The FoXS Suite of SAXS Modeling Tools

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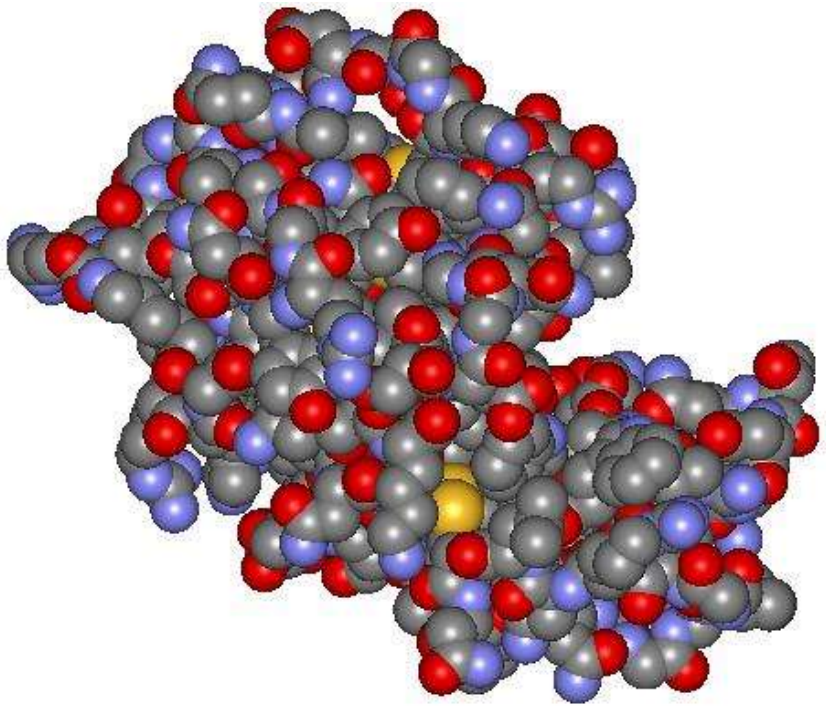
All applications developed by Dina Schneidman

Caveats to Structural Modeling

- All models are wrong. Some models are useful.

  - George Box

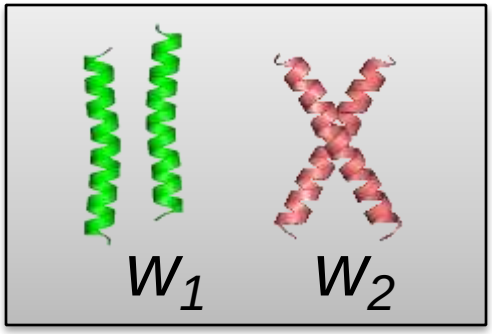
- Crystal Structures are models
  - Crystal packing artifacts
  - Lack of binding partners
  - No dynamic information

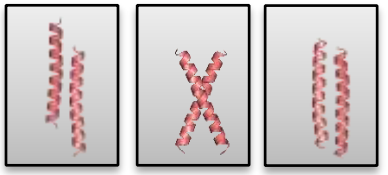


- Models derived from SAXS data may contain heterogeneity

Heterogeneous Samples Require Multi-state Models

Heterogeneous sample	compositional or conformational heterogeneity in the sample used to generate the data
Multi-state model	a model that specifies two or more co-existing structural states and values for any other parameter
Ensemble of models	an ensemble of (good scoring) single or multi-state models





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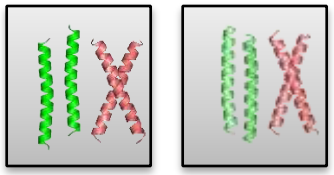

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The FoXS Suite of SAXS Analysis Tools

	Web server + examples	Code + executables	UCSF Chimera
FoXS	Fast profile calculation	salilab.org/foxs	salilab.org/imp 	
MultiFoXS	Multi-state modeling from single structure	salilab.org/multifoxs	salilab.org/imp	
FoXS Dock	protein-protein docking	salilab.org/foxsdock	salilab.org/imp	
FoXS	comparative modeling, conformational sampling	salilab.org/allosmod-foxs	salilab.org/modeller	

Tutorial Workflows

- Single Structure Fits to SAXS Profile
  - For stable, homogenous systems

- Multiple Structure Fits to SAXS Profile + MES
  - Multiple crystal structures in different conformations

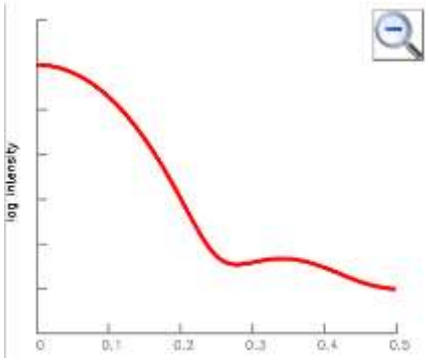
- SAXS with Flexible Fitting
  - If your single structure fit does not work

- Predicting PPI interfaces with SAXS
  - Crystal structures of two protein components and SAXS profiles of their complex

- Automated Comparative Model fitting to SAXS profiles
  - If you have no crystal structure

