

Electronic Supplementary Information

An integrated, peptide-based approach to site-specific protein immobilization for detection of biomolecular interactions.

Ilmar C. Kruis,^{t,†} Dennis W.P.M. Löwik,[†] Wilbert C. Boelens,[†] Jan C.M. van Hest[‡] and Ger J.M. Pruijn^{†}*

[†]Radboud University, Department of Biomolecular Chemistry, Institute for Molecules and Materials and Radboud Institute for Molecular Life Science, Nijmegen, The Netherlands. Electronic address: g.pruijn@ncmls.ru.nl.

[‡]Radboud University, Department of Bioorganic Chemistry, Institute for Molecules and Materials, Nijmegen, The Netherlands. Electronic address: j.vanhest@science.ru.nl.

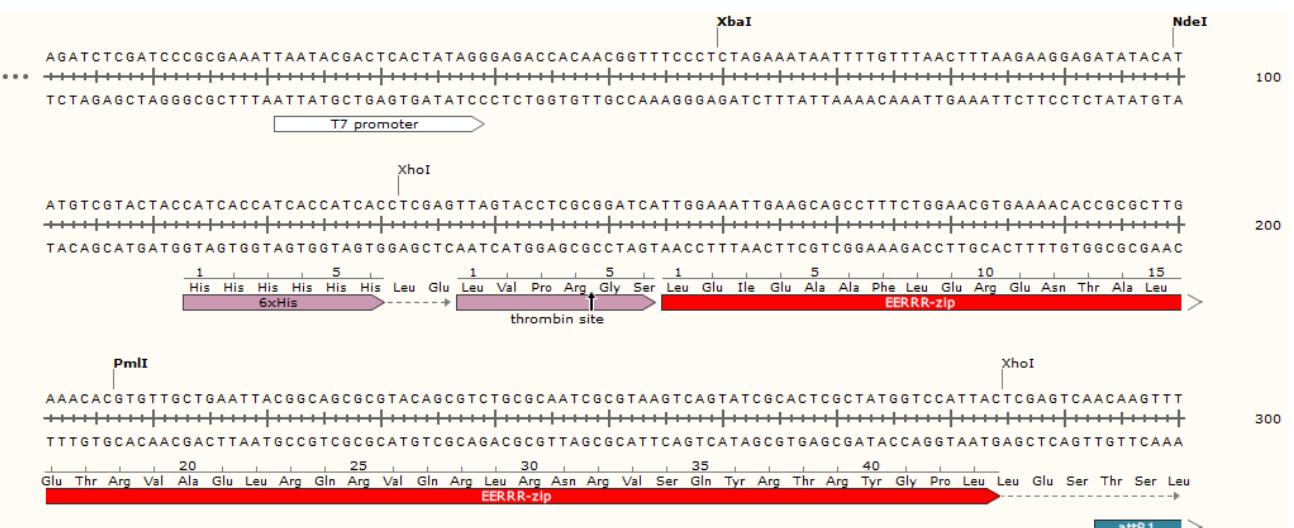
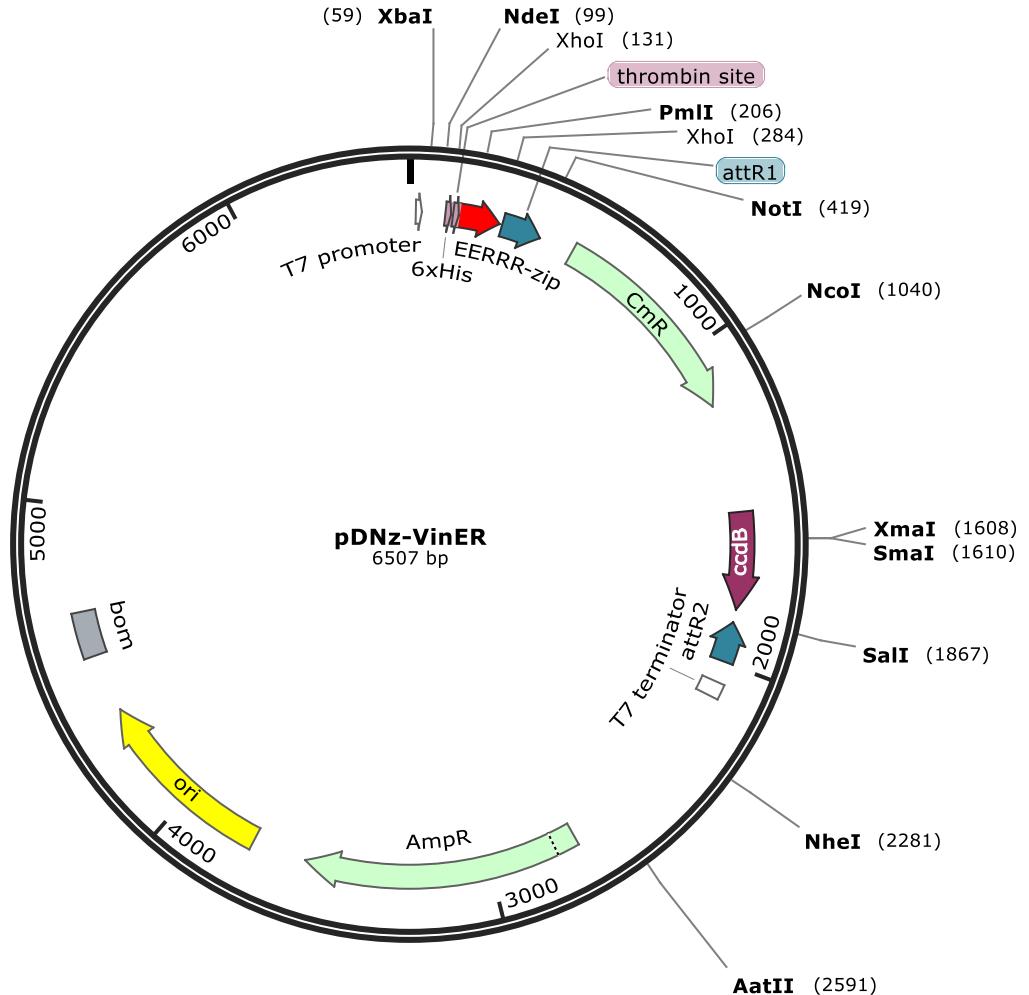


