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Mapping protein-protein interactions and architecture of large protein complexes using mass spectrometry.

Jie Luo, PhD
Research Scientist
Institute for Systems Biology

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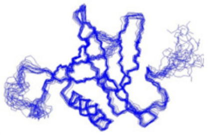
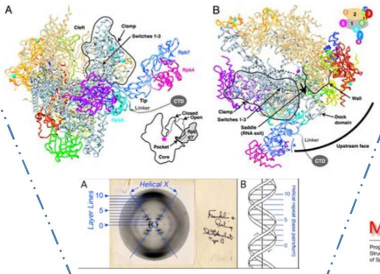
Protein-Protein interactions & Protein structure

NMR

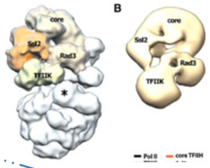


X-ray
Crystallography

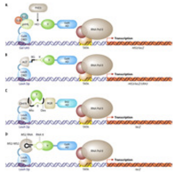


Computer
modeling
Homology
/ab initio

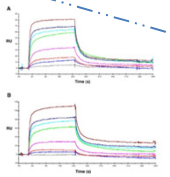
EM



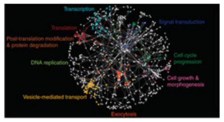
Yeast 2
hybrid



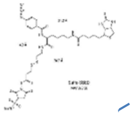
SPR

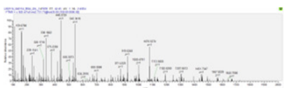


Genetics/
mutagenesis

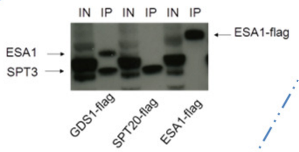


Chemical/ Enzymatic
labeling /crosslinking

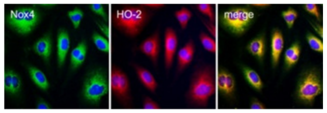




Mass spectrometry
AP-MS
Native MS
IM-MS
H/D exchange MS
CXMS



Co-IP
Chromatography



FRET/
Co-localization

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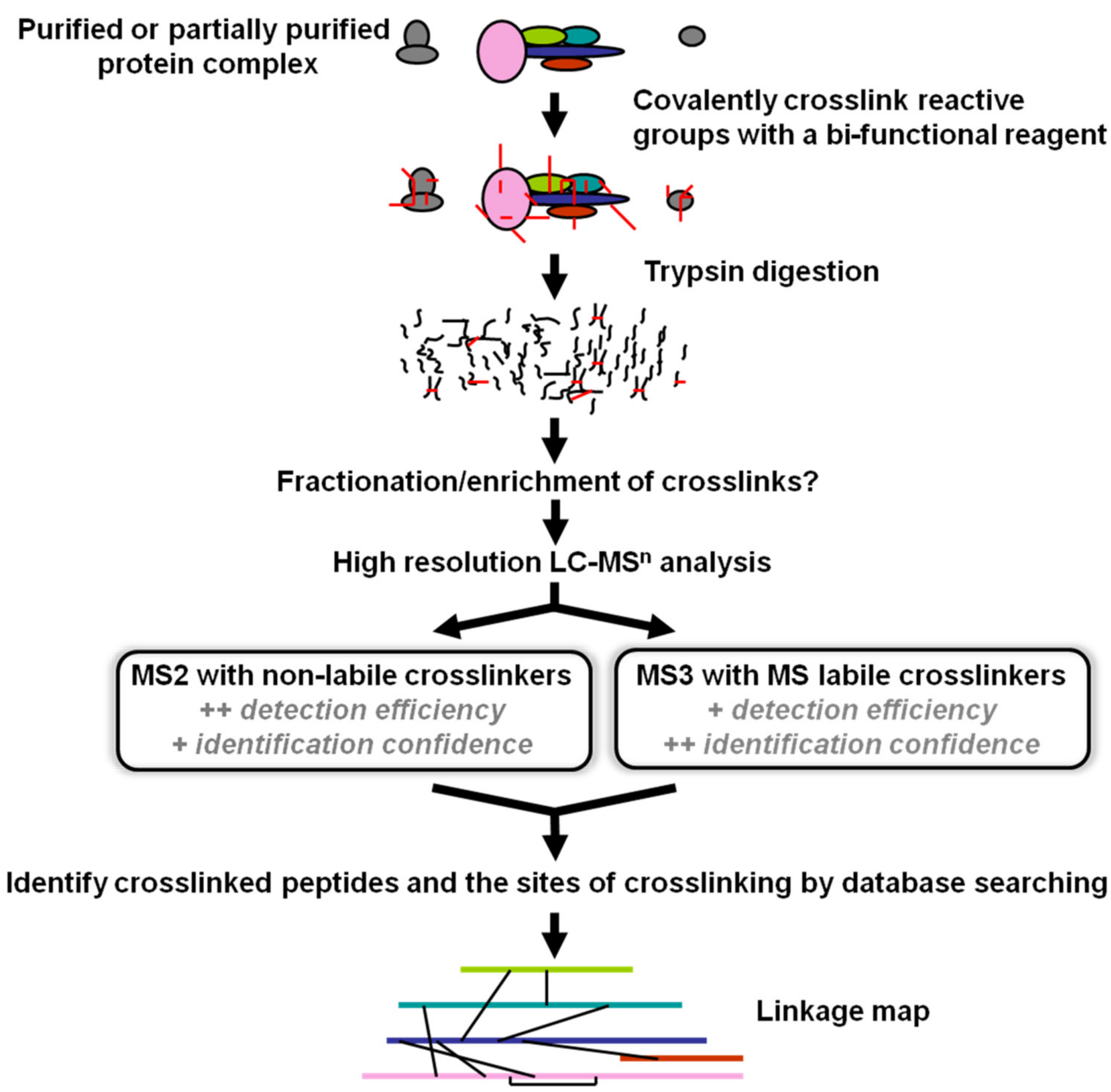