The Basics of FoXS

- Calculate theoretical scattering profile from atomic coordinates



$R_g = 13.97$

Lysozyme: 6lyz

- Debye summation

$$I_m(q) = \sum_{j=1}^{N_A} \sum_{j=1}^{N_A} f_i(q) f_j(q) \frac{\sin(qd_{ij})}{qd_{ij}}$$

f = atomic form factors
q = Momentum transfer
d = distance

The Basics of FoXS

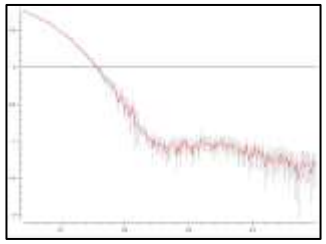
- Chi Function fit to an experimental profile

$$\chi = \sqrt{\frac{1}{M}\sum_{i=1}^{M}\left(\frac{I_{exp}(q_i) - cI(q_i)}{\sigma(q_i)}\right)^2}$$

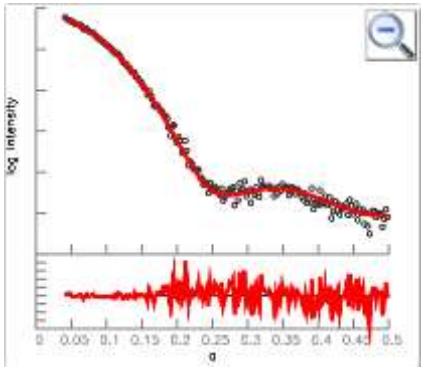
σ = Experimental error
c = Scaling parameter
M = Model data point

- **Fit to a single structure**

Lysozyme: 6lyz



FoXS



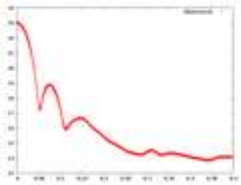
$R_g = 13.97$

$\chi = 0.45$

https://modbase.compbio.ucsf.edu/foxs/index.html

FoXS

Fast SAXS Profile Computation with Debye Formula



• About FOXS • Web Server • Help • FAQ • Download • Sali Lab • IMP • Links

Type PDB code of input molecule or upload files in PDB format (zip file with several PDBs can be uploaded):

Input molecule: (PDB:chainId e.g. 6lyz:A) **or** upload file: Choose File No file chosen

Experimental profile: Choose File No file chosen (optional) sample input

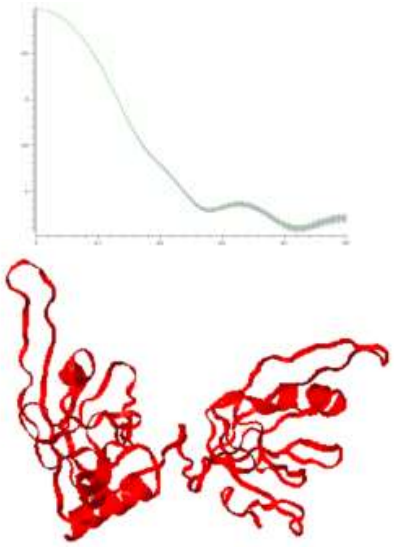
e-mail address: (optional, the results are sent to this address)

Submit Form Clear

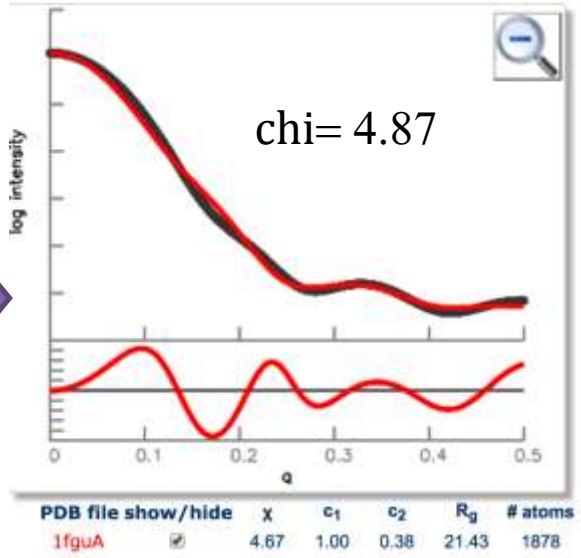
Advanced Options

Maximal q value	0.5	
Profile size	500	# of points in the computed profile
Hydration layer	☑	use hydration layer to improve fitting
Excluded volume adjustment	☑	adjust the protein excluded volume to improve fitting
Implicit hydrogens	☑	implicitly consider hydrogen atoms
Residue level computation	☐	perform coarse grained profile computation for Ca atoms only
Background adjustment	☐	adjust the background of the experimental profile
Offset	☐	use offset in profile fitting
MODEL reading	All MODELs into a single structure	determine how to read PDB files with MODEL records

SAXS fits to crystal structures are generally poor



1FGU:A
Repl. Protein A



- SAXS observes entire conformational ensemble
  - Total protein signal is a linear combination of the signal from all conformations

