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Protocol status: Working

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 α -catenin by AFM. Sci Rep 2018;8: 1–8. doi:10.1038/s41598-018-20115-8

MATERIALS

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⊗ MilliQ Water **Contributed by users**

⊗ (3-Aminopropyl)triethoxysilane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #440140**

Created: Jun 26, 2018

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13372

⊗ Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9647**

⊗ Maleimide-C3-NTA **Contributed by users Catalog #M035**

⊗ 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**

⊗ Hyaluronic Acid Binding Protein, Bovine Nasal Cartilage, Biotinylated **Merck Millipore (EMD Millipore) Catalog #385911**

⊗ Trizma® hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T2319**

STEP MATERIALS

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- 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

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

⊗ (3-Aminopropyl)triethoxysilane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #440140**

⊗ MilliQ Water **Contributed by users**

⌚ 00:15:00 (3-Aminopropyl)triethoxysilane

Safety information

Please refer to SDS for (3-Aminopropyl)triethoxysilane

[https://www.sigmaaldrich.com/catalog/product/aldrich/440140?](https://www.sigmaaldrich.com/catalog/product/aldrich/440140?lang=en@ion=GB&utm_medium=referral&utm_source=pubchem&utm_campaign=pubchem_2017)

[lang=en@ion=GB&utm_medium=referral&utm_source=pubchem&utm_campaign=pubchem_2017](https://www.sigmaaldrich.com/catalog/product/aldrich/440140?lang=en@ion=GB&utm_medium=referral&utm_source=pubchem&utm_campaign=pubchem_2017)

- 2 After washing, the cantilevers are submerged in 6 mM Maleimide-C₃-NTA / Tris-HCl buffer for 30 minutes to expose NHS esters

⊗ Maleimide-C₃-NTA **Contributed by users Catalog #M035**

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

⊗ Trizma® hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T231**

⌚ 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

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

IM 1 Mass / % volume Bovine Serum Albumin / ultra-pure water

⊗ Hyaluronic Acid Binding Protein, Bovine Nasal Cartilage, Biotinylated **Merck Millipore (EMD Millipore) Catalog #385911**

⊗ Trizma® hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T231**

⊗ MilliQ Water **Contributed by users**

⊗ Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9647**

⌚ 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3148**

⊗ MilliQ Water **Contributed by users**

⌚ 00:01:00 2-mercaptoethanol

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

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

⊗ MilliQ Water **Contributed by users**