Purified or partially purified protein complex

Covalently crosslink reactive groups with a bi-functional reagent

Trypsin digestion

Fractionation/enrichment of crosslinks?

High resolution LC-MS^n analysis

MS2 with non-labile crosslinkers
++ detection efficiency
+ identification confidence

MS3 with MS labile crosslinkers
+ detection efficiency
++ identification confidence

Identify crosslinked peptides and the sites of crosslinking by database searching

Linkage map

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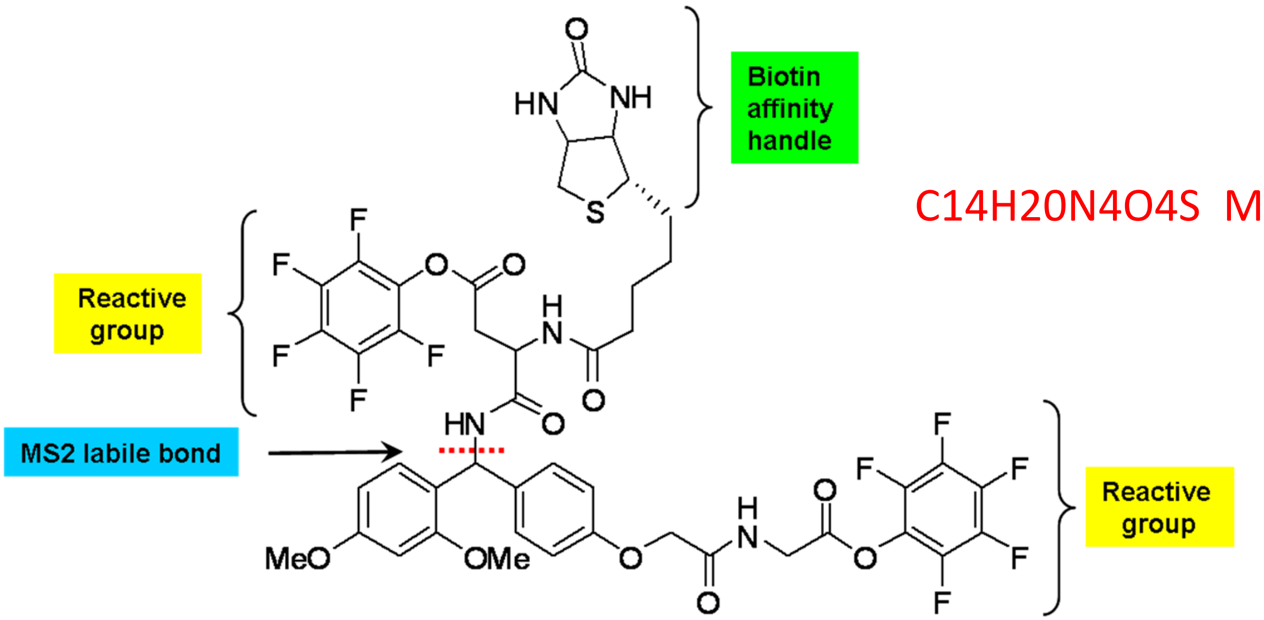
BDRG crosslinker



