Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Comparison of alternative nucleophiles for Sortase A-mediated bioconjugation and application in neuronal cell labelling

Samuel Baer[a], Julie Nigro[a,b], Mariusz P. Madej[a], Rebecca M. Nisbet[a,b], Randy Suryadinata[a], Gregory Coia[a], Lisa P. T. Hong[a], Timothy E. Adams[a], Charlotte C. Williams[*a†], Stewart D. Nuttall[a,b†].

[a]CSIRO Materials Science and Engineering, 343 Royal Parade, Parkville, Victoria, 3052, AUSTRALIA. [b]Preventative Health Flagship, 343 Royal Parade, Parkville, Victoria, 3052, AUSTRALIA.

*Correspondence to: Charlotte C. Williams (Charlotte.Williams@csiro.au) at CSIRO Materials Science and Engineering, 343 Royal Parade, Parkville, Victoria, 3052, AUSTRALIA; Ph: +61 3 9662 7100).

† These authors contributed equally to the manuscript

Supplementary item	Relationship to main items
Experimental	Method for expression and purification of SrtA.
Figure S1	Results describing determination of affect of nucleophile and SrtA enzyme concentrations on progress of reactions. Important for optimization of reaction conditions used for testing of 5 nucleophiles against 5 different proteins and peptides described in the figure.
Figure S2	Supporting data demonstrating that SrtA-mediated labelling is LPETG recognition sequence specific.
Figure S3	Supporting data demonstrating SrtA-mediated labelling time-course for GGG-EDA-TAMRA; Lysine-EDA-TAMRA; and Cadaverine-TAMRA.

SUPPLEMENTARY EXPERIMENTAL

Expression and purification of SrtA

For protein expression, pET28a–SrtA plasmid DNA was transformed into *E. coli* BL21(DE3) and a starter culture (2YT medium/Kanamycin 30 µg/mL) diluted into fresh medium and grown at 37°C/ 200 RPM to A_{600nm} = 0.7. Protein expression was induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 3 h at 37°C. *E. coli* cells were harvested by centrifugation (Beckman JA-14/5,524 g/10min/4°C) and a 10 g pellet was re-suspended in 100 ml cellular extraction buffer (50 mM Tris, 150 mM NaCl, 10 mM imidazole, 10 % Glycerol, 2 mM $MgCl_2$, pH 7.4). To facilitate cellular lysis and reduce viscosity prior to cell crushing, lysozyme (0.25 mg/ml) and Benzonase (Novagen Cat No. 71205 at 0.0025 u/µl) were added. The lysate was passed three times through an EmulsiFlex-C5 cell crusher (Avestin), (15,000 psi/ 4ºC), centrifuged (Beckman JA 25.5/48,384 g/15 min/4ºC), and the clarified lysate was filtered through a syringe filter (Millipore Millex SV-5µM). Recombinant hexahistindine-tagged SrtA (predicted molecular weight 18,891 Da) was purified from the clarified lysate by IMAC chromatography (HisTrap HP 5 ml, ÄKTA Purifier, GE Healthcare), with a step gradient (20 mM sodium phosphate, 500 mM NaCl, 250 mM imidazole, pH 7.4), and further polished by gel filtration chromatography on a HiLoad Superdex 200 26/60 Prep Grade column (GE Healthcare) pre-equilibrated in 1 × TBS (50 mM Tris, 150 mM NaCl, 10% Glycerol, pH 7.4). Protein yields were routinely of the order of 35 mg/L. Aliquots of SrtA protein were stored as aliquots at -20°C in the presence of 10 % glycerol.

