The Abdus Salam International Centre for Theoretical Physics

United Nations Educational, Scientific and Cultural Organization

IAEA International Atomic Energy Agency

2332-7

School on Synchrotron and FEL Based Methods and their Multi-Disciplinary Applications

19 - 30 March 2012

Adjusting Shape and Size of Pores

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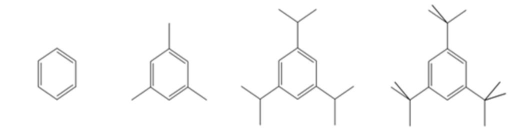
University of Graz (Austria)

Adjusting Shape and Size of Pores

Size and shape of the template micelle can be altered by adding organic molecules:

→ **‚swelling'**

Used aromatic compounds in this work:



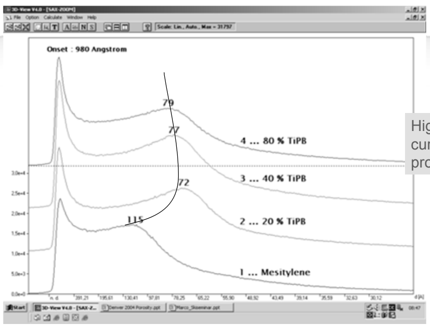
Benzene (78.1 g/mol)

Mesitylene (120,2 g/mol) 1,3,5-Trimethylbenzene

TiPB (204.4 g/mol) 1,3,5-Triisopropylbenzene

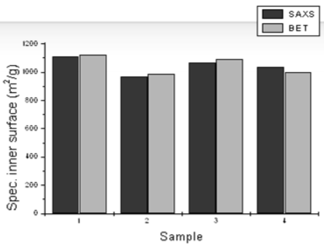
TtBB (246.4 g/mol) 1,3,5-Tri-tert.-butylbenzene

Mesoporous Silica



High Resolution SAXS curves of calcinated products

Mesoporous Silica: Inner surface from SAXS and BET



1 ... Mesitylene
2 ... TiPB (tri-isopropylbenzene) 20 %
3 ... TiPB 40 %
4 ... TiPB 80 %

Inner surface

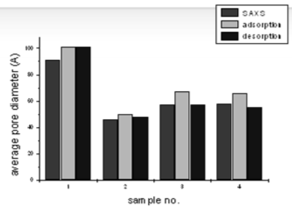
Independent measurements:

SAXS : IBR Graz

BET : ETH Zurich

Variance: less than 3%

Mesoporous Silica: Pore sizes from SAXS and BET



Average pore diameter:

SAXS (IBR Graz)

BdB-FHH (ETH Zurich)

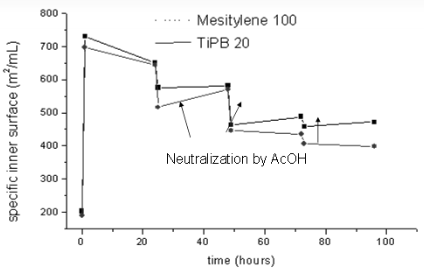
BdB-FHH according to Lukens et al. Langmuir 15 (1999) 5403

1 ... Mesitylene
2 ... TiPB (tri-isopropylbenzene) 20 %
3 ... TiPB 40 %
4 ... TiPB 80 %

Mesoporous Silica: Product Parameters

	100 Mes	20 TiPB	40 TiPB	80 TiPB
S_i SAXS (m²/g)	1131	977	1064	1029
S_i BET (m²/g)	1124	986	1090	998
Pore size SAXS (A)	94	46	57	58
Pore size BdB-FHH (A) adsorption	101	50	67	57
Pore size BdB-FHH (A) desorption	101	48	57	55
Wall thickness SAXS	16	19	17	17
d_{Bragg} (A)	115	72	77	79

Mesoporous Silica: Measuring the inner surface during reaction – *in situ in the reaction mixture*



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Conclusion:

• Specific inner surface values and pore sizes from SAXS and BET are in agreement (except for very small BET S_i-values)

• SAXS has the advantage of being much faster: results within minutes, lower costs per analysis

• SAXS gives information about the inner surface also in the humid or wet state – time-resolved reaction monitoring

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The inner surface between crystalline and amorphous domains, rather than their percentage, determines important technological properties:

•Mechanical stability, compactability

•Chemical stability, solubility, dissolution rate

•Thermal stability

•Water vapor sorption

•Chemosorption

•Active compound release

•Bioavailability

•...