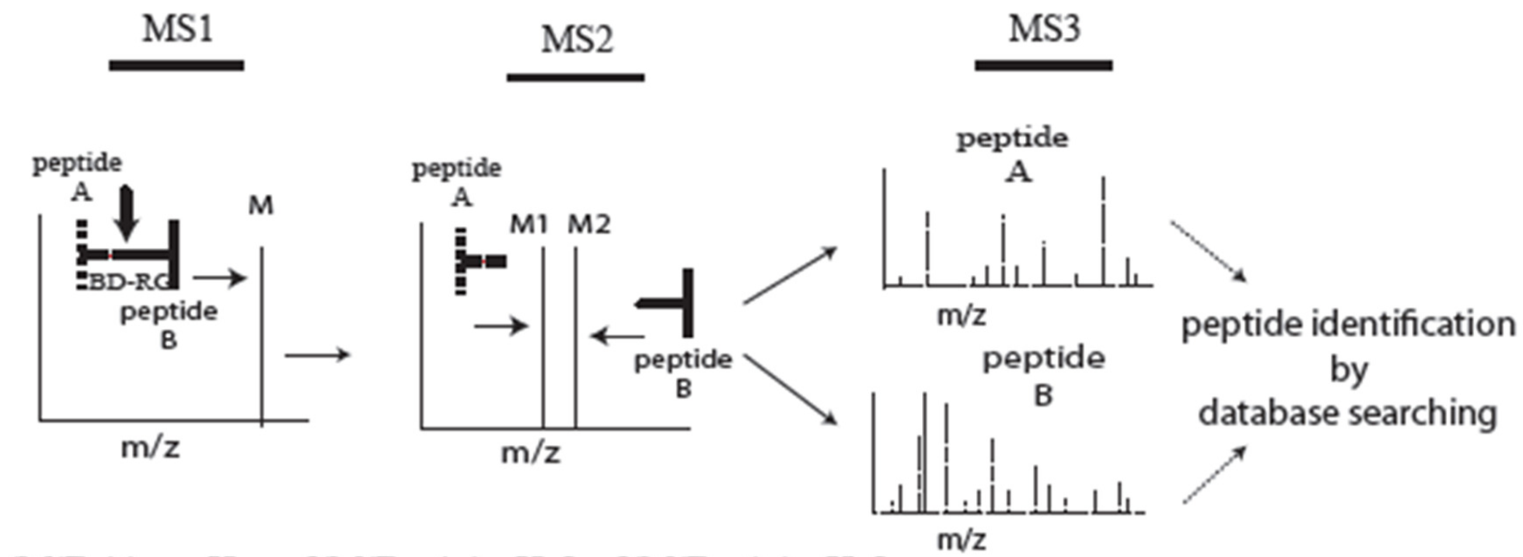
C14H20N4O4S MW: 340.12

Chemical Formula: $C_{45}H_{39}F_{10}N_5O_{11}S$
Exact Mass: 1047.22

C19H19NO5 MW: 341.12

Ranish and Luo, US Patent No. 8,535,948

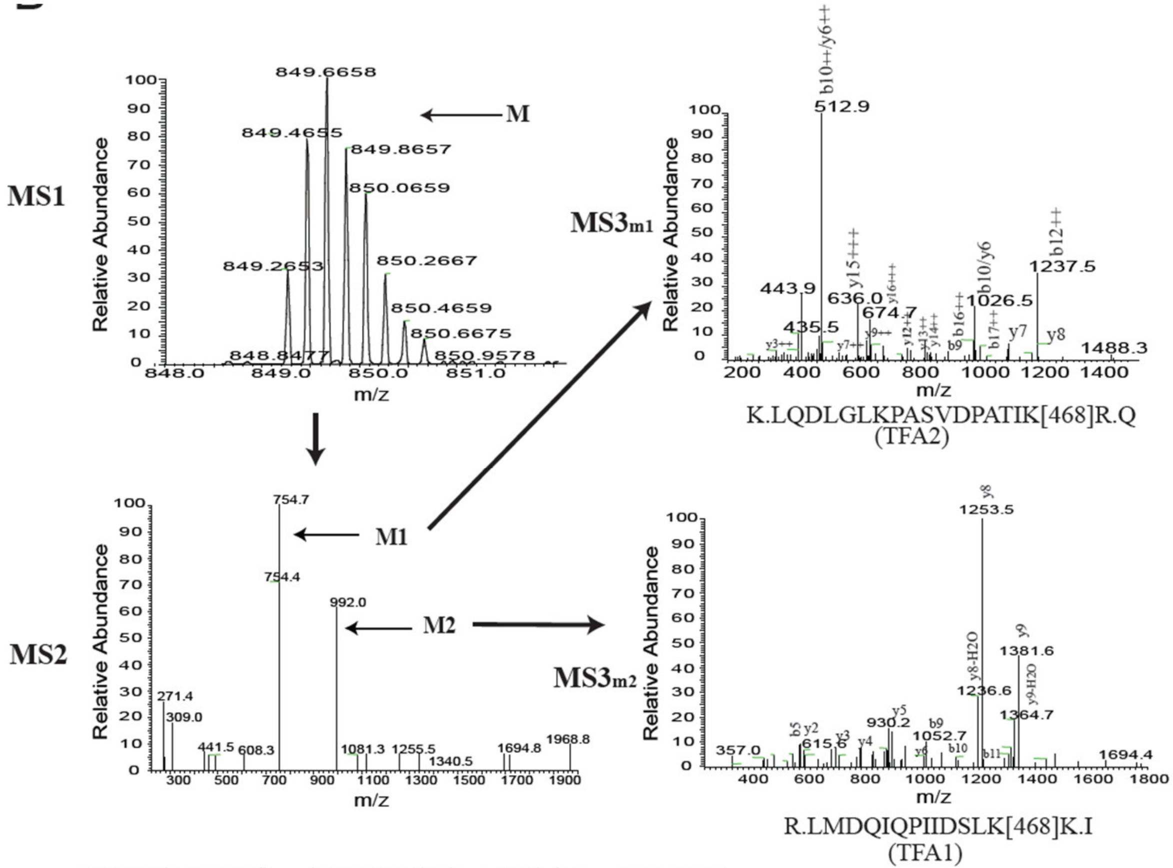
Identification of Crosslinked peptides



$$\underset{\text{MW pepride A-B}}{(M/Z_m)*n - nH+} = \underset{\text{MW