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Structure, Folding, and Interactions of the Fibroblast Growth Factors

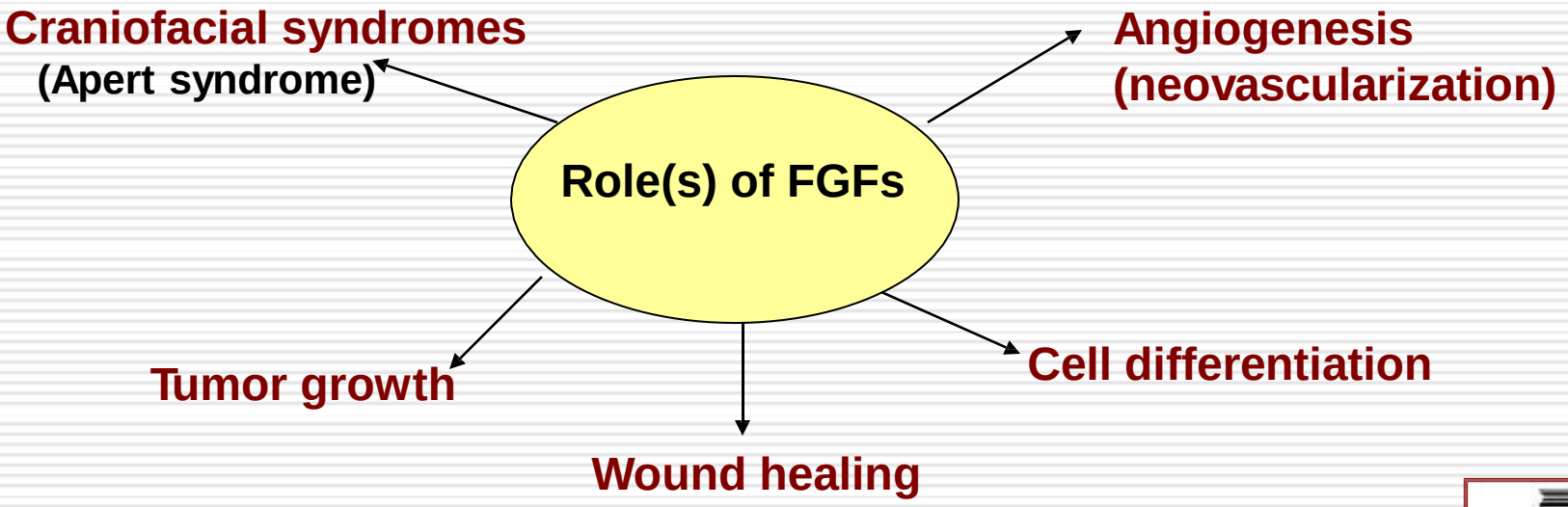
T. K. S. Kumar

Department of Chemistry & Biochemistry

University of Arkansas, Fayetteville, AR

Chemistry and biology of FGFs

- FGFs are ~17 kDa proteins that bind to heparin.
- contain 3 conserved cysteine residues but lack disulfide bonds.
- contain a conserved tryptophan residue which can be used as an optical probe to monitor global structural changes in the protein.



Three-dimensional solution structure of acidic fibroblast growth factor (FGF-1)

Determination of the 3D solution structure Of proteins involves two major steps.

1. **Spatial assignment of all atoms in the protein**

2. **Establishment of the spatial distance between various atoms.**

Spectra

1D

optimized

Magnet pulse sequence

2D

3D

^{13}C

Computer

(Aria, cyana)

^{15}N

3D structure

Molmol/Insight II

^{1}H

Adapted from http://structbio.vanderbilt.edu/chazin/

1D NMR spectrum of FGF-1

The resolution obtained in a 1D proton spectrum is not sufficient to identify all the protons in the protein.

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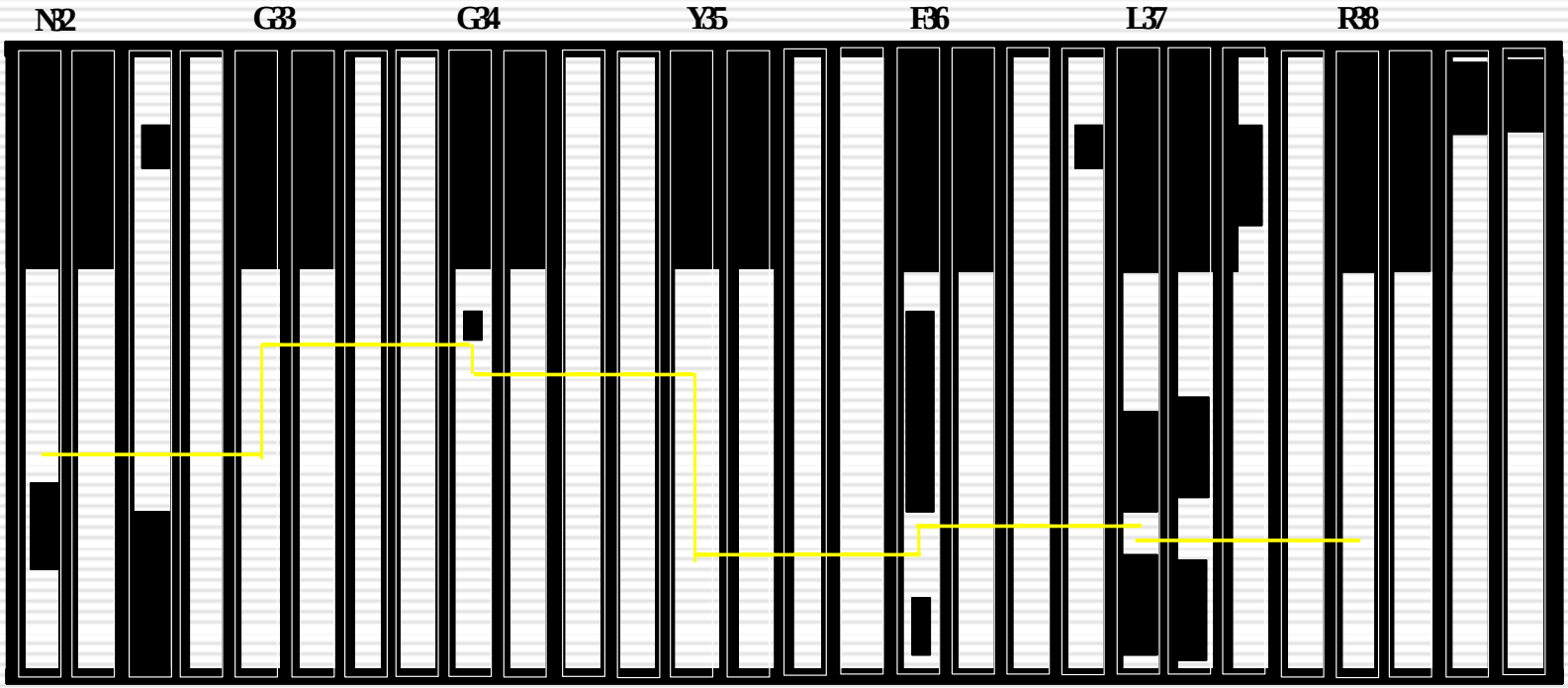
3D NMR data on FGF-1

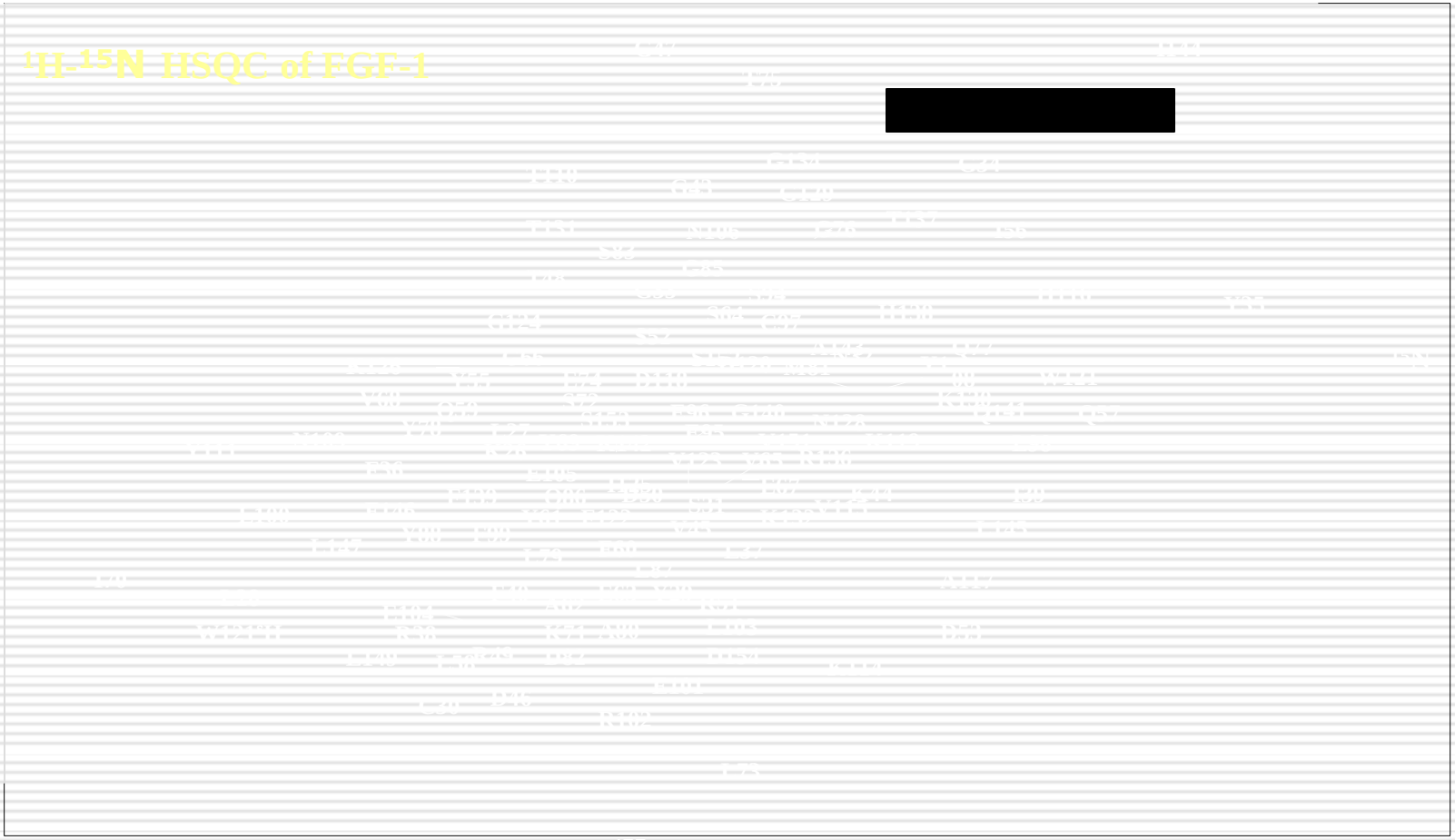
Sample – ^{15}N, ^{13}C FGF-1

HNCA-HNCOCA-CBCACONH-HNCACB



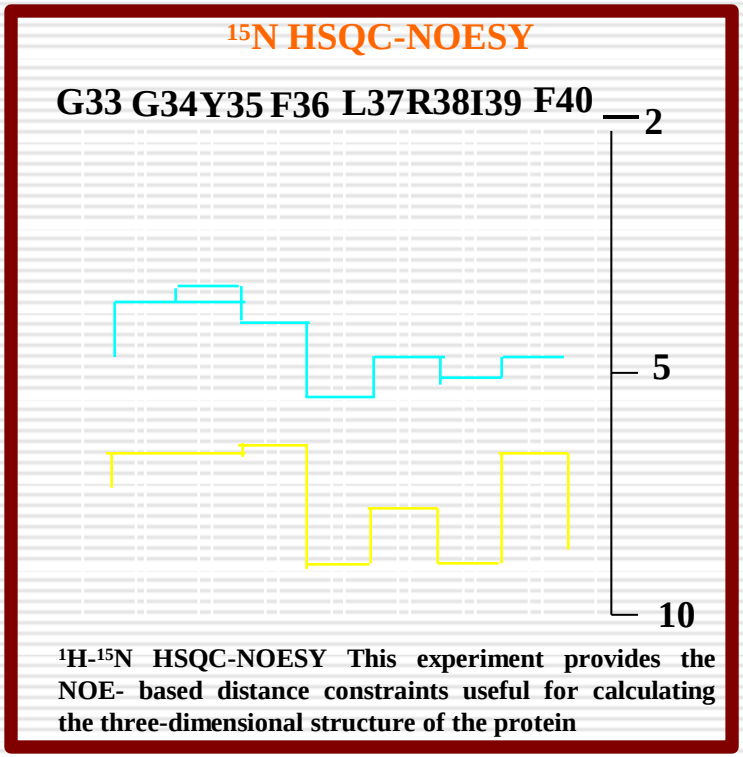
Triple resonance data provide through-bond connectivity between various atoms

Complete resonance assignment of FGF-1



^{1}H-^{15}N HSQC spectrum represents the finger-print of the backbone conformation of a protein

NOE data of FGF-1

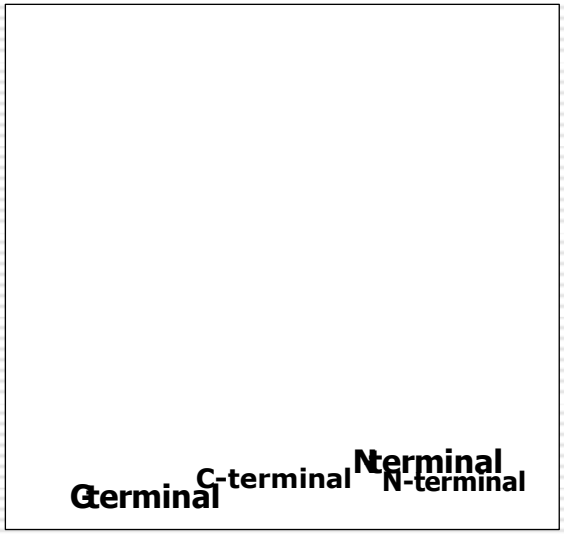


^{1}H-^{15}N HSQC-NOESY This experiment provides the NOE- based distance constraints useful for calculating the three-dimensional structure of the protein

2D-NOESY, 3D ^{15}N HSQC-NOESY & ^{13}C HSQC-NOESY data provide through-bond and through-space connectivity between various atoms in a protein.

3D solution structure of FGF-1

- FGF-1 consists of 12 antiparallel β-strands arranged into a beta-trefoil structure
- The β-strands are arranged in three layers enclosing a hydrophobic core.



