A Protein-Nanoparticle Conjugate Platform for Simplified and Sensitive Protease Activity Assays

Masafumi Sakono[1]*, Kazuki Higashi[1], Mitsuki Nakamura[1], Naomi Sakono[2]

[1] Department of Applied Chemistry, Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama, Toyama 930-8555, Japan

[2] Department of Applied Chemistry and Chemical Engineering, National Institute of Technology, Toyama College, 13 Hongo, Toyama, Toyama 939-8630, Japan

*Corresponding author: Masafumi Sakono

Department of Applied Chemistry, Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama, Toyama 930-8555, Japan

E-mail: msakono@eng.u-toyama.ac.jp

Tel: +81-76-476-6845

Figure S1 Full image of the electrophoresis gel as shown in Figure 2.

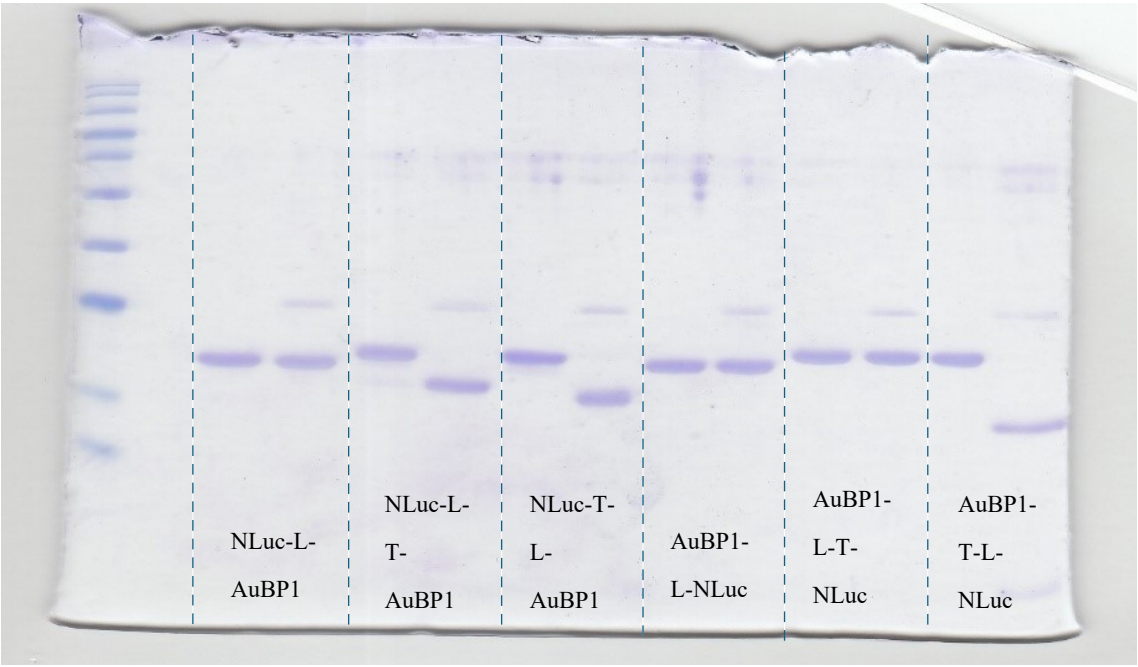

Table S1 The numerical data shown in the graph presented in Figure 3.

	Intensity	Error
NLuc	609.8	99.781
NLuc-(G4S)2-AuBP1	208.57	22.736
NLuc-TEVsub-(G4S)2-NLuc	69.803	3.6172
NLuc-(G4S)2-TEVsub-AuBP1	182.43	10.938
AuBP1-(G4S)2-NLuc	551.5	38.14
AuBP1-TEVsub-(G4S)2-NLuc	372.6	40.465
AuBP1-(G4S)2-TEVsub-NLuc	360	35.388

Table S2 The numerical data shown in the graph presented in Figure 4(a).

wavelength(nm)	Abs
600	0.068151
599	0.070032
598	0.071476
597	0.075012
596	0.077754
595	0.078199
594	0.080316
593	0.082929
592	0.084756
591	0.088029
590	0.089998
589	0.092264
588	0.094081
587	0.098172
586	0.1015
585	0.10527
584	0.10748
583	0.11032
582	0.11462
581	0.1181
580	0.12117
579	0.12412
578	0.12867
577	0.13295
576	0.13658
575	0.13962
574	0.14339
573	0.14897
572	0.15372
571	0.15845
570	0.16239
569	0.16668

