(1) Quantitative Analysis of Footprinting

(2) Looking at Estrogen Receptor from Small Angles

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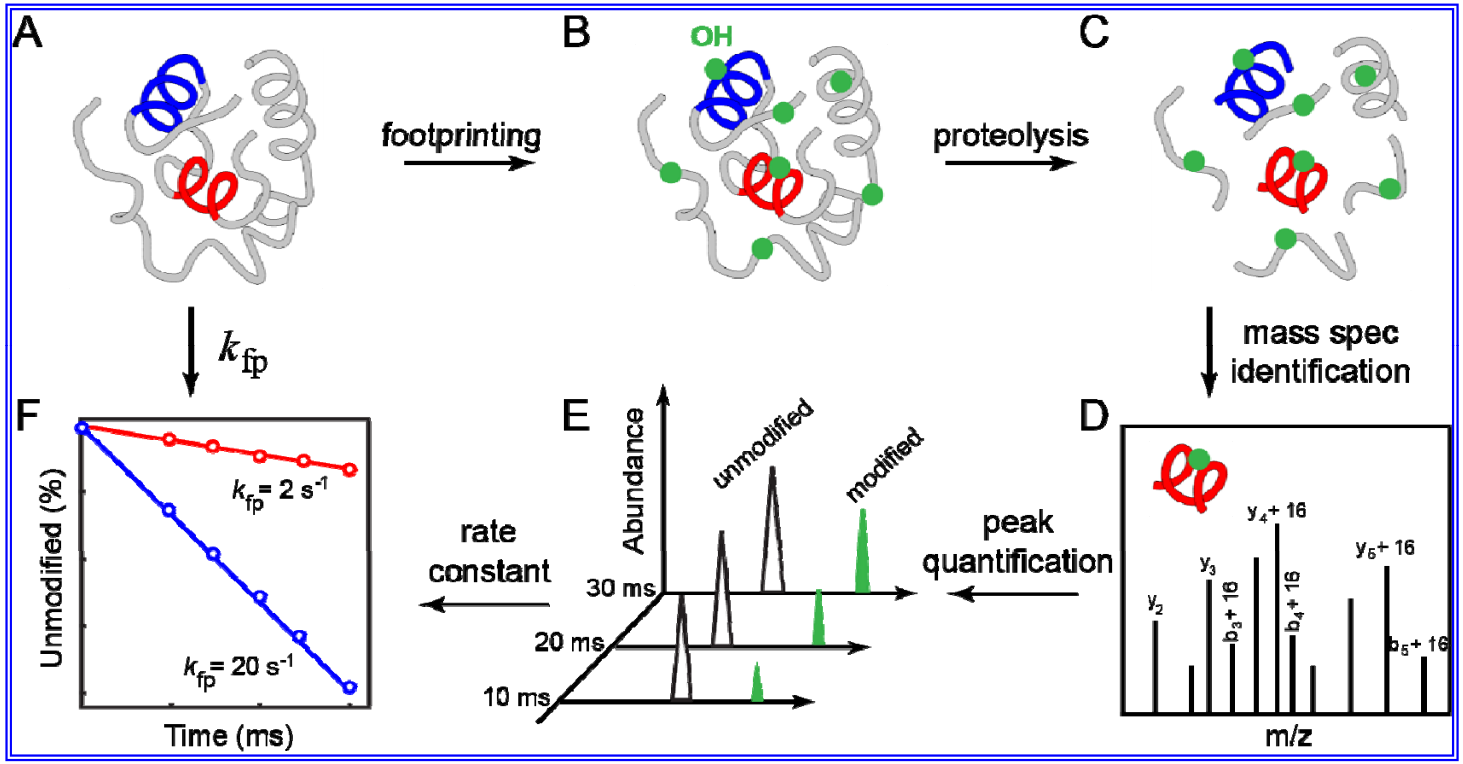
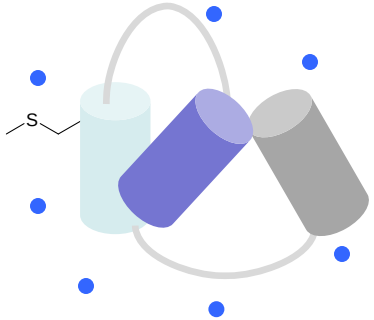
Schematic of Protein Footprinting Experiments

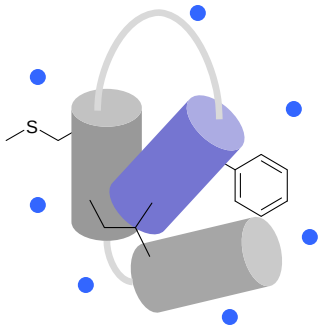


- Rate constants (k_{fp}) for different protein regions
- Similar to H/D exchange (backbone vs. sidechain)

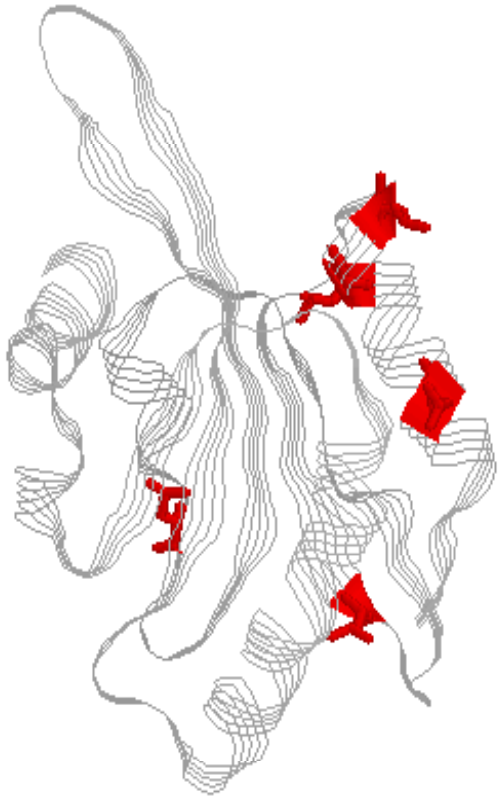
Traditional analysis of footprinting and Challenges