Figure S1: Vector map and zipper sequence of pDNz-VinER.

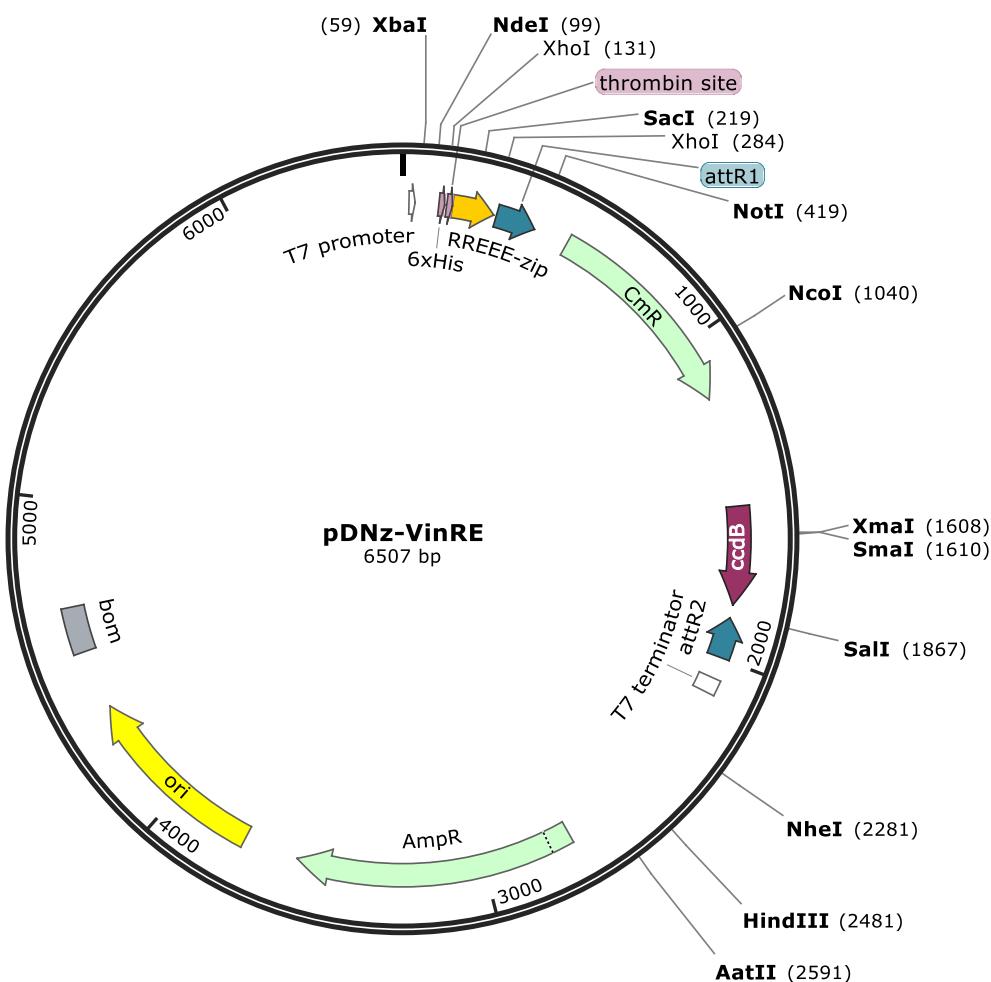


Figure S2: Vector map and sequence of pDNz-VinRE.

