International Spring School Statistical Thermodynamics, Santiago de Chile
Tuesday, November 28, 2017
Lecture 17

On comparing simulated with experimental data

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ETH Zürich, Switzerland

On comparing molecular modelling results with experimental data

A. The experimental problem

1. Experimental data Q^{exp} are averages
2. Insufficient number of experimental data
3. Insufficient accuracy of experimental data
4. Experimental data Q^{exp} may be inconsistent

B. Six aspects

1. Measured (primary) versus derived (secondary) data
2. How to handle averaging
3. Sensitivity of $<Q>_{sim}$ or $<Q>_{exp}$ to the conformational distribution
4. Compensation of (simulation/experimental) errors
5. Biasing of the simulation towards experiment
6. Identity of calculated versus measured quantities or systems

C. Interpretation of experimental data using simulation

1. Relation between average $<Q>$ and conformational distribution P(r)
2. Four reasons for agreement between $<Q>_{sim}$ and $<Q>_{exp}$
3. Five reasons for disagreement between $<Q>_{sim}$ and $<Q>_{exp}$

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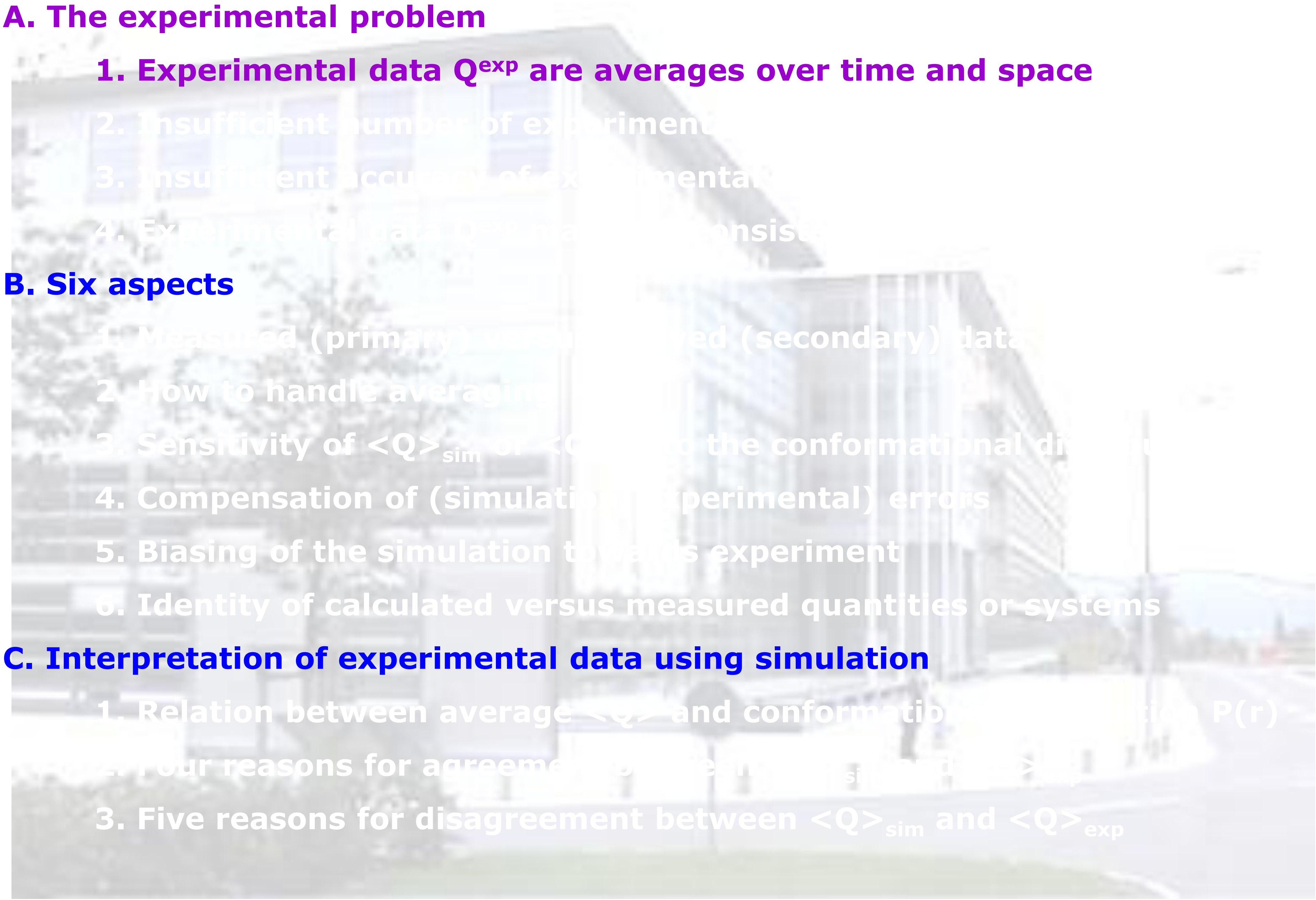

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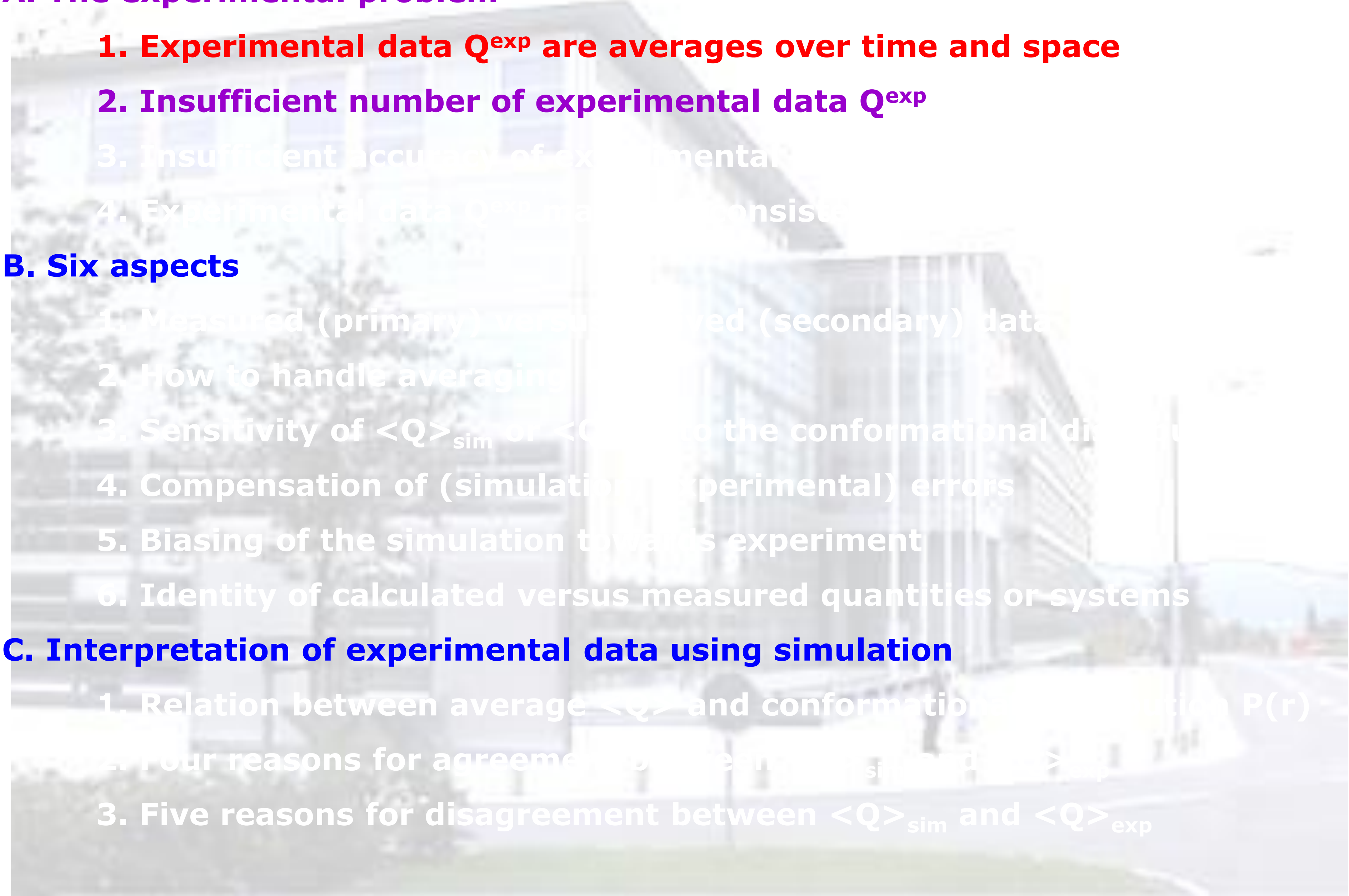

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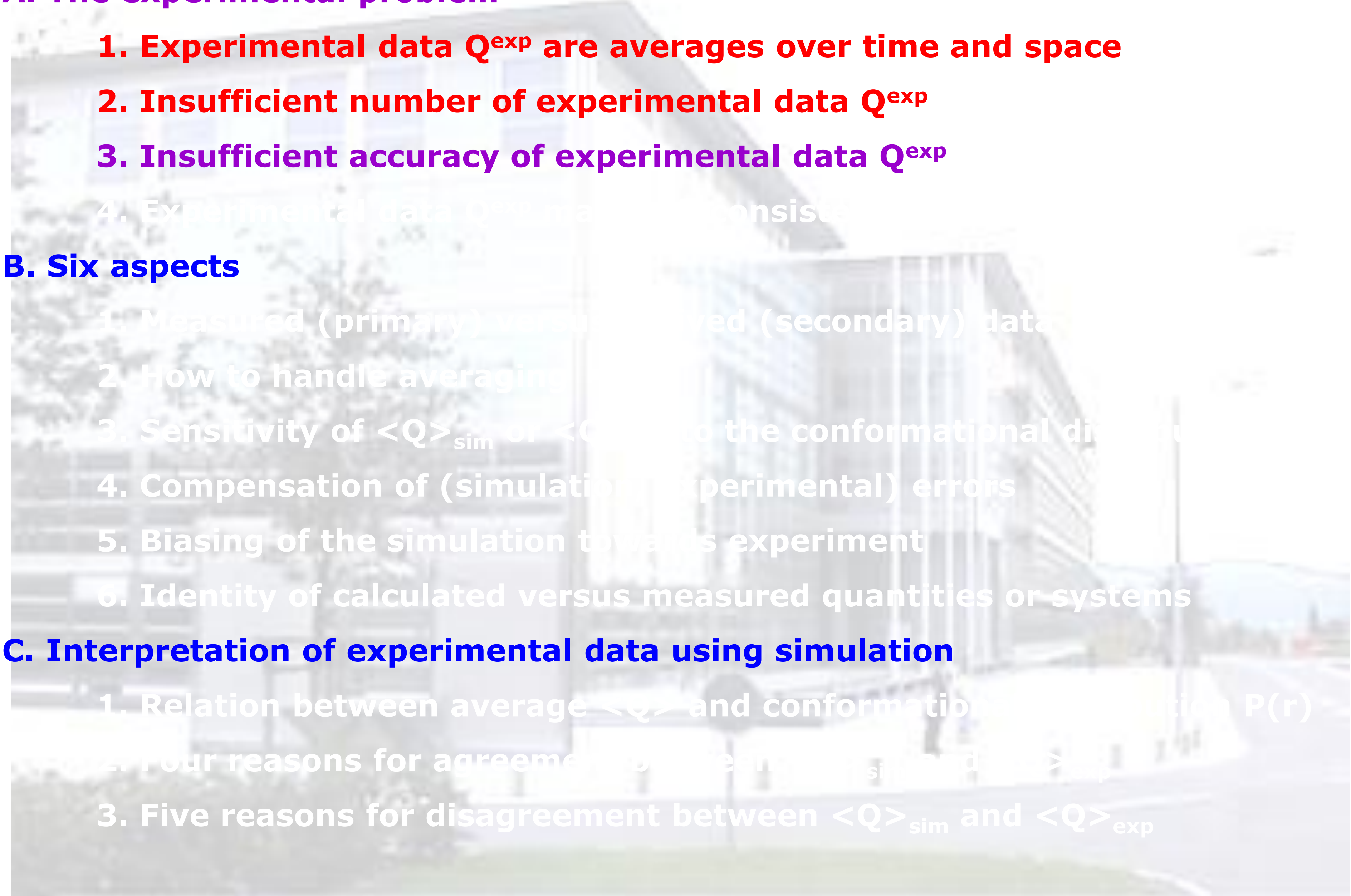

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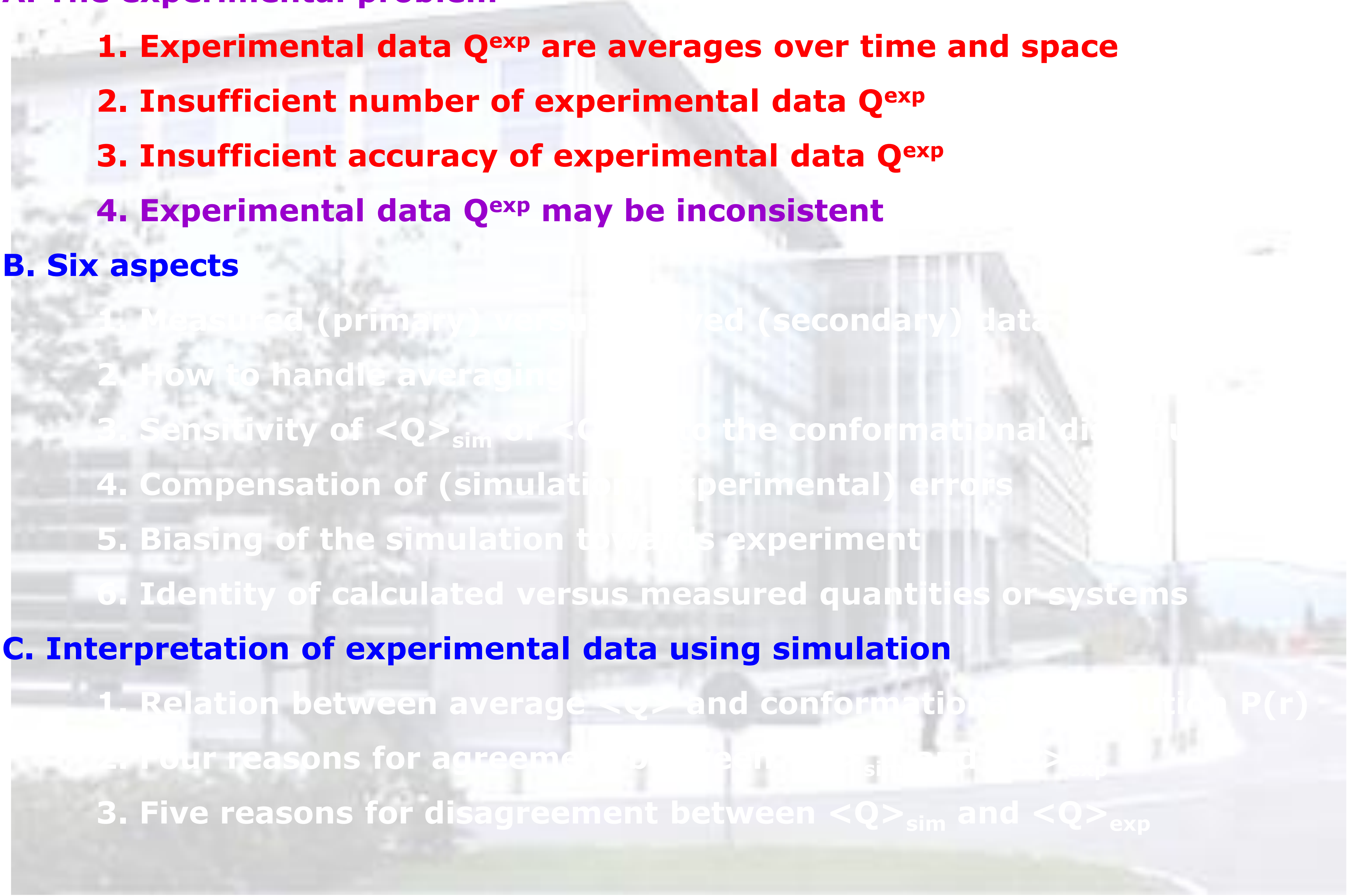

