Inhibiting the assembly of protein–protein interfaces

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Protein–protein association is found throughout mechanisms of cellular growth and differentiation, and viral replication. Inhibiting the assembly of protein complexes, therefore, presents itself as a novel means of inhibition for a wide variety of cellular and viral events. Peptides and small molecules that modify the overall quaternary structure of a selection of receptor–ligand interactions and oligomeric viral enzymes have been developed recently.

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Abbreviations

ADR	β_2-adrenergic receptors
bHLH	basic-helix-loop-helix
HSV DP	herpes simplex virus DNA polymerase
cGMP PDE	cyclic guanine monophosphate phosphodiesterase
GPIIβIIIa	glycoprotein IIβIIIα

Introduction

Protein assembly is a widespread mechanism used for growth and cellular control. For instance, many enzymes, viral proteins, and receptor–ligand interactions are comprised of or involve oligomeric protein complexes [1•,2•]. Such protein assemblies are essential elements in allosteric control [3], signal transduction [4], and viral assembly and replication [5].

The ubiquitous nature of protein–protein interactions in essential cellular processes leads to the possibility of developing novel control mechanisms based on inhibition of active protein assemblies. Indeed, nature uses this mechanism to control cellular processes by forming inactive heterodimeric complexes, as illustrated by the protein ΔFos-B that forms heterodimers with Jun and results in a loss of Jun transcriptional activity [6], and the basic-helix-loop-helix (bHLH) protein Id that forms inactive heterocomplexes with other bHLH transcription factors [7]. Dominant negative approaches have been developed on the basis of this strategy to inhibit a wide variety of proteins, such as the dimeric enzyme HIV-1 protease [8] and the growth hormone–receptor trimeric complex [9]. Recent developments in the past three years using peptides and small molecules to inhibit protein–protein interactions in viral enzymes and receptors have met with varying degrees of success, and these will be the focus of this review.

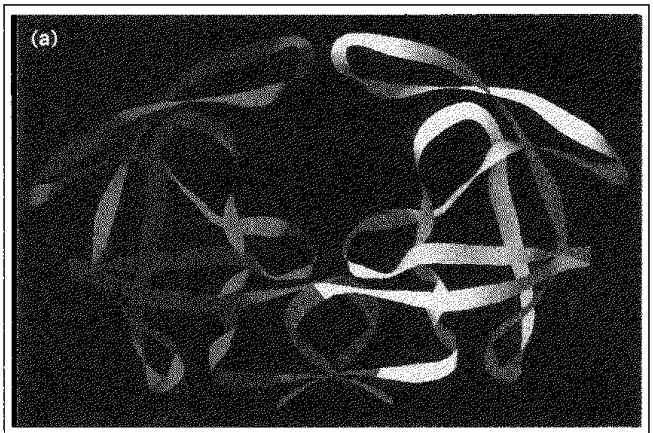
Viral enzymes

Viruses often use oligomeric proteins as enzymes, presumably because this method is a more economical use of genetic information [5]. The three enzymes of HIV, protease, integrase and reverse transcriptase, for instance, are all homodimers or pseudohomodimers. In each case the substrate binding site and the active site are produced from residues derived from each monomer in the dimeric complex. Because of the structural information available (for HIV), and the importance of these enzymes for viral replication, a growing number of dimerization inhibitors for these enzymes are being reported.

HIV protease is a dimeric enzyme whose active site is composed of residues from each monomer (Figure 1a, in blue) [10]. Monomers of HIV protease are, therefore, intrinsically inactive because the shared active site is lacking. Agents have been designed to disrupt the dimer and/or bind to a protease monomer by targeting the four stranded βsheet interface of protease (Figure 1a). Schramm *et al.* [11••] have developed mutated sequences of HIV protease interface peptides that act as inhibitors of protease. For instance, they developed a hexapeptide inhibitor that had an IC_{50} value of 12 μM (10 nM protease) which was enhanced 24-fold by adding hydrophobic moieties to the amino terminus of the peptide (Figure 1b) [11]. The proof that these agents act by inhibiting the dimerization interface was obtained through kinetic analysis by Zhang *et al.* [12], although small changes in the ionic strength of the buffer induced a mechanistic switch from dimerization inhibition to competitive inhibition.

