

Kinetic mechanisms of fast glutamate sensing by fluorescent protein probes

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Running title: *Fast glutamate sensor kinetics*

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**SUPPORTING MATERIAL**

Global fitting/simulation of association kinetic data using Dynafit.

Note that the units when considering the rate constants, the time scale of the data is either in scale seconds or ms. Concentration unit is micromolar.

### iGlu-E25A (iGlu<sub>n</sub>) Scheme 1 (two fluorescent states)

Rate constant assignments correspond to those in the main text as follows:

k1 ( $k_{+1}$ ); k2 ( $k_{-1}$ ); k3 ( $k_{+2}$ ); k4 ( $k_{-2}$ );

[task]

data = progress

task = fit

[mechanism]

Glu + iGlucomplete <===> Glu.iGlucomplete : k1 k2

Glu.iGlucomplete <===> Glu.iGlucomplete\* : k3 k4

[constants]; micromolar scale

k1 = 210 , k2 = 53500

k3 = 1243 , k4 = 42

[responses]

Glu.iGlucomplete = 0.89

Glu.iGlucomplete\* = 0.92

[concentrations]; micromolar concentrations

iGlucomplete = 1

[data]

directory ./E25A\_ass\_fit\_new/Data/

extension txt

mesh from 0.00001 to 0.020 step 0.0001

error constant 0.001

**Note the correction to concentrations for best fit.**

file Glu0 | conc Glu = 9

file Glu1 | conc Glu = 21

file Glu2 | conc Glu = 75

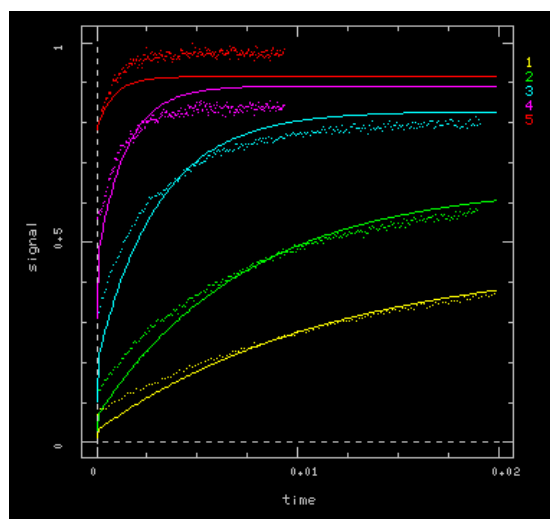
file Glu3 | conc Glu = 274

file Glu4 | conc Glu = 2000

[output]

directory ./E25A\_ass\_fit\_new/Output/

[end]



## iGlu-T92A (iGlu) Scheme 2

Rate constant assignments correspond to those in the main text as follows:

k1 ( $k_{+3}$  ; k2 ( $k_{-3}$ ) ; k3 ( $k_{+4}$ ) ; k4 ( $k_{-4}$ ) ;

[task]

data = progress

task = fit

[mechanism]

GluBP <====> GluBP\* : k1 k2

Glu + GluBP\* <====> Glu.GluBP\*\* : k3 k4

[constants] ; micromolar scale

k1 = 0.4 , k2 = 0.8

k3 = 0.002, k4 = 85

[responses]

Glu.GluBP\*\* = 0.7

[concentrations] ; micromolar concentrations

GluBP = 1.7

GluBP\* = 0.95

[data]

directory ./T92A\_ass\_fit\_new/Data/

extension txt

mesh from 0.001 to 10 step 0.01

error constant 0.01

Note the correction to concentrations for best fit.

file Glu0 | conc Glu = 19870

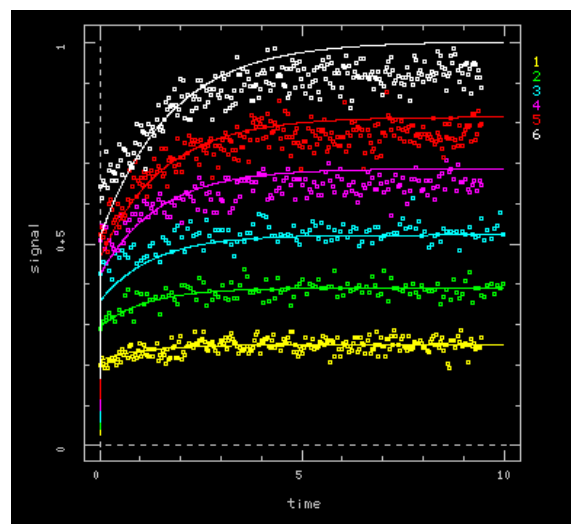
file Glu1 | conc Glu = 34000

file Glu2 | conc Glu = 50000

file Glu3 | conc Glu = 75000

file Glu4 | conc Glu = 100000

file Glu5 | conc Glu = 150000



[output]

directory ./T92A\_ass\_fit\_new/Output/

[end]

## iGlu-R24K (iGlu<sub>m</sub>) Scheme 2

Rate constant assignments correspond to those in the main text as follows:

k1 ( $k_{+3}$ ); k2 ( $k_{-3}$ ); k3 ( $k_{+4}$ ); k4 ( $k_{-4}$ );

[task]

data = progress

task = fit

[mechanism]

GluBP  $\rightleftharpoons$  GluBP\* : k1 k2

Glu + GluBP\*  $\rightleftharpoons$  Glu.GluBP\*\* : k3 k4

[constants]; micromolar scale

k1 = 0.436, k2 = 0.238

k3 = 0.006, k4 = 18

[responses]