568	0.17045
567	0.17638
566	0.18221
565	0.18625
564	0.1906
563	0.19621
562	0.20119
561	0.2076
560	0.21187
559	0.21842
558	0.22397
557	0.22827
556	0.23463
555	0.24065
554	0.24741
553	0.25243
552	0.25662
551	0.26197
550	0.26765
549	0.27313
548	0.27821
547	0.2839
546	0.28951
545	0.29654
544	0.30066
543	0.30638
542	0.31229
541	0.31727
540	0.32156
539	0.32493
538	0.3299
537	0.33578
536	0.34035
535	0.3447
534	0.34617

533	0.34953
532	0.35291
531	0.35521
530	0.35868
529	0.36047
528	0.36367
527	0.36573
526	0.36712
525	0.36759
524	0.36761
523	0.36897
522	0.37001
521	0.37061
520	0.37098
519	0.36834
518	0.36793
517	0.36691
516	0.36588
515	0.3635
514	0.36066
513	0.358
512	0.35467
511	0.3532
510	0.35277
509	0.34821
508	0.34445
507	0.34023
506	0.33599
505	0.33179
504	0.32778
503	0.32514
502	0.32225
501	0.31826
500	0.31356
499	0.30688

498	0.30369
497	0.30107
496	0.29697
495	0.29422
494	0.28904
493	0.28523
492	0.28113
491	0.27851
490	0.27511
489	0.27174
488	0.26914
487	0.26516
486	0.26158
485	0.25956
484	0.25547
483	0.25241
482	0.25058
481	0.24834
480	0.24645
479	0.24371
478	0.24257
477	0.24114
476	0.23952
475	0.23745
474	0.23511
473	0.23324
472	0.23085
471	0.23024
470	0.22995
469	0.22802
468	0.2263
467	0.22628
466	0.22462
465	0.22251
464	0.22205

463	0.22149
462	0.22027
461	0.22009
460	0.21987
459	0.21787
458	0.2196
457	0.21838
456	0.2171
455	0.21556
454	0.21577
453	0.21514
452	0.21345
451	0.21341
450	0.21422
449	0.21234
448	0.21258
447	0.21408
446	0.21197
445	0.2128
444	0.21347
443	0.21328
442	0.21388
441	0.21332
440	0.21195
439	0.21173
438	0.2118
437	0.21203
436	0.21191
435	0.21287
434	0.21208
433	0.21316
432	0.21354
431	0.21298
430	0.21359
429	0.21232

428	0.21384
427	0.21371
426	0.21275
425	0.21258
424	0.2135
423	0.21288
422	0.21319
421	0.21434
420	0.21306
419	0.21529
418	0.21187
417	0.21542
416	0.21437
415	0.21447
414	0.21705
413	0.21393
412	0.21681
411	0.21492
410	0.2162
409	0.21766
408	0.21684
407	0.21908
406	0.21617
405	0.2161
404	0.21844
403	0.21931
402	0.22144
401	0.22278
400	0.22231

Table S3 The numerical data shown in the graph presented in Figure 4(b).

nm	Number	ratio
0-2	0	0
2-4	0	0
4-6	0	0
6-8	0	0
8-10	3	1.363636
10-12	7	3.181818
12-14	100	45.45455
14-16	24	10.90909
16-18	67	30.45455
18-20	7	3.181818
20-22	12	5.454545
total	220	100

Table S4 The numerical data shown in the graph presented in Figure 4(c).

