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## INTRODUCTION

Continues advancement and rapid development of techniques operating at the nanoscale open new opportunities to revise and question commonly accepted **nucleation** and **crystal growth** theories. **Atomic Force Microscopy** (AFM) has been successfully involved in various aspects of active pharmaceutical ingredient (API) characterization including crystal growth, stability of solid dispersions, surface morphology, phase changes and dissolution [1]. Recent studies conducted on proteins crystallization at nanoscale show new evidence disproving generally accepted **Classical Nucleation Theory** (CNT) (Fig.1 a) [2]. Currently, **'dense liquid droplets'** seen in protein crystallisation and 'pre-nucleation clusters' (Fig.1 b) [3] seen mostly in inorganic salt crystallisation, are two main concepts of non-classical nucleation theory, although no significant progress has been made towards better understanding of mechanisms controlling heterogeneous nucleation in **small organic molecules** systems, what is in particular interest, as an epitaxial ordering phenomenon is frequently used to enhance nucleation rates and control properties of materials.

Our studies present a new light on heteronucleation and the **epitaxial growth** mechanisms based epitaxial growth of olanzapine dihydrate D on the surface of olanzapine form I (OZPN I) both in high humidity conditions and water solution. Results obtained from **Peak Force Quantitative Nanomechanical Mapping Atomic Force Microscopy** (PF-QNM-AFM) [4] indicate the presence of intermediate dense liquid-like phase in process of dihydrate D nucleation.

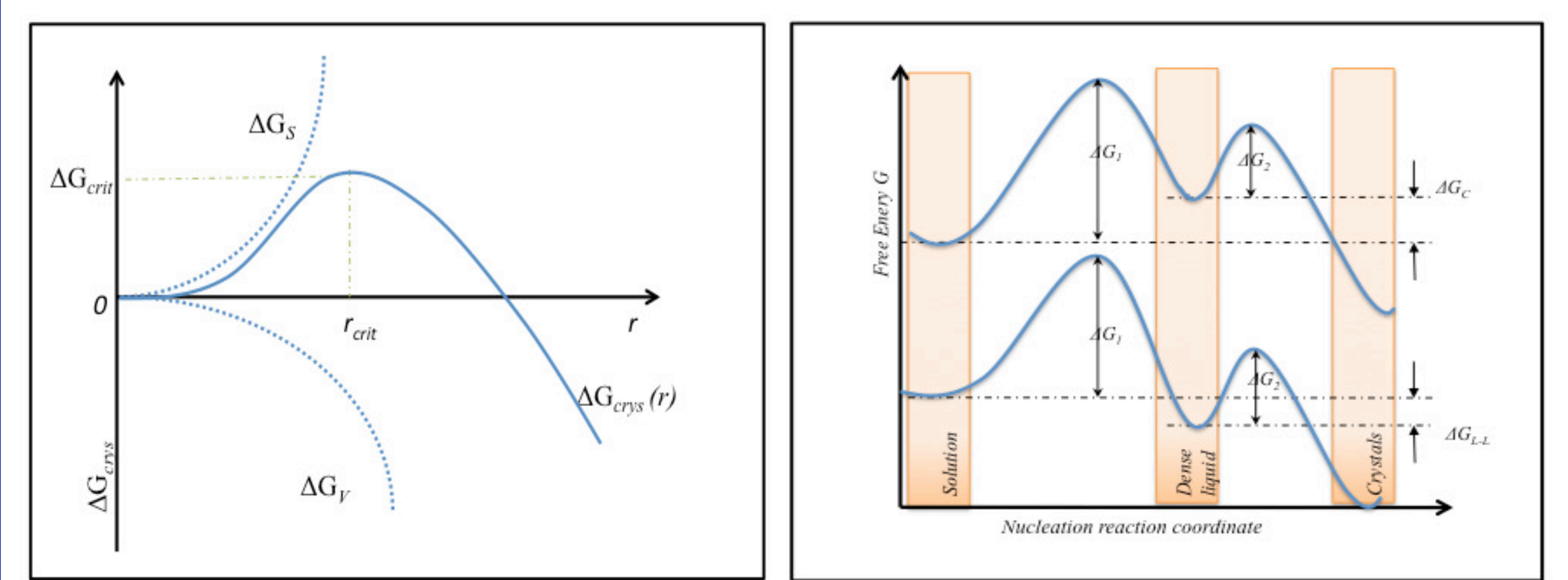


Fig. 1 Free energy diagram according to a) Classical nucleation theory, b) two-step nucleation theory [2].

