Supplementary material

We next explain all formulas and equations, starting from assumptions on the initial concentrations and how they were established, followed by the kinetic parameters and their assessment, and then driving inputs. Finally, the different formulas are combined for the kinetic rates.

Initial concentrations (µM):

The components are listed on the left, followed by their molecular name. For receptors we consider in addition whether they are activated or inhibited version. This is followed by the concentration plus a reference confirming the value assumed. Default values were zero ("manually set").

x[1]: IP (IP receptor)	1	relatively determined by RNA-quantification
x[2]: EP3 (EP3 receptor)	0.06	relatively determined by RNA-quantification
x[3]: EP4 (EP4 receptor)	0.034	relatively determined by RNA-quantification
x[4]: DP1 (DP1 receptor)	0.096	relatively determined by RNA-quantification
x[5]: P2Y12 (ADP receptor P2Y12)	0.1	relatively determined by RNA-quantification
x[6]: P2Y12_act (activated form of P2Y12)	0	manually set
x[7]: IP_act (activated form of IP)	0	manually set
x[8]: DP1_act (activated form of DP1	0	manually set
x[9]: DP1_BWA (inhibited form of DP1)	0	manually set
x[10]: EP3_act (activated form of EP3)	0	manually set
x[11]: EP3_L798 (inhibited form of EP3)	0	manually set
x[12]: EP4_act (activated form of EP4)	0	manually set
x[13]: EP4_L161 (inhibited form of EP4)	0	manually set
x[14]: AC (adenylate cyclase)	0.59	determined by mass-spectometry [6]
x[15]: aAC (activated form of adenylate cyclase)	0	manually set
x[16]: Fors_AC (forskulin-bound form of adenylate cyclase)	0	manually set
x[17]: cAMP (cyclic adenosine monophosphate)	3.4	determined by mass-spectometry [6]
x[18]: AMP (adenosine monophosphate)	0	manually set
x[19]: VASP (vasodilitator stimulated phosphoprotein)	10.34	determined by mass-spectometry [6]
x[20]: pVASP (phosphorylated form of VASP)	0	manually set
x[21]: PDE3 (phosphodiesterase 3)	0.31	determined by mass-spectometry [6]
x[22]: aPDE3 (activated form of phosphodiesterase 3)	0	manually set
x[23]: iPDE3 (inhibited form of phosphodiesterase 3)	0	manually set
x[24]: PDE2 (phosphodiesterase 2)	0.077	determined by mass-spectometry [6]
x[25]: PKA (protein kinase A)	2.04	determined by mass-spectometry [6]
x[26]: R (regulatory subunit of PKA)	0	manually set
x[27]: C (catalytic subunit of PKA)	0	manually set
x[28]: PKA_cAMP (PKA bound to single cAMP)	0	manually set
x[29]: PKA_cAMP_cAMP (PKA bound to two cAMPs)	0	manually set
x[30]: PKA_cAMP_cAMP_cAMP (PKA bount to three cAMPs)	0	manually set
x[31]: PKA_cAMP_cAMP_cAMP_cAMP (PKA bound to four cAMPs)	0	manually set
x[32]: R_cAMP_cAMP (regulatory subunit bound to two cAMPs)	0	manually set
x[33]: PKA_cBIMPS (PKA bound to one cBIMPS)	0	manually set
x[34]: PKA_cBIMPS_cBIMPS (PKA bound to two cBIMPSs)	0	manually set
x[35]: PKA_cBIMPS_cBIMPS_cBIMPS (PKA bound to three cBIMPSs)	0	manually set
x[36]: PKA_cBIMPS_cBIMPS_cBIMPS_cBIMPS (PKA bound to four cBIMPSs)	0	manually set
x[37]: R_cBIMPS_cBIMPS (regulatory subunit bound to two cBIMPSs)	0	manually set

Kinetic parameters:

The model parameters are listed on the left, followed by a short description. The second column contains the respective values. The third column states, whether the parameter was fitted along the data ("estimated"), taken from literature (reference number, e.g. "8"), or has a default value ("manually set").

