

A practical introduction to Small Angle X-ray Scattering (SAXS)

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January, 2010

SAXS observables and interpretables

- **Warning: there are few standardized metrics for validating SAXS results**
 - Remain diligent toward possibility of systematic / internal errors
 - Seek agreement in calculated parameters from variety of methods
 - E.g., R_G from both Guinier and $P(r)$
 - E.g., volume from MW from $I(0)$, from Porod and from *ab initio* 3D reconstruction
- **R_G – Radius of gyration (not the radius of particle)**
 - For estimating size and shape
 - Should not vary with sample concentration
 - Obtained from Guinier plot: $\log(I)$ vs. q^2
 - Using small q values such that $qR_G \leq 1.3$ (globular) or ≤ 0.8 (elongated)
 - Obtained from $P(r)$ as well
 - More accurate since entire scattering profile is used
 - $R_G(\text{Globular}) = \sqrt{3/5} \cdot \{MW(\text{Da}) / (0.44 \text{ Da}/\text{\AA}^3) \cdot (3/4\pi)\}^{1/3}$
- **$I(0)$ – Intensity at zero scattered momentum**
 - For estimating MW and therefore concentration-dependent interactions
 - $I(0)/c$ scales with MW but may deviate (undesirable!)
 - $I(0) = Nm^2(1-\rho_0\psi)^2$ with m = Number of electrons
 - Must use reference sample to calibrate $I(0)/c$ vs. MW curve first though!
 - Obtained from Guinier plot: $\log(I)$ vs. q^2
 - Using small q values such that $qR_G \leq 1.3$ (globular) or ≤ 0.8 (elongated)
 - From low- q values (extrapolate I at $q \rightarrow 0$)
 - Obtained from $P(r)$ as well
 - More accurate since entire scattering profile is used
- **Pair-distribution function $P(r)$**
 - Auto-correlation function of spatial distribution of electronic density
 - Obtained from Fourier transform of scattering curve
 - This describes inter-electronic distances in the molecule
 - For learning about shape of molecule
 - $P(r)$ with long tail may indicate elongated or unfolded sample
 - Provides another estimate for R_G and $I(0)$
 - Calculated iteratively using GNOM program
 - $P(r)$ can be calculated from a high-resolution structural model (e.g., PDB)
- **D_{Max} or R_{Max} – largest distance across molecule**
 - Difficult to accurately determine value and error
 - From fitting $P(r)$ since $P(r > R_{\text{max}}) = 0$

- Calculated iteratively using GNOM program
- Data should be measured at $q \geq 2\pi/D_{\max}$
- **Porod volume of macromolecule (Porod, 1982)**
 - $V = 2\pi I(0)/Q$ with $Q = \int(0 \rightarrow \infty) q^2 I(q) dq$
 - Obtained from fitting $I(q)$ with relative or absolute $I(q)$ function
 - Must discard large-angle portions of curve ($q > 0.2 \text{ /Å}$)
 - Estimate $V_{\text{porod}}(\text{nm}^3) \approx 2 * MW(\text{kDa})$
 - Very inaccurate for asymmetric and/or small proteins
- **Shape and foldedness of particle**
 - SAXS can assay folding, assembly, aggregation (like DLS in this regard)
 - $P(r)$ indicates globular vs. extended
 - Kratky plot: $I * q^2$ vs. q indicates foldedness
 - Porod's law: $I(q) \sim q^{-df}$
 - $df = 4, 1, 2, (5/3)$ for sphere, oblate (flat), prolate (needle), random coil
- **Calculating scattering profiles**
 - Difference between $\{q(\text{molecule}) + q(\text{hydration-layer})\}$ and $q(\text{solvent in place of molecule and its hydration layer})$
 - Ordered water layer ($\sim 3 \text{ Å}$) is 15% denser than bulk solvent
 - Considered too computationally expensive to calculate $P(r)$ directly
 - SolX does this though
 - This problem is unsolved for high-resolution ($q > 0.4 \text{ /Å}$) data
 - Instead, use iterative approximation-refinement procedure
 - CRY SOL, ORNL_SAS, FOXS
 - FOXS web-server: <http://modbase.compbio.ucsf.edu/foxs/>
 - Once $I(q)$ is obtained for high-resolution model, check it!
 - Check $P(r)$, R_G , D_{\max} , Kratky, Guinier, etc.
 - If the high resolution model is homogeneous in solution, then this should agree with experimental interpretation