## MOTIVATION

**Olanzapine (OZPN, Zyperxa®)** (Fig. 2) is a BCS class II drug (low solubility, high permeability) used in schizophrenia (bipolar disorder) treatment. OZPN exhibits rich solid state diversity, so far 60 distinct forms were identified including 3 polymorphic forms (I, II, III), 52 crystalline solvates, 3 polymorphic **dihydrates B, D, E**, and **disordered higher hydrate** plus an amorphous form [5].

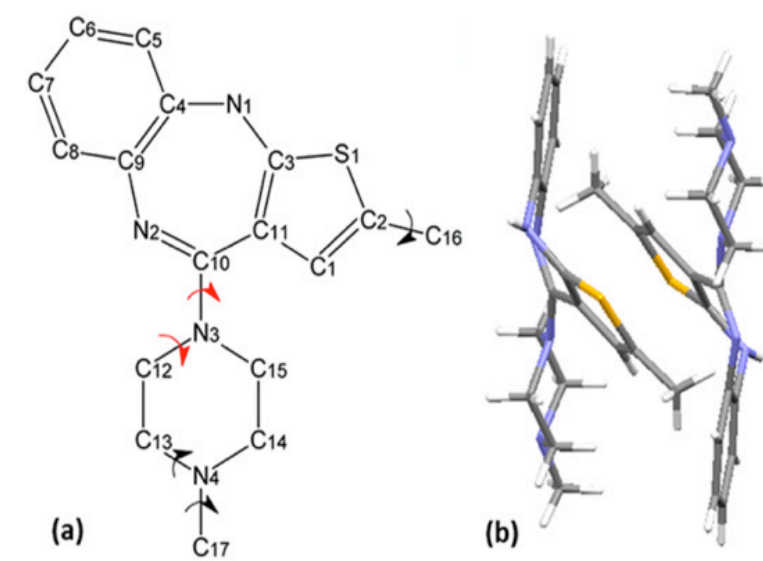


Fig. 2. Structure of Olanzapine [5].

OZPN I is stable from under ambient conditions, although significant surface changes were observed when OZPN I single crystal was stored for 2 days in quiescent water. **Epitaxial growth of a new form** was observed on (100) OZPN I face.

The **main objective** is to **characterise new form** growing on the surface of (100) OZPN I face and **study nucleation and growth mechanism**.

