# ForceRobot® 300

## The Gold Standard in Single Molecule Force Spectroscopy

### Automated Force Spectroscopy


- Fully automated force spectroscope
- Highest flexibility and precision
- Combination with single molecule fluorescence using JPK's DirectOverlay™ software
- Automated laser and detector alignment
- Automated cantilever drift compensation
- Powerful software for control & processing
- Molecular recognition mapping

JPK Instruments

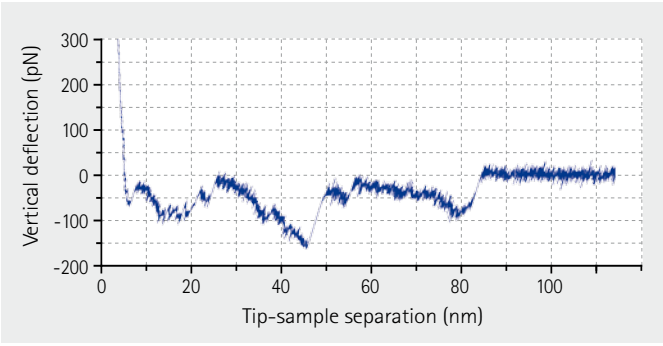
*Nanotechnology for Life Science*

# Where Nanotechnology Meets Molecular Analytics

## Automated force spectroscopy – a revolution in molecular analysis

Force spectroscopy is a label-free single molecule technique that allows the real-time study of molecular interactions on the nano-scale. Originating from the broad field of Atomic Force Microscopy (AFM), force spectroscopy directly addresses the measurement of forces between and within molecules. The sensitivity is high enough to characterize molecular interactions such as the unfolding forces of single proteins or forces of a single molecular bond.

The key to obtaining meaningful results from single molecule techniques such as force spectroscopy is statistics, and this is where the new ForceRobot® technology is revolutionary. On the one side, the automated setup and continuous adjustments provide huge improvements in the efficiency of data collection. On the other side, the integration with optical techniques allows targeted measurements where the molecules of interest are located. These factors, combined with highest data quality and stability, open the field of single molecule force spectroscopy to a new level of results.



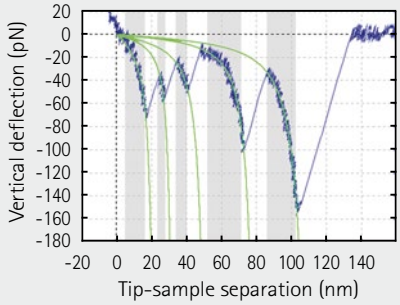

Force spectroscopy curve of a membrane protein unfolding, data courtesy of Zocher, Fung, Kobilka and Müller. The structure and kinetics of the G protein-coupled receptor (human $\beta_2$ adrenergic receptor $\beta_2$AR) were investigated for a variety of ligands (Structure 20, 1391-1402, 2012).


## ForceRobot® 300 applications

- Protein (un)folding and receptor-ligand interactions
- Elastic response or melting of DNA
- Single molecule mechanical properties, e.g. muscle proteins, synthetic biopolymers, carbohydrates or spider silk protein
- Localization of binding of small molecules on proteins (e.g. inhibitors on membrane proteins)
- Quantification of kinetics, affinity and energy landscapes of biological interactions
- Colloidal probe and Nanoindentation experiments
- Analysis of adhesion forces of single macromolecules for surface chemistry and polymer science

## What makes the difference?

Until now, single molecule force spectroscopy was a complicated procedure. The requirement of frequent manual calibrations and alignments as well as the need for permanent instrument attention made it a hard task. Useful data output was very low, with only a few suitable force curves over many hours. To make use of the full potential force spectroscopy offers, you need a dedicated tool. The ForceRobot® is built to perfectly address all the demands of a cutting edge force spectroscopy experiment.




**WLC fit including:**

- Contour length
- Persistence length
- Fit quality
- Breaking force
- Loading rate

Force spectroscopy curve of filamin unfolding, with worm-like chain fits marked. Multiple events are automatically recognized, fitted and a full set of fit parameters is generated, ready to export using batch processing.
ddFLN sample courtesy group of M. Rief, TU München.

# ForceRobot® 300 – the Automated Force Spectroscope

## Faster time-to-results

The basis for a meaningful interpretation of force spectroscopy experiments is a large number of reproducible data sets. The ForceRobot® overcomes the limitations of traditional force measurements by automating routine procedures and providing intelligent software for experimental design, data acquisition and evaluation. Tens of thousands of force curves are generated in a matter of hours in unattended mode, and force curves without events are automatically filtered out before processing. An integrated CCD camera ensures easy alignment of the laser detection system, and also allows probe and sample observation to find sample spots or monitor tip or sample changes. At the end the user can focus on the experimental results while the system delivers high quality data in unattended operation.

