School of Food Science and Nutrition
FACULTY OF MATHEMATICS AND PHYSICAL SCIENCES

UNIVERSITY OF LEEDS

CRYSTAL ENGINEERING APPROACHES FOR THE DESIGN OF FOOD AND PHARMACEUTICAL FORMULATIONS

Dr Elena Simone

Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom

*e.simone@leeds.ac.uk

Formative formulation

18th March 2019

University of Cambridge, Cambridge, UK

Overview of the presentation

Crystal engineering: motivation and some experimental strategies

Engineering crystals for the complex soft food structures

- Multiphase formulations for food and pharma applications
- Particle stabilization (Pickering)
- Crystal properties and implications for Pickering particles

Modelling Methodology

- Model compound: quercetin and its hydrates
- Attachment energy model for the prediction of intermolecular interactions and crystal anisotropy

Results and Discussion

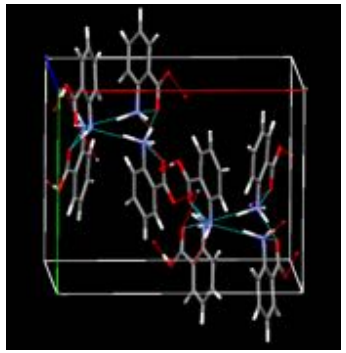
- Bulk intermolecular interactions and surface chemistry for the three quercetin polymorphs
- Experimental techniques for model validation

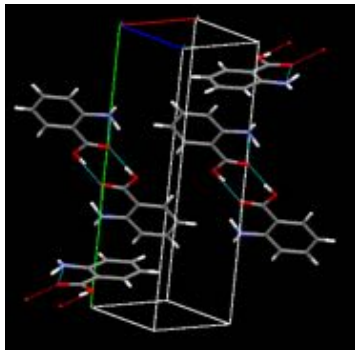
Conclusions and future developments

Main crystal properties

- ❑ **Size**
- ❑ **Shape**
- ❑ **Structure** (polymorphs etc.)
- ❑ **Purity**

- *Same compound but different structures*
- ***Different properties*** *(thermodynamic, kinetics, mechanical, surface)*
- *Problem with stability when metastable forms are produced*





CONTROL IS THE KEY!

How to engineer crystals

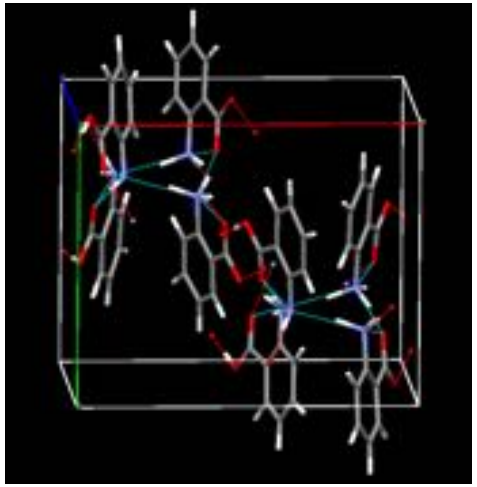
The Crystal Engineering Approach utilises the understanding of the **intermolecular interactions** within crystalline materials to design specifically tailored solid materials in terms of *shape, size and polymorphism*

Choice of solvent

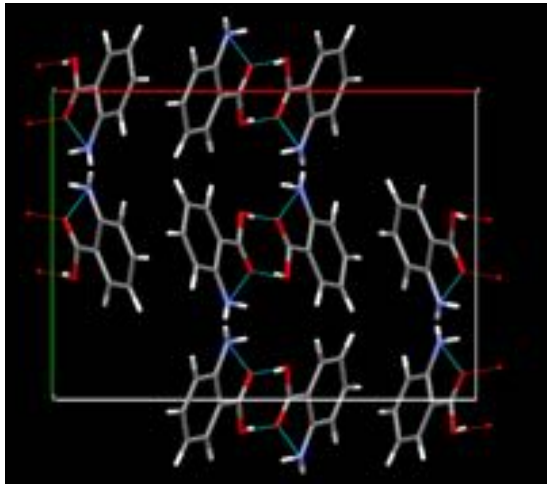
Temperature profile

Use of additives

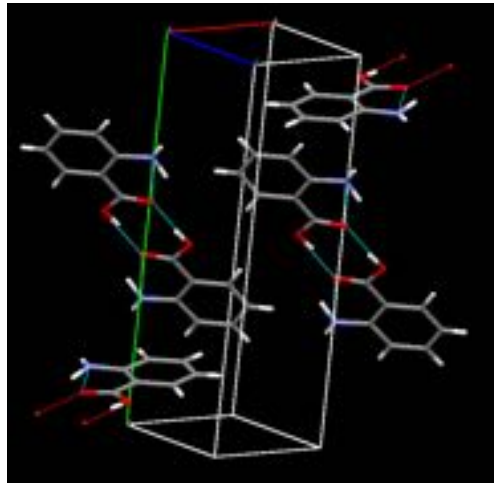
Model compound: Ortho-aminobenzoic acid (OABA)



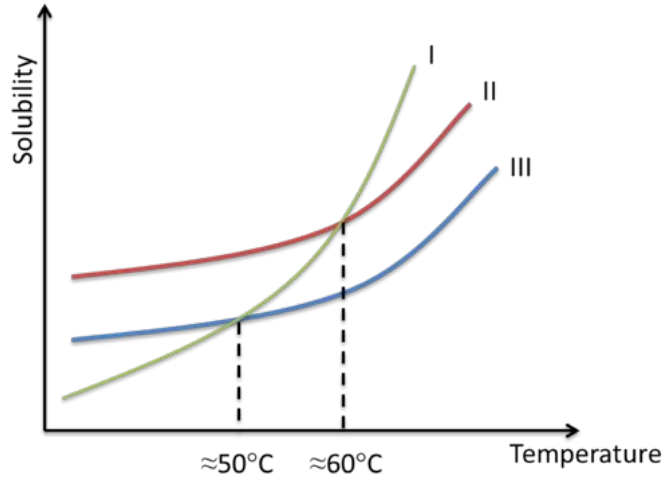
Form I: orthorhombic cell, **zwitterions** and neutral molecules (1:1 ratio)



Form II: orthorhombic cell, **dimers** of neutral molecules



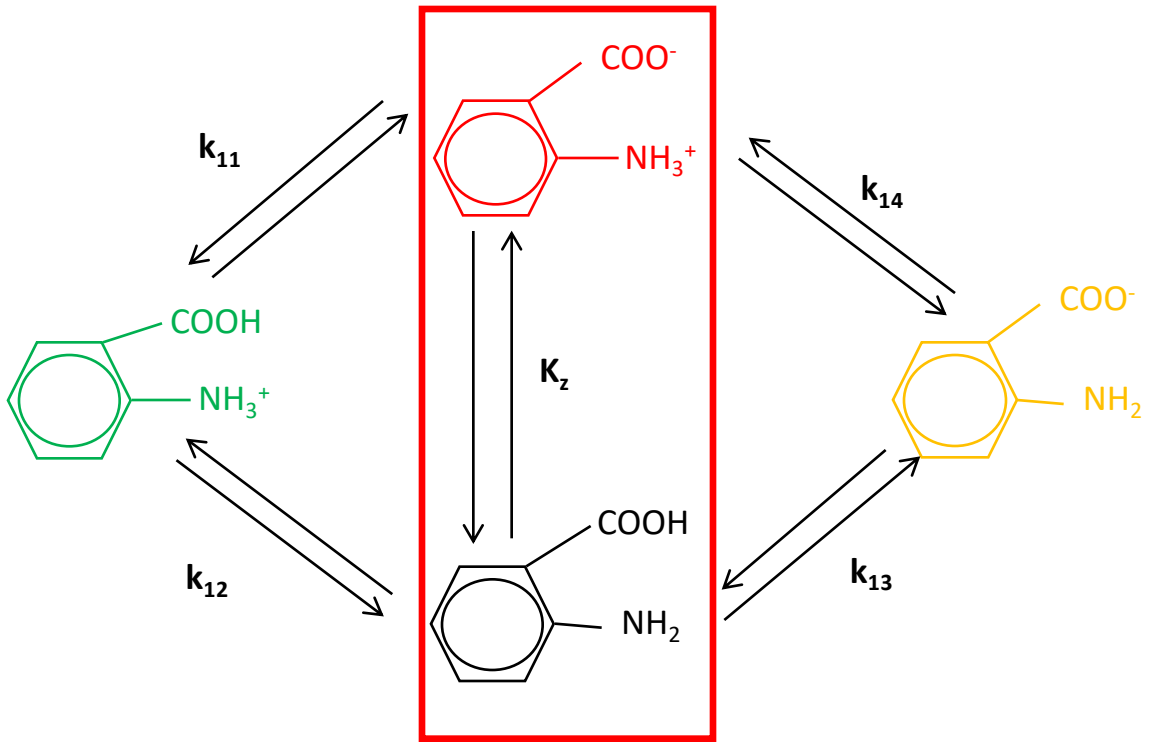
Form III: monoclinic cell, **dimers** of neutral molecules