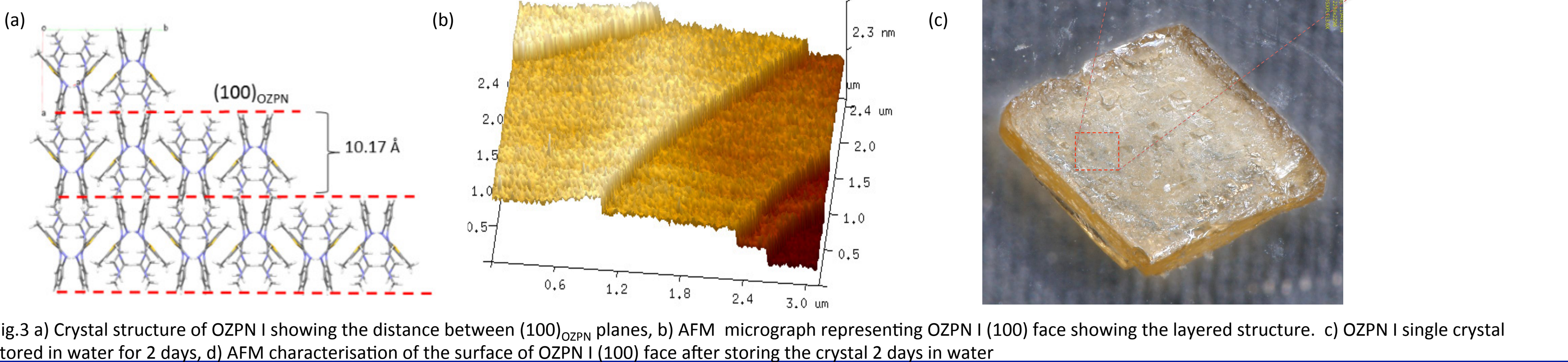


Fig. 3 a) Crystal structure of OZPN I showing the distance between (100)<sub>OZPN</sub> planes, b) AFM micrograph representing OZPN I (100) face showing the layered structure, c) OZPN I single crystal stored in water for 2 days, d) AFM characterisation of the surface of OZPN I (100) face after storing the crystal 2 days in water

## Quantitative Analysis of Observed Droplets

**Dense nanodroplets (ND)** visible on the surface of (100) OZPN I face in 70 % RH were characterised by PF-QNM-AFM. Also the same measurements for OZPN I crystal placed in water were conducted. Nanomechanical characterisation of ND by PF-QNM AFM reveals that:

- Three different phases can be distinguished (OZPN I, new crystalline form and dense droplets), droplets are the softest phase
- ND separate from water as a **new stable denser phase**,
- ND undergo **transformation** to a new solid phase.
- Growing new crystalline form has also different **nanomechanical properties** then OZPN I and additional results from Raman spectroscopy shows that new crystalline form is OZPN **dihydrate D**.