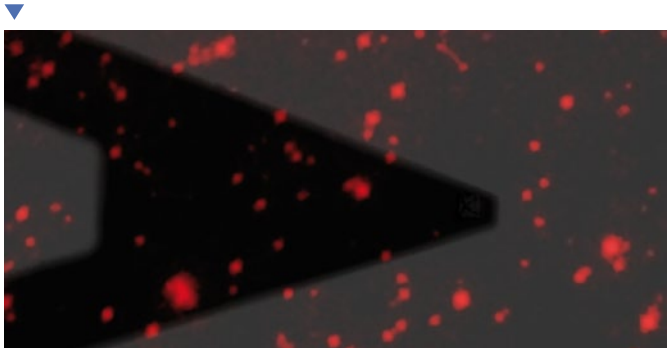
## Higher data quality and quantity

The quality of the force spectroscopy data is vital for successful analysis. The lowest electronic noise floor and the most rigid mechanical design are essential. The highest accuracy and stability of the instrument is ensured by integrated capacitive position sensors with exceptional performance. For minimized drift, the system design is symmetric. For the detection of smallest variations in force curves JPK improved the sensitivity and increased the detection bandwidth without compromising the noise levels. The high sampling rate and virtually unlimited number of data points per force curve complete this unique instrument.

## Perfect environmental control

The mechanical properties of single molecules are strongly influenced by their environment. The unique environmental control of ForceRobot® is tailored to this requirement, with heating and cooling available over a wide range of temperatures. This enables the study of biological samples at physiologically relevant temperatures or polymer samples in phase transition.

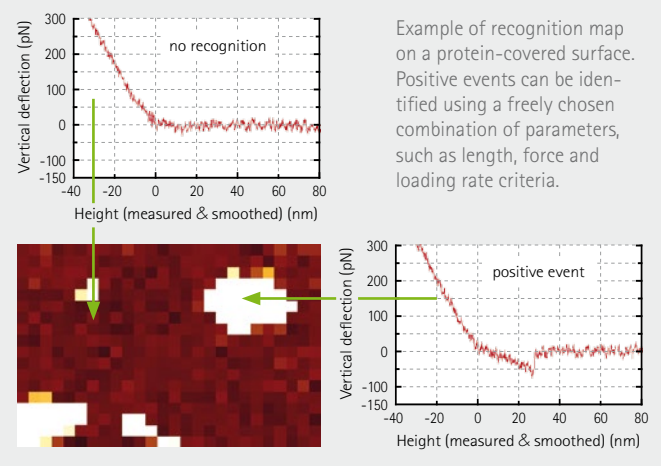
Inverted optical microscope image of the cantilever located above fluorescently labelled protein patches with JPK's DirectOverlay™ software. Brightfield and fluorescence images merged.



The JPK FluidicsModule™ allows the design and performance of experiments with different buffer composition, pH value or chemical agents. The ForceRobot® is fully compatible with a wide range of different JPK liquid cells and temperature stages.

## Automated calibration & drift correction

The ambiguity of classic calibration procedures is removed by a standardized, automated spring constant and deflection sensitivity calibration. During measurements over hours, cantilever bending drift plays a significant role for the data quality. To overcome this, JPK implemented an automated correction to readjust the beam deflection system between force curve cycles. Intelligent auto-calibration and the automated drift correction together with a rigorously driftminimized mechanical design enable long term measurements with consistent data quality.




Example of recognition map on a protein-covered surface. Positive events can be identified using a freely chosen combination of parameters, such as length, force and loading rate criteria.

## State of the art software

The ForceRobot® software package is characterized by its completely automated data acquisition but open enough to give the user full experimental control. The system comes with an intuitive user interface – a new way to structure the data acquisition and analysis. The ExperimentPlanner™ increases productivity with enhanced experimental flow control. The user can easily design experiments with temperature ramps, position movements, different force ramps or liquid exchange. For maximum flexibility, the user can program their own scripts. Hand in hand with this, the RampDesigner™ was developed to offer a new level of experimental flexibility. The user can freely combine segments of a force-distance cycle to customize advanced force curve procedures. Intelligent user-defined curve classification and fitting algorithms standardize the usual ‚manual' procedures. The fast batch processing routines ensure highest data quality.

# ForceRobot® 300
# Features & Specifications

**ForceRobot® 300 available in 2 configurations for sample positioning or mapping utilizing the same head**

1) ForceRobot® 300 MotorizedStage
   - Travel range of 2 × 2cm²
   - Step Size (Resolution) better than 1 µm
   - Repeatability better than 1 µm
2) ForceRobot® 300 PrecisionMappingStage
   - Travel range of 100 × 100 µm² under closed loop control
   - Position noise better than 0.3 nm closed loop

Both configurations can be used with inverted research microscopes or as stand-alone systems and work with JPK's DirectOverlay™ feature.

