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US-SOMO HPLC-SAXS module: dealing with capillary fouling and extraction of pure component patterns from poorly resolved SEC-SAXS data

Emre Brookes, Patrice Vachette, Mattia Roccoc and Javier Pe´rezd

Presented by: Zachary Robinson

Overview

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- SAXS Background
- Capillary Fouling
- Proposed Solutions
- Poor SEC Resolution
- Proposed Solutions
- Future Directions

SEC-SAXS

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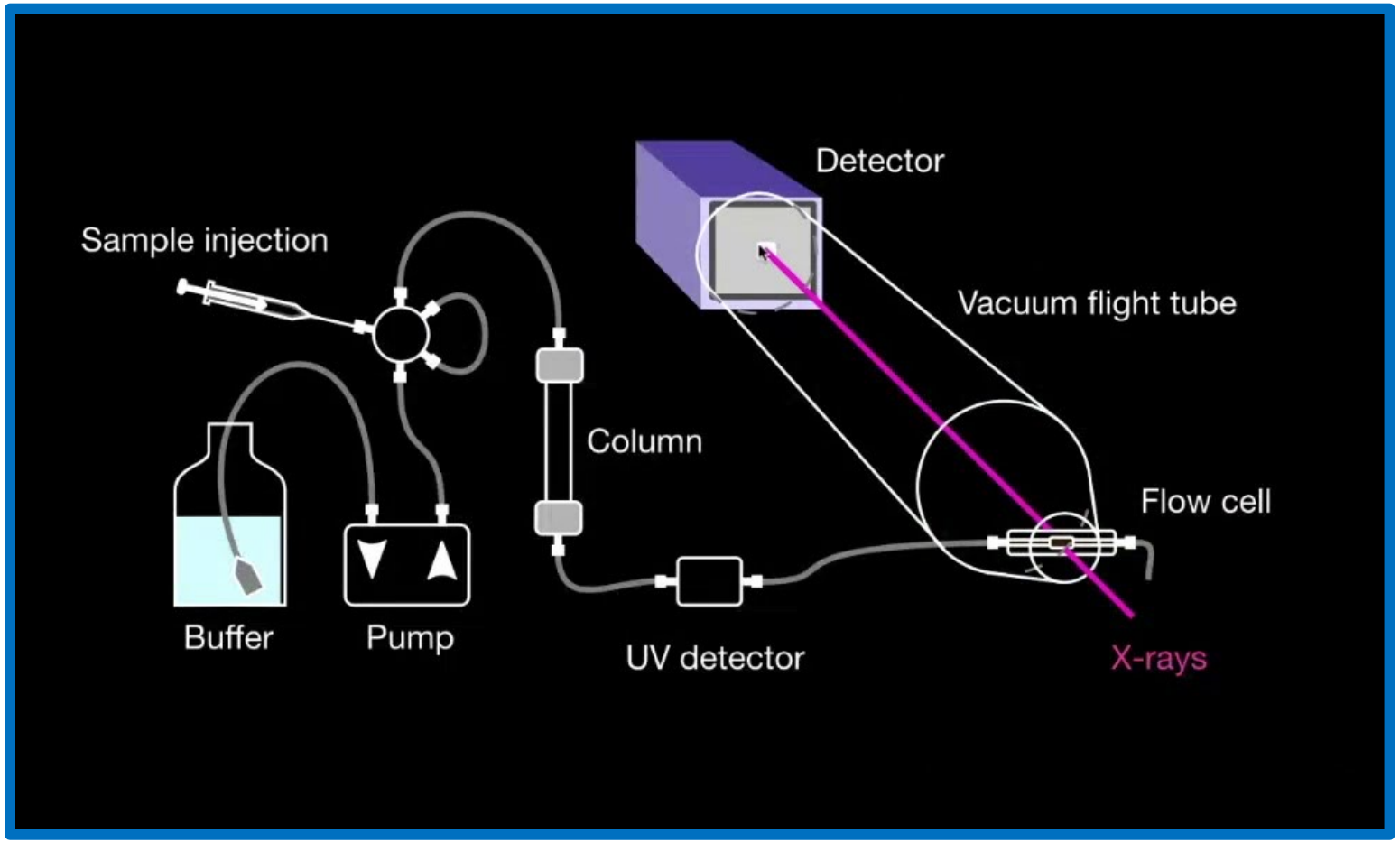
- Sample is pushed through a size-exclusion column to separate out different species before it is passed through a capillary for SAXS measurements to be taken.



Size Exclusion Chromatography coupled SAXS (SEC-SAXS), (BioCAT, 2021)

US SOMO HPLC SAXS Module

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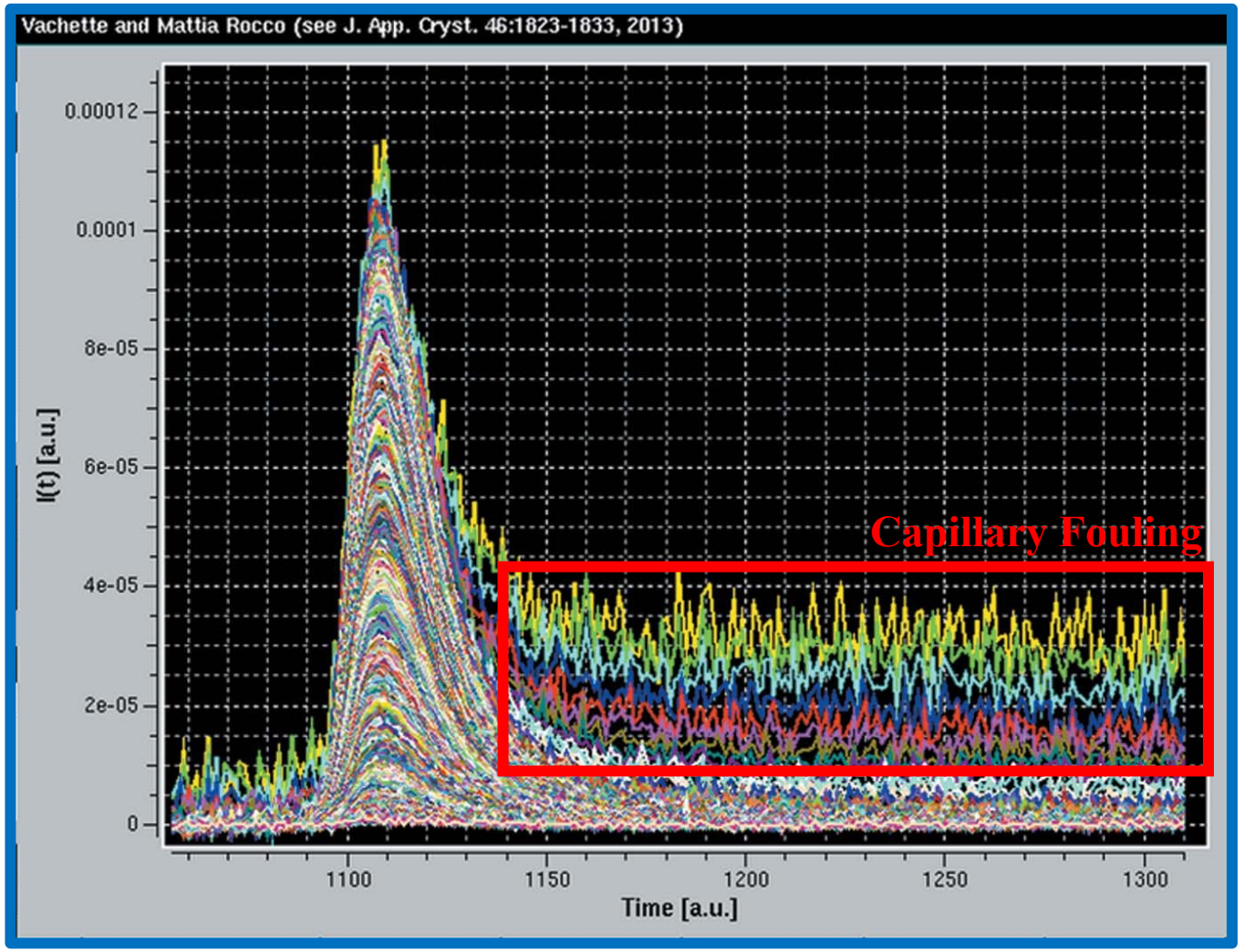
Step 1