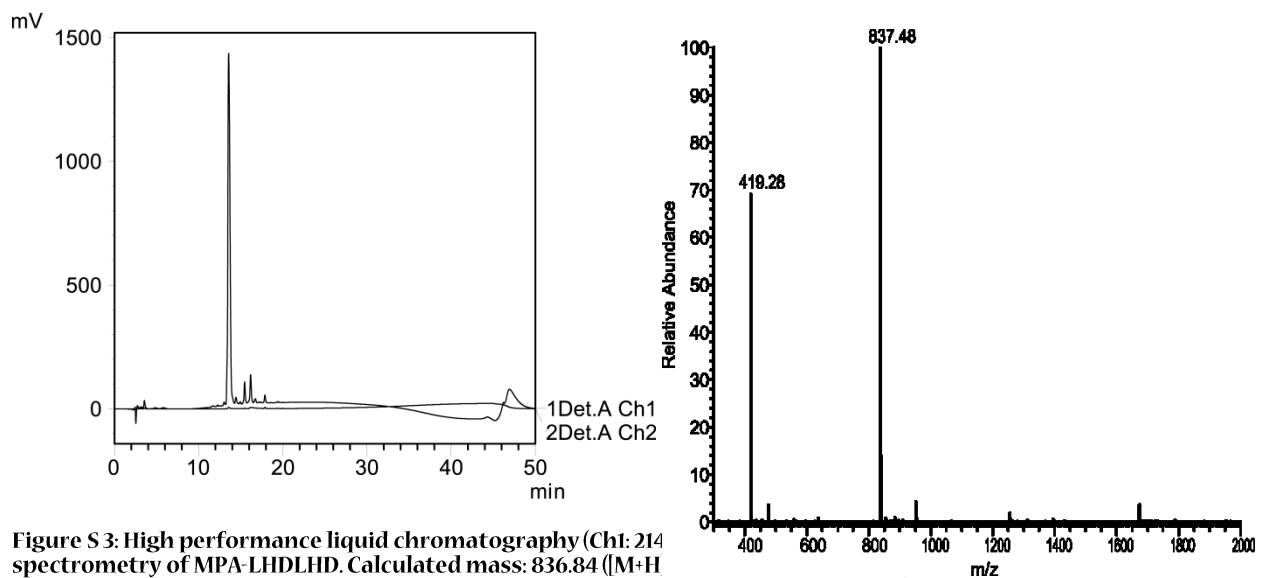


Figure S3: High performance liquid chromatography (Ch1: 214 nm) and mass spectrometry of MPA-LHDLHD. Calculated mass: 836.84 ($[M+H]^+$)