RMSD for structured region - 0.60Å
RMSD for the whole protein – 0.97Å

Kumar et al., J. Biol. Chem. (**2000**) 275, 39444-39450

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Role of heparin in the mitogenic activity of FGF-1

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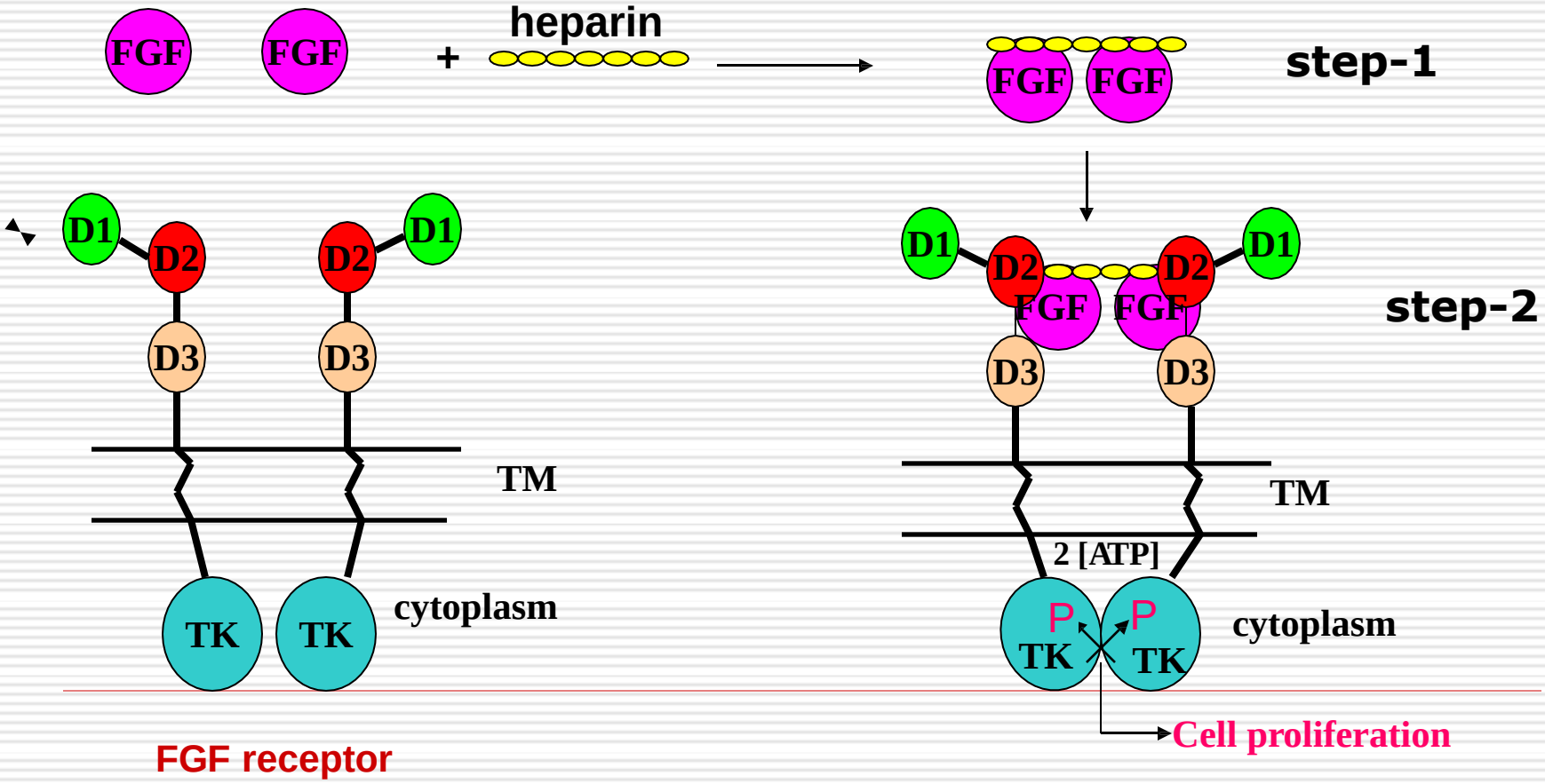
FGF-1 exhibits its cell proliferation activity by binding to its cell surface receptor

- Heparin is believed to be the secondary receptor for FGF-1.
- Dimerization induced by heparin is considered to be pre-requisite for FGF-1 action



Is the heparin-induced oligomerization of FGF mandatory for its mitogenic activity?

- **FGF exhibits mitogenic activity even in mutant Chinese Hamster Ovary Cells deficient in heparin (Roghani et al. *J. Biol. Chem*. (1997) 269, 3976-84)**

- **Mutants of FGF which lack heparin binding affinity exhibit mitogenic activity in NIH3T3 cells (*J. Vasc Surg*. (1998) 31, 382-90).**

These studies suggest that heparin *per se* is not required for the cell proliferation activity of FGF

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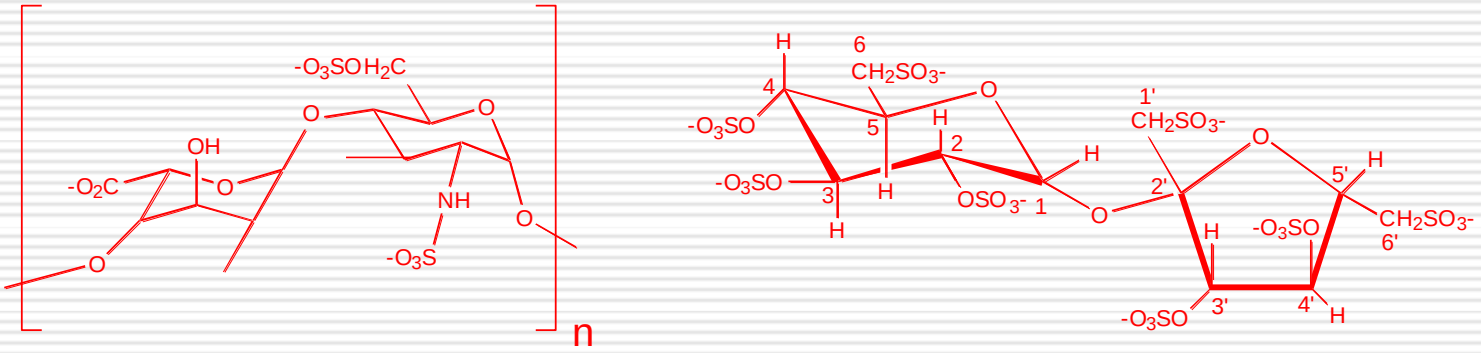
Structures of heparin and sucrose octasulfate (S0S)

SOS has been shown to be good structural and functional mimic of heparin



Heparin **Sucrose octasulfate**

Sulfated glucosamine-iduronic acid polymer

Homogeneous heparin samples are difficult to prepare.

Volkin *et al* (1993) *Biochim. Biophys. Acta.* 1203, 18.

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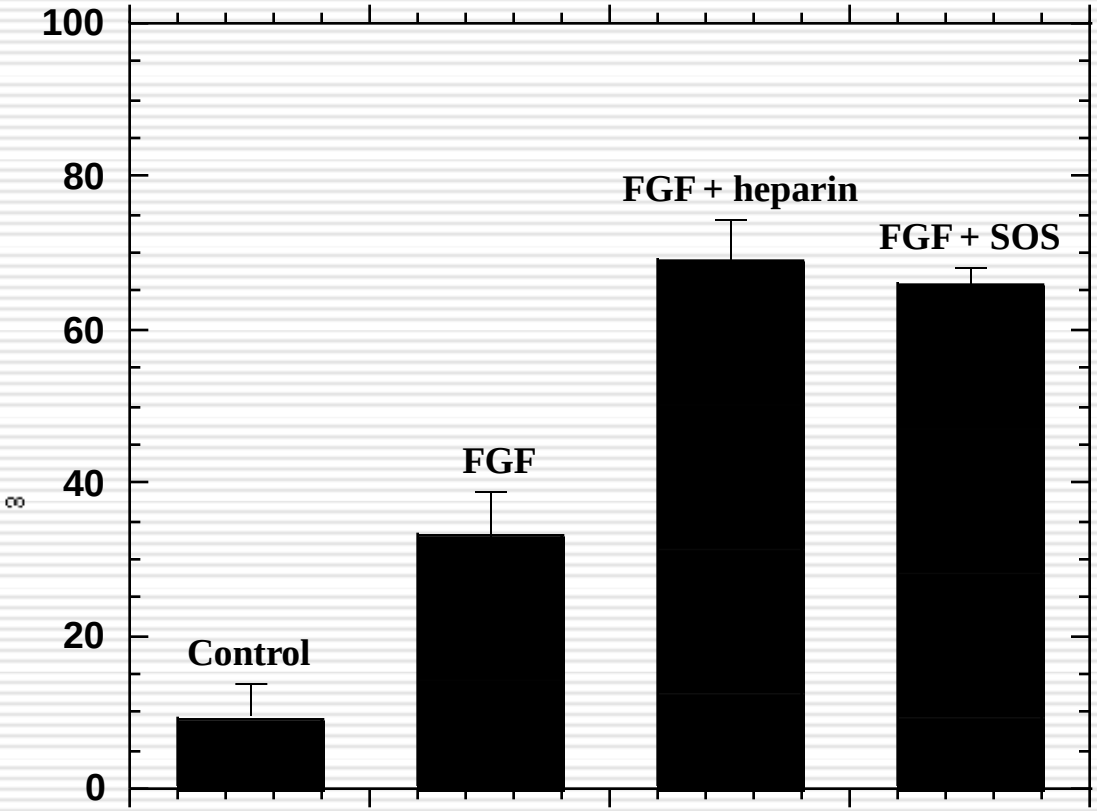
SOS supports the mitogenic activity of FGF-1 on NIH3T3 cells treated with heparinase



Control –deheparinized cells grown in 5% calf serum

Kumar et al, Protein Sci. (**2002**) 11, 11050-11061

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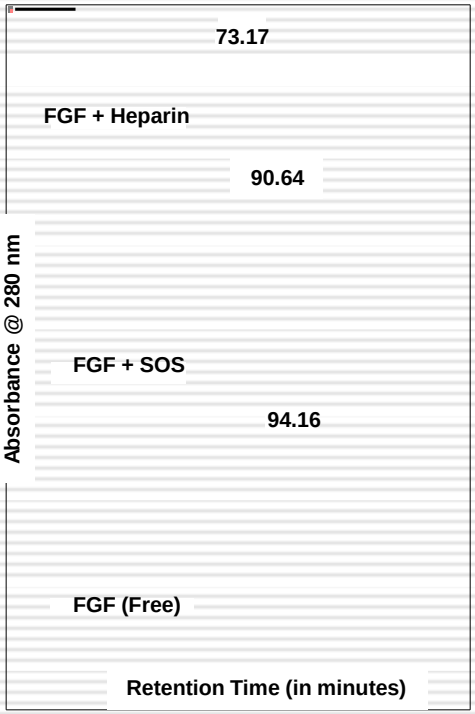
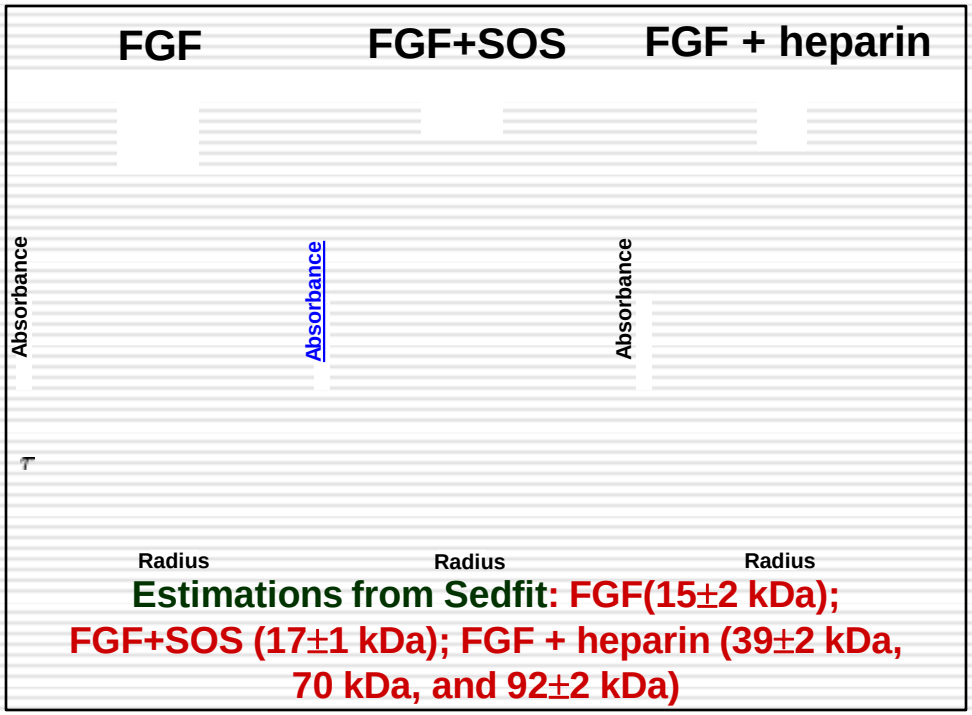
FGF-1 is a monomer in the presence of SOS

Size exclusion data



Sedimentation velocity data



Estimations from Sedfit: FGF(15±2 kDa); FGF+SOS (17±1 kDa); FGF + heparin (39±2 kDa, 70 kDa, and 92±2 kDa)

NMR relaxation data

Overall correlation time (τ_m) = T_1/T_2

τ_m= 9.35±0.1 ns (FGF)

τ_m = 10.55±0.2 ns (FGF + SOS)

τ_m = 24.13±0.4 ns (FGF + heparin)

Oligomerization of FGF-1 induced heparin is not mandatory for its cell proliferation activity

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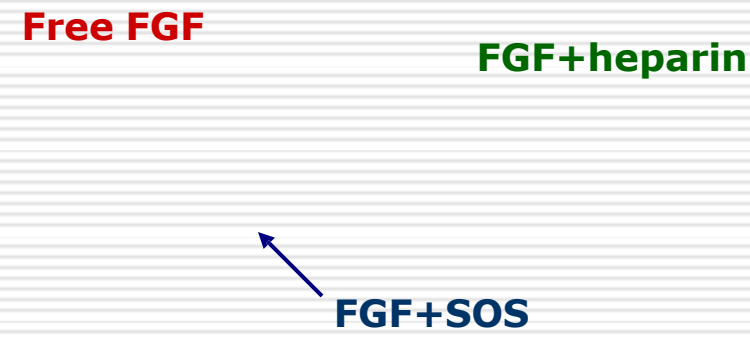
Both heparin and SOS stabilize FGF-1

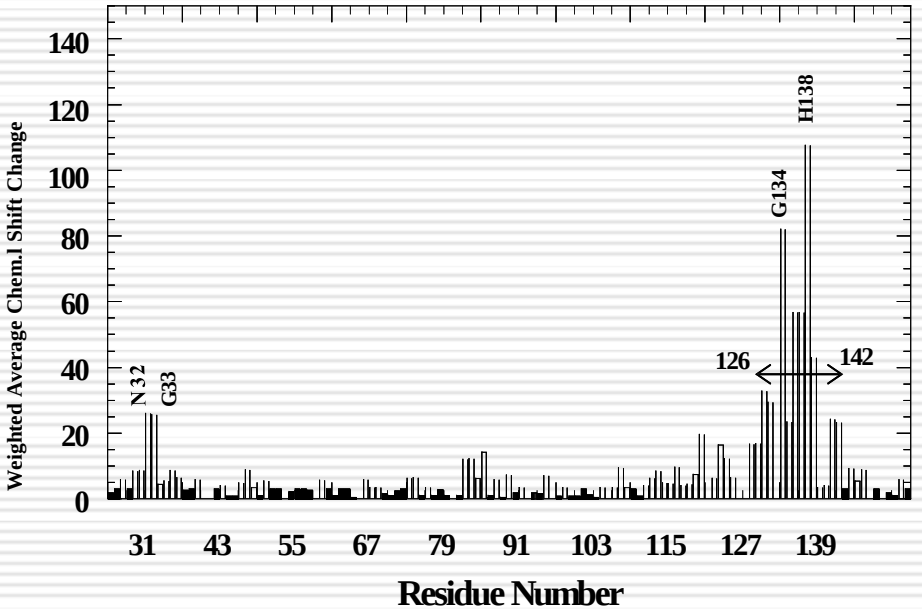


Kumar et al, Protein Sci. (2002) 11, 11050-11061

SOS and heparin appear to support the mitogenic activity of FGF-1 by protecting it against proteolytic enzymes active at the cell surface

SOS binding sites in FGF-1

SOS binding sites are spread between residues, 126-142.



