Combination of scattering and biophysical methods

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Overview

Examples

• Use of HBM + model building to interpret complex SV data
• Simple model-independent investigation of a hetero-association
• SAXS revealed what the crystal did not....
• AUC & SAXS validate cyroEM structure
Example: use of HBM + model building to interpret complex SV data
Oligomerisation of synthetic polyvalent integrin α5β1 ligands

α5β1 ligands used to immobilise cells on surfaces via
• 9th type III FN domain synergy site (PHSRN)
• 10th type III FN domain RGD site

α5β1 ligand oligomers facilitate increased binding

Oligomerisation accomplished via 5 heptad repeats based on GCN4 leucine zipper
• I/L placed variously @ a and d positions to promote di-, tri- & tetramerisation

Thiol-linked immobilisation to surface achieved via C-terminal Cys

Question: do the ligands oligomerise as designed?
Construction of hydrodynamic bead models

- From vector (including His-tag) – too short for e.g. SWISSMODEL
- FN III 9-10 domain pair homology model (SWISSMODEL)
- Synthesised “missing beads”
- Coiled-coil (42 a.a.) – SWISSMODELs generated for underlined segment

Oligomer models generated

linear monomer
$s = 1.7 \text{ S}$

bent monomer
$s = 1.8 \text{ S}$

linear dimer
$s = 2.7 \text{ S}$

bent dimer
$s = 2.5 \text{ S}$

Oligomer models generated

linear trimer
s = 3.9 S

bent trimer
s = 3.1 S

Oligomer models generated

- Linear tetramer $s = 4.7 \text{ S}$
- Bent tetramer $s = 3.7 \text{ S}$
- Linear hexamer $s = 5.0 \text{ S}$
- Disulphide bridge

Oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands - AUC SV: c(s) reveals complex composition

AUC SV + DTT: \( c(s) \) composition simplified

Example: simple model-independent investigation of a hetero-association
Human PDC core complex comprises E2 & E3BP to which E1 and E3 bind

The workers
What is the stoichiometry & structure of E3BP:E3 subcomplex?

- E3 forms tight dimer
- E3 binds to E3BP
- 2:1 or 1:1?
Native PAGE: stoichiometry is 2:1
ITC: stoichiometry is 2:1

- E3 titrated into E3BP-DD
- 0.5 E3 bind / E3BP-DD
  - equivalent to 2 E3BP-DD/E3

Alan Cooper, Mischa Smolle
Smolle et al., JBC 281 19771-80 (2006)
SV: stoichiometry is 2:1

Mischa Smolle
Smolle et al., JBC 281 19771-80 (2006)
SE titration: stoichiometry is 2:1

Mischa Smolle
Smolle et al., JBC 281 19771-80 (2006)
SAXS data characteristic of asymmetric elongated E3BP:E3 complex

Mischa Smolle
Smolle et al., JBC 281 19771-80 (2006)
Only 1 \textbf{E3BP-DD} lipoyl domain is docked into \textbf{E3}

Brautigam et al. Structure (2006) 14, 611–21

Mischa Smolle
Smolle et al., JBC 281 19771-80 (2006)
Plausible E3BP-DD:E3 models
Example: SAXS revealed what the crystal did not....
Novel bacteriocin mechanisms

Rhys Grinter  Kesha Josts  Catriona Thompson  Dan Walker
Translocation of colicin across bacterial outer membrane: role of the IUTD

Housden et al., Science, 2013
Colicin-like pectocin M2 has no IUTD - how does it cross the bacterial outer membrane?

M-class cytotoxic domain

plant-like ferredoxin domain

Rhys Grinter & Kesha Josts
Grinter, Josts et al., Molecular Microbiology, 2014, 93, 234-46
DESY X33 SAXS: PM2 solution conformation inconsistent with crystal structure ($\chi^2 = 1.362$)
US-SOMO DMD: generation of fleximers
US-SOMO DMD + GAJOE: bimodal ensemble of models that better fit SAXS data ($\chi^2 = 0.827$)

- US-SOMO DMD: 5,000 models created
- GAJOE: best fit ensemble selected
PM2 re-crystallisation in space group P3$_1$21 confirms extended structure predicted by SAXS

Rhys Grinter & Kesha Josts
Grinter, Josts et al., Molecular Microbiology, 2014, 93, 234-46
Hypothesis: P3_{1,21} conformation permits PM2 to fit through a TonB-like receptor barrel domain
CSP analysis of PM1$_{fer}$ HSQC spectra in presence of FusA allows mapping of FusA-binding surface

Grinter, Josts et al., Nature Comms, 2016, DOI: 10.1038/ncomms13308
Fer interacts with FusA b-wall & plug loop & is positioned centrally in HADDOCK docking simulations

Grinter, Josts et al., Nature Comms, 2016, DOI: 10.1038/ncomms13308
PM2+FusA – binding observed by AUC SV

Kesha Josts, unpublished
SANS solution structure of FusA

Catriona Thompson, unpublished
Example: AUC & SAXS validate cyroEM structure
AdhE – a virulence target of *E. coli* O157

Ji-Joon Song  
Andy Roe  
Gijeong Kim  
Liyana Azmi

Colleagues at eBIC & beamline B21 at Diamond Light Source, UK
Microbial resistance to antibiotics

There is a high correlation between antibiotic use and resistance.

