biological knowledge from multiple databases to complement the often sparse and noisy data during reconstruction.
The DBN-MCMC algorithm was first examined with multi-perturbation data at discrete time points synthesized from pseudo gene networks (50-100 genes). We also reconstructed a well-studied pathway (60-70 mapped genes) from two types of perturbation data: two single KOs and a time-course RNAi knock-down. In all cases, the learned networks were highly accurate (sensitivity>0.9, specificity>0.8). In addition, the DPM-guided sampling converges significantly faster than the unguided one (~50% decrease in MC steps). For larger networks where unguided sampling is increasingly less efficient, we expect more pronounced benefits from the DPM sampling. Results demonstrate that the DBN-MCMC algorithm is able to efficiently depict gene interactions at a pathway level complexity (~hundred genes) from multi-perturbation data, a step forward towards dissecting the genetic relationships of a complete biology system.

1745-Pos Board B655
A novel Wavelet-Based Clustering Algorithm for Analysis of Gene Expression Patterns
Eitan Gross.
Most computer clustering programs available today use global de-noising algorithms to filter raw gene expression pattern data. However, many gene expression time series data contain interval-dependent noise where the second moment statistics of the noise are non-stationary. To address this issue we developed a new wavelet-based algorithm (Wave-SOM) that uses a localized filtering method (wavelets) to remove noise from the data while preserving local time events in the gene expression patterns. We employed a discrete Hilbert transform thresholding technique to compare the size of the signal component relative to the noise level at each wavelet transform level by creating a complex-valued analytic vector from which an amplitude vector was defined. Using various wavelet transformations, raw data are first de-noised by decomposing the time-series into low and high frequency wavelet coefficients. Following thresholding, the coefficients are fed as an input vector into a two-dimensional Self-Organizing-Map clustering algorithm. Transformed data are then clustered by minimizing the Euclidean (L^2) distance between their corresponding fluctuation patterns. A multi-resolution analysis by Wave-SOM of expression data from the yeast *Saccharomyces cerevisiae,* exposed to oxidative stress and glucose-limited growth identified 29 genes with correlated expression patterns that were mapped into 5 different nodes. This ordered clustering of yeast genes by Wave-SOM illustrates the fact that the same set of genes (encoding ribosomal proteins) can be regulated by two different environmental stresses, oxidative stress and starvation. Using an adjusted Rand index measure to cluster expression patterns of yeast' cell-cycle genes as test data sets, our algorithm outperformed the Cluster 3.0, MCLUST, CurveSOM , SSClust and GENECLUSTER clustering algorithms.

Calorimetry

1746-Pos Board B656
Probing the Ion-Binding Site of the Holliday Junction
Jacob Litke, Ishita Mukerji.
Holliday Junctions are important structures for the pathways of homologous DNA repair and recombination. Strand migration during DNA recombination is an important source of chromosome diversity but can only occur when the junction exists in the open conformation. Previous studies have shown that the binding of monovalent and divalent ions induces formation of the stacked conformation, in which strand migration is prohibited. The open-to-stacked transition has been measured in vitro through various methods, including gel mobility shift assays and fluorescence resonance energy transfer, and several ions have been shown to induce this transition. The location of ions needed to induce the transition and the thermodynamics particular to ion-binding are the focus of this study. Currently, isothermal titration calorimetry (ITC) has been employed to measure changes in DNA conformation as a function of ion concentration. Preliminary studies have focused on intramolecular-triplex folding induced by magnesium ion binding and have indicated an equilibrium constant of 463 M-1 an entropically-driven $\Delta G = -3.63$ kcal/mol. Similar ITC experiments have been used to examine the thermodynamic parameters of magnesium-binding to Holliday Junctions and conformational change, indicating an equilibrium constant of 2.44E4 M-1 and an entropically-driven $\Delta G = -5.7$ kcal/mol. This increase in entropy is attributed to release of water from ions during the folding process. The hydration state of ions will also be probed using lanthanide fluorescence spectroscopy by determining whether ion-binding is occurring through direct or indirect interactions. Results from UV resonance Raman measurements that investigate ion coordination and explicitly explore which functional groups, if any, of the DNA bases are involved in ion-binding will also be discussed.

