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Nucleating the Assembly of Macromolecular Complexes

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Abstract

Nature constructs intricate complexes containing numerous binding partners in order to direct a variety of cellular processes. Researchers have taken a cue from these events to develop synthetic molecules that can nucleate natural and unnatural interactions for a diverse set of applications. These molecules can be designed to drive protein dimerization or to modulate the interactions between proteins, lipids, DNA, or RNA and thereby alter cellular pathways. A variety of components within the cellular machinery can be recruited with or replaced by synthetic compounds. Directing the formation of multicomponent complexes with new synthetic molecules can allow unprecedented control over the cellular machinery.

Keywords

Synthetic nucleators; Molecular recognition; Scaffold-directed assembly; Small molecule-protein interaction; Small molecule-nucleic acid interaction; Cooperative effects

Macromolecular Complex Formation

The formation of macromolecular complexes is an elaborate process carried out in nature. The interaction between binding partners can serve to stabilize a desired conformation of the components for a specific purpose, help present a new binding face that additional components may bind to and can even alter the location and function of cellular factors. These types of interactions are seen throughout nature in the form of protein-protein interactions and protein-nucleic acid interactions, among others.

A variety of cellular processes are controlled by macromolecular complexes using various scaffolds, from interactions at the cell surface to nucleation of protein complexes inside a cell (Figure 1). At the cell surface, signaling cascades are comprised of a network of interactions that are carefully regulated and spatially distributed.[7] The cellular responses to signaling events can range from cell proliferation and survival to stress responses and apoptosis, with the potential for cross talk between different pathways depending on the complexes formed.[8] From cell surfaces to deep within the nucleus, many of the steps in gene expression are also directed by macromolecular complex formation. Transcription is regulated by a number of protein and DNA interactions, including the formation of a large

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complex containing RNA polymerase II, general transcription factors, and additional proteins that are necessary to activate transcription of a gene.[9] In eukaryotes, the nascent transcripts are bound and processed by protein complexes to generate mature transcripts that are transported to the cytoplasm. There, the ribosome, comprised of four ribosomal RNA molecules and 70–80 proteins engages the transcript for protein synthesis.[10] Conversely, the proteosome is comprised of two copies each of fourteen different subunits[11] and it degrades targeted proteins with the aid of additional multimeric complexes.[1–2, 12] In essence, macromolecular complexes govern every aspect of cellular function.

The study of small molecules and their utility in regulating complex formation in natural systems is an important facet of chemical biology.[13] A multitude of disease states occur due to improper interactions within multimeric complexes. Problems arise when important interactions fail to occur or when new detrimental interactions are formed.[14] Several small molecules have been developed to inhibit aberrant interactions.[15] However, the development of *synthetic nucleators*, or molecules that are able to nucleate the formation of macromolecular complexes using a variety of scaffolds, represents a unique challenge. In addition to requiring high-specificity and high-affinity for the binding partners in a macromolecular complex, synthetic nucleators must participate in macromolecular interactions that may be transient or stable over time,[16] and the same binding surface can potentially be used to dock with different partners at different locations or times.[17] For example, different sub-complexes of the chromatin remodeling and transcriptional machineries are chosen from multiple, mutually exclusive components during differentiation of stem cells.[18] The intricacy involved in forming these complexes must be taken into account when devising molecules to either stabilize a complex or take the place of one of the binding partners. Lessons found in natural systems can be used toward this goal.

Small molecules that nucleate macromolecular complexes in nature

In nature, complexes containing combinations of proteins, nucleic acids and co-factors are constructed with incredible precision. These complexes can be nucleated through the introduction of a small molecule to the system. The specificity of the small molecule for its binding target is critical for triggering a specific signaling pathway in the midst of a milieu of potential targets.

In addition to bridging interacting partners, small molecules are capable of changing the structure of a protein through the opening of an active site or by enhancing a desired protein dimerization event.[19] The AraC protein, for instance, dimerizes in such a way that two distantly separated DNA binding sites are bound by the protein dimer, forming a DNA loop that represses transcription.[20] In the presence of an aldopentose, arabinose, the structure of the AraC dimer changes and the DNA binding domains attach to adjacent DNA sites; this change in AraC-DNA association permits access to DNA sequence information that promotes transcription via the association and recruitment of a multisubunit bacterial RNA polymerase.

In eukaryotes, a particularly important role for small molecules is seen with nuclear receptors, a major class of drug targets. A small molecule hormone binds to a nuclear

receptor protein, which induces an allosteric change.[21] The new structure enables the small molecule-protein complex to bind to high affinity sites in chromatin and sequentially recruit multiple macromolecular complexes to modulate gene expression. Alternatively, the hormone can bind to a receptor and activate signaling pathways rather than transcription, as in the case of nongenomic steroid action. In this case, hormones nucleate the formation of non-DNA macromolecular complexes to elicit a cellular response.[22]

The specific interactions between hormones and their receptors have been exploited by researchers to alter the transcription levels of desired genes. Ligand analogues as well as protein mutations have been developed to introduce nonnative interactions to a system.[23] For example, a library of ~380,000 ligand binding domain mutants of the nuclear receptor RXR were screened for the ability to bind to a designed ligand in an engineered cell signaling pathway.[24]

