Laboratory of Computational Biophysical Chemistry

Electrostatic interactions *in* and *between* biomolecules:

From fundamentals concepts to applications

Part 3

STAMiNA Global Network

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March15, 2020

124

Comparison with experiments

Table 1 Calculated and experimental pKa values of three proteins.

Residue	Experiment	GB	All-atom CpHMD			Implicit method
		0-1	0-5	5-10	0-10	
HP36						
Asp44	3.10(1)	3.2(1)	2.0	3.0	2.6(5)	3.7
Glu45	3.95(1)	3.5(1)	4.3	4.5	4.4(1)	4.5
Asp46	3.45(12)	3.5(1)	2.4	3.7	3.1(6)	3.8
Glu72	4.37(3)	3.5(1)	4.4	4.4	4.4(0)	3.5
BBL						
Asp129	3.88(2)	3.2(0)	2.2	3.2	2.7(5)	3.7
Glu141	4.46(4)	4.3(0)	4.0	4.4	4.2(2)	3.8
His142	6.47(4)	7.1(0)	5.9	5.8	5.8(0)	6.5
Asp162	3.18(4)	3.4(3)	2.9	3.0	3.2(3)	3.2
Glu164	4.50(3)	4.5(1)	5.7	4.6	5.2(6)	3.8
His166	5.39(2)	5.4(1)	4.4	4.4	4.4(0)	6.0
HEWL						
Glu7	2.6(2)	2.6(1)	3.6	3.4	3.5(1)	3.3
His15	5.5(2)	5.3(5)	5.1	5.1	5.1(0)	5.6
Asp18	2.8(3)	2.9(0)	2.5	3.3	2.9(5)	2.8
Glu35	6.1(4)	4.4(2)	8.5	8.7	8.6(1)	3.5
Asp48	1.4(2)	2.8(2)	0.1	1.1	0.6(6)	3.4
Asp52	3.6(3)	4.6(0)	5.4	5.6	5.5(1)	3.3
Asp66	1.2(2)	1.2(4)	0.6	0.8	0.3(7)	3.0
Asp87	2.2(1)	2.0(1)	0.8	2.1	1.5(7)	3.2
Asp101	4.5(1)	3.3(3)	6.1	5.7	5.9(2)	2.9
Asp119	3.5(3)	2.5(1)	3.0	3.3	3.2(1)	3.2
Maximum absolute deviation		1.8	2.4	2.6	2.5	2.6
Average absolute deviation (RMS deviation)		0.5	1.0	0.6	0.7	0.5

Faster, reasonable well, but it can be improved!

nputational
nistry

FPTS

JSP
2020

125

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New CpH MD scheme

Simulation of protein conformational freedom as a function of pH: constant-pH molecular dynamics using implicit titration

António M. Baptista, Paulo J. Martel & Steffen B. Petersen

PROTEINS: Structure, Function, and Genetics 27:523–544 (1997)

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Fast constant-pH computational scheme

A coarse-grained protein force field for folding and structure prediction

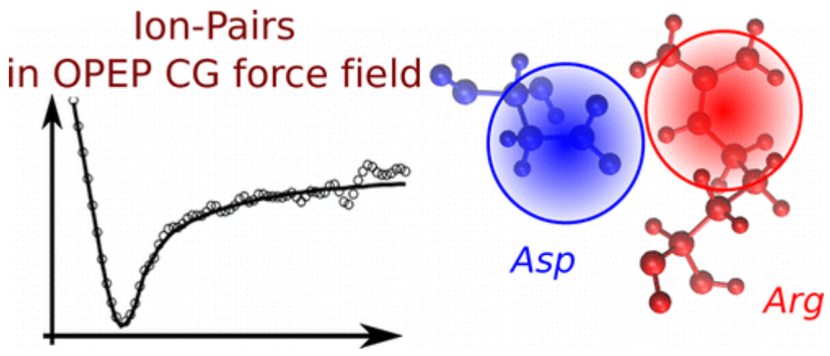
Julien Maupetit,[1] P. Tuffery,[1] and Philippe Derreumaux[2]* Proteins 2007; 69:394–408.

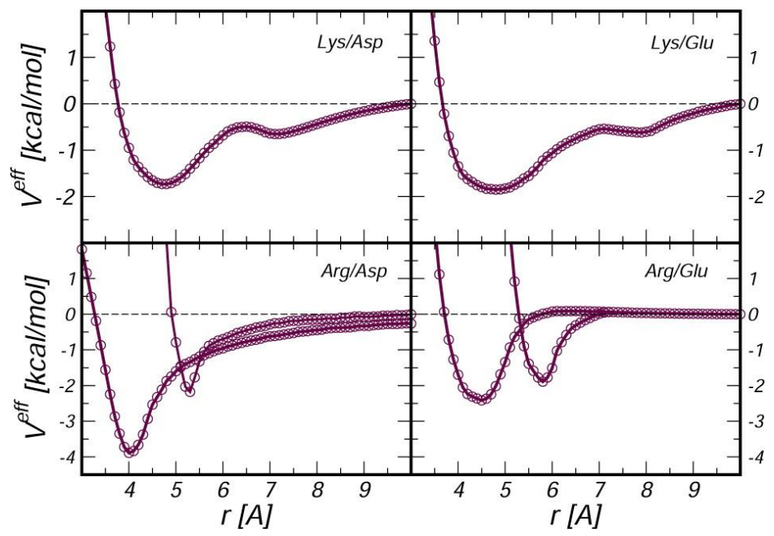
Optimized **P**otential for **E**fficient protein structure **P**rediction (OPEP) force field

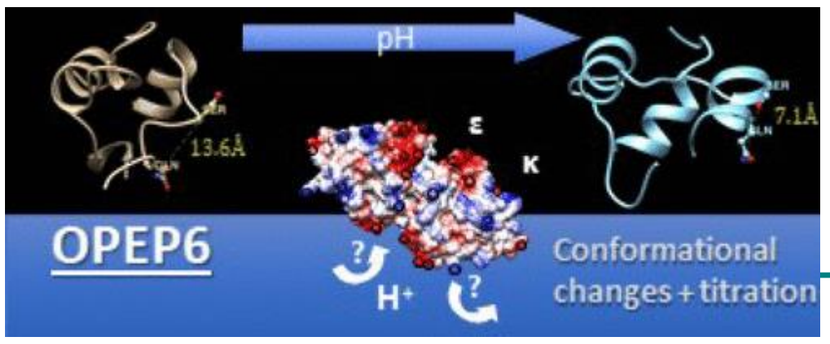
Importance of the Ion-Pair Interactions in the OPEP Coarse-Grained Force Field: Parametrization and Validation

Fabio Sterpone*†, Phuong H. Nguyen†, Maria Kalimeri†, and Philippe Derreumaux†‡
† Laboratoire de Biochimie Théorique, IBPC, CNRS, UPR9080, Université Paris Diderot, Sorbonne Paris Cité, 13 rue Pierre et Marie Curie, 75005, Paris, France
‡ Institut Universitaire de France, Bvd St Michel, 75005, Paris, France

J. Chem. Theory Comput., **2013**, *9* (10), pp 4574–4584







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128

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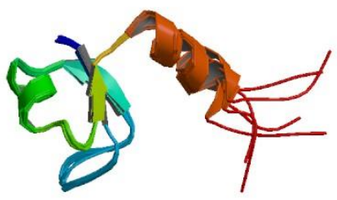

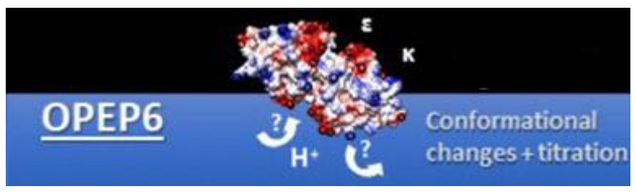

increases cpu costs

increases accuracy

Calculated and Experimental pK_a Values of the NTL9[f]

residue	expt[a]	GB[b]	all-atom REX-CpHMD[b]	SBM[c,d]	propKa	FPTS$_{rigid}$[d,e]	OPEP6[d,e]
ASP8	3.0	3.19(20)	2.83(7)	1.9	3.84	2.61	3.04(51)
GLU17	3.6	3.67(13)	3.57(14)	3.0	4.47	2.91	2.69(99)
ASP23	3.1	2.11(11)	2.75(16)	3.2	2.83	3.58	3.63(14)
GLU38	4.0	3.70(19)	3.38(30)	3.6	4.71	4.07	3.25(83)
GLU48	4.2	3.74(20)	3.47(17)	3.5	4.62	3.53	3.55(15)
GLU54	4.2	3.64(8)	3.65(22)	3.4	4.57	3.83	3.63(16)
MAXe		1.0	1.2	1.1	0.9	0.7	0.9
AAD		0.4	0.4	0.6	0.6	0.4	0.6
RMSE		0.5	0.6	0.7	0.6	0.5	0.6

[a]Data taken from ref 79. [b]Data taken from ref 66. [c]Data taken from ref 38. [d]Tyrosines were not free to titrate as a function of solution pH. [e]The data for OPEP6 is the average from 10 independent 10 ns trajectories. [f]Salt concentration is 100 mM.

(FLBDS, FS & PD, JCTC 2019)

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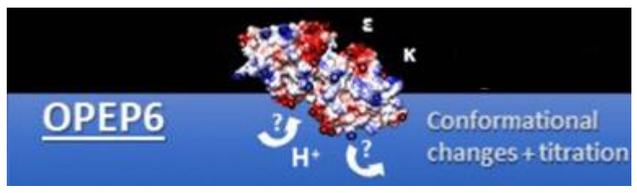
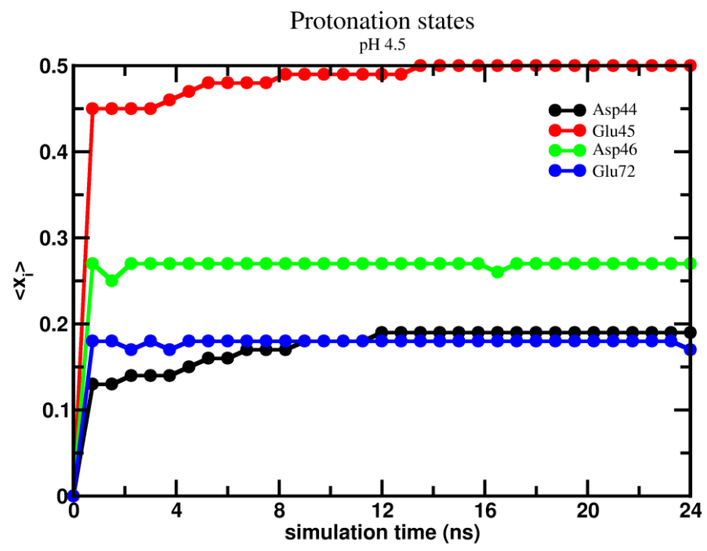
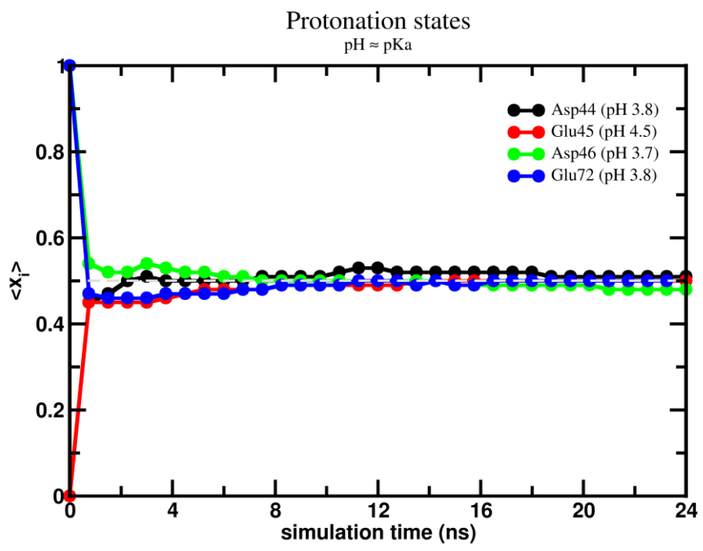
129

Convergence



Calculated and experimental pK$_a$ values of HP36.
Salt concentration is 150 mM.





(FLBDS, FS & PD, JCTC 2019)

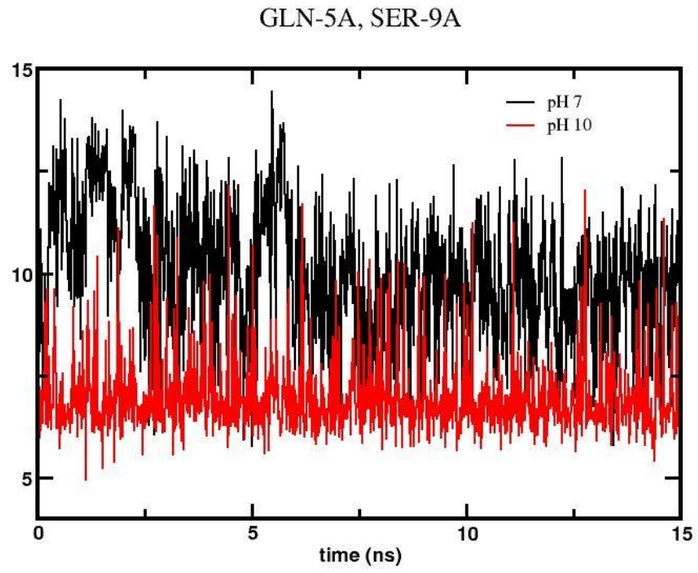
Conformational changes in cubic insulin crystals in the pH range 7–11

Olga Gursky, John Badger, Youli Li, and Donald L. D. Caspar

Biophys. J. © Biophysical Society
Volume 63 November 1992 1210–1220

TABLE 3 Relative weights of multiple protein conformers at different pH and solvent cation concentrations for protein groups from four regions: (*1*) A5 Gln and A9 Ser that gates cation binding in the cavity between the insulin dimers; (2) A4 Glu and B30 Ala carboxyls interacting with A1 Gly α-amino group; (*3*) B10 His that can compete for a binding site with a cation; (*4*) B25 Phe and A21 Asn paired across the dimer dyad

Cation concentration	pH	Regions with variable weights of alternate protein conformers: A5 Gln, A9 Ser-cavity		B30, A4 Glu, A1 α-amino	B10 His-Na^+ site	B25 Phe, A21 Asn
M						
0.1 Na or K	7	Open	0.7			
		Closed	0.3			
	8	Open	0.3			
		Closed	0.7			
	9	Closed	>0.7			
	10	Closed	1.0			
1.0 Na	7	Closed	>0.7			
	9	Closed	1.0			
	11	Closed	1.0			



(FLBDS, FS & PD, JCTC 2019)

Electrostatic interactions

So simple?!

Peculiar phenomena 1

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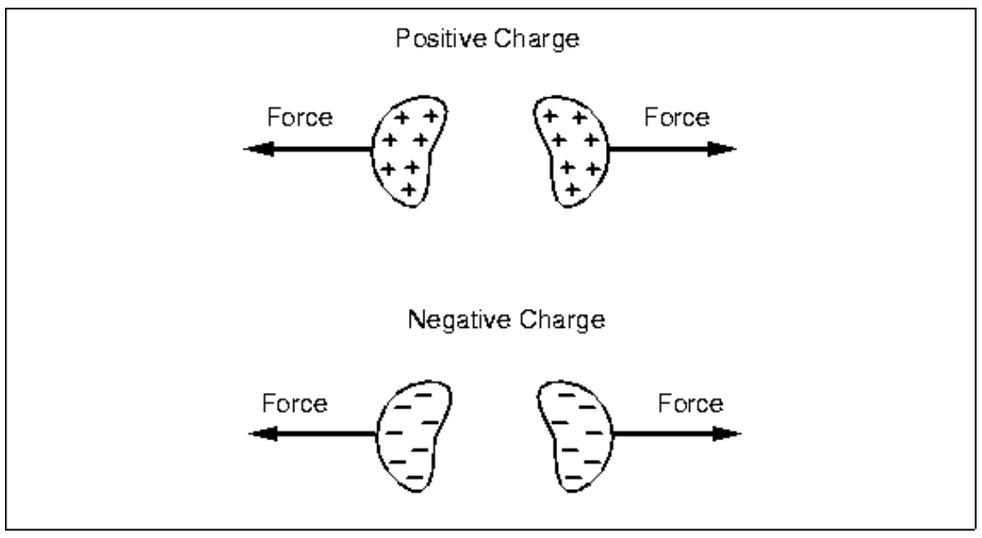
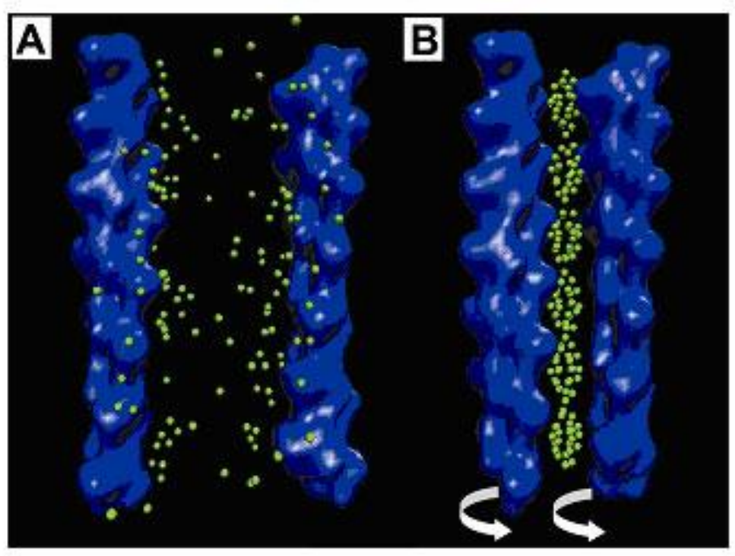
Fundamental interactions

Like charges should repel each other?



Nature shows this is not true!



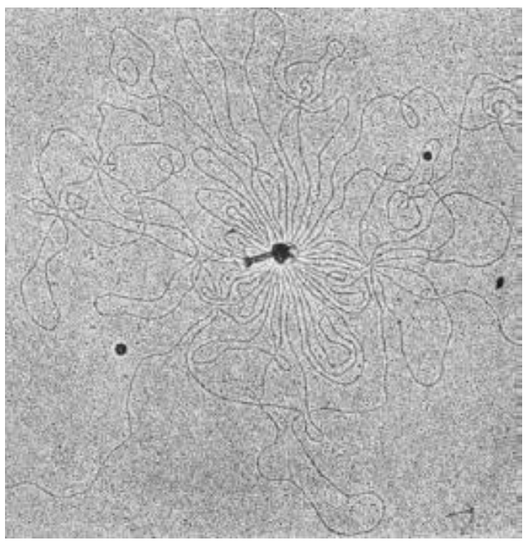
Schematic representations of uncondensed and condensed F-actin. (A) At low multivalent ion concentrations, two F-actin filaments maintain their native 13/6 symmetry and are unbound. (B) At high multivalent ion concentrations, the ions collectively form a CDW and bundle F-actin filaments.

