Functional diversity and structural disorder in human ubiquitination pathway

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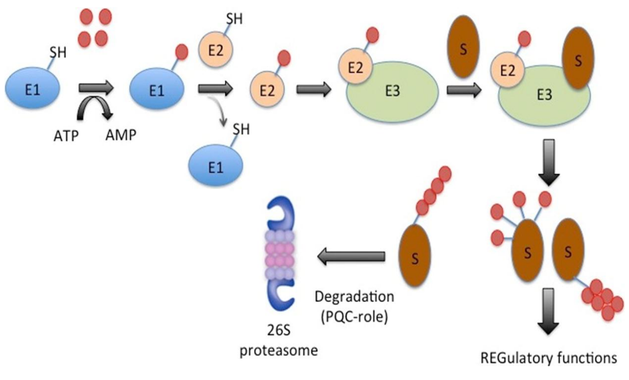
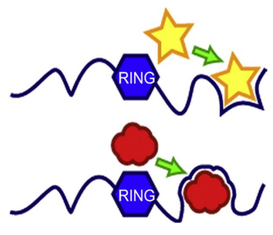
Introduction

Ubiquitination: an important cellular control mechanism



Conformational plasticity model

(Rosenbaum et al (2011), Molecular Cell 41: 93-106)



Ubiquitination system

- Recognizes multiple shapes/types of misfolded substrates
- Intrinsically disordered proteins capable of binding plasticity

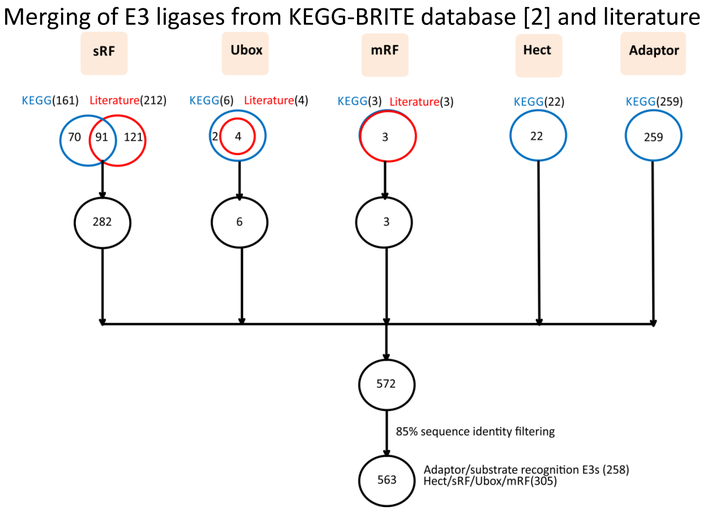
Objectives

- Intrinsically disordered proteins (IDPs) play important roles in molecular recognition, signaling and regulatory pathways; implicated in diseases [1]
- Large-scale bioinformatics analysis of structural disorder in ubiquitin system
- Analysis of functional importance of intrinsic protein disorder in E3 enzymes
- Study the role of structural disorder in substrate-recognition functions of E3s, and in the mechanism of ubiquitination by enabling large conformational changes

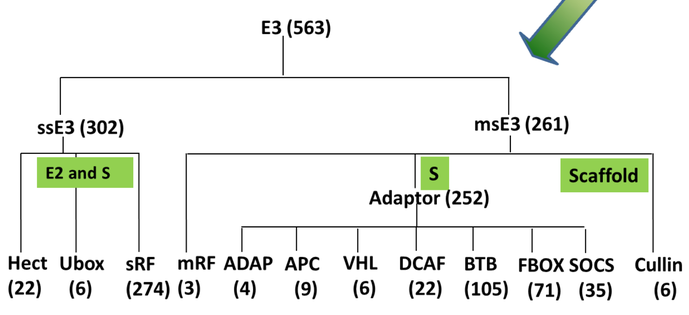
Methods

I. Dataset creation

Merging of E3 ligases from KEGG-BRITE database [2] and literature [3]



Enzyme class	E1	E2	E3
No. of entries	2	29	563



II. Prediction of structural disorder from protein sequence

IUPred [4], FoldIndex [5] and DisProt-VSL2 [6] disorder predictors were used

Calculations: Residue-specific disorder scores, number and ratio of disordered residues, length of longest consecutive disordered segment

III. Interaction classification

E3 Protein-protein interaction data from two sources (Literature [3] & STRING database [7]).