Directing protein interactions to control cell signaling and output

Cell-surface receptors recognize extracellular signaling molecules and coordinate the cell's internal machinery to regulate the flow of information into the cell. Often, these receptors must dimerize or oligomerize, either on their own or with other proteins, in order to carry out their functions; frequently, ligands are used to regulate the association. Multivalent interactions are prevalent among surface receptors.[25] These interactions have been exploited to create multivalent synthetic ligands, such as dendrimers and polymers, that drive the associations of desired receptors.[26] In one example, a synthetic molecule displaying multiple copies of sulfated galactose residues, developed by Kiessling and coworkers, clusters L-selectin at the cell surface and targets it for release from the cell membrane through the action of proteases.[27]

Synthetic molecules are not only used to bring receptors together outside of a cell. Many ligands have been developed to dimerize proteins intracellularly. Signaling pathways may be modified or completely altered, depending on the protein targets and ligands chosen. Small molecules have been used as chemical inducers of dimerization (CIDs), a subject that has been well-reviewed.[28] This CID technique was first established by Schreiber, Crabtree, and coworkers.[29] A small molecule, FK1012, was developed that has two protein binding moieties and is capable of simultaneously binding to two copies of the protein FKBP12. Signal transmission from a modified T cell antigen receptor (TCR), containing just the cytoplasmic domain, was restored with the addition of FK1012 when TCR was expressed as a chimera with FKBP12. The formation of this complex led to subsequent oligomerization of the TCR domain and cell signaling. Protein association and subcellular localization,[30] as well as programmed cell death,[31] were a few of the cellular processes controlled with this technique.

CIDs have been studied using numerous different small molecule-protein partners for a variety of applications. The method has been extended to include utilization of a coumermycin molecule to oligomerize a Raf-1 serine/threonine kinase-GyrB fusion protein at the plasma membrane (Figure 2A). The Raf-1 protein complex dimerized with a membrane-bound human interferon-γ receptor fused to GyrB and activated the MAP kinase

cascade.[3, 32] Raf-1 dimers were also formed when two Raf-1 serine/threonine kinase-GyrB fusion proteins were brought together via coumermycin-driven dimerization. Raf-1 oligomerization, with or without membrane association, was sufficient for activating the protein and stimulating the MAP kinase cascade. Alternatively, bifunctional synthetic molecules can bring two different proteins together to form a heterodimer (ex. Figure 2B).[4, 28f]

In addition to nucleating complexes, small molecules can stabilize transient complexes and perturb signaling pathways that require dynamic association. For example, the small molecule Brefeldin A traps an intermediate of the Arf-Sec7 protein complex by binding at the Arf-GDP/Sec7 interface. This binding event prevents the complex from proceeding to nucleotide dissociation, offering a unique therapeutic interruption of signaling pathways that employ small G-proteins and are involved in various human diseases.[33]

A more dramatic rewiring of cellular signaling pathways can be accomplished by reengineering the protein scaffolds. Alterations to the domains within a scaffold can be used to activate a variety of unrelated pathways while holding the initial chemical activator constant. Lim and coworkers report an Ste5 scaffold protein in the MAP kinase pathway capable of acting as a platform for engineering synthetic positive and negative feedback loops.[34] Ste5 was fused to a basic leucine zipper, which interacted with an acidic zipper fused to a positive or negative modulator of the pathway. The same mating pheromone (α-factor) stimulation activated different loops that lead to an increase or decrease in pathway output, depending on the modulator chosen.

Altering cellular networks and cascades using macromolecular complex nucleation can be accomplished by modifying the protein scaffolds used as the templates for these complexes, as shown above. Controlling intracellular processes, such as transcription, is another interesting area where similar concepts have been applied. In the assembly of transcriptional machinery, DNA scaffolds are used to template the formation of macromolecular complexes.

Nucleating the assembly of the transcriptional machinery using small molecules

Assembly of the transcriptional machinery at a transcription start site requires the engagement of numerous multiprotein complexes.[35] An important step in this process includes a transcription factor binding to a specific region of DNA through its DNA binding domain (DBD). The regulatory domain (RD) of the transcription factor can then recruit the appropriate machinery, including chromatin modifying enzymes, mediator proteins and RNA polymerase II, as depicted in Figure 1.[36] These two functional domains occur on most transcription factors; however transcription can be stimulated even when the DBD and RD are associated through noncovalent interactions.[37] Transcription activation with synthetic components has relied on this modular nature of transcription factors to devise “three-hybrid” approaches or to incorporate of synthetic analogues of natural domains (a synthetic DBD or RD).

The three-hybrid system, a variant of CIDs, has been used in a variety of contexts to study the formation of protein complexes with other proteins, DNA, or RNA.[28f, 38] In this system, two components of the complex are brought together through interactions with an intermediary bridging molecule to achieve a readable output, such as gene expression. This technique was pioneered by Wickens and coworkers to study RNA-protein interactions *in vivo*.[39] An example of a three-hybrid system has been developed by Cornish and coworkers that uses a bifunctional small molecule, dexamethasone-methotrexate or the structurally related dexamethasone-trimethoprim, to bring together a DBD and an RD. The bifunctional compound organizes the interaction between a DBD-dihydroxyfolate reductase fusion and a RD-gucocorticoid receptor fusion.[40] Recently, this system was extended to the development of a biocompatible system for modulating Golgi proteins, such as fucosyltransferase VII, in mammalian cells.[41]