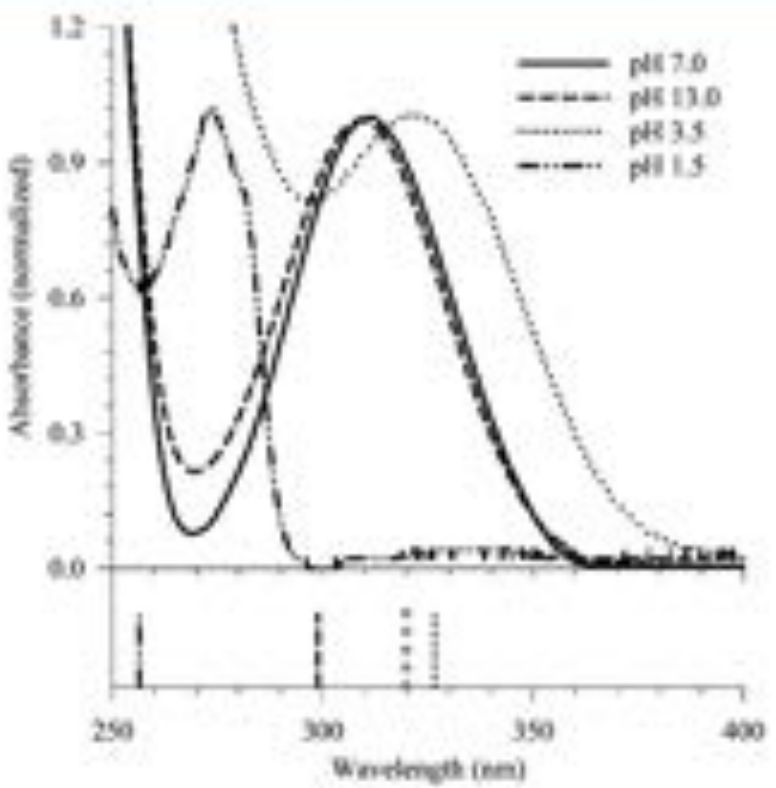


- Three known forms of OABA
- Form I and II are normally nucleated from solution
- Form III is very difficult to nucleate

Equilibrium of OABA in water

- OABA can exist in **solution** as a neutral molecule, with positive or negative charge or as a **zwitterion**
- Different forms of OABA presents different UV/Vis spectra



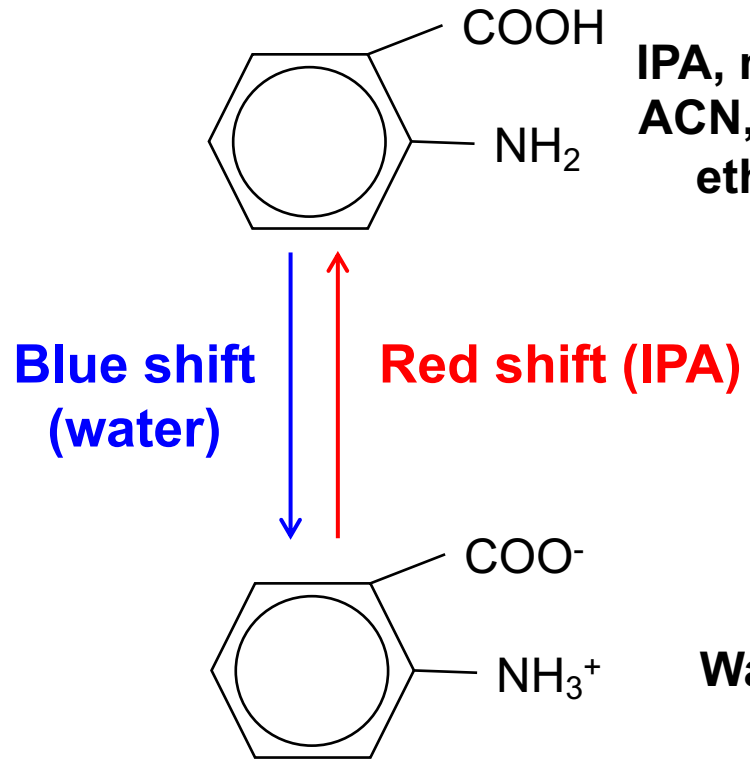


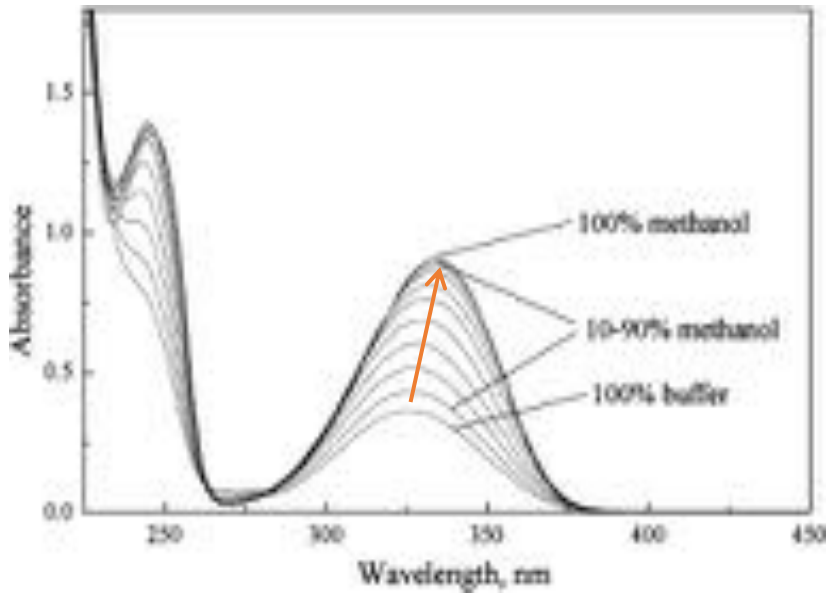
Abou-Zied et al., *The Journal of Physical Chemistry*, **2009**

Zapala et al., *Biophysical Chemistry*, **2013**

Equilibrium of OABA in mixtures of water and organic solvents

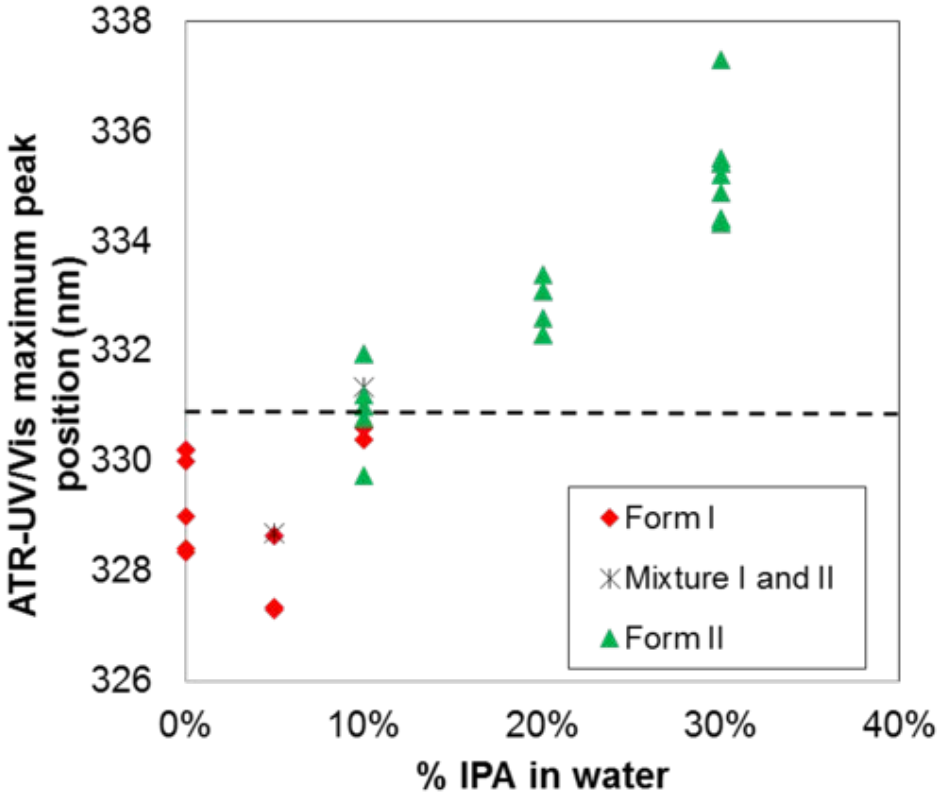
- Changes in the **UV/Vis spectrum** of OABA solutions can be associated with ***different amount of zwitterions in solution***