Where can SAXS help in biology ?

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- Drug Discovery – Proteomics – Structural Biology
- Drug Development – Formulation – Process Control
- Biomaterials - Hair, Skin, Bone, Tissue Engineering
- Biochem / Biophys Basic Research

Biochem/Biophys Basic Research

The most important applications of SAXS in basic research relate to:

- Macro- / supramolecular interactions in solution with small molecules, salts , (Hofmeister series …)
- Supramolecular complex formation in solution (protein-lipid, protein-nucleic acid,…)
- Self-assembly of amphiphilic compounds (Micelle formation , coagulation…)

Laboratory SAXS/SWAXS/GISAXS become complementary standards to spectroscopic and thermodynamic tools in biophysics/biochemistry. Powerful instruments provide the necessary speed of measurement.

Proteomics – Structural Biology

Isotropic SAXS – no preferred orientation – 1D-scattering curve

Q: Drug binding effect on enzyme structure in solution ?
- SAXS in dilute solution – radius of gyration (R_G) , molecular weight , max. dimension

Q: Search for optimum crystallization conditions ?
- SAXS in different salt or polymer solutions – size (R_G) monitors attractive interactions

Q: Differences between X-ray crystal structure and solution structure ?
- SAXS curve fitting with crystal date (e.g. from PDB data bank)

Q: Oligomerization / multi-protein assembly in solution ?
- SAXS under different protein concentrations

ALL POSSIBLE WITH LABORATORY INSTRUMENTS – NO SYNCHROTRON REQUIRED

SAXS in Structural Proteomics and Drug Discovery

Genomics → Protein Synthesis → Candidate Selection → Protein Structure → Rational drug design

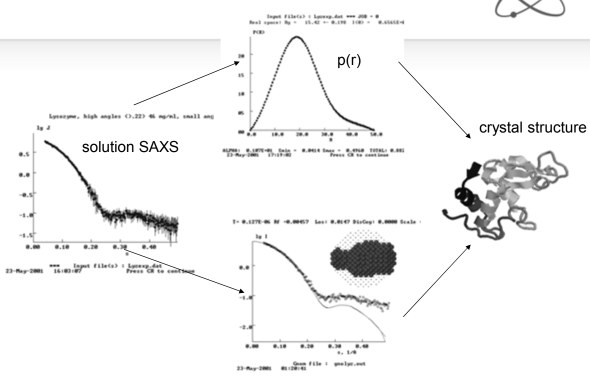
Protein sizing to determine:

• State of aggregation in solution (monodisperse/polydisperse)?

• Suitability for crystallization?

• Effects of salts, additives, ligands?

SOLUTION *vs.* CRYSTAL STRUCTURE

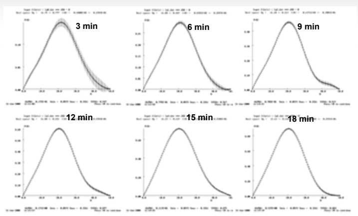


How long does it take?

Protein Size and Shape Within Minutes by Lab-SAXS

(Bruker MICROPix™)

Pair Distance Distribution



Stable results after < 12 min exposure for the entire solution structure

Size (Radius of Gyration) to < +/- 3 % within 3 min

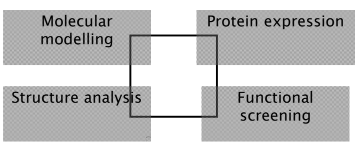
Sample volume < 3 µl; concentration 0.1%

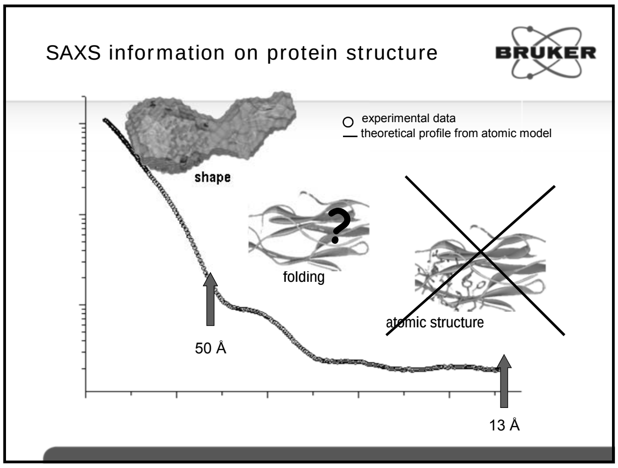
Advantage of SAXS over MALDI: True state in solution

