

Where are the protons? Measuring and modelling
proton equilibria in complex macromolecular
systems.

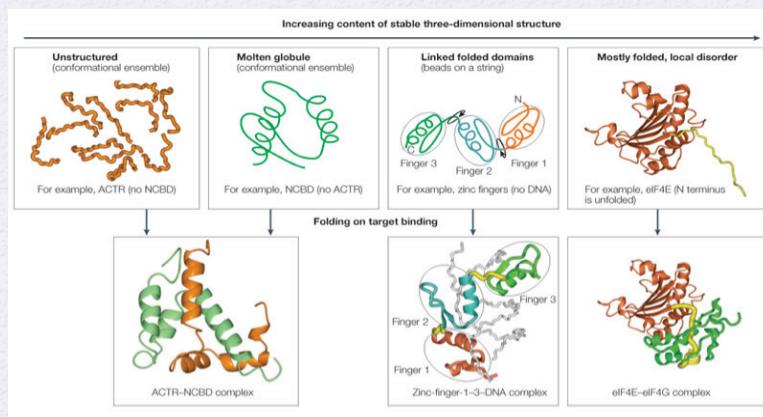


Lecture 4
Application of NMR spectroscopy
to study electrostatics in random coils



Intrinsically disordered proteins

- ✓ Lack of ordered structure under physiological conditions



Wright PE *Nat Rev Mol Cell Biol*. 2005;6(3):197-208

α -synuclein: a great model IDP for studying electrostatics

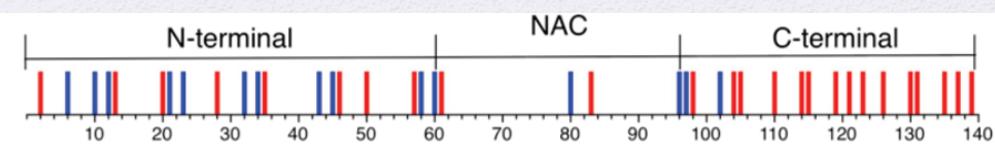


image from: Wu & Baum
J Am Chem Soc (2010)

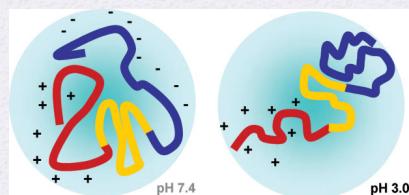
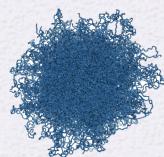
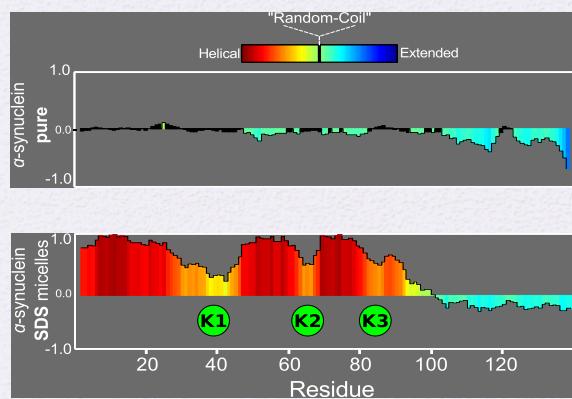


image from: Cho et al. *Prot Sci* (2009)

α -synuclein, as observed by NMR



Ullman & Stoltz,
JACS 2011

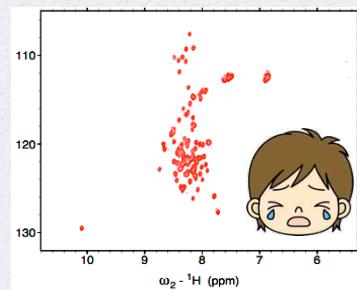
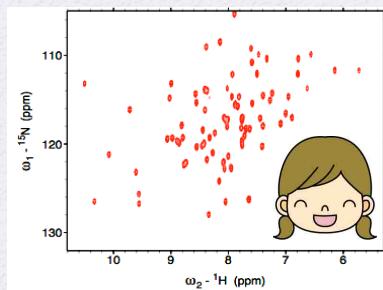
Ulmer et al. JBC 2005