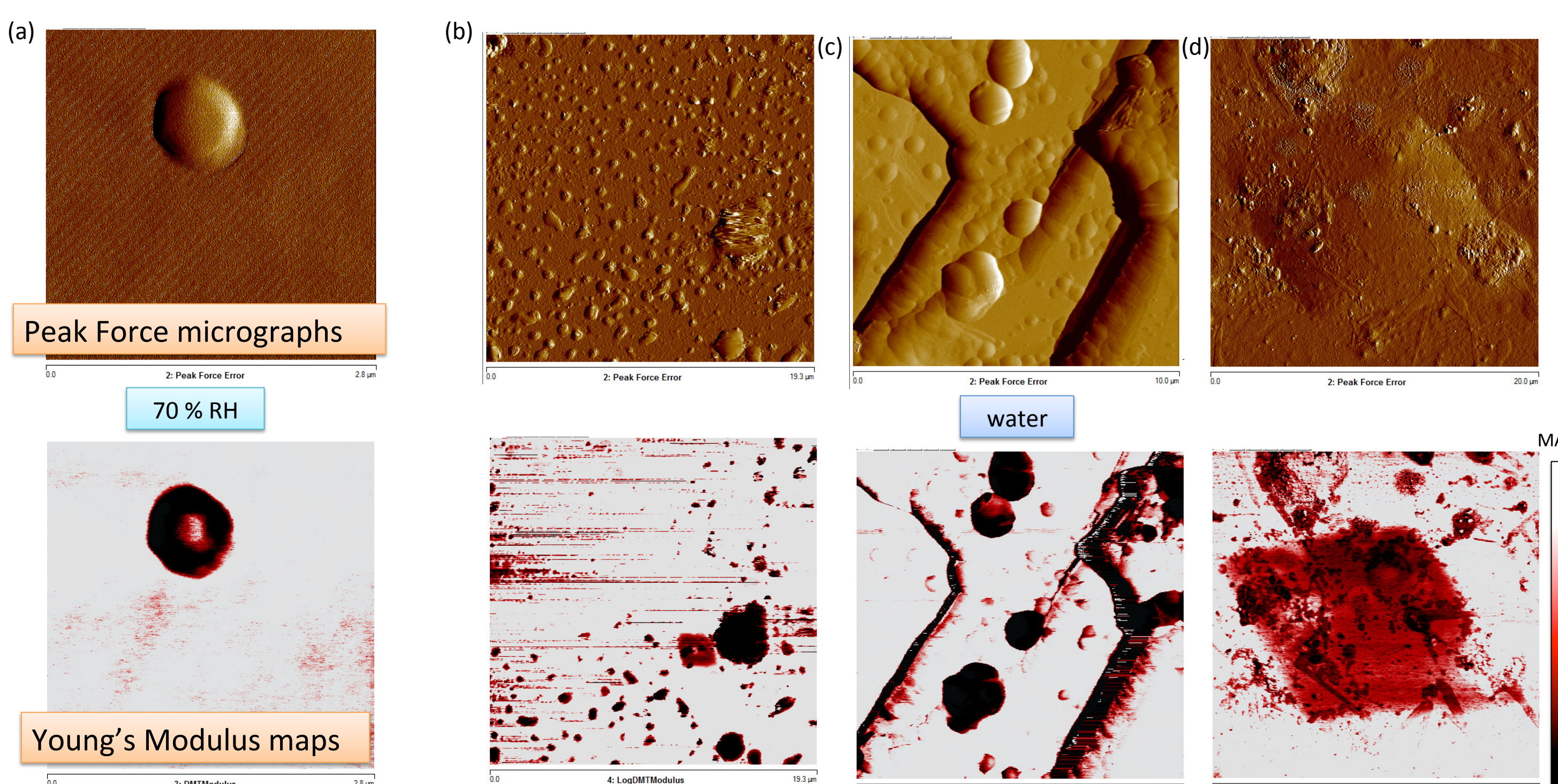


Fig. 6 AFM micrographs and corresponding Young's Modulus Maps for a) Dense droplet observed in 70% RH conditions; b, c) dense droplets observed in water; d) new crystalline form growing on the surface of OZPN I (100) face.

## METHODS

**PeakForce-QNM-AFM** opens new opportunities for nanocharacterisation of the mechanical surface properties. This mode enables force curves separation in order to obtain **Young's modulus**, adhesion force between the tip and the sample, energy dissipation, and maximum deformation (Fig. 4) [4].

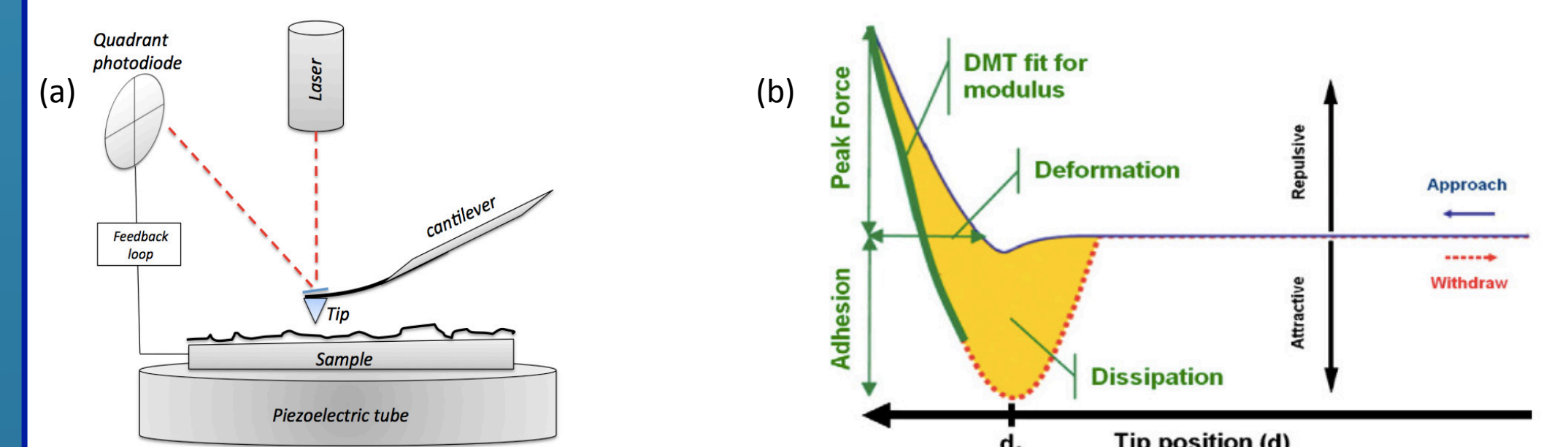


Fig. 4 a) Principle of AFM operation, detecting the bending of the cantilever with a photodetector and laser beam, b) Diagram presenting a force vs. separation curve for one cycle of the peak-force tapping AFM