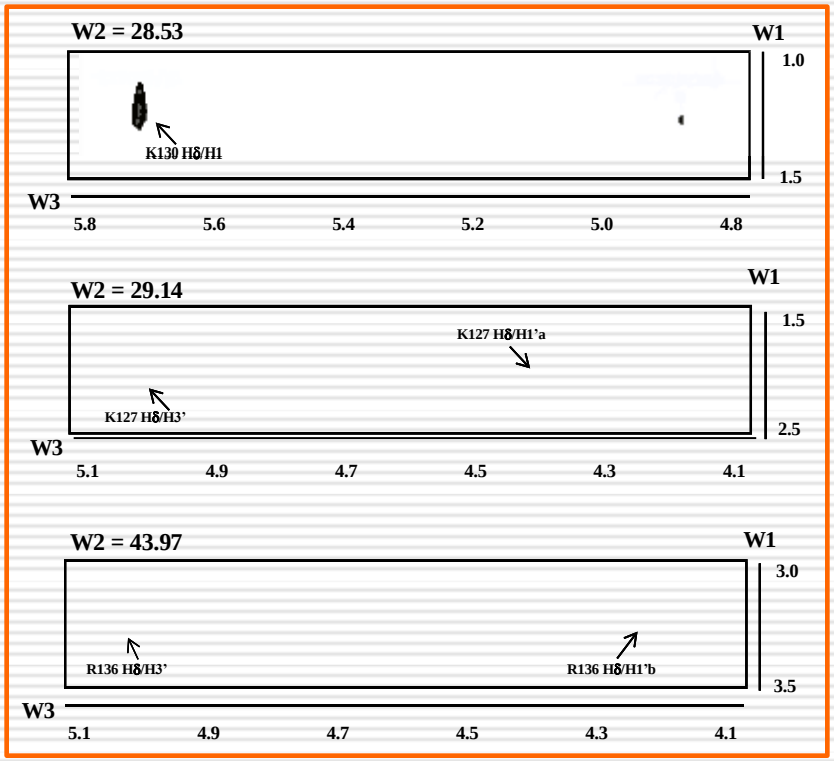
Cyan –FGF Red – FGF +SOS

Residues located at the c-terminal are involved in binding to SOS

Kumar et al, Protein Sci. (2002) 11, 11050-11061

SOS binds to FGF-1 at the heparin binding site

Intermolecular NOEs from 3D ^{1}H-^{13}C isotope filtered NOESY



Lys126 C H-H`; Lys130 C H-H3`; Lys130C H-H2, and Lys142C H-H3`

Structure of the FGF-SOS complex

FGF exists as a monomer upon binding to SOS

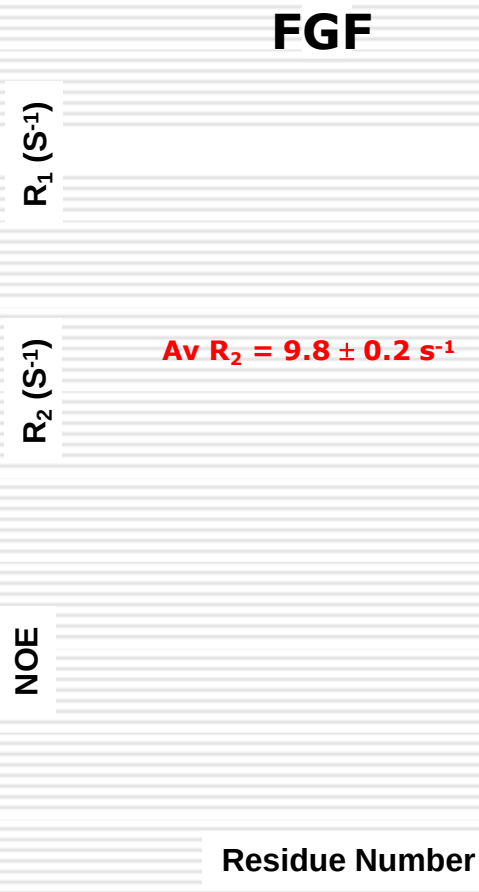
3D ^{1}H-^{13}C isotope filtered NOESY provides NOE information between ^{13}C-^{1}H (in FGF) and ^{12}C-$_{1}H$ atoms (in SOS).

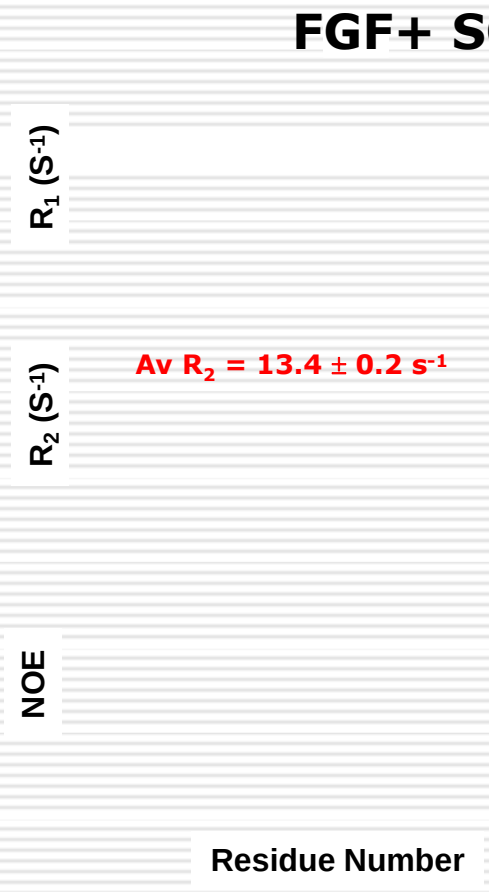
*Kumar et al., J. Biol. Chem. (**2006**) 277, 47507-46516*

Backbone dynamics of FGF-1

Protein molecules experience 'breathing" motions

Backbone dynamics of proteins can be studied based on R1, R2 and NOE relaxation measurements





The average conformational flexibility of FGF appears to decrease in the presence of SOS.

Kumar et al., J Biol Chem. (2003)278, 17701-9

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SOS stabilizes the structure of FGF-1

The order parameter S^2 defines the degree of flexibility of residues in the protein

FGF

Average S^2 = 0.73± 0.1

FGF + SOS

Average S^2 = 0.81± 0.1

SOS decreases the overall flexibility of the FGF molecule

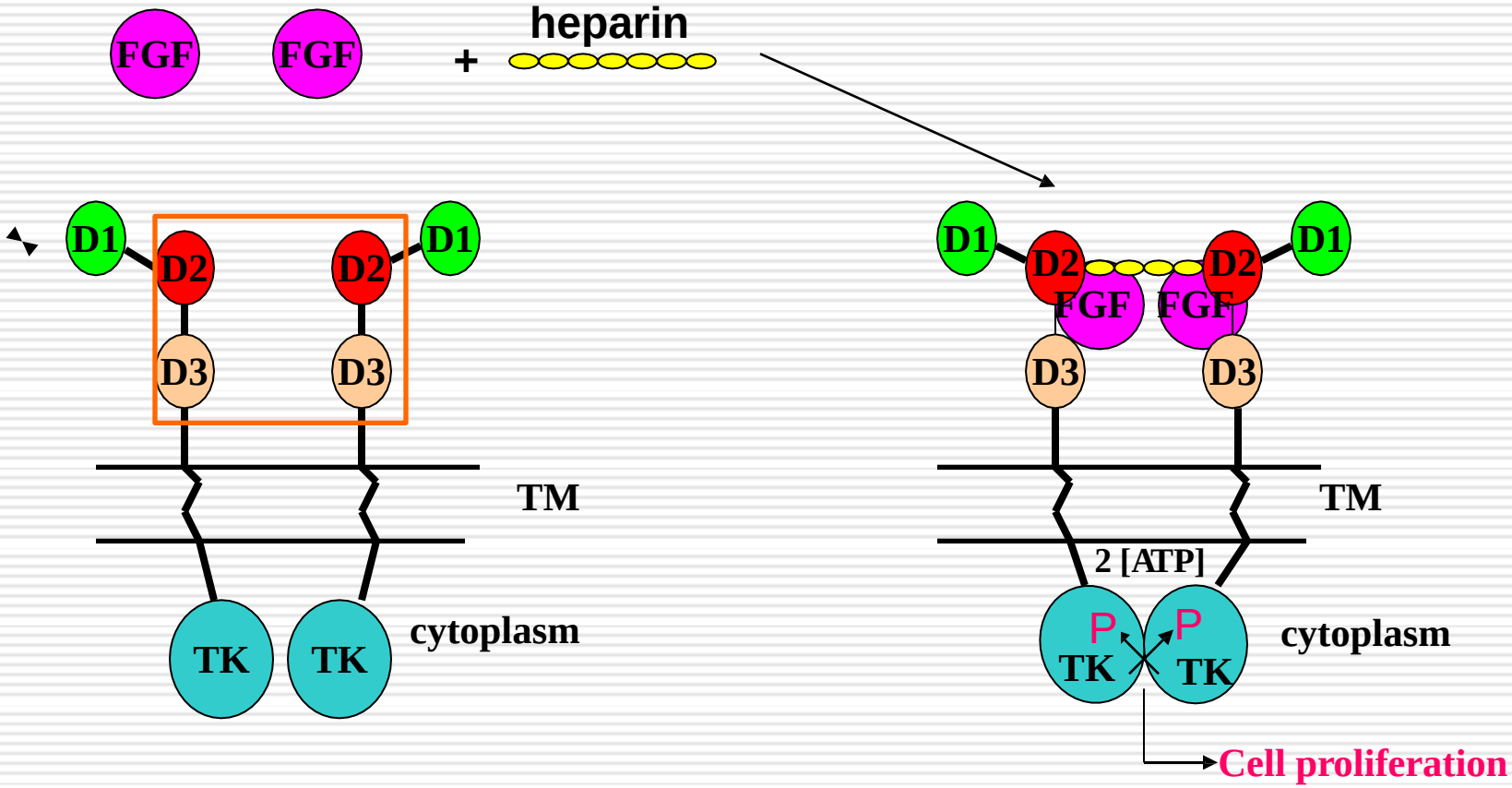
J Biol Chem. (2010)285, 34220-34230

Conclusions

- SOS is both a structural as well as a functional mimic of heparin.
- FGF exists as a monomer in the presence of FGF.
- Heparin-induced oligomerization of FGF is not a pre-requisite for its cell proliferation activity.
- Both SOS and heparin appear to support the cell proliferation activity by stabilizing FGF.
- SOS binds to the putative heparin binding site in FGF.

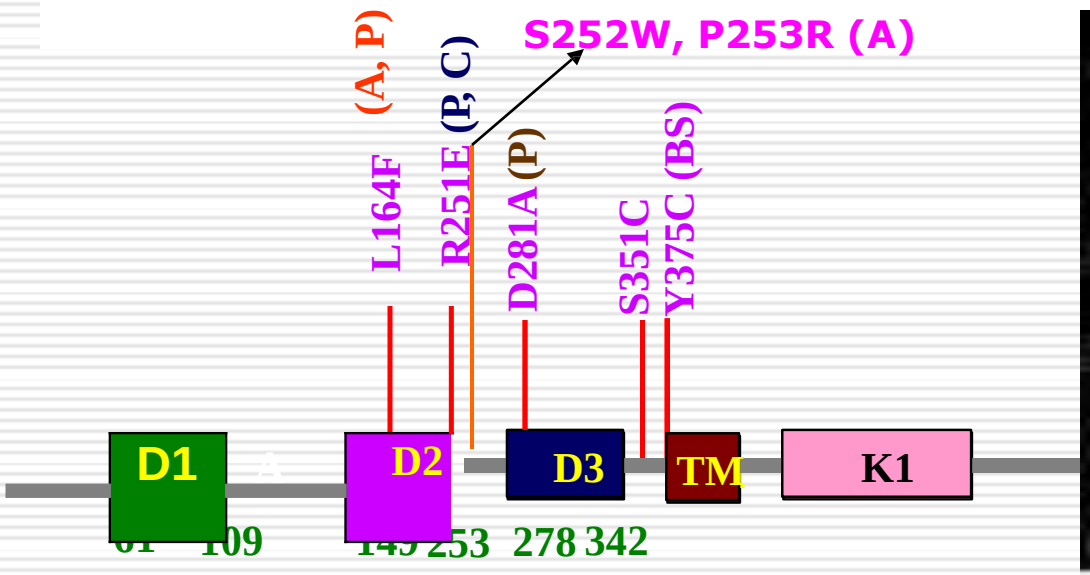
Structure of the FGF receptor

- FGF exhibits its cell proliferation activity by binding to its cell surface receptor.
- D2, D3 modules of the extracellular domain of FGFR are sufficient for the FGF signaling process.



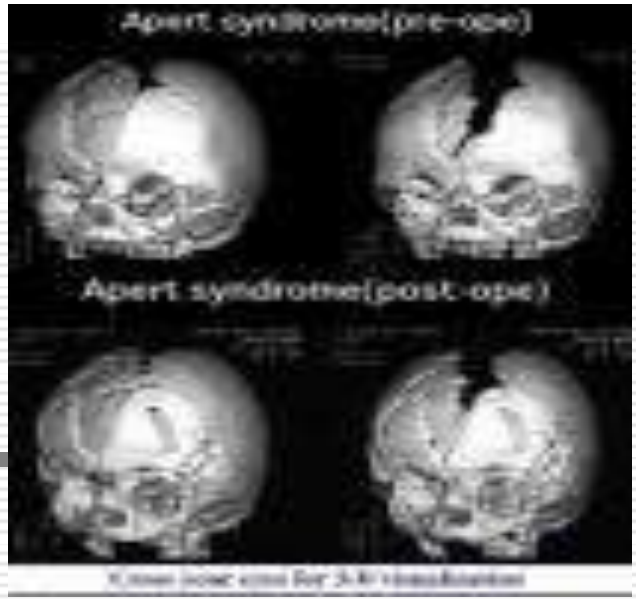
Mutations in FGFR are linked to craniofacial syndromes and urinary bladder cancers

Mutations in FGFR lead to gain of FGF function



Functional domains of FGFR

www.id.yamagata-u.ac.jp/



Cranial defects in Apert syndrome

A – Apert syndrome; C.S – Cronzon syndrome; P-Pfeiffer syndrome; BS Beare-Stevenson syndrome

Structure of the D2 domain

D2 domain consists of 103 amino acids (residues,149 to 253)
Resonance assignments were accomplished using a variety of triple resonance NMR experiments