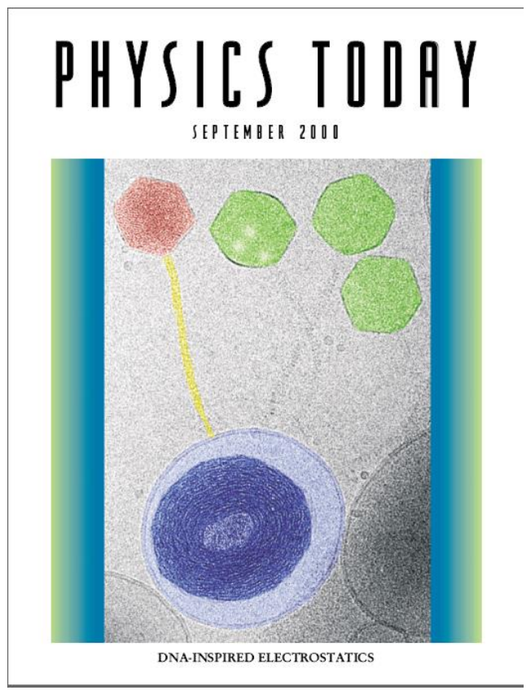
Angelini T. E. et.al. PNAS 2003;100:8634-8637

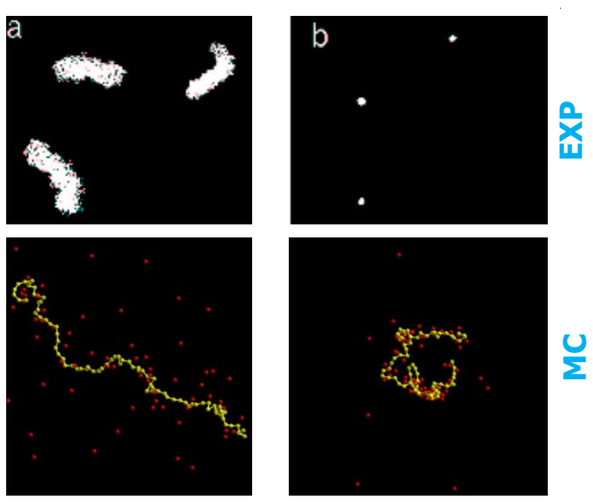
DNA-INSPIRED ELECTROSTATICS

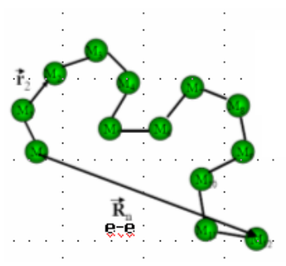
Not just the repository of our genetic information, DNA is also a fascinating, shape-shifting molecule whose behavior in solution counters our intuition and challenges our physical understanding.

William M. Gelbart, Robijn F. Bruinsma, Philip A. Pincus, and V. Adrian Parsegian

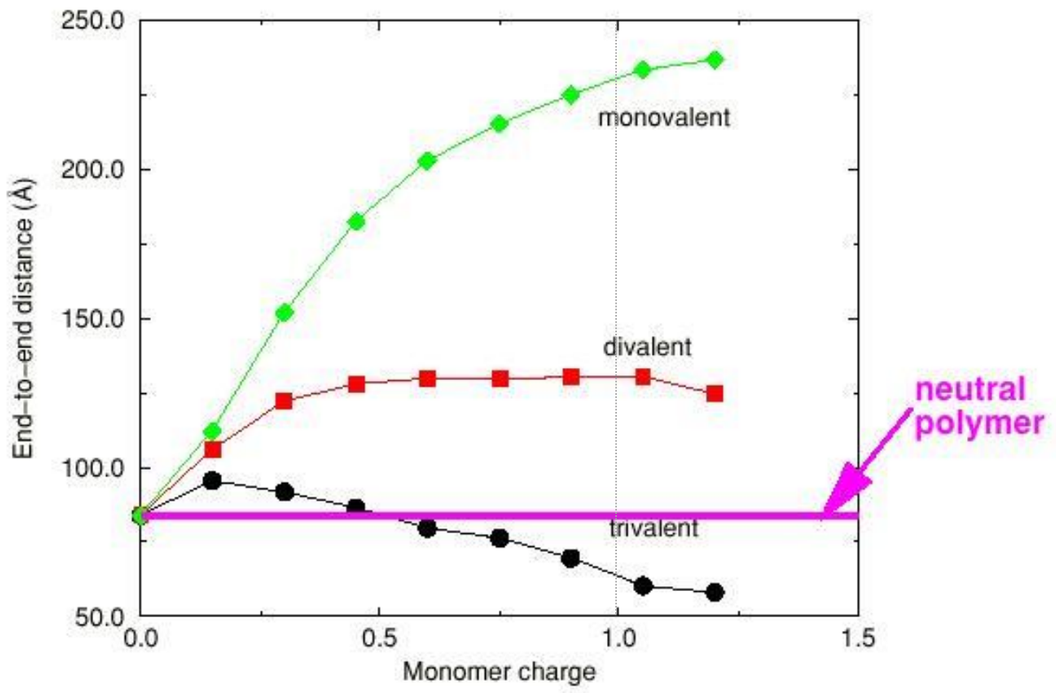








"Protein" folding



The end-to-end distance as a function of the monomer charge, for different valency of the counterions. **(Khan & Jönsson, 1999)**

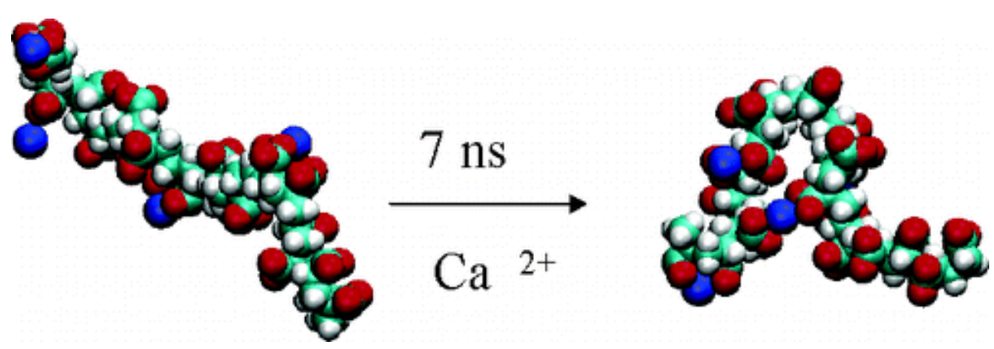
"Like-Charge Attraction" between Anionic Polyelectrolytes: Molecular Dynamics Simulations

Ferenc Molnar* and Jens Rieger

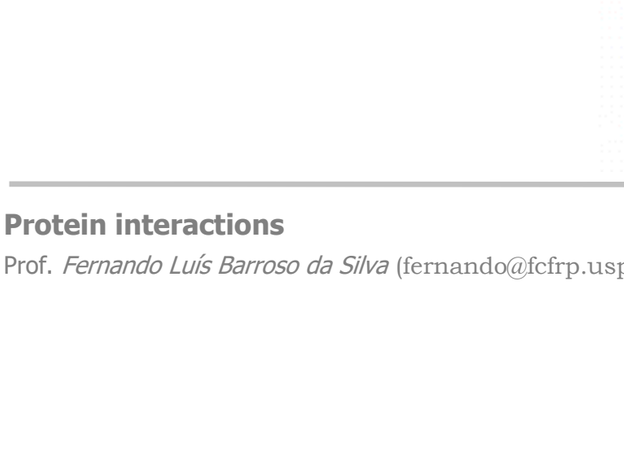
BASF Aktiengesellschaft, Polymer Physics, Carl-Bosch Str. 38, 67056 Ludwigshafen, Germany

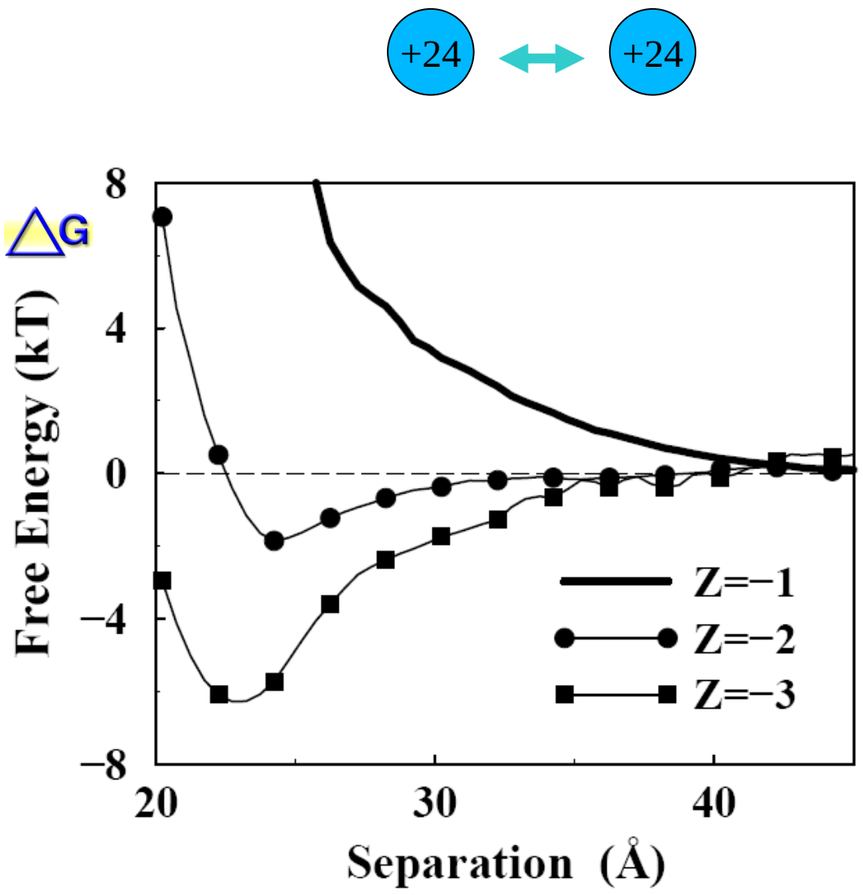
Received August 2, 2004. In Final Form: October 14, 2004

"Like-charge attraction" is a phenomenon found in many biological systems containing DNA or proteins, as well as in polyelectrolyte systems of industrial importance. "Like-charge attraction" between polyanions is observed in the presence of mobile multivalent cations. At a certain limiting concentration of cations, the negatively charged macroions cease to repel each other and even an attractive force between the anions is found. With classical molecular dynamics simulations it is possible to elucidate the processes that govern the attractive behavior with atomistic resolution. As an industrially relevant example we study the interaction of negatively charged carboxylate groups of sodium polyacrylate molecules with divalent cationic Ca^{2+} counterions. Here we show that Ca^{2+} ions initially associate with single chains of polyacrylates and strongly influence sodium ion distribution; shielded polyanions approach each other and eventually "stick" together (precipitate), contrary to the assumption that precipitation is initially induced by intermolecular Ca^{2+} bridging.



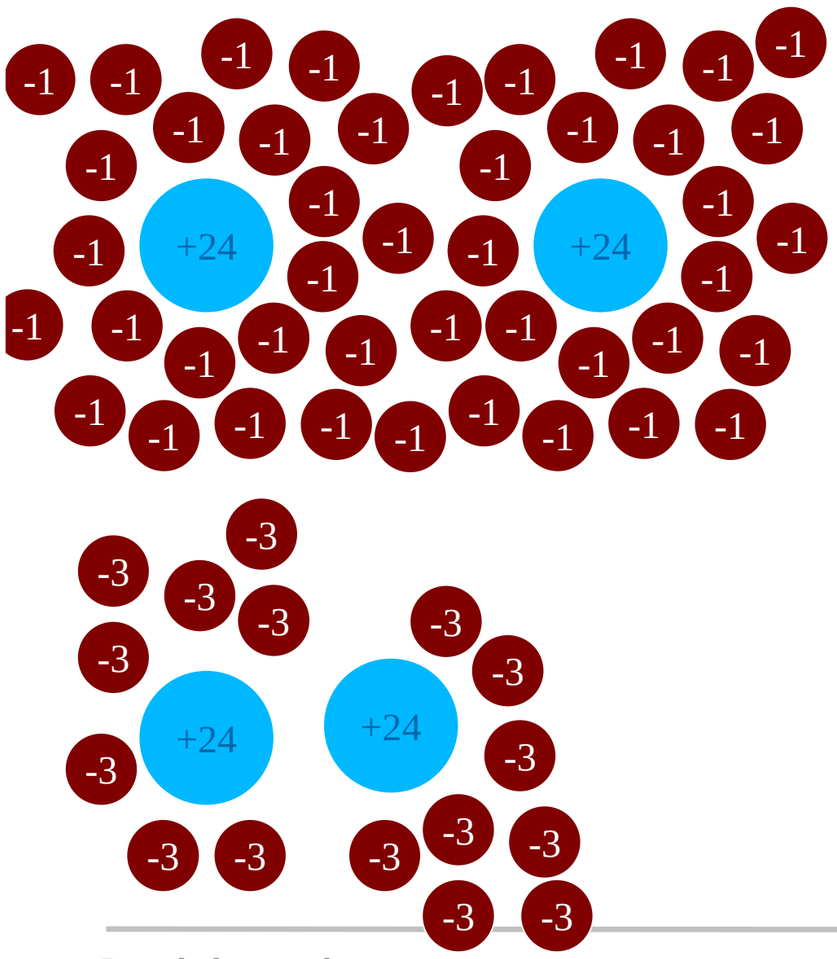
Protein-protein interaction

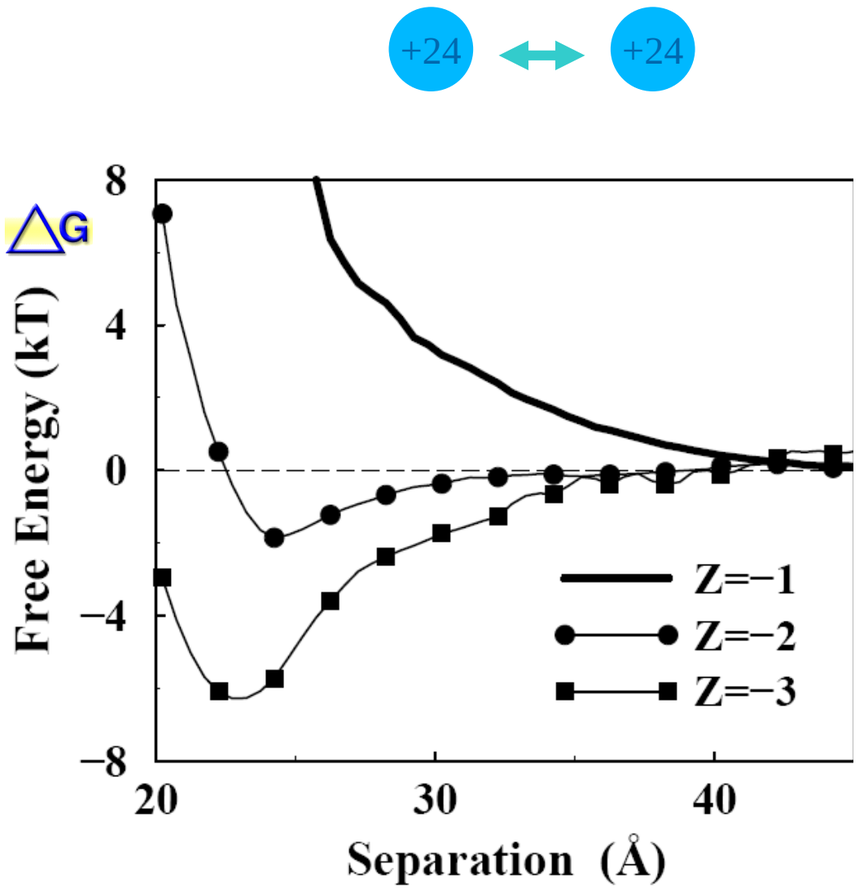




(Jönsson, Lund & Da Silva, 2007)

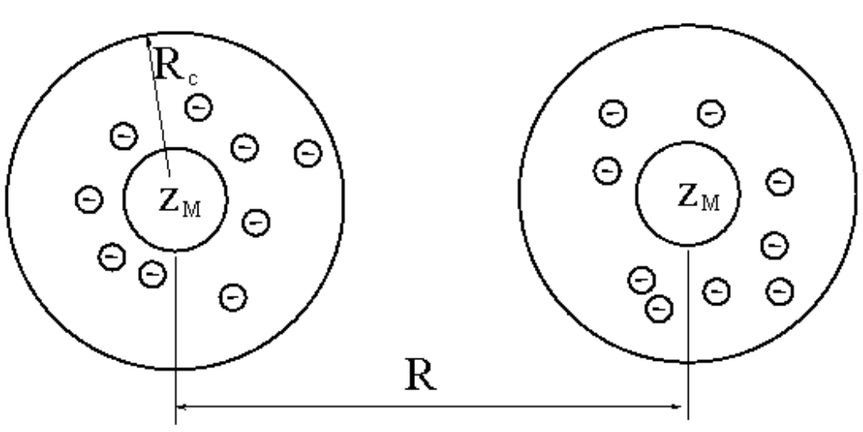
Protein-protein interaction





(Jönsson, Lund & Da Silva, 2007)

Ion-ion correlation



$$\Delta A = k_B T \ln\left\langle \exp\left[-\Delta V(R)/k_B T\right]\right\rangle_0$$

$$\Delta A = -\frac{3k_B T \alpha_k \alpha_l}{(4\pi\varepsilon_0\varepsilon_r)^2 R^6}$$

From Biological Physics to Material Sciences

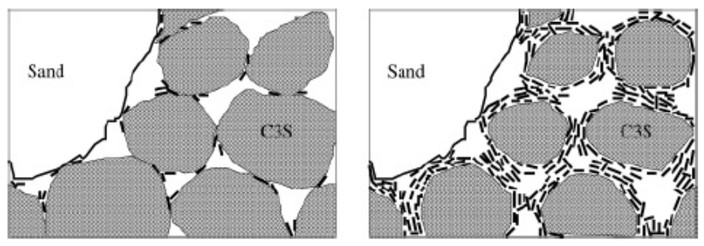


Figure 1. Schematic drawing of early cement paste with sand, C_3S particles drawn as hatched, approximately spherical objects, and C−S−H platelets drawn as black bars. (Left) At an early stage, water has been added, and the C_3S particles have formed a weak network. C_3S has also started to go into solution, and the precipitation threshold of C−S−H has been reached. The C−S−H particles form preferentially close to the contact points of the C_3S particles. (Right) At a later stage, a significant portion of C_3S has dissolved, and a corresponding amount of C−S−H has been created. The contact points between the C_3S grains mediated by the C−S−H particles has increased in number, and so has the cohesion of the paste.