## RESULTS

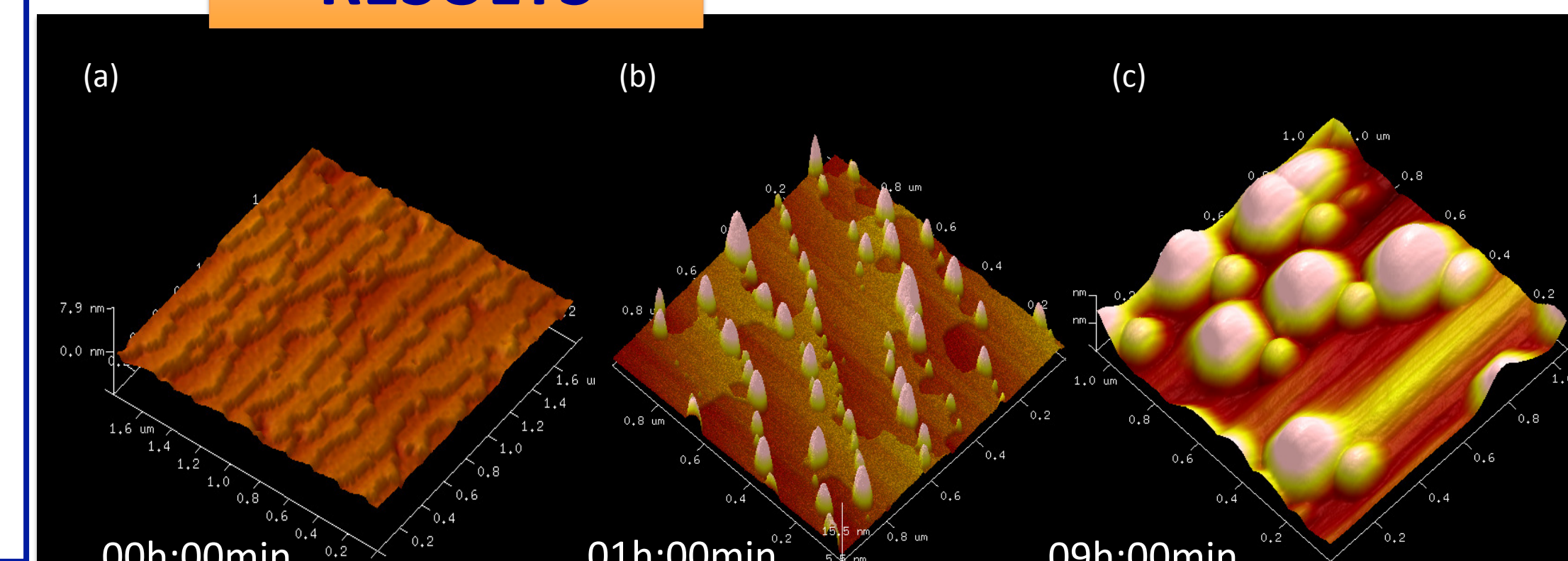


Fig. 5 AFM height mode micrographs of OZPN I surface (100) face in 70 % RH, room temperature conditions, a) 0 min, b) 1 hour, c) 9 hours.

Surface of OZPN I (100) face was observed in 70 % RH conditions using PF-QNM-AFM. Surface of (100)<sub>OZPN</sub> with visible ledges becomes covered with large number of **small nanodroplets**.

## Geometric real-space analysis of crystal epitaxy

Geometric real-space analysis of crystal epitaxy (GRACE) [6] calculations revealed significant **2-D lattice registry** between (100)<sub>OZPN</sub> and (001)<sub>D</sub>, that is responsible for **nucleation and epitaxial growth of dihydrate D** on the surface of OZPN I