Today‘s X-ray optics and detector technology make it possible to perform these studies

in the normal laboratory – without synchrotron

– side-by-side to the protein isolation and modification.This is essential for an efficient ‚structural proteomics‘ approach:




SAXS information on protein structure
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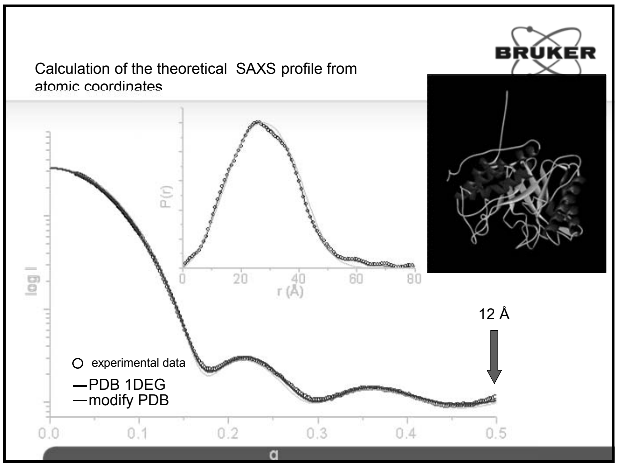
experimental data
theoretical profile from atomic model
shape
folding
atomic structure
50 Å
13 Å


Calculation of the theoretical SAXS profile from atomic coordinates
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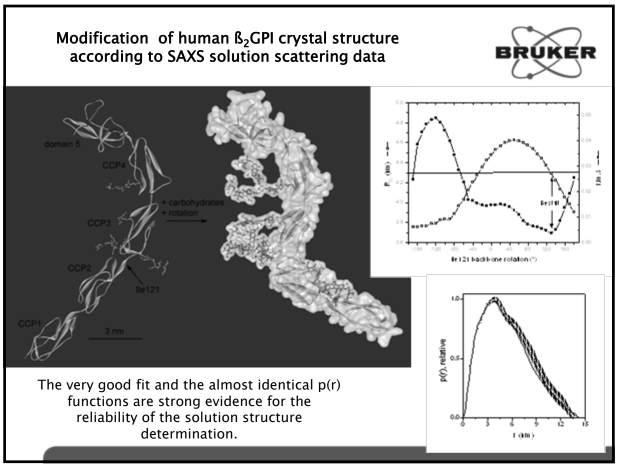
P(r)
0
20
40
60
80
r (Å)
log I
12 Å
experimental data
—PDB 1DEG
—modify PDB
0.0
0.1
0.2
0.3
0.4
0.5
q


Modification of human ß$_2$GPI crystal structure according to SAXS solution scattering data
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domain 5
CCP4
+ carbohydrates
+ rotation
CCP3
CCP2
Ile121
CCP1
3 nm
The very good fit and the almost identical p(r) functions are strong evidence for the reliability of the solution structure determination.

Solution structure of proteins revealed by small angle X-ray scattering (SAXS)

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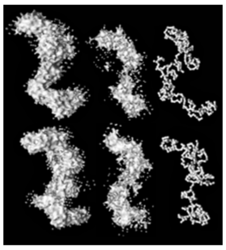
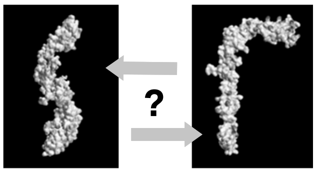
β2-Glycoprotein I

Low resolution models



Dammin Dalai_ga Gasbor

Comparison to crystal structure



Ab initio modeling

15- Lipoxygenase

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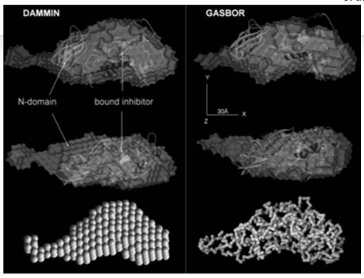
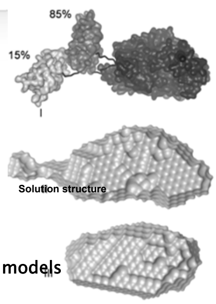