free *vs.* ligand-bound

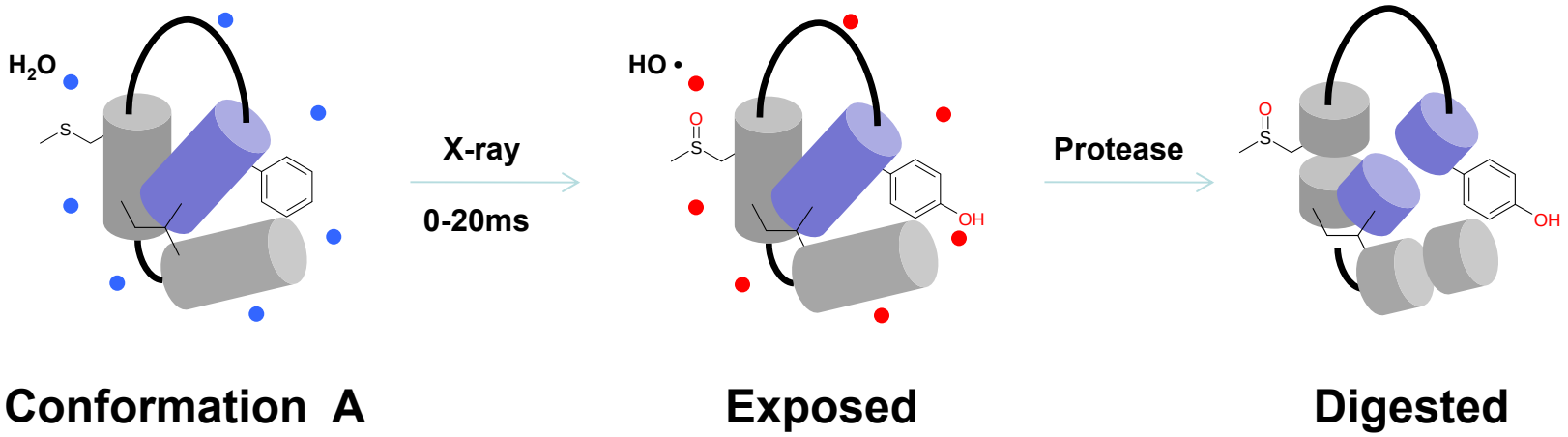


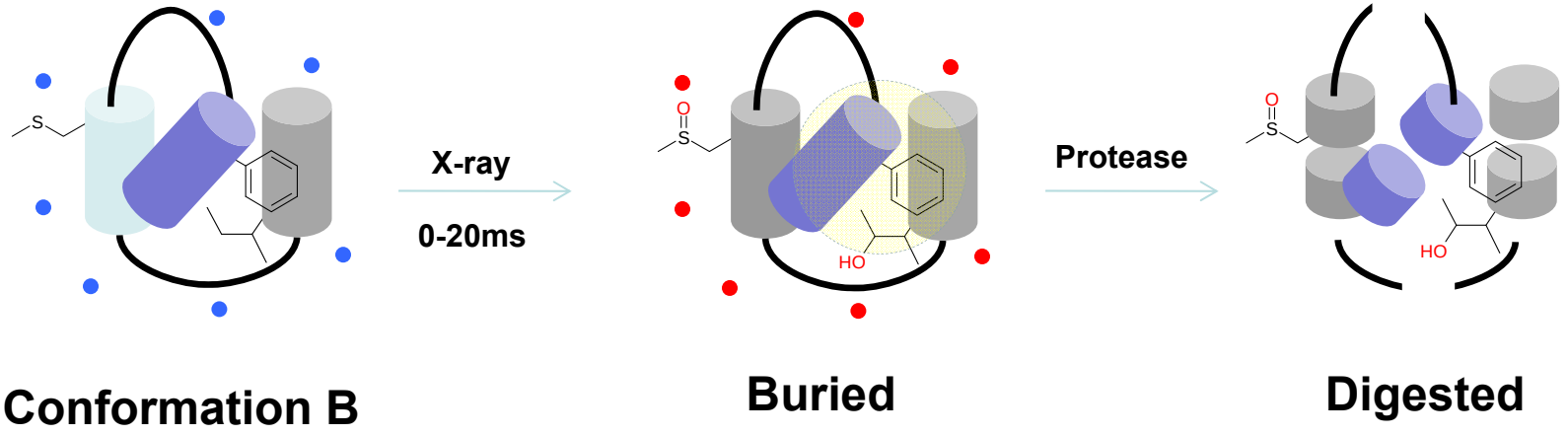
Cross-state comparison



Cross-site comparison?
(within each functional state)

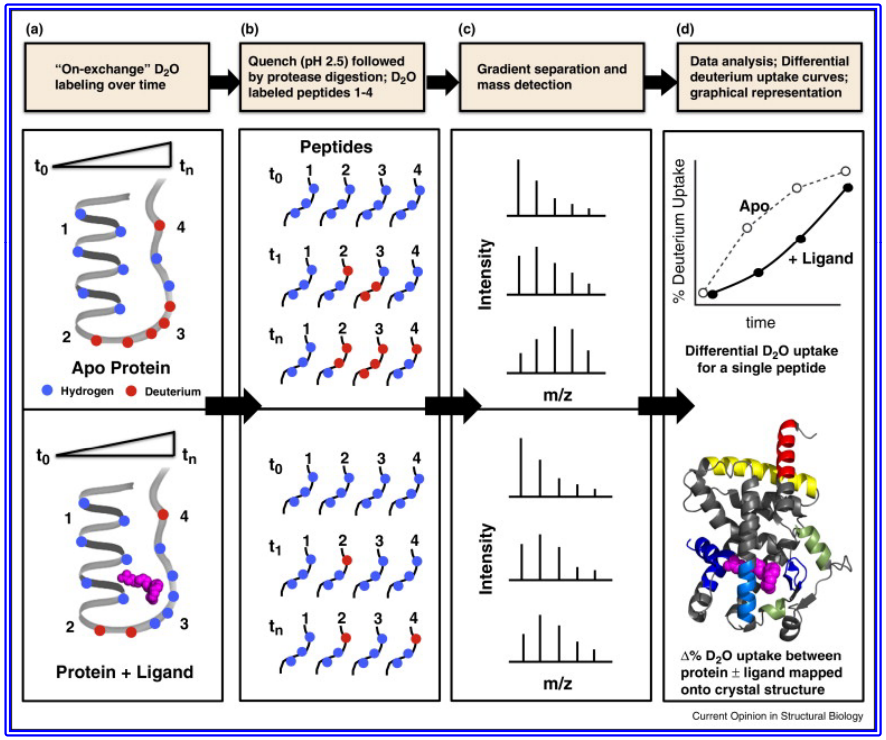
Traditional analysis of footprinting data



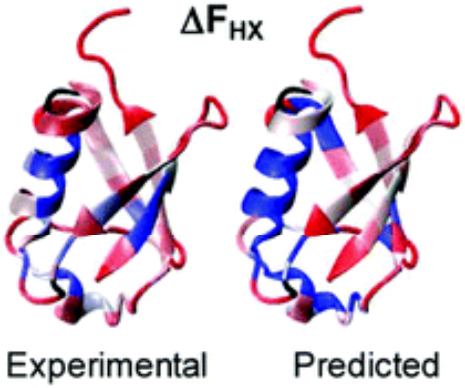


From Janna

H/D exchange and the Protection Factor analysis

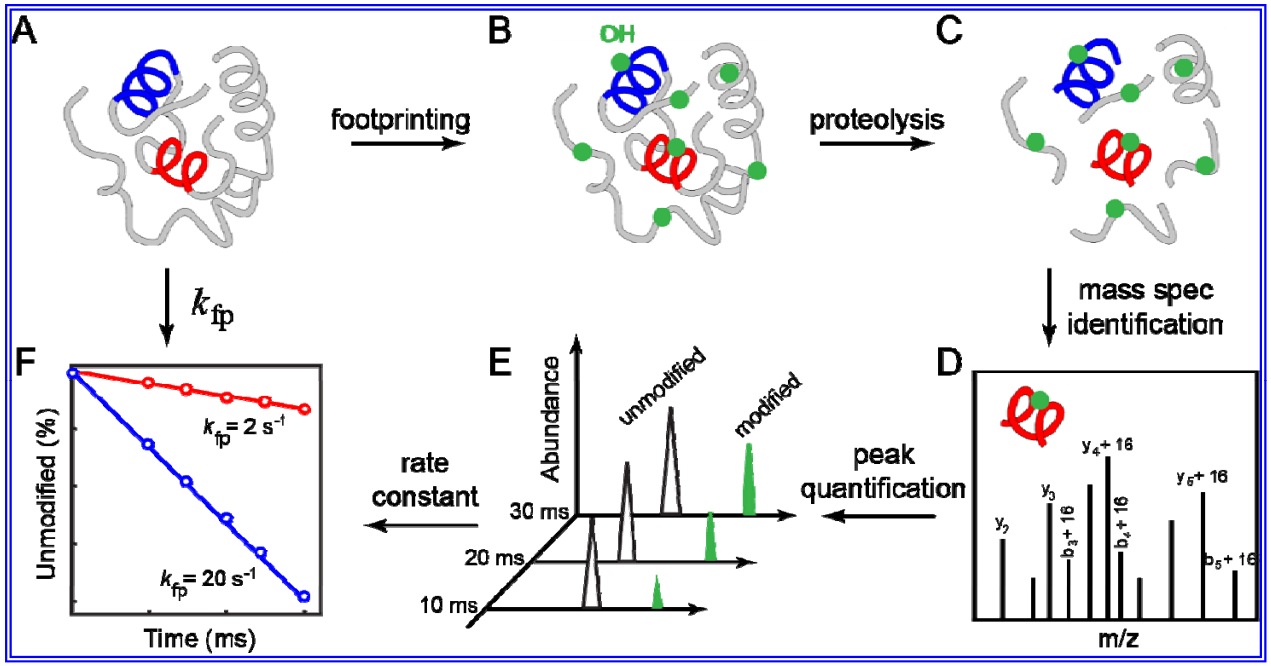


$$PF = \frac{k_{int}}{k_{ex}}$$



Bai and Englander 1993; Craig *et al* (2011); Marciano *et al* (2014)

A protection factor analysis for protein footprinting?



$$\mathrm{PF} = \frac{\sum_i k_{\mathrm{int}}^i}{k_{\mathrm{fp}}} \qquad \text{OR} \qquad \mathrm{PF} = \frac{k_{\mathrm{int}}}{k_{\mathrm{fp}}}$$

(peptide-level) (single-residue)

$^{\bullet}OH$ Intrinsic Reactivity of Amino Acids

Table 1. Rate Constants for Reaction of Amino Acids with Hydroxyl Radical and Hydrated Electrons[a]

