Karlsruher Institut für Technologie

Institute of Functional Interfaces
Chemistry of oxydic and organic Interfaces

HGBP1 as a model system investigated by several surface techniques

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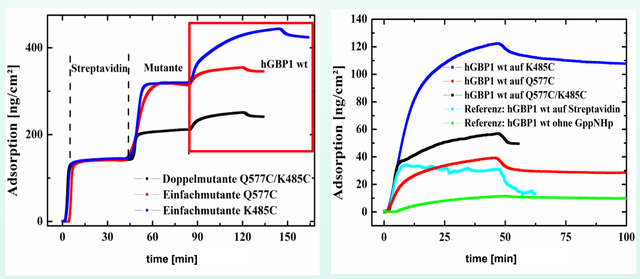
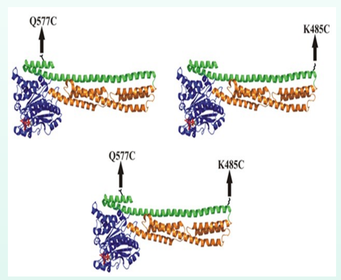
Introduction

In medical technology concerning the surface immobilization of proteins in a defined orientation, maintaining their activity is a critical aspect. This protein, hGBP1 bearing GTPase catalytic activity, is furnished either with one biotin anchor at one end of the molecule or with two biotin anchors opposing each other at the long ends. Various complementary methods (SPR, AFM and QCM) yield the same conclusion: (1) the double-anchored hGBP1 is oriented alongside and (2) the single-anchored protein is oriented upright. In addition, the catalytic activity of hGBP1 reveals full integrity of the surface bound protein and it also shows that the maximum activity of hGBP1 is not reached because immobilization prevents hGBP1 from homo dimer formation in the required orientation.

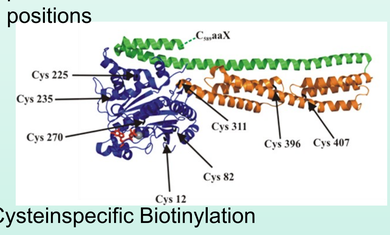
Dimerisation of hGBP1



Comparison of hGBP1 mutants in SPR



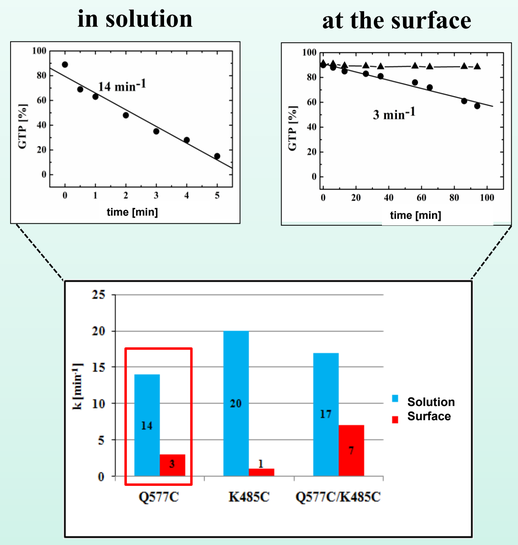
Crystal structure of hGBP1 in ribbon presentation with different anchor positions



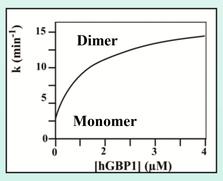
Cysteinspecific Biotinylation

- ✓ K485C : hGBP1 wt → 1 : 0,5
- ✓Q577C/K485C : hGBP1 wt → 1 : 0,8
- – Q577C : hGBP1 wt→ no dimerisation

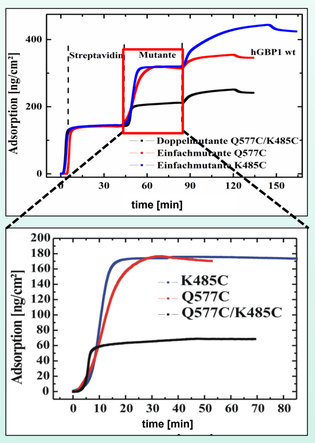
Tests of activity on the surface with SPR



- Solution: Dimer activity
- Surface: two possibilities for reduced activity
  - → Diffusion limitiation
  - → Monomer activity
- Exception: Q577C/K485C
  - → Dimerisation at the surface

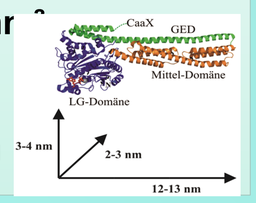


Immobilisation of hGBP1-mutants



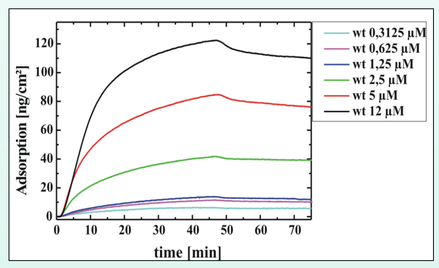
- Single mutants go on surface in comparison to the double mutants at the molar ratio of 3:1
- Double and Single mutants have different ***Footprints***