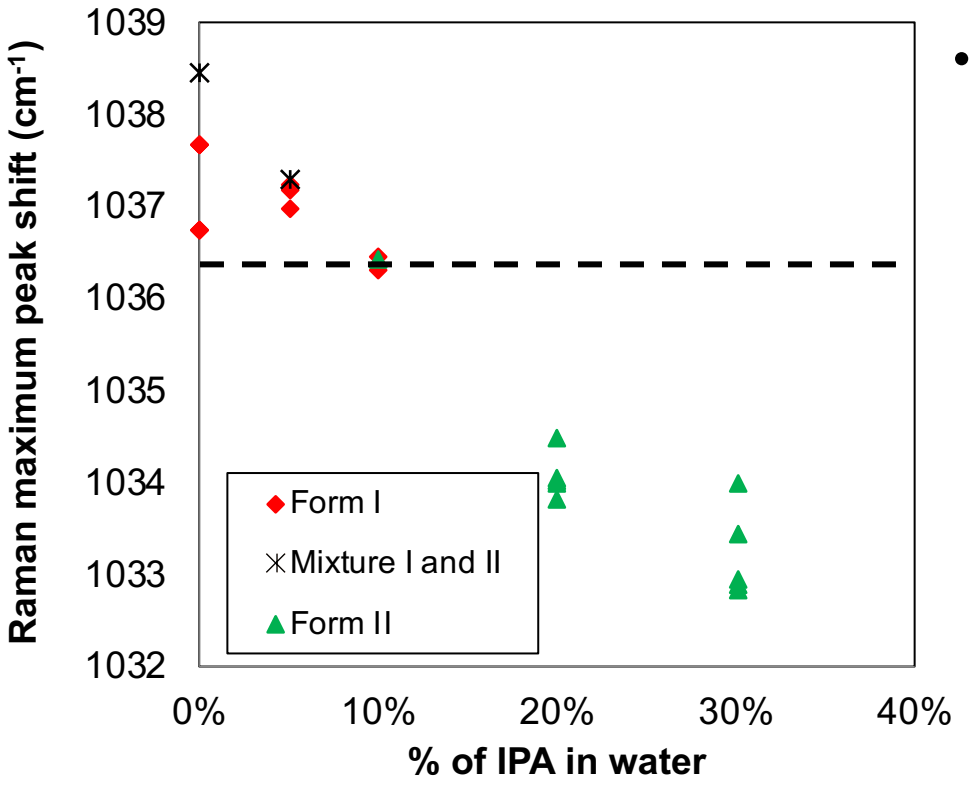




Zapala et al., *Biophysical Chemistry*, **2009**
Murugan et al., *PCCP Communication*, **2011**
Abou-Zied et al., *The Journal of physical Chemistry*, **2013**

Experimental results





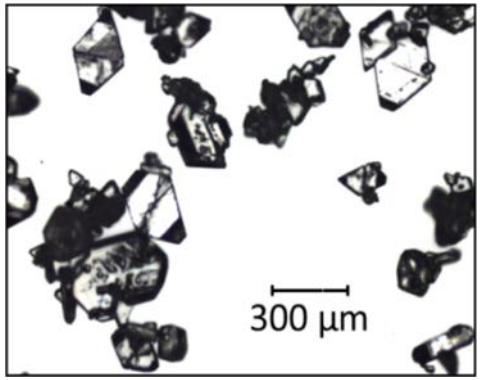
- In water and IPA mixtures a limiting peak position for UV/Vis and Raman (**benzene ring vibration**) was found over which only form II nucleates

Simone & Nagy, *CrystEngComm*, 2015,**17**, 6538-6547

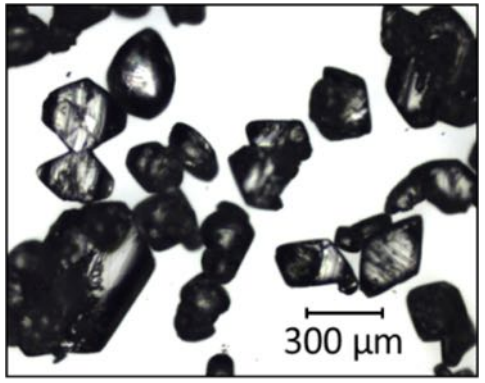
Shape manipulation of succinic acid (SA) via temperature cycling

- SA in water (**20 °C saturation temperature**)
- Initial cooling from 30 °C to 10 °C at -0.5 °C/min
- Temperature cycling varying **heating/cooling rate** and **cycles' amplitude**
- Sampling and off-line analysis (**Optical** and **Raman** spectroscopy, **single crystal XRD**) during the experiments

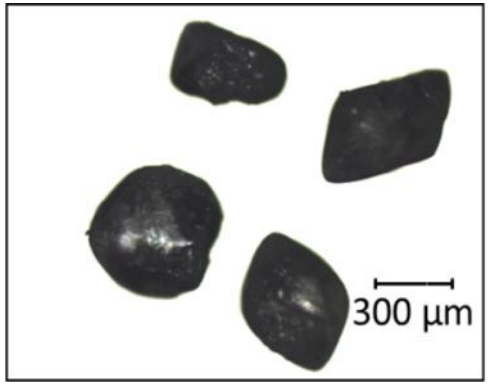
Experiment n°	Cycles amplitude (°C)	Heating/ Cooling rates (°C/min)
1	4.5	±0.3
2	6	±0.3
3	7.5	±0.3
4	4.5	±0.1
5	4.5	±0.5



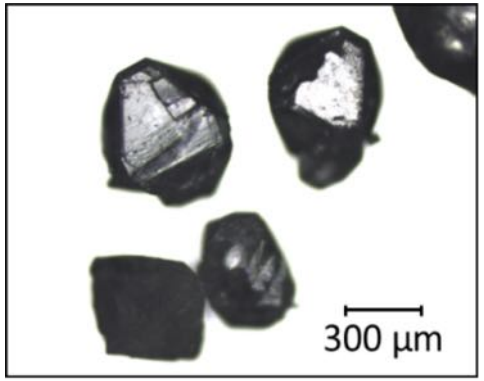
120 min (cooling)



270 min (heating)

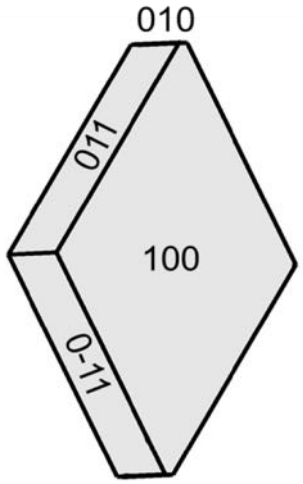


1260 min (cooling)

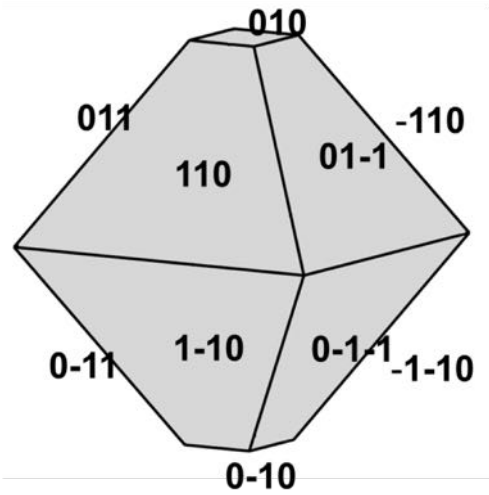


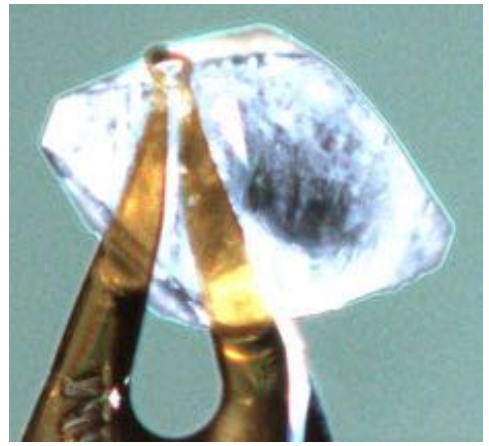
3000 min (cooling)

Face indexing (single crystal XRD)

