Experiment and Modelling in Structural NMR

November 28th – December 2nd 2011
INSTN – CEA Saclay, France

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Modelling protein assemblies
combining
Bioinformatics and NMR

[03003]

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Modelling protein assemblies combining bioinformatics and NMR

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School "experiment and modelling NMR"

30th of November 2011

Outline

1. Insights into interactomes
2. Modelling *in silico* the structures of macromolecular complexes
3. Using NMR data for docking

1. **Insights into interactomes**
2. Modelling *in silico* the structures of macromolecular complexes
3. Using NMR data for docking

Deciphering the Sociology of the cell

McGuffee SR, Elcock AH, 2010. *PLoS Comput Biol* 6(3): e1000694.

Technological advances to map interactomes at large scale

What can we learn from 3D structures protein complexes ?

Asf1 is at the center of a network of transient interactions

What can we learn from 3D structures protein complexes ?

Dissection of the molecular logic underlying interactomes functions

How to obtain the structures of the complexes ?

How to obtain the structures of the complexes *without* exp. data?

Protein surfaces : complex mosaics of various physico-chemical textures

1. Insights into interactomes
2. **Modelling *in silico* the structures of macromolecular complexes**
3. Using NMR data for docking

Docking strategies.

Prediction of protein assembly modes

[Convergence and combination of methods in protein-protein docking.](#) Vajda S, Kozakov D. Curr Opin Struct Biol. 2009

A hierarchical approach to docking

STEP1 : Coarse-Grained Sampling

A hierarchical approach to docking

STEP2 : Refinement

How to identify relevant models after Coarse-grain filtering ?
... Looking for the funnel

Small perturbations
around selected models

- Monte-carlo Simulations
- Side-chains and loops flexible
- Full-atom scoring functions

Funnel-like shape are a typical properties of native structures
→ Account for the cooperativity of the binding process

Small perturbations
Side-chain optimisation
Full-atom description

Analysis on known X-ray structures of complexes

Native
structure

Scored using FoldX (*Guerois et al JMB 2002*) or Rosetta (*Baker's group*)

Integrating flexibility during docking

Explicit flexibility :

- Before STEP1 (coarse grained) by sampling multiple initial structures using molecular dynamics, normal mode analysis or hinge guided moves.
- During STEP2 (refinement step), allow for backbone and sidechain moves

Implicit flexibility :

- Allow for bumps and try to relax them later on.

Example of prediction : A complex involved in DNA repair

Example : XRCC4-Cernunnos two partners involved

in the NHEJ repair pathways

XRCC4 (*H. sapiens*)

➔ Identify residues at the interface to specifically perturb the complex ?

Coll. JB Charbonnier (LBSR)

Conserved sites give hint for interaction site

Combining Evolution and Energy Calculations XRCC4-Cernunnos in the NHEJ DNA repair

Coll. JB Charbonnier (LBSR)

Malivert et al, JBC (2010)

The predicted model provided useful functional insights

- ➔ Interface mutations were successfully designed to dissect *in vivo* the role of this interaction (1)
- ➔ A low resolution structure was obtained one year later (Xray at 5Å) (2)

Coll. JB. Charbonnier (1) Malivert et al JBC 2010, (2) Ropars et al PNAS 2011

How to evaluate the best methods The CAPRI experiment (2009)

- **15** Targets to predict
- All in all, **70%** were correctly predicted
- **Major failures :**
Large conformational moves

	Group	Total / High / Medium
1	S. Vajda (Boston University, Boston, MA) – PIPER	6/4***/2**
2	M. Zacharias (Jacobs University, Bremen, Germany)	6/4***/1**
3	X. Zou (University of Missouri, Columbia, MO)	6/3***/2**
4-5	M. Eisenstein (Weizmann Institute, Rehovot, Israel)	6/3***/1**
	H. Wolfson (Tel Aviv University, Tel Aviv, Israel)	
6-7	Z. Weng (U. of Massachussets, Worcester, MA) - ZDOCK	6/2***/2**
	H-X. Zhou (Florida State U., Tallahassee, FL)	
8	A. Bonvin (Utrecht University, Netherlands) - HADDOCK	6/1***/4**
9	CLUSPRO (server, Vajda group, Boston University, MA)	5/1***/3**
10	J. Fernandez-Recio (Barcelona Supercomputing C., Spain)	5/2**
11	J. Gray (John Hopkins U., Baltimore, MD) - Rosetta	4/2***/1**
12-14	P. Bates (Cancer Research Inst., London, UK)	4/1***/1**
	C. Camacho (University of Pittsburgh, PA)	
	HADDOCK (server, Bonvin group, Utrecht University)	
15-16	H. Nakamura (Osaka University, Osaka, Japan)	3/2***/1**
	D. Baker (U. of Washington, Seattle, WA)	
17	C-X. Wang (Beijing U. of Technology, Beijing, China)	3/1***/1**
18	D. Ritchie (INRIA, Nancy, France)	3/3**
	GRAMM-X (server, Vakser group, U. of Kansas, KS)	2/2***
19-21	M. Takeda-Shitaka (Kitasato University, Tokyo, Japan)	