HSQC of D2

Kumar et al., J. Biomol. NMR. (**2004**) 30, 99-100

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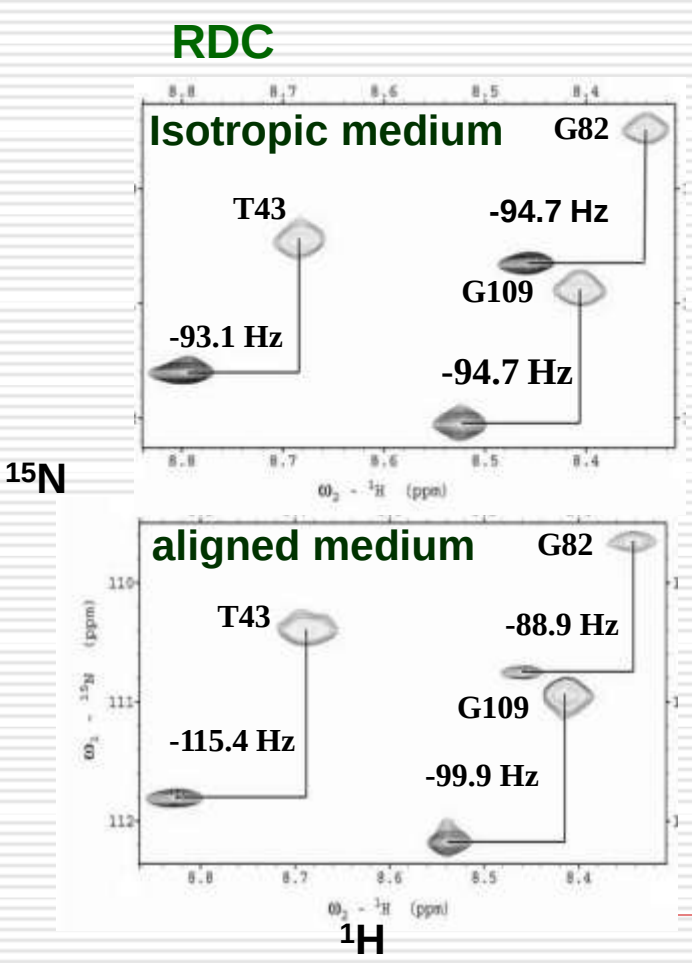
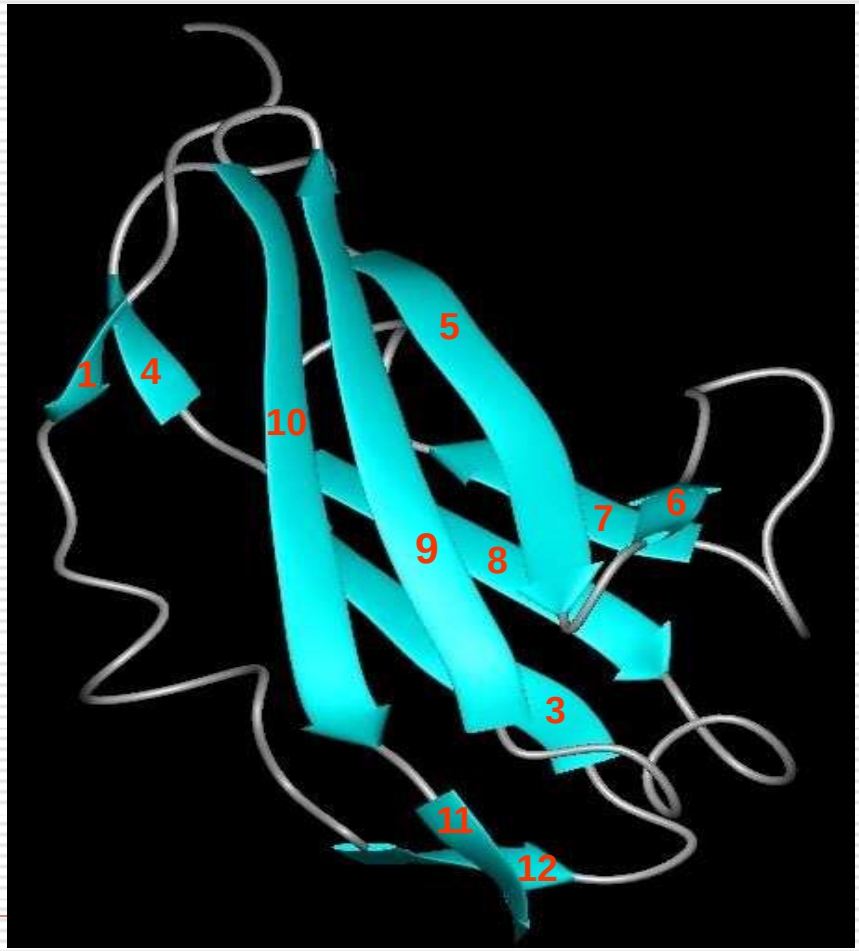
3D solution structure of the D2 domain of FGFR

Backbone RMSD based on NOE constraints ~ 0.54 Å
Backbone RMSD based on NOE + RDC constraints ~ 0.45 Å

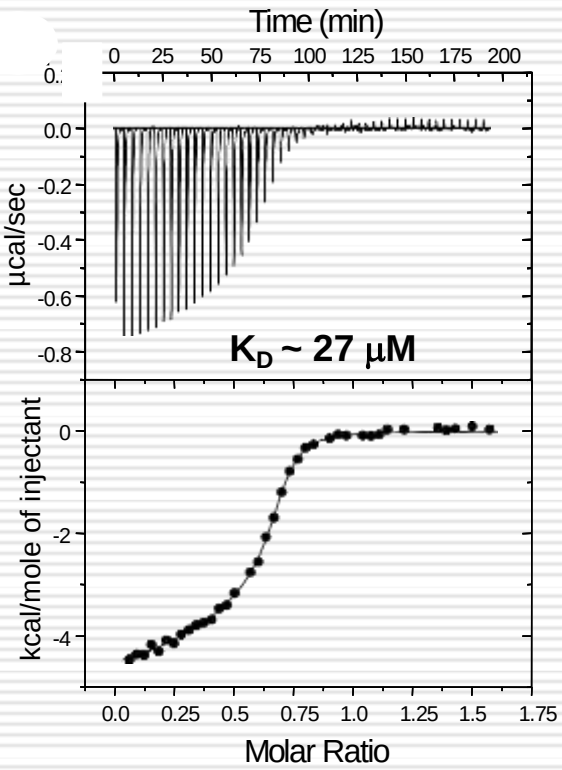




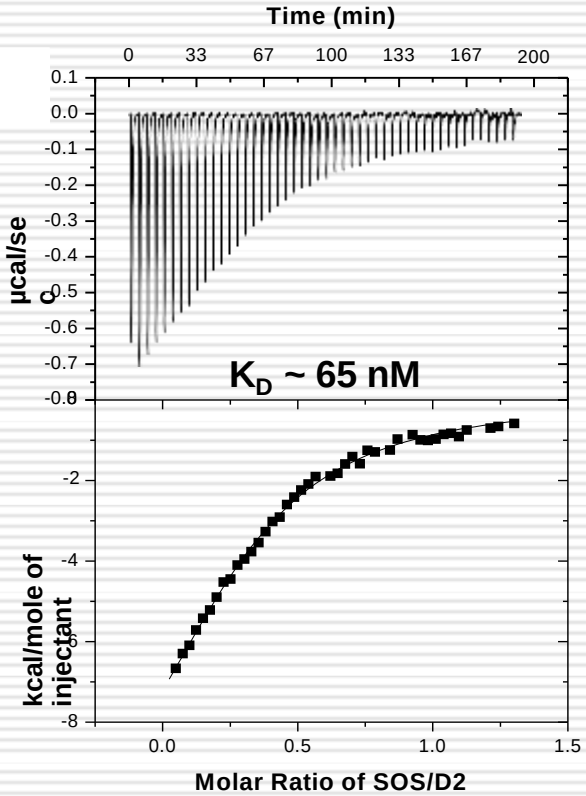
Kumar et al., Biochemistry (**2005**) 44, 15787-98

Isolated D2 domain is functional

D2 domain vs SOS



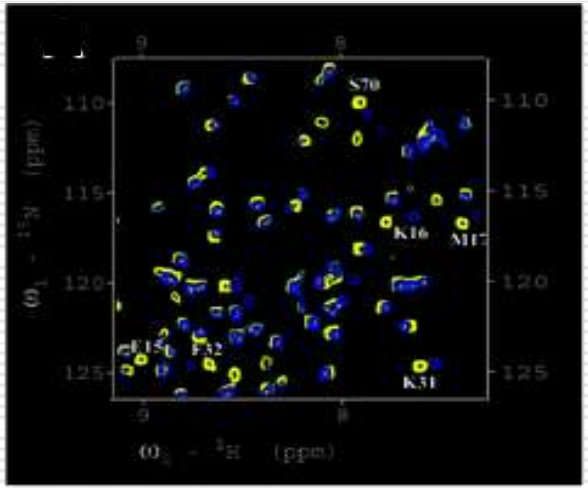
D2 domain vs FGF

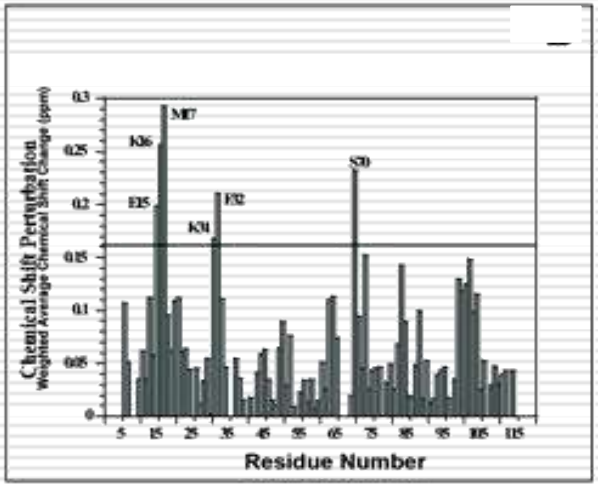


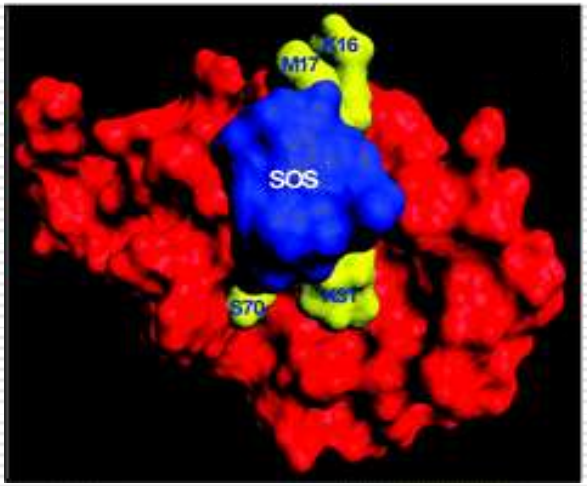
Kathir & Kumar, unpublished results

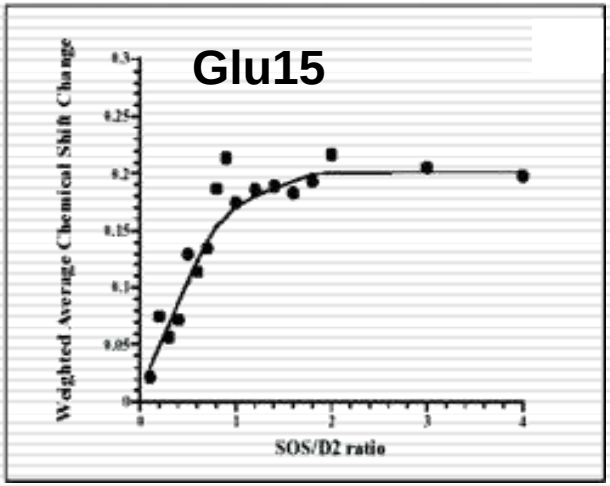
D2 domain binds to both SOS (heparin) and FGF. The growth factor does not *per se* require heparin for binding to its cell surface receptor

Mapping the SOS (heparin) binding sites in D2 domain





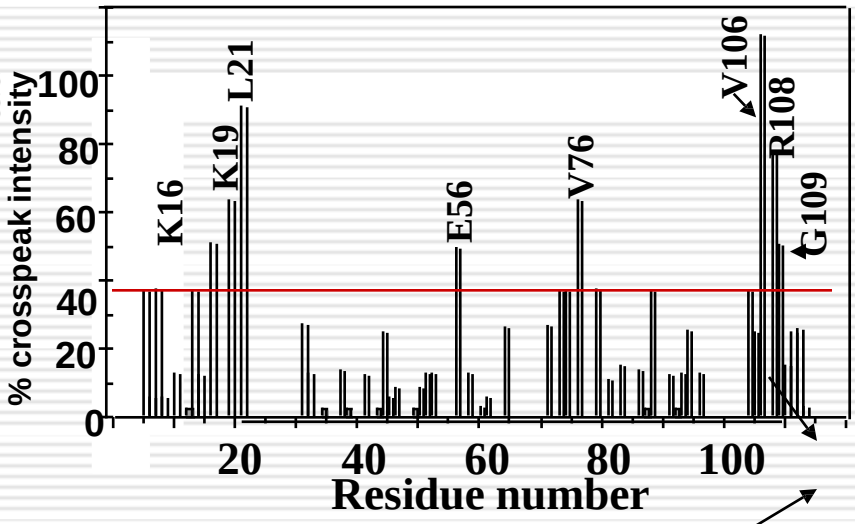




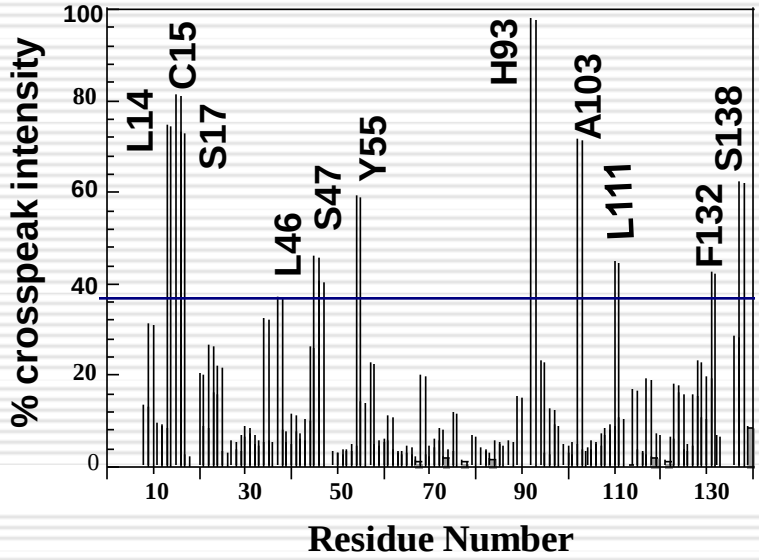
- D2 domain binds to SOS in a 1:1 stoichiometry. *Kumar et al., Biochemistry* (**2010**) 44, 15787-98
- The residues in D2 domain that possibly bind to SOS include E15, K16, M17, K31, F32 and S70