Zutshi *et al.* [13••] have described cross-linked interfacial peptides that mimic the dimerization interface of HIV protease (Figure 1c). These agents contained both of the HIV protease interface peptides that were tethered together with flexible alkyl-moieties, and provided a class of molecules with good inhibitory activity (IC_{50} values of 2.0 μM and 380 nM at 100 nM and 25 nM protease, respectively). The agents were rigorously demonstrated to inhibit the dimerization of HIV protease using size-exclusion chromatography, chemical cross-linking and fluorescence spectroscopy. Truncations have also recently been made at the amino termini of these agents, along with specific modifications, to generate low molecular weight, dimerization inhibitors of HIV protease (Figure 1c) (Zhang L, Chmielewski J, submitted). Since the four-stranded βsheet interface of HIV protease is highly conserved among HIV-1 isolates [14] and HIV mutants exists that are resistant to active-site protease inhibitors [15], dimerization inhibition may ultimately play a significant role in HIV therapeutic strategies.

Figure 1

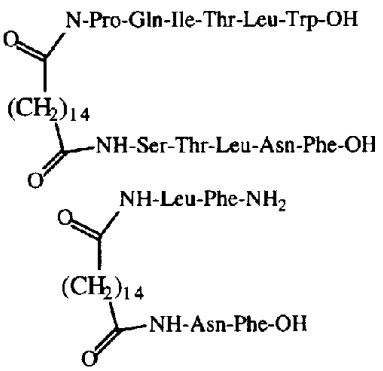


(b)

Peptide	IC_{50} (μM)
TVSYEL	12
Ibu- TVSYEL	6
Bio-Cap- TVSYEL	3
Pam- TVSYEL	0.5

Bio - biotin, Cap - aminocaproyl, Pam - palmitoyl

(c)



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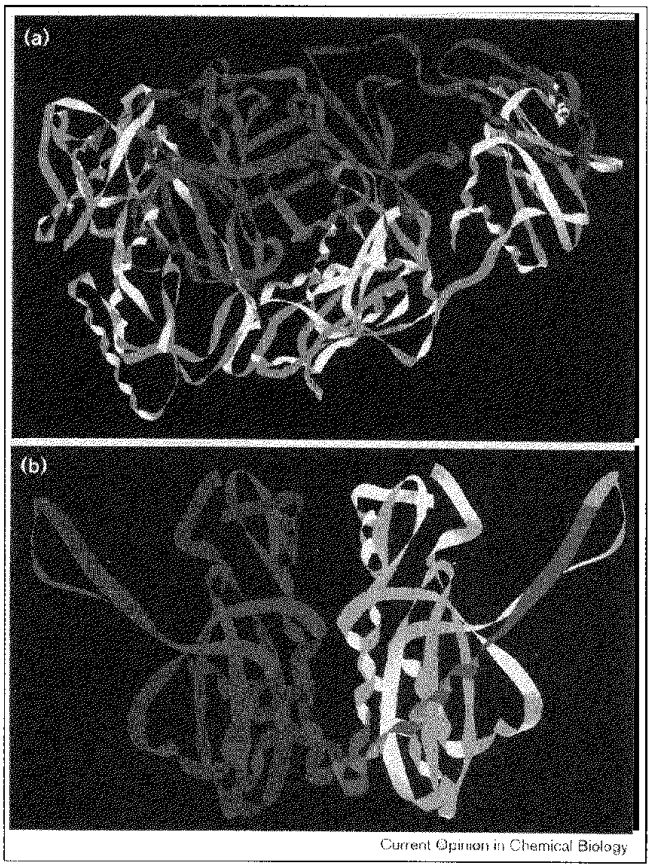
(a) The structure of HIV-1 protease [10] showing the individual monomers in white and red, and the 4-stranded βsheet interface in blue. **(b)** Inhibitors of Schramm *et al.* [11••], and **(c)** inhibitors developed by Zutshi *et al.* [13••].

Figure 2



The structures of **(a)** HIV reverse transcriptase [16] with the 66 kDa subunit in white, the 51 kDa subunit in blue and the peptide inhibitor in red. **(b)** HIV integrase [18]. Individual monomers are shown in white and blue, the peptide inhibitor in pink.