Schematic movement of the N-terminal domain



DAMMIN model derived from the crystal structure

Superposition of the averaged low resolution models with the crystal structure

The features of the catalytic domain are preserved, but the N-terminal domain is streched and flexible

Lipidic Formulations – Membrane Biophysics

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Powder SAXS – no preferred orientation – 1D-scattering pattern

Q: Polymorphic structure and transitions of liposomal formulations ?
- Simultaneous small-and wide-angle scattering (SWAXS) in T-/c-/p-scanning mode

Q: Interactions between lipid model systems and drugs ?
- Dose-dependent SWAXS scanning

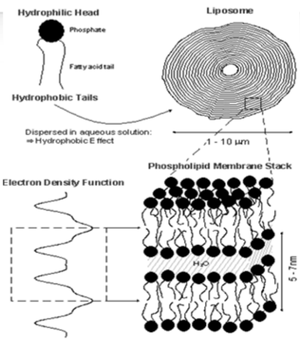
Q: Simultaneous calorimetric and structural measurement ?
- Integrated SWAXS – scanning microcalorimetry

Q: Solid-supported thin lipid films ? **ORIENTED ! 2D-pattern**
- Grazing-incidence SAXS (GISAXS)

SWAXS for screening
of membrane – active drugs

Starting from the components:

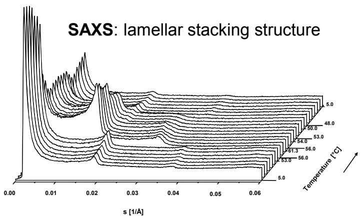
Lipid self-assembly



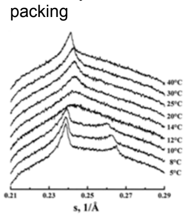
Thermal phase transitions SWAXS T-scans.

Dipalmitoyl-PC (DPPC)

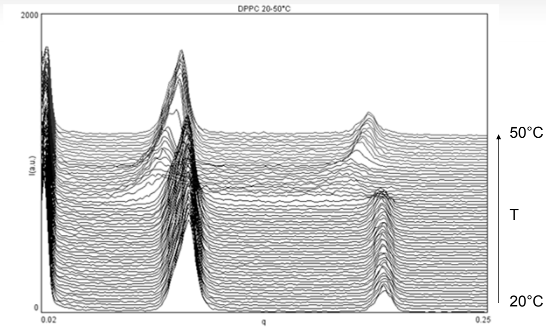
SAXS: lamellar stacking structure



WAXS: hydrocarbon chain packing

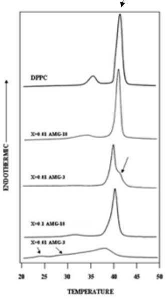


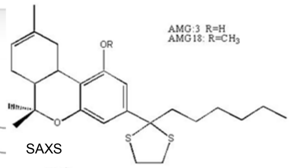
DPPC-heating-scan: 20-50°C
0.5°/min, 1 frame/min
every frame shown,
Phases: $L_{\beta'}$, $P_{\beta'}$, L_{α}



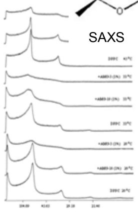
The transition can be used to study the interaction with drugs

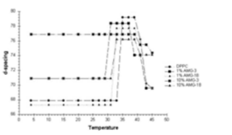
DSC-trace





SAXS

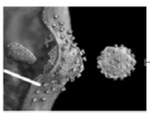




Searching targets for anti-viral therapy

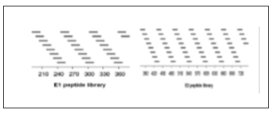
Q: Which parts of the protein are responsible for fusion ?

How can viral fusion with the cell membrane be suppressed ?