peptide A}}{[(M/Z_{m1}*n)-nH+]} + \underset{\text{MW peptide B}}{[(M/Z_{m2}*n)-nH+]}$$

1. Single differential search on Lys residue.
2. Only two major fragments for either A---B or B---A linkage.

Example of a crosslinked peptide



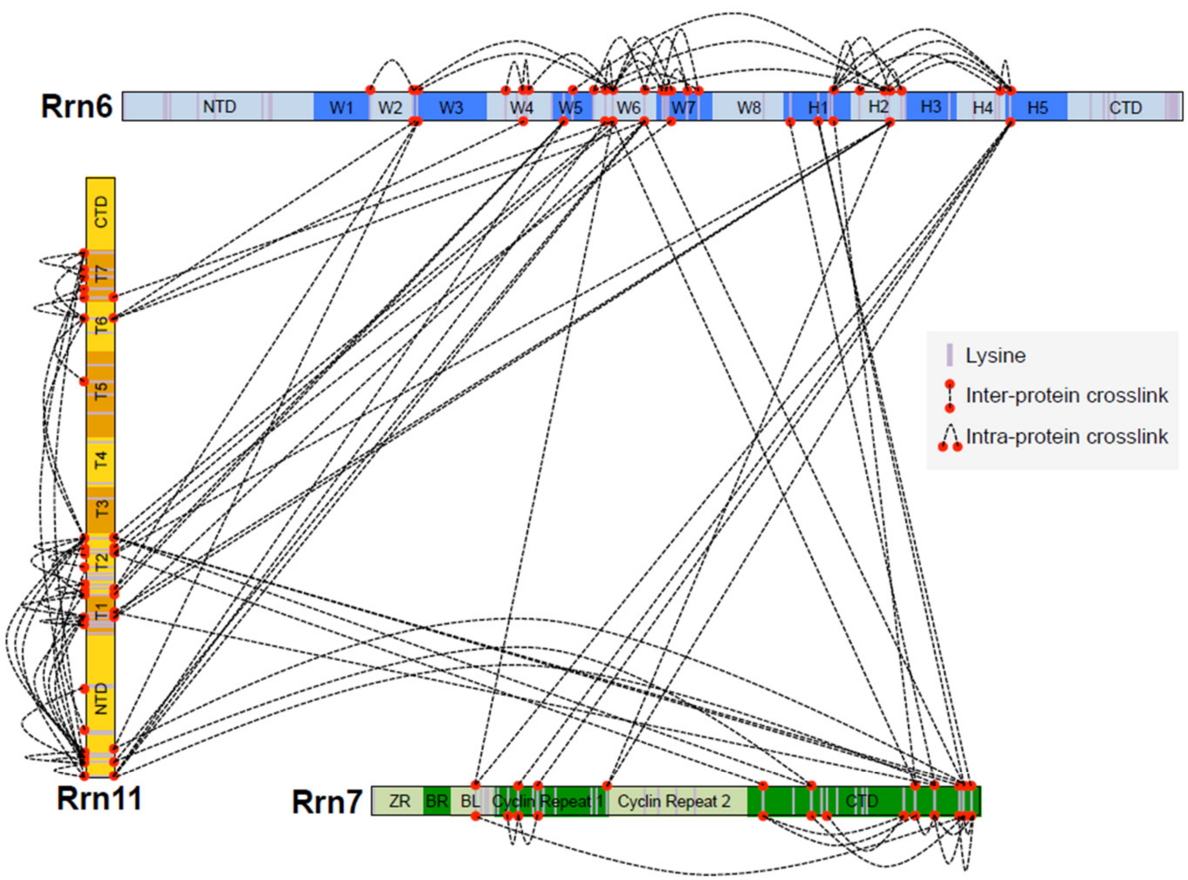
MW M(measured) = (849.2653 (5+) - 1.007) * 5 = 4241.2899