Synthetic compounds that act as nucleators of the transcriptional machinery are known as artificial transcription factors (ATF). In an early example of this concept, the Dervan and Ptashne labs developed a bifunctional DNA-binding hairpin polyamide-peptide RD (Figure 3).[42] Kodadek and coworkers developed a peptoid RD mimic conjugated to a polyamide that was able to stimulate transcription in living cells.[5a] Small molecule RDs such as isoxazolidines and wrenchnolol, when tethered to DNA, also nucleate the assembly of the transcriptional machinery, as shown by the Mapp and Uesugi labs.[5b, 5c, 43] Similarly, the Ansari and Dervan labs have combined a DNA-binding polyamide and a 5-residue or 2-residue peptide to generate synthetic Hox mimics that recruit the natural binding partner (Exd protein) as the first step in the assembly of the transcriptional machinery.[5d, 44] However, it must be noted that this is not a complete list of the available components of artificial transcription factors. Zinc finger proteins are also commonly used as DBDs due to the number of DNA sequences that can be recognized with these proteins.[45]

Small molecule control of protein structure and stability

The usefulness of small molecules is not only in bringing biological macromolecules together in order to seed complex formation, as previously explained. It is also possible for a small molecule to direct protein degradation through macromolecular complex formation. Alternatively, small molecules may be use to directly control the structure and stability of a protein to achieve these goals.

Several chemical methods have been developed to specifically target a protein for degradation, bringing in the cellular machinery required for proteolysis.[46] Crews and Deshaies and coworkers constructed Proteolysis Targeting Chimeric molecules (PROTACS), which coupled a ligand for the protein targeted for degradation to a ligand for the E3 ubiquitin ligase (Figure 4).[6, 47] Upon addition of the PROTAC molecule, the targeted protein was post-translationally modified with a growing polyubiquitin chain and consequently degraded by the 26S proteasome, a multicatalytic protease complex. A parallel method was established by Kanemaki and coworkers. The auxin-inducible degron (AID) system, found in plant cells, was modified to achieve the directed degradation of a protein in animal cells.[48] The target protein was fused to the indole-3-acetic acid (IAA) 17 degron that then formed a complex with the TIR1 protein in the presence of auxin, a family of plant

hormones. The interaction acted as an E3 ubiquitin ligase and recruited an E2 ligase to polyubiquitylate the IAA17 degron, thereby degrading the degron and the target protein

A protein can alternatively be targeted directly to the proteosome for degradation without ubiquitination. Church and coworkers expressed two protein constructs, where a protein to be degraded was fused to Tor1 while the proteosome subunit was fused to Fpr1. Heterodimerization of Tor1 and Fpr1 was achieved through the addition of rapamycin and the target protein was successfully degraded, bypassing the ubiquitination step of degradation.[49]

Conversely, a preferable protein structure, stabilized with the addition of a small molecule, can serve as a scaffold to nucleate the desired macromolecular complex. Dickey and coworkers have identified molecules that allosterically bind to Hsp70, one component in the chaperone system, and force the “lid” of the Hsp70 protein to remain in its open conformation.[50] This restriction in “lid” movement prevents the conversion of ATP to ADP and appears to allow the Hsp70 protein to target for degradation the microtubule-associated protein tau, a protein structure implicated in disease states such as Alzheimer’s. The use of small molecules in governing protein structure and stability for complex formation represents a complementary approach to directly nucleating macromolecular assemblies.

Conclusions and Outlook

Numerous important cellular processes are directed through the interactions between proteins, DNA, and RNA in macromolecular complexes. Researchers have used this as the inspiration for a fascinating avenue of research, where small molecules can bring together many interacting partners using different scaffolds to accomplish specific objectives. These molecules can even drive the formation of new complexes using existing biological components that do not typically associate.

Controlling macromolecular complex formation with synthetic components is a simple but powerful approach to modulating biological function that can be used to increase available research tools as well as expand the range of therapeutic targets (Figure 5). Molecules have been designed to bring together two proteins (such as CIDs) or multiple copies of a protein (using multivalent ligands). The same small molecule can even activate the signaling of different pathways depending on the proteins chosen for interaction with natural or unnatural scaffolds. The transcriptional machinery is a complex multiprotein system that can be assembled using rationally engineered synthetic molecules. Furthermore, small molecules can direct the fate of proteins, either stabilizing desirable proteins for interaction in a complex or targeting a protein for degradation by the proteolytic machinery.

The variety of synthetic approaches to drive macromolecular complex formation can be condensed roughly into two complementary classes. A systems-level approach uses small molecules to probe the network of interactions that underlie a given cellular process or to selectively direct that natural process. Alternatively, synthetic molecules can be designed to assemble orthogonal partners and thereby direct information flow in a desired manner.[51]

The ability to assemble orthogonal complexes would borrow from the underlying principles of cooperative assembly on natural scaffolds that facilitate appropriate macromolecular complex formation. Typically, the interaction between any two components is relatively weak (on the μM or mM scale).[44b, 52] However, on a scaffold these interactions work in concert through coupled equilibria to hold together a large number of components within a complex.[52] This cooperativity is essential for maintaining the complex with the desired binding partners over other potential components available within a cell. Each scaffold contains information embedded in a specific pattern that is decoded by the binding partners, which are weakly but cooperatively associated (Figure 6A & B). Often, depending on the signal, the scaffold permits alternate assemblies of partners to dictate different outcomes. Such combinatorial control is used from cellular signaling to gene expression.[53] The ability to harness the assembly of molecules on scaffolds has been demonstrated in examples described above.