	HO^-		e_{aq}^{-1}	
substrate	rate (M^{-1} s^{-1})	pH	rate (M^{-1} s^{-1})[b]	pH
Cys	3.5×10^{10}	7.0	1.0×10^{10}	−7
Trp	1.3×10^{10}	6.5−8.5	3.0×10^{8}	7.8
Tyr	1.3×10^{10}	7.0	2.8×10^{8}	6.6
Met	8.5×10^{9}	6−7	4.5×10^{7}	7.3

Cys[1]	Met[2]	Trp	Tyr	Phe	His	Leu[3]	Ile[3]	Arg	Lys
29.2	20.5	17.4	12.0	11.2	10.0	9.3	4.4	2.9	2.2
Val	Thr	Ser	Pro	Glu	Gln	Asn	Asp	Ala	Gly
1.9	1.6	1.4	1.0	0.69	0.66	0.44	0.42	0.14	0.04

Thr	5.1×10^{8}	6.6	2.0×10^{7}	7.0
Lys	3.5×10^{8}	6.6	2.0×10^{7}	7.4
Ser	3.2×10^{8}	~6	$<3 \times 10^{7}$	6.1
Glu	2.3×10^{8}	6.5	$1\text{--}2 \times 10^{7}$	5.7−7
Ala	7.7×10^{7}	5.8	1.2×10^{7}	7.4
Asp	7.5×10^{7}	6.9	1.8×10^{7}	7.0
Asn	4.9×10^{7}	6.6	1.5×10^{8}	7.3
Gly	1.7×10^{7}	5.9	8.0×10^{8}	6.4

[a] http://allen.rad.nd.edu/browse compil.html. [b] Davies, M. J.; Dean, R. T. *Radical-mediated protein oxidation: from chemistry to medicine*; Oxford University Press: 1997; pp 44−45.

Taken from Xu and Chance (2005)

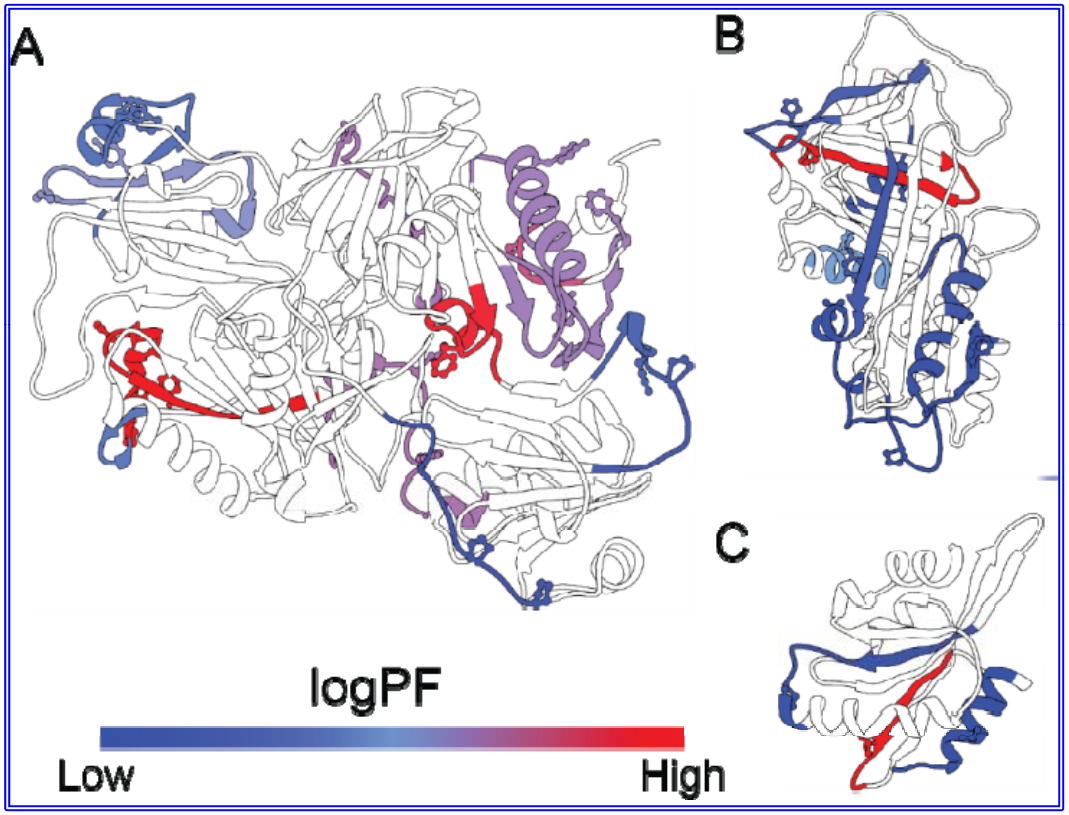
Examples of the footprinting-based PF analysis

TABLE 2. A list of peptides from human gelsolin with k_{fp} , logPF and $\mathcal{S}$ values.

	Peptides	k_{fp} (s.d.) (unit: s^{-1})	logPF (s.d)	$\mathcal{S}_{CG}$ (s.d)	$\mathcal{S}_{all\text{-}atom}$ (s.d.)
1	E_{38}PGLQIWR$_{45}$	0.44 (0.09)	4.27 (0.20)	9.0 (0.5)	10.1 (0.5)
2	F_{49}DLVPVPTNLYGDFFTGDAYVILK$_{72}$	1.47 (0.09)	4.12 (0.06)	7.6 (0.2)	8.7 (0.3)
3	Y_{87}WLGNECSQDESGAAAIFTVQLDDYLNGR$_{115}$	1.86 (0.13)	4.12 (0.07)	6.7 (0.4)	8.3 (0.3)
4	E_{121}VQGFESATFLGYFK$_{135}$	0.69 (0.05)	4.46 (0.07)	6.6 (0.3)	7.9 (0.2)
5	G_{143}GVASGFK$_{150}$	0.48 (0.03)	3.57 (0.06)	4.7 (0.7)	6.6 (0.7)
6	H_{151}VVPNEVVVQR$_{161}$	0.80 (0.06)	3.42 (0.08)	2.7 (0.2)	4.9 (0.6)
7	P_{251}ALPAGTEDTAK$_{262}$	0.58 (0.05)	3.27 (0.09)	2.8 (0.3)	3.2 (0.3)
8	D_{371}PDQTDGLGLSYLSSH$_{386}$	0.68 (0.07)	4.16 (0.10)	6.4 (0.2)	7.5 (0.5)
9	R_{424}IEGSNKVPVDPATY$_{438}$	0.78 (0.09)	3.72 (0.12)	6.7 (0.4)	6.8 (0.4)
10	V_{431}PVDPATYGQFYGGDSYIILYNYR$_{454}$	1.05 (0.10)	4.54 (0.10)	8.9 (0.2)	9.6 (0.3)
11	T_{571}PSAAYLWVGTGASEAEK$_{588}$	0.84 (0.09)	4.03 (0.11)	9.4 (0.3)	9.8 (0.4)
12	A_{600}QPVQVAEGSEPDGFWEALGGK$_{621}$	1.17 (0.04)	3.69 (0.03)	6.8 (0.5)	7.2 (0.3)
13	Q_{722}GFEPPSFVGWFLGWDDDYWSVDPLDR$_{748}$	1.91 (0.15)	4.16 (0.08)	6.9 (0.3)	8.2 (0.3)

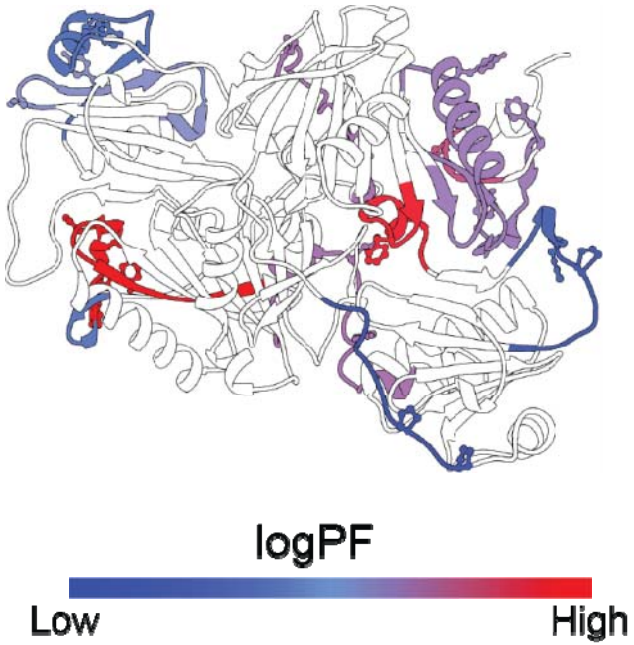
Data of k_{fp} from Kiselar *et al* (2003)

