chromophores to single points with anisotropic polarizability and all nonchro-
monic aliphatic atoms to points with isotropic polarizability; all other atoms are
ignored. By determining interactions among the chromophoric and non-
chromophoric 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. DInaMo 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 contain-
ing less than 300 residues are energy minimized using NAMD. Hydrogens
attached to certain groups (e.g., CH2, CH, or CH3 groups) are then deleted pro-
grammatically. 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

3103-Pos Board B795

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 nucle-
otide 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
reactions reversibly upon visible light (VIS) and ultra-violet (UV) light irriga-
tion. Therefore, it is expected that Ras can be also regulated using photo-
chromic 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 fluo-
rescent intensity during their binding to Ras. Second, we synthesized a new
photochromic molecule, iodoacetyl spiropyran (IASP) and iodoacetyl fulgi-
ride (IAFR) that are capable of structural complementation. We dissected PE3
on HPLC. We have designed three kinds of Ras mutants Y32C, I36C, and
Y64C. The mutants were prepared using photochromic molecules.

3104-Pos Board B796

Structural Complementation of the Catalytic Domain of Pseudomonas Exotoxin A

Re-emphasized split 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 ribo-

somal 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 character-
ized 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 sys-
tems, 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.

3105-Pos Board B797

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 fibrin-
ogen 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 fibers was done using the AFM to laterally
stretch individual fibers suspended over 13.5µm wide groves 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.

3106-Pos Board B798

Principles for the Rational Design of Allosterically Cooperative Biomole-
cular 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 nor-
mally non-cooperative biomolecular receptors. My approach to this end
employs the 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
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
covalent connectivity of my redesigned receptors is quantitatively dependent on the equi-
librium constant \( K_d \) describing the switch between the tense and relaxed (e.g.,
folded and unfolded) states. Effectively maximal sensitivity (i.e., a Hill coeffi-
cient within error of the theoretically expected 2 and a 9-fold increase in sensi-
tivity) 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 behaviors is of value.