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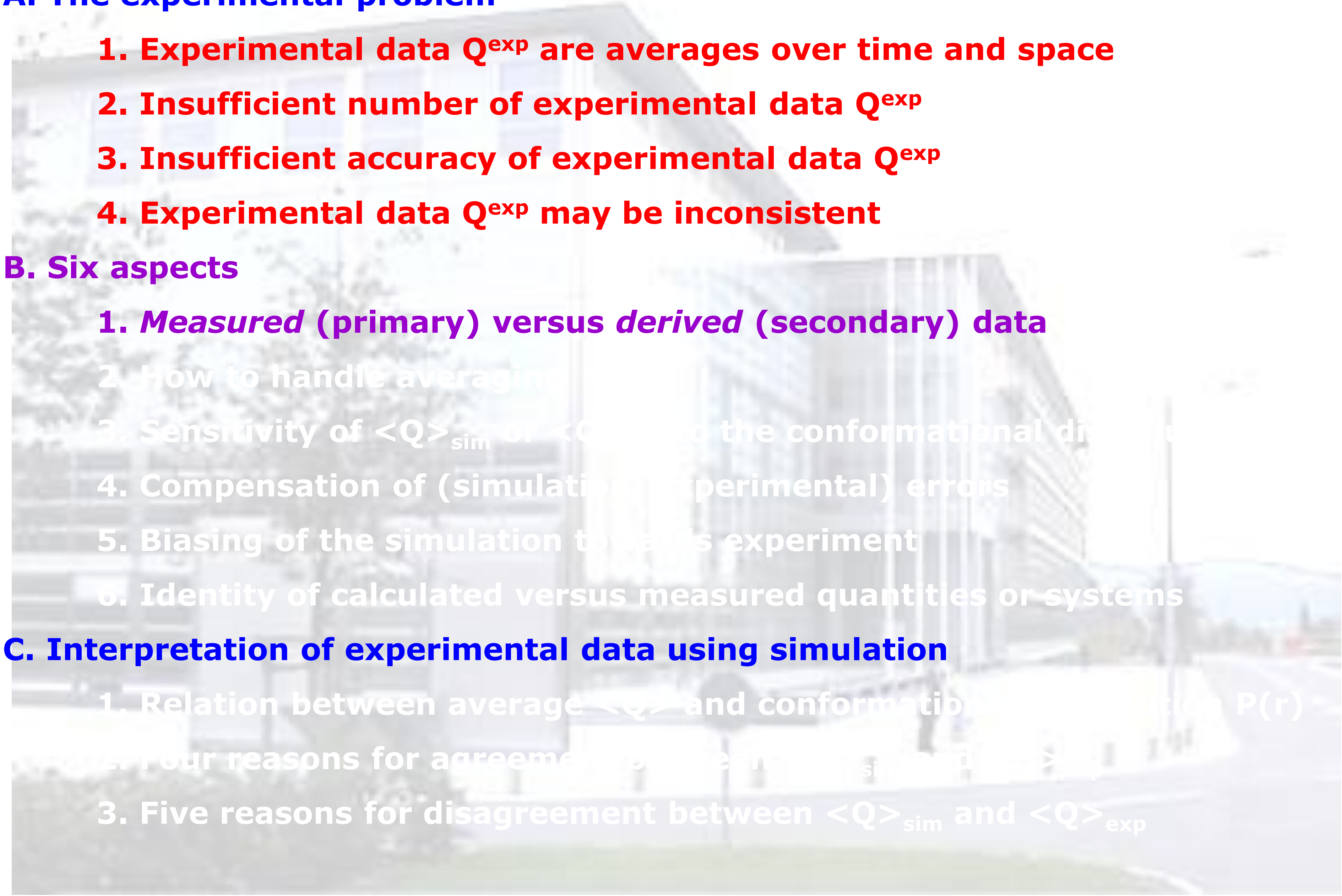

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Comparison with Experimental Data for a Quantity Q

$\langle Q \rangle_{sim} \quad \leftrightarrow \quad \langle Q \rangle_{exp}$

W.F. van Gunsteren, J. Dolenc, &
A.E. Mark, Curr. Opin. Struct. Biol.,
*18 (**2008**) 149-153*

Distinguish between:

1. primary experimental data $\mathbf{Q^{measured}}$: *observable* quantities Q that are ***directly measured***

   Examples: peak location and intensity from X-ray diffraction or NMR spectroscopic measurements (a.o. ^{3}J–values)

2. secondary (***derived using a model***) "experimental" data $\mathbf{Q^{derived}}$: quantities Q for which (*non-observed*) values are ***derived from*** (observed) values of primary experimental data $\mathbf{Q^{measured}}$ by applying a particular **procedure f**: $\mathbf{Q^{derived} = f\,(Q^{measured})}$ which involves assumptions and approximations

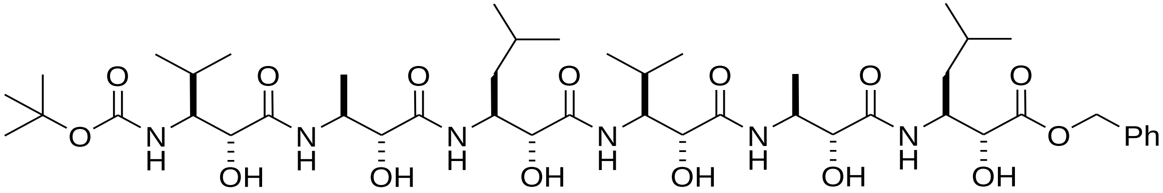
   Examples: *molecular structures* (a.o. torsional angle values) NMR order parameters

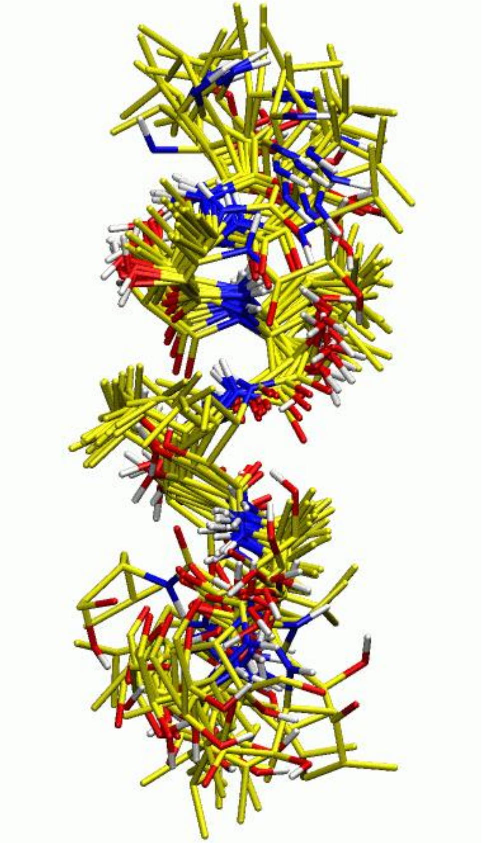
Comparison of		may reflect the quality of:
a. $\langle Q^{measured} \rangle_{sim}$	with $\langle Q^{measured} \rangle_{exp}$	the simulation
b. $\langle Q^{derived} \rangle_{sim}$	with $\langle Q^{derived} \rangle_{exp} = f\,(\langle Q^{measured} \rangle_{exp})$	the procedure f

In reality $\langle Q^{derived} \rangle_{exp}$ may carry little experimental information

A β-hexapeptide

Two non-overlapping conformational ensembles reproduce the experimental data: Which one is realistic?





- β-hexapeptide with hydroxyl groups attached to the α-carbons
- NMR single-structure refinement *based on NOE and* ^{3}*J-coupling data* suggests the formation of a 2_8-***P***-helix
- MD simulation from totally extended conformation at two different temperatures (298 K & 340K) using the GROMOS 45A3 force field *without any NOE-distance or* ^{3}*J-value restraining* suggests the formation of a 2.5_{12}-***P***-helix

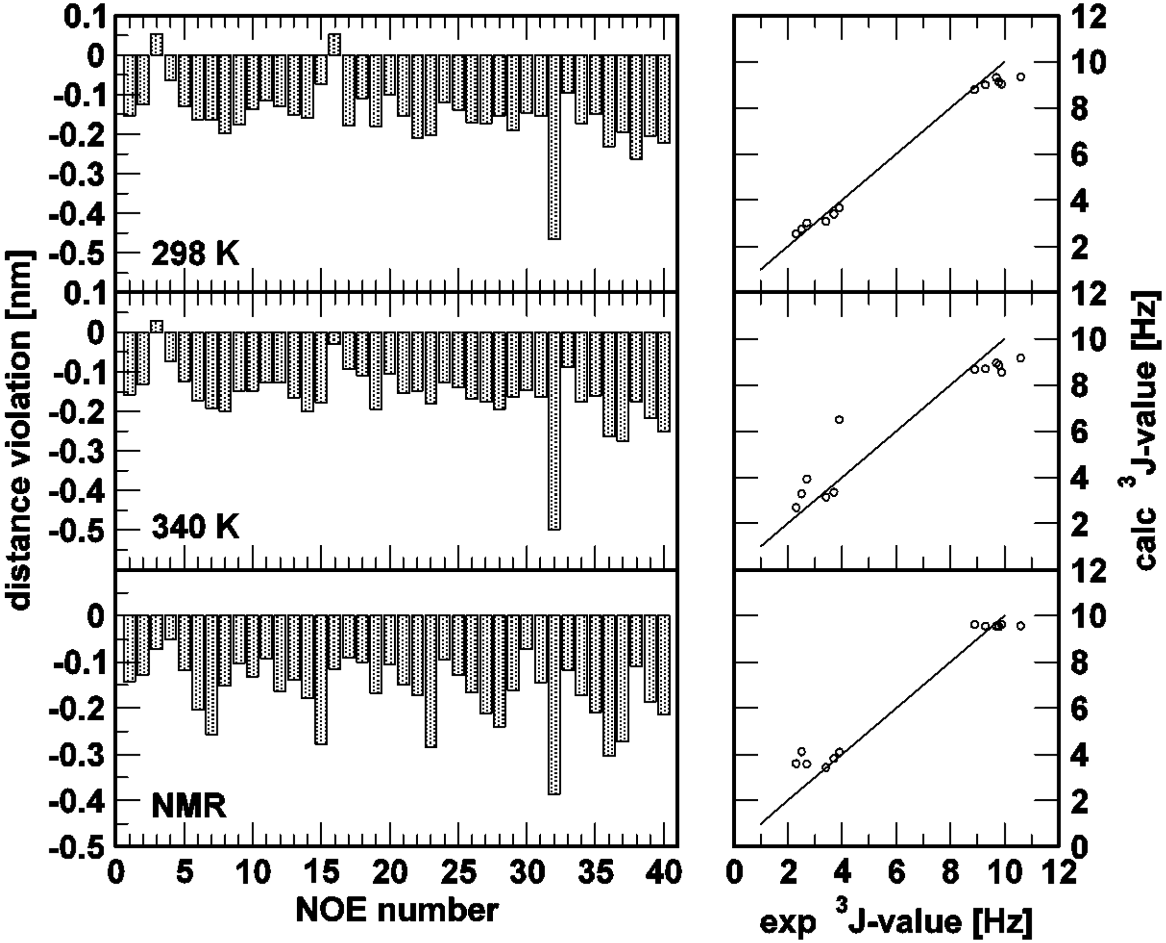
Bundle of 20 NMR model structures

(protection groups not shown)

*Glaettli & van Gunsteren, Angew. Chem. Int. Ed. Engl. 43 (**2004**) 6312*

*Gademann et al., Angew. Chem. Int. Ed. Engl. 42 (**2003**) 1534*

NOE Distance Violations & Backbone ^{3}J-values



- MD at 298 K
  2 violations (~0.05 nm)

  average deviation from exp. J-values: 0.44 Hz

- MD at 340 K
  1 violation (~ 0.03 nm)

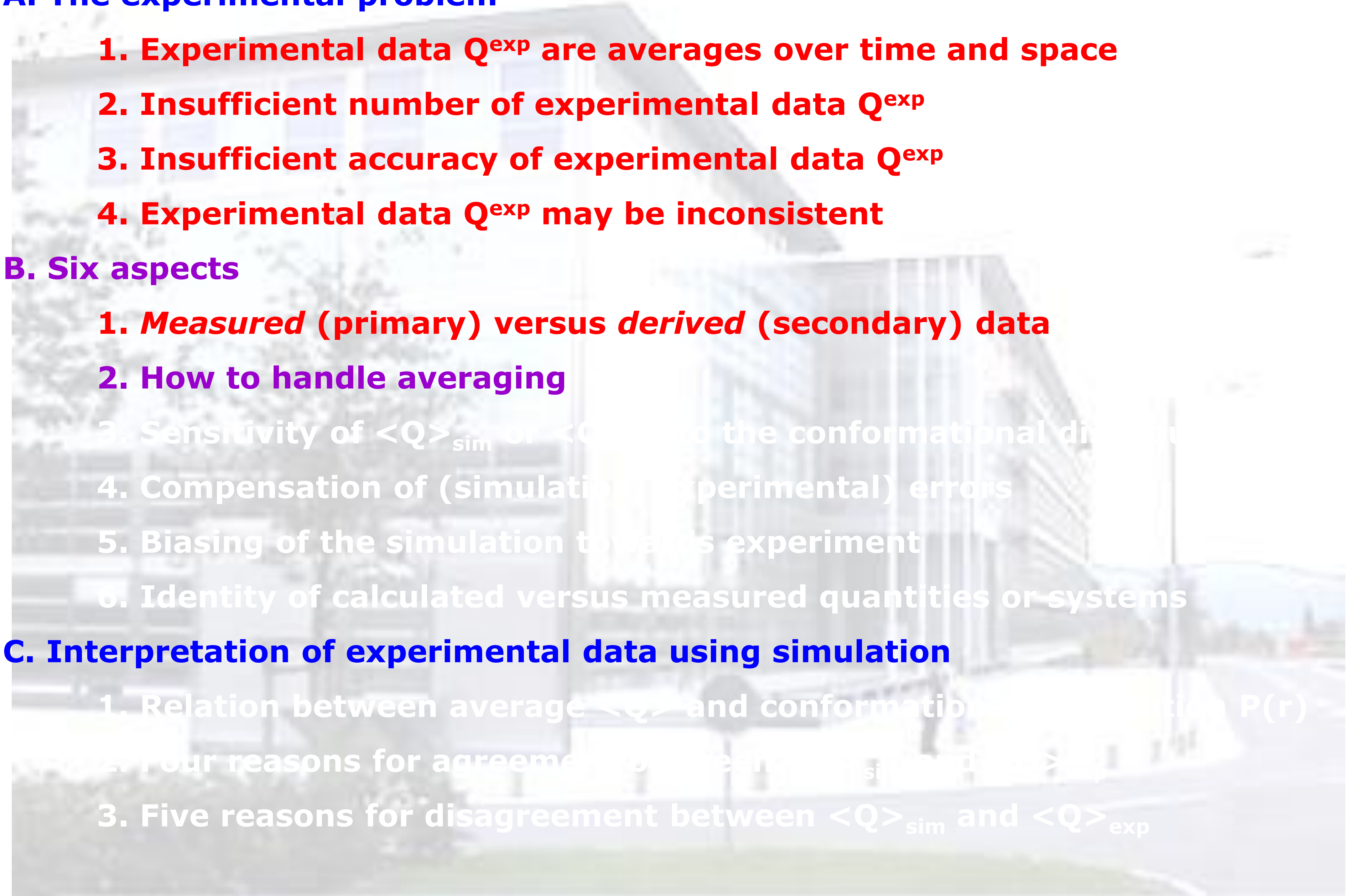
  average deviation from exp. J-values: 0.91 Hz

- NMR set of structures
  no violation (0.0 nm)

  average deviation from exp. J-values: 0.57 Hz

**Two different methods to derive a set of peptide structures produce non-overlapping ensembles that each reproduce the *measured* data.
However MD simulation (*ensemble*) predicts a well known 2.5_{12}-helix, whereas the NMR *single-structure* refinement predicts an unknown 2_8-helix**

On comparing molecular modelling results with experimental data



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The Molecular Modelling Approach

How to calculate a quantity or observable $Q(\vec{r})$?

