**Prof. Ehud Gazit’s Research**

**Molecular Structure and Self-Assembly at the Nano-Scale**

The central dogma in the study of protein folding suggests that the thermodynamically-favorable state of proteins under physiological conditions is their folded one. However, there are number of cases in which the favorable states of proteins are rather unfolded, partially folded (e.g., “molten globular”), or misfolded (e.g., nonspecific aggregates or amyloid fibrils). These observations lead to much interest in the significance and mechanism of formation of such unfolded, misfolded or partially folded structures in physiological as well as pathological conditions.

 In our lab we utilize a variety of biochemical, biophysical and molecular biology methodologies to study the mechanism and significance of protein unfolding and misfolding. The experimental systems used are diverse and the partial list includes several bacterial toxin-antidote systems, type II diabetes-related amyloidogenic proteins and peptides, and the VHL tumor suppressor protein. We also study the mechanism of non-specific aggregation of generic unrelated proteins. Another line of research is directed toward the elucidation of the mechanism of action of chemical chaperons and their effect on folding, aggregation and amyloid formation.

**Amyloid formation and degenerative disease**

The formation of well-ordered amyloid fibrils is the hallmark of several diseases of unrelated origin. A partial list includes Alzheimer’s disease, Type II diabetes, and prion disease such as bovine spongiform encephalopathy (BSE) and Creutzfeldt Jakob Disease (CJD). In spite of its grave clinical consequence, the mechanism of amyloid fibrils formation is not fully understood. We suggest, based on experimental and bioinformatical analysis, that aromatic stacking interactions may provide energetic contribution as well as order and directionality in the self-assembly of ordered amyloid structures. Indeed, using rational probing and systematic peptide array screens we had identify several novel and very short (as short as tetrapeptide) amyloidogenic motifs that include aromatic moieties. This is in line with the well-known central role of aromatic stacking interactions in self-assembly of many supramolecular structures. Therefore, it appears that the molecular recognition and self-assembly process that lead to the formation of order structures is being mediated by small structural elements. In the path of our reductionist approach toward the identification of the shortest motifs that mediate the self-assembly of the fibrils, we demonstrated that the diphenylalanine core-recognition motif of the Alzheimer’s beta-amyloid polypeptide contains all the molecular information needed for efficient self-assembly into well-ordered, stiff, and elongated nanotubes. We also revealed the important of early intermediates that are small in size and transient in the membrane permeating activity of amyloidogenic proteins. Therefore, the inhibition of the very early step of recognition appears to be crucial. To address this issue, we had developed novel inhibition methodologies that are based on hetero-aromatic interaction using small-molecule and peptide-derived compounds. We also demonstrated the potent inhibitory activity of non-natural beta-breakers such as the alpha-aminoisobutyric acid.

**Nano-scale Peptide Assemblies**

Following our mechanistic studies on amyloid fibril formation, we demonstrated that the diphenylalanine recognition motif of the Alzheimer’s beta-amyloid polypeptide self-assembles into ordered peptide nanotubes with a remarkable persistence length and mechanical strength. It was also demonstrated that these peptide nanotubes could serve as a mold for the fabrication of metals and building blocks of novel electrochemical platform. We also reveal that diphenylglycine, a similar analogue and the simplest aromatic peptide, forms spherical nanometric assemblies. Both the nanotubes and nanospheres assemble efficiently and have remarkable stability. The formation of either nanotubes or closed-cages by fundamentally similar peptides is consistent with a two-dimensional layer closure, as described both for carbon and inorganic nanotubes and their corresponding buckminsterfullerene and fullerene-like structures. These properties of the peptide nanostructures, taken together with their biological compatibility and remarkable thermal and chemical stability, may provide very important tools for future nanotechnology applications.  
  
Applications, methodologies, and theories that were applied to the study of carbon and inorganic nanostructures should be of great importance for future exploration and utilization of the peptide nanostructures. We have recently demonstrated the potential use of the peptide nanotubes for biosensors applications. We also revealed the remarkable rigidity of the peptide nanotubes, which could be utilized in novel composite biomaterials. These properties of the peptide nanostructures, taken together with their biological compatibility and remarkable thermal and chemical stability, may provide very important tools for future nanotechnology applications.