E3s grouped into **hubs** (H, k = 25), **intermediately connected proteins** (ICP, 4 = k = 24) and **non-hubs** (NH; k =3)

k= no. of partners (i.e., connectivity)

IV. Structural information on E3 interactions

Protein Data Bank (PDB): all structures where human E3s were in complex with any other human protein

V. Conformational change to understand mechanism of Ub transfer

Normal mode (El Nemo webserver, www.igs.cnrs-mrs.fr/elnemo/) and **molecular dynamics** (GROMACS) analyses

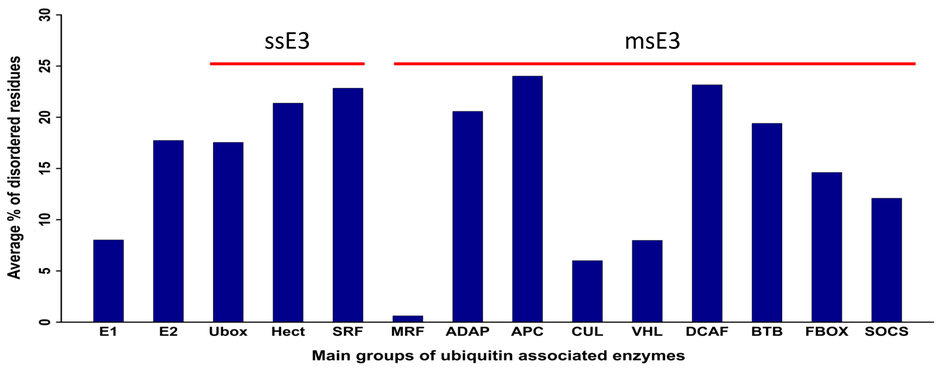
Results

I. Predicted disorder tendency of Ub system

	IUPred	VSL2	FoldIndex
E1	5.97	18.10	20.10
E2	17.74	37.51	32.90
E3	20.03	39.59	33.02

Predicted disorder: E3 > E2 > E1

II. Enzyme sub-families and predicted disorder

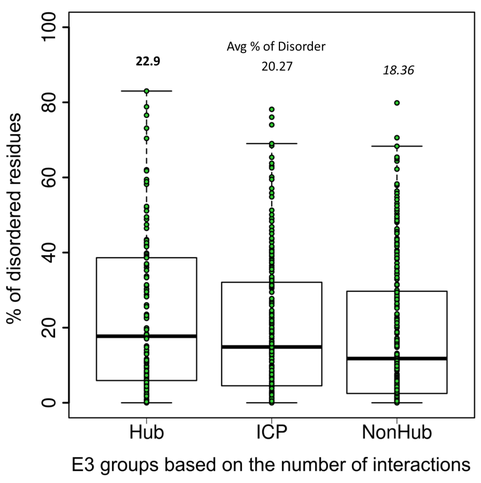


III. Disorder for E2-binding and non-E2-binding regions in E3s

E3 Family	Avg disorder (%)		p-value[c]
	E2-binding domain[a]	Non-E2-binding domain[b]	
Ubox	21.0	19.5	0.7
Hect	1.0	28.2	1.33e-07
SRF	0.43	23.8	< 2.2e-16
MRF	0.0	28.5	0.098
Total	1.03	24.2	< 2.2e-16

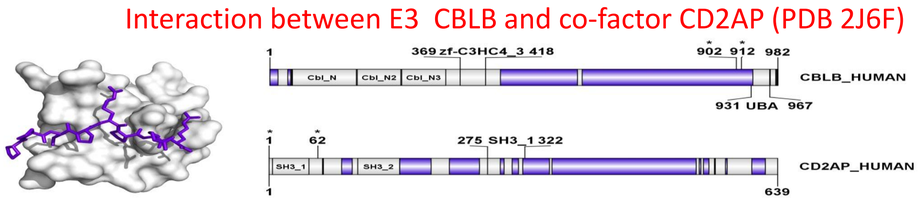
a E2-binding domains include RING/U-box/HECT domains taken from Pfam
b All Pfam regions excluding RING/U-box/HECT domains
c P-values from the one-tailed Mann-Whitney U-test corresponding to the hypothesis that non-E2-binding domains are significantly more disordered that E2-binding domains.

IV. E3 disorder and connectivity

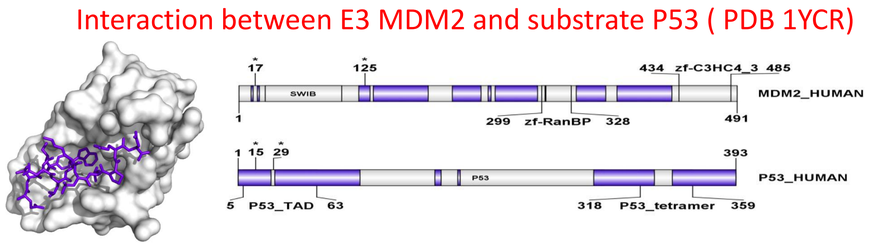


V. E3s use disordered regions/segments for interaction

Interaction between E3 CBLB and co-factor CD2AP (PDB 2J6F)