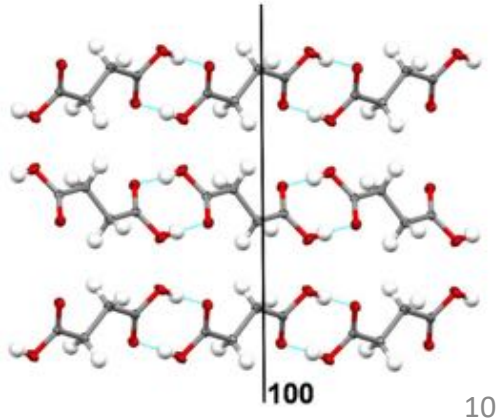


T cycling





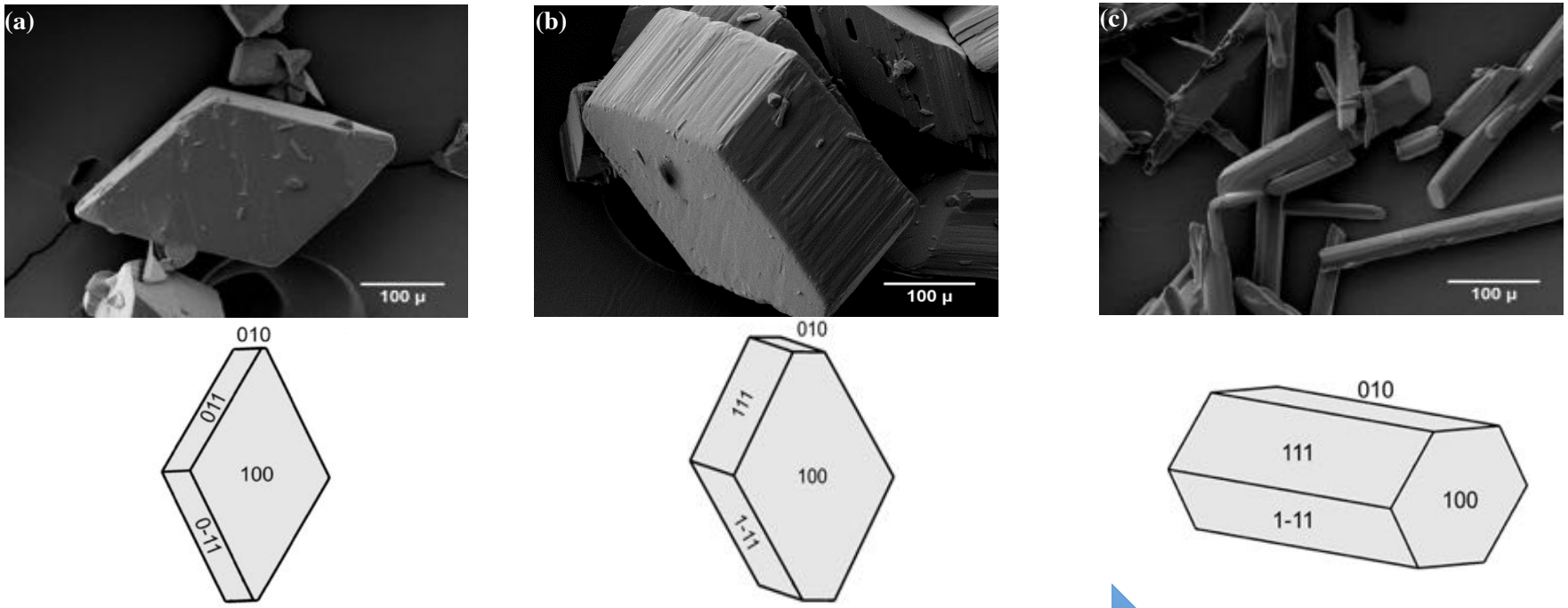
- The (100) face intercepts chains of succinic acid molecules linked **by hydrogen bonded carboxylic acid dimers (polar face)**
- Water molecules interact with the (100) face, inhibiting growth along the direction perpendicular to this face
- Temperature cycling generates a **diamond shape**
- Face (110) and (01-1) **outgrow face (100)**



Simone et al., *Crystal Growth and Design*, **2017**

Effect of Pluronic P123 on succinic acid crystal shape

- Pluronic P123 inhibit growth of the side faces of the crystal leading to a **needle-like shape** along the (100) direction



Klapwijk et al., *Crystal Growth and Design*, **2016**

Multiphase formulations: emulsions and foams

- Multiphase formulations are widely used for several applications within the **food**, **cosmetic** and **pharmaceutical** industries;
- Used for oral or topical **controlled delivery** of poorly water soluble drugs and nutraceuticals;
- Extremely common food structures;
- Common formulations for cosmetic products, fast adsorption and low greasiness;
- They can be **thermodynamically unstable** and undergo phase separation over time (limited shelf-life);
- Reducing the **interfacial tension** using surfactants or solid particles (Pickering) can improve long term stability.

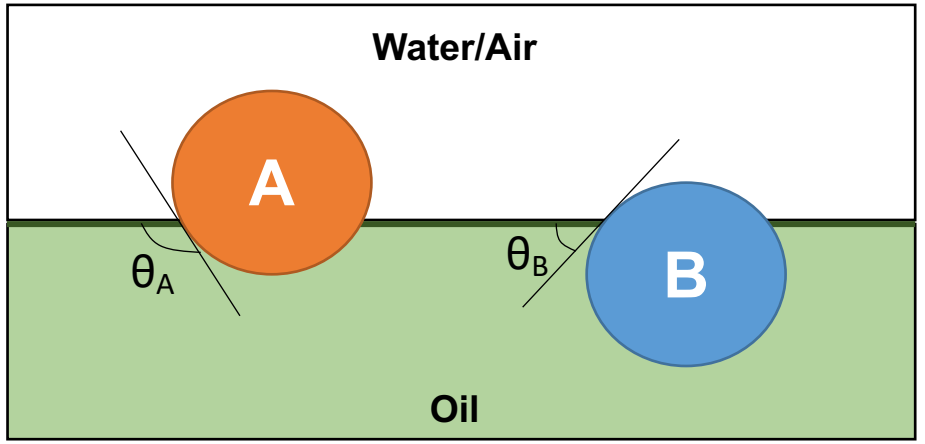
Particle stabilization of multiphase systems (Pickering)

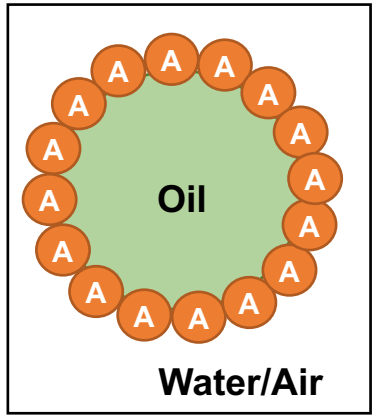
- Pickering formulations are more stable than surfactant based ones because particles adsorb **more strongly** at the interface;
- Less adverse effects, possibility to use **biocompatible**, **naturally** sourced particles (consumer acceptability);
- The free energy (E) required to desorb a spherical particle from an interface can be expressed as:

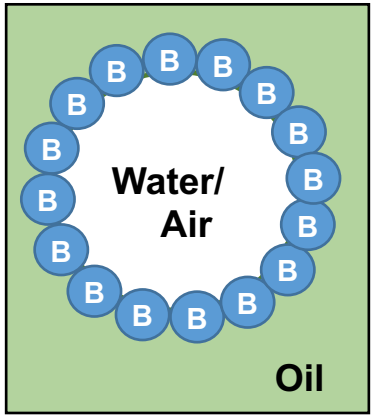
$$E = \gamma \pi r^2 (1 - |cos\theta|)^2$$

Where γ is the interfacial tension between the two phases, r is the particle radius and θ is the contact angle.

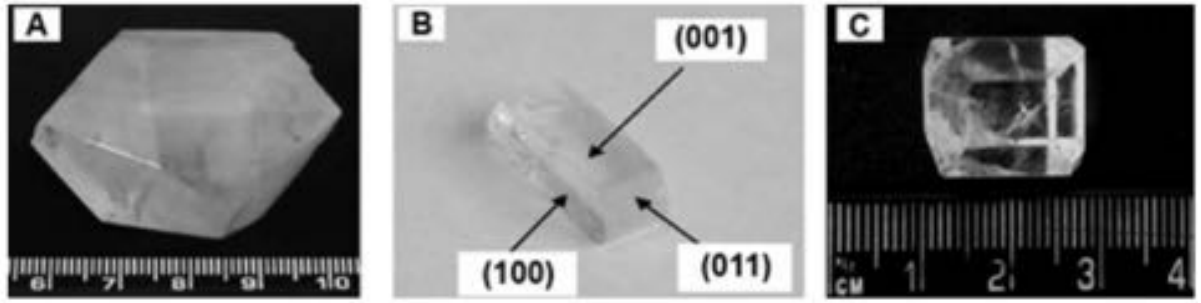
Particle **stability**, **solubility** and **wettability** are critical properties for Pickering particles!







Faceted crystals as Pickering particles: the issue of anisotropy

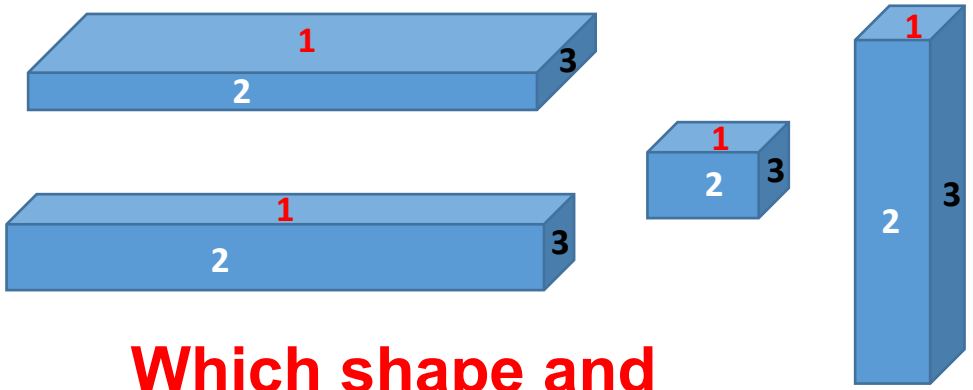


Macroscopic crystals of (a) paracetamol, (b) aspirin, and (c) S-(+)-ibuprofen.

- Most solid particles are not spherical but they are faceted;
- Different composition and surface chemistry on each face;
- Anisotropy can affect Pickering stabilization!

	Advancing Contact Angle, θ_a (Deg)				
Facet	Paracetamol Form I	Paracetamol Form II	Aspirin	Racemic Ibuprofen	S-(+)-Ibuprofen
(201)	38.1 ± 4.6	—	—	—	—
(001)	15.9 ± 3.1	**64.5 ± 3.5***	60.7 ± 3.5	68.5 ± 4.8	64.5 ± 3.9
(011)	29.8 ± 5.7	—	42.9 ± 4.8	46.9 ± 5.5	—
(110)	50.8 ± 4.9	16.6 ± 1.4	—	—	48.4 ± 4.0
(010)	**67.7 ± 2.5***	17.9 ± 2.5	—	—	—
(100)	—	—	**52.9 ± 2.5***	**77.2 ± 4.0***	**70.7 ± 3.1***

*Bolded data are values for the weakest attachment energy facet. (—) indicates that no such facet was present in the macroscopic crystal.