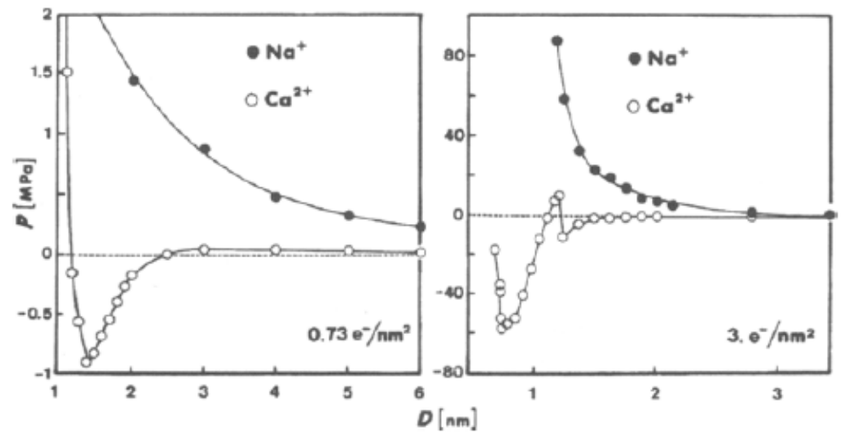


Fig. 4 - Primitive model calculation of the pressure between two negatively charged walls separated by sodium or calcium ions in water. The surface charge density is that of a smectite clay on the left and that of a Tobermorite-like *C-S-H* with all its OH groups ionized on the right. The repulsive osmotic pressure dominates the overall interaction in the case of sodium, whereas it is more than compensated by the attractive correlations forces, at short separation distances, in the case of calcium. Note that in this pressure *vs* distance representation, the equilibrium positions do not correspond to the minima, but at the roots (P = 0) where the first derivative dP/dx is negative.

Materials and Structures / Concrete Science and Engineering, Vol. 37, January-Febrtmry 2004, pp 3-14

Langmuir **2005,** *21,* 9211−9221

Controlling the Cohesion of Cement Paste

Bo Jönsson,*,† A. Nonat,‡ C. Labbez,‡ B. Cabane,§ and H. Wennerström||

Theoretical Chemistry, Chemical Center, POB 124, S-221 00 Lund, Sweden, LRRS, UMR CNRS 5313, Universite de Bourgogne, F-21078 Dijon Cedex, France, PMMH, ESPCI, 10 rue Vauquelin, F-75231 Paris Cedex 05, France, and Physical Chemistry 1, Chemical Center, POB 124, S-221 00 Lund, Sweden

Received April 19, 2005. In Final Form: July 8, 2005

The main source of cohesion in cement paste is the nanoparticles of calcium silicate hydrate (C−S−H), which are formed upon the dissolution of the original tricalcium silicate (C_3S). The interaction between highly charged C−S−H particles in the presence of divalent calcium counterions is strongly attractive because of ion−ion correlations and a negligible entropic repulsion. Traditional double-layer theory based on the Poisson−Boltzmann equation becomes qualitatively incorrect in these systems. Monte Carlo (MC) simulations in the framework of the primitive model of electrolyte solution is then an alternative, where ion−ion correlations are properly included. In addition to divalent calcium counterions, commercial Portland cement contains a variety of other ions (sodium, potassium, sulfate, etc.). The influence of high concentrations of these ionic additives as well as pH on the stability of the final concrete construction is investigated through MC simulations in a grand canonical ensemble. The results show that calcium ions have a strong physical affinity (in opposition to specific chemical adsorption) to the negatively charged silicate particles of interest (C−S−H, C_3S). This gives concrete surprisingly robust properties, and the cement cohesion is unaffected by the addition of a large variety of additives provided that the calcium concentration and the C−S−H surface charge are high enough. This general phenomenon is also semiquantitatively reproduced from a simple analytical model. The simulations also predict that the affinity of divalent counterions for a highly and oppositely charged surface sometimes is high enough to cause a "charge reversal" of the apparent surface charge in agreement with electrophoretic measurements on both C_3S and C−S−H particles.

The electrostatic world of (biological) molecules

Weak coupling (monovalent ions)

- "Screening" long-range interactions
- Condensation at short distances ($Z \rightarrow Z_{eff}$)
- DVLO (**repulssion**): ~ exp(-κr)/r

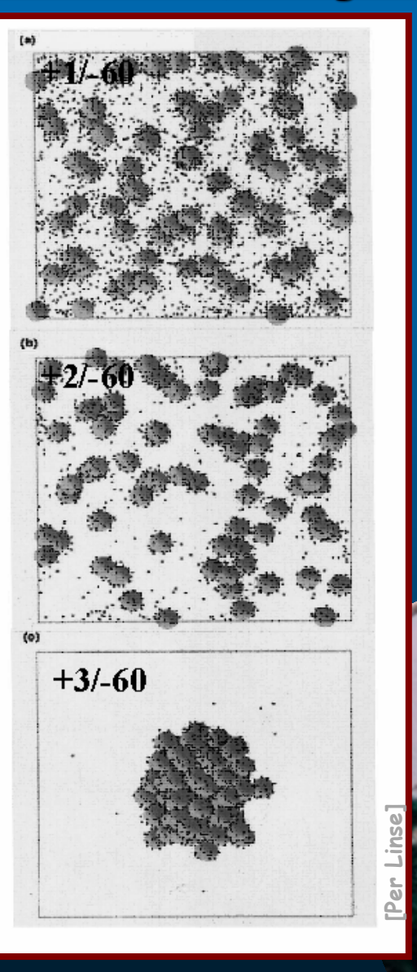
$$\Xi = 2\pi\, z_k^3 l_b^2 \sigma_s$$

STRONG coupling

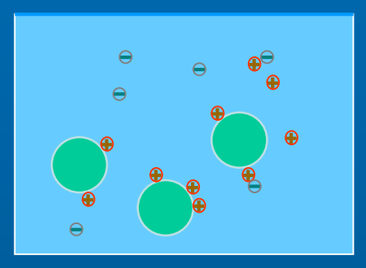
(multivalent ions)

- Charge reversal/"overcharging"
- Colloidal Attraction

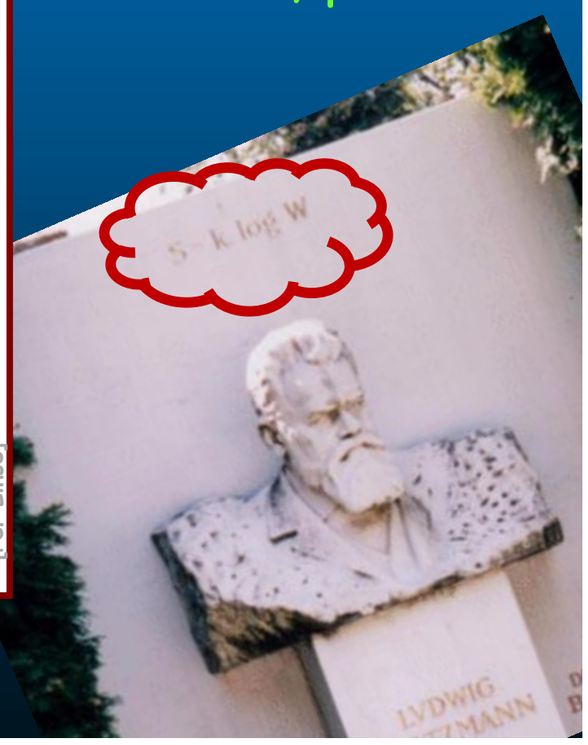
valence



[Per Linse]



Coulomb law, pka and kT



Protein interactions

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144

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Peculiar phenomena 2

Fernando Barroso, FCFRP/USP

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145

Complexation "on the wrong side"

Food Chemistry 156 (2014) 197–203

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cold-set whey protein microgels as pH modulated immobilisation matrices for charged bioactives

Thelma Egan, Dolores O'Riordan, Michael O'Sullivan, Jean-Christophe Jacquier *

Institute of Food and Health, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

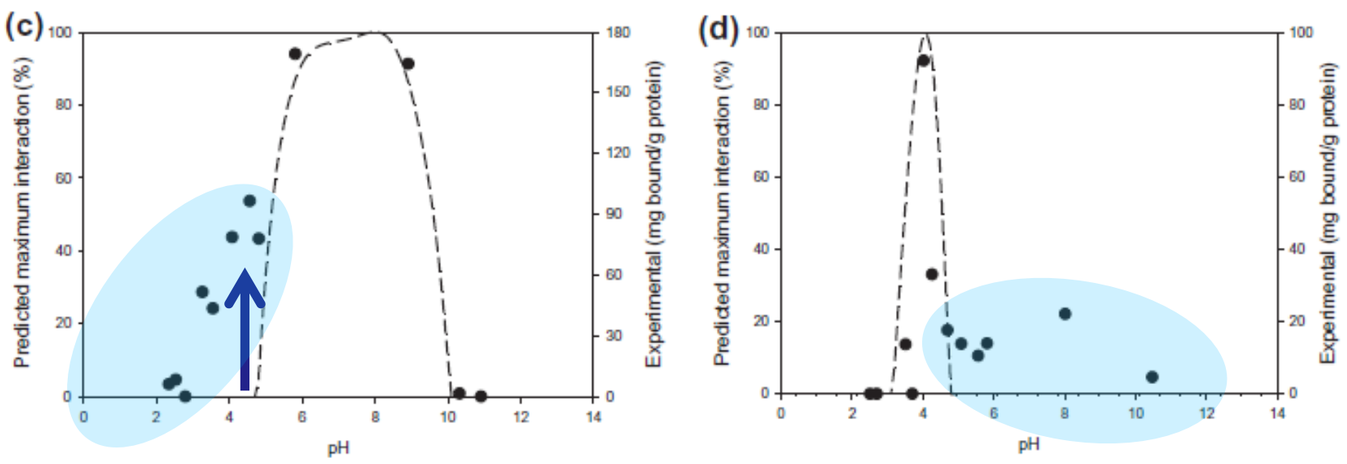
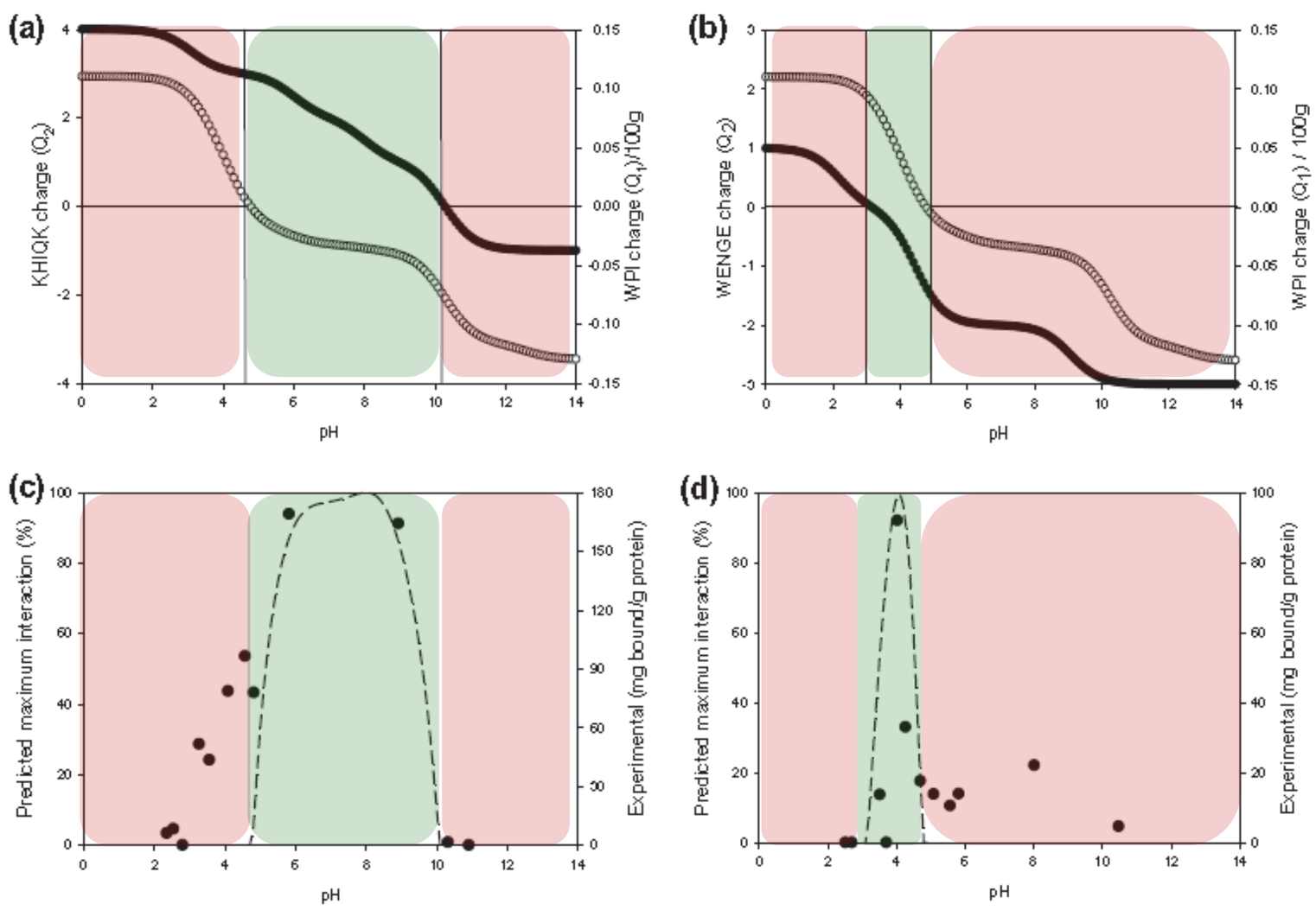


Fig. 6. The pH charge profile of (a) KHIQK and (b) WENGE peptide. Peptide (●) WPI (○). The uptake of (c) KHIQK peptide and (d) WENGE by the WPI microgels over a pH range. Experimental data (●) and predicted data (-).

146

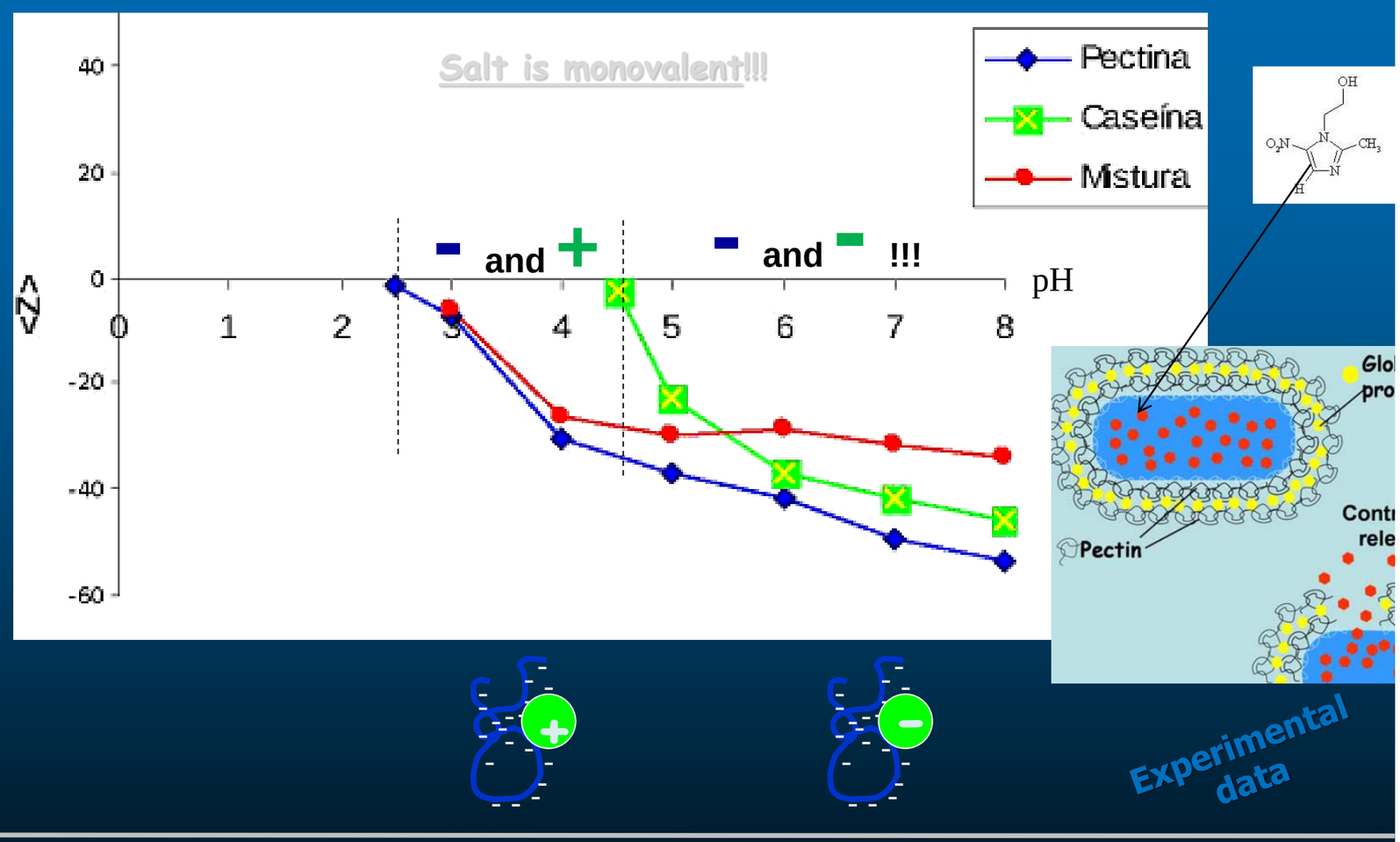
Complexation "on the wrong side"



147

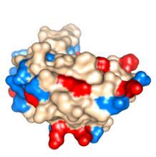
Protein-polysaccharide interaction

Complexation "on the wrong side"



$$\Xi = 2\pi\, z_k^3 l_b^2 \sigma_S \approx 0\,!!!$$

Complexation "on the wrong side"

