

Supporting Information

High affinity binding of an engineered, modular peptide to bone tissue

Sabrina H. Brounts, Jae Sung Lee, Sean Weinberg, Sheeny K. Lan Levengood, Everett L. Smith, William L. Murphy*

Table S1. P values from Student's t-test between the amount of mBMP bound to cortical bone by incubating in peptide solution with various concentrations. The amount was quantified by measuring the change of incubating solution after peptide binding.

		mBMP ($\mu\text{g/mL}$)				
		300	200	100	50	0
0.5 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-
		200	0.009	-	-	-
		100	< 0.001	< 0.001	-	-
		50	< 0.001	< 0.001	0.083	-
1 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-
		200	0.008	-	-	-
		100	< 0.001	0.008	-	-
		50	< 0.001	0.003	0.030	-
2 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-
		200	0.008	-	-	-
		100	< 0.001	0.068	-	-
		50	< 0.001	0.010	0.010	-
3 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-
		200	0.049	-	-	-
		100	< 0.001	0.091	-	-
		50	< 0.001	0.022	0.001	-

Table S2. P values from Student's t-test between the fluorescence intensity of modular peptide bound to cortical bone by incubating in peptide solution with various concentrations.

		mBMP ($\mu\text{g/mL}$)					mBMP-mut ($\mu\text{g/mL}$)	
		300	200	100	50	0	200	100
0.5 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-	-	-
		200	0.073	-	-	-	-	-
		100	0.060	0.253	-	-	-	-
		50	0.003	< 0.001	0.023	-	-	-
		0	0.001	< 0.001	0.006	0.001	-	-
	Rhodamine	50	0.002	< 0.001	0.014	0.087	0.019	0.227
	mBMP-mut ($\mu\text{g/mL}$)	200	-	0.004	-	-	-	0.020
		100	-	-	0.005	-	-	-
1 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-	-	-
		200	0.682	-	-	-	-	-
		100	0.046	0.003	-	-	-	-
		50	0.002	< 0.001	0.262	-	-	-
		0	< 0.001	< 0.001	0.009	0.003	-	-
	Rhodamine	50	< 0.001	< 0.001	0.098	0.156	0.002	0.688
	mBMP-mut ($\mu\text{g/mL}$)	200	-	< 0.001	-	-	-	0.572
		100	-	-	0.106	-	-	-
2 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-	-	-
		200	0.072	-	-	-	-	-
		100	0.011	0.089	-	-	-	-
		50	< 0.001	0.004	0.163	-	-	-
		0	< 0.001	< 0.001	0.005	0.001	-	-
	Rhodamine	50	< 0.001	0.004	0.125	0.687	0.002	0.120
	mBMP-mut ($\mu\text{g/mL}$)	200	-	< 0.001	-	-	-	0.300
		100	-	-	0.027	-	-	-
3 hour	mBMP ($\mu\text{g/mL}$)	300	-	-	-	-	-	-
		200	0.494	-	-	-	-	-
		100	0.657	0.312	-	-	-	-
		50	0.002	< 0.001	0.011	-	-	-
		0	< 0.001	< 0.001	0.001	< 0.001	-	-
	Rhodamine	50	0.005	0.004	0.030	0.103	0.001	0.504
	mBMP-mut ($\mu\text{g/mL}$)	200	-	< 0.001	-	-	-	0.533
		100	-	-	0.008	-	-	-

Table S3. P values from Student's t-test between the fluorescence intensity of modular peptide bound to cortical bone by incubating in peptide solution for various time periods.

		1 hour	2 hour	3 hour
mBMP 50 µg/mL	0.5 hour	0.175	0.045	< 0.001
	1 hour	-	0.212	0.035
	2 hour	-	-	0.705
mBMP 100 µg/mL	0.5 hour	0.294	0.770	0.046
	1 hour	-	0.253	0.019
	2 hour	-	-	0.081
mBMP 200 µg/mL	0.5 hour	0.014	0.027	0.002
	1 hour	-	0.199	0.009
	2 hour	-	-	0.093
mBMP 300 µg/mL	0.5 hour	0.585	0.107	0.151
	1 hour	-	0.029	0.159
	2 hour	-	-	0.424
mBMP-mut 100 µg/mL	0.5 hour	0.044	0.043	0.090
	1 hour	-	0.631	0.725
	2 hour	-	-	0.489
mBMP-mut 200 µg/mL	0.5 hour	0.961	0.746	0.498
	1 hour	-	0.775	0.462
	2 hour	-	-	0.325

Table S4. P values from Student's t-test between the fluorescence intensity of mBMP bound to trabecular bone by culturing in an *ex vivo* bone reactor circulating 100 µg/mL mBMP solution.

	2 hour	4 hour	6 hour	8 hour	10 hour
2 hour	-	-	-	-	-
4 hour	< 0.001	-	-	-	-
6 hour	0.013	0.528	-	-	-
8 hour	0.002	0.303	0.112	-	-
10 hour	< 0.001	0.528	0.201	0.404	-
Control	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

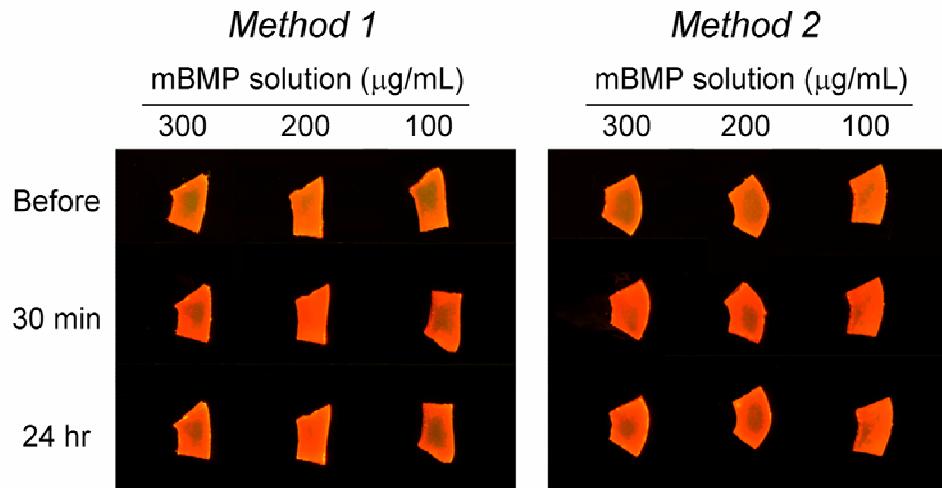


Figure S1. Fluorescence images of mBMP-bound cortical bone after treating with sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS). The peptide was bound to cortical bone by incubating in mBMP solution with various concentrations. The mBMP-bound bone was treated with 0.2 N NaOH solution for 7 min and neutralized with hydrochloric acid (HCl). The resulting bone piece was subsequently incubated in absence (method 1) or presence (method 2) of 1% SDS under mild rotation.