Choose:

1. (essential) degrees of freedom $\vec{r}$

   ↳ for $Q(\vec{r})$ electronic
   atomic
   solvent

2. interaction function $V^{phys}(\vec{r})$

   between degrees of freedom (force field, e.g. GROMOS)

3. equations of motion or sampling method

   to generate a Boltzmann-weighted ensemble of conformers:

   probability $P(\vec{r}) = \exp(-V^{phys}(\vec{r})/k_B T) / \int \exp(-V^{phys}(\vec{r})/k_B T)\ d\vec{r}$

4. function $Q(\vec{r})$ (contains approximations and assumptions)

Ensemble averages

$$\langle Q \rangle_{\vec{r}} \equiv \int Q(\vec{r}) P(\vec{r}) d\vec{r}$$

are to be compared:

$$\langle Q \rangle_{sim}$$

is to be compared to

$$\langle Q \rangle_{exp} \equiv Q^{exp}$$

If

1. $V^{phys}(\vec{r})$ and $Q(\vec{r})$ ***are correct***
2. ***infinite*** sampling

problem solved

Otherwise

make other choices and repeat

Effects of Ensemble (Motional) Averaging

$$\left\langle Q(\vec{r})\right\rangle_{\vec{r}} \equiv \int Q(\vec{r})\, P(\vec{r}) d\vec{r} \equiv \frac{\int Q(r) e^{-V^{phys}(\vec{r})/k_B T}\, d\vec{r}}{\int e^{-V^{phys}(\vec{r})/k_B T}\, d\vec{r}}$$

$$\left\langle Q(\vec{r})\right\rangle_{\vec{r}} \neq Q(\vec{r})$$

averaged Q — single structure Q

$$\neq Q\left(\left\langle \vec{r}\right\rangle_{\vec{r}}\right)$$

mean structure Q

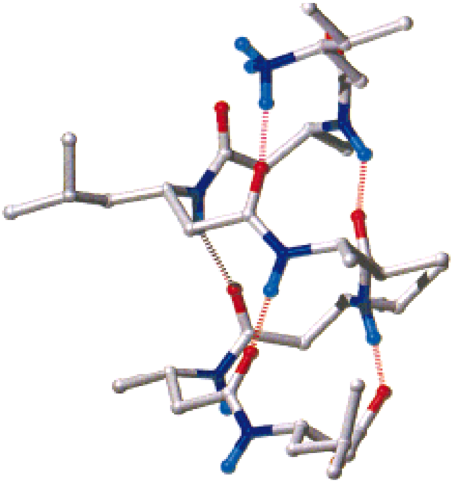
Examples of (*observable*) quantities *Q(r)*:

- NOE intensities (NMR)
- ^{3}J-coupling constants (NMR)
- Residual dipolar couplings (NMR)
- Chemical shifts (NMR)
- Structure factors (amplitudes) (X-ray)
- CD spectra (CD)
- ……..

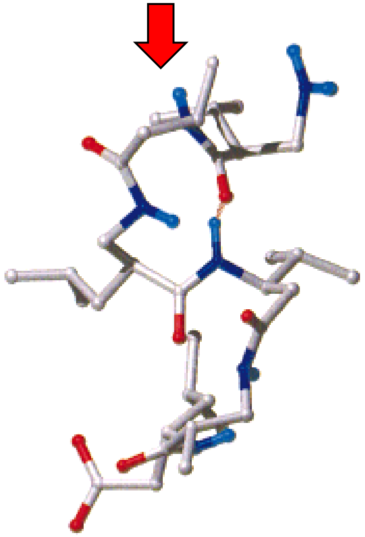
Boltzmann weighting is non-linear
Function Q(r) may be non-linear

Effect of Ensemble (Motional) Averaging

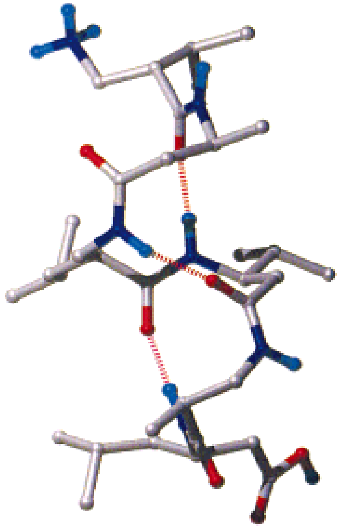
The average structure $<\mathbf{r}>_r$ is highly strained
for a 6-ß-peptide in methanol: 34 NOE's



left-handed **3_{14}-helix**
of a similar peptide in MeOH
H-bonds: NH(i) – O(i+2)



average structure:
distorted right-handed helix in MeOH only one
H-bond: NH(4) – O(1)



right-handed **helix in pyridine**
H-bonds: NH(i) – O(i+1, i-3)

due to 3 NOE's characteristic → not observed in pyridine
for a left–handed 3_{14}-helix

MD simulation: - satisfies all NOE bounds
- shows 35% *right*-handed helix
1.3% *left*-handed helix

Conclusion: - average structure may be meaningless
- use ***primary*** (***observed***) exp. data (NOE's),
not secondary (***derived***) exp. data (structures) ***to compare with***

*X. Daura et al., Angew. Chem. Int. Ed. 38 (**1999**) 236-240*

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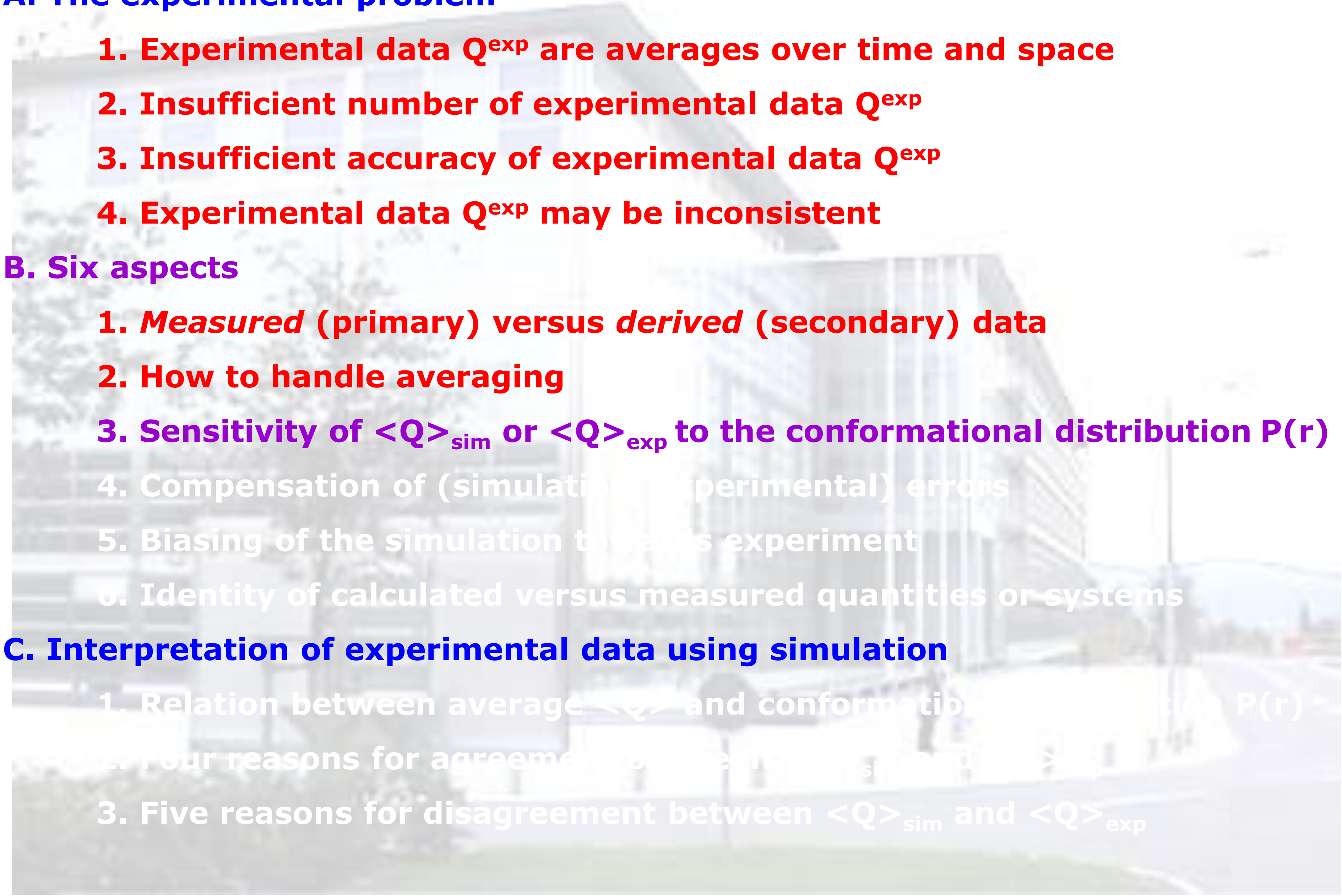

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Relation between average $\langle Q \rangle$ and conformational distribution $P(\vec{r})$

When relating the *average* of a property over a given conformational distribution P(r), whether from a simulation (<Q>$_{sim}$) or measured experimentally (<Q>$_{exp}$), to the conformational distribution itself, ***three general cases*** can be distinguished:

Q1 <Q> does not reflect the shape of P(**r**) as <Q> is ***insensitive*** to conformation

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Q3 <Q> ***does reflect*** the dominant conformations of P(**r**)

Only in case **Q3** can <Q>$_{sim}$ carry information relevant to the interpretation of <Q>$_{exp}$ at a molecular level

*W.F. van Gunsteren, J. Dolenc, & A.E. Mark, Curr. Opin. Struct. Biol., 18 (**2008**) 149-153*

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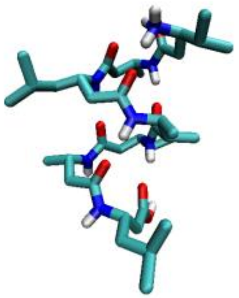
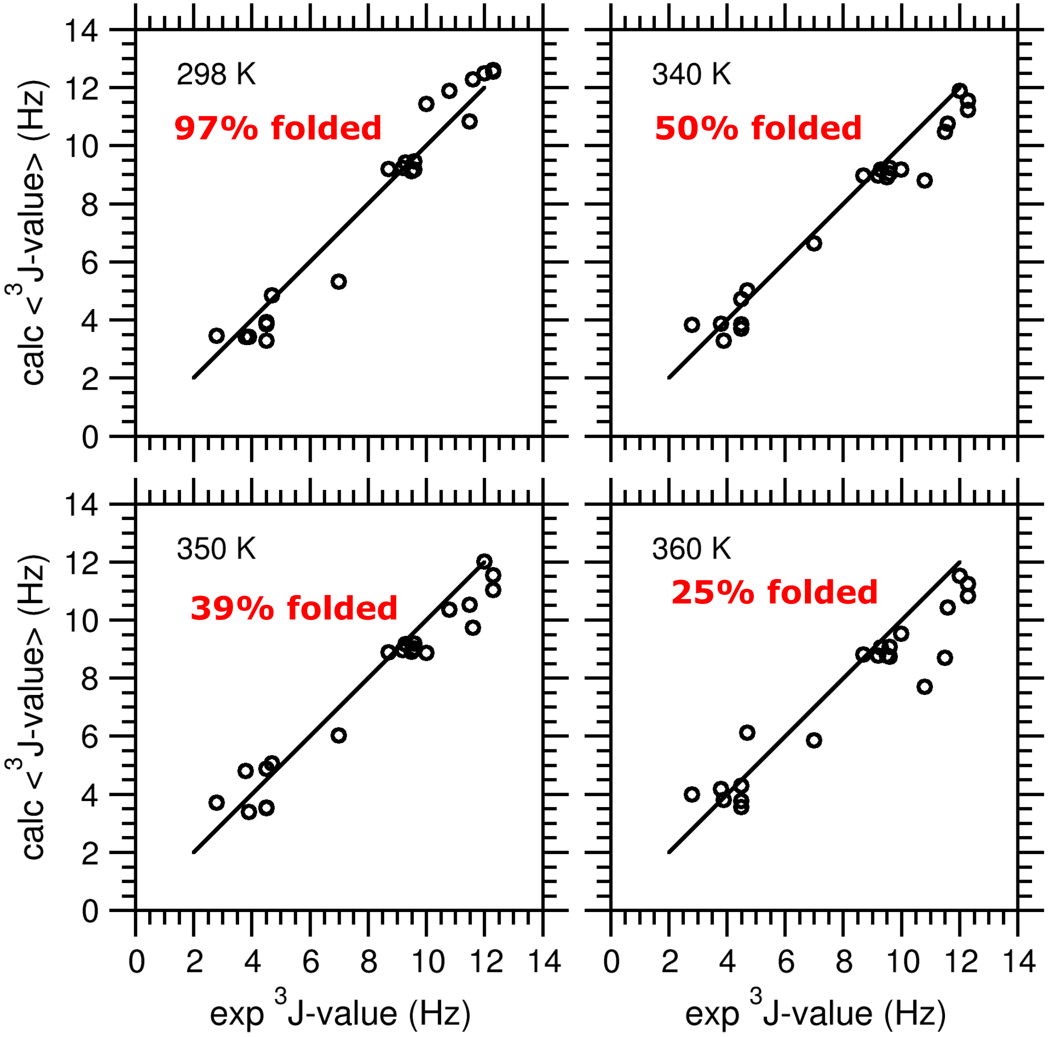
Different Ensembles of a 7-β-peptide in solution