As our understanding of natural systems increases, the opportunities for directing entirely new complexes using synthetic molecules become vast. Molecules can be designed to engage orthogonal scaffolds and direct information flow through desired cellular signaling or gene regulatory networks. The scaffolds used as templates for macromolecular complex formation can be expanded upon for exciting applications. New molecules could be engineered to utilize information that is present in several different scaffolds to assemble novel complexes controlling desired but orthogonal biological outputs. Synthetic nucleators could potentially bring together two unrelated protein scaffolds through one specific interaction that can then turn on a new cell signaling event (Figure 6C). Similarly, a synthetic molecule can be designed to bring together two different pieces of the genome (DNA scaffold) bearing complementary pieces of information that trigger the desired gene expression (Figure 6D).

Using synthetic nucleators to couple a variety of scaffolds, and therefore direct the formation of macromolecular complexes, researchers can channel information flow through orthogonal gene, cellular, or metabolic networks. In fact, pioneering experiments show that this is a tangible goal.[54] For example, receptor protein clustering at the cell surface using a multivalent ligand has been shown to promote inter-receptor communication and thereby affect cell signaling.[55] Furthermore, altering one receptor protein can alter the function of other related proteins,[56] a point which could be exploited with small molecules. Targeting new scaffolds to different locations within the cell, based on the different known localization sequences, could also offer opportunities beyond those that are currently being explored.[57]

Macromolecular complex formation can be directed with exquisite control using specifically engineered synthetic molecules, making this a powerful technique with exciting future applications. With past successes in controlling binary interactions, the next challenge is to design synthetic nucleators that integrate information embedded in scaffolds to template the assembly of multiprotein complexes toward a variety of applications. These complexes could be used to control a significant flow of information to affect any desired outcome within a cell.

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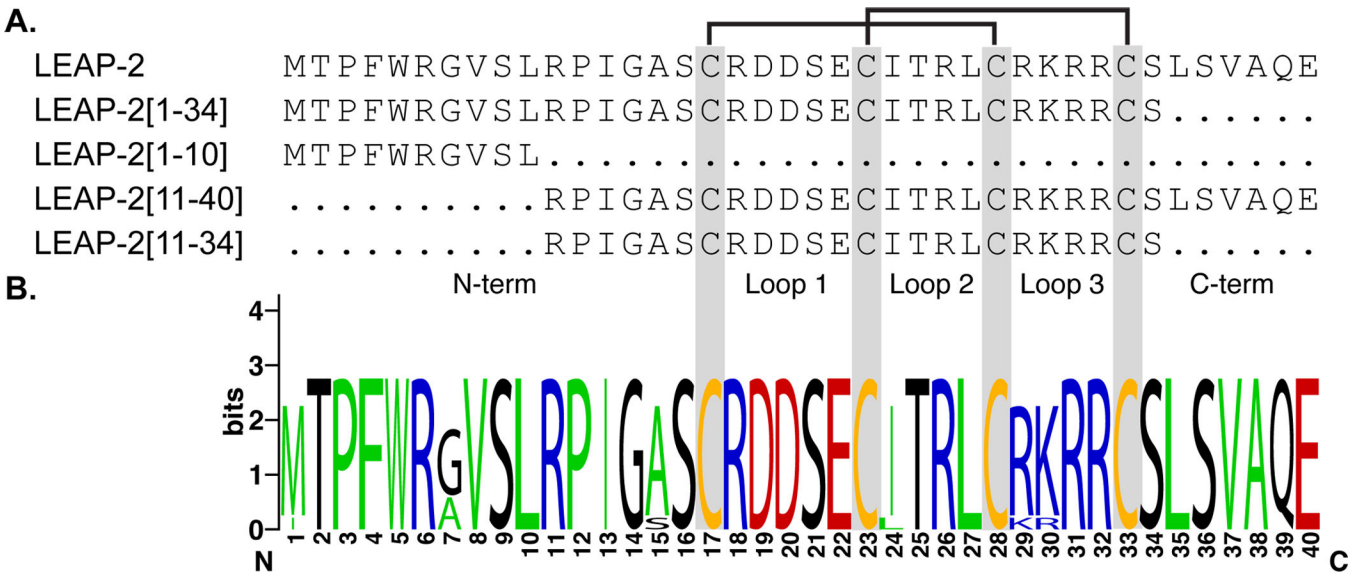

Figure 1.

Macromolecular complexes in biological systems. In **signaling cascades**, a ligand binds to a cell-surface receptor to begin the cascade events. Phosphorylation (P) of the intracellular kinase leads to the phosphorylation of downstream proteins and elicits a desired cellular response. With **transcription**, a transcription factor DNA binding domain (DBD) associates with a specific sequence of DNA while the regulatory domain (RD) recruits the transcription machinery, including mediator (Med) proteins and RNA polymerase II (RNA Pol II), which initiates gene expression. The **ribosome** is comprised of two large multi-protein complexes that join together to synthesize new proteins (figure adapted from reference [1]). Proteins are degraded in the 20S core particle of the 26S **proteosome**, which is capped by a 19S regulatory particle on one or both ends (figure adapted from reference [2])