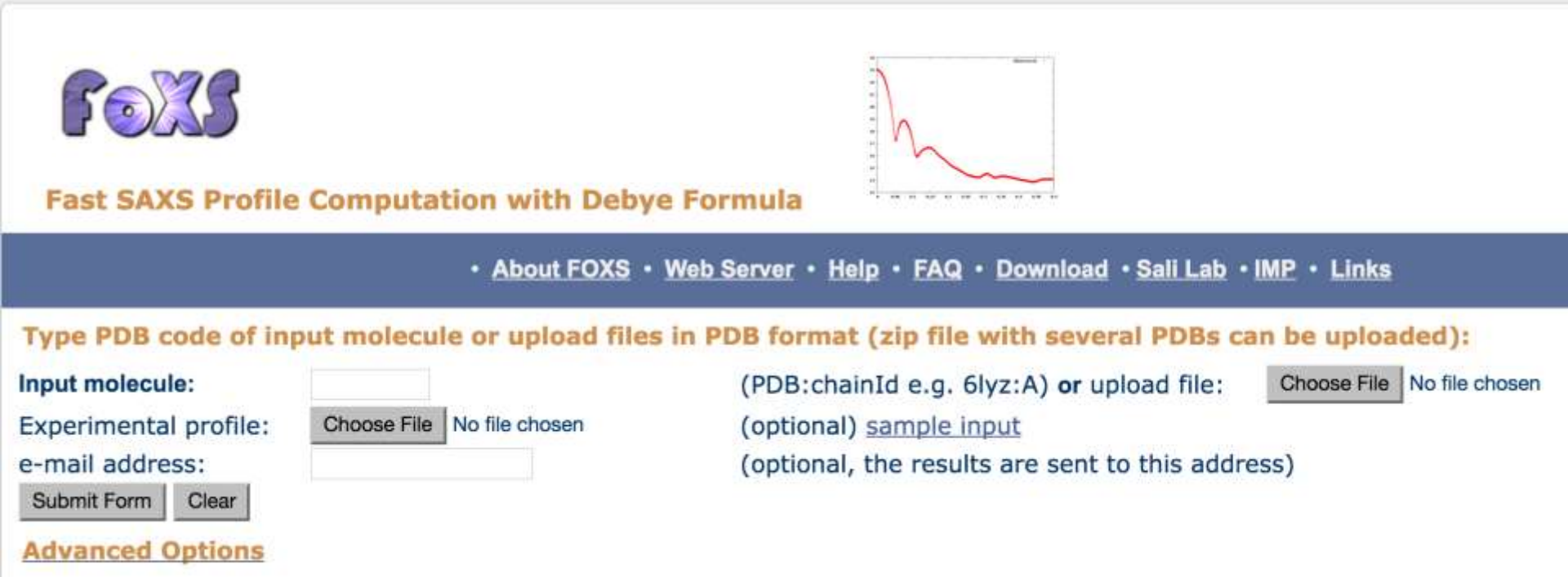
$$I_{\text{prot}}(q) = \sum_{c}^{N_c} a_c I_c(q)$$

Fitting Multiple Crystal Structures to SAXS

- Individual and Ensemble Fits

FoXS + MES

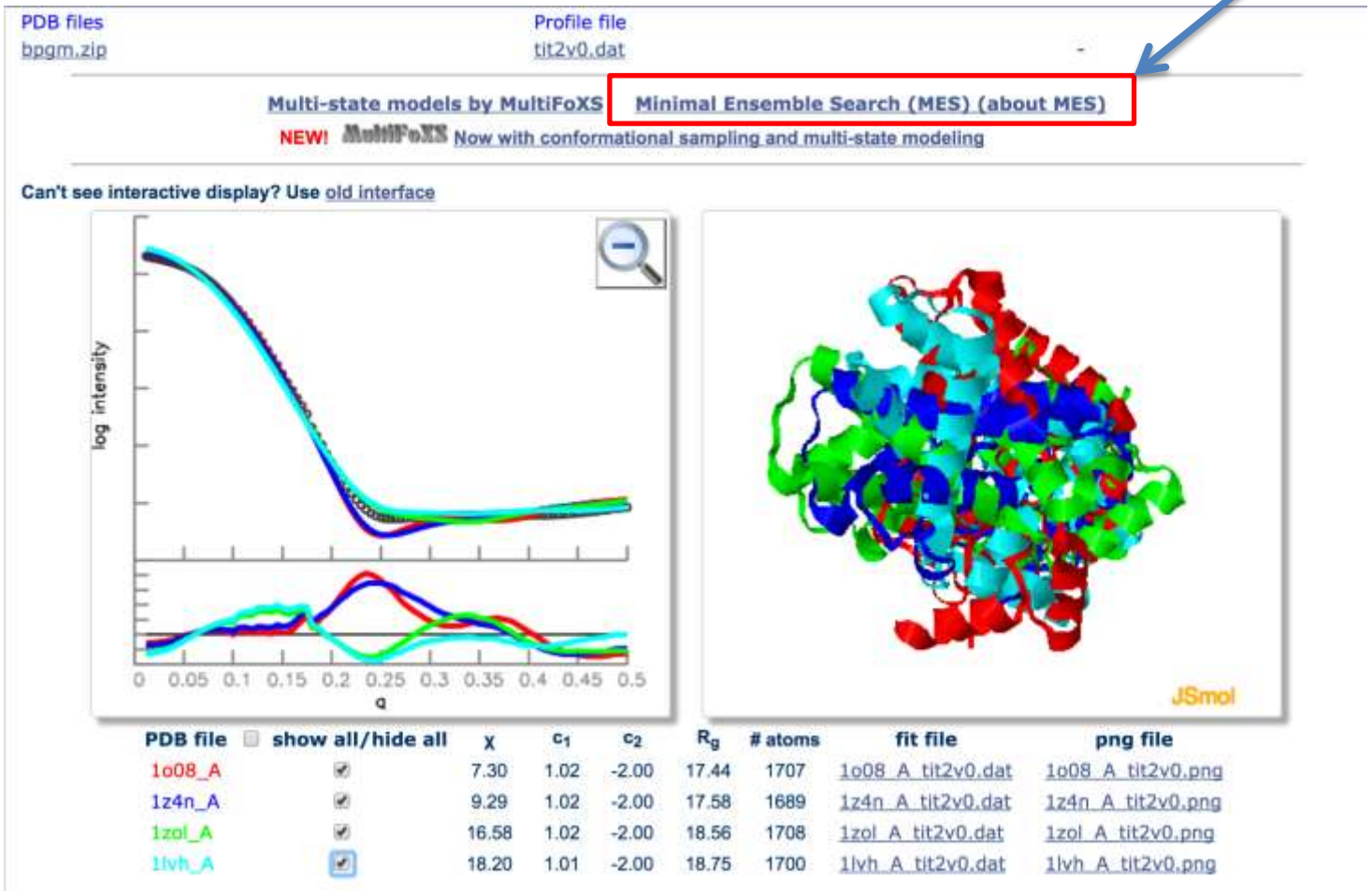
- Input:
  - .zip file of multiple PDB structures
  - SAXS curve