3_{14}-L-helix



$^3J(H_N\text{-}H_{\alpha\text{ or }\beta})$-couplings are *insensitive* to the conformational distribution

*X. Daura et al., Proteins 36 (**1999**) 542-555*

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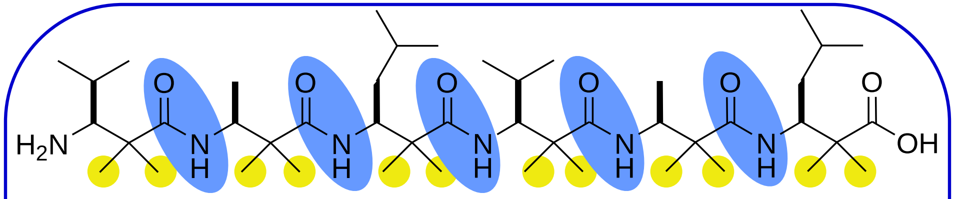
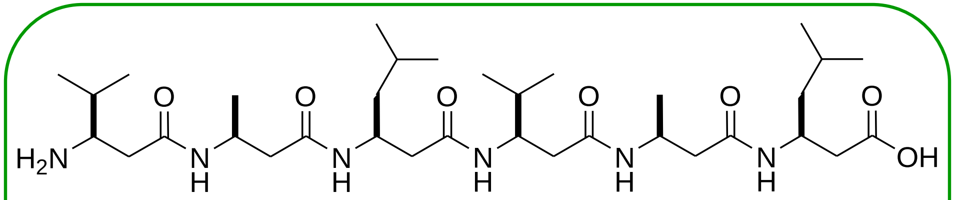
Calculation of Circular Dichroism (CD) Spectra

Two molecules with *similar* CD spectra, but cannot have a *similar* dominant stucture



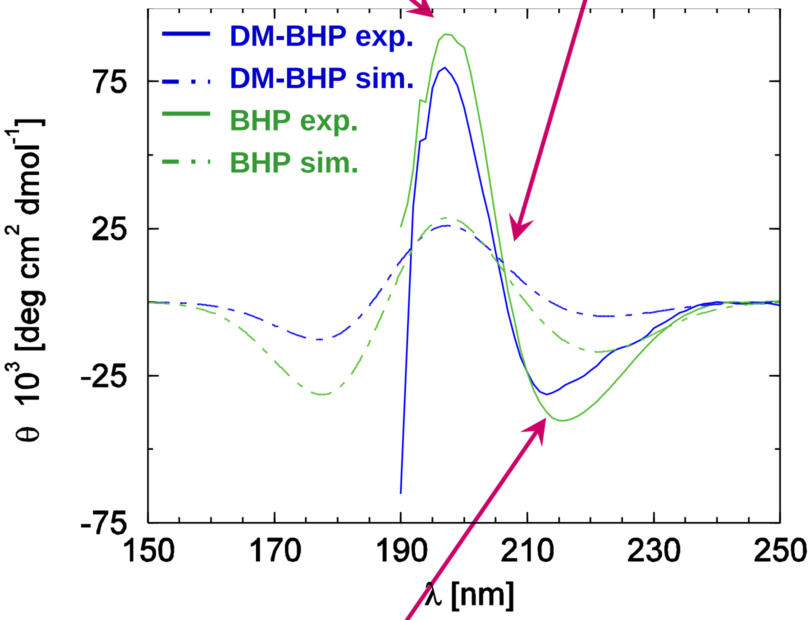
Peptide A: DM-BHP *(methyls (yellow))*

- geminal dimethylation inhibits the formation of a 3_{14} helix
- no NMR data available
- CD spectrum shows a pattern, which is “typical” for a 3_{14} helix



Peptide B: BHP *(no methyls)*

- can adopt a 3_{14} helix, confirmed by NMR experiments, CD spectrum similar



positive Cotton effect at ~200 nm

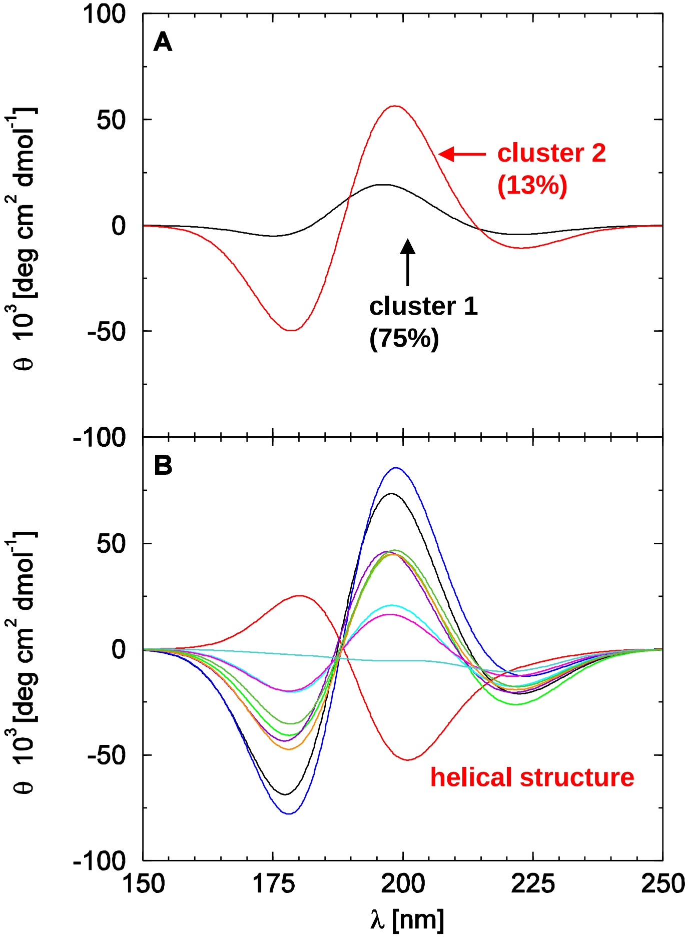
zero crossing between 205 and 210 nm

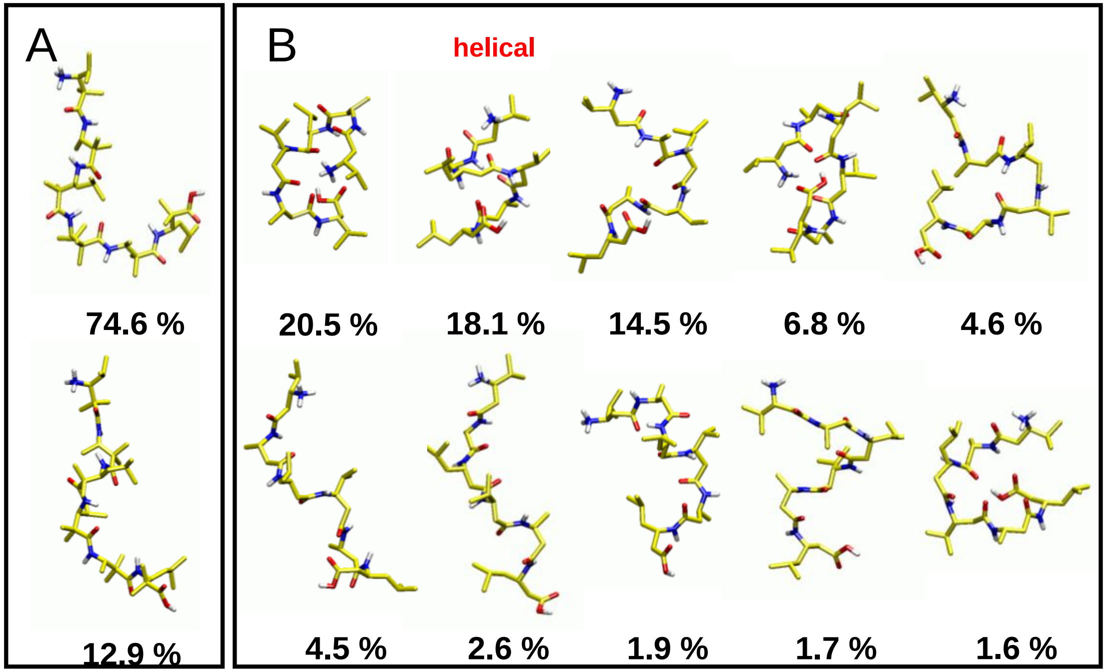
negative Cotton effect between 215 and 220 nm

*A. Glaettli et al., JACS 124 (**2002**) 12972-12978*

CD Spectra per Conformational Cluster

Similarity criterion: backbone RMSD ≤ 0.09nm
10000 structures, 10 psec apart





Non-helical conformers exhibit the CD pattern assigned to the 3_{14} helix, the “helical” conformer doesn’t.

→ virtually NO OVERLAP between the conformational ensembles of both molecules, which have similar CD spectra !

→ spectrum *not representative* for the dominant conformation !

*A. Glaettli et al., JACS 124 (**2002**) 12972-12978*

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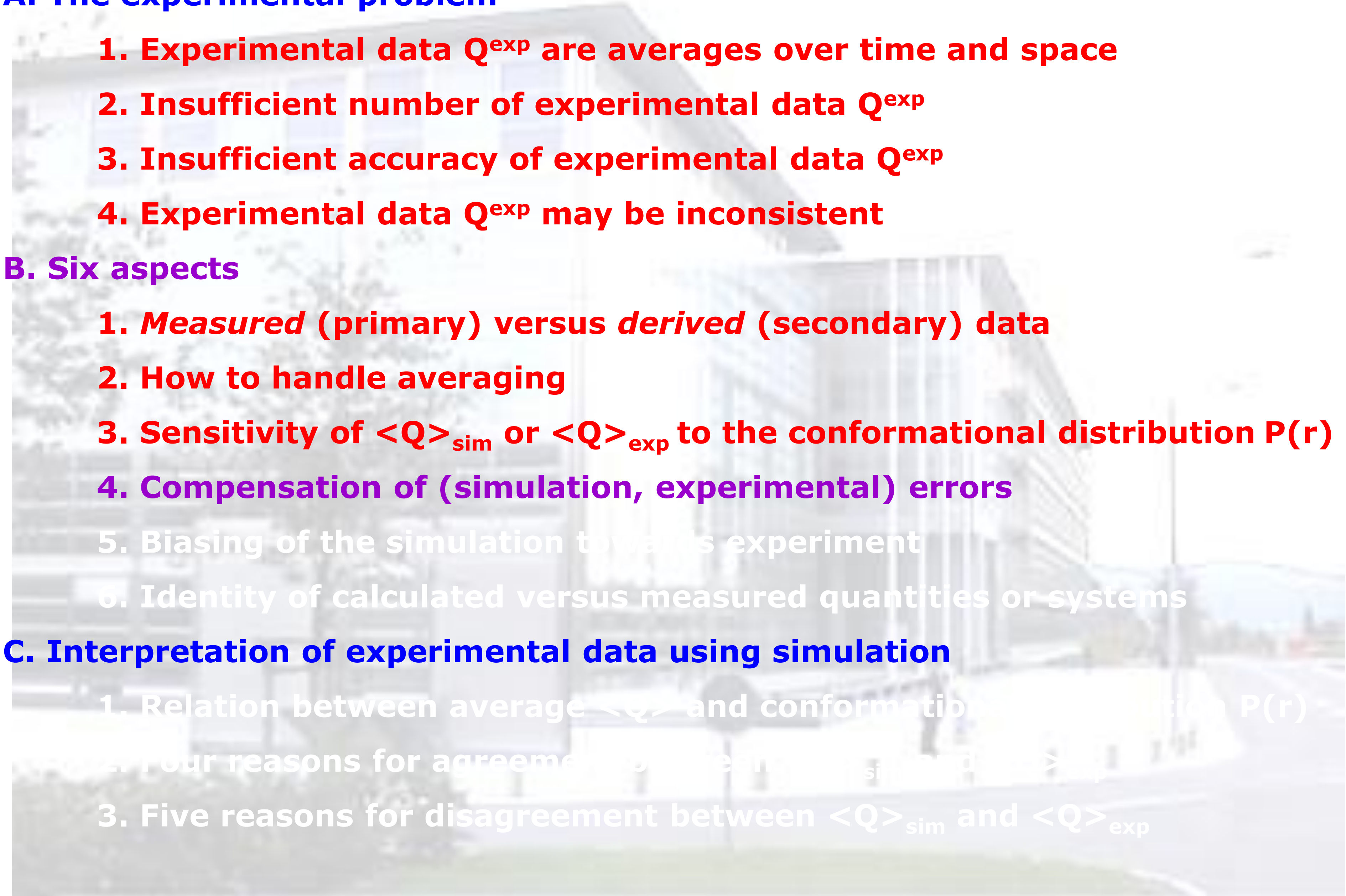

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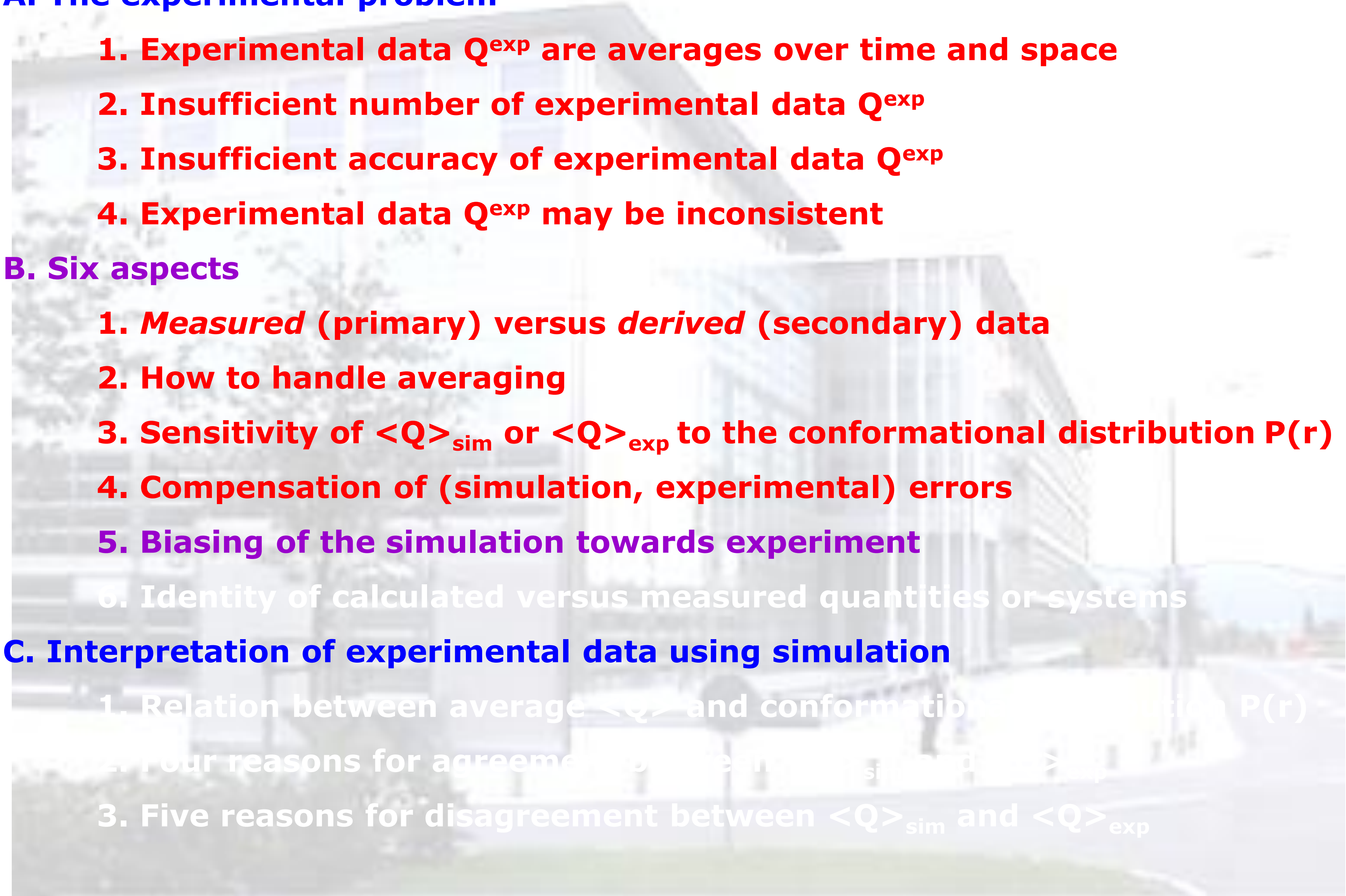