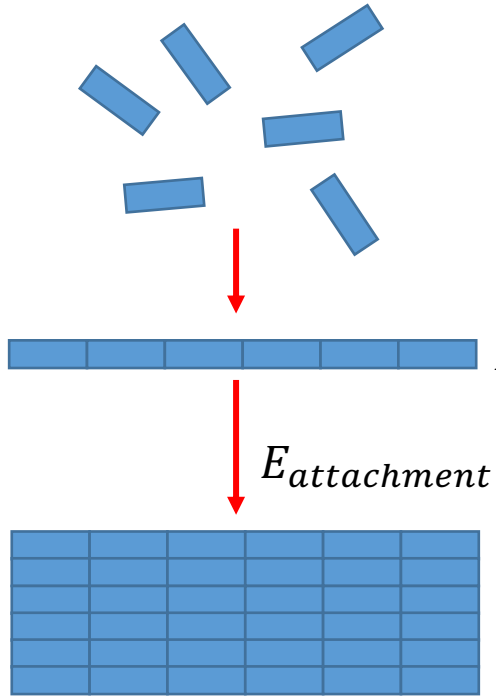


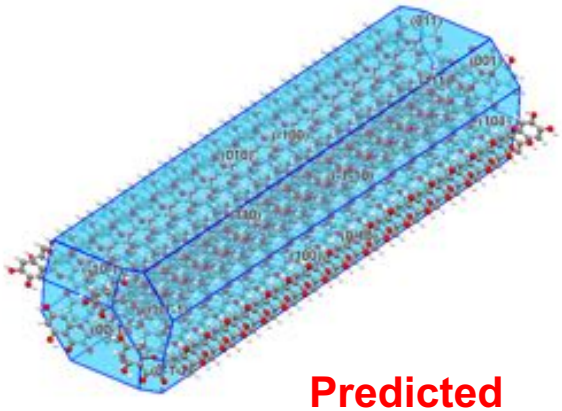
Which shape and polymorph work best?

Heng *et al*. (**2006**) *AAPS PharmSciTech*

Molecular modelling for crystal properties prediction

- The intermolecular interaction energies within the crystal structure can be calculated using an interatomic potential (Momany);
- The calculated interactions can be **ranked** based on their **contribution** to the total lattice energy;
- The **Attachment Energy Model** identifies which intermolecular interactions contribute to the growth of the specific crystal faces (*hkl*);
- Assumes a **slice of d_{hkl} thickness** added to the surface and the energy of the interactions between the new slice and surface proportional to **growth rate**;
- The chemistry of the interactions dominating the growth of a particular surface **(e.g. H-bonding, dispersive etc.)** can give an indication of crystal properties.





Predicted morphology

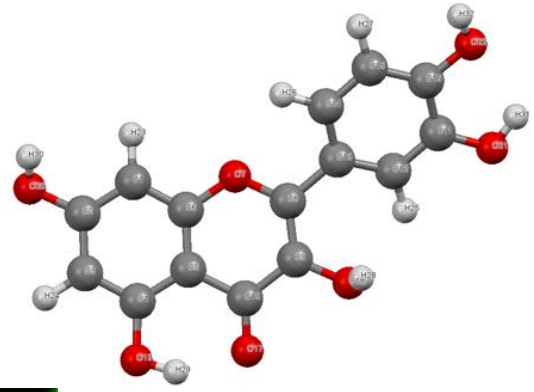
$$E_{crystal} = E_{slice} + E_{attachment}$$

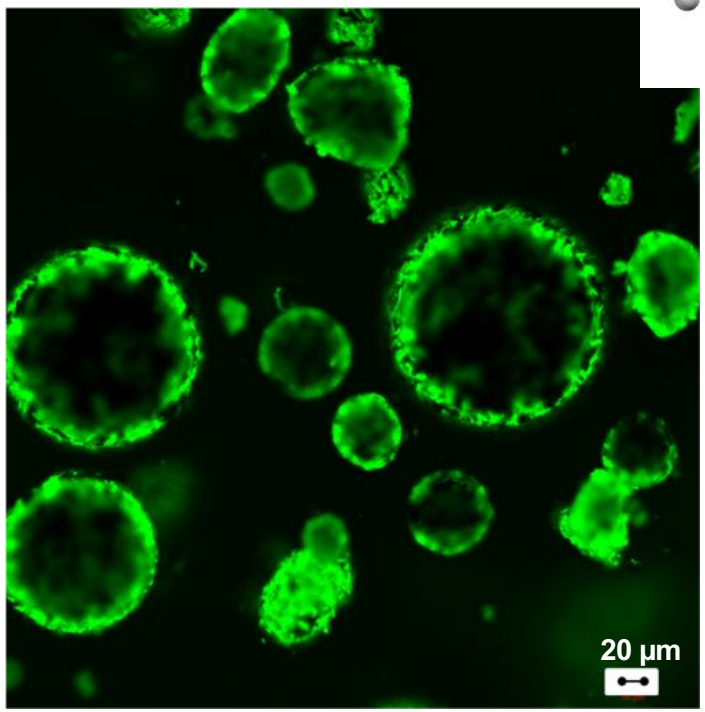
$$E_{attachment} \propto Surface\ Growth\ Rate$$

Momany et al. (**1974**) JACS
Rosbottom *et al*. (**2015**) *CrystEngComm*

Model compound: quercetin

- Quercetin is **flavonoid** molecule found in fruits and vegetables;
- It is known to be an **antitumor agent** and to exhibit **antiallergic**, **anti-inflammatory** and **antioxidant** activity;
- Due to the wide range of health benefits, quercetin finds use in **nutraceuticals** and **food supplements**;
- Three different structures: anhydrous (QA), monohydrate (QMH) and dihydrate (QDH);
- Poorly soluble in both water and oil → good candidate particle for Pickering water in oil emulsions.

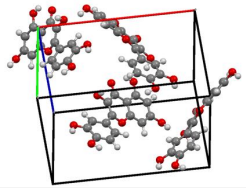
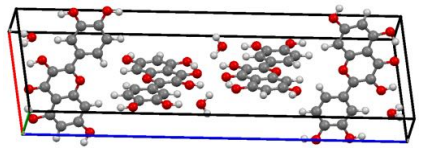
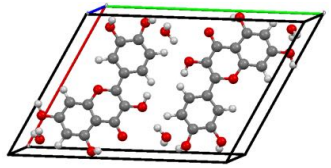




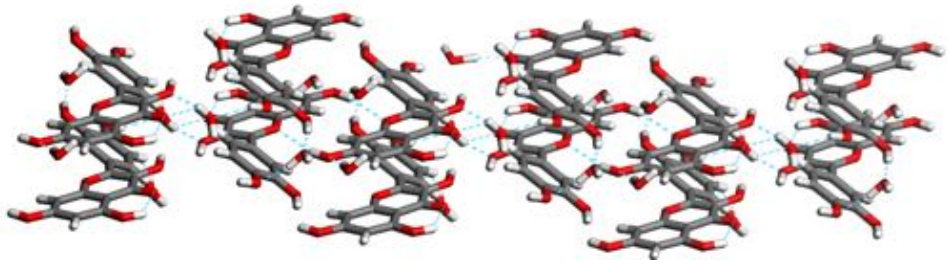
Zembyla e al., *Langmuir,* **2018**

Molecular modelling methodology

- The crystal structures of the three quercetin polymorphs were retried from the **Cambridge Crystallographic Data Centre** (CCDC);
- The **Mercury** software was used to image each calculated intermolecular interaction;

	Quercetin anhydrous	Quercetin monohydrate	Quercetin dihydrate
Formula	$C_{15}H_{10}O_7$	$C_{15}H_{10}O_7.H_2O$	$C_{15}H_{10}O_7.2H_2O$
Space Group	P n 2_1 a Orthorhombic	P 2_1/c Monoclinic	P 1 Triclinic
Cell Volume (Å^3)	1721.6	1272.61	701.931
Cell Density (g/ Å^3)	0.702	1.007	0.964

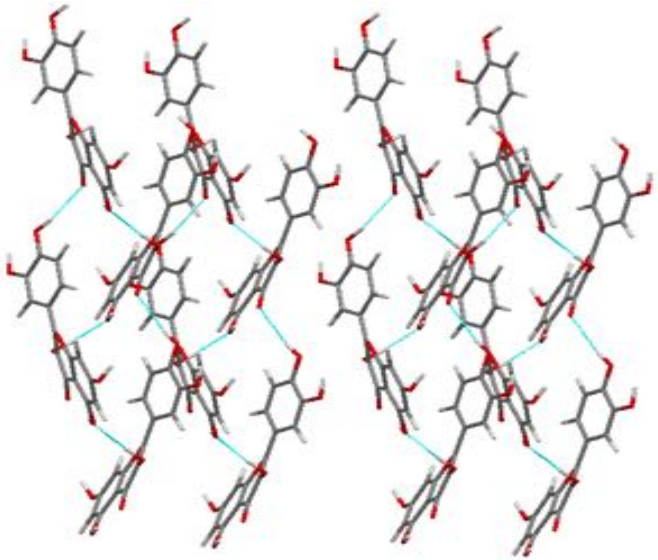


- The **Habit 98** software (developed in Leeds), was used to predict strength, directivity and dispersive nature of the intermolecular interactions in the crystal structure (Momany force-field);