k[1]: IP_activation_Ilo (affinity of IP to Iloprost)	7.7663	estimated
k[2]: DP1_activation_PGE (affinity of DP1 for PGE2)	0.0303	estimated
k[3]: DP1_inhibition (affinity of DP1 for BWA)	0.1539	estimated
k[4]: EP3_activation_PGE (affinity of EP3 for PGE2)	0.0271	estimated
k[5]: EP3_activation_Sul (affinity of EP3 for sulproston)	0.0086	estimated
k[6]: EP3_inhibition (affinity of EP3 for L798)	0.0033	estimated
k[7]: EP4_activation_PGE (affinity of EP4 for PGE2)	0.0113	estimated
k[8]: P2Y12_activation (affinity of P2Y12 for ADP)	9.5612e-05	estimated
k[9]: EP4_inhibition (affinity of EP4 for L161)	0.0043	estimated
k[10]: ATP (concentration of ATP)	5000	manually set
k[11]: AC_Vmax (Vmax of adenylate cyclase)	689.5944	estimated
k[12]: AC_km (Michaelis-Menten constant of adenylate cyclase)	0.021	8
k[13]: AC_k (deactivation constant of adenylate cyclase)	113.5769	estimated
k[14]: Fors_AC (activation rate of forskulin for adenylate cyclase)	1.3567e-05	estimated
k[15]: IP_AC (signalling rate of IP)	1.8319	estimated
k[16]: DP1_AC (signalling rate of DP1)	28.5	estimated
k[17]: EP3_AC (signalling rate of EP3)	335.9116	estimated
k[18]: EP4_AC (signalling rate of EP4)	293.3162	estimated
k[19]: P2Y12_AC (signalling rate of P2Y12)	18.2939	estimated
k[20]: PKA_AC (inhibition rate of PKA on AC)	0.0718	estimated
k[21]: pVASP_k (dephosphorylation rate of VASP)	0.0376	estimated
k[22]: PDE3_Vmax (Vmax of phosphodiesterase 3)	6.1	5,7
k[23]: PDE3_km (Michaelis-Menten constant of phosphodiesterase 3)	0.2	5,7
k[24]: PDE2_Vmax (Vmax of phosphodiesterase 2)	120	5,7
k[25]: PDE2_km (Michaelis-Menten constant of phosphodiesterase 2)	50	5,7
k[26]: aPDE3_k (dephosphorylation constant of phosphodiesterase 3)	0.0049	estimated
k[27]: iPDE3_k (inhibition rate of cilostamid on phosphodiesterase 3)	0.0063	estimated
k[28]: PKA_Vmax (Vmax of PKA)	19.6	25
k[29]: PKA_km (Michaelis-Menten constant of PKA)	17.4	25
k[30]: PKA_cAMPgain (binding rate of cAMP to PKA)	1915.4642	estimated
k[31]: PKA_cAMPloss (dissociation rate of cAMP to PKA)	8651.3628	estimated
k[32]: PKA_diss (dissociation rate of the PKA holoenzyme)	0.0111	estimated
k[33]: PKA_RcAMPdiss (dissociation rate of cAMP from regulatory subunit)	0.6151	estimated
k[34]: PKA_ass (association rate of PKA subunits)	19403.9022	estimated
k[35]: PKA_cBIMPSgain (bindig constant of cBIMPS to PKA)	7.7088e-005	estimated
k[36]: PKA_cBIMPSloss (dissociation constant of cBIMPS from PKA)	0.0116	estimated
k[37]: PKA_RcBIMPSdiss (dissociation rate of cBIMPS from reg. subunit)	0.0026	estimated
k[38]: error_rel (relative error)	0.06	estimated
k[39]: error_reltomax (error relative to the maximum)	0.03	estimated

Driving inputs:

Abbreviation used in the model, followed by full name.

u[1]: PGE (Prostglandin E2)
u[2]: Ilo (Iloprost)
u[3]: L161 (L161)
u[4]: Fors (Forskulin)
u[5]: Cilo (Cilostamid)
u[6]: L798 (L798)
u[7]: Sul (Sulproston)

u[8]: BWA (BWA 868C)
u[9]: ADP (Adenosine diphosphate)
u[10]: cBIMPS (Sp-5,6-DCl-cBIMPS)

Rates:

Kinetic rates represented by a formula consisting of players, parameters and driving inputs (v = f (x,k,u)).

```
v[ 1]  = k[1]*x[1]*u[2];
v[ 2]  = k[2]*x[4]*u[1];
v[ 3]  = k[3]*x[4]*u[8];
v[ 4]  = k[4]*x[2]*u[1];
v[ 5]  = k[7]*x[3]*u[1];
v[ 6]  = k[9]*x[3]*u[3];
v[ 7]  = k[8]*x[5]*u[9];
v[ 8]  = (x[14]*((x[7]*k[16]) + (x[8]*k[17]) + (x[12]*k[19])))/(1.0 + (k[18]*x[10]) + (k[20]*x[6]));
v[ 9]  = k[13]*x[15];
v[10]  = k[15]*x[14]*u[4];
v[11]  = (k[10]*k[11]*x[15])/((k[12] + k[10])*(1.0 + (x[27]/k[14])));
v[12]  = (k[10]*k[11]*x[16])/((k[12] + k[10])*(1.0 + (x[27]/k[14])));
v[13]  = k[30]*x[17]*x[25];
v[14]  = k[30]*x[17]*x[28];
v[15]  = k[30]*x[17]*x[29];
v[16]  = k[30]*x[17]*x[30];
v[17]  = k[31]*x[31];
v[18]  = k[31]*x[30];
v[19]  = k[31]*x[29];
v[20]  = k[31]*x[28];
v[21]  = k[32]*x[31];
v[22]  = k[35]*x[25]*u[10];
v[23]  = k[33]*x[32];
v[24]  = k[35]*x[33]*u[10];
v[25]  = k[35]*x[34]*u[10];
v[26]  = k[35]*x[35]*u[10];
v[27]  = k[36]*x[36];
v[28]  = k[36]*x[35];
v[29]  = k[36]*x[34];
v[30]  = k[36]*x[33];
v[31]  = k[32]*x[36];
v[32]  = k[37]*x[37];
v[33]  = k[34]*x[26]*x[26]*x[27]*x[27];
v[34]  = (x[17]*k[22]*x[22])/(k[23] + x[17]);
v[35]  = (x[21]*k[28]*x[27])/(k[29] + x[21]);
v[36]  = k[26]*x[22];
v[37]  = k[27]*x[21]*u[5];
v[38]  = (x[19]*k[28]*x[27])/(k[29] + x[19]);
v[39]  = k[21]*x[20];
v[40]  = k[5]*x[2]*u[7];
v[41]  = k[6]*x[2]*u[6];
v[42]  = (x[17]*k[24]*x[24])/(k[25] + x[17]);
```

ODEs:

Differential equations built of kinetic rates.

```
dxdt[ 1] =  - v[1];
dxdt[ 2] =  - v[4] - v[40] - v[41];
dxdt[ 3] =  - v[5] - v[6];
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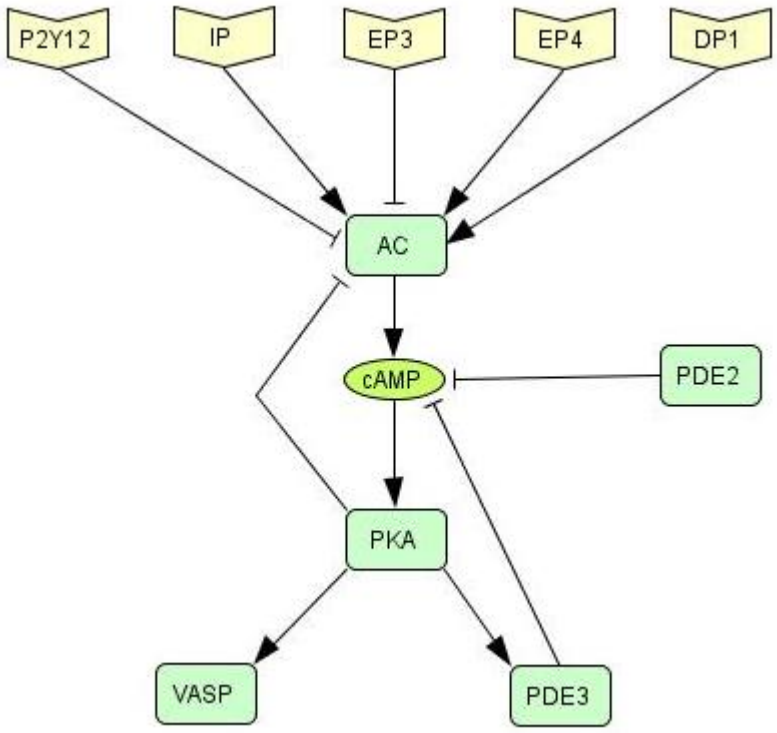
```
dxdt[ 4] =  - v[2] - v[3];
dxdt[ 5] =  - v[7];
dxdt[ 6] = v[7];
dxdt[ 7] = v[1];
dxdt[ 8] = v[2];
dxdt[ 9] = v[3];
dxdt[10] = v[4] + v[40];
dxdt[11] = v[41];
dxdt[12] = v[5];
dxdt[13] = v[6];
dxdt[14] =  - v[8] + v[9] - v[10];
dxdt[15] = v[8] - v[9];
dxdt[16] = v[10];
dxdt[17] = v[11] + v[12] - v[13] - v[14] - v[15] - v[16] + v[17] + v[18] + v[19] + v[20] + 2.0*v[23] - v[34] - v[42];
dxdt[18] = v[34] + v[42];
dxdt[19] =  - v[38] + v[39];
dxdt[20] = v[38] - v[39];
dxdt[21] =  - v[35] + v[36] - v[37];
dxdt[22] = v[35] - v[36];
dxdt[23] = v[37];
dxdt[24] = 0.0;
dxdt[25] =  - v[13] + v[20] - v[22] + v[30] + v[33];
dxdt[26] = v[23] + v[32] - 2.0*v[33];
dxdt[27] = 2.0*v[21] + 2.0*v[31] - 2.0*v[33];
dxdt[28] = v[13] - v[14] + v[19] - v[20];
dxdt[29] = v[14] - v[15] + v[18] - v[19];
dxdt[30] = v[15] - v[16] + v[17] - v[18];
dxdt[31] = v[16] - v[17] - v[21];
dxdt[32] = 2.0*v[21] - v[23];
dxdt[33] = v[22] - v[24] + v[29] - v[30];
dxdt[34] = v[24] - v[25] + v[28] - v[29];
dxdt[35] = v[25] - v[26] + v[27] - v[28];
dxdt[36] = v[26] - v[27] - v[31];
dxdt[37] = 2.0*v[31] - v[32];
```



Supplementary Figure 1: Summary of the signal flow

Experimental considerations:

The experimental data is limited to time-course and dose-dependent measurements of cAMP and phosphorylated VASP. Determination of other signaling components is basically possible but not in a reliable quantitative fashion. Quantification of cyclic nuleotide concentrations and VASP phosphorylation has been established and verified by the authors with unmatched precision. For other parameters no method with comparable accuracy is available. While VASP and cAMP are recognized meaningful parameters reflecting platelet regulation by nucleotides and prostaglandins, other signaling components are known to be regulated by other pathways as well, thus limiting their applicability to this problem. Direct quantification of G-protein activation is essentially possible, however Gi or Gs protein activity is controlled by numerous enzymes unrelated or distal to cyclic nucleotide regulation. Monitoring PKA activity can be achieved by quantification of free catalytic PKA (PKAc) subunits. As we could show, PKAc subunita are in platelets not necessarily bound to the regulatory PKA subunits but may also be liberated from a NFkappaB complex. Consequently determination of free PKAc can not provide reliable information on the PKA activation state.