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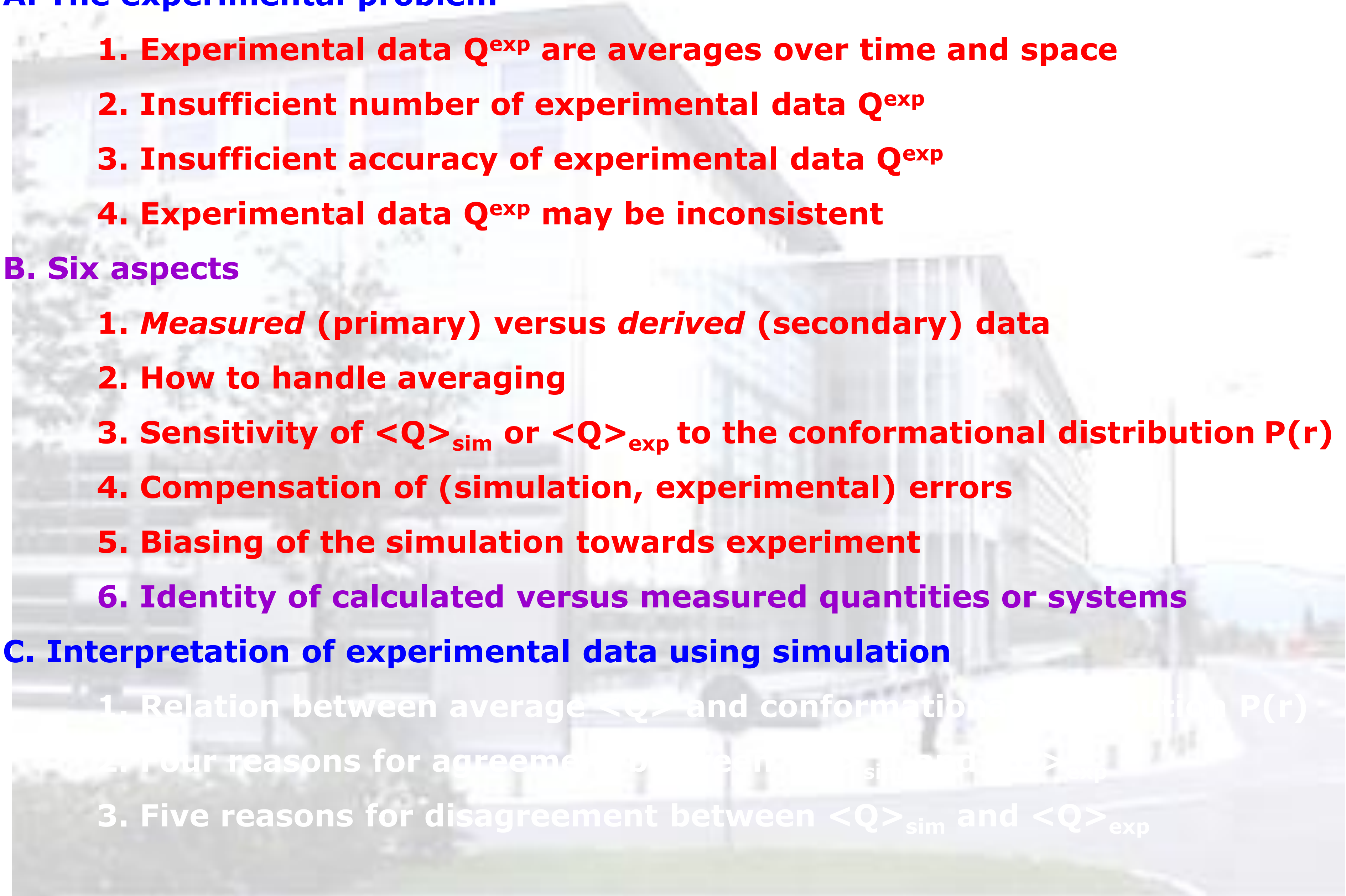

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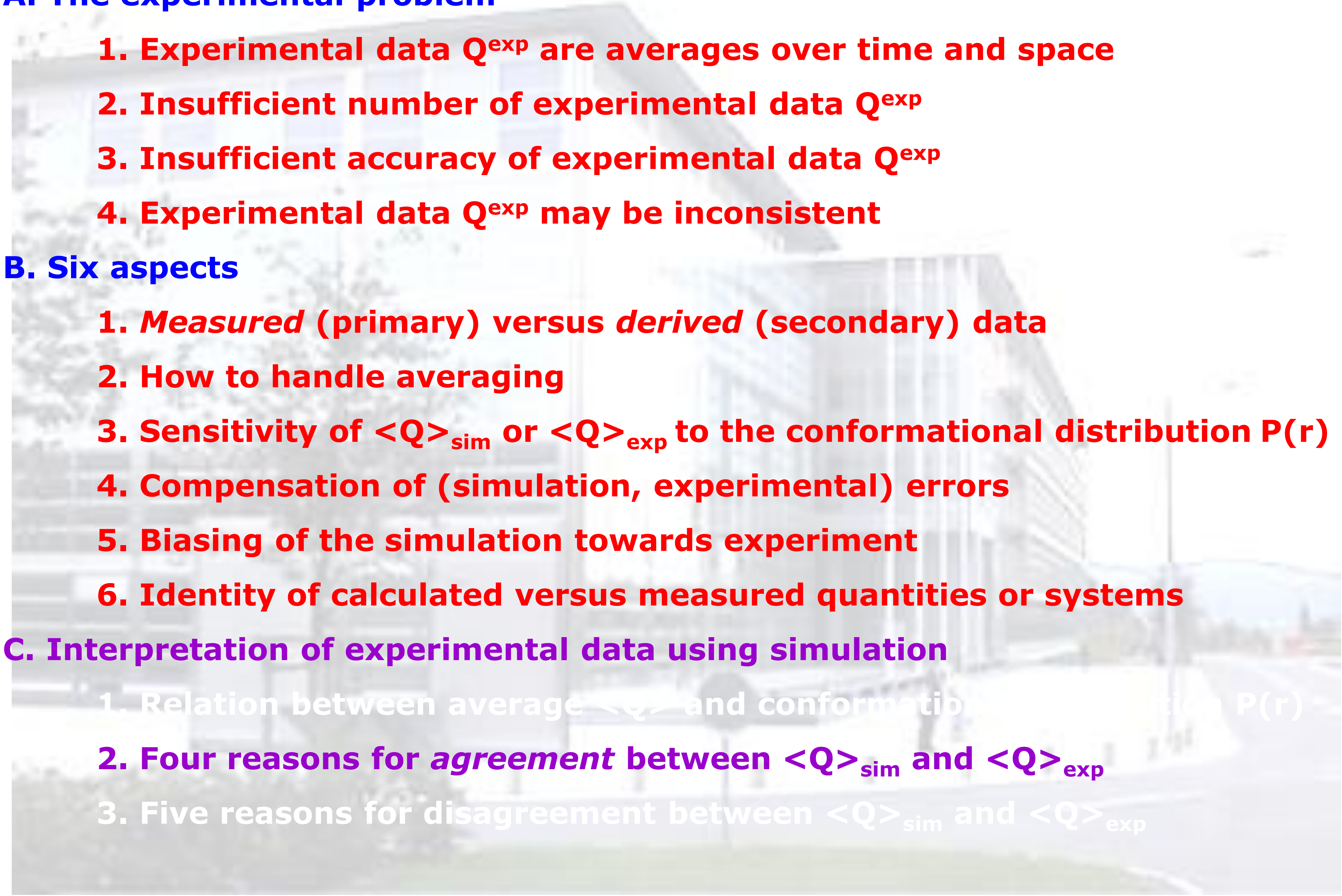

A. The experimental problem

1. Experimental data Q^{exp} are averages over time and space
2. Insufficient number of experimental data Q^{exp}
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B. Six aspects

1. *Measured* (primary) versus *derived* (secondary) data
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3. Sensitivity of $<Q>_{sim}$ or $<Q>_{exp}$ to the conformational distribution P(r)
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C. Interpretation of experimental data using simulation

1. Relation between average $<Q>$ and conformational distribution P(r)
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Interpretation of Experimental Data using Simulation

Reasons for ***agreement*** between $\langle Q \rangle_{sim}$ and $\langle Q \rangle_{exp}$

Agreement between $<Q>_{sim}$ and $<Q>_{exp}$ may be obtained if:

A1 <Q> is insensitive to P(**r**), i.e. $<Q>_{sim}$ matches $<Q>_{exp}$ irrespective of the conformational distribution P(**r**) simulated

A2 There are compensating errors in the simulation model, procedure or experimental set-up

A3 The experimental data of interest, $<Q>_{exp}$, has been used to bias the simulation

A4 $<Q(\mathbf{r})>_{sim}$ is sensitive to the distribution P(**r**)

Only in the case **A4** can the degree of agreement between $<Q>_{sim}$ and $<Q>_{exp}$ be used to validate the simulation and/or to interpret the experimental results

*W.F. van Gunsteren, J. Dolenc, & A.E. Mark, Curr. Opin. Struct. Biol., 18 (**2008**) 149-153*

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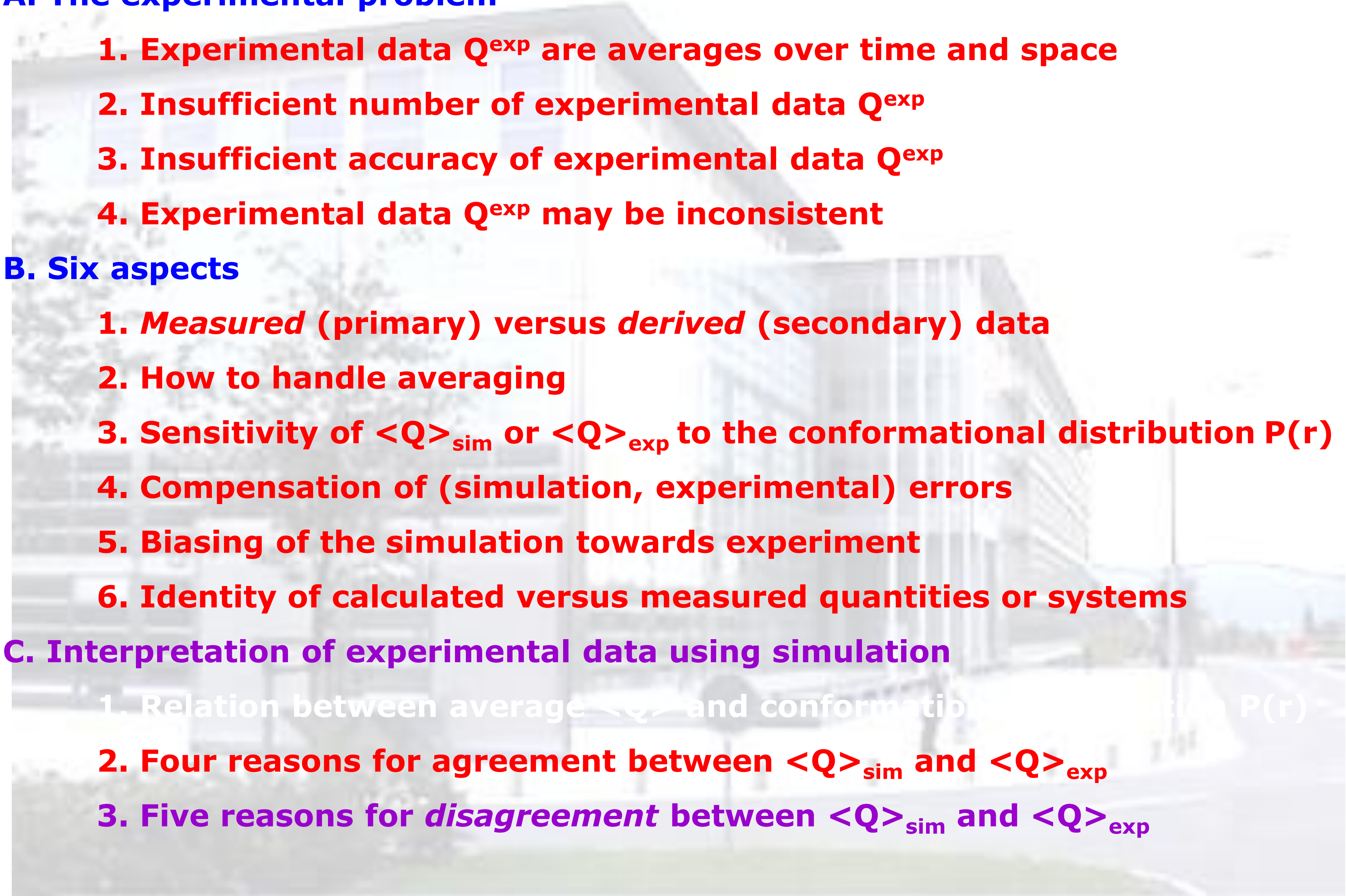

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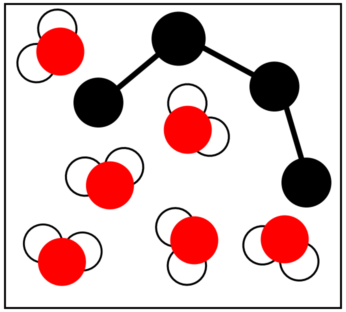
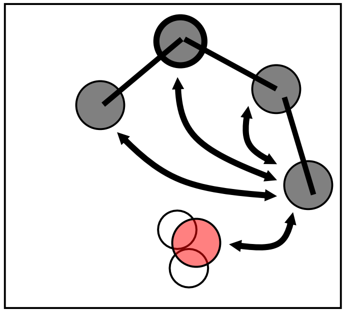
*W.F. van Gunsteren, J. Dolenc, & A.E. Mark, Curr. Opin. Struct. Biol., 18 (**2008**) 149-153*