nIDP: Tamiola, Acar & Mulder J Am Chem Soc (2010)

ncSPC: Tamiola & Mulder Biochem Soc Trans (2012) www.protein-nmr.org

Intrinsically disordered proteins

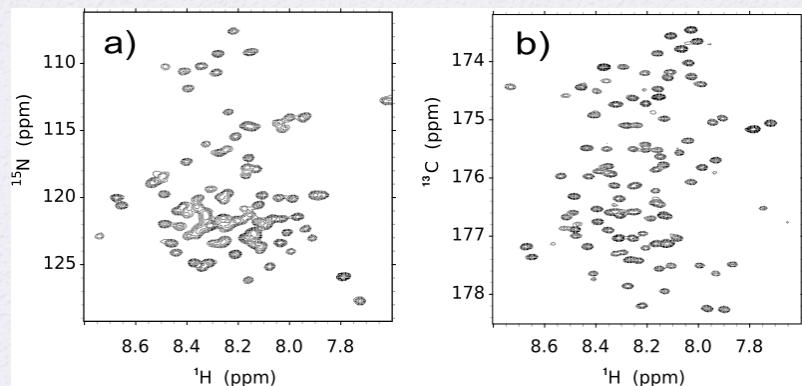
- Limited dispersion of the NMR signals
- Long coherence life times



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$^{13}\text{C}'$ backbone shifts are probably a good choice for IDPs: e.g. hNlg3cyt

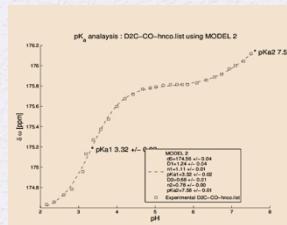


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Yao, Dyson & Wright *FEBS Lett* (1997)
Otten et al. *J Biomol NMR* (2009)

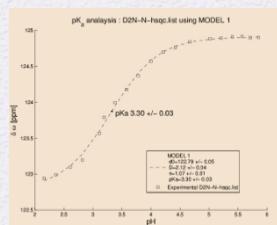
Accurate side-chain pK_a values can be measured from backbone chemical shifts

$^{13}\text{C}'$



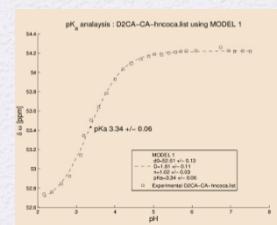
$$\text{p}K_a = 3.32 \pm 0.02$$

^{15}N



$$\text{p}K_a = 3.30 \pm 0.03$$

^{13}Ca

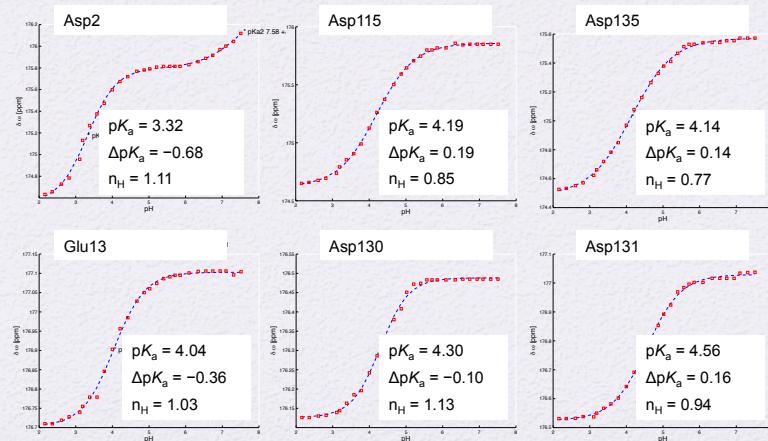


$$\text{p}K_a = 3.34 \pm 0.06$$

α Syn Asp2

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experimental pK_a values are fully consistent with calculation



Can titration curves be calculated from electrostatic theories?