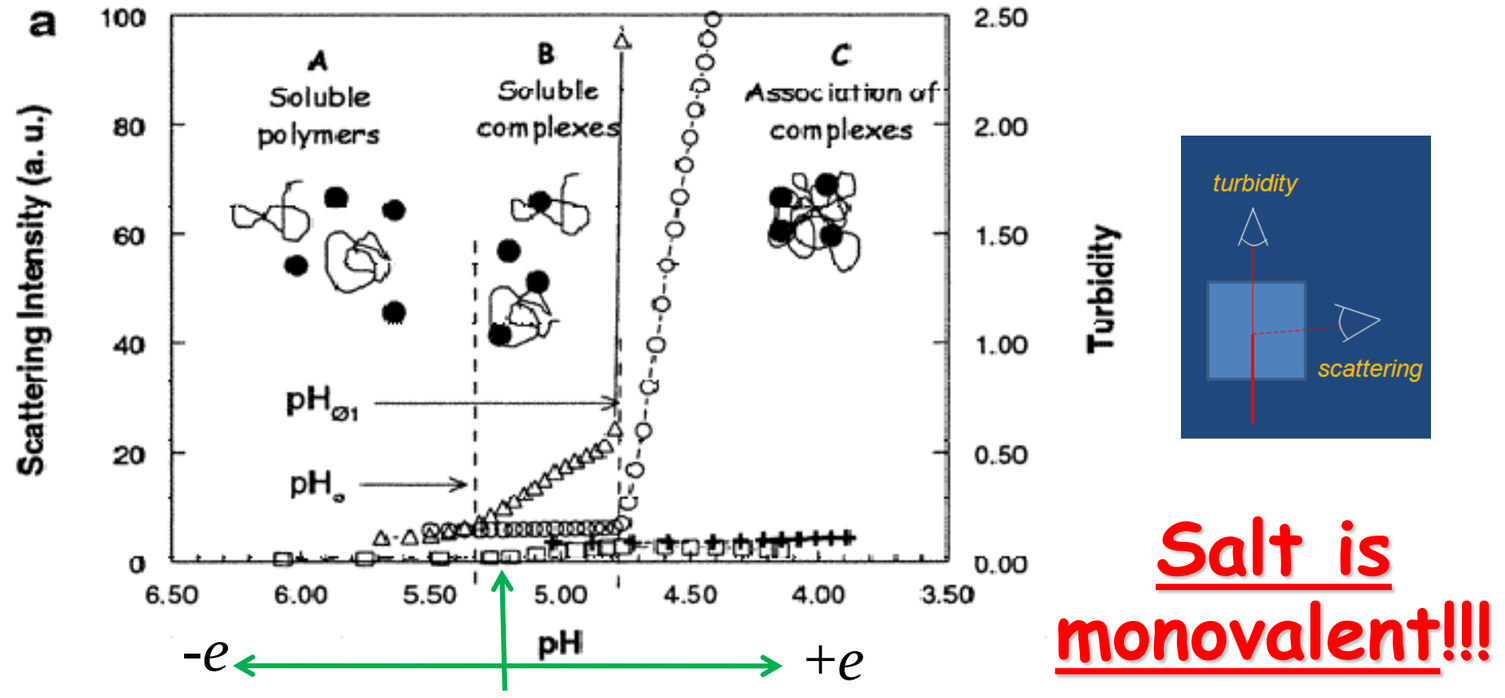


Complex Coacervation of Whey Proteins and Gum Arabic

F. Weinbreck, R. de Vries, P. Schrooyen, and C. G. de Kruif*

Biomacromolecules, 4 (2), 293 -303, 2003

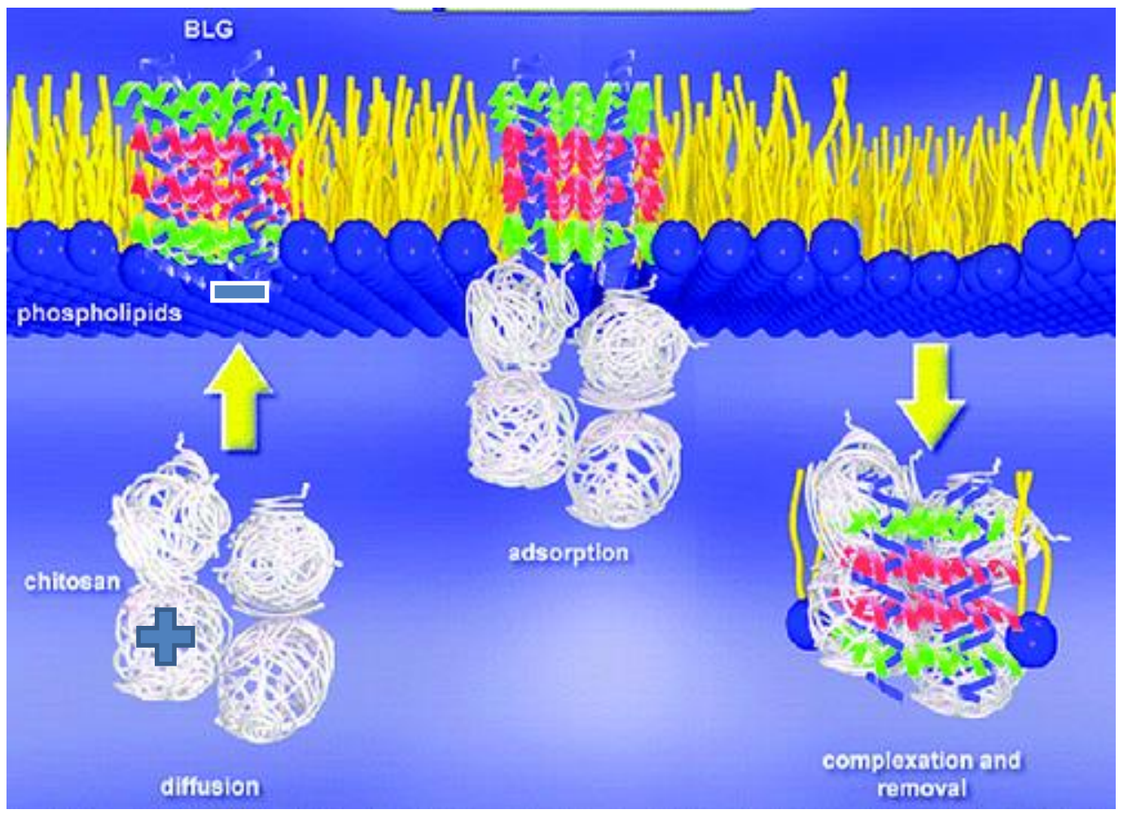
Experimental data



Salt is monovalent!!!

Salt is monovalent!!!

Complexation "on the wrong side"



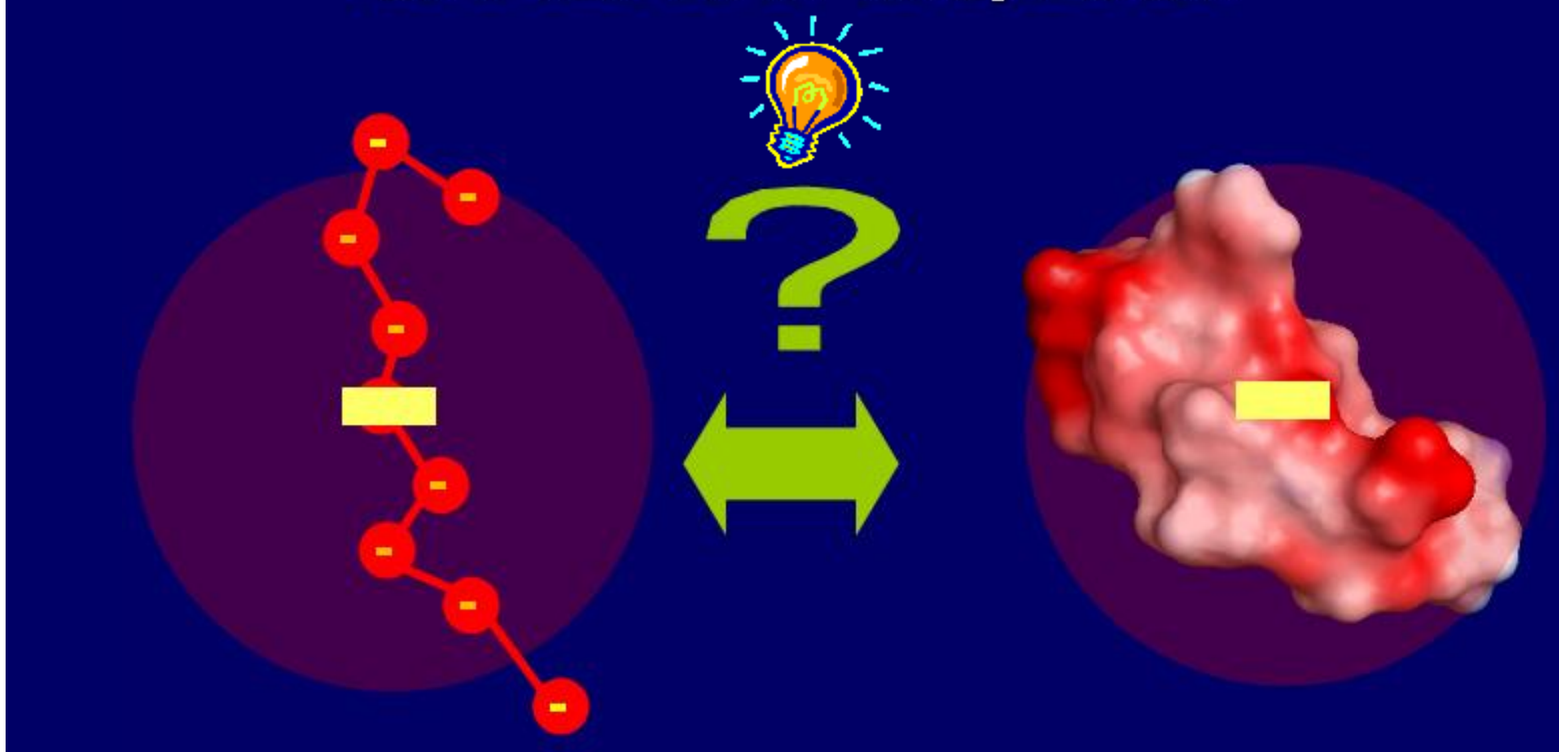
[L. Caseli et al; *Langmuir* **2008,** 24, 4150-4156]

Experimental behaviour

Fernando Barroso, FCFRP/USP
SAIFR, March 9-15, 2020

Laboratory of Computational Biophysical Chemistry

Association of complexes?



But, salt is monovalent!!!

Weak coupling (monovalent ions)

- Screening long-range interactions
- Short-range condensation($Z \rightarrow Z_{eff}$)
- DVLO (**repulsion**): ~ exp(-κr)/r

$$\Xi = 2\pi\, z_k^3 l_b^2 \sigma_s$$

STRONG coupling (multivalent ions)

- Charge reversal/"overcharging"
- Coloidal Attraction
- ❑ **Ion-ion correlation**

How to explain it?

Old controversial issue...

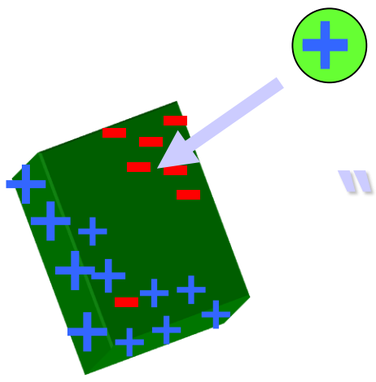
Emmanuel TRIZAC

Professor London has informed us that he also obtained an approximate expression for the interaction energy of two colloidal particles which is similar to ours. [...] He got the peculiar result that on the basis of Debye-Hiickel theory, two similarly charged colloidal particles **attract each other at large distances.** [...] His calculations have, so far, not been published due to certain objections raised by H. Kallmann, which we cannot consider to be valid any longer.

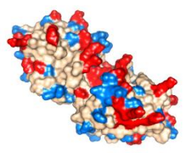
S. Levine et G. Dube, 1939

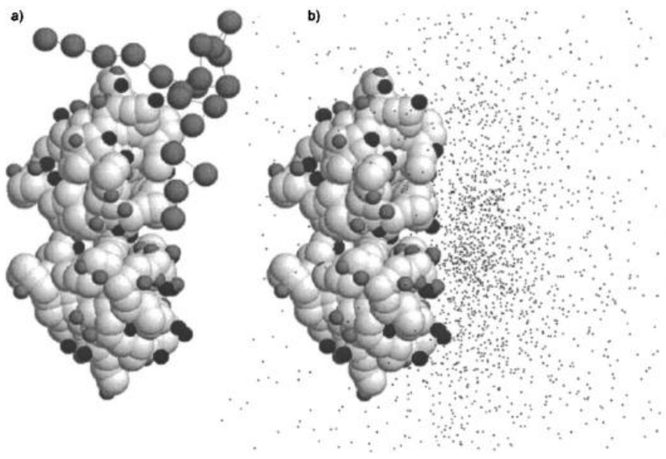
In a series of papers, Levine and Dube treated of the interactions of spherical particles [...] They derived –unfortunately using a wrong method– equations for the potential energy of interaction. [...] The attraction at relatively great distances is a result of a fallacious expression for the free energy. The general principles [...] were erroneous.

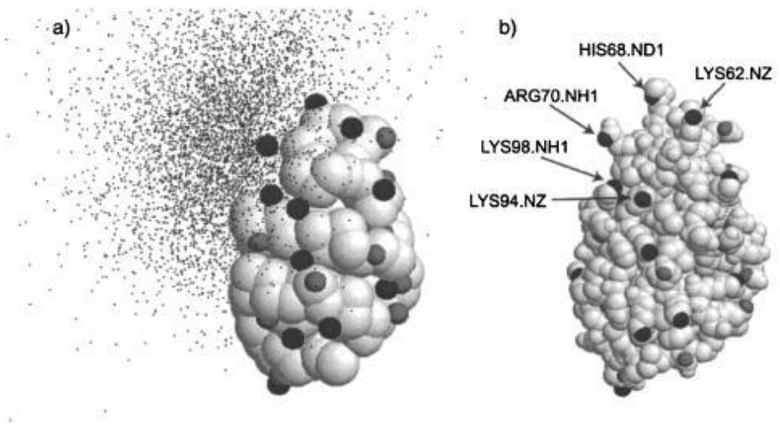
E. Verwey et J. Overbeek, 1948 |



"Patches" (on the wrong side)







3480 J. Chem. Phys., Vol. 120, No. 7, 15 February 2004 R. de Vries

154

PNAS -- Tabl

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Home

Previous

Table

Harlow Sha
Gal
PNA

Warren P.
Nat
PNA

A. J. Hodg
A N
Sol
PNA

John G. Ki
The
Sol
PNA

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PNA

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FORCES BETWEEN PROTEIN MOLECULES IN SOLUTION ARISING FROM FLUCTUATIONS IN PROTON CHARGE AND CONFIGURATION*

BY JOHN G. KIRKWOOD AND JOHN B. SHUMAKER

STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Communicated July 23, 1952

The forces between protein molecules in solution are in large part electrostatic in origin. Evidence for the dominant role of electrostatic forces is provided by the marked effect of ionic strength on the thermodynamic interaction of proteins. Thus, the commonly observed reduction in interaction produced by the screening action of the statistical space charge of the ions of an electrolyte would only be effective on that part of the force between protein molecules, which is electrostatic in origin. At values of pH departing from the isoionic points of the molecules in question, a simple Coulomb force, determined by their net electric charges, is predominant. This force is non-specific and is sensitive to molecular structure only in so far as structure influences the net charges. When one or both of a pair of protein molecules are at their isoionic points, structure sensitive electrostatic forces come into play. These forces have been

155

excess chemical potential at protein concentrations of the order of several per cent.

The present analysis of the attractive force between proteins arising from fluctuations in charge and configuration of mobile protons is necessarily schematic because of lack of knowledge of the details of protein structure. Although it is a force of long range at low ionic strength, it appears to exhibit specificity only through the influence of structure on the fluctuations. It is clear, however, that highly specific interactions might well arise from the mechanism of interaction, which has been described. In favorable orientations, steric matching of a constellation of basic groups on one molecule with a complementary constellation on the other could conceivably produce a redistribution of protons leading to a strong and specific attraction depending upon the local structural details of the complementary constellations. As an extreme example it is possible to imagine proton fluctuations to be frozen in such a manner as to produce an intermolecular zwitterion, with matching areas of positive and negative charge on complementary areas of the two molecules. Such considerations relating to specificity of the fluctuation force must necessarily remain speculative until detailed knowledge of structure is available.

In conclusion, the authors wish to acknowledge their indebtedness to Julian M. Sturtevant for interesting discussions during the course of this investigation.

* This investigation was in part supported by the office of Naval Research.

[1] Kirkwood, J. G., and Shumaker, J. B., these PROCEEDINGS **38**, 855–862 (1952).

156

*FORCES BETWEEN PROTEIN MOLECULES IN SOLUTION ARISING FROM FLUCTUATIONS IN PROTON CHARGE AND CONFIGURATION**

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The forces between protein molecules in solution are in large part electrostatic in origin. Evidence for the dominant role of electrostatic forces is provided by the marked

$$C \equiv \langle Q^2 \rangle - \langle Q \rangle^2 \alpha - \frac{1}{\ln 10} \frac{\partial Q}{\partial \mathrm{pH}}$$

$$\beta A(R) \approx \frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R} - \frac{l_B^2}{2R^2} \left[C_A C_B + C_A \langle Q_B \rangle^2 + C_B \langle Q_A \rangle^2 \right]$$

direct Coulomb term

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At values of pH departing

question, a simple Coulomb

is predominant. This force is non-specific and is sensitive to molecular structure only in so far as structure influences the net charges. When one or both of a pair of protein molecules are at their isoionic points, structure sensitive electrostatic forces come into play. These forces have been

157

Charge Regulation of Proteins

Theory

$$\beta A(R) \approx \frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R} - \frac{l_B^2}{2R^2}\left[\langle Q_A^2 \rangle \langle Q_B^2 \rangle - \langle Q_A \rangle^2 \langle Q_B \rangle^2\right]$$

"Charge Polarizability" (or Capacitance)

$$C \equiv \langle Q^2 \rangle - \langle Q \rangle^2 \alpha - \frac{1}{\ln 10}\frac{\partial Q}{\partial \mathrm{pH}}$$

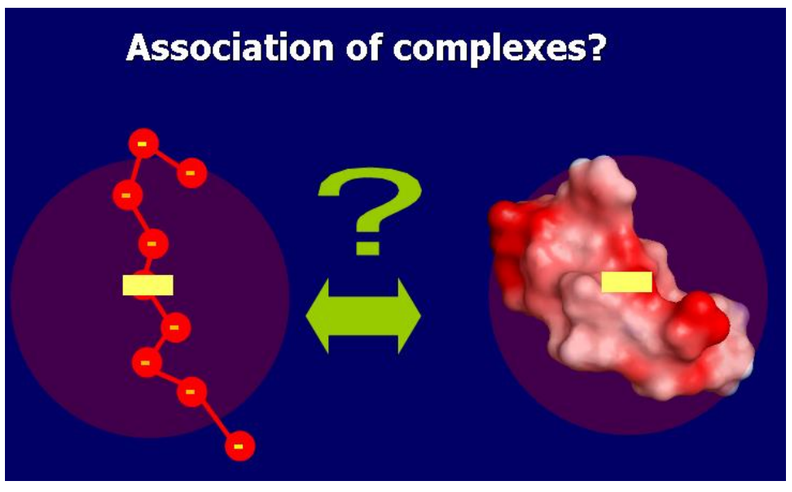
(1)