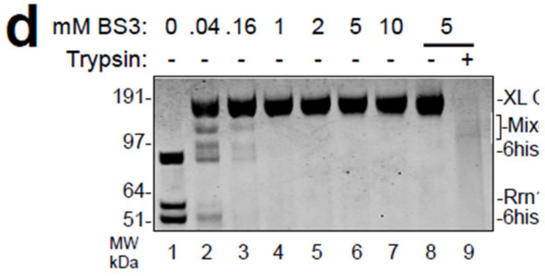
MW M1(theoretical) + MW M2 (theoretical) = 2260.2124 + 1981.0356 = 4241.2480 (10 ppm mass error)

1. IDed modified peptides from the same precursors.
2. Independent verification of crosslinks by the high accurate masses.

Luo et al., Mol Cell Proteomics, 2012 mcp.M111.008318

Crosslinking map of RNA Pol I Core factor





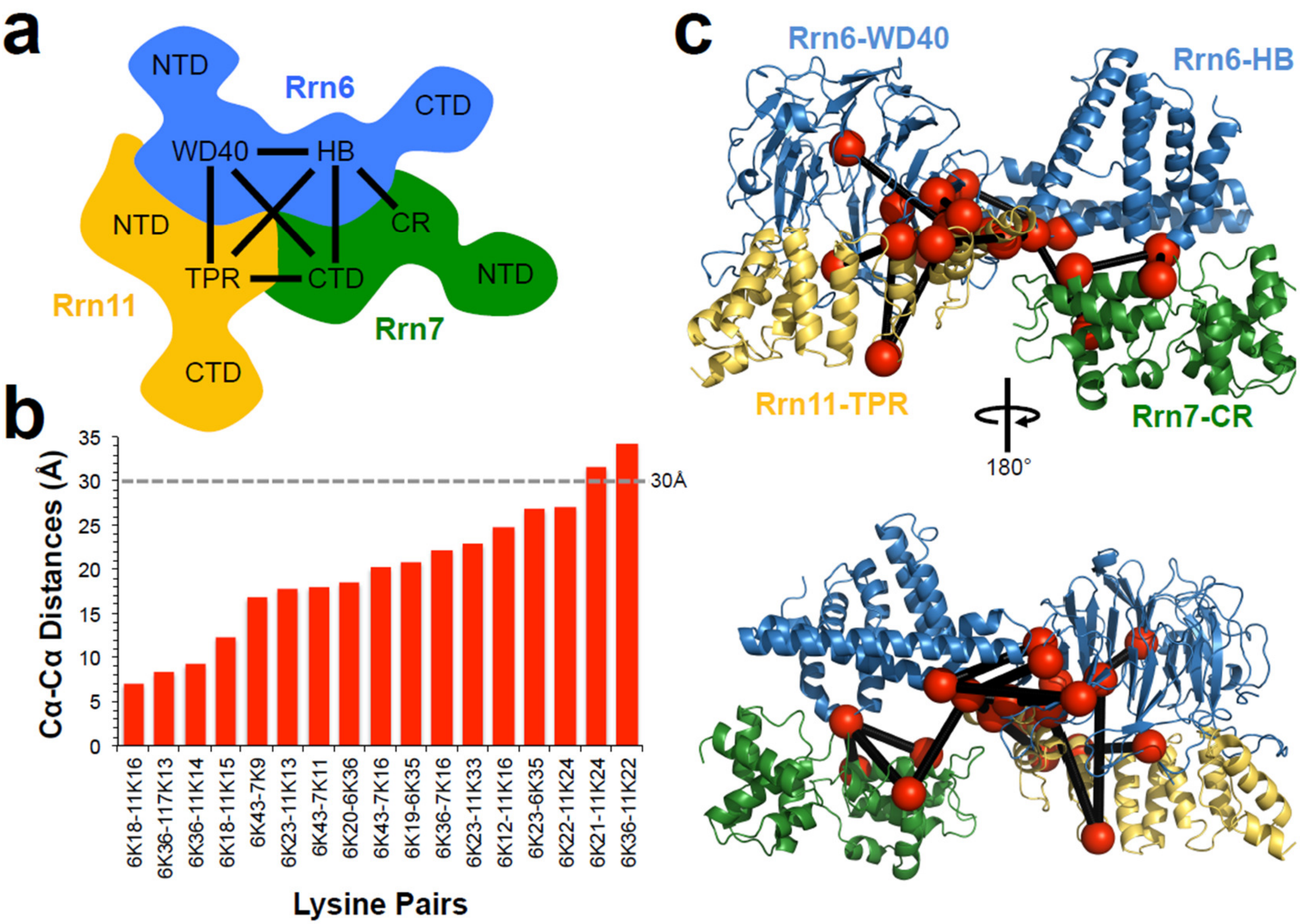
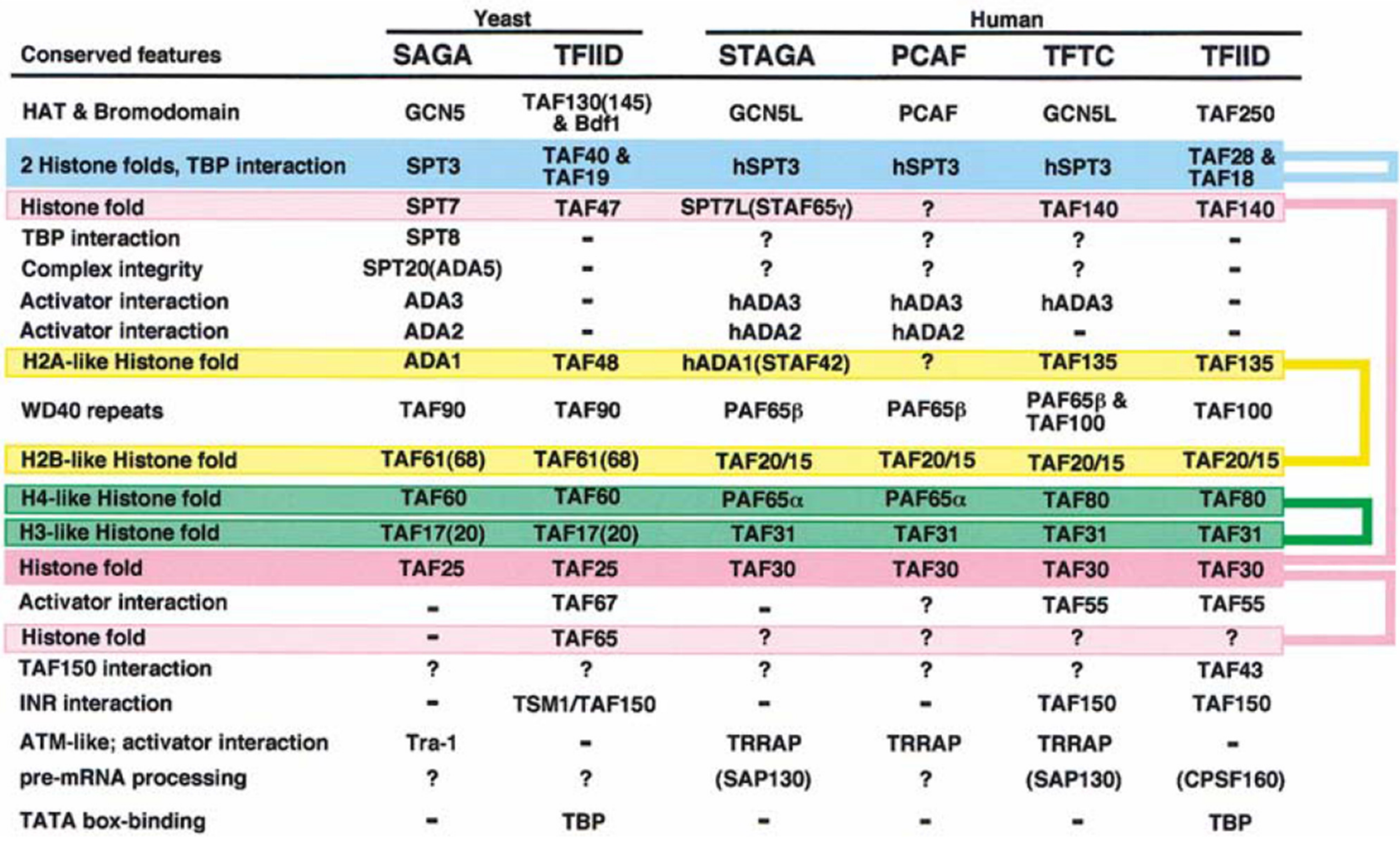