Tanford-Roxby theory is the simplest approach for a flexible poly-electrolyte

$$pK_i = pK_{\text{intr},i} - \frac{1}{2.303kT} \sum_{k=1}^N W_{ik}(q_k^0 + \theta_k) \quad (1a)$$

with

$$\log \frac{\theta_i}{1 - \theta_i} = pK_i - pH \quad (1b)$$

where:

- the pK_i of a residue *i* can be calculated from the sum of all pairwise charge-charge interactions (1a)
- the probability θ_i that site *i* is protonated (1b)

Can titration curves be calculated from electrostatic theories?

The probability θ_i that site i is protonated is calculated as

$$\theta_i = \frac{\sum_{\{x\}} x_i e^{-\beta \Delta G(x) - \nu(x) 2.303 \text{pH}}}{\sum_{\{x\}} e^{-\beta \Delta G(x) - \nu(x) 2.303 \text{pH}}} \quad (5)$$

where

$$\Delta G(x) = \sum_{j=1}^N \Delta G_{\text{intr},j} x_j + \frac{1}{2} \sum_{j,k=1}^N W_{j,k} (q_j^0 + x_j)(q_k^0 + x_k) \quad (3)$$

With model pK_a constants, and an expression for W we can then calculate the pH-dependent protonation states of all side chains in unfolded proteins

Note that this is a 2^N problem! All permutations in (5) need to be considered.

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Titration curves can be calculated from DH theory and Gaussian Chain statistics

One approach was suggested by H.-X. Zhou:

- Two charged residues have an interaction energy given by Debye-Hückel
- A Gaussian Chain (GC) describes the distance distribution between charges; $d = b \times \sqrt{l} + s$
(l = number of peptide bonds, b = effective bond length, s = side chain shift)
- This then yields an expression for the interaction energies:

$$W_{ij} = 332 \int_0^\infty dr p(r) \exp(-\kappa r) / \epsilon r,$$
$$= 332(6/\pi)^{1/2} [1 - \pi^{1/2} x \exp(x^2) \operatorname{erfc}(x)] / \epsilon d,$$

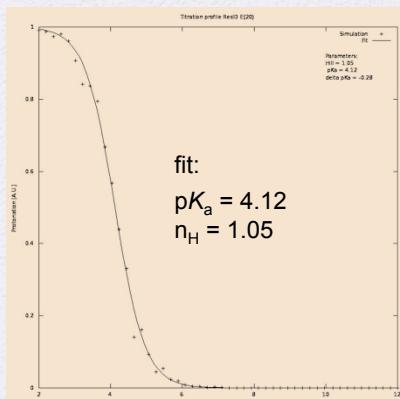
- Use Monte Carlo method to simulate the average protonation at each site

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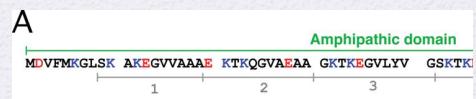
Zhou Proc Natl Acad Sci USA (2002)

Limitations of the Zhou method



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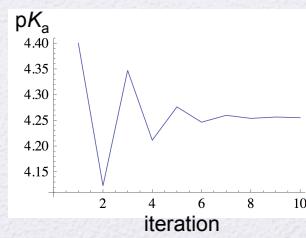
- Example: Glu20 in the α -synuclein N-terminus
- 15 titratable groups:
- Zhou (2002) GC model
- 327680 MC steps
- calculated data is noisy
- 2^N scaling prevents its use for larger systems

Titration curves can be calculated from DH theory and Gaussian chain statistics

Alternative approach. At each pH do:

- Calculate all pairwise interaction energies for a small subset of charges. For example, the nearest 5 charges, i.e. 2^5 interaction energies.
- Calculate the pK -shifts due to all other charges in the system, using their current average charge.
- Move the calculation segment along the protein chain
- Update the charges for the segment
- Iterate to conversion

Typically takes a few seconds to calculate, and about five cycles to reach conversion with subsystems of 4 explicit charge interactions

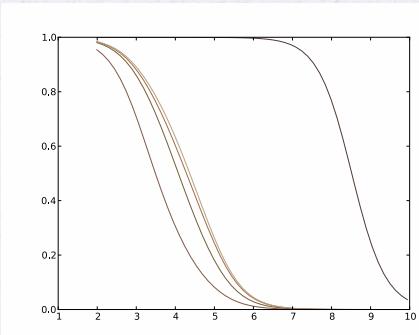


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Smooth titration curves calculated from Debye-Hückel theory and GC statistics

nDDDc ... I=0.0 M



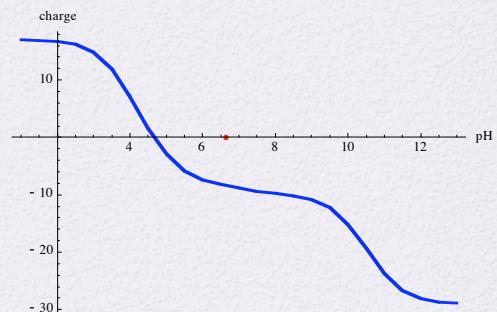
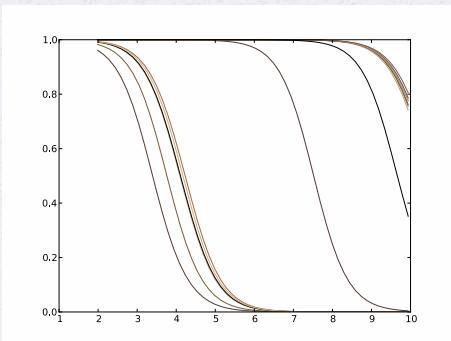
site:	pK _a	ΔpK _a	n _H
n-term:	8.564	1.064	0.999
D1:	4.082	0.082	0.725
D2:	4.279	0.279	0.693
D3:	4.355	0.355	0.701
c-term:	3.529	0.029	0.751

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Smooth titration curves calculated from Debye-Hückel theory and GC statistics

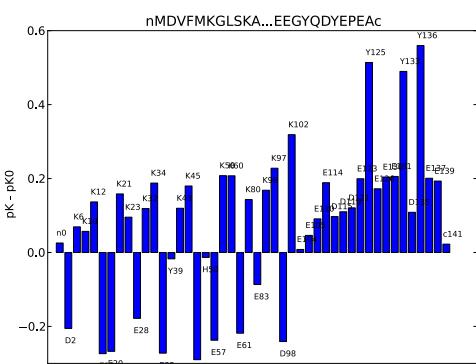
alpha-syn[1-40] ; I=0.1 M



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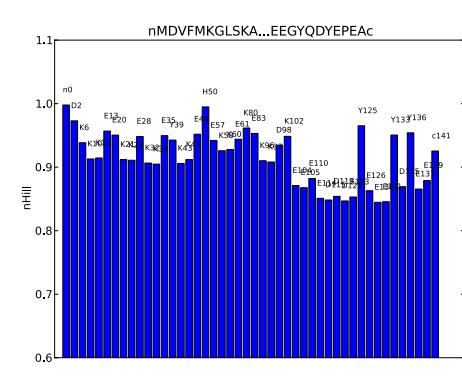
pK_a shifts for alpha-synuclein using the Gaussian Chain model



- Example calculation for 46 sites in α -synuclein
- $s = 0.5 \text{ nm}$; $b = 0.89 \text{ nm}$
- All acids in N-domain have down-shifted pK_a, whereas those in the C-domain are all up-shifted

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Cooperativity for alpha-synuclein using the Gaussian Chain model



- Example calculation for
- $s = 0.5 \text{ nm}$; $b = 0.89 \text{ nm}$
- Acid side chains experience negative cooperativity for proton dissociation, and this effect is stronger in the C-terminal part

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How much compaction in an unfolded protein is required to explain pKa shifts?