- Plot m$I_q(t)$ vs t
- t corresponds to time
- m corresponds to the number of q values at that time
- $I_q(t)$ is scattering intensity as a function of time.
- **This plot allows for detection of capillary fouling.**



Brookes et al., 2016

Experimental Methods

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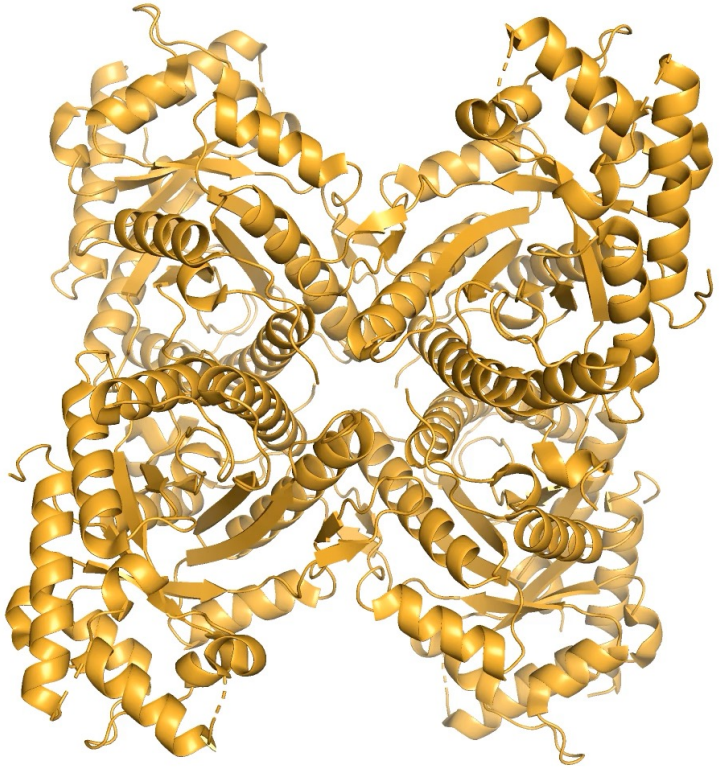
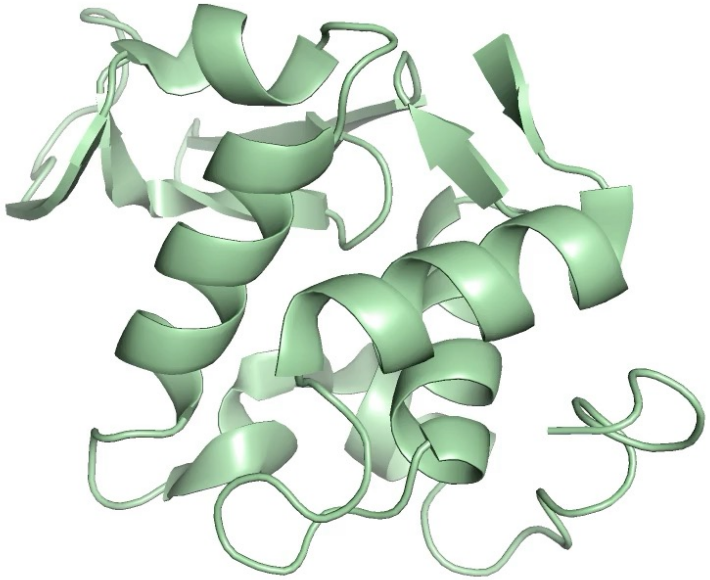
- Buffer conditions previously shown to exhibit high capillary fouling rates in lysozyme were chosen.



(Wisniewski *et al, Unpublished*)



(Harata *et al,* 1993)

Assumptions

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1. Capillary fouling deposits are proportional to sample concentration while exposed to the beam.

2. The buffer nor make/model of the instrument used contribute to the extent of fouling that occurs.

3. No “cleaning” occurs while the sample is eluting. (First approximation.)

4. The proportionality constant is species independent.

Steady-State End Signal

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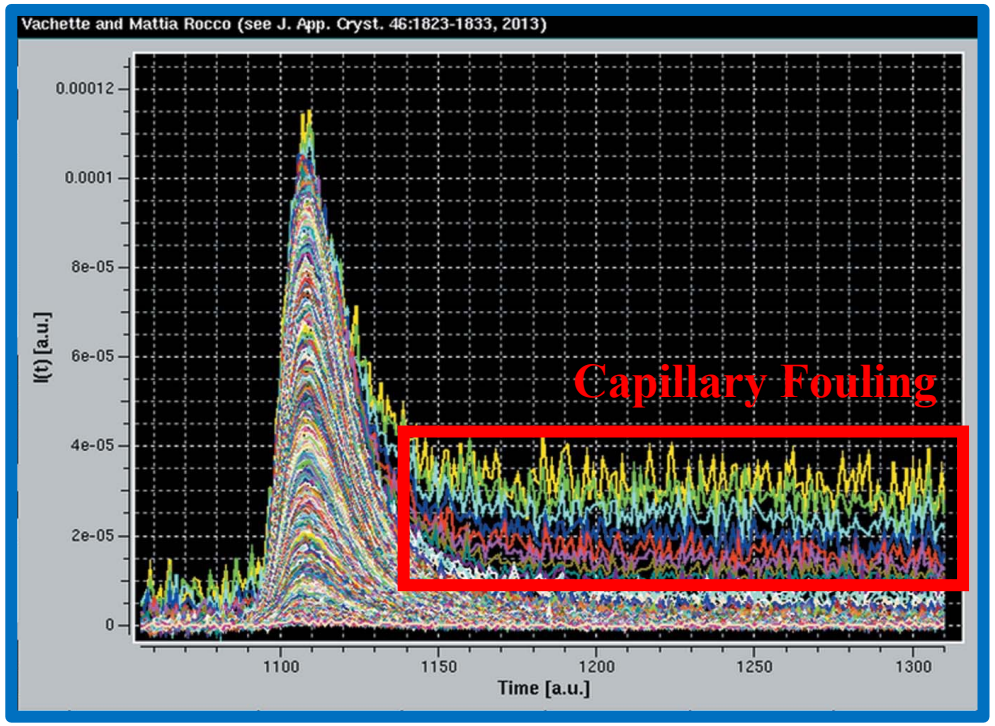
- First, calculate average intensity for a region post-elution.
- If the value is close to 0, then no further steps are necessary. If lower than zero, other experimental issues.
- If greater than zero, capillary fouling is likely.



