Molecular-level assessment of disease-relevant mechanisms by AFM

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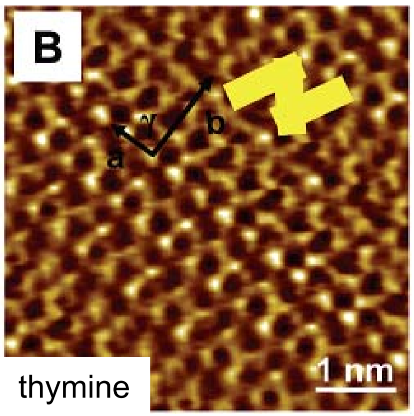
Biomolecules at surfaces

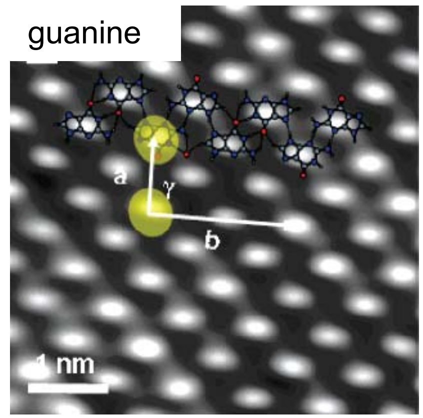
Biomolecules at the solid-liquid interface

The presence of surfaces has a strong effect on biomolecules, i.e. DNA, proteins, and peptides.

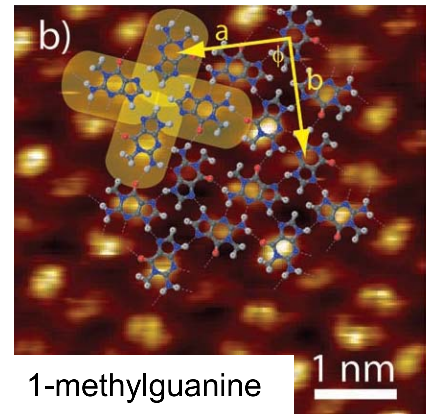
→ **exploited for the self-assembly of supramolecular nanopatterns**



Mamdouh *et al.*, *J. Am. Chem. Soc.* (2006), **128**, 13305



Mamdouh *et al.*, *J. Am. Chem. Soc.* (2008), **130**, 695



Bald *et al.*, *Small* (2011), **7**, 939

However -

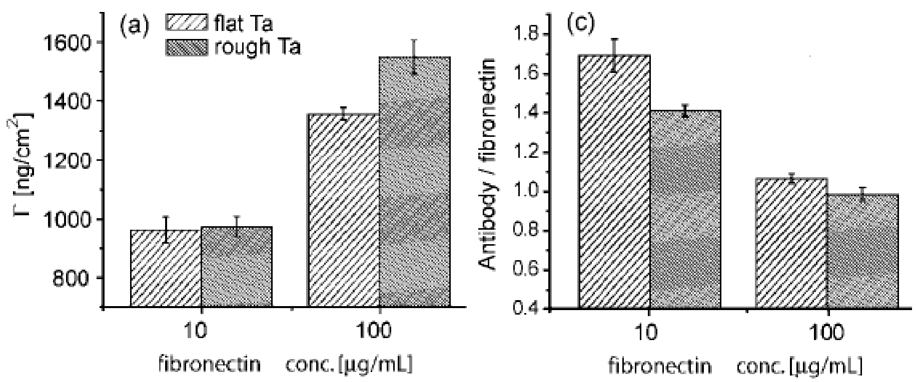
also in the physiological environment, different surfaces interact with biomolecules:
cell membranes, blood vessels, surfaces of bones, implants, tubing, …

→ **How do surfaces influence the physiological action of biomolecules?**

→ ***in-vitro studies using biological model surfaces***

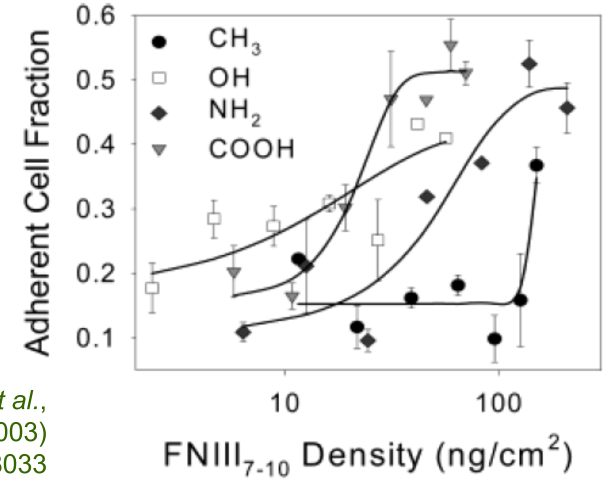
Surface properties influence protein adsorption