→Single mutants: approx. **12 nm²**
→Double mutants: approx. **39 nm²**



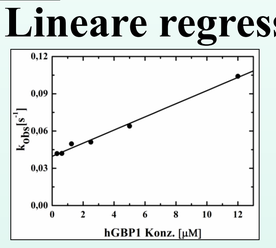
crystal structure of hGBP1 in ribbon presentation

Studies of kinetiks of dimerisation

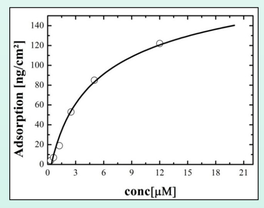


Studies of kinetiks of dimerisation of hGBP1

Lineare regression



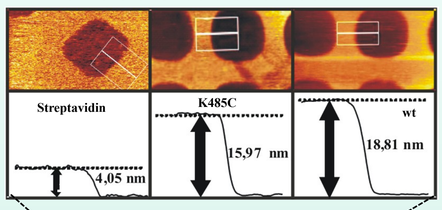
Plateau value

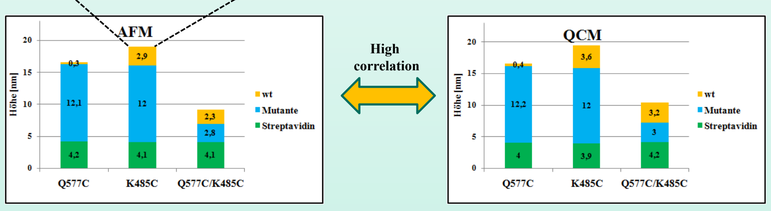


- linear regression: - calculation of k_{on} out of slope - $k_{off}/k_{on} = K_D$
  →K_D = 7,45 µM
  (k_{off} out of y-intercept: 6,32 µM)
- Plateau value (Monomer-Dimer-equilibrium): → K_D = 8,89 µM

Analysis of thickness with the AFM

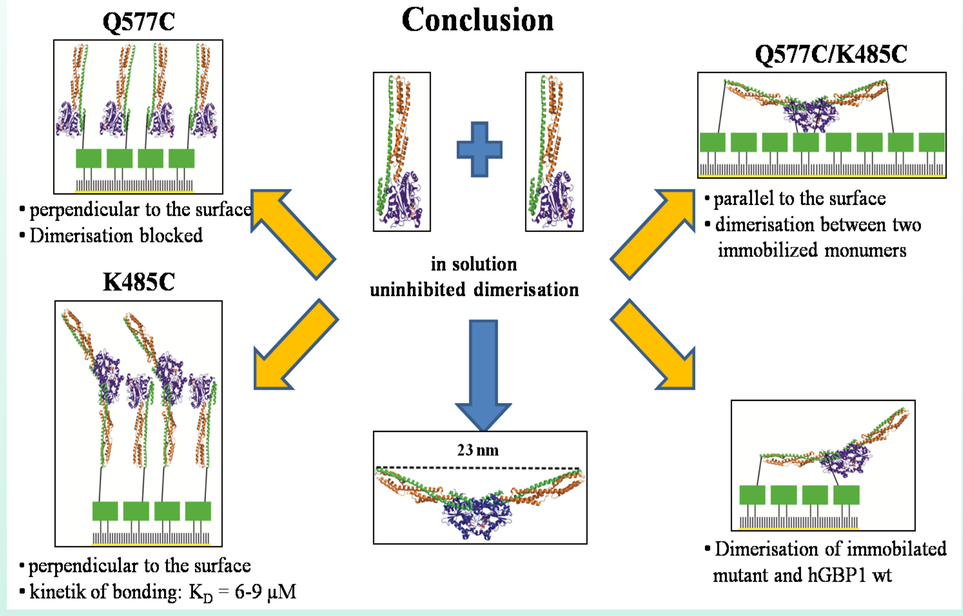
AFM on lateral structured surfaces





High correlation

- Orientation:
  →Single mutant: perpendicular to surface
  →Double mutant: parallel to surface
- Dimerisation:
  →(-) Single mutant: Q577C
  →(+) Single mutant: K485C
  →(+) Double mutant: Q577C/K485C



A. Kerstan, T. Ladnorg, C. Grunwald, T. Vopel, D. Zacher, C. Herrmann, C. Wöll, *Biointerphases* ***2010, 5, 131.***

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