SUPPLEMENTARY FIGURES

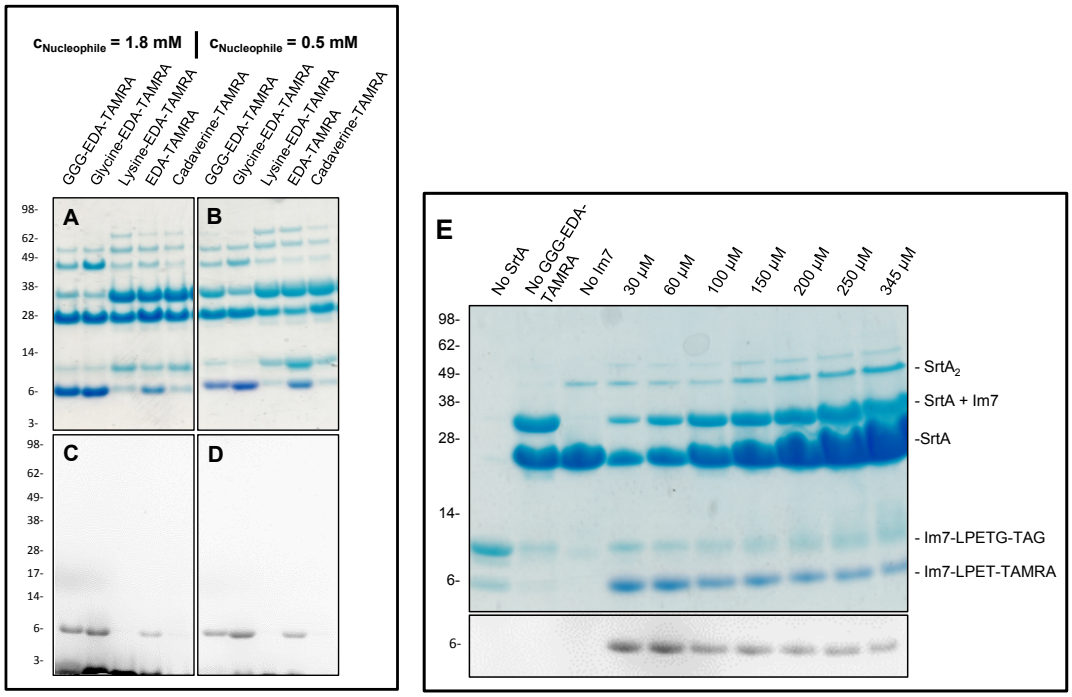

Fig. S1. Affect of nucleophile and SrtA concentration on SrtA-mediated labelling. (**A, B**) Coomassie stained non-reducing SDS-PAGE gel. (**C, D**) Image of same gels measured at 605 nm to detect fluorescence. (**A**) SrtA-mediated labelling in the presence of 1.8 mM nucleophile; (**B**) SrtA-mediated labelling in the presence of 0.5 mM nucleophile; (**C**) Fluorescent image of (**A**), bands at the bottom of the gel are due to excess, unreacted rhodamine-labelled compound; (**D**) Fluorescent image of (**B**); (**E**) Effect of increasing concentration of SrtA (30 – 345 µM) on labelling of Im7-LPETGG, illustrated by Coomassie brilliant blue staining (top panel) and fluorescence imaging (bottom panel).