Figure 6. Molecular model of Core Factor

Knutson, Luo, Ranish and Hahn, Nature Struct Mol Biol., 2014

Study the architecture of the SAGA complex



	Yeast		Human			
Conserved features	SAGA	TFIID	STAGA	PCAF	TFTC	TFIID
HAT & Bromodomain	GCN5	TAF130(145) & Bdf1	GCN5L	PCAF	GCN5L	TAF250
2 Histone folds, TBP interaction	SPT3	TAF40 & TAF19	hSPT3	hSPT3	hSPT3	TAF28 & TAF18
Histone fold	SPT7	TAF47	SPT7L(STAF65γ)	?	TAF140	TAF140
TBP interaction	SPT8	-	?	?	?	-
Complex integrity	SPT20(ADA5)	-	?	?	?	-
Activator interaction	ADA3	-	hADA3	hADA3	hADA3	-
Activator interaction	ADA2	-	hADA2	hADA2	-	-
H2A-like Histone fold	ADA1	TAF48	hADA1(STAF42)	?	TAF135	TAF135
WD40 repeats	TAF90	TAF90	PAF65β	PAF65β	PAF65β & TAF100	TAF100
H2B-like Histone fold	TAF61(68)	TAF61(68)	TAF20/15	TAF20/15	TAF20/15	TAF20/15
H4-like Histone fold	TAF60	TAF60	PAF65α	PAF65α	TAF80	TAF80
H3-like Histone fold	TAF17(20)	TAF17(20)	TAF31	TAF31	TAF31	TAF31
Histone fold	TAF25	TAF25	TAF30	TAF30	TAF30	TAF30
Activator interaction	-	TAF67	-	?	TAF55	TAF55
Histone fold	-	TAF65	?	?	?	?
TAF150 interaction	?	?	?	?	?	TAF43
INR interaction	-	TSM1/TAF150	-	-	TAF150	TAF150
ATM-like; activator interaction	Tra-1	-	TRRAP	TRRAP	TRRAP	-
pre-mRNA processing	?	?	(SAP130)	?	(SAP130)	(CPSF160)
TATA box-binding	-	TBP	-	-	-	TBP

Martinez, *Plant Molecular Biology, 2002*

Figure 2. BS3 intermolecular crosslinking map of SAGA-TBP complex.