surface roughness

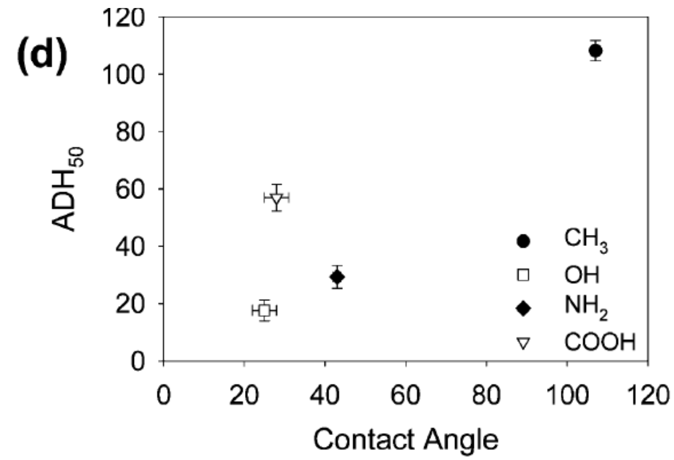


Hovgaard *et al.*, *J. Phys. Chem. B* (2008), **112**, 8247

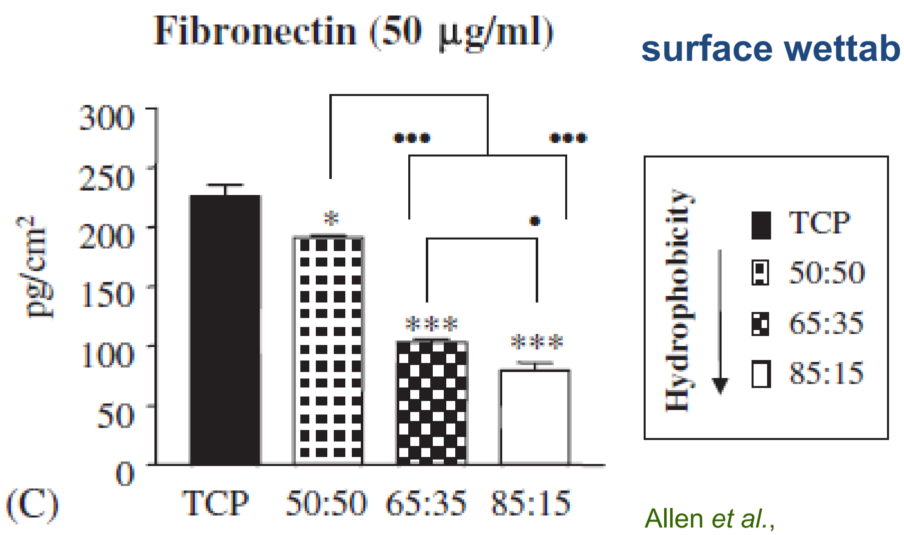
surface chemistry



Michael *et al.*, *Langmuir* (2003) **19**, 8033



surface wettability

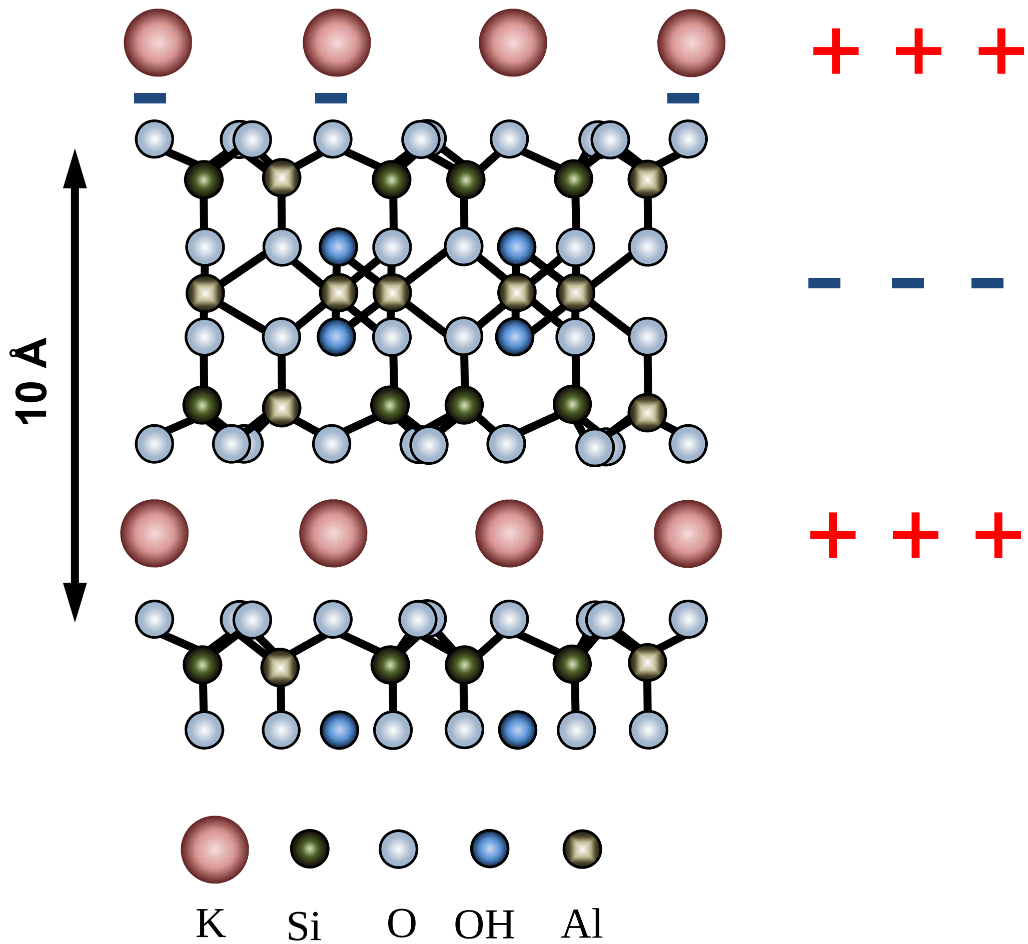


Allen *et al.*, *Biomaterials* (2006) **27**, 3096

→ tailored model surfaces for in-vitro studies

Tuning the hydrophobicity of mica surfaces by hyperthermal Ar ion irradiation

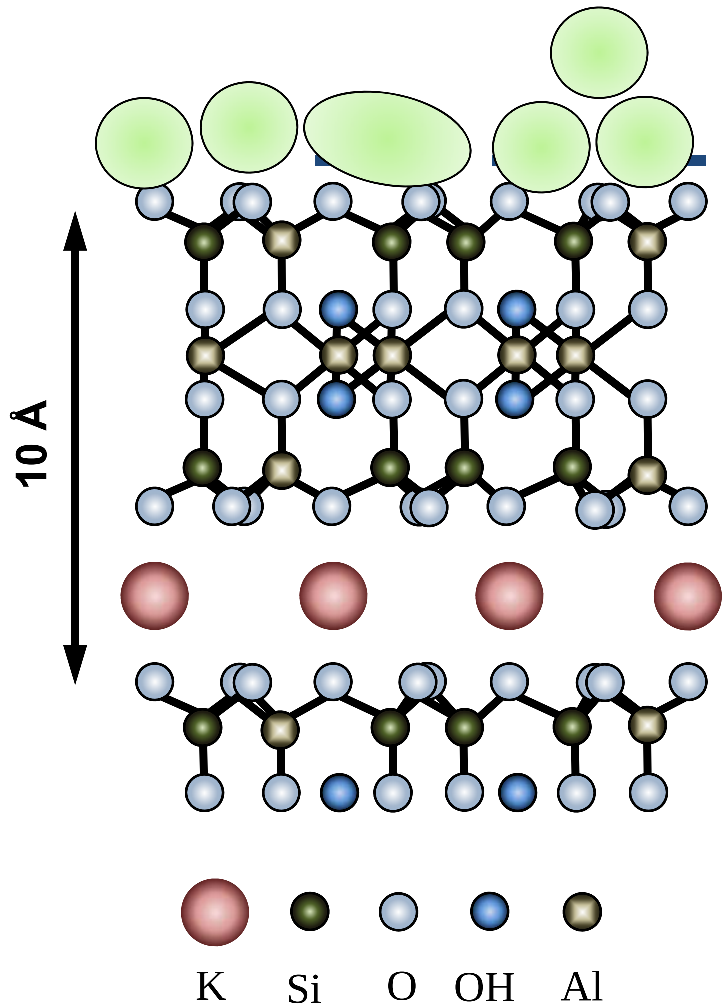
The structure of mica surfaces



- layered structure:
  - negatively charged alumino-silicate sheets
  - alternating layers of K^+ ions
  - distance between layers 1 nm
- cleaving (Scotch tape)
  → atomically flat terraces
  → great for AFM!
- aqueous solution:
  - K^+ ions exchanged into solution
  - surface exhibits a negative net charge

→ ***hydrophilic surface***