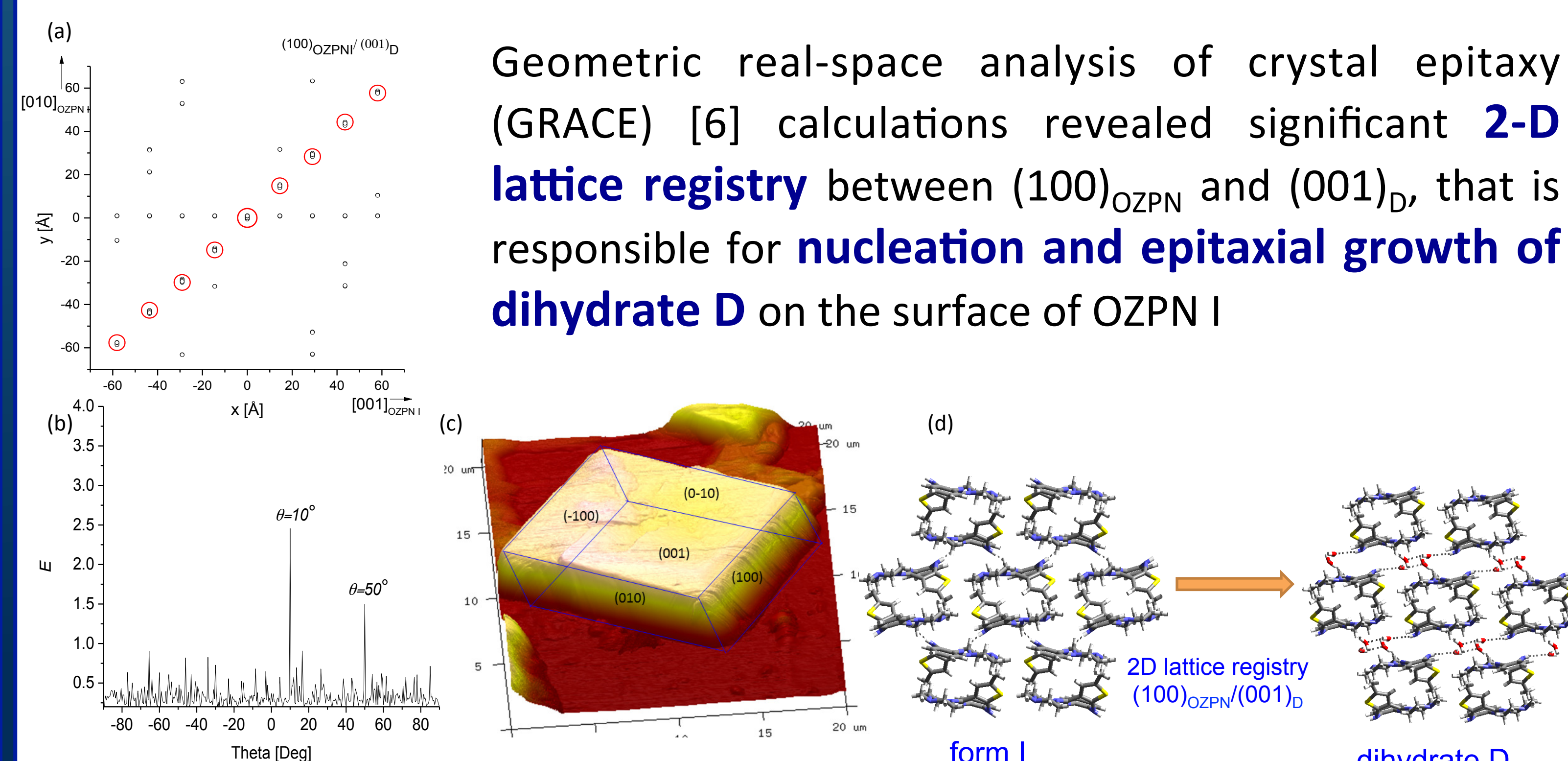


Fig. 7 a) Moiré patterns between (100)<sub>OZPN</sub>/(001)<sub>D</sub> showing 2D epitaxial match, b) Epitaxy score between (100)<sub>OZPN</sub>/(001)<sub>D</sub>, c) Face indexed Dihydrate D crystal growing on the surface of (100)<sub>OZPN</sub>, d) crystal structure of OZPN I and OZPN dihydrate D.

## CONCLUSIONS

Obtained information about observed nanodroplets both in **water and 70% RH** agrees with the **two-step nucleation** theory described by Vekilov and co-workers **via dense-liquid droplets** [2]. New form growing on (100) OZPN I face was characterised as **dihydrate D** by Raman spectroscopy.