induced by suramin binding. Equilibrium unfolding experiments monitored by fluorescence spectroscopy reveal that the D2 domain is significantly stabilized by suramin. $^{1}H$-$^{15}N$ chemical shift perturbation data shows that the suramin binding sites are mostly composed of residues located at the N- and C-terminal ends of the D2 domain and is supported by site-directed mutagenesis experiments. Interestingly, some of the residues that bind to suramin are located at the FGF-D2 domain interface. A structural model of the suramin D2 domain complex is generated from the experimental data. It appears that suramin inhibits cell proliferation activity of FGF by preventing its interaction with FGFR. The results of this study are expected to pave the way for a rational design of drugs against FGF-induced tumors.

**3094-Pos Board B141**

**Rapid Discovery of Molecular Recognition Elements from Combinatorial Libraries of Peptoids**

**Alexander N. Asanov**, Olga S. Asanov.
TIRF Technologies, Morrisville, NC, USA.

Molecular Recognition Elements (MRE) are in the heart of bioassays for molecular diagnostics and biosensing. Antibodies are the gold standard of MRE for detection of proteins. Bioassays based on antibodies exhibit high specificity and affinity. However, natural antibodies are not reproducible and do not withstand temperature and other environmental factors. Synthetic MRE made of nonnatural sequence-specific heteropolymers is a valuable alternative to antibodies. Peptoid oligomers are of particular interest for creating synthetic MRE because of ease of synthesis and their chemical and biological stability. Several laboratories have shown a wide variety of potent biological activities of peptoids, including antibody-like molecular recognition functions. In this paper, we report on the development of novel method for the rapid discovery of synthetic MRE from one-bead-one-compound (OBOC) combinatorial libraries of peptoids. The approach employs total internal reflection fluorescence (TIRF) combined with electrochemistry and electric field control (TIRF-EC). Target protein is immobilized at the TIRF surface, and the OBOC library is injected into TIRF flow cell. TIRF allows for instantaneous detection of MRE-target interactions and real-time monitoring of their association and dissociation. EC allows for accelerating mass transfer of the beads and stimulating dissociation of bound beads for identification of the peptoids.

**3095-Pos Board B142**

**Biophysical Characterization of FGF Signaling Complex**

**Dakshinamurthy Rajalingam**, Suresh Kumar Krishnaswamy Thallapuranam.
University of arkansas, Fayetteville, AR, USA.

Fibroblast growth factors are important heparin binding proteins involved in the regulation of key cellular processes such as angiogenesis, wound healing and differentiation. Heparin is believed to play a major role in the interaction of FGFs to their receptors, FGFRs. Most of the FGF binding sites are localized on the extracellular D2 domain of the receptor. In the present study, we characterize the structure of the minimalistic FGF-D2 domain interface using a variety of biophysical techniques, including multidimensional NMR spectroscopy and X-ray crystallography. Using sucrose octasulfate, a structural analogue of heparin, we examine the role of heparin in the formation of the FGF-receptor complex. Results of the isothermal titration calorimetry experiments indicate that the human acidic FGF-1 binds to the D2 domain with high affinity both in the presence ($K_{d(appa)} \sim 10^{-7}$ M) and absence of SOS ($K_{d(appa)} \sim 10^{-8}$ M). Far-UV CD and pulse proteolysis experiments, thermal denaturation experiments monitored by far-UV CD reveal that both FGF-1 and the D2 domain undergo subtle conformational changes upon binding and also reveal that SOS stabilizes a preformed 1:1 FGF-D2 domain binary complex. The X-ray structure of a minimalistic fibroblast factor signaling complex, consisting of D2 domain and FGF-1 and sucrose octasulfate (SOS) forms a 2:2:2 symmetrical ternary assemblage. Using $^{1}H$-$^{15}N$ chemical shift perturbation data, the SOS and the FGF binding sites on the D2 domain have been successfully mapped. NMR spectroscopy data is more consistent with the minimalistic ternary complex than the other crystallographic models of the FGF signaling complexes. Results of this study clearly suggest that the primary role of heparin in the FGF signaling process is merely limited to conferring stability to the FGF-receptor complex. Results obtained herein appear to challenge the existing view of the structural events leading to FGF-induced cell proliferation.

**3096-Pos Board B143**

**Designing Fibroblast Growth Factor with Higher Heparin Binding Affinity**

**Ivy Fitzgerald**, Dakshinamurthy Rajalingam, Suresh Kumar Krishnaswamy Thallapurnam.
University of arkansas, Fayetteville, AR, USA.