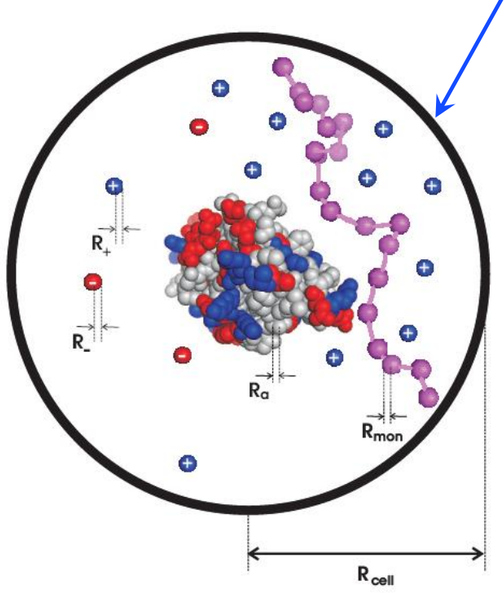
(2)

$$\beta A(R) \approx \underbrace{\frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R}}_{\text{direct Coulomb term}} - \underbrace{\frac{l_B^2}{2R^2}\left[C_A C_B + C_A \langle Q_B \rangle^2 + C_B \langle Q_A \rangle^2\right]}_{\text{induced charge-induced charge charge-induced charge terms}}$$

[F. L. B. da Silva et al; *J. Phys. Chem. B* **2006,** 110, 4459-4464]

CpH model for protein-polyelectrolyte





$$\Delta E = \Delta E_C \pm kT \ln 10 (pH - pK_0)$$

(GLU: K_0=4.4; ASP: K_0=4.0)

- **Hamiltonian - Particle *i* and *j***

$$u_{ij}(r_{ij}) = \begin{cases} \infty & , \text{if } r_{ij} \le (R_i + R_j) \\ \dfrac{z_i z_j e^2}{4\pi\,\varepsilon_0 \varepsilon_S r_{ij}} & , \text{otherwise} \end{cases}$$

- **Cell boundary constraint**

$$v^{ex}(r_i) = \begin{cases} 0, \text{ if } (R_i + R_P) \le r_i \le R_{Cell} \\ \infty, \text{ otherwise} \end{cases}$$

- **Bond constraint**

$$u^{\text{Bond}}_{i,i+1}(r_{i,i+1}) = \frac{k}{2}\left(|r_i - r_{i+1}|^2\right)$$

(K ≡ K(b); b=5Å and K=11.0 10^{-3}N/m)

- **Full configurational energy**

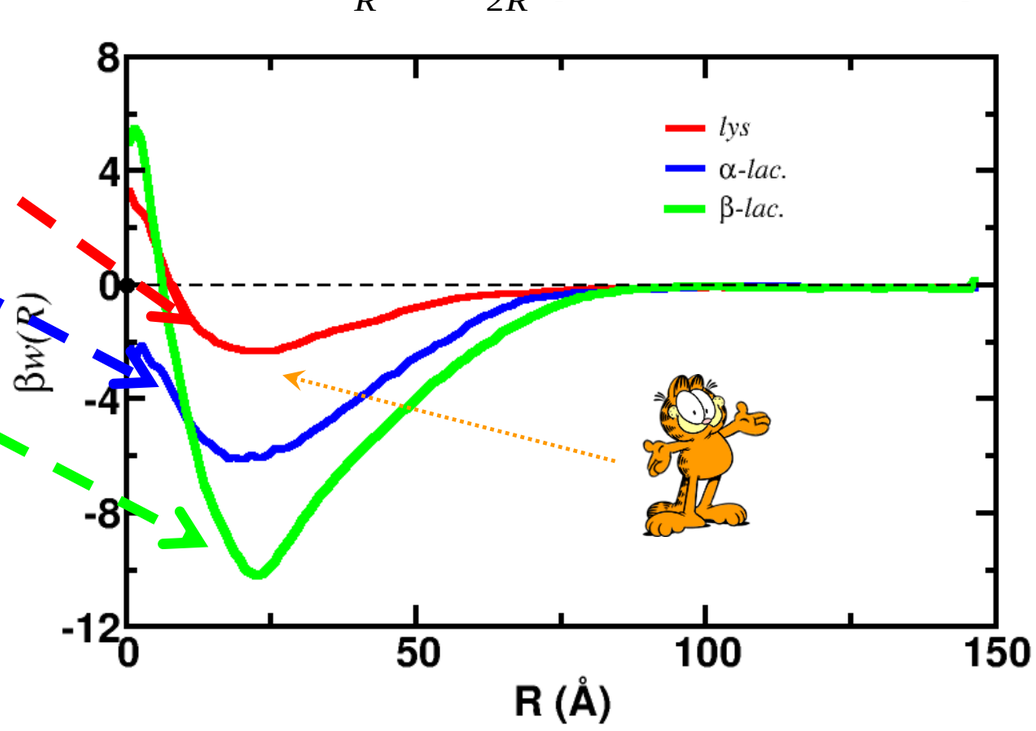
$$U(\{r_k\}) = \sum_{i=1}^{N_c+N_S+N_L} v^{ex}(r_i) + \frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N} u_{ij}(r_{ij}) + \sum_{i=N_P+N_S+N_c}^{N_L} u^{\text{Bond}}_{i,i+1}(r_{i,i+1})$$

Laboratory of Computational Biophysical Chemistry

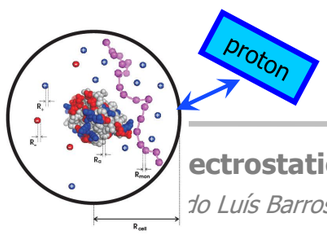
Can we observe the complex formation at pI ?

$$\beta A(R) \approx \frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R} - \frac{l_B^2}{2R^2}\left[C_A C_B + C_A \langle Q_B \rangle^2 + C_B \langle Q_A \rangle^2\right]$$

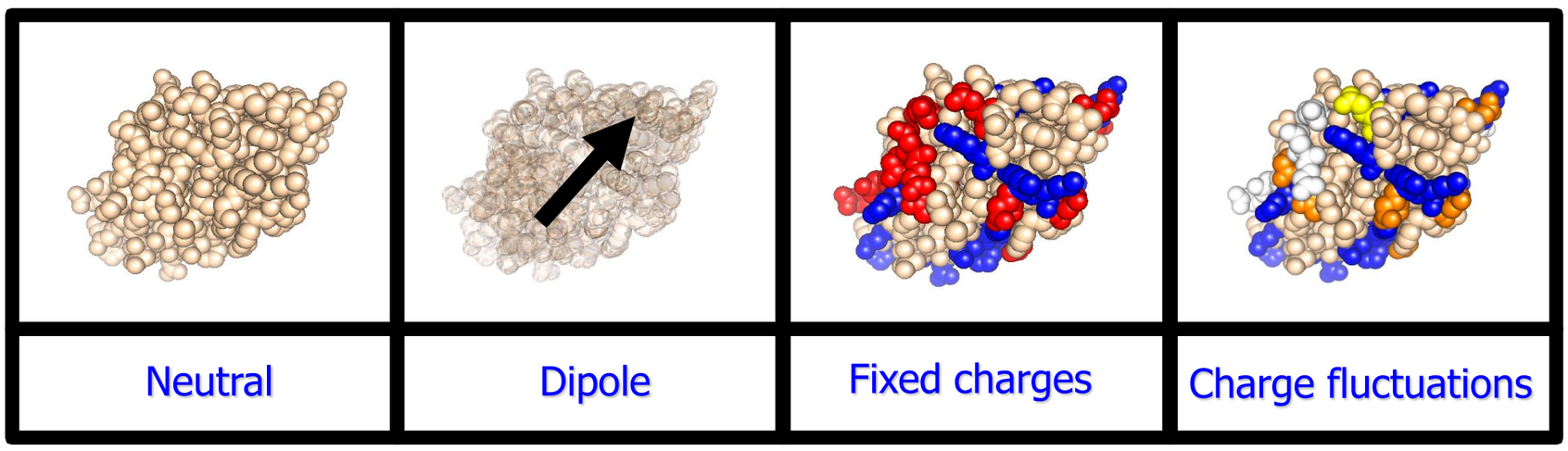
C	μ
1.7	23
1.0	82
4.6	130



	pI
lysozyme	10.9
α-lactalbumin	5.4
β-lactoglobulin	4.5

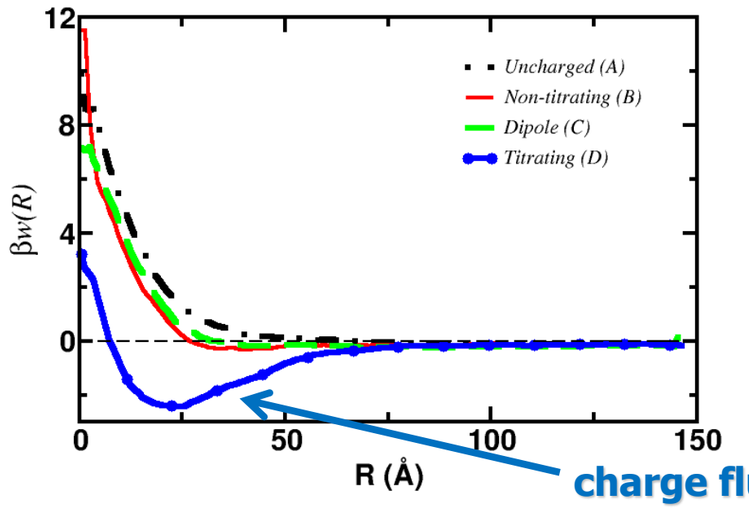


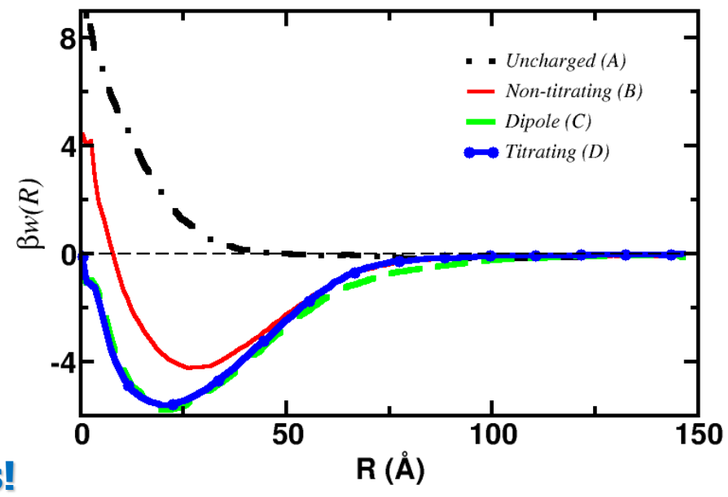
(Barroso Da Silva, Lund & Jönsson, JPCB 110, p. 4459, 2006)



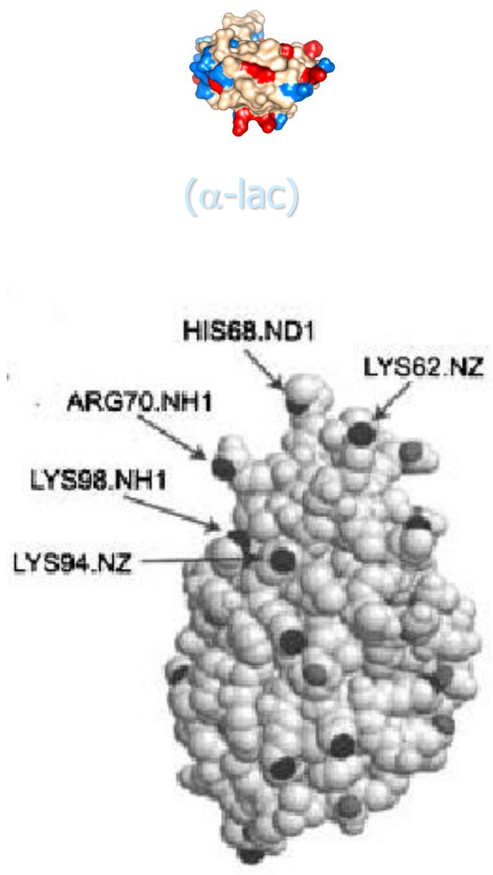
μ = 23 (lys)

μ = 82 (α-lac)



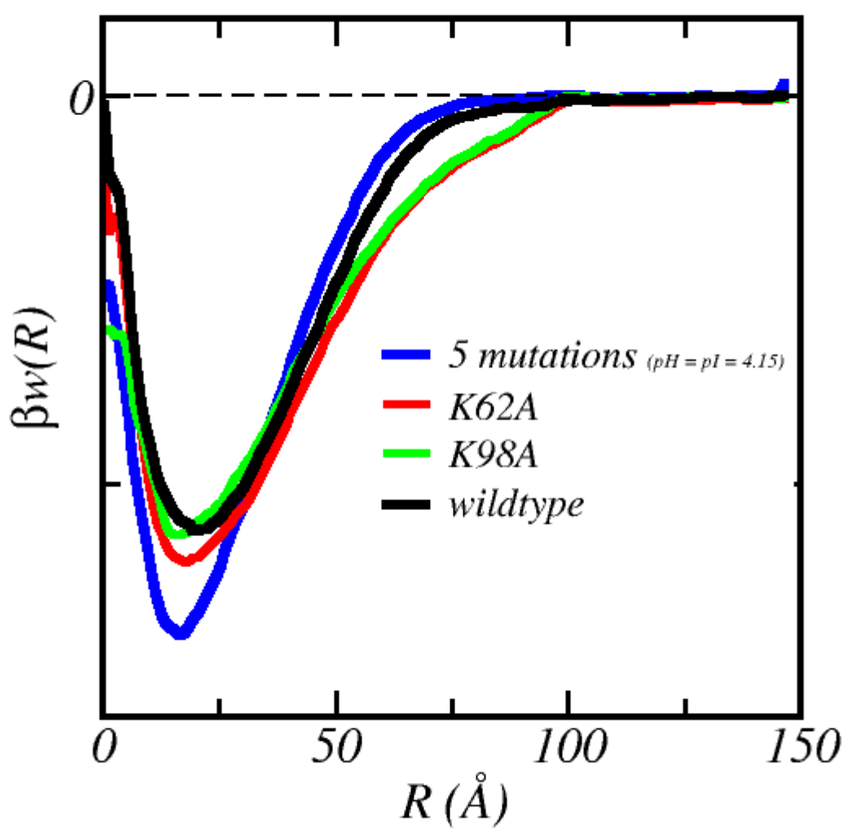


162

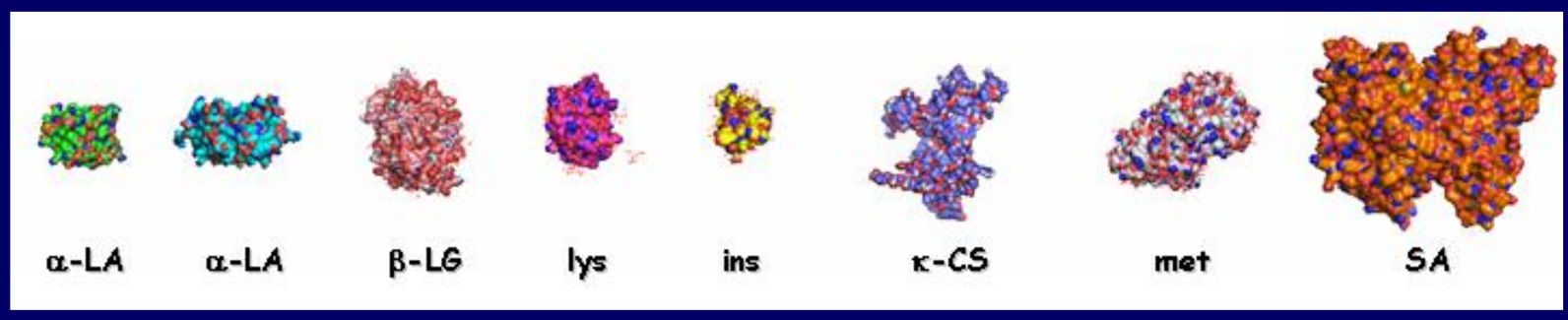


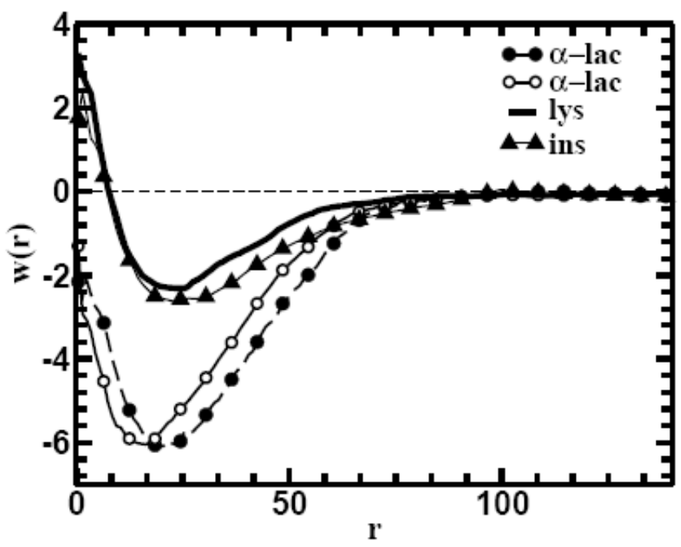
R. de Vries
J. Chem. Phys., Vol. 120, No. 7, 15 February 2004

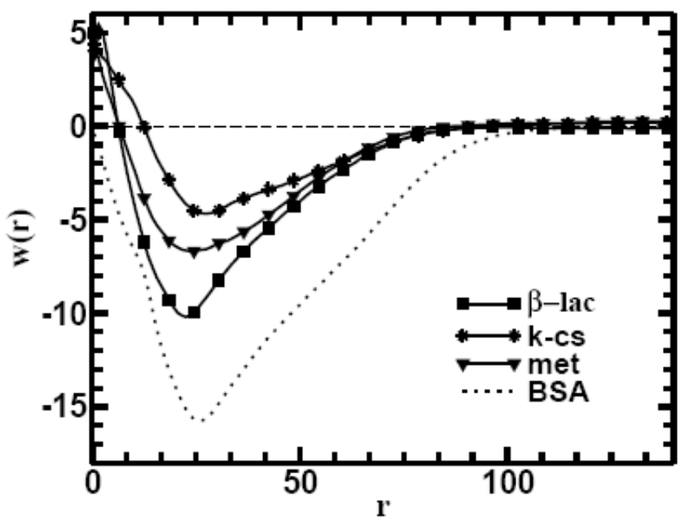
(α-lac)



[Da Silva & Jönsson, SM 5(15) 2862 (2009)]







(Da Silva & Jönsson, *Soft Matter*, 2009)

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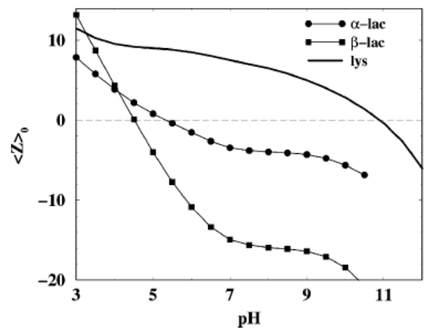
Protein Electrostatics
Fernando Barroso (fernando@fcfrp.usp.br)