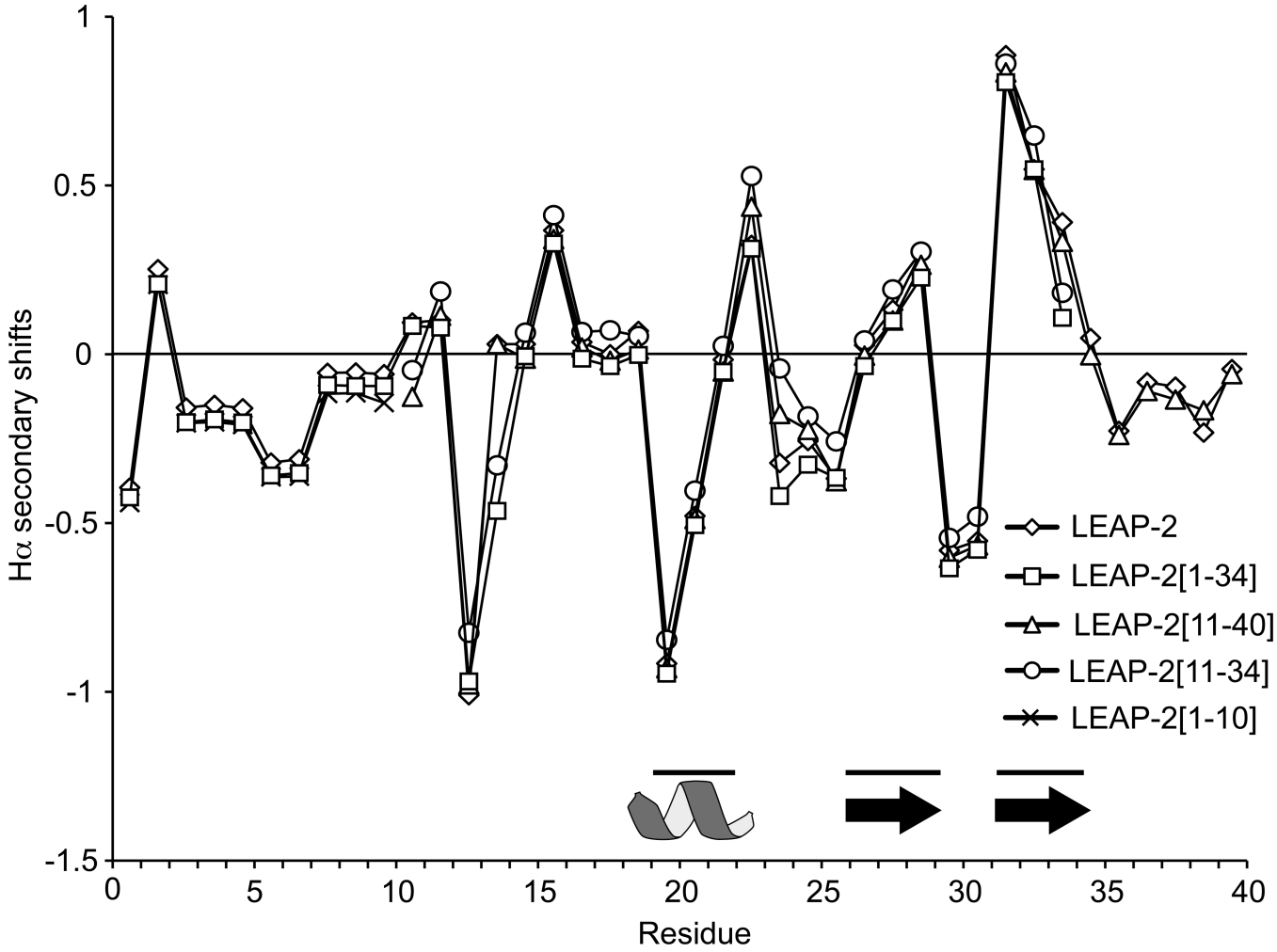

Figure 2.

Chemical inducers of dimerization. A) Homodimerization – the small molecule, coumermycin, induces the dimerization of Raf-1 (adapted from reference [3]), B) Heterodimerization – a bifunctional molecule guides the complex formation between CD22 and anti-nitrophenol IgM.[4]