Viral entry:
Membrane
Fusion

Viral Fusion Protein



Viral Fusion Protein: Membrane-Peptide Screening

with Ana J. Perez Berna and Jose Villalain, 2007

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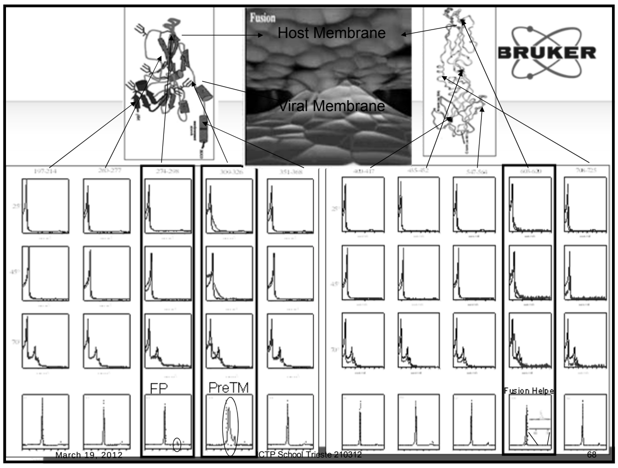
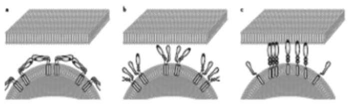
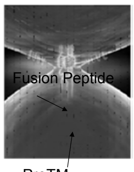


Screening of Peptide Library with Differential scanning calorimetry and Small-angle x-ray diffraction (SAXS) are suitable techniques for finding regions that promote non-lamellar phases likely to be involved in fusion.





PreTM

the region 603-620, fusion helper, 274-298, fusion peptide, and 309-326, PreTransmembrane domain are entry targets against HCV (hepatitis C virus).

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Biomaterials : Hair , Skin, Bone, Wood, Coal…

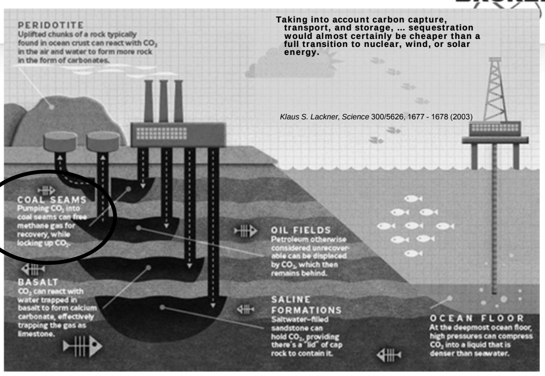
Small- and wide-angle patterns of oriented samples – 2D detection / nanography

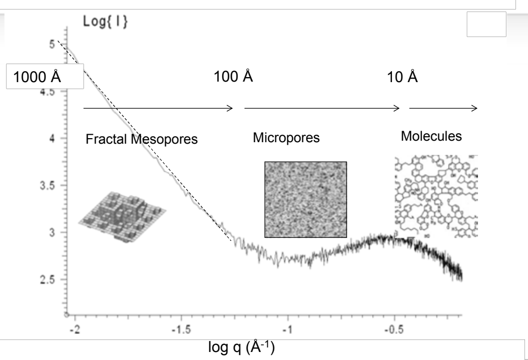
SAXS on Gas- / Vapor- Sorption

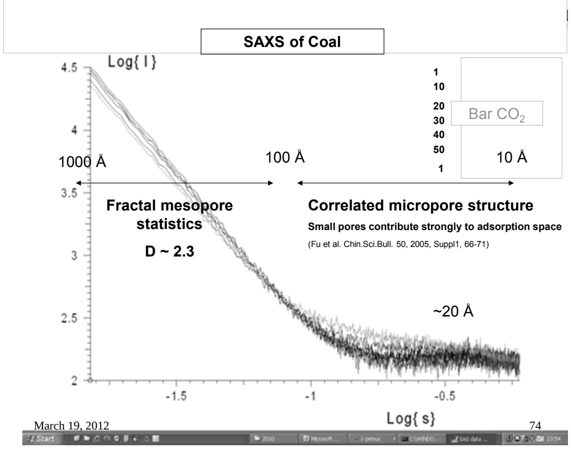
In-situ characterisation of nanostructure by SAXS upon reaction with gases / vapor