9424

Biochemistry 1995, 34, 9424–9433

p_{K_a} Values of Carboxyl Groups in the Native and Denatured States of Barnase:
The p_{K_a} Values of the Denatured State Are on Average 0.4 Units Lower Than
Those of Model Compounds

Mikael Oliveberg, Vickery L. Arcus, and Alan R. Fersht*

Cambridge Centre for Protein Engineering, Hills Road, Cambridge CB2 2QH, England, U.K.

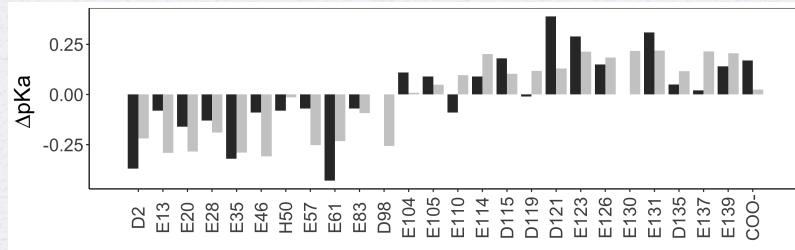
Received January 23, 1995; Revised Manuscript Received May 4, 1995®

"The results have direct implications for calculations of the energetics of proton equilibria and suggest that the acid/thermally denatured state is not an extended coil where the residues are isolated from one another by the intervening solvent but is compact and involves intramolecular charge repulsion."

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How much compaction in an unfolded protein is required to explain pKa shifts?

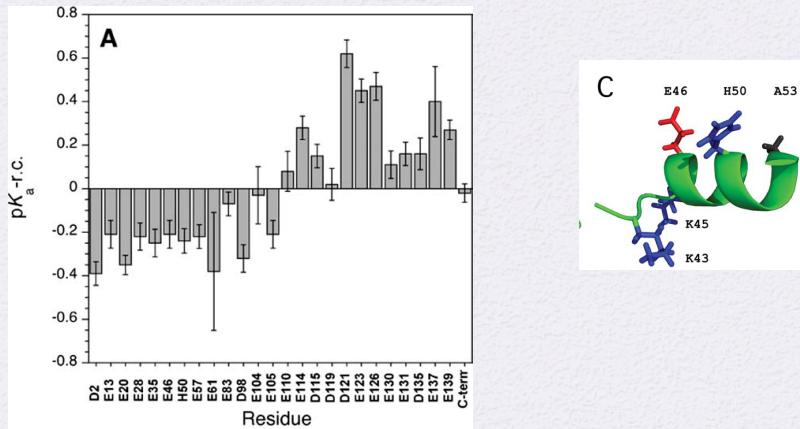


Well, Prof. Sir Alan Fersht, the Gaussian Chain comes already very close, but quite possibly the remaining differences can be explained by local preferences, and hence these p_{K_a} values carry structural information!

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Are certain pK_a shifts due to specific salt-bridges?



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Croke et al. *Prot Sci* (2010)

So, finally ...

1. It is possible to compute pH-dependent protonation states of all charged side chains in full-length IDPs
2. It is possible to obtain high-quality titration data for IDPs from backbone chemical shifts, (e.g. ¹³C')
3. Calculated and experimental titration curves look very similar, and we predict pK_a shifts and negative cooperativity parameters that are in quite good agreement with experiment. The remaining differences may be meaningful.

Ps. Pulse sequences and pK_a calculation tools (will) feature on www.protein-nmr.org (where you also find the IDP random coil chemical shift calculation tool)



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