Brookes et al., 2016

$$I_{BL}(q) = \sum_{k=1,m} \frac{I(q, t_{sk})}{m}$$

Iterative Approach

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Step #1: Set the initial baseline to zero: $B_0(q, t) = 0$

Step #2: Loop from i = 0 to the desired number of iterations.

$$D(q, t_k) = \gamma(q)[I(q, t_{k-1}) - B(q, t_{k-1})]$$

Iterative Approach 2

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$$I_{TOT_i}(q) = \sum_{k=1}^{n-m} I(q, t_k) - B(q, t_k) \qquad I_{BL}(q) = \sum_{k=1,m} \frac{I(q, t_{sk})}{m}$$

$$\gamma_i(q) = \frac{I_{BL}(q)}{I_{TOT_i}(q)} \qquad |\gamma_i(q) - \gamma_{i-1}(q)| < \varepsilon$$

Iterative Approach 3

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$$\gamma_i(q) = \frac{I_{BL}(q)}{I_{TOT_i}(q)}$$

$$D_i(q, t_k) = \gamma_i(q)[I(q, t_{k-1}) - B_i(q, t_{k-1})]$$

$$B_{i+1}(q, t) = \sum_{t'=1}^{t} D_i(q, t')$$

Lysozyme SEC-SAXS Experiment Analysis

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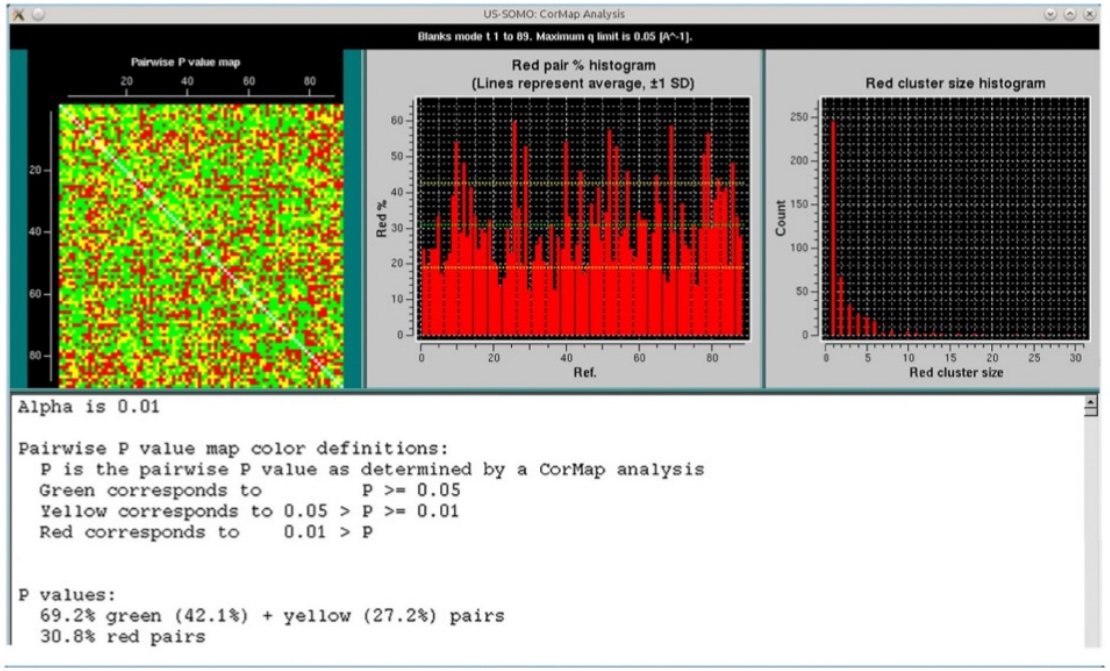
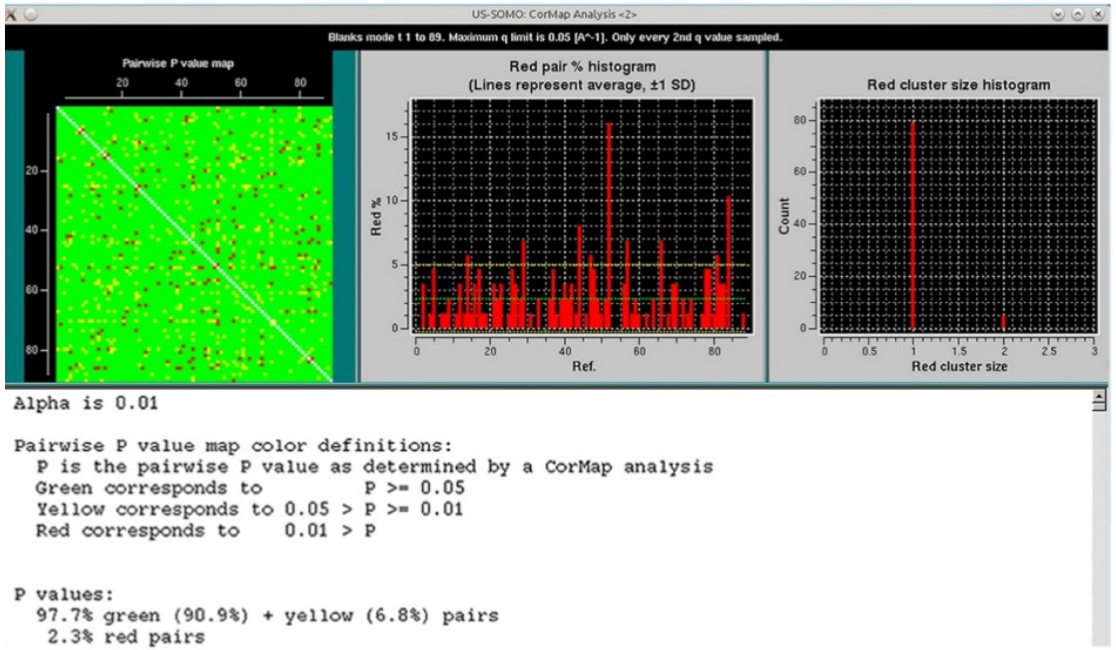
- Initially, buffer frames were shown to be significantly different in a control.