Strategy and tactics for SAXS experiments

- **Buffer conditions**

- SAXS is a contrast technique, so maximize ρ_e for solute and minimize ρ_e for solvent
- Match buffers as well as possible (dialysis, micro-dialysis or centrifugal filter)
 - Even μM differences in salt may be problematic at high- q
- Minimize salt (but $< 1\text{M}$ is OK)
- Avoid detergents
- Include free radical scavengers such as DTT (2-10 mM), TCEP (1-2 mM), glycerol (~5%), Tris, HEPES
- Select the buffer conditions such that the sample is stable and monodisperse
 - For protein/ligand binding, work high above K_D if possible
- Aggregation is most common problem
 - DLS and native PAGE can assay this
 - Use analytical ultracentrifugation, gel filtration, centrifugation through high-MW cutoff membrane

- **Acquisition**

- Sample quantity for each condition to be tested
 - $5\text{-}10\text{ g/L} \times 60\text{ }\mu\text{L} = 300\text{-}600\text{ }\mu\text{g}$ sample $\rightarrow 30\text{-}60\text{ nmol}$ for 10 kDa molecule
 - RNA concentration can be $\sim 5\times$ lower than protein for same S/N
- Each sample condition then requires the following ~ 15 min acquisition process
 - Buffer before (1 sec frame, 10 sec frame, 1 sec frame)
 - 10x dilution (1 sec frame, 10 sec frame, 1 sec frame)
 - 5x dilution (1 sec frame, 10 sec frame, 1 sec frame)
 - 2x dilution (1 sec frame, 10 sec frame, 1 sec frame)
 - 1x dilution (1 sec frame, 10 sec frame, 1 sec frame)
 - Buffer after (1 sec frame, 10 sec frame, 1 sec frame)
 - Frame = scattering profile
- Each sample run will be subtracted from the buffer run
 - (Sample - Buffer(before)) and (Sample - Buffer(after)) should be the same
- Scattering is normalized using beam line intensity at time of acquisition
 - This may vary $10^3\text{-}10^4\times$ between frames!
- Check for radiation damage (compare the two 1 sec exposures for each)
 - Damage addressed via cooling, diluting, addition of free radical scavengers (5% glycerol), flow cells, summation of short exposures on multiple fresh samples
- Entire scattering curve should scale with concentration
 - $I(q)/c$ should overlay for all concentrations used (e.g., $c = 1, 2, 5, 10\text{ g/L}$)
 - Usually discrepancies lie in low- q region of plot
 - Aggregation $\rightarrow I(0)/c$ increases with increasing concentration
 - Repulsion forces $\rightarrow I(0)/c$ decreases with increasing concentration
 - Long-range interactions may be attenuated by increasing [salt]
 - Data at multiple concentrations can extrapolate to $c \rightarrow 0$
- Proceed with data free of aggregation, radiation damage and long-range interactions

- **Beam line 12.3.1 at the Advanced Light Source (ALS) in Berkeley, CA**

- Wavelength fixed at $1\text{ }\text{\AA}$
- Data acquired in range $q = 0.01\text{-}0.32\text{ }\text{\AA}^{-1}$ with S/N $\sim 1\%$
- Detector usually set for one fixed q -range
 - Want to get low- q data $< \pi/D_{\text{max}}$ and high- q data $> 2\pi/D_{\text{max}}$

- For large molecules ($D_{\text{max}} > 250 \text{ \AA}$), low-q data may be difficult
- For small molecules, high-q data may be difficult

Structure modeling with SAXS

• **Structure modeling**

- *Ab initio* 3D shape via DAMMIN, DALAI_GA, SAXS3D, GA_STRUCT
 - If number of residues and/or symmetry is known, GASBOR may be superior
 - Compute multiple candidate structures to assay variance
- If high resolution structure model exists
 - Compare with theoretical SAXS curve
 - FOXS, CRY SOL ($\chi^2 < 3.0$ is not uncommon)
 - Overlay *ab initio* shape onto high-resolution model using SUPCOMB, SITUS
- Theoretical and experimental SAXS curves may disagree
 - If heterogeneous multimerization exist (e.g., AB, A2B, AB2, A2B2, etc.)
 - OLIGOMER calculates fraction of each multimer in solution
 - Good fit with OLIGOMER and poor fit with CRY SOL imply heterogeneous multimerization
 - Flexibility and truncated loops (e.g., in crystal structure)
 - Generate a variety of candidate model conformers and fit data with OLIGOMER
 - Multimeric assembly of rigid-docking subunits (e.g., 4xAT3 or TRAP+AT3)
 - MASSHA, SASREF