1. Insights into interactomes
2. Modelling *in silico* the structures of macromolecular complexes
- 3. Using NMR data for docking**

How do we go about studying interactions using NMR ?

- Chemical shift mapping may be possible even in intermediate exchange regime.
- Relative orientation of components can be included using Residual Dipolar Couplings.
- Distance information can be extracted using transferred NOE.

Mapping of protein/protein interactions by NMR

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Example of chemical shift mapping application

Modelling the complex between **Sgt1** and the chaperone **Hsp90** ?

Sgt1 has a modular organisation

Shown to bind Hsp90
But very weak interaction !
> 20 μM
Is it biologically relevant ?????

Plants seem to resist infection by pathogenic agents thanks to Sgt1 and Hsp90... Is their interaction involved ???

Coll. Group of
Ken Shirasu
(RIKEN plant, Japan)

K. Shirasu PNAS 2002

How do Sgt1 and Hsp90 assemble ?

NMR chemical shift mapping + docking

Where are the
interaction surfaces ?

Domaine N-HSP90

How do Sgt1 and Hsp90 assemble ?

NMR chemical shift mapping + docking using HADDOCK

What are the interaction surfaces ?

Domaine N-HSP90

ratio N-HSP90 : CS

1 : 0
1 : 0.1
1 : 0.2
1 : 0.4
1 : 0.6
1 : 0.8
1 : 1
1 : 1.2
1 : 1.5
1 : 2.0
1 : 3.0

● G94 : $K_d = 24 \mu\text{M} \pm 2.5$
▲ L89 : $K_d 27 \mu\text{M} \pm 4.3$
■ L93 : $K_d 29.7 \mu\text{M} \pm 3.6$

How do Sgt1 and Hsp90 assemble ?

NMR chemical shift mapping + docking using HADDOCK

What are the interaction surface ?

Domaine N-HSP90

Domaine CS
SGT1

Which positions are in contact ???
 ➔ Molecular docking using ambiguous restraints (HADDOCK)

Using Chemical Shift Mapping Data for Docking (HADDOCK)

- All the residues significantly affected by binding are defined as « active ».
- Their periphery is defined as « passive »
- Docking under Ambiguous Restraint :

Any atom from « active » in SGT1 with any « active » or « passive » in Hsp90.

$$d_{iAB} = \left(\sum_{m_{iA}=1}^{N_{\text{atoms}}} \sum_{k=1}^{N_{\text{res}}^B} \sum_{n_{kB}=1}^{N_{\text{atoms}}} \frac{1}{d^{6_{m_{iA}n_{kB}}}} \right)^{(-1/6)}$$

Dominguez et al (2003) J Am Chem Soc 125 1731-1737

HADDOCK calculation strategy

- (i) Randomization of orientations and rigid body energy minimization
- (ii) Semi-rigid simulated annealing
- (iii) Refinement with explicit solvent

- Models clustered by interaction energies (Eelec, EvdW, EAIR) and average buried surface area
- Lowest energy cluster with the highest buried surface area assumed to be correct
- Can also add RDCs, inter-molecular NOEs and radius of gyration term to prevent expansion at the interface

How do Sgt1 and Hsp90 assemble ?

NMR chemical shift mapping + docking using HADDOCK

Boter*, Amigues* et al. (2007) Plant Cell
Kadota et al. (2008) EMBO Reports

In vivo assessment of the model

Design of a compensatory mutant between SGT1 and HSP90

Kadota et al. (2008) EMBO Reports

Even very weak interactions can play crucial roles *in vivo*

Numerous advantages :

- Best for weak interactions
- Fast to implement
- Can even address very large systems

SAT saturation transfer

Takahashi et al, NSB (2000)

ASF1

Chemical shift
mapping
by NMR

~150 intermolecular NOEs
Obtained using
filtered NMR exp.

ASF1

H3
C-ter

Structure of the complex
by NMR