Brookes et al., 2016



Brookes et al., 2016

Holm-Bonferroni Adjustment

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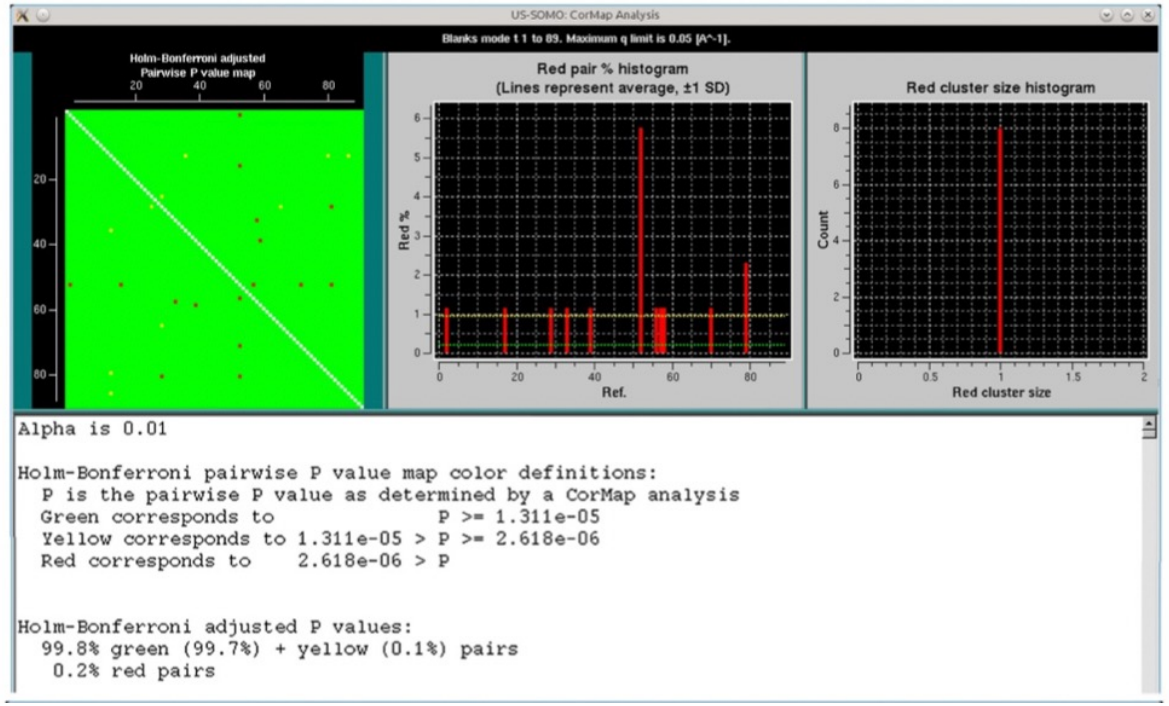
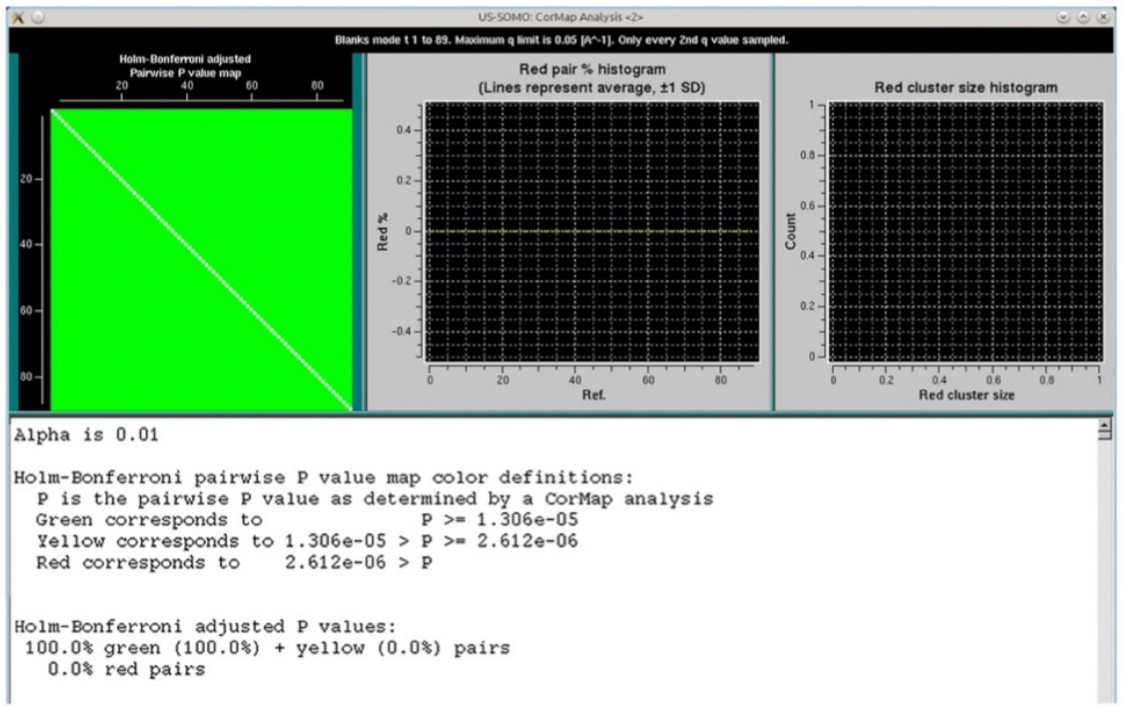


Brookes et al., 2016



Brookes et al., 2016

Identification of the Baseline Region

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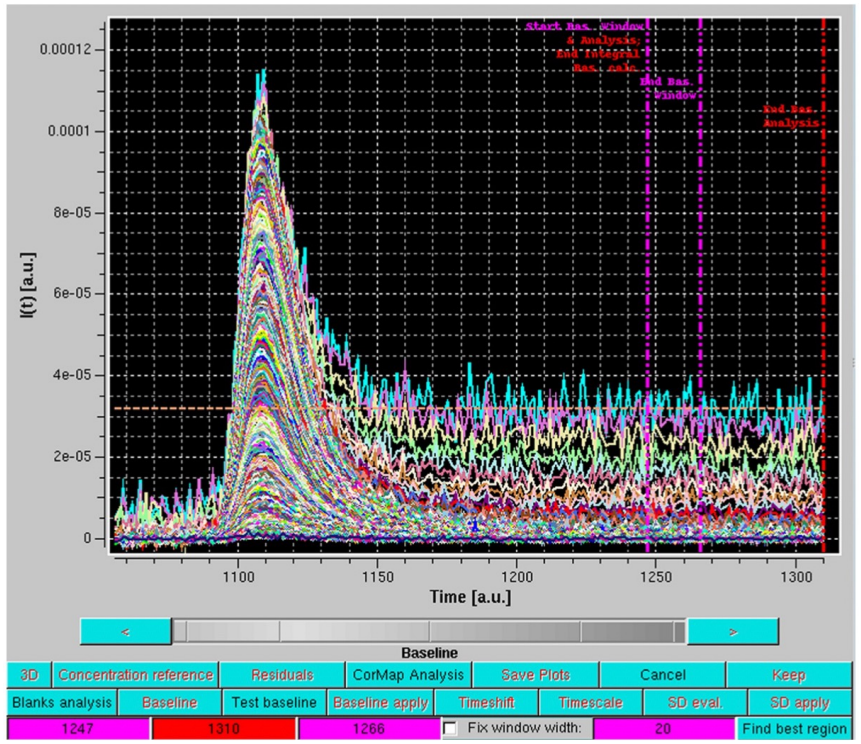
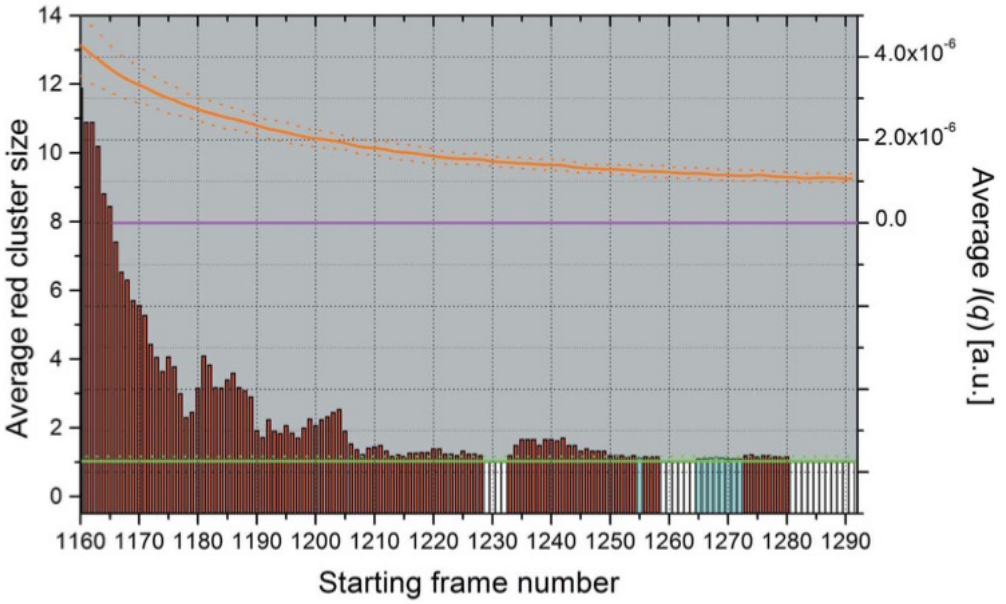
- Identification of a stable final baseline region is identified using correlation map.



