Cantilever functionalisation for AFM single molecule force spectroscopy

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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

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

MATERIALS

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

STEPS MATERIALS

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(3-Aminopropyl)triethoxysilane	440140	Sigma Aldrich
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Hyaluronic Acid Binding Protein, Bovine Nasal Cartilage, Biotinylated	385911	Merck Millipore
Trizma® hydrochloride solution	T2319	Sigma Aldrich
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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

(3-Aminopropyl)triethoxysilane
by Sigma Aldrich
Catalog #: 440140

MilliQ Water

00:15:00 (3-Aminopropyl)triethoxysilane

SAFETY INFORMATION

Please refer to SDS for (3-Aminopropyl)triethoxysilane
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-C_3-NTA / Tris-HCl buffer for 30 minutes to expose NHS esters

Maleimide-C3-NTA
Catalog #: M035

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

Trizma® hydrochloride solution
by Sigma Aldrich
Catalog #: T2319

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

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

00:01:00 **2-mercaptoethanol**

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