Glu.GluBP\*\* = 1.12

[concentrations]; micromolar concentrations

GluBP = 0.455

GluBP\* = 0.7

[data]

directory ./R24K\_ass\_fit\_new/Data/

extension txt

mesh from 0.0001 to 14 step 0.01

error constant 0.001

**Note the correction to concentrations for best fit.**

file Glu0 | conc Glu = 3560

file Glu1 | conc Glu = 6250

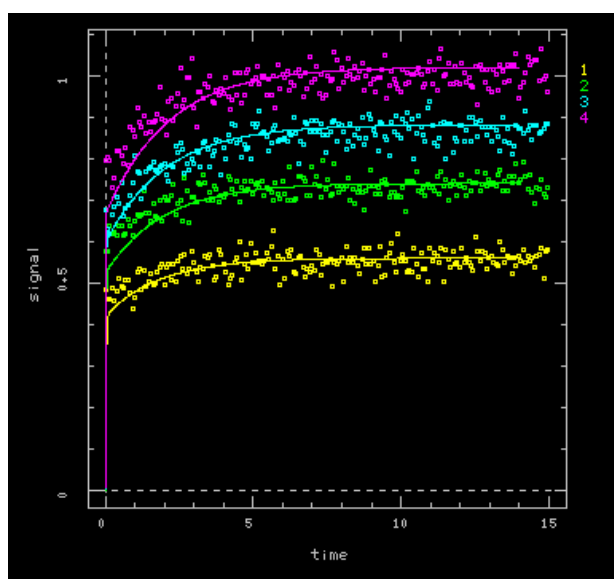
file Glu2 | conc Glu = 10000

file Glu3 | conc Glu = 17500

[output]

directory ./R24K\_ass\_fit\_new/Output/

[end]



### iGlu-R24K (iGlu<sub>m</sub>) Scheme 1 (two fluorescent states)

Rate constant assignments correspond to those in the main text as follows:

k1 ( $k_{+1}$ ) ; k2 ( $k_{-1}$ ) ; k3 ( $k_{+2}$ ) ; k4 ( $k_{-2}$ ) ;

[task]

data = progress

task = fit

[mechanism]

Glu + GluBP  $\rightleftharpoons$  Glu.GluBP : k1 k2

Glu.GluBP  $\rightleftharpoons$  Glu.GluBP\* : k3 k4

[constants] ; micromolar scale

k1 = 0.004 , k2 = 16

k3 = 0.135 , k4 = 0.365

[responses]

Glu.GluBP = 0.9

Glu.GluBP\* = 1.47

[concentrations] ; micromolar concentrations

GluBP = 1.1

[data]

directory ./R24K\_ass\_fit\_new/Data/

extension txt

mesh from 0.0001 to 14 step 0.01

error constant 0.001

**Note the correction to concentrations for best fit.**

file Glu0 | conc Glu = 2840

file Glu1 | conc Glu = 6250

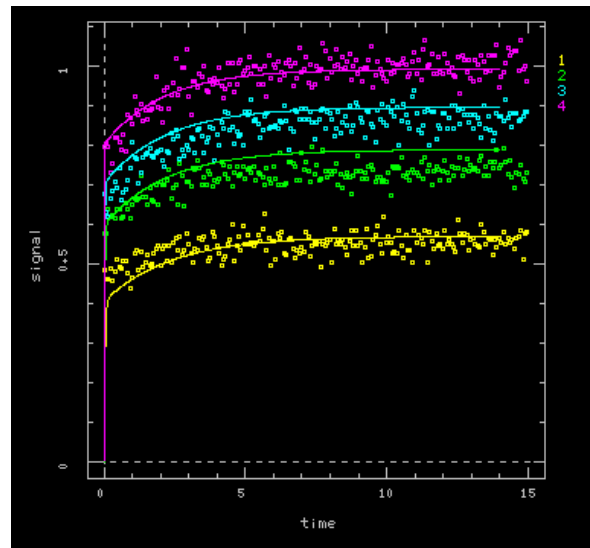
file Glu2 | conc Glu = 10000

file Glu3 | conc Glu = 17500

[output]

directory ./R24K\_ass\_fit\_new/Output/

[end]



### FI-GluBP Scheme 1 synthetic

Rate constant assignments correspond to those in the main text as follows:

$k_1 (k_{+1}) ; k_2 (k_{-1}) ; k_3 (k_{+2}) ; k_4 (k_{-2}) ;$

[task]

data = progress

task = fit

[mechanism]

$\text{Glu} + \text{GluBP} \rightleftharpoons \text{Glu.GluBP} \quad : \quad k_1 \quad k_2$

$\text{Glu.GluBP} \rightleftharpoons \text{Glu.GluBP}^* \quad : \quad k_3 \quad k_4$

[constants] ; micromolar scale

$k_1 = 1 , k_2 = 216$

$k_3 = 2.22 , k_4 = 0.230$

[responses]

$\text{Glu.GluBP} = 2$

$\text{Glu.GluBP}^* = 0.8$

[concentrations] ; micromolar concentrations

$\text{GluBP} = 1$

[data]

directory ./IANBD-T133C\_GluBP\_ass\_new/Data/

extension txt

mesh from 0.0001 to 8 step 0.01

error constant 0.001

Note the correction to concentrations for best fit.

file Glu0 | conc Glu = 3.6

file Glu1 | conc Glu = 5.6

file Glu2 | conc Glu = 10.5

file Glu3 | conc Glu = 14

file Glu4 | conc Glu = 25

file Glu5 | conc Glu = 60

file Glu6 | conc Glu = 80

file Glu7 | conc Glu = 115

[output]

directory ./IANBD-T133C\_GluBP\_ass\_new/Output/

[end]

