Penetration of Cell Membranes and Synthetic Lipid Bilayers by Nanoprobes

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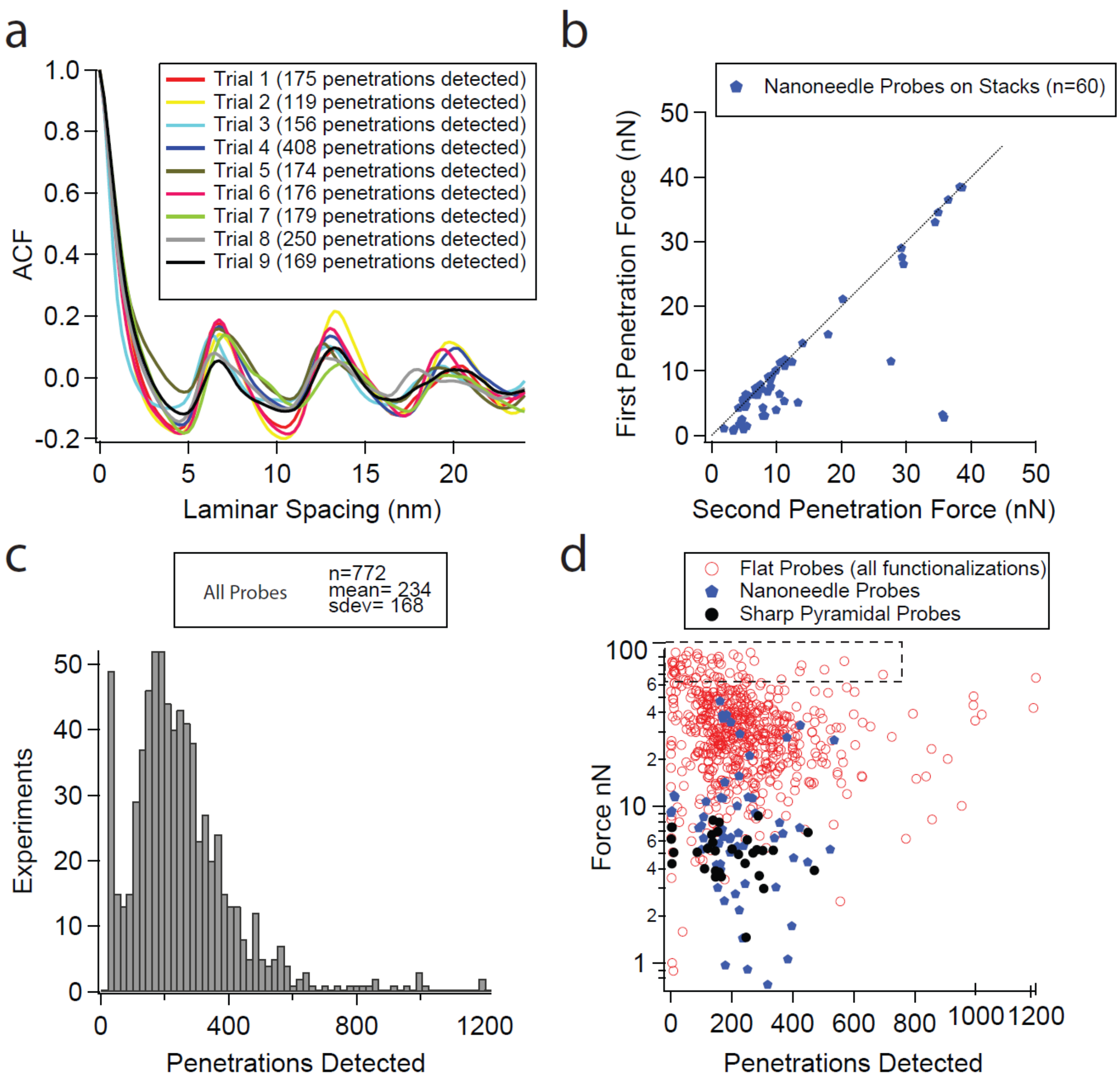
Supporting Material:



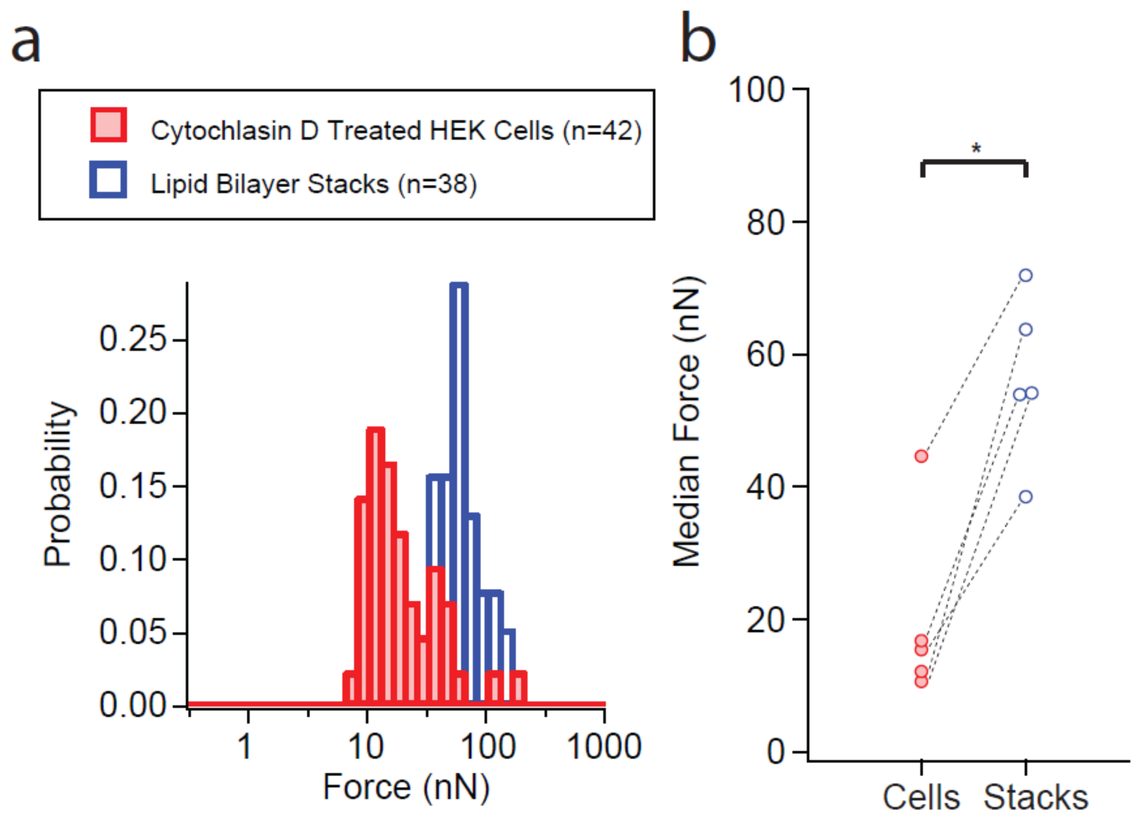
Supplementary Figure 1: On Lipid Stacks

a) Autocorrelation function for the tip position over time. Shows regular, laminar spacing between 6-7 nm. These 9 trials are representative of the entire dataset and were drawn from the nanoneedle experiments.

b) Comparison of first and second breakthrough forces for all experiments performed in lipid stacks using nanoneedle probes. Second breakthrough forces were not analyzed for the other sets of experiments.

c) Histogram of the number of penetrations detected in each lipid stack experiment for all lipid stack experiments (all probe types).

d) Plot of penetration force versus number of penetrations. The variation in thickness has no obvious effect on penetration force. The boxed region indicates where the penetration force is very high. Some of these points may be underestimating the true thickness of the stack if the end-trigger ~100 nN was reached prior to complete penetration of the stack. This shouldn't appreciably effect our conclusion that the stacks are ~200 bilayers thick.



Supplementary Figure 2: Synthetic Oligonucleotide
From a total of 5 functionalized tips.
(*a*) Histogram of all first penetration force in cells and lipid stacks.
(*b*) Pairwise Comparison of median penetration force in cells and lipid stacks for each tip. *Paired T-test P< 0.005.