	Intensity	Error
NLuc	4.587	0.25138
AuBP1-(G4S)2-NLuc	56.863	1.247

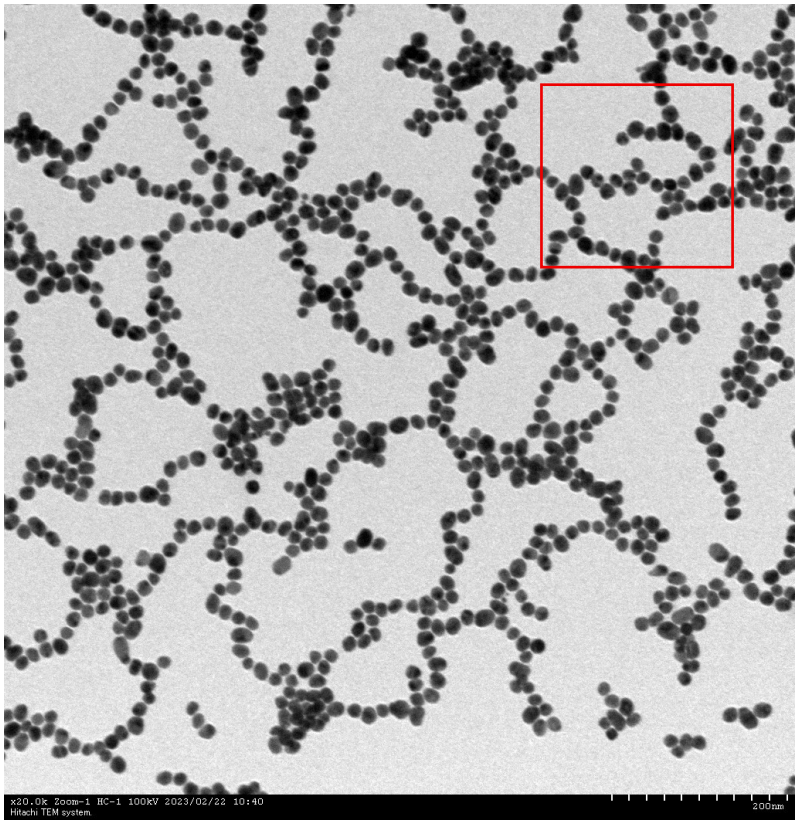

Figure S2 Full electrophoresis gel image corresponding to the inset in Figure 4(b).

Table S5 The numerical data shown in the graph presented in Figure 5.

(a)

	Relative intensity	error
AuBP1-L-Nluc	1.356	0.070449
AuBP1-L-T-Nluc	26.246	2.2839
AuBP1-T-L-Nluc	82.312	11.492

(b)

	Relative intensity	error
AuBP1-L-Nluc	1.664	0.088058
AuBP1-L-T-Nluc	11.15	0.89307
AuBP1-T-L-Nluc	31.846	1.8956

Table S6 The numerical data shown in the graph presented in Figure 6.

(a)

Conc. of TEVp	Intensity
0.000	1
0.002	7.767208
0.011	15.42026
0.044	28.59422
0.066	50.15497
0.087	63.40258
0.109	86.98868
0.131	87.36613
0.153	96.66431
0.197	109.0926
0.253	122.3494
0.404	137.2633
0.505	142.6949
0.607	149.6916
0.911	152.0852
1.518	155.9518

(b)

0.000	1
0.001	1.729447
0.005	3.165051
0.011	4.018633
0.022	9.874732
0.043	16.07075
0.054	17.25859
0.065	21.49062
0.076	22.41596
0.087	23.41685
0.098	23.92673
0.143	28.34571
0.172	29.49767
0.200	27.77918
0.229	30.29082

0.286	29.85648
0.316	30.36636
0.421	30.78182
0.526	31.7827
0.632	31.63163
0.842	28.68564
1.053	29.04444

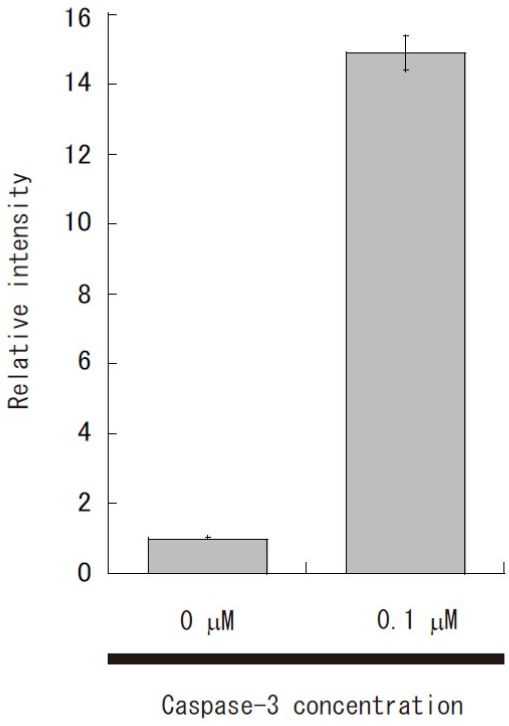

Figure S3 Detection of caspase-3 activity using the AuNP-conjugated luciferase system. A substrate protein was constructed by replacing the TEVp recognition sequence (T) in AuBP1-T-L-NLuc with the caspase-3 substrate sequence DEVD. The reaction conditions, including protein concentration and incubation time, were identical to those used for TEVp measurements. Relative luminescence intensity was calculated by normalizing the luminescence signal to that of the corresponding sample without caspase-3 addition.