Rosbottom *et al*. (**2018**) *Phys Chem Chem Phys*

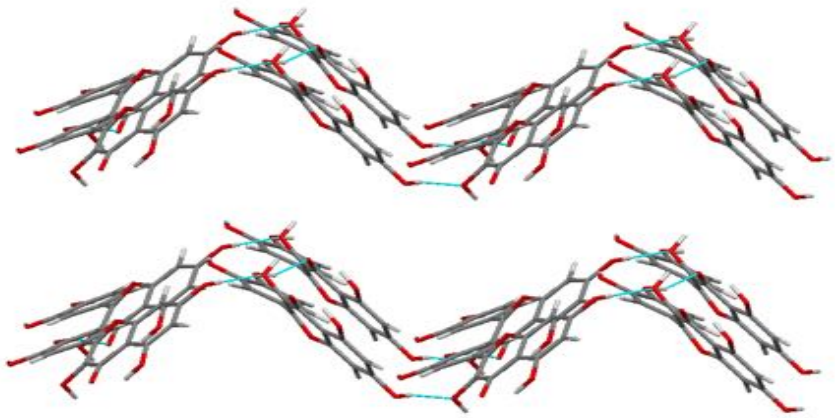
QA structure and main intermolecular interactions in the crystal structure

1



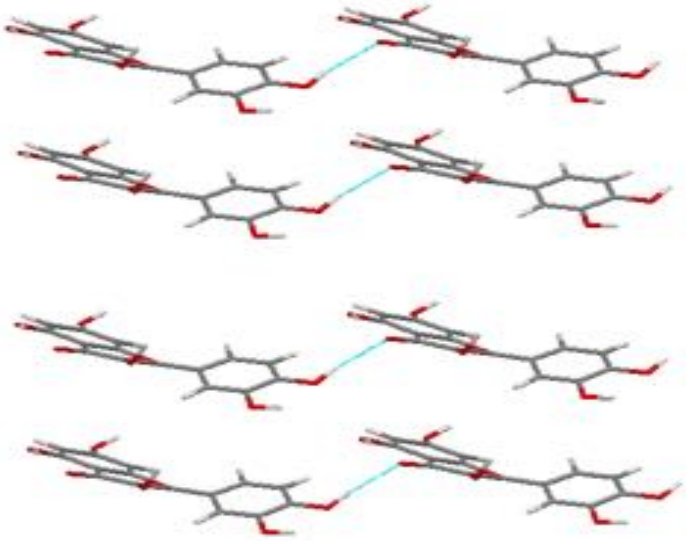
- **First strongest interaction (38.4% of lattice energy):** **Hydrogen bonds** between OH groups and permanent **dipole-dipole interactions**

2



- **Second strongest interaction (25.8% of lattice energy):** **Zig-zag chain** structure formed by **hydrogen bonds** between OH groups and permanent **dipole-dipole interactions**

3



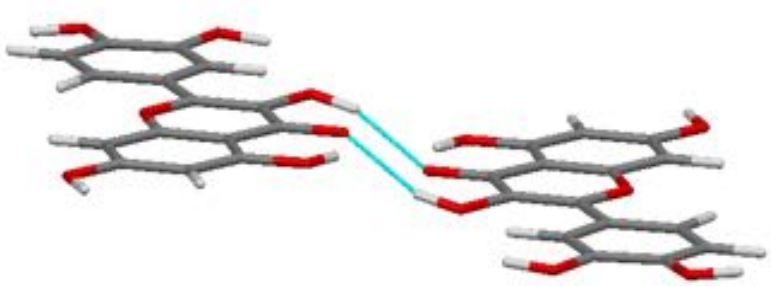
- **Third strongest interaction (14.1% of lattice energy):** Permanent dipole-dipole interactions from COOH groups, **dimers** arrangement

QMH structure and main intermolecular interactions

1

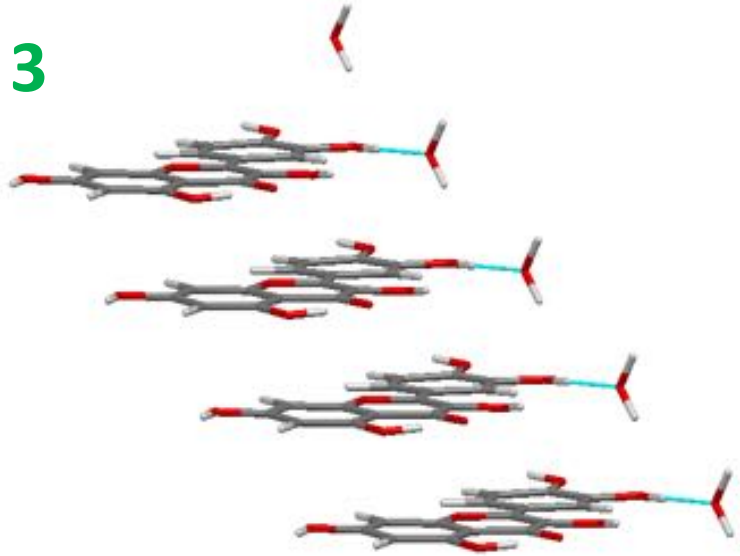
- **First strongest interaction (24.5% of lattice energy): π-π stacking** interactions between quercetin-quercetin molecules

2



- **Second strongest interaction (10.2% of lattice energy): double hydrogen bonding** interaction between two quercetin molecules

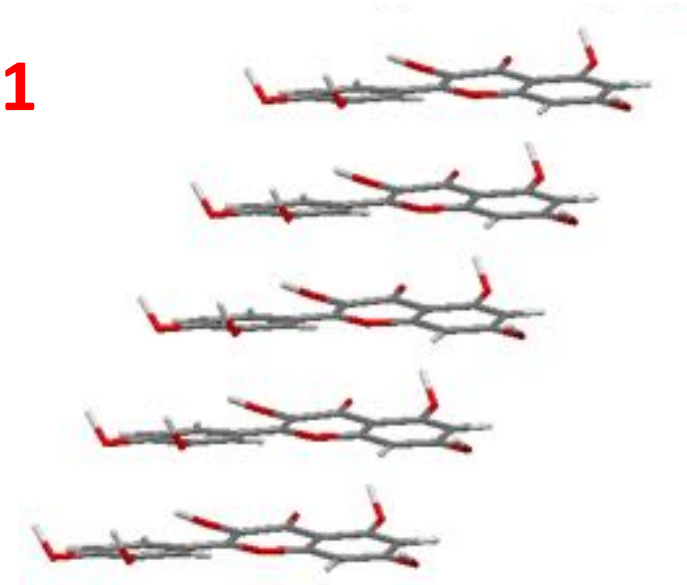
3



- **Third strongest interaction (9.8% of lattice energy): hydrogen bonding** between quercetin-water molecules

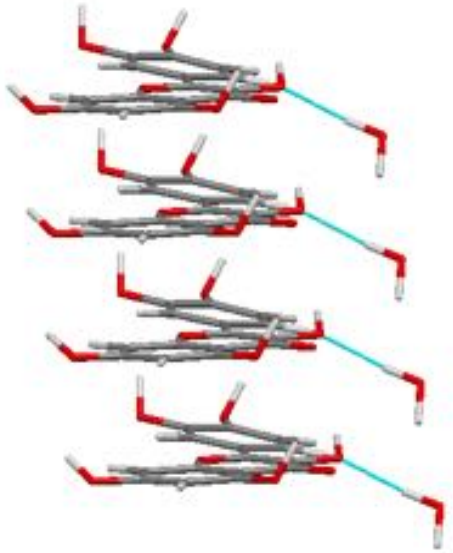
QDH structure and main intermolecular interactions

1



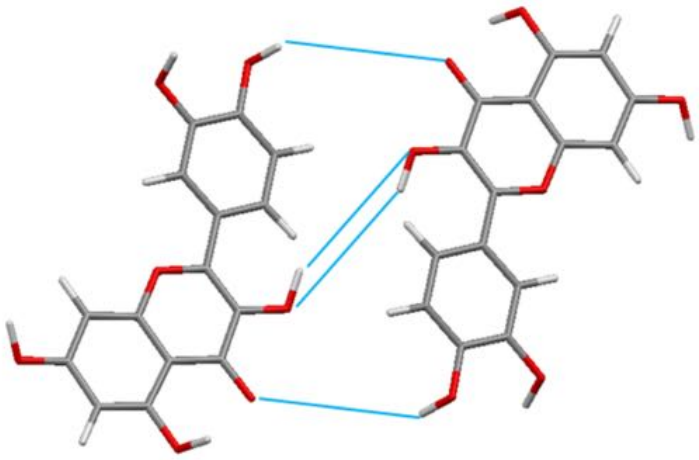
- **First strongest interaction (37.8% of lattice energy): π-π stacking** interactions between quercetin-quercetin molecules, **uninterrupted** stack

2



- **Second strongest interaction (7.9% of lattice energy): hydrogen bonding** interaction between water and quercetin molecules

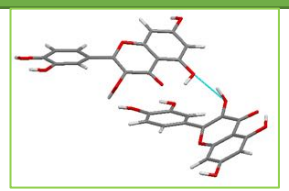
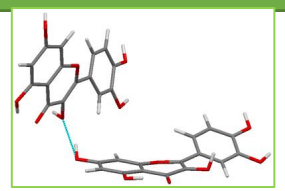
3



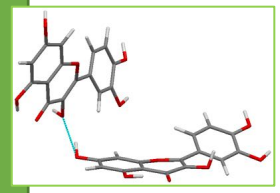
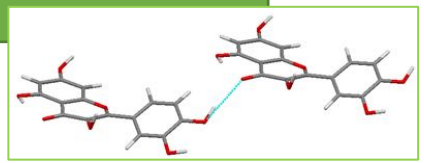
- **Third strongest interaction (3.5% of lattice energy): permanent dipole-dipole interaction** quercetin-quercetin (**dimer**)