Fitting Multiple Crystal Structures to SAXS

- Individual and Ensemble Fits
  - Four structures fit to xtal

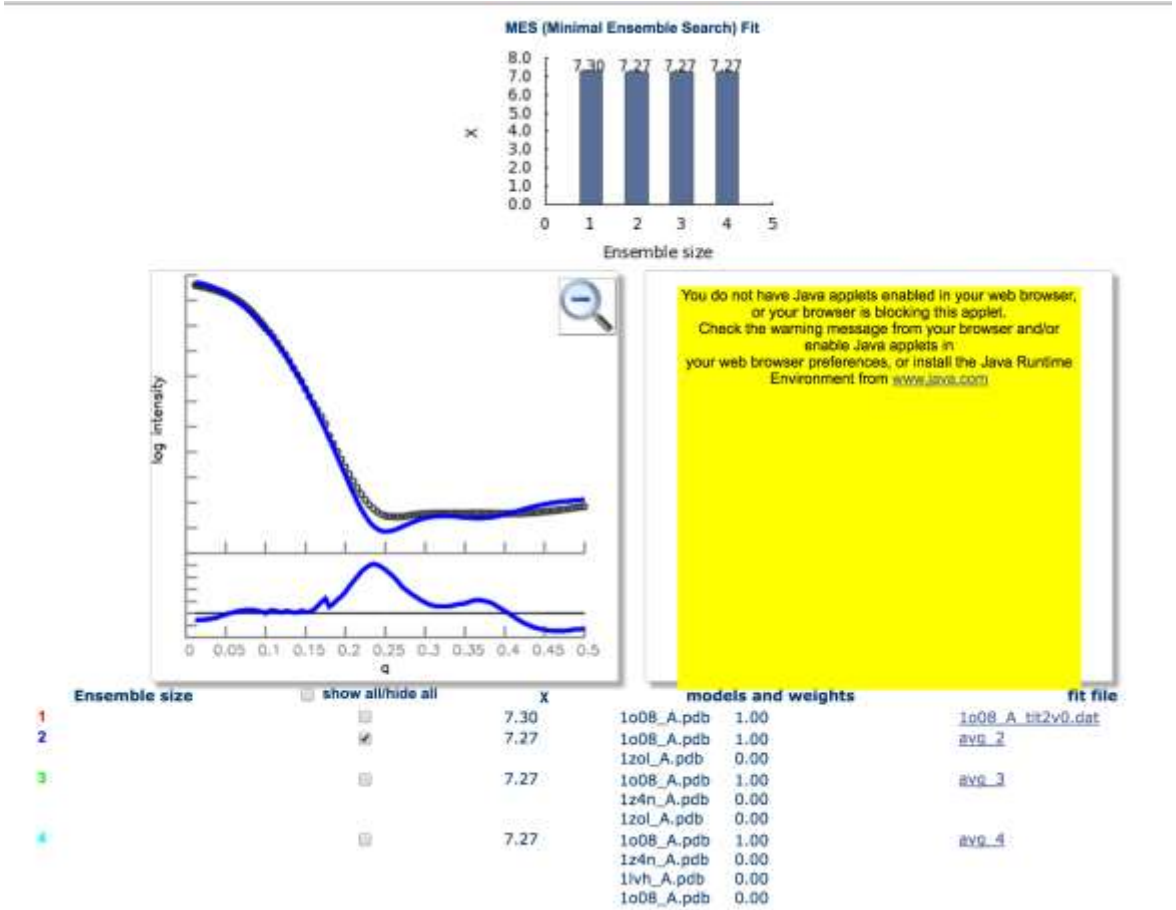
FoXS + MES



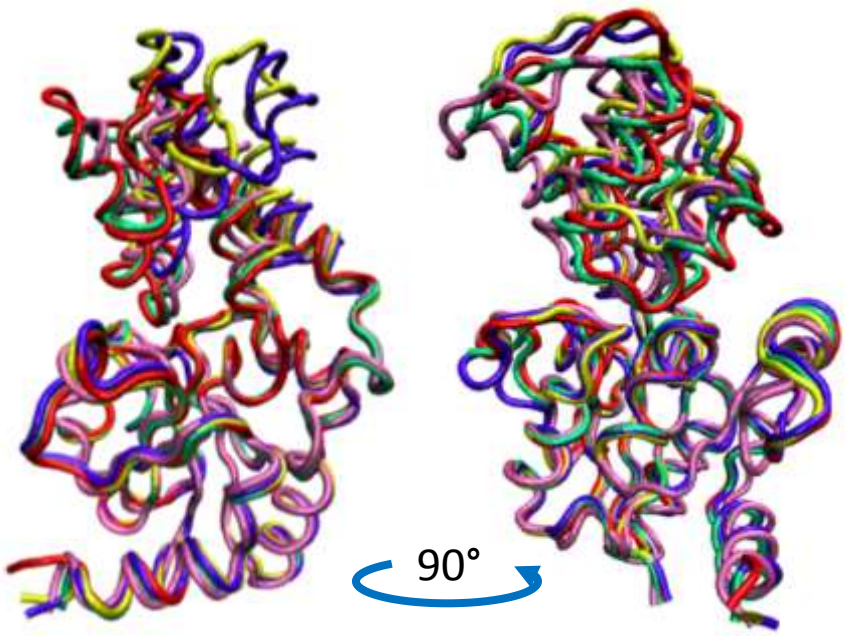
Fitting Multiple Crystal Structures to SAXS