Similarly, a 19 amino acid peptide derived from residues 389–407 of HIV reverse transcriptase has been used as an inhibitor of this enzyme [16]. Reverse transcriptase is a pseudohomodimer (K_d 0.4 nM) composed of a 66 kD subunit and its 51 kD truncated counterpart (Figure 2a) [17]. The dimeric interface of the two subunits covers an extremely large surface area; the peptide inhibitor is derived from a helical segment within the dimerization interface (Figure 2a). Although the peptide does not actively dissociate the dimer of reverse transcriptase, only 50% of the active heterodimer of reverse transcriptase reformed after denaturation in the presence of 1.2 μM of the peptide, as determined by size-exclusion chromatography.

The dimeric enzyme HIV integrase (K_d 25 μM) has a dimerization interface composed of four α helices and one β strand from each monomer (Figure 2b) [18]. A peptide corresponding to residues 147–175 of HIV integrase, a segment which only minimally contains residues from the dimerization interface, has been studied as an inhibitor [19]. Although the peptide exists mainly in a random coil conformation as determined by circular dichroism, it did inhibit *in vitro* integration activity, albeit at concentrations in the low mM regime. No experiments have been performed to support the hypothesis that inhibition was due to disrupting the dimerization of HIV integrase.

Herpes simplex virus (HSV) ribonucleotide reductase, the enzyme that converts ribonucleotide diphosphates into deoxyribonucleotides, is composed of two distinct homodimeric subunits (large and small) [20]. The carboxy terminus of the small subunit of ribonucleotide reductase is essential for the assembly of the complex and peptides corresponding to this sequence inhibit subunit association [21]. In an impressive study, Liuzzi *et al.* [22] redesigned a hexapeptide inhibitor of the

small subunit of ribonucleotide reductase (IC_{50} 58 μM) to produce a peptidomimetic that is greater than six orders of magnitude more potent than the 'starting' peptide (Figure 3). This agent also suppresses the replication of HSV-1, HSV-2 and an acyclovir-resistant strain of HSV in cultured cells.

HSV DNA polymerase consists of a catalytic subunit (Pol) and a smaller subunit (UL42); the crucial residues for the interaction lie at the carboxy terminus of Pol [23]. A 36 amino acid peptide corresponding to the carboxyl terminus of Pol and an 18 amino acid truncated peptide both inhibited long chain DNA polymerization with IC_{50} values of 2 μM and 30 μ, respectively [24]. Direct experiments to assess the effect of these peptides on the dimerization of HSV DNA polymerase have not been described.

Receptors

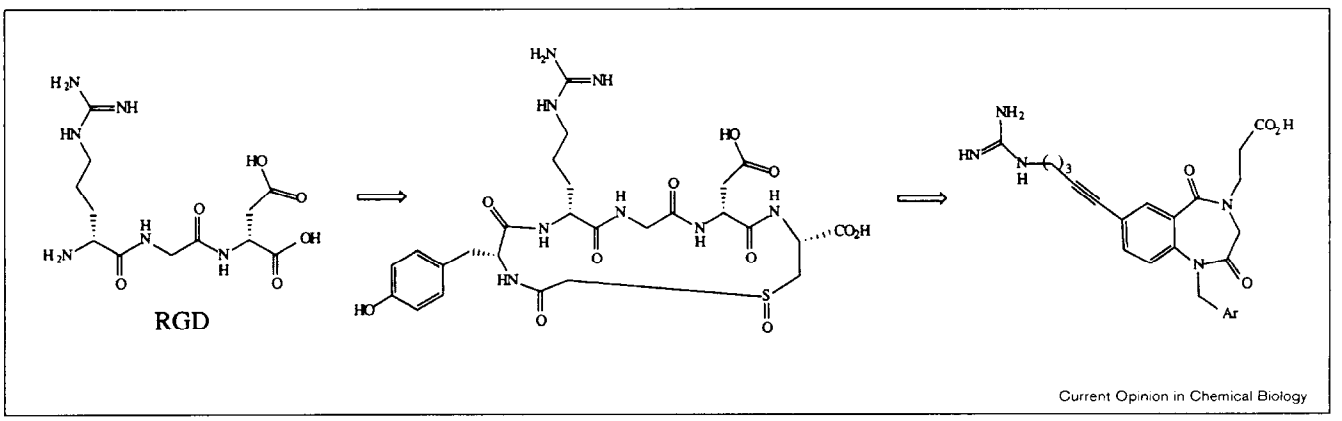
Polypeptide hormones, growth factors and cytokines regulate cellular growth and differentiation [4], generally by binding to cell-surface receptors. The binding event often activates the dimerization or oligomerization of the receptor, an event that leads to transduction of the appropriate signals to the interior of the cell. Receptor oligomerization and receptor–polypeptide ligand interactions have also been the target of protein assembly inhibition studies as a means of regulating signal transduction.