• **Using SAXS to validate/falsify high-resolution models**

- Challenging and ill-defined to estimate accuracy
 - No consensus on a best goodness of fit metric
- Domains considered static objects and are rotated and translated into place to generate reasonable scattering profile to match experimental profile
 - DAMMIN, GASBOR (if number of residues are known)
- Important to explore uniqueness of scattering curve
 - E.g., Relative orientation of RNaseP S and C domains
 - E.g., TRAP donut vs. TRAP double-donut (stacked)
 - E.g., AT3 vs. AT12
- Resolution of SAXS data
 - Nominal resolution $d = \lambda / (2\sin(\theta)) = 2\pi/q$ contains info in range of 10-1000 \AA

• **Finding homologous structures**

- SAXS data and unknown structure → DARA → PDB structures with similar calculated SAXS curves
 - Like searching a low-resolution version of the PDB: <http://dara.embl-hamburg.de/>

Tutorial using *B. stearothermophilus* WT TRAP + Trp

(0) Select desired data set

- All data are already buffer-subtracted
- Holo TRAP
 - Large protein ~92 kDa oblate donut-shaped molecule with C11 symmetry
 - Good sample with tested results
- Apo TRAP
 - Large protein ~92 kDa oblate donut-shaped molecule with C11 symmetry
 - Shows radiation damage
- AT
 - Small protein ~15 kDa, globular shape with C3 symmetry
 - Data have not yet been examined

(1) Process data and load into Primus

- Data are already buffer-subtracted
- Load all data into Primus
 - **Tools**...opens window
 - **Select**...load all 12 data sets in spots #1 through #12

(2) Check for radiation damage

- *Goal: Compare first and second 1 sec acquisitions to probe for radiation damage*
- *In absence of radiation damage, the first and second profiles overlay*
- **De-select** all 10 sec acquisitions (#2,5,8,11)
- **Plot**
- Range for plotting...1 to 150...**Plot Range**
- **File...Save Screen...01-TRAP-Holo-Radiation_check.bmp**
- *It appears there is no significant radiation damage (only minor difference in scattering curves for sample D at 2 g/L)*
- **Now we no longer make use of the second 1 sec acquisitions**

(3) Determine concentration-dependence of scattering at low-q

- *Goal: scale low-q region of each scattering curve to see if there is any anomalous concentration-dependent behavior*
- *All curves should overlay in absence of anomalous concentration-dependent behavior*
- Select all four 1 sec frames (#1,#4,#7,#10)
- Range for plotting **1** to **100**; un-check **Full range**; click **Plot Range**
- Enter value for (1/concentration) in **Multiplier** column for each of the 4 frames (#1=1.0, #4=0.5, #7=0.2, #10=0.1)
 - This will normalize each curve to that from 1 g/L
- **File...Save Screen...02-TRAP-Holo-Concentration_check.bmp**
- *In the absence of undesirable concentration-dependent scattering and pipetting error, these should overlay within the S/N of the instrument*
- *In this case, the shapes of the curves are similar and the offsets may reflect some pipetting error*

(4) Fit low-q data to Guinier equation using Guinier plot

- *Goal: Describe aggregation-state, size and shape using Guinier plot and the fitted R_G and $I(0)$*
 - *Note: can also use program autoRg_NEW.exe for this step instead of Primus*

- Select only first 1 sec frame (#1)
- Click **Full range** and **Plot Range**
- Click **Guinier**
 - This will fit the plotted range to the Guinier equation
 - The Guinier approximation is only valid for $q \leq 1.3 \cdot R_G$
- Iteratively reduce the plotting range until the fitted value sR_g only goes up to 1.3
 - Uncheck **Full range**, set plotting 1 to {100,50,25,etc.}, click **Plot Range**, click **Guinier**
- When this is done (around 50 points) save the figure **File...Save Screen...03-TRAP-Holo-Guinier-{C,D,E,F}.bmp**
- Repeat this process for each additional concentration (#4, #7, #10)