- Individual and Ensemble Fits
  - Four structures fit to xtal
  - This fit is bad due to high concentration (20mg/mL)

FoXS + MES



Partially Bound Scattering Comparison to Xtal Data



Crystal Structures

PDB	Ligand State
1ZOL	None
1LVH	Phospho-Asp8 in Xfer
1Z4N	Gal1P in Distal Site
1O03	Intermediate/Transition State
4GIB	Phosphate in Distal and Xfer

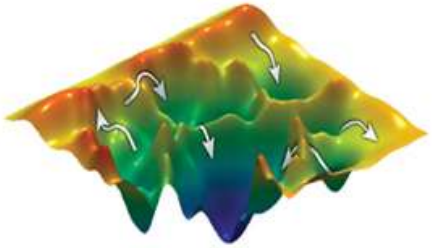
Computed SAXS profiles generated with FoXS (Schneidman-Duhovny, Nucleic Acid Science, 2010)

Ensemble fits performed using Minimum Ensemble Search (Pelikan, General Physiology and Biophysics, 2009)

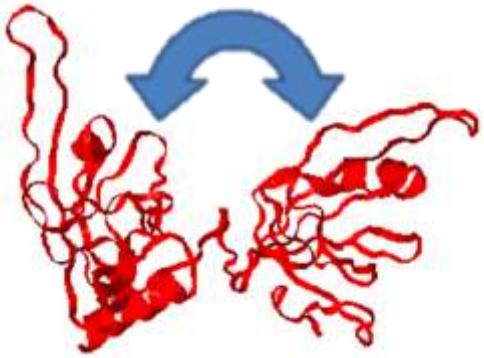
Ligand	PDB	χ^2
None	100% 1ZOL	2.14
WO_4^{2-}	61% 4GIB, 39% 1LVH	0.99
G6P	53% 1Z4N, 47% 1ZOL	0.98
Gal1P	62% 1Z4N, 38% 1ZOL	1.01
G6P+VO_3^-	54% 1003, 46% 1Z4N	1.01

Generating Conformational Ensembles

- Long MD Simulations
  - Can search complete landscape
  - Slow
  - Thousands to millions of structures to analyze



- Coarse-grained simulations
  - Faster
  - Lose atomic precision



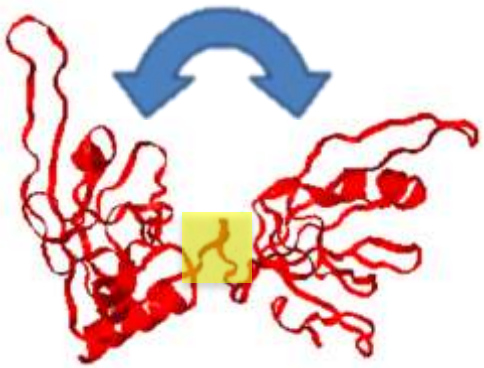
- Reduced sampling methods
  - Generally searches part of a landscape
  - Much faster
  - Good when you are sure motion is contained in specific DOF

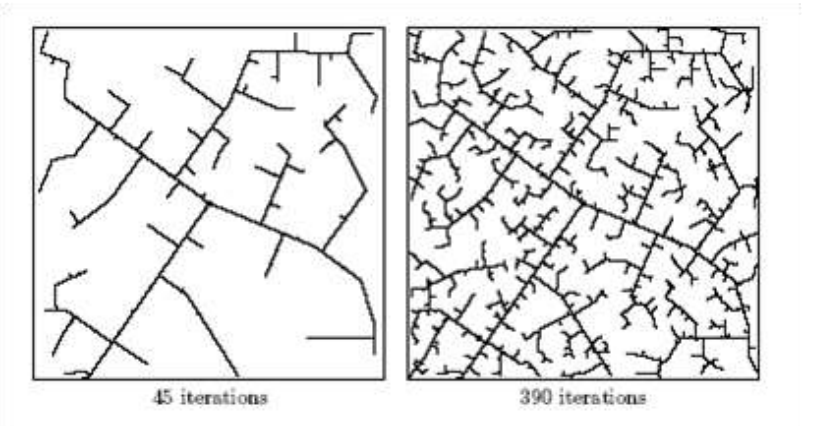
SAXS with Flexible Fitting

- Utilize Rapidly-exploring Random Trees to quickly search conformational space of linked domains

MultiFoXS

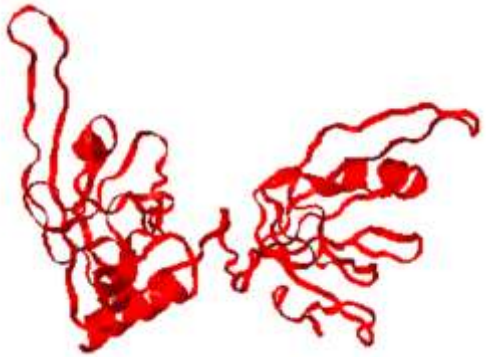
- Input:
  - Single PDB Structure
  - List of Flexible Residues
  - SAXS Profile