Brookes et al., 2016



Brookes et al., 2016

Results of Baseline-Correction Procedure

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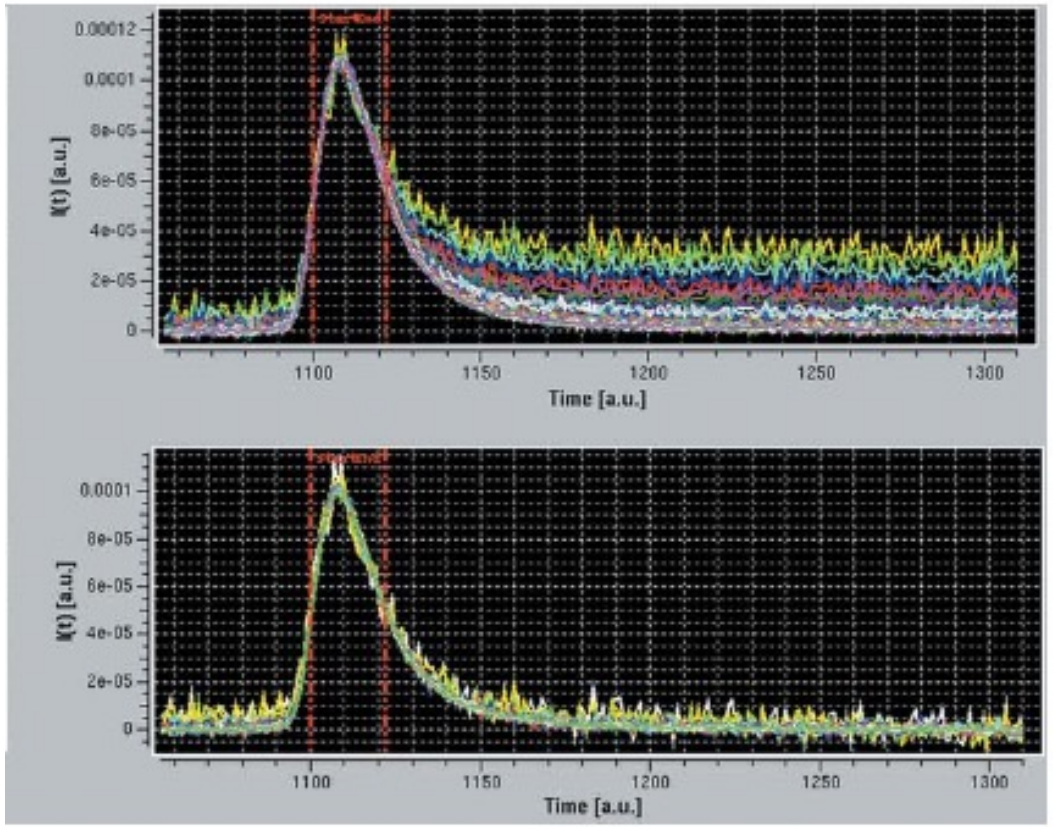
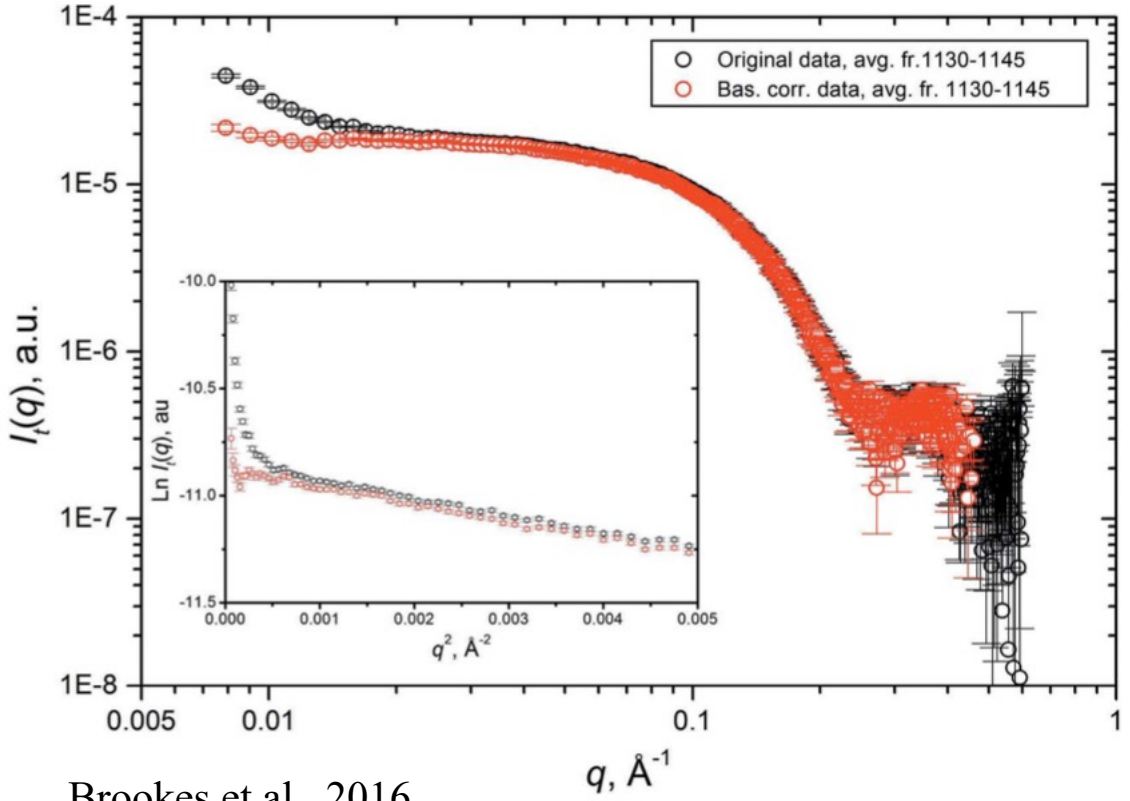


Brookes et al., 2016



Brookes et al., 2016

Poor HPLC Resolution

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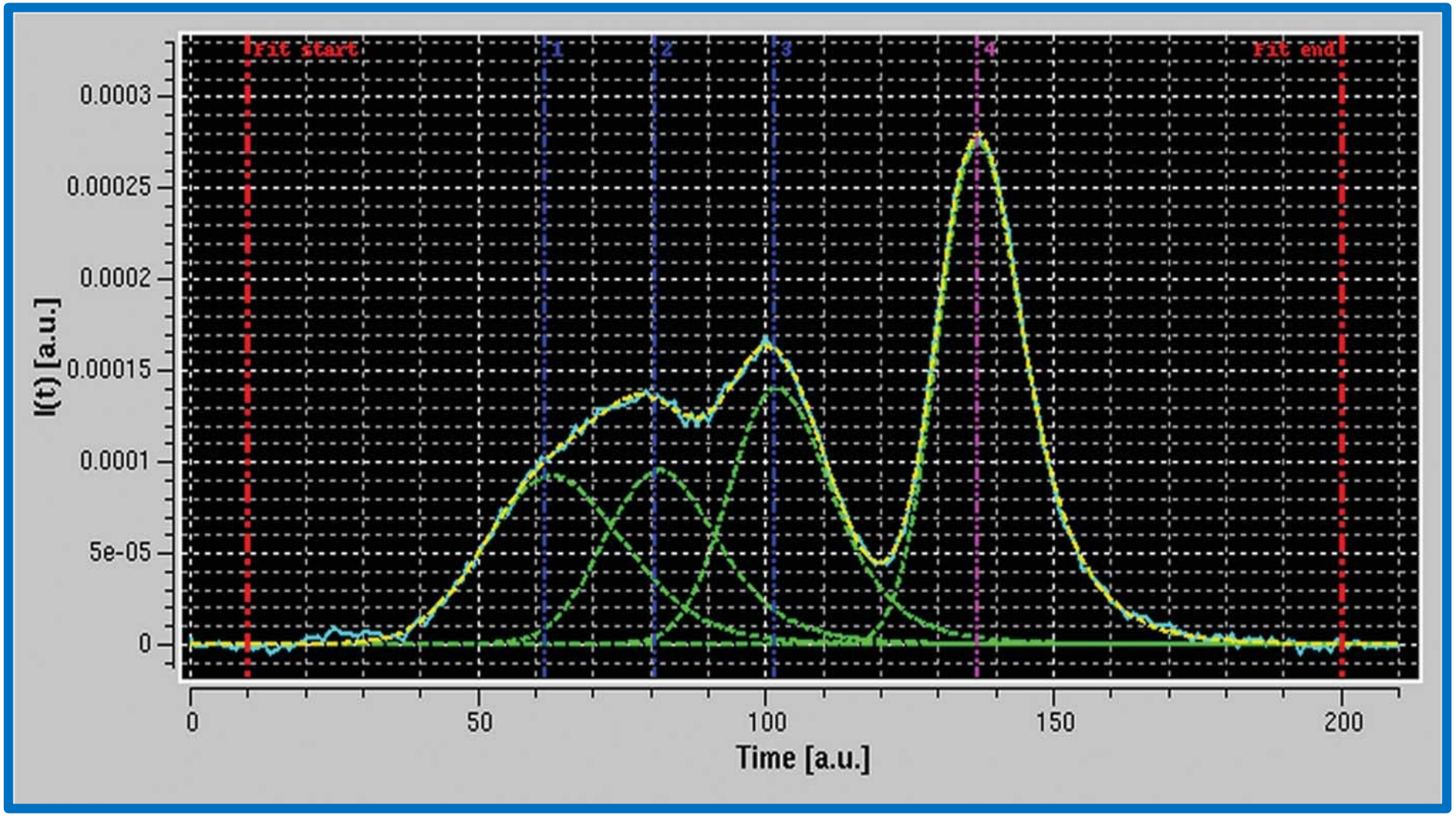
- Different species may not be completely resolved by the size exclusion column.