QA calculated morphology and face specific surface chemistry

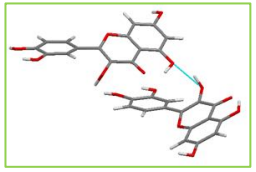
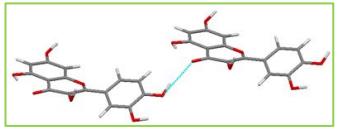
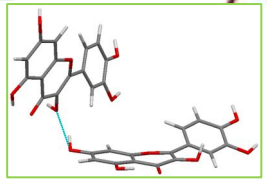
Faces 011 / 01-1 / 0-11 / 0-1-1, ε_{hkl}*=52.5%,*
Dominant surface
Quercetin-quercetin H-bonding & Van der Waals interactions from phenyl & pyrone rings

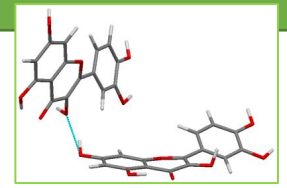
Faces 101 / 10-1 / -101 / -10-1, ε_{hkl}*=71.2%,*
Dominant surface
Quercetin-quercetin H-bonding, -OH and C=O contributions, Polar interactions

Faces 111 / 11-1 / -111 / -11-1 / 1-11 / 1-1-1 / -1-11 / -1-1-1, ε_{hkl}*=40.9%*
Faces 210 / -210 / 2-10 / -2-10, ε_{hkl}*=29.4%*
Faces 020 / 0-20, ε_{hkl}*=24.9%*
Non-Dominant surfaces
Quercetin-quercetin H-bonding & Van der Waals interactions

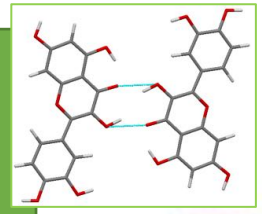




Faces 200 / -200, ε_{hkl}*=59.8%,*
Dominant surface
Quercetin-quercetin H-bonding, zig-zag stacking of quercetin molecules, Polar interactions

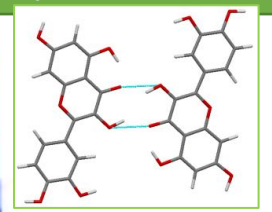


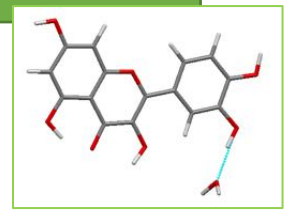
QMH calculated morphology and face specific surface chemistry

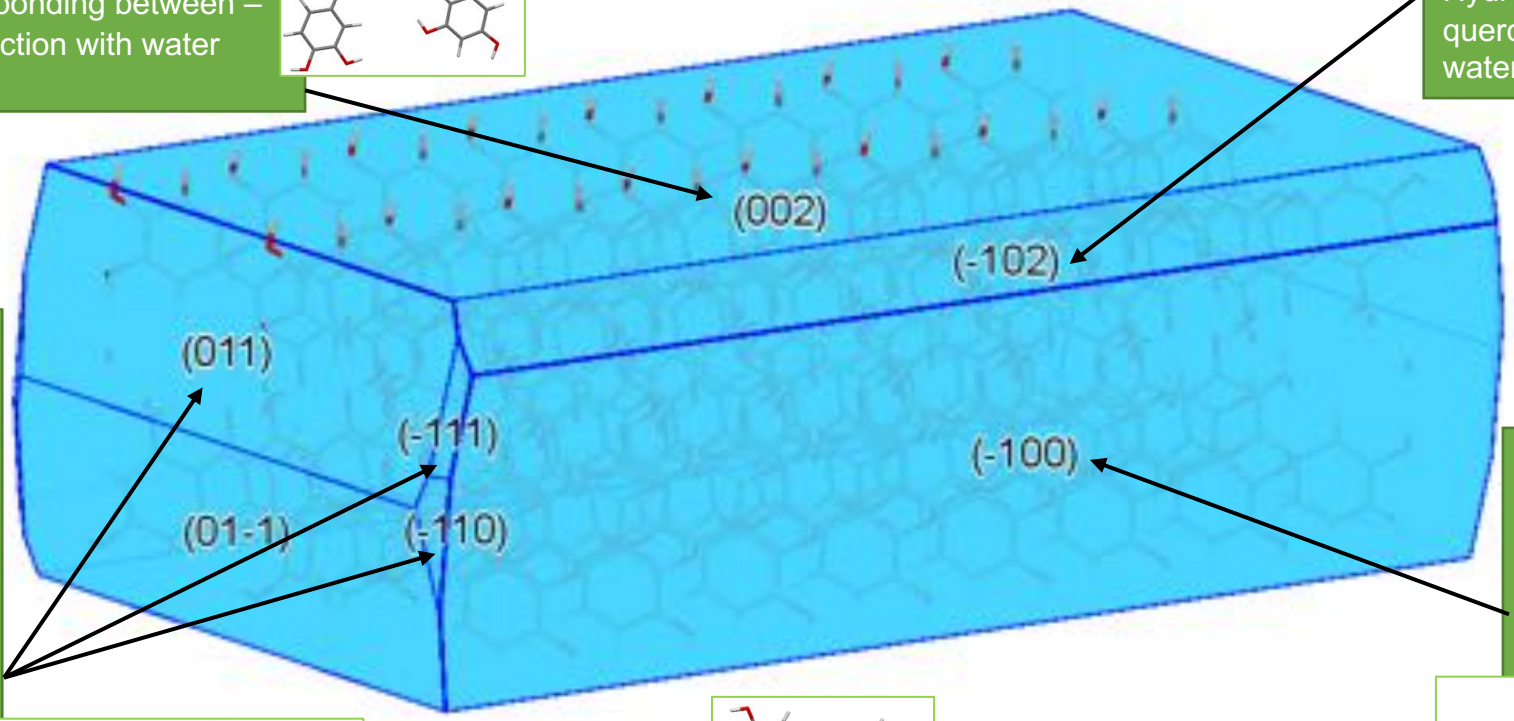
Faces 002 / 00-2, ε_{hkl}*=85.6%,*
Dominant surface
Quercetin-quercetin double H-bonding between –OH and C=O groups, no interaction with water molecule, Polar interactions



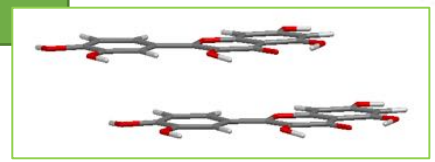
Faces -102 / 10-2, ε_{hkl}*=58.2%,*
Dominant surface
Hydrogen bonding between quercetin-quercetin and quercetin-water, polar interactions

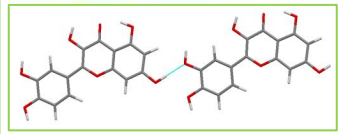


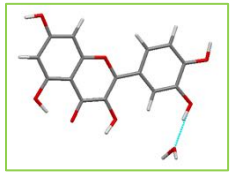




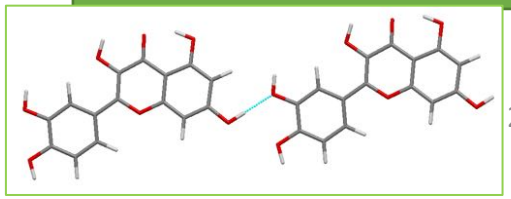
Faces 11-1 / 1-1-1 / -111 / -1-11, ε_{hkl}*=32.7%,*
Faces 110 / 1-10 / -110 / -1-10, ε_{hkl}*=31.2%,*
Faces 011 / 0-11 / 01-1 / 0-1-1, ε_{hkl}*=27.5%,*
Non-Dominant surfaces
Apolar π-π stacking interactions, hydrogen bonds between quercetin-quercetin and quercetin-water







Faces 100 / -100, ε_{hkl}*=69.6%,*
Dominant surface
Quercetin-quercetin H-bonding between –OH groups, no interaction with water molecule, mostly polar interactions



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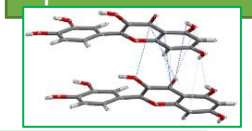
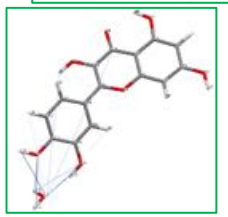
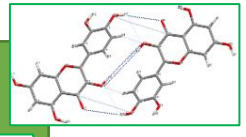
QDH calculated morphology and face specific surface chemistry

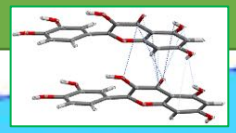
Faces 10-1 / -101, ε_{hkl}***=34.9%,***
Both quercetin-quercetin and quercetin-water apolar interactions



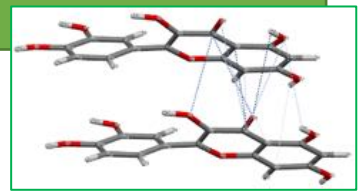


Faces 01-1 / 0-11, ε_{hkl}=21.6%,
Combination of polar and non polar interactions (both quercetin-quercetin and quercetin-water)