S	Conc	$R_G(\text{\AA})$	$I(0)$	$I(0)/c$
C	1 g/L	$32.3 \pm 0.199 \text{\AA}$	44.315 ± 0.182	44.315
D	2 g/L	$34.8 \pm 0.142 \text{\AA}$	103.27 ± 0.301	51.635
E	5 g/L	$35.5 \pm 0.094 \text{\AA}$	198.48 ± 0.394	39.696
F	10 g/L	$35.9 \pm 0.069 \text{\AA}$	397.70 ± 0.575	39.770

- (1) *Aggregation: data appear to be well-described by Guinier plot (linear) which does not indicate evidence for aggregation*
- (2) *Radius of gyration R_G increases somewhat with increasing concentration*
 - *This may indicate aggregation*
- (3) *$I(0)/c$ is indicator of MW of molecule*
 - *Sample D (2g/L) has very large $I(0)/c$*
- *As stated above, the shapes of the curves are similar and the offsets may reflect some pipetting error*

(5) Merge low-q and high-q regions at each concentration

- *Goal: obtain 4 clean scattering profiles across the entire q range scattered momentum*
- *1 sec frame contains information at low- q , 10 sec frame contains information at high- q*
- Set **Multiplier** back to 1.0 for each frame
- Select 1 sec and 10 sec frames for the first concentration (#1 and #2)
- Check **Full range** and **Plot Range**
 - *Note: 10 sec exposure has much higher S/N at high- q (here, $q > 0.15 \text{\AA}^{-1}$)*
- Adjust plot points **nBeg** and **nEnd** on 1 sec and 10 sec such that the entire scattering curve is produced with ~20 point overlap
 - *The 1 sec frame is more accurate, so favor that dataset until the point at which the 1 sec and 10 sec $I(q)$ values agree but simply the S/N is higher in the 10 sec frame*
 - *In this case: try #1 from 1 \rightarrow 120 and #2 from 100 \rightarrow 507*
- Click **Plot** (not Plot Range) to plot subset of data defined by nBeg and nEnd
- Once the desired merge is ready, **File...Save Screen...04-TRAP-Holo-Merge-{C,D,E,F}.bmp**
- Click **Merge** and a new dataset appears shown at the bottom **Merge00.dat**
- In the file explorer, rename this **Merge00.dat** \rightarrow **TRAP-Holo-Merge-C.dat**
- Un-check the **OUT** at the bottom of the Primus window as Merge00.dat no longer exists
- Repeat the merging procedure for the other concentrations (#4&5, #7&8, #10&11)
 - Set limits, save screen, merge data, rename Merge00.dat file
- **At this point, only the merged data are of further interest**
- Click **Clear** button to clear all data from Primus
- Load each merged scattering profile into #1, #2, #3 and #4

- Overlay all four plots and **File...Save Screen...04-TRAP-Holo-Merge-All.dat**
- Set multipliers (1/concentration) to normalize frames and **File...Save Screen...04-TRAP-Holo-Merge-All-Norm.bmp**
 - *It appears that data set D has anomalous scattering at high-q*
 - **Do not trust data set D any more**

(6) Normalize each scattering curve to 1 g/L

- *Goal: for future comparisons with other data sets, normalize all merged data to 1 g/L*
- Enter concentration in **Conc** for each data set
- Display only one data set
- Click **Divcst** button to divide each $I(q)$ point by the concentration
- Rename output data file **Divcst00.dat** → **TRAP-Holo-Merge-{C,D,E,F}-Norm.dat**
- Repeat this for each data set (recall, set D appears to be invalid)

(7) Calculate Porod volume

- *Goal: Fit Porod equation to scattering data to obtain estimates of R_G , $I(0)$ and macromolecule volume V*
- Plot full range of data and click **Porod**
- Iteratively reduce the plotting range until the Porod fit yields a sigmoid curve with a flat region at high q
 - *Note: This is a similar iterative process used for fitting Guinier plot*
 - *For data set F, use points 1 through 130*
- **File...Save Screen...05-TRAP-Holo-Porod-{C,D,E,F}.bmp**

S	Conc	$R_G(\text{\AA})$	$I(0)/c$	$V_{\text{porod}} (\text{\AA}^3)$
C	1 g/L	0	0	146.59e3
D	2 g/L	Bad data at high-q	-	-
E	5 g/L	35.8	39.861	146.59e3
F	10 g/L	35.9	39.764	147.67e3