Supporting Table:

The following table explains the details of the 18 functionalization reagents. It has been split onto two pages and color-coded for display in the merged document. Several of the cell-penetrating peptides were co-functionalized with mercaptopropanol as a competitor (see Concentration). The competitor concentration was based on titration experiments measuring the height of the nitrogen peak from x-ray photoelectron spectroscopy. Concentrations were chosen such that peptide density was non-saturating but > 50%. The rationale for this was to prevent overpacking and steric hindrance. All references in the Supporting Table (1-9) appear elsewhere in the main text.

Supplementary Table 1. Functionalization Reagents for Modifying AFM Tip Surface Chemistry.

Type	Reagent	Sequence	Source	Rationale/Description	Concentration
Peptide	PrPsegement	KTNMKHMAGAAAAGAVVGGLGGRSRGC	Lin et al 1997 [1]	Membrane-inserting (Lin et al 1997) [1]	0.1 mg/mL
	TMD_AR	LSVLRRAVQYLRSLRGSGC	(NP_758513)	Homology to motiff found by Cruz et al 2013 [3]	0.1 mg/mL
	TMD_VTP	LLRGGPACGRLLRGSGC	(NP_080282)	Homology to motiff found by Cruz et al 2013 [3]	0.1 mg/mL
	TMD_Kchan	LLRAGKLRLLRGPGSGC	(NP_001177302)	Homology to motiff found by Cruz et al 2013 [3]	0.1 mg/mL
	Melittin	GIGAVLKVLTTGLPALISWIKRKRQQGSGSGGC	Pubchem CID 16133648	Membrane-inserting (Sessa 1969) [4]	0.1 mg/mL Melittin with 150 μM mercaptopropanol
	TAT	CGGGRKKRRQRRR	PM_12417587	Known CPP (Fawell 1994) [5	0.1 mg/mL TAT with 1 mM mercaptopropanol
	Penetratin	CGGGRQIKIWFQNRRMKWKK	PM_16476052	Known CPP (Derossi et al 1994) 6]	0.1 mg/mL Penetratin with 2 μM mercaptopropanol
	MAP	CGGGKLALKLALKALKAALKLA	PM_10323198	Known CPP (Oehlke et al 1998) [7]	0.1 mg/mL MAP with 10 μM mercaptopropanol
	Lear	LSSLLSLLSSLLSLLSSLLSLGSGC	Lear et al 1988 [2]	Membrane-inserting (Lear et al 1988) [2]	0.1 mg/mL Lear with 1 μM mercaptopropanol
	Propincher	AGRWPPPPPPPWRRRRGSGGSGC		Hydrophobic mismatch (de Jesus 2013) [8]	0.1 mg/mL
	Flexpincher	AGRWLALALALALAWRRRRGSGGSGC		Hydrophobic mismatch (de Jesus 2013) [8]	0.1 mg/mL
	PolyArginine	RRRC		Interaction with phospholipids	0.1 mg/mL PolyArginine with 50 μM mercaptopropanol

Type	Reagent	Sequence	Source	Rationale/Description	Concentration
Nucleotide	DNP-PEG-SPRM	XXXWYWYWY (see Description)	Designed by M. Angle	Amphipathic Oligomer X=DTPA (GlenRes10-1937-xx) Y=DNP (GlenRes 10-1985-xx) W=Spermine (GlenRes 10-1939-xx) From Stanford Pan Oligo (nucleotides from GlenRes)	est. 200 μM

Type	Reagent	Source	Rationale/Description	Concentration
Small molecule	Mercaptopropanol (CAS 19721-22-3)	Sigma	Hydrophillic	100 μM mercaptopropanol
	Dodecanethiol (CAS 112-55-0)	Sigma	Hydrophobic	100 μM 1-decanethiol
	Perfluordecanethiol (CAS 34143-74-3)	Sigma	Very Hydrophobic	100 μM perfluorodecanethiol
	Phenylethanethiol (CAS 4410-99-5)	Sigma	Aromatic	100 μM phenylethanethiol
	Benzophenonethiol	Synthesized by Herbert Zimmerman (MPImF, Heidelberg)	Thiol analog of silane from Angle and Schaefer 2012 [9]	100 μM benzophenonethiol

Supporting References:

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9. Angle, M. R., and A. T. Schaefer. 2012. Neuronal recordings with solid-conductor intracellular nanoelectrodes (SCINEs). *PLoS ONE* 7.