



### Cantilever functionalisation for AFM single molecule force spectroscopy

PLOS One

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#### ABSTRACT

Four different samples were employed for experiments, designed as follows:

- 1. HABP/HA: cantilever functionalised with hyaluronic acid binding protein, untreated cell sample;
- 2. BSA/HA: cantilever functionalised with bovine serum albumin, untreated cell sample;
- 3. untreated/HA: non-functionalised cantilever, untreated cell sample;
- 4. HABP/HAase: cantilever functionalised with hyaluronic acid binding protein, cell sample treated with HAase

The steps of activation are listed in this protocol and were the same for Sample 1 (HABP/HA, functionalisation molecule: hyaluronic acid binding protein), Sample 2 (BSA/HA, functionalisation molecule: bovine serum albumin) and Sample 4 (HABP/HAase, functionalisation molecule: hyaluronic acid binding protein). The cantilevers used to test cells in Sample 3 (untreated/HA) were not treated.

Similar protocols were described in:

- \* Maki K et al. Mechano-adaptive sensory mechanism of α-catenin under tension. Sci Rep 2016;6: 24878. doi:10.1038/srep24878
- \* Maki K et al. Real-time TIRF observation of vinculin recruitment to stretched  $\alpha$ -catenin by AFM. Sci Rep 2018;8: 1–8. doi:10.1038/s41598-018-20115-8

**EXTERNAL LINK** 

https://doi.org/10.1371/journal.pone.0206056

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Marcotti S, Maki K, Reilly GC, Lacroix D, Adachi T (2018) Hyaluronic acid selective anchoring to the cytoskeleton: An atomic force microscopy study. PLoS ONE 13(10): e0206056. doi: 10.1371/journal.pone.0206056

**PROTOCOL STATUS** 

## Working

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|       | NAME V  | CATALOG # | VENDOR V        |
|-------|---|-----------|-----------------|
|       | MilliQ Water  |           |                 |
|       | (3-Aminopropyl)triethoxysilane  | 440140    | Sigma Aldrich   |
|       | Bovine Serum Albumin  | A9647     | Sigma Aldrich   |
|       | Maleimide-C3-NT A   | M035      |                 |
|       | 2-Mercaptoethanol   | M3148     | Sigma Aldrich   |
|       | Hyaluronic Acid Binding Protein, Bovine Nasal Cartilage, Biotinylated | 385911    | Merck Millipore |
|       | Trizma® hydrochloride solution  | T2319     | Sigma Aldrich   |
| _E(5) | Themae hydrochionae solution  | 1 2 3 1 9 | Olgina Alanon   |

STEPS MATERIALS

NAME V CATALOG # V VENDOR V

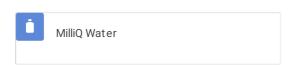


| NAME Y  | CATALOG # | VENDOR V        |
|---|-----------|-----------------|
| (3-Aminopropyl)triethoxysilane  | 440140    | Sigma Aldrich   |
| MilliQ Water  |           |                 |
| Maleimide-C3-NTA  | M035      |                 |
| Trizma® hydrochloride solution  | T2319     | Sigma Aldrich   |
| Hyaluronic Acid Binding Protein, Bovine Nasal Cartilage, Biotinylated | 385911    | Merck Millipore |
| Trizma® hydrochloride solution  | T2319     | Sigma Aldrich   |
| MilliQ Water  |           |                 |
| Bovine Serum Albumin  | A9647     | Sigma Aldrich   |
| 2-Mercaptoethanol   | M3148     | Sigma Aldrich   |
| MilliQ Water  |           |                 |
| MilliQ Water  |           |                 |

1 AFM cantilevers (OMCL-TR400PSA; spring constant, 0.02 N/m; curvature radius of tip, 15 nm; Olympus Co.) are oxidised using an ozone cleaner and submerged in 2% w/w (3-Aminopropyl)triethoxysilane / ultra-pure water for 15 minutes to depose (-SH) groups on the probe surface

2 Mass Percent (3-Aminopropyl)triethoxysilane / ultra-pure water





**७**00:15:00 (3-Aminopropyl)triethoxysilane

# **▲**SAFETY INFORMATION

Please refer to SDS for (3-Aminopropyl)triethoxysilane

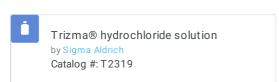
https://www.sigmaaldrich.com/catalog/product/aldrich/440140?

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 $2 \quad \text{After washing, the cantilevers are submerged in 6 mM Male imide-} \\ \text{C}_{3}\text{-NTA/Tris-HCl buffer for 30 minutes to expose NHS esters}$ 



\*0.006 Molarity (M) Maleimide-C3-NTA / Tris-HCl buffer



## © 00:30:00 Maleimide-C3-NTA

3 After washing, the functionalisation molecule is bound to the exposed NHS ester groups by submerging the cantilever in 100 nM Hyaluronic Acid Binding Protein / Tris-HCl buffer solution (Sample 1 HABP/HA and Sample 4 HABP/HAase) or 1% w/v Bovine Serum Albumin / ultra-pure water (Sample 2 BSA/HA) for 1 hour

\*0.0000001 Molarity (M) Hyaluronic Acid Binding Protein / Tris-HCl buffer

◆1 Mass/Volume Percent Bovine Serum Albumin / ultra-pure water

Hyaluronic Acid Binding Protein, Bovine
Nasal Cartilage, Biotinylated
by Merck Millipore
Catalog #: 385911

Trizma® hydrochloride solution
by Sigma Aldrich
Catalog #: T2319

MilliQ Water

Bovine Serum Albumin
by Sigma Aldrich
Catalog #: A9647

**७**01:00:00 Functionalisation molecule

4 The excess maleimide is quenched with 50 mM 2-Mercaptoethanol / ultra-pure water by submerging the cantilevers for 1 minute

2-Mercaptoethanol
by Sigma Aldrich
Catalog #: M3148

MilliQ Water

 $\bigcirc$  00:01:00 2-mercaptoethanol

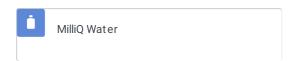
\$0.05 Molarity (M) 2-Mercaptoethanol / ultra-pure water

**A**SAFETY INFORMATION

Please refer to SDS for 2-Mercaptoethanol

https://www.sigmaaldrich.com/catalog/product/sigma/m3148?lang=en®ion=GB

5 After a final wash, the functionalised cantilevers are kept submerged in ultra-pure water until mounting on the AFM holder



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