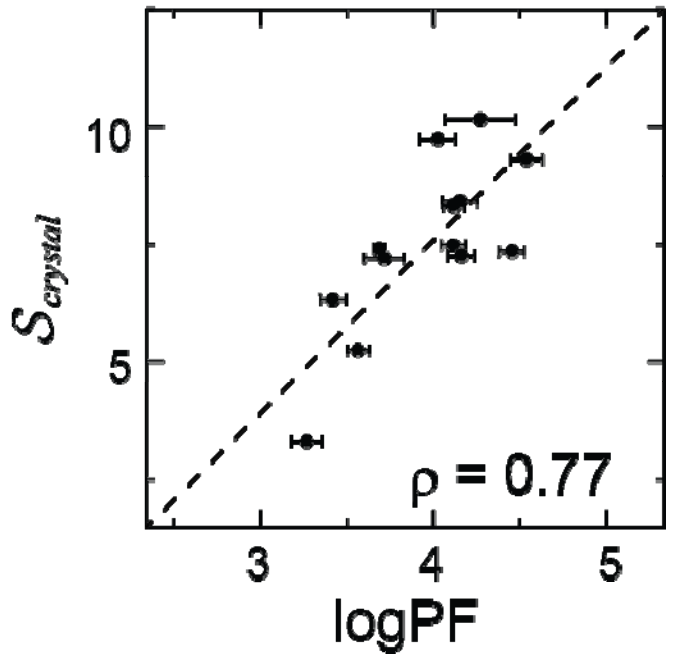
Absolute quantification and Structural mapping enabled by the PF analysis



Low PF – exposed *vs* **high PF - buried**

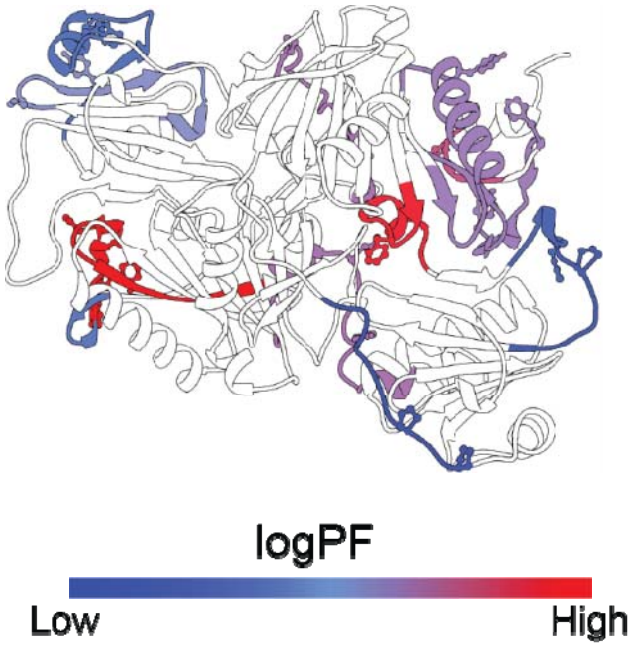
Strong correlation of PFs with Protein structure