FGF-D2 domain binding interface

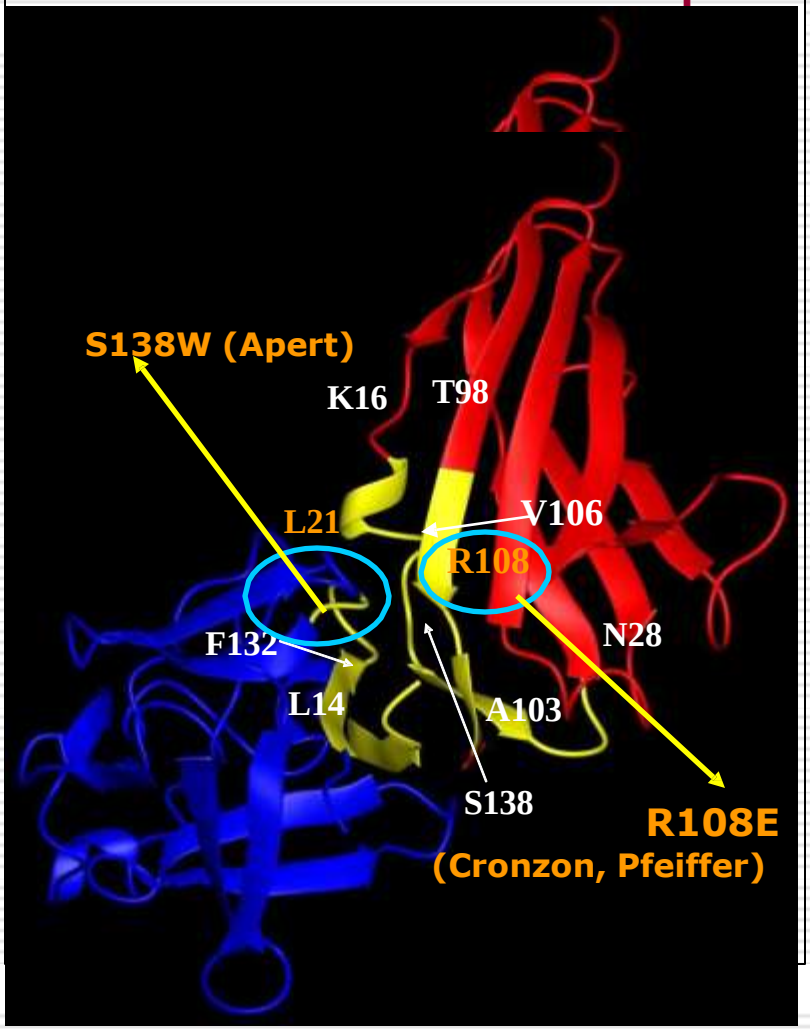
^{15}N D2 vs unlabeled FGF



^{15}N FGF vs unlabeled D2

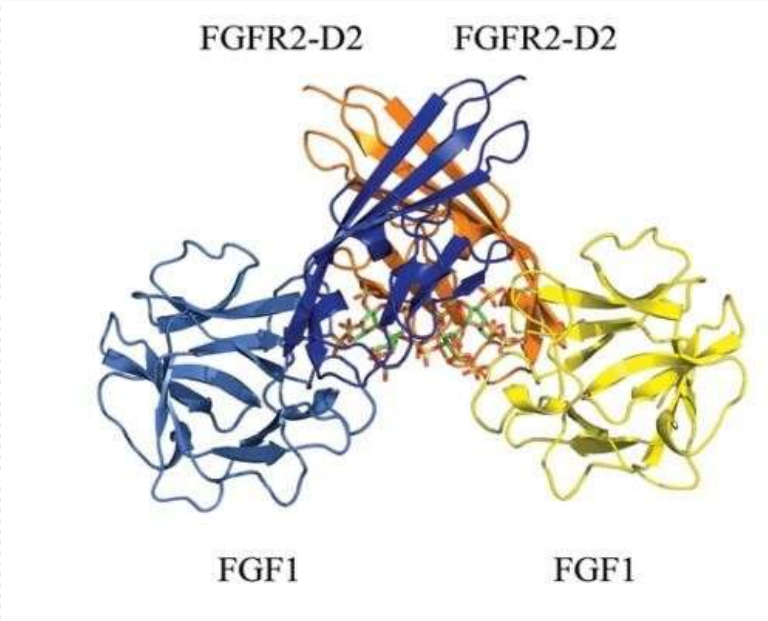


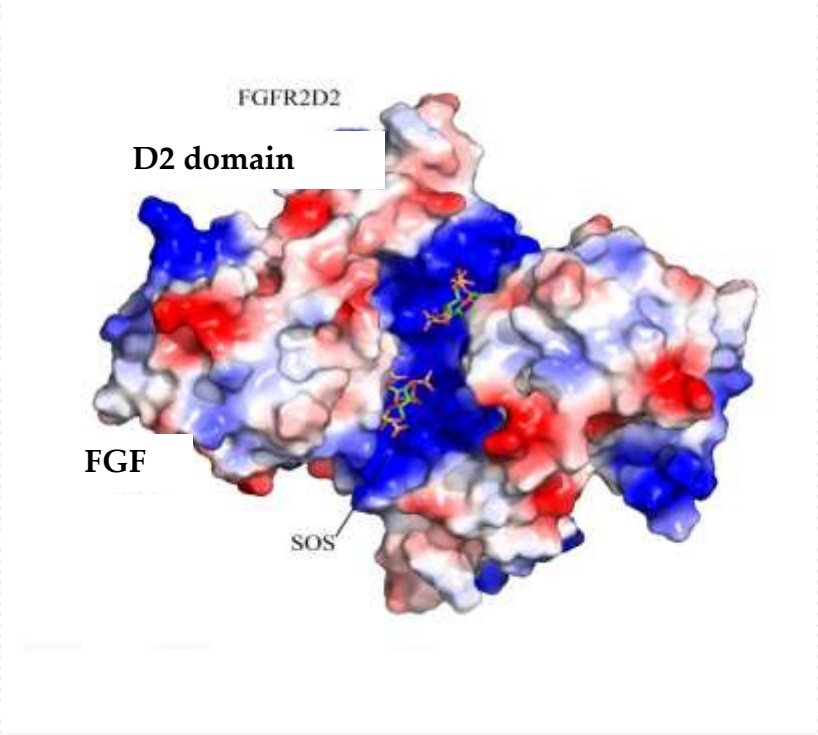
Structure of the D2-FGF complex



Kathir & Kumar, unpublished results

3D crystal structure of the FGF-receptor-SOS ternary complex





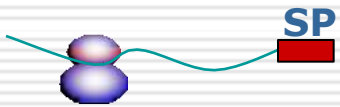
Kumar & Sakon, unpublished results

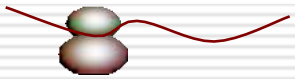
Conclusions

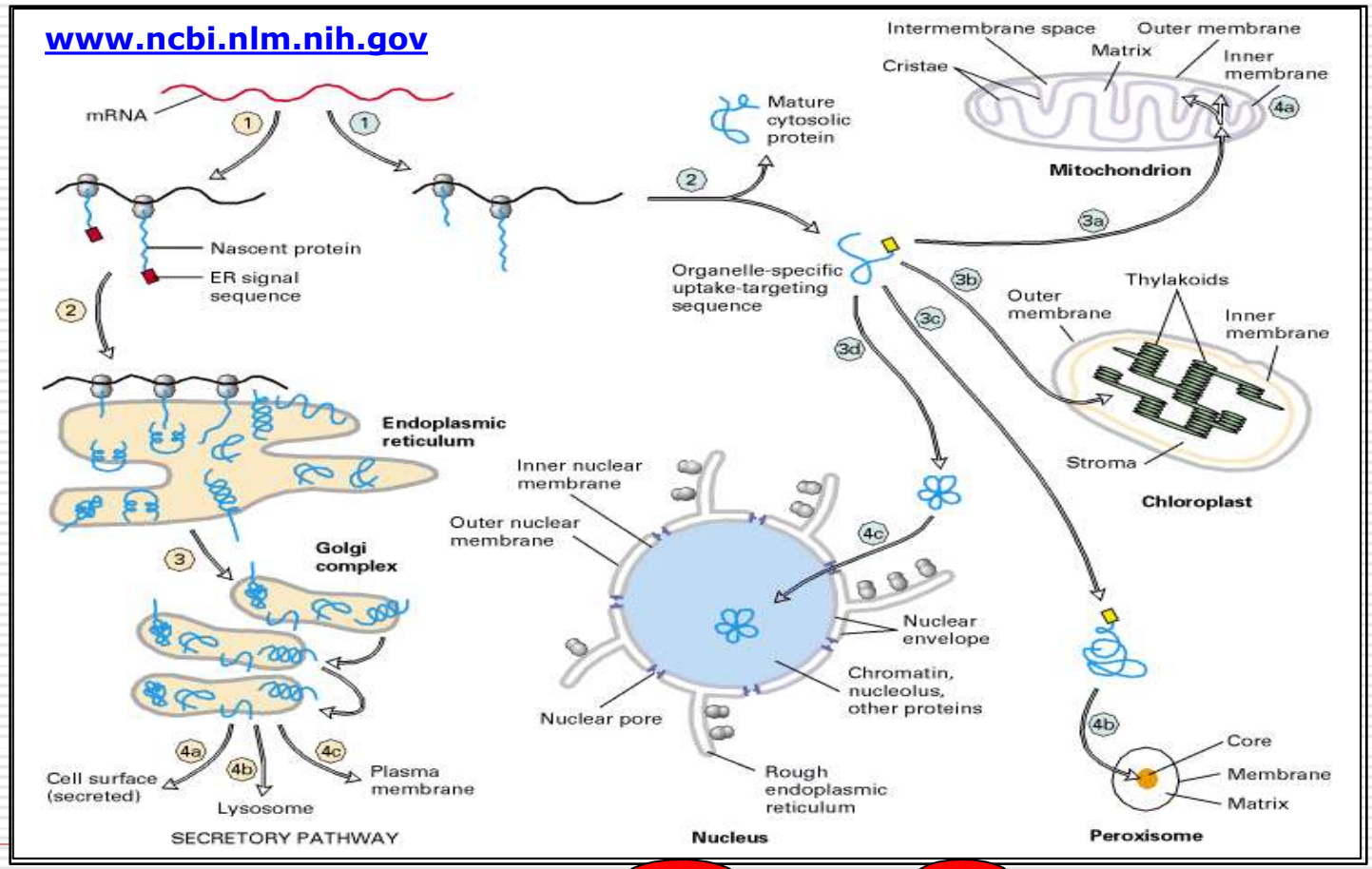
- The 3D solution structure of the D2 domain of the FGFR has been solved at high resolution.
- The SOS (heparin) binding sites on D2 have been mapped
- The structure of the FGF- D2 domain binding interface has been characterized

Overview of sorting of nuclear-encoded proteins in eukaryotic cells

Proteins secreted by the classical ER-Golgi apparatus secretion pathway typically contain N-terminal signal peptides. FGF-1 lacks N-terminal signal peptide.







List of signal peptide-less proteins

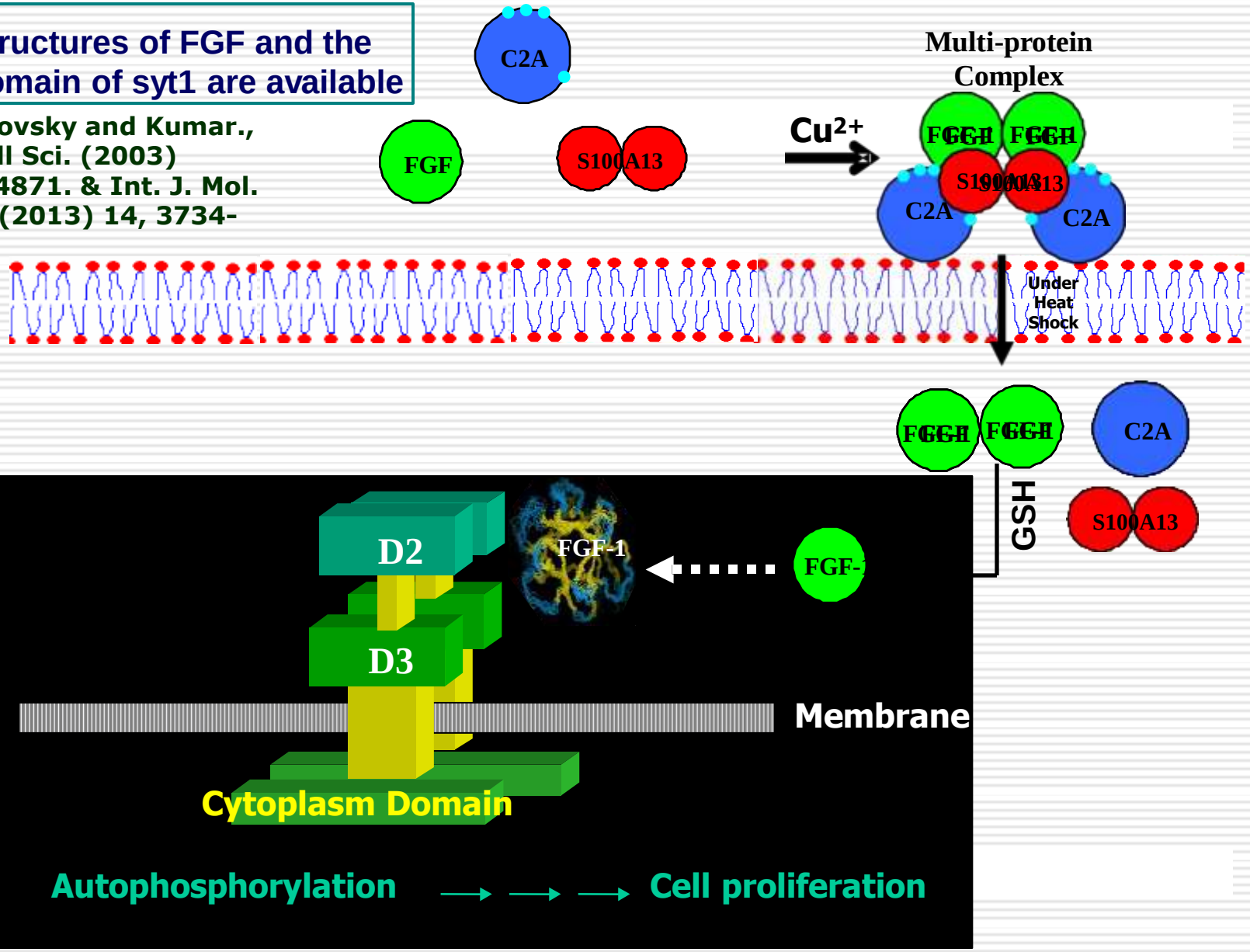
Secretory transglutaminase
Thioredoxin
p40 synaptotagmin
Interleukins
Fibroblast growth factors
Spingosine kinase
Annexins
TAT-HIV integrase;
Engrailed-2
Herpes VP 22 protein
HM GB1
Galectins
Foamy virus bet protein
Leishmania HASPB protein.

Prudovsky & Kumar et al.(2003) *J. Cell Sci*., 116, 4871-81; Kumar et al (2013) Int. J. Mol. Sci., 14, 3734-72 .

Proposed mechanism for the non-classical release of FGF-1

3D structures of FGF and the C2A domain of syt1 are available

Prudovsky and Kumar., J. Cell Sci. (2003) 116,4871. & Int. J. Mol. Sci., (2013) 14, 3734-72



Open Questions

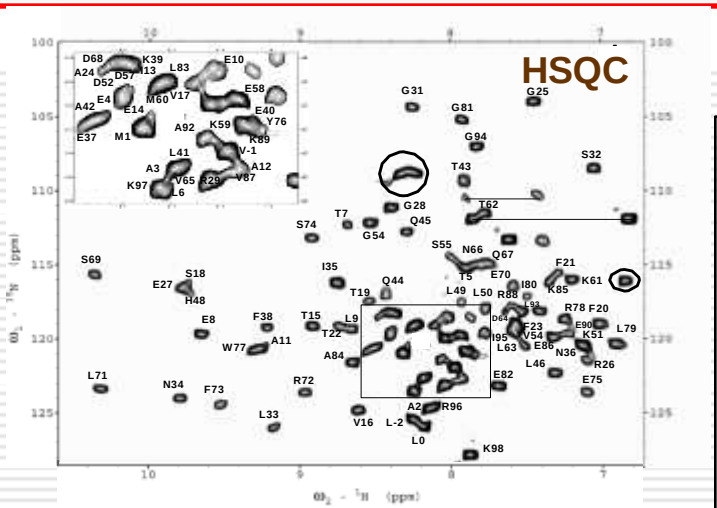
1a. What is the structure of the FGF multiprotein complex?
b. What are the sequence of structural events leading to the formation of the FGF release complex?

