

**Cell Adhesive Gradient Substrates**

<b>Substrate</b>	<b>Component 1</b>	<b>Component 2</b>	<b>Ligand</b>	<b>Gradient Slope Range</b>	<b>Characterized by:</b>
Au - 30-50 nm	MUA	MUD	Fn	0-100% mm <sup>-1</sup>	Fluor(Ab), Fluor (nano), SPR, CV, MALDI
Au - 30-50 nm	MUA	OT	Fn	0-100% mm <sup>-1</sup>	Fluor(Ab), Fluor (nano), SPR, CV, MALDI
Au - 30-50 nm	MUA	PEG	Fn	0-100% mm <sup>-1</sup>	Fluor(Ab), IRRAS, SPR, CV, MALDI
Au - 30-50 nm	1:5 MUA:PEG	PEG	Fn	0-20% mm <sup>-1</sup>	Fluor(Ab), IRRAS, SPR, CV, MALDI
Au - 30-50 nm	1:10 MUA:PEG	PEG	Fn	0-10% mm <sup>-1</sup>	Fluor(Ab), IRRAS, SPR, CV, MALDI
Au - 30-50 nm	MUA	PEG	RGD	0-100% mm <sup>-1</sup>	IRRAS, SPR, CV, MALDI
Au - 30-50 nm	1:5 MUA:PEG	PEG	RGD	0-20% mm <sup>-1</sup>	IRRAS, SPR, CV, MALDI
Au - 30-50 nm	1:10 MUA:PEG	PEG	RGD	0-10% mm <sup>-1</sup>	IRRAS, SPR, CV, MALDI
Au - 30-50 nm	1:10 MUA:MUD	MUD	Fn	0-10% mm <sup>-1</sup>	Fluor(Ab), SPR
Au - 30-50 nm	1:100 MUA:MUD	MUD	Fn	0-1% mm <sup>-1</sup>	Fluor(Ab), SPR
Au - 30-50 nm	1:10 MUA:OT	OT	Fn	0-10% mm <sup>-1</sup>	Fluor(Ab), SPR
Au - 30-50 nm	1:100 MUA:OT	OT	Fn	0-1% mm <sup>-1</sup>	Fluor(Ab), SPR
Au - 30-50 nm	MUA	MUD	RGD	0-100% mm <sup>-1</sup>	Fluor (nano), SPR, XPS, IRRAS
Au - 30-50 nm	MUA	OT	RGD	0-100% mm <sup>-1</sup>	Fluor (nano), SPR, XPS, IRRAS
Au - 30-50 nm	1:10 MUA:MUD	MUD	RGD	0-10% mm <sup>-1</sup>	SPR
Au - 30-50 nm	1:100 MUA:MUD	MUD	RGD	0-1% mm <sup>-1</sup>	SPR
Au - 30-50 nm	1:10 MUA:OT	OT	RGD	0-10% mm <sup>-1</sup>	SPR
Au - 30-50 nm	1:100 MUA:OT	OT	RGD	0-1% mm <sup>-1</sup>	SPR
Au - 20 nm	MUA	PEG	Fn	0-100% mm <sup>-1</sup>	Fluor(Ab), IRRAS, SPR, MALDI
Au - 20 nm	1:5 MUA:PEG	PEG	Fn	0-20% mm <sup>-1</sup>	Fluor(Ab), IRRAS, SPR, MALDI
Au - 20 nm	1:10 MUA:PEG	PEG	Fn	0-10% mm <sup>-1</sup>	Fluor(Ab), IRRAS, SPR, MALDI
Au - 20 nm	MUA	PEG	RGD	0-100% mm <sup>-1</sup>	IRRAS, SPR, MALDI
Au - 20 nm	1:5 MUA:PEG	PEG	RGD	0-20% mm <sup>-1</sup>	IRRAS, SPR, MALDI
Au - 20 nm	1:10 MUA:PEG	PEG	RGD	0-10% mm <sup>-1</sup>	IRRAS, SPR, MALDI
Au - 30-50 nm	1:5 MUA:PEG	PEG	10:1 RGD(E)	0-20% mm <sup>-1</sup>	IRRAS, SPR, MALDI
Au - 30-50 nm	1:10 MUA:PEG	PEG	10:1 RGD(E)	0-10% mm <sup>-1</sup>	IRRAS, SPR, MALDI
Au - 30-50 nm	MUA	PEG	hEGF	0-100% mm <sup>-1</sup>	IRRAS, SPR, XPS
Au - 30-50 nm	1:5 MUA	PEG	hEGF	0-20% mm <sup>-1</sup>	IRRAS, SPR, XPS
Au - 30-50 nm	1:10 MUA	PEG	hEGF	0-10% mm <sup>-1</sup>	IRRAS, SPR, XPS

1. Au substrate thickness may be made to order in the range  $d > 20$  nm. Films thicker than 50 nm present light transmission difficulties.
2. MUA = mercaptoundecanoic acid  
MUD = mercaptoundecanol  
OT = octanethiol  
PEG = ethylene glycol oligomer
3. Fn = fibronectin  
RGD = H<sub>2</sub>N-GGGGGRGDSPAA-CO<sub>2</sub>H  
hEGF = human epidermal growth factor
4. Gradient slope range is typical. Gradient slopes within the range, *e.g.* 0-30% in the MUA/PEG/Fn system, are also possible. Substrates with defined surface coverages are special cases, or “zero-slope” gradients.
5. Fluor(Ab) = fluorescence imaging with an antibody-based sandwich assay  
Fluor(nano) = fluorescence imaging with fluorescent nanospheres (prior to Fn immobilization)  
SPR = surface plasmon resonance characterization of individual component assemblies  
CV profiling = spatially dependent electrochemical profiling  
XPS = x-ray photoelectron spectroscopy  
IRRAS = Fourier transform infrared reflection-absorption spectroscopy  
MALDI = matrix-assisted laser desorption-ionization mass spectrometry  
XPS = x-ray photoelectron spectroscopy (C<sub>1s</sub> and N<sub>1s</sub>)