Definition of a model for molecular simulation



Degrees of freedom: atoms are the elementary particles

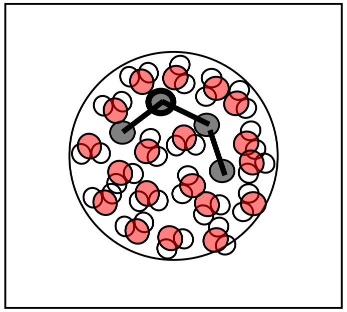
Forces or interactions between atoms



Force field = physico-chemical knowledge

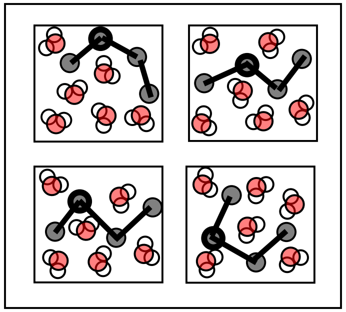
MOLECULAR MODEL

Boundary conditions



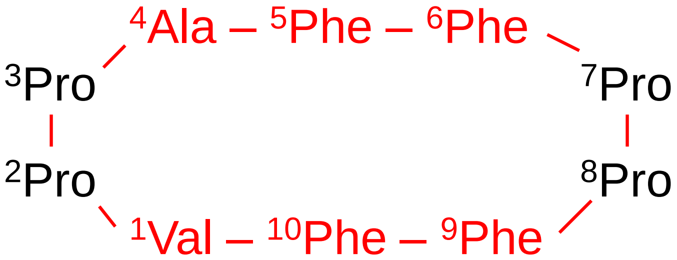
system
temperature
pressure
walls
external forces

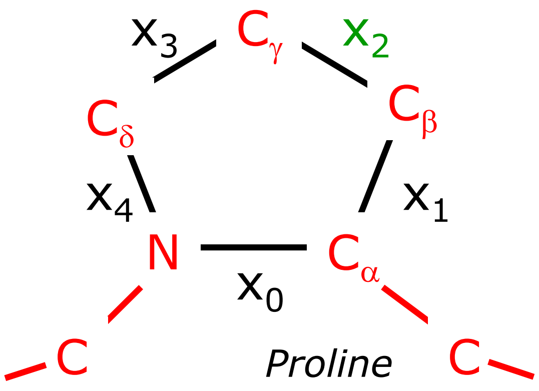
Methods to generate configurations of atoms: Newton



Conformational Dynamics of Proline Residues in Antamanide:

Effect of explicit solvent versus continuum: missing degrees of freedom





Experiment : NMR [**^{13}C relaxation** / **E COSY**] ***R.R. Ernst***

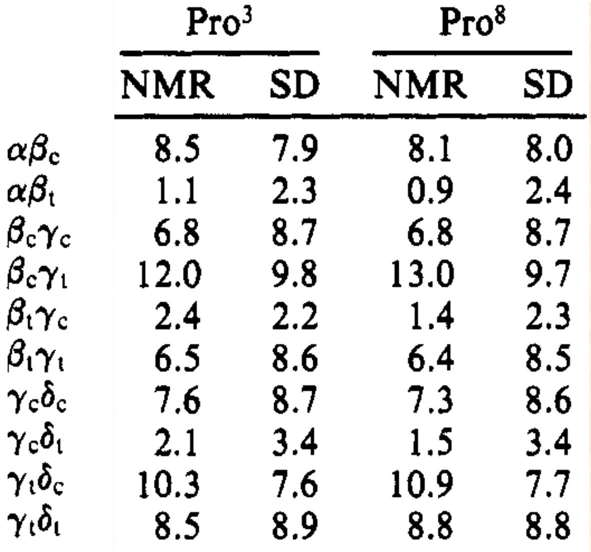
3/8 Pro: rigid

2/7 Pro: 2 conformers (time constant ~ 30ps)

Simulation: stochastic dynamics (500ps) SD

Comparison of $^3J_{HH}$ coupling constants (in Hz) from NMR

Rms deviation simulation-experiment 1.5 Hz



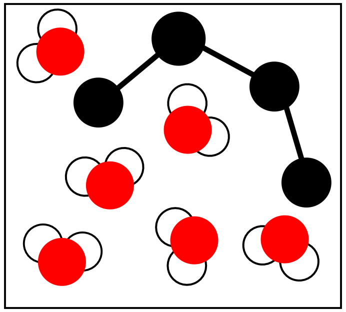
	Pro^3		Pro^8	
	NMR	SD	NMR	SD
$\alpha\beta_c$	8.5	7.9	8.1	8.0
$\alpha\beta_t$	1.1	2.3	0.9	2.4
$\beta_c\gamma_c$	6.8	8.7	6.8	8.7
$\beta_c\gamma_t$	12.0	9.8	13.0	9.7
$\beta_t\gamma_c$	2.4	2.2	1.4	2.3
$\beta_t\gamma_t$	6.5	8.6	6.4	8.5
$\gamma_c\delta_c$	7.6	8.7	7.3	8.6
$\gamma_c\delta_t$	2.1	3.4	1.5	3.4
$\gamma_t\delta_c$	10.3	7.6	10.9	7.7
$\gamma_t\delta_t$	8.5	8.9	8.8	8.8

Dynamics	**GROMOS force field change**	**Friction coefficient *ps^{-1}***	**Residence time *ps***
Experiment			**≈ 30**
SD mean solvent	**-**	**19**	**3**
SD mean solvent	**-**	**1000**	**25**
SD mean solvent	**torsion x kT up**	**19**	**25**
MD explicit solvent	**-**	**-**	**24**

Solvent degrees of freedom are essential for dynamics

*R.M. Brunne et al., JACS, 115 (**1993**) 4764-4768*
*J.W. Peng et al., J. Biomol. NMR 8 (**1996**) 453-476*

Definition of a model for molecular simulation



Degrees of freedom:
atoms are the
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Forces or
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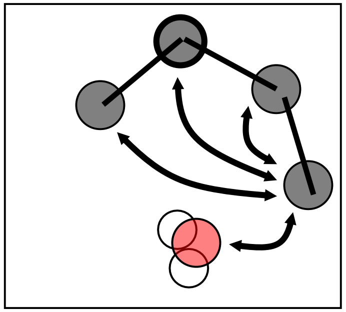

Force field =
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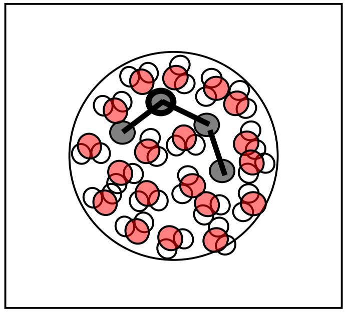

system
temperature
pressure
walls
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Methods to generate
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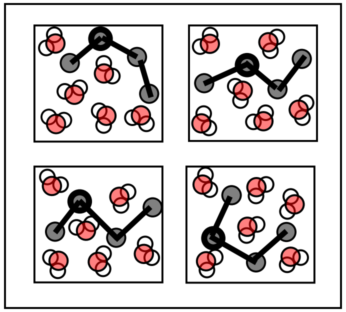
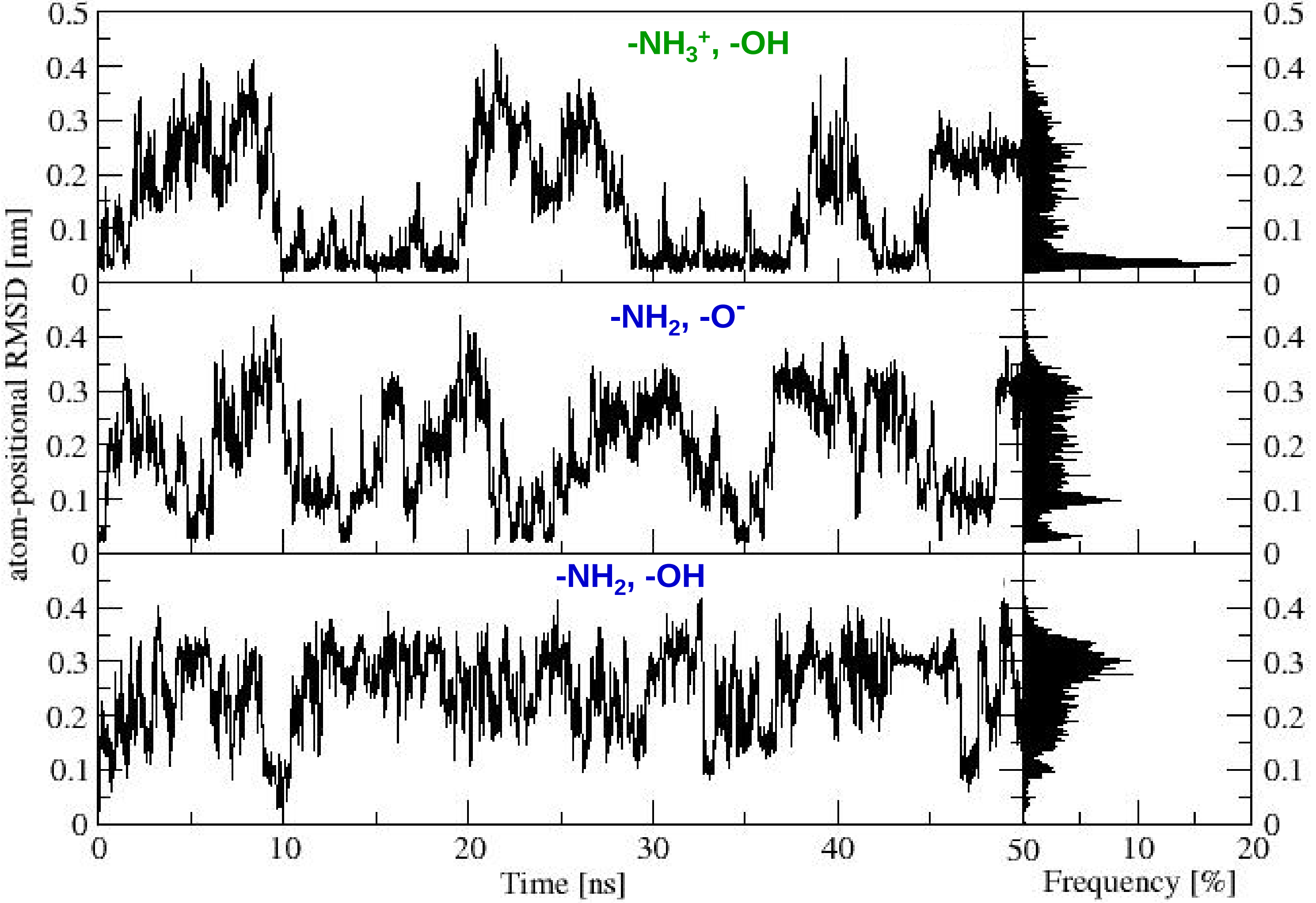


pH Dependence of the folding equilibrium of a β-peptide in methanol solvent

Backbone atom-positional RMSD from the helical fold



Low pH:
Helix dominant
Corresponds to experiment

High pH:
Bit of helix present

Intermediate pH:
No helix present

Thermodynamic conditions chosen in a simulation may influence the result, i.c. the folding equilibrium

*P.J. Gee & W.F. van Gunsteren, Proteins 63 (**2006**) 136-143*

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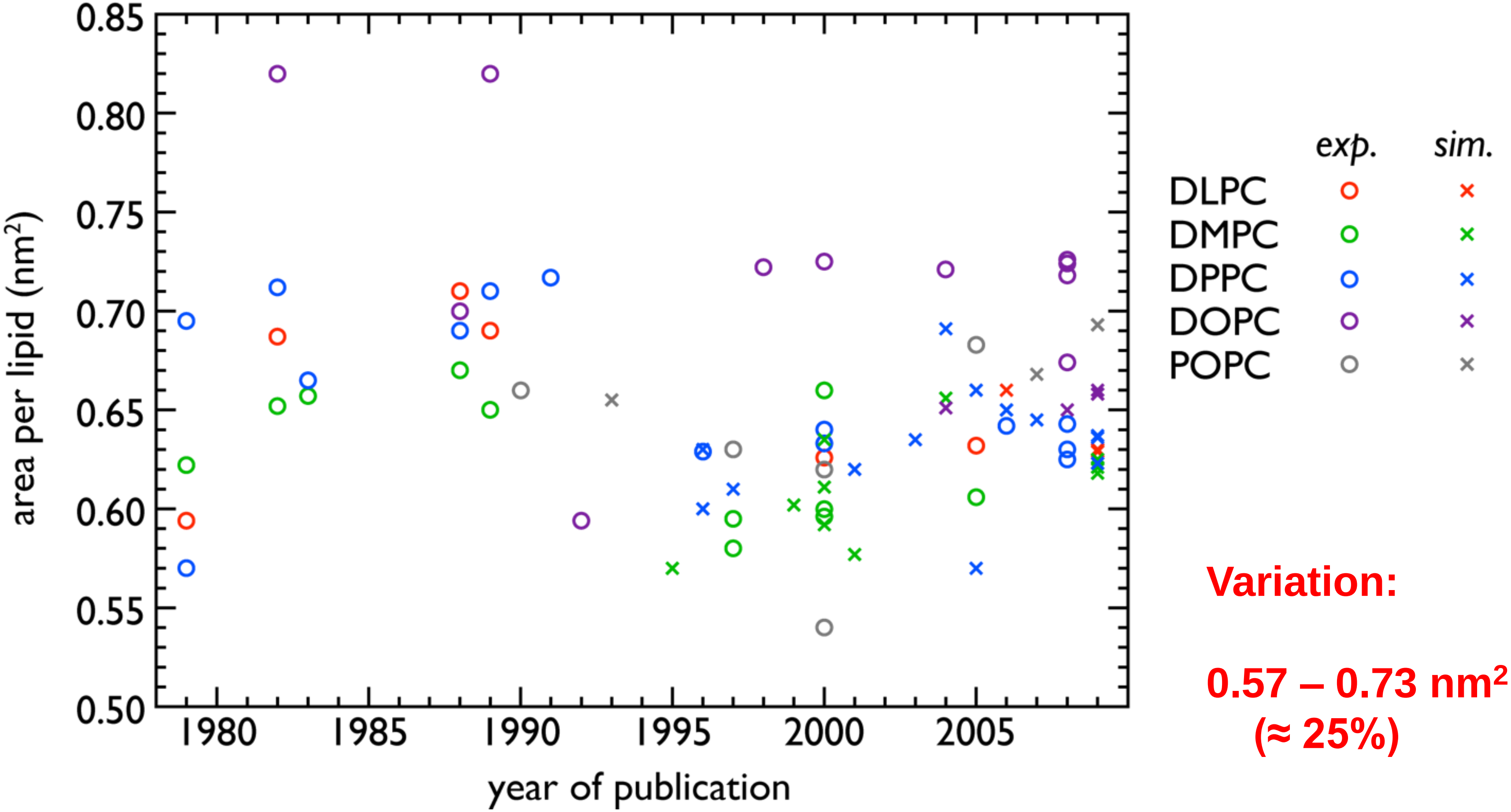
*W.F. van Gunsteren, J. Dolenc, & A.E. Mark, Curr. Opin. Struct. Biol., 18 (**2008**) 149-153*

Modelling a specific membrane: area per lipid

Area per lipid:

*D. Poger & A.E. Mark, JCTC 6 (**2010**) 325-226*

1. Most commonly used experimental quantity to validate a lipid model.
2. Difficult to measure directly.
3. Often inferred from NMR relaxation data.
4. Depends on measurement conditions.
5. Few research groups generate data.