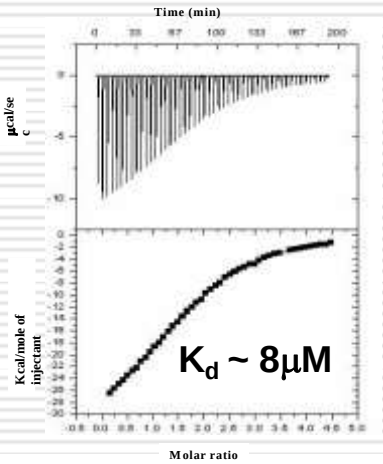
2a. What is the exact role of Cu^{2+} in the organization of the multiprotein complex?
b. Which of the protein components in the release complex bind to Cu^{2+}?

3. How is the FGF release complex transported across the cell membrane?

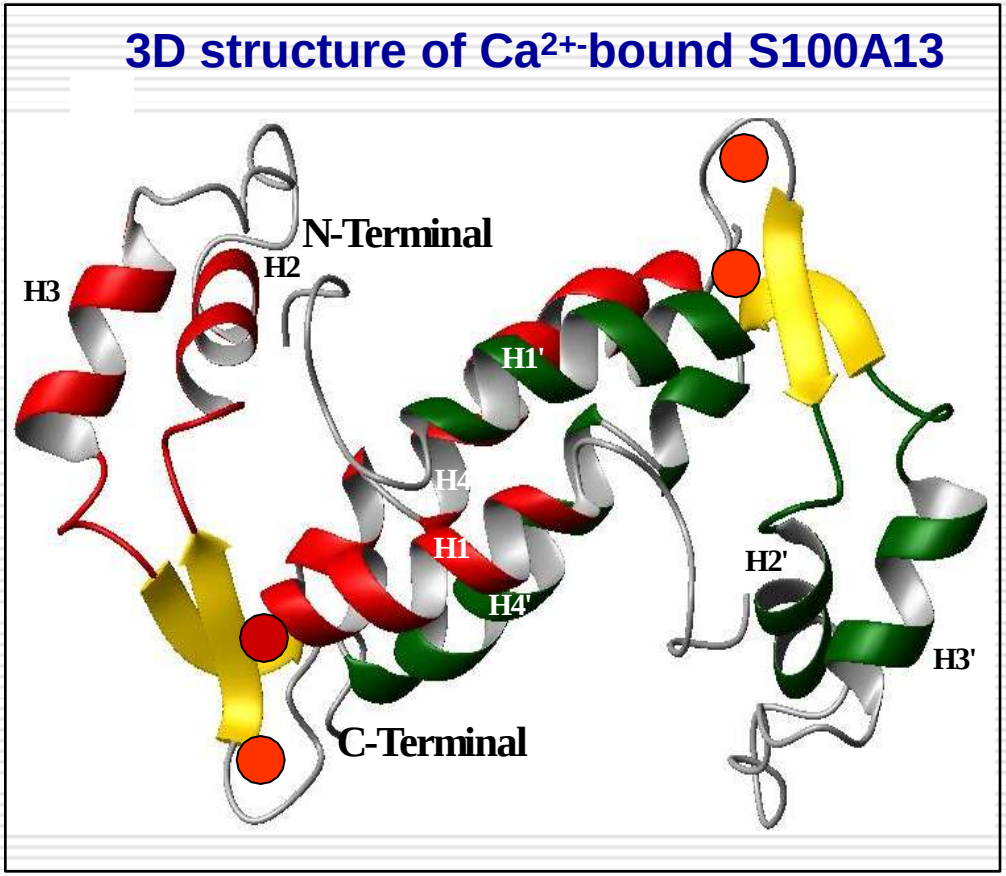
3D solution structure of S100A13

S100A13 is a ~25 kDa homodimer consisting of two calcium binding EF hands. Each monomer of S100A13 consists of four helices





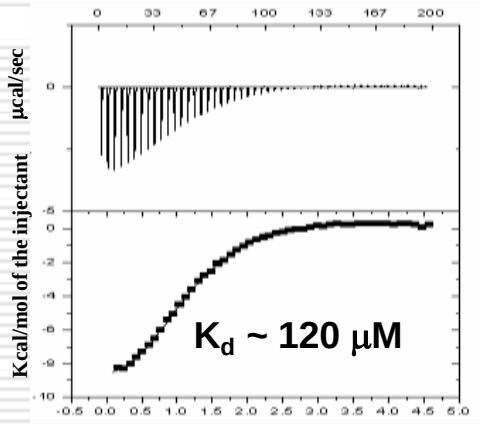
Titration of S100A13 with Ca^{2+}



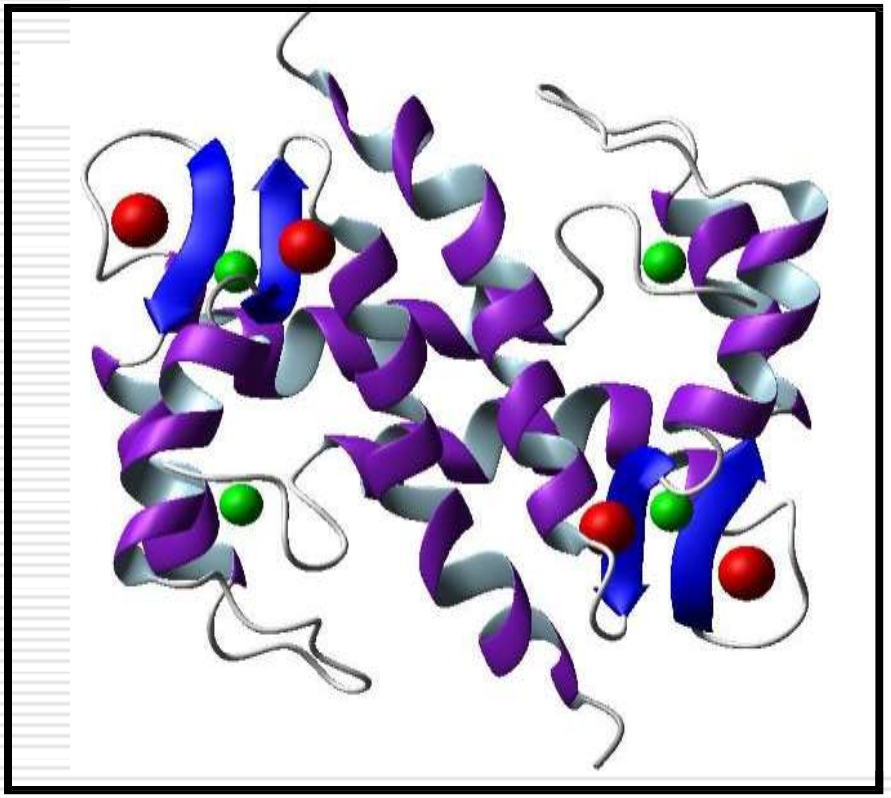
Kumar et al.,J. Biomol. NMR (**2005**) 32, 257-8; **Biophys. J (2006) 91, 1832-43**

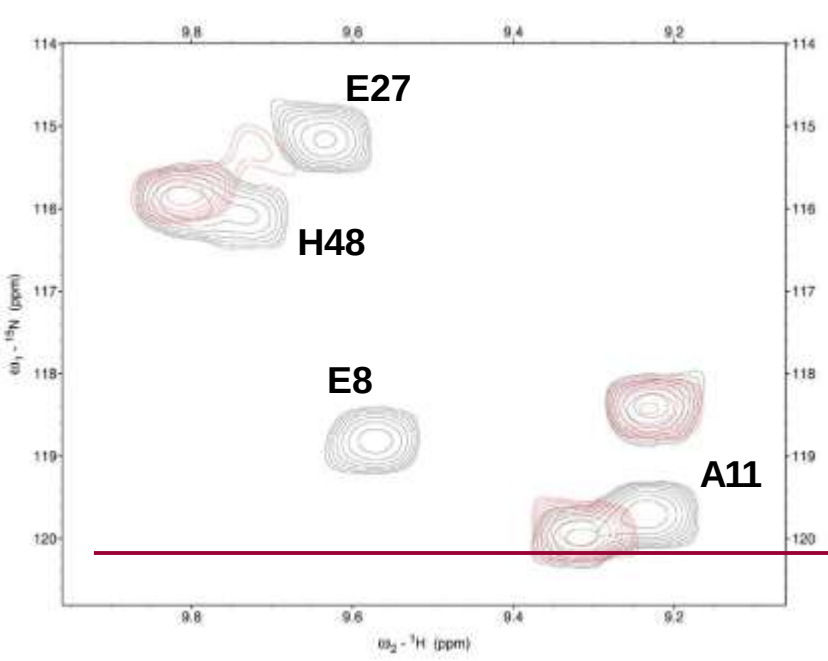
S100A13 binds to Cu2+

Cu $^{2+}$ -S10013 titration

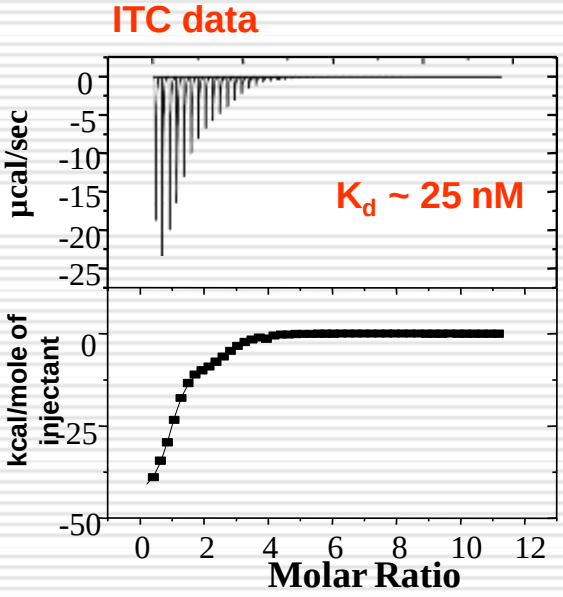


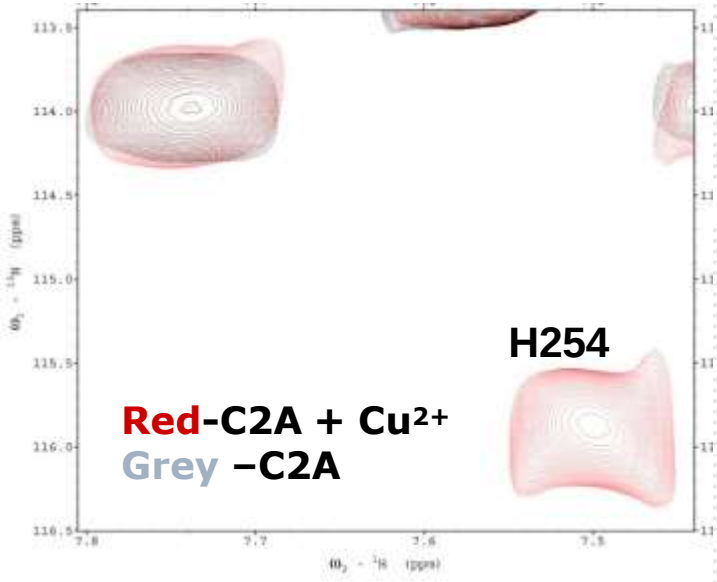
Cu $^{2+}$ binding sites are distinct from the Ca $^{2+}$ binding sites in S100A13.