The cascade of processes induced by absorption of light in retinal rod cells is the result of a carefully orchestrated series of protein–protein interactions [25]. The photoreceptor rhodopsin, upon activation by light, undergoes a conformational change which promotes its interaction with the membrane-bound G protein transducin (G_t). Gt then interacts with a cGMP phosphodiesterase (PDE), a heterotetramer, leading to hyperpolarization of the membrane. A light-dependent phosphorylation of rhodopsin, and its subsequent binding to the protein 'visual arrestin', inactivates the receptor and stops further signal transduction events. Hamm and coworkers [26••] have developed peptide inhibitors to interfere with this network of protein interactions. The carboxyl terminus of the α subunit of G proteins is generally recognized as essential for receptor binding. A combinatorial library of peptide sequences related to the carboxyl terminus of G_t, residues 340–350, identified rhodopsin-binding peptide sequences that have 2- to 300-fold higher affinity for rhodopsin than the natural sequence (IC_{50} competition values of 0.64 – 110 μM) [26••]. These peptides also stabilized the active form of rhodopsin with EC_{50} values ranging from 0.76 - 152 μM, which is up to 290 times more efficient at binding rhodopsin than the natural peptide sequence.

Peptide sequences were also identified that inhibited the interaction of the α subunit of G_t and PDE, and the heterotetrameric form of PDE itself. A peptide corresponding to residues 63–87 of the γ-subunit of PDE bound to G_t with a K_d of 2.5 μM and truncation of this peptide to residues 68–87 led to a 15-fold reduction in its binding [27]. Similarly, both peptides completely inhibited a trypsin-activated form of PDE with a K_i value of 0.8 μM, indicating that residues 68–87 are essential for the interaction of G_t with PDE.

Peptides were also obtained to interfere with the final stage in rhodopsin inactivation: the formation of the rhodopsin–arrestin complex. Peptides derived from the proposed cytoplasmic loops of rhodopsin were used as inhibitors of arrestin binding [28]. The third loop of rhodopsin was the most effective inhibitor of complex formation with an IC_{50} value of 34 μM, whereas the first cytoplasmic loop had a 30-fold lower inhibitory activity than the third loop. These studies, therefore, have shed light on the regions of ligands and receptors responsible for specific binding.

Figure 3



The overall design of Arg–Gly–Asp (RGD) peptidomimetics beginning with the RGD structure, development of cyclic peptide analogs of RGD, and the benzodiazepinedione-containing agent [30].

Hormonal transduction is also receptor-mediated, and the β2-adrenergic receptor (ADR) is one of the best characterized hormone receptors. ADR is a homodimeric receptor, and its transmembrane domain VI (TM VI) is believed to reside at the dimerization interface [29•]. A peptide containing most of TM VI (residues 276–296) was prepared and found to substantially reduce the amount of membrane-bound ADR dimer in a time-dependent fashion: at a concentration of 75 μM the TM VI peptide reduced the amount of dimer by 69% after 30 min. A control hydrophobic peptide derived from the transmembrane domain VII of a dopamine receptor did not inhibit dimerization in a similar assay, thereby confirming the specific nature of the ADR–TM VI peptide interaction.

The Arg–Gly–Asp (RGD) sequence is a common motif in a variety of adhesion proteins, including fibrinogen, which mediates binding to glycoprotein IIbIIIa, GPIIbIIIa, a protein on the surface of platelets. McDowell *et al.* [30] accomplished a stunning feat in the design of nonpeptide RGD analogs. A cyclic peptide analog of RGD (Figure 3) was developed that was a highly potent inhibitor of the fibrinogen–GPIIbIIIa association with an IC_{50} value of 150 nM [31]. A nonpeptide, rigid, benzodiazepinedione scaffold was then designed to display the sidechain functionality of RGD in a geometry believed to be required for GPIIbIIIa binding (Figure 3) [30]. Novel inhibitors of fibrinogen- GPIIbIIIa association were obtained that were approximately 20-fold more potent inhibitors than their peptide counterparts and, unlike the peptides, the benzodiazepinedione compounds have oral bioavailibility.