Brookes et al., 2016

Non-Symmetrical Gaussian Functions

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Classical Gaussian Function:

$$y = \frac{a_0}{2\pi^{\frac{1}{2}} a_2} e^{\left[-\frac{1}{2}\left(\frac{x-a_1}{a_2}\right)^2\right]}$$

Exponentially Modified Gaussian Function:

$$y = \frac{a_0 e^{\left[\frac{1(x-a_1)^2}{2\ a_3^2+a_2^2}\right]}\left\{1 + erf\left[\frac{a_3(x-a_1)}{2^{\frac{1}{2}} a_2 (a_3^2+a_2^2)^{\frac{1}{2}}}\right]\right\}}{(2\pi)^{\frac{1}{2}}(a_3^2+a_2^2)^{\frac{1}{2}}}$$

Half-Gaussian Modified Gaussian Function:

$$y = \frac{a_0}{2a_3} e^{\left(\frac{a_2^2}{2a_3^2}+\frac{a_1-x}{a_3}\right)}\left[erf\left(\frac{x-a_1}{2^{\frac{1}{2}} a_2} - \frac{a_2}{2^{\frac{1}{2}} a_3}\right) + \frac{a_3}{|a_3|}\right]$$

Exponentially Modified Gaussian + Half-Gaussian Modified Gaussian Function:

$$y = \frac{a_o}{4a_3} e^{\left(\frac{2a_1a_3-2a_3x+a_2^2}{a_3^2}\right)} erfc\left(\frac{a_1a_3 - a_3x + a_2^2}{2^{\frac{1}{2}} a_2 a_3}\right) + \left[\frac{a_0}{2(2\pi)^{\frac{1}{2}}(a_2^2+a_4^2)^{\frac{1}{2}}}\right] e^{\left[\frac{1(a_1-x)^2}{2\ a_2^2+a_4^2}\right]} erfc\left[\frac{a_4(a_1-x)}{2^{\frac{1}{2}} a_2 (a_2^2+a_4^2)^{\frac{1}{2}}}\right]$$

Goodness of Fit Estimation

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- χr_σ is used as a noise independent goodness of fit estimator.

$$r_\sigma^2 = \frac{1}{n}\sum_{i=1}^{n}\left[\frac{\sigma_{exp}(q_i)}{I_{exp}(q_i)}\right]^2$$

- Correlation Map statistical analysis was also used.

$$\chi^2 = \frac{1}{n}\sum_{i=1}^{n}\left[\frac{I_{exp}(q_i) - I_{calc}(q_i)}{\sigma_{exp}(q_i)}\right]^2$$

Efficacy of SVD with Different Gaussian Functions

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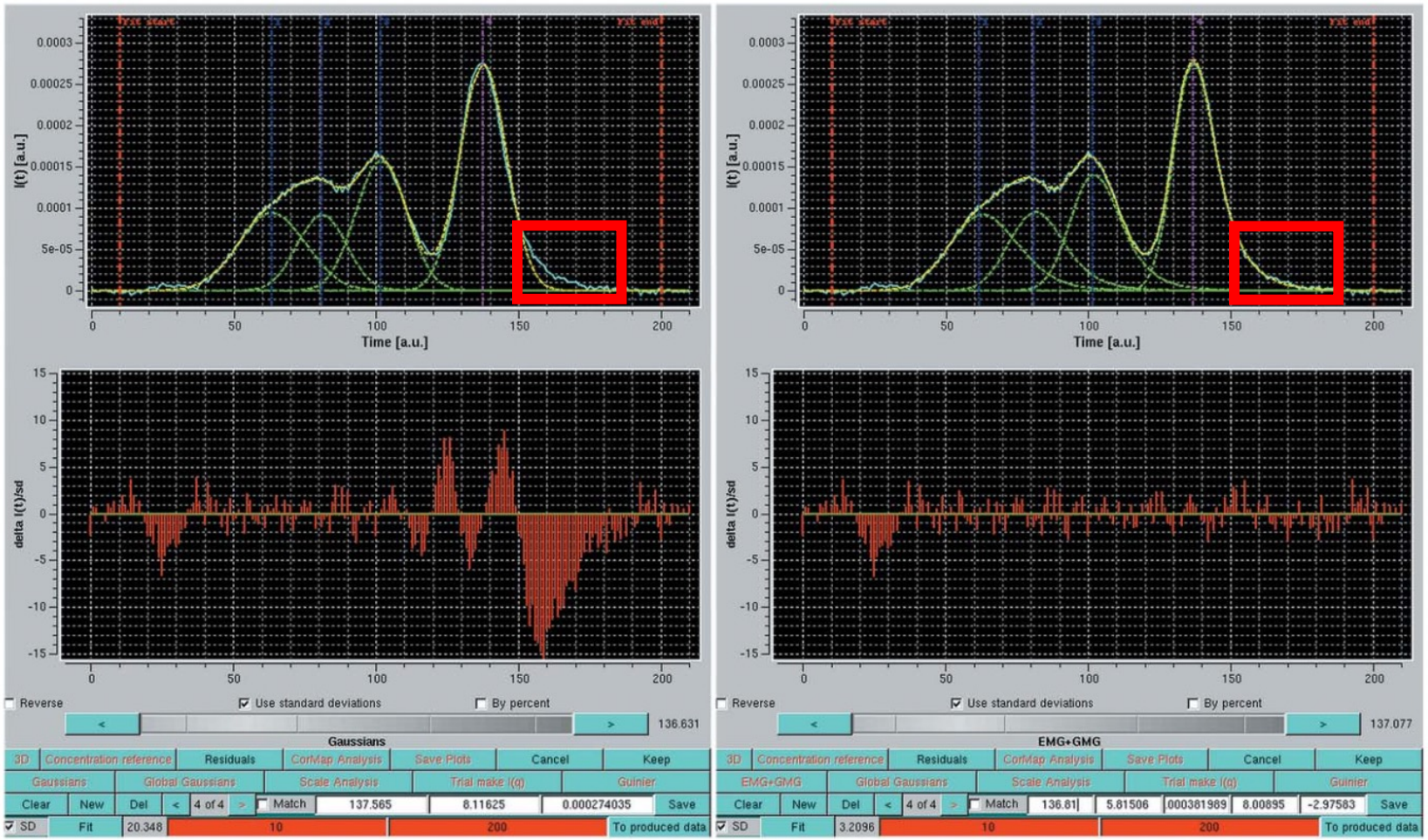