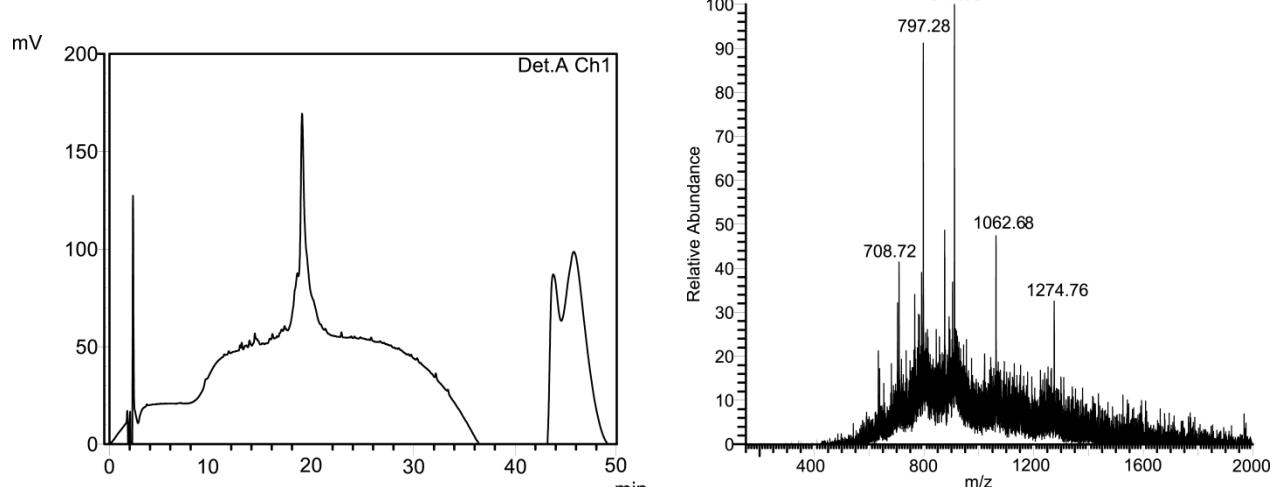


Figure S4: High performance liquid chromatography (Ch1: 214 nm) and electron spray mass spectrometry of ER leucine zipper peptide. Calculated mass: 6370.14 ($[M+5H]^{5+}$ 1274.76, $[M+6H]^{6+}$ 1062.68, $[M+7H]^{7+}$ 911.08, $[M+8H]^{8+}$ 797.28, $[M+9H]^{9+}$ 708.72).

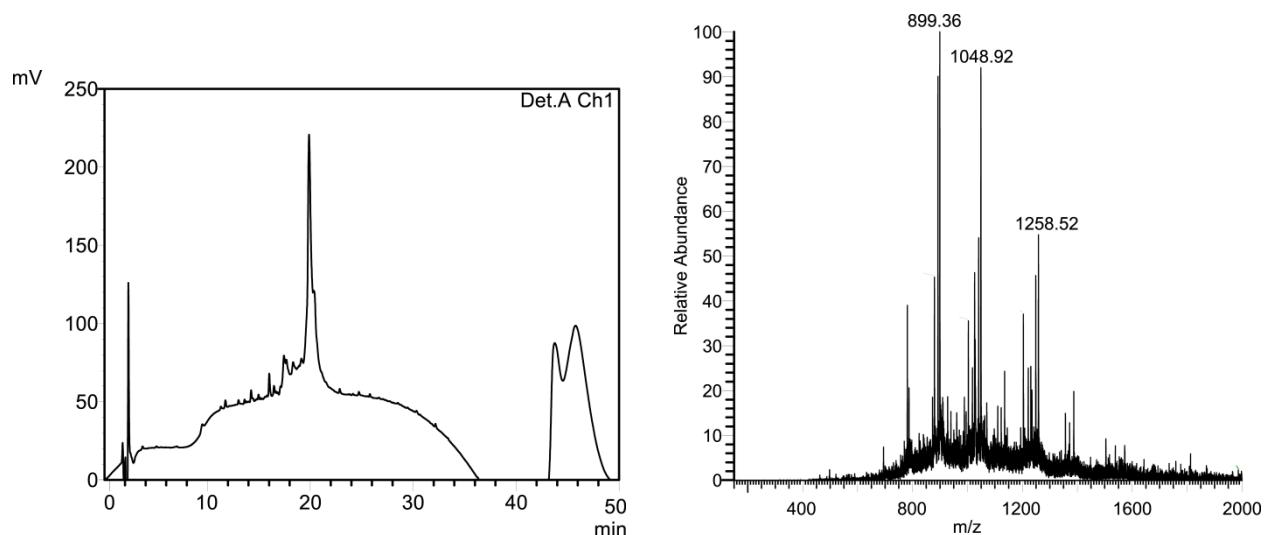


Figure S5: High performance liquid chromatography (Ch1: 214 nm) and electron spray mass spectrometry of RE leucine zipper peptide. Calculated mass: 6287.94 ($[M+5H]^{5+}$) 1258.52, $[M+6H]^{6+}$ 1048.92, $[M+7H]^{7+}$ 899.36).

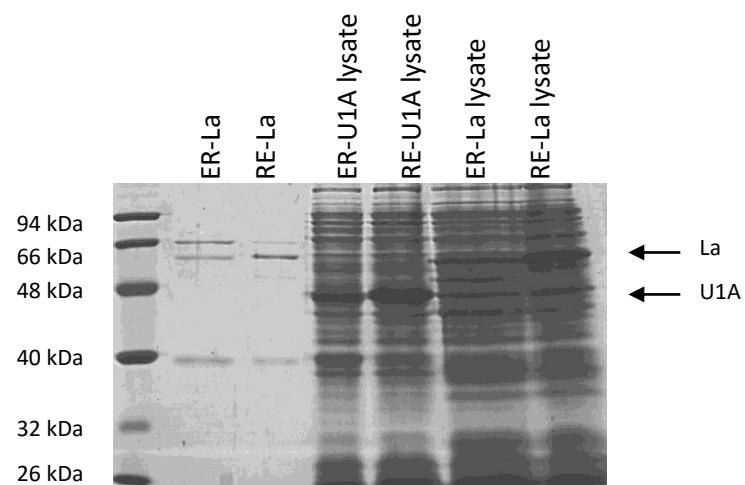


Figure S6: SDS-PAGE with coomassie brilliant blue protein stain of leucine zipper-tagged La solutions and lysates containing leucine zipper-tagged La and U1A proteins.