Ion-beam modification of mica surfaces



25 eV Ar^+ → mica:

- negligible effect on crystal structure
- very low sputter yield
  → *negligible effect on topography*
  → *negligible effect on surface composition*
- no new chemical species
- *very effecient removal of outermost K^+ ions*

→ underlying alumino-silicate sheet exposed

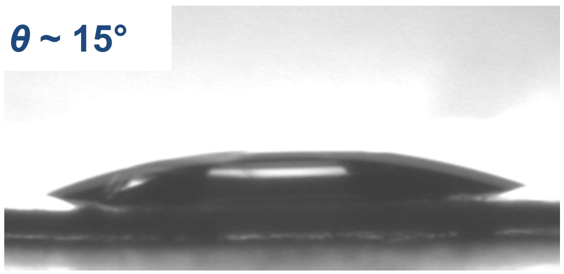
→ silicate tetrahedra act as adsorption sites for C

→ ***increased adsorption of hydrocarbons from the environment***

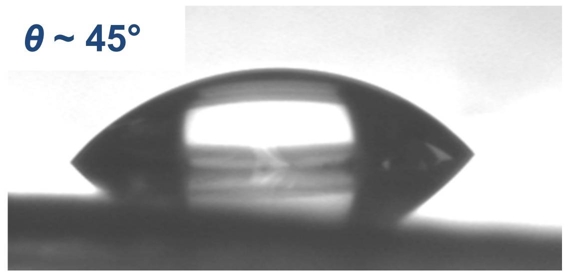
→ ***hydrocarbons are hydrophobic***

Ion-induced surface hydrophobicity

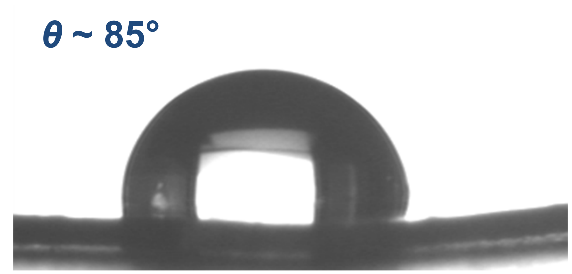
untreated
aged 7 days

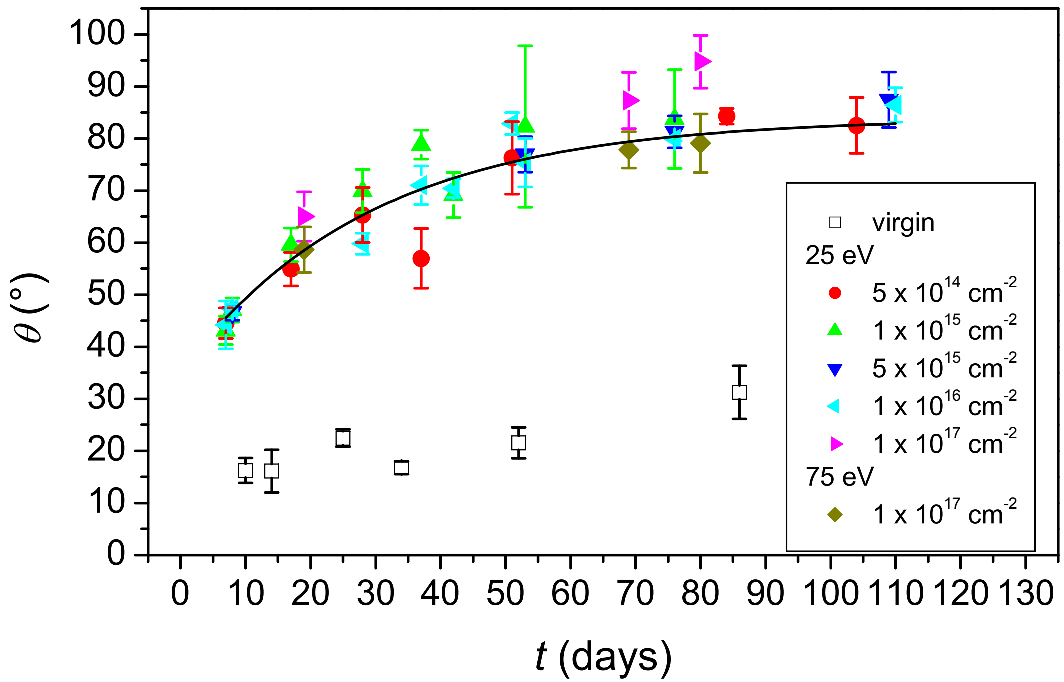


25 eV Ar^+, 5 x 10^{14} cm^{-2}
aged 10 days



25 eV Ar^+, 5 x 10^{14} cm^{-2}
aged 84 days





→ continuous increase of contact angle with age

→ increase independent of irradiation conditions!