- $V_{\text{porod}} \approx 2MW$ (not good for asymmetric molecules though)
- *MW of TRAP donut = 92 kDa which yields Porod volume of $2 * MW(\text{kDa}) = 184e3 \text{\AA}^3$ but since TRAP is not globular this may not be a reasonable estimate. So the value of $\sim 146e3 \text{\AA}^3$ may or may not be reasonable.*

(8) Display Kratky plots to examine foldedness

- *Goal: Produce Kratky plot to seek foldedness of molecule*
- Run program **sasplot.exe**
- Click **File** to load data **TRAP-Holo-Merge-{C,D,E,F}-norm.dat**
 - *This will make two plots: one for data "1" and one for error in data "2"*
 - *Ignore the plots of the error as they are not meaningful here*
- Set view to Kratky plot using **View...Y * s^2 :: X**
 - *This sample appears to be folded based on the parabolic shape of Kratky plot*
 - *Unfolded sample increases but doesn't decrease all the way back to $q^2 I(q) \sim 0$*
 - *See figure 24 in Putnam, et al. for example of unfolded sample*
- Save the figure using **printscreen 06-TRAP-Holo-Kratky-All.png**
 - Open Microsoft Paint (or similar) to paste image, crop and save
- **Exit** the program

(9) Calculate pair-distribution function $P(r)$ using GNOM

- *Goal: Calculate $P(r)$ function for estimate of R_G , $I(0)$ and to extrapolate $I(q \rightarrow 0)$*

- Run program **gnom45qw.exe**
 - *This program may not work in Windows and unix-based version must be used*
- GNOM settings used are default unless otherwise specified
 - *Enter ? to get more information at any prompt in gnom*
 - *To save output plots from GNOM copy the "gnu-capture" file in unix to save the **most recently closed** plot*
 - *gnuplot may need to be set up first, see GNOM documentation*
 - http://www.embl-hamburg.de/ExternalInfo/Research/Sax/manual_gnom.html
- Select file to be opened: *TRAP-Holo-Merge-F-norm.dat*
 - *May have to use shorter file name (e.g., trap_holo.dat)*
- angular scale (1/2/3/4) *1*
 - $s = 4\pi\sin(\theta)/\lambda$ (1/Å)
- Plot input data: *Yes*
 - *Verify this is the TRAP scattering curve*
- Rmax for evaluating p(r) *Try 250, 200, 180, 150, 130, 100*
 - *Goal is to have non-negative P(r) values with smooth decay to zero at Rmax*
 - *Find smallest Rmax=0 such that this is true starting with large values and trying successively smaller ones*
- Check that fit to I(q) is reasonable
 - *Save the output figure 07-TRAP-Holo-Gnom-Iq-###.ps*
 - *### = Value of R_{Max} used (250, 200, etc.) to help tabulate all fitting attempts*
- Check that P(r) is reasonable
 - *Use final plot of P(r) with computed errors (you are asked at end)*
 - *Save the output figure 07-TRAP-Holo-Gnom-Pr-###.ps*
- Rename the output log file gnom.out → gnom-###.out
- Record the R_G, I(0) and Dmax values reported on figure or in gnom.out file
- Compare R_G and I(0) from P(r) to that from Guinier (above)

Num	Rmax	R _G *	I(0)/c*	Dmax	Est	Comment
1	250	41.19	41.38	200	0.418**	-
2	200	40.6	41.3	200	0.418**	-
3	180	39.6	41.0	180	0.430**	-
4	150	37.6	40.3	150	0.502	Match R _G to prior
5	130	36.3	39.6	130	0.594	Match R _G to prior
6	100	33.0	37.0	100	0.652	-

*Use R_G and I(0) estimates from Real space

**Suspicious solution indicated in gnom.out log file

Results from Guinier (above)

S	Conc	R _G (Å)	I(0)	I(0)/c
C	1 g/L	32.3 ± 0.199 Å	44.315 ± 0.182	44.315
D	2 g/L	34.8 ± 0.142 Å	103.27 ± 0.301	51.635
E	5 g/L	35.5 ± 0.094 Å	198.48 ± 0.394	39.696
F	10 g/L	35.9 ± 0.069 Å	397.70 ± 0.575	39.770

Results from Porod (above)

S	Conc	R _G (Å)	I(0)/c	Vporod (Å ³)
C	1 g/L	0	0	146.59e3
D	2 g/L	Bad data at high-q	-	-
E	5 g/L	35.8	39.861	146.59e3