1747-Pos Board B657
Functional Reconstitution of Membrane Proteins by Isothermal Titration Calorimetry
Sandro Keller, Nadin Jahnke, Oxana Krylova.
Membrane proteins make up roughly 30% of all proteins encoded by the human genome and represent about 50% of drug targets in the human body. They fulfil vital functions as receptors and signal transducers, channels and transporters, motors and anchors. Many of these functions are amenable to biochemical and biophysical investigation only after the membrane protein of interest has been extracted, purified, and reconstituted into artificial liposomes. Extraction from the host-cell membrane and chromatographic purification are usually performed with the aid of detergents. However, detergent micelles do not allow the study of vectorial functions such as solute transport or signal transduction. Therefore, numerous membrane proteins need to be reconstituted from a purified, detergent-solubilised state into liposomes in order to regain their native structures and activities.
Unfortunately, functional reconstitution has remained one of the main bottlenecks in the handling of membrane proteins. In particular, gauging the success of reconstitution experiments has thus far been limited to trial-and-error approaches. To address this problem, we have established high-sensitivity isothermal titration calorimetry (ITC) as a powerful method for monitoring the reconstitution of membrane proteins into liposomes. ITC has previously been employed for characterising liposome solubilisation and reconstitution in the absence of protein. Here we show that ITC is also excellently suited for tracking the complex process of membrane-protein reconstitution in a non-invasive and fully automated manner. The approach is exemplified for the prokaryotic potassium channel KcsA, which we first purified in detergent micelles and then reconstituted into stable proteoliposomes at very high protein densities. Electrophysiological experiments performed in planar lipid membranes confirmed that KcsA regained its functional activity upon ITC-guided reconstitution.

Cryo Electron Microscopy & Reconstruction

1748-Pos Board B658
Accurate Flexible Fitting of High-Resolution Protein Structures into cryo-Electron Microscopy Maps Using Coarse-Grained Pseudo-Energy Minimization
Wenjun Zheng.
Cryo-electron microscopy (cryo-EM) has been widely used to explore conformational states of bio-molecular assemblies including various motor proteins like myosin and kinesin. The detailed interpretation of cryo-EM data requires the flexible fitting of a known high-resolution protein structure into a low-resolution cryo-EM map. To this end, we have developed a novel method based on a two-bead-per-residue protein representation, and a modified form of the elastic network model (ENM) that allows large-scale conformational changes while maintaining pseudo-bonds and secondary structures. Our method minimizes a pseudo-energy which linearly combines various terms of the modified ENM energy with a cryo-EM-fitting score and a collision energy that penalizes steric collisions. Unlike previous flexible fitting efforts using the lowest few normal modes, our method effectively utilizes all normal modes so that both global and local structural changes can be fully modeled without overfitting. We have validated our method for a diverse set of 10 pairs of protein structures using simulated cryo-EM maps with a range of resolutions and in the absence/presence of random noise. The final root mean squared deviation (RMSD) of the fitted models (relative to the target structures) ranges from 0.5 to 1**Å.** We have shown that our method is more accurate than alternative techniques, and its performance is robust to the addition of random noise. Our method is also shown to work well for the flexible fitting of an experimental cryo-EM map of myosin motor protein. It is currently being used to model the biochemical states of myosin and kinesin motor proteins when they are strongly bound to actin or microtubule filaments.

1749-Pos Board B659
A modified Cross Correlation Method for Reference-Free Image Alignment in Single-Particle Electron Microscopy
Wooram Park, Gregory S. Chirikjian.
In single-particle electron microscopy, the cross correlation method has been used widely for aligning noisy images from a given class. In this method, aligned images are averaged to generate a high signal-to-noise SNR)