Keller *et al.*, *J. Chem. Phys.* (2011) **134**, 104705

Chemical analysis by XPS

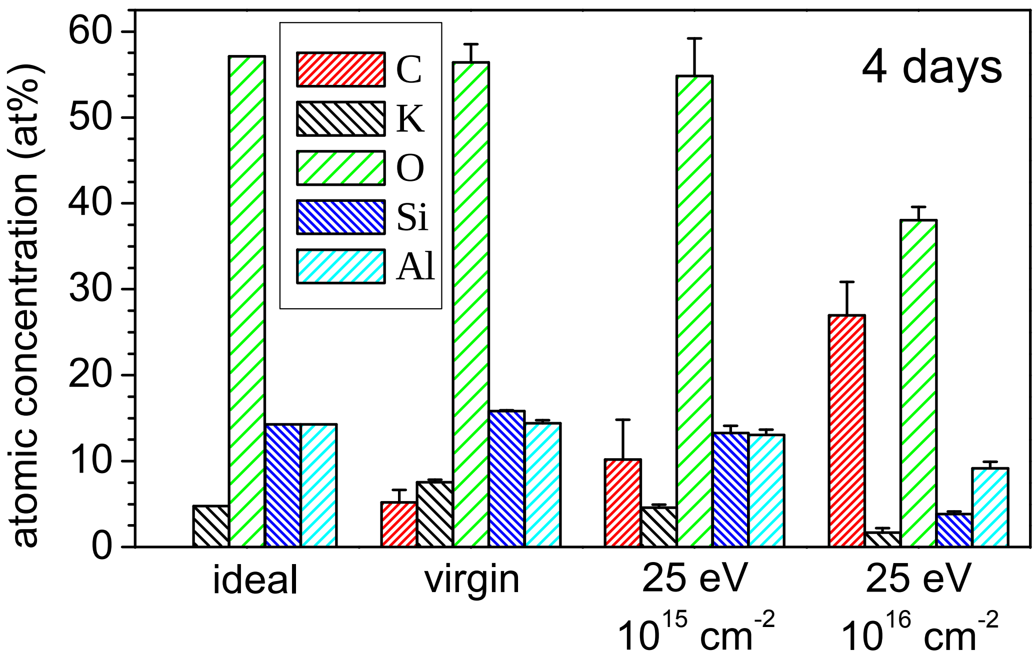


TABLE I. Elemental concentrations of K and C (in at.%) of virgin and irradiated mica surfaces aged for different times.

	Virgin			25 eV, 10^{15} cm^{-2}	
Element	10 min	4 days	64 days	4 days	67 days
K	7.3 ± 0.1	7.6 ± 0.3	6.1 ± 1.6	4.6 ± 0.4	2.2 ± 0.3
C	6.1 ± 0.1	5.2 ± 1.4	10.5 ± 3.7	10.2 ± 4.6	18.4 ± 0.6

- increased C content on bombarded samples
  → *thin HC film*
- high fluences: preferential sputtering
- C content increasing with age
- HR XPS: composition of HC films independent of age and treatment
- *contact angle not (solely) determined by amount of HCs on the surface*

Keller *et al.*, *J. Chem. Phys.* (2011) **134**, 104705

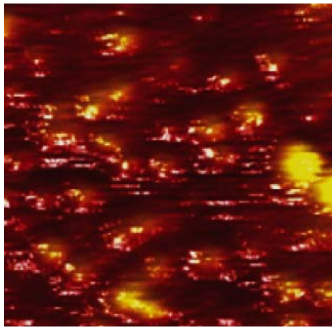
Influence of hydrophobicity on the surface-catalyzed assembly of the Islet Amyloid Polypeptide

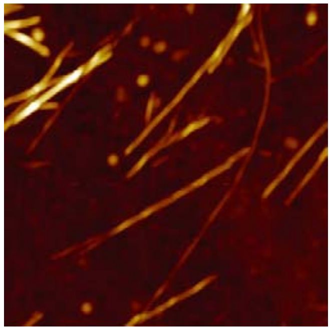
Amyloid aggregation

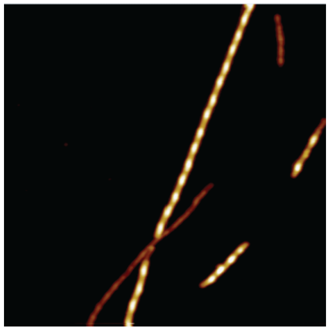
- Polymerization of misfolded peptides/proteins in solution or at interfaces

- Self-assembly into nanostructures:

oligomeric particles　　protofibrils　　higher-order fibrils







glucagon

Dong *et al.*, *Nanotechnology.* (2006), **17**, 4003

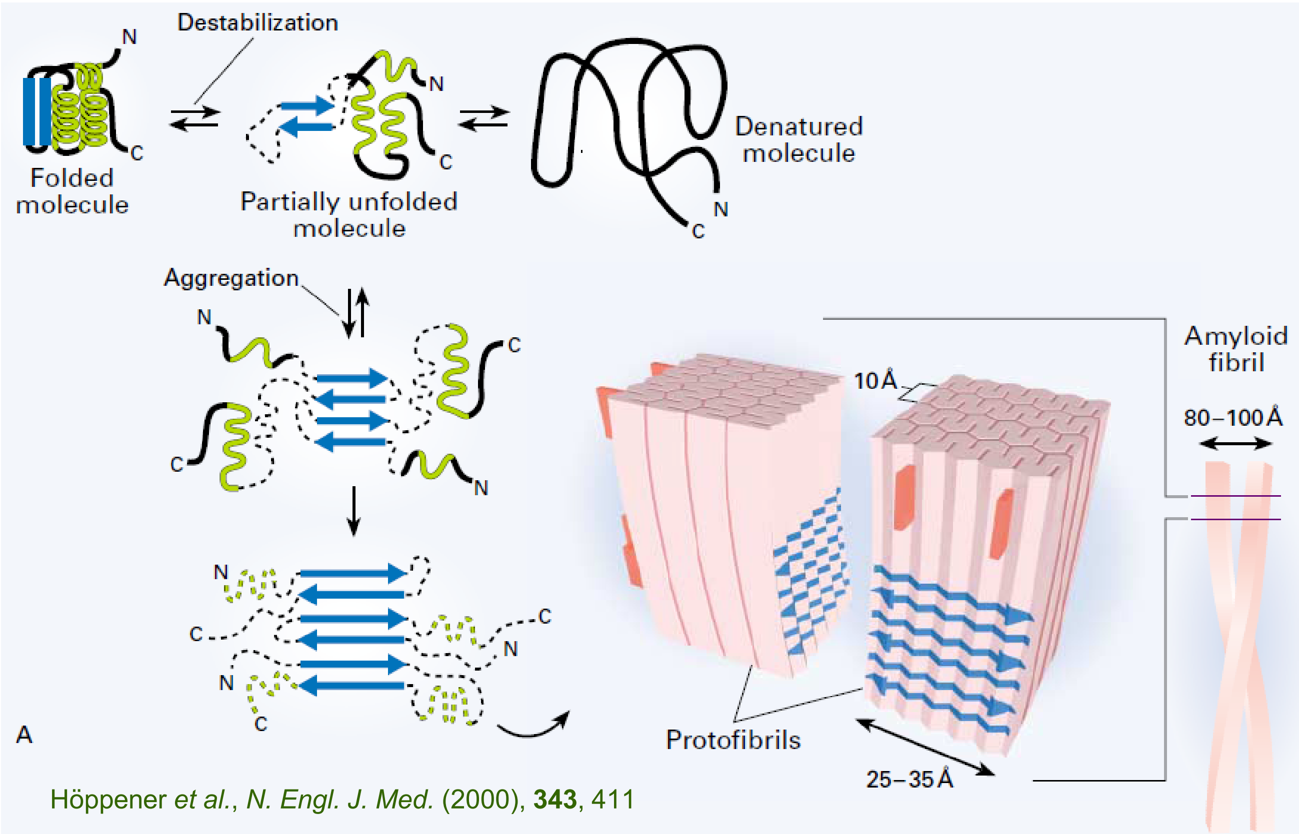
- In principle, *ANY* protein or polypeptide can form amyloid aggregates!

- Amyloid aggregation related to the development of various diseases, e.g. *Alzheimer's disease*, *Parkinson's disease*, *prion disease*, …

The Islet Amyloid Polypeptide (IAPP)

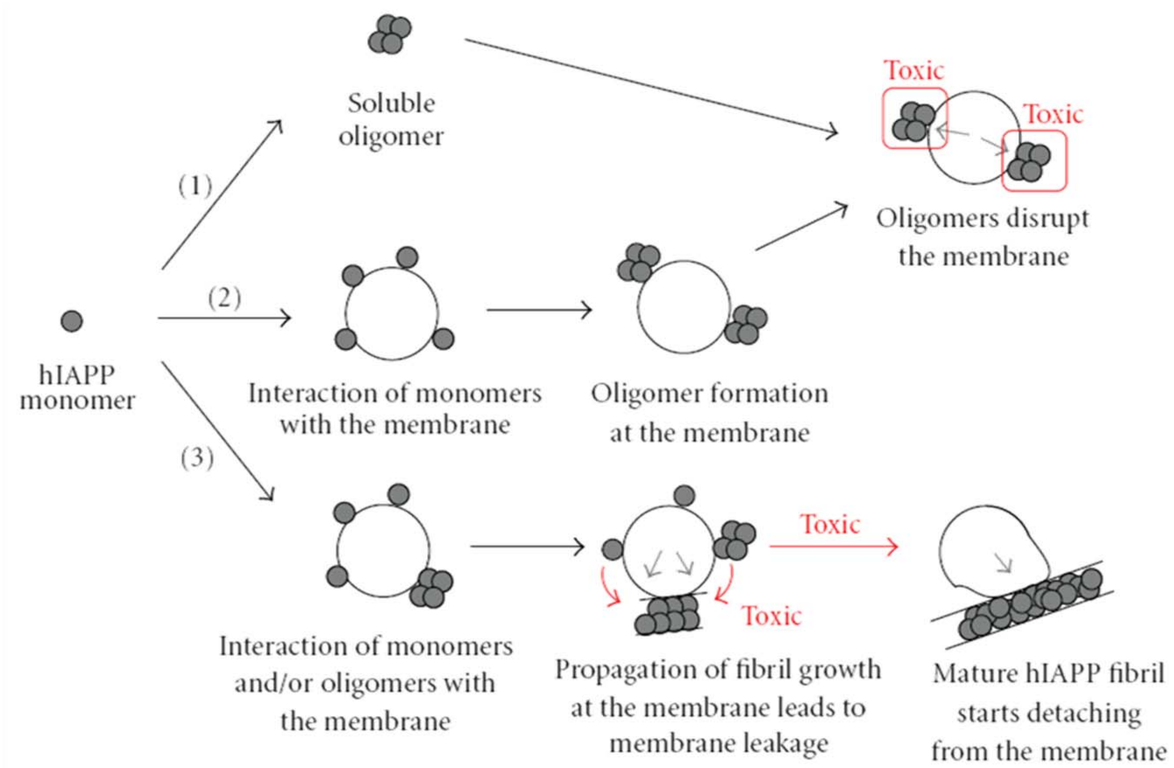
- Islet Amyloid Polypeptide (IAPP):
  - hormonal factor secreted from the pancreatic *β*-cells together with insulin
  - reduces insulin sensitivity
  - islet amyloid deposits present the pancreas of > 90% of type II diabetes patients

- IAPP fibrillation:

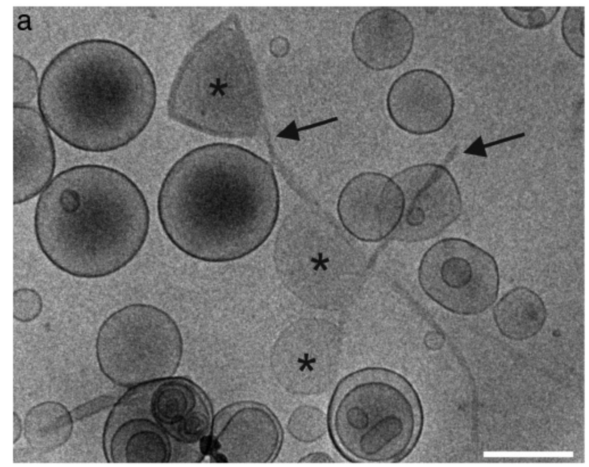


Höppener *et al.*, *N. Engl. J. Med.* (2000), **343**, 411

Cytotoxicity of IAPP aggregates



Khemtéourian *et al.*, *Exp. Diabetes Res.* (2008), 421287



Engel *et al.*, *Proc. Natl. Acad. Sci. USA.* (2008), **105**, 6033

→ islet amyloid can induce apoptotic cell-death in insulin-producing *β*-cells

→ relevant to the development of type II diabetes

Amyloid aggregation at surfaces

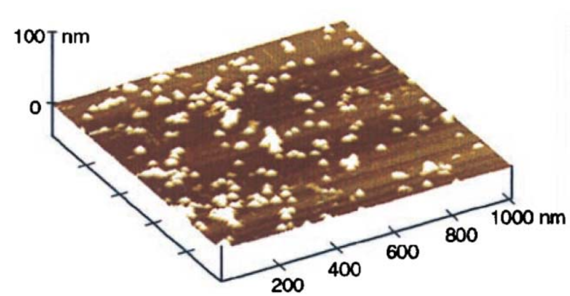
The presence of a surface may:

- induce the formation of initial oligomers (nucleation)
- influence the assembly rate and lag time
- affect the structure of the aggregates

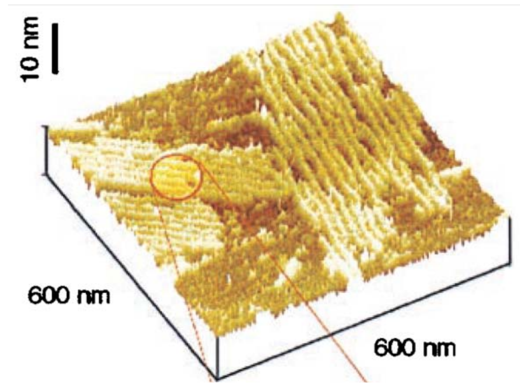
Physicochemical surface properties have a strong effect on amyloid aggregation!

Amyloid β

Mica: hydrophilic

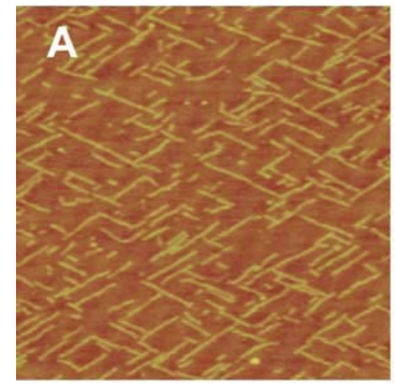


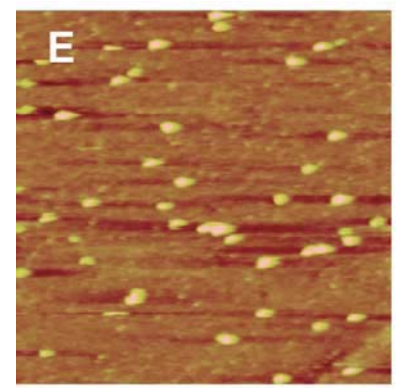
Graphite: hydrophobic



Kowalewski *et al.*,
Proc. Natl. Acad. Sci. USA. (1999), **96**, 3688

α-synuclein





Hoyer *et al.*,
J. Mol. Biol. (2004), **340**, 127

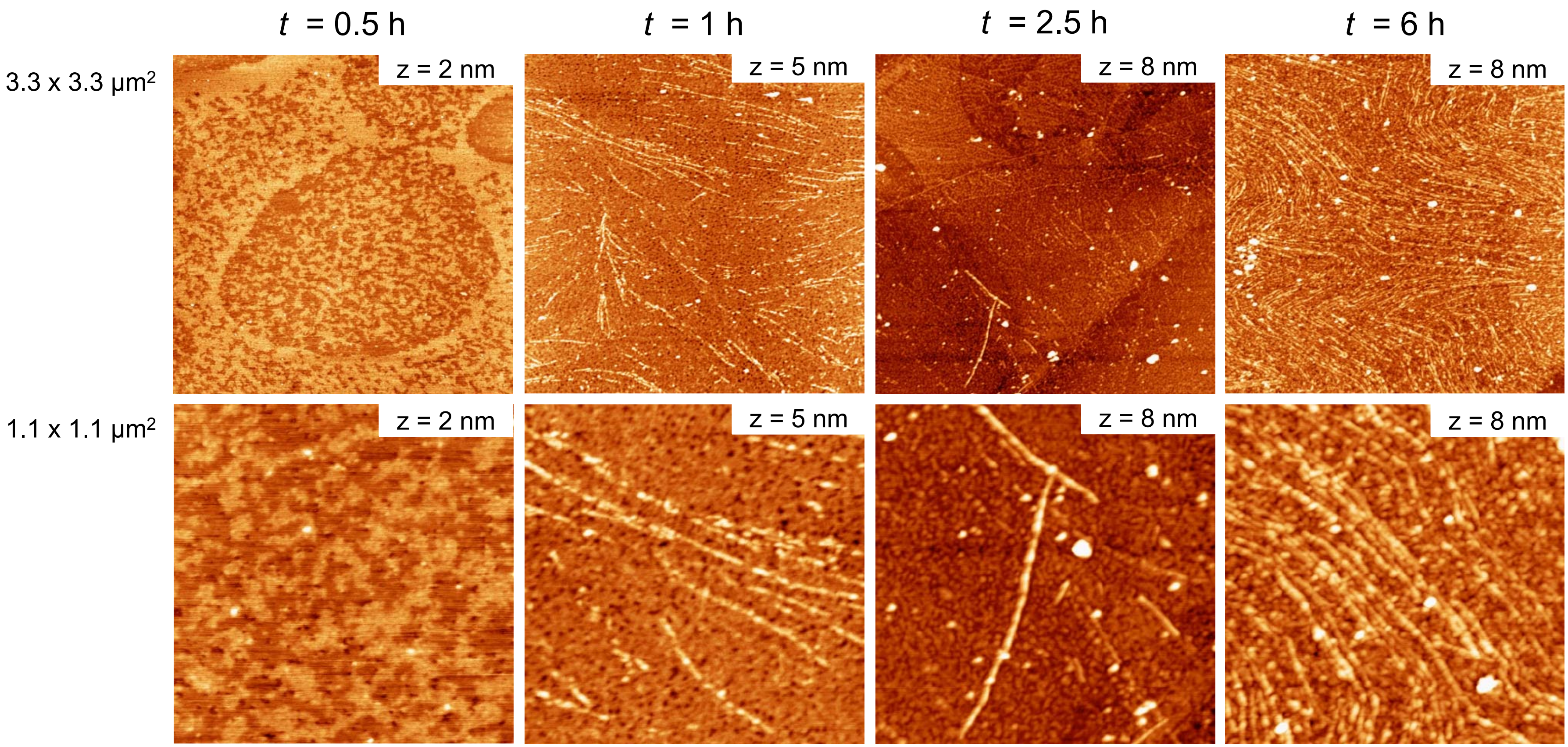
Time-lapse study of IAPP aggregation on HC films