Cyan represents the original curve, yellow is the generated curve, and green represents the individual Gaussian distributions generated. (Brookes et al., 2016)

Efficacy of SVD with Different Gaussian Functions 2

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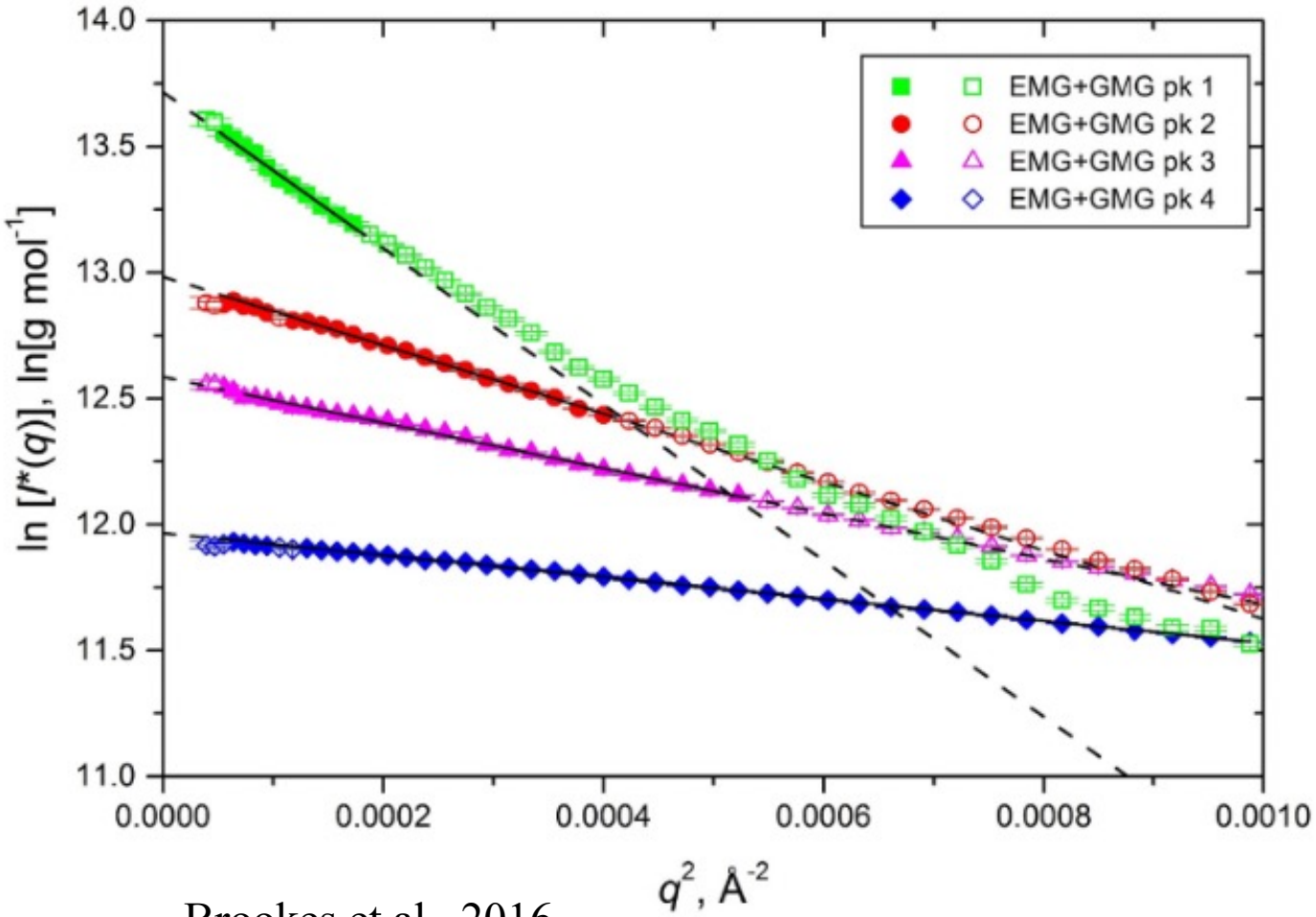

Brookes et al., 2016

Efficacy of SVD with Different Gaussian Functions 3

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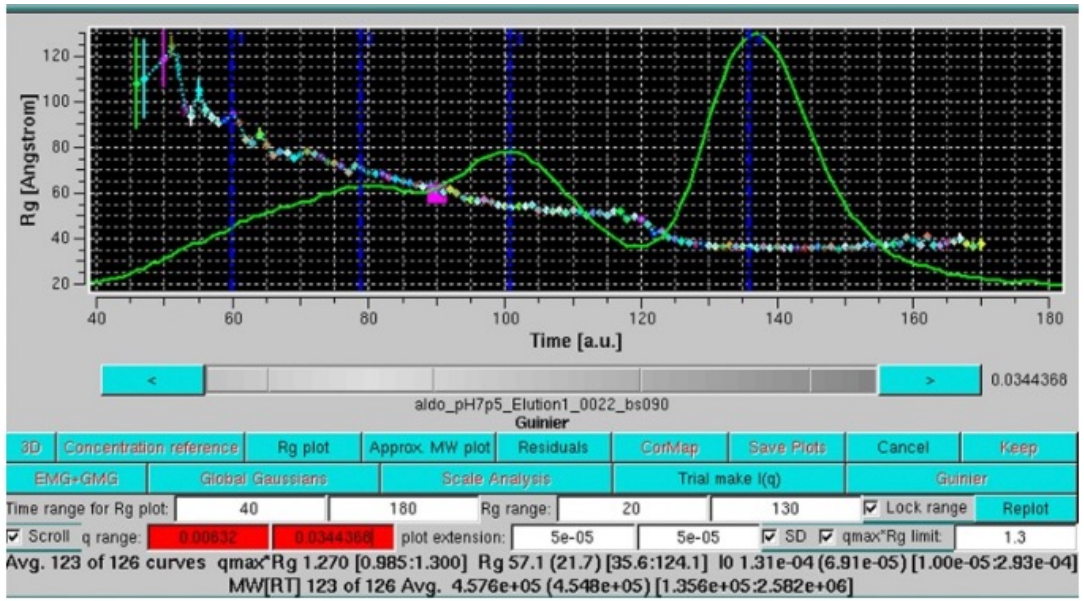
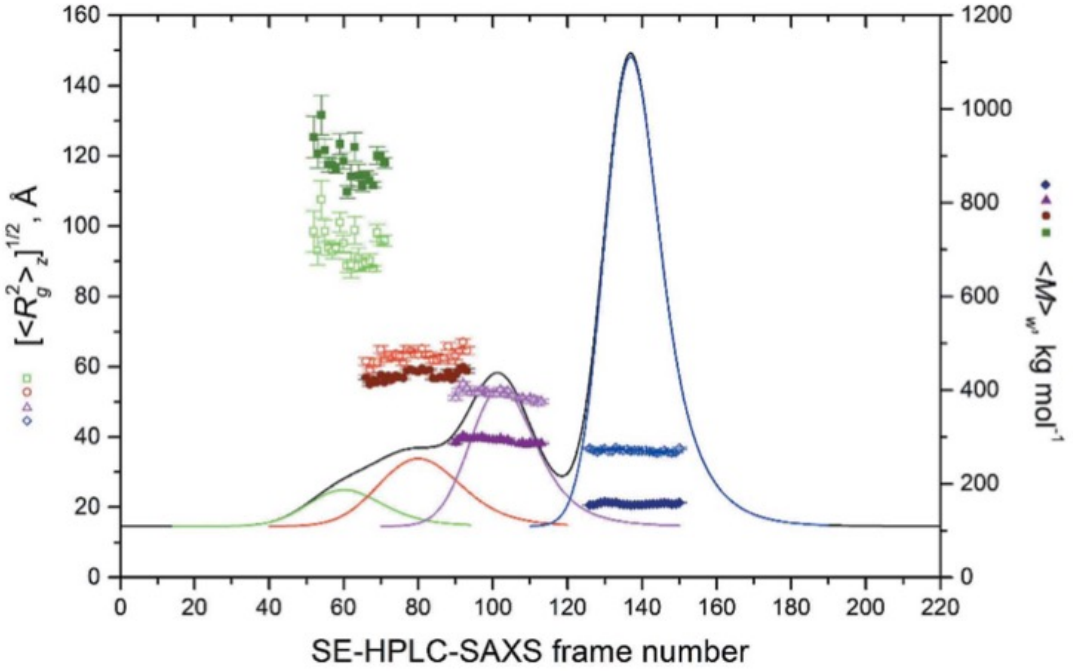