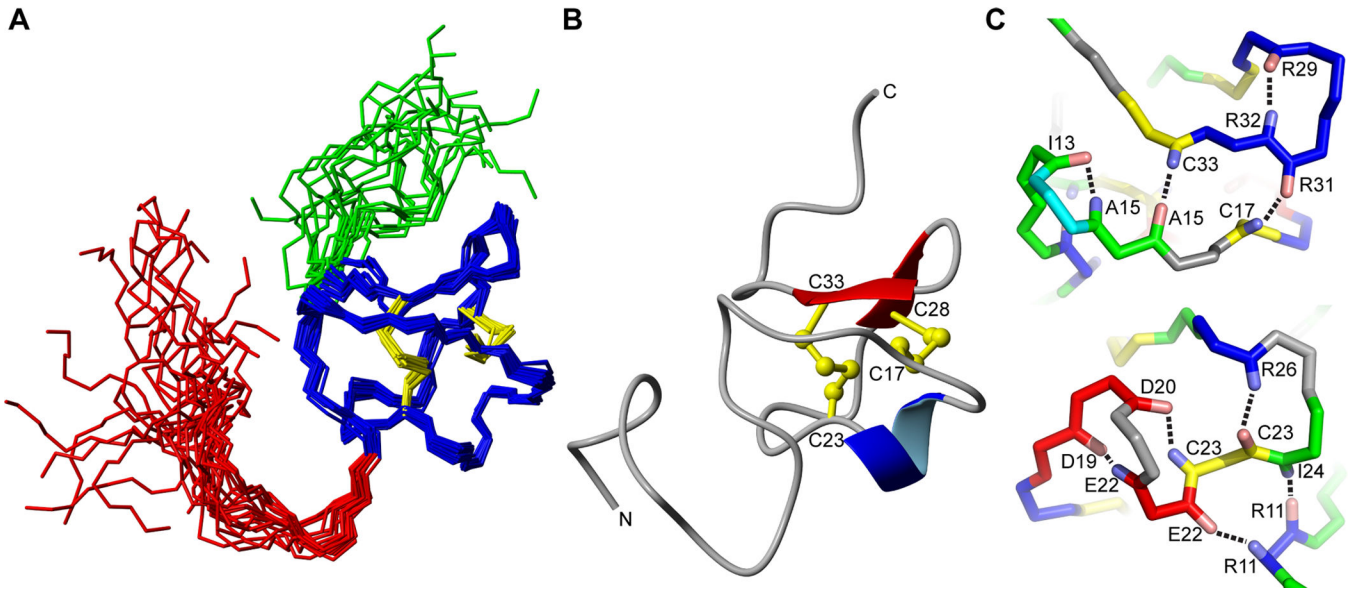

Figure 3.

Artificial transcription factors (adapted from references[5] and **PDB**:1ZAA). These are a few examples of the synthetic RDs and DBDs that have been developed for transcription activation.

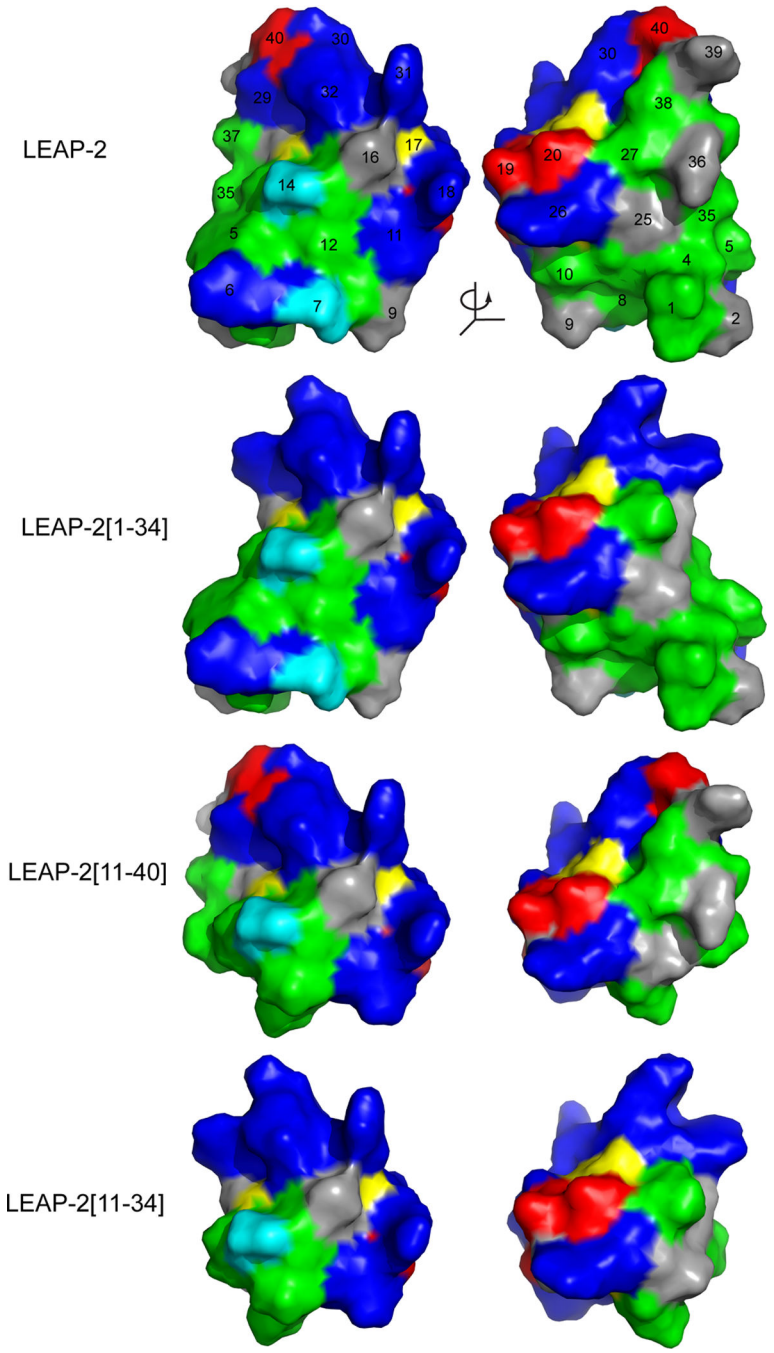

Figure 4.

Complex formation for protein degradation nucleated by the PROTAC molecule (adapted from reference [6]). A PROTAC molecule brings together the target protein and the ubiquitin ligase complex. The association causes the target protein to be polyubiquitylated and targeted for degradation by proteases.