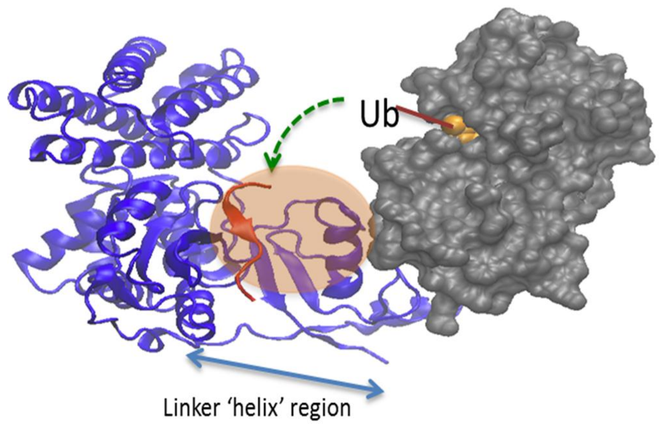
Interaction between E3 MDM2 and substrate P53 (PDB 1YCR)



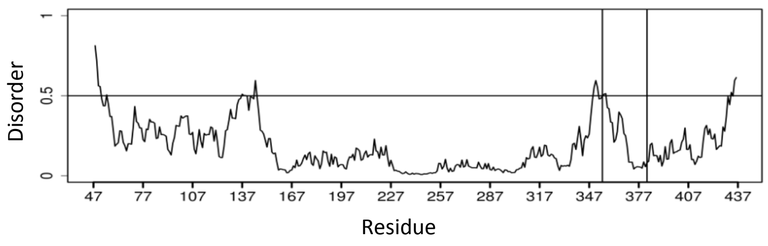
VI. Structural organization and molecular dynamics analysis of an E2-E3-substrate complex

Case study for studying long-range conformational changes

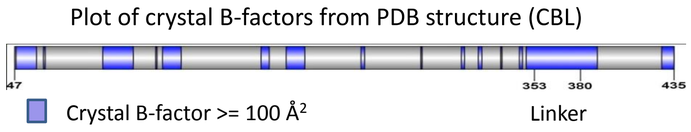
PDB 4A4C [E2 (UbcH5B), E3 ligase (CBL), Substrate (Tyr kinase ZAP-70)]

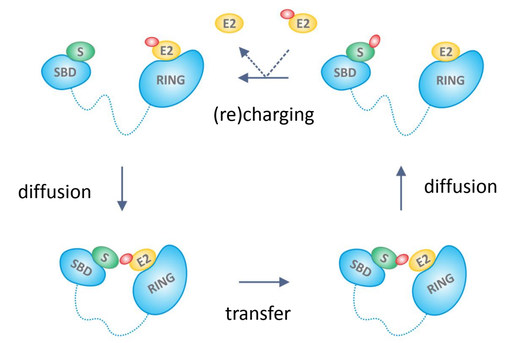


Predicted disorder for E3 (CBL) sequence

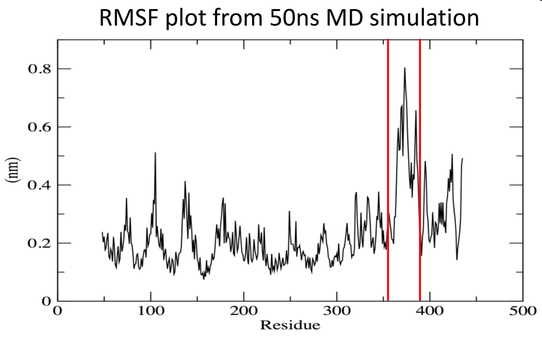


Plot of crystal B-factors from PDB structure (CBL)

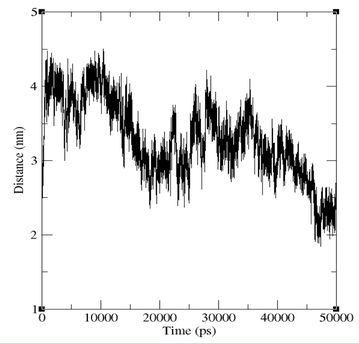




RMSF plot from 50ns MD simulation



Dist (E2 cat. CYS and Zap70 N-term) from MD



Conclusions

1) Predicted disorder : E3>E2 >E1
2) Disorder : Substrate/adaptor binding region > RING/scaffold domain
3) Level of disorder ∞ connectivity.
4) Disorder of E3 increases with 'hubness'.
5) E3: Disordered segments bind substrate
6) ssE3s : Disordered linker allows conformational change for Ub transfer

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