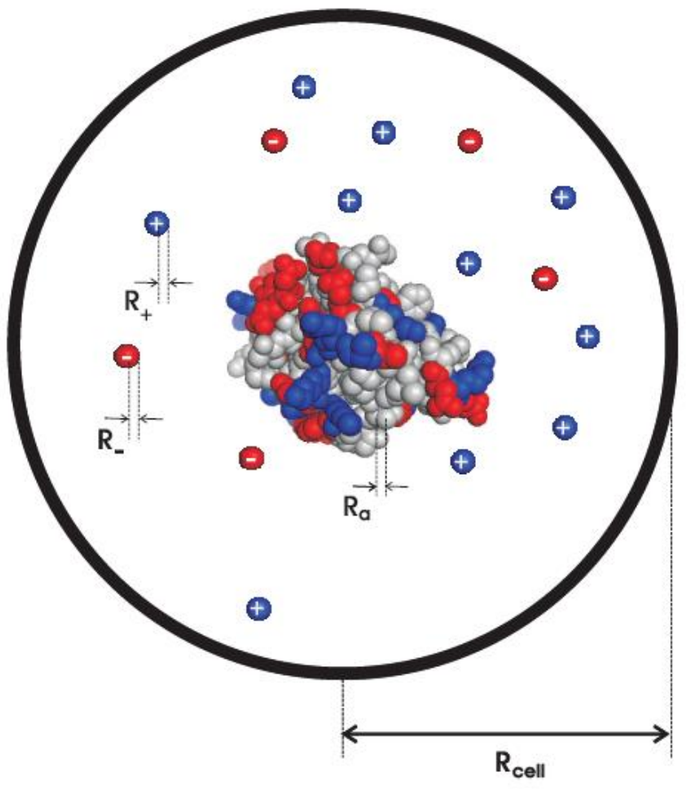
B.P.C. – DCBM/FCFRP - USP
SAIFR - March 9-15, 2020

164

Complex formation at pI

	residues	pI	C	μ
albumin	585	5.5	3.2	297
α-lactalbumin[a]	123	4.8	1.5	101
α-lactalbumin[b,c]	123	5.4	1.0	82
β-lactoglobulin[c,*]	324	4.5	4.5	128
insulin	41	5.4	0.4	49
k-casein[d]	169	5.9	1.3	151
lysozyme[c]	129	10.9	1.7	24
pectin methylesterase	319	9.5	2.4	60
α-lac[b] (mutation K62)	123	5.0	1.2	78
α-lac[b] (mutation K94)	123	5.0	1.3	69
α-lac[b] (mutation K98)	123	5.0	1.2	65
α-lac[b] (mutation H68)	123	5.0	1.2	74
α-lac[b] (mutation R70)	123	5.0	1.2	65
α-lac[b] (all 5 mutations)	123	4.1	2.5	61





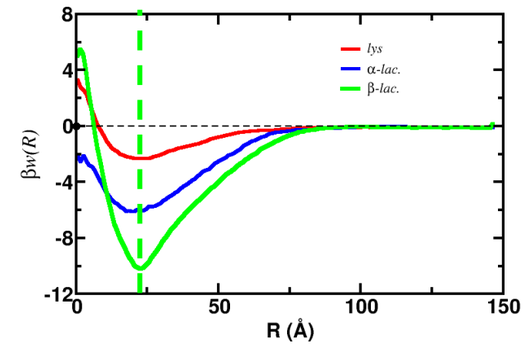
(Da Silva & Jönsson, *Soft Matter*, 2009)

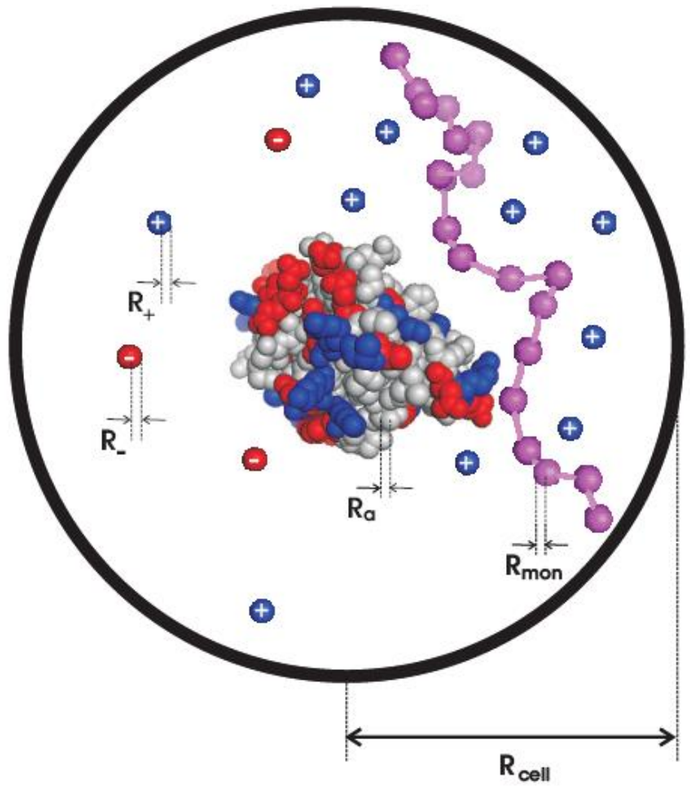
165

Complex formation at pI

	residues	pI	C	μ	$-\beta A(R)$	R
albumin	585	5.5	3.2	297	15.8	25
α-lactalbumin^{a}	123	4.8	1.5	101	6.0	16
α-lactalbumin^{b,c}	123	5.4	1.0	82	6.1	19
β-lactoglobulin^{c,*}	324	4.5	4.5	128	10.2	22
insulin	41	5.4	0.4	49	2.6	22
k-casein^{d}	169	5.9	1.3	151	4.7	27
lysozyme^{c}	129	10.9	1.7	24	2.3	25
pectin methylesterase	319	9.5	2.4	60	6.7	24
α-lac^{b} (mutation K62)	123	5.0	1.2	78	4.1	21
α-lac^{b} (mutation K94)	123	5.0	1.3	69	3.4	22
α-lac^{b} (mutation K98)	123	5.0	1.2	65	2.7	24
α-lac^{b} (mutation H68)	123	5.0	1.2	74	3.6	19
α-lac^{b} (mutation R70)	123	5.0	1.2	65	3.5	19
α-lac^{b} (all 5 mutations)	123	4.1	2.5	61	6.9	16

166





(Da Silva & Jönsson, *Soft Matter*, 2009)

Complex formation at pI

$$\beta A(R) \approx l_B Z_\alpha \left(\frac{\langle Z\rangle_0}{R}\right) - l_B^2 Z_\alpha^2 \left(\frac{C}{2R^2} + \frac{\langle \mu\rangle_0^2}{6R^4}\right)$$

	residues	pI	C	μ	$-\beta A(R)$	R	$R_p + R_{pe}$	$-\beta A_{reg}$	$-\beta A_{dip}$	A_{reg}/A_{dip}
albumin	585	5.5	3.2	297	15.8	25	81	5.5	7.7	0.7
α-lactalbumin^{a}	123	4.8	1.5	101	6.0	16	57	5.0	3.3	1.5
α-lactalbumin^{b,c}	123	5.4	1.0	82	6.1	19	58	3.3	2.2	1.5
β-lactoglobulin^{c,*}	324	4.5	4.5	128	10.2	22	73	9.4	2.1	4.4
insulin	41	5.4	0.4	49	2.6	22	51	1.7	1.3	1.3
k-casein^{d}	169	5.9	1.3	151	4.7	27	84	2.0	1.7	1.2
lysozyme^{c}	129	10.9	1.7	24	2.3	25	58	5.6	0.2	29.8
pectin methylesterase	319	9.5	2.4	60	6.7	24	68	5.8	0.6	9.2
α-lac^{b} (mutation K62)	123	5.0	1.2	78	4.1	21	58	4.0	2.0	2.0
α-lac^{b} (mutation K94)	123	5.0	1.3	69	3.4	22	58	4.3	1.6	2.8
α-lac^{b} (mutation K98)	123	5.0	1.2	65	2.7	24	58	4.0	1.4	2.9
α-lac^{b} (mutation H68)	123	5.0	1.2	74	3.6	19	58	4.0	1.8	2.2
α-lac^{b} (mutation R70)	123	5.0	1.2	65	3.5	19	58	4.0	1.4	2.9
α-lac^{b} (all 5 mutations)	123	4.1	2.5	61	6.9	16	58	8.3	1.2	6.8

(Da Silva & Jönsson, *Soft Matter*, 2009)

167

Complex formation at pI

$$\beta A(R) \approx l_B Z_\alpha \left(\frac{\langle Z\rangle_0}{R}\right) - l_B^2 Z_\alpha^2 \left(\frac{C}{2R^2} + \frac{\langle \mu\rangle_0^2}{6R^4}\right)$$

	residues	pI	C	μ	$-\beta A(R)$	R	$R_p + R_{pe}$	$-\beta A_{reg}$	$-\beta A_{dip}$	A_{reg}/A_{dip}
albumin	585	5.5	3.2	297	15.8	25	81	5.5	7.7	0.7
α-lactalbumin[a]	123	4.8	1.5	101	6.0	16	57	5.0	3.3	1.5
α-lactalbumin[b,c]	123	5.4	1.0	82	6.1	19	58	3.3	2.2	1.5
β-lactoglobulin[c,*]	324	4.5	4.5	128	10.2	22	73	9.4	2.1	4.4
insulin	41	5.4	0.4	49	2.6	22	51	1.7	1.3	1.3
k-casein[d]	169	5.9	1.3	151	4.7	27	84	2.0	1.7	1.2
lysozyme[c]	129	10.9	1.7	24	2.3	25	58	5.6	0.2	29.8
pectin methylesterase	319	9.5	2.4	60	6.7	24	68	5.8	0.6	9.2
α-lac[b] (mutation K62)	123	5.0	1.2	78	4.1	21	58	4.0	2.0	2.0
α-lac[b] (mutation K94)	123	5.0	1.3	69	3.4	22	58	4.3	1.6	2.8
α-lac[b] (mutation K98)	123	5.0	1.2	65	2.7	24	58	4.0	1.4	2.9
α-lac[b] (mutation H68)	123	5.0	1.2	74	3.6	19	58	4.0	1.8	2.2
α-lac[b] (mutation R70)	123	5.0	1.2	65	3.5	19	58	4.0	1.4	2.9
α-lac[b] (all 5 mutations)	123	4.1	2.5	61	6.9	16	58	8.3	1.2	6.8

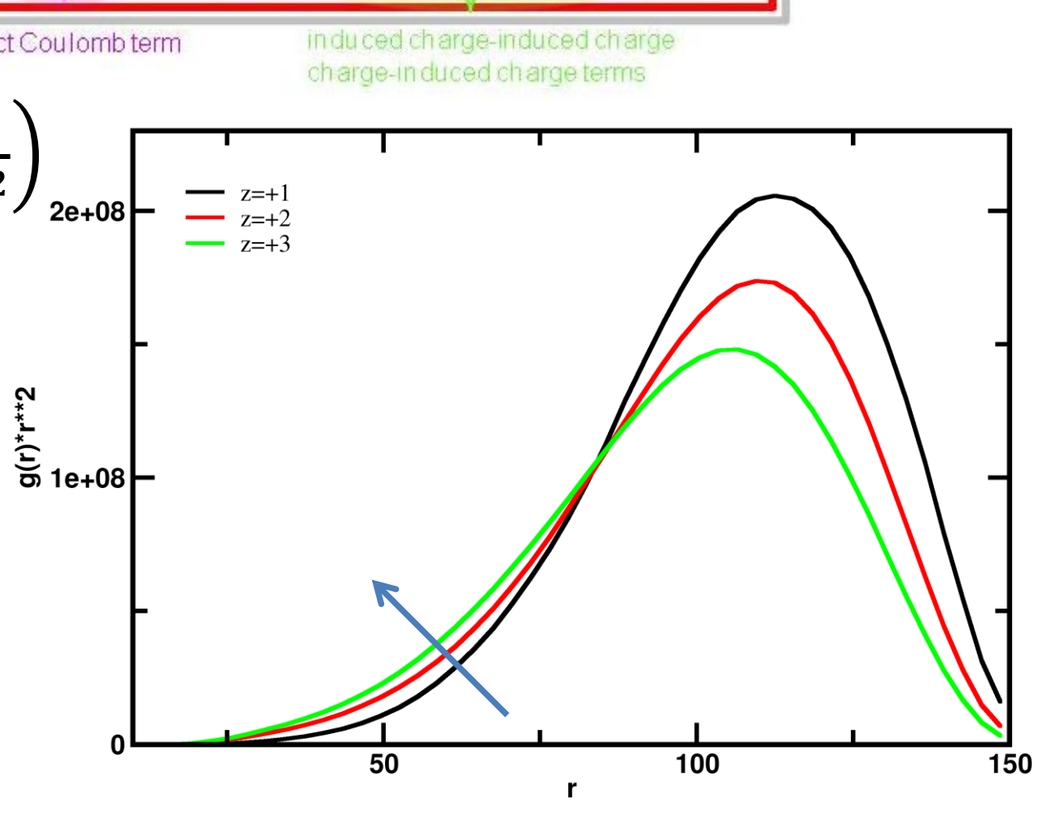
(Da Silva & Jönsson, *Soft Matter*, 2009)

168

Charge regulation: the charge contribution

$$\beta A(R) \approx \underbrace{\frac{l_B \langle Q_A\rangle\langle Q_B\rangle}{R}}_{\text{direct Coulomb term}} - \underbrace{\frac{l_B^2}{2R^2}\left[C_A C_B + C_A\langle Q_B\rangle^2 + C_B\langle Q_A\rangle^2\right]}_{\text{induced charge-induced charge terms}}$$

$$\beta A(R) = -l_B^2 \, \boldsymbol{Z^2} \left(\frac{C}{2R^2}\right)$$



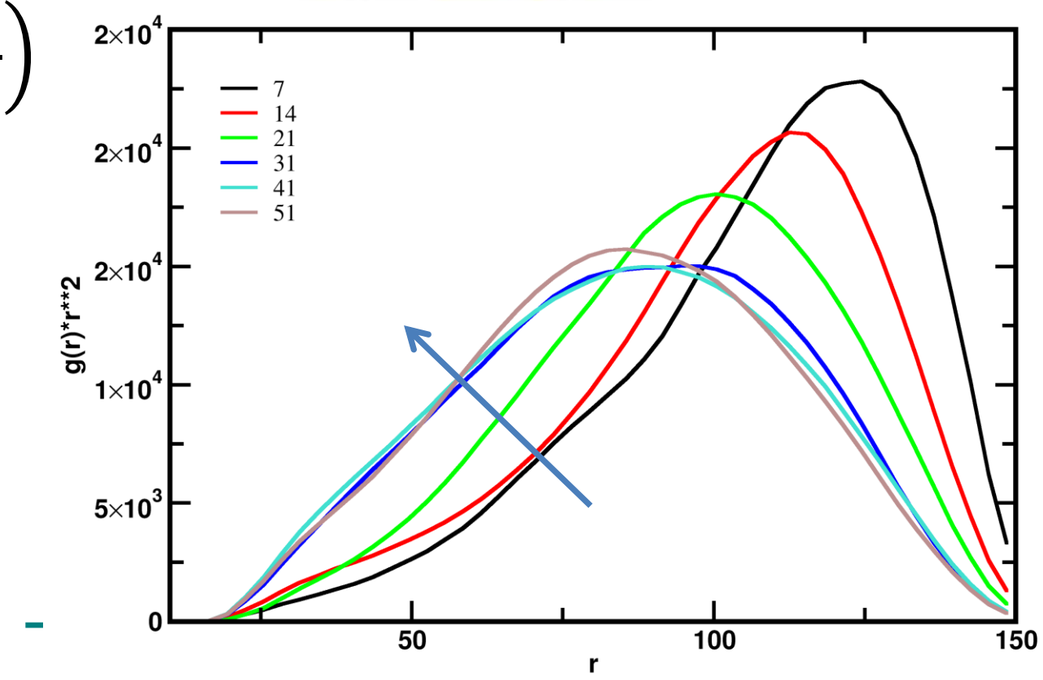
(Acken, Santiso and Barroso da Silva, in preparation)

169

Charge regulation: the charge contribution

$$\beta A(R) \approx \underbrace{\frac{l_B \langle Q_A \rangle \langle Q_B \rangle}{R}}_{\text{direct Coulomb term}} - \underbrace{\frac{l_B^2}{2R^2}\left[C_A C_B + C_A \langle Q_B \rangle^2 + C_B \langle Q_A \rangle^2\right]}_{\text{induced charge-induced charge charge-induced charge terms}}$$

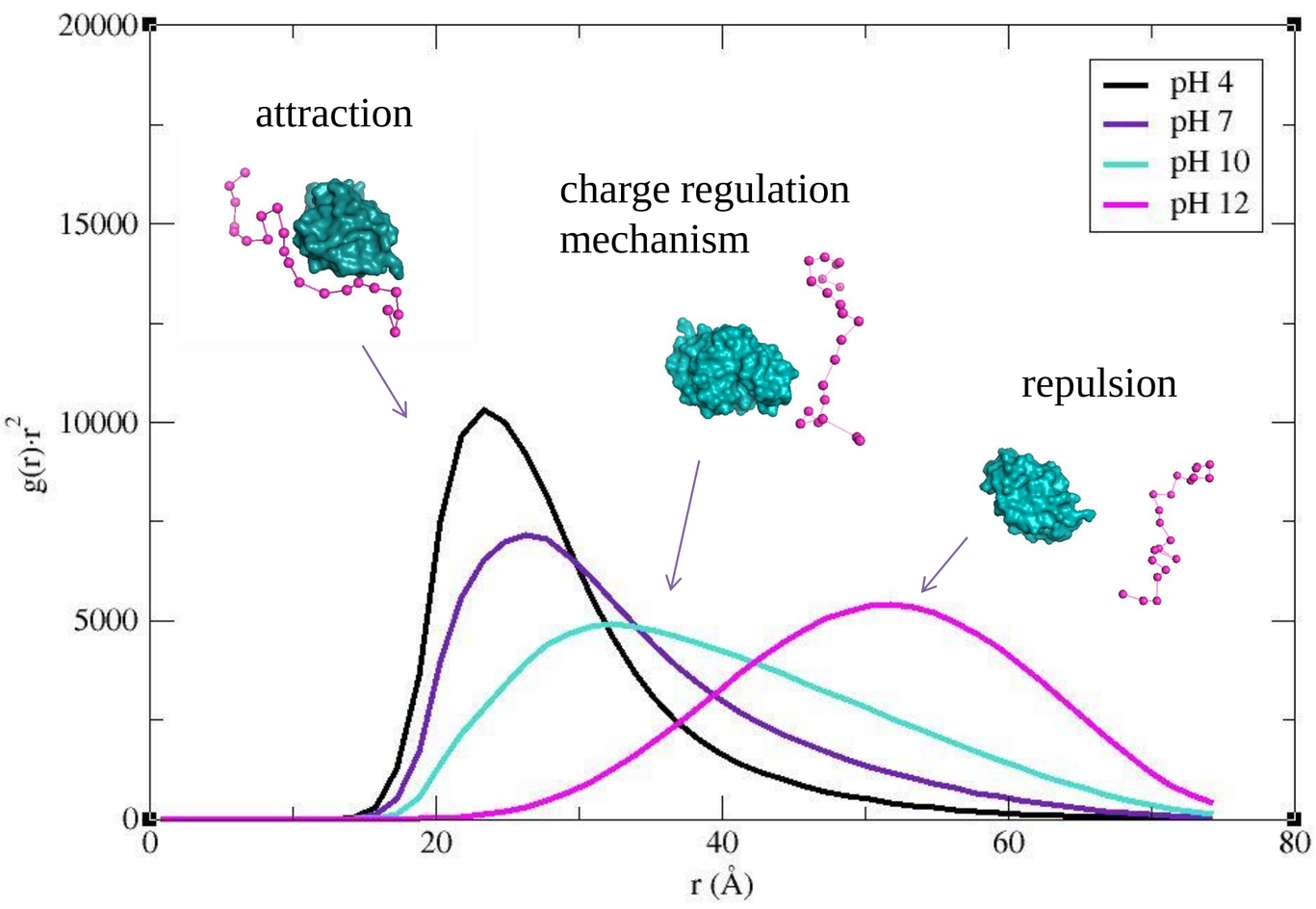
$$\beta A(R) = -l_B^2 \, Z^2 \left(\frac{C}{2R^2}\right)$$