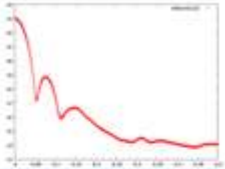
http://modbase.compbio.ucsf.edu/multifoxs/

- Input:
  - Single PDB Structure
  - List of Flexible Residues
  - SAXS Profile



MultiFoXS

Multi-state modeling with SAXS profiles



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Type PDB code for protein or upload file in PDB format sample input files

Input protein (PDB:chainId e.g. 2kai:AB) or upload file: Choose File No file chosen

Flexible residues Choose File No file chosen

SAXS profile Choose File No file chosen

e-mail address (the results are sent to this address, optional)

Submit Clear

Advanced Parameters

Job name

Connect rigid bodies Choose File No file chosen

Number of conformations 100 Use 100 to test your setup, 10,000 for final calculation

Submit Clear

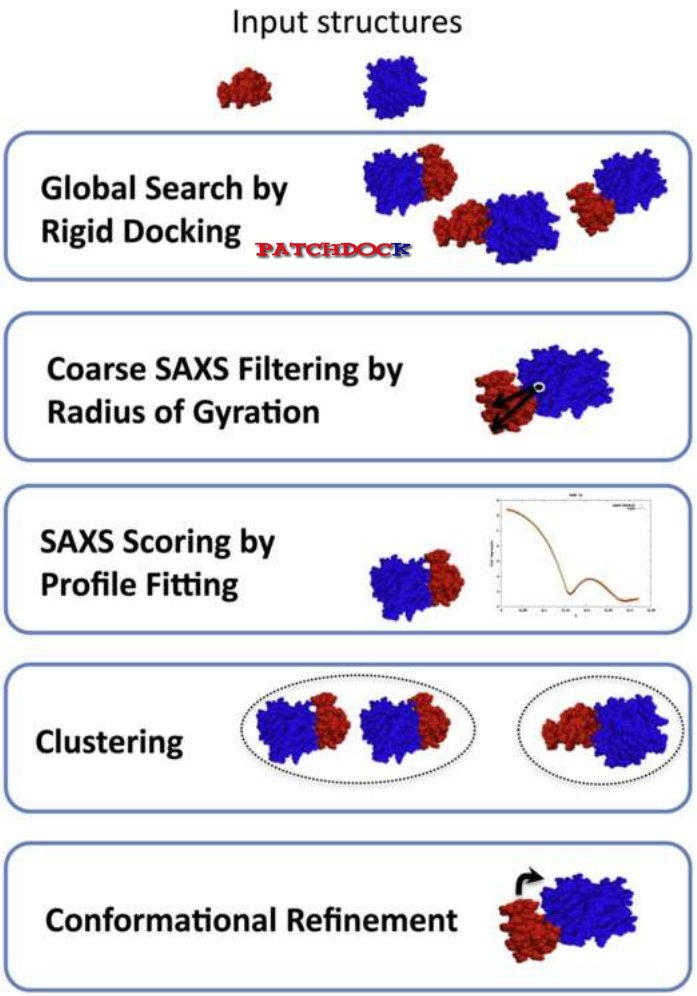
Contact: dina@salilab.org

link to output

Finding PPI Docking Interfaces with SAXS

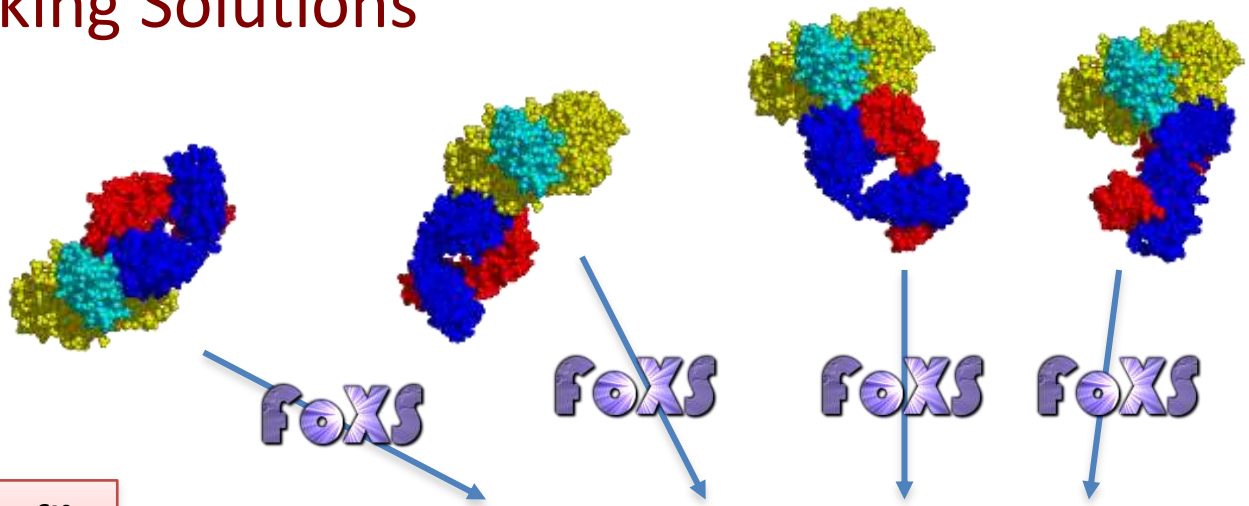
FoXS Dock = FoXS + PATCHDOCK