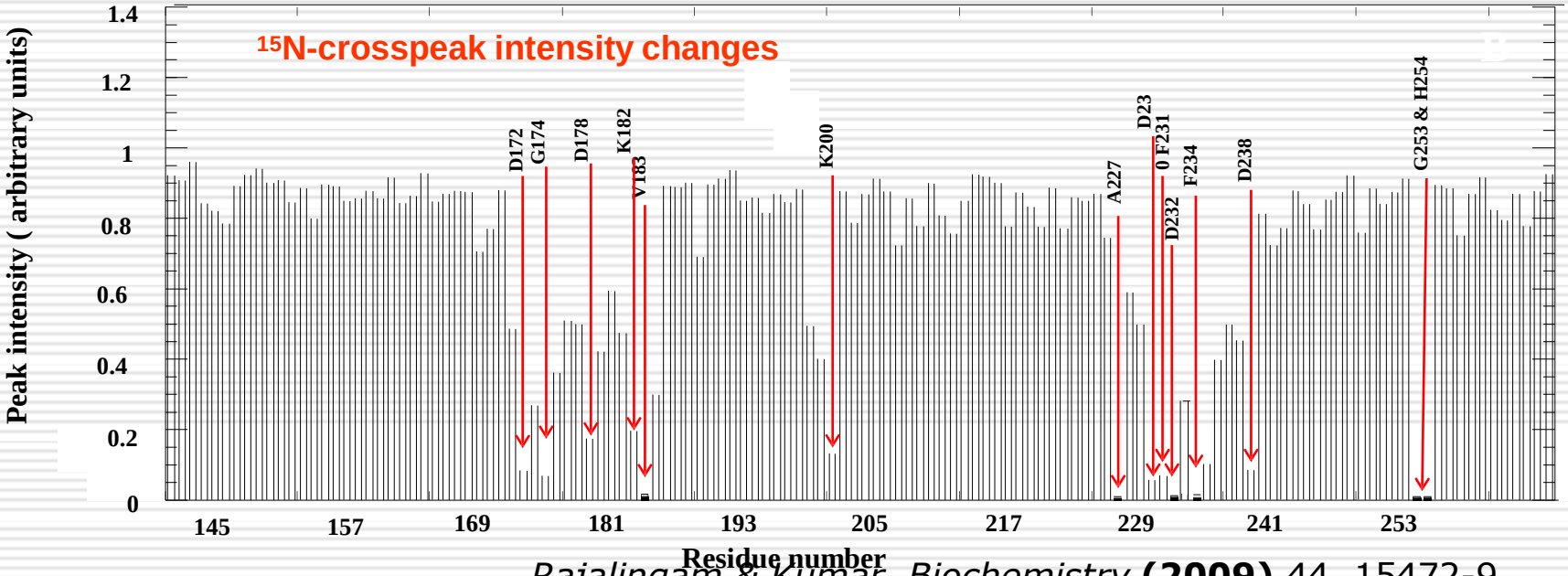




Sivaraja and Kumar, Biophys. J. (2006) 91(5):1832-43

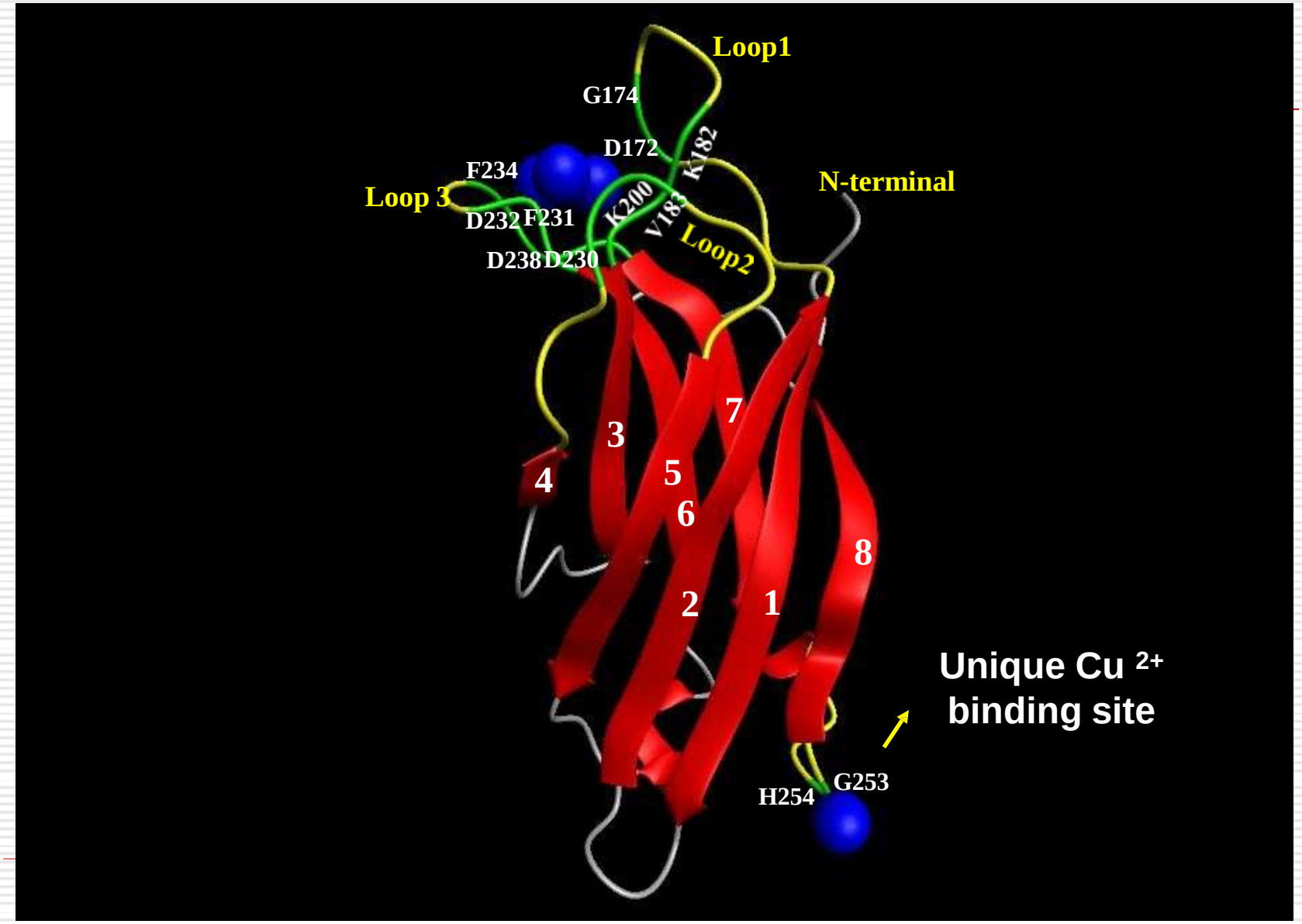






Rajalingam & Kumar, Biochemistry **(2009)** 44, 15472-9

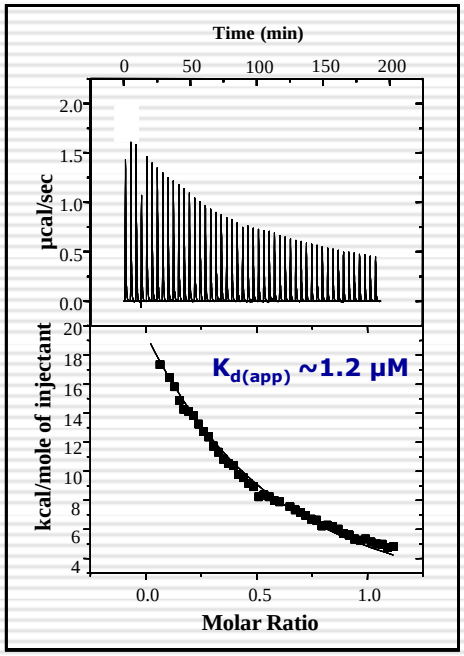
C2A domain binds to 4 Cu2+ ions

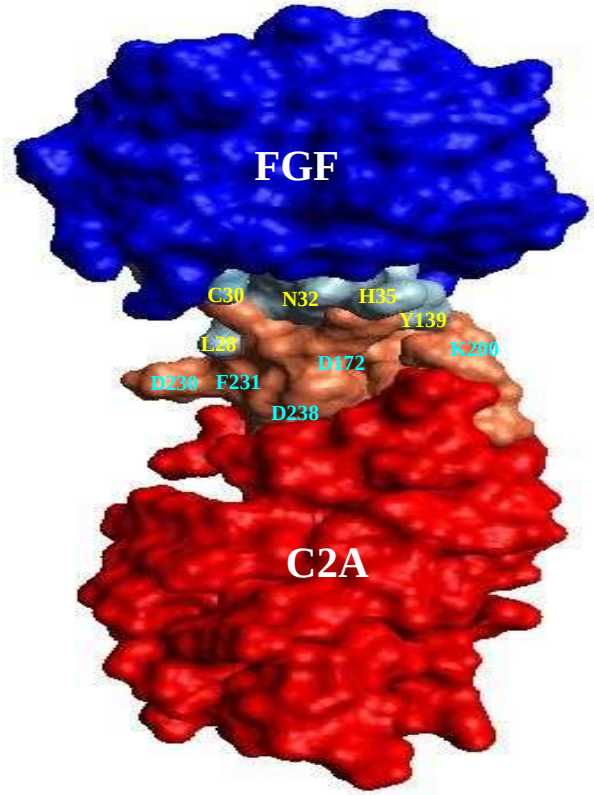


Rajalingam and Kumar Biochemistry **(2009)** 44, 15472-9

FGF-C2A binding interface

FGF-C2A Titration

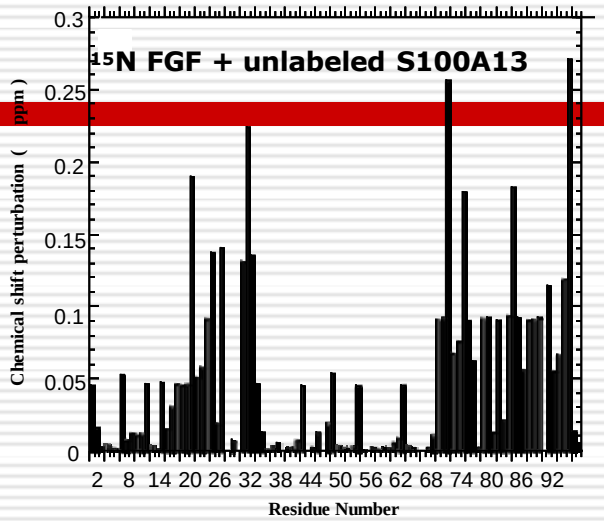


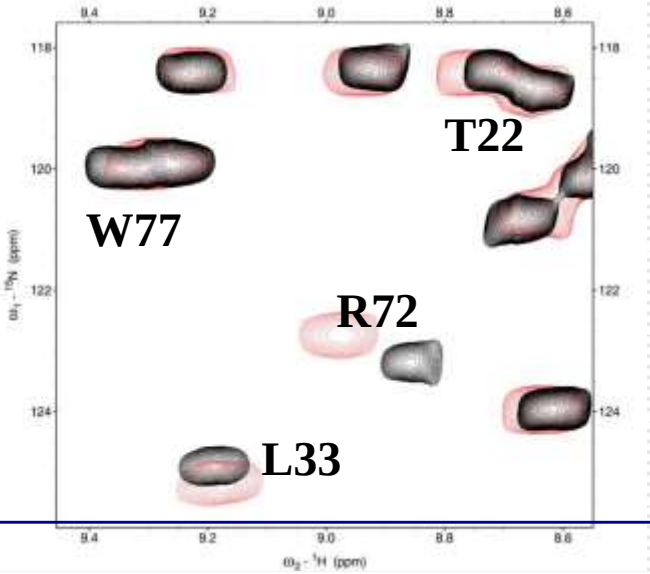


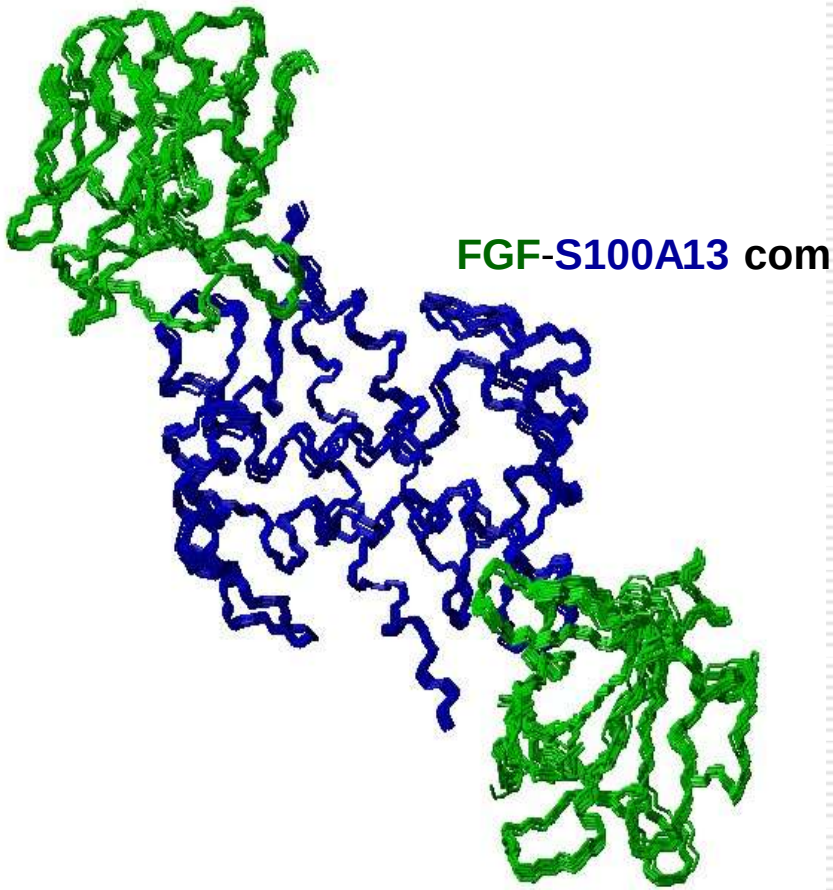
Rajalingam and Kumar, unpublished results

Structure of the S100A13-FGF binary complex

K_d ~ 100 μM estimated from ITC experiments







FGF-S100A13 complex

Kathir and Kumar (2009) J. Mol. Biol., 381, 49-60

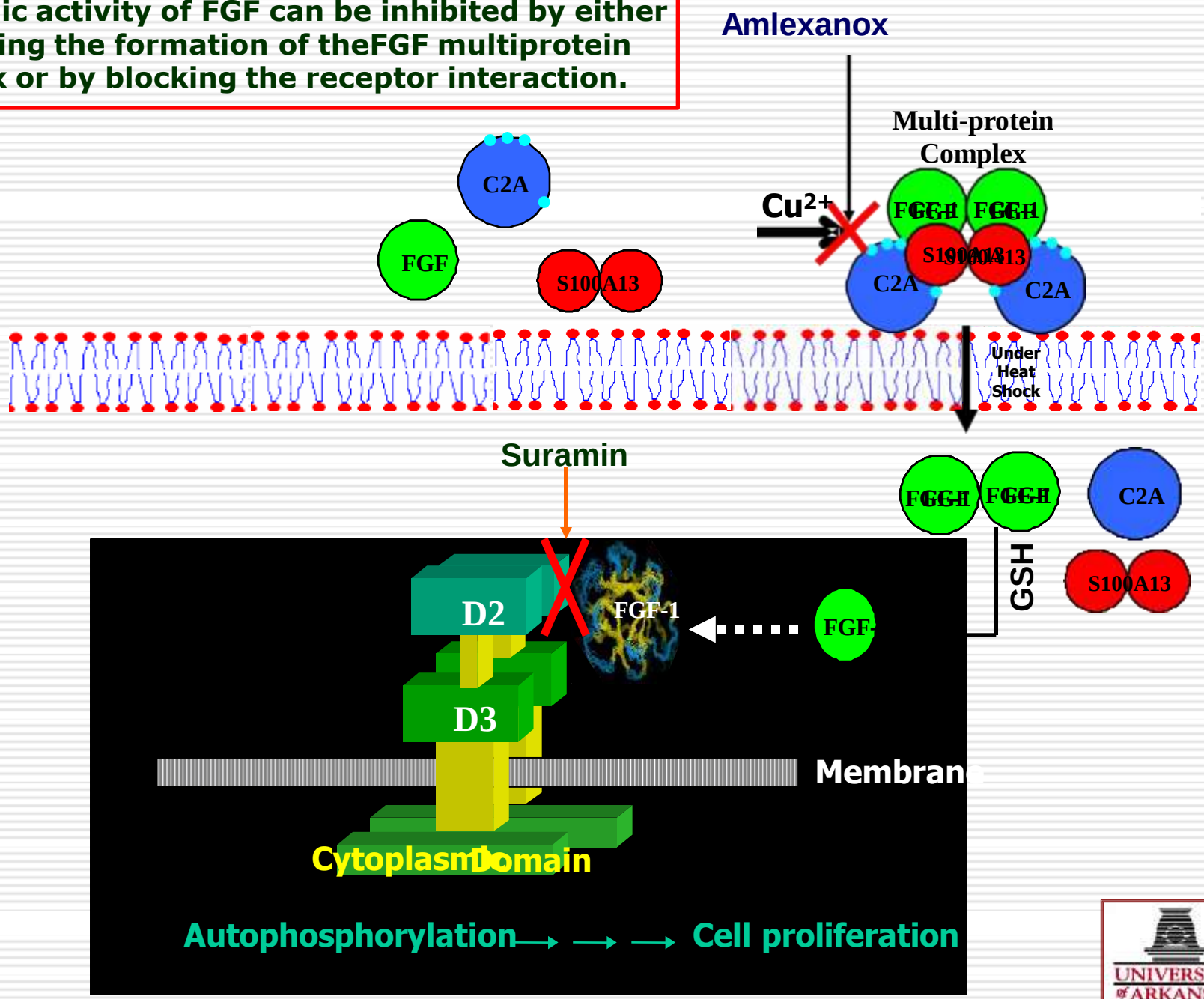
Black – ^{15}N-S100A13 Red – ^{15}N S100A13 + unlabeled FGF

Conclusions

- A high resolution 3D solution structure of S100A13 has been determined.
- The Cu^{2+}-binding sites in S100A13 and the C2A domain of Syt1 have been mapped.
- FGF binds to both S100A13 and the C2A domain of Syt1. The FGF-S10013 and FGF-C2A binding interfaces have been characterized.

