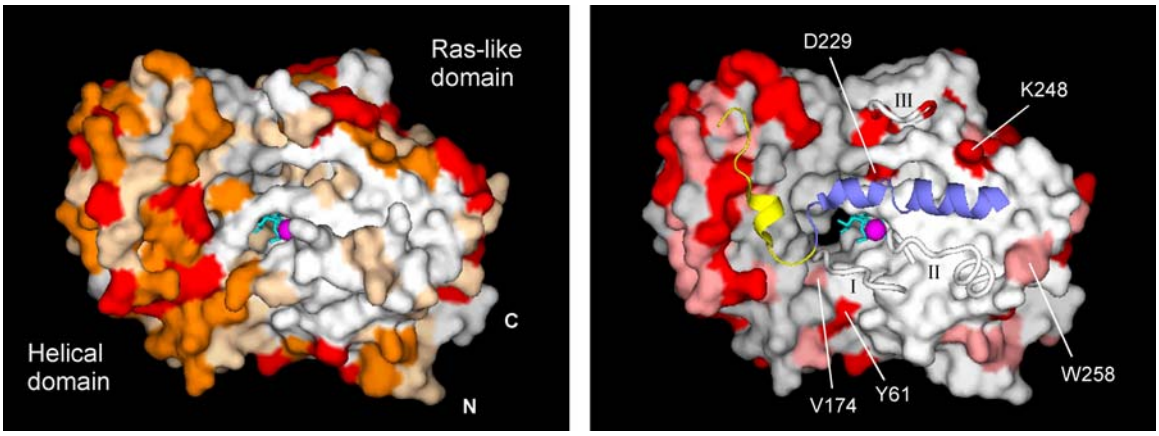
Ras-like
domain
Helical
domain
C
N
D229
III
K248
II
I
V174
Y61
W258

Figure 3