Brookes et al., 2016



Brookes et al., 2016

Efficacy of SVD with Different Gaussian Functions 4

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EMG + GMG peak No.	Frame(s)	$[\langle R_g^2 \rangle_z]^{1/2}$ (Å)	Guinier $\langle M \rangle_w$ (kg mol^{-1})	$q_{min} - q_{max}$ (Å^{-1})	χ^2	*SAXS-MoW* $\langle M \rangle_w$ (kg mol^{-1})
1	60 (top)	101.1 ± 2.8	925 ± 22	0.00743–0.01257	0.0205	n.d.
1	Average of frames 53–72 results	94.4 ± 5.1	886 ± 40	n.a.	n.a.	n.d.
1	Average frame of frames 53–72	94.8 ± 1.4	897 ± 11	0.00743–0.01372	0.0126	1000 ± 3
2	80 (top)	63.6 ± 0.9	441 ± 4	0.00800–0.01886	0.0152	n.d.
2	Average of frames 67–94 results	63.1 ± 1.7	432 ± 10	n.a.	n.a.	n.d.
2	Average frame of frames 67–94	63.4 ± 0.3	434 ± 1	0.00800–0.01886	0.0058	462 ± 1
3	102 (top)	53.5 ± 0.6	297 ± 2	0.00857–0.02229	0.0127	n.d.
3	Average of frames 90–113 results	52.1 ± 1.4	292 ± 5	n.a.	n.a.	n.d.
3	Average frame of frames 90–113	52.0 ± 0.3	292 ± 1	0.00857–0.02286	0.0065	306 ± 4
4	137 (top)	36.4 ± 0.1	156 ± 0	0.00857–0.03143	0.0015	
4	Average of frames 126–150 results	36.1 ± 0.4	157 ± 2	n.a.	n.a.	n.d.
4	Average frame of frames 126–150	36.0 ± 0.1	157 ± 0	0.00857–0.03143	0.0031	154 ± 1

Brookes et al., 2016

Theoretical molecular weight of an aldolase homotetramer: 157kDa

Theoretical molecular weight of dimer of homotetramers: 314kDa

Theoretical molecular weight of trimer of homotetramers: 471kDa

Theoretical molecular weight of hexamer of homotetramers: 942kDa

Modelling of Identified Complexes

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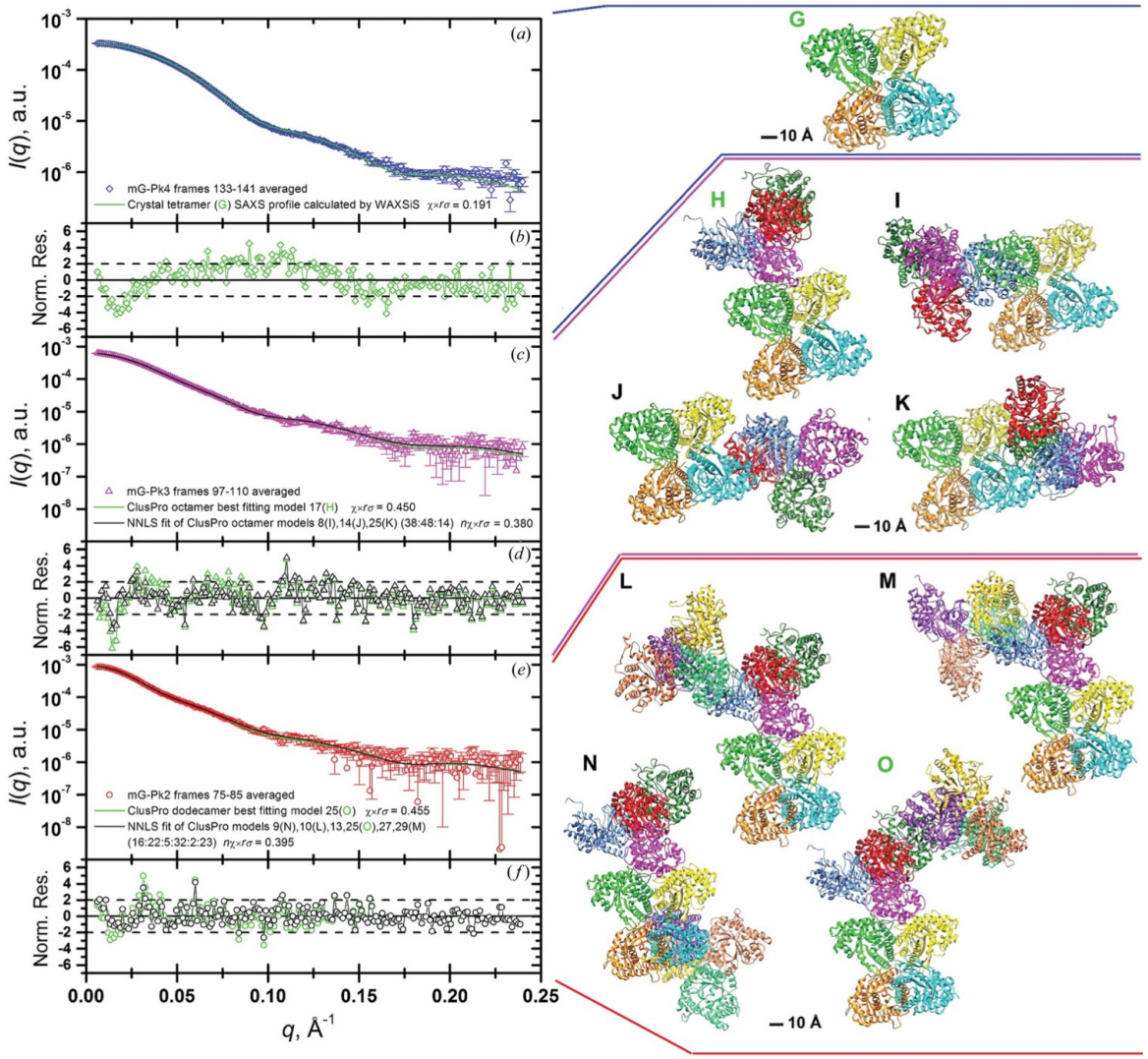
- Molecular docking experiments were carried out to try to visualize what the observed complexes would look like structurally.



Brookes et al., 2016

Conclusions/Future Directions

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- The new analysis methods are effective in reducing the affect of capillary fouling and poorly resolved SEC peaks on experimental SAXS data.

- The analysis methods presented are more efficacious than most alternatives available at the time.

- Currently, the software cannot be considered a high-throughput tool, due to the number of manual adjustments that must be made. The authors may release a subsequent edition of the software, automating several steps in a future release.

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Questions?

Literature Cited

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