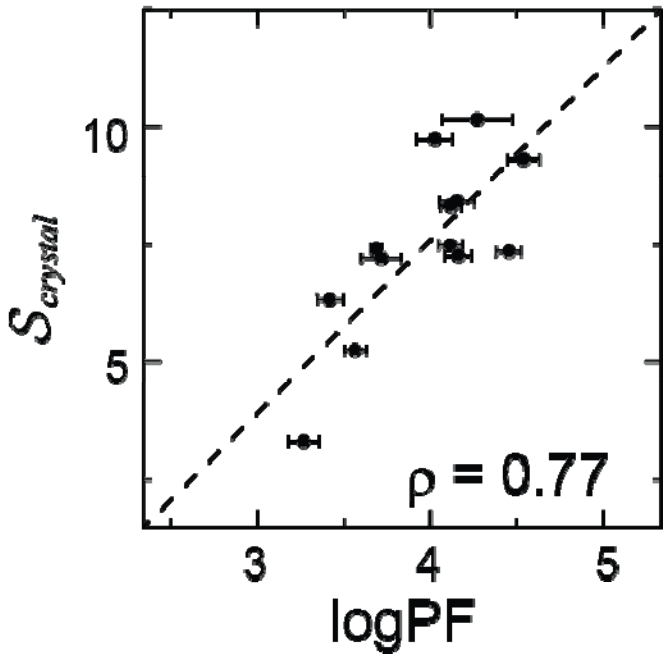




* little direct correlation with k_{fp}'s

Strong correlation of PFs with Protein structure

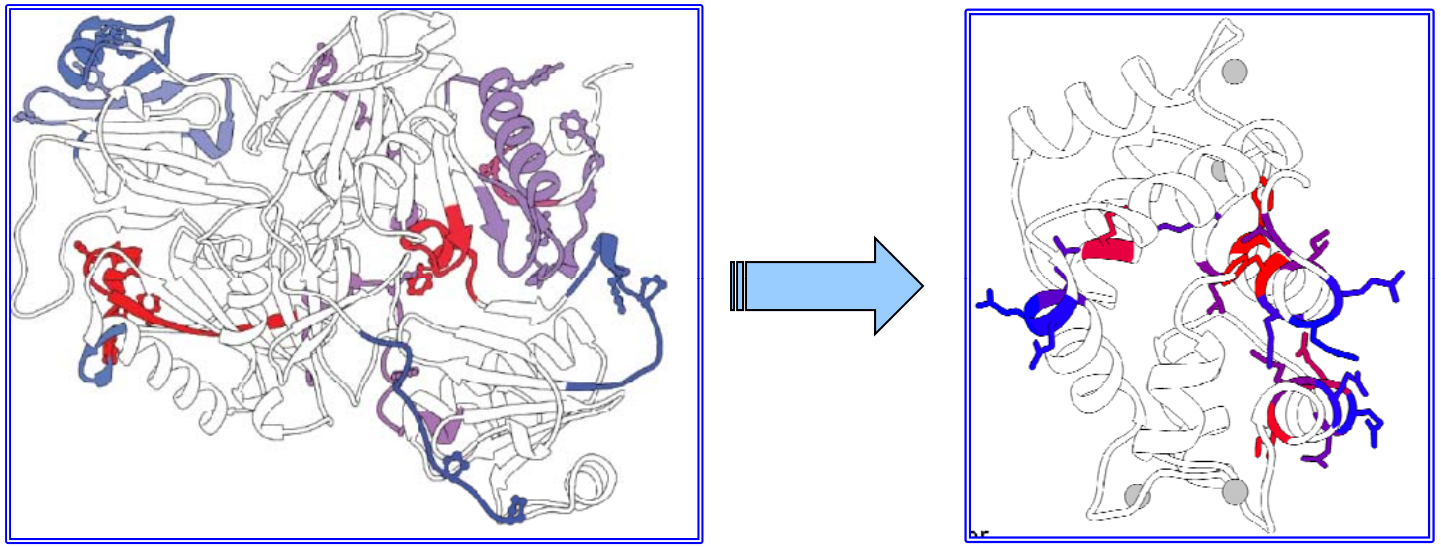




- little direct correlation with k_{fp}'s

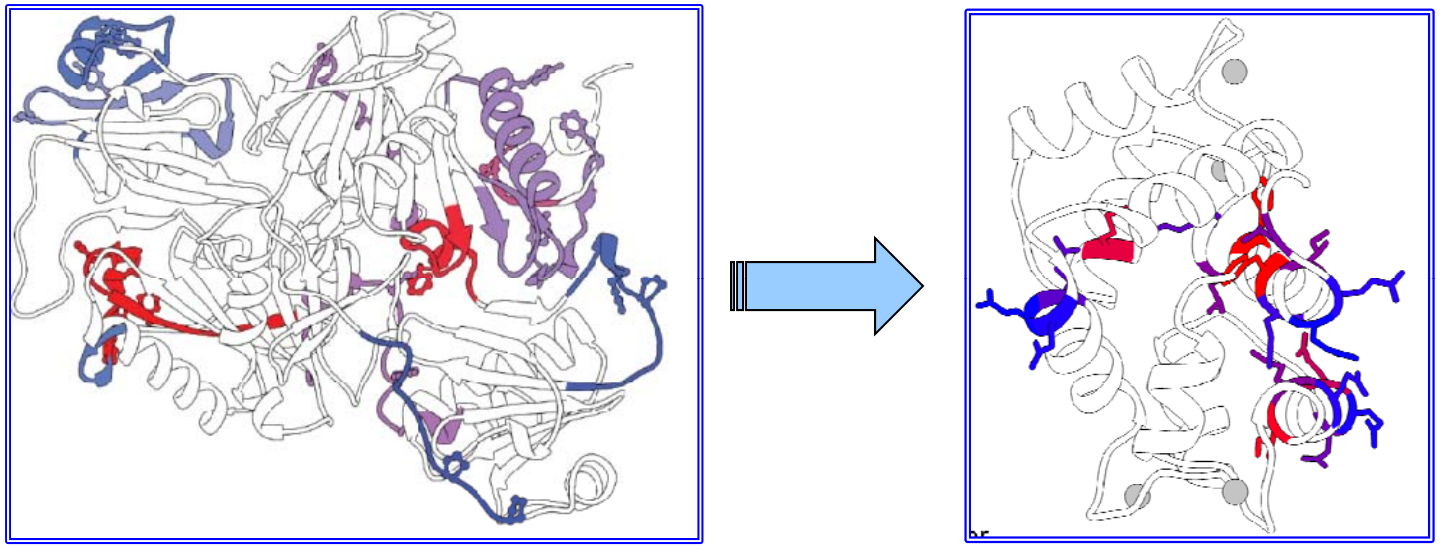
- Used single-residue; use SASA and S both

Going to Single-Residue Resolution



Huang, Ravikumar, Chance, Yang (2014) & Kaur *et al (2015)*

Going to Single-Residue Resolution



- The very same PF analysis (as to peptide-level)
- Identify interaction sites (or non-interacting)
- Broadly applicable to protein-protein complexes

Huang, Ravikumar, Chance, Yang (2014) & Kaur *et al (2015)*

Looking at Estrogen Receptor from Small Angles

Some Facts about BC:

- **Incidence: 1 in 8 (today) *vs.* 1 in 11 (1975)**

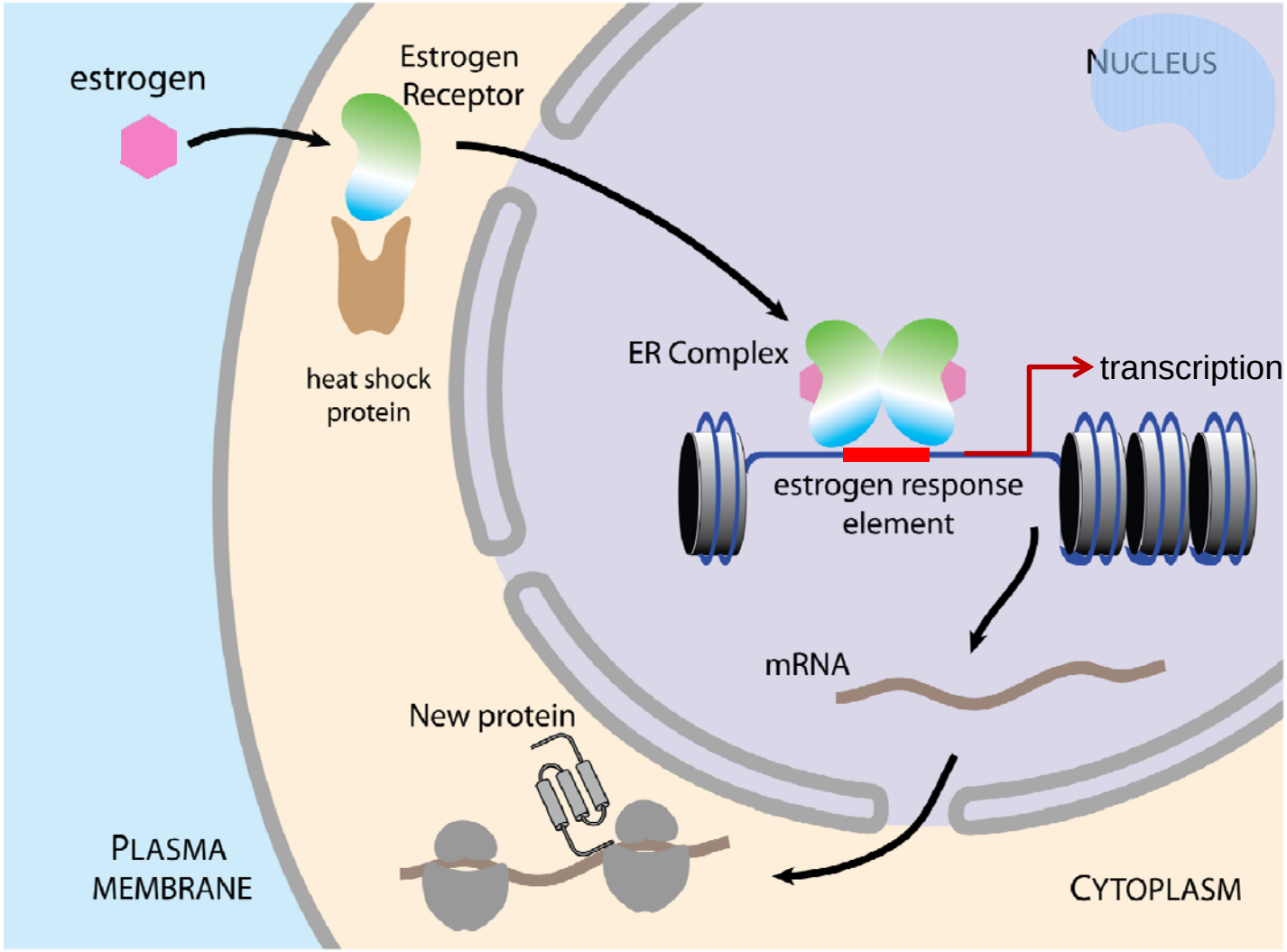
  39,620 (women) and 410 (men) in US (2013)