Example CO_2 sorption in natural coal between 1-50 bar

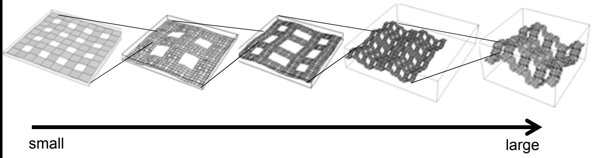
Strategies to Sequester CO_2







Fractal Surface



Measured surface depends on the length scale

Brownian surface D_S = 2.5

Human brain D_S ~ 2.8

Human lung D_S ~ 3

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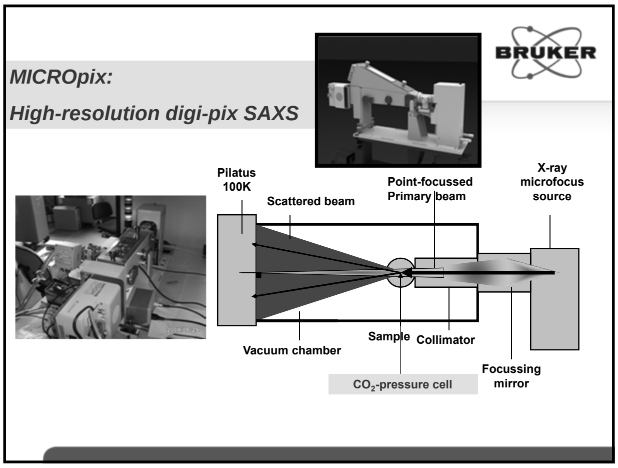
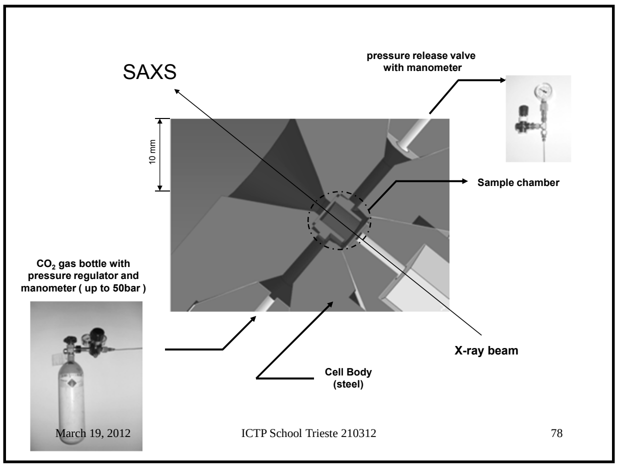
CO_2 - SEQUESTRATION

> 50% of the sequestration capacity in coal mines: adsorption

Little is known about CO_2-coal interaction phenomena such as:

- **Swelling**
- **Adsorption vs. molecular absorption**
- **Density and density distribution of CO_2 confined in the pores**
- **Effects of coal heterogeneity**


BRUKER
MICROpix:
High-resolution digi-pix SAXS
Pilatus 100K
Scattered beam
Point-focussed Primary beam
X-ray microfocus source
Sample
Collimator
Vacuum chamber
Focussing mirror
CO2-pressure cell


SAXS
pressure release valve with manometer
10 mm
Sample chamber
CO2 gas bottle with pressure regulator and manometer (up to 50bar)
X-ray beam
Cell Body (steel)
March 19, 2012
ICTP School Trieste 210312
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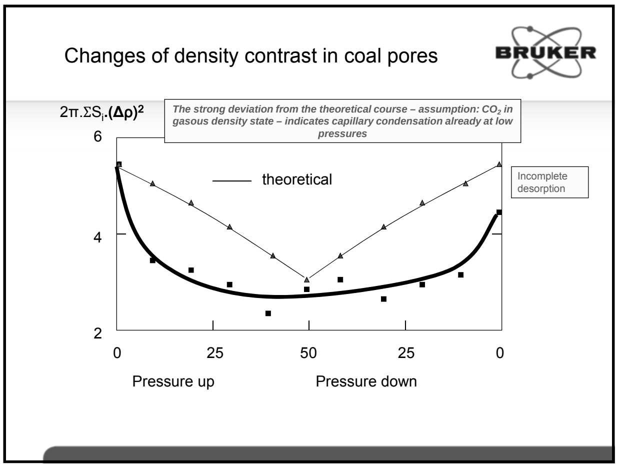
Changes of density contrast in coal pores
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2π.ΣSi.(Δρ)²
The strong deviation from the theoretical course – assumption: CO2 in gasous density state – indicates capillary condensation already at low pressures
theoretical
Incomplete desorption
6
4
2
0
25
50
25
0
Pressure up
Pressure down

Fractal Dimension of Coal DPB3

DPB3 coal shows fractal structure with mass fractal dimension between 2.4 and 2.6, nonlinearly decreasing with CO_2 pressure, reversibly.