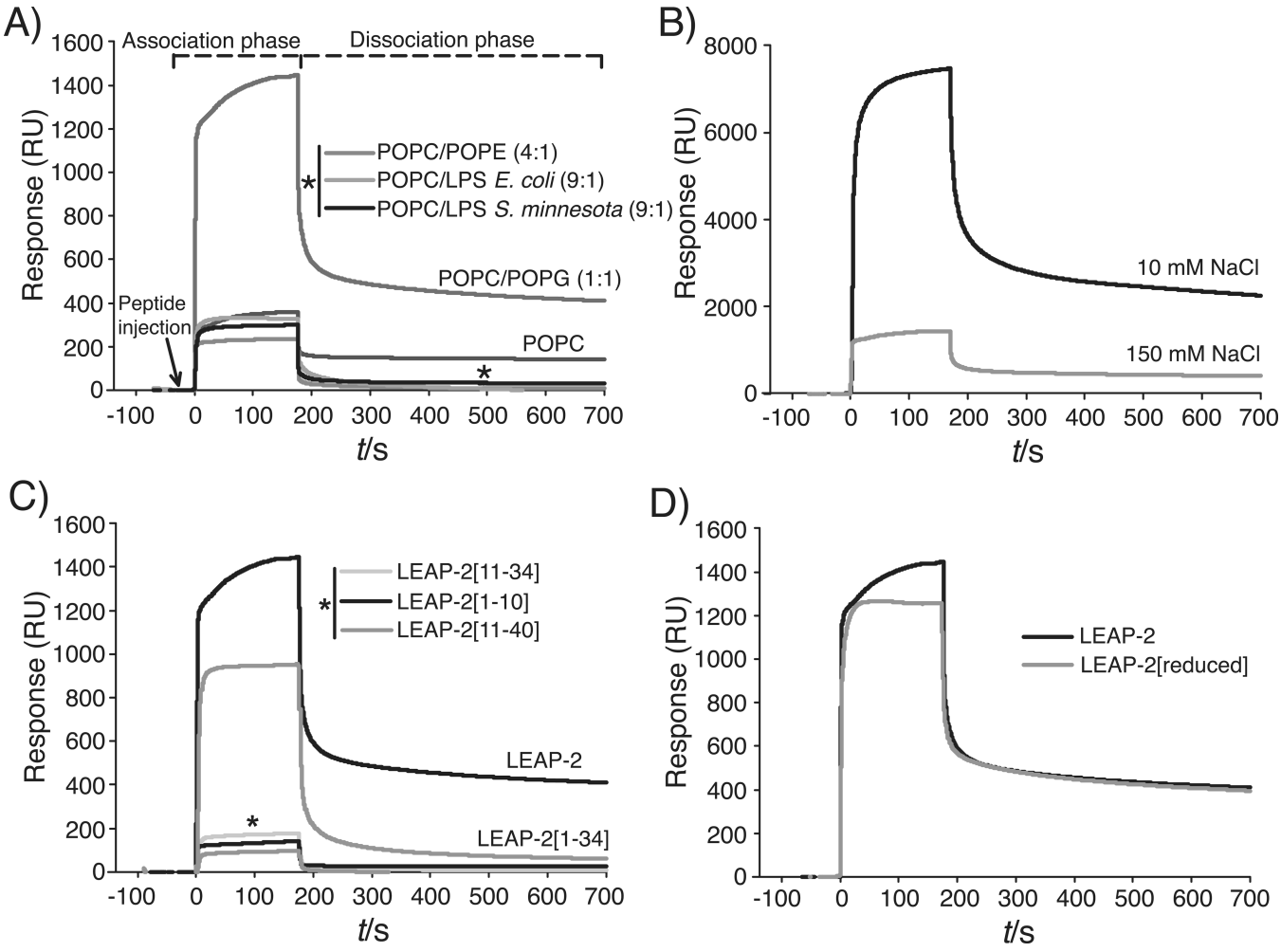

Figure 5.

Potential targets for small molecule nucleation of macromolecular complexes. A small molecule, shown as a circle, can be designed to recruit many different cell-surface receptors (shown at the top) to bring together numerous protein scaffolds for cell signaling. Engineering of the intracellular domains of these receptors can lead to activation of different cell signaling pathways. A small molecule can be added to stabilize a desired protein conformation (shown in dark grey toward the lower left) that can direct other proteins, such as tau, to the protease machinery. Finally, a small molecule can be designed as a transcriptional factor mimic (shown in the lower right) to recruit the transcriptional machinery and turn on gene expression.

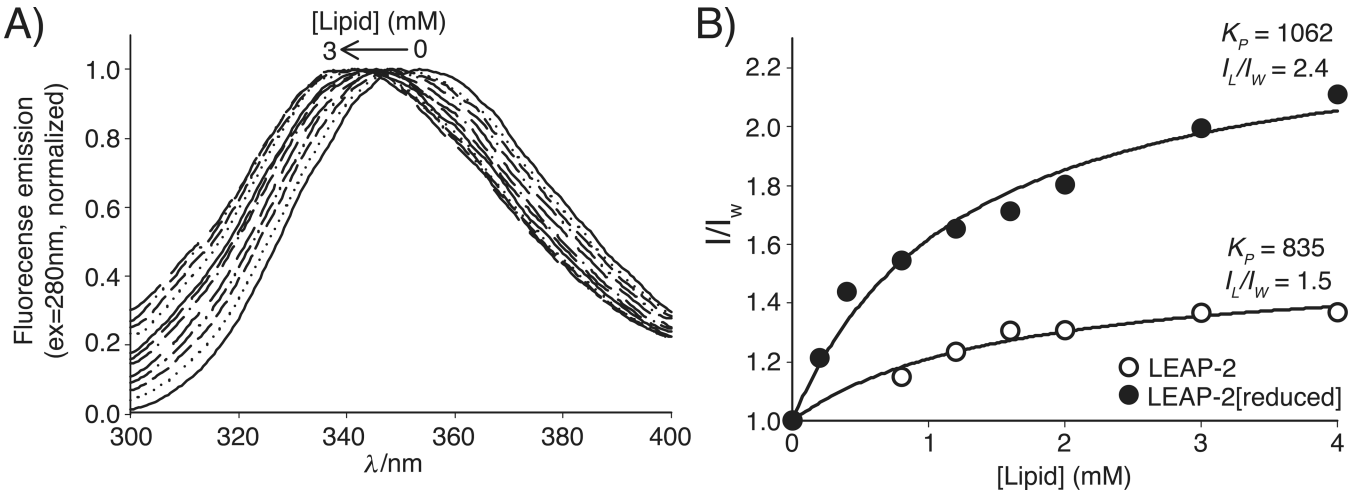

Figure 6.

Cooperativity and multivalency utilized by scaffolds to assemble macromolecular complexes. A) Weak interactions couple multiple docking partners onto a protein scaffold to direct cell signaling, B) Weak interactions govern associations between components of the transcription factors and machinery on the DNA scaffold, C) Two orthogonal protein scaffolds can be brought together with a designed small molecule (in green) for engineering signaling pathways, D) Two DNA scaffolds can be brought into close proximity to allow the transcription factors from the second DNA scaffold to recruit the transcription machinery onto the first scaffold for gene expression.

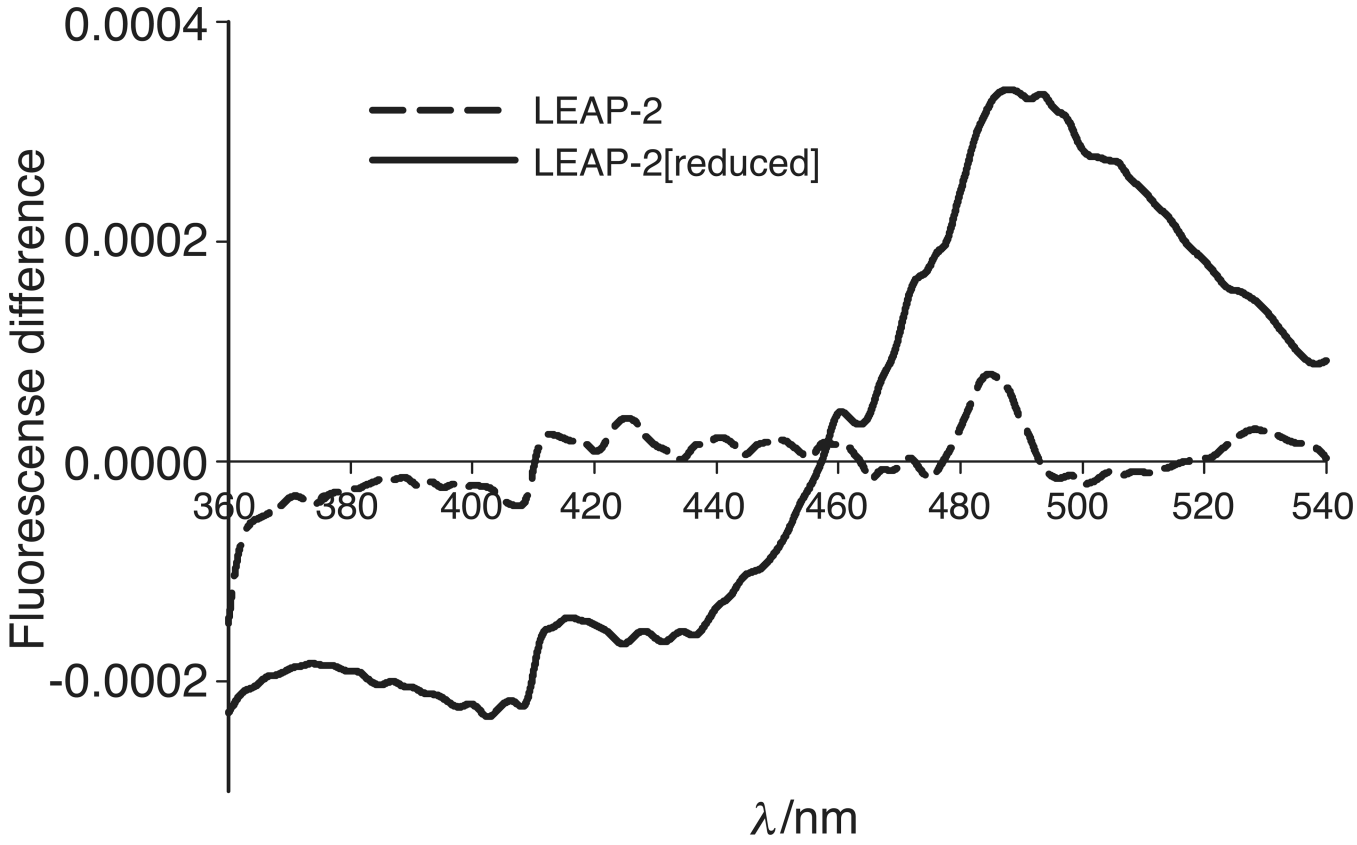

Figure 7.