- **Preparation of mica surfaces**
  - sub-100 eV ion bombardment
  - exposure to lab atmosphere
  - comparing aggregation on surfaces with different contact angles

- **IAPP incubation**
  - 13 µM IAPP in *water*
  - *room temperature*
  - incubation time 0.5 to 6 h
  - samples dried in N_2 stream

- ***Ex-situ* AFM:**
  tapping mode in air

IAPP on a mica/HC surface with ***θ* = 23°**

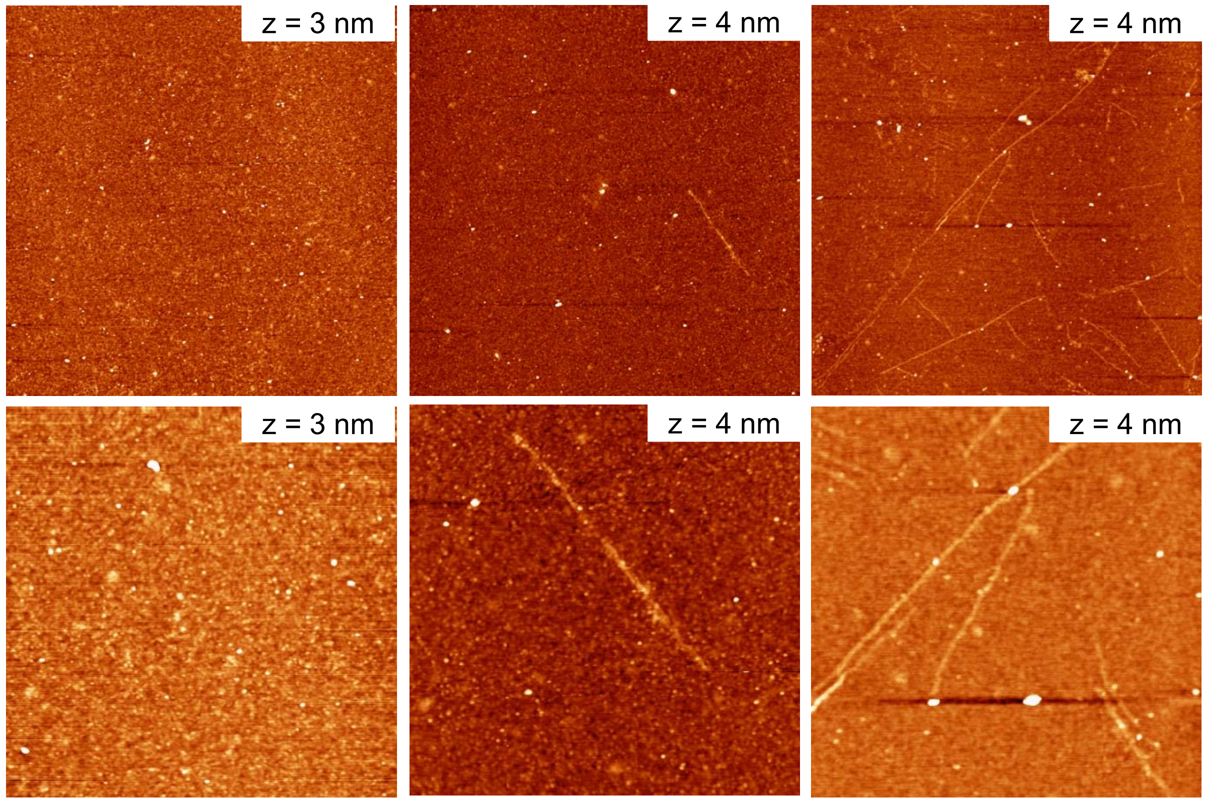


protofibrils
height: (2.0 ± 0.5) nm

fibrils
height: (4.5 ± 0.5) nm
twist: (28.4 ± 3.7) nm

Keller *et al.*, *ACS Nano* (2011), DOI: 10.1021/nn1031998

IAPP on a mica/HC surface with ***θ* = 38°**



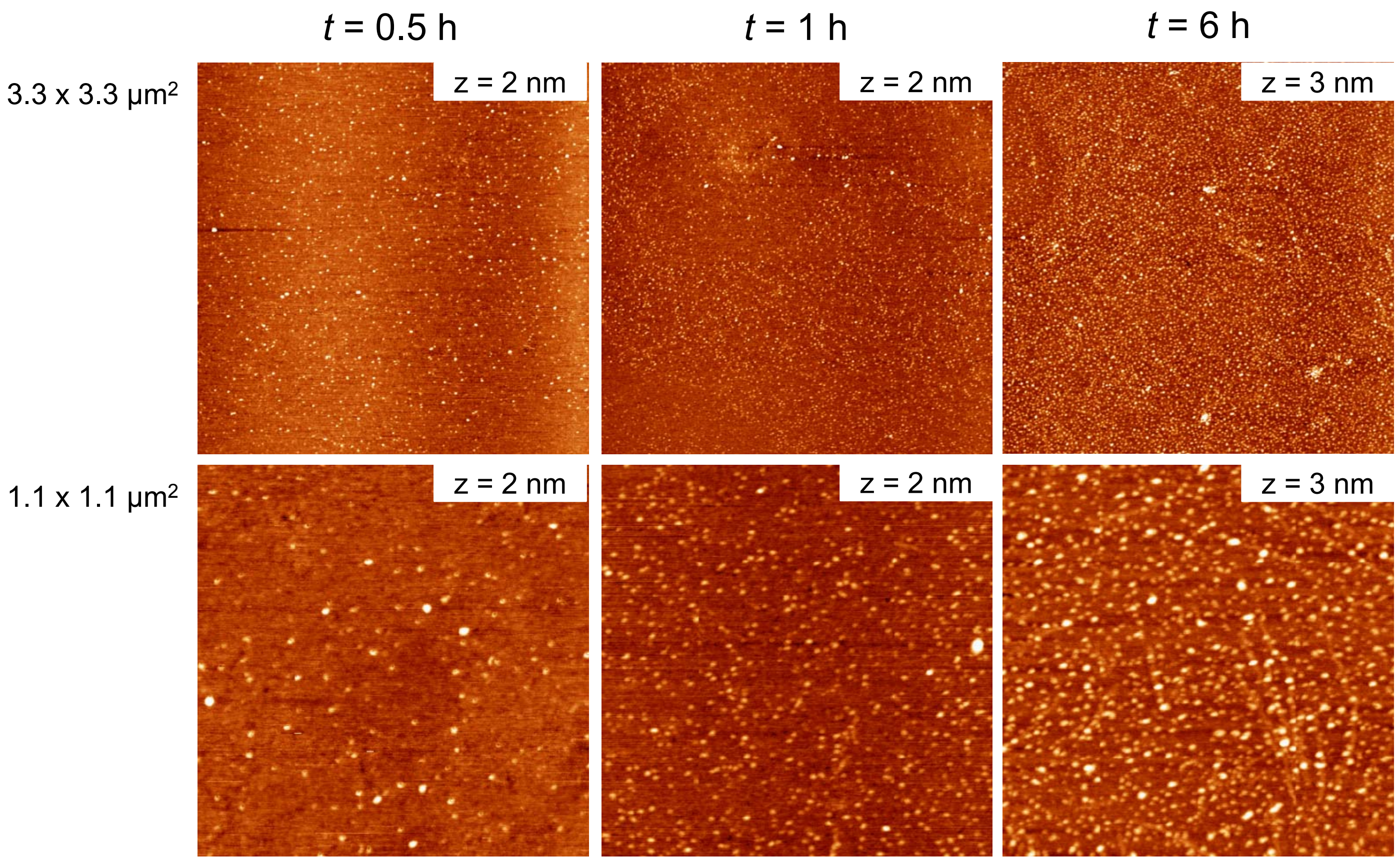
protofibrils
height: (0.9 ± 0.2) nm
23°: ~ 2 nm

fibrils
height: ~ 1.8 nm
twist: ~ 20 nm

23°:
~ 4.5 nm
~ 28 nm

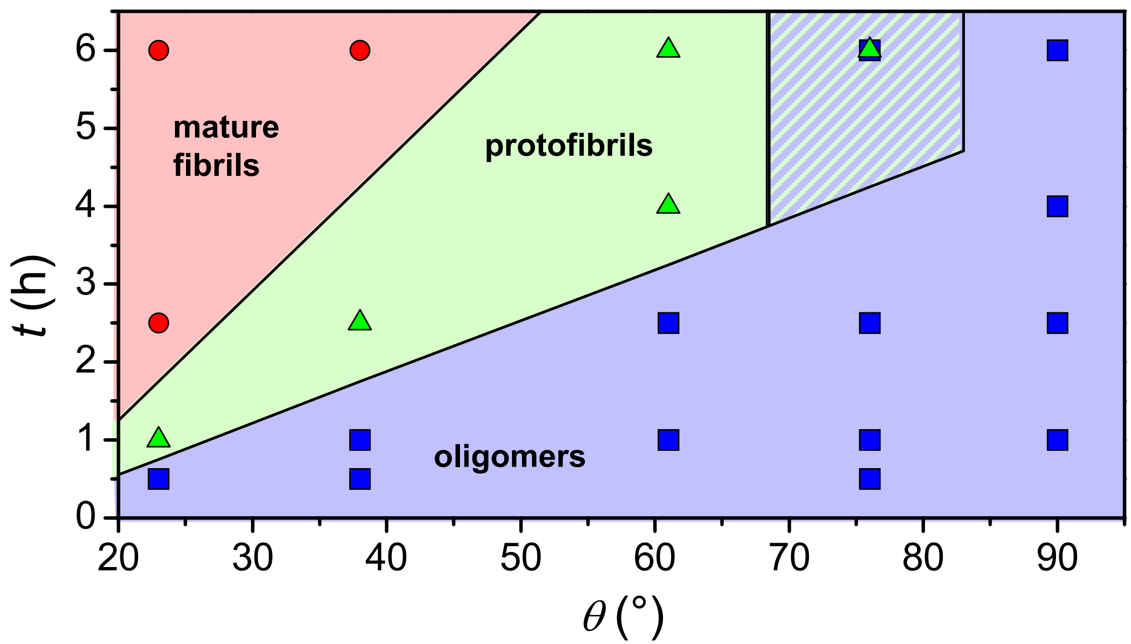
Keller *et al.*, *ACS Nano* (2011), DOI: 10.1021/nn1031998

IAPP on a mica/HC surface with $\boldsymbol{\theta}$ = 76°



Keller *et al.*, *ACS Nano* (2011), DOI: 10.1021/nn1031998

Influence of hydrophobicity on IAPP aggregation



Interplay between electrostatic and hydrophobic interactions with the substrate:

→ **determines conformation of adorbed monomers**

→ ***dictates the pathway of aggregation***

IAPP: 37 amino-residue polypeptide

KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY

\+ +

amyloidogenic region

Keller *et al.*, *ACS Nano* (2011), DOI: 10.1021/nn1031998

Conclusion

- Surface-catalyzed processes play an important role in the physiological environment and strongly affect how biomolecules behave.

- Disease-relevant aggregation of proteins and peptides can follow completely different pathways when occurring at surfaces.

- The physicochemical surface properties dictate the type of interaction with the molecule: *electrostatic vs. hydrophobic interactions*

  → ***biological model surfaces with tunable properties needed!***

- Low-energy ion-beam irradiation allows the fabrication of novel model surfaces with tunable properties.

- IAPP aggregation (lag time, aggregation rate, morphology) at surfaces is driven by the interplay between electrostatic and hydrophobic interactions.

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