- **75% is Estrogen Receptor (ER) positive;**

  most deaths occur in ER+ women

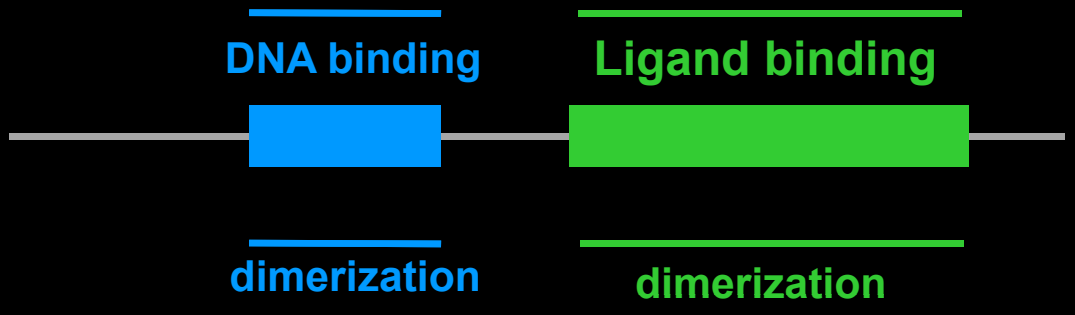
- **No cure if advanced**

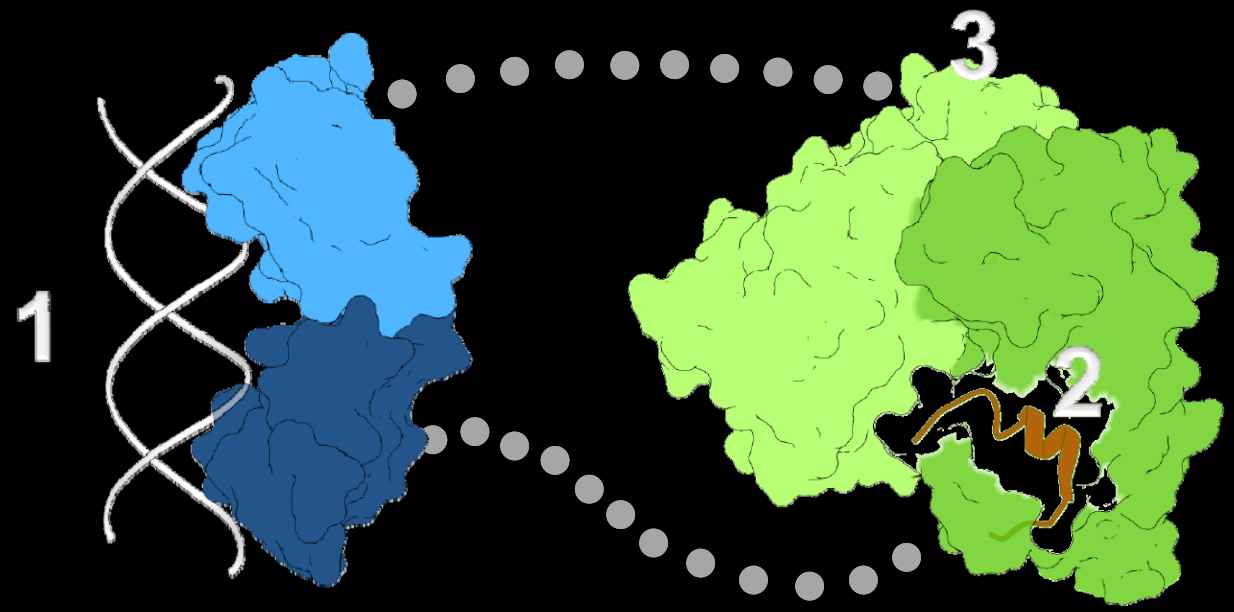
No cure but wish to know: **Transcriptional regulation by ER**



ER dynamics: **1. DNA binding 2. Ligand binding** 3. Ligand independence

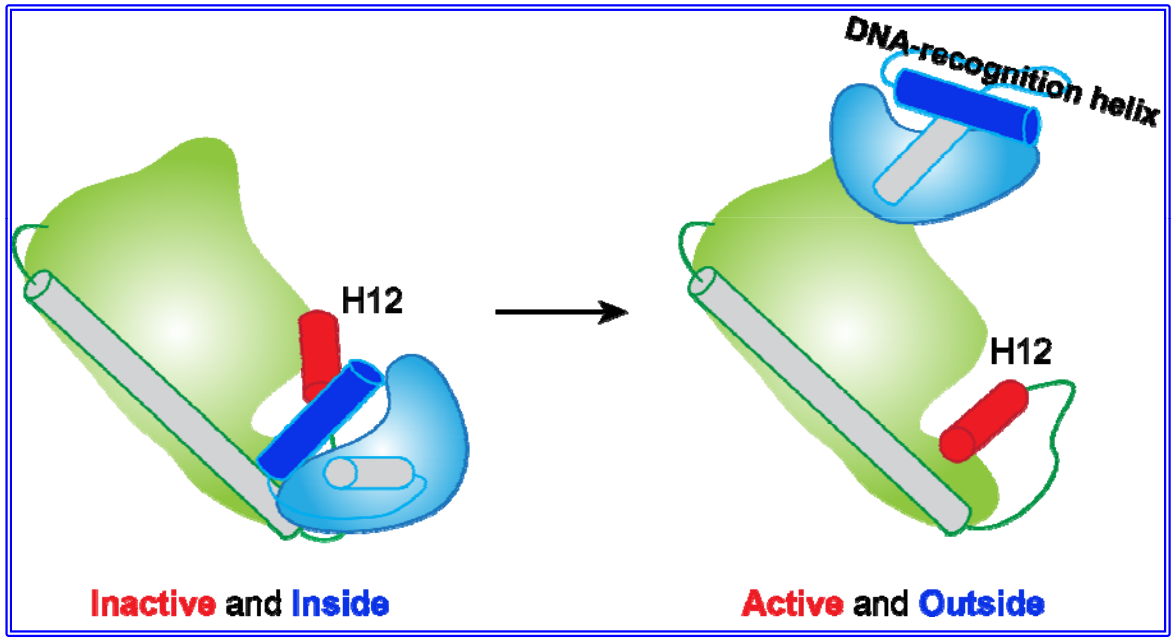
Individual Domains of ER Organization





Sites for binding
1. DNA
2. Ligands
3. CoR

Computation as a tool for hypothesis generation: INSIDE-OUT for DNA binding



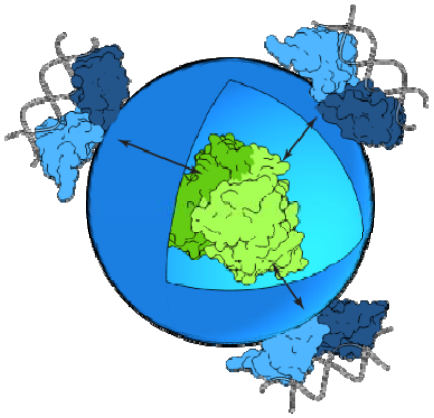
- Very different molecular shapes
- Very different interaction modes/sites

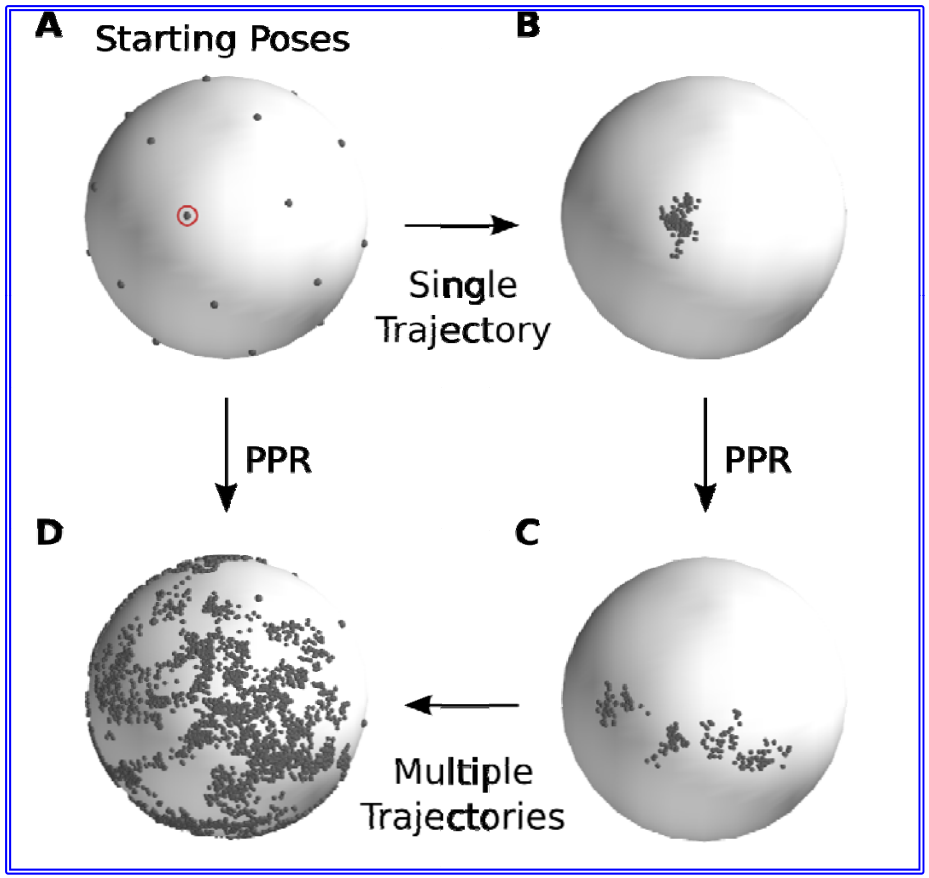
Computation as a tool for hypothesis generation:
(1) Coarse-grained Simulations

- Two crystal structures of the ER ligand-binding domain
- Accurately reproduce the known transition

Ravikumar, Huang, Yang, Biophys J. (2012) & Huang, Ravikumar, Yang, JCTC (2014)