Conclusions

The importance of protein association as a means of mediating cellular events, and in the etiology of a wide variety of disease states, makes such interactions prime targets for therapeutic intervention. With the demonstration that interfacial peptides can block protein interfaces by binding to monomers and preventing dimer formation, and the advances in designing peptidomimetics from peptide sequences, this previously unexplored mechanism for inhibition of protein–protein interaction holds great promise as the next generation of therapeutic agents.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Stites W: **Protein-protein interactions: interface structure, binding thermodynamics, and mutational analysis.** *Chem Rev* 1997, **97**:1233-1250.
A thorough review of the multitude of interactions in protein interfaces.

2. • Jones S, Thornton JM: **Principles of protein-protein interactions.** *Proc Natl Acad Sci USA* 1996, **93**:13-20.
A thorough review of the multitude of interactions in protein interfaces.

3. Frieden C: **Protein–protein interaction and enzymatic activity.** *Annu Rev Biochem* 1971, **40**:653-665

4. Heldin C-H: **Dimerization of cell surface receptors in signal transduction.** *Cell* 1995, **80**:213-223.

5. Gibson W: **Structure and assembly of the virion.** *Intervirology* 1996, **39**:389-400.

6. Nakabeppu Y, Nathans D: **A naturally occurring truncated form of FosB that inhibits Fos/Jun transcriptional activity.** *Cell* 1991, **64**:751-759.

7. Benezra R, Davis R, Lockshon D, Turner D, Weintraub H: **The protein Id: a negative regulator of helix-loop-helix DNA binding proteins.** *Cell* 1990, **61**: 49-59.

8. Babe LM, Pichuantes S, Craik CS: **Inhibition of HIV protease activity by heterodimer formation.** *Biochemistry* 1991, **30**:106-111.

9. Fuh G, Cunningham BC, Fukunaga R, Nagata S, Goeddel DV, Wells JA: **Rational design of potent antagonists to the human growth hormone receptor.** *Science* 1992, **256**:1677-1680.

10. Wlodawer A, Miller A, Jaskolski M, Sathyanarayana B, Baldwin E, Weber I, Selk L, Clawson L, Schneider J, Kent S: **Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease.** *Science*, 1989, **245**:616-621.

11. •• Schramm HJ, Boetzel J, Buttner J, Fritsche E, Gohring W, Jaeger E, Konig S, Thumfart O, Wenger T, Nagel NE, Schramm W: **The inhibition of human immunodeficiency virus proteases by 'interface peptides'.** *Antiviral Res* 1996, **30**:155-170.
A large data set of small peptide inhibitors of HIV-1 protease dimerization and activity.

12. Zhang Z-Y, Poorman RA, Maggiora LL, Heinrikson RL, Kezdy FJ: **Dissociation inhibition of dimeric enzymes. Kinetic characterization of the inhibition of HIV1 protease by its COOH-terminal tetrapeptide.** *J Biol Chem* 1991, **266**:15591-15594.

13. •• Zutshi R, Franciskovich J, Shultz M, Schweitzer B, Bishop P, Wilson M, Chmielewski J: **Targeting the dimerization interface of HIV-1 protease: inhibition with crosslinked interfacial peptides.** *J Am Chem Soc* 1997, **119**:4841-4845.
Mimics of the dimerization interface of HIV-1 PR are described, in addition to a variety of assays to monitors protease dissociation.

14. Gustchina A, Weber IT: **Comparative analysis of the sequences and structures of HIV-1 and HIV-2 proteases.** *Proteins* 1991, **10**:325-331.

15. Deeks SG, Smith M, Holodniy M, Kahn JO: **HIV-1 protease inhibitors.** *J Am Med Assoc* 1997, **277**:145-153.

16. Divita G, Restle T, Goody RS, Chermann J-C, Baillon JG: **Inhibition of human immunodeficiency virus type 1 revrse transcriptase dimerization using synthetic peptides derived from the connection domain.** *J Biol Chem* 1994, **269**:13080-13083.

17. Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA: **Crystal structure at 3.5 Å resolution of HIV reverse transcriptase complexed with an inhibitor.** *Science* 1992, **256**:1783-1790.

18. Bujacz G, Alexandratos J, Zhou-Liu Q, Cläment-Mella C, Wlodawer A: **The catalytic domain of human immunodeficiency virus integrase: ordered active site in the F185H mutant.** *FEBS Lett* 1996, **398**:175-178.

19. Sourgen F, Maroun RG, Frere V, Bouziane M, Auclair C, Troalen F, Fermandjian S: **A synthetic peptide from the human immunodeficiency virus type-1 integrase exhibits coiled-coil properties and interferes with the *in vitro* integration activity of the enzyme.** *Eur J Biochem* 1996, **240**:765-773.

20. Filatov D, Ingemarson R, Graslund A, Thelander L: **The role of herpes simplex virus ribonucleotide reductase small subunit carboxyl terminus in subunit interaction.** *J Biol Chem* 1992, **267**:15816-15822.

21. Krogsrud RL, Welchner E, Scouten E, Liuzzi M: **A solid phase assay for the binding of peptidic subunit association inhibitors to the herpes simplex virus ribonucleotide reductase large subunit.** *Analyt Biochem* 1993, **213**:386-394.

22. Liuzzi M, Deziel R, Moss N, Beaulieu P, Bonneau A-M, Bousquet C, Chafouleas JG, Garneau M, Jaramillo J, Krogsrud RL

et al.: **A potent peptidomimetic inhibitor of HSV ribonucleotide reductase with antiviral activity *in vivo*.** *Nature* 1994, **372**:695-698.

23. Digard P, Bebrin W, Weisshart K, Coen DM: **The extreme C terminus of herpes simplex virus DNA polymerase is crucial for functional interaction with processivity factor UL42 and for viral replication.** *J Virol* 1993, **67**:398-406.

24. Digard P, Williams KP, Hensley P, Brooks IS, Dahl CE, Coen DM: **Specific inhibition of herpes simplex virus DNA polymerase by helical peptides corresponding to the subunit interface.** *Proc Natl Acad Sci USA* 1995, **92**:1456-1460.

25. Hargrave PA, McDowell JH: *Molecular Biology of Receptors and Transporters: Receptors.* Edited by Friedlander M, Mueckler M. San Diego: Academic Press; 1992:49-98.

26. •• Martin EL, Rens-Domiano S, Schatz PJ, Hamm HE: **Potent peptide analogues of a G protein receptor-binding region obtained with a combinatorial library.** *J Biol Chem* 1996, **271**:361-366.
A combinatorial approach is described to identify peptide sequences that bind to rhodopsin with high affinity.

27. Skiba NP, Artemyev NO, Hamm HE: **The carboxyl terminus of the γ-subunit of rod cGMP phosphodiesterase contains distinct sites of interaction with the enzyme catalytic subunits and the γ-subunit of transducin.** *J Biol Chem* 1995, **270**:13210-13215.

28. Krupnick JG, Gurevich VV, Schepers T, Hamm HE, Benovic JL: **Arrestin-rhodopsin interaction.** *J Biol Chem* 1994, **269**:3226-3232.

29. • Hebert TE, Moffet S, Morello J-P, Loisel TP, Bichet DG, Barret C, Bouvier M: **A peptide derived from a β_2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation.** *J Biol Chem* 1996, **271**:16384-16392.
Demonstration that the β_2-adrenergic receptors homodimerize, and that the transmembrane domain (TM) VI of the receptor is at the interface. Peptides derived from TM VI were shown to inhibit receptor dimerization.

30. McDowell RS, Blackburn BK, Gadek TR, McGee LR, Rawson T, Reynolds ME, Robarge KD, Somers TC, Thorsett ED, Tischler M, Webb RR II, Venuti MC: **From peptide to non-peptide. 2. The *de novo* design of potent, non-peptidal inhibitors of platelet aggregation based on a benzodiazepinedione scaffold.** *J Am Chem Soc* 1994, **116**:5077-5083.

31. McDowell RS, Gadek TR: **Structural studies of potent constrained RGD peptides.** *J Am Chem Soc* 1992, **114**:9245-9253.