F 10 g/L 35.9 39.764 147.67e3

- Conclusions
 - R_G matches Porod and Guinier at $\sim 36 \text{ \AA}$ for $R_{\text{Max}} = 130 \text{ \AA}$
 - $I(0)/c$ agrees well for all computations at around 39-40
 - The $P(r)$ shape predicted here in run 5 ($R_{\text{Max}} = 130 \text{ \AA}$) is consistent with a donut
 - Plateau around two $P(r)$ maxima with dip in middle, and symmetric decrease on each side
 - Trailing values of $P(r)$ at large r may reflect some aggregation or extended structure
- Estimates for TRAP R_g
 - TRAP as ellipsoid: $a = 40 \text{ \AA}$, $b = 40 \text{ \AA}$, height $c = 15 \text{ \AA}$
 - $R_G = \sqrt{(a^2 + b^2 + c^2)/5} = 26 \text{ \AA}$
 - Double TRAP as ellipsoid: $a = 40 \text{ \AA}$, $b = 40 \text{ \AA}$, height $c = 30 \text{ \AA}$
 - $R_G = \sqrt{(a^2 + b^2 + c^2)/5} = 28 \text{ \AA}$

(10) Calculate scattering curve from TRAP XRCS 1QAW

- Compare to experimental curve using FOXS web utility
 - <http://modbase.compbio.ucsf.edu/foxs/>
- *Note: can also run FOXS or CRY SOL on local machine*

(11) Calculate *ab initio* envelope for 3D structure

- Use ALS 12.3.1 cluster and web interface
 - <http://www.saxier.org/forum/index.php>
 - Login: ikleckner
 - Pass: (Foster lab pass twice in a row, no spaces, all lowercase)
- Can use ATSAS web interface
 - <http://www.embl-hamburg.de/ExternalInfo/Research/Sax/atsas-online/>
 - Must register with email to receive password
- I have not gotten either web interface to work as of 2010/01/25 :-/

Description of data files used

TRAP-0AT_C1_1.mccd.dat	1 g/L 1 sec	TRAP + Trp
TRAP-0AT_C1_2.mccd.dat	1 g/L 10 sec	TRAP + Trp
TRAP-0AT_C1_3.mccd.dat	1 g/L 1 sec	TRAP + Trp
TRAP-0AT_D1_1.mccd.dat	2 g/L 1 sec	TRAP + Trp
TRAP-0AT_D1_2.mccd.dat	2 g/L 10 sec	TRAP + Trp
TRAP-0AT_D1_3.mccd.dat	2 g/L 1 sec	TRAP + Trp
TRAP-0AT_E1_1.mccd.dat	5 g/L 1 sec	TRAP + Trp
TRAP-0AT_E1_2.mccd.dat	5 g/L 10 sec	TRAP + Trp
TRAP-0AT_E1_3.mccd.dat	5 g/L 1 sec	TRAP + Trp
TRAP-0AT_F1_1.mccd.dat	10 g/L 1 sec	TRAP + Trp
TRAP-0AT_F1_2.mccd.dat	10 g/L 10 sec	TRAP + Trp
TRAP-0AT_F1_3.mccd.dat	10 g/L 1 sec	TRAP + Trp

Resources

- Biological small angle scattering group
 - Download ATSAS software (Primus, gnom, crysol, etc.)
 - <http://www.embl-hamburg.de/ExternalInfo/Research/Sax/software.html>
 - <http://www.embl-hamburg.de/ExternalInfo/Research/Sax/index.html>
- Saxier on line forum
 - <http://www.saxier.org/forum/index.php>
 - <http://www.saxier.org/>
- SAXS 12.3.1 beam line at Advanced Light Source (ALS)
 - <https://bl1231.als.lbl.gov/saxs/loginForm.jsp>
 - Login: ikleckner
 - Pass: (Foster lab pass twice in a row, no spaces, all lowercase)
- Putnam, CD; Hammel, M; Hura, G; Tainer, JA. "X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution". *Quart. Rev. Biophys.* (2007).
- Greg L Hura, Angeli L Menon, Michal Hammel, Robert P Rambo, Farris L Poole II, Susan E Tsutakawa, Francis E Jenney Jr, Scott Classen, Kenneth A Frankel, Robert C Hopkins, Sung-jae Yang, Joseph W Scott, Bret D Dillard, Michael W W Adams & John A Tainer. "Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS)", *Nature Methods* 6:606-612 (2009).