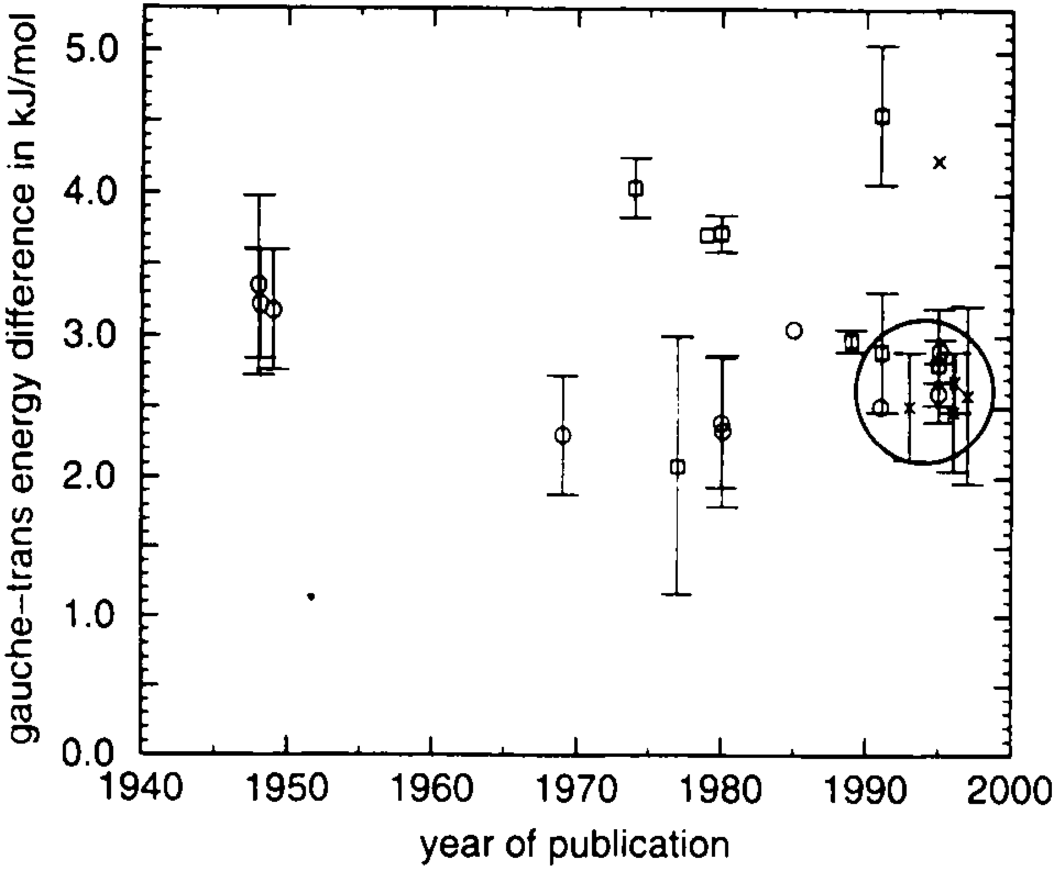
Variation:

0.57 – 0.73 nm^2 (≈ 25%)

Experimental data vary with time

Variation over time in the experimental data regarding the trans-gauche energy difference in aliphatic chains

This quantity will influence the structure and mobility of lipid chains



Variation:
2.0 to
3.2 – 4.5 kJ/mol

Experimental data vary with time

*Schuler & van Gunsteren, Mol. Simulation 25 (**2000**) 301-319*

Test of Force Field and NMR Data for Hen Egg White Lysozyme

Experimental Data

(Smith et. al., 1991, 1993; Buck et. al., 1995; Schwalbe et. al., 2001, both Oxford)

1158 NOE's derived inter-proton distances (set1 1993)

1525 NOE's derived inter-proton distances (set2 2001)

95 $^3J_{HN\alpha}$-coupling constants

100 $^3J_{\alpha\beta}$-coupling constants

124 backbone and 28 side-chain order parameters

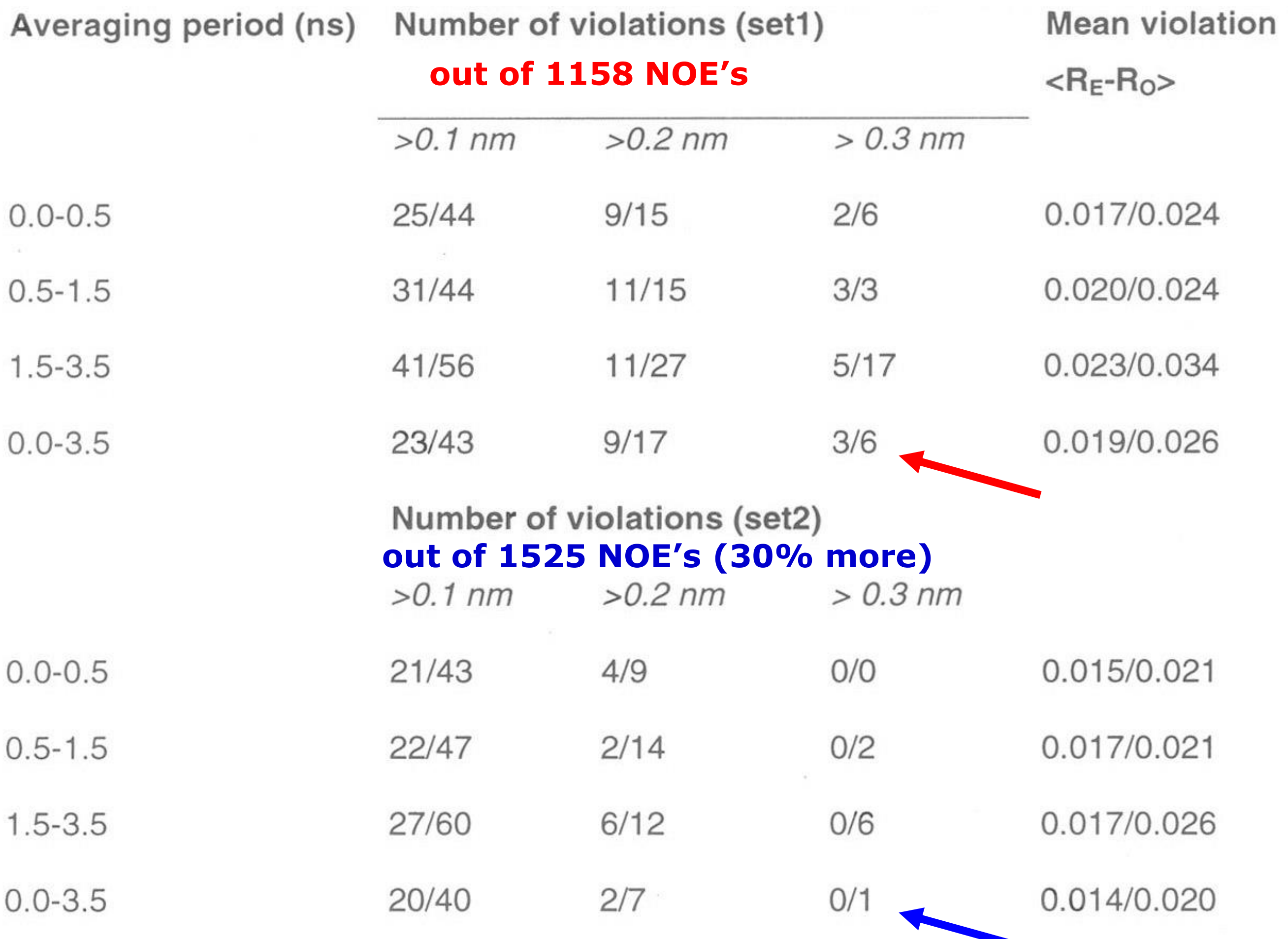
X-ray coordinates (PDB 1aki, 1.5 Å)

NMR coordinates (PDB 1e8l, set of 50 structures)

*Soares et al., J. Biomol. NMR 30 (**2004**) 407-422*
*van Gunsteren et al., Angew. Chemie Intl. Ed. 45 (**2006**) 4064-4092*

NOE distance bound violations in HEWL

NOE bound violations computed from MD trajectories (43A1(1996)/45A3(2001)) **against *two sets of experimental NOE distance bounds*** from Smith *et. al.* (set1, 1993) and from Schwalbe *et. al.* (set2, 2001)



Averaging period (ns)	Number of violations (set1) out of 1158 NOE's			Mean violation $<R_E-R_O>$
	>0.1 nm	*>0.2 nm*	*> 0.3 nm*	
0.0-0.5	25/44	9/15	2/6	0.017/0.024
0.5-1.5	31/44	11/15	3/3	0.020/0.024
1.5-3.5	41/56	11/27	5/17	0.023/0.034
0.0-3.5	23/43	9/17	3/6	0.019/0.026
	Number of violations (set2) out of 1525 NOE's (30% more)			
	>0.1 nm	*>0.2 nm*	*> 0.3 nm*	
0.0-0.5	21/43	4/9	0/0	0.015/0.021
0.5-1.5	22/47	2/14	0/2	0.017/0.021
1.5-3.5	27/60	6/12	0/6	0.017/0.026
0.0-3.5	20/40	2/7	0/1	0.014/0.020

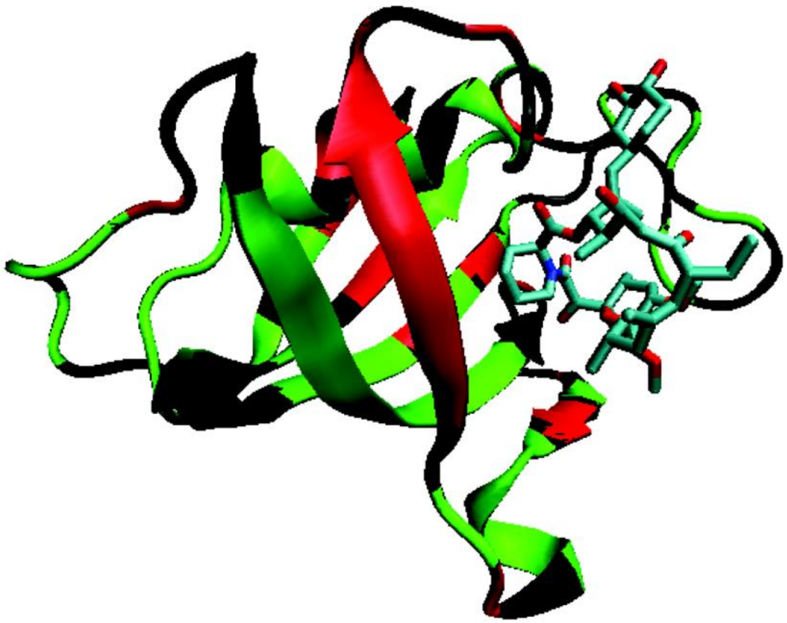
1993 set

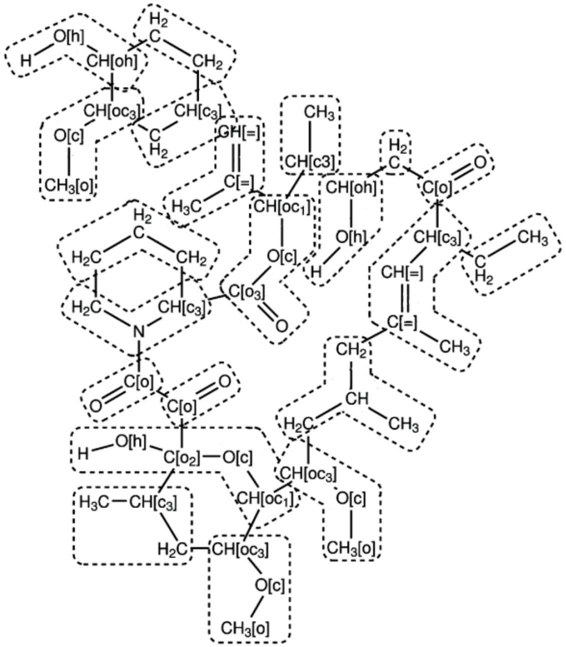
2001 set

Over time (1993 → 2001) the experimental data converged towards simulated ones

FKBP (107 residues) + ascomycin

inconsistent experimental data





The protein is coloured according to whether or not there is a range of χ_1 dihedral angle values corresponding to the experimental ^3J-coupling data (±1 Hz variation, distribution analysis):

black: no data (no ^3J-couplings : 38; one ^3J-coupling : 7 residues)

green: there is a single, continuous range of angle values that satisfies all of the experimental data (39 out of 62 residues: 63%)

red: there is no such solution (23 out of 62 residues: 37 %): ***inconsistent ?***

yes, for 5 residues

103 $^3J_{N\text{-}H\beta}$ and 94 $^3J_{H\alpha\text{-}H\beta}$-values

*Allison & van Gunsteren, ChemPhysChem 10 (**2009**) 3213-3228*

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D4 Related but different quantities are compared, e.g. differently defined free energies of folding

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*W.F. van Gunsteren, J. Dolenc, & A.E. Mark, Curr. Opin. Struct. Biol., 18 (**2008**) 149-153*

Reasons for disagreement between <Q>$_{sim}$ and <Q>$_{exp}$

Related but different quantities are compared

$$\langle Q \rangle_{sim} \Leftrightarrow \langle Q' \rangle_{exp}$$

1. $Q'(\vec{r})$ is a **free energy change**

$$\Delta G_{\text{folding}} = G_{\text{fold}} - G_{\text{denatured}}$$

of folding or renaturation,

as derived from experiment by ***changing the thermodynamic conditions***:

- *temperature change* ⟵
- pH change
- ionic strength or *co-solvent change* ⟵

Different solute stabilities
or
free energies of folding Q'(r)