Fibroblast growth factors (FGFs) play significant roles in the regulation of cell proliferation, angiogenesis, differentiation, tumor formation, embryonic growth, invasion, inflammation, and tissue repair. FGFs are also able to improve wound healing caused by metabolic diseases such as obesity, diabetes, infection, chronic liver failure, malnutrition, and second-degree burns. Studies have indicated that FGFs produce biological responses by binding to two types of receptors on the cell surface. The first class is a high-affinity family of transmembrane tyrosine kinase receptors called FGFRs. The second class of receptors is the family of heparan sulfate proteoglycans (HSPGs), which have a low affinity for FGFs. The macromolecular interactions of the growth factors, HSPGs, and FGFRs that lead to signal transduction are key to signaling by this important class of molecules. Proteoglycans, such as heparin, are required for the cell proliferation activities of FGFs. Additionally heparin helps to increase the affinity and half-life of the FGF-FGFR complex, which is crucial for signal transduction. The three-dimensional structure of the FGF-heparin complex show that the sulfate groups in the proteoglycans contribute significantly to binding. Residues involved in heparin binding correspond to amino acid 126 to 142 of the human FGF-1 sequence. In this context, we examined the role of additional lysine residues in the putative heparin-binding region of wild-type FGF-1, as introduced by single and double -site mutagenesis on binding, stability, and structure using various biophysical techniques including multi-dimensional NMR spectroscopy. The results clearly indicate that the introduction of lysine residues in three different positions in the heparin-binding pocket significantly increases binding to sucrose octasulfate (SOS, a heparin analog) and conformational stability. In addition, results of this study provide a valuable basis for novel therapeutics targeting this interaction.

**3097-Pos Board B144**

**Quantitative Analysis of Water Dynamics in and near Proteins**

Oliver Beckstein[1,2], Naveen Michaud-Agrawal[1], **Thomas B. Woolf**[1].
[1]Johns Hopkins University, Baltimore, MD, USA, [2]Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

Proteins exist in aqueous solution. Hydration can be viewed as a description of how the protein disturbs the structure and dynamics of water. Water molecules in the vicinity of proteins are generally seen as either external or internal water molecules. Internal water molecules occupy cavities, exchange on a time-scale of 0.1–10 microseconds with bulk water, are almost as conserved as amino acids, and are therefore likely to be important for function. External water molecules tend to be found in protein crevices and are typically not conserved, even between crystal structures of the same protein. We introduce a method to analyze the behavior of water molecules in molecular dynamics (MD) simulations in terms of graphs. The graph encodes a simple hopping model: Nodes in the graph correspond to hydration sites, typically defined from the density in computer simulations or observed water sites in crystal structures. Directed edges correspond to transitions (''hops'') between sites, with transition rates computed from MD simulations. We apply this analysis to the water-filled cavity of intestinal fatty acid binding protein (I-FABP) in its apo and holo (palmitate-bound) state. This demonstrates how ligand binding influences the well-defined set of hydration sites in and around the protein's cavity. The ligand displaces a number of hydration sites but does not affect others close by. The parameters extracted from the network model allow us to model the movement of water molecules with a Markov Chain Monte Carlo model. The graphical construct reproduces the average site occupancy found in the MD simulations and the fluctuations of the occupancy.

This approach suggests new types of sampling and analysis that can be applied to extend the range of molecular dynamics models and the role of water in ligand binding.

## Physical Chemistry of Protein & Nucleic Acids

**3098-Pos Board B145**

**Polarizable Force Fields for Protein Simulations**

**George Kaminski**.
Worcester Polytechnic Institute, Worcester, MA, USA.

Computer simulations have become a widely used tool in biophysical and biochemical research. Fixed-charges empirical force fields have the advantage of computational speed, but explicit treatment of electrostatic polarization as a way to represent many-body interactions is often necessary if accurate energetic results are desired. Examples of such results include protein-ligand binding energies and acidity constants. We have demonstrated that using a polarizable force field permits achieving a ca. 0.6 pH units accuracy in calculating protein pKa values and qualitatively successful predictions of protein-ligand complex stabilities which are predicted as unstable by fixed-charges force fields. Moreover, we are developing a fast version of a complete polarizable force field for proteins, which is expected to speed up these accurate calculations by about an order of magnitude. Furthermore, the development of our