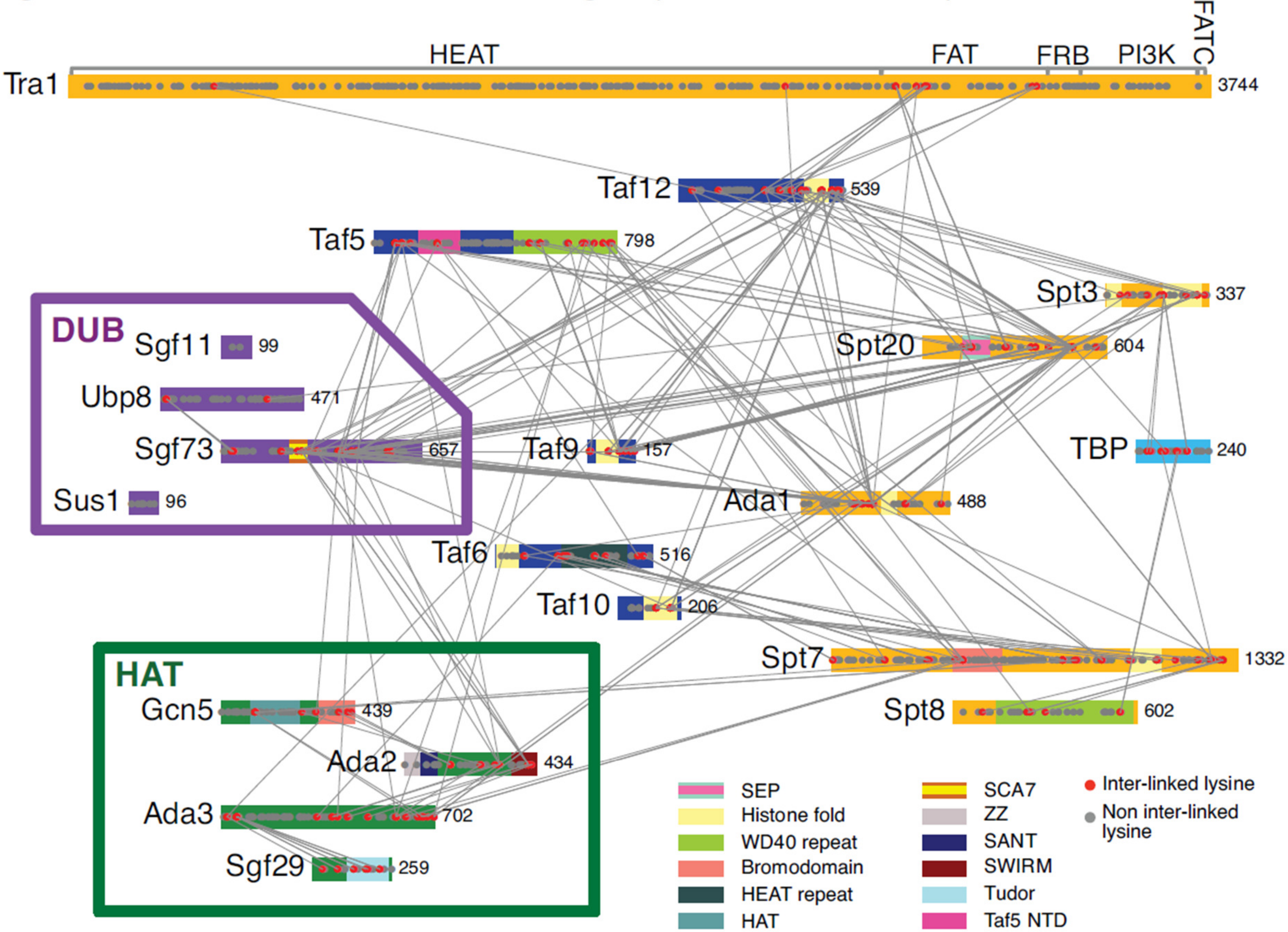


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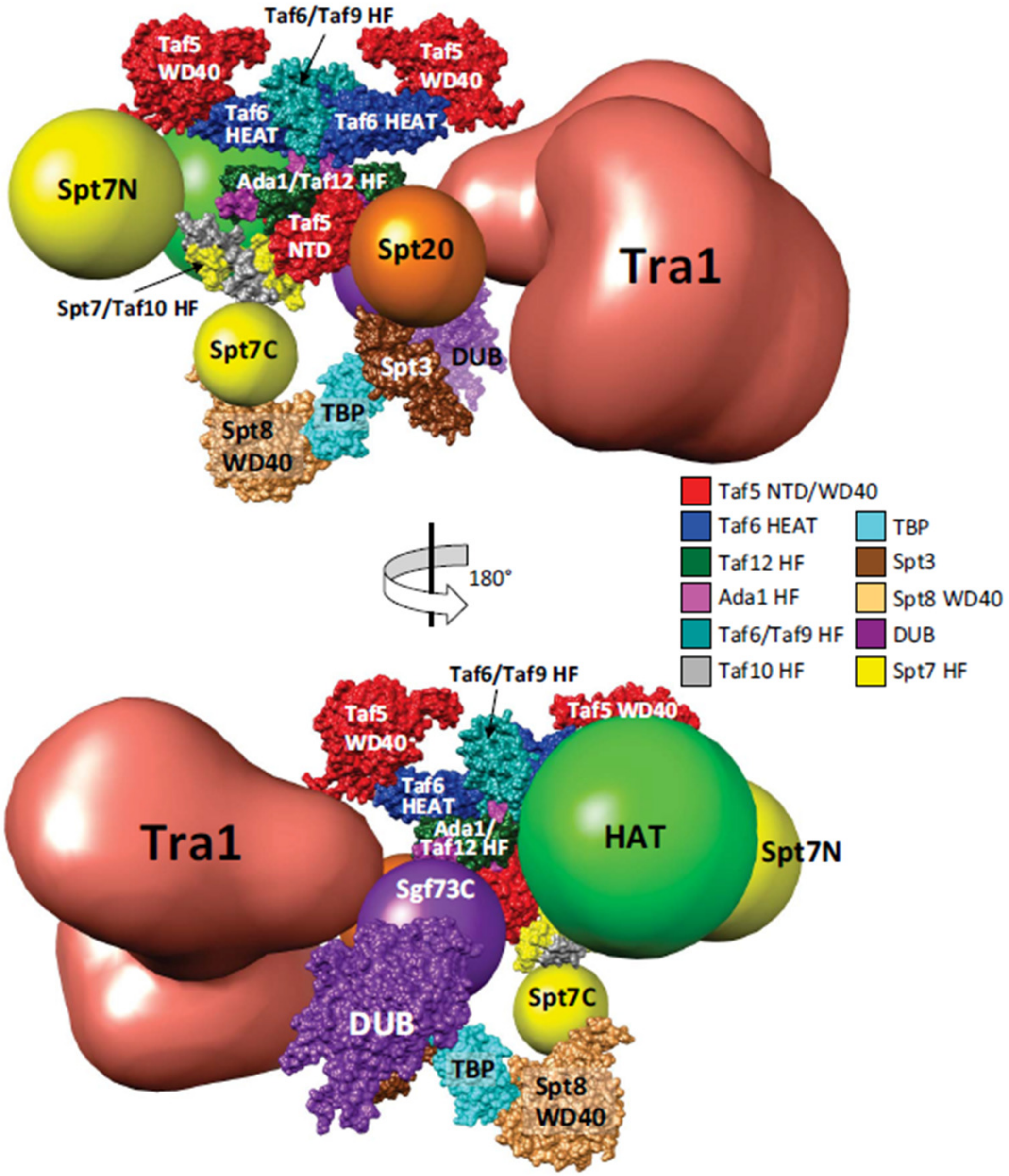
Figure 7. Model for the molecular architecture of the SAGA complex.



Yan, Luo, Ranish and Hahn, EMBO J, 2014

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Wei Yan (now Shanghai Jiaotong U)

Hahn Lab, FHCRC

Bruce Knutson
Yan Han

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