- Input:
  - PDB file of both proteins
  - SAXS envelope of complex

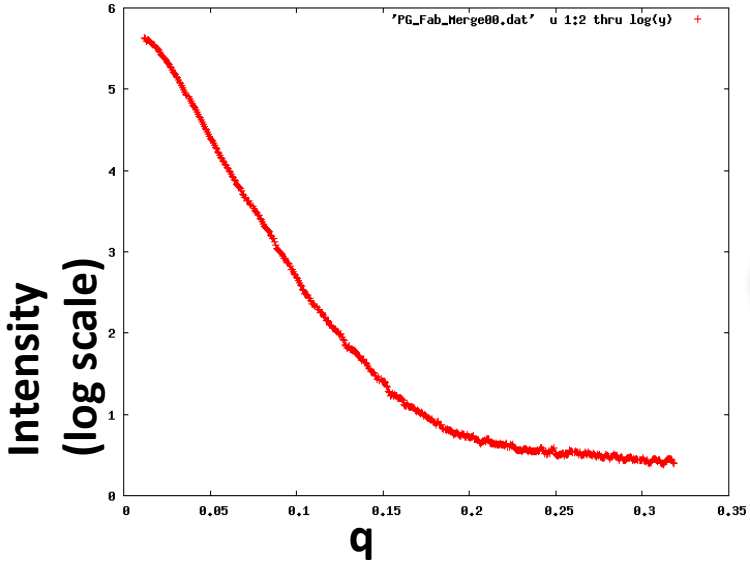


Finding PPI Docking Interfaces with SAXS

- Proposed Docking Solutions

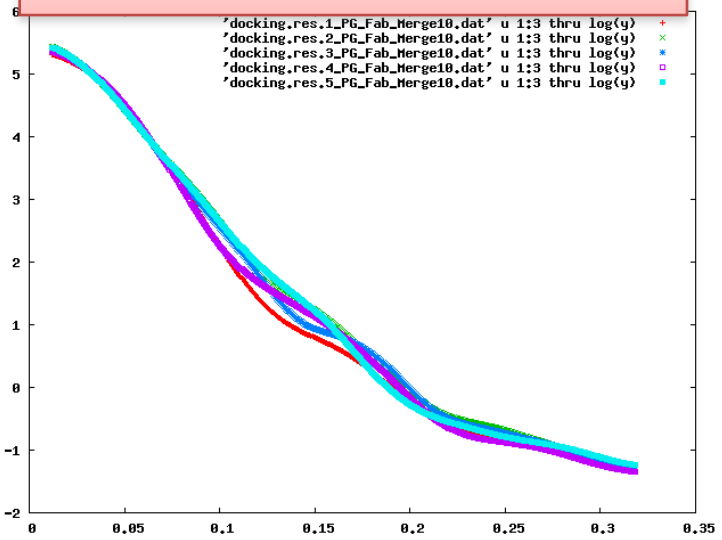


Experimental SAXS profile

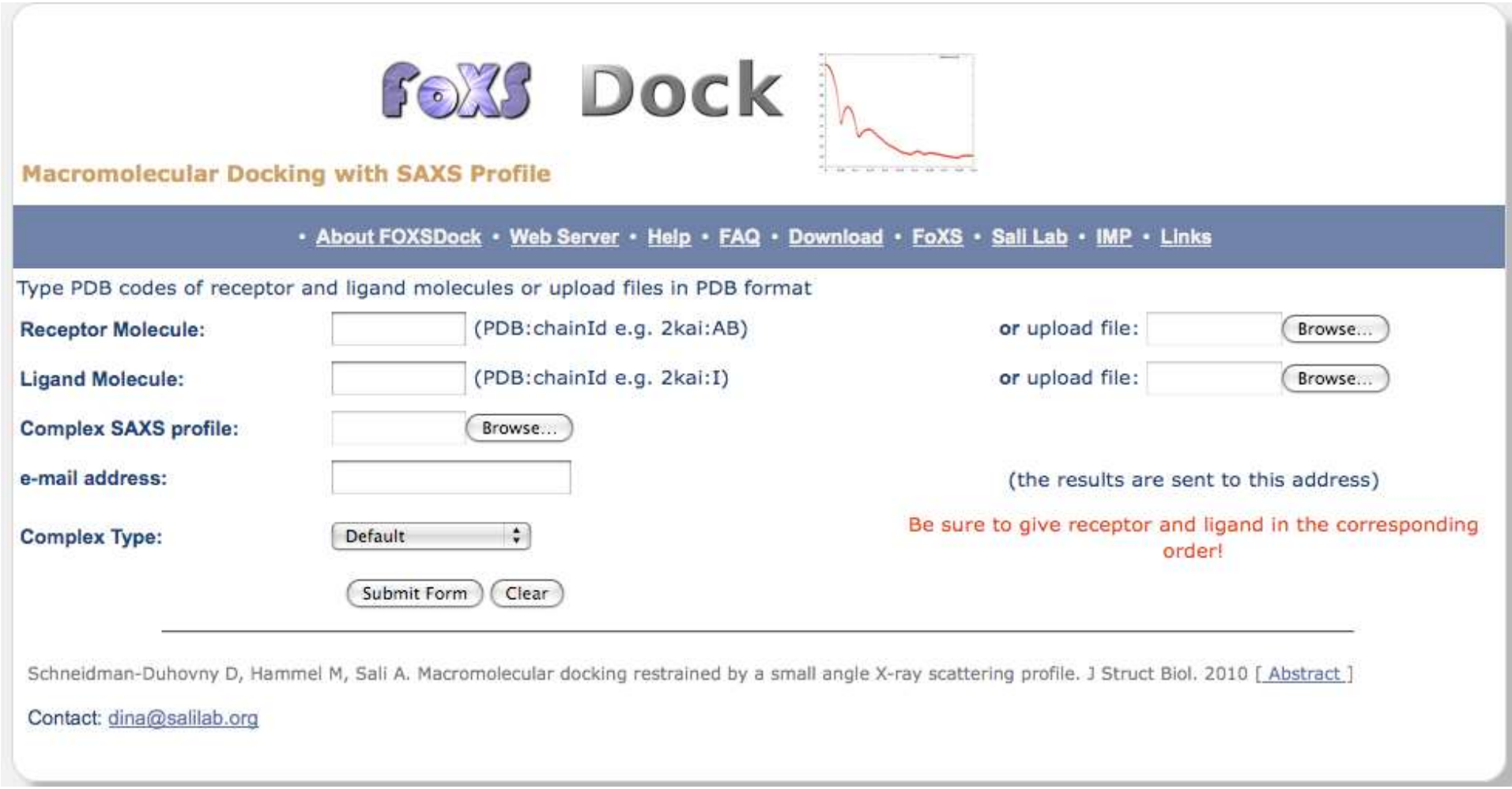


compare

Computed SAXS profiles



https://modbase.compbio.ucsf.edu/foxsdock/

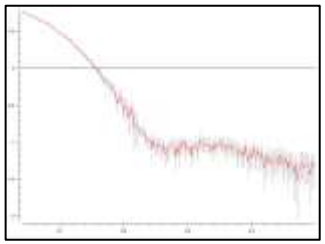


“Receptor” is static molecule
“Ligand” is moving molecule

When you don't have a crystal structure…

- Use AllosMOD to create ensembles of homology models

- Input:
  - SAXS Profile or protein
  - Sequence of protein
    - The construct in your SAXS experiment
  - Template structure(s)
    - pblast of the PDB
    - MODELLER
    - PHYRE2



Automated Comparative Modeling and SAXS Fitting

AllosMod-FoXS: Structure Generation and SAXS Profile Calculations