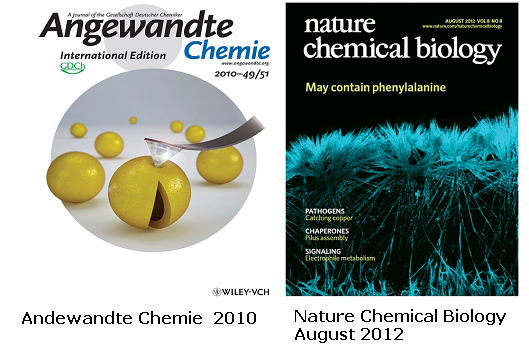
**The Role of Protein Folding and Stability in Type I VHL Syndrome**

The recent crystal structure of pVHL-Elongin C-Elongin B complex revealed that many of the cancer-associated mutations in pVHL (most notably the mutations that lead to type I VHL syndrome) are mapped to the hydrophobic core of the protein and do not take part in the molecular interaction of pVHL with other molecules. This result clearly indicates that abnormal folding and stability play a central role in malfunction of many pVHL mutants. Nevertheless, basic information about the molecular details of the folding reaction of pVHL is still missing. There is also a lack of information on the physiological and thermodynamic stability of pVHL and the effect of cancer-related mutations on these properties. The aim of our project is to study the folding, stability, and molecular dynamics of wild-type and mutants pVHL proteins. For that purpose wild-type VHL was expressed as a GST fusion protein and was purified to >90% purity using Glutaionine Sepharose chromatography followed by Gel Filtration chromatography. As the structure of the VHL was know only at the crystal form and under cryo conditions, our first experiments were aimed toward a basic characterization of the secondary structure at physiological temperature and the thermal stability of the wild-type VHL protein using circular dichroism (CD) spectroscopy. The far UV spectrum of VHL at 37 °C is consisted with a well-folded protein with a content of both -helices and -sheets. The thermal melt of the VHL protein is also consistent with a stable protein have a Tm well above 37 °C. In parallel, we prepared site-directed mutants of the VHL protein at locations that are known to induce type I and type II VHL syndrome. The site-directed mutants were prepared using unique site elimination (USE) techniques. The next step of our study will include the purification of the mutant protein followed by further assessment of the folding process and the thermodynamic stability of the wild-type and mutant proteins by a variety of spectroscopic methods and by the use of calorimetry.

**Molecular Characterization of a Putative Novel Toxin-Antidote System**

Addiction systems are composed of pairs of toxin and antidote proteins that act in a plasmid-encoded mechanism that causes the death of their cured hosts. The mechanism of addiction is achieved by a differential stability of the toxin and antidote proteins. While the toxin proteins are stable, the antidotes are labile proteins that undergo rapid degradation. Upon plasmid curing, only the stable toxins are presence while the labile antidotes undergo degradation. This eventually leads to the death of cured cells. The differential physiological stability of the toxins and antidotes is correlated with a differential thermodynamic Stability at least in two cases: Phd and Doc, and ParE and ParD proteins. While the toxin molecules are well-folded and stable proteins, the antidote proteins are instable, partially or fully unfolded proteins. Toxin-antitoxin proteins were initially found in the context of plasmids, but genes coding addiction proteins are also present in bacterial chromosomes. It has been speculated that they may have a role in transcription-translation control or even participate in programmed cell death. Using bioinformatics tools, we have identified a novel pair of putative addiction genes in the *E. coli*chromosome, only one to be previously annotated, that show homology to known and predicted addiction systems. Furthermore, the pair of genes resemble classical addiction systems in its gene structure and organization. The aim of the project is to study this new operon -its function, interactions, and structure and Stability parameters. As a first step, the antidote and toxin genes were cloned together and separately under induciable T7 promoter in order to achieve high quantity levels of the proteins and assess their effect. In parallel, putative addiction genes were cloned under temperature sensitive promoter. Recently, the antidote putative protein has been partially purified using-ion exchange chromatography. The next steps of our study will include physiological characterization of putative addiction operon using the temperature sensitive clone, and biophysical characterization of the putative toxin-antitoxin proteins.

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