Computation as a tool for hypothesis generation: (2) An exhaustive search

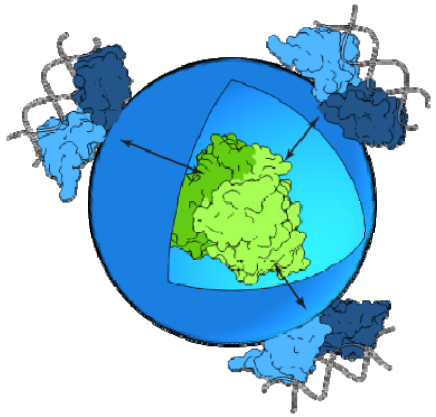


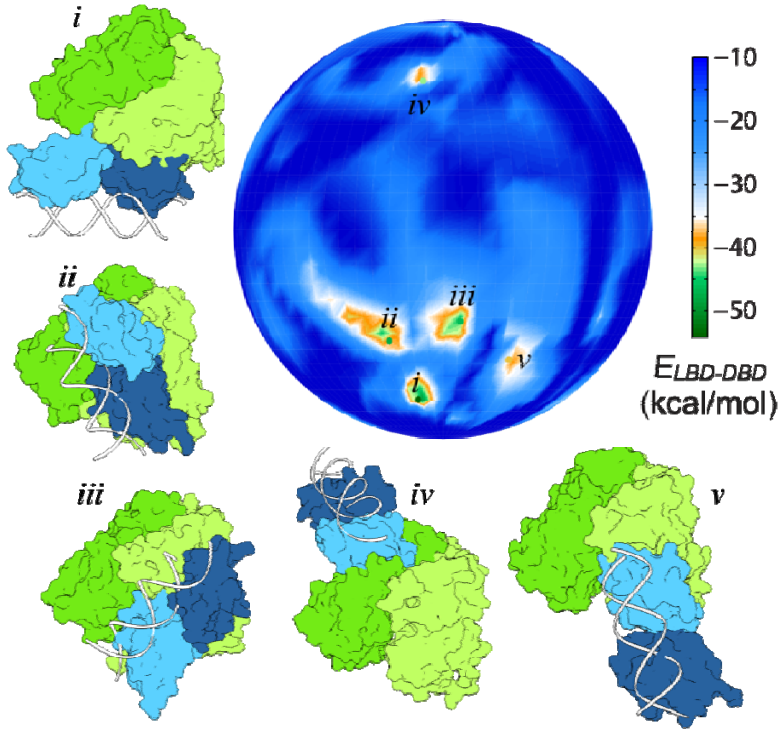


Exhaustively search all six degrees of freedom (inter-domain)

Ravikumar, Huang, Yang, Biophys J. (2012); Ravikumar et al (TBS)

Computation as a tool for hypothesis generation:
(3) Putative ER Conformations

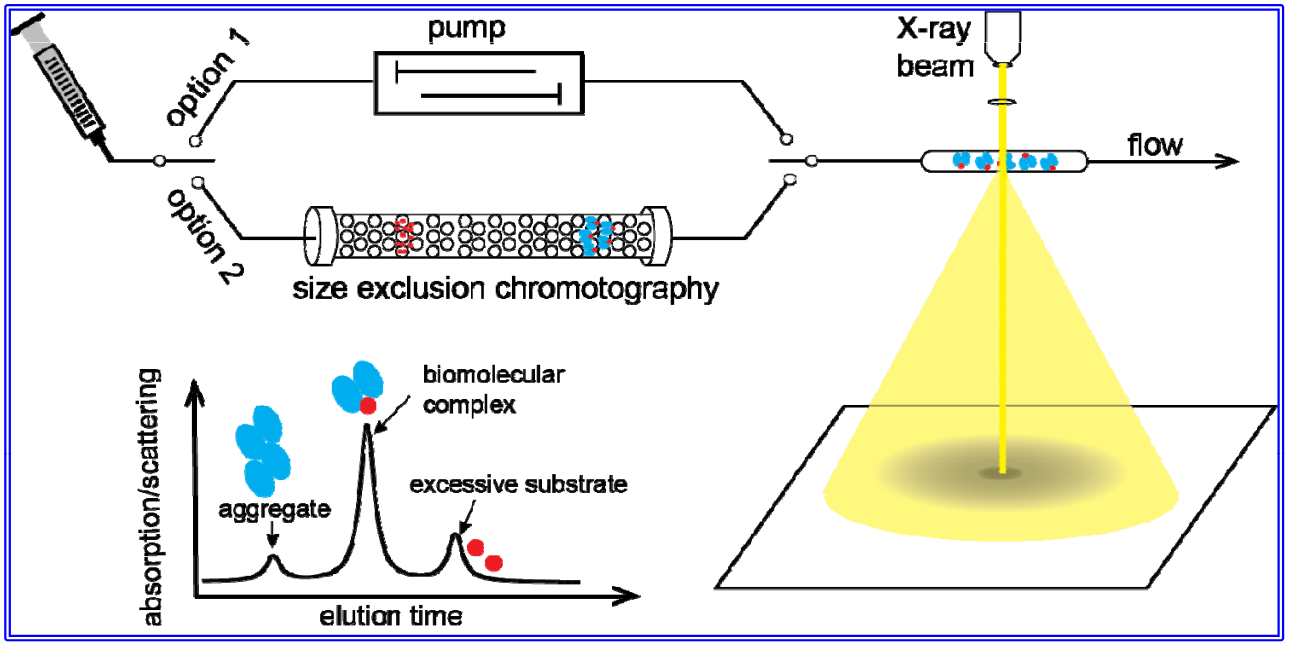




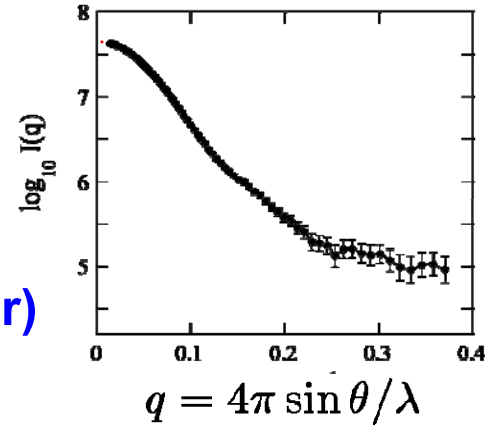
1. Interact via a critical H12 helix; consistent with experiment (truncation of a H12-containing region alters function)
2. Conformation-*iv* is similar to a new HNF complex (Chandra *et al*, Nature 2013)

Huang, Ravikumar, Greene, Yang, Proteins (2013); Huang et al (TBS)