AllosMod-FoXS combines the AllosMod and FoXS web servers. Our combined server allows various sampling algorithms from AllosMod to generate structures that are directly inputed into FoXS for small angle X-ray scattering profile calculations. The server supports modeling of protein, DNA, RNA, and glycosylation. For help, click here.

Job name

Email (Optional)

PDB code or upload PDB file Choose File No file chosen

Add more structures

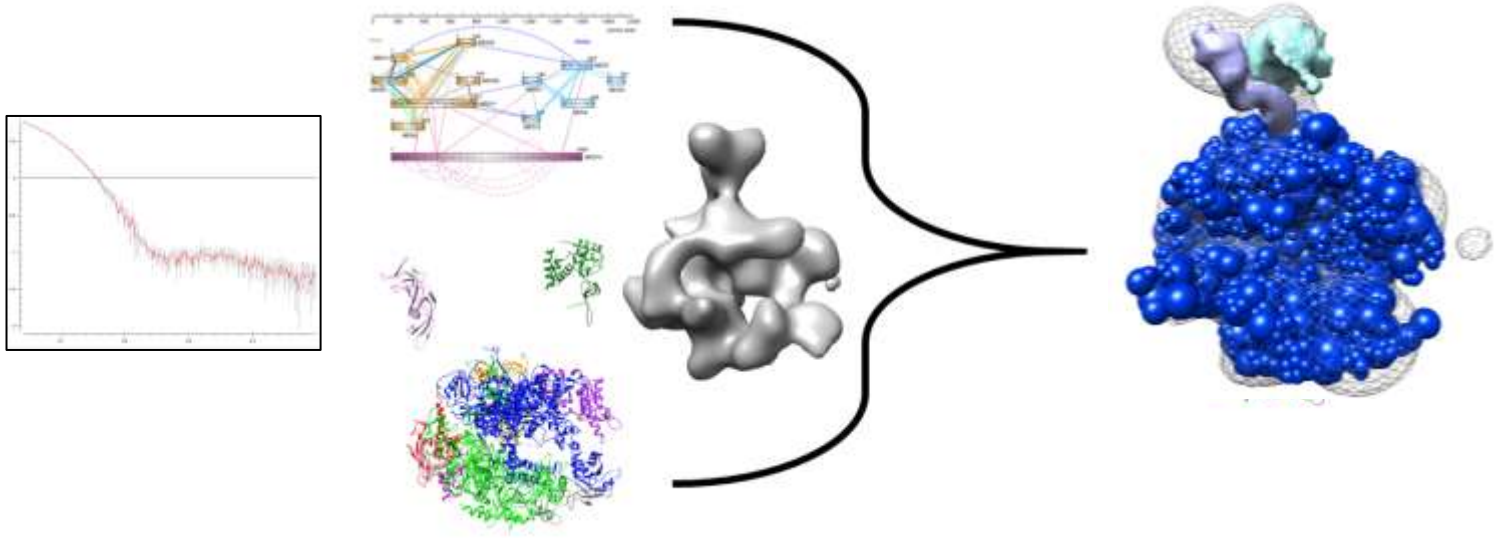
Sequence to be used in simulation (specify protein and DNA/RNA, input sugar in adv. opt., see help page)

Submit

If you have more than just SAXS data….

- Integrative Modeling Platform (IMP) www.integrativemodeling.org
  - Model building with SAXS in conjunction with other experimental and physics-based restraints
  - Requires python scripting

  - Contact me/salilab if you have any questions



Thanks!

Dina Schneidman - Tel Aviv University

UCSF

SJ Kim

Andrej Sali

NSLS(-II!)

Lin Yang

Marc Allaire (now ALS)

SSRL

Tsutomu Matsui

Thomas Weiss

Hiro Tsuruta