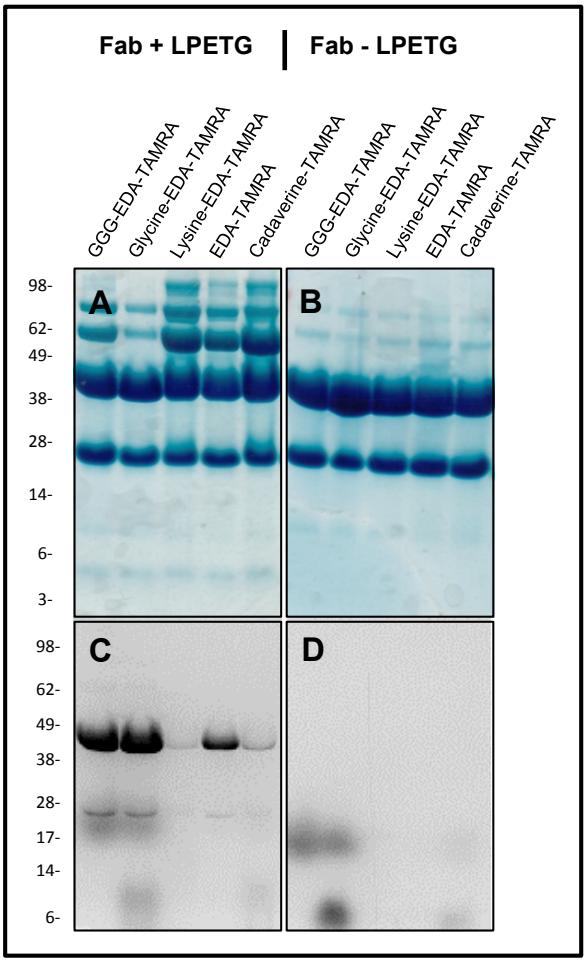

Fig. S2. SrtA-mediated labelling is LPETG recognition sequence specific. (**A, B**) Coomassie stained non-reducing SDS-PAGE gel. (**C, D**) Image of the same gels measured at 605 nm to detect fluorescence. (**A**) Antibody Fab + LPETG tag labelled using SrtA; (**B**) Antibody Fab alone labelled using SrtA; (**C**) Fluorescent image of (A); (**D**) Fluorescent image of (**B**).

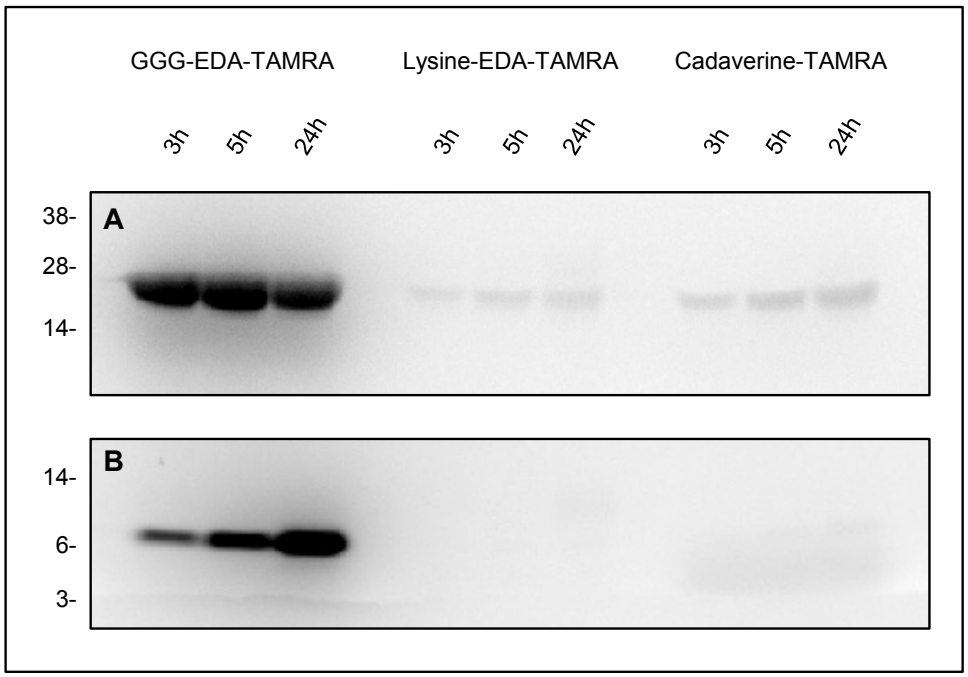

Fig. S3. Affect of incubation time and nucleophile on SrtA-mediated labelling. Images of non-reducing SDS-PAGE gels measured at 605 nm to detect fluorescence. (**A**) Rhodamine fluorescence image of SrtA-mediated labelling of scFv antibody fragment with GGG-EDA-TAMRA, Lysine-EDA-TAMRA, or Cadaverine-EDA-TAMRA. (**B**) As for (A) except labelling of Im7 protein scaffold. Approximate molecular weights (in kDa) are shown to the left.