Global: A failure to address the problem of antibiotic resistance could result in:
10 million deaths by 2050
Costing £66 trillion


Slide from Liyana Azmi
Pathogenesis of EHEC

*Escherichia coli* O157:H7

- Host cell
- Type three secretion system (T3SS)
- Outer membrane
- Periplasm
- Inner membrane
- Cytosol

Image by Steven Lewis, PhD Student in Stephanie Schuller’s Group

Slide from Liyana Azmi
Deletion of adhE: the link to virulence

- Suppressed TTSS expression
- No bacterial motility
- Overexpressed flagella

AdhE is a 2-domain, bifunctional enzyme

Structure predicted using Phyre2

aldehyde dehydrogenase (ALDH)

alcohol dehydrogenase (ADH)

Lactobacillus brevis AdhE spirosomes

Kawata, Masuda and Ueki, 1976
E. coli AdhE forms heterogeneous spirosomes

Geobacillus thermoglucosidasius spirosome model – 7 monomers/turn

Extance et al., 2013
Aim – determine AdhE atomic resolution structure for drug design
Predicted structure includes significant areas of **negative** and **positive** potential

Spirosomes are salt-sensitive solution species – but not monomerisable

3D reconstruction of AdhE:
60,000 particles, 22.7 Å resolution

But heterogeneity too great for high resolution structural determination

Study individual ADH & ALDH
ALDH is a monomer in solution

\[ s_{20,w}^0 = 3.04 \text{ S} \]
SAXS @ Diamond Light Source, UK
ALDH SAXS data on SASBDB: SASDCK3

SASDCK3 – Aldehyde dehydrogenase

Aldehyde-alcohol dehydrogenase
ALDH SAXS data on SASBDB: SASDCK3
ALDH predicted structure is incorrect or flexible

Predicted $\omega_{20,w}^0 = 3.70$ S

Experimental $\omega_{20,w}^0 = 3.04$ S

DAM $\omega_{20,w}^0 = 3.40$ S

ADH mostly dimeric in solution - s consistent with experimentally determined value
ADH crystal structure matches DAM – D2 is a dimer – interface for spirosome formation?

Fractionation reduces heterogeneity

Kim et al., Nature Comms, 10, 4527 (2019)
Fraction 1: 3.5 Å cryo-EM structure

Kim et al., Nature Comms, 10, 4527 (2019)
How does AdhE make spirosomes?
1: Dimer formation

Kim et al., Nature Comms, 10, 4527 (2019)
2: ADH domains from dimer interact tail-tail: 4 AdhE / helical turn

Kim et al., Nature Comms, 10, 4527 (2019)
11.2 Å helical reconstruction of AdhE spirosome

Kim et al., Nature Comms, 10, 4527 (2019)
Spirosome consistent with SAXS data

Kim et al., Nature Comms, 10, 4527 (2019)
Spirosome topologically separates ALDH & ADH activities

Kim et al., Nature Comms, 10, 4527 (2019)
Spirosomes change conformation + NADH cofactor

Kim et al., Nature Comms, 10, 4527 (2019)
ADH F670 inserts into h-phobic pocket in other ADH domain

Kim et al., Nature Comms, 10, 4527 (2019)
F670VAE mutants disrupt spirosomes

Kim et al., Nature Comms, 10, 4527 (2019)
F670VAE mutants are still polydisperse

Kim et al., Nature Comms, 10, 4527 (2019)
F670VAE mutants include AdhE dimers & monomers

$s_{\text{spirosome dimer}} > s_{\text{solution dimer}}$

Kim et al., Nature Comms, 10, 4527 (2019)
SEC-SAXS of F670VAE mutants

$$S_{\text{spirosome dimer}} > S_{\text{solution dimer}}$$

$$S_{\text{SOMO}} = 8.5 \text{ S}$$
$$S_{\text{exp}} = 7.9 \text{ S}$$

SAXSMoW 202.1 kDa
dimer 192.2 kDa

Kim et al., Nature Comms, 10, 4527 (2019)
Solution dimer more extended than within spirosome

\[ S_{\text{SOMO}} = 8.0 \, S \]
\[ s_{\text{exp}} = 7.9 \, S \]

Kim et al., Nature Comms, 10, 4527 (2019); Submitted to SASBDB: ID SASDGN2
What we know

- Atomic resolution structure of AdhE and its spirosome!
- Spirosome formed from arm-crossing dimers that then associate via hydrophobic interactions
- Excellent agreement with Extance et al. model
- Spirosome is found in solution
- Spirosome topologically separates ALDH & ADH activities
- Spirosomes change conformation + NADH cofactor
- Mutation of F670 disrupts spirosome
- Dimer relaxes in solution
- Spirosome structure is required for AdhE activity
Where next?

- Now have atomic resolution structure of AdhE
- Can try spirosomes + drug candidates to observe binding site & understand more about mechanism
- Can also optimise AdhE for bio-ethanol production
- What controls spirosome length?
- Investigate substrate channeling
Questions?

I MUSTACHE YOU

A QUESTION

Why the hell isn't the iPhone's battery life called Apple Juice?

Are you childish?

☑️ yes

☒ no

If a dog wore pants would he wear them like this or like this?

If Apple made a car... would it have Windows!? 

What would your pro-wrestler name be?