2. $Q(\vec{r})$ is a **free energy change**

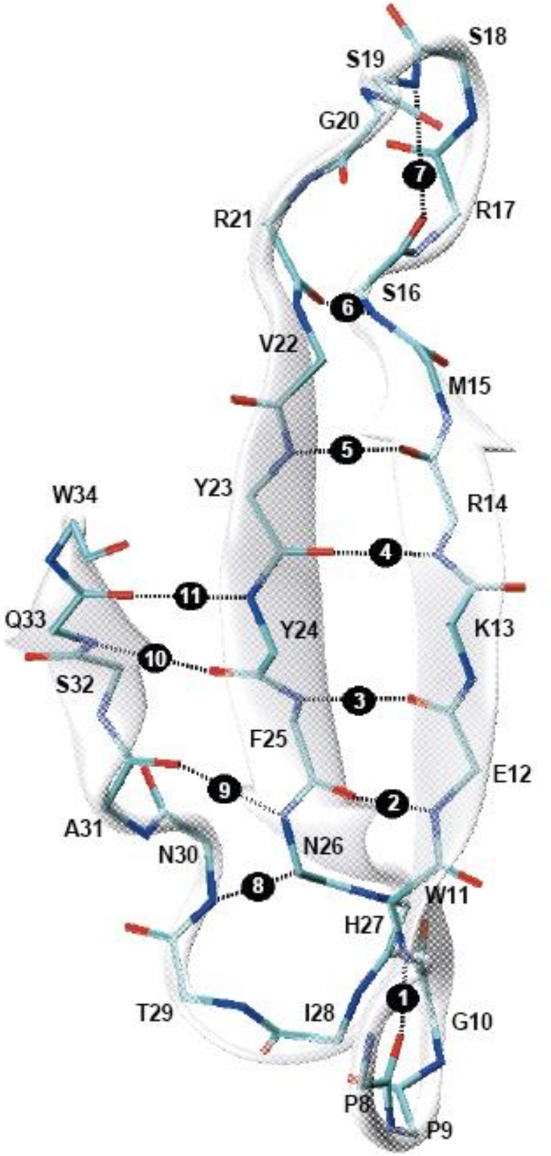
$$\Delta G_{\text{folding}} = G_{\text{fold}} - G_{\text{unfolded}}$$

of folding ***as derived from*** one ***simulation*** at one given thermodynamic state point by ***counting the ratio of folded versus unfolded conformations***

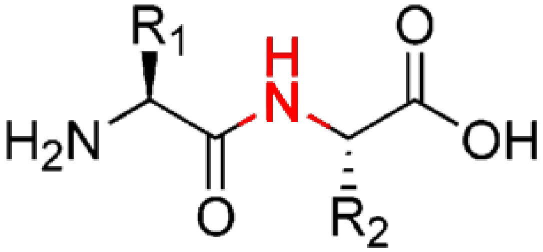
Comparison has only limited value: $\Delta G_{folding}\left(Q'\right) \neq \Delta G_{folding}\left(Q\right)$

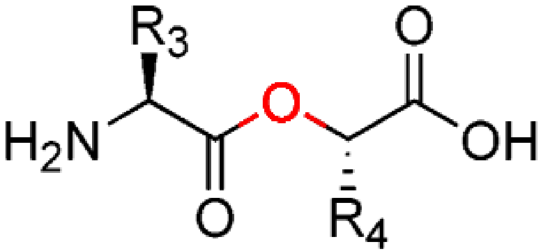
The WW domain of the PIN1 protein:

Contribution of backbone hydrogen bonding to β-sheet stability



11 backbone-backbone hydrogen bonds, distributed over three β-strands, *each NH individually replaced by Oxygen*





$R_1 = R_3$ and $R_2 = R_4$

Deechongkit et al., JACS 126 (2004) 16762

20 single residue mutants synthesised and their stability measured by experiments:

1. Thermal denaturation
2. Chaotrope denaturation (Gd-Cl)

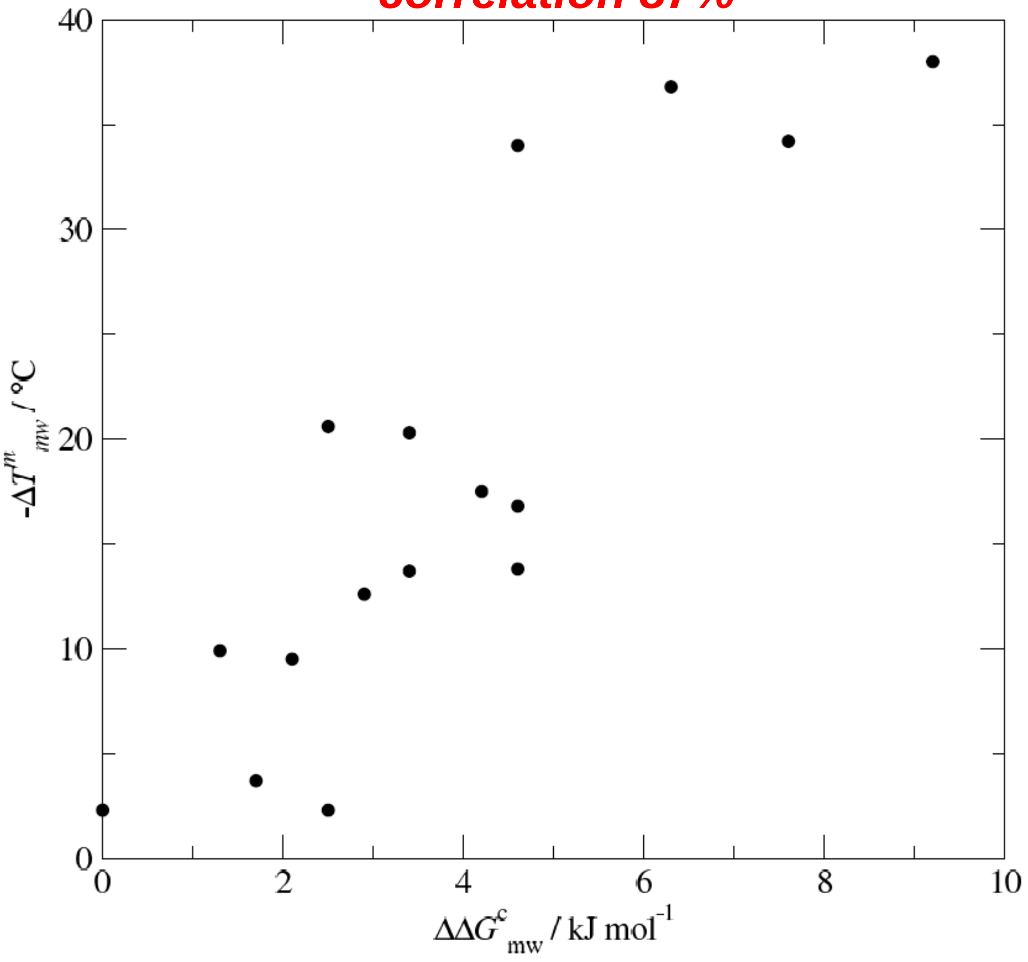
The WW domain of the PIN1 protein:

Contribution of backbone hydrogen bonding to β-sheet stability

Deechongkit et al., JACS 126 (2004) 16762

Temperature versus chaotrope denaturation

correlation 87%



Dominant fold or structure of the 16 mutants verified by:

1. far-UV CD spectroscopy
2. fluorescence spectroscopy
3. 1D ^{1}H NMR spectroscopy
4. ligand-binding assay

R14ρ, F25φ, N26ν, Q33θ
only stable upon addition of TMAO (trimethylamine N-oxide), no melting temperature reported

Different quantities reflecting fold stability need not show high correlation

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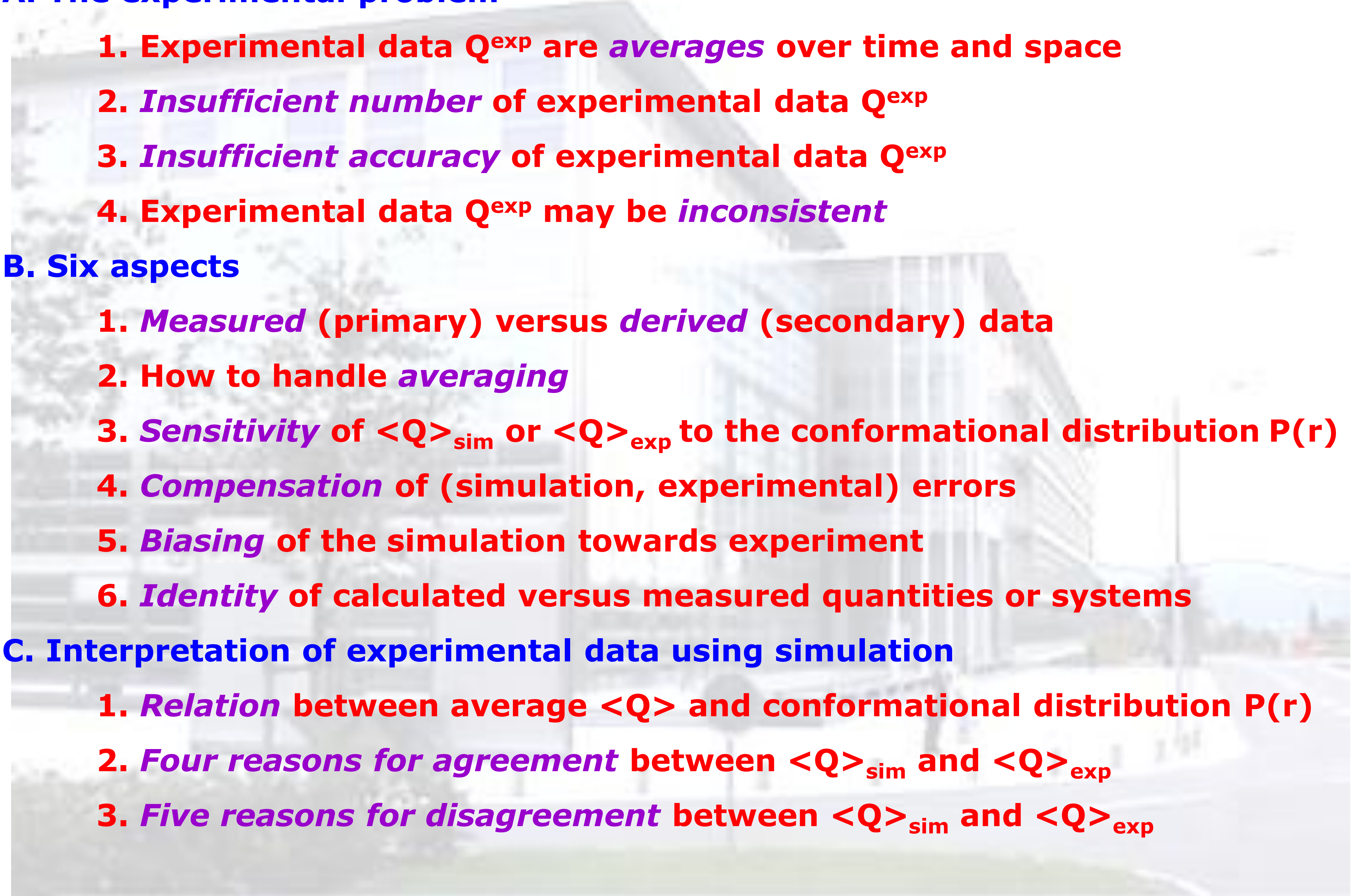

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1. Experimental data Q^{exp} are *averages* over time and space
2. *Insufficient number* of experimental data Q^{exp}
3. *Insufficient accuracy* of experimental data Q^{exp}
4. Experimental data Q^{exp} may be *inconsistent*

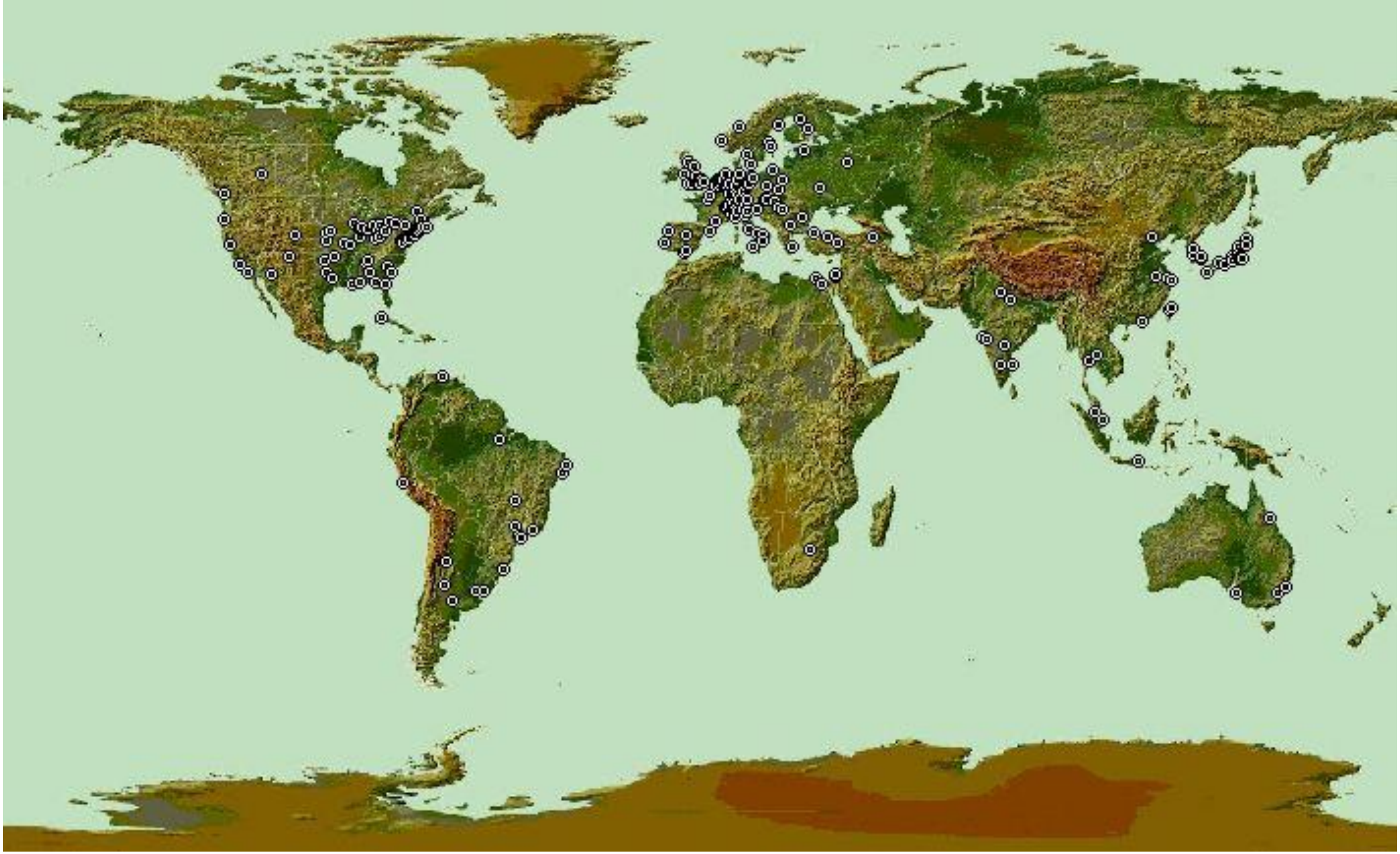
B. Six aspects

1. *Measured* (primary) versus *derived* (secondary) data
2. How to handle *averaging*
3. *Sensitivity* of $\langle Q \rangle_{sim}$ or $\langle Q \rangle_{exp}$ to the conformational distribution P(r)
4. *Compensation* of (simulation, experimental) errors
5. *Biasing* of the simulation towards experiment
6. *Identity* of calculated versus measured quantities or systems

C. Interpretation of experimental data using simulation

1. *Relation* between average $\langle Q \rangle$ and conformational distribution P(r)
2. *Four reasons for agreement* between $\langle Q \rangle_{sim}$ and $\langle Q \rangle_{exp}$
3. *Five reasons for disagreement* between $\langle Q \rangle_{sim}$ and $\langle Q \rangle_{exp}$

Spatial distribution of licences
GROMOS biomolecular simulation software



GROMOS = Groningen Molecular Simulation + GROMOS Force Field

Generally available: http://www.gromos.net