**ForceRobot® 300 can be operated:**

1) On top of an inverted research microscope for Single Molecule Force Spectroscopy (SMFS) simultaneously with Fluorescence Microscopy
   - Find a measurement spot optically on your sample by fluorescent labelling
   - Combine SMFS with advanced optical techniques such as FCS, FRET, TIRF or optical tweezers
   - Exact positioning and overlay of optical and force spectroscopy data with the JPK DirectOverlay™ software module
   - Fits to microscope based from
     - Zeiss (Axio Observer, AxioVert 200)
     - Olympus (IX line)
     - Nikon (TE 2000, Ti)
     - Leica (DMI line)
2) Stand-alone system
   - Maximum flexibility even if no fluorescence is needed (only 1 minute to mount the stage on an optical microscope)
   - Free access to the sample area

**Highest data quality and output**

- 200,000 curves per 24 hours in unattended mode while varying parameters such as temperature or loading rate
- Highest data density with virtually unlimited points per force curve

**Automate your measurements**

- Intelligent and automated approach for soft landing even with functionalized tips
- User-friendly automated laser and detector alignment eliminates cantilever drift for long term measurements
- Automated sample positioning or mapping with high precision sample stages
- Full remote instrument operation through internet

**Head**

- Rigid low-noise construction and drift-minimized mechanics based on our proven NanoWizard® design
- Liquid-safe concept with integrated vapour barrier, special encapsulated piezo drives and tip moving design
- Intelligent and automated approach with user defined parameters for different experiments
- Automated laser and detector alignment for long term experiments
- IR detection light source with low coherence
- High detector bandwidth of 8 MHz for high speed signal capture
- 860 nm wavelength detection light source for undisturbed fluorescence or Raman experiments
- Built-in optical filters for fluorescence without crosstalk with the beam deflection detection
- Built-in CCD camera for viewing the probe and sample
- High-speed flexure stage with ultrafast z-response and closed loop control
- Piezo options:
  - 6.5 µm z-range with fast response
  - 15 µm z-range for long pulling ranges (optional)
- Z-sensor noise level: 0.06 nm RMS at 0.1-1 kHz bandwidth
- Laser safety class 1

**Control electronics**

- 4 high speed 16 bit ADCs with 60 MHz
- High-speed data capture with optional burst mode
- Modular analog and digital design with latest PPC technology (PowerPC @ 660 MHz)
- Discrete analog high-speed high voltage amplifiers
- Gigabit Ethernet interface for fast data link
- Number of data points that can be captured continuously: restricted only by HDD
- Thermal noise acquisition up to 3.25 MHz
- Optional Signal Access Module (SAM) with analog and digital connectors for maximum experimental freedom

**Fluid cell options**

The JPK SmallCell™ is the perfect solution for smallest volume experiments in a hermetically sealed environment.

- Fluid or gas exchange
- Optimized flow with reduced dead volumes
- Prevention of cross-contamination
- Compatible with a wide range of cantilevers, including Olympus BioLever

The SmallCell™ is available in 2 versions and is compatible with JPK temperature control systems including HTHS™ and HCM™

1) SmallCell™ small volume version for aqueous solutions
   - Closed fluid cell for minimized volumes (60 µl)
   - Optimized for aqueous solutions
   - 3 easily accessible sample ports, 2 for buffer exchange and 1 for adding chemical agents
   - Cell materials: COC, steel or gold, and silicone
   - Easy to clean, also in ultrasonic bath
   - Autoclavable
2) SmallCell™ glass version for harsh environments
   - Multi-purpose closed fluid cell for small volumes (150 µl)
   - Inlet and outlet ports for perfusion experiments
   - Solvent, acid and chloroform resistant for polymer applications
   - Cell materials: glass, steel or gold, and resistive seal
   - Easy to clean, also in ultrasonic bath
   - Autoclavable

**Fluidics module**

- JPK FluidicsModule™ with up to 8 different liquids such as buffer solutions
- Software-controlled flow rate
- Automated incubation or adding of chemicals
- Comprehensive safety package

**Temperature control options**

- RT - 200 °C temperature range with 0.1 °C precision with the JPK High Temperature Heating Stage (HTHS™) or
- -30 °C - 120 °C temperature range with 0.1 °C precision with the JPK Heating Cooling Module (HCM™) (~0 °C - ~80 °C for experiments in buffer solution)
- All heaters and heating cooling solutions are software controlled

**Flexibility integrated**

- Compatible with most JPK NanoWizard® accessories
- Different stages for every application
- Large choice of add-ons such as temperature controls, liquid cells even for aggressive solvents
- Sample size up to 20 × 20 × 10 mm³
- JPK's ForceWheel™ handheld accessory for most sensitive experiment control
- Full experimental control by scripting functionality

**Software**

- Fully automated data acquisition
- Highest density of data points per force curve
- Fully automated sensitivity and spring constant calibration
- Automated re-calibration and cantilever drift compensation
- JPK ExperimentPlanner™ for designing a dedicated measurement workflow
- JPK RampDesigner™ for custom designed force curve segments
- Advanced spectroscopy modes such as
  - Various force clamp modes
  - User-defined temperature ramps, pulling speed or force feedback
- Enhanced force mapping capabilities
- Automated online and offline advanced filtering of curves, based on multiple criteria e.g. force, length and loading rate ranges
- Powerful batch processing including WLC, FJC, step fitting and other analysis
- JPK's DirectOverlay™ feature as an option
- Molecular recognition mapping

The ForceRobot® 300 mounted on a Zeiss AxioObserver inverted optical microscope. It is compatible with all major research-level inverted optical microscope platforms, and all the stages can also be used unmounted for stand-alone operation.



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