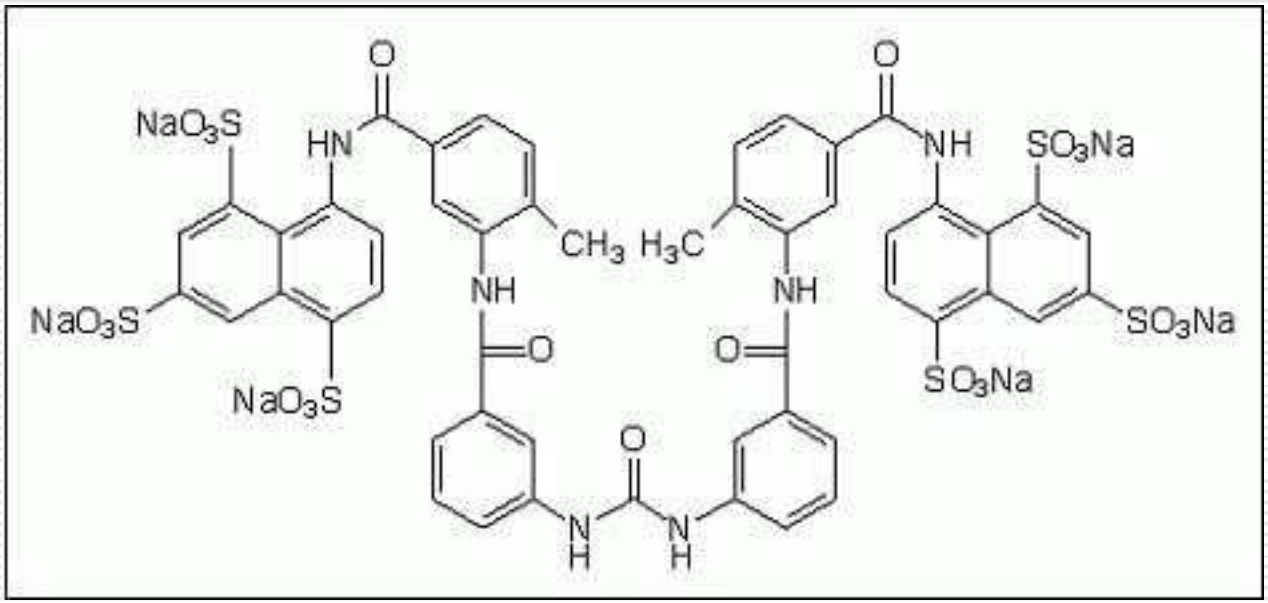
FGF - Inhibitor interactions

Mitogenic activity of FGF can be inhibited by either preventing the formation of theFGF multiprotein complex or by blocking the receptor interaction.



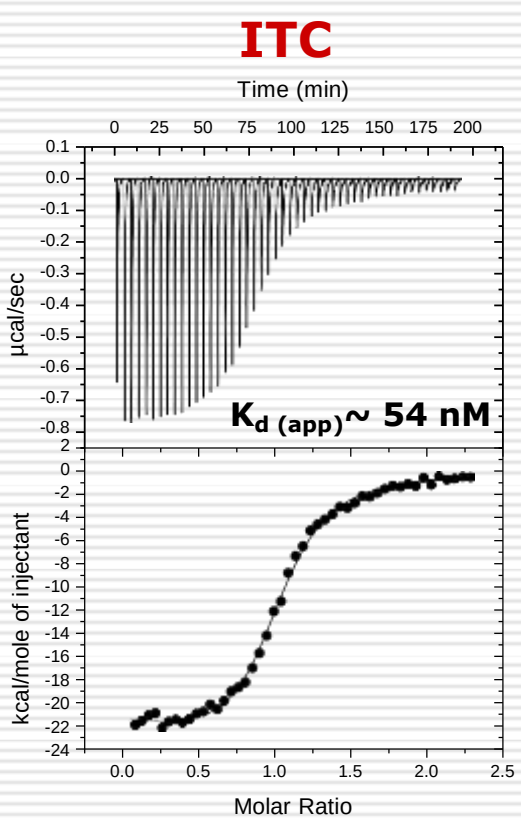
FGF-suramin interactions

Suramin is a well known mitogenic agent. Suramin has been shown to inhibit the mitogenic activity of FGF. However, the exact mode of action of suramin was previously not known

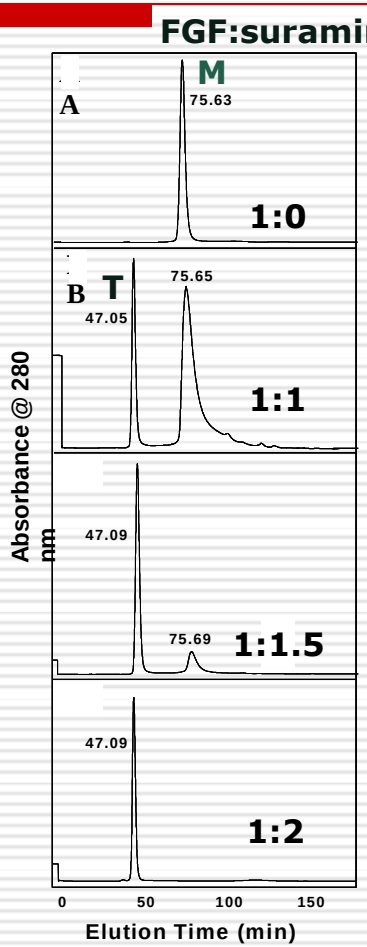


Structure of suramin

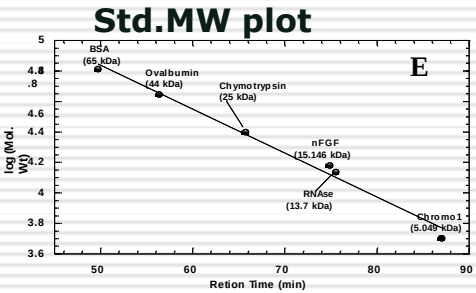
Suramin binds to FGF



Two molecules bind per molecule of FGF



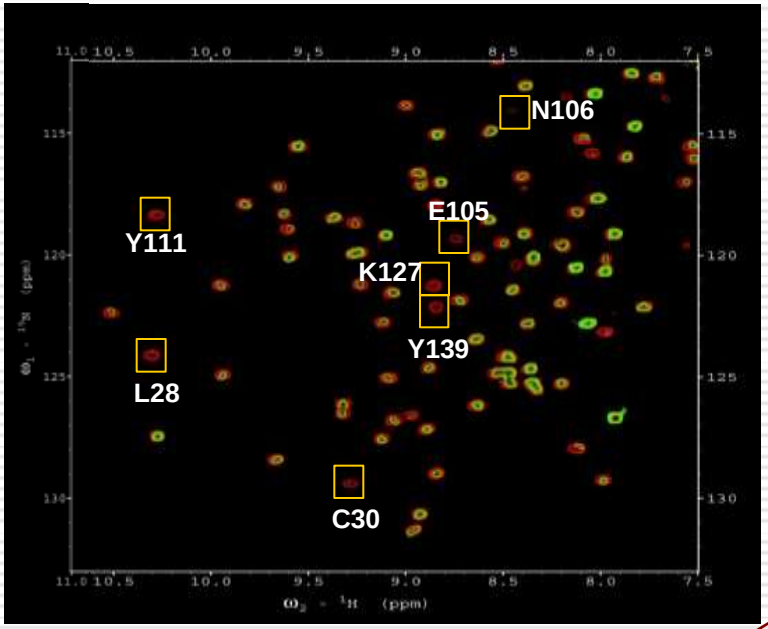
Size exclusion chromatography

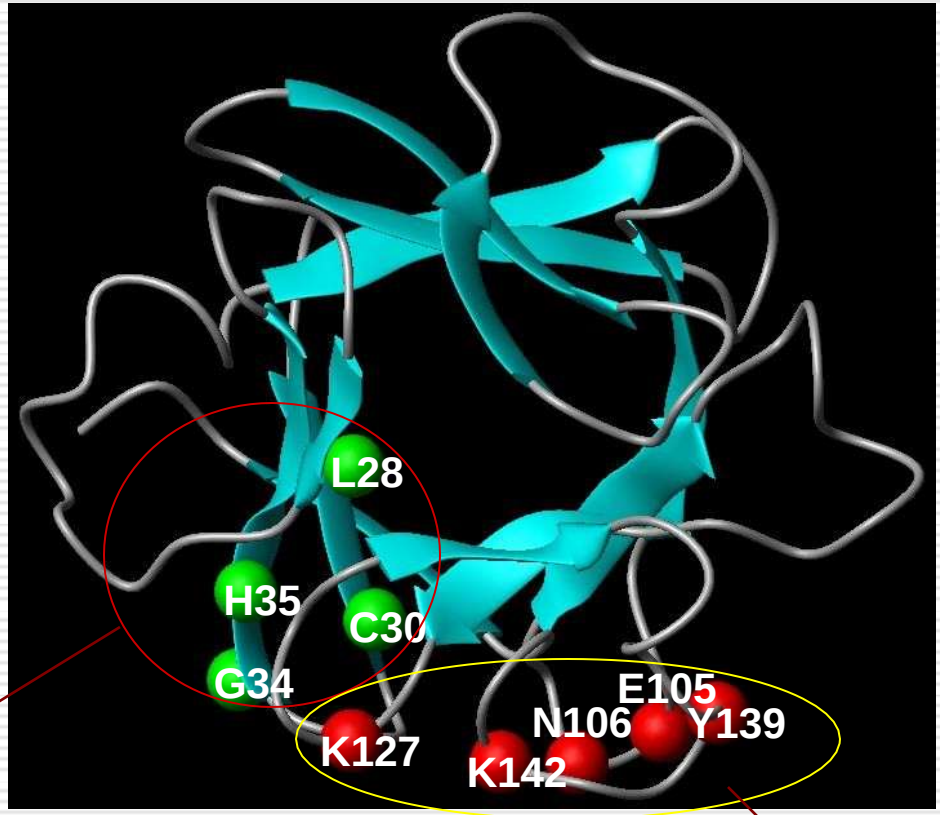


FGF oligomerizes to a tetramer upon binding to suramin

Kathir & Kumar, Biochemistry **(2006)** 45, 899-906

Suramin binding sites on FGF



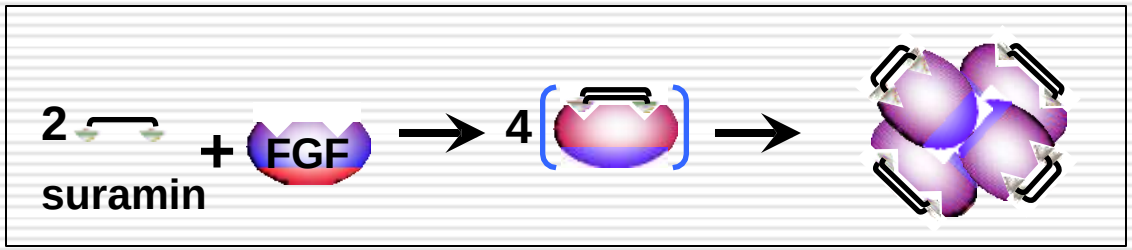


Heparin binding residues

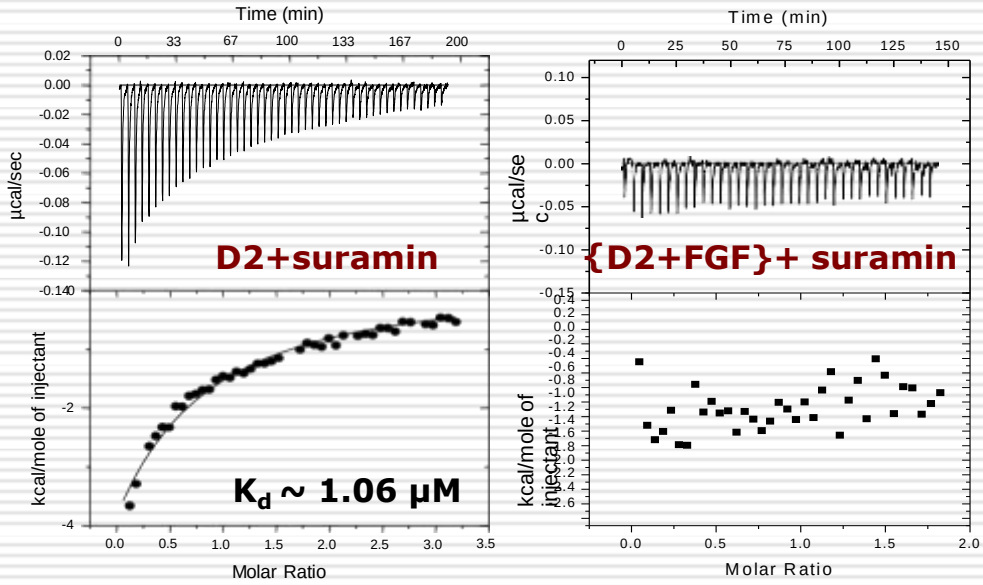
Receptor binding residues

Suramin binds to both heparin and receptor binding residues

Modes of action of suramin



Inhibition by oligomerization

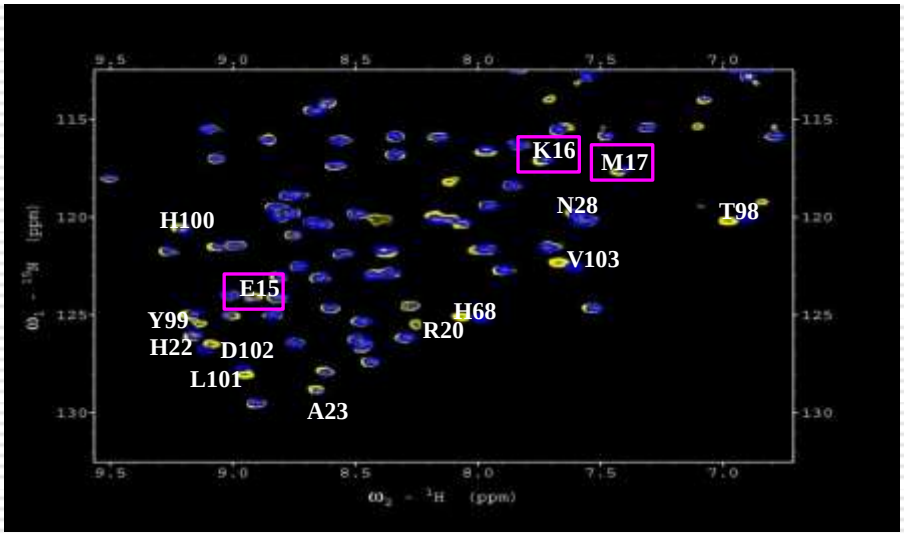


suramin also binds to the D2 domain of the receptor

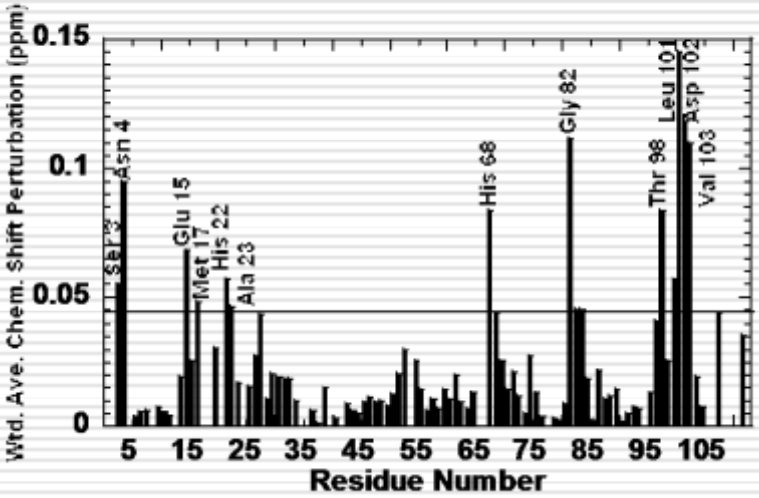
Kathir & Kumar, Biochemistry (2013) in press

Suramin inhibits the mitogenic activity of FGF by also binding at the FGF-receptor interface

Suramin binding sites on the D2 domain



Residues that bind to suramin are those that are located at the FGF-receptor interface



Kathir & Kumar, unpublished results

Conclusions on FGF-suramin interaction

- **Suramin binds to both FGF and the D2 domain of the receptor**
- **Suramin induces oligomerization (tetramer) of FGF.**
- **Suramin binds at the FGF-D2 binding interface.**
- **Suramin inhibits the mitogenic activity of FGF by inducing oligomerization of FGF and also by blocking FGF-receptor interaction**

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