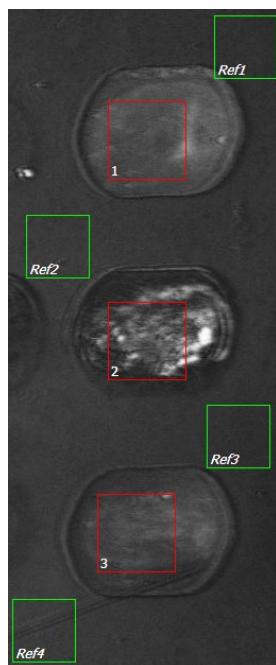


Figure S7: Example of a view through the iSPR camera. Three microarray-spots, part of the measurements depicted in figure 4b, are visible with regions of interest defined in red (1: RE-La, 2: covalent, 3: ER-La) and adjacent reference regions in green.

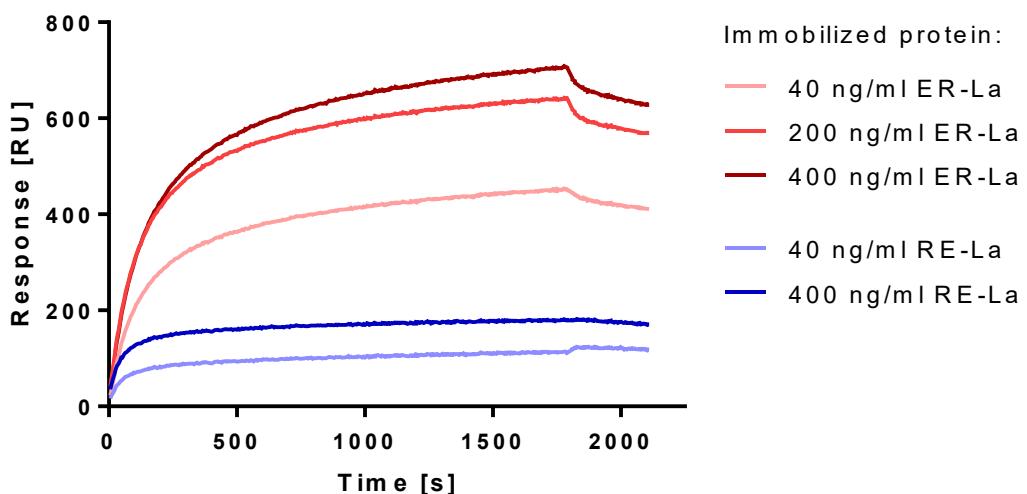


Figure S8: Sensorgram visualizing binding of 0.4 µg/mL anti-La monoclonal antibody SW5 to the La protein, tagged with a leucine zipper complementary (ER) or non-complementary (RE) to the sensor surface, immobilized using various concentrations. Binding was performed in PBS containing 0.075% Tween-80 for 1800 s, after which dissociation conditions (buffer wash) were applied for 480 s. Non-specific binding signals to reference areas of the surface were subtracted. RU: response units.

Table S1: Oligonucleotides used in cloning

Mutational primers for insertion of Xhol site in pDEST17	forward: CACCATCACCATCACCTCGAGTCAACAAGTTGTACAA reverse: TTGTACAAACTTGTGACTCGAGGTGATGGTGATGGTG
RE zipper oligonucleotide	forward: GCATCTCGAGTTAGTACCTCGCGGATCACTGAAATTGGCAGCC TTTTTACGCCAGCGGAATACTGCTTCGCACTGAAGTTGCAGAGCTCG reverse: GAGCCTCGAGTAAAGGTCCATACCGCGTTCTACTGACTAACTTC GTTTCCAGGCCTGCACTCCTGTTGAGCTCTGCAACTTCAGTGC
ER zipper oligonucleotide	forward: GTCGCTCGAGTTAGTACCTCGCGGATCATTGAAATTGAAGCAGCCTT CTGGAACGTGAAAACACCGCGTTGAAACACGTGTTGC reverse: GCTGCTCGAGTAATGGACCATAGCGAGTGCCTACTGACTTACCGGATTGCG CAGACGCTGTACCGCTGCCGTATTCAAGAACACGTGTTCAAGCGCGG