(Acken, Santiso and Barroso da Silva, under preparation)

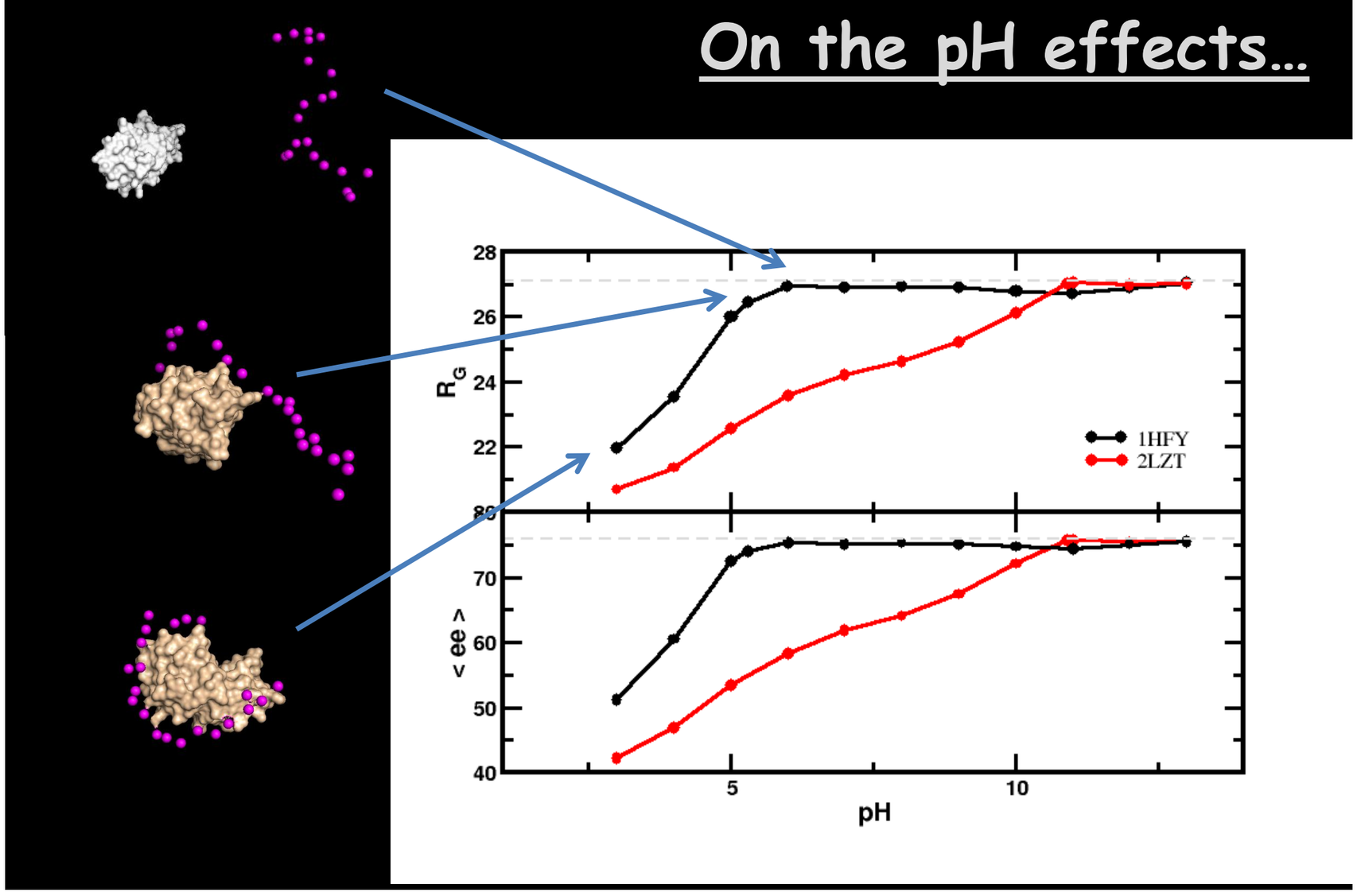
170

pH effects



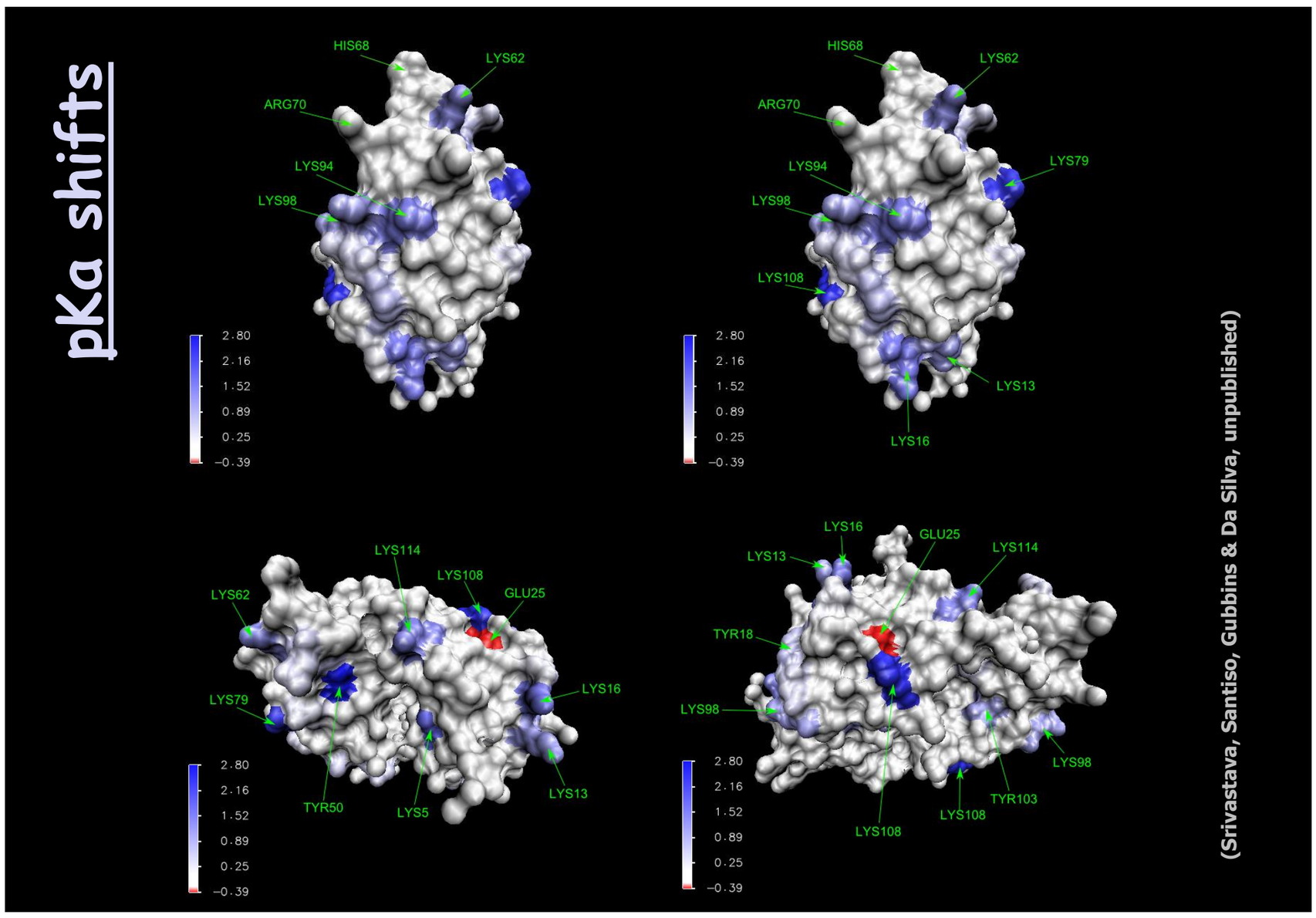
(Acken, Santiso and Barroso da Silva, under preparation)

On the pH effects…

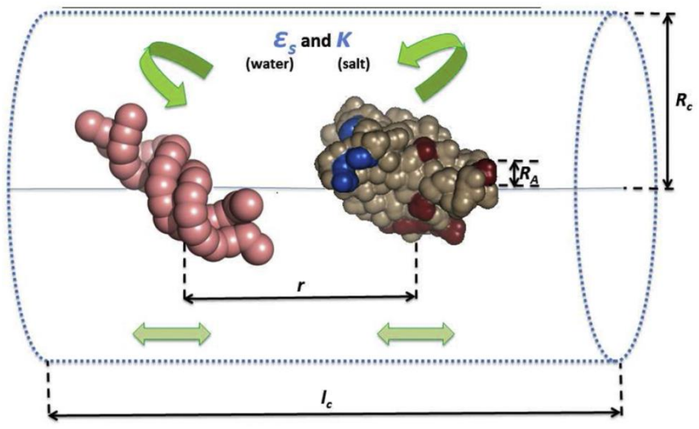


172

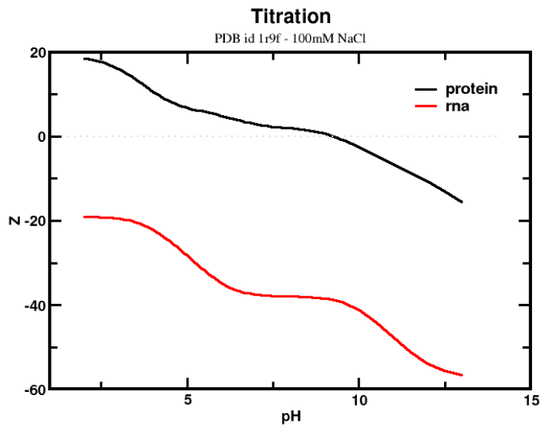
pKa shifts



173

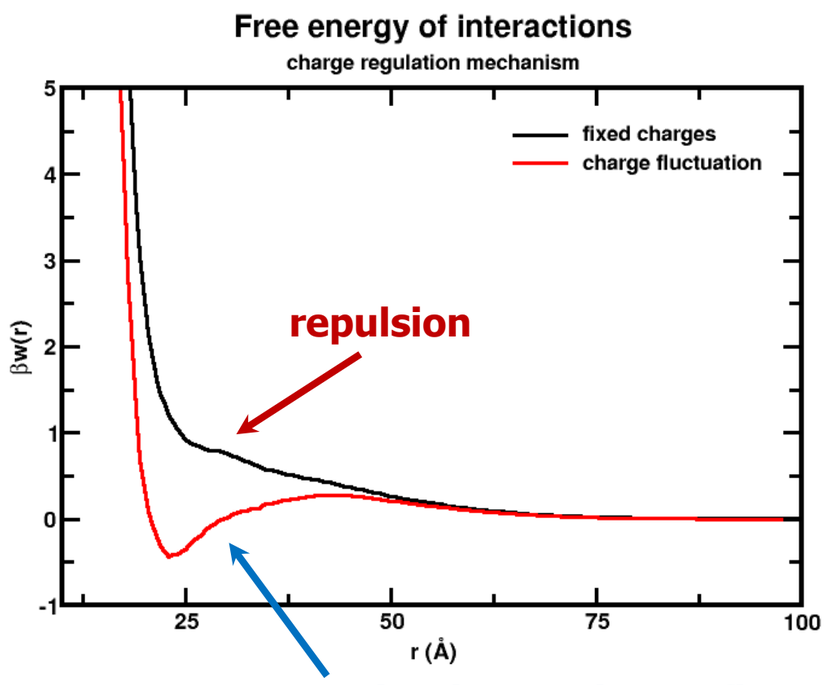


p19 viral protein and the 19-bp small interfering RNA



Protein-RNA complexation driven by the charge regulation mechanism

FLB da Silva, P Derreumaux, S Pasquali
Biochemical and biophysical research communications 498 (2), 264-273, 2018



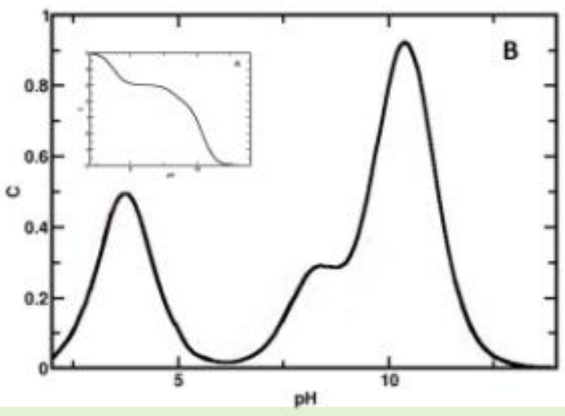
attraction **due to charge fluctuations!**

Charge regulation

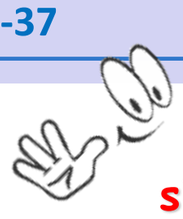
Investigating the pH and ionic strength effects on the affinity of a cationic peptide to anionic bilayer

POPC:POPG 20:80 and **pH = pI** (10.5)

Antibacterial peptide L1A (IDGLKAIWKKVADLLKNT-NH2)



	Experimental data			Simulation data	
Salt (mM)	K_P	Zeta (mV)	- ln K/K_{ref}	ΔpK(fixed ch)	ΔpK(charge fluctuation)
10	55300	-64			
150	15800	-37	1.3	0.2	1.2



Only constant-pH simulation can capture this mechanism!!!

[Viegas, Ruggiero & Da Silva, in preparation]

Other similar studies...

Langmuir **2004**, *20*, 2785−2791

Electrostatic Free Energy of Weakly Charged Macromolecules in Solution and Intermacromolecular Complexes Consisting of Oppositely Charged Polymers

P. Maarten Biesheuvel* and Martien A. Cohen Stuart

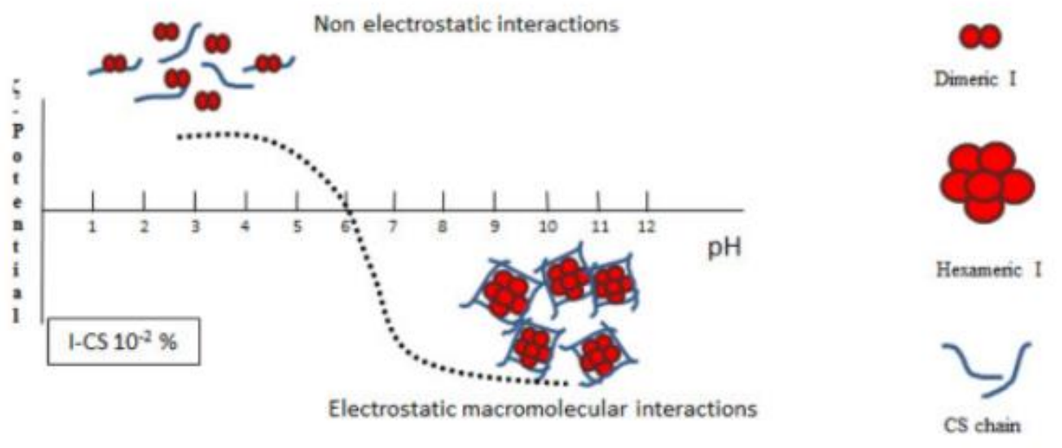
Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands

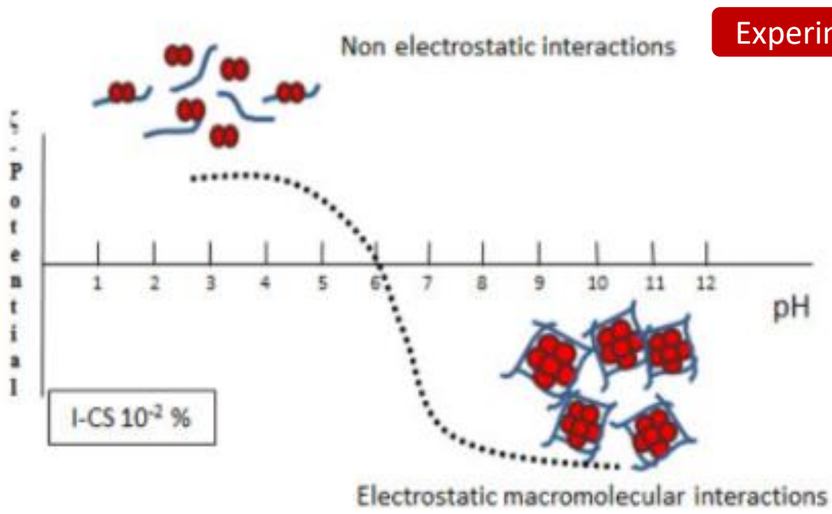
Received November 24, 2003. In Final Form: January 28, 2004

When oppositely charged polyelectrolytes are mixed in water, attraction between oppositely charged groups may lead to the formation of polyelectrolyte complexes (associative phase separation, complex coacervation, interpolymer complexes). Theory is presented to describe the electrostatic free energy change when ionizable (annealed) (macro-)molecules form a macroscopic polyelectrolyte complex. The electrostatic free energy includes an electric term as well as a chemical term that is related to the dissociation of the ionic groups in the polymer. An example calculation for complexation of polyacid with polybase uses a cylindrical diffuse double layer model for free polymer in solution and electroneutrality within the complex and calculates the free energy of the system when the polymer is in solution or in a polyelectrolyte complex. Combined with a term for the nonelectrostatic free energy change upon complexation, a theoretical stability diagram is constructed that relates pH, salt concentration, and mixing ratio, which is in qualitative agreement with an experimental diagram obtained by Bungenberg de Jong (1949) for complex coacervation of arabic gum and gelatin. The theory furthermore explains the increased tendency toward phase separation when the polymer becomes more strongly charged and suggests that complexation of polyacid or polybase with zwitterionic polymer (e.g., protein) of the same charge sign (at the "wrong side" of the iso-electric point) may be due (in part) to an induced charge reversal of the protein.

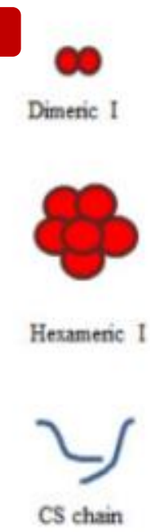
pH-dependent responsive mechanisms

- Insuline-chitosan nanocomplexation driven by pH solution.



