Experiment

Date: 07-04-2015

Aim: *purification of CsgG constriction mutant*

Production conditions

Strain: CsgG-ΔPYPA Cloop mutant in pASK-IBA3+ (amp) in BL21(DE3) cells

Medium: TB

Induction: O/N @ 25°C when OD600=0.6 with anhydrotetracycline

Buffer: 50 mM Tris pH 8.0, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 0.1 mg AEBSF/ml buffer, Dnase, 0.1 mg/ml lysozyme, leupeptine

Strep chromatography

Loading buffer: 25 mM Tris pH 8, 500 mM NaCl, 1 mM EDTA, 1 mM DTT

Elution buffer: 100 mM Tris pH 8.0, 500 mM NaCl, 1 mM DTT, 2.5 mM Desthiobiotin, **5 mM LDAO, 0.5% C8E4**

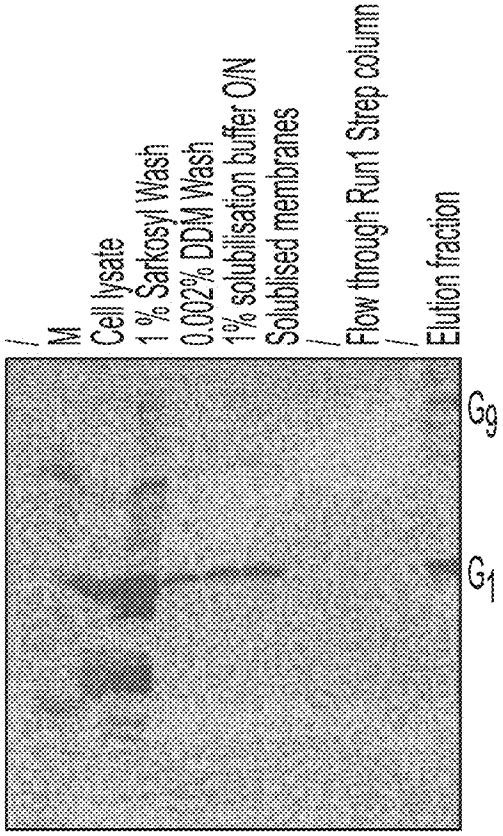
Western Blot

Anti-Strep

M: PageRuler prestained protein ladder

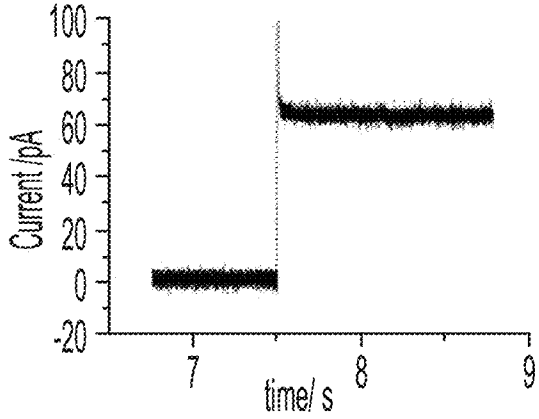
Purification and channel properties of CsgG-ΔPYPA

CsgG with mutation of $P^{50}Y^{51}P^{52}A^{53}$ to GG



G_1: CsgG-ΔPYPA monomer

G_g: CsgG-ΔPYPA nonamer



CsgG-ΔPYPA current trace at +50 mV

Figure 57