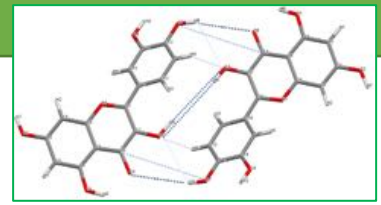




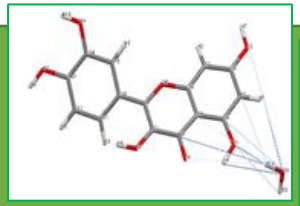
Faces 0-10 / 010, ε_{hkl}=91.0%
Dominant Surface
Mostly growing via quercetin-quercetin apolar interactions ($\pi - \pi$ stacking)

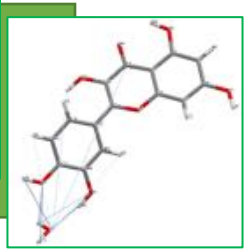


Faces -110 / 1-10, ε_{hkl}=73.2%
Dominant Surface
Polar quercetin-quercetin interactions contributes to growth

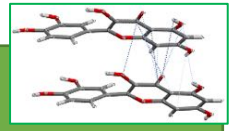


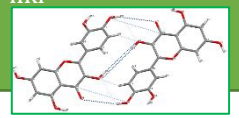
Faces 100 / -100, ε_{hkl}***=69.6%***
Dominant Surface
Polar water-quercetin interactions (including hydrogen bonds) contributes to growth





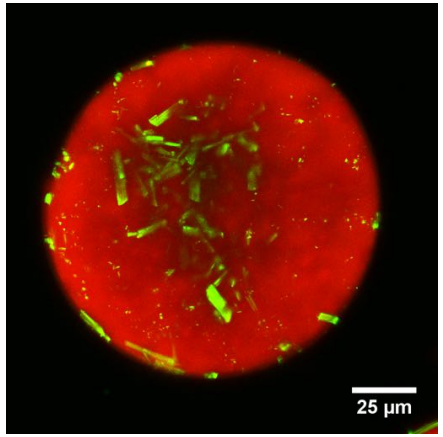
Faces 00-1 / 001, ε_{hkl}=19.6% and ***Faces -111 / 1-1-1,*** ε_{hkl}=33.1%
Combination of polar and non polar interactions (both quercetin-quercetin and quercetin-water)



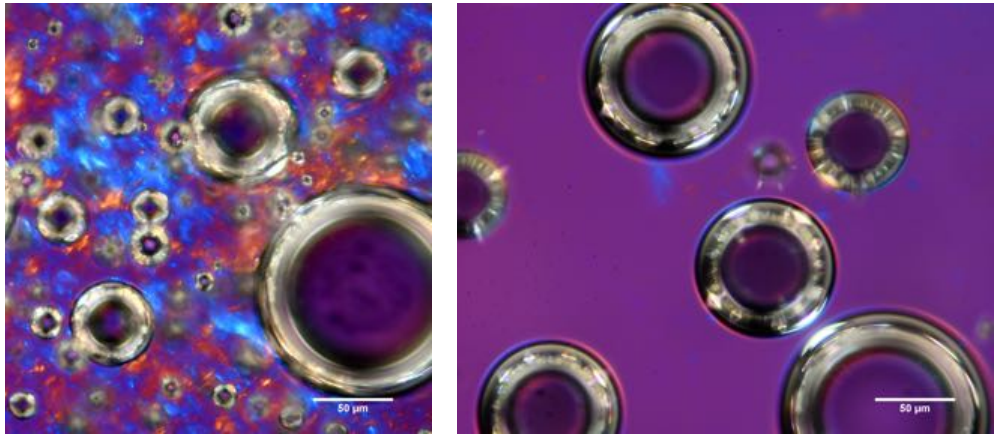


Experimental validation

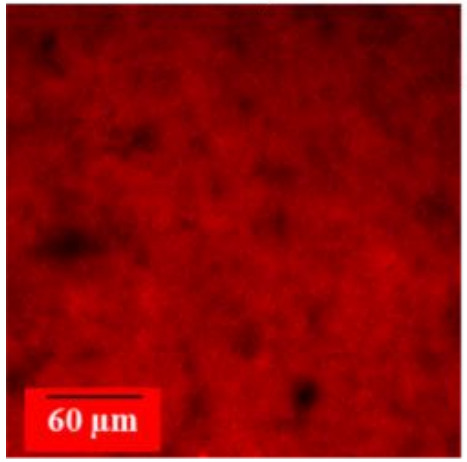
- Face specific wettability can be measured via **contact angle** and single crystal XRD face indexing;
- This information is **NOT** enough to understand the effect of crystal anisotropy;
- **Advance imaging** for better understanding of interface orientation and interaction.

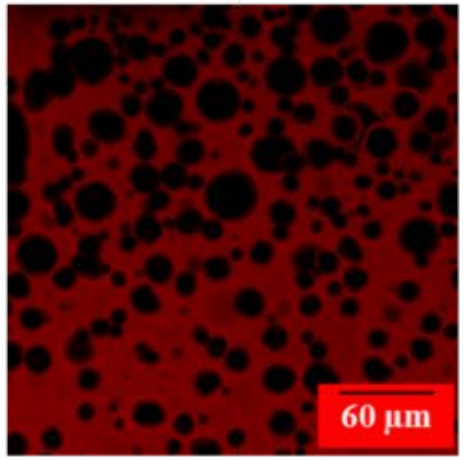


Confocal Coherent Anti-Stokes Raman Scattering



Polarized optical microscopy: orientation at interfaces





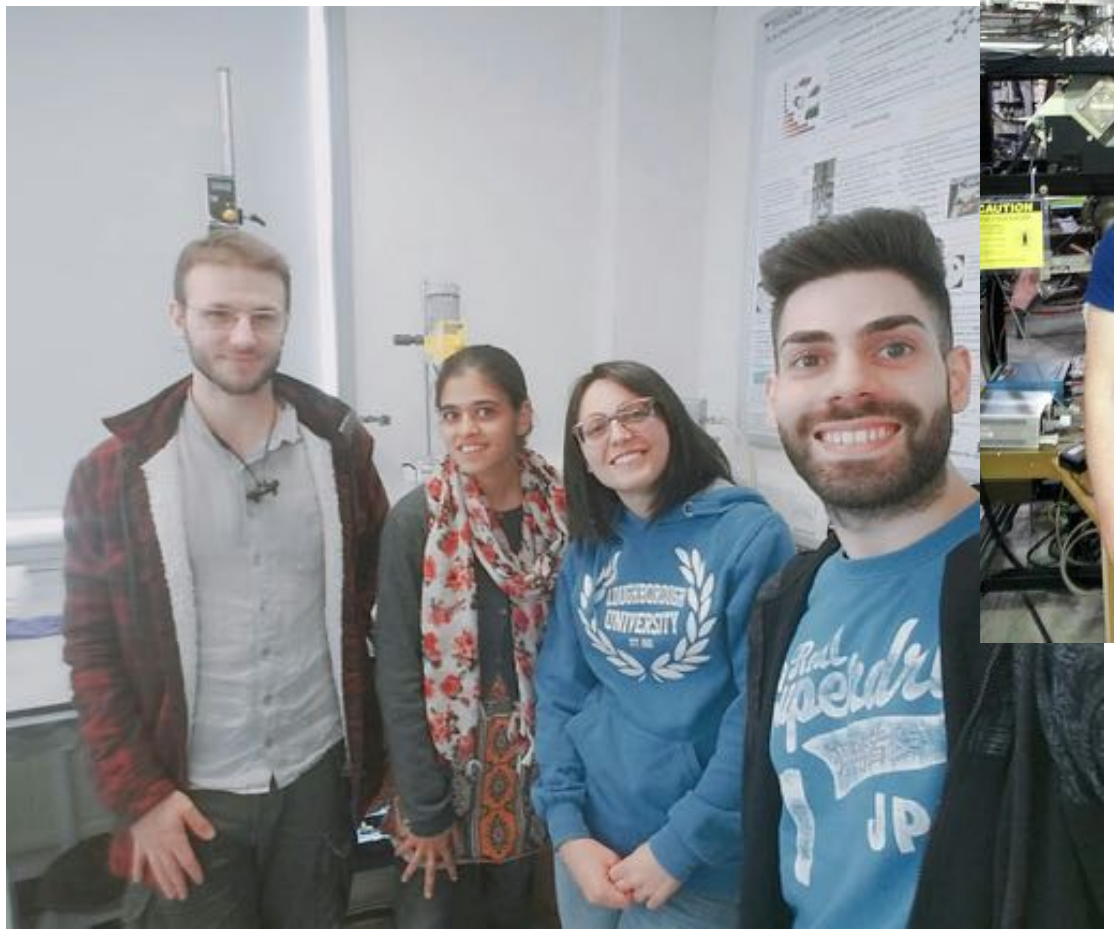
Confocal Microscopy

X-ray tomography

Conclusions and future work

- Molecular modelling using the Attachment Energy Model is a promising tool to **estimate face specific chemical properties** of biocompatible crystals;
- These information can help in the design of tailor made crystals for **Pickering stabilization**;
- **Experimental validation** is still needed (advance microscopy, contact angle, single crystal XRD face indexing);
- Better understanding of the relation between **crystal anisotropy** and **orientation** at the interface is necessary for efficient particle design.

Acknowledgements



Thank you for the attention!

PhD students:
Mr Panayiotis Klitou
Mr Lorenzo Metilli

Collaborators:
Dr Ian Rosbottom