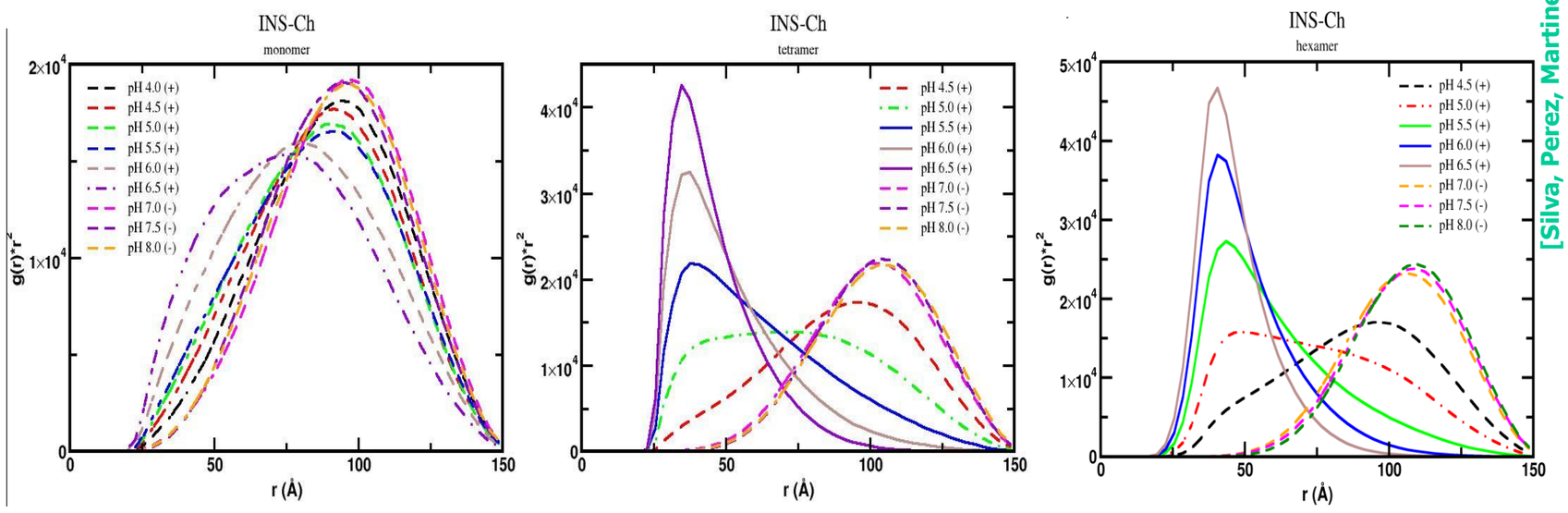


Experimental data



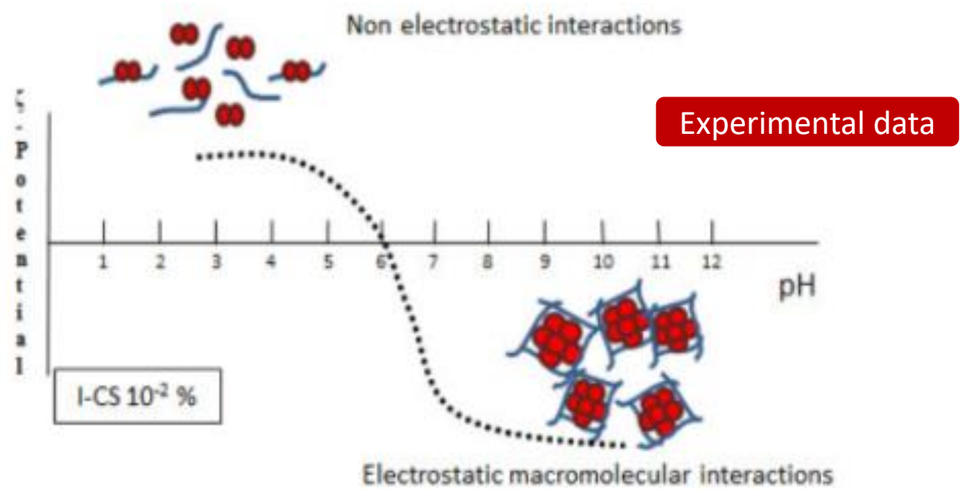
pH	INS	CS
3	20.6±2.1	5.8±2.8
4	17.4±1.3	6.8±0.7
5	**-9.5±0.7**	**6.5±0.5**
6	**-32.4±2.2**	**5.5±0.7**
8	-39.1±3.3	-17.9±0.8
9	-44.6±1.9	-20.5±1.6

Simulation data

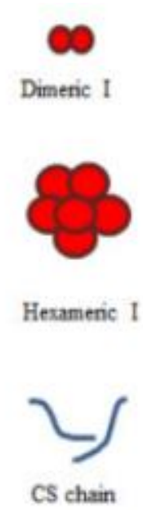


[Silva, Perez, Martinez & Barroso Da Silva, JCTM 2020]

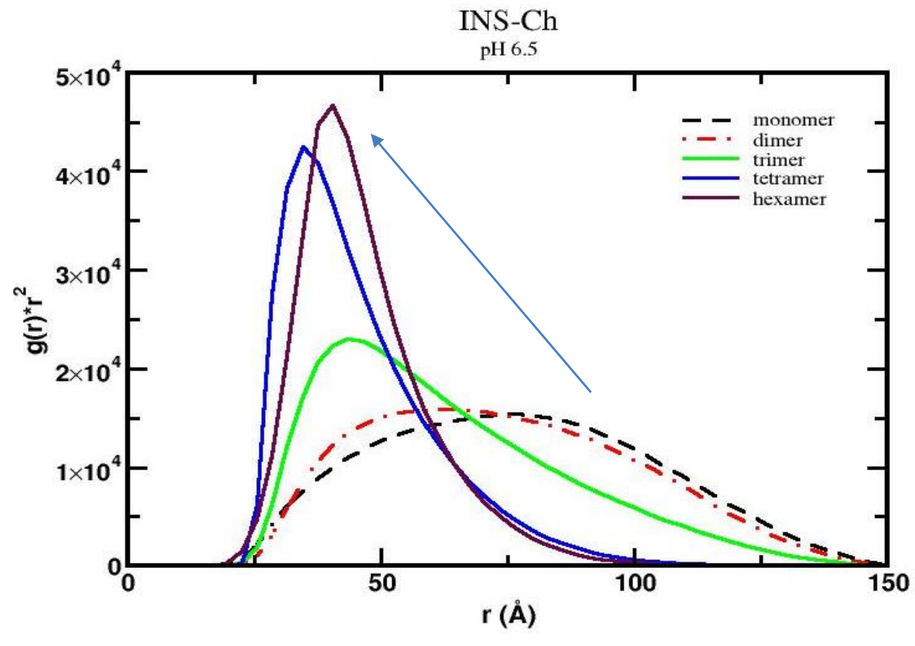
178



Experimental data



[Silva, Perez, Martinez & Barroso Da Silva, JCTM 2020]



Simulation data

179

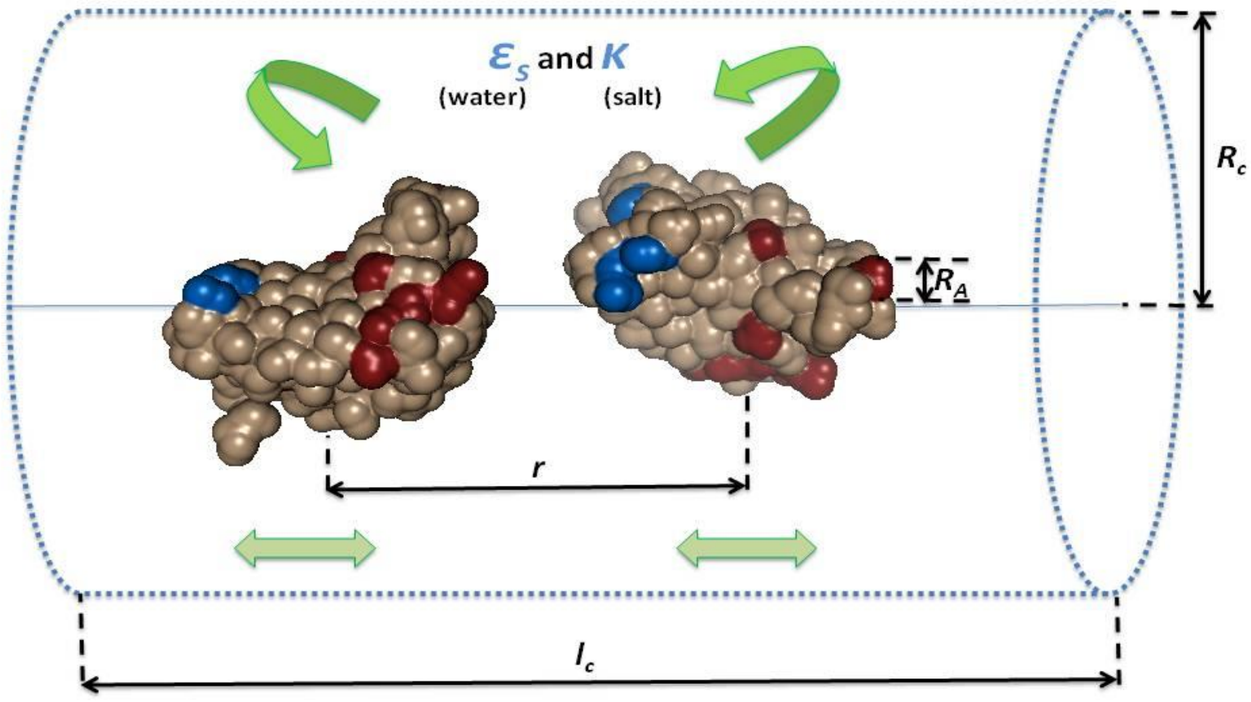
Constant pH simulations

[AART, ML & **FLBDS**. *J. Chem. Theory Comput.*, **2010**]

$$w_{TK} \approx \frac{e^2}{4\pi\varepsilon_0\varepsilon_r}\left(\sum_{i>j}^{n}\frac{z_i z_j}{r_{ij}} - \frac{Z_P^2\kappa}{2(1+\kappa b)}\right)$$

$$\Delta E = \Delta w_{TK} \pm kT\ln 10(pH - pK_0)$$

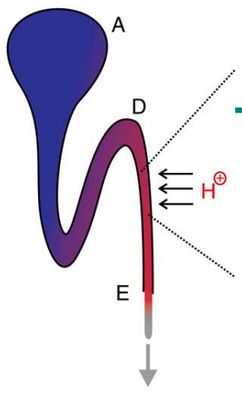
Titration

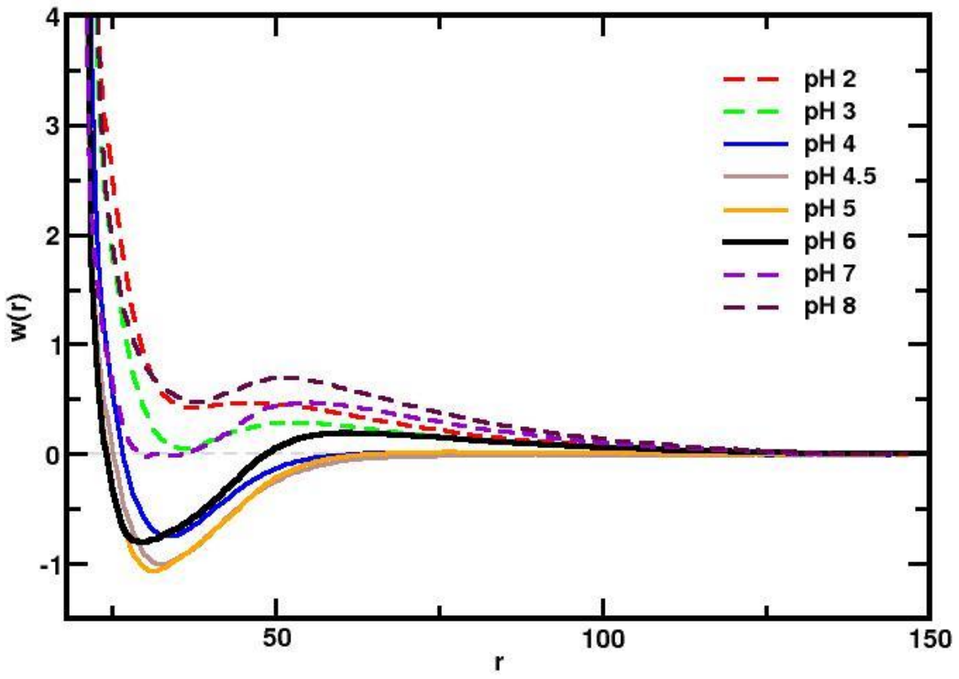


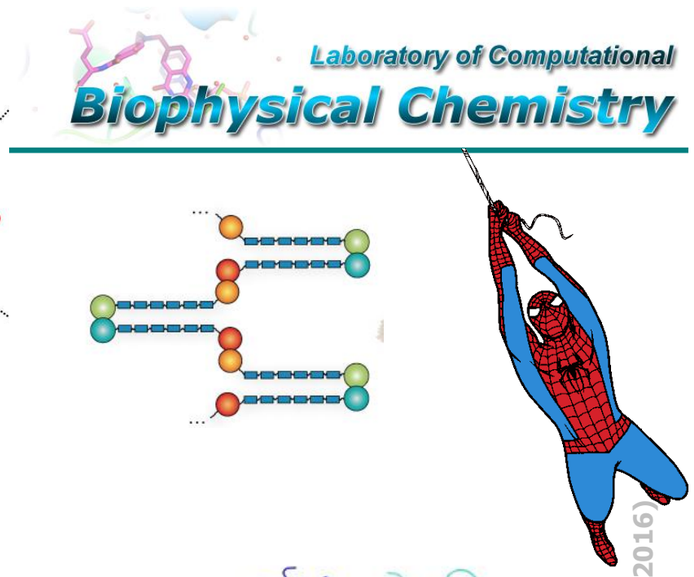
180

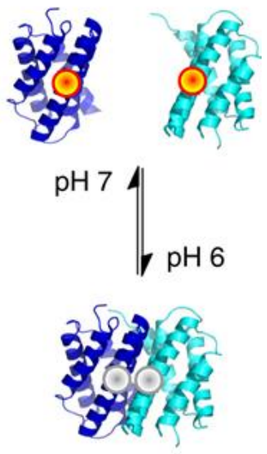
Spidroins

Reproduce experimental behaviour!







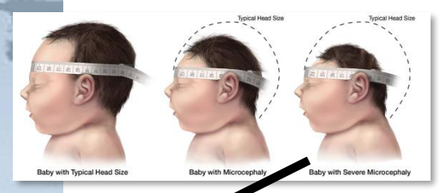


Fernando Barroso, FCFRP/USP
NCSU - June 5, 2019

(Da Silva, Pasquali, Derreumaux & Dias, SM, 2016)

181

Zika Virus



http://www.dailymail.co.uk/health/article-3433577/Brazilian-health-institute-says-discovered-Zika-saliva-urine.html

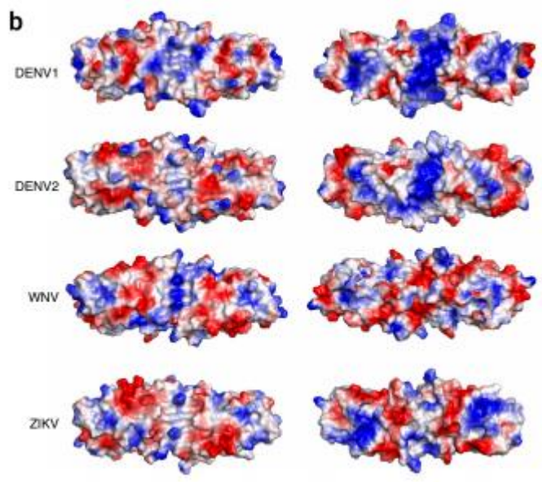
182

Zika virus NS1 structure reveals diversity of electrostatic surfaces among flaviviruses

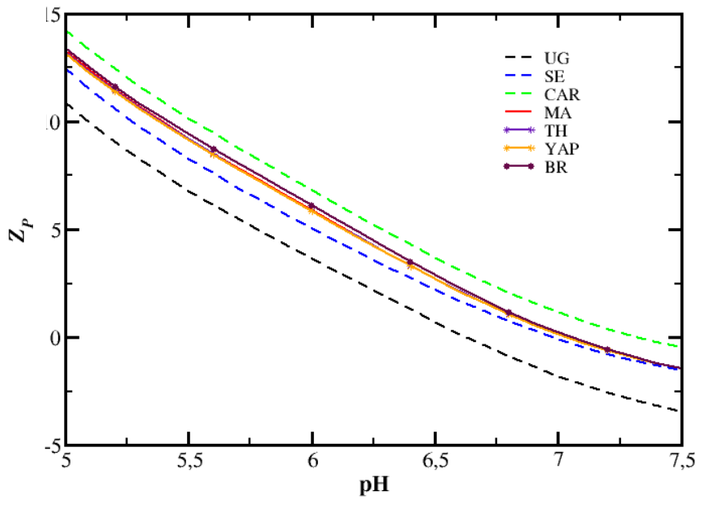
Hao Song[1,2], Jianxun Qi[1], Joel Haywood[1], Yi Shi[1–5] & George F Gao[1–6]

The association of Zika virus (ZIKV) infections with microcephaly has resulted in an ongoing public-health emergency. Here we report the crystal structure of a C-terminal fragment of ZIKV nonstructural protein 1 (NS1), a major host-interaction molecule that functions in flaviviral replication, pathogenesis and immune evasion. Comparison with West Nile and dengue virus NS1 structures reveals conserved features but diverse electrostatic characteristics at host-interaction interfaces, thus possibly implying different modes of flavivirus pathogenesis.

(Nature structural & molecular biology 2016; doi:10.1038/nsmb.3213)



This inspired us!



(Cuevas, Etchebest & Barroso Da Silva, 2018)

183

Laboratory of Computational Biophysical Chemistry

Highlights

- Simplified models can aid the understanding of complex molecular mechanisms.

- They can also be used to design new rational applications.

- **Electrostatic selectivity** in biomolecular systems is possible.

- The **charge fluctuation** is a *key* mechanism for the molecular complexation particularly at low ionic strength.

Fernando Barroso, FCFRP/USP
March 9-15, 2020

184

185

Many thanks to...

- Dr. João Ruggiero Neto (Unesp)
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- Dr. Deepti Srivastava (NCSU)
- Katie Acken (NCSU)
- Dr. Fabio Sterpone (IBPC)
- Prof. Philippe Derreumaux (IBPC)
- Dr. Cecilia Prudkin Silva (UBA)
- Dr. Oscar E. Pérez (UBA)
- Dr. Karina D. Martínez (UBA)
- Dr. Donal MacKernan (UCD)
- Prof. Bo Jonsson (LU)
- Dr. Mikael Lund (LU)
- Profa. Catherine Etchebest (UP)