Acquisition of SAXS data: Chromatography-coupled

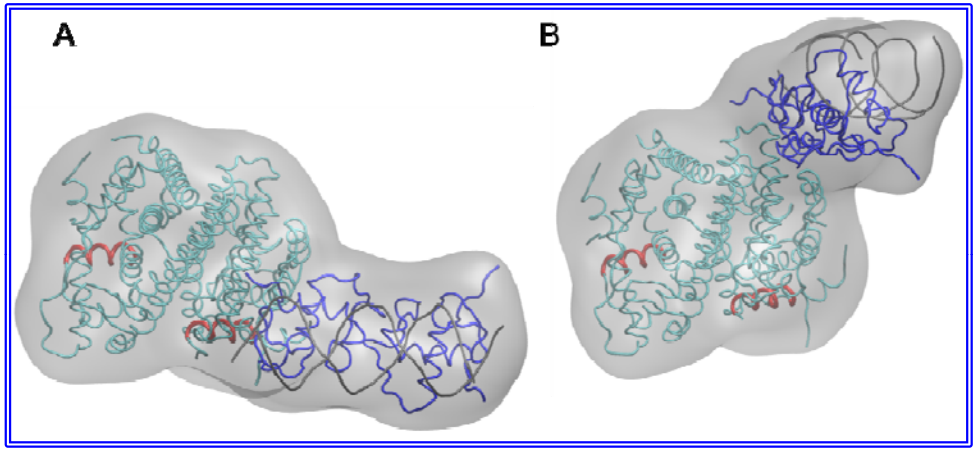


1. Remove unwanted “aggregates”
2. Remove excess DNA (strong scatter)



S. Yang, Advanced Materials (2014)

Examples of ER Shape Models using SAXS data

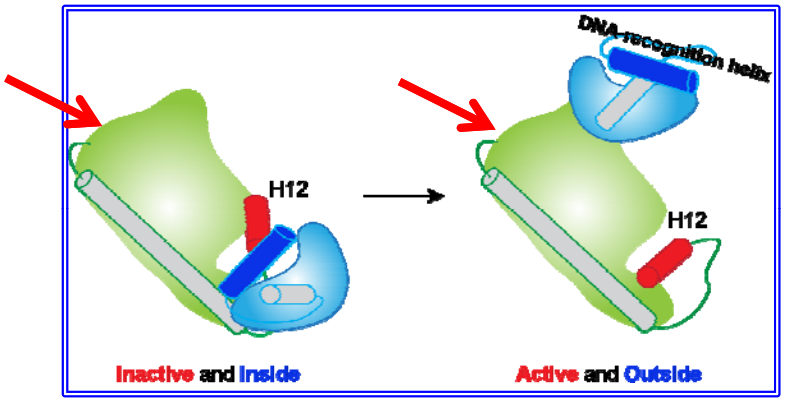


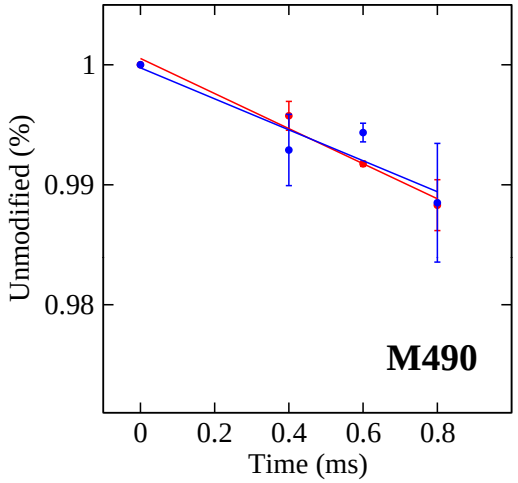
Model for ER•DNA

Two Different Approaches of Modeling:

- Traditional: SAXS data as a source of input (“backward”)
- Here: Plausible conformations to best-fit SAXS (“forward”)

Hot-off-the-oven: Footprinting data of ER ± DNA



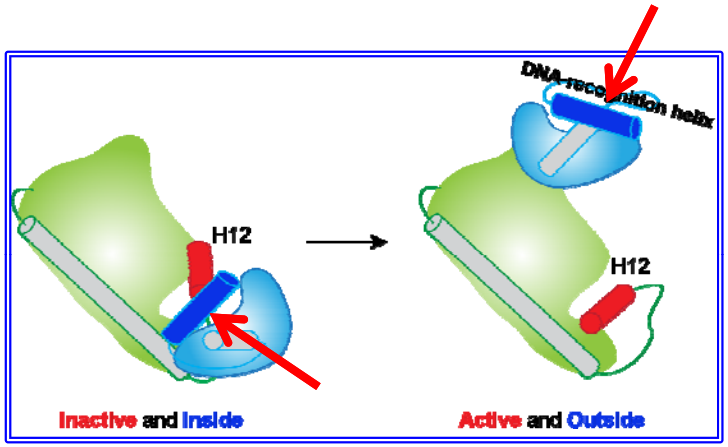


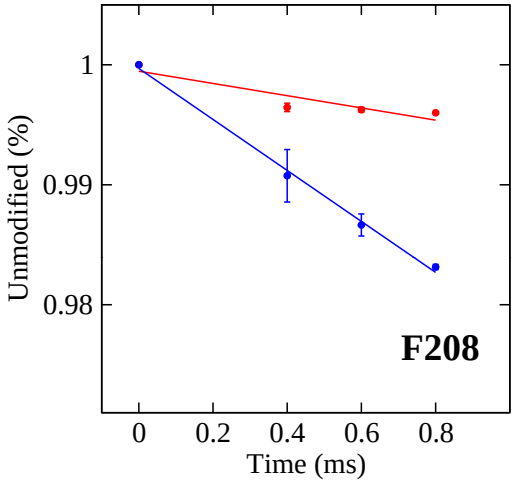
Protection Factor

Residue	+DNA	-DNA
M490	1.48	1.53

Control: M490 has very similar rates (or PFs)

Hot-off-the-oven: Footprinting data of ER ± DNA

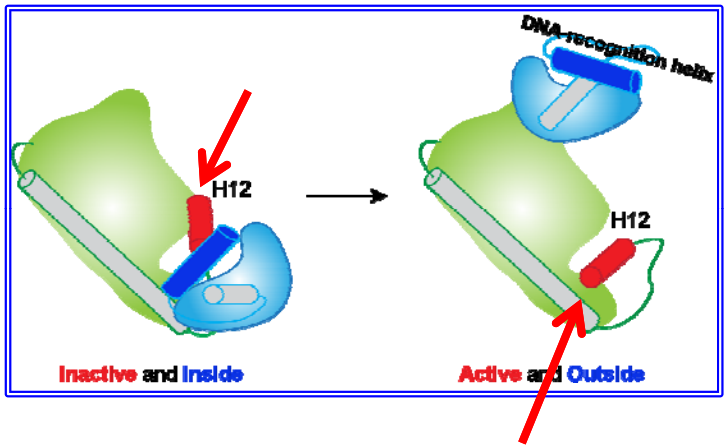


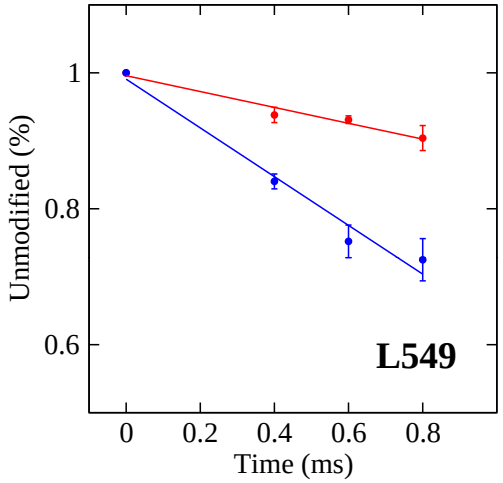


Residue	+DNA	-DNA
F208	1.90	1.95
M490	1.48	1.53

F208 at DNA-binding sites:
more protected in the absence of DNA
despite having different rates

Hot-off-the-oven: Footprinting data of ER ± DNA



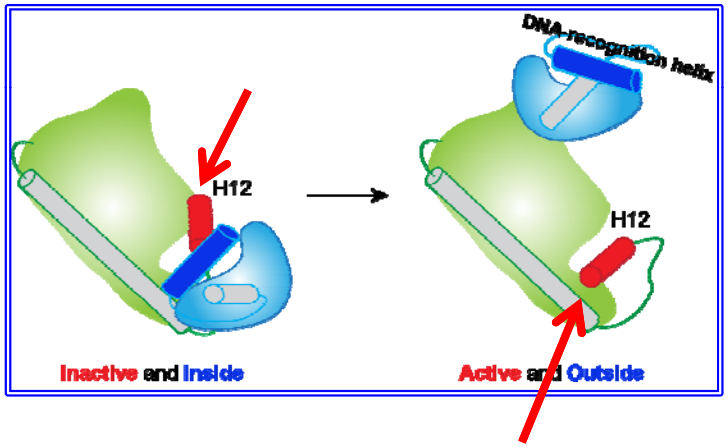


C-terminal: L549 is well exposed (even compared to the control)

Residue	+DNA	-DNA
F208	1.90	1.95
M490	1.48	1.53
L549	0.07	0.02

Highly promising for complete structural determination: More MS data + SAXS

Hot-off-the-oven: Footprinting data of ER ± DNA



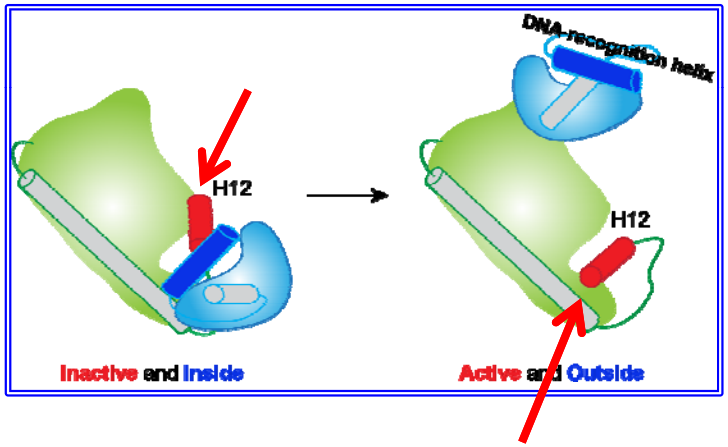
Protection Factor

Residue	+DNA	-DNA
F208	0.64 (±0.05)	-0.67 (±0.02)
M490	0.39 (±0.02)	0.43 (±0.06)
L549	-2.63 (±0.16)	-3.84 (±0.16)

C-terminal: L549 is well exposed (even compared to control)

More MS analysis coming soon

Hot-off-the-oven: Footprinting data of ER ± DNA



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