chromophores to single points with anisotropic polarizability and all nonchromophoric aliphatic atoms to points with isotropic polarizability; all other atoms are ignored. By determining interactions among the chromophoric and nonchromophoric parts of the molecule using empirically derived polarizabilities, the rotational and dipole strengths are determined leading to the calculation of the CD spectrum for each molecule. DnMa software is being modified to reduce computational demand by collapsing hydrogens into the atoms to which they are bound (i.e., united atom). Crystal structures of proteins containing less than 300 residues are energy minimized using NAMD. Hydrogens attached to certain groups (e.g., C2H, CH2, or CH groups) are then deleted programmatically. Deleted hydrogen polarizabilities are either ignored or added to the atom to which they are bound. Theoretically predicted CD for a variety of proteins (26 different structures, examples pictured below) are compared with synchrotron radiation CD data. Theory agrees with experiment showing bands with similar morphology and absorption maxima for the $\pi-\pi^*$ transitions.

Bioengineering

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Photoregulation of Small G Protein Kras using Photochromic Molecules
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Ras is one of small G-proteins known as a molecular switch mediating cellular signalling. Switching ON state of Ras is induced by exchange of bound GDP for GTP and OFF state is by hydrolysis of GTP to GDP. Interestingly, the core nucleotide-binding motif of Ras is considerably conserved with the ATP driven motor proteins, myosin and kinesin. Therefore, it is believed that these bio-molecular machines share common molecular mechanism utilizing nucleotide hydrolysis cycle. Previously, we have incorporated photochromic molecules, 4-phenylazophenyl maleimide (PAM), into the functional site of kinesin as a photo-switching device and succeeded to regulate kinesin ATPase activities reversibly upon visible light (VIS) and ultra-violet (UV) light irradiation. Therefore, it is expected that Ras can be also regulated using photochromic molecules.

In this study, we performed basic study to control the function of Ras reversibly using photochromic molecules upon VIS and UV light irradiations. First, in order to monitor the exchange of bound GDP for GTP, we synthesized a new fluorescent GTP analogue, NBD-GTP and NBD-GDP, which change their fluorescent intensity during their binding to Ras. Second, we synthesized a new photochromic molecule, iodoacetyl spiriyran (IASP) and iodoacetyl fulgimide (IAFI) that are incorporated into cytochrome residue specifically. And the GTPase activity of Ras was monitored by the quantitative analysis of GTP and GDP in the active site of Ras using reverse phase column chromatography on HPLC. We have designed three kinds of Ras mutants Y32C, I36C, and Y64C. The mutants were prepared using E.coli expression system and modified with PAM and IASP stoichiometrically. It was suggested that the GTPase activities of the Ras mutants modified with PAM were reversibly alternated upon VIS and UV light irradiations. The Ras mutants modified with IASP and IAFI were also examined.

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Structural Complementation of the Catalytic Domain of Pseudomonas Exotoxin A
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Pseudomonas exotoxin A (PE3) is a specific ADP-ribosyltransferase for eukaryotic elongation factor 2 (eEF2). ADP-ribosylation of eEF2 arrests ribosomal protein synthesis and potently induces cell death by apoptosis. A single molecule of functional homologs of exotoxin A, such as Diphtheria toxin, is known to be sufficient to kill a cell. We have designed and characterized catalytically inactive fragments of PE3 that are capable of structural complementation. We dissected PE3 at an extended loop and fused each fragment to one subunit of a hetero-specific coiled coil. In vitro ADP-ribosylation and protein translation assays demonstrate that the resulting fusions—supplied exogenously as genetic elements or purified protein fragments—had no significant catalytic activity or effect on protein synthesis individually, but in combination catalyzed the ADP-ribosylation of eEF2 and inhibited protein synthesis. Although complementing PE3 fragments are less efficiently catalytic than intact PE3 in cell-free systems, co-expression in live cells transfected with transgenes encoding the toxin fusions inhibits protein synthesis and causes cell death comparably as intact PE3. Split PE3 offers a direct extension of the immunotoxin approach to generate bispecific agents that may be useful to target complex phenotypes.

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Fibrin Fibers: Blocking the B:B Knob-Pocket Interaction
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Fibrin clot formation has been studied to determine the mechanical properties of fibrin fibers modified by blocking the B:b knob-pocket interaction. Synthetic B:b knob peptides AHRPYAAC or AHRPYAAC-Peg have been added to a fibrinogen solution to allow for binding to the b:pockets prior to clot formation. After fibrin clot formation, a combined atomic force microscopic (AFM)/optical microscopic technique was used to study the properties of individual fibrin fibers in buffer. Mechanical testing of fibrils was done using the AFM to laterally stretch individual fibers suspended over 13.5μm wide grooves in a transparent substrate. The optical microscope, located below the sample, was used to monitor the stretching process. We found that the density and lateral aggregation of fibers was hindered by blocking the b:pockets with the synthetic B:knobs.

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Principles for the Rational Design of Allosterically Cooperative Biomolecular Receptors
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Allosteric cooperativity is used ubiquitously throughout nature to generate steeper, more responsive input-output behaviors. The focus of my work has been to rationally introduce this valuable property into several classes of normally non-cooperative biomolecular receptors. My approach to this end employs classical population-shift (between "tense" and "relaxed" states) mechanism underlying the cooperative oxygen binding of hemoglobin. Specifically, I have designed receptors that equilibrate between two conformations, the more stable of which binds the target ligand only weakly and the less stable of which exposes two high-affinity ligand binding sites. The binding of the first of the ligand shifts this conformational equilibrium towards the later, higher-affinity state, improving the affinity of the second binding event and thus producing a steeper, highly cooperative response. One approach to this end involves receptors that equilibrate between an intrinsically disordered conformation and a folded conformation containing two target-binding sites. The folding of the entire receptor upon binding the first copy of target molecule improves the affinity of the second, leading to cooperative binding. The observed cooperativity of my redesigned receptors is quantitively dependent on the equilibrium constant $K_d$, describing the switch between the tense and relaxed (e.g., folded and unfolded) states. Effectively maximal sensitivity (i.e., a Hill coefficient $n$) is achieved as $K_d$ falls below 0.0025. The enhanced responsiveness of these cooperative receptors should improve their utility in applications, such as biosensors, biomolecular logic gates, and "